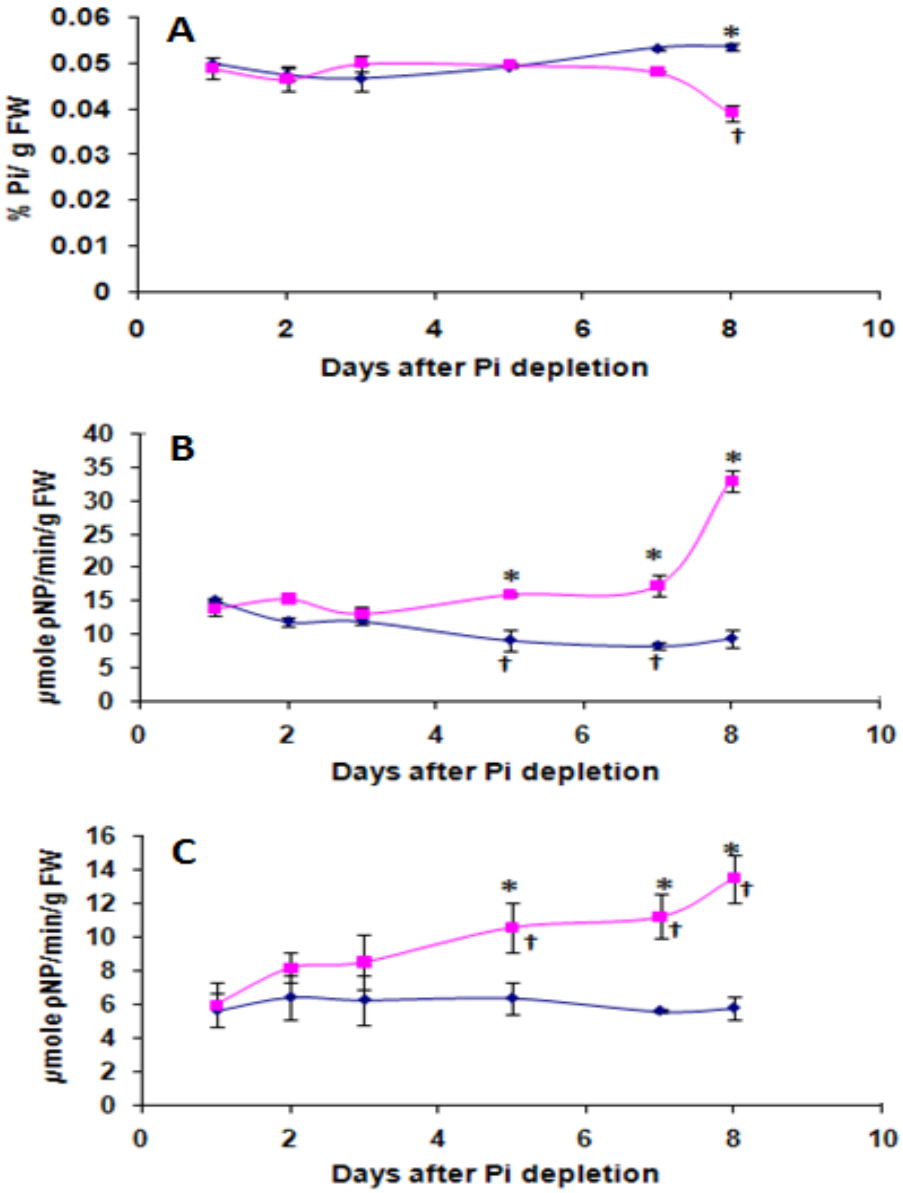

Figure S1 Cellular effects of Pi supply in roots of wild-type white clover

The effects of P treatment [1 mM Pi, P+ (-♦-); 10 µM Pi, P- (-■-)] on total leaf Pi content per gram fresh weight (% Pi/g FW) (A), acid phosphatase activity (µmole ρNP/min/g FW) in the soluble (B) and cell-wall-enriched fractions (C) in rooted stolon cuttings of genotype 10F, cv. Grasslands Challenge. Values are means ± SE, n = 3. * indicates significant differences between treatments ($P \leq 0.05$); † indicates significant differences within a treatment from day 1 ($P \leq 0.05$).

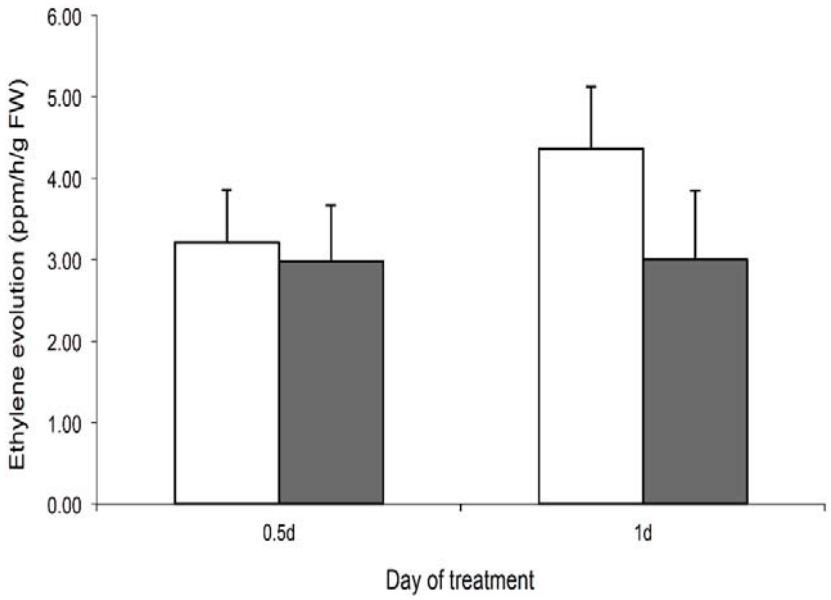

Figure S2 Ethylene evolution from whole roots of white clover in response to Pi supply

Ethylene evolution (expressed as ppm/hr/g FW) from whole roots of white clover nodal explants maintained hydroponically in half-strength Hoagland's media containing either 1 mM Pi (clear bars) or 10 µM Pi (shaded bars) for the time intervals listed. Values are means, ± SE, of three biological replicates where the ethylene production of six pooled nodal roots (primary and lateral roots combined) represents one biological replicate.

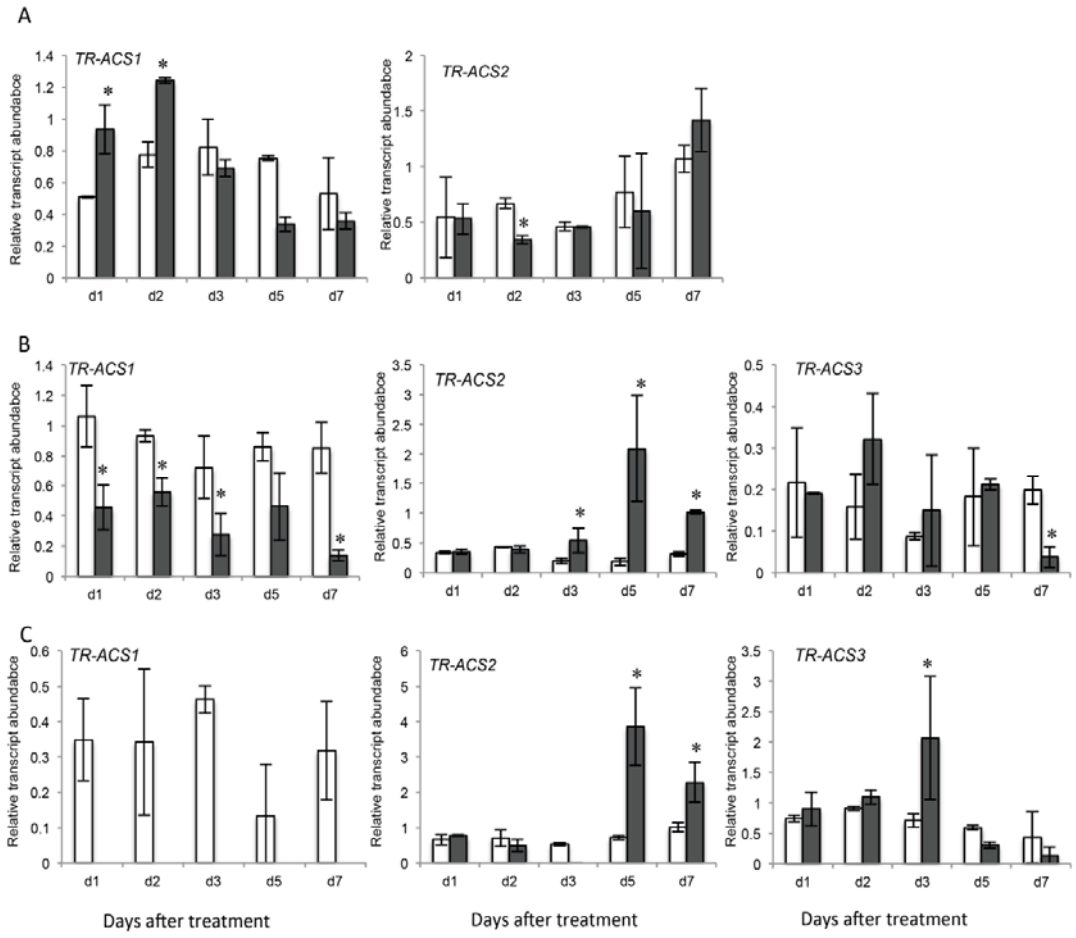

Figure S3 Changes in transcript abundance of the *TR-ACS* gene family in roots in response to Pi supply

Relative transcript abundance of *TR-ACS1, TR-ACS2* and *TR-ACS3,* as indicated in the EZ (A), VL (B) and MR (C) regions of the primary roots of nodal explants maintained hydroponically in half-strength Hoagland's media containing either 1 mM Pi (clear bars) or 10 µM Pi (shaded bars) for the time intervals indicated. Relative transcript abundance was determined by qRT-PCR and transcription was normalized against two internal reference genes, *TR-β-actin* and *TR-GAPDH.* Values are means ± SE, for two biological replicates each of which was derived from means of three technical replicates. ***** indicates significant differences between the 1 mM Pi and the 10 µM Pi treatments ($P \leq 0.05$).

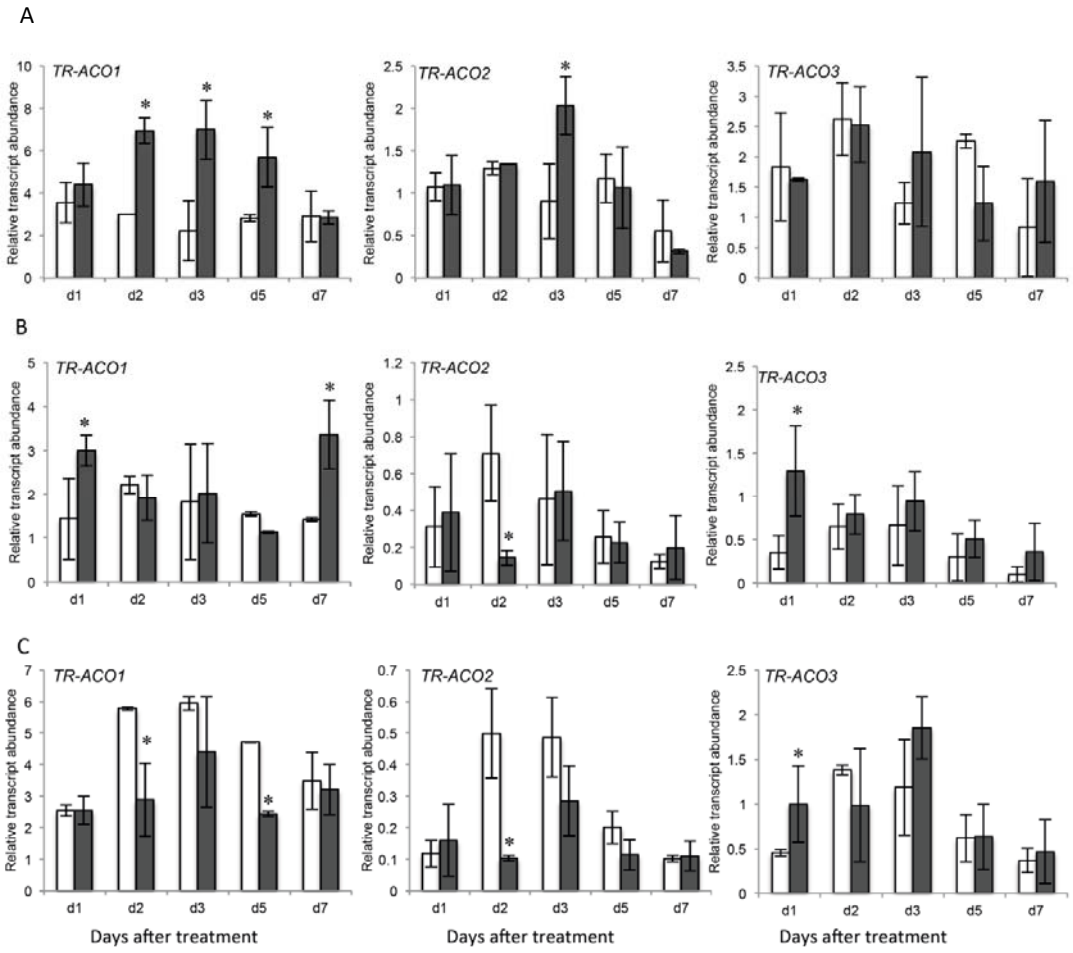

Figure S4 Changes in transcript abundance of the *TR-ACO* gene family in roots in response to Pi supply

Relative transcript abundance of *TR-ACO1, TR-ACO2* and *TR-ACO3,* as indicated in the EZ (A), VL (B) and MR (C) regions of the primary roots of nodal explants maintained hydroponically in half-strength Hoagland's media containing either 1 mM Pi (clear bars) or 10 µM Pi (shaded bars) for the time intervals indicated. Relative transcript abundance was determined by qRT-PCR and transcription was normalized against two internal reference genes, *TR-β-actin* and *TR-GAPDH.*Values are means ± SE, for two biological replicates each of which was derived from means of three technical replicates. ***** indicates significant differences between the 1 mM Pi and the 10 µM Pi treatments ($P \leq 0.05$).

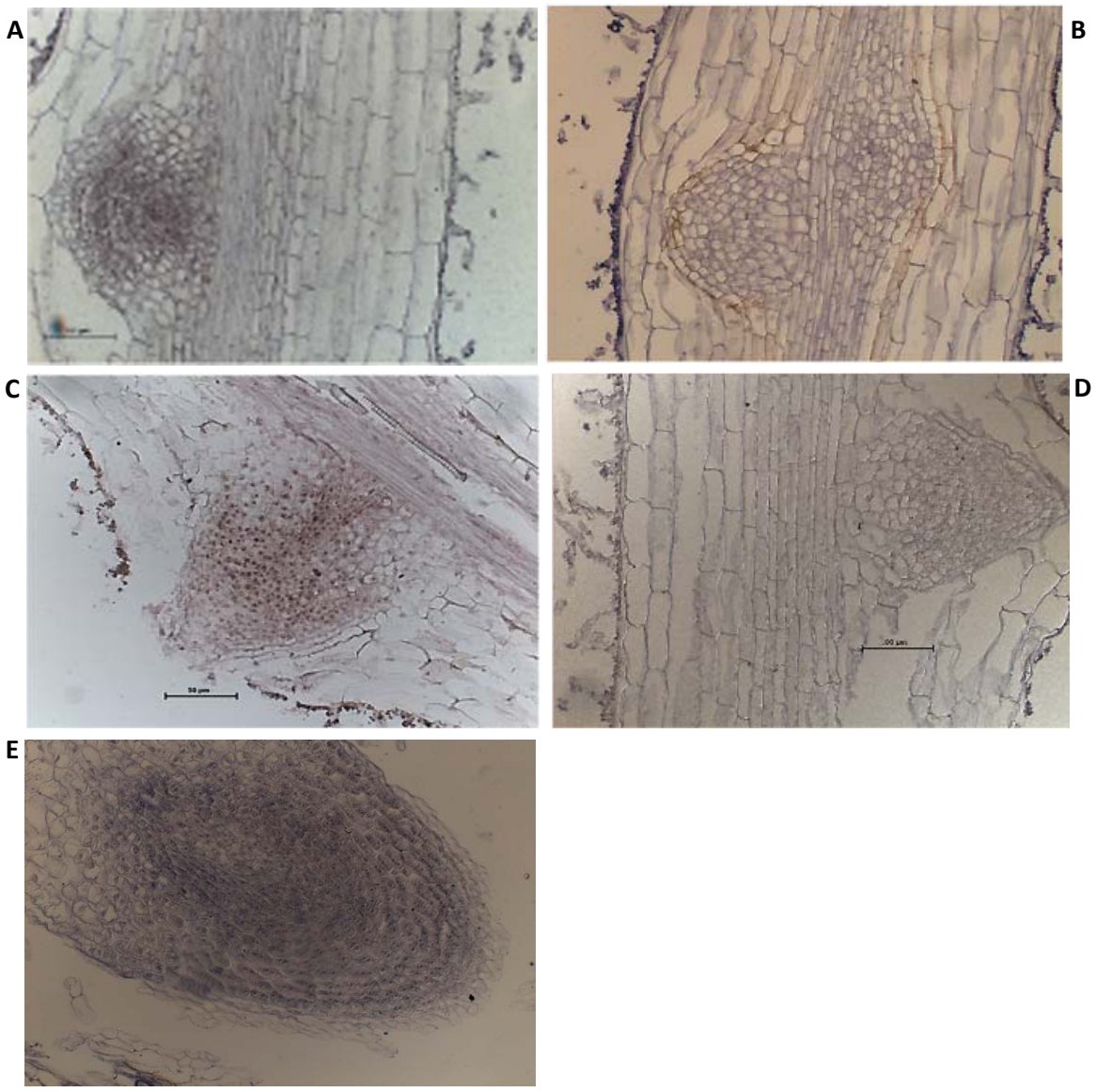

Figure S5 Localization of *TR-ACO1* transcripts in Pi-sufficient roots using *in situ* hybridization

Localization of *TR-ACO1* transcripts in Pi-sufficient roots of white clover. A,C, localization in lateral root primordia at different developmental stages in the VL region revealed using the antisense *TR-ACO1* probe*;* B,D, localization in lateral root primordia at different developmental stages in the VL region revealed using the sense *TR-ACO1* probe; E, localization in the primary root tip using the antisense *TR-ACO1* probe. The bars represent 50 μm.

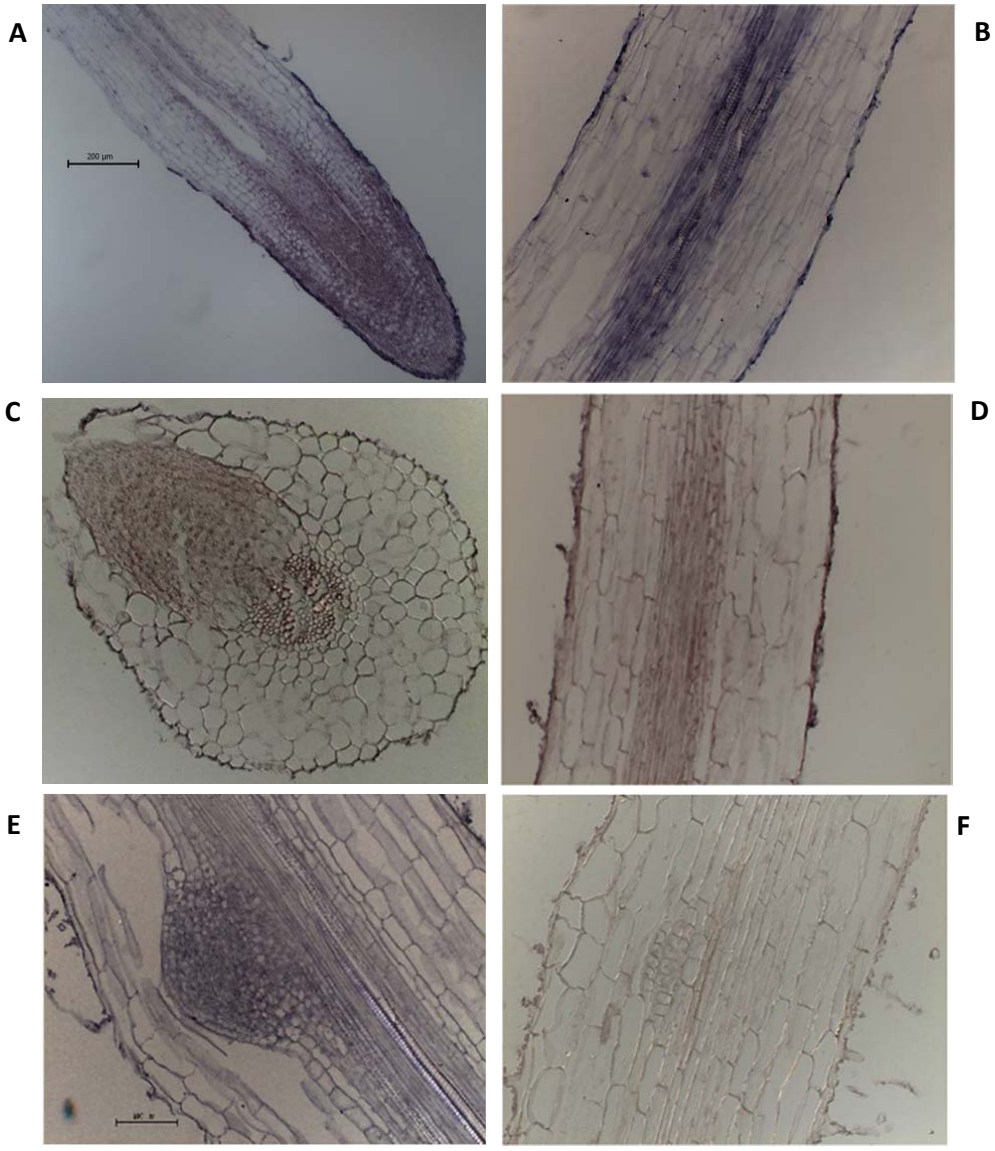

Figure S6 Localization of *TR-ACO2* and *TR-ACO3* transcripts in Pi-sufficient roots using *in situ* hybridization

Localization of *TR-ACO2* transcripts in the primary root tip (A), and in the vascular tissue in the VL (B) revealed using the anti-sense *TR-ACO2* probe. Localisation of *TR-ACO2* transcripts in the lateral root primordia (C) and in the vascular tissue (D) in the VL revealed using the sense *TR-ACO2* probe. Localization of *TR-ACO3* transcripts in the lateral root primordia in Pi-sufficient roots of white clover in the VL revealed using the anti-sense *TR-ACO3* probe (E) or the sense *TR-ACO3* probe (F). The bars represent 50 µm.

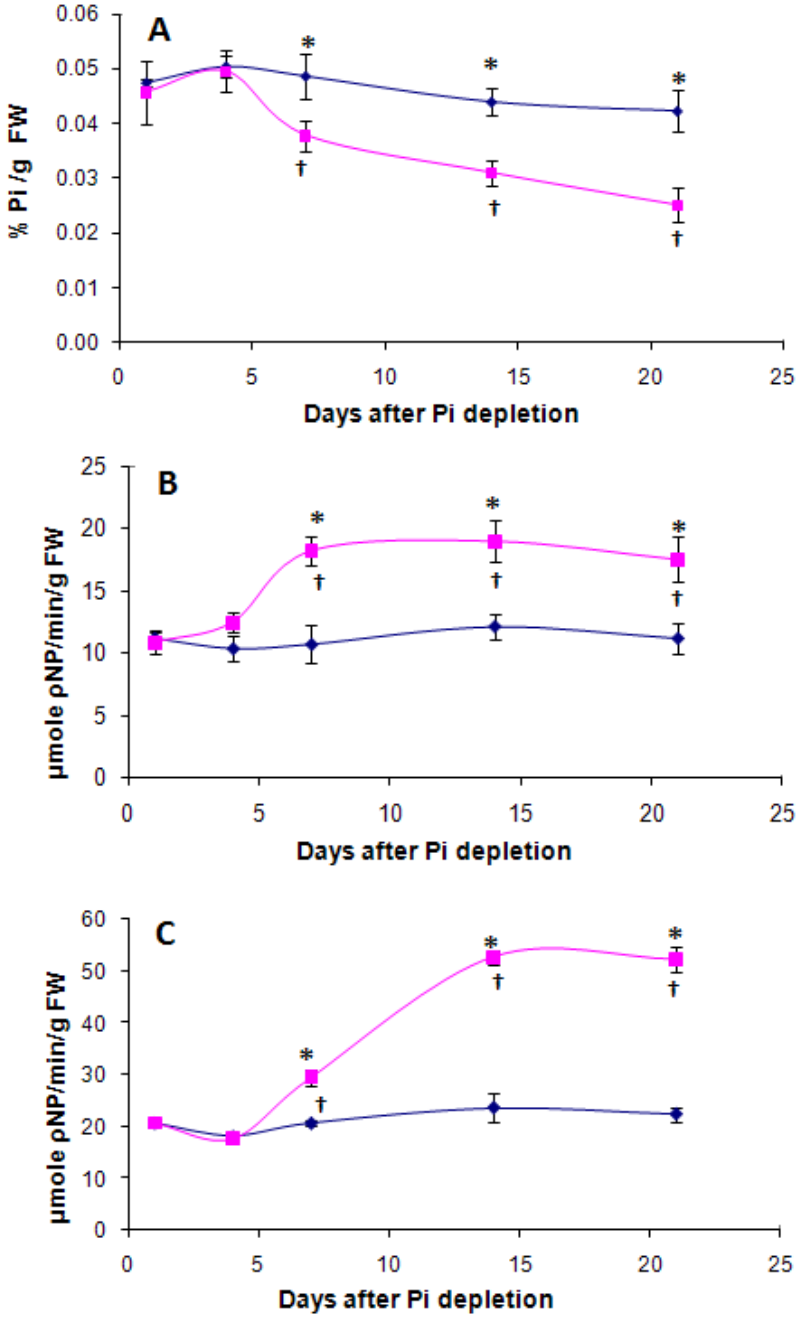

Figure S7 Cellular effects of Pi supply in roots of the *TR-ACO1p::mGFP-ER* white clover (line TR2-1)

The effects of Pi treatment [1 mM Pi, P+ (- ♦ -); 10 µM Pi, P- (- ■ -)] on total leaf Pi content (A), acid phosphatase activity in the cell-wall-enriched (B) and soluble fractions (C) in the transgenic white clover (*TR-ACO1p::mGFP-ER)* line TR2-1. Values are means ± SE, n = 3 (A), n = 4 (B,C) * indicates significant differences between treatments ($P \leq 0.05$); † indicates significant differences within a treatment from day 1($P \leq 0.05$).

Table S1 List of primer sequences used in this study

Gene	Sense	Antisense
Degenerate primers used for cloning *TR-PT1*		
DegTR-PT1	CAATTGTGATAGCWGGAATGGG	CCTGCAGCAGCWGAGATTC
Sequences of primers used for qRT-PCR		
TR-β-actin	CGTATGAGCAAGGAGATCACTG	CATCTGCTGGAAGGTGCT
TR- GAPDH	TCCAGTATTGAACGGTAAATTGAC	TCTGATTCCTCCTTGATAGCAG
TR-ACS1	AGGTTTCGATCGAGATTTGA	TTGGTTCTGTCCATAACTGTG
TR-ACS2	GAGAACCGGTGTTGAGATT	GACTTTAAGGTTGCGGTCT
TR-ACS3	TGTTCGTGATTTGTGTTGGA	TGTTCCTAGAGGGTTTGATG
TR-ACO1	GTGTTGATGTGGGACCAGTAG	CCAAACCAAACACTAATAATCGC
TR-ACO2	CTTGTAAAAGGTCTCCGAGCAC	GAGGAACATCTACCCATTTACCAT
TR-ACO3	AGCATCATTCTACAACCCTGG	CAAACACAAATTTAGGATACACATTGG
TR-PT1	GAATGCGAAACAGGCTACTG	CTGAACAAGCCATACTGATTTCT

Table S2 Accession numbers of genes examined in this study

Gene Name	GenBank Accession
TR-ACS1	KM881530
TR-ACS2	KM881531
TR-ACS3	KM881532
TR-ACO1	DQ112347
TR-ACO2	DQ112348
TR-ACO3	DQ112349
TR-PT1	KF022212