Supplemental Tables

Table I: PHT1 transporter family comparison at the amino acid level. BLAST program was used to obtain percentage of similarity and identity (in bold).

	PHT1;1	PHT1;2	PHT1;3	PHT1;4	PHT1;5	PHT1;6	PHT1;7	PHT1;8	PHT1;9
PHT1;1	**100%**	100%	96%	87%	86%	80%	86%	63%	64%
PHT1;2	**99%**	**100%**	96%	87%	86%	80%	86%	64%	64%
PHT1;3	**94%**	**94%**	**100%**	88%	86%	80%	88%	64%	64%
PHT1;4	**78%**	**78%**	**79%**	**100%**	88%	81%	94%	65%	66%
PHT1;5	**77%**	**76%**	**78%**	**78%**	**100%**	78%	88%	63%	63%
PHT1;6	**68%**	**68%**	**67%**	**67%**	**64%**	**100%**	81%	61%	63%
PHT1;7	**78%**	**78%**	**79%**	**90%**	**78%**	**69%**	**100%**	64%	65%
PHT1;8	**51%**	**52%**	**51%**	**49%**	**47%**	**49%**	**49%**	**100%**	87%
PHT1;9	**49%**	**49%**	**48%**	**48%**	**47%**	**47%**	**49%**	**79%**	**100%**

Table II: List of the primers used in qRT-PCR experiments.

Name or nb	AGI	reverse	forward
PHT1;1/1;2	At5g43350/ At5g43370	CAACTTGAGGAGGCGTTGA	GGTTTTGGTTGGGATTTGG
PHT1;4	At2g38940	CCATCACAGCTTTTGGCTCATG	CCTCGGTCGTATTTATTACCACG
PHT1;5	At2g32830	CTACCGGAATTTGCCACAGT	CGTTGTTGATGCTTGCTTGT
PHT1;6	At5g43340	TGAAAAAGTAACCGGGAACG	CCTTCGGCTAAAACCATGAA
PHT1;7	At3g54700	GTTCGTTCTCACCGGACATT	TCCCTCATTGTTTTGGGTGT
PHT1;8	At1g208606	AAACGCCACCAAGAATCAAG	TCCCGGCTAGGTTTAGGTCT
PHT1;9	At1g764306	ACTCATTCGCAGGAGACGAC	AATCGCGTTTTTGATCCTTG
1	At1g67810	TCATCCTCCCAAACTCATCC	TCACGGGATGTACGACTCAA
2	At5g67080	ACACCGATGCGTAACAACAA	AGTGGTAAAACGGCATGGAG
3	At2g04460	GAGAATCTTTGCCGTCCAGA	GATCGGTTTGGCCTCTACAA
4	At3g02870	TTACCGGATGGATCAAAGATG	CTTCTACGAGCTCGGTTTCG
5	At5g01220	TGTGAGAGTTCATCGCCTTG	TACCTGAAGCTCGGATTGCT
6	At1g08650	GATCGAAACAGAGGGATGGA	CTCTCTCCGACGACCTTGAC
7	At1g13750	AAACTGGTGCGTTTGGATTC	AGCGCCTGTCAAGTTTCAAT
8	At1g17710	CAACGAGAGCAACCATGAGA	TTAGGGTGCGAGCTGAGAAT
9	At1g71130	AGCAACCTCAGAAGCTCCAA	TCGTCTCTGGCTGGGTACTT
10	At1g73010	GACGACACGTGGATGAATTG	TCATGATCAAGGCAAAACCA
11	At2g11810	CATCAGAGGATGCACGCTAA	AGAGGCCGGTTTAATGGAGT
12	At2g27190	CAACGAACCTTCCCCAAGTA	TGAGATGAATCCGGGAAAAG
13	At2g30540	GGCTTTCTCGATCTCACGAC	CAAGAGCTCGTGTTGCATGT
14	At2g45130	CCTCCTCTTGTTCGCTTGTC	ATGGCGAAATGGTTCTGCTA
15	At3g02040	GTCGTCGGTTGCGACGCTCCACTT	ACGGGCCACAGAGGGATACAGGCA
16	At3g05630	TTTTGAAGCCGTTTCTTGCT	TGCATTGCTGGAGACAAAAG
17	At3g43110	TTGTCCCCAACATGTCTCAA	CGTGTACGTAACGGTGGTTG
18	At3g47420	AGCAAAACCGTACCATCAGG	CGCTGCAAGAAAAACAACAA
19	At4g03960	CCTCTTGGAGCATGAGAAGG	GCAGCTGCGAAAGCTAGAAT
20	At4g33030	AGGCTCAAGTCCAAGTTCCA	AGCTTGGGCTAGACGTGAAA
21	At5g20150	GCGGCAATGAAAACACACTA	CGGGTTTTGAAGGAGATCAG
22	At5g20410	TGGTCCAGCTTTTGTGATGA	ACAAGAAATTGGCATCTGCAT
23	At5g20790	TATTAACGGCTCCGTTTTCG	CGTCTCGAACCGAAATCAAT
24	At3g19970	ACGCAGCTGAGAAACCTGAT	CGTCTTTCACACCTTCAGCA
25	At3g51860	GGCGATACCACCAAAGAAGA	GCGTTGGCCAATAACAAAGT
26	At5g64000	CGCAGATCTCCAGTTTCCTC	CAGCTGATTACGGCTCACAA
27	At3g55970	TGCTCAGCATCTGAATTTGG	TCGGTATCTCTCCGCACTCT
28	At5g58310	GCTGCTTGAAGGAGGAATTG	GACAGGCAAGGACCATTTGT
29	At4g14090	CGTTTGAACCACATCGTTTG	TCTCTGCTCACCGACGTATG
30	At1g52940	GCGCTTGGTTCCACAAACCGGCCGTA	TCGAACCCGAAAGGCCAAGCGGTGC
31	At1g35910	CAATGGGGGATAATGTACCG	CCCGTCAGCTTTAACCATGT
32	At4g10120	TGAGATCCGCCATTCTCTTT	GCCTCCACAAAACCATCATT
33	At1g03170	CCACTCTCGCTTCCTGTTTC	AGCTTATGTTCACCCGATGG
34	At2g42690	AAGATCCTGGCACCAAACAC	TAGCTGGATGGAATGGGAAG
35	At4g15210	CATAGCAACCCCGTACGTTT	GGCATTTGCAACTTTAAGAGG
36	At1g68740	GCCACCACAAGACAGAGCCAACCAAGGC	AGCAATGCAGTGTGCAAGGAGGTGGT
37	At1g20860	AAACGCCACCAAGAATCAAG	TCCCGGCTAGGTTTAGGTCT
38	At5g17340	CGGGTGGAAGCCGGCTGGGATTGG	TGGCCATTCCCCACCACCGGAAAGG
39	At4g30320	GCTCCTTGCTTCAGACAACC	CCAATGGACCCTATGGTGAG
40	At3g09922	CGAAGCTTGCCAAAGGATAG	TGAAGACTGCAGAAGGCTGA

Supplemental Figure Legends

Figure 1. Pi content, qPCR, ^{33}P uptake and partitioning in triple mutant lines. A. Pi dosage content in leaves (grey bars) and roots (white bars) of 11 dpg plantlets grown in +P. Standard deviations are shown. At least two independent experiments gave similar results. **B**. Relative gene expression level (qRT-PCR) in roots of single *pht1;1* and triple mutant lines grown in -P, including a WT control grown in +P. **C**. ^{33}P uptake capacity for single and triple mutant lines, grown in -P. **D**. ^{33}Pi partitioning for single and triple mutant lines, grown in -P. **E**. Relative gene expression (qRT-PCR) in *mut3* lines in +P conditions, including a WT control in -P.

Figure 2. Pi content, ^{31}P-NMR spectra and mineral content in in *mut4* and *mut5* lines. A. Pi dosage content in leaves (grey bars) and roots (white bars) with 11 dpg plantlets grown in +P. Standard deviations are shown. **B**. *In vivo* proton-decoupled ^{31}P-NMR spectra reveal a dramatic reduction in cytoplasmic and vacuolar P in roots of *mut5* lines. Acquisition time was 1 h (6,000 scans). Peak assignments are as follows: ref, methylenediphosphonate reference used to measure chemical shifts and for quantifications; glc-6-P, Glucose-6-phosphate; cyt-Pi, cytoplasmic Pi; vac-Pi, vacuolar Pi; NTP, nucleoside triphosphate; UDP-glc, uridine-5'-diphosphate-α-D-glucose. **C**. ICP analysis of *mut5* lines reveals a selective drop in total P in leaves (L) and roots (R) of 2-week-old plantlets. For each panel, data shown are from a representative experiment; experiments were performed in triplicate.

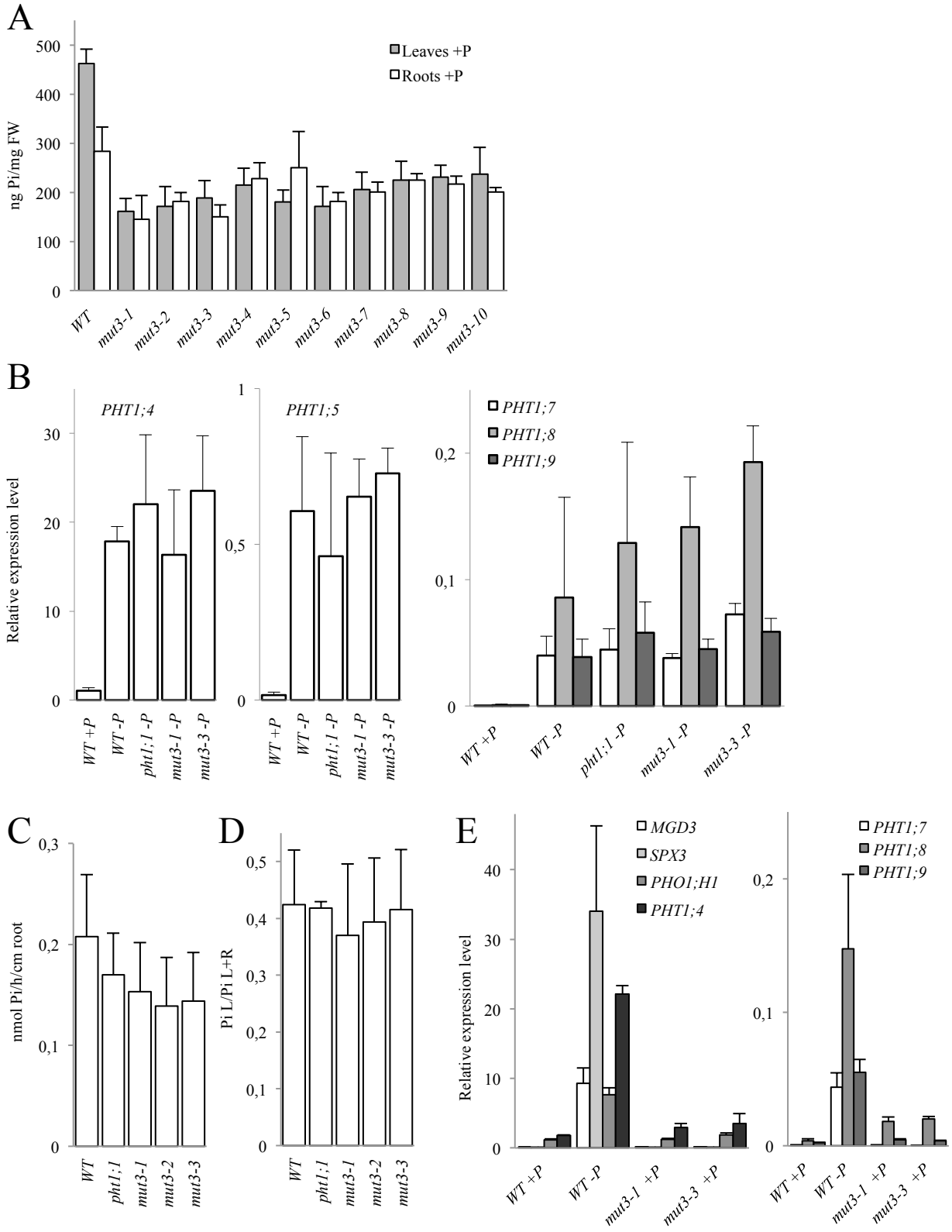
Figure 3. qRT-PCR of *mut5* lines reveals a general induction of PSI genes. qRT-PCR of 40 Pi starvation-induced genes in two independent *mut5* lines and in WT grown in +P and -P conditions. Corresponding ATG numbers and primer sequences are provided in Supplemental Table II. Biological triplicates were performed and all samples were analyzed with technical triplicates. Standard deviations are shown.

Figure 4. *In vitro* phenotype of *mut4* and *mut5* lines on -P medium containing 10 µM Fe reveals full perception of P deficiency. A. Growth arrest of the primary root is observed in all lines when grown in -P medium with 10 µM Fe. Photographs were taken at 7 dpg. Scale bar is 1 cm. **B**. Detail of the root tips of plantlets photographed in A. Scale bar is 1 mm. **C**. Primary root length in mm. **D**. Number of root hair in 5 mm region from primary root tip. **E**. Length from primary root tip to the youngest root air, in mm. For C to E panels, measurements were done at 7 dpg in +P (white bars) and –P (grey bars) media, using 20 plants per genotype and condition. Bilateral T-tests were conducted to assess statistical differences between WT and the other genotypes, comparing the +P growing plants with +P growing WT, and –P growing plants to –P growing WT. * stands for p value < 0.01. **F**.

Anthocyanin content (arbitrary units) in leaves of *mut4* and *mut5* lines show Pi deficiency perception in standard +P conditions and reestablishment of WT anthocyanin levels in VHPi conditions.

Figure 5. Flowering time, grain yield and seed size are affected in quintuple mutant lines. A. Flowering time delay in *mut5* lines. Two independent *mut5* lines are shown (in the right) in comparison with 8 WT plants (on the left) grown on the same support and conditions. **B**. Seed yield is reduced in *mut5* lines. **C**. Seed size and weight are reduced in *mut5* lines. Average seed weight was calculated as an average, using 200 seeds per genotype, whereas seed size was measured by light microscopy (n = 30 to 45 seeds per genotype). White triangle: WT. Pale grey square: *mut5-1*. Dark grey lozenge: *mut5-2*.

Figure 6. CCCP addition inhibits both ^{33}Pi uptake and roots-to-leaves translocation. Plants were grown for 10 days (WT) or 14 days (*mut5*) on -P before ^{33}P uptake in control (white bars), +CCCP (grey bars) and 1 µL/mL DMSO (hatched bars) conditions. Pi uptake capacity was calculated as nmol Pi/h/cm of root. Data shown are from a representative experiment; experiments were performed in triplicate. Each bar corresponds to 20 to 24 plants. Error bars represent standard error.



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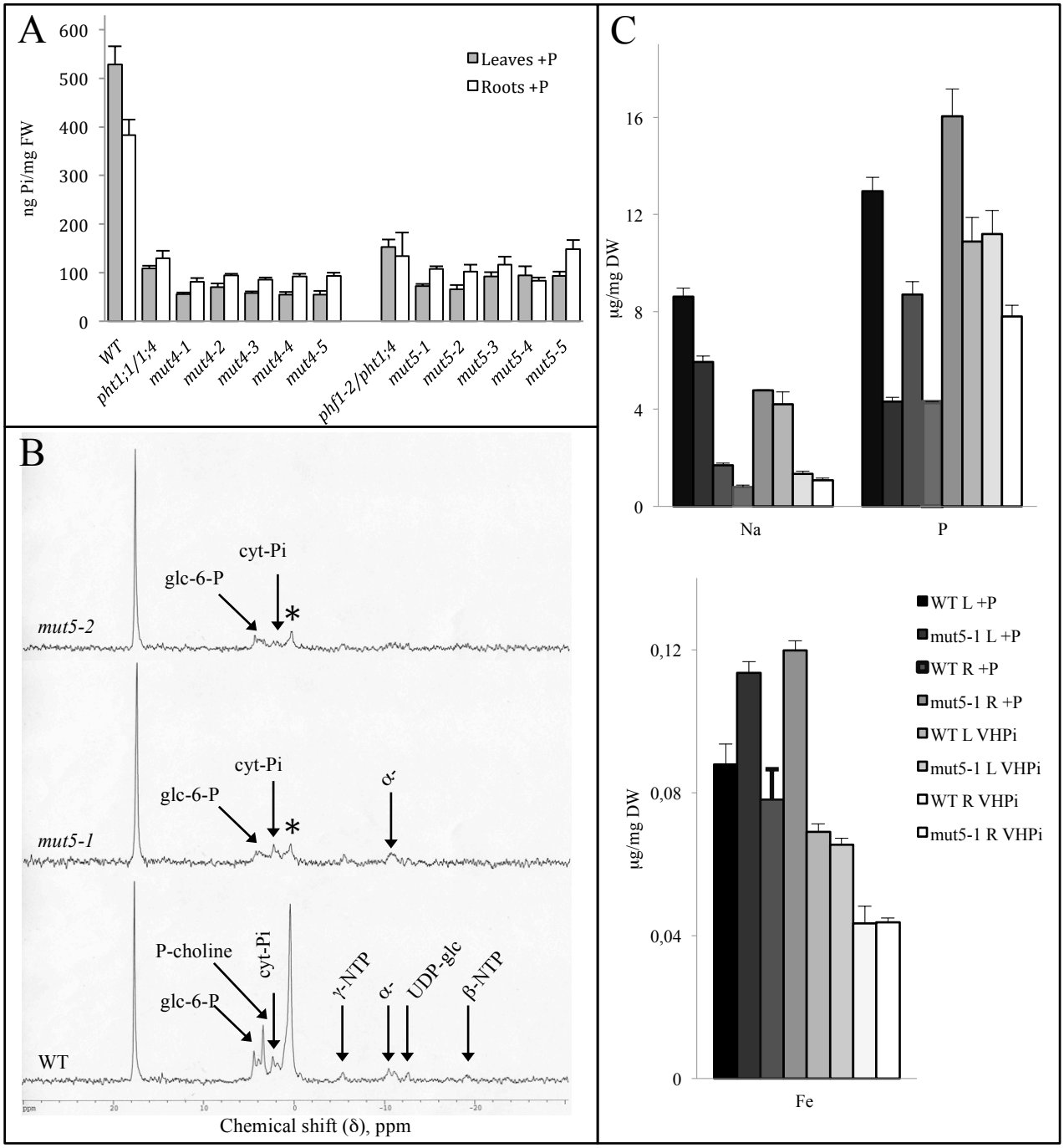

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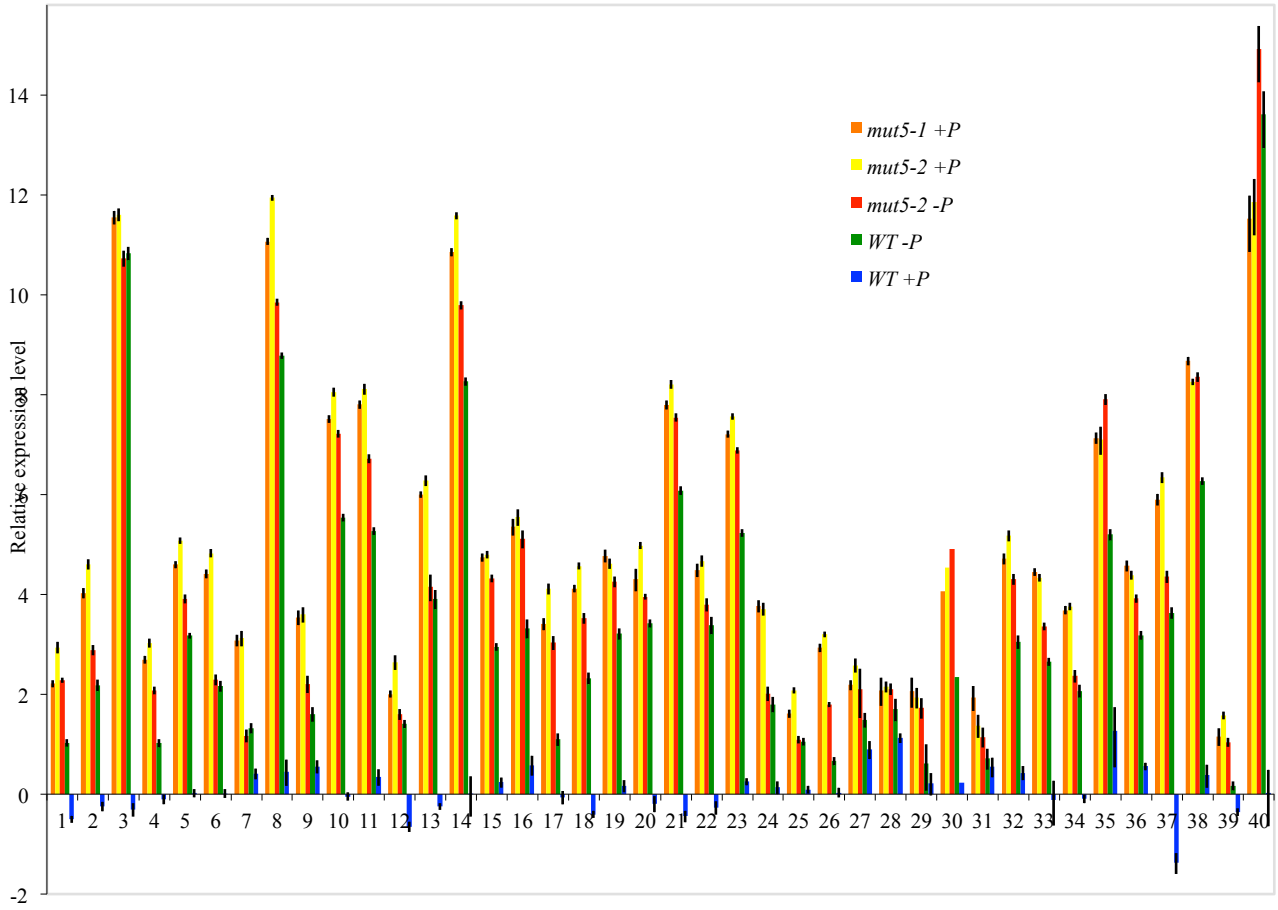

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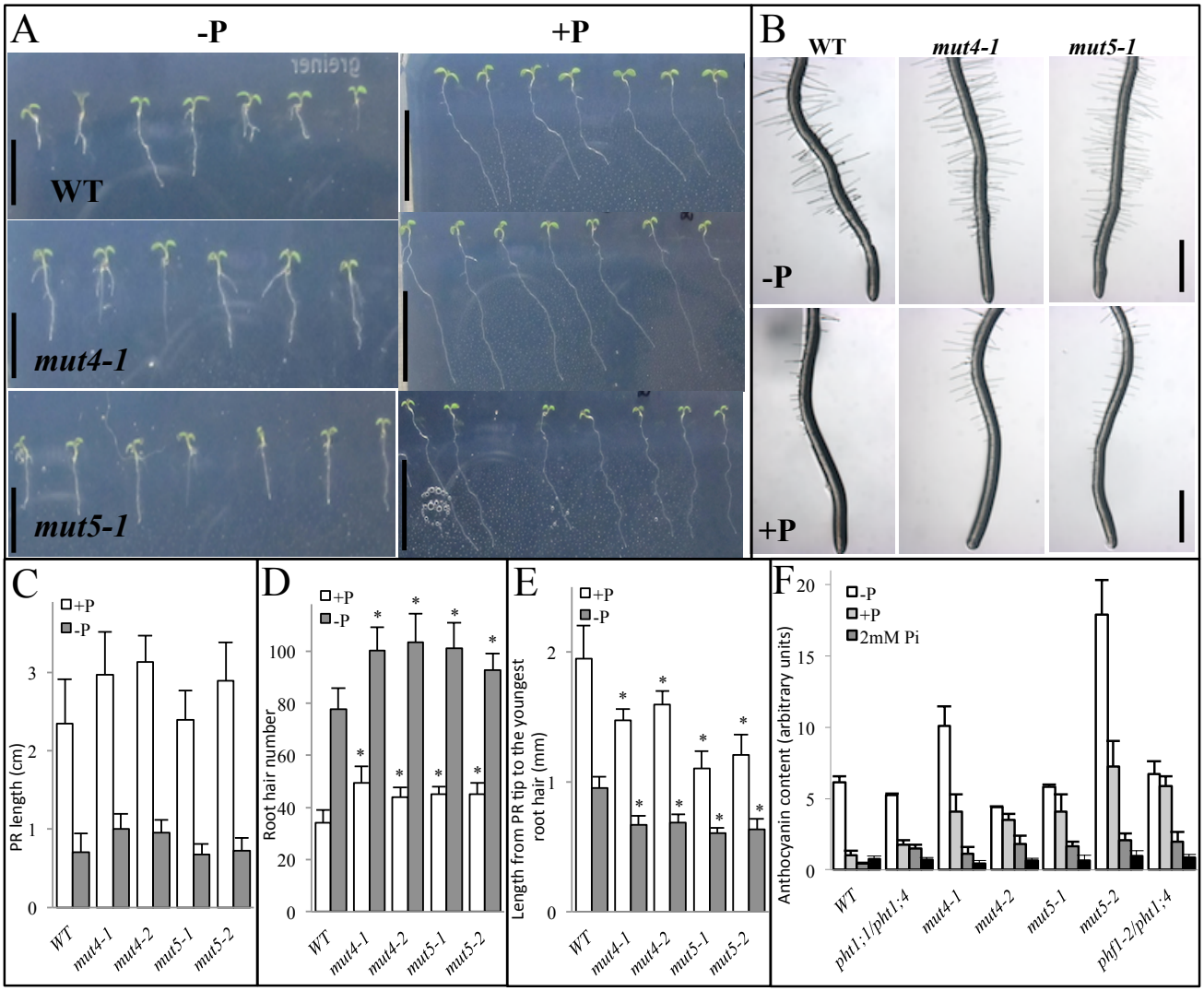

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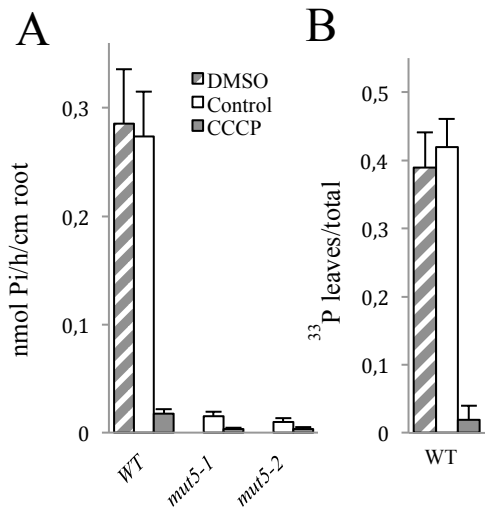

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