10.1071/FP19374_AC

Supplementary Material: *Functional Plant Biology*, 2020, 47(12), 1041–1052.

Supplementary Material

Micronutrient homeostasis and chloroplast iron protein expression is largely maintained in a chloroplast copper transporter mutant

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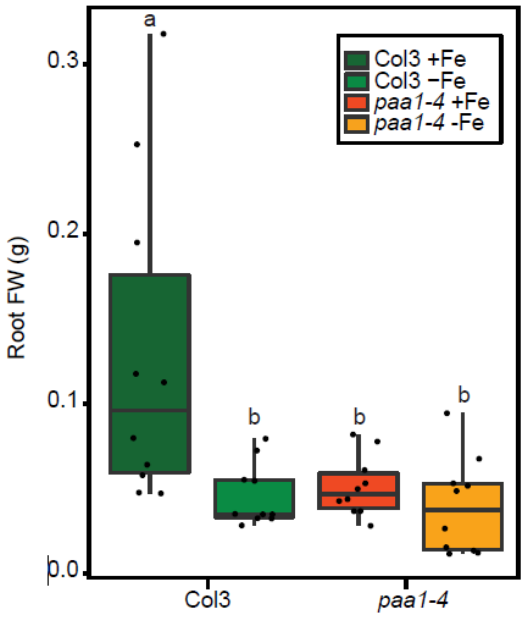

Fig. S1. Root biomass of hydroponically grown *paa1-4*. Fresh weight of root systems of hydroponically grown Col3 and *paa1-4* in response to Fe deficiency. Roots were used to measure Ferric Reductase Activity ($n = 10$). Box plots represent the first and third quartiles of data with the median denoted by horizontal line in the box. Whiskers on boxes represent spread of data from lowest to highest value. Dots are individual data points, and dots above or below whisker spread are outliers.

Table S1. *Paa1* genotyping

PCR primers and restriction digest procedures for genotyping

PAA1-1F: 5'-TACTGCAAGGGATATTCTCATTCA-3'
PAA1-1R: 5'-CCTGCGACCTGTAGTTGC-3'
Digest with Hinf1. *WT*: four fragments, with a main band at 281 bp; *paa1-1*: three fragments with a main band at 422 bp
PAA1-3F: 5'-CCGTCTTTCAGGAGTATCTCAAG-3'
PAA1-3R: 5'- GCAACCATTCTTTGAGACAGAAC-3'
Digest with Taq1. *WT*: 168 + 262 bp; *paa1-3*: 420 bp
PAA1-4F: 5'-CAGGAGTTAAACCAGCTGAG-3'
PAA1-4R: 5'-GTGGGGATAATATGCGAAACAT-3'
Digest with Nde1. *WT*: 217 bp; *paa1-3*: 22 + 195 bp
PAA1-6F: 5'-GCCAGATTTAGTTCCTGCATC-3'
PAA1-6R: 5'-GCTTCACGGTCTTCATGG-3'
Digest with Mse1. Six bands are produced in *WT*, seven in *paa1-6.* A 182 bp fragment is present in *WT* but cleaved in *paa1-6*.

Table S2. Quantitative PCR primers

AT4G04770	**SufB-F:** 5'-CCTTACATCCAGGTAAAGAATCCA-3' **SufB-R:** 5'-CAGAAACCAGAGATCATTGCC-3'
AT1G60950	**FD2-F:** 5'-CAGTCTCCGTTCCCTTCCAT-3' **FD2-R:** 5'-CCAGCTTCCTCAGCAGCATC-3' Lui *et al.* (2013)
AT4G03280	**Rieske-F:** 5'-ATTCCAGCAGACAGAGTTCC-3' **Rieske-R:** 5'-CTACATCGTTTCCAAGGGCA-3'
AT3G18780	**TUB2-F:** 5'-GTTCTCGATGTTGTTCGTAAG-3' **TUB2-R:** 5'-TGTAAGGCTCAACCACAGTAT-3' Quin *et al.* (2005)
AT4G05320	**UBQ10-F:** 5'-CGTTAAGACGTTGACTGGGAAAACT-3' **UBQ10-R:** 5'-GCTTTCACGTTATCAATGGTGTCA-3' Lasanthi-Kudahettige *et al.* (2007)

Table S3. Elemental analysis of Col3 and *paa1-4*

Elemental concentration ($mg \cdot kg^{-1}$ DW) of Col3 and *paa1-4* rosettes after 7 days of Fe deficiency treatment. For each element, values represent mean ± s.e. Statistical significance is denoted by asterisk

	Col3 10 μM Fe	Col3 10 nM Fe	*paa1-4* 10 μM Fe	*paa1-4* 10 nM Fe
Ca	$428 \times 10^2 \pm 15.3 \times 10^2$	$445 \times 10^2 \pm 12.9 \times 10^2$	$467 \times 10^2 \pm 18.5 \times 10^2$	$406 \times 10^2 \pm 16.6 \times 10^2$
Cu	14 ± 1.7	48 ± 1.6*	32 ± 3.2	57 ± 4.0*
Fe	59 ± 4.2	42 ± 2.7*	52 ± 4.0	24 ± 2.3*
K	$444 \times 10^2 \pm 16.5 \times 10^2$	$507 \times 10^2 \pm 10.8 \times 10^2$	$479 \times 10^2 \pm 31.3 \times 10^2$	$472 \times 10^2 \pm 16.9 \times 10^2$
Mg	$587 \times 10^2 \pm 20.5 \times 10^2$	$695 \times 10^2 \pm 12.9 \times 10^2$	$690 \times 10^1 \pm 30.5 \times 10^2$	$650 \times 10^2 \pm 11.6 \times 10^2$
Mn	78.0 ± 2.70	70.3 ± 3.02	116 ± 12.9	86.7 ± 11.4
Mo	2.9 ± 0.1	3.3 ± 0.2	5.5 ± 1.1	3.4 ± 0.7
P	$855 \times 10^1 \pm 27.9 \times 10^1$	$877 \times 10^1 \pm 15.7 \times 10^1$	$937 \times 10^1 \pm 21.6 \times 10^1$	$814 \times 10^1 \pm 12.7 \times 10^1$
S	$779 \times 10^1 \pm 25.2 \times 10^1$	$1260 \times 10^1 \pm 35.3 \times 10^1$*	$847 \times 10^1 \pm 26.2 \times 10^1$	$1080 \times 10^1 \pm 43.7 \times 10^1$*
Zn	55 ± 3.7	49 ± 2.8	67 ± 3.1	53 ± 6.2

References

Lasanthi-Kudahettige R, Magneschi L, Loreti E, Gonzali S, Licausi F, Novi G, Beretta O, Vitulli F, Alpi A, Perata P (2007) Transcript profiling of the anoxic rice coleoptile. *Plant Physiology* **144**, 218–231.

Liu J, Wang P, Liu B, Feng DR, Zhang J, Su JB, Zhang Y, Wang J, Wang H (2013) A deficiency in chloroplastic ferredoxin 2 facilitates effective photosynthetic capacity during long-term high light acclimation in Arabidopsis thaliana. *Plant Journal* **76**, 861–874.

Qin G, Gu H, Zhao Y, Ma Z, Shi G, Yang Y, Pichersky E, Chen H, Liu M, Chen Z, Qu L-J (2005) An indole-3-acetic acid carboxyl methyltransferase regulates *Arabidopsis* leaf development. *The Plant Cell* **17**, 2693–2704.