The Combined Strategy for iron uptake is not exclusive to domesticated rice (*Oryza* sativa)

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Supplementary Material

Supplementary Table Legends

Supplementary Table 1. Full transcriptome data sets revealed by RNAseq in roots of *Oryza sativa* after five days under Fe-deficiency.

Supplementary Table 2. Full transcriptome data sets revealed by RNAseq in roots of *Oryza rufipogon* after five days under Fe-deficiency.

Supplementary Table 3. Gene-specific PCR primers used for RT-qPCR and PCR efficiencies.

Locus	Gene name	Forward Primer $5' \rightarrow 3'$	Reverse Primer $5' \rightarrow 3'$	PCR amplification efficiencies	
Oryza sativa/ O. rufipogon ^A				Eff target gene / endogenous O. sativa	Eff target gene / endogenous O.rufipogon
OS01G0952800/ORUFI01G47560	IRO2	CGGATTTGGGAACAGGACA	GTTCCTGACGACTTTCTCCA	1.939027/1.886871	1.931052/1.86818
OS03G0667500/ORUFI03G29910	IRT1	ACTGGTGCCCATTCTGC	GCGAGGATGGGGATGG	1.877963/1.882419	1.912471/1.936508
OS01G0605100/ORUFI01G24790	BCS1	CTGGAACTCAACCCTTCGA	CCTCTGAAGGATTGGATTTGG	1.917836/1.889704	1.937038/1.906191
OS10G0370500	Cellulase	CACCCTGAGGTCGAACG	GGCGAACCGGACGAG	1.838172/1.862471	1.848612/1.860437
OS01G0323600/ORUFI01G14960	S- adenosylmethionine synthetase 2	ACGCCACCTTGCTGTC	GACGTTCCTCTTCACCTCC	1.888845/1.936181	1.886621/1.891841
OS07G0258400/ORUFI07G09420	NRAMP1	CATGCTGCTCTACGTCGTC	CAGCTCACGACGAGACAC	1.946169/1.886966	1.957729/1.953282
OS03G0751100/ORUFI03G35800	OPT7	AGTGTGAAGGCGCCG	ATCTTCTTCTTCGCGAGCTT	1.796674/1.825561	1.799743/1.816699
OS11G0134900	ZIFL4-TOM1	TGTGATTGAATTAATTGGACTTGC	GGGGTGCTATTCCAGCTTCT	1.935136/1.866774	1.93262/1.956244
OS03G0237100/ORUFI03G09980	DMAS	CCTGGACATCGTCGGAT	GTCGTCGAGCGACTTGTAG	1.883525/1.911072	1.892687/1.898705
OS09G0133200	Reductase SDR Family	GAGCTTATTGACAGGAGCACA	AGCAACAACAATAGTTTCAGCTG	1.944826/1.909023	1.899864/1.93218
OS02G0650300/ ORUFI02G27480	YSL15	GGTGCGGGGGATGATTTG	CCATACAAACTTGTCATGCTG	1.949223/1.902821	1.925499/1.871946
OS12G0133100	ZIFL12	CCCAAACTGTTGAAGCTTTGG	GGACATCAAGGGCCAATTTC	1.894337/1.888208	1.884827/1.885545
OS01G0328400	UBQ5	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT	-	-
Zea mays				Eff target gene	Eff endogenous gene
Zm00001d033446	IRT1	GCTTCTACAACCGGAGGAAG	GTCCTCGGGCTTGTCAG	1.940012	1.909559
Zm00001d017429	YS1	GTCTGGGAGAAGGTGAACAG	GGCCAGAGCGAGAATGG	1.894495	1.892154
Zm00001d028360	DMAS	GAGCTTCGACGAGGGG	GTGCTCGGAGACGTAGC	1.920767	1.879584
Zm00001d041111	TOM1	CTTTCCTTGTGGACCGTAAGT	ACAAATGAACATACCTGCAATCG	1.917432	1.861516

Supplementary Table 3. Gene-specific PCR primers used for RT-qPCR.

Zm00001d052434	TOM2	GACCCTACACAAGCACAGA	GGAGGGAGAAGACGCAGTA	1.865249	1.861516
Zm00001d005001	ТОМЗ	GCCCACCAGAGTGACTAC	CTGAATAAGCCATATCATGGAAGG	1.897926	1.861516
Zm00001d042062	IRO2	GCCTCAACGACACGGAG	CCCAAAGGTGGAGTAGGTT	1.843356	1.892154
Zm00001d029339	IRO3	CTTTCCAGAAGTGGCATCTG	GCTTGTGCTGCTACTGC	1.866295	1.905458
Zm00001d053834	UBQ	CGGCAAGCAGCTGGAG	GCGCAGCACCAGGTG	-	-
Zm00001d025205	FIT	CCGAACGGCGGGTTATT	CACTGCTGGTAGTGGTCG	1.846875	1.879584
Zm00001d002006	MHA2	GTTCCTTGGGTTCATGTGGA	GGAGTTGATAACCAAGAGCACG	1.865067	1.905458
Zm00001d005479	NRAMP1	CTCATTGGCATTGTCGTGTT	CACGAACTTGATGGTCTCC	1.862461	1.768098
Sorghum bicolor				Eff target gene	Eff endogenous gene
SORBI_001G142900	IRT1	GACCGCAACCTCTTCGT	GGTGGTGAAGGAGAAATCGG	1.958446	1.958171
SORBI_003G428800	IRO2	CGGAGATCATGGTTCAGGT	CTTGTTCTCGAAGGTGGAGT	1.948199	1.913552
SORBI_002G095600	NRAMP1	GCATCATCGTGTTCCCTCTTAT	CATCGGCAACCTTTGATTTGTC	1.924862	1.914071
SORBI_004G299500	YS1	GAAGTACGCGAGGTTTGTG	CAGCCTCCTTCTTGTTCATC	1.913064	1.924862
SORBI_004G299500 SORBI_3001G112600	YS1 ACT1	GAAGTACGCGAGGTTTGTG GGCAACATCGTCCTCTCT	CAGCCTCCTTCTTGTTCATC	1.913064	1.924862 -

Genes for which O. rufipogon loci is not shown were identified as differentially expressed by RNAseq only in O. sativa.

^A IRT1, NRAMP1, IRO2, and YSL15 primers are the same used for all species.

Supplementary Figure 1. Transcriptomic analysis of *Oryza sativa* and *Oryza rufipogon* roots from plants grown in control (CC) and Fe deficiency (-Fe) conditions for five days. Differential gene expression analysis of RNAseq from CC versus -Fe for (A) *O. sativa* and (B) *O. rufipogon*. Genes identified as differentially expressed by Kallisto (FDR < 0.05) are represented by red (up-regulated) and blue (down-regulated) lines. The *y*-axis represents a dendrogram from hierarchical clustering presented as transcripts per million reads (TPM). CC1: library 1 in control condition; CC2: library 2 in control condition; -Fe1: library 1 in iron deficiency condition.



Supplementary Figure 2. Shared Gene Ontology (GO) terms in the response to Fe deficiency between cultivated (*Oryza sativa*) and wild (*Oryza rufipogon*) rice. The figure presents the terms regulated in common and with high level of significance for both species. (A) Up-regulated processes. (B) Down-regulated processes. Blue columns refer to *O. sativa*. Orange columns refer to *O. rufipogon*.

А Upregulated processes in both species llular amino acid biosynthetic process glycosyl compound metabolic process sulfur amino acid biosynthetic process nucleoside metabolic process ion transmembrane transport methionine metabolic process single-organism biosynthetic process methionine biosynthetic process small molecule biosynthetic process small molecule biosynthetic process carbohydrate metabolic process response to iron ion S-adenosylmethionine biosynthetic process L-methionine biosynthetic process alpha-amino acid biosynthetic process L-methionine salvage amino acid salvage L-methionine salvage from methylthioadenosyne ion transport alpha-amino acid metabolic process metal ion transport zinc II ion transmembrane transport carboxylic acid biosynthetic process organic acid biosynthetic process oxidation-reduction process zinc II ion transport iron ion transport O. sativa O. rufipogon 0.0 1.0 2.0 3.0 Significance level (-log10[p value]) В Downregulated processes in both species organophosphate catabolic process membrane lipid biosynthetic process single-organism process sulfate transmembrane transport glycolipid biosynthetic process regulation of defense response sulfolipid biosynthetic process sulfolipid biosynthetic process glycosylceramide metabolic process cellular response to starvation single-organism metabolic process response to starvation single-organism metabolic process gleosylceramide metabolic process cellular response to starvation single-organism metabolic process response to extracellular stimulus cellular response to nutrient levels O. sativa O. rufipogon 0.0 0.5 1.0 1.5 2.0 2.5 3.0



Supplementary Figure 3. Unique Gene Ontology (GO) terms in the response to Fe deficiency between cultivated (*Oryza sativa*) and wild (*Oryza rufipogon*) rice. Exclusive GO terms up and down-regulated by Fe deficiency in (A) *O. sativa* and (B) *O. rufipogon*. The figure presents the terms with high degree of significance for *O. sativa* and *O. rufipogon*. Blue bars represent terms up-regulated, and red bars represent terms down-regulated.



Supplementary Figure 4. Syntenic block search for the *OsIRT1* gene from *Oryza sativa*. A genomic block of *OsIRT1*, composed of 41 genes (20 genes up and 20 genes downstream of *OsIRT1*, plus *OsIRT1*), was employed to search the complete genomes of *Oryza meridionalis* (A) and *Oryza rufipogon* (B). In the *O. meridionalis* genome, no potential syntenic blocks were found (no peak occurs on the *y*-axis). When the *O. rufipogon* genome was considered as a positive control, potential syntenic blocks were found, indicated by a peak at the protein relative position 3000 (B).



Supplementary Figure 5. Collinearity analyses of Fe deficiency-related genes in *Oryza spp.*, maize, and sorghum. Graphs displaying the results from the collinearity analysis, performed through the MCSCanX tool, across the *Oryza* genus, *Zea mays*, and *Sorghum bicolor* gene orthologs. The analyses were performed on genomic segments containing 41 genes. Arrows present the orientation of genes. Numbers represent the genes inside the genomic segments. The target gene (*YSL15, IRO2, NRAMP1*, or *IRT1*) is represented by number 0. (A) Collinearity analysis for *IRT1*. (B) Collinearity analysis for *YSL15*. (C) Collinearity analysis for *IRO2*. (D) Collinearity analysis for *NRAMP1*.

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Chr 3 O.glaberrima	4 4 - 19 - 1 4 - 4 - 4 13 - 4 - 3 - 2 - 4 0 1 2 - 4 - 4 - 4 - 4 - 4 - 9 10 11 12 - 4 15	
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- Chr 3 O.nivara	3 (+-2020)—18-17 -14 (—-12 -11 (+) (+-) (+-) (+-) (+-) (5 3 3 (+-) (- 7 8 (+-) (+) (+-) (+-) (+-) (+-) (+-) (+-)	+++
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+ Chr 3 O.rufipogon	-18-17 -14 -12-11 -11 -11 -11 -11 -11 -11 -11 -11	
+ Chr.3 O sativa in		C
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Chr 7 O.rufipogon		
Chr 7 O.sativa jp.		
		10Kb

Supplementary Figure 6. Chlorophyll concentration in leaves of *Zea mays* plants grown under control (CC) or Fe deficiency (-Fe) conditions. Chlorophyll concentration (μ g/mg DW). The *x*-axis represents days after the onset of -Fe treatment. Values are the averages of four samples \pm SE (n = 4, 3 plants each). Asterisks indicate statistical difference between plants grown under CC and -Fe conditions (Student *t*-test, **P-value < 0.01). DW = dry weight.



Supplementary Figure 7. Evaluation of Fe-deficiency symptoms when plants of *Sorghum bicolor* were grown under control (CC) or Fe deficiency (-Fe) conditions. (A) Chlorophyll concentration (μ g/mg DW), (n = 4, 3 plants each). (B) Root dry weight (g) (n = 10). (B) Shoot dry weight (g) (n = 10). The *x*-axis represents days after the onset of -Fe treatment. Values are the averages of four or ten samples ± SE. Asterisks indicate statistical difference between plants grown under CC and -Fe conditions (Student *t*-test, *P-value < 0.05, **P-value < 0.01, ****P-value < 0.0001).

