Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning

Supporting information

Figure S1. Effect of different mineral nutrient solutions on mycorrhizal colonization and plant fresh weight. Control (C) and mycorrhizal (AM) *P. hybrida* plants were grown for 5 weeks with a basic fertiliser (low Pi) or with this fertiliser supplemented with 5 mM of different mineral nutrients. (a) Mycorrhizal colonization and (b) fresh weight of shoots (white columns) or roots (black columns) were determined. Shown are mean values ± SD ($n = 3$). Different letters indicate significant differences (Student's t-test, $p \leq 0.05$).

Figure S2. Gene Ontology (**GO**) **classification of the EST sequences from petunia roots and petals.** Gene sequences were assigned to GO annotation adopting the GO slim nomenclature. The relative frequencies of GO hits for *petunia* unigenes was partitioned in the GO functional categories Biological Process, Molecular Function, and Cellular Component, as defined for the *Arabidopsis* proteome.

Figure S3. Expression of symbiotic phosphate transporter genes in mycorrhizal roots treated with 5 mM KH_2PO_4. Petunia plants were inoculated and grown for 4 weeks before the treatment with 5 mM KH_2PO_4 for additional 4 weeks. Root samples (same samples as in Figure 7) were taken at the indicated times for gene expression analysis of *PhPT3, PhPT4,* and *PhPT5.*.

Table S1. Sequences clustered prior to microarray design. Accessed at http://pgrc.ipk-gatersleben.de/petunia_array. For each individual sequence, it lists the sequence identifier, the consensus sequence to which it contributed (corresponding to the 'Array sequence ID' in Table 2, and Tables S2–S7), a GenBank accession number (where available), the length of the sequence, the number of individual EST reads that contributed to it, the results from BLAST searches to the non-redundant Genbank database NRPEP, and the sequence itself. Table S1 features all the sequences generated in this study and the sequences retrieved from the public domain. The clustered sequences from roots and petals are also accessible at http://est.molgen.mpg.de/plantDR/archiv/ (User: DidierReinhardt; Password: Al8JJ9mt; Database: drPoolB).

Table S2. Expression analysis for all 24'816 IDs represented on the microarray. Listed are the array sequence ID, the results of a BlastX search against the protein database at NCBI, as well as the results from BlastN searches against fungal and plant EST databases at NCBI. Control expression levels at 5 weeks, 7 weeks, and 8 weeks (controls, low P), expression ratios for mycorrhizal roots at 5 weeks, 7 weeks, and 8 weeks after inoculation with *G. intraradices* (AM, low P), or with high phosphate supply (5 mM KH_2PO_4) in the absence (high P) or presence of the fungus (AM, high P) are listed.

Table S3. List of genes regulated by AM and P_i sorted according to their expression pattern. Gene lists were extracted by FIRE2.2 analysis according to their expression levels in mycorrhizal and Pi-treated roots, relative to controls. Control expression level and expression ratios for roots at 5, 7, and 8 weeks after inoculation with *G. intraradices* in the presence of low phosphate (30 µM KH_2PO_4) (AM, low P), or with high phosphate supply (5 mM KH_2PO_4) in the absence or presence of the fungus (high P; AM, high P) is shown.

Table S4. Comparison of AM-regulated genes of petunia with AM-regulated homologues of *M. truncatula, L. japonicus and O. sativa*.

Table S5 Expression of genes encoding putative components of symbiotic signaling. Annotations, expression levels (controls) and expression ratios are shown for genes that encode homologues of symbiosis signalling components known from legumes. The color code corresponds to Table S3.

Table S6. qPCR analysis of the effect of P_i on the expression of selected genes. The relative expression level of selected genes was determined by quantitative real time PCR (qPCR) in three independent experiments (qPCR1, qPCR2, qPCR3). Gene expression ratios were determined five weeks after inoculation in mycorrhizal roots treated with 30 mM KH_2PO_4, (AM), roots treated with 5 mM KH_2PO_4, (P_i), and mycorrhizal plants treated with 5 mM KH_2PO_4, (AM-P_i). The values from array analysis (see Table 3) are indicated for comparison (Array 1, Array 2). Functional class (FC), and expression ratios are given. Repression of gene expression by P_i is indicated as the ratio of the expression values from inoculated roots at low versus high P_i levels (AM / AM-P_i). Repression values >25-fold are indicated in bold face. Genes repressed >25-fold in at least two independent qPCR experiments are shaded in grey. These genes are considered to be repressed more than expected if they were expressed proportional to the level of root colonization.

Table S7. Primers used for qPCR analysis. Indicated are the array sequence identifier (ID) on the array, the gene annotation, the forward (extension F) and reverse (extension R) primers.

Appendix S1.*Generation of subtractive root libraries and EST sequencing.* Substractive cDNA libraries were constructed with mRNA from roots inoculated with *G. intraradices* or treated with 5 mM KH_2PO_4 each for 2, 4, or 8 weeks. To ensure that early and late AM- and P_i-responsive genes were equally represented in the substractive libraries, equal amounts of RNA of the three time points were combined for each of the three treatments to give the RNA samples for substraction. Substractions of RNA from control (c), mycorrhizal (AM), and P_i-treated roots was performed in both directions (AM minus c; c minus AM; P_i minus c; c minus P_i). This strategy promised to yield a maximum diversity of genes up- and downregulated in the symbiosis and by P_i treatment. From each of the four substractive libraries, approximately 4500 clones were sequenced in both directions. The suffix dsr21 in EST names (Table 1) stands for the subtraction of control (30 µM KH_2PO_4) from AM sample (with 30 µM KH_2PO_4), dsr12 stands for the reverse subtraction. dsr31 stands for high P_i (5 mM KH_2PO_4) minus control (30 µM KH_2PO_4), and dsr13 stands for the reverse subtraction. From normalized libraries of petunia roots and petals approximately 6000 clones were sequenced each in both directions.

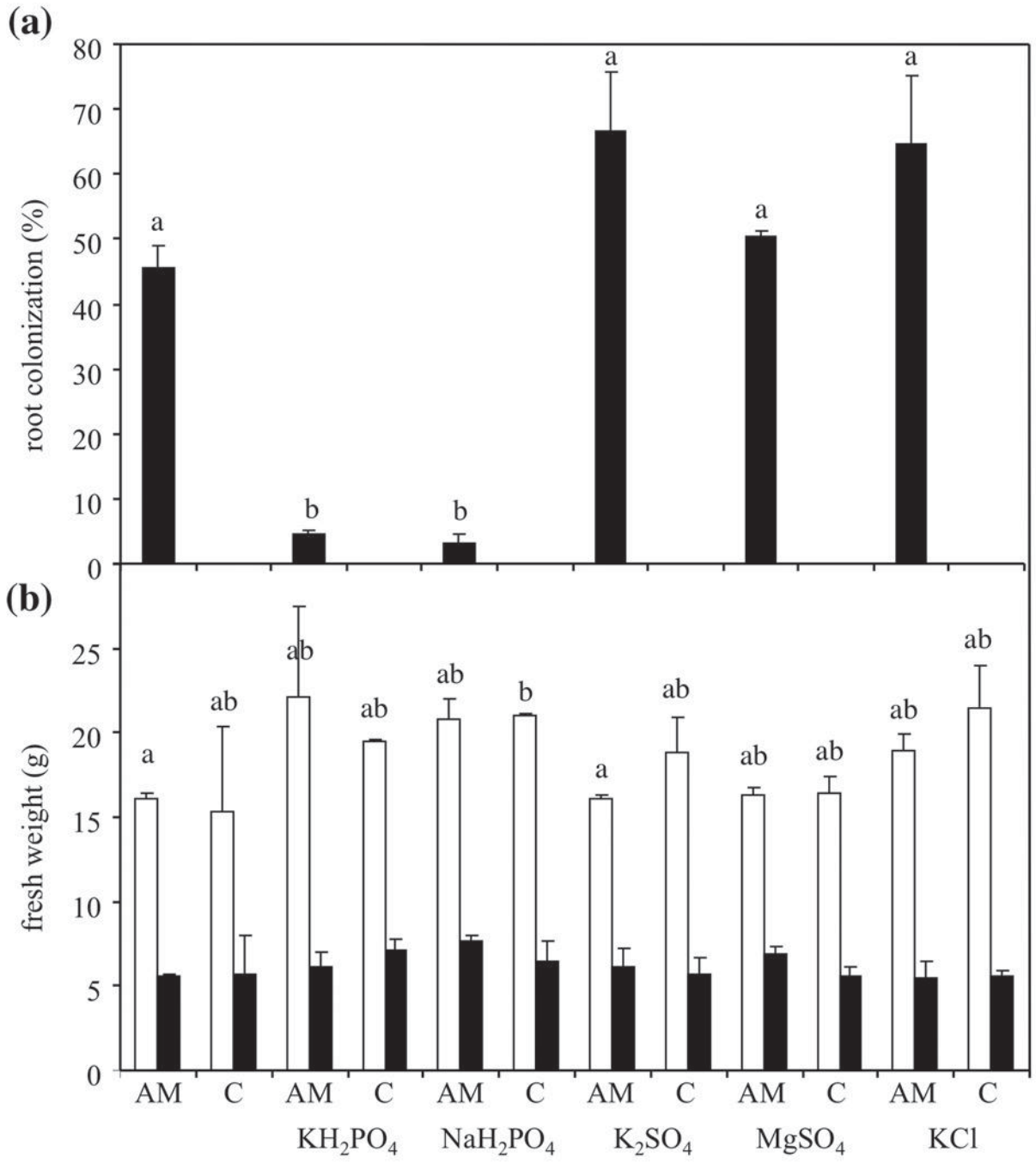
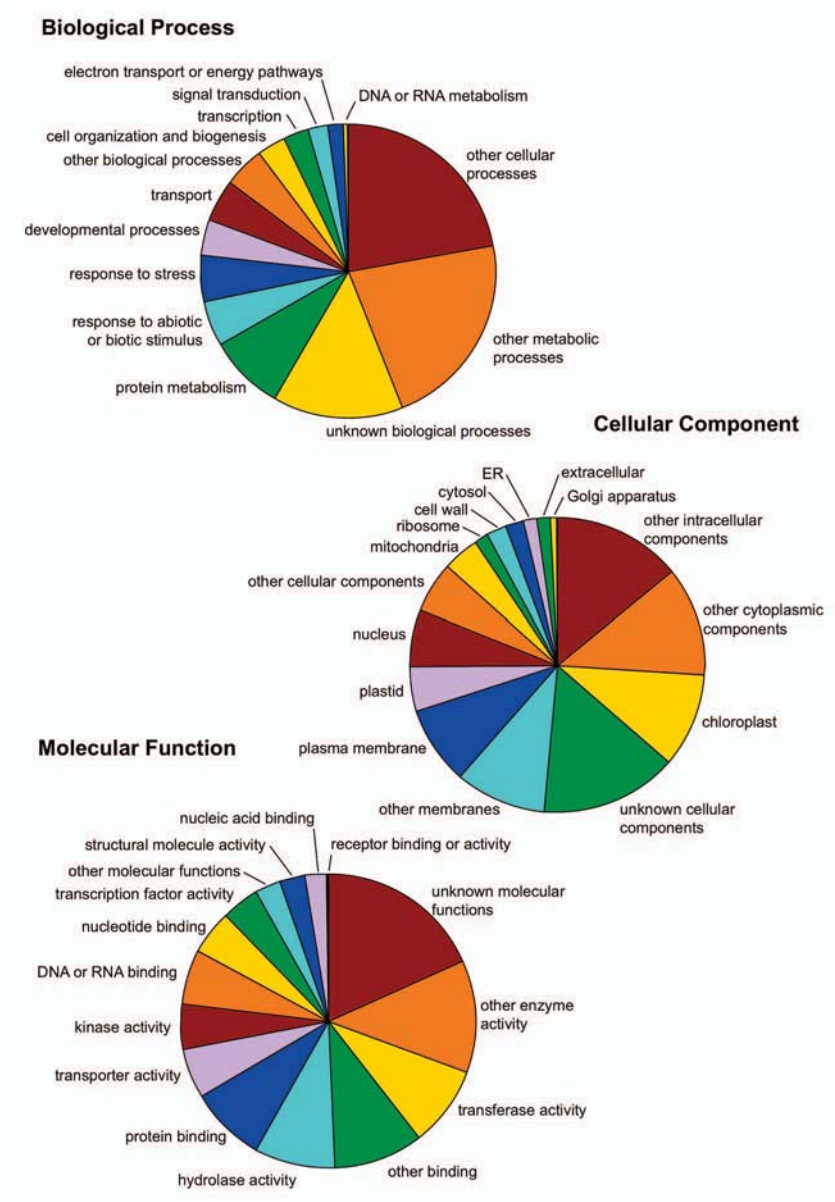
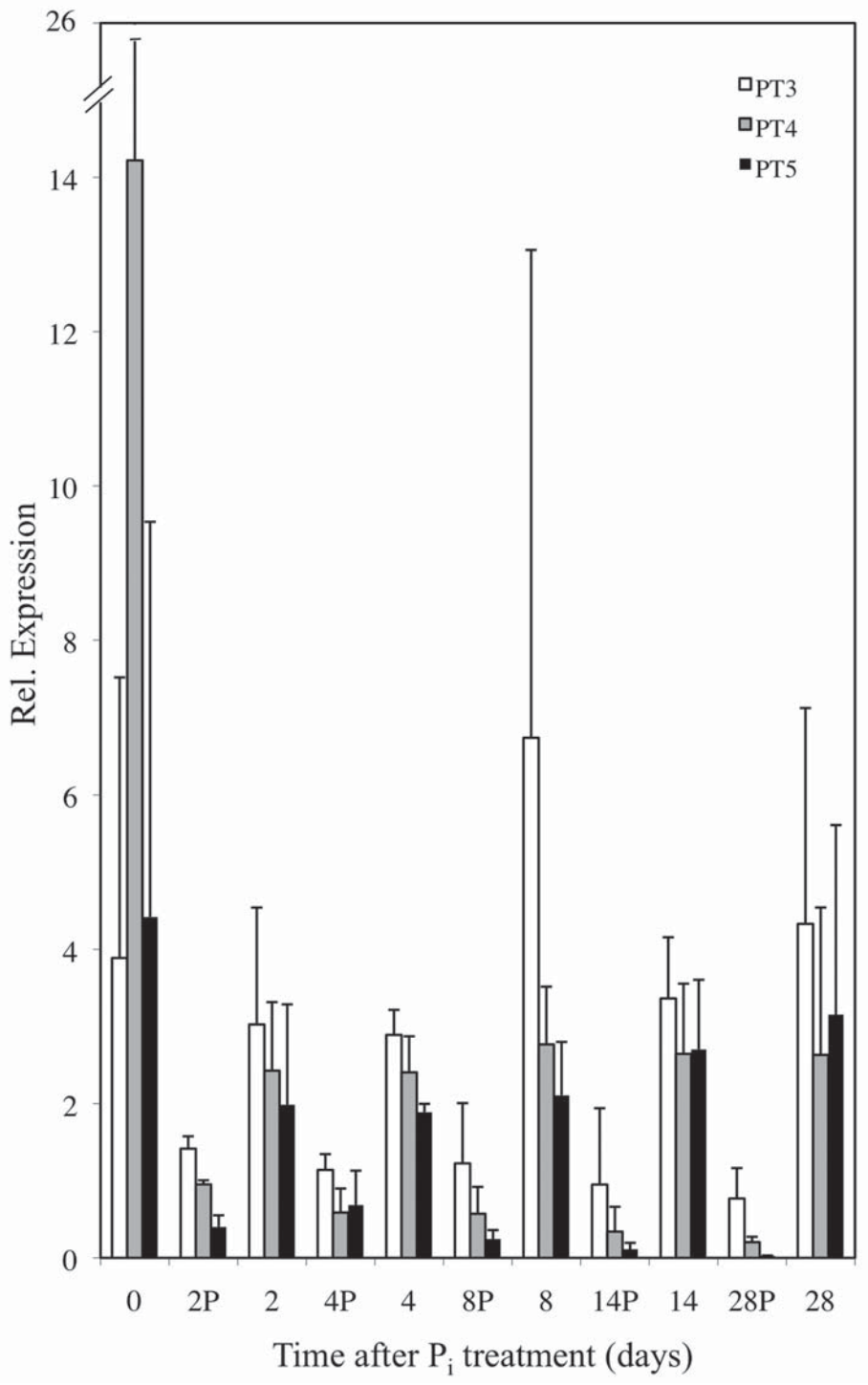


Figure S1. Effects of various salt solutions on AM root colonization in petunia.


Biological Process
electron transport or energy pathways
signal transduction
transcription
cell organization and biogenesis
other biological processes
transport
developmental processes
response to stress
response to abiotic or biotic stimulus
protein metabolism
DNA or RNA metabolism
other cellular processes
other metabolic processes
unknown biological processes
Cellular Component
ER
extracellular
cytosol
Golgi apparatus
cell wall
ribosome
mitochondria
other intracellular components
other cellular components
other cytoplasmic components
nucleus
plastid
chloroplast
plasma membrane
other membranes
unknown cellular components
Molecular Function
nucleic acid binding
structural molecule activity
receptor binding or activity
other molecular functions
transcription factor activity
nucleotide binding
DNA or RNA binding
kinase activity
transporter activity
protein binding
hydrolase activity
unknown molecular functions
other enzyme activity
transferase activity
other binding


26
14
12
10
8
6
4
2
0
Rel. Expression
PT3
PT4
PT5
0
2P
2
4P
4
8P
8
14P
14
28P
28
Time after P$_i$ treatment (days)