SUPPLEMENTAL MATERIAL

Table S1. ATH1-Genechip® Data. See separate MS-EXCEL file.

Table S2. Specifications and primer sequences of mapping markers and the *pho2*-1 dCAPS marker.

Marker Name	Chr. 2 Position (Mb)	BAC Clone	Polymorphism (Name & Type)	Primer Sequences (5' → 3')	Product Length (bp) Col / Ler / *pho2-1*	[Mg^{2+}] (mM) T_a (°C)
nga1126	11.703	F12K2	CER448824 INDEL3	CGCTACGCTTTTCGGTAAAG GCACAGTCAAAGTCACAACC	191 / 199	2.0 / 55
nga361	13.229	T16B12	not found	ACATATCAATATATTAAAGTAGC AAAGAGATGAGAATTTGGAC	114 / 120	2.0 / 55
mpi11	13.733	T32F6	CER460447 INDEL9	CTATCCACATTCGGTAAATCC AAACACTTACTTGCGACTCCT	248 / 239	2.0 / 55
mpi13	14.014	T21L14	CER459346 INDEL17	TTTTAACTCGGTCGGATCAA AATTACCTTCTCAATCGCAGTTT	211 / 194	2.0 / 55
mpi15	14.276	T1B8	CER458994 INDEL10	CCTTTCCCCTAAACTAGTAAACTTC GAAATCAACGAGGCTTATCAAG	188 / 178	2.0 / 55
pho2-1 dCAPS	14.286	T1B8	*pho2*-1 SNP	TGAAGAAAGTCC<u>CA</u>CAAGAA TCGTGTCTGTTCATCACAA	197 / 214	2.0 / 50
mpi19	14.305	T1B8	CER459006 INDEL45	TCGCAAACCAAATATCAACT AGCTGATGAACAAAAGACTGA	203 / 158	2.0 / 50
mpi20	14.327	T1B8	CER459010 INDEL18	ACATTGAAAGTTCCCGATTCT CAACAGATTTTCTTTGACCCA	90 / 72	2.0 / 50
mpi18	14.388	T14G11	CER458319 INDEL26	AGGGAATCGCAGAGTCAATAG GGCTCTGATGTCATTGTTACG	96 / 70	2.0 / 50
mpi16	14.484	F13P17	CER449098 INDEL9	CGGATCTTACTACTCGAAGAA GGGTATTGCTTTTGATGTATT	120 / 111	1.5 / 55
mpi14	14.800	T4C15	CER460568 INDEL8	TTGAGGTTACTCCTTAAATCGG AGCGACGAGTGTATATGCAG	172 / 164	2.0 / 50
mpi12	15.152	F11F19	CER448624 INDEL12	TTCCAGAGAGAAGAAAACGAAA AGATGTTGAGAGCGGGTGT	165 / 153	2.0 / 55
nga168	16.299	T7F6	CER461057 INDEL16	GAGGACATGTATAGGAGCCTCG TCGTCTACTGCACTGCCG	151 / 135	1.5 / 55
mpi17	17.622	T24P15	CER459672 INDEL12	CATTGCTGAAGTTCTTTTTC TTTTCCACATTTCTTTAGGC	175 / 163	2.5 / 53

Mb: megabase; bp: base pair; Col: WT Columbia-0; L*er*: WT Landsberg *erecta*; Ta: annealing temperature used for PCR amplification. Mismatched nucleotides in the dCAPS marker forward primer are underlined.

Table S3. Primers used for qRT-PCR analysis.

AGI	Forward Primer (5'→3')	Reverse Primer (5'→3')
At1g73220	CGGATCTCTTTTCGGTTCTGGTGT	GCAAAGGCCGTGACGAAAGTTAA
At3g02040	CGATGTTCAGGTCACCAGAGATGG	TGACCCTCTTCTCGTACACCACTC
At3g17790	AGTGGTACAGTGTTTTGGGAAACC	TCTCTACCAACTCTGCATCAACGA
At3g47420	GCCATACAGCGATTGAAGGCGAA	CCAGCCATGATTCCACCAACCA
At5g20150	CCAATTGTTAGAAGACGAGTTGGA	CCATTGAATCCTTAGCTTTCGCAA
At5g20790	AGCTACGGAGGAGGTTTGGGTAAT	CTCATCCTCACCACTCCATCCCAT
At3g09922 AtIPS1	AGACTGCAGAAGGCTGATTCAGA	TTGCCCAATTTCTAGAGGGAGA
At5g03545 AT4	CTGAAGCTCAAGAACCCTCTGAA	CCTCTCAAAACCCTTTATTGGTGA
At2g33770_1	AATCTGGTTTACTTCGCATGAGCT	CATTCACGCATACTCCCACAAG
At2g33770_2	AGGTTTGAAGCTCCACCCTCA	CCCAAGATGTGATTGGAGTTCC
At2g33770_3	CCCCTTTGAAGTTTATCCAACTGG	AGGTGAGCCAACTGAGGACTCC
At2g33770_4	AGCTGACCGTTGCTGTCAGTC	CGGTGTAGCTTCCCTGTTTGTT
At2g33770_5	GTGAAGGACCATTTTACGCACC	CCATATAAGCCTTGCACGCAG
At4g05320 UBQ10	GGCCTTGTATAATCCCTGATGAATAAG	AAAGAGATAACAGGAACGGAAACATAGT
At5g43350 Pht1;1	GAGCTCTAGGAAATGGCCGAAC	TGACAATCGCCGTGAAATGA
At5g43370 Pht1;2	AAGGTGGATGCAGGATACCCA	GAACACCAAGCACGATCAATGA
At5g43360 Pht1;3	GCTCAGTTGCTTCCGGTCTTT	ACCCGAGCCAAAACCTGAA
At2g38940 Pht1;4	TCAATGGCGTTGCCTTCTGT	ATCACCAAGCCACCCGAAA
At2g32830 Pht1;5	GCCGCAAGAAAGTTTACGGTAT	GATAGACCAGACCCGAGAGAACA
At5g43340 Pht1;6	CGGACTCCACTTACTCGGAACA	GCTGTAGAAAGCGATGTCGAGG
At3g54700 Pht1;7	ATGTTTCTTCCGGTTCTGGCTT	CGTGGCGGATAACGGATAATC
At1g20860 Pht1;8	ACTGCAGAAAACGTCTACGACG	CAGCGATGATGGCTCCTAATTC
At1g76430 Pht1;9	CGTCGGTGAAAAGTCCCATTC	CGCAGCGAGGATACAGTGGTA
At3g26570 Pht2;1	GCAGCTGGAACTTGGTTACAGG	CCAACCATTGATCCGACGATAC
At5g14040 Pht3;1	TCGTTTCTCATCCAGCAGACAA	AATCTTCTTCACCGCATCTCCA
At3g48850 Pht3;2	TTTAGCTGGATTGCCAACCACT	TACAGATGGAGCAAGCGCAGT
At2g17270 Pht3;3	ATGTGTTGCAGGCTGTGAGGA	TGATCCGAACAGGAAGGCTTC
Ath-miR399a	AGGGTAAGATCTCTATTGGCAGGAAAC	GCAGAAGAATTACAGGGCAAATCTCC
Ath-miR399b	TCTCCATTGGCAGGTCCTTTACTTCC	TCAGGGCAACTCTCCTTTGGCAG
Ath-miR399c	CATCTTTCTATTGGCAGGCGACTTGG	AAGCAGTGACAGGGCAACTCTCC
Ath-miR399d	AATACTCCTATGGCAGATCGCATTGG	TCCTTTGGCAGAGAAGCATTTTACTTG
Ath-miR399e	CTCTATTGGCAGTGGAAGTTGATGACC	ACGTTAGTGAAGCATTGCGAGGC
Ath-miR399f	GCATTACAGGGCAAGATCACCATTGG	GCGCAAGAGAATTACCGGGCAAATC
Ath-miR393a	GGGATCGCATTGATCCTAATTAAGGTG	TCCAAAGAGATAGCATGATCCAAAACC
Ath-miR395a	GAGTTCCTCTGAGCACTTCATTGGG	CGTTGAATGGGTCCGGGAGTTC
Ath-miR159a	TTCAAACATGAGTTGAGCAGGGTAAAG	AGGGCAAGTTAAAGCTCCTGAGATATG
Ath-miR164a	CGAAATCCGTCTCATTTGCTTATTTGC	AGCTCATGTTGGAGAAGTTAAGTACG
Ath-miR170	TGATATTGGCCTGGTTCACTCAGATTC	AATACGAGAGAAGCGACGAGAGAGG
Ath-miR171a	TTCTCACTTCTCCTCCTCACACTTCAC	GCCAATATCAAAGGGACTCTCTCATGC
Os1g52230	TCTTCATCGACACCATCCTCG	GGTTGGTGTTGATCTCGGAGAA
Os1g59150	GGAGTCACATGCTGCCTAAGGTT	TCACTGCCAGCTTACGGAGG
Os-miR399a	GCTGGAAATGATGCTGGTAGC	CTCCTTTGGCACGAGATCTGT
Os-miR399d	GGTGGCCTTTGATAGACCATCA	GCAGGCCGTTTTGGTGAAT
Os-miR399f	GGCAGAGGTGATCAGATTGCA	GGCAAATCTCCTTTGGCAGAG
Os-miR399j	GGAGCATGTGAAGTCTTTTGTAGC	GGCAACTCTCCTTTGGCAGA
GUS	GCCGTTTTCGTCGGTAATCAC	TGAACAACGAACTGAACTGGCA

Fig. S1. Fine mapping of the *PHO2* locus.

(A) Representation of Arabidopsis chromosome 2 in the region between 12.5 and 16.5 megabases, showing the positions of markers m429 and as1, that were found previously to be linked to the *pho2* mutation (Delhaize and Randall, 1995), and those of SSLP markers nga361, mpi11-mpi16 and nga168. AGI-BAC clones containing the markers are represented by white boxes. The number of recombination events (meiotic breakpoints) found for each marker in a total of 7872 examined chromosomes is given. Recombination events upstream or downstream of *PHO2* are shown in the upper or lower row, respectively. **(B)** Close-up of the region between flanking SSLP markers mpi15 (CER458994) and mpi16, showing the names, the non-overlapping (open boxes) and overlapping parts (hatched boxes) of BAC clones, as well as the position and the number of recombinants found for three additional SSLPs (Table S2). Using these markers the *PHO2* gene was mapped between polymorphisms CER458994 and CER459010. **(C)** The *pho2*-1 point mutation are detectable by PCR using a co-dominant dCAPS marker that exploits a *Msl*I restriction site ($CAPyN_4PuTG$) which is created with a mismatched primer (Table S3). Primer sequences are underlined and exon sequence is shown in capital letters. While the WT PCR product can be digested by *Msl*I resulting in a 197 bp fragment, the *pho*2 mutant product remains uncleaved. *pho*2 mutants complemented with genomic WT cosmids C23 and C3 display a heterozygous PCR genotype.

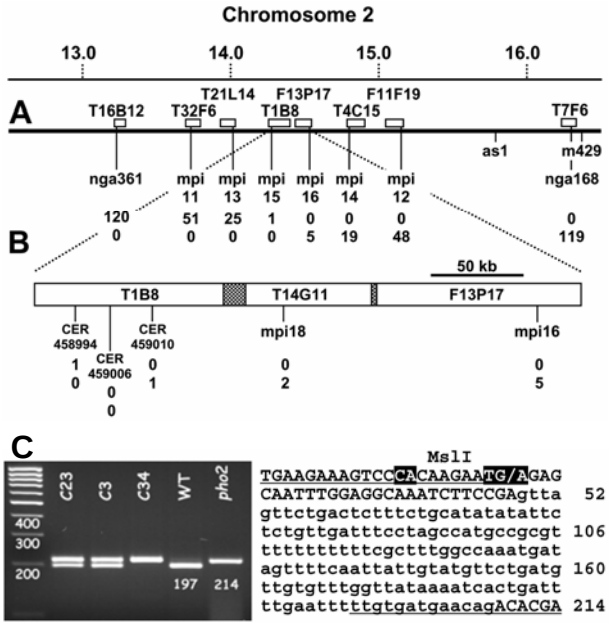


Fig. S2. ATH1 genechip® expression profile of the *PHO2* gene.

(A) Developmental series. A full description of samples #1-#101 is available at http://www.weigelworld.org/resources/microarray/AtGenExpress/. **(B, C)** Abiotic stress series for (B) shoots and (C) roots. A detailed description of the samples and plant growth conditions is available at http://web.uni-frankfurt.de/fb15/botanik/mcb/AFGN/ atgenextable2.htm. **(D)** Hormone series. WT Col-0 seedlings were grown in liquid MS medium for seven days at 23°C treated with IAA, zeatin, GA3, ABA, MJ, ACC or BL for 30 minutes, 1 or 3 hours. **(E)** Nutrient starvation and re-addition series. WT Col-0 seedlings were grown as described (*S1*). Full nutrition (FN); sulfur, nitrogen, phosphorus or carbon starvation (-S, -N, -P, -C, respectively); 30 min or 3 hrs sulfate (SO4), nitrate (NO3), phosphate (PO4) or sucrose (suc) re-addition. **(F)** Diurnal series / extended night. WT Col-0 plants were grown and harvested as described (*48*). **(G)** Light series. A detailed description of the samples is available at http://web.uni-frankfurt.de/fb15/botanik/mcb/AFGN/atgenextable3.htm. **(H)** Biotic stress series. A detailed description of the samples is available at http://web.uni-frankfurt.de/fb15/botanik/mcb/AFGN/atgenextable3.htm.

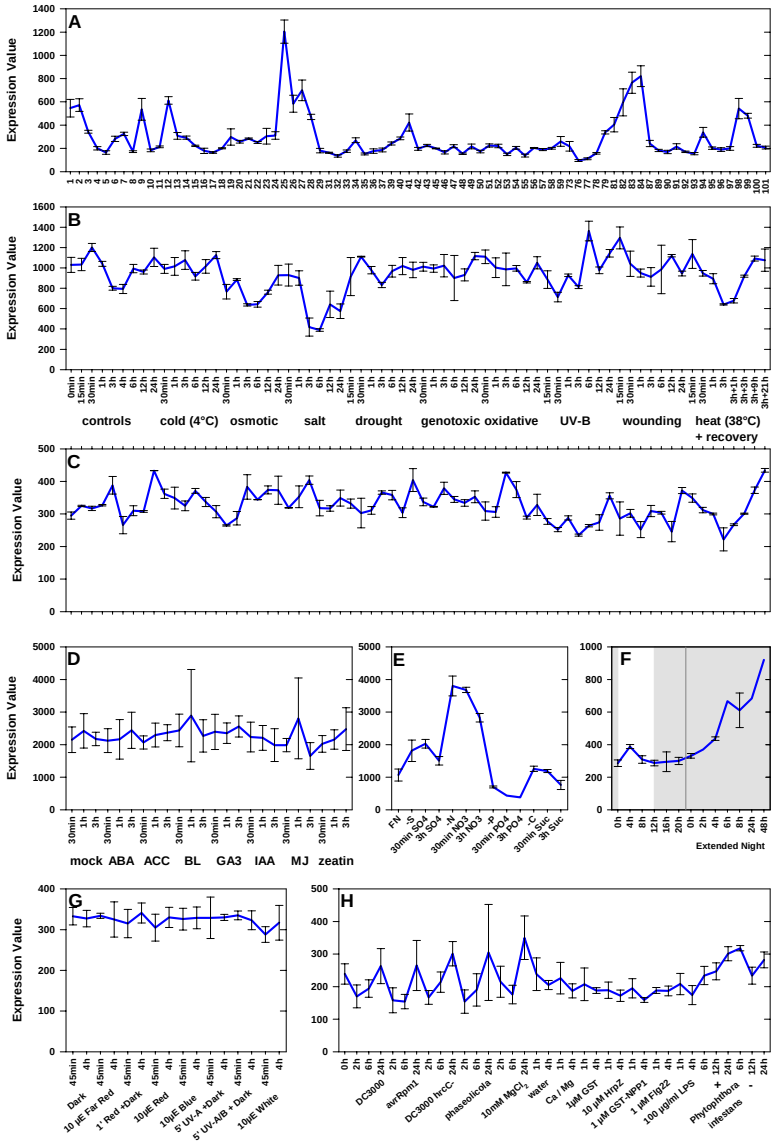


Fig. S3. Expression of *GUS* under control of ~1.8kb *PHO*2 upstream sequence.

GUS driven by ~1.8kb of *PHO*2 upstream sequence is expressed throughout development and in all organs of Pi-replete grown Arabidopsis plants. **(A)** Five day old seedling, **(B)** Magnification of the primary root of a five day old seedling, **(C)** 20 days-old plantlet grown on an agar plate, **(D)** 30 days-old plant grown in soil, **(E)** Magnified view of a flower from a 30 days-old soil-grown plant.

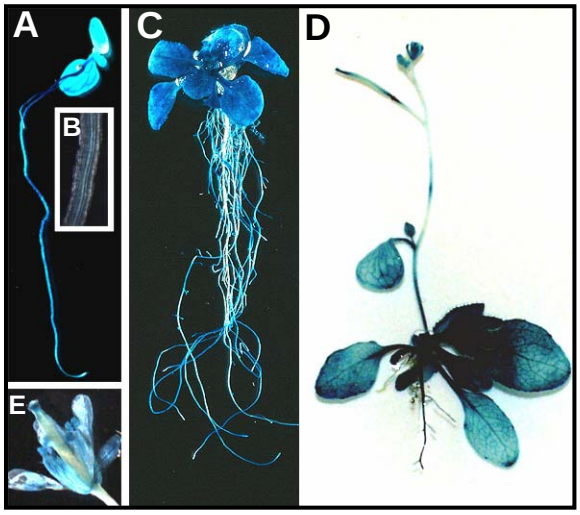


Fig. S4. Depiction of *PHO2* gene structure and positions of qRT-PCR primers.

(A) *PHO2* gene structure. Exons are shown as boxes. UTRs are shaded yellow, and coding regions are shown in shades of blue and red (UBC domain). The ticks in the second exon of the 5'-UTR depict the position of the five miR399 binding sites (miR BS). The sequence of the region around the five miR399 binding sites and a miR399 consensus sequence are shown below. **(B)** Depiction of the *PHO2* cDNA sequence. Binding sites for miR399 are shaded yellow, and qRT-PCR primer pairs (cf. Table S3), used for *PHO2* transcript/cDNA quantification, are shown as colored arrows.

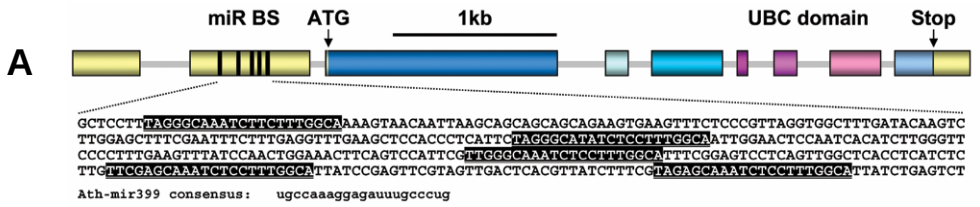


Fig. S5. qRT-PCR expression levels of *PHO2* and miR399 PTs.

Results are shown for shoots and roots of WT seedlings grown in Pi-replete and Pi-deprived conditions (see the legend in the graph). Expression of the miR399e/f PT was investigated with two qRT-PCR primer pairs (indicated by the bracket) designed on the stem-loop structures of the miR399e and miR399f precursors. Expression levels are given on a log scale as described in the legend to Fig. 3.

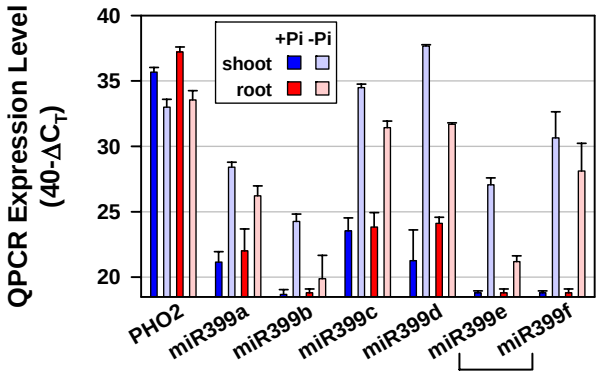


Fig. S6. qRT-PCR expression levels of 64 Pi-responsive transcripts in Pi-deprived and -replete WT, Pi-deprived *phr1* mutant and Pi-replete *pho2* seedlings.

The expression levels (40-ΔC_T; mean ± SD, n=4) are given on a log scale as described in the legend to Fig. 3. Transcript level was considered altered if $\Delta\Delta C_T$>1.

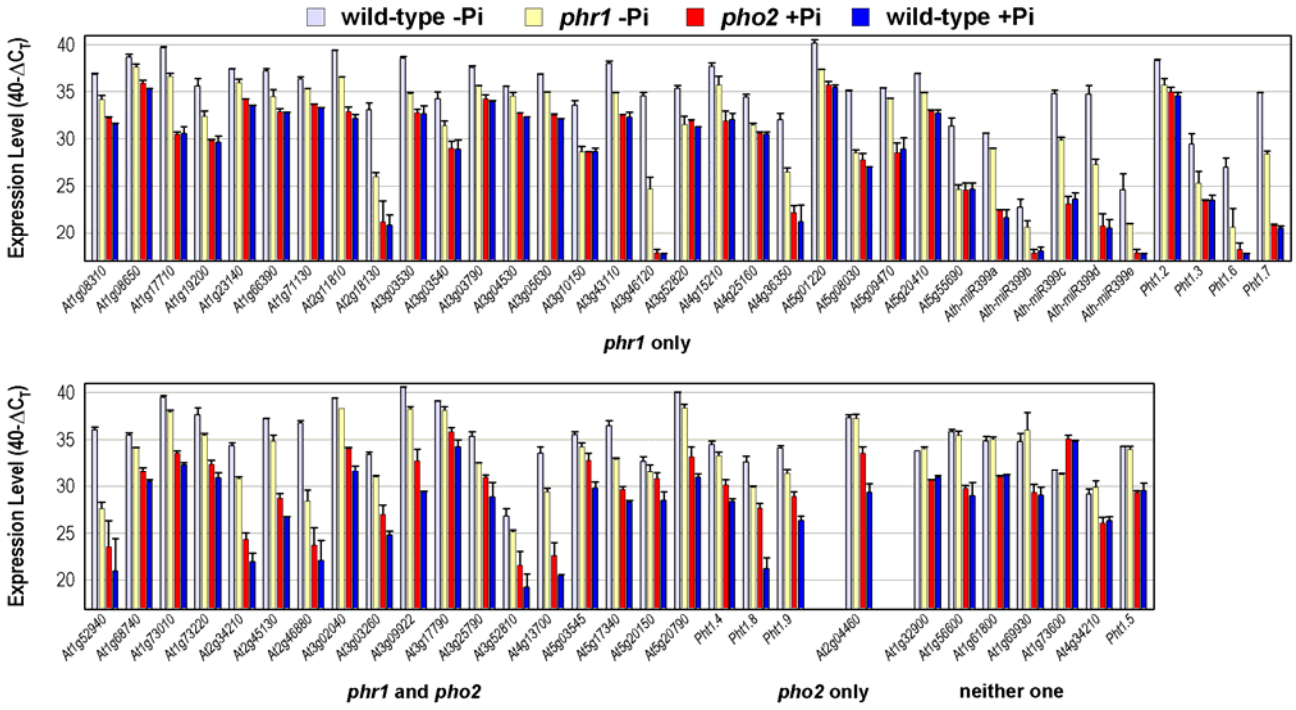
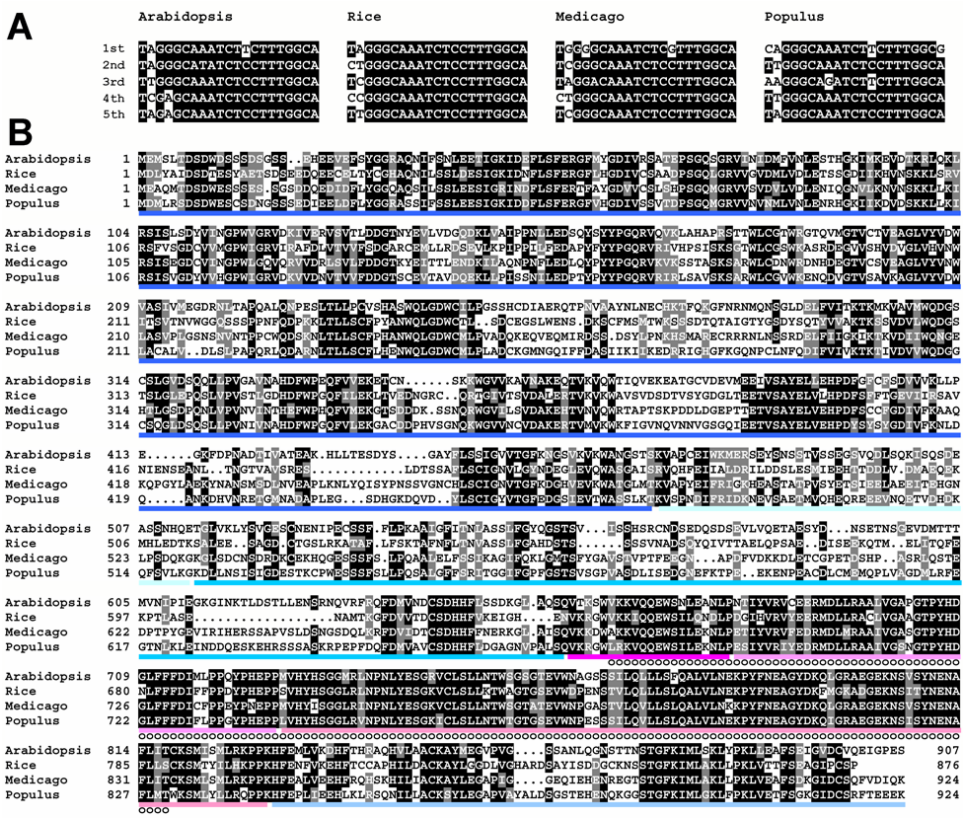


Fig. S7. Protein alignment of *AtPHO2* and three potential plant orthologs.

(A) Sequences of the five miR399 binding sites in the 5'-UTRs of the four potential orthologs. **(B)** Protein alignment. Colored lines depict the regions encoded by the various exons as shown in Fig. 6D. The UBC domain is underlined with circles. **(C)** Protein identity and similarity of PHO2 and its potential rice, *Medicago* and poplar orthologs. Similarity and identity values (%) for pair-wise comparisons are shown with blue and yellow background, respectively. Values for the entire protein, the UBC domain, the N-terminal region (amino acids 1-250) and the C-terminal region are given in black, red, blue and green numbers, respectively.



C

	Arabidopsis		Rice		*Medicago*		Poplar	
Arabidopsis			56.8 83.1	57.7 65.9	60.9 88.9	65.1 70.7	59.9 87.7	68.8 65.4
Rice	47.7 76.0	47.8 59.8			58.6 85.6	63.1 63.9	58.9 85.7	62.2 59.3
Medicago	53.3 83.7	55.6 62.2	49.0 75.8	52.8 60.2			64.3 89.5	70.1 74.4
Poplar	52.0 81.2	60.4 55.6	51.7 77.3	56.6 54.7	56.0 83.0	61.0 68.3		