

Review article

Genetic regulation of homeostasis, uptake, bio-fortification and efficiency enhancement of iron in rice

S.K. Pradhan^{a,*}, E. Pandit^{a,1}, S. Pawar^a, A. Pradhan^a, L. Behera^a, S.R. Das^b, H. Pathak^a^a ICAR-National Rice Research Institute, Cuttack, Odisha, India^b Orissa University of Agriculture and Technology, Bhubaneswar, India

ARTICLE INFO

Keywords:

Biofortification
 Fe-deficiency
 Fe-toxicity
 Homeostasis
 QTLs
 Rice
 Uptake efficiency

ABSTRACT

Soils of large areas of the globe are affected by deficiency or toxicity of iron (Fe), making it one of the major limitations to higher productivity of rice. Deficiency of Fe, an essential micronutrient for growth and development of rice, produces grain with low Fe-content. Consumption of low-Fe rice causes malnutrition affecting human health. Biofortification is an easy and low-cost way to enhance Fe content in rice, the staple food of more than half of the global population. Identification of relevant quantitative trait loci (QTLs) and genes controlling the stresses are needed for developing tolerant genotype(s). Fe deficiency is commonly observed in alkaline and aerobic soils, while toxicity is seen in low pH soils of lowland rice ecology. Rice plants cope up under deficiency or toxicity conditions through various morphological, physiological and differential gene expression strategies. Rice plant uses various transporter genes like *OsNAS1*, *OsNAS2*, *OsIRT1*, *OsIRT2*, *OsNRAMP1*, *OsYSL15* and *OsYSL16* under deficiency stress while *OsIRT1*, *OsFRO2*, *OsVIT1*, *OsVIT2*, *OsNRAMP6*, *OsNAAT1*, *OsNAS3*, *OsNAC4*, *OsNAC5* and *OsNAC6* under toxicity condition are involved for Fe homeostasis. Several QTLs including *qFe3-1*, *qFe3-2*, *qFe7-1*, *qFe9-1*, *qFe9-2*, *qFe10-1*, *Fe11-1*, *qFe3.3* and *qFe7.3* associated with grain-Fe content have been identified. Many Fe binding and transporters genes like *OsZIP1*, *OsHMA4*, *OsACA2*, *OsZIP2*, *OsCNGC*, *OsZIP3*, *OsZIP5*, *OsZIP9*, *OsHma2*, ABC transporter, *OsNAS3*, heavy metal transporter, Chy zinc finger and *OsACA9* have been identified to improve grain-Fe content. Donor lines for grain-Fe content have been identified from rice germplasms showing even up to $147 \mu\text{g g}^{-1}$ in brown rice. Fe content in rice grain has been enhanced to many folds using ferritin genes of soybean and common bean, NAS gene and mugenic acid synthase genes (*HvNAS1* and *HvNAAT-A,-B* or *IDS3*) of barley, nicotianamine transporter gene (*OsYSL2*) and nicotianamine synthase genes (*OsNAS1*, *OsNAS2* and *OsNAS3*) through transgenic approach. The paper analyses the mechanisms of tolerance to Fe-deficiency and toxicity, identification of genes/QTLs responsible for tolerance under the stresses and helping for biofortification, assesses the stress affected symptoms, reviews the screening and summarizes the efforts for breeding programs for improving tolerance to Fe-deficiency and toxicity in rice.

1. Introduction

Iron (Fe) is an essential micronutrient for growth, development and higher grain yield of rice (Wu et al., 2014; Aung et al., 2019; Zhang et al., 2018; Pradhan et al., 2020). It plays an important role in chloroplast development, chlorophyll synthesis, photosynthesis, nitrogen metabolism, respiration, enzymatic redox reactions and acts as an important electron donor (Taiz and Zeiger, 1991; Marschner, 1995; Sahrawat, 2005; Kobayashi and Nishizawa, 2012; Rout and Sahoo, 2015; Zhang et al., 2017). Abiotic stresses due to deficiency or excess of available Fe in soil cause nutritional disorder in rice plants. Fe-related soil stresses are commonly seen in many rice growing countries of the

world. Deficiency of this nutrient in human also results serious health issues. More than two billion people suffer from Fe deficiency annually, which results in mortality of about 0.8 million people in the world (Stoltzfus and Dreyfuss, 1998; Wessells and Brown, 2012; Arcanjo et al., 2013; Simbauranga et al., 2015; Freitas et al., 2016; Trijatmiko et al., 2016; WHO, 2016; Dos Santos et al., 2017; Swamy et al., 2018). More than half of the world population, mostly from developing countries, is suffering from bioavailable micronutrient deficiencies (Seshadri, 2001; Shahzad et al., 2014; Mahender et al., 2016; Sarma et al., 2018). Micronutrient supplementation, food fortification and biofortification are the common ways to overcome this deficiency related problems. Fe-biofortification in rice will be a useful strategy to solve the problem of

* Corresponding author.

E-mail address: pradhancrri@gmail.com (S.K. Pradhan).¹ Authors contributed equally.

Fe-deficiency for the poor people in developing countries, for whom rice is the staple food (Nakandalage et al., 2016; Swamy et al., 2016; Trijatmiko et al., 2016).

High yielding modern rice varieties are poor in grain-Fe content (Zimmermann and Hurrell, 2002; Brinch-Pedersen et al., 2007; Mahender et al., 2016). However, genetic variations exist for Fe content in grains of rice germplasm (Yang et al., 1998; Jiang et al., 2008; Jahan et al., 2013; Zeng et al., 2010; Mahender et al., 2016; Trijatmiko et al., 2016). Commonly grown rice varieties contain only 7–8 $\mu\text{g g}^{-1}$, however, few germplasm lines contain up to 147 $\mu\text{g g}^{-1}$ in brown rice (Zeng et al., 2010). Fe content in rice grain can be enhanced many folds using transgenic approach. Once stable transgenic donor line is obtained, the increased Fe-content can be transferred to high yielding varieties through molecular breeding approaches (Masuda et al., 2013).

The deficiency of Fe is a major constraint for achieving higher yield in rice under alkaline calcareous soil with higher pH (Marcher, 1995; Kim and Guerinot, 2007). About 30% of the global soils are alkaline in nature which suffer from Fe-deficiency. Rice grown in such soils produces low yield with poor quality (Abadia et al., 2011; Swamy et al., 2018). Low availability of Fe in soils often causes leaf chlorosis and less photosynthesis, leading to reduction in yield and quality of rice. However, tolerant rice plants cope up with the soil deficiency condition through various morphological, physiological and differential gene expression strategies. Under such insufficient soil Fe condition, two absorption strategies have been developed by rice plants. Rice root shows symptoms of cluster roots and swelling of apical root tips under deficiency of iron (Schmidt et al., 2000; Schikora and Schmidt, 2002). Again, the practice of consuming polished rice reduces Fe content in the grains.

Accumulation in higher concentration of Fe is toxic to rice plant (Howeler, 1973; Prade et al., 1993; Jugsujinda and Patrick, 1993; Genon et al., 1994; Audebert, 2006; Becker and Asch, 2005; Chérif et al., 2009; Audebert and Fofana, 2009; Stein et al., 2009; Sikirou et al., 2015, 2016; Zhang et al., 2015, 2016; Zhang et al., 2018). Rice rhizosphere is considered as the first line of defence against excess Fe-uptake. Toxicity is commonly observed in lowland rice ecology showing abundance of soluble ferrous (Fe^{2+}) in soil. It generates reactive oxygen species (ROS) and hydroxyl radicals (OH) under the toxic situation. These compounds damage the rice plants there by reduce grain yield. The problem is very severe in West and Central African countries (Sikirou et al., 2015; Oort, 2018). The toxicity is also observed in Burundi, Benin, Ivory Coast, Burkina Faso, Niger, Gambia, Guinea, Guinea-Bissau, Liberia, Nigeria, Senegal, Sierra Leone, Togo, India, China, Indonesia, Malaysia, Thailand, Philippines, Sri Lanka, Colombia, Vietnam and Brazil (Moorman and Van Breeman, 1978; Oort, 2018). The area coverage with Fe-rich soils in Africa is estimated to be about 427 million ha (Oort, 2018). Rice yield loss due to this problem in Africa varies from 12% to 100% (Audebert and Sahrawat, 2000). In India, around 2 million ha of rice area are affected by Fe-toxicity (Prasad et al., 2020). Parts of Odisha, Kerala, Tamil Nadu and north-eastern states of India face this problem (Pawar et al., 2017).

Biofortification usually refers to the process of enriching food grains for nutritive elements in crop plants through genetic approaches. This way of enriching food grains is an easy, effective and cheaper way to supplement micronutrient deficiencies in cereal crops and much cheaper to grains fortification. The popular high yielding varieties are poor in essential micronutrients and need to be biofortified to enhance the nutritive value. Adequate variability for Fe-content exists in natural rice population. By over-expressing *ferritin* gene, Fe-content was increased to many folds in transgenic rice lines compared to the control genotypes. Transgenic rice lines with higher Fe-content derived from other crops are already available for successful crop improvement programme (Goto et al., 1999; Lee et al., 2009; Ogo et al., 2011; Kobayashi and Nishizawa, 2012; Lee et al., 2012; Paul et al., 2012; Bashir et al., 2013; Masuda et al., 2013; Slamet-Loedin et al., 2015; Trijatmiko et al., 2016; Boonyaves et al., 2017). These transgenic lines

may be useful as potential donors by the rice breeders for enhancing of grain-Fe content in popular rice varieties through molecular breeding approach. Micronutrients improvement may not be antagonistic to grain yield enhancement in rice as there is the presence of separate genomic regions controlling micronutrient traits in major crops (Welch and Graham, 2004; Pradhan et al., 2019, 2020).

2. Fe uptake mechanisms in rice plants under deficiency and toxicity conditions

2.1. Fe uptake mechanisms from rhizosphere to rice roots

Fe is commonly available in ferric (Fe^{3+}) and ferrous (Fe^{2+}) forms in soil. This element is abundantly found in aerobic soils as Fe^{3+} polymeric insoluble form, which is not available for uptake by rice plants and causing Fe-deficiency. Under alkaline soil, Fe^{2+} oxidizes to Fe^{3+} during electron transfer process. Deficiency of this element results in reduction of non-heme Fe-proteins involved in photosynthesis, N_2 fixation and respiration (Taiz and Zeiger, 1991). It also reduces functioning of Fe-containing enzymes that generates less chlorophyll and ultimately produces leaf chlorosis (Reinbothe et al., 2006). Expression analysis of affected rice tissues showed down regulation of chlorophyll-binding proteins and other photosynthetic components under low supply of this nutrient (Thimm et al., 2001). Chloroplasts are main target site of Fe-deficiency, which results low photosynthetic activity and pigments formation under such conditions (Winder et al., 1995).

Solubility of Fe^{3+} is higher in lowland rice ecology wherein Fe^{3+} is converted to Fe^{2+} , a form available to plants. Under such conditions, excess uptake of Fe causes Fe-toxicity to rice plant (Howeler, 1973; Jugsujinda and Patrick, 1993; Prade et al., 1993; Genon et al., 1994; Becker and Asch, 2005; Audebert, 2006; Audebert and Fofana, 2009; Chérif et al., 2009; Stein et al., 2009; Sikirou et al., 2015, 2016; Zhang et al., 2018). The toxicity increases with increase in Fe^{2+} content in acid soil with low redox potential. Fe toxicity is commonly seen in heavy soils, peat, acidic clay, acid sulphate, poorly drained sandy soils, infertile and light-textured soils (Sahrawat, 2005; Becker and Asch, 2005). Leaf bronzing occurs because of accumulation of poly phenol oxides in leaves in the affected plants. The Fe^{2+} ions are almost immobile in the plant tissues, accumulation of which increases bronzing in the leaves. Soil with Fe concentration of 10 to 500 ppm produces toxicity in rice depending on other favorable factors in the soil (Tanaka and Loe, 1966; Bode et al., 1995). Soil acidity, increased content of Fe^{3+} and its reactivity, poor drainage system in the field, low temperature of soil, salt content, inflow of Fe^{3+} or Fe^{2+} from adjacent fields are main factor of Fe toxicity in rice (Ponnamperuma, 1976). High Fe toxicity is also reported during dry period because of high vapor pressure deficit than that of wet period (Sahrawat and Singh, 1998; Asch et al., 2005). Under Fe-toxicity condition, precipitation of Fe^{2+} occurs in the root epidermis of rice plant, which decline the uptake of other minerals like magnesium, calcium, potassium and phosphorus (Fang and Kao, 2000; Sahrawat, 2005). The soluble Fe available in the rhizosphere is taken up by root cortex intracellular space and casparian strips transporting the ions to xylem vessels (Stephan, 2002). Localization of high Fe^{2+} concentration produces hydrogen peroxide in cell organelles like mitochondria and chloroplast that accelerates the Fenton reaction to generate reactive hydroxyl radicals and hydroxides (Bacana et al., 1998; Quinet et al., 2012). These reactive radicals damage biomolecules and their metabolic products, inactivate enzymes, damage membranes, peroxidation of lipid and modify the nucleic acid strands resulting in cell death (Bode et al., 1995; Quinet et al., 2012).

There are two main strategies for Fe uptake, namely reducing strategy and chelating strategy (Fig. 1). The major differences in both the strategies are oxidation state of absorbed Fe: ferrous (Fe^{2+}) is for strategy I and ferric (Fe^{3+}) for strategy II (Romheld and Marschner, 1986). Non-leguminous plants adapt to the strategy I in which Fe^{3+} reduces to Fe^{2+} performed by FRO (Ferric reductase oxidase) activity,

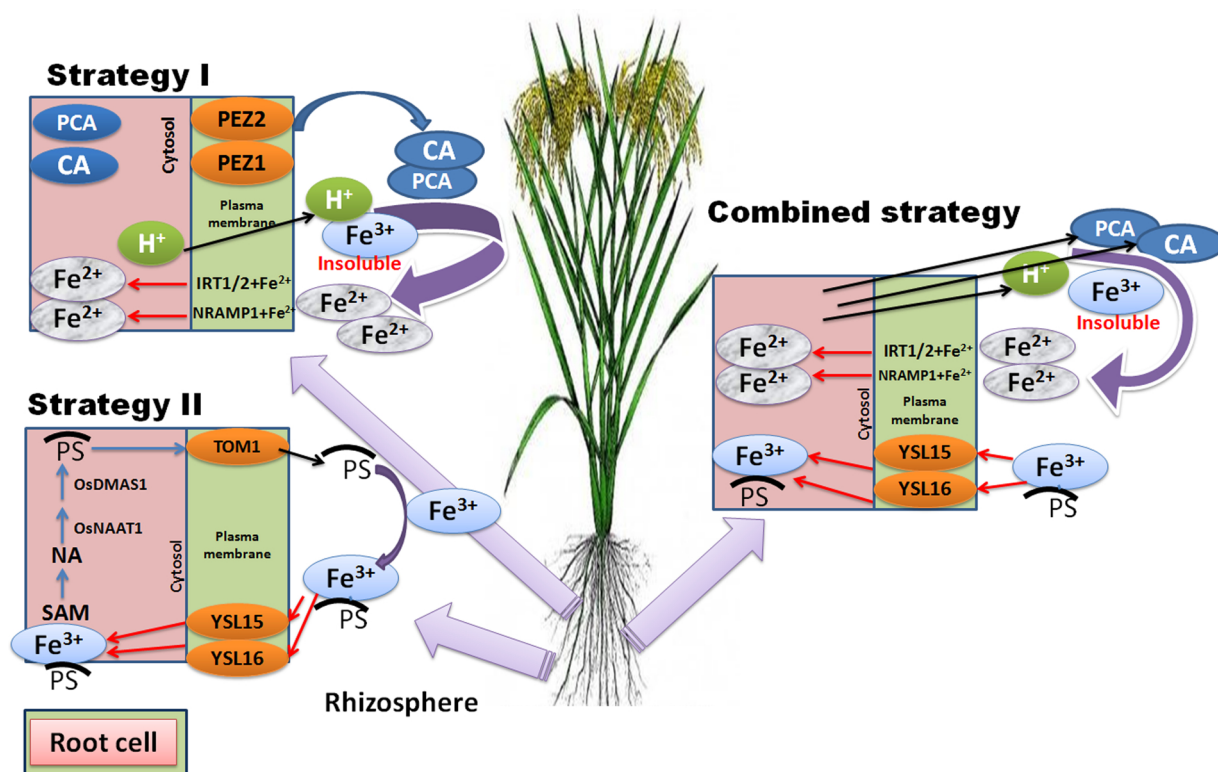


Fig. 1. Schematic diagram for Fe uptake mechanisms in roots through strategy I, Strategy II and combined strategy in rice. Part of the figure is adopted from Bashir et al., 2013 and Kobayashi and Nishizawa, 2014. PS: Phytosiderophores; IRT: Fe-regulated transporter; Tom: Transporter of mugineic acid; SAM: S-adenosyl methionine; DMA: 2-deoxy-mugineic acid; YSL15: Yellow stripe-like 15; YSL16: Yellow stripe-like 16; PCA: Protocatechuic acid; CA: Caffeic acid.

induced on the root surface under Fe deficiency situations and the uptake takes place through the plasma membrane of roots. In rice, very low ferric-chelate reductase activity is observed in response to Fe deficiency conditions (Ishimaru et al., 2006). The crop is adapted to anaerobic conditions wherein Fe²⁺ is abundant and direct uptake of this form occurs without active ferric-chelate reduction. Expression analysis reveals the role of many Fe²⁺ transporters in the plasma membrane of rice roots, namely *OsIRT1*, *OsIRT2*, *OsNRAMP1* and *OsNRAMP5* (Ishimaru et al., 2006; Takahashi et al., 2011; Ishimaru et al., 2012; Ogo et al., 2014). Amongst the Fe-transporters, *OsIRT1* is the main transporter for Fe uptake in rice (Ishimaru et al., 2006). Transcriptome analysis shows up regulation of *OsIRT1*, *OsIRT2* and *OsNRAMP1*, but not *OsNRAMP5* under Fe deficiency in soil. High expression of IRT1 and FRO is observed in root epidermal layer of rice plant (Vert et al., 2002; Dinneny et al., 2008). These divalent cation transporters belong to zinc and Fe permease family (ZIP) (Eckhardt et al., 2001; Guerinot, 2001). Rice plant produces protons and many phenolic compounds in the rhizosphere under Fe deficiency which increases Fe solubility and FRO activity under strategy I (Romheld and Marschner, 1986; Rodríguez-Celma et al., 2013; Fourcroy et al., 2014; Schmidt and Eickhorst, 2014). Rice plant has phenolics efflux transporters (*PEZ1* and *PEZ2*) response for secretion of phenolics under Fe deficiency in soil (Bashir et al., 2011; Ishimaru et al., 2011). Higher expression of transporter *PEZ2* is observed in the plasma membrane of root epidermis and associated with secretion of protocatechuic acid and caffeic acid into rice root rhizosphere (Bashir et al., 2011; Ogo et al., 2014). These phenolic compounds possess chemical properties for chelating and reducing Fe³⁺ to Fe²⁺ for its uptake (Yoshino and Murakami, 1998).

In strategy II, rice roots absorb Fe and transport it through symplast, xylem and phloem. The chelating molecules are attached to the oxidized Fe in symplast (Miroslav, 1998; Kim and Guerinot, 2007). Chelating molecules viz., mugineic acid (MA), nicotianamine and citrate

facilitate transportation of Fe. High expression of nicotianamine molecule was detected in the phloem tissue suggesting the role of the compound for Fe transport. The MAs are present near the periphery of root surface and are strong Fe chelators called phytosiderophores (PS) which are secreted by roots in the rhizosphere and binds to Fe³⁺ ions (Takagi, 1976; Takagi et al., 1984; Takagi et al., 1988). The Fe³⁺-PS complex is further transported to root cells by different tissue specific transporters (Römheld and Marcher, 1986; Von Wiren et al., 1994). There are few protein transporter molecules namely, ITP (Fe transport protein) and dehydrins (DHN) which ligate to Fe³⁺ but not Fe²⁺ to facilitate transfer of non-soluble Fe complex (Hell and Stephan, 2003; Morrissey and Guerinot, 2009). Transporter of mugineic acid (TOM1) mediates the 2-deoxy-mugineic acid (DMA) secretion through the plasma membrane (Nozoye et al., 2011). The Fe³⁺-DMA complex formed in the rhizosphere is taken up into root cells by *OsYSL15* transporter (Fig.1; Table 1) (Inoue et al., 2009; Lee et al., 2009). *OsYSL16* is another Fe³⁺-DMA transporter expressed in the plasma membrane of root epidermis of rice suggesting its role in Fe³⁺-DMA uptake from the rhizosphere (Kakei et al., 2012; Lee et al., 2012). All these transporters and enzymes for DMA-based Fe uptake are highly up regulated under Fe deficiency conditions to meet the higher Fe requirement (Table 1; Fig. 3). However, in contrast to the strong induction of *OsYSL15*, expression of the *OsYSL16* gene is observed to be constitutive and induced under Fe deficiency, indicating that Fe³⁺-DMA uptake is mainly mediated through *OsYSL15*. In addition to Fe (III)-DMA uptake, rice plant also synthesizes non-proteinogenic amino acids by three molecules of S-adenosyl methionine (SAM). Rice plants adopt combined strategy of both the strategies I and II while grass family adopt only strategy II for Fe uptake (Fig. 1). However, in a comparative transcription analysis using *Oryza* genus revealed about the adoption of the combined strategy by *Oryza sativa* group containing AA genome only (Wairich et al., 2019). Ethylene production is not detected in strategy II for Fe uptake while it plays important role in strategy I and

Table 1
Genes/QTLs useful for higher Fe uptake and transport in rice plant under soil Fe-deficiency condition.

Sl.	QTLs/ transporters	Name	Function	Reference
1	<i>OsNAS1</i>	Nicotianamine synthase	DMA biosynthesis for Fe(III)-DMA uptake/translocation	Higuchi et al., 2001; Inoue et al., 2003; Bashir et al., 2006; Cheng et al., 2007
2	<i>OsNAS2</i>	Nicotianamine synthase	DMA biosynthesis for Fe(III)-DMA uptake/translocation	Higuchi et al., 2001; Inoue et al., 2003; Bashir et al., 2006; Cheng et al., 2007
3	<i>OsNAS3</i>	Nicotianamine synthase	DMA biosynthesis for Fe(III)-DMA uptake/translocation	Higuchi et al., 2001; Inoue et al., 2003; Bashir et al., 2006; Cheng et al., 2007
4	<i>OsNAAT1</i>	Nicotianamine aminotransferase	DMA biosynthesis for Fe(III)-DMA uptake/translocation	Higuchi et al., 2001; Inoue et al., 2003; Bashir et al., 2006; Cheng et al., 2007
5	<i>OsDMAS1</i>	Deoxymugic acid synthase	DMA biosynthesis for Fe(III)-DMA uptake/translocation	Higuchi et al., 2001; Inoue et al., 2003; Bashir et al., 2006; Cheng et al., 2007
6	<i>TOM1</i>	DMA efflux transporter	Transporters for Fe(III)-DMA uptake/translocation	Nozoye et al., 2011
7	<i>OsYSL15</i>	Fe(III)-DMA transporter	Transporters for Fe(III)-DMA uptake/translocation	Inoue et al., 2009; Lee et al., 2009
8	<i>OsYSL16</i>	Fe(III)-DMA transporter	Transporters for Fe(III)-DMA uptake/translocation	Kakei et al., 2012;
9	<i>OsSAMS1</i>	S-adenosyl-L-methionine synthetase	Methionine cycle for Fe(III)-DMA uptake/translocation	Lee et al., 2012
10	<i>OsSAMS2</i>	S-adenosyl-L-methionine synthetase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
11	<i>MTN</i>	Methylthioadenosine/S-adenosyl Homocysteine nucleosidase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
12	<i>OsMTK1</i>	Methylthioribose kinase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
13	<i>OsMTK2</i>	Methylthioribose kinase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
14	<i>OsID2</i>	Methylthioribose-1-phosphate isomera	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
15	<i>DEP</i>	Methylthioribulose-1-phosphate dehydratase-enolase-phosphatase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
16	<i>OsDII/OsARD2</i>	Acireductonedioxygenase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
17	<i>OsDIII/OsARD1</i>	Acireductonedioxygenase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
18	<i>OsD14</i>	Aminotransferase catalyzing the synthesis of methionine	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
19	<i>OsAPT1</i>	Adenine phosphoribosyltransferase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
20	<i>PRPPS</i>	Phosphoribosyl pyrophosphate synthetase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
21	<i>RPI</i>	Ribose 5-phosphate isomerase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
22	<i>FDH</i>	Formate dehydrogenase	Methionine cycle for Fe(III)-DMA uptake/translocation	Kobayashi and Nishizawa, 2014
23	<i>OsIRT1</i>	Ferrous Fe transporter	Transporters for ferrous Fe uptake/translocation	Ishimaru et al., 2006; Ogo et al., 2014
24	<i>OsIRT2</i>	Ferrous Fe transporter	Transporters for ferrous Fe uptake/translocation	Ishimaru et al., 2006; Ogo et al., 2014
25	<i>OsNRAMP1</i>	Ferrous Fe transporter	Transporters for ferrous Fe uptake/translocation	Ishimaru et al., 2006; Ogo et al., 2014
26	<i>OsNRAMP5</i>	Ferrous Fe/manganese/cadmium transporter	Transporters for ferrous Fe uptake/translocation	Ishimaru et al., 2006; Ogo et al., 2014
27	<i>PEZ2</i>	Phenolics efflux transporter	Transporters for Fe translocation	Ishimaru et al., 2011; Bashir et al., 2011
28	<i>OsYSL2</i>	Fe(II)/manganese(II)-NA transporter	Transporters for Fe translocation	Koike et al., 2004;
29	<i>ENA1</i>	NA efflux transporter	Transporters for Fe translocation	Ishimaru et al., 2010
30	<i>ENA2</i>	NA efflux transporter	Transporters for Fe translocation	Nozoye et al., 2011
31	<i>OsFRL1</i>	Citrate efflux transporter	Transporters for Fe translocation	Nozoye et al., 2011
32	<i>PEZ1</i>	Phenolics efflux transport	Transporters for Fe translocation	Yokosho et al., 2009
33	<i>OsVT1</i>	Fe transporter into vacuole	Transporters for subcellular Fe sequestration	Ishimaru et al., 2011; Bashir et al., 2011a
34	<i>OsVT2</i>	Fe transporter into vacuole	Transporters for subcellular Fe sequestration	Zhang et al., 2012
35	<i>OsHRZ1</i>	Ubiquitination activity and accumulate more Fe in shoot and grain	negative regulator of various Fe deficiency-inducible genes	Zhang et al., 2012
36	<i>OsHRZ2</i>	Ubiquitination activity and accumulate more Fe in shoot and grain	negative regulator of various Fe deficiency-inducible genes	Kobayashi et al., 2013
37	<i>OsRub6a</i>	Small GTPase for Fe acquisition	Modulator for Fe acquisition by up-regulation of OsIRO2, OsIRT1, OsNAS1 and OsNAS2	Yang and Zhang, 2016
38	<i>OsMT2D</i>	Metallothionein gene		Desalvosa et al., 2018

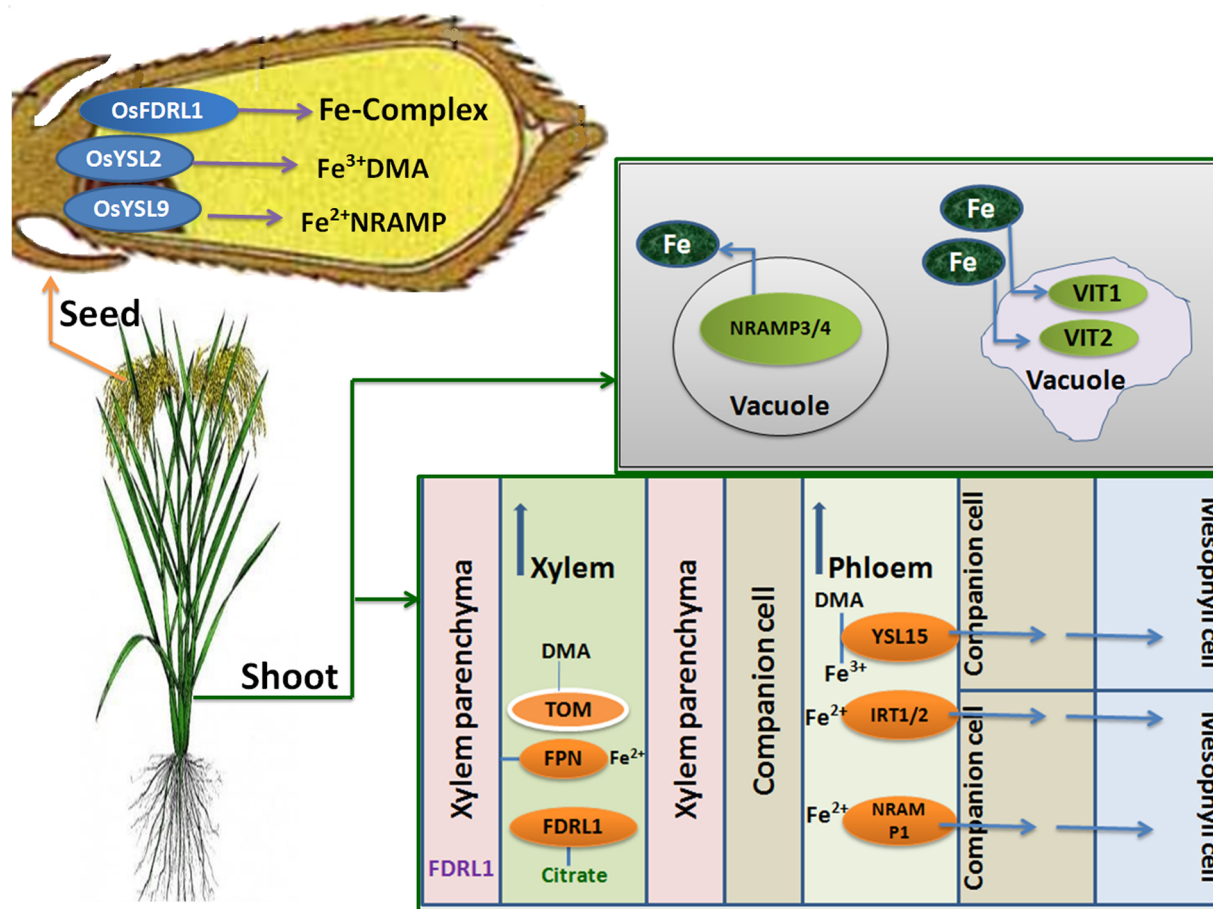


Fig. 2. Schematic diagram for Fe- transport from roots to shoots and grains in rice plant. Involvement of Ferric Reductase Defective, Ferroportins and nicotianamines for transport from roots to shoots through xylem. Fe is transported to vacuoles by VIT and efflux from vacuole by NRAMP3/4. Fe-complex transport to seeds is mediated by Yellow Stripe-Like transporters.

its production increases during Fe deficiency condition to roots (Romera et al., 1999, 2011).

2.2. Fe uptake mechanisms from roots to shoots and other plant parts

The upward movement of Fe ions from root cytosol to shoots and other plant parts takes place through xylem and phloem (Fig. 2). Few organic acids of xylem namely, citrate, malate, and succinate increase its content in response to Fe-deficiency situations (López-Millán et al., 2000). The transport of iron through xylem to shoots mainly happens in the form of Fe^{3+} -citrate complex (Von Wiren et al., 1999; Durrett et al., 2007; Rellán-Álvarez et al., 2008; Rellán Álvarez et al., 2010). The movement of Fe-citrate complex through xylem mediated by transporter, FRDL1 which is an ortholog of ferric reductase defective 3 (FRD3) in *Arabidopsis* (Yokosho et al., 2016). Pericycle and adjoining cells near to vascular bundle contain the ferric reductase defective enzyme which is essential for movement of the Fe-complex (Green and Rogers, 2004). The mutant rice plants with *Osfrdl* showed less citrate and Fe content in the xylem vessels and also the phenotype of the plant was like that under the Fe-deficiency condition (Yokosho et al., 2009). Ferroportins (FPN1) mediates the loading of iron into xylem vessel (Morrissey et al., 2009). The translocation of Fe through xylem occurs in the form of Fe-nicotianamine and Fe-MAs which also contributes to the precursors of PS (Inoue et al., 2003; Bonneau et al., 2016).

Movement of Fe from xylem, it travels via apoplastic space to reach leaves. In the phloem-based transfer of Fe, the complex forms of NA and DMA are also observed (Curie et al., 2009). The movement of Fe from apoplast to symplast is mediated by the metal-NA transporters, namely

AtYSL1, *AtYSL2* and *AtYSL3* (Waters et al., 2006; Didonato et al., 2004). These transporter genes show in the higher expression in the parenchymatous cells of vascular bundles in leaves (Waters et al., 2006; Didonato et al., 2004). *OsYSL2* transporter is involved in transfer of the Fe-NA chelate to shoots and seeds (Koike et al., 2004; Ishimaru et al., 2010). The transport of Fe (III)-MA is facilitated by *OsYSL16* (Kakei et al., 2012). The other Fe-complex transport is performed in the reproductive organs and phloem of lamina joints by *OsYSL18* (Aoyama et al., 2009). However, Fe (II)-NA and Fe (III)-DMA complex forms are also reported to be transported to seeds by *OsYSL9* (Senoura et al., 2017). It is also presumed that the recirculation of Fe ions from leaves to developing tissues are made by oligo peptide transporter 3 (OPT3) transporter (Wintz et al., 2003; Mendoza-Cózatl et al., 2014).

3. Strategies of maintaining Fe-toxicity tolerance by rice plants

Increased metabolism of scavengers protein in tolerant plants helps avoiding oxidative stress in the plants. For scavenging of hydroxyl radicals and ROS, plant produces wide varieties of phenolic compounds scavengers such as tannins, lignin and flavonoids (Blokchina et al., 2003). Plant store Fe^{2+} in leaf sheath which is harmful for photosynthesis and yield (Engel et al., 2012). Excess metal ions are stored in the vacuole and are released during deficiency condition (Moore et al., 2014). The following Fe-toxicity tolerance mechanisms have been adopted by rice plants.

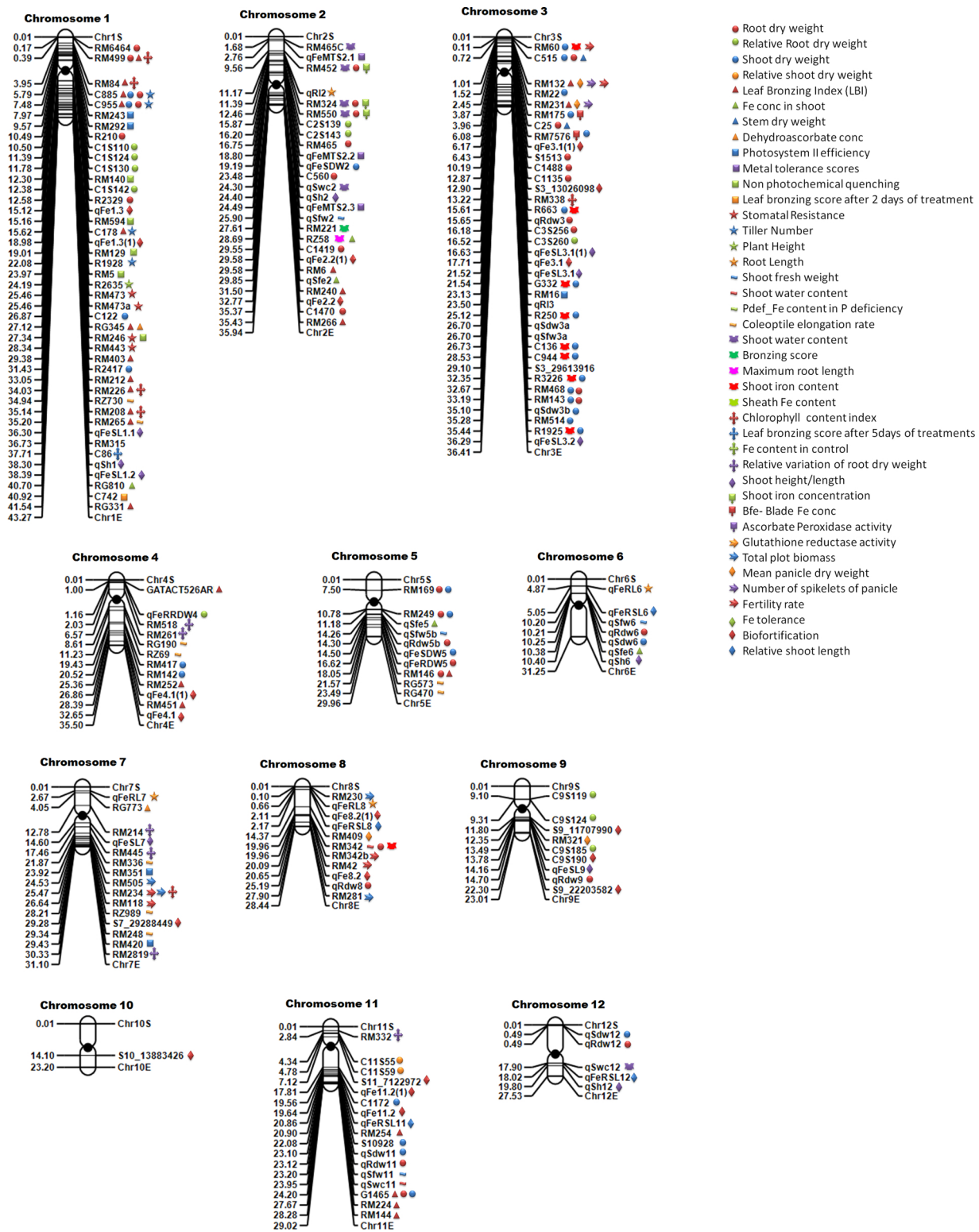


Fig. 3. Physical position of genes/QTLs involved in deficiency, toxicity and biofortification for homeostasis of Fe in rice.

3.1. Oxidation of Fe²⁺ at root surface

It favours low availability of Fe²⁺ in soil solution. This type of mechanism is exclusion of Fe²⁺ by rhizosphere. Root oxidizing power by the tolerant genotype by release of oxygen or enzymatic oxidation is responsible for precipitation of Fe²⁺ in the rhizosphere and excludes excess Fe²⁺ uptake into rice plants (Green and Etherington, 1977; Ando et al., 1983). The root oxidation power of the tolerant plant is more

than susceptible one due to the presence of larger primary root and shoot diameter of the pith cavity in tolerant one that favors better oxygen transport from root to shoot (Wu et al., 2014). This increases the absolute volume of aerenchyma that facilitates the process of oxygen transport. Presence of higher density of lateral fine roots increase the root oxidation power thereby better exclusion of Fe²⁺ in tolerant genotype. This is already established fact that aerenchyma helps in oxygen movement by creating low resistance for air movement within

the roots (Colmer, 2002). A QTL (*qFETOX-3*) is involved in controlling the Fe exclusion from rice roots (Wu et al., 2014). In strategy I, the movement of Fe^{2+} from rhizosphere is mediated by *OsIRT1* for acquisition of the micronutrient (Ishimaru et al., 2006). This Fe-regulated transporter gene, *OsIRT1* is located within the QTL, *qFETOX-3* region on chromosome 3. This gene ID, Os03g0667500 could be annotated from in this QTL region from rice annotation database (RAP-DB, <http://rapdb.dna.affrc.go.jp/index.html>).

3.2. Exclusion of Fe inside the root surface

This mechanism favors inclusion of Fe but stores it in a non-reactive form as in case of 'Ferritin' molecule in rice. Ferritin is a candidate protein that keeps upto 4000 Fe atoms in a safe and bio available form (Briat et al., 1999, Briat et al., 2010). This helps in avoidance of transport of Fe storing it internally. Presence of Fe storage in plant tissues as high-molecular-mass may be a possible mechanism found in the tolerant plants (Stein et al., 2009; da Silveira et al., 2009). The QTL on chromosome 1, *qFETOX-1-2* showed shoot based tolerance and two genes having IDs Os01g0878800 and Os01g0878900 are annotated for molecular functions for Fe^{2+} binding by rice annotation database.

3.3. Retention of Fe by roots

In this step of tolerance mechanism, there is retention of Fe in roots and declines their translocation to different plant parts. Decline in Fe^{2+} uptake may possibly to avoid production of reactive oxygen species (ROS) by Fenton reaction due to accumulation of more Fe^{2+} . By this retention strategy, the genes controlling Fe-uptake and transport viz., *OsIRT1*, *OsIRT2*, *OsNRAMP1*, *OsYSL2*, and *OsYSL15*, especially in roots are suppressed during Fe-toxicity condition. The transporter *TOM1*, *TOM1*-like and *ENA1* were highly suppressed in roots. *ENA1*, an efflux transporter of NA and MA transporter genes (*OsNAS1*, *OsNAS2*, *OsNAAT1*, and *OsDMAS1*) exhibited clear suppression in the roots (Aung et al., 2018). The vacuolar transporter, *OsVIT2* and the oxygen and electron transfer related genes are highly induced in root tissues under excess Fe conditions (Aung et al., 2018). Fe-regulated transporter-like protein (*ZIP*) and *OsZIP5* genes are also induced in roots under Fe excess conditions (Aung et al., 2018). Thus, Fe uptake is restricted during iron toxicity situation of the rice field. However, the scavenging molecules like ascorbate, reduced glutathione and antioxidants namely, superoxide dismutase (SOD), catalase, peroxidase and poly phenol oxidase (PPO) generated by rice plants protects them from the ROS fatal effects (Bode et al., 1995; Fang and Kao, 2000; Fang et al., 2001; Becker and Asch, 2005; Gallie, 2013). Studies on contrasting genotypes for Fe-toxicity tolerance conducted revealed that lower dehydroascorbate reductase and higher ascorbate oxidase are detected in tolerant genotypes during Fe-toxicity stress (Wu et al., 2016).

3.4. Enzymatic detoxification of elevated Fe^{2+} within symplast

Reactive oxygen species scavengers like SOD are prominent reducer of high Fe toxicity effects (Majerus et al., 2009). The cofactor of SOD oxidizes the process for dismutation of super oxide radicals. Reduction of one electron generates O_2 and H_2O_2 at the molecular level. Concentration of O_2 and H_2O_2 determines the concentration of SOD (Muscoli et al., 2003; Selote et al., 2004). The excessive production of H_2O_2 may affect SOD activity under stress condition viz., Fe-SOD and Cu/Zn-SOD isoform (Alscher et al., 2002). High concentration of O_2 and H_2O_2 indicates the presence of tolerance mechanism. Under Fe-toxicity condition, *ENA1* was strongly suppressed in roots, but *ENA2* was not suppressed. NA synthesized by *OsNAS3* and transported by *ENA2* protects the cell from oxidative damage and plays an important role in scavenging the Fe detoxification (Von Wiren et al., 1999; Aung et al., 2018). The transcription factors viz., *OsNAC4*, *OsNAC5* and *OsNAC6* are induced in rice roots at higher Fe levels in the field. *OsNAC4* controls

programmed cell death and induces under excess Fe. Over expression of *OsNAC5* confers drought, cold, high salinity, abscisic acid (ABA), and methyl jasmonic acid stress tolerance in rice (Takasaki et al., 2010). In addition, *OsNAC6* is induced by drought, high salinity, and ABA treatment (Nakashima et al., 2014). Thus, *OsNAC4*, *OsNAC5* and *OsNAC6* genes are responsive to Fe toxicity stress and play role in Fe homeostasis in rice.

4. Enhancing Fe uptake in rice using genes/QTLs under Fe-deficiency situations

Fe-deficiency stress to plants can be tolerated by improving the transporter genes. Two transporter genes, *OsIRT1* and *OsYSL15* are used to increase the Fe concentrations in seeds produced under low Fe availability condition by over expression approach (Table 1). Expression of transporter gene, *OsYSL15* increases the level of Fe^{3+} -MAs under Fe-deficiency situations to increase the uptake of Fe (Curie et al., 2001; Lee et al., 2009; Ueno et al., 2009; Li et al., 2016). Nicotianamine aminotransferase gene (*naat1*) expresses to increase the production of 2-deoxymugineic acid (DMA) for more Fe^{3+} uptake under Fe-deficiency conditions (Cheng et al., 2007). Two Fe^{2+} transporter genes, *OsIRT1* and *OsIRT2* in rice plants are highly expressed under soil Fe-deficiency condition (Ishimaru et al., 2006). Increase of ethylene in soil also indicates more acquisition of Fe^{2+} ions (Romera and Alcantara, 1994; Lucena et al., 2006; Waters et al., 2006; Giehl et al., 2009; Romera et al., 2011).

OsIRO2 (*Oryza sativa* Fe regulator 2), a transcription factor gene is up regulated during low Fe concentration and combines with expression of other genes for controlling Fe homeostasis during germination and grain filling (Ogo et al., 2007; Hindt and Guerinot, 2012; Li et al., 2016). Fe deficiency responsive elements (IDE) are positive regulator and binds to the specific sequence that promotes Fe regulation in plants. Positive Fe regulation role of IDE1 and IDE2 has been established (Kobayashi et al., 2003). Homologous sequences to IDE1 are detected in other Fe deficiency inducible promoters of genes viz., *OsNAS1*, *OsNAS2*, *OsIRT1*, *AtFRO2* and *AtIRT1*. IDE1 and IDE2 have sequences which match to *OsDEF1* and *OsDEF2* (Table 1; Fig. 3). *OsDEF1* induces positively for Fe uptake and Fe governing genes in rice viz., *OsIRO2*, *OsYSL15*, *OsYSL2*, *OsIRT1*, *OsNAS1*, *OsNAS2* and *OsNAS3* (Kobayashi et al., 2010; Li et al., 2016).

The transgenic plants obtained by insertion of *SoyFERH2* gene using *OsGLUB1* and *OsGLB* rice promoters and barley genes *Nicotianamine aminotransferase* (*HvNAAT-A* and *HvNAAT-B*) and mugineic acid synthase (*IDS3*) were tolerant to Fe-deficiency situation (Table 1). Peanut *AhIRT1*, a key transporter gene of Fe under limiting condition was transferred to rice plant using Fe-deficiency-inducible artificial promoter (Xiong et al., 2013). The transgenic lines showed more Fe uptakes from soil under Fe deficiency condition. The transgenic line containing *AtIRT1*, *AtNAS1* and *PvFERRITIN* (*PvFER*) genes in a single cassette expressed together showed increased Fe content in rice under Fe deficiency condition (Boonyaves et al., 2017).

5. Approaches for enhancing Fe tolerance under iron toxicity condition in rice

5.1. Genes/QTLs useful for enhancing tolerance under Fe toxicity condition

Genes governing tolerance to Fe toxicity have been reported in rice using various mapping populations (Table 2). Using Azucena and IR64 derived population, it was observed that leaf bronzing index (LBI) is controlled by QTL present on chromosome 1 (Dufey et al., 2009). Seven QTLs were detected for LBI on chromosome 1, 2, 7, 8 and 12 showing 99% of phenotypic variation by analyzing the mapping population of Gimbozu and Kasalath (Shimizu, 2009). These chromosomal regions were also detected earlier for leaf bronzing tolerance in rice (Wu et al., 1998; Wan et al., 2003a). Three QTLs for leaf bronzing tolerance were

Table 2
Genes/QTLs useful for Fe transport and toxicity tolerance in rice.

Sl.	Trait/	QTLs	Chromosome	Markers	Population used	Reference
1	LBI		1	RG345/RG381	BC ₁ F ₁ (Azucena) of Azucena/IR64	Wu et al., 1997
2	LBI, RDWS		1	RG220B	Azucena/IR64	Wu et al., 1997, Wu et al., 1998
3	RDWS		8	RM42	Azucena/IR64	Wu et al., 1997
4	LBI		1,3,8	C955/C885	Nipponbare/Kasalath	Wan et al., 2003a
5	LBI, RDWS		3,6,9,11	STSH163	Asominori/IR24	Wan et al., 2003b
6	LBI		1	RM315-RM212	Longza8503/IR64	Wan et al., 2005
7	LBI		2	RM6-RM240	Longza8503/IR64	Wan and Wan, 2005
8	LBI		4	RM252-RM451	Longza8503/IR64	Wan and Wan, 2005
9						Kim et al., 2006
10	LBI		1	RM034-RM246	Azucena and IR64	Dufey et al., 2009
11	LBI		1	RM443-RM403	Azucena and IR64	Dufey et al., 2009
12	LBI		1	RM265-RM315	Azucena and IR64	Dufey et al., 2009
13	LBI		2	RM254-RM224	Azucena and IR64	Dufey et al., 2009
14	LBI		2	RM224-RM144	Azucena and IR64	Dufey et al., 2009
15	Ferroporins	OsVIT1 OsVIT2	4	Os04g0463400		Dufey et al., 2009
			9	Os09g0396900		Zhang et al., 2012
16	NRAMP genes	OsNRAMP1 to OsNRAMP8	7,3,6,7,12			Victoria et al., 2012
17	Ferroporins	OsFPN1 OsFPN2				Mehlot et al., 2014
18	LBI	qFETPX-1-1	1	id1008684	IR29/Pokkali	Wu et al., 2014
19	LBI	qFETPX-1-2	1	id1021920	IR29/Pokkali	Wu et al., 2014
20	LBI		1	id1023158	IR29/Pokkali	Wu et al., 2014
21	LBI	qFETOX-2	2	id2013434	IR29/Pokkali	Wu et al., 2014
22	LBI	qFETOX-4-1	4	id4002852	IR29/Pokkali	Wu et al., 2014
23	LBI		4	id4002913	IR29/Pokkali	Wu et al., 2014
24	LBI		4	id4003259	IR29/Pokkali	Wu et al., 2014
25	LBI		4	id4003727	IR29/Pokkali	Wu et al., 2014
26	LBI	qFETOX-4-2	4	id4005867	IR29/Pokkali	Wu et al., 2014
27	LBI	qFETOX-7	7	id7000519	IR29/Pokkali	Wu et al., 2014
28	LBI	qFETOX-12	12	id12010050	IR29/Pokkali	Wu et al., 2014
29	LBI, RfE, SDW, RDW	Glutathione-S-transferase	10	RM184-RM258	O.sativa/O.glaberrima	Dufey et al., 2015a; Dufey et al., 2015b
30	LBI			LOC_Os01g49710 and LOC_Os01g49720		Mathus et al., 2015
31	LBI	OsNAATI OsYSL1	2			Finatto et al., 2015
32	Relative root dry weight	qRRDW1-1	1	C1S110-C1S124 10,514,742-11389991	Minghui 63 and Shanyou 63	Li et al., 2016
33	Relative root dry weight	qRRDW1-2	1	C1S130-C1S142 11,788,361-12387454	Minghui 63 and Shanyou 63	Li et al., 2016
34	Relative root dry weight	qRRDW2	2	C2S139-C2S143 15,875,115-1620220	Minghui 63 and Shanyou 63	Li et al., 2016
35	Relative shoot dry weight	qRRSDW11	11	C11S55-C11S59 4,342,500-4788363	Minghui 63 and Shanyou 63	Li et al., 2016
36	Relative root dry weight	qRRDW3	3	C3S256-C3S260 16,186,851-16524031	Minghui 63 and Shanyou 63	Li et al., 2016
37	Relative root dry weight	qRRDW9-1	9	C9S119-C9S124 9,104,463-9312653	Minghui 63 and Shanyou 63	Li et al., 2016
38	Relative root dry weight	qRRDW9-2	9	C9S185-C9S190 13,496,238-13788797	Minghui 63 and Shanyou 63	Li et al., 2016
39	LB	id1000223			Indonesian rice varieties	Nugraha et al., 2016
40	Shoot dry weight	qSdw3a	3	LOC_Os03g47360; LOC_Os03g47370	Genome-wide association study	Zhang et al., 2017
41	Shoot dry weight	qSdw3b	3	LOC_Os03g62050; LOC_Os03g62060; LOC_Os03g62170	Genome-wide association study	Zhang et al., 2017
42	Shoot dry weight	qSdw12	12		Genome-wide association study	Zhang et al., 2017
43	Root dry weight	qRdw3	3	LOC_Os03g47370	Genome-wide association study	Zhang et al., 2017
44	Root dry weight	qRdw5b	5		Genome-wide association study	Zhang et al., 2017
45	Root dry weight	qRdw6	6		Genome-wide association study	Zhang et al., 2017
46	Root dry weight	qRdw8	8		Genome-wide association study	Zhang et al., 2017
47	Root dry weight	qRdw9	9		Genome-wide association study	Zhang et al., 2017
48	Root dry weight	qRdw11	11		Genome-wide association study	Zhang et al., 2017
49	Root dry weight	qRdw12	12	LOC_Os09g24700	Genome-wide association study	Zhang et al., 2017
50	Shoot iron content	qSFe5	5		Genome-wide association study	Zhang et al., 2017
51	Fe storage protein & vacuolar transporter	OsVIT2	9		Genome-wide association study	Aung et al., 2018

(continued on next page)

Table 2 (continued)

Sl.	Trait/	QTLs	Chromosome	Markers	Population used	Reference
52	NAS	OsNAS3	7			Aung et al., 2019

Note: LBI- leaf bronzing index; NAS-nicotinamine synthase; RDW-root dry weight; RfFe-root plaque iron concentration; RL-root length; RDWS- relative dry weight of shoot; RRDW-relative root dry weight; RSDW-relative shoot dry weight; SFe-shoot iron concentrations; SDW-shoot dry weight; SFe-shoot fresh weight; SF-shoot height and SWC-shoot water content.

detected on chromosome 1, 3 and 8 in the backcross population of Nipponbare and Kasalath showing 11.6–18.6 % of phenotypic variation (Wan et al., 2003a). Another 3 QTLs controlling LBI were tagged from the population of Asominori/ IR24 in the marker interval of C515-XNpb279, R2638-C1263 and G1465-C950 on chromosome 3, 9 and 11, contributing 16.45%, 11.16% and 28.02% to the variation, respectively (Wan et al., 2003b). QTLs for LBI were detected in the marker region of RM315-RM212 on the 1, RM6-RM240 on the chromosome 2 and RM252-RM451 on the chromosome 4 (Wan and Wan, 2005). Another seven QTLs were reported for bronzing tolerance present on chromosome 1, 2, 4, 7 and 12 explaining 9.2–18.7% of the phenotypic variation using the mapping population of IR29/Pokkali F₈ recombinant inbred lines (Wu et al., 2014). QTLs controlling LBI, SDW and RDW were reported in the marker interval of RM184-RM258 on chromosome 10 by analyzing *O. sativa/O. glaberrima* population (Dufey et al., 2015a; Dufey et al., 2015b).

Fe toxicity tolerance is controlled by several QTLs which are induced by the surrounding Fe concentration in the rhizosphere. Research findings from earlier study reported few genes for Fe toxicity regulation viz., *OsIRT1*, *OsIRT2*, *OsFRO2*, *OsIRO2*, *OsYSL15*, *OsYSL2*, *OsNAS1*, *OsNAS2* and *OsNAS3* in rice (Narayanan et al., 2007; Banerjee et al., 2011; Aung et al., 2018) (Fig. 3). *OsIRT1*, a Fe transporter involves in the metabolic pathways of Fe uptake controls the Fe concentration and expression in roots, leaves and stems of the plant. Twelve homologs of this transporter are present in the rice genome under this ZIP (Zinc Iron Permease) family genes (Guerinot, 2000; Ishimaru et al., 2005; Lee et al., 2010). Among these genes, *OsZIP8* (Os07g0232800), *OsZIP10* (Os06g0566300) and *OsZIP14* (Os08g0467400) were up-regulated under iron toxicity condition (Finatto et al., 2015). *OsFRO2* is up regulated by high concentration of Fe²⁺ and plays role in Fe uptake and mobilization (Finatto et al., 2015). Fe storage protein & vacuolar transporter gene *OsVIT2* divert and store the excess iron in rice (Aung et al., 2018). *OsNAS3* plays important role for Fe-toxicity and induced under excess Fe conditions in the older leaves (Aung et al., 2019).

The chelation and movement of Fe³⁺ ions are facilitated by phloem nicotianamine (NA) to maintain metal homeostasis (Takahashi et al., 2003). Transportation of Fe throughout the plant is governed by *OsNAS* (Inoue et al., 2003). Natural resistance-associated macrophage protein (NRAMP) family molecules play important role in Fe transportation throughout the plant. Eight NRAMP genes, namely *OsNRAMP1* to *OsNRAMP8* are reported in rice. These macrophage protein genes use to regulate the Fe transport at sub-cellular level and later transport to vacuoles and plastids. Out of these eight genes, *OsNRAMP1* is expressed in roots; *OsNRAMP2* in leaves and *OsNRAMP3* in both roots and leaves (Belouchi et al., 1997; Curie et al., 2000; Victoria et al., 2012). Among these genes, *OsNRAMP6* (Os01g0503400) was up regulated under Fe-toxicity condition (Finatto et al., 2015). Transport of Fe to vacuole is facilitated by ferroportin (FPN) and the molecules of Vacuolar Iron Transporter (VITs) family (Morrissey et al., 2009; Zhang et al., 2012). Two transporter FPN genes were identified as *OsFPN1* and *OsFPN2* in rice (Bashir et al., 2011; Merlot et al., 2014). These ferroportins are attached to vacuolar membrane and express in seeds, embryo and young seedlings (Kim et al., 2006). Ferroportin- genes, *OsVIT1* and *OsVIT2* initiate storage of Fe in flag leaves and suppression of these genes produces more Fe concentration in the seed (Zhang et al., 2012).

A linkage disequilibrium study on Fe toxicity tolerance has revealed significant association of single nucleotide polymorphism (SNPs) with leaf bronzing score. SNP id1016768 was located close to two putative glutathione-S-transferase genes locus viz. LOC_Os01g49710 and LOC_Os01g49720. Also, SNP id2015632 and SNP id1015380 were found to be associated with tolerance but at a lower significance level (Mathus et al., 2015). Transcriptome analysis showed up-regulation of *OsNAAT1*, *OsYSL1* and *OsYSL17* under Fe stress condition (Finatto et al., 2015). These induced three genes belong to multi drug and toxic compounds extrusion (MATE) family. It may govern the reduction of ROS synthesis in mitochondria.

5.2. Managing Fe toxicity tolerance through agronomic practice

Rice rhizosphere is considered as the first line of defence against excess Fe^{2+} uptake. Rice roots produce more ethylene that helps in longer with more laterals roots. This increases aerenchymatous cells in the roots which facilitates diffusion of oxygen into roots under submerged condition. These aerenchyma cells occupy about 20% to 50% of the total root volume of rice under submerged condition for exchange of gases (Armstrong, 1979; Kawase, 1981). Rhizosphere collects oxygen from atmosphere for oxidation of Fe with the help of aerenchyma (Harahap et al., 2014). The mechanism behind the root growth under the Fe toxic condition might be related to decrease in mineral uptake at severe Fe^{2+} toxic situation (Liao et al., 2001; López-Bucio et al., 2002). Some conventional practices decrease the Fe-toxicity are good drainage to oxidize Fe, applications of P, K, Zn or Si fertilizers and lime to increase pH of soil solution (Ramirez et al., 2002; Abdoul, 2006; Fageria et al., 2008; Elec et al., 2013; Prasetyo et al., 2013; Dufey et al., 2014). But these methods are neither economically good nor practically improved. Farmers usually prefer Fe toxicity tolerant rice varieties than management practices due to more cost and lower rate of success.

6. Approaches for Fe biofortification in rice

6.1. Genes/QTLs identified for enhancing grain Fe content

A wide genotypic variation for grain Fe content is available in rice germplasms (Qui et al., 1995; Ahmed et al., 1998; Graham et al., 1999; Gregorio et al., 2000; Zhang et al., 2005; Anuradha et al., 2012a, b; Mahender et al., 2016; Swamy et al., 2016). Increasing the iron content in rice grain through breeding approach is a cheaper way and an easier option. Knowledge on genes/QTLs controlling grain Fe is pre-requisite for enhancement of the trait. Few reports on mapping results on grain-Fe content are available. Three loci on chromosomes 7, 8, and 9 explaining 19–30% phenotypic variance (PV) for Fe content were reported by Gregorio et al. (2000). A QTL on chromosome 2 exhibiting 16.5% of PV for the trait was reported from a DH population (Stangoulis et al., 2007). Another QTL on the same chromosome close to the marker RM6641 was also detected (Garcia-Oliveira et al., 2008). Using a DH population, James et al. (2007) reported three Fe linked QTLs on chromosomes 2, 8 and 12, explaining 17, 18 and 14% of the total PV, respectively. Norton et al. (2010) reported *qFe-1* explaining 25.81% PV with LOD score of 7.66. Anuradha et al. (2012a, b) identified 14 QTLs for Fe and Zn from unpolished rice of Madhukar/Swarna RILs. Nawaj et al., 2015 have identified many Fe binding and transporters. These loci genes viz., *OsZIP1*, *OsHMA4*, *OsACA2*, *OsZIP2*, *OsCNGC*, *OsZIP3*, *OsZIP5*, *OsZIP9*, *OsHma2*, ABC transporter, *OsNAS3*, heavy metal transporter, Chy zinc finger and *OsACA9* are useful for Fe enhancement (Table 3; Fig. 3). Seven QTLs, namely *qFe3:1*, *qFe3:2*, *qFe7:1*, *qFe9:1*, *qFe9:2*, *qFe10:1* and *Fe11:1* were detected through GWAS for Fe enhancement in rice (Descalsota et al., 2018). Recently, two novel QTLs, *qFe3.3* and *qFe7.3* controlling grain Fe-content have been detected through LD mapping (Pradhan et al., 2020).

6.2. Transgenic approach for Fe biofortification

Genetic enhancement of Fe content in rice seeds through transgenic approach seems to be highly effective compared to other methods (Table 4; Fig. 3). Goto et al. (1999) first reported 3-fold increase in grain-Fe content in transgenic rice than recipient genotype by soyabean *ferritin* gene transfer. Lucca et al. (2001) enhanced 2-fold Fe content by transfer of ferritin from common bean to rice. Vasconcelos et al. (2003) achieved 3-fold increase in Fe by transferring the soyabean ferritin gene to rice. Khalekuzzaman et al. (2006) claimed 2-fold increase in Fe content in transgenic rice lines. Many researchers have reported enhancement of the micronutrient by over expression of Fe-uptake genes from root, leaf shoot to grains, and by more storage of Fe in proteins

(Lee et al., 2009; Ogo et al., 2011; Kobayashi and Nishizawa, 2012; Lee et al., 2012; Bashir et al., 2013; Masuda et al., 2013; Slamet-Loedin et al., 2015). Nicotianamine synthase genes viz., *OsNAS1*, *OsNAS2* and *OsNAS3* are expressed in rice and works as transporter genes. Transfer of NAS gene from *Hordeum vulgare* to rice enhanced 2- to 3-fold iron content in polished rice (Masuda et al., 2009). Therefore, overexpression of these NAS genes is useful to enhance the Fe content in rice (Lee et al., 2009; Lee et al., 2010; Zheng et al., 2010). Overexpression of NAS genes increased Fe accumulation to five-fold in rice grain (Zheng et al., 2010). Also, previous overexpression study using three NAS homologous proteins increased two-fold Fe-content in polished rice (Johnson et al., 2011). Similarly, by overexpressing *ferritin* gene, Fe content was increased many folds in transgenic rice lines (Paul et al., 2012). Overexpressing nicotianamine transporter gene, *OsYSL2* increased iron content in rice (Koike et al., 2004; Ishimaru et al., 2010; Masuda et al., 2013). Transgenic rice lines containing mugineic acid synthase genes, namely *HvNAS1* and *HvNAAT-A*, *B* or *IDS3* transferred from barley have resulted higher concentration of Fe in grains (Higuchi et al., 2001; Kobayashi et al., 2001; Masuda et al., 2008; Suzuki et al., 2008). Higher translocation of 1.4-fold iron in rice grain was achieved from leaves to seeds by knockdown of transporter genes *OsVIT1* and *OsVIT2* in rice (Zhang et al., 2012). Similarly, knockdown of *OsVIT2* showed 1.3-fold and 1.8-fold higher Fe content in brown and polished rice, respectively (Bashir et al., 2013). Transgenic rice containing nicotianamine synthase (*OsNAS2*) and soybean ferritin (*SferH-1*) genes enhanced 6-fold higher endosperm Fe without yield penalty or change in grain quality (Trijatmiko et al., 2016). The engineered plant with *AtIRT1*, *AtNAS1* and *PvFERRITIN* (*PvFER*) genes expressed together showing increased grain iron content in rice (Boonyaves et al., 2017).

6.3. Fe biofortification through agronomic practices

Iron biofortification in rice through agronomic practices by application of Fe fertilizers/nutrients to enhance the nutritional status and health of crops (Prasad et al., 2020). This method improves the nutritional quality for the plant temporarily. Soil application of Fe fertilizers namely ferrous sulphate to the soil soon gets converted to ferric form and cannot be available to rice crop. Foliar application of Fe fertilizers is the preferred method than soil application for Fe fortification (Prasad et al., 2020). Grain Fe content of rice could be increased by 20–40% by this foliar application approach (Yadav et al., 2013; Prasad and Shivay, 2018a, Prasad and Shivay, 2018b). Supplementation of ferrous sulfate to germinating rice increases Fe concentration in the germinated brown rice up to 15.6 folds (Yuan et al., 2013). However, the agronomic approach has specific application method, environmental condition as well as effect on the environment. This may be practicable in developed countries as this approach require more cost than other approaches.

7. Iron deficiency and toxicity symptoms in rice

The Fe deficiency symptoms are commonly seen in young and emerging leaves because of its immobile nature. Interveinal yellowing and chlorosis of leaves are usually found but turn pale and plant die under severe deficiency situation (Kobayashi and Nishizawa, 2014). The roots may be affected and converted into chimeric and necrotic epidermis (Morrissey and Guerinot, 2009; Giehl et al., 2012; Gruber et al., 2013). Under deficiency of this nutrient, chlorophyll content reduces which decreases photosynthetic rate thereby result in stunted growth and finally produces low grain yield. Upland soils, with a pH of more than 6.5 are generally deficient in available Fe. The severity of the problem increases with increase in soil pH.

Bronzing, the Fe-toxicity symptom in leaves starts from the tip and progresses towards basal region in older leaves by formation of tiny brown spots (Tanaka and Loe, 1966; Dufey et al., 2009, Dufey et al., 2015a; Dufey et al., 2015b; Aung et al., 2018). These tiny spots rapidly amplify and coalesce under higher toxicity condition giving a purple

Table 3
Genes/QTLs responsible for iron biofortification in rice grain.

Sl.	QTLs	Chromosome	Markers	Population used	Reference
1	<i>qFe1.1</i>	1	RM243-RM488	RILs Madhukar/Swarna	Anuradha et al., 2012a,b
2	<i>qFe1.2</i>	1	RM488-RM490	RILs	
3	<i>qFe1</i>	1	RM259-RM243	RILs Zhenshan 97/Minghui 63	Kaiyang et al., 2008
4	<i>qFe2-1</i>	2	RM6641	ILs <i>O. rufipogon</i> /Teqing	Garcia-Oliveira et al., 2008
5	<i>qFe.2</i>	2	RM53-RM300	DHs of IR64/Azucena	James et al., 2007
	Fe conc	1	STS1163		Wan et al., 2003a, Wan et al., 2003b
6	<i>qFe.2</i>	2	RM452	LT/TL-RILs Lemont/Teqing	Zhang et al., 2015
7	<i>qFe5.1</i>	5	RM574-RM122	RILs of Madhukar/Swarna	Anuradha et al., 2012a,b
8	<i>qFe7.1</i>	7	RM234-RM248	RILs	
9	<i>qFe.8</i>	8	RM137-RM325A	DHs IR64/Azucena	James et al., 2007
10	<i>qFe.11</i>	11	RZ536-TEL3	RILs Zhenshan 97/Minghui 63	Kaiyang et al., 2008
11	<i>qFe.12</i>	12	RM270-RM17	DHs	
12	<i>qFe12.2</i>	12	RM260-RM7102	RILs Madhukar/Swarna	Anuradha et al., 2012a,b
13	<i>qFe12.1</i>		RM17-RM260	RILs	
14		1	RM246-RM5461		Du et al., 2013
15	Gene and locus: <i>OSZIP1</i> Os01g0972200	1	RM431		Nawaz et al., 2015
16		1	C949+0		Norton et al., 2010
17		3	RM5864 and RM3400		Nawaz et al., 2015
18		3	RM8203		Nawaz et al., 2015
19		2	RM279 and RM555		Nawaz et al., 2015
20		6	RM340-RM494		Du et al., 2013
21		3	R1618-7		Norton et al., 2010
22		4	RM3217		Nawaz et al., 2015
23		5	RM161		Nawaz et al., 2015
24		6	RM162		Nawaz et al., 2015
25	Gene and locus: Os10g0456800 CHY zinc finger family protein	10	RM271		Nawaz et al., 2015
26	Gene and locus: <i>OsACA9</i> Os12g0136900	12	RM19		Nawaz et al., 2015
27	<i>qFe3.1</i>	3	S3_13026098	MAGIC Plus population	Descalsota et al., 2018
28	<i>qFe3.1</i>	3	S3_29613916	MAGIC Plus population	Descalsota et al., 2018
29	<i>qFe7.1</i>	7	S7_29288449	MAGIC Plus population	Descalsota et al., 2018
30	<i>qFe9.1</i>	9	S9_11707990	MAGIC Plus population	Descalsota et al., 2018
31	<i>qFe9.2</i>	9	S9_22203582	MAGIC Plus population	Descalsota et al., 2018
32	<i>qFe10.1</i>	10	S10_13883426	MAGIC Plus population	Descalsota et al., 2018
33	<i>qFe11.1</i>	11	S11_7122972	MAGIC Plus population	Descalsota et al., 2018
34	<i>qFe3.3</i>	3		Genome-wide association study	Pradhan et al., 2020
35	<i>qFe7.3</i>	7		Genome-wide association study	Pradhan et al., 2020

brown appearance and ultimately leaf dries. The symptoms are localized due to immovability of Fe to the emerging tissues and leaves. Leaf tip yellowing and then bronzing occurs particularly in older leaves and the symptom increases under high transpiration rate (Yamanouchi and Yoshida, 1981; Dufey et al., 2015a; Dufey et al., 2015b; Zhang et al., 2018). Fe toxicity stress increases inter-venal brown spots and the leaf may turn brown-yellow and finally senescence (Fairhurst and Witt, 2002). The symptoms of Fe toxicity in rice are generally observed during active tillering and heading stages of the crop, but may be seen at any growth stages depending on mobilization of Fe²⁺ ions in the plant tissue. The sensitive genotypes also show shortening of roots, appearance of brown coating with damaged root system and many non-functional roots (Sahrawat, 2005). Increase in Fe toxicity decreases the number of secondary roots in rice (Pawar et al., 2017). Growth and yield reduction are also observed in few cases without appearance of proportionate leaf bronzing (Sahrawat, 2005; Onaga et al., 2013). Fe imbalance in rice delays flowering, increases sterility and reduces grain yield from 10 to 100% (Audebert and Fofana, 2009; Masuda et al., 2013a, b; Abifarin, 1988, 1989). A reduction in shoot biomass is observed under Fe toxicity condition (Onaga et al., 2013). Formation of Fe plaque, a Fe-complex in the rhizosphere under Fe-toxicity prevents uptake of other nutrients by roots causing nutritional disorder for P, Ca, K, Mg, Mn, Zn (Genon et al., 1994; Sahrawat, 2005). Imbalance in uptake of these nutrients produces negative effect on the diversity of microbes and total microbial biomass of soil solution (Zhang et al., 2005). Presence of more Fe in available form in lowland soil shows reddish scum on the surface of water (Motomura and Yokoi, 1969).

8. Screening strategies for Fe deficiency and toxicity tolerance in rice

The commonly followed phenotypic screenings for Fe deficiency and toxicity tolerance in rice by researchers are performed in hotspot locations, potted plants and under hydroponic screening approaches. Hotspot screening is the most common screening method in a place where Fe toxicity or deficiency spots or locations exist naturally. The stress symptoms are easily observed in susceptible rice varieties. In pot screening, toxic or deficient soils for Fe ions are supplied in pots for screening of the germplasm lines. However, in hydroponics approach, a soil-free screening method is followed using nutrients solution wherein excess or very low Fe concentration is maintained to screen the germplasm lines for Fe toxicity or deficiency in rice, respectively. Many nutrient solutions are being used for screening of Fe toxicity or deficiency tolerance in rice as per the sources and amount of Fe, pH of solution and chelators used (Mori et al., 1991; Wang and Peverly, 1998; Shimizu et al., 2005; Dufey et al., 2009; Shi et al., 2012; Elec et al., 2013).

9. Progress of Fe toxicity tolerance and Fe Biofortification improvement in rice

9.1. Breeding for enhancing Fe toxicity tolerance in rice

Various breeding programs were started systematically in African countries since 1974. Initially, rice varieties were introduced for testing and evaluation in Western African countries. Few good varieties were

Table 4
Fe enhancement in rice grain by deployment of genes through transgenic approach.

Sl.No.	Gene	Fold increase of Fe concentration	Reference
1	<i>Osnas2</i>	4.2-fold Fe	Johnson et al., 2011
2	<i>Gm ferritin, Afphytase, and Osnas1</i>	4 to 6.3-fold Fe	Wirth et al., 2009
3	<i>Activation tagging of Osnas3</i>	2.9-fold Fe	Lee et al., 2009
4	<i>Ferritin gene</i>	4.4-fold Fe	Vasconcelos et al., 2003
5	<i>Nicotianamine synthase (NAS) gene</i>	2.0-fold Fe	Masuda et al., 2009
6	<i>Camv35spro-OsNAS1, OsNAS2, and OsNAS3</i>	4.0-fold Fe	Johnson et al., 2011
7	<i>Barley genes</i>	1.40-fold Fe	Masuda et al., 2013a,b
8	<i>SoyferH1</i>	3.0-fold Fe	Goto et al., 1999
9	<i>SoyFerH1</i>	3.0-fold Fe	Qu et al., 2005
10	<i>PyFerritin, rgMT</i>	2.0-fold	Lucca et al., 2002
11	<i>OsIRO2</i>	2.8-fold Fe	Ogo et al., 2011
12	<i>OsYSL15</i>	1.3-fold Fe	Lee et al., 2009
13	<i>HvNAS1, HvNAS1, HvNAAT, and IDS3</i>	1.2-fold Fe	Suzuki et al., 2008
14	<i>OsNAS1</i>	1.0-fold Fe	Zheng et al., 2010
15	<i>SoyFerH1</i>	2.5-fold Fe	Paul et al., 2012
16	<i>OsNAS2</i>	3.0-fold Fe	Lee et al., 2012
17	<i>HvNAS1</i>	2.5-fold Fe	Higuchi et al., 2001
18	<i>OsYSL2</i>	4.4-fold Fe	Ishimaru et al., 2010
19	<i>AtNAS1, Pvferritin, and Afphytase</i>	6.3-fold Fe	Wirth et al., 2009
20	<i>SoyFerH2, HvNAS1, and OsYSL2</i>	3.4-fold Fe	Aung et al., 2013
21	<i>SoyFerH2, HvNAS1, HvNAAT-A, -B and IDS3 genome fragments</i>	2.5-fold Fe	Masuda et al., 2013
22	<i>OsNAS3-D1</i>	1.7-fold Fe in shoots, 1.6-fold in Fe roots	Lee et al., 2009
23	<i>Ferritine gene</i>	2.0-fold Fe	Khalekuzzaman et al., 2006
24	<i>Osfer2</i>	2.09-fold Fe	Pal et al., 2012
25	<i>HvYS1 (Transporter gene)</i>	1.5 fold Fe in leaves	Gomez-Galera et al., 2012
26	<i>OsNAS2 & SoyferH-1 construct</i>	6 fold	Trijatmiko et al., 2016.
27	<i>Promoter-OsGluB1- SoyferH2</i>	6 fold	Masuda et al., 2012
28	<i>Promoter- OsGlb1- SoyferH2</i>		
	<i>AtNAS1, PvFERRITIN and ZmPSY</i>	2.5-3.3 fold	Singh et al., 2017

Table 5
Donor germplasm lines for biofortification, iron deficiency and toxicity tolerance in rice.

Trait	Donor germplasm lines	Reference
Iron deficiency tolerance	Prasanna	Hoan et al., 1993
	IET7613	
	Cauvery, ARC 10372, Tuljapur, Akashi, WBPB25	Hoan et al., 1993
	Prabhavati (PBN1)	Nerkar et al., 1984
	Pusa-33	Nogiya et al., 2016
	IR36	Naidu et al., 1981
	Dongdao-4	
	Suakoko 8	Li et al., 2016
	WITA3 (TOX3100-32-2-3-5)	Virmani, 1977
	FKR	WARDA, 1998
Iron toxicity tolerance	WITA4 (TOX3100-44-1-3-3)	Ouedraogo and Ouedraogo, 2003
	CK 4	Gridley et al., 2006
	CK73	Abdoul, 2006
	TOX-4216-25-2-3-1-3	
	WAT 1059-B-51-2	Gridley et al., 2006
	WAT1282-B-3-3	
	WAT1131-B-26-2-1-2	
	Nerica-L19	
	KD-2-6-3, Guwahati, Shahsarang, Pyzum and Phougak	Dramé et al., 2010
	IR61246-3B-15-2-2-3	Devi et al., 2018
IR61612-3B-16-2-2-1	Elec et al., 2013	
IR61640-3B-14-3-3-2		
WITA 7		
Mahanadi, Ghanteswari, Bhanja, Surendrra, Sankar, Rajeswari, Lalat, Daya, Tejaswini, Khandagiri, Bhuban, Udaygiri, Uphar, Rudra, Manika	Rout et al., 2014	
EPAGRI 108		
Iron biofortification	MSE-9, Kalanamak, Kanchana, Karjat, Chittimutyalu, Udayagiri, Jyothi, VRM 7, Matta Triveni, Varsha	Da Silveira et al., 2007
	Enghi, Dakh Shail, Moina Moti, Hamai, Patnai, Khak Shail, Lal Gotal, Ful Kainja, Kakua Binni, Sada Binni, BRR1 dhan5, BRR1 dhan 28 & BRR1 dhan 29	Babu, 2013
	Annada, ASD16, CH-45, Nagina 22, IR-29,	Jahan et al., 2013
	Pusa Sugandha-1, IRGC-106187, IR68144-3B-2-2-3, IRGC-105320, IRGC-105320, IRGC-86476, CH-45, Jyoti, HKR-126, Jalmagna, Zuchem, Kalabath, Pusa Basmati, Noothipattu, Pitchavari, Thanu, TKM-9, NDR-6279, and Aghonibora.	Mahender et al., 2016
	Chittimutylu, BPT5204, Kalanamak, ET25450, IET 24775, IET 24760, IET 24316, BPT5204, IET 23832, IET25441, IET25465, IET 23,829 and IET 24779	
		Pradhan et al., 2020

released from this program. Suakoko 8, a variety released for Liberia, become very popular and till date is being used as national check for Fe toxicity tolerance. Another introduced variety ROK 24 was released for Sierra Leone. These introduced varieties were good for Fe toxicity tolerance but longer in duration and low yielder. Therefore, many Fe toxicity tolerant breeding programs were continued and many varieties were developed to overcome yield loss due to high Fe toxicity (Abifarin, 1989; Masuda et al., 2013a, b; Winslow et al., 1989; Gregorio et al., 2000; Gridley et al., 2006; Ruskandar et al., 2011; Sikirou et al., 2015). Large germplasm screenings have been performed and good donor lines were reported to be tolerant to Fe-toxicity and deficiency in rice for various countries across the globe (Table 5). Initially organizations like IITA, WARDA and AfricaRice along with many national partners were involved in the breeding programs. Many promising lines were developed for cultivation in African countries particularly WITA3, WITA4, WITA8, FKR19, CK73 and CK92. Despite a lot of breeding efforts, these materials could not be so popular like earlier varieties, namely Suakoko 8 and CK4. The reasons for non-adoption of new varieties were due to higher GxE interactions and soil heterogeneity. However, the development of NERICAs (New Rice for Africa) could be a moderate success particularly with the lowland NERICAs like NERICA-L19 and CG14 varieties. Recent years, AfricaRice and its collaborators have a new breakthrough in new plant type rice in the name of ARICA (Advanced rice varieties for Africa) of next generation rice exhibiting higher grain yield and more stress tolerant compared to NERICA-L19 variety. Recent rice variety ARICA6 (IR75887-1-3-WAB1) released in Guinea and ARICA7 (WAS 21-B-B-20-4-3-3) in Senegal are tolerant to Fe toxicity and higher yielder than NERICA-L19. ARICA 7 and ARICA 8 (WAT 1046-B-43-2-2-2) were released in the rainfed and irrigated lowland ecologies of Burkina Faso, Ghana and Guinea. On the other hand, ARICA 6 was released for rainfed lowland environments of Ghana. Many ARICA varieties have been developed for African countries. Success of these varieties will depend on farmers 'adoption and consumers' acceptance in the Sub-Saharan African countries.

9.2. Breeding for higher Fe content in rice

Enhancing grain Fe content through molecular breeding approach is the most reliable and suitable approach in rice. The genetic mechanism of Fe-content inheritance is complex in nature. The trait is controlled by many QTLs and genes of which majority were identified in recent years. The genes, QTLs and various transporters useful for Fe enhancement are well characterized (Bashir et al., 2010; Conte and Walker, 2011; Garg et al., 2018; Majumder et al., 2019). Many germplasm lines containing high grain Fe content are screened and identified from rice gene pool (Babu, 2013; Jahan et al., 2013; Mahender et al., 2016; Pradhan et al., 2020). In addition, the transgenic donor lines are also available to be used in molecular breeding programs (Higuchi et al., 2001; Kobayashi et al., 2001; Lucca et al., 2001; Vasconcelos et al., 2003; Koike et al., 2004; Khalekuzzaman et al., 2006; Suzuki et al., 2008; Masuda et al., 2009; Lee et al., 2009; Lee et al., 2010; Zeng et al., 2010; Ishimaru et al., 2010; Ogo et al., 2011; Johnson et al., 2011; Pal et al., 2012; Kobayashi and Nishizawa, 2012; Lee et al., 2012; Zhang et al., 2012; Bashir et al., 2013; Masuda et al., 2013a, b; Slamet-Loedin et al., 2015; Trijtmiko et al., 2016; Boonyaves et al., 2017). Development of nutrient dense rice containing high Fe, Zn and protein through molecular breeding is a priority research area of ICAR-National Rice Research Institute, Cuttack, India, ICAR-Indian Institute of Rice Research, Hyderabad and International Rice Research Institute, Philippines. The major advantage of molecular breeding is that the presence of the target gene is checked through foreground selection by using linked or gene specific molecular markers in each backcross generation. This affirms the presence of the trait in the end product along with highest genome recovery of the recurrent rice variety which gets biofortified for Fe content. Pyramiding the major QTLs governing grain Fe content with Ferritin /IDS3/YSL/VIT genes can be good strategy for enhancing grain

Fe content. For this purpose, multiple donor lines having the target genes can be used. Transgenic rice containing nicotianamine synthase (*OsNAS2*) and soybean ferritin (*SferH-1*) genes enhanced 6-fold higher endosperm Fe without yield penalty or change in grain quality (Trijtmiko et al., 2016). The two Fe enhancing genes were crossed with popular varieties from the Philippines (NSic Rc222), Indonesia (Cihorang), and Bangladesh (BR29). These two genes are very useful for enhancing Fe content in different popular varieties through molecular breeding approach.

10. Conclusion

Many genes/QTLs responsible for Fe-uptake from rhizosphere, transport from roots to shoots and other plant parts and their regulation are now known. Those reported genes/QTLs for the stress tolerance are still not properly validated and utilized in the breeding programs. Additionally, more genes/QTLs contributing towards high phenotypic variance to these stresses tolerance need to be identified. Hence, efforts are needed for inclusion of modern techniques like genome editing, genome wide-association mapping and transgenics in the current research programs. A thorough understanding of the genetic dissections and the physiological mechanisms of those QTLs need to be examined. The relationship between rice rhizosphere to uptake right quantity of Fe from soils under stress condition and the genetic background of rice plant need to be studied more. The Fe dynamics in various tissues and cells of rice plants need more understanding. Genes/QTLs controlling Fe-content in grain endosperm need to be explored and deployment in Fe improvement programs to manage the iron loss due to milling.

Biofortification is an easier and cheaper way to enhance the content in rice grain. QTLs and genes controlling Fe-content are essential in the breeding programs. Transgenic rice carrying ferritin gene from other crops with many folds Fe content is available with us. The transgenic method of fortification is facing many challenges related to ethical issues. However, these lines may serve as potential source of donor parent in marker-assisted and genomic-assisted breeding programs to transfer high Fe- content into popular high yielding varieties. Understanding on regulation of homeostasis, uptake and efficiency enhancement under low or excess of the nutrient in soil are important. The consistent QTLs/genes involved during the stresses are needed for developing tolerant genotype (s) to the stress. More transcriptome studies are required though in the recent years much progress has been made for understanding and unravelling major gene actions under these stresses. Proteomic and epigenetic studies should be undertaken to understand more on tolerance to toxicity, Fe-deficiency and biofortification. Identification and understanding of path-ways related to the regulation of iron metabolism and the molecular markers associated with QTLs for these pathways will be useful to enhance yield under these stresses. Development of cheaper, effective and less polluting agro-techniques may also be explored for redressal of these stresses.

Author contributions

SKP conceived the idea for preparation of the manuscript and outlined the content. SKP, EP, SP, AP, HP helped in writing of the manuscript. SKP, EP, LB, SRD, HP revised the manuscript.

Funding

No funding involved in this article.

Declaration of Competing Interest

The authors declare that the review article was submitted without any commercial or economic interest that could be construed as a potential conflict of interest.

References

- Abadia, J., Vazquez, S., Rellan-Alvarez, R., El-Jendoubi, H., Abadia, A., Alvarez-Fernandez, A., et al., 2011. Towards a knowledge-based correction of iron chlorosis. *Plant Physiol. Biochem.* 494, 71–482. <https://doi.org/10.1016/j.plaphy.2011.01.026>.
- Abdoul, K.C., 2006. Testing and developing tolerant rice varieties to iron toxicity in lower guinea (CRA Kilissi and Koba). In: Audebert, A., Narteh, L.T., Kiepe, P., Millar, D., Beks, B. (Eds.), *Iron Toxicity in Rice-Based System in West Africa*. WARDA, Cotonou, pp. 64–74.
- Abifarin, A.O., 1989. Progress in breeding rice for tolerance to iron toxicity. In: WARDA (Ed.), *WARDA Annual Report for 1990*. West Africa Rice Development Association, Bouaké, pp. 34–39.
- Abifarin, A.O., 1988. Grain yield loss due to iron toxicity. *WARDA Technical Newsletter* 8 (1), 1–2.
- Ahmed, S.A., Borua, I., Das, D., 1998. Chemical composition of scented rice. *Oryza* 35 (2), 167–169.
- Asch, F., Becker, M., Kpong, D.S., 2005. A quick and efficient screen for resistance to iron toxicity in lowland rice. *J. Plant Nutr. Soil Sci.* 168, 764–773.
- Aoyama, T., Kobayashi, T., Takahashi, M., Nagasaka, S., Usuda, K., Kakei, Y., Ishimaru, Y., Nakanishi, H., Mori, S., Nishizawa, N.K., 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.
- Ando, T., Yoshida, S., Nishiyama, I., 1983. Nature of oxidizing power of rice roots. *Plant Soil* 72, 57–71. <https://doi.org/10.1007/BF02185094>.
- Anuradha, K., Agarwal, S., Batchu, A.K., Babu, A.P., Swamy, B.P.M., Longvah, T., Sarla, N., 2012a. Evaluating rice germplasm for iron and zinc concentration in brown rice and seed dimensions. *J. Phytol.* 4 (1), 19–25.
- Anuradha, K., Agarwal, S., Rao, Y.V., Rao, K., Viraktamath, B., Sarla, N., 2012b. Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar × Swarna RILs. *Gene* 508, 233–240.
- Arcanjo, F.P.N., Santos, P.R., Arcanjo, C.P.C., 2013. Daily and weekly iron supplementations are effective in increasing haemoglobin and reducing anemia in infants. *J. Trop. Pediatr.* 59, 175–179.
- Armstrong, W., 1979. Aeration in higher plants. In: In: Woolhouse, H.W. (Ed.), *Advances in Botanical Research Vol. 7*. Academic Press, London, pp. 225–332.
- Audebert, A., 2006. Iron toxicity in rice – environmental conditions and symptoms. In: Audebert, A., Narteh, L.T., Kiepe, P., Millar, D., Beks, B. (Eds.), *Iron Toxicity in Rice-Based System in West Africa*. WARDA, Cotonou, pp. 18–33.
- Audebert, A., Fofana, M., 2009. Rice yield gap due to iron toxicity in West Africa. *J. Agron. Crop Sci.* 195, 66–76.
- Audebert, A., Sahrawat, K.L., 2000. Mechanisms for iron toxicity tolerance in lowland rice. *J. Plant Nutr.* 23, 1877–1885.
- Aung, M.S., Masuda, H., Kobayashi, T., Nakanishi, H., Yamakawa, T., Nishizawa, N.K., 2013. Iron biofortification of Myanmar rice. *Front Plant Sci.* 4, 158. <https://doi.org/10.3389/fpls.2013.00158>.
- Aung, S.S., Masuda, H., Kobayashi, T., Naoko, K., Nishizawa, N.K., 2018. Physiological and transcriptomic analysis of responses to different levels of iron excess stress in various rice tissues. *Soil Sci. Plant Nutr.* 64 (3), 370–385.
- Aung, M.S., Masuda, H., Nozoye, T.T., Jong-Seong, J., An, G., Nishizawa, N.K., 2019. Nicotianamine Synthesis by OsNAS3Is Important for Mitigating Iron Excess Stress in Rice. *Front. Plant Sci.* <https://doi.org/10.3389/fpls.2019.00660>.
- Babu, R.V., 2013. Importance and advantages of rice biofortification with iron and zinc. An Open Access Journal published by ICRISAT. *SAT eJ* 11, 1–6.
- Bashir, K., Ishimaru, Y., Shimo, H., Nagasaka, S., Fujimoto, M., Takanashi, H., 2011. The rice mitochondrial iron transporter is essential for plant growth. *Nat. Commun.* 2, 322–329.
- Bashir, K., Inoue, H., Nagasaka, S., Takahashi, M., Nakanishi, H., Mori, S., Nishizawa, N.K., 2006. Cloning and characterization of deoxymugineic acid synthase genes from graminaceous plants. *J. Biol. Chem.* 281, 32395–32402.
- Banerjee, S., Chandel, G., Mandal Meena, N.M., Saluja, T., 2011. Assessment of nutritive value in milled rice grain of some indian rice landraces and their molecular characterization. *Bangladesh J. Agril. Res.* 36 (3), 369–380.
- Becker, M., Asch, F., 2005. Iron toxicity in rice-conditions and management concepts. *J. Plant Nutr. Soil Sci.* (1999) 168, 558–573.
- Belouchi, A., Kwan, T., Gros, P., 1997. Cloning and characterization of the OsNramp family from *Oryza sativa*, a new family of membrane proteins possibly implicated in the transport of metal ions. *Plant Mol. Biol.* 33, 1085–1092.
- Bashir, K., Ishimaru, Y., Nishizawa, N.K., 2010. Iron Uptake and Loading into Rice Grains. *Rice* 3, 122–130. <https://doi.org/10.1007/s12284-010-9042-y>.
- Bashir, K., Nozoye, T., Ishimaru, Y., Nakanishi, H., Nishizawa, N.K., 2013. Exploiting new tools for iron bio-fortification of rice. *Biotechnol. Adv.* 31, 1624–1633.
- Bode, K., Döring, O., Lüthje, S., Neue, H.U., Böttger, M., 1995. The role of active oxygen in iron tolerance of rice (*Oryza sativa* L.). *Protoplasma* 184, 249–255.
- Briat, J.F., Lobreaux, S., Grignon, N., Vansuyt, G., 1999. Regulation of plant ferritin synthesis: how and why. *Cell Mol. Life Sci.* 56, 155–166.
- Briat, J.F., Duc, C., Ravet, K., Gaynard, F., 2010. Ferritins and iron storage in plants. *Biochim Biophys Acta Gen Subj* 1800, 806–814.
- Blokhina, O., Virolainen, E., Fagerstedt, K.V., 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.* 91, 179–194.
- Bonneau, J., Baumann, U., Beasley, J., Li, Y., Johnson, A.A.T., 2016. Identification and molecular characterization of the nicotianamine synthase gene family in bread wheat. *Plant Biotechnol. J.* 14, 2228–2239.
- Boonyaves, K., Wu, T.-Y., Gruijssem, W., Bhullar, N.K., 2017. Enhanced Grain Iron Levels in Rice Expressing an IRON-REGULATED METAL TRANSPORTER, NICOTIANAMINE SYNTHASE, and FERRITIN Gene Cassette. *Front. Plant Sci.* 8, 130. <https://doi.org/10.3389/fpls.2017.00130>.
- Brinch-Pedersen, H., Borg, S., Tauris, B., Holm, P.B., 2007. Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. *J. Cereal Sci.* 46, 308–326.
- Chérif, M., Audebert, A., Fofana, M., Zouzou, M., 2009. Evaluation of iron toxicity on lowland irrigated rice in West Africa. *Tropicultura* 27, 88–92.
- Colmer, T.D., 2002. Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, paddy and deep-water rice (*Oryza sativa* L.). *Ann. Bot.* 91 (2), 301–309.
- Conte, S.S., Walker, E.L., 2011. Transporters contributing to iron trafficking in plants. *Mol. Plant* 4, 464–476. <https://doi.org/10.1093/mp/ssp015>.
- Curie, C., Alonso, J.M., Le Jean, M., Ecker, J.R., Briat, J.F., 2000. Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. *Biochem. J.* 347, 749–755.
- Curie, C., Cassin, G., Couch, D., Divol, F., Higuchi, K., Le Jean, M., Misson, J., Schikora, A., Czernic, P., Mari, S., 2009. Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters. *Ann. Bot.* 103, 1–11.
- Curie, C., Panaviene, Z., Loulergue, C., Dellaporta, S.L., Briat, J.F., Walker, E.L., 2001. Maize yellow stripe1 encodes a membrane protein directly involved in Fe (III) uptake. *Nature* 409, 346–349.
- Cheng, L., Wang, F., Shou, H., Huang, F., Zheng, L., He, F., Li, J., Zhao, F.J., Ueno, D., Ma, J.F., Wu, P., 2007. Mutation in nicotianamine aminotransferase stimulated the Fe (II) acquisition system and led to iron accumulation in rice. *Plant Physiol.* 145, 1647–1657.
- da Silveira, V.C., Fadanelli, C., Sperotto, R.A., Stein, R.J., Basso, L.A., Santos, D.S., Vaz, I.S., Dias, J.F., Fett, J.P., 2009. Role of ferritin in the rice tolerance to iron overload. *Sci. Agric.* 66 (4), 549–555.
- Da Silveira, V.C., de Oliveira, A.P., Sperotto, R.A., Espindola, L.S., Amaral, L., Dias, J.F., da Cunha, J.B., Fett, J.P., 2007. Influence of iron on mineral status of two rice (*Oryza sativa* L.) cultivars. *Braz. J. Plant Physiol.* 19, 127–139.
- Devi, A.G., Rangappa, K., Yadav, G.S., Devi, H.L., Barman, K.K., Kandpal, B.K., Ngachan, S.V., 2018. Physiological tolerance mechanism of selected rice germplasm of North east India to iron toxicity. *Indian Journal of hill farming* 31 (1), 75–81.
- Descalosa, G., Swamy, M., Zaw, H., Inabangan-Asilo, M.A., Amparado, A., Mauleon, R., Mohanty, P.C., Emily, C., Arocena, Raghavan, C., Leung, H., Hernandez, J.E., Lalusin, A.B., Mendiolo, M.S., Diaz, M.G.Q., Reinke, R., 2018. Genome-wide association mapping in a rice MAGIC plus population detects QTLs and genes useful for biofortification. *Front. Plant Sci.* 9, 1347.
- Didonato, R.J., Roberts, L.A., Sanderson, T., Easley, R.B., Walker, E.L., 2004. *Arabidopsis* Yellow Stripe-Like2 (YSL2): A metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. *Plant J.* 39, 403–414.
- Dinneny, J.R., Long, T.A., Wang, T.Y. et al. 2008. Cell identity mediates the response of *Arabidopsis* roots to abiotic stress. *Science* 320, 942–945.
- Dos Santos, R.S., de Araujo Júnior, A.T., Pegoraro, C., de Oliveira, A.C., 2017. Dealing with iron metabolism in rice: from breeding for stress tolerance to biofortification. *Genet. Mol. Biol.* 40 (1), 312–325.
- Dramé, K.N., Saito, K., Koné, B., Chabi, A., Dakouo, D., Ebenezer, Annan-Afful, E., Monh, S., Abo, E., Sié, M., 2010. Coping with iron toxicity in the lowlands of sub-Saharan Africa: experience from Africa rice center. In: *AfricaRice* (Ed.), *Innovation and Partnerships to Realize Africa's Rice Potential*. Proc. 2nd Africa Rice Congress. 22-26 March, Bamako.
- Du, J., Zeng, D., Wang, B., Qian, Q., Zheng, S., Ling, H.Q., 2013. Environmental effects on mineral accumulation in rice grains and identification of ecological specific QTLs. *Environ. Geochem. Health* 35, 161–170. <https://doi.org/10.1007/s10653-012-9473-z> PMID:22760687.
- Dufey, I., Draye, X., Lutts, S., Louerix, M., Martinez, C., Bertin, P., 2015a. Novel QTLs in an interspecific backcross *O.sativa*/O.gLaberrima for resistance to iron toxicity in rice. *Euphytica*. <https://doi.org/10.1007/s10688-014-1342-7>.
- Dufey, I., Mathieu, A.-S., Draye, X., Lutts, S., Bertin, P., 2015b. Construction of an integrated map through comparative studies allows the identification of candidate regions for resistance to ferrous iron toxicity in rice. *Euphytica* 203, 59–69.
- Dufey, I., Hakizimana, P., Draye, X., Lutts, S., Bertin, P., 2009. QTL mapping for biomass and physiological parameters linked to resistance mechanisms to ferrous iron toxicity in rice. *Euphytica*. 167, 143–160.
- Durrett, T.P., Gassmann, W., Rogers, E.E., 2007. The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. *Plant Physiol.* 144, 197–205.
- Eckhardt, T.U., Mas Marques, A., Buckhout, T.J., 2001. Two iron-regulated cation transporters from tomato complement metal uptake-deficient yeast mutants. *Plant Mol. Biol.* 45, 437–448.
- Elec, V., Quimio, C.A., Mendoza, R., Sajise, A.G.C., Beebout, S.E.J., Gregorio, G.B., Singh, R.K., 2013. Maintaining elevated Fe²⁺ concentration in solution culture for the development of a rapid and repeatable screening technique for iron toxicity tolerance in rice (*Oryza sativa* L.). *Plant Soil* 372, 253–264.
- Engel, K., Asch, F., Becker, M., 2012. *In vivo* staining of reduced iron by 2, 2' bipyridine in rice exposed to iron toxicity. *J. Plant Nutr.* 175, 548–552.
- Fageria, N.K., Santos, A.B., Barbosa, M.P., Guimaraes, C.M., 2008. Iron toxicity in lowland rice. *J. Plant Nutr.* 31 (7–9), 1676–1697.
- Fairhurst, T.H., Witt, C., 2002. Rice: A practical guide to nutrient management. In: Audebert, A., Narteh, L., Kiepe, P., Millar, D., Beks, B. (Eds.), *Iron toxicity in rice-based system in West Africa*. WARDA, International Rice Research Institute, Manila p. 25.
- Fang, W.C., Kao, C.H., 2000. Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. *Plant Sci.* 158, 71–76.
- Fang, W.C., Wang, J.W., Lin, C.C., Kao, C.H., 2001. Iron induction of lipid peroxidation and effects on antioxidative enzyme activities in rice leaves. *Plant Growth Regul.* 35,

- 75–80.
- Finatto, T., de Oliveira, A.C., Chaparro, C., da Maia, L.C., Farias, D.R., Woyann, L.G., Mistura, C.C., Soares-Bresolin, A.P., Lauro, C., Panaut, O., et al., 2015. Abiotic stress and genome dynam- ics: specific genes and transposable elements response to iron excess in rice. *Rice* 8, 13.
- Freitas, B.A., Lima, L.M., Moreira, M.E., Priore, S.E., Henriques, B.D., Carlos, C.F., Sabino, J.S., Franceschini, S.C., 2016. Micronutrient supplementation adherence and influence on the prevalence of anemia and iron, zinc and vitamin A deficiencies in preemies with a corrected age of six months. *Clinics* 71 (8), 440–448.
- Fourcroy, P., Siso-Terraza, P., Sudre, D., Saviron, M., Rey, G., Gaymard, F., et al., 2014. Involvement of the ABCG37 transporter in secretion of scopoletin and derivatives by *Arabidopsis* roots in response to iron deficiency. *New Phytol.* 201, 155–167. <https://doi.org/10.1111/nph.12471>.
- Gallie, D.R., 2013. The role of l-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. *J. Exp. Bot.* 64, 433–443. <https://doi.org/10.1093/jxb/ers330>.
- Garcia-Oliveira, A.L., Tan, L., Fu, Y., Sun, C., 2008. Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. *J. Integr. Plant Biol.* 1744–7909.
- Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., et al., 2018. Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Front. Nutr.* 5, 12. <https://doi.org/10.3389/fnut.2018.00012G>.
- Genon, J.G., Dehepsee, N., Duffy, J.E., Delvaux, B., Hennebert, P.A., 1994. Iron toxicity and other chemical soil constraints to rice in Highland Swamps of Burundi. *Plant Soil* 166.
- Green, L.S., Rogers, E.E., 2004. FRD3 controls iron localization in *Arabidopsis*. *Plant Physiol.* 136, 2523–2531.
- Giehl, R.F.H., Lima, J.E., von Witrén, N., 2012. Localized iron supply triggers lateral root elongation in *Arabidopsis* by altering the AUX1-mediated auxin distribution. *Plant Cell* 24, 33–49.
- Giehl, R.F.H., Meda, A.R., von Wire, N., 2009. Moving up, down, and everywhere: signalling of micronutrients in plants. *Curr. Opin. Plant Biol.* 12, 320–327.
- Goto, F., Yoshihara, T., Shigemoto, N., Toki, S., Takaiwa, F., 1999. Iron fortification of rice seed by the soybean ferritin gene. *Nat. Biotechnol.* 17, 282–286.
- Gomez-Galera, S., Sudhakar, D., Pelacho, A.M., Capell, T., Christou, P., 2012. Constitutive expression of a barley Fe phytosiderophore transporter increases alkaline soil tolerance and results in iron partitioning between vegetative and storage tissues under stress. *Plant Physiol/Biochem* 53, 46–53.
- Graham, R., Senadhira, D., Beebe, S., Iglesias, C., Monasterio, I., 1999. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Res.* 60, 57–80.
- Gregorio, G.B., Senadhira, D., Htut, H., Graham, R.D., 2000. Breeding for trace mineral density in rice. *Food Nutr Bull* 21 382–386.
- Green, M.S., Etherington, J.R., 1977. Oxidation of ferrous iron by rice roots: a mechanism for water logging tolerance. *J. Exp. Bot.* 28, 678–690.
- Gridley, H.E., Efiuse, A., Tolou, B., Bakayako, T., et al., 2006. Breeding for tolerance to iron toxicity at WARDA. In: Audebert (Ed.), *Iron Toxicity in Rice-Based System in West Africa*. WARDA, Cotonou, pp. 96–111.
- Gruber, B.D., Giehl, R.F.H., Friedel, S., von Witrén, N., 2013. Plasticity of the *Arabidopsis* root system under nutrient deficiencies. *Plant Physiol.* 163, 161–179. <https://doi.org/10.1104/pp.113.218453>.
- Guerinot, M.L., 2001. Improving rice yields—ironing out the details. *Nat. Biotechnol.* 19, 417–418.
- Guerinot, M.L., 2000. The ZIP family of metal transporters. *Biochem. Biophys. Acta.* 1465, 190–198.
- Harahap, S.M., Ghulamahdi, M., Aziz, S.A., Sutandi, A., 2014. Relationship of ethylene production and aerenchym formation on oxidation ability and root surfaced-iron (Fe²⁺) accumulation under different iron concentrations and rice genotypes. *Int. J. Appl. Sci.* 4 (1), 186–194.
- Hell, R., Stephan, U.W., 2003. Iron uptake, trafficking and homeostasis in plants. *Planta* 216, 541–551.
- Higuchi, K., Watanabe, S., Takahashi, M., Kawasaki, S., Nakanishi, H., Nishizawa, N.K., Mori, S., 2001. Nicotianamine synthase gene expression differs in barley and rice under Fe-deficient conditions. *Plant J.* 25, 159–167.
- Hoan, N.T., Rao, U.P., Siddiq, E.A., 1993. Genetics of tolerance to iron chlorosis in rice. *Genetic Aspects of Plant Mineral Nutrition*. Springer, Dordrecht, Netherland, pp. 327–333.
- Hindt, M.N., Guerinot, M.L., 2012. Getting a sense for signals: regulation of the plant iron deficiency response. *Biochim. Biophys. Acta* 1823, 1521–1530.
- Howler, R.H., 1973. Iron-induced orange disease of rice in relation to physicochemical changes in a flooded Oxisol. *Soil Sci Soc Am Proc.* 37, 898–903.
- Jahan, G.S., Hassan, L., Begum, S.N., Islam, S.N., 2013. Identification of Iron rich rice genotypes in Bangladesh using chemical analysis. *J. Bangladesh Agril. Univ.* 11 (1), 73–78.
- Inoue, H., Higuchi, K., Takahashi, M., Nakanishi, H., Mori, S., Nishizawa, N.K., 2003. Three rice nicotianamine synthase genes, *OsNAS1*, *OsNAS2*, and *OsNAS3* are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. *Plant J.* 36, 366–381.
- Inoue, H., Kobayashi, T., Nozoye, T.M., Kakei, Y., Suzuki, K., et al., 2009. Rice *OsYSL15* is an iron-regulated Iron(III)-deoxy mugineic Acid Transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. *J. Biol. Chem.* 284, 3470–3479.
- Ishimaru, Y., Bashir, K., Nakanishi, H., Nishizawa, N.K., 2011. The role of rice phenolics efflux transporter in solubilizing apo-plasmic iron. *Plant Signal. Behav.* 6, 1624–1626.
- Ishimaru, Y., Masuda, H., Bashir, K., Inoue, H., Tsukamoto, T., Takahashi, M., Nakanishi, H., Aoki, N., Hirose, T., Ohsugi, R., et al., 2010. Rice metal-nicotianamine transporter, *OsYSL2*, is required for the long-distance transport of iron and manganese. *Plant J.* 62, 379–390.
- Ishimaru, Y., Suzuki, M., Kobayashi, T., Takahashi, M., Nakanishi, H., Mori, S., Nishizawa, N.K., 2005. *OsZIP4*, a novel zinc-regulated zinc transporter in rice. *J. Exp. Bot.* 56, 3207–3214. <https://doi.org/10.1093/jxb/eri317>.
- Ishimaru, Y., Suzuki, M., Tsukamoto, T., Suzuki, K., Nakazono, M., Kobayashi, T., 2006. Rice plants take up iron as a Fe³⁺ + phytosiderophore and as Fe. *Plant J.* 45, 335–346. <https://doi.org/10.1111/j.1365-313X.2005.02624.x>.
- Ishimaru, Y., Takahashi, R., Bashir, K., Shimo, H., Senoura, T., Sugi-moto, K., et al., 2012. Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport. *Sci. Rep.* 2, 286.
- Jiang, S., Wu, J., Thang, N., Feng, Y., Yang, X.E., Shi, C.H., 2008. Genotypic variation of mineral elements contents in rice (*Oryza sativa* L.). *Eur. Food Res. Technol.* 228, 115–122.
- Johnson, A.A.T., Kyriacou, B., Callahan, D.L., Carruthers, L., Stan-goulis, J., Lombi, E., Tester, M., 2011. Constitutive over-expression of the *OsNAS* gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS One* 6, e24476.
- James, C.R., Huynh, B.L., Welch, R.M., Choi, E.Y., Graham, R.D., 2007. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica* 154 (3), 289–294.
- Jugsujinda, A., Patrick, J.W.H., 1993. Evaluation of toxic conditions associated with orange symptoms of rice in a flooded oxisol in Sumatra, Indonesia. *Plant Soil* 152, 237–243.
- Kaiyang, L., Lanzhi, L., Xingfei, Z., Zhang, Z., Mou, T., Zhongli, H., 2008. Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains. *J. Genet.* 87 (3), 305–310.
- Kakei, Y., Ishimaru, Y., Kobayashi, T., Yamakawa, T., Nakanishi, H., Nishizawa, N.K., 2012. *OsYSL16* plays a role in the allocation of iron. *Plant Mol. Biol.* 79, 583–594.
- Kawase, M., 1981. Anatomical and morphological adaptation of plants to waterlogging. *HortScience* 16, 30–34.
- Khalequzzaman, M., Datta, K., Oliva, N., Alam, M.F., Joarder, I., Datta, S.K., 2006. Stable inheritance, expression and inheritance of the ferritin gene in transgenic elite indica rice cultivar BR29 with enhanced iron level in the endosperm. *Indian J.* 5, 26–31.
- Kim, S.A., Guerinot, M.L., 2007. Mining iron: iron uptake and transport in plants. *FEBS Lett.* 581, 2273–2280.
- Kim, S.A., Punshon, T., Lanzirrotti, A., Li, L., Alonso, J.M., Ecker, J.R., Kaplan, J., Guerinot, M.L., 2006. Localization of iron in *Arabidopsis* seed requires the vacuolar membrane trans- porter VIT1. *Science* 314, 1295–1298.
- Kobayashi, T., Nakanishi, H., Takahashi, M., Kawasaki, S., Nishizawa, N.K., Mori, S., 2001. *In vivo* evidence that *Id3* from *Hordeum vulgare* encodes a dioxygenase that converts 2'-deoxymugineic acid to mugineic acid in transgenic rice. *Planta* 212, 864–871.
- Kobayashi, T., Nakayama, Y., Itai, R.N., Nakanishi, H., Yoshihara, T., Mori, S., 2003. Identification of novel cis-acting elements, IDE1 and IDE2, of the barley *IDS2* gene promoter conferring iron-deficiency-inducible, root-specific expression in heterogeneous tobacco plants. *Plant J.* 36, 780–793.
- Kobayashi, T., Nagasaka, S., Senoura, T., Itai, R.N., Nakanishi, H., Nishizawa, N.K., 2013. Iron-binding haemerythrin RING ubiquitin ligases regulate plant iron responses and accumulation. *Nat. Commun.* 4, 2792. <https://doi.org/10.1038/ncomms3792>.
- Kobayashi, T., Nishizawa, N.K., 2012. Iron uptake, translocation, and regulation in higher plants. *Annu. Rev. Plant Biol.* 63, 131–152.
- Kobayashi, T., Nishizawa, N.K., 2014. Iron sensors and signals in response to iron deficiency. *Plant Sci.* 224, 36–43.
- Kobayashi, T., Ogo, Y., Aung, M.S., Nozoye, T., Itai, R.N., Nakanishi, H., Yamakawa, T., Nishizawa, N.K., 2010. The spatial expression and regulation of transcription factors IDEF1 and IDEF2. *Annals Bot* 105, 1109–1117.
- Koike, S., Inoue, H., Mizuno, D., Takahashi, M., Nakanishi, H., Mori, S., Nishizawa, N.K., 2004. *OsYSL2* is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant J.* 39, 415–424.
- Lee, S., Chiecko, J.C., Kim, S.A., Walker, E.L., Lee, Y., Guerinot, M.L., An, G., 2009. Disruption of *OsYSL15* leads to iron inefficiency in rice plants. *Plant Physiol.* 150, 786–800.
- Lee, S., Jeong, H.J., Kim, S.A., Lee, J., Guerinot, M.L., An, G., 2010. *OsZIP5* is a plasma membrane zinc transporter in rice. *Plant Mol. Biol.* 73, 507–517.
- Lee, S., Ryoo, N., Jeon, J.S., Guerinot, M.L., An, G., 2012. Activation of rice *Yellow Stripe1-Like 16* (*OsYSL16*) enhances iron efficiency. *Mol. Cell* 33, 117–126.
- Liao, H., Rubio, G., Yan, X., Cao, A., Brown, K.M., Lynch, J.P., 2001. Effect of phosphorus availability on basal root shallowness in common bean. *Plant Soil* 232, 69–79.
- Li, X., Zhang, H., Ai, Q., Liang, G., Yu, D., 2016. Two bHLH transcription factors, bHLH34 and bHLH104, regulate iron homeostasis in *Arabidopsis thaliana*. *Plant Physiol.* 170, 2478–2493.
- López-Bucio, J., Hernández-Abreu, E., Sánchez-Calderón, L., Nieto-Jacobo, M.F., Simpson, J., Herrera-Estrella, L., 2002. Phosphate availability alters architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. *Plant Physiol.* 129, 244–256.
- López-Millán, A.F., Morales, F., Abadía, A., Abadía, J., 2000. Effects of iron deficiency on the composition of the leaf apoplasmic fluid and xylem sap in sugar beet. Implications for iron and carbon transport. *Plant Physiol.* 124, 873–884.
- Lucca, P., Hurrell, R., Potrykus, I., 2001. Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. *Theor. Appl. Genet.* 102, 392–397.
- Lucca, P., Hurrell, R., Potrykus, I., 2002. Fighting iron deficiency anemia with iron-rich rice. *J. Am. Coll. Nutr.* 21, 184–190. <https://doi.org/10.1080/07315724.2002.10719264>.

- Lucena, C., Waters, B.M., Romera, F.J., Garcia, M.J., Morales, M., Alcantara, E., et al., 2006. Ethylene could influence ferric reductase, iron transporter, and H⁺-ATPase gene expression by affecting FER (or FER-like) gene activity. *J. Exp. Bot.* 57, 4145–4154. <https://doi.org/10.1093/jxb/erl189>.
- Mahender, A., Anandan, A., Pradhan, S.K., Pandit, E., 2016. Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. *SpringerPlus* 5, 2086.
- Majerus, V., Bertin, P., Lutts, S., 2009. Abscisic acid and oxidative stress implications in overall ferritin synthesis by African rice (*Oryza glaberrima* Steud.) seedlings exposed to short term iron toxicity. *Plant Soil* 324, 253–265.
- Majumder, S., Datta, K., Datta, S.K., 2019. Rice Biofortification: High Iron, Zinc, and Vitamin-A to Fight against "Hidden Hunger" *Agronomy* 9, 803. <https://doi.org/10.3390/agronomy9120803>.
- Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2nd edn. Academic press, London.
- Masuda, H., Aung, M.S., Nishizawa, N.K., 2013b. Iron biofortification of rice using different transgenic approaches. *Rice* 6, 40.
- Masuda, H., Suzuki, M., Morikawa, K.C., Kobayashi, T., Nakanishi, H., Takahashi, M., et al., 2008. Increase in iron and zinc concentrations in rice grains via the introduction of barley genes involved in phytosiderophore synthesis. *Rice* 1, 100–108.
- Masuda, H., Kobayashi, T., Ishimaru, Y., Takahashi, M., Aung, M.S., Nakanishi, H., et al., 2013a. Iron-bio-fortification in rice by the introduction of three barley genes participated in mugineic acid biosynthesis with soybean ferritin gene. *Front. Plant Sci.* 4, 132.
- Masuda, H., Usuda, K., Kobayashi, T., Ishimaru, Y., Kakei, Y., Takahashi, M., et al., 2009. Overexpression of the barley nicotianamine syn- thase gene HvNAS1 increases iron and zinc concentrations in rice grains. *Rice* 2, 155–166.
- Mathus, E., Wu, L., Veda, Y., Hollen, S., Becken, M., Frei, M., 2015. Loci genes and mechanism associated with tolerance to ferrous iron toxicity in rice (*Oryza sativa* L.). *Theor. appl. Genet.* 128, 2085–2098.
- Masuda, H., Ishimaru, Y., Aung, M.S., Kobayashi, T., Kakei, Y., Takahashi, M., Higuchi, K., Nakanishi, H., Nishizawa, N.K., 2012. Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. *Sci. Rep.* 2, 534.
- Mendoza-Cózar, D.G., Xie, Q., Akmakjian, G.Z., Jobe, T.O., Patel, A., Stacey, M.G., Song, L., Demoin, D.W., Jurisson, S.S., 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.
- Merlot, S., Hannibal, L., Martins, S., Martinelli, L., Amir, H., Lebrun, M., Thomine, S., 2014. The metal transporter PgIREG1 from the hyperaccumulator *Psychotria gabriellae* is a candidate gene for nickel tolerance and accumulation. *J. Exp. Bot.* 65, 1551–1564.
- Miroslav, M., 1998. The role of the redox system in uptake and translocation of iron by higher plants. *Iugoslav Physiol Pharmacol Acta* 34, 479–489.
- Moore, K.L., Chen, Y., Van de Meene, A.M.L., Hughes, L., Liu, W., Geraki, T., Mosselmans, F., McGrath, S.P., Grovenor, C., Zhao, F.J., 2014. Combined Nano SIMS and synchrotron X-ray fluorescence reveal distinct cellular and subcellular distribution patterns of trace elements in rice tissues. *New Phytol.* 201, 104–115.
- Moorman, F.R., Van Breeman, N., 1978. Rice: Soil, Water and Land (IRRI Philippines).
- Mori, S., Nishizawa, N., Hayashi, H., Chino, M., Yoshimur, E., Ishihara, J., 1991. Why are young rice plants highly susceptible to iron deficiency? *Plant Soil* 130, 143–156.
- Morrissey, J., Baxter, I.R., Lee, J., Li, L., Lahner, B., Grotz, N., Kaplan, J., Salt, D.E., Guerinot, M.L., 2009. The ferroportin metal efflux proteins function in iron and cobalt homeostasis in *Arabidopsis*. *Plant Cell* 21, 3326–3338.
- Morrissey, J., Guerinot, M.L., 2009. Iron uptake and transport in plants: the good, the bad, and the ionome. *Chem. Rev.* 109, 4553–4567.
- Motomura, S., Yokoi, H., 1969. Fractionation method of ferrous iron in paddy soil. *Soil Sci. Plant Nutr.* 15 (1), 28–37. <https://doi.org/10.1080/00380768.1969.10432778>.
- Muscoli, C., Cuzzocrea, S., Riley, D.P., Zweier, J.L., Thiemermann, C., Wang, Z.-Q., Salvemini, D., 2003. On the selectivity of superoxide dismutase mimetics and its importance in pharmacological studies. *Br. J. Pharmacol.* 140, 445–460.
- Naidu, B.S., Mahadevappa, M., Inamdar, S.S., Maharudrapa, K., 1981. Performance of IR36 in Karnataka. *India. Int. Rice Res. Newsl* 6, 4.
- Nakashima, K., Yamaguchi-Shinozaki, K., Shinozaki, K., 2014. The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. *Front. Plant Sci.* 5, 170.
- Nakandalage, N., Nicola, S.M., Norton, R.M., Hirotsu, N., Milham, P.J., Seneweera, S., 2016. Improving rice zinc biofortification success rates through genetic and crop management approaches in a changing environment. *Front. Plant Sci.* 7, 1–13.
- Narayanan, N.N., Vasconcelos, M.W., Grusak, M.A., 2007. Ex- pression profiling of *Oryza sativa* metal homeostasis genes in different rice cultivars using a cDNA microarray. *Plant Physiol. Biochem.* 45, 277–286.
- Nawaz, Z., Kakar, K.U., Li, X.B., Zhang, B., Shou, H.X., Shu, Q.Y., 2015. Genome-wide association mapping of quantitative trait loci (QTLs) for contents of eight elements in brown rice (*Oryza sativa* L.). *J. Agric. Food Chem.* 63, 8008–8016.
- Nerker, Y.S., Misal, M.B., Marekar, R.V., 1984. PBN 1, a semidwarf upland rice cultivar tolerant of iron deficiency. *Int. Rice Res. Newsl.* 9, 15–16.
- Nogiya, M., Pandey, R.N., Singh, B., 2016. Physiological basis of iron chlorosis tolerance in rice (*Oryza sativa*) in relation to the root exudation capacity. *J. Plant Nutr.* 39, 1536–1546.
- Norton, G.J., Deacon, C.M., Xiong, L., Huang, S., Meharg, A.A., Price, A.H., 2010. Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. *Plant Soil* 329, 139–153.
- Nozoye, T., Nagasaka, S., Kobayashi, T., Takahashi, M., Sato, Y., Sato, Y., Uozumi, N., Nakanishi, H., Nishizawa, N.K., 2011. Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. *J. Biol. Chem.* 286, 5446–5454.
- Nugraha, Y., Dwinita, W., Rosdianti, U.I., Ardie, S., Ghulamahdi, M., Suwarno, Aswiddinnoor, H., 2016. *Biodiversitas* 17 (2), 753–763.
- Ogo, Y., Itai, R.N., Kobayashi, T., Aung, M.S., Nakanishi, H., Nishizawa, N.K., 2011. OsIRO2 is responsible for iron utilization in rice and improves growth and yield in calcareous soil. *Plant Mol. Biol.* 75, 593–605.
- Ogo, Y., Kakei, Y., Itai, R.N., Kobayashi, T., Nakanishi, H., Takahashi, H., Nakazono, M., Nishizawa, N.K., 2014. Spatial transcriptomes of iron-deficient and cadmium-stressed rice. *New Phytol.* 201, 781–794.
- Ogo, Y., Nakanishi, Itai, R., Nakanishi, H., Kobayashi, T., Takahashi, M., Mori, S., Nishizawa, N.K., 2007. The rice bHLH protein OsIRO2 is an essential regulator of the genes involved in Fe uptake under Fe-deficient conditions. *Plant J.* 51, 366–377.
- Onaga, G., Edema, R., Asea, G., 2013. Tolerance of rice germplasm to iron toxicity stress and the relationship between tolerance, Fe²⁺, P and K content in the leaves and roots. *Arch. Agron. Soil Sci.* 59 (2), 213–229. <https://doi.org/10.1080/03650340.2011.622751>.
- Oort, P.A., Jvan., 2018. Mapping abiotic stresses for rice in Africa: drought, cold, iron toxicity, salinity and sodicity. *Field Crops Res.* 219, 55–75.
- Paul, S., Ali, N., Gayen, D., Datta, K., 2012. Molecular breeding of Osfer2 gene to increase iron nutrition in rice grain. *GM Crops Food* 3 (4), 310–316. <https://doi.org/10.4161/gmcr.22104>.
- Pawar, S., Pandit, E., Arjun, P., Wagh, M., Bal, D., Panda, S., Bastia, D.N., Pradhan, S.K., Mohanty, I.C., 2017. Genetic variation and association of molecular markers for Fe toxicity tolerance in rice. *Oryza* 54 (4), 356–366. <https://doi.org/10.5958/2249-5266.2017.00066.2>.
- Ponnamperuma, F.N., 1976. Temperature and chemical kinetics of flooded soils. *Climate and Rice*. The International Rice Research Institute, Los Baños, Philippines.
- Prade, K., Ottow, J.C.G., Jacq, V.A., 1993. Excessive Iron Uptake (Iron Toxicity) by Wetland Rice (*Oryza Sativa* L.) on an Acid Sulphate Soil in the Casamance/Senegal. International Institute for Land Reclamation and Improvement, Wageningen, The Netherlands, pp. 150–162.
- Pradhan, S.K., Pandit, E., Pawar, S., Bharati, B., Chatopadhyay, K., Singh, S., Dash, P., Reddy, J.N., 2019. Association mapping reveals multiple QTLs for grain protein content in rice useful for biofortification. *Mol. Genet. Genom.* <https://doi.org/10.1007/s00438-019-01556-w>.
- Pradhan, S.K., Pandit, E., Pawar, S., Naveenkumar, R., Barik, S.R., Mohanty, S.P., Nayak, D.K., Ghritlahre, S.K., Rao, D.S., Reddy, J.N., Patnaik, S.S.C., 2020. Linkage disequilibrium mapping for grain Fe and Zn enhancing QTLs useful for nutrient dense rice breeding. *BMC Plant Biol.* <https://doi.org/10.1186/s12870-020-2262-4>.
- Prasad, R., Shivay, Y.S., 2018. Iron fertilization of cereal crops for human health—A new concept. *Ind. J. Fertilisers.* 14 (5), 68–77.
- Prasad, R., Shivay, Y.S., Kumar, D., 2020. Current status, challenges and opportunities in rice production. *Rice Production Worldwide.* <https://doi.org/10.1080/01904167.2020.1738464>.
- Prasetyo, T.B., Ahmad, F., Saidi, A., 2013. Humic acid and water management to decrease Ferro (Fe²⁺) solution and increase productivity of established new rice field. *J. Trop. Soil* 17 (1), 9–17. <https://doi.org/10.5400/jts.2012.17.1.9>.
- Ouedraogo, S., Ouedraogo, M., 2003. Evaluation économique de l'impact de la recherche et de la vulgarisation sur le riz au Burkina- Faso. Mini-report. Institut de l'Environnement et des Recherches Agricoles (INERA), Ouagadougou, pp. 1–24.
- Qui, L.C., Pan, J., Dan, B.W., 1995. The mineral nutrient component and characteristic of color and white brown rice. *Chinese journal of rice science* 7, 95–100.
- Quinet, M., Vromman, D., Clippe, A., Bertin, P., Lequeux, H., Dufey, I., Lutts, S., Lefe'vre, I., 2012. Combined transcriptomic and physiological approaches reveal strong differences between short and long term response of rice (*Oryza sativa*) to iron toxicity. *Plant Cell Environ.* 35 (10), 1837–1859.
- Ramirez, L.M., Claassen, N., Werner, H., Moawad, A.M., 2002. Effect of phosphorus, potassium and zinc fertilizers on iron toxicity in wetland rice (*Oryza sativa* L.). *Plant Soil* 239 (197-), 206. <https://doi.org/10.1023/A:1015099422778>.
- Reinbothe, C., Bartsch, S., Eggink, L.L., Hooper, J.K., Brusslan, J., Andrade-Paz, R., Monnet, J., Reinbothe, S., 2006. A role for chlorophyllide a oxygenase in the regulated import and stabilization of light-harvesting chlorophyll a/b proteins. *Proc. Natl. Acad. Sci. U. S. A.* 103, 4777–4782.
- Rellán-Álvarez, R., Abadía, J., Álvarez-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.
- Rellán Álvarez, R., Giner Martínez Sierra, J., Orduna, J., Orera, I., Rodríguez Castrillón, J.Á., García Alonso, J.I., 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.
- Rodríguez-Celma, J., Lin, W.D., Fu, G.M., Abadía, J., López-Millán, A.F., Schmidt, W., 2013. Mutually exclusive alterations in secondary metabolism are critical for the uptake of insoluble iron compounds by *Arabidopsis* and *Medicago truncatula*. *Plant Physiol.* 162, 1473–1485.
- Romera, F.J., Alcantara, E., 1994. Iron-deficiency stress responses in cucumber (*Cucumis sativus* L.) roots (A possible role for ethylene?). *Plant Physiol.* 105, 1133–1138. <https://doi.org/10.1104/pp.105.4.1133>.
- Romera, F.J., Alcántara, E., de la Guardia, M.D., 1999. Ethylene production by Fe-deficient roots and its involvement in the regulation of Fe-deficiency stress responses by Strategy I plants. *Ann. Bot.* 83, 51–55. <https://doi.org/10.1006/anbo.1998.0793>.
- Romera, F.J., García, M., Alcántara, E., Pérez-Vicente, R., 2011. Latest findings about the interplay of auxin, ethylene and nitric oxide in the regulation of Fe deficiency responses by Strategy I plants. *Plant Signal. Behav.* 6, 167–170. <https://doi.org/10.4161/psb.6.1.14111>.
- Romheld, V., Marschner, H., 1986. Evidence for a specific uptake system for iron

- phytosiderophore in roots of grasses. *Plant Physiol.* 80, 175–180.
- Rout, G.R., Sahoo, S., 2015. Role of iron in plant growth and metabolism. *Cab Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* 3, 1–24.
- Rout, G.R., Sunita, S., Das, A.B., Das, S.R., 2014. Screening of iron toxicity in rice genotypes on the basis of morphological, physiological and biochemical analysis. *J. Exp. Biol. Sci.* 2, 567–582.
- Ruskandar, A., Rustiati, T., Wardana, P., 2011. Adopsi varietas unggul baru dan keuntungan usahatani padi di lahan rawa lebak. Seminar Nasional Hasil Penelitian Padi. Sukamandi (ID): Balai Besar Penelitian Padi.
- Sahrawat, K.L., 2005. Iron toxicity in wetland rice and the role of other nutrients. *J. Plant Nutr.* 27, 1471–1504. <https://doi.org/10.1081/PLN-200025869>.
- Sahrawat, K.L., Singh, B.N., 1998. Seasonal differences in iron toxicity tolerance of lowland rice cultivars. *Int. Rice Res. Notes* 23 (1), 18–19.
- Sarma, R., Swamy, H.V., Shashidhar, H.E., 2018. Dealing with zinc and Iron deficiency in rice: combine strategies to fight hidden hunger in developing countries. *Int. J. Curr. Microbiol. App. Sci.* 7, 1887–1895.
- Schmidt, H., Eickhorst, T., 2014. Detection and quantification of native microbial populations on soil-grown rice roots by catalyzed reporter deposition-fluorescence in situ hybridization. *FEMS Microbiol. Ecol.* 87, 390–402.
- Schikora, A., Schmidt, W., 2002. Formation of transfer cells and H (+)-ATPase expression in tomato roots under P and Fe deficiency. *Planta* 215, 304–311. <https://doi.org/10.1007/s00425-002-0738-0>.
- Schmidt, W., Titte, J., Schikora, A., 2000. Role of hormones in the induction of iron deficiency responses in Arabidopsis roots. *Plant Physiol.* 122, 1109–1118. <https://doi.org/10.1104/pp.122.4.1109>.
- Selote, D.S., Bharti, Khanna-Chopra, S., R., 2004. Drought acclimation reduces O₂ @! accumulation and lipid peroxidation in wheat seedlings. *Biochem. Biophys. Res. Commun.* 314, 724–729.
- Senoura, T., Sakashita, E., Kobayashi, T., Takahashi, M., Aung, M., Masuda, H., Nakanishi, H., Nishizawa, N.K., 2017. The iron-chelate transporter OsYSL9 plays a role in iron distribution in developing rice grains. *Plant Mol. Biol.* 95, 375–387.
- Seshadri, S., 2001. Prevalence of micronutrient deficiency particularly of iron, zinc and folic acid in pregnant women in South East Asia. *Br. J. Nutr.* 85 (Suppl. 2), S87–S92.
- Shimizu, A., Guerta, C.Q., Gregorio, G.B., Kawasaki, S., Ikehashi, H., 2005. QTLs for nutritional content of rice seedlings (*Oryza sativa* L.) in solution cultures and its implication to tolerance to iron-toxicity. *Plant Soil* 275. <https://doi.org/10.1007/s11104-004-4683-5>.
- Shi, R., Hao, H., Fan, X., Karim, R., Zhan, F., Zou, G., 2012. Responses of aerobic rice (*Oryza sativa* L.) to iron deficiency. *J. Integr. Agric.* 11, 938–945.
- Shahzad, Z., Rouached, H., Rakha, A., 2014. Combating mineral malnutrition through Iron and zinc biofortification of cereals. *Food Sci. Food Safety* 13. <https://doi.org/10.1111/1541-4337.12063>.
- Shimizu, A., 2009. QTL analysis of genetic tolerance to iron toxicity in rice (*Oryza sativa* L.) by quantification of bronzing score. *J. New Seeds* 10, 171–179.
- Sikirou, M., Saito, K., Achigan-Dako, E.G., Dramé, K.N., Ahanchédé, A., Venuprasad, R., 2015. Genetic improvement of Iron toxicity tolerance in Rice-Progress, challenges and prospects in West Africa. *Plant Prod. Sci.* 18 (4), 423–434.
- Sikirou, M., Saito, K., Drame, K.N., Saidou, A., Dieng, I., Ahanchede, A., Venuprasad, R., 2016. Soil-based screening for iron toxicity tolerance in rice using pots. *Plant Prod. Sci.* 19, 489–496.
- Simbauranga, R.H., Kamugisha, E., Hokororo, A., Benson, R., Kidenya, Makani, J., 2015. Prevalence and factors associated with severe anaemia amongst under-five children hospitalized at Bugando Medical Centre, Mwanza, Tanzania. *BMC Hemato.* 15, 13. <https://doi.org/10.1186/s12878-015-0033-5>.
- Singh, S.P., Gruijssem, W., Bhullar, N.K., 2017. Single genetic locus improvement of iron, zinc and β -carotene content in rice grains. *Sci. Rep.* 7, 6883.
- Slamet-Loedin, I.H., Johnson-Beebout, S.E., Impa, S., Tsakirpaloglou, N., 2015. Enriching rice with Zn and Fe while minimizing Cd risk. *Front. Plant Sci.* 6 (121), 1–9.
- Stangoulis, J.C.R., Huynh, B.L., Welch, R.M., Choi, E.Y., Graham, R.D., 2007. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica* 154, 289–294.
- Stein, R.J., Ricachenevsky, F.K., Fett, J.P., 2009. Differential regulation of the two rice ferritin genes (*OsFER1* and *OsFER2*). *Plant Sci.* 177, 563–569. <https://doi.org/10.1016/j.plantsci.2009.08.001>.
- Stephan, U.W., 2002. Intra- and intercellular iron trafficking and subcellular compartmentation within roots. *Plant and Soil* 241 (1), 19–25. <https://doi.org/10.1023/A:1016086608846>.
- Stoltzfus, R.J., Dreyfuss, M.L., 1998. Guidelines for the Use of Iron Supplements to Prevent and Treat Iron Deficiency Anemia. ILSI Press, Washington, DC.
- Suzuki, M., Morikawa, K.C., Nakanishi, H., Takahashi, M., Saigusa, M., Mori, S., Nishizawa, N.K., 2008. Transgenic rice lines that include barley genes have increased tolerance to low iron availability in a calcareous paddy soil. *Soil Sci. Plant Nutr.* 54, 77–85.
- Swamy, B.P.M., Kaladhar, K., Anuradha, K., Batchu, A.K., Longvah, T., Sarla, N., 2018. QTL analysis for grain iron and zinc concentrations in two O. Nivara derived backcross populations. *Rice Sci.* 25 (4), 197–207.
- Swamy, M.B.P., Rahman, M.A., Inabangan-Asilo, M., Amparado, A., Manito, C., Mohanty, C., Reinke, R., Slamet-Loedin, I.H., 2016. Advances in breeding for high grain Zn in rice. *Rice* 9, 49–57.
- Taiz, L., Zeiger, E., 1991. *Plant Physiology*. pp. 565. The Benjamin/Cummings Publ. Co. Inc., Redwood City, Calif., USA.
- Takagi, S., 1976. Naturally occurring iron-chelating compounds in oat-and rice-root washings: I. Activity measurement and preliminary characterization. *Soil Sci. Plant Nutr.* 22, 423–433.
- Takasaki, H., Maruyama, K., Kidokoro, S., Ito, Y., Fujita, Y., Shinozaki, K., Yamaguchi-Shinozaki, K., Nakashima, K., 2010. The abiotic stress-responsive NAC-type transcription factor OsNAC5 regulates stress-inducible genes and stress tolerance in rice. *Mol. Genet. Genomics* 284, 173–183.
- Tagaki, S., Kamei, S., Yu, M.H., 1988. Efficiency of iron extraction from soil by mugineic acid family phytosiderophores. *J. Plant Nutr.* 11, 643–651.
- Takagi, S., Nomoto, K., Takemoto, T., 1984. Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. *J. Plant Nutr.* 7, 469–477.
- Takahashi, R., Ishimaru, Y., Senoura, T., Shimo, H., Ishikawa, S., Arai, T., Nakanishi, H., Nishizawa, N.K., 2011. The OsNRAMP1 iron transporter is involved in Cd accumulation in rice. *J. Exp. Bot.* 62, 4843–4850.
- Takahashi, M., Terada, Y., Nakai, I., Nakanishi, H., Yoshimura, E., Mori, S., Nishizawa, N.K., 2003. Role of nicotianamine in the intracellular delivery of metals and plant reproductive development. *Plant Cell* 15, 1263–1280.
- Tanaka, A., Loe, R., 1966. Some mechanisms involved in the development of iron toxicity symptoms in the rice plant. *Soil Sci. Plant Nutr.* 4, 32–36. <https://doi.org/10.1080/00380768.1966.10431951>.
- Thimm, O., Essigmann, B., Kloska, S., Altmann, T., Buckhout, T.J., 2001. Response of Arabidopsis to iron deficiency stress as revealed by microarray analysis. *Plant Physiol.* 127, 546–556.
- Trijatniko, K.R., Dueñas, C., Tsakirpaloglou, N., Torrizo, L., Arines, F., Adeva, C., Balindong, J., Oliva, N., et al., 2016. Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. *Sci. Rep.* 6, 19792. <https://doi.org/10.1038/srep19792>.
- Qu, L.Q., Yoshihara, T., Ooyama, A., Goto, F., Takaiwa, F., 2005. Iron accumulation does not parallel the high expression level of ferritin in transgenic rice seeds. *Planta* 222, 225–233. <https://doi.org/10.1016/j.taap.2007.04.009>.
- Ueno, D., Yamaji, N., Ma, J.F., 2009. Further characterization of ferric phytosiderophore transporters ZmYS1 and HvYS1 in maize and barley. *J. Exp. Bot.* 60, 3513–3520.
- Vasconcelos, M., Datta, K., Oliva, N., Khalekuzzaman, M., Torrizo, L., Krishnan, S., et al., 2003. Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. *Plant Sci.* 164, 371–378.
- Vert, G., Grotz, N., Dédaldéchamp, F., Gaymand, F., Guerinot, M., Briat, J., et al., 2002. IRT1, an arabidopsis transporter essential for iron uptake from the soil and for plant growth. *Plant Cell* 14, 1223–1233.
- Victoria, F.D.C., Bervald, C.M.P., da Maia, L.C., de Sousa, R.O., Panaud, O., de Oliveira, A.C., 2012. Phylogenetic relationships and selective pressure on gene families related to iron homeostasis in land plants. *Genome* 55, 883–900.
- Virmani, S.S., 1977. Varietal tolerance of rice to iron toxicity in Liberia. *Int. Rice Res. News.* *Int. Rice Res. Inst.* 2, 4–5.
- Von Wiren, N., Klair, S., Bansal, S., Briat, J.F., Khodr, H., Shioiri, T., Leigh, R.A., Hider, R.C., 1999. Nicotianamine chelates both FeIII and FeII. Implications for metal transport in plants. *Plant Physiol.* 119, 1107–1114.
- Von Wiren, N., Mori, S., Marschner, H., Romheld, V., 1994. Iron inefficiency in maize mutant ys1 (*Zea mays* L. Cv Yellow-Stripe) caused by a defect in uptake of iron phytosiderophores. *Plant Physiol.* 106, 71–77.
- Wan, J.L., Zhai, H.Q., Wan, J.M., Ikehashi, H., 2003a. Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice, *Oryza sativa* L. *Euphytica* 131, 201–206.
- Wan, J.L., Zhai, H.Q., Wan, J.M., Yasui, H., Yoshimura, A., 2003b. Mapping QTL for traits associated with resistance to ferrous iron toxicity in rice (*Oryza sativa* L.), using japonica chromosome segment substitution lines. *Yi Chuan Xue Bao* 30 (10), 893–898.
- Wan, J.L., Zhai, H.Q., Wan, J.M., 2005. Mapping of QTLs for ferrous iron toxicity tolerance in rice (*Oryza sativa* L.). *Acta Genetica Sinica* 32 (11), 1156–1166.
- Wairich, A., De oliveira, B.H., Arend, E.B., Duarte, G.L., Ponte, L.R., Sperotto, R.A., Ricachenevsky, F.K., Palma, F.J., 2019. The combined strategy for iron uptake is not exclusive to domesticated rice (*Oryza sativa*). *Sci. Rep.* 9, 16144. <https://doi.org/10.1038/s41598-019-52502-0>.
- Waters, B.M., Chu, H.H., DiDonato, R.J., Roberts, L.A., Eisleys, R.B., Lahner, B., Salt, D.E., Walker, E.L., 2006. Mutations in Arabidopsis Yellow Stripe-Like1 and Yellow Stripe-Like3 reveal their roles in metal ion homeostasis and loading of metal ions in seeds. *Plant Physiol.* 141, 1446–1458.
- WARDA, 1998. Annual Report 1997. West Africa Rice Development Association, Bouaké, pp. 1–71.
- Welch, R.M., Graham, R.D., 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J. Exp. Bot.* 55, 353–364.
- Wang, T., Peverly, J.H., 1998. Screening a selective chelator pair for simultaneous determination of iron(II) and iron(III). *Soil Sci. Soc. Am. J.* 62, 611–617.
- Wessells, K., Brown, K., 2012. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS One*. <https://doi.org/10.1371/journal.pone.0050568>.
- WHO, 2016. Iron Deficiency Anaemia. Available at: <http://www.who.int/nutrition/topics/ida/en/>.
- Wintz, H., Fox, T., Wu, Y.-Y., Feng, V., Chen, W., Chang, H.-S., Zhu, T., Vulpe, C., 2003. Expression profiles of Arabidopsis thaliana in mineral deficiencies reveal novel transporters involved in metal homeostasis. *J. Biol. Chem.* 278, 47644–47653.
- Wirth, J., Poletti, S., Aeschlimann, B., Drosse, B., Osorio, S., Tohge, T., Fernie, A.R., Günther, D., Gruijssem, W., Sautte, C., 2009. *Plant Biotechnol. J.* 7, 718.
- Winslow, M.D., Yamauchi, M., Alluri, K., Masajo, T.M., 1989. Reducing iron toxicity in rice with resistant genotype and rigid planting. *Agron. J.* 81, 458–460.
- Wu, P., Hu, B., Liao, C.Y., Zhu, J.M., Wu, Y.R., Senadhira, D., Paterson, A.H., 1998. Characterization of tissue tolerance to iron by molecular markers in different lines of rice. *Plant Soil* 203, 217–226.
- Wu, P., Luo, A., Zhu, J., Yang, J., Huang, N., Senadhira, D., 1997. Molecular markers linked to genes underlying seedling tolerance for ferrous iron toxicity. *Plant Soil* 196, 317–320.
- Wu, L., Shhadi, M.Y., Gregorio, G., Matthus, E., Becker, M., Frei, M., 2014. Genetic and physiological analysis of tolerance to acute iron toxicity in rice. *Rice* 7, 8. <https://doi.org/10.1007/s12230-014-9211-1>.

- [org/10.1186/s12284-014-0008-3](https://doi.org/10.1186/s12284-014-0008-3).
- Wu, L., Ueda, Y., Lai, S., Frei, M., 2016. Shoot tolerance mechanisms to iron toxicity in rice (*Oryza sativa* L.). *Plant Cell Environ.* <https://doi.org/10.1111/pce.12733>.
- Xiong, H., Kakei, Y., Kobayashi, T., Guo, X., Nakazono, M., Takahashi, H., et al., 2013. Molecular evidence for phytosiderophore-induced improvement of iron nutrition of peanut intercropped with maize in calcareous soil. *Plant Cell Environ.* 36, 1888–1902. <https://doi.org/10.1111/pce.12097>.
- Yadav, G.S., Shivay, Y.S., Kumar, D., Babu, S., 2013. Enhancing iron density and uptake in grain and straw of aerobic rice through mulching and rhizo-foliar fertilization of iron. *Afr. J. Agric. Res* 8, 5447–5454.
- Yamanouchi, M., Yoshida, M.S., 1981. Physiological mechanisms of rice's tolerance for iron toxicity. In: *Proceedings of IRRI Seminar*. International Rice Research Institute, Philippines. pp. 1–8.
- Yang, A., Zhang, W., 2016. A Small GTPase, OsRab6a, is involved in the regulation of iron homeostasis in rice. *Plant Cell Physiol.* 57 (6), 1271–1280.
- Yang, X., Ye, Z., Shi, C., Zhu, M., Graham, R.D., 1998. Genotypic differences in concentrations of iron, manganese, copper, and zinc in polished rice grains. *J. Plant Nutr.* 21, 1453–1462.
- Yokosho, K., Yamaji, N., Ma, J.F., 2016. OsFRDL1 expressed in nodes is required for distribution of iron to grains in rice. *J. Exp. Bot.* 67, 5485–5494.
- Yokosho, K., Yamaji, N., Ueno, D., Mitani, N., Ma, J.F., 2009. OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice. *Plant Physiol.* 149, 297–305.
- Yoshino, M., Murakami, K., 1998. Interaction of iron with polyphenolic compounds: application to antioxidant characterization. *Anal. Biochem.* 257, 40–44. <https://doi.org/10.1006/abio.1997.2522>.
- Yuan, L., Wu, L., Yang, C., Quin, L.V., 2013. Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. *J. Sci. Food Agric.* 93 (2), 254–261.
- Zeng, Y., Zhang, H., Wang, L., Pu, X., Du, J., Yang, S., Liu, J., 2010. Genotypic variation in element concentrations in brown rice from Yunnan landraces in China. *Environ. Geochem. Health* 32, 165–177.
- Zhang, J., Chen, K., Pang, Y., Naveed, S.A., Zhao, X., Wang, X., Wang, Y., Dingkuhn, M., Pasuquin, J., Li, Z., Xu, J., 2017. QTL mapping and candidate gene analysis of ferrous iron and zinc toxicity tolerance at seedling stage in rice by genome-wide association study. *BMC Genomics* 18, 828. <https://doi.org/10.1186/s12864-017-4221-5>.
- Zhang, J., Liu, B., Li, M., Feng, D., Jin, H., Wang, P., Liu, J., Xiong, F., Wang, J., Wang, H.B., 2015. The bHLH transcription factor bHLH104 interacts with IAA-LEUCINE RESISTANT3 and modulates iron homeostasis in Arabidopsis. *Plant Cell* 27, 787–805.
- Zhang, M.W., Guo, B.J., Peng, Z.M., 2005. Genetics effects on grain characteristics of indica black rice and their uses on indirect selections for some mineral element content in grains. *Genet. Resour. Crop Evol.* 52, 1121–1128.
- Zhang, Y., Xu, Y.H., Yi, H.Y., Gong, J.M., 2012. Vacuolar membrane transporters OsVIT1 and OsVIT2 modulate iron translocation between flag leaves and seeds in rice. *Plant J.* 72, 400–410.
- Zhang, L., Li, G., Wang, M., Di, D., Sun, L., Kronzucker, H.J., et al., 2018. Excess iron stress reduces root tip zone growth through nitric oxide-mediated repression of potassium homeostasis in Arabidopsis. *New Phytol.* 219, 259–274. <https://doi.org/10.1111/nph.15157>.
- Zheng, L., Ying, Y., Wang, L., Wang, F., Whelan, J., Shou, H., 2010. Identification of a novel iron regulated basic helix-loop-helix protein involved in Fe homeostasis in *Oryza sativa*. *BMC Plant Biol.* 10, 166.
- Zimmermann, M.B., Hurrell, R., 2002. Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Curr. Opin. Biotechnol.* 13, 142–145.