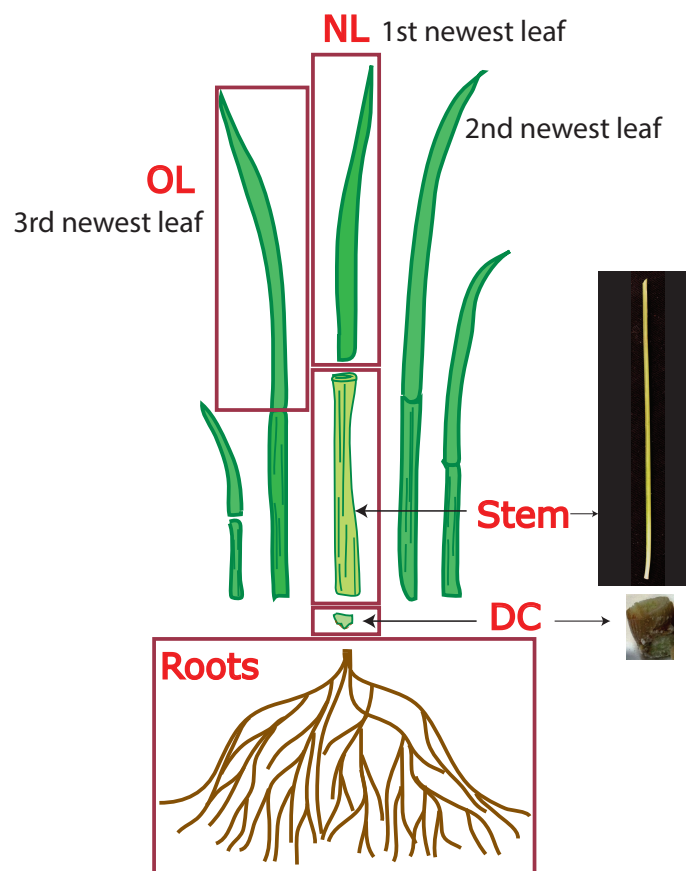


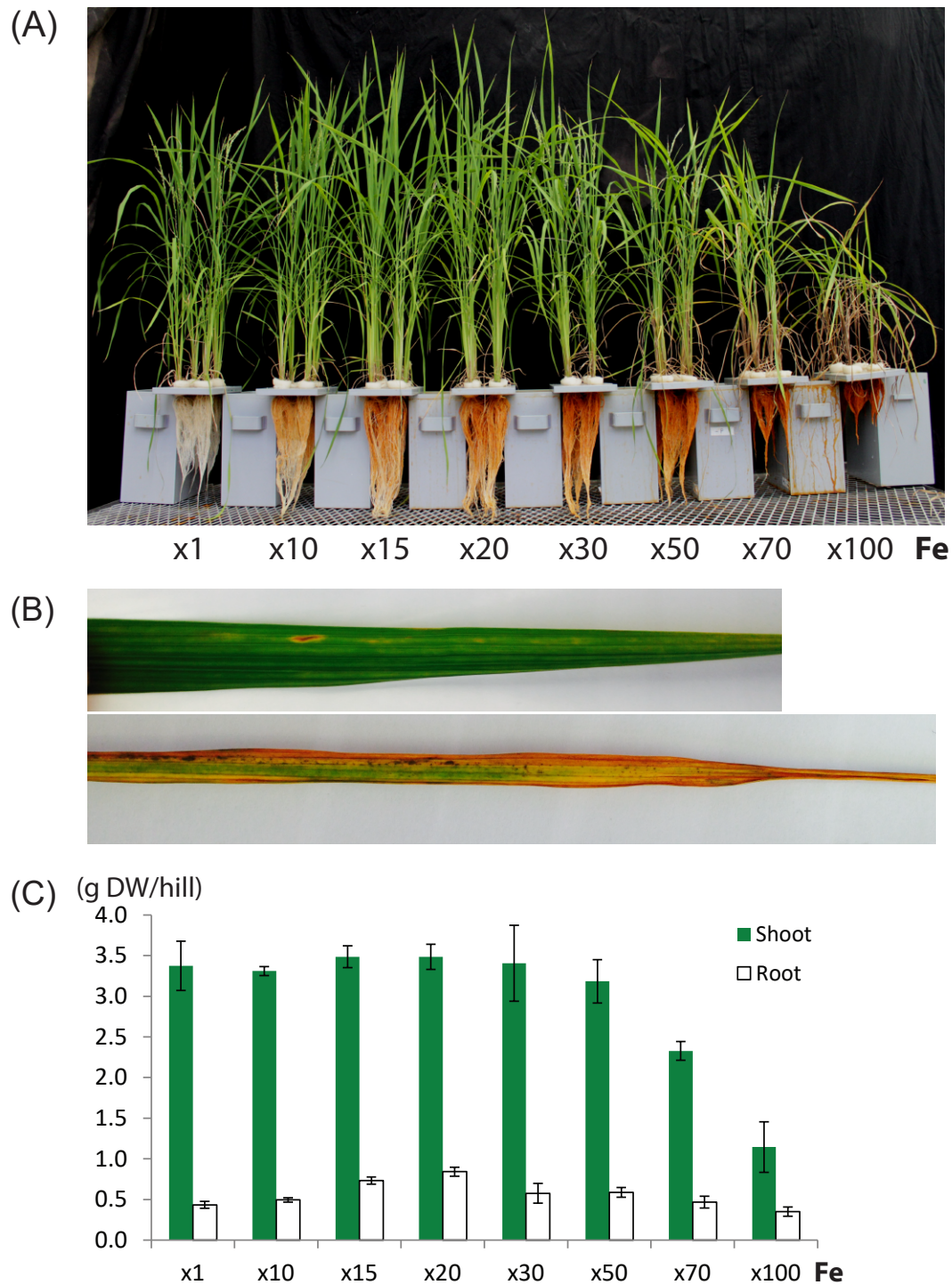
Physiological and transcriptomic analysis of responses to different levels of iron excess stress in various tissues of rice

May Sann Aung, Hiroshi Masuda, Takanori Kobayashi, Naoko K. Nishizawa

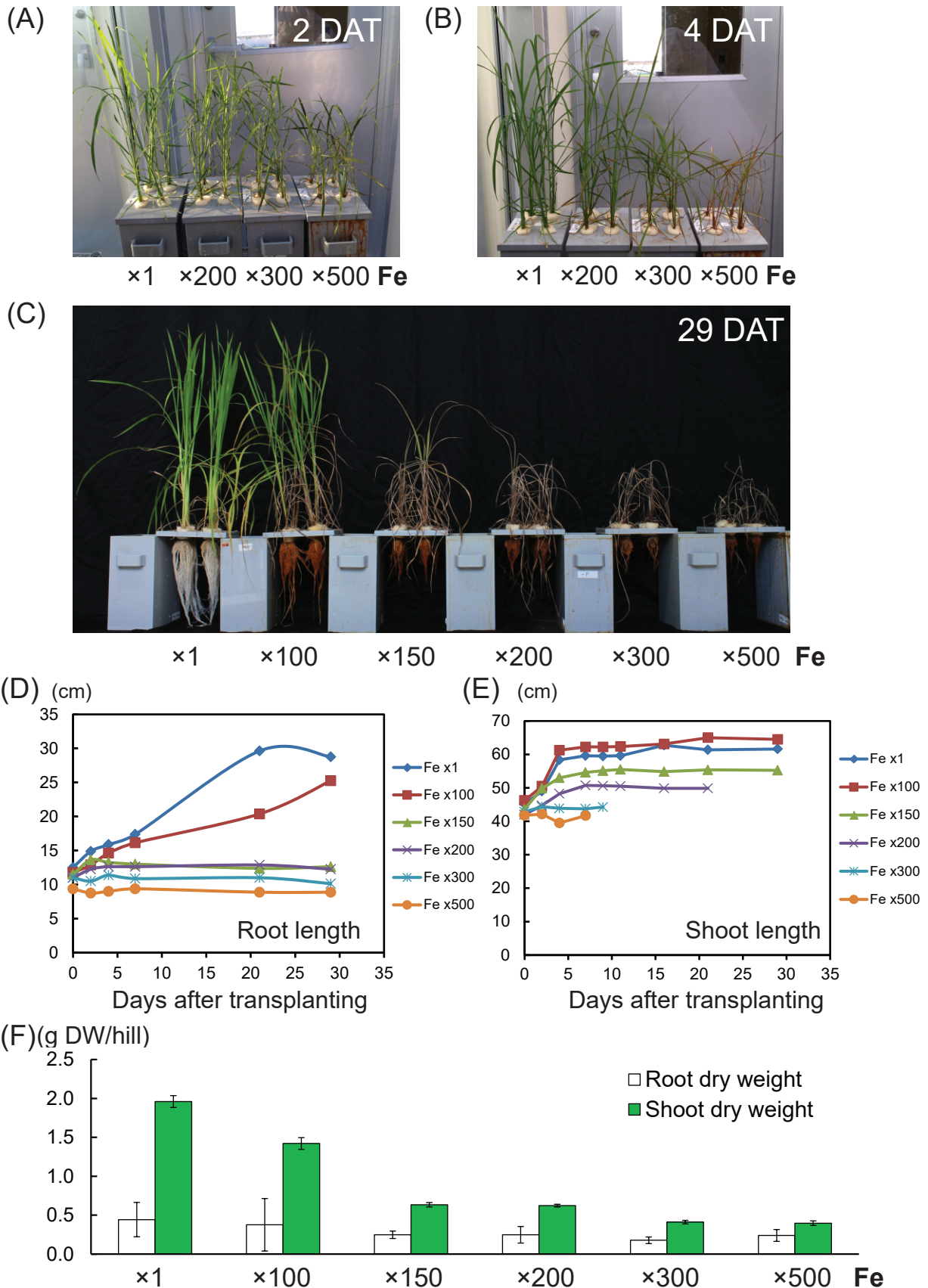
Ishikawa Prefectural University, Suematsu 1-308, Nonoichi, Ishikawa, 921-8836, Japan



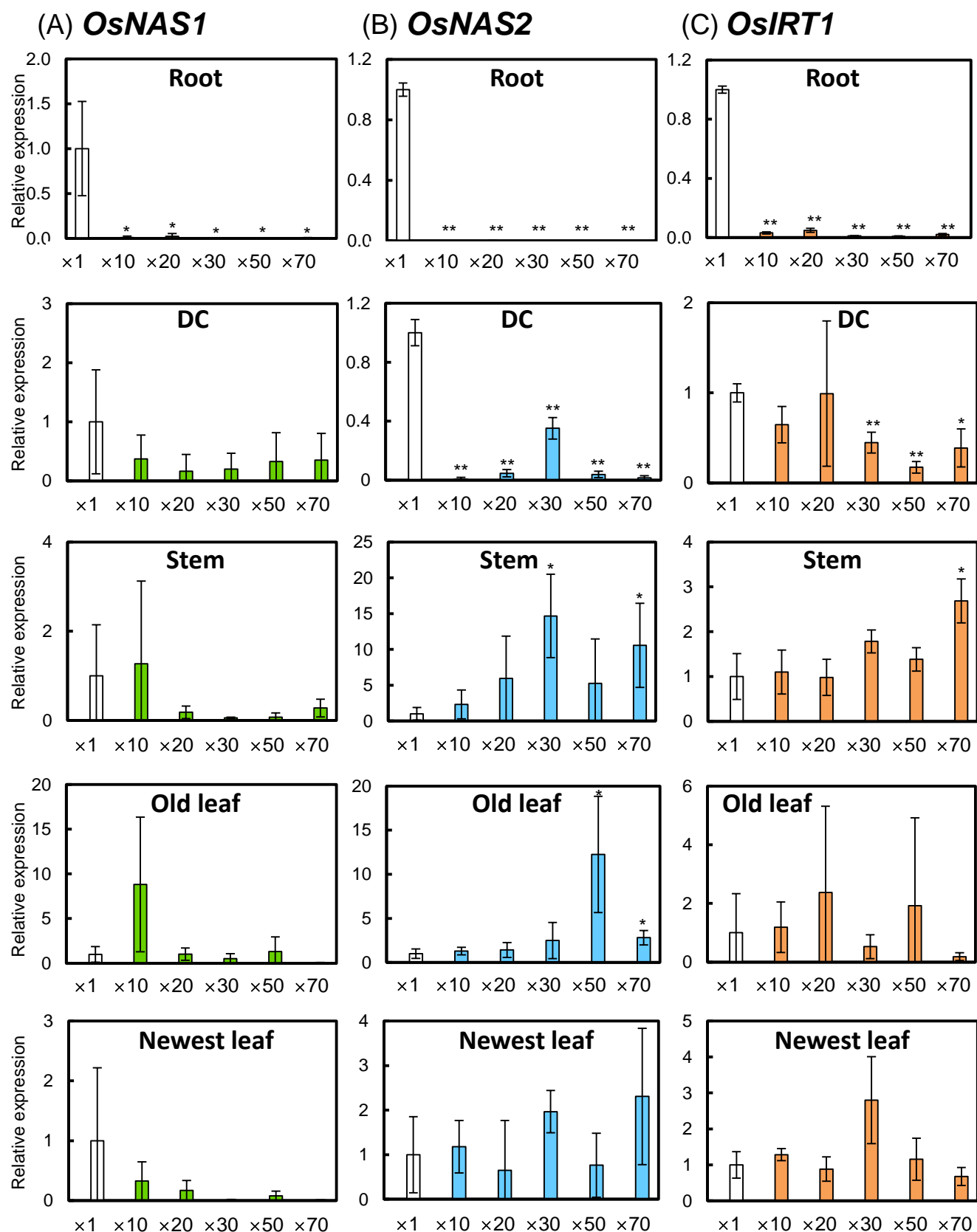
Supplemental Figure 1. Various tissues of rice divided and used for microarray analysis. DC: discrimination center or the junction between root and shoot; NL: newest leaf; OL: older leaf (3rd newest leaf).



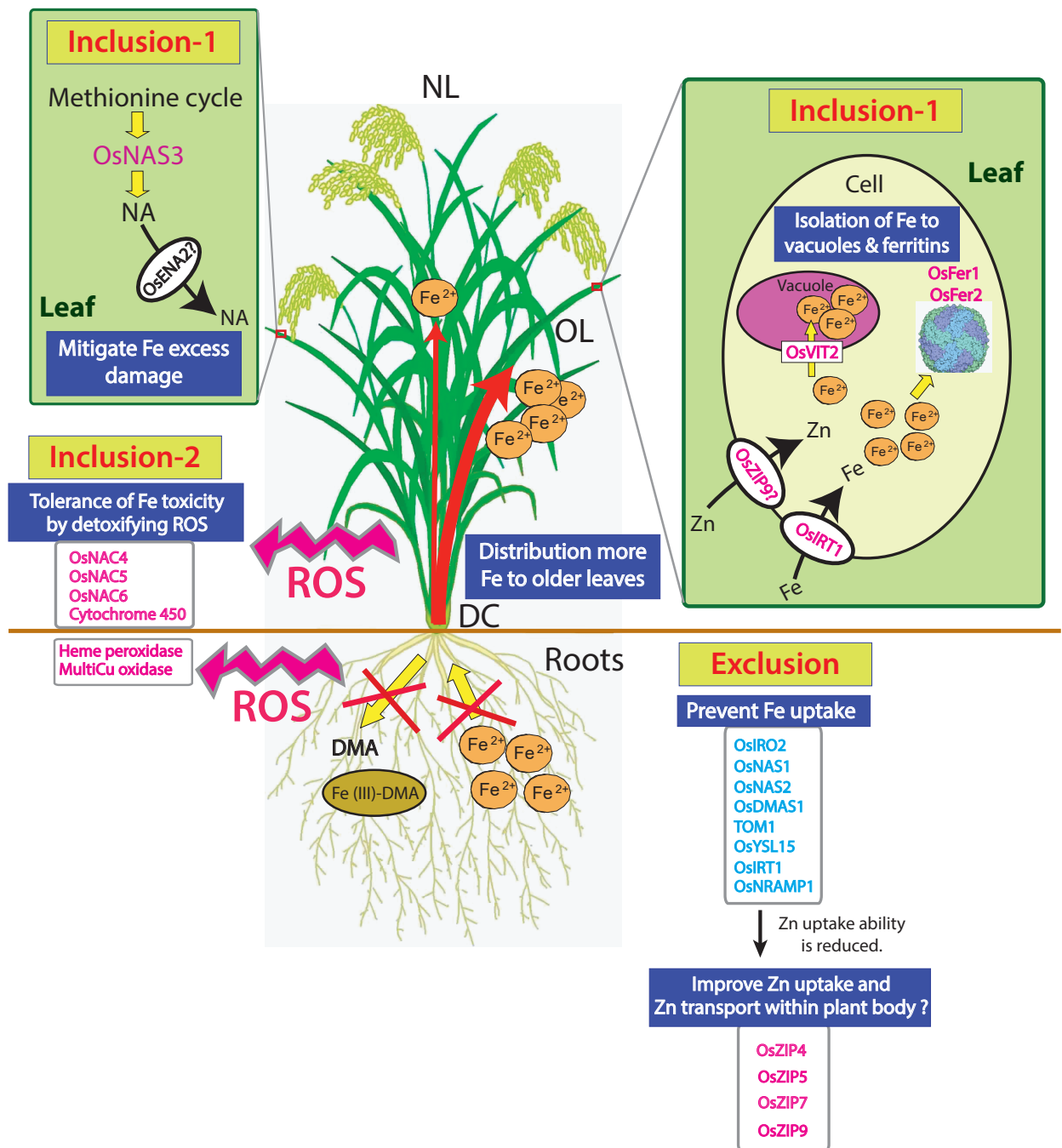
Supplemental Figure 2. Another trial of plant growth test on various levels of Fe excess stress. (A) shoot and root growth at 41 days after Fe excess exposure, (B) newest leaf (Upper) and old leaf (Lower) of x70 Fe plant at 19 days after Fe exposure and (C) shoot and root dry weight. Error bars represent \pm SD of biological replicates, $n = 3$.



Supplemental Figure 3. Plant growth and symptoms after 29 days of Fe excess exposure with severe levels of Fe intensities. (A, B, C) Plant appearance and symptoms, (D) Root growth, (E) Shoot growth and (F) Root and shoot dry weight. DAT: Days after transplanting. NL: Newest leaf; OL1: Old leaf 1 (3rd newest leaf); OL2: Old leaf 2 (4th newest leaf). Error bars represent \pm SD of biological replicates, $n = 4$.



Supplemental Figure 4. Expression of (A) *OsNAS1*, (B) *OsNAS2* and (C) *OsIRT1* genes in various tissues of rice confirmed by real time RT-PCR. DC: discrimination center. The numbers shown on horizontal axis of the graphs indicate the levels of Fe excess. The Error bar shows the technical variation, SD; n = 3. Data were normalized to the observed expression levels of *alpha-Tubulin* and presented as relative gene expression in each tissue (x1 Fe = 1). Asterisks above the bars indicate significant differences (* $P < 0.05$; ** $P < 0.01$) compared with the control (x1 Fe).



Supplemental Figure 5. Hypothetical model of Fe excess tolerance mechanism.

DC: Discrimination Center; OL: Older leaf; NL: Newest leaf; NA: Nicotianamine; DMA: Deoxymugineic acid; Pink letters: highly induced genes; Blue letters: highly repressed genes. The ferritin picture was kindly provided by Dr. David S. Goodsell (Scripps Research Institute, La Jolla, CA, U.S.A.) and the RCSB PDB.

Supplemental Table 1. Primer list used in Real Time RT-PCR.

Primers	Sequences
alpha- <i>Tubulin</i> forward	5'-TCT TCC ACC CTG AGC AGC TC-3'
alpha- <i>Tubulin</i> reverse	5'-AAC CTT GGA GAC CAG TGC AG-3'
<i>OsIRT1</i> forward	5'-CGT CTT CTT CTT CTC CAC CAC GAC-3'
<i>OsIRT1</i> reverse	5'-GCA GCT GAT GAT CGA GTC TGA CC-3'
<i>OsNAS1</i> forward	5'-GTC TAA CAG CCG GAC GAT CGA AAG G-3'
<i>OsNAS1</i> reverse	5'-TTT CTC ACT GTC ATA CAC AGA TGG C-3'
<i>OsNAS2</i> forward	5'-TGA GTG CGT GCA TAG TAA TCC TGG C-3'
<i>OsNAS2</i> reverse	5'-CAG ACG GTC ACA AAC ACC TCT TGC-3'
<i>OsNAS3</i> forward	5'-CGA TGA CTG CTT CCA TCG CTT-3'
<i>OsNAS3</i> reverse	5'-GGC ATG CAT TCA TGC ATG ACT GC-3'
<i>OsFer1&2</i> forward	5'- GTG AAG GGC AGT AGT AGG TTT CG -3'
<i>OsFer1&2</i> reverse	5'- CGC GCG ACA TAC ACA TGA TTC TG -3'
<i>OsVIT2</i> forward	5'- GTT TGA GTT GGG ACT GGA GAA G-3'
<i>OsVIT2</i> reverse	5'- CCT TGA CAT AGC CGA AGA AGA G-3'

Supplemental Table 2. Ratio increased in Fe concentrations in newest leaves (NLs), old leaves (OLs) and roots of the plants cultivated under various Fe excess levels compared to those of control plants.

	x10/x1	x20/x1	x30/x1	x50/x1	x70/x1	x100/x1	x150/x1
NL	1.9	2.3	3.5	4.1	5.9	11.8	24.7
OL1	2.9	3.6	4.4	7.4	10.9	25.6	48.1
Root	4.0	8.7	9.1	9.9	9.6	6.7	7.9

Supplemental Table 3 Expression ratios of other Fe-related genes

<0.2 <0.5 0.5 – 2 >2 >5

Locus	Gene	Description	Root				DC				Stem				Old leaf				Newest leaf			
			× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1	× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1	× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1	× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1	× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1
Other Fe related transporters																						
Os04g0578600	OsFRO2	Ferric reductase oxidase 2	0.74	0.73	0.81	0.75	0.45	0.27	0.07	0.04	0.10	0.04	0.08	0.04	0.42	0.75	0.73	0.65	0.36	0.58	0.40	0.29
Os03g0571900	PEZ1	Phenolic Efflux Transporter 1	0.41	0.60	0.71	0.89	0.79	0.60	1.19	0.73	0.44	0.70	1.00	4.13	0.60	0.75	1.44	1.83	0.57	0.73	1.24	1.67
Os03g0572900	PEZ2	Phenolics efflux transporter 2, OsCIM4L	0.99	0.74	0.63	0.68	1.14	0.69	0.57	0.57	2.10	1.03	1.31	1.51	1.14	0.91	0.63	0.56	0.82	0.90	0.84	0.94
Os01g0279400	OsZIFL2	(Zinc-induced facilitator –like) family genes	1.14	0.96	0.76	0.53	0.53	0.64	0.31	0.51	0.54	2.04	1.02	0.87	0.30	0.66	0.16	0.10	0.40	0.55	0.23	0.22
Os12g0133300	OsZIFL13	(Zinc-induced facilitator –like) family genes	0.76	0.86	0.68	0.79	0.80	0.59	1.73	0.79	0.99	0.91	0.69	0.88	0.79	0.78	0.94	0.96	1.30	0.83	0.74	0.76
Os01g0304100	OsCCC2	Putative cation: chloride co-transporter	0.86	1.35	1.17	0.98	0.77	0.81	0.33	0.65	0.81	0.62	0.58	0.62	0.81	0.99	1.19	2.97	1.00	0.91	0.93	0.66
Os09g0440700	OsCOPT7	Putative copper cation transporter	0.69	0.67	0.97	1.06	0.96	0.77	1.10	1.24	0.84	0.82	0.95	1.43	0.77	0.84	1.33	1.50	0.89	0.86	0.92	0.90
Os03g0216700	OsFRDL1	Citrate efflux transporter	1.65	1.44	1.59	2.54	1.17	1.40	1.84	2.11	1.17	1.17	1.32	0.93	1.82	0.87	2.14	2.35	1.37	1.14	0.80	1.17
Os10g0206800	OsFRDL2	Citrate efflux transporter (Putative)	1.41	1.20	3.19	3.76	1.26	1.09	1.91	1.77	1.49	1.03	1.21	1.52	1.23	0.91	1.01	0.98	1.02	1.04	1.09	1.28
Os02g0833100	OsFRDL3	Citrate efflux transporter (Putative)	1.11	0.99	1.00	1.03	1.08	0.92	0.98	0.90	1.14	0.90	1.14	0.71	0.96	0.89	0.70	0.67	1.52	1.23	1.07	1.11
Other Fe related homeostasis genes																						
Os03g0288000	OsMT1b	Similar to Metallothionein	2.25	1.72	1.55	1.52	1.50	1.36	1.64	1.72	1.54	1.14	0.65	3.65	0.47	0.67	9.82	25.53	0.56	0.67	1.05	1.40
Os05g0202800	OsMT3b	Similar to Metallothionein-like protein 3B	0.36	0.24	0.32	0.50	1.14	0.92	1.01	1.11	3.67	1.81	1.66	1.72	0.72	1.17	3.29	3.07	0.25	0.68	0.65	1.22
Os12g0567800	OsMT1f	Plant metallothionein, family 15 protein	1.06	0.70	0.49	0.57	1.26	0.81	1.52	0.51	3.54	0.82	1.21	1.97	0.76	0.59	0.79	3.32	1.66	1.26	0.69	0.85
Os12g0571000	OsMT1g	Metallothionein-like protein type 1	0.80	0.51	0.24	0.34	1.23	0.82	1.15	0.45	5.63	1.45	2.07	13.98	1.68	1.78	1.24	7.64	0.40	1.12	0.57	2.18
Other Fe related transcription factors																						
Os01g0816100	OsNAC4	Transcription Factor; related to cell death	0.50	0.64	1.63	2.05	0.69	0.63	0.71	0.95	0.43	0.70	0.42	2.64	0.79	1.06	1.51	3.50	0.75	0.76	0.90	1.28
Os11g0184900	OsNAC5	Transcription Factor; Fe and Zn remobilization	1.23	0.91	1.96	2.77	0.77	0.66	1.45	1.34	0.77	0.73	0.82	1.53	0.91	0.89	2.93	4.13	0.63	0.49	1.27	1.30
Os01g0884300	OsNAC6	Transcription Factor; related to stress induce genes	0.55	0.68	0.93	1.15	0.94	0.59	1.09	1.18	0.88	0.74	0.94	1.68	0.56	0.87	1.29	3.31	0.84	0.90	0.83	1.00
Os03g0339100	OsPRL1	Transcription Factor	0.89	0.77	0.68	0.80	0.97	0.88	0.40	0.99	1.10	1.22	1.15	1.19	0.80	0.92	1.32	1.54	0.88	0.97	0.91	1.02
Os03g0860100	OsEBP1	Ethylene responsive element binding protein encoding gene	1.59	0.31	0.52	0.67	0.90	0.43	0.59	0.69	0.12	0.36	0.25	2.19	1.07	2.25	9.37	20.71	0.72	1.41	1.98	3.24
Other metal transporters																						
Os12g0228800		Calcium-binding protein	1.82	2.01	1.17	1.26	1.49	1.14	0.93	0.83	1.16	0.69	1.02	0.85	1.47	0.87	0.88	0.70	1.64	1.22	1.15	0.54
Os01g0604500		Calcium-binding protein	0.75	0.75	0.73	0.69	0.71	0.48	0.46	0.77	0.96	0.94	0.56	1.06	1.20	0.94	0.52	0.53	0.79	0.70	0.65	0.64
Os06g0190800		Glutamate receptor protein, calcium ion homeostasis	1.10	0.87	1.29	1.28	0.88	0.93	0.89	0.87	0.79	0.86	0.90	0.80	1.20	0.82	0.78	0.67	0.70	0.74	0.78	0.73
Os10g0418100		Putative Ca ²⁺ –transporting ATPase	0.25	0.47	0.34	0.48	0.60	1.10	0.30	0.72	1.07	0.94	2.07	0.97	0.28	1.14	1.85	2.10	1.76	1.35	1.46	1.32
Os04g0581800		Copper-binding protein-related	1.06	0.75	1.56	1.24	0.99	0.83	0.50	0.63	0.83	1.78	0.62	0.58	0.55	0.91	1.08	1.10	2.05	2.17	0.83	1.19
Os02g0245800		Potassium Channel	0.54	0.27	0.31	0.45	1.07	1.42	1.75	1.14	1.13	1.31	0.94	1.17	1.43	1.25	0.75	0.63	0.67	1.03	0.58	0.56
Os09g0448200	HAK4	Potassium ion transmembrane transporter	0.65	0.25	0.38	0.28	0.34	0.27	0.23	0.64	0.55	0.65	1.38	2.90	0.94	1.04	0.77	0.84	1.07	1.00	1.27	1.31
Os04g0401700	HAK1	Potassium transporter	1.63	1.29	1.34	1.39	0.75	0.51	1.10	1.38	0.64	0.75	2.73	18.28	0.79	0.92	2.57	3.21	1.03	1.72	2.59	6.51
Os04g0186400	OsPT1	Phosphate transpoter	0.79	0.90	0.74	0.67	0.70	0.46	0.58	0.35	1.67	1.00	1.99	3.12	1.11	1.32	1.77	2.22	1.21	1.55	1.30	1.28
Os04g0186400	Pht1–2	Phosphate transpoter	0.91	0.98	0.87	0.81	0.89	0.62	0.78	0.51	1.74	1.15	1.97	2.29	1.07	1.19	1.60	1.97	1.29	1.58	1.33	1.29
Os03g0226400		Manganese transporter	0.88	0.87	0.89	0.81	2.41	4.43	3.74	2.40	3.13	1.04	1.26	0.73	3.25	1.24	0.72	0.30	1.61	1.48	0.67	0.53
Os07g0518100		Sulphate transporter	0.66	0.88	1.00	0.70	0.57	0.61	0.32	0.71	1.72	1.15	1.37	7.53	1.05	0.91	2.89	6.13	0.90	0.78	0.77	1.41

Supplemental Table 4 Expression ratios of other important genes

<0.2	<0.5	0.5 – 2	>2	>5
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Locus	Gene	Description	Root				DC				Stem				Old leaf				Newest leaf			
			× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1	× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1	× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1	× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1	× 10/ × 1	× 20/ × 1	× 50/ × 1	× 70/ × 1
The genes related to oxidative stress																						
Os08g0561700	CuZnSOD	OsSOD4, OsCSD4, Similar to Superoxide dismutase	0.99	1.03	1.01	0.93	0.95	0.99	0.90	0.86	1.16	0.87	1.27	0.92	0.96	1.14	1.65	2.12	3.15	2.20	2.05	2.00
Os07g0665300		Similar to Superoxide dismutase	0.78	1.00	0.99	1.09	1.04	1.13	1.14	1.13	0.98	1.12	1.11	1.01	1.00	1.08	1.06	0.95	0.84	0.95	0.97	0.92
Os03g0285700	OsAPX1	APXa, OsAPx01, Similar to L-ascorbate peroxidase	1.53	1.48	1.65	1.54	1.25	1.14	1.25	1.20	0.57	1.04	0.74	1.42	0.84	1.03	1.68	2.21	0.95	0.92	1.09	1.28
Os10g0415300	OsGR3	GR3, Similar to Chloroplastic glutathione reductase	1.19	1.13	1.57	1.99	0.96	0.72	0.52	1.05	0.33	0.98	0.57	1.73	0.82	1.07	1.73	2.42	0.51	0.82	1.08	1.11
Os02g0280700		Similar to iron/ascorbate-dependent oxidoreductase	1.00	1.55	1.04	0.81	0.95	1.05	1.15	1.77	1.88	1.05	1.43	0.75	0.84	0.77	1.56	1.41	3.59	3.30	2.19	2.42
Os07g0531400		Peroxidase	50.45	32.76	91.13	116.12	4.49	6.66	49.60	38.73	1.03	0.89	2.25	22.45	0.76	0.12	0.46	12.21	0.70	0.65	0.73	0.74
Os11g0112200		Cationic peroxidase	0.68	0.63	1.37	1.82	0.56	0.33	1.41	1.39	1.35	1.02	2.47	11.49	0.87	1.61	19.32	28.85	0.70	0.71	0.65	3.19
Os01g0327100		Haem peroxidase family protein	0.69	0.69	0.45	0.36	0.75	0.54	0.57	0.65	1.54	1.22	2.29	2.16	2.88	4.00	26.98	155.14	5.78	3.54	2.89	3.58
Os10g0527400	OsGSTU3	Similar to Tau class GST protein 3	0.30	0.30	0.17	0.27	0.72	0.36	0.61	1.15	0.62	0.33	1.11	16.87	0.28	0.79	1.69	12.53	2.53	1.28	3.62	2.91
The genes involved in oxygen and electron transfer																						
Os05g0529700		Heat shock protein DnaJ family protein	0.84	0.49	0.65	0.75	1.68	0.65	0.74	0.78	2.78	0.87	0.89	1.78	0.88	0.80	1.39	1.98	0.82	0.83	0.85	0.89
Os06g0549900		Reticuliline oxidase-like protein	0.40	0.53	0.27	0.34	1.39	1.09	1.11	1.27	1.18	1.12	7.18	92.56	0.75	0.78	3.17	81.41	1.78	2.06	1.30	1.54
Os02g0218700		Similar to Allene oxide synthase	0.35	0.37	0.38	0.55	0.86	0.38	0.60	0.67	1.74	2.11	2.15	7.61	0.97	1.12	12.55	25.40	0.95	1.19	1.09	1.89
Os04g0600300		Alternative oxidase	0.24	0.29	0.17	0.22	0.79	0.31	0.62	0.70	0.32	0.51	3.03	35.70	0.50	0.96	7.05	36.75	0.83	0.91	2.05	3.50
Os08g0105700		Cytochrome P450; Similar to Bx2-like protein	0.74	0.53	0.71	0.81	0.75	0.65	0.79	0.83	0.90	1.13	0.96	2.03	1.12	1.73	2.50	14.28	1.89	1.04	1.42	1.56
The genes involved in cytochrome P450 family proteins																						
Os06g0294600		Cytochrome P450 family protein	0.59	1.13	0.49	0.34	0.48	0.48	0.39	0.40	1.34	1.30	2.10	4.53	1.63	0.83	2.90	6.10	7.15	5.84	3.59	4.36
Os03g0594100		Cytochrome P450 family protein	0.54	0.86	0.31	0.36	0.41	0.12	0.22	0.56	0.65	0.94	3.94	16.96	0.84	1.83	5.85	21.79	0.74	0.96	1.21	4.72
Os03g0570100		Cytochrome P450 79A1 (EC 1.14.13.41)	0.31	0.48	0.07	0.21	0.54	0.18	0.22	0.93	0.15	1.01	0.61	8.11	0.75	0.74	4.00	28.72	0.67	0.71	0.71	1.01
Os02g0571900		Cytochrome P450 family protein	0.54	0.63	0.09	0.16	0.32	0.14	0.21	0.55	1.18	0.48	3.56	29.36	1.49	2.32	13.60	14.11	1.86	1.23	2.62	5.44
Os02g0569900		Cytochrome P450 family protein	0.33	0.46	0.31	0.39	0.27	0.10	0.21	0.67	0.60	1.01	5.23	50.31	0.25	0.41	0.72	11.80	0.78	1.77	2.69	5.22
Os10g0439800		Cytochrome P450 family protein	0.27	0.35	0.11	0.18	0.28	0.34	0.20	0.47	0.30	0.29	1.79	23.68	0.75	0.74	8.96	31.92	0.67	0.64	0.70	1.04
Other genes induced in DC																						
Os07g0531400		Heme Peroxidase	50.45	32.76	91.13	116.12	4.49	6.66	49.60	38.73	1.03	0.89	2.25	22.45	0.76	0.12	0.46	12.21	0.70	0.65	0.73	0.74
Os08g0434100	OsRNS3	S-like ribonuclease (RNase PD2)	1.90	1.64	1.55	1.37	2.48	2.31	2.46	2.01	1.45	1.23	1.55	1.46	1.02	1.16	1.34	1.00	1.26	1.40	1.68	2.10
Os09g0537700	OsRNS4	S-like ribonuclease (RNase PD2)	1.82	0.75	0.95	0.71	21.95	36.94	40.01	6.70	2.66	1.99	1.54	1.34	3.57	4.22	2.50	0.48	1.87	1.32	2.62	1.10
Os01g0127000		MultiCu oxidase	10.48	7.25	9.23	10.31	2.90	3.01	9.13	8.99	1.61	1.10	1.79	1.14	1.20	1.16	0.98	0.69	1.21	1.05	1.65	1.00
Os11g0151700		Purple acid phosphatase (IRO2-regulated)	1.82	2.82	4.69	6.39	4.31	4.73	5.30	3.65	2.45	1.33	1.61	1.07	1.16	1.28	1.63	1.71	1.37	1.39	1.34	1.19
Os07g0468200		Hypothetical protein	1.52	1.17	1.84	2.64	5.17	2.02	4.85	4.46	1.77	1.19	1.31	2.95	1.67	0.99	2.82	7.41	0.72	0.77	0.78	0.92
Os06g0486900	FDH	Formate dehydrogenase, mitochondrial precursor	1.59	1.49	1.86	1.38	9.44	5.22	2.01	6.23	0.59	0.69	0.42	3.08	0.76	0.50	0.75	3.82	1.34	1.05	1.00	0.78

Gene Annotation: Gene functions are as according to RAP-DB and Quinet *et al.* 2012.