

SUPPLEMENTARY INFORMATION

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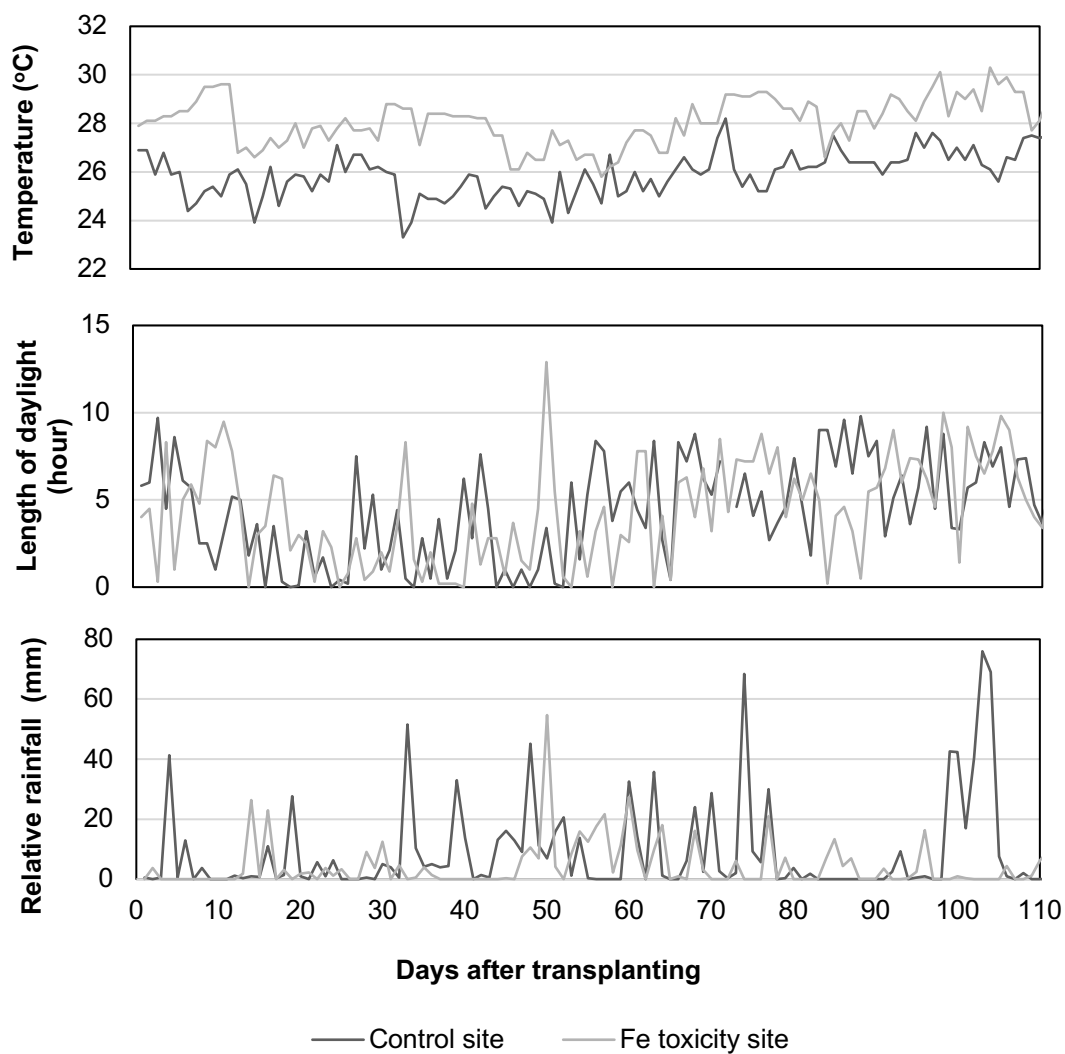


Fig. S1 | Weather data during field trials

Daily mean temperature, length of daylight, and precipitation during the growth period. Transplanting dates were January 5 and January 11, 2023, at the control and Fe-toxic sites, respectively

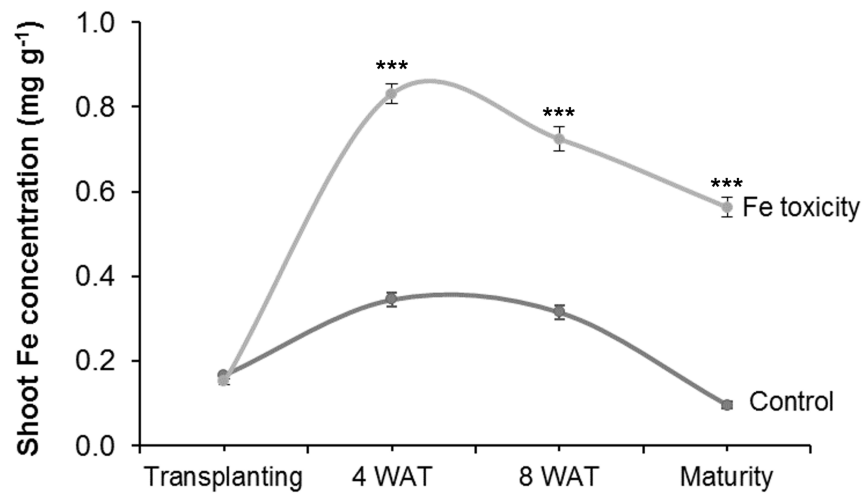


Fig. S2 | Shoot Fe concentrations at different growth stages at control and Fe-toxic field sites

Plants were sampled at transplanting, 4 weeks after transplanting (WAT) during the vegetative stage, 8 WAT during the reproductive stage, and before harvest. Data are means $\pm$ standard errors ($n = 12$; 4 replicates from 3 genotypes). A Student's *t*-test was conducted, and asterisks (***) indicate significant differences between the control and Fe-toxic sites ($P < 0.001$)

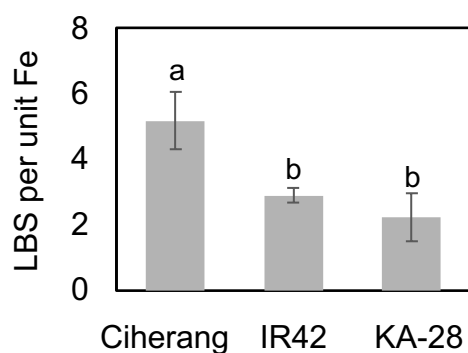


Fig. S3 | Comparison of LBS per unit Fe in the hydroponic experiment

The LBS obtained at 10 DAT was divided by the Fe concentration ($\mu\text{g mg DW}^{-1}$) for each sample. Values of different groups were compared by Tukey's HSD test, with significant differences indicated by different alphabets

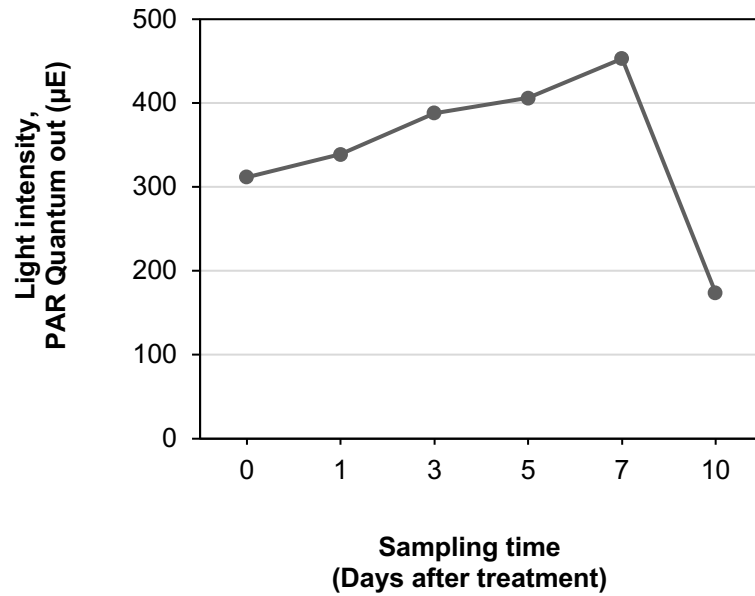


Fig. S4 | Light intensity during photosynthesis measurement

Light intensity was recorded by the LI-6400XT portable photosynthesis system during the photosynthesis measurement in the hydroponic experiment. The experiment was performed in a glasshouse with natural light, which was supplemented by LED lamps

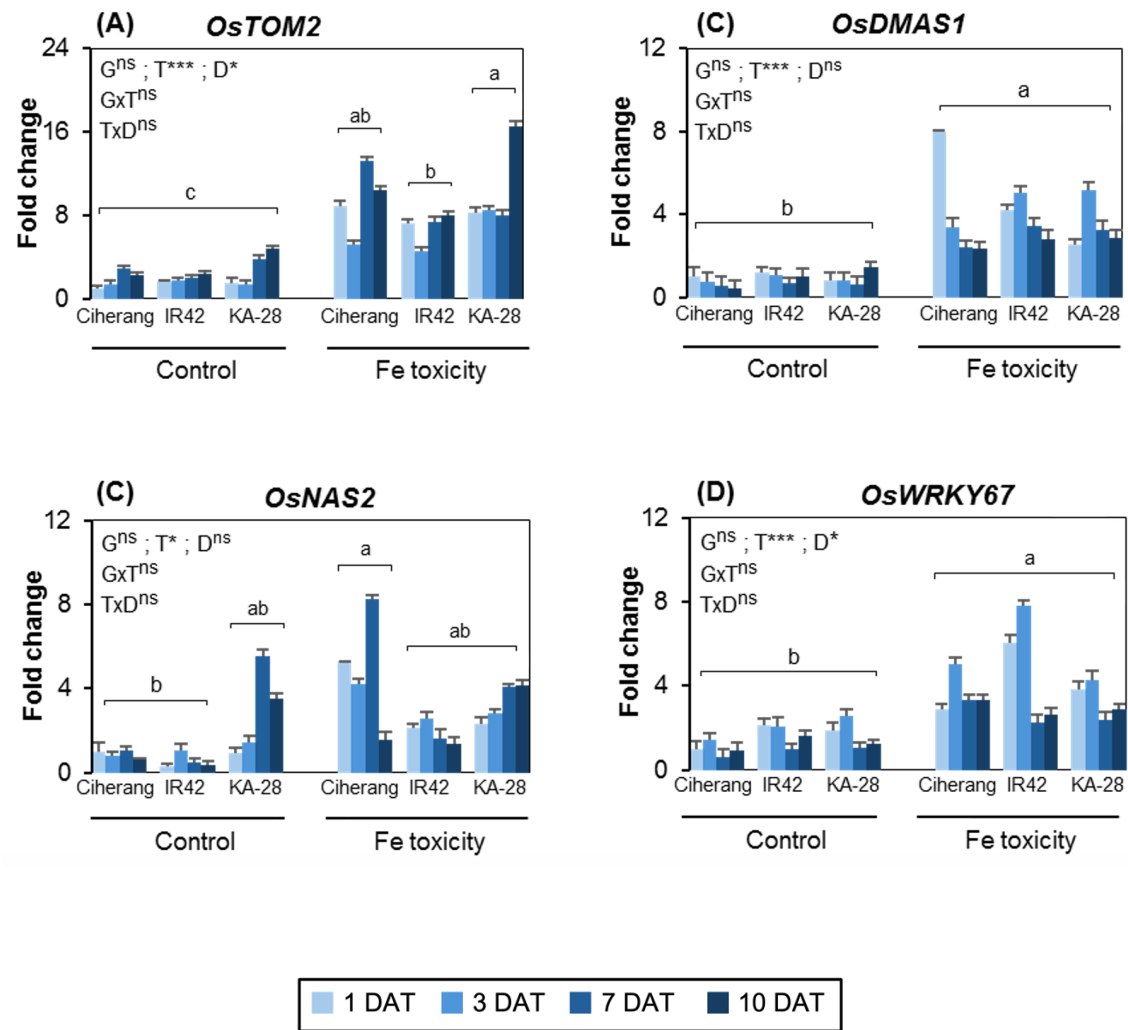


Fig. S5 | Gene expression analysis in the hydroponic experiment

Gene expression profiles for *OsTOM2* (A), *OsDMAS1* (B), *OsNAS2* (C), and *OsWRKY67* (D) in young leaves in the hydroponic experiment at 1, 3, 7, and 10 DAT. Fold change values relative to Ciherang under control conditions at 1 DAT are shown. Data are means $\pm$ standard errors ($n = 4$). A three-way ANOVA was conducted to analyze the effects of genotype (G), treatment (T), day (D), and their interactions, and significance levels are denoted with asterisks. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, $P > 0.05$. Common letters indicate no significant difference between genotypes by Tukey's HSD test

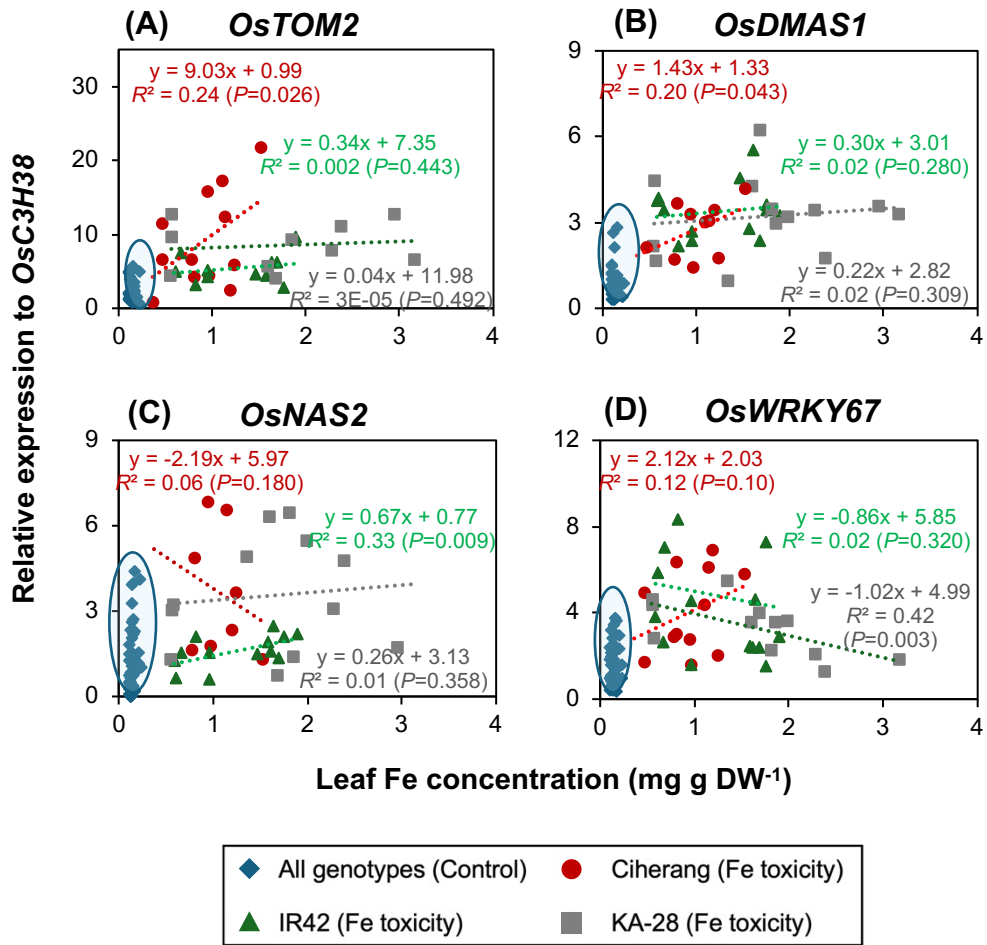


Fig. S6 | Differential correlation between Fe concentration and gene expression

The correlation between Fe concentrations and gene expression of *OsTOM2* (A), *OsDMAS1* (C), *OsNAS2* (D), and *OsWRKY67* (D) in young leaves. Data derived from 4 replicates of plants for each genotype from 1, 3, 7, and 10 days after treatment

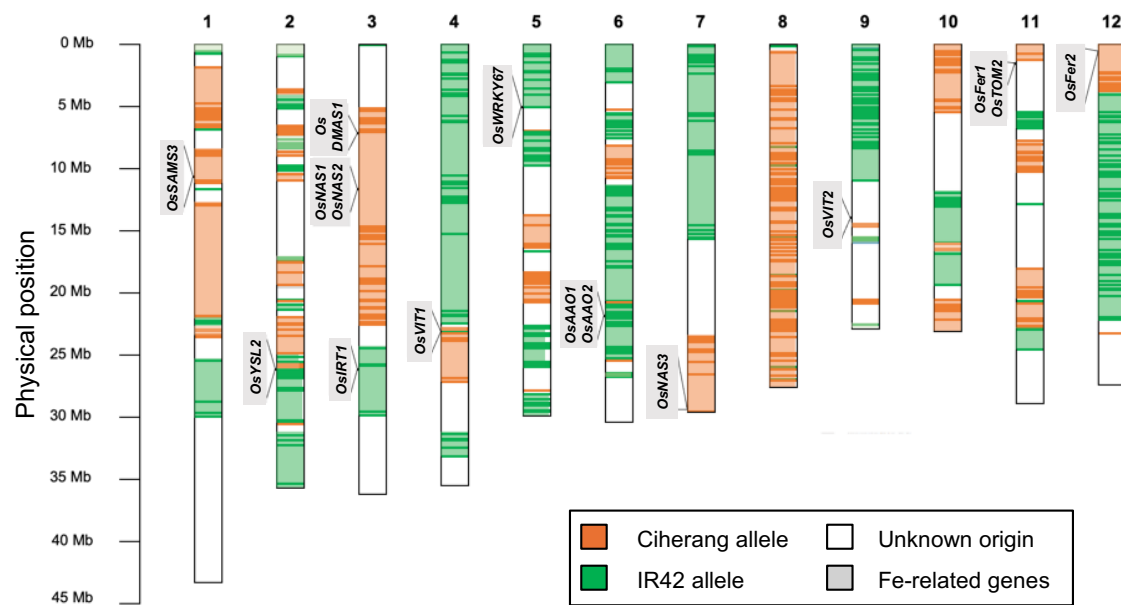
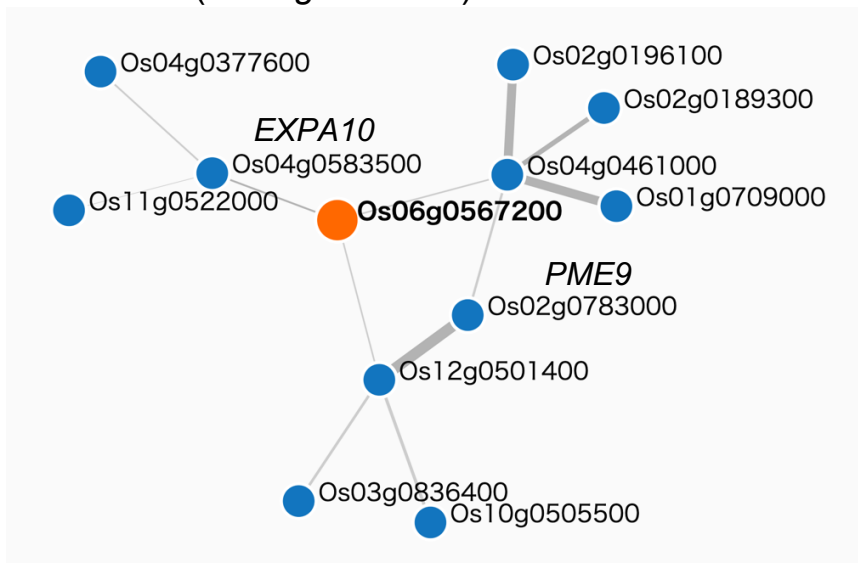


Fig. S7 | Composition of the KA-28 genome analyzed by RAD-Seq

Orange and green vertical lines indicate the position of markers that KA-28 shares with Ciherang and IR42, respectively. Regions flanked by markers commonly deriving from Ciherang and IR42 are colored in pale orange and green, respectively.

OsAAO1 (*Os06g0567200*)



OsAAO2 (*Os06g0567900*)

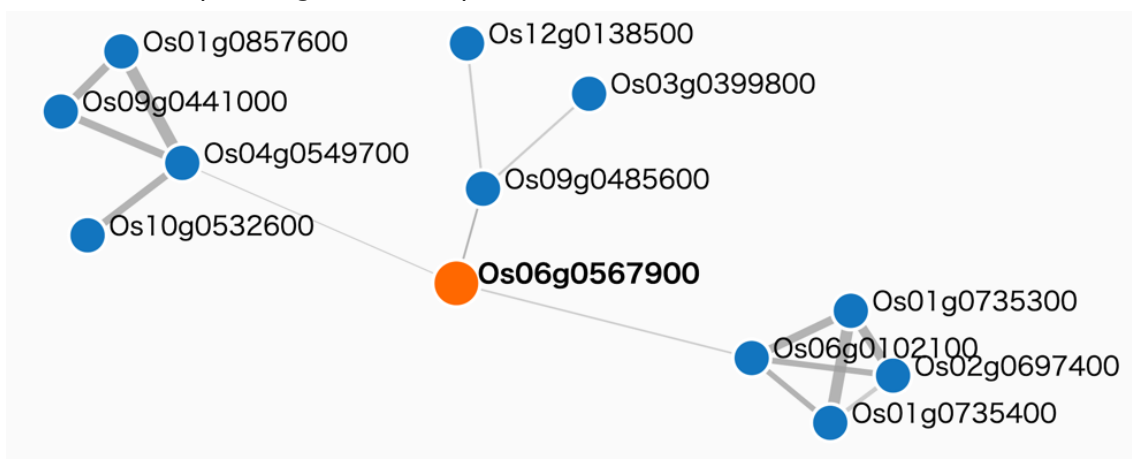


Fig. S8 | Genes co-expressed with *OsAAO1* and *OsAAO2*

Genes co-expressed with respective genes were retrieved from the RAP-DB website (<https://rapdb.dna.affrc.go.jp/index.html>). Each circle represents a gene, and co-expressed genes are connected by a gray line.

Table S1 | Physicochemical properties of the soils at two experimental sites

Properties	Unit	Control site (Muara, Bogor)	Fe-toxic site (Taman Bogo, Lampung)
pH H ₂ O	%	5.54	4.33
pH KCl	%	5.13	4.19
Fe	ppm	23.65	551.01
FeS ₂	%	n.d	0.16
Organic-C	%	1.69	0.57
N	%	0.17	0.13
C/N ratio		9.94	4.38
Available P	ppm	6.22	5.14
P ₂ O ₅	Potential	79.00	42.77
K ₂ O	(mg/100g)	4.80	3.57
K-dd		0.88	0.07
Na-dd		0.07	0.20
Ca-dd		7.67	0.49
KTK	cmol(+)/Kg	24.31	5.91
Al-dd		n.d	1.41
H-dd		1.24	0.75
	Sand	15.84	45.22
Texture (%)	Silt	29.07	16.36
	Clay	55.09	38.42

Soil samples were collected before tillage by forming composite samples from five spots in the outer and middle areas

n.d. = not detected

Table S2 | Primer sequences for gene expression analysis

Gene name	Gene ID	Primer Sequence (5' to 3')	
		Forward	Reverse
<i>OsC3H38</i>	Os05g0564200	CACAACAGGCCAACTGTGTC	GAGGGTCTCAACCTCACCAA
<i>OsIRT1</i>	Os03g0667500	CGTCTTCTTCTTCTCCACCACGAC	GCAGCTGATGATCGAGTCTGACC
<i>OsIRT2</i>	Os03g0667300	AGGAATCGCGTCATTGTGCAG	GGATCGTGCACACGTTCTG
<i>OsNAS2</i>	Os03g0307200	TGAGTGCGTGCATAGTAATCCTGGC	CAGACGGTCACAAACACCTCTTGC
<i>OsNAS3</i>	Os07g0689600	CGATGACTGCTTCCATCGCTT	GGCATGCATTCATGCATGACTGC
<i>OsDMAS1</i>	Os03g0237100	AGGTACATCGAGCGTGTCAA	AAGCTTTTCCTTGGCTCGGC
<i>OsTOM2</i>	Os11g0135000	AAACATGCACCATTCCAGGTG	TCTCACAGACCCAAAGCAGAAC
<i>OsSAMS3</i>	Os01g0293000	CAAGTGGGAGGAGCCTTCTG	TGCCTGCTACTAAGGCAGTG
<i>OsYSL2</i>	Os02g0649900	TCTGCTGGCTTCTTTGCATTTTCTG	ACCATGTCGAACTCAGCATCCAGGA
<i>OsFER1-OsFER2^a</i>	Os11g0106700	AGTGTGGCATCAAGGTGCAAT	TTGATGGCTTCAACCCAAGT
	Os12g0106000		
<i>OsAOO1</i>	Os06g0567200	CGGCATCAGACAGATCGGGA	CGAAGCGATAGGTGAAGGTTTC
<i>OsAOO2</i>	Os06g0567900	TGACGTGGGACGTGGAGTA	GCGTGCCAAACTGTCTGATG
<i>OsVIT1</i>	Os04g0463400	TCCTCAAGCTTGGCTCGACTTCAT	ATGGCGTTCTGTGCTGTGGAGATA
<i>OsVIT2</i>	Os09g0396900	GTTTGAGTTGGGACTGGAGAAG	CCTTGACATAGCCGAAGAAGAG
<i>OsWRKY67</i>	Os05g0183100	CCGTAGGACTGAACCTGAAG	CGTGTAGCTGGCCATGAAAG

^aThe primer pair does not distinguish *OsFER1* and *OsFER2*

Table S3 | Candidate genes related to Fe uptake, Fe storage, and defense mechanisms to Fe toxicity stress

Locus	Gene	Gene annotation	Biological function
Os03g0667500	<i>OsIRT1</i> ^{1,4,6,16}	Fe(II) transport protein 1	Fe ²⁺ transporter, uptake of iron
Os03g0667300	<i>OsIRT2</i> ^{1,4,6}	Fe(II) transport protein 2	Fe ²⁺ transporter
Os03g0307200	<i>OsNAS2</i> ^{1,3,6,8,12,15}	Nicotianamine synthase 2; S-adenosyl-L-methionine	Upstream-translocation of Fe(III); Fe/Zn uptake
Os07g0689600	<i>OsNAS3</i> ^{1,3,6,12,15}	Nicotianamine synthase 3	Upstream-translocation of Fe(III); mitigation of excess Fe stress, Fe detoxification, transport of iron
Os11g0135000	<i>OsTOM2</i> ^{1,10}	Transporter of mugineic acid family phytosiderophores 2	Exporter of mugineic acid, efflux transporter of phytosiderophores, metal transport
Os01g0293000	<i>OsSAMS3</i> ¹	S-adenosyl-L-methionine synthetase	Involved in synthesis of mugineic acid
Os03g0237100	<i>OsDMASI</i> ^{1,9}	Deoxymugineic acid synthase 1	Synthesis of deoxymugineic acid
Os02g0649900	<i>OsYSL2</i> ^{1,5,6,7,11,12,13,15}	Iron-phytosiderophore transporter, Iron homeostasis	Response and sensitivity to the Zn ion
Os11g0106700	<i>OsFER1-OsFER2</i> ^{1,6,12,15,18}	Ferritin-1, chloroplast	Fe storage protein; Fe homeostasis
Os12g0106000		precursor	
Os04g0463400	<i>OsVIT1</i> ¹⁹	Vacuolar Iron Transporter 1	Vacuolar membrane transporter; Fe and Zn translocation between flag leaves and seeds
Os09g0396900	<i>OsVIT2</i> ^{1,2,6,15,19}	Metal-nicotianamine (NA) transporter	Fe absorption and Zn ion transport; Fe and Mn transport to the endosperm
Os06g0567200	<i>OsAOO1</i> ^{14,15,17}	L-ascorbate oxidase precursor	Pro-oxidant; abiotic stress response
Os06g0567900	<i>OsAOO2</i> ^{14,15,17}	L-ascorbate oxidase precursor	Pro-oxidant; cold, salt, drought stress response
Os05g0183100	<i>OsWRKY67</i>	WRKY transcription factor	Positive regulation of blast and bacteria blight resistance

References:

¹Aung et al. (2018) *Soil Sci Plant Nutr* 64, 370–385; ²Finatto et al. (2015) *Rice* 8, 13; ³Inoue et al. (2003) *Plant J* 36, 366–381; ⁴Ishimaru et al. (2006) *Plant J* 45, 335–346; ⁵Ishimaru et al. (2010) *Plant J* 62, 379–390; ⁶Kar et al. (2021) *Plant Cell Physiol* 62, 624–640; ⁷Koike et al. (2004) *Plant J* 39, 415–424; ⁸Lee et al. (2012) *Mol Cells* 33, 269–275; ⁹Nozoye et al. (2011) *J Biol Chem* 286, 5446–5454; ¹⁰Nozoye et al. (2015) *J Biol Chem* 290, 27688–27699; ¹¹Paul et al. (2013) *Plant Cell Tissue Org Cult* 113, 363–373; ¹²Regon et al. (2022) *Front Plant Sci* 13, 798580; ¹³Swamy et al. (2016) *Rice* 9, 49; ¹⁴Ueda et al. (2015) *Plant Physiol* 169, 873–889; ¹⁵Wairich et al. (2021) *J Exp Bot* 72, 2242–2259; ¹⁶Wu et al. (2014) *Rice* 7, 8; ¹⁷Wu et al. (2017) *Nat Plants* 3, 16203; ¹⁸Wu et al. (2017) *Plant Cell Environ* 40, 570–584; ¹⁹Zhang et al. (2012) *Plant J* 72, 400–410

Table S4 | Gene expression analysis at 10 DAT in the hydroponic experiment

<0.2 0.2 - 0.5 0.5 - 2 2 - 5 >5						
Gene	Control			Fe toxicity		
	Ciherang	IR42	KA-28	Ciherang	IR42	KA-28
Fe(II) transporter)						
OsIRT1	1.0	1.3	1.1	0.7	1.0	1.0
OsIRT2	1.0	0.9	0.5	0.7	0.8	0.6
Long distance transport and Fe homeostasis						
OsNAS2	1.0	0.6	5.6	2.5	2.2	6.7
OsNAS3	1.0	1.7	2.0	0.4	1.2	1.0
OsSAMS3	1.0	1.1	1.3	1.0	1.0	1.3
OsTOM2	1.0	1.1	2.2	4.7	3.5	7.4
OsDMAS1	1.0	2.4	3.5	5.8	6.9	7.0
OsYSL2	1.0	0.8	1.0	0.9	1.0	1.3
Fe storage						
OsFer1/2	1.0	1.6	1.9	39.0	81.9	53.5
OsVIT1	1.0	0.8	0.9	0.7	0.7	0.7
OsVIT2	1.0	1.9	0.8	66.0	62.1	48.8
ROS scavenging						
OsAAO1	1.0	0.5	0.9	1.6	1.3	2.4
OsAAO2	1.0	0.7	1.9	0.8	0.4	1.0
OsWRKY67	1.0	1.7	1.3	3.6	2.8	3.1

The expression of genes in each sample group is shown as relative values to Ciherang under the control condition