Table 1 Suppl. Primers used in this study.

Gene	Acc. No.	Primer sequence (5' to 3')	Amplicon size [bp]	Application	Species
AtPHT1;4	AB016166	F: GTACGTCGACGAATAAACGTAGGTAGTGGC R: CGTCCCGGGCACGTCAAGTGCATTCAACAC	3060	promoter cloning	*A. thaliana*
AtPHT1;4	pCN2	F: TCATAGGCAGCTTCAACG R: CTTAATTGCGGACACACC	530	PCR	*T. aestivum*
GUS	pCN2	F: GGTGGGAAAGCGCGTTACAAG R: TTTACGCGTTGCTTCCGCCA	1200	PCR	*T. aestivum*
Bar	pAL51	F: GTCTGCACCATCGTCAACC R: GAAGTCCAGCTGCCAGAAAC	440	PCR	*T. aestivum*
GUS	pCN2	F: GGAGGCAAACAATGAATCAACAAC R: CCCATCTCATAAATAACGTCATGC	227	RT-PCR	*T. aestivum*
18S rRNA	AY049040	F: GAGGGACTATGGCCGTTTAGG R: CACTTCACCGGACCATTCAATCG	160	RT-PCR	*T. aestivum*
GUS	pCN2	F: CAGTGAAGGGCCAACAGTTC R: GGCACAGCACATCAAAGAGA	670	Southern blot	*T. aestivum*
GUS	pCN2	F: GTTGGCGGTAACAAGAAAGG R: TAGCAATTCCCGAGGCTGTAG	178	RT-qPCR	*T. aestivum*
TaPHT1;2	AJ344240	F: CCGAGACCGGCTACTCAC R: CGATTGTGCAAGTACACCTGC	206	RT-qPCR	*T. aestivum*
Ta26S	M37274	F: TGCCGTCCGATGGGTACACG R: ACGGCTTTGATGACTCGACG	154	RT-qPCR	*T. aestivum*
TaACT	AB18199	F: TTGGCACAATTGCATGGCCT R: ACCACACAATGTCGCTTAGGCA	92	RT-qPCR	*T. aestivum*
TaGPDH	EF592180	F: CTCAAGGGCATTTTGGGTTA R: GCTGTATCCCCACTCGTTGT	156	RT-qPCR	*A. thaliana*
TaTUB	DQ435673	F: TGGCCACTACACTGTTGGAA R: CACCAACAGCATTGAACACC	116	RT-qPCR	*A. thaliana*
AtPHT1;4	AT2G38940	F: TCGTTGTGCCGGCCGAAATCTT R: ATCCTGCGTCGGTCTTGTCCTTGT	150	RT-qPCR	*A. thaliana*
AtERF1	AT4G17500	F: CACCCTTAATCTTCGCTGCTC R: TTGCATGATCCATGTTTGG	171	RT-qPCR	*A. thaliana*
AtEF1α	ATIGO7940	F: GTTGCTTTCACCCTTGGTGT R: TCCCTCGAATCCAGAGATTG	144	RT-qPCR	*A. thaliana*

Table 2 Suppl. Estimation of the transgene copy number in transgenic wheat lines IIA5 and IIA292 based on 1C value = 16.4 pg DNA for *Triticum aestivum* [1]. The Ct value varied from 16.6 to 29.1 for 5×10^5 to 5×10^1 plasmid copy number mm^{-3}, respectively. PCR efficiency = 109.4 %, $r^2 = 0.99$. [2]Calculated from the standard curve of plasmid pCN2 using the cycle threshold (Ct) value after real-time PCR. [3]Calculated by dividing the real-time PCR copy number by the initial number of haploid genome (Ahmad *et al.* 2005). Values are the means ± SD ($n = 3$ biological replicates).

Transgenic lines	DNA [ng]	Initial haploid genome [number of molecules mm^{-3}][1]	Ct value	Real time PCR copy number [number of molecules mm^{-3}][2]	Estimated copy number per haploid genome [number of molecules mm^{-3}][3]
IIA5	50	152.4	22.42 ± 0.37	6274 ± 1701	41.2 ± 11.2
IIA292	50	152.4	22.06 ± 0.40	8205 ± 2361	53.8 ± 15.5

Table 3 Suppl. The 40-ΔCt values of transcript abundance from Fig. 5. The 40-ΔCt value represents the expression level of a gene or gene construct, where ΔCt is the difference in RT-qPCR threshold cycle number between the respective gene and the reference gene *Actin*, and 40 corresponds to the cycle number on which the PCR run stopped. Values are given on a log2 scale, where each unit represents a two-fold change in transcript abundance (*e.g.*, a value of 34 represents 16-fold lower expression than a value of 38) (Bari *et al*. 2006). Values are averaged over Pi treatments (mean ± SD, *n* = 6).

Gene	Gene construct	Hydroponics		Potted plants	
		14 DAT	21 DAT	14 DAT	21 DAT
TaPHT1;2	Endogenous gene	39.3 ± 3.1	38.5 ± 4.3	40.0 ± 0.7	41.0 ± 1.4
IIIA35	*TaPHT1;2-GUS*	36.5 ± 0.8	36.8 ± 0.7	36.5 ± 1.0	35.9 ± 0.7
IIA5	*AtPHT1;4-GUS*	31.2 ± 1.2	31.2 ± 1.1	37.2 ± 0.6	35.2 ± 0.7
IIA292	*AtPHT1;4-GUS*	34.6 ± 0.2	37.1 ± 0.5	37.9 ± 0.8	35.0 ± 3.4

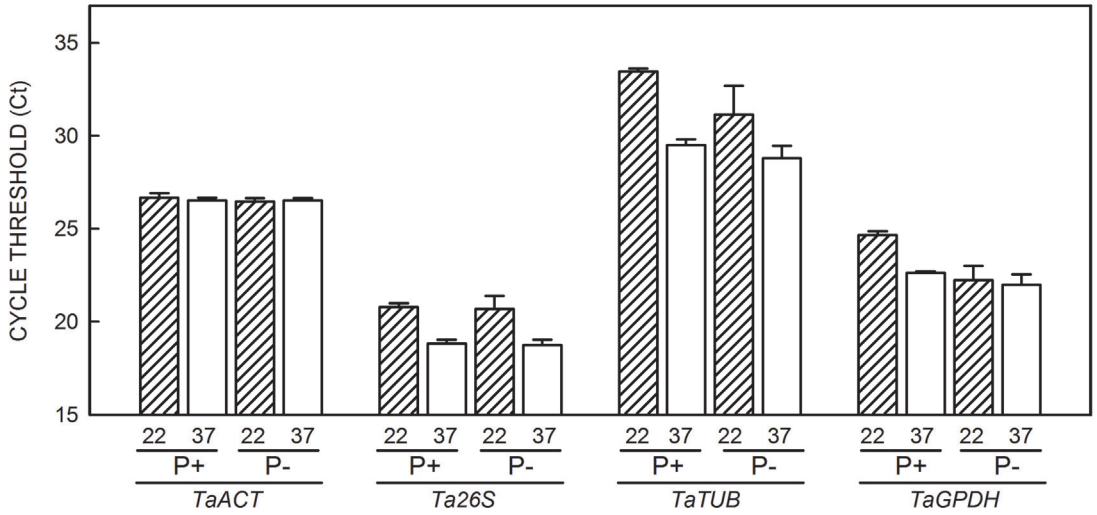

Fig. 1 Suppl. Cycle threshold (Ct) values of four reference genes. Transgenic wheat (line IIA292) was grown in complete nutrient solution with 0.1 mM P for 7 d, followed by a 7-d period without P (P-), or with 0.5 mM P (P+). Half of the plants from each Pi treatment were then kept at 37 °C (37) for 2 h, whereas the other half was maintained at 22 °C (22). Ct values are the means ± SD of three biological replicates for the endogenous *Actin* (*TaACT*), *26S rRNA* (*Ta26S*), *α-Tubulin* (*TaTUB*), and *GPDH* (*TaGPDH*) genes.

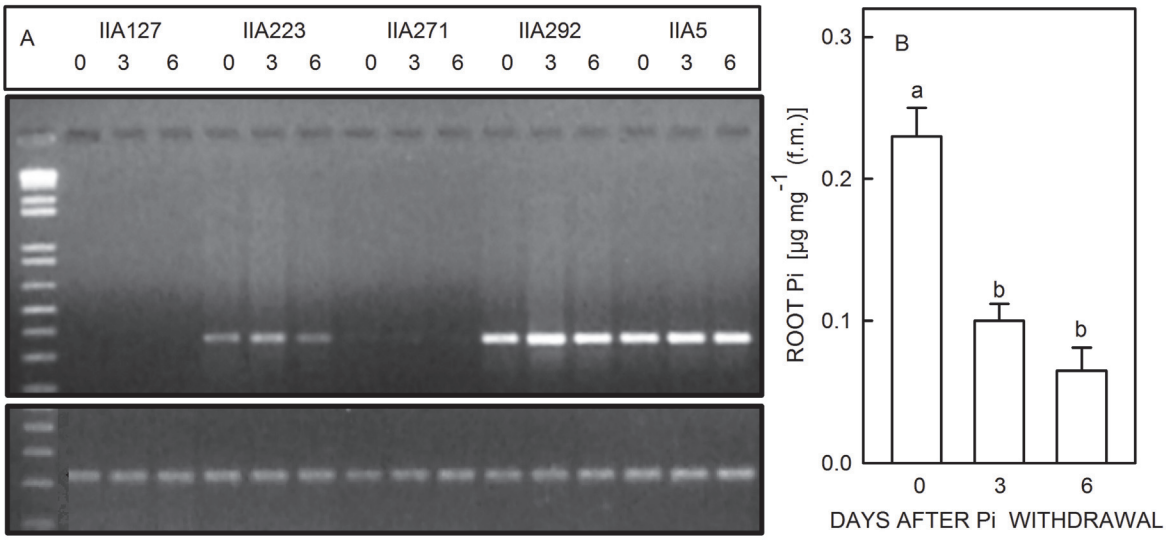

Fig. 2 Suppl. *GUS* expression in roots of transgenic wheat lines harboring the *AtPHT1;4-GUS* construct (*A*). Plants were grown in complete nutrient solution with 0.1 mM P for 7 d, followed by a 6-d period without Pi. RT-PCR was performed in roots sampled at 0, 3, and 6 d after Pi withdrawal. Bottom bands in (*A*) correspond to ethidium bromide-stained 18S rRNA. *B* - The root Pi content (mean ± SE, $n = 5$) averaged over genotypes at each sampling date. Different letters above the bars indicate significant differences, as determined by Tukey's test at $P \leq 0.05$.

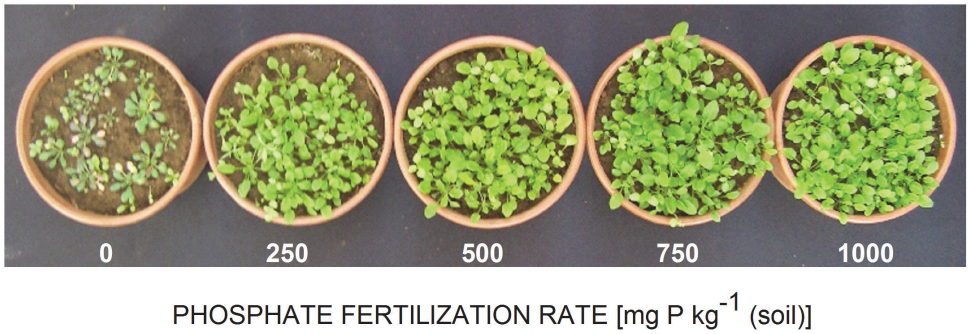

Fig. 3 Suppl. Top view of roots of *Arabidopsis* plants grown for 50 d in an acidic soil under different phosphate fertilization rates. From left to right, the available P at the end of the experiment was 18, 41, 63, 92, and 129 mg(P) kg^{-1}(soil).