

IRON TOXICITY, TOLERANCE AND QUANTITATIVE TRAIT LOCI MAPPING IN RICE; A REVIEW

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Abstract. Rice being a big staple food, faces many abiotic stresses, resulting in a yield reduction. Iron (Fe) has many key roles for plants in sustaining growth and production, however, Fe toxicity is a big threat to rice production worldwide. There are many regulation mechanisms in rice to ensure an adequate supply of Fe to plant and to prevent deleterious effects. Rice is adopted many physiological and molecular mechanisms to cope with Fe toxicity in flooded soils, however, rice tolerance to Fe toxicity varies greatly and this mechanism has not fully understood. Efforts are being made to unfold the genetic basis of Fe toxicity tolerance in rice. There are many regulators discussed here, which are responsible for Fe uptake and transport from rhizosphere to the plants. Toxic effects of Fe on rice, the genes advocating the regulation of Fe, and many quantitative traits loci governing Fe toxicity tolerance in rice are discussed here. Many putative QTL involved in rice tolerance to Fe toxicity are presented in this review. The identification of QTL and regulators for Fe toxicity tolerance would be more helpful in regulating Fe toxicity and developing Fe tolerant lines in rice. More efficient breeding techniques are required to screen Fe tolerant rice genotypes. This review focused on some possible ways to improve Fe toxicity tolerance in rice and provide a strong theoretical base for future research.

Keywords: *iron; toxicity, genes, QTL, regulation, rice, mapping population*

Abbreviations: AB: Abscisic Acid, NSP: Number of spikelet's/plant, BIL: Backcross inbred lines, OA: Organic Acid, BRILs: Backcross recombinant inbred lines, QTL: Quantitative trait loci, CC: Chlorophyll content, RIL: Recombinant inbred lines, Chr: Chromosomes, RL: Root length (cm), Cm: Centimeter, ROS: Reactive oxygen species, CSSL: Chromosomal segment substitution lines, DH: Double haploid, RDW: Root dry weight (mg), Fe: Iron, SWC: Shoot water content, GW: Grain weight, SL: Shoot length (cm), IL: Introgression line, LBI: Leaf bronzing index, MG: Magic population.

Introduction

Rice is the main cereal and staple crop for almost 50% of the world's population (Mahender et al., 2019; Rasheed et al., 2020a). Rice yield is expected to increase by 100 tons to feed the 9.1 billion population of the world by 2050 (Jaggard et al., 2010). Rice crop is facing many abiotic and biotic stresses including drought, heat, salinity, cold and nutrient deficiencies and most importantly the Fe toxicity (Mahender et al., 2019; Rasheed et al., 2020b). Fe toxicity is one of the leading restrictions for rice growth in many soil and a lot of studies have been conducted on understanding the genetic base of this stress (Bashir et al., 2014; Mahender et al., 2019).

Fe has many significant functions in rice-like, photosynthesis, homeostasis, and mitochondrial respiration (Nakanishi et al., 2006; Kim and Guerinot, 2007; Li et al., 2017). About 18% of soils globally are suffering from Fe toxicity and Fe deficiency (Saikia and Baruah, 2012; Das and Roychoudhury, 2014; Dufey et al., 2015), which results in a change in soil pH, soil fertility status and many other alterations in soil properties (Audebert and Sahrawat, 2000; Audebert, 2006). In many crops 50% reduction in grain yield has been reported owing to Fe toxicity, however, complete crop failure has also been observed during the early growth stage. Fe toxicity usually affects rice shoot length (SL), root length (RL) and also results in leaf bronzing which is a primary symptom of Fe toxicity in rice (Dufey et al., 2009).

Fe toxicity often occurs in alkaline soils, which leads to rise in pH, an increase in the amount of calcium carbonate and nitrate, a change in temperature and poor aeration (Kobayashi et al., 2014; Mongon et al., 2017). Several factors such as poor drainage, soil organic matter content, more hydrogen sulfides, low soil fertility and genotypes lead to an increase in available forms of Fe in soil (Chandel et al., 2010; Mahender et al., 2019). Rice plants adopted several mechanisms to cope with Fe toxicity in soil, like reducing Fe uptake and chelation through chelating agents, trafficking and storing in less responsive type, but most important is the expression of resistance genes (Dufey et al., 2009; Zhang et al., 2017).

QTL mapping is one of the powerful approaches to identify the genes of interest on the chromosome. In order to locate the gene of interest on chromosome, we need to screen genotypes of rice against Fe toxicity. A lot of QTL have been reported in rice for Fe toxicity tolerance using several mapping populations (Jain and Connolly, 2013; Zhang et al., 2017; Meng et al., 2017). Breeding Fe resistant varieties is an economically important approach to enhance rice production under Fe toxicity stress and Fe toxicity is controlled by many genes in rice (Dufey et al., 2012; Wainaina et al., 2018). A lot of genes have been reported in rice which is responsible for rice tolerance to Fe toxicity and these genes belong to five major protein families (OsFROs and OsFERS) (Chandel et al., 2010). Most of QTL reported in rice against Fe toxicity tolerance belongs to easily measurable traits, like seedling length, root length, seedling fresh and dry weight (Zhao et al., 2013; Dufey et al., 2015; Liu et al., 2016; Meng et al., 2017). The understanding of the Fe tolerance mechanism in rice is important to develop rice varieties tolerant to Fe toxicity. In this review we discussed the recent advancements on rice tolerance to Fe toxicity and ways to improve rice production in Fe affected soils and moreover, a view on identified QTL for Fe toxicity tolerance in rice is also discussed here.

Role of Fe in plants and rice

Fe has many roles in plants such as nutrients transport, mitochondrial respiration, photosynthesis, regulation of several enzymes and nitrogen assimilation (Bashir et al., 2010; Wu et al., 2014; Brumbarova et al., 2015). The regulation of protein stability is also performed by Fe and it also takes part in many chemical reactions such as, hydration, dehydration, redox-dependent catalysis, photo redox catalysis which detoxify excessive Fe, thus, Fe is one of the essential mineral element for plants (Zhang et al., 2013; Jain and Connolly, 2013; Dufey et al., 2015). Fe accelerates many antioxidant defense mechanisms such as, superoxide dismutase, catalase, polyphenol oxidase which protects rice plants from oxidative damage, which helps to screen Fe tolerant rice

cultivars (Saikia and Baruah, 2012; Pennock et al., 2015). The photosynthesis process is facilitated by Fe; owing to the fact 90% of Fe is present in plastids to maintain the structural integrity of the thylakoid membrane (Rout and Sahoo, 2015; Mahender et al., 2019).

Iron toxicity in rice

In the case of Fe toxicity, cell division occurs and leaves turned into white and results in stunted growth (Vejchasarn et al., 2016; Banakrt et al., 2017). An excessive amount of Fe results in cellular oxidative damage and leads to several changes in morpho-physiological and yield traits of rice (Hell and Stephan, 2003; Sikirou et al., 2015). Iron toxicity lead to blockage of important nutrients essential for rice growth (Audebert and Sahrawat, 2000; Nughara et al., 2016) and complete crop failure can occur if Fe toxicity becomes more severe (Audebert, 2006; Li et al., 2016). A surplus quantity of Fe is firstly responsible for Fenton reaction and it produces hydroxyl radicals ($-OH$) and reactive oxygen species (ROS), induce permanent injury to the membrane lipid, protein and genetic material. The ROS resulting in oxidized chlorophyll and successively decrease the photosynthesis, and leads to chlorosis (a major yield-reducing factor) (Mengel, 1995; Onaga et al., 2016).

How Fe toxicity arises is soil

Fe in soil under anaerobic condition converted from Fe^{3+} to Fe^{2+} due to low pH and becomes toxic for plants. Fe prevalently occurs in the soluble and reduced ferrous form (Fe^{2+}) due to low soil redox potential arising from anaerobic conditions, which are developed when soil microorganisms and plant roots deplete oxygen by respiration. The excessive Fe molecules are transported via xylem flow to the shoot leading to Fe toxicity which is one of the main nutrient disorder in rice (Frei et al., 2016; Van Ort, 2018).

Iron uptake, transport and assimilation in rice grains

Fe uptake, transport from root to shoot and grain are essential for normal plant growth. Fe can be transported in various forms through xylem and phloem including Fecitrate, DMA-Fe (III), and NA-Fe (II). *OsFRDL1* is involved in Fe homeostasis in rice via xylem. There are 18 putative *YSL* family genes in rice, out of them *OsYSL5-7*, *-14* and *-17* are mainly expressed at epidermis, cortex and stele of the Fe sufficient and Fe deficient rice roots. The expression of *OsYSL1-4*, *9-11* and *-18* was not studied in roots. *OsYSL12* is expressed in cortex and stele in both Fe deficient and sufficient condition whereas, *OsYSL12* is expressed in the cortex and stele under Fe sufficient *OsYSL6* was expressed in the epidermis in under Fe-sufficient condition only (Inoue et al., 2008). Among these mutant genes, *OsYSL2*, *OsYSL-15* and *OsYSL18* have been studied in detail (Aoyama et al., 2009; Ishimaru et al., 2010). *OsYSL15* transports Fe^{2+} -DMA from the rhizosphere to the roots and is involved in internal Fe homeostasis. *OsYSL15* promoter driven GUS expression was only observed in leaf tissue but also at the flowering stage (Inoue et al., 2009). These all studies indicated that *OsYSL15* is involved in Fe transport and assimilation in rice grains. *OsYSL2* is also involved in Fe assimilation in rice seeds (Koike et al., 2004). *OsYSL15* and *OsYSL12* contributed

widely for Fe translocation during germination. Some of the studies suggested that *OsYSL2* is important for Fe translocation in seeds (Nozoye et al., 2007). Fe distribution and localization in grains is shown in *Figure 1*.

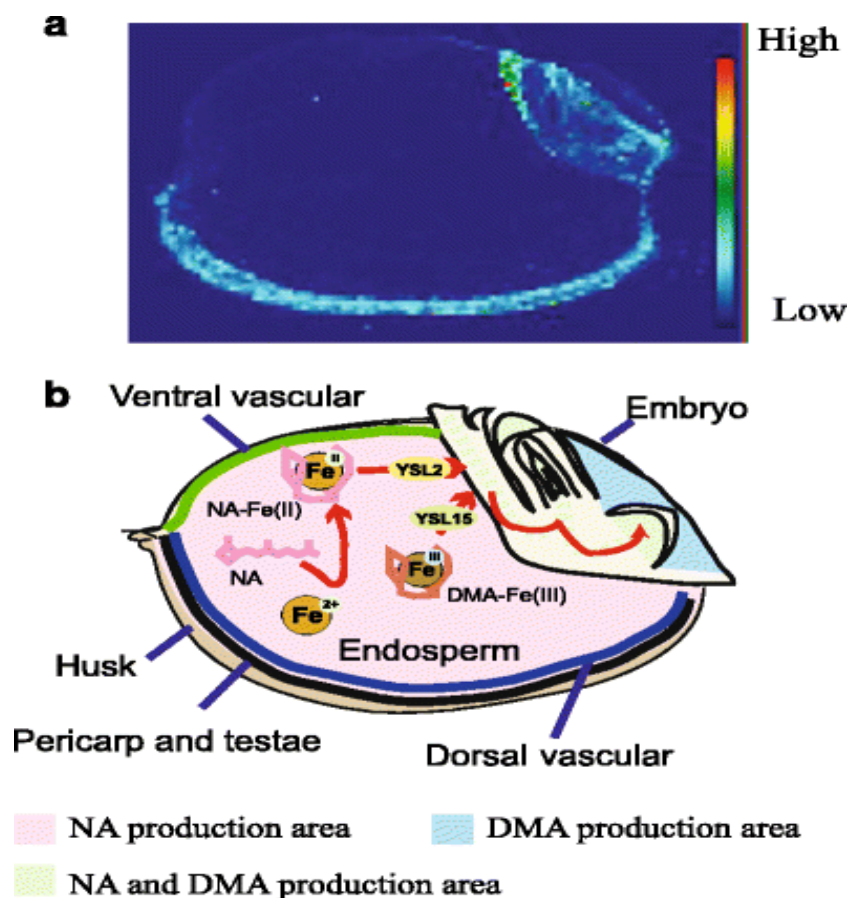


Figure 1. Fe distribution, localization in seeds, and production of DMA, and NA, and iron distribution during germination. Taken from (Bashir et al., 2010)

Iron toxicity tolerance mechanism in rice

Some studies give a shred of evidence; rice can be grown under Fe toxicity conditions without any loss in yield (Onaga et al., 2013; Mahender et al., 2019). There are three possible mechanisms of rice tolerance to Fe toxicity are documented; exclusion of Fe from root, storage in plant parts and tissues (Wan et al., 2003; Müller et al., 2015). The genotypes adopted three types of procedures to exclude Fe from roots, oxidation of Fe from root which result in low concentration of Fe in nutrient solution, elimination of Fe at root surface, and most remarkable strategy adopted by rice genotypes is formation of aerenchyma and lateral roots which provided low resistant pathway for oxygen transport into rhizosphere where iron is oxidized into less toxic form (Wu et al., 2019; Mahender et al., 2019). Many studies evidenced the tissue tolerance mechanism for rice tolerance against Fe toxicity (Hossenli et al., 2012; Muller et al., 2015; Frei et al., 2016), which is regulated by nitrous oxide signaling pathway, signaling storage proteins and enzymes, reactive oxygen species (ROS) and hormones etc. ROS are neutralized by nitrous oxide by its function (Darbani et al., 2013; Onaga et

al., 2016). Nitrous oxide also regulates, kinase protein, calcium, cycling GMP, cyclic ADP-Rib, via S-nitrosylation of Cys deposits (Besson-Bard et al., 2009). N₂O also mediates Fe storage protein (ferritin) at both messenger RNA and protein level and an ARFAT an auxin-responsive element described which represented in 400 up-regulated genetic factors below Fe harmfulness (White and Brown, 2010). This mechanism is still unclear that either Fe regulates this auxin signaling protein or it is independent of Fe modulation and further studies are required on this aspect.

Iron storage in sub-cellular compartments

Once Fe is entered into the cell, there are certain steps inside the cell to stop Fe or mediate its toxicity. Many scavenging elements like nitrous oxide, DMA act as chelators of Fe and started scavenging of Fe at a sub-cellular level by forming a complex (Onaga et al., 2016). In rice the transporters of metals at inter and intracellular levels are categorized as in natural resistance-related protein (macrophage *NRAMP*) for cation carrier (Takahashi et al., 2011, Tan et al., 2019). *OsNRAMP1* is up-regulated by deficiency of Fe and *OsNRAMP7&8* showed a negative association with Fe concentration in shoots. *OsNRAMP1* most likely work as metal efflux carrier contributing in the transfer of metals from the section of vacuole to cytosol and, *OsNRAMP7*, *OsNRAMP8* play role as metals influx protein which worked as sequestration of metals in the vacuole (Ogo et al., 2014). *OsNRAMP6* is regulated with *OsVIT1* in rice grown under surplus iron (Vivitha et al., 2017; Mahender et al., 2019). This showed that *NRAMP6* is responsible for transfer of Fe from cell to vacuoles and chloroplast, thereby increasing Fe toxicity tolerance in rice. *OsVIT2* is up-regulated in rice shoot and root in reaction to excessive Fe (Bashir et al., 2014), proposing that *OsVIT1* & *OsVIT2* had a function in the compartmentalization of Fe (Wu et al., 2019). The transporter *VIT1* has been studied to be functional by organizing with *AtNRAMP3* and *AtNRAMP4*; the two isologs of Arabidopsis on the surface of the vacuolar membrane of roots and shoots under Fe deficiency (Onaga et al., 2016). *OsVIT1* and *OsVIT2* are more functional in rice when rice is grown under Fe toxicity stress. *OsVIT1* and *OsVIT2* are congested in rice under Fe shortage (Zhang et al., 2013). The unpredictable function of transporter (*VIT1*) in Arabidopsis and rice showed that *VIT1* also interacts with other regulators carry Fe in the vacuole, and function as diverting the release of Fe into cells of vacuole in absence of Fe through modifying the action of *AtNRAMP3* and *AtNRAMP4* (Zhao et al., 2013; Onaga et al., 2016). The transporter *OsNRAMP5*; is existing in the plasma membrane and it is involved in reducing Fe concentration in roots and shoots through xylem (Ishimaru et al., 2012). Many plants carrying *OsNRAMP5i* store less Fe in shoot and xylem sap, indicating that this transporter cooperates with different other Fe transporters to regulate iron in shoots of rice (Morrissey and Guerinot, 2009; Wu et al., 2019; Mahender et al., 2019).

Chloroplast

Fe toxicity is also regulated in the chloroplast, where *FRO7* a member of *FRO* localized in plastids showed that Fe is reduced by *AtFRO7* in rice and Arabidopsis and then taken by Fe²⁺ carrier. Inner membrane-localized permease is considered to be the main site for Fe distribution into chloroplast (Zhang et al., 2017). *OsFRO1* is connected to *AtFRO7*, and may show the same role converting Fe³⁺ to Fe²⁺, in preparations for transport into the chloroplasts (Sperotto et al., 2010). The genes regulating ferritin have

been recognized in various species of plants mainly in rice (Matthus et al., 2015). The up-regulation of genes in reaction to surplus Fe (Bashir et al., 2014; Onaga et al., 2016; Onyango et al., 2019) reveals that ferritin is effective paths of intracellular Fe in the rice (Onaga et al., 2016).

Mitochondrial chelation of Fe

The Fe can also be deposited in mitochondrial section where protein ferritin exists. (Lin et al., 2011) exhibited that over expression of a mitochondrial Fe carrier, mitochondrial RNA splicing (*MRS3*), in yeast over-whelms the expansion of Fe toxicity by declining cytosolic Fe via mitochondrial Fe increase. Orthologue of (*MSR3*) is a Fe carrier in mitochondria which was recognized as accountable for carrying Fe into mitochondria (Bashir et al., 2011; Mahender et al., 2019). The suppression of *MIT* lead to decrease in aconitase activity, specifying that mitochondrial Fe transporter is important for Fe s-cluster biogenesis in cytoplasm and mitochondria. Gross et al. (2003) and Bashir et al. (2014) have discovered the contradictory track for these genetic factors. The regulation of (*OsNAS1*, *OsNAS2*, *OsNAAT1*, and *OsNRAMP1*) was witnessed, possibly to avoid unnecessary uptake and toxicity of Fe. Five major protein families of Fe tolerance genes are *OsNRAMPs*, *OsFROs*, *OsZIPs*, *OsFERS* and *OsYSLs*. Fe toxicity and deficiency up and down regulated genes in rice are shown in *Figure 2*.

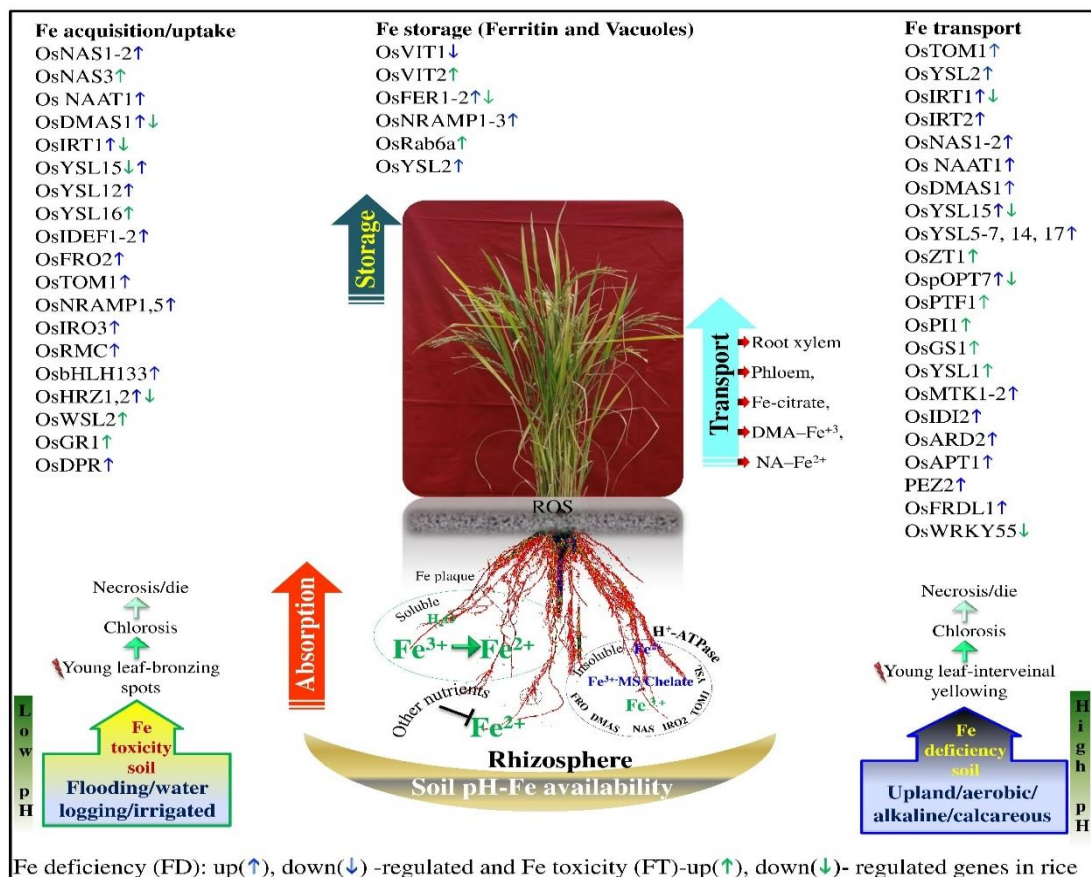


Figure 2. Fe toxicity and deficiency up and down regulated genes showing alterations in levels of transcription through microarray and transcriptomics studies (Adopted from Mahender et al., 2019)

Regulation of Fe uptake

The regulation of Fe uptake is done by many *TFs* transcription factors and regulators including, signal transducers. Plants adopt strategy II in response to Fe deficiency, like enhance biosynthesis along with secretion of Fe³⁺ chelator named Mas (mugenic acid). Some enzymes, which are main target of transcription factors contributed to mugenic acids (Inoue et al., 2008). A gene (*OsFRO2*), cool with *OsNAATI* and *OsVIT1* and protein *OsRMC* work as receptor, involved in Fe regulation in rice (Finatto et al., 2015; Onaga et al., 2016; Wu et al., 2019). The transcription factors prompted by Fe deficiency, (*OsIRO3* & *OsbHL133*) were characterized in rice (Wang et al., 2012). Some other genes like (*IDEF1* & *OsHRZ1-2*) were studied to catch Fe signals through binding of Fe and zinc and avoid surplus Fe uptake under sufficient Fe availability (Liu et al., 2016). Most meaningful is to conclude that there are some genes which can interact in vivo with *OsHRZ* and to find that this post-transcriptional modification can be helpful to enhance Fe toxicity tolerance in rice (Onaga et al., 2016; Mahender et al., 2019). Post-transcriptional regulation of Fe in rice has not fully understood, therefore, it will be significant to unfold the natural variation for *OsRT1* with stable disparity deposit replacement in its loop that may enhance tolerance to Fe toxicity in rice. The studies on link between amid the residue replacements in *OsIRT1* with action of *OsHRZ* and *IDEF1* can add significant benefit in studying post-translational alteration of Fe uptake in rice (Onaga et al., 2016). The earlier studies described the role of ABA (abscisic acids) and brassinosteroids, in regulation of IDE1 modulation of downstream target in Fe homeostasis single transductions mechanisms need to be unfold (Gallie, 2012; Pereira et al., 2014). Rice has a number of strategies to switch unnecessary Fe uptake.

Fe storage genes which are encoding *VOT*, *FPN2* transporter are well regulated and thus Fe may engage in old leaves. The similar genetic factors could be controlled in shoot of include that positively stock Fe in plant aerial parts. Additionally, genes encoding *YSL4* and *YSL6* like Fe efflux carriers, could be controlled to release spare Fe from chloroplast, to stop oxidative damage. It would be valuable to detect important genes, with constant influence in reaction to the dissimilar kinds of Fe harmfulness, to use for breeding's schemes. The only one author reported the confirmed effects of Fe nutrition gene (*OsFRO1*) in tolerance of Fe toxicity (Mahender et al., 2019), despite lot of research work has been accomplished. Transporters identified regarding Fe regulated genes and their function are shown in *Table 1*.

Table 1. Transporters identified regarding Fe regulated genes and their function

Genes	Position	Role	Reference
<i>OsZIP4</i>	Root	Zinc transporting proteins, Fe transport and homeostasis	(Quinet et al., 2012)
<i>OsYSL2</i>	Root	Fe-NA transport, Fe accumulation in seeds and translocation in grains	(Koike et al., 2004)
<i>OSPIC1</i>	Chloroplast	Transport Fe from root to chloroplast	(Zhang et al., 2012)
<i>OsFRO2</i>	Root	Alter Fe oxidation state	(Stein et al., 2009)
<i>OsVIT1,2</i>	Leaves/Seeds	Vacuolar Fe transporter	(Zhang et al., 2012)
<i>OsMIR</i>	Shoot	Fe homeostasis	(Ishimaru et al., 2010)
<i>OsFER1</i>	Aleurone layer	Vacuolar Fe transport and homeostasis	(Bashir et al., 2013)

QTL identified for rice tolerance to Fe toxicity

The identification and isolation of QTL linked to Fe tolerance is a powerful way to improve the rice tolerance to Fe toxicity (Dramé et al., 2011; Mahender et al., 2019). A lot of mapping populations are used in many studies, which have identified many QTL in rice. Here we discussed some QTL identified previously and their role in iron toxicity tolerance. Wu et al. (2014) used a mapping population of RIL (recombinant inbred lines) derived from IR29/Pokkali and identified two putative QTL for leaf bronzing score *qFETOX-1-1* and *qFETOX-1-2* between the markers with 10.6% and 12% phenotypic variation. The LBI indicated the large genetic variation among the parental genotypes for tolerance to Fe toxicity. A variety of traits like, SL, RL, shoot dry weight (SDW) and root dry weight (RDW) are also used for estimation of Fe toxicity tolerance in rice. Two QTL for SL and RL were reported by Meng et al. (2017) using 873 RIL (recombinant inbred lines) derived from MAGIC populations. QTL *qSL-1* and *qRL-8* were identified with 18% phenotypic variation.

This trait indicated large genetic variability for Fe tolerance. Many genes were reported behind these QTL controlling Fe toxicity tolerance in rice. Dufey et al. (2010) used 164 RIL (recombinant inbred lines) population evaluated in multiple environment, and identified one putative QTL *qNSP-3* for number of spikelet's in rice which showed large variation for iron toxicity tolerance. Likewise, Dufey et al. (2012) identified two more QTL using same population for 1000 grain *qGW-1* weight and chlorophyll content *qCCI-7* with varying ratio of variance. Dufey et al. (2015) conducted a hydroponic experiment and evaluated 220 BC3DH (double haploid) and identified QTL, for shoot water content (SWC) *qSWC-3*, one for shoot dry weight (SDW) *qSDW-3* and one for leaf bronzing index (LBI) *qLBI-1* which strongly demonstrated that all of these QTL had significant role in rice response to Fe toxicity tolerance. Liu et al. (2016) identified two QTL using IL (Introgression lines) population derived from japonica (02428) and indica (Minghui63) and reported two QTL, *qRSDW-11*, *qRRDW-2* independent of genetic background. A QTL *qSDW-5* was identified recently which expressed under both experimental conditions with similar additive effects suggesting the genetic overlap between Fe toxicity tolerance and zinc toxicity tolerance in rice (Zhang et al., 2013). Zhang et al. (2017) identified a putative QTL *qSFW-2* with a positive additive effect which showed that the genes behind this QTL were contributed from the donor parent. These all are reported QTL for Fe toxicity tolerance and more breeding strategies and efficient screening techniques are required for the identification of tolerant genotypes of rice. Some of the putative QTL identified under Fe stress in rice are shown below in *Table 2*. *Figure 3* showed the way of novel phenotypic screening techniques to enhance Fe toxicity tolerance in rice genotypes.

Novel breeding and screening techniques for Fe toxicity tolerance in rice

Several molecular breeding techniques are used by researchers to improve Fe tolerance in rice. Molecular breeding aims to identify and transfer genes for improving heavy metals tolerance is more reliable technique. Marker assisted selection (MAS) includes using of markers for construction of linkage map to identify the putative genes and to clone the genes for speed up molecular breeding to enhance Fe tolerance in rice. This breeding approach is becoming more and more beneficial which more efficient and time saving approach is as compare to conventional breeding approaches. Use of backcross recombinant inbred lines (BRILs) populations is ideal population for targeted

gene cloning and to speed up molecular breeding for improving Fe tolerance in rice (Rasheed et al., 2020a). An effective screening technique is required to characterize the resistance of genotypes at seedling stage. Seedling stage is ideal stage to identify the genotypes against different metals stress. One of the best way is to use preliminary screening technique to evaluate the genotypes against different levels of Fe toxicity. Parents and population should be grown in hydroponic condition and expose to different level of stress at seedling stage. In this way most effective dose of stress would be determined and that can be further used to screen the genotypes. Resistant genotypes could be selected for different seedling traits which are indicators of metals tolerance (Rasheed et al., 2020a). Resistant genotypes could be used for QTL mapping which lead to MAS selection. Therefore, this screening technique is needed for effective QTL mapping.

Table 2. Putative QTL identified in rice during exposure to Fe stress

Parents	Population	Traits	Marker	Chr	QTL	PVE%	Reference
glaberrim/ Caiapo	220 BC3DH	SWC	RM-251- RM238	3	<i>qSWC-3</i>	5.1	(Dufey et al., 2015)
glaberrim/ Caiapo	220 BC3DH	SDW	RM-60- RM22	3	<i>qSDW-3</i>	5.3	(Dufey et al., 2015)
glaberrim/ Caiapo	220 BC3DH	LBI	RM208- RM266	1	<i>qLBI-1</i>	4.7	(Dufey et al., 2015)
Azucena/IR64	164RIL	1000GW	RM034- RM246	1	<i>qGW-1</i>		(Dufey et al., 2012)
Azucena/IR64	164RIL	CCI	RM324- RM118	7	<i>qCCI-7</i>	33.9	(Dufey et al., 2012)
Azucena/IR64	164RIL	NSP	RM132- RM231	3	<i>qNSP-3</i>		(Dufey et al., 2010)
MP	873RIL	SL	SNP	1	<i>qSL-1</i>	18	(Meng et al., 2017)
MP	873RIL	RL	SNP	8	<i>qRL-8</i>		(Meng et al., 2017)
IR29/Pokkali	RIL/CSSL	LBI	173SNP/83SSR	1	<i>qFETOX-1-1</i>	10.6	(Wu et al., 2014)
IR29/Pokkali	RIL/CSSL	LBI	173SNP/83SSR	1	<i>qFETOX-1-2</i>	12	(Wu et al., 2014)
02428/Minghui63	IL	RSDW	384 SNP	11	<i>qFRSDW-11</i>	10.95	(Liu et al., 2016)
02428/Minghui63	IL	RRDW	384 SNP	2	<i>qFRRDW-2</i>	8.68	(Liu et al., 2016)
Lemont/Teqing	BIL	SDW	308SNP	5	<i>qSDW-5</i>		(Zhang et al., 2013)
Indica	222 Indica- accessions	SFW	395,553 SNP	2	<i>qSFW-2</i>	10.2	(Zhang et al., 2017)

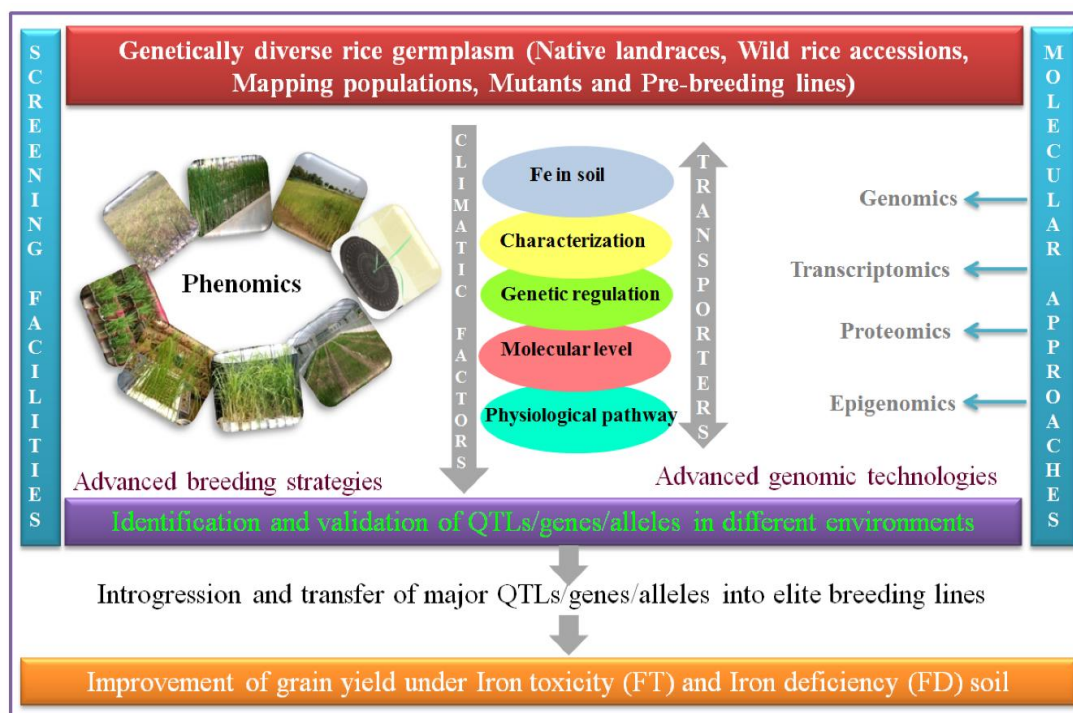


Figure 3. A schematic way of presentation of innumerable phenotypic screening techniques and omics-based approaches to increase iron toxicity tolerance in rice cultivars (Taken from (Mahender et al., 2019))

Wild relatives as potential source for Fe tolerance in rice

Wild relative constitutes a valuable gene pool that can be used to breed novel rice genotypes which can be tolerated to abiotic stresses like Fe toxicity. By transferring genes from these wild parents we can efficiently improve Fe toxicity tolerance in rice to sustain rice production. In a study conducted by Bierschenk et al. (2020) screened 75 rice genotypes, including 16 local genotypes, one glaberrima, and 58 wild genotypes which representing 21 species to study Fe toxicity tolerance. Plants were evaluated in green house and were treated with control and Fe stress during vegetative growth stage. Foliar Fe treatment were indicators of Fe toxicity during both stress treatments. Plants with chronic stress reduced yield due to spike fertility. Both wild and local genotypes showed variation in their response to Fe toxicity. Some of the wild relatives performed higher in individual traits towards Fe toxicity. These results showed that Fe toxicity can be improved by domestication of wild parents and to transfer their genes into domestic cultivars.

Conclusion

Fe toxicity is a polygenetic trait in rice, and there are many genetic and physiological basis of Fe toxicity tolerance. Moreover, many regulators are involved in Fe regulation at various levels. Here we provided a strong theoretical base of rice response to excessive Fe and genetic basis of Fe regulation in rice, genes and QTLs involving in regulation and tolerance of Fe toxicity. This review concluded that the use of molecular markers, high-resolution population, rice gene pool and wild relatives is a potential

strategy to improve Fe toxicity tolerance in rice crop. Secondly many identified QTL discussed here are needed to transfer into susceptible lines through QTL pyramiding. Many Fe transporter is needed to identify to unfold their role in Fe toxicity and its regulation and more efficient breeding techniques are required to screen Fe tolerant rice genotypes.

Future perspectives

Fe is an essential element for rice and regulates rice growth when supplied in optimum concentration, but higher concentrations of Fe lead to induction of many deleterious changes in the crop which significantly reduces growth and production. There are certain levels in the cell, where Fe deficiency is regulated by using metabolic pathways. The physiological and biochemical basis of Fe regulation are clearly described in many plants but the molecular basis of Fe toxicity tolerance has not fully characterized. Here we provided a strong theoretical base of rice response to excessive Fe and genetic basis of Fe regulation in rice and genes involved in Fe toxicity tolerance. Many genes described here are associated with previously identified QTL and some of them are regulators that are controlled by *VIT*, *FNP2* like carriers, *MIT PIC1*, *FPN2* like carriers, *MIT*, *PIC1* transporters, ferritin, *OsFRO1*, *OsIRO3* & *OsHLL133*, and *OsNRAMP7* & *OsNRAMP8*. Many molecules responsible for Fe intra and inter cellular movement are still unidentified, linked to the huge number of unidentified genes in rice. Recently 3000 rice genomes were studied which highlighted the possibilities of validating these unannotated genes and identify their expression. The genome sequence for candidate gene can be accessed, moreover, the use of molecular markers that help in screening the Fe tolerant genotypes from susceptible one would be more helpful to accelerate genes pyramiding. The morphological, physiological and molecular basis of Fe toxicity tolerance would facilitate successful breeding if they are targeted to a large extent. The wild relatives of rice should be focused to screen out new sources of Fe toxicity tolerance and to transfer the novel genes in rice that could be exploited in rice breeding.

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REFERENCES

- [1] Aamer, M., Hassan, M. U., Abid, A., Su, Q., Liu, Y., Rasheed, A., Muhammad, A. U. K., Tahir, A. K., Huang, G. (2018): Foliar application of glycinebetaine (GB) alleviates the cadmium (Cd) toxicity in spinach through reducing Cd uptake and improving the activity of antioxidant systems. – *Applied Ecology and Environment Research* 16(6): 7575-7583.
- [2] Aoyama, T., Kobayashi, T., Takahashi, M., Nagasaka, S., Usuda, K., Kakei, Y. (2009): OsYSL18 is a rice iron (III)-deoxymugineic acid transporter specifically expressed in reproductive organs and phloem of lamina joints. – *Plant Molecular Biology* 70: 681-692.

- [3] Audebert, A., Sahrawat, K. L. (2000): Mechanisms for iron toxicity tolerance in lowland rice. – *Journal of Plant Nutrition* 23(11-12): 1877-1885.
- [4] Audebert, A. (2006): Iron toxicity in rice–environmental conditions and symptoms. – *Iron Toxicity in Rice-Based System in West Africa*, WARDA, Cotonou 18: 33.
- [5] Bashir, K., Ishimaru, Y., Nishizawa, N. K. (2010): Iron uptake and loading into rice grains. – *Rice* 3: 122-130.
- [6] Bashir, K., Ishimaru, Y., Shimo, H., Nagasaka, S., Fujimoto, M., Takanashi, H., Tsutsumi, N., Nakanishi, H., Nishizawa, N. K. (2011): The rice mitochondrial iron transporter is essential for plant growth. – *Nature Communications* 2: 1-7.
- [7] Bashir, K., Hanada, K., Shimizu, M., Seki, M., Nakanishi, H., Nishizawa, N. K. (2014): Transcriptomic analysis of rice in response to iron deficiency and excess. – *Rice* 7: 1-15.
- [8] Besson-Bard, A., Gravot, A., Richaud, P., Auroy, P., Duc, C., Gaymard, F., Taconnat, L., Renou, J. P., Pugin, A., Wendehenne, D. (2009): Nitric oxide contributes to cadmium toxicity in *Arabidopsis* by promoting cadmium accumulation in roots and by up-regulating genes related to iron uptake. – *Plant Physiology* 149: 130-131.
- [9] Bierschenk, B., Tagele, M. T., Ali, B., Ashrafuzzaman, M. D., Wu, L., Becker, M., Frei, M. (2020): Evaluation of rice wild relatives as a source of traits for adaptation to iron toxicity and enhanced grain quality. – *Plos One* 15(1): 1-17.
- [10] Brumbarova, T., Bauer, P., Ivanov, R. (2015): Molecular mechanisms governing *Arabidopsis* iron uptake. – *Trends Plant Science* 20: 124-133.
- [11] Chandel, G., Banerjee, S., Verulkar, S. (2010): Expression profiling of metal homeostasis related candidate genes in rice (*Oryza* spp.) using semi quantitative RT-PCR analysis. – *Rice Genetics Newsletter* 25: 44-47.
- [12] Darbani, B., Briat, J. F., Holm, P. B., Husted, S., Noeparvar, S., Borg, S. (2013): Dissecting plant iron homeostasis under short and long-term iron fluctuations. – *Biotechnology Advances* 31: 1292-1307.
- [13] Das, K., Roychoudhury, A. (2014): Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. – *Frontiers in Environmental Science* 2: 1-13.
- [14] Drame, K. N., Saito, K., Kone, B., Chabi, A., Dakouo, D., Annan-Afful, E., Monh, S., Abo, E., Sie, M. (2011): Coping with iron toxicity in the lowlands of subSaharan Africa: experience from Africa Rice Center. – *Proceedings of the second Africa Rice Congress, Bamako, Mali. Innovation and partnerships to realize Africa's Rice potential.*
- [15] Dufey, I., Hakizimana, P., Draye, X. S., Lutts, L., Bertin, P. (2009): QTL mapping for biomass and physiological parameters linked to resistance mechanisms to ferrous iron toxicity in rice. – *Euphytica* 167: 143-160.
- [16] Dufey, I., Hiel, M. P., Hakizimana, P., Draye, X., Lutts, S., Kone, B., Drame, K., Konate, K., Sie, M., Bertin, P. (2012): Multienvironment quantitative trait loci mapping and consistency across environments of resistance mechanisms to ferrous iron toxicity in rice. – *Crop Science* 52: 539-550.
- [17] Dufey, I. X., Draye, S., Lutts, M., Lorieux, C., Martinez Bertin, P. (2015): Novel QTLs in an interspecific backcross *Oryza sativa* × *Oryza glaberrima* for resistance to iron toxicity in rice. – *Euphytica* 204: 609-625.
- [18] Finatto, T., Oliveira, A. C., Chaparro, C., Maia, L. C., Farias, D. R., Woyann, L. G., Mistura, C. C., Soares-Bresolin, A. P., Llauro, C., Panaud, O. (2015): Abiotic stress and genome dynamics: specific genes and transposable elements response to iron excess in rice. – *Rice* 8: 1-18.
- [19] Frei, M., Tetteh, R. N., Razafindrazaka, A. L., Fuh, M. A., Wu, L. B., Becker, M. (2016): Responses of rice to chronic and acute iron toxicity: genotypic differences and biofortification aspects. – *Plant and Soil* 408: 149-161.
- [20] Gallie, D. R. (2012): The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. – *Journal of Experimental Botany* 64: 433-443.

- [21] Gross, J., Stein, R. J., Fett-Neto, A. G., Fett, J. P. (2003): Iron homeostasis related genes in rice. – *Genetics and Molecular Biology* 26: 477-497.
- [22] Hell, R., Stephan, U. W. (2003): Iron uptake, trafficking and homeostasis in plants. – *Planta* 216: 541-551.
- [23] Hosseini, M., Houshmand, S., Mohamadi, S., Tarang, A., Khodambashi, M., Rahimsoroush, H. (2012): Detection of QTLs with main, epistatic and QTL× environment interaction effects for rice grain appearance quality traits using two populations of backcross inbred lines (BILs). – *Field Crops Research* 135: 97-106.
- [24] Inoue, H., Takahashi, M., Kobayashi, T., Suzuki, M., Nakanishi, H., Mori, S., Nishizawa, A. (2008): Identification and localisation of the rice nicotianamine aminotransferase gene OsNAAT1 expression suggests the site of phytosiderophore synthesis in rice. – *Plant Molecular Biology* 66: 193-203.
- [25] Inoue, H., Kobayashi, T., Nozoye, T., Takahashi, M., Kakei, Y., Suzuki, K., Nakazono, M., Nakanishi, H., Mori, S., Nishizawa, N. K. (2009): Rice OsYSL15 is an iron-regulated iron(III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. – *Journal of Biological Chemistry* 284: 3470-3479.
- [26] Ishimaru, Y., Masuda, H., Bashir, K., Inoue, H., Tsukamoto, T., Takahashi, M. (2010): Rice metal–nicotianamine transporter, OsYSL2, is required for long distance transport of iron and manganese. – *Plant Journal* 62: 379-390.
- [27] Ishimaru, Y., Takahashi, R., Bashir, K., Shimo, H., Senoura, T., Sugimoto, K., Ono, K., Yano, M., Ishikawa, S., Arao, T. (2012): Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport. – *Scientific Reports* 2: 1-8.
- [28] Jaggard, K. W., Qi, A., Ober, E. S. (2010): Possible changes to arable crop yields by 2050. – *Philosophical Transactions of the Royal Society B: Biological Science* 365: 2835-2851.
- [29] Jain, A., Connolly, E. L. (2013): Mitochondrial iron transport and homeostasis in plants. – *Frontiers in Plant Science* 4: 1-7.
- [30] Kim, S. A., Guerinot, M. L. (2007): Mining iron: iron uptake and transport in plants. – *FEBS letters* 581: 2273-2280.
- [31] Kobayashi, T., Itai, R. N., Nishizawa, N. K. (2014): Iron deficiency responses in rice roots. – *Rice* 7: 1-11.
- [32] 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. – *The Plant Journal* 39: 415-424.
- [33] Li, G., Kronzucker, H. J., Shi, W. (2016): Root developmental adaptation to Fe toxicity: mechanisms and management. – *Plant Signaling Behaviour* 11: 1117-1122.
- [34] Li, W., Ping, L. (2017): The understanding of the plant iron deficiency responses in Strategy I plants and the role of ethylene in this process by omics approaches. – *Frontiers in Plant Science* 8: 1-15.
- [35] Lin, H., Li, L., Jia, X., Ward, D. M., Kaplan, J. (2011): Genetic and biochemical analysis of high iron toxicity in yeast iron toxicity is due to the accumulation of cytosolic iron and occurs under both aerobic and anaerobic conditions. – *Journal of Biological Chemistry* 286: 3851-3862.
- [36] Liu, H., Soomro, A., Zhu, Y., Qiu, X., Chen, K., Zheng, T., Yang, I., Xing, E. D., Xu, J. (2016): QTL underlying iron and zinc toxicity tolerances at seedling stage revealed by two sets of reciprocal introgression populations of rice (*Oryza Sativa* L.). – *The Crop Journal* 4: 280-289.
- [37] Mahender, A., Swamy, B., Anandan, A., Ali, J. (2019): Tolerance of iron-deficient and toxic soil conditions in rice. – *Plants* 8: 1-34.
- [38] Matthus, E., Wu, L. B. W., Höller, Y., Becker, S. S., Michael, M. F. (2015): Loci, genes, and mechanisms associated with tolerance to ferrous iron toxicity in rice (*Oryza sativa* L.). – *Theoretical and Applied Genetics* 128: 2085-2098.

- [39] Meng, L., Wang, B., Zhao, X., Ponce, K., Qian, Q., Ye, G. (2017): Association mapping of ferrous, zinc, and aluminum tolerance at the seedling stage in indica rice using MAGIC populations. – *Frontiers in Plant Science* 8: 1-15.
- [40] Mengel, K. (1995): Iron availability in plant tissues-iron chlorosis on calcareous soils. – In: Abadia, J. (ed.) *Iron Nutrition in Soils and Plants*. *Plant and Soil Sciences* 59: 389-397.
- [41] Mongon, J., Chaiwong, N., Bouain, N., Prom, U., Thai, C., Secco, D., Rouached, H. (2017): Phosphorus and iron deficiencies influences rice shoot growth in an oxygen dependent manner: insight from upland and lowland rice. – *International Journal of Molecular Sciences* 18: 1-7.
- [42] Morrissey, J., Guerinot, M. L. (2009): Iron uptake and transport in plants: the good, the bad, and the ionome. – *Chemical Reviews* 109: 4553-4567.
- [43] Muller, C., Kuki, K. N., Pinheiro, D. T., Souza, L. R., Silva, A. L., Loureiro, M. E., Oliva, M. A., Almeida, A. A. (2015): Differential physiological responses in rice upon exposure to excess distinct iron forms. – *Plant and Soil* 391: 123-138.
- [44] Nakanishi, H., Ogawa, I., Ishimaru, Y., Mori, S., Nishizawa, N. K. (2006): Iron deficiency enhances cadmium uptake and translocation mediated by the Fe²⁺ transporters OsIRT1 and OsIRT2 in rice. – *Soil Science and Plant Nutrition* 52: 464-469.
- [45] Nozoye, T., Inoue, H., Takahashi, M., Ishimaru, Y., Nakanishi, H., Mori, S. (2007): The expression of iron homeostasis-related genes during rice germination. – *Plant Molecular Biology* 64: 35-47.
- [46] Nugraha, Y., Ardie, S. W., Ghulamahdi, M., Aswidinnoor, H., Suwarno, A. H. (2016): Generation mean analysis of leaf bronzing associated with iron toxicity in rice seedlings using digital imaging methods. – *SABRAO Journal of Breeding and Genetics* 48: 453-464.
- [47] Ogo, Y., Kakei, Y., Nakanishi Itai, R., Kobayashi, T., Nakanishi, H., Nishizawa, N. K. (2014): Tissue-specific transcriptional profiling of iron-deficient and cadmium-stressed rice using laser capture microdissection. – *Plant Signaling Behaviour* 9(8): 1-4.
- [48] Onaga, G., Egdane, J., Edema, R., Abdelbagi, I. (2013): Morphological and genetic diversity analysis of rice accessions (*Oryza sativa* L.) differing in iron toxicity tolerance. – *Journal of Crop Science and Biotechnology* 16: 53-62.
- [49] Onaga, G., Drame, K. N., Ismail, A. M. (2016): Understanding the regulation of iron nutrition: can it contribute to improving iron toxicity tolerance in rice. – *Functional Plant Biology* 43: 709-726.
- [50] Onyango, D. A., Entila, F., Dida, M., Ismail, A. M., Drame, K. N. (2019): Mechanistic understanding of iron toxicity tolerance in contrasting rice varieties from Africa: 1. Morpho-physiological and biochemical responses. – *Functional Plant Biology* 46: 93-105.
- [51] Pennock, D., McKenzie, N. (2015): *Montanarella* L. Status of the World's Soil Resources. – FAO: Rome, Italy.
- [52] Pereira, M., Santos, P., Gomes, C., Vasconcelo, M. W. (2014): Cultivar variability of iron uptake mechanisms in rice (*Oryza sativa* L.). – *Plant Physiology and Biochemistry* 85: 21-30.
- [53] Quinet, M., Vromman, D., Clippe, A., Bertin, P., Lequeux, H., Dufey, I., Lutts, S., Lefèvre, I. (2012): Combined transcriptomic and physiological approaches reveal strong differences between short- and long-term response of rice (*Oryza sativa* L) to iron toxicity. – *Plant Cell and Environment* 35: 1837-1859.
- [54] Rasheed, A., Fahad, S., Hassan, M. U., Tahir, M. M., Aamer, M., Wu, Z. M. (2020a): A review on aluminum toxicity and quantitative trait loci mapping in rice (*Oryza sativa* L.). – *Applied Ecology and Environmental Research* 18(3): 3951-3961.
- [55] Rasheed, A., Fahad, S., Aamer, M., Hassan, M. U., Tahir, M. M., Wu, Z. M. (2020b): Role of genetic factors in regulating cadmium uptake, transport and accumulation mechanisms and quantitative trait loci mapping in rice. a review. – *Applied Ecology and Environmental Research* 18(3): 4005-4023.

- [56] Rout, G. R., Sahoo, S. (2015): Role of iron in plant growth and metabolism. – *Agricultural Science Review* 3: 1-24.
- [57] Saikia, T., Baruah, K. K. (2012): Iron toxicity tolerance in rice (*Oryza sativa* L) and its association with anti-oxidative enzyme activity. – *Journal of Crop Science* 3: 90.
- [58] Sikirou, M., Saito, K., Achigan-Dako, E. G., Drame, K. N., Ahanchede, A., Venuprasad, R. (2015): Genetic improvement of iron toxicity tolerance in rice-progress, challenges and prospects in West Africa. – *Plant Production Science* 18: 423-434.
- [59] Sperotto, R. A., Boff, T., Duarte, G., Santos, L. S., Grusak, M. A., Fett, J. P. (2010): Identification of putative target genes to manipulate Fe and Zn concentrations in rice grains. – *Journal of Plant Physiology* 167: 1500-1506.
- [60] Stein, R. J., Duarte, G. L., Spohr, M. G., Lopes, S. I. G., Fett, J. P. (2009): Distinct physiological responses of two rice cultivars subjected to iron toxicity under field conditions. – *Annual Applied Biology* 154: 269-277.
- [61] Takahashi, R., Ishimaru, Y., Senoura, T., Shimo, H., Ishikawa, S., Arao, T., Nakanishi, H., Nishizawa, N. K. (2011): The OsNRAMP1 iron transporter is involved in Cd accumulation in rice. – *Journal of Experimental Botany* 62: 4843-4850.
- [62] Tan, L., Zhu, Y., Fan, T., Peng, C., Wang, J., Sun, L., Chen, C. (2019): OsZIP7 functions in xylem loading in roots and inter-vascular transfer in nodes to deliver Zn/Cd to grain in rice. – *Biochemical and Biophysical Research Communications* 512: 112-118.
- [63] Van Oort, P. A. J. (2018): Mapping abiotic stresses for rice in Africa: Drought, cold, iron toxicity, salinity and sodicity. – *Field Crops Research* 219: 55-75.
- [64] Vejchasarn, P., Lynch, J. P., Brown, K. M. (2016): Genetic variability in phosphorus responses of rice root phenotypes. – *Rice* 9: 1-16.
- [65] Vivitha, P., Raveendran, M., Vijayalakshmi, D. (2017): Introgression of QTLs controlling spikelet fertility maintains membrane integrity and grain yield in improved white Ponni derived progenies exposed to heat stress. – *Rice Science* 24: 32-40.
- [66] Wainaina, C. M., Makihara, D., Nakamura, M., Ikeda, A., Suzuki, T., Mizukami, Y., Nonoyama, T., Doi, K., Kikuta, M., Samejima, H. (2018): Identification and validation of QTLs for cold tolerance at the booting stage and other agronomic traits in a rice cross of a Japanese tolerant variety, Hananomai, and a NERICA parent, WAB56-104. – *Plant Production Science* 21: 132-143.
- [67] Wan, J. L., Zhai, H. Q., Wan, J. M., Yasui, H., Yoshimura, A. (2003): 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, Acta Genetica Sinica* 30(10): 893-898.
- [68] Wang, L., Ying, Y., Narsai, R., Ye, L., Zheng, J., Tian, J., Whelan, K., Shou, H. (2012): Identification of OsbHLH133 as a regulator of iron distribution between roots and shoots in *Oryza sativa*. – *Plant Cell and Environment* 36: 224-236.
- [69] White, P., Brown, P. (2010): Plant nutrition for sustainable development and global health. – *Annals of Botany* 105: 1073-1080.
- [70] Wu, L. B., Shhadi, M. Y., Gregorio, G., Matthus, E., Becke, M., Frei, M. (2014): Genetic and physiological analysis of tolerance to acute iron toxicity in rice. – *Rice* 7(1): 8.
- [71] Wu, L. B., Holtkamp, B. F., Wairich, A., Frei, M. (2019): Potassium ion channel gene OsAKT1 affects iron translocation in rice plants exposed to iron toxicity. – *Frontiers in Plant Science* 10: 579.
- [72] Zhang, J., Soomro, A. A., Chai, L., Cui, Y., Wang, X., Zheng, T., Xu, J., Li, Z. (2013): Mapping of QTL for iron and zinc toxicity tolerance at seedling stage using a set of reciprocal introgression lines of rice. – *Acta Agronomica Sinica* 39: 1754-1765.
- [73] Zhang, J., Chen, K., Pang, Y., Naveed, S. A., Zhao, X., Wang, X., Wang, Y., Dingkuhn, M., Pasuquin, J., Li, Z. (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: 1-15.

- [74] Zhao, C., Zhou, L., Ding, D., Zhang, Y., Zhao, Q., Yu, X., Zhu, Z., Chen, T., Yao, S., Wang, C. (2013): Mapping of quantitative trait loci associated with ferrous iron toxicity tolerance at seedling stage based on chromosome segment substitution line in rice. – *Jiangsu Journal of Agricultural Science* 29: 461-467.