Journal of Experimental Botany, Vol. 59, No. 9, pp. 2479–2497, 2008
doi:10.1093/jxb/ern115 Advance Access publication 23 May, 2008

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Transcript profiling of *Zea mays* roots reveals gene responses to phosphate deficiency at the plant- and species-specific levels

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Received 24 January 2008; Revised 4 March 2008; Accepted 27 March 2008

Abstract

Maize (*Zea mays*) is the most widely cultivated crop around the world; however, it is commonly affected by phosphate (Pi) deficiency in many regions, particularly in acid and alkaline soils of developing countries. To cope with Pi deficiency, plants have evolved a large number of developmental and biochemical adaptations; however, for maize, the underlying molecular basis of these responses is still unknown. In this work, the transcriptional response of maize roots to Pi starvation at 1, 3, 6, and 10 d after the onset of Pi deprivation was assessed. The investigation revealed a total of 1179 Pi-responsive genes, of which 820 and 363 genes were found to be either up- or down-regulated, respectively, by 2-fold or more. Pi-responsive genes were found to be involved in various metabolic, signal transduction, and developmental gene networks. A large set of transcription factors, which may be potential targets for crop breeding, was identified. In addition, gene expression profiles and changes in specific metabolites were also correlated. The results show that several dicotyledonous plant responses to Pi starvation are conserved in maize, but that some genetic responses appear to be more specific and that Pi deficiency leads to a shift in the recycling of internal Pi in maize roots. Ultimately, this work provides a more comprehensive view of Pi-responses in a model for economically important cereals and also sets a framework to produce Pi-specific maize microarrays to study the changes in global gene expression between Pi-efficient and Pi-inefficient maize genotypes.

Key words: Abiotic stress, maize, microarrays, phosphate, root.

Introduction

Maize (*Zea mays*) is the most widely cultivated crop constituting a major component in the diet of many developing countries and considered as one of the crops with most biotechnological potential for energy production and other industrial applications (McLaren, 2005).

Low Pi availability is one of the major constraints for maize production worldwide, since this crop is largely grown in areas in which soils with low Pi availability are predominant, such as the acid soils of tropical and subtropical regions and the calcareous soils of temperate regions. These soils account for more than half of the area cultivated with maize worldwide (Fairhurst *et al*., 1999; Fixen, 2002; Leakey *et al*., 2006; Pingali and Pandey, 2001). Pi availability is critical in the early developmental stages (Barry and Miller, 1989; Hajabbasi and Schumacher, 1994; Plénet *et al*., 2000) and, therefore a direct constraint for maize production, particularly under agricultural conditions where intensive fertilization is not affordable (Lynch, 1995).

Plants have evolved a wide array of molecular and biochemical adaptive strategies to optimize Pi uptake and utilization that include the release of soil Pi from organic and inorganic sources that are not readily available for plant uptake, the employment of high affinity Pi transporters (Raghothama, 1999), the modification of root system architecture to increase the exploratory capacity

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and reach Pi-rich patches in the soil (Hodge, 2004; López-Bucio *et al*., 2002), the establishment of symbiotic relations with arbuscular-mycorrhizal fungi (Bucher, 2007), and the recycling and mobilization of internal Pi as well as the optimization of the exploitation of a wide range of structural and metabolic compounds (Duff *et al*., 1994; Essigmann *et al*., 1998; Theodorou and Plaxton, 1993; Usuda and Shimogawara, 1991).

Although macro/microarray studies have contributed enormously to demonstrate the transcriptional regulation of some genes related to biochemical adaptations and an integral reprogramming of major metabolism under Pi-starvation, i.e. carbohydrate mobilization, nitrate assimilation, lipid recycling, and secondary metabolism in plants (Wasaki *et al*., 2003; Misson *et al*., 2005; Hernandez *et al*., 2007; Morcuende *et al*., 2007), the impact of Pi-starvation on the expression of genes encoding proteins that mediate such pathways in roots remains to be determined. Although previous analyses have also shown that nitrogen metabolism is modified mainly through the down-regulation of nitrate assimilation, protein synthesis (Misson *et al*., 2005; Morcuende *et al*., 2007) and changes in particular metabolite contents (including some amino acids and sugars; Hernandez *et al*., 2007; Morcuende *et al*., 2007), little is known about the impact of Pi starvation on the equilibrium between carbohydrate and nitrate pools as mediated by amino acid metabolism. Moreover, the knowledge of Pi-starvation responses in economically important crops is still limited since most studies have been done using *Arabidopsis thaliana* (*Arabidopsis*) as a model system. In the case of monocotyledonous, only a partial characterization of transcriptional responses to Pi starvation in rice (*Oryza sativa*) has been carried out (Wasaki *et al*., 2003, 2006). Despite the economic and nutritional importance of maize, little is known about the molecular adaptations to Pi starvation in this species. Li *et al*. (2007), using a proteomic approach with root tips, reported changes in some specific proteins in response to long-term Pi-starvation treatment, indicating modifications in the alteration of the balance of carbohydrate [glycolisis and tricarboxylic acid (TCA) cycle mainly], protein, nucleotide, and secondary metabolism (Li *et al*., 2007). Nevertheless, a detailed analysis of the effects of Pi-starvation on the expression of maize genes involved in different metabolic pathways is still lacking.

The wide variety of modifications in metabolic, developmental, and global gene expression observed in Pi-deprived plants show that the Pi-deficiency response in plants is quite complex and probably mediated by several local and systemic signalling pathways (Franco-Zorrilla *et al*., 2004). Among the transcription factors (TFs) involved in low Pi-responses in *Arabidopsis*, the MYB transcription factor PHR1 stands out as a central regulator of downstream starvation-induced genes (Rubio *et al*., 2001). Further analyses in *Arabidopsis* have contributed to identify additional TFs involved in the *Arabidopsis* response to Pi starvation (Hammond *et al*., 2003; Wu *et al*., 2003; Misson *et al*., 2005; Devaiah *et al*., 2007*a*, *b*; Morcuende *et al*., 2007). For monocotyledons, the current information of TFs that mediate the mechanisms behind Pi starvation responses is scarce. To date, only the gene orthologues to *SCARECROW 8* (*SCR 8*; Wasaki *et al*., 2003) and *OsPTF1* (Yi *et al*., 2005) have been identified in rice as TF genes responsive to Pi starvation. Interestingly, *OsPTF1* overexpression conferred enhanced tolerance to Pi-starvation (Yi *et al*., 2005). These latter results highlighted the importance of research carried out directly on economically important crop species since *Arabidopsis* genes do not always represent the respective orthologue genes in cereal genomes. Although the identification of TFs involved in the Pi-deficiency response could be important targets for breeding, no TFs responsive to Pi-starvation have been identified in maize.

In this work, a genomic-level approach was undertaken using a Pi-starvation-tolerant genotype of maize L3×228-3 in order to identify Pi-responsive genes and to obtain an overview of molecular modifications of different biochemical, cellular, and developmental processes which occur as a result of Pi-starvation in maize roots. Transcriptional profiling of maize roots was carried out by analysing global gene expression alterations, at four time points to identify both early and late Pi responsive genes by employing an oligonucleotide microarray platform representing about 56 600 maize genes. As a result, a comprehensive view of Pi starvation responses in maize roots was obtained. The robustness of this study permitted us to identify (i) genes involved in biochemical processes temporarily affected by Pi starvation and a large set of TFs differentially regulated by Pi availability, and (ii) a comprehensive catalogue of differentially expressed genes for detailing novel and conserved virtual metabolic pathways affected by Pi starvation in plants. In addition, the impact of gene expression modifications as a result of Pi-starvation on metabolite profiles was corroborated by determining the changes in lipid composition and anthocyanin content.

Materials and methods

Plant material and growth conditions

For all experiments, the P-utilization efficient hybrid *Zea mays* L3×228-3 (kindly supplied by EMBRAPA, Brazil) was used. Sterile seeds without endosperm were germinated in 0.1× MS medium in a growth room at 28 °C with a 16/8 h light/dark cycle for 3 d under sterile conditions followed by 2 d of growth in pots with perlite as a solid support. Seedlings were then carefully transferred into a hydroponic system for 5 d in individual pots with 3.0 l of a nutrient solution at pH of 5.5 [NH_4NO_3 0.3 mM, $Ca(NO_3)_2$ 0.75 mM, $CaCl_2$ 0.25 mM, $MgSO_4$ 1 mM, K_2SO_4 0.5 mM, H_3BO_3 46 μM, $MnSO_4$ 9 μM, $ZnSO_4$ 0.8 μM, Na_2MoO_4

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0.8 μM, $CuSO_4$ 0.3 μM, FeEDTA 75 μM, and 200 μM $Ca(H_2PO_4)_2$ as a source of P. After 5 d of adaptation, seedlings were transferred to a fresh nutrient solution containing optimal (0.2 mM) or limiting (0.002 mM) P. Calcium in low-P solutions was added as $CaCl_2$ to a final concentration of 0.45 mM. Roots and shoots were collected separately at 1, 3, 6, and 10 d after the onset of stress (AOS).

Experimental design and microarray platform

For microarray analyses, a dye balanced modified loop design was implemented. Four biological replicates representing each sampling point were obtained by pooling the whole root system of eight randomly chosen plants. This experiment involved a total of 16 sets of microarray hybridizations, including direct and dye swap comparisons between treatments as well as across time points for the same treatment. This design allowed us to determine differences in gene expression between P-depleted and control roots (P availability effect) and whether the differences were time dependent (P×time effect). The Maize Oligonucleotide Array (MOA) from www.maizearray.org was used to carry out this study. The MOA contains about 57 000 individual spots on two slides (A and B) and putatively contains all maize genes identified to date. Array annotation and composition is available at www.maizearray.org.

RNA isolation, labelling, hybridization, and image processing

Total RNA was isolated from roots using the Trizol reagent (Invitrogen) and re-purified with the RNeasy kit (Qiagen) following the manufacturer's instructions. Purified total RNA was then labelled according to the protocols recommended at www.maizearray.org. Briefly, for each treatment×time combination, four biological replicates were used for probe synthesis. For each sample, 1.5 μg total RNA were amplified in the presence of aminoallyl-dUTP (Ambion) using the Aminoallyl Message Amp II kit (Ambion). Resulting amplified RNA probes were further labelled with fluorescent Cy3 and Cy5 dyes (Amersham). The fluorescent dye-labelled probes were then purified using RNeasy columns (Qiagen). For hybridization, probes were mixed, concentrated by precipitation, and resuspended in the hybridization solution (50% formamide, 5× SSC, 0.1% SDS, 0.4 μg μl^{-1} tRNA, and 0.2 μg μl^{-1} Salmon Sperm DNA) for 14 h. Slides were washed for 5 min in each of the following solutions: (i) 2× SSC, 0.1% SDS/42 °C, (ii) 0.1× SSC/RT, and two final washes with 0.05× SSC/RT.

Slides were scanned with an Axon GenePix 4100 scanner at a resolution of 10 μm adjusting the laser and gain parameters to obtain similar levels of fluorescence intensity in both channels. Spot intensities were quantified using Axon GenePix Pro 5.1 image analysis software. The mean of the signals and the median of backgrounds were used for further analysis. The design, protocols, and microarray data can be found at the *Zea mays* Microarray Gene Expression Database (ZEAMAGE), www.maizearray.org/maize_study.shtml.

Normalization and data analysis

Raw data were imported into the R 2.2.1 software (http://www.R-project.org). Background correction was done using the RMA algorithm (Irizarry *et al*., 2003) and normalization of the signal intensities within slides was carried out using the 'printtiploess' method (Yang *et al*., 2002) using the LIMMA package (Smyth *et al*., 2003, at www.bioconductor.org). Normalized data were log2 transformed and then fitted into mixed model ANOVAs (Wolfinger *et al*., 2001; Gibson and Wolfinger, 2004) using the Mixed procedure (SAS 9.0 software, SAS Institute Inc., Cary, NC, USA) with two sequenced linear models considering as fixed effects the dye, time, Pi-treatment, and time×Pi-treatment. Array and array×dye were considered as random effects. The Type 3 *F*-tests and *P*-values of the time×*P*-treatment and *P*-treatment model terms were explored and significance levels for those terms were adjusted for by the False Discovery Rate (FDR) method (Benjamini and Hochberg, 1995). Estimates of the expression differences were calculated using the mixed model. Based on these statistical analyses, the spots with tests with an FDR less than or equal to 5% and with changes in signal intensity between Pi-depleted and control roots of 2.0-fold or higher were considered as differentially expressed.

Real-time quantitative RT-PCR (qRT-PCR)

Genes whose expression was considered as regulated by Pi-deficiency in the microarray analysis were selected with the aims of both validating the expression patterns found and also to gain further biological information through selecting only annotated genes. Genes known to be Pi-deficiency-regulated in other plant species were also included. Primer design (T_m, 60–65 °C) was performed according the guidelines recommended in the Primer Express Software, Version 3 (Applied Biosystems) using as template the original target sequences from which the oligonucleotides printed in the array were designed. Oligonucleotide sequences for qRT-PCR are shown in Supplementary Table S1 at *JXB* online.

Total RNA for qRT-PCR was isolated from 1 g frozen root tissue based on the protocol of the Trizol reagent (Invitrogen) and further purified using Qiagen RNeasy columns according to the manufacturer's protocol (Qiagen). cDNA was first synthesized using 10 μg total RNA with SuperscriptIII reverse transcriptase (Invitrogen), according to the manufacturer's instructions and used for performing qRT-PCR (7500 Real Time PCR System, Applied Biosystems). qRT-PCR of *POLIUBIQUITIN2* (*UBQ2*, TC305418) was performed for normalization. SYBR Green PCR Master Mix was used for the PCRs according to the manufacturer's protocol. Gene expression was normalized to that of the control *UBQ2* gene by subtracting the C_T value of *UBQ2* from the C_T value of the gene of interest. –P to +P average expression ratios were obtained from the equation $(1+E)2^{\Delta\Delta C_T}$ where $\Delta\Delta C_T$ represents $\Delta C_T(–P)–\Delta C_T(+P)$, and *E* is the PCR efficiency according to protocol reported by (Czechowski *et al*., 2004).

Metabolite determinations

Total anthocyanin content was measured from about 300 mg of root tissue from both low-Pi and optimal growth conditions at 6 d and 10 d AOS. The optical density from acidified methanolic extracts (5% HCl) was measured and corrected by using $OD530_{nm}$–0.25 $OD657_{nm}$ as described in (Pietrini *et al*., 2002) using a Beckman DU® 650 spectrophotometer. Anthocyanin content was calculated as cyanidine-3-glucoside by using 29 600 ($L\ mol^{-1}\ cm^{-1}$) as the molar extinction coefficient and 449.2 as the molecular weight.

Lipids from root tissues were extracted by homogenization in a chloroform solution and isolated by the TLC technique by employing a solvent system of acetone/toluene/water (91:30:7, by vol.; Welti *et al*., 2002). For quantification, individual lipids were isolated from TLC plates and used to prepare fatty acid methyl esters. The corresponding methyl esters were quantified by GC-MS with myristic acid as an internal standard control (Hartel *et al*., 2000). Two independent replicates of each sample were analysed with similar results (R^2=0.966).

Clustering, functional annotation, and metabolic pathway analysis

For comparison of transcriptome profiles, the significant genes according to the selected parameters (FDR <0.05 and Fold ±2) were clustered and visualized with the standard correlation measure using Genespring 7.0 software (Silicon Genetics, Redwood City,

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CA). To visualize the gene lists in the figures, the Excel (Microsoft) FiRe 2.2 macro (Garcion *et al*., 2006) was used. To gain further information of the biological relevance of the differentially expressed transcripts, BLAST alignments of the consensus sequences were performed from which the oligonucleotide array was designed against the TIGR Plant Transcript Assemblies, release 02/06/2007 (Childs *et al*., 2006) and the NCBI non-redundant database release (03/08/2007). These BLAST results provided us with a new annotation for each sequence based on protein similarity. If additional information was found using an E-value ≤1.0E–10 as a threshold, the original annotation was replaced.

As the functional annotation of maize sequences is still limited, the functional classification implemented in the mapping files that structure the *Arabidopsis* genes from the Affymetrix ATH1 array into distinct metabolic and cellular processes from the MapMan program (Thimm *et al*., 2004) was used. To associate the maize differentially expressed genes with a functional annotation, a BLAST alignment was performed against the TAIR Arabidopsis database release 6.0 (www.arabidopsis.org), the annotations of the mapping files for the best match to the TAIR protein database (with at least an E-value of 1.0E–10) were applied to the corresponding maize orthologue, in the case of double or more category assignments, the most informative was chosen. When possible, the genes without a metabolic or cellular annotation were further assigned a putative function based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Ogata *et al*., 1999).

To show the comparisons between the maize and *Arabidopsis* P-responsive genes (see Supplementary Fig. S2 at *JXB* online), the MapMan software was used (Thimm *et al*., 2004). The averaged signals of each time point for differentially expressed sequences were expressed relative to the control for *Arabidopsis* –P to +P (see Supplementary Table S4 in Morcuende *et al*., 2007), *Arabidopsis* root (see Supplementary Table 7 in Misson *et al*., 2005), and maize root (day 6 AOS) experiments. Ratios were expressed in a log2 scale for importing into the software and then drawn into the diagrams with a false colour scale for each gene in an experiment. Each square represents a single gene.

Results

The Pi deficiency treatment consisted in growing maize plants on media containing sufficient (0.2 mM) or limiting (0.002 mM) Pi levels. In order to examine changes in global gene expression that may precede the appearance of Pi-starvation symptoms but also to identify late responsive genes, roots were collected from plants grown for 1, 3, 6, and 10 d AOS. Seedlings at the onset of the treatment had one fully developed leaf and the primary root system was already completely developed, with shoot-borne roots at the first and second phytomers. Phenotypic differences between Pi sufficient and depleted plants were visible from day 6 AOS (Fig. 1). Pi-depleted plants presented anthocyanin pigmentation in the oldest leaves with decreased shoot weight (Fig. 1A) and leaf area. A delay in the appearance of shoot-borne roots was also observed (Fig. 1B). By day 10 AOS, Pi-depleted plants also presented longer shoot-borne roots (Fig. 1B) and a higher anthocyanin content in roots and leaves. Despite the decrease in shoot weight in Pi-depleted plants, a relative maintenance of C translocation to roots was observed, as reflected by the increase of the root-to-shoot

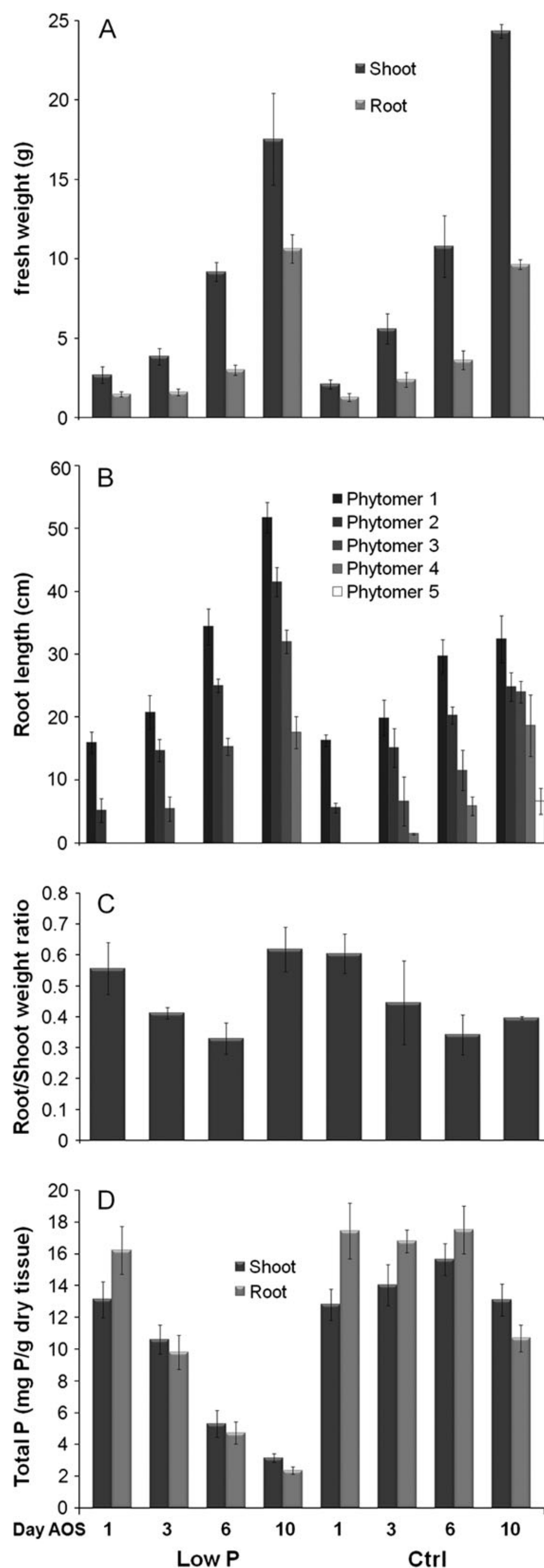

Fig. 1. Phenotypic responses of maize to Pi starvation. Maize seedlings were grown hydroponically under 2 μM (limiting) or 200 μM (optimal) Pi concentrations and collected at 1, 3, 6, and 10 d after the onset of the stress. Mean values ±SE of fresh weight of roots and shoots (A), length of shoot-borne roots (B), root-to-shoot weight ratios (C), and total P contents (D) are presented.

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weight ratio, as compared to Pi-sufficient plants (Fig. 1C). Pi deficiency also led to a significant decrease in P-accumulation in both shoot and roots starting in the first days of Pi starvation (Fig. 1D).

Transcriptome profiling of root response to Pi deficiency

A microarray platform containing about 56 600 maize gene oligonucleotides was used, so that the transcriptome profiling of maize roots under Pi starvation using most of the currently available genes and potentially covering most of the maize transcriptome was determined. The differences in gene expression between Pi-depleted versus Pi-sufficient roots (the overall P availability effect) were identified and also the differences caused by the P availability by time interaction (Time×P effect). According to the stringency levels (FDR ⩽0.05 and Fold ±2), a total of 1179 genes showed differential expression in at least one of the four sampled time points (see Supplementary Table S2 at *JXB* online).

Over time, for both induced and repressed genes, the fold-change in expression of Pi-responsive genes gradually increased from the first to the sixth day AOS. At the sixth and tenth days AOS, the greatest fold changes in gene expression were observed (Fig. 2A). Principal component analysis showed that this was the main trend and accounted for 86% of the differentially expressed genes. Common differentially expressed genes were found mainly between days 6 and 10 AOS, whereas only nine induced and four repressed genes were found to be differentially expressed over all four sampled time points (Fig. 2B) indicating that at early time points (days 1 and 3 AOS) the response is represented by a distinct set of differentially expressed genes compared with those identified at later time points.

Annotation and functional classification

As the functional annotation of maize sequences is still limited, functional classification was implemented by comparison (using an expected value of 1E-10 as threshold) with the *Arabidopsis* genomic data implemented in the MapMan program (Thimm *et al.*, 2004). Remaining sequences without annotation were further classified based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Ogata *et al.*, 1999). Five hundred and sixty genes were automatically annotated with MapMan and 214 sequences were further manually annotated using the KEGG database, however, 407 sequences had no significant correlation to any annotated database (see Supplementary Table S2 at *JXB* online). Among the annotated genes, the signalling, hormone, and transcription related genes accounted for about 10% of the Pi-responsive genes, whereas both N metabolism (including that of amino acids and proteins)

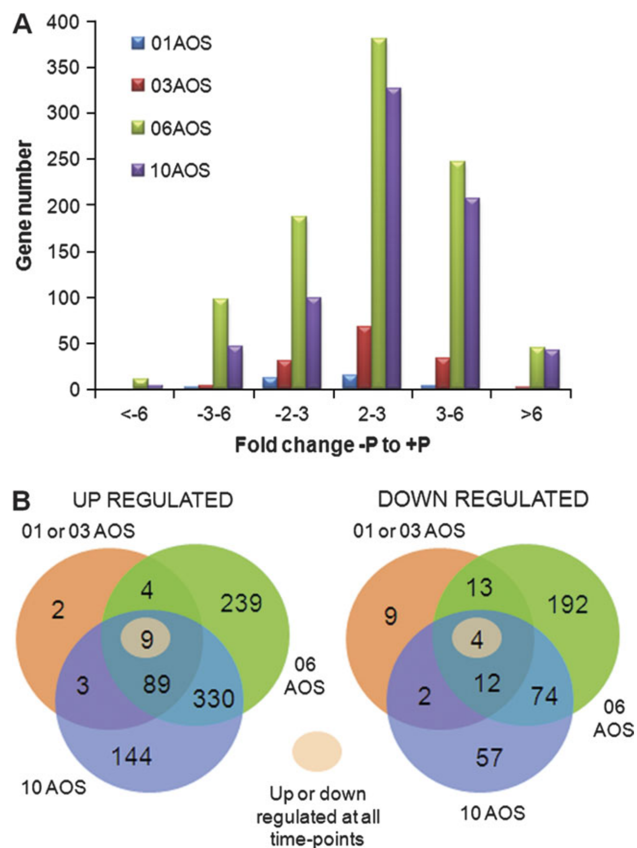

Fig. 2. Overview of Pi-deficiency regulated genes in maize roots at 1, 3, 6, and 10 d AOS. A total of 1179 genes were identified according to our thresholds (fold change of at least ±2 at any time point and FDR ⩽0.05). (A) Distribution of the change in expression levels for all 1179 genes. (B) Venn diagrams showing common or distinct regulated genes over the sampled time points.

and transport systems were the most affected processes since they each accounted for around 7% of the Pi-responsive genes (see Supplementary Fig. S1 at *JXB* online).

Table 1 shows the 50 most strongly differentially expressed genes. Among them, a strong induction of Pi-starvation-related genes was observed (encoding Pi transporters, a putative acid phosphatase, and a SPX domain protein), for which differential expression was initiated at day 3 AOS. These results suggest that maize roots sense Pi-starvation prior to the development of visible symptoms and developmental modifications. Interestingly, four genes related to hormone/signalling showed strong differential expression at day 1 AOS (Table 1), suggesting that genes involved in sensing/signal transduction mechanisms are activated rapidly after the onset of the stress.

Expression analyses by real-time quantitative RT-PCR (qRT-PCR)

Differential expression was corroborated for 18 genes using qRT-PCR. Ten orthologues of genes previously

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Table 1. *The 50 most differentially expressed genes in maize roots in at least one time point of Pi-deficiency*

Target accession[a]	Identifier[b]	Annotation	Functional category	Expression ratio –P to +P			
				01 AOS	03 AOS	06 AOS	10 AOS
TC310218	MZ00019635	ABC transporter, similar to P-glycoprotein	Transport	1.28	2.89	9.14	14.81
CD963962	MZ00053670	NA	ND	1.16	3.73	13.19	10.46
TC291514	MZ00023032	Putative SPX domain containing protein	P responsive	1.88	3.98	11.34	12.71
TC316025	MZ00004285	Unknown protein	ND	1.12	2.06	10.28	12.39
AZM5_45963	MZ00049722	Putative MDR-like ABC transporter	Transport	1.49	1.46	11.99	9.48
TC312468	MZ00019914	NA	ND	1.09	1.21	8.78	11.67
AZM5_4343	MZ00002324	Inorganic phosphate transporter 5	P transport	1.50	1.30	9.45	11.19
TC302253	MZ00022483	Terpene synthase 9	Secondary MET	0.98	1.24	11.15	4.70
TC283710	MZ00041712	Glutathione *S*-transferase GST 8	Transferase	1.45	0.96	1.79	10.94
AW267592	MZ00001433	MDR-like ABC transporter	Transport	1.19	1.58	10.27	9.60
BG842460	MZ00036725	NA	ND	1.28	5.38	9.91	4.75
TC288826	MZ00027556	Putative glycerol-3-phosphate transporter	Transport	2.63	6.83	9.90	4.91
TC283557	MZ00048157	Hypothetical protein	ND	3.96	1.91	9.75	5.01
DN224579	MZ00030636	Hypothetical protein	ND	2.34	5.72	9.57	5.19
TC326784	MZ00000520	NA	ND	1.51	1.98	9.24	9.43
TC193450	MZ00041713	Glutathione *S*-transferase GST 8	Transferase	1.48	0.94	2.31	9.28
TC296017	MZ00027485	Putative NADPH dehydrogenase	Electron transport	0.90	1.39	7.79	9.01
BG836783	MZ00004115	NA	ND	1.68	1.40	8.87	8.64
TC317481	MZ00032776	Putative disease resistance response protein	Stress	1.29	2.94	7.72	8.81
TC307414	MZ00031671	Calcineurin-like phosphoesterase family protein	ND	1.14	3.18	6.91	8.68
TC279283	MZ00025934	Putative phosphate transporter 3	P transport	1.61	6.20	8.65	6.19
TC279827	MZ00019402	Catalase isozyme 2	Redox	1.84	1.99	3.60	8.41
CD997929	MZ00039365	Putative retrotransposon RIRE1 poly protein	Transposon	1.00	1.17	4.40	8.26
CD947059	MZ00053174	NA	ND	1.30	4.00	6.05	8.19
CF059395	MZ00011838	NA	ND	1.15	1.54	5.72	8.14
CF632788	MZ00012780	3-β-hydroxysteroid dehydrogenase	ND	1.76	3.05	8.10	7.31
TC328226	MZ00021935	Putative dual-specific kinase DSK1	Signalling	1.23	3.13	7.14	8.03
CO526902	MZ00001782	Hypothetical protein At4g32810 like	Hormone/development related	1.20	5.07	8.01	3.31
TC321119	MZ00023351	C2 domain domain-containing protein-like	Protein degradation	1.01	1.13	4.60	7.88
CF623138_root	MZ00033570	At3g07350 like	ND	1.50	2.24	3.77	7.80
TC313893	MZ00039150	Putative retrotransposon RIRE1 poly protein	Transposon	1.03	1.13	3.67	7.59
AZM5_11519	MZ00036021	Putative xyloglucan fucosyltransferase	Cell wall synthesis	1.15	0.98	4.53	7.55
AZM5_89028	MZ00001699	Acyl-CoA ligase-like	ND	0.96	2.90	6.51	7.48
TC330126	MZ00032932	Putative taxane 14β-hydroxylase	ND	1.20	1.18	7.41	7.02
BM380776	MZ00037128	NA	ND	1.34	1.48	6.51	7.35
AZM5_16433	MZ00050103	Pleiotropic drug resistance protein 15	Transport	1.55	5.67	7.28	4.76
TC302179	MZ00024914	Kaurene synthase A	Hormone related	0.89	2.56	7.26	3.86
AZM5_18438	MZ00002902	Putative replication factor	DNA synthesis/chromatin	1.85	4.05	7.25	5.70
TC290375	MZ00019908	Putative acid phosphatase	P recycling	1.19	2.97	7.17	4.91
TC319983	MZ00004936	Putative serine/threonine protein kinase	Signalling	1.43	1.98	1.89	7.15
AI691724	MZ00000607	Kelch repeat-containing protein	RNA regulation	1.19	0.95	4.86	7.11
TC303242	MZ00042780	Hypotetical protein	ND	2.34	5.36	7.04	3.31
TC310047	MZ00027962	Endonuclease/nucleic acid binding	DNA synthesis/chromatin	1.02	1.47	7.02	4.84
TC299213	MZ00041197	Gypsy-like retrotransposon	Transposon	0.88	0.57	0.09	0.55
TC292365	MZ00031519	NA	ND	0.73	0.35	0.09	0.17
TC310514	MZ00016926	Putative calcium-dependent protein kinase	Signalling	0.42	0.43	0.09	0.71
TC323200	MZ00009976	Putative pepper esterase	ND	0.96	0.66	0.13	0.56
TC310106	MZ00056992	NA	ND	0.42	0.33	0.13	0.60
CF055761	MZ00040180	Major facilitator superfamily antiporter	Transport	0.37	0.52	0.14	0.20
TC310257	MZ00013727	NA	ND	0.93	0.66	0.14	0.95

[a] Gene identifier.
[b] Maize oligonucleotide array identifier.
[c] False discovery rate values from *P*-Values obtained in both P*TIME and P statistical tests.
NA, not annotated; ND, not determined.

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reported as Pi-responsive in other plant species and eight that were not previously known to be regulated by Pi were included. The expression pattern observed in the microarray experiments was confirmed for the genes analysed (Fig. 3A); however, several genes presented a higher level of induction when determined by qRT-PCR than estimated by microarray analyses (Fig. 3B). Notably, genes encoding a sulpholipid synthase and a putative acid phosphatase showed the highest values of expression (fold values of 455 and 541, respectively). This suggests that microarray experiments are underestimating the levels of changes in expression. Similar quantitative differences have also been reported previously (Morcuende *et al*., 2007).

Metabolite determinations

To evaluate the degree to which changes in gene expression in maize roots are reflected in the corresponding metabolite products, lipid composition and anthocyanin content was measured at 6 d and 10 d AOS, because these metabolic pathways were among the most affected in both the level of differential expression and the number of affected genes. Pi-deprived maize roots showed a decrease of 30–50% in phospholipid content [phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC)]. By contrast, an increase of 2–4-fold was determined for sulphoquinovosyldiacylglycerol (SQDG), monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). Total anthocyanin content was also significantly increased in the roots of Pi-deprived maize plants (Table 2). These determinations demonstrate that the changes in expression are truly reflected at the metabolite level.

Metabolic pathway analysis

Analysis of the kinetics of gene induction and repression during Pi-deprivation led to the identification of genes

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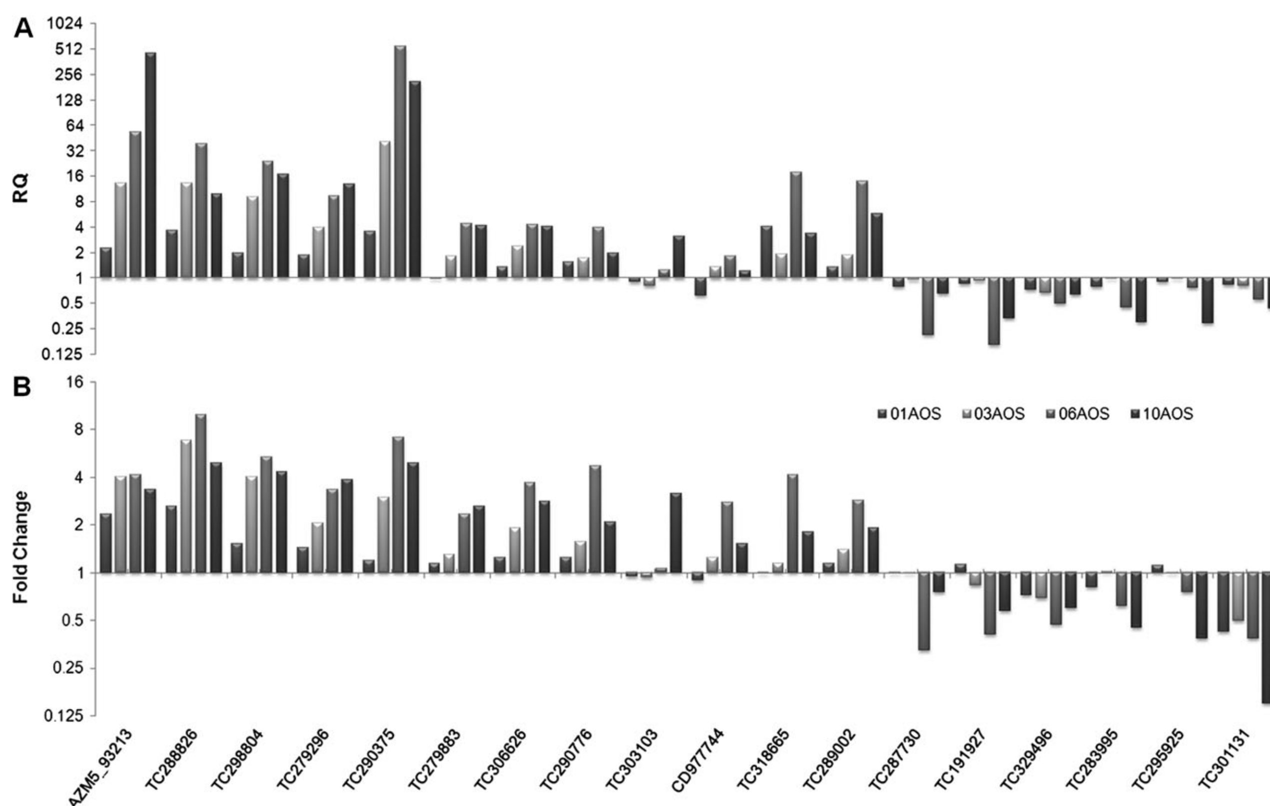

Fig. 3. Validation of the oligonucleotide microarray results by qRT-PCR. Fold change (–P to +P) for some selected genes at 1, 3, 6, and 10 d AOS in a log2 scale are shown. (A) Expression ratios obtained by qRT-PCR. RQ (relative quantification number) was obtained from the equation $(1+E)2^{\Delta\Delta C_T}$ where $\Delta\Delta C_T$ represents $\Delta C_T(-P)-\Delta C_T(+P)$, and E is the PCR efficiency. Each C_T was previously normalized using the expression levels of *UBQ2* (TC305418) as internal reference. Expression levels were obtained from four replicates. (B) Expression ratios obtained by microarray experiments. Estimates of the differences of expression levels were calculated using the mixed model as described in the Materials and methods. All indicated genes were selected to corroborate the expression patterns obtained in the microarray analysis and also within the threshold limits (FDR $\leqslant$5% and fold change $\pm$2 in any sampled time point). AZM5_93213, sulpholipid synthase. TC288826, glycerol-3-phosphate transporter. TC298804, SPX domain-containing protein. TC279296, inorganic phosphate transporter 1. TC290375, putative acid phosphatase. TC279883, inositol monophosphatase 3. TC306626, putative F-box protein family member. TC290776, putative laccase. TC303103, putative phosphoethanolamine methyltransferase. CD977744, putative phosphoenolpiruvate carboxylase. TC318665, chalcone synthase. TC289002, putative inorganic pyrophosphatase. TC287730, putative malate synthase. TC191927, auxin-repressed protein. TC329496, putative zinc finger transcription factor. TC283995, bZIP transcription factor family member. TC295925, high-affinity potassium transporter. TC301131, Cen-like protein FDR1.

Table 2. *Changes in metabolite levels (lipid and anthocyanin) contents of maize roots under Pi-deficiency. Lipid composition analyses were carried out for two independent experiments and anthocyanin contents for five replicates with similar results*

Metabolite	Low-P		High-P	
	06 AOS	10 AOS	06 AOS	10 AOS
Lipids (mol %)				
Monogalactosyldiacylglycerol	7.6	10.7	3.5	3.7
Digalactosyldiacylglycerol	18.6	23.4	10.1	8.7
Sulphoquinovosyldiacylglycerol	12.4	12.7	3.7	4.4
Phosphatidylethanolamine	14.9	15.5	27.6	29.3
Phosphatidylglycerol	5.8	4.9	7.0	10.2
Phosphatidylcholine	40.6	32.7	47.9	43.6
Anthocyanin[a] (mg g^{-1} dry root)	0.36	0.39	0.19	0.20

[a] Total anthocyanin content was determined from acidified methanolic extracts and expressed as cyanidine-3-glucoside.

involved in a large number of metabolic, developmental and signalling pathways (see Supplementary Fig. S1 at *JXB* online). We will focus on describing the most prominent specific pathways and categories affected by Pi starvation in maize roots. In addition, those responses are illustrated through drawing virtual metabolic pathways.

Carbon metabolism

In addition to previously reported Pi-starvation-responsive pathways, the list of differentially expressed genes was enriched in genes involved in carbohydrate, amino acid, and lipid metabolism (see Supplementary Fig. S2 at *JXB* online).

In terms of genes related to photosynthate partitioning it was found that the sucrose synthase 2 gene (*SUS2*) was up-regulated at day 1 AOS suggesting that an increase in the C supply toward the roots is one of the first responses of maize to Pi-deprivation (see Supplementary Fig. S3 at *JXB* online). Starch synthesis appears to be enhanced since two genes encoding ADP-glucose pyrophosphorylase (AGPase) and one encoding a glucose 6P/P translocator are induced at days 6 and 10 AOS, although a gene encoding the enzyme that participates in the last step of starch degradation, α-glucosidase 1, is also induced. Glycolysis is modified through bypassing reactions that require ATP, as reflected by the increased expression of genes encoding phosphoglycerate mutase (PGM), phosphoenol pyruvate carboxylase (PEPCase), and PEPcase kinase (PEPK), probably to supply the carbon skeletons necessary for the next intermediary reactions in C metabolism (i.e. the TCA cycle). Interestingly, eight PEPcase/PEPK encoding genes were induced at day 6 AOS, indicating that these enzymes may serve as key check points to direct carbon flow under Pi limitation in maize (see Supplementary Fig. S3 at *JXB* online). In addition, it was found that two glyoxysomal malate synthase (MS) encoding genes were significantly repressed; indicating that the carbon flow from β-oxidation (Cornah *et al.*, 2004) is attenuated in maize roots (see Supplementary Fig. S3 at *JXB* online).

Nitrogen metabolism

A significant repression of three nitrate reductase (*NR*) genes and one glutamine synthetase (GS1) encoding gene starting at day 3 AOS was observed in maize roots (Fig. 4). The effect of Pi-starvation on N metabolism was also reflected in a dynamic modification of both amino acid catabolism and anabolism. A degree of specificity for synthesis and/or degradation of amino acids during the Pi-deprivation progress was observed. Ten genes encoding enzymes involved in degradation of Pro, Tyr, Leu, Val, Trp, Gly, and Ser were found to be down-regulated, in parallel with an increase in the transcript levels of genes involved in the synthesis of Trp, Phe, Pro, Ser, and Cys. In addition, a constant down-regulation of genes involved in the synthesis of Leu, Ile, Val, Tyr, Hys, and Gly was observed (Fig. 4).

Protein synthesis and activation was less affected by Pi limitation: only one gene encoding the translation initiation factor 2B and one for a proline-tRNA ligase were down-regulated starting at day 1 AOS, whereas four genes (encoding a tRNA synthetase, a translation factor-like protein, and two 60S acidic P2 and S6 kinase-homolog ribosomal proteins) were up-regulated at day 6 AOS. The expression pattern of several genes encoding proteins related to post-translational modifications were also altered (see Supplementary Fig. S4 at *JXB* online).

Lipid metabolism

Figure 5 shows changes in the expression level of genes related to the lipid metabolism. In *Arabidopsis*, recycling of internal Pi involves sulpho/galactolipid synthesis and phospholipid degradation (Hammond *et al.*, 2004). Such degradation is mediated by a combination of phospholipases C (PLC) and D (PLD; Cruz-Ramirez *et al.*, 2006). Despite the fact that the microarray contained 36 oligos targeting PLD genes and five targeting PLC genes, no accumulation of these transcripts was observed. Instead, a strong induction was found of one gene encoding a phospholipase A2 and six glycerophosphodiesterases (GPDEs), all involved in phospholipid degradation (Fig. 5).

The impact of Pi limitation on lipid metabolism in maize roots is further reflected by the altered expression of 30 additional sequences corresponding to another set of unspecified lipases/hydrolases as well as other proteins related to fatty acid (FA) synthesis, β-oxidation, PA synthesis, TAG degradation, and lipid transfer activities (Fig. 5A). In total, the modification in expression of 59 putative lipid-related genes was identified. Under long-term

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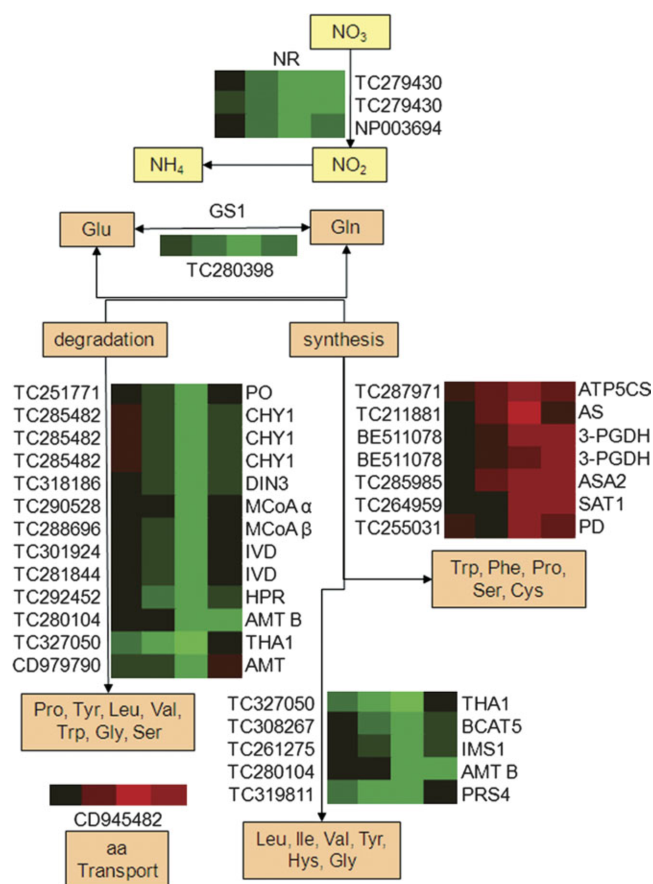

Fig. 4. Effects of Pi-deficiency on the transcript accumulation for genes encoding enzymes involved in primary pathways of nitrogen assimilation and amino acid metabolism in maize roots. Gene expression was monitored at 1, 3, 6, and 10 d AOS. Genes encoding the represented enzymes were identified by searching the functional annotation for *Arabidopsis* homologues using the MapMan software {Thimm, 2004, no. 66}. Gene identifiers correspond to the accession numbers from the corresponding databases as reported in the Maize Oligonucleotide Array Annotation GAL Files version 1.9 (http://www.maizearray.org/maize_annotation.shtml). Microarray data (ratio, –P to +P) were visualized using the FiRe 2.2 Excel macro {Garcion, 2006, no. 151}. A ratio ⩾10 is shown in red, a ratio ⩽0.1 in green. and no change (ratio=1) in black. Each four-square horizontal bar represents one maize accession number and the squares represent the ratios found at days 1, 3, 6, and 10 AOS, from left to right. NR, nitrate reductase. GS1, glutamine synthetase. PO, proline oxidase. CHY1, 3-hydroxyisobutyryl-CoA hydrolase/CoA-thioester hydrolase. DIN3, branched chain α-ketoacid dehydrogenase E2 subunit. MCoA-α, methylcrotonyl-CoA carboxylase α chain. MCoA-β, methylcrotonyl-CoA carboxylase β chain. IVD, isovaleryl-CoA-dehydrogenase. HPR, hydroxypyruvate reductase. THA1, L-allo-threonine aldolase. AMT, aminomethyl transferase. ATP5CS, delta-l-pyrroline-5-carboxylate synthetase. AS, asparagine synthase. 3-PGDH, 3-phosphoglycerate dehydrogenase. ASA2, anthranilate synthase. SAT1, serine *O*-acetyltransferase. PD, prephenate dehydratase. BCAT5, branched-chain amino acid transaminase 5. IMS1, 2-isopropylmalate synthase. AMT B, nicotianamine aminotransferase B. PRS4, phosphoribosyl diphosphate synthetase 4.

Pi starvation, maize roots seem also to be using P-inositol as a source of Pi (Fig. 5B). An increase in the transcript abundance of genes encoding inositol monophosphatase, phosphoinositide phosphatase, inositol-1,4, 5-trisphosphate-5-phosphatase-like proteins, and two myo-inositol-3-phosphate synthase enzymes was observed (Fig. 5B). The increased expression of these genes suggests that P-inositol could be degraded to release Pi to support other metabolic activities.

Transport and other enzyme families

Further metabolic modifications under Pi limitation are reflected by the activation and repression of several genes encoding members of protein families involved in secondary metabolism, such as several members of the large gene families encoding cytocrome P450, UDP-glucosyltransferase, peroxidases, and different oxidases (see Supplementary Table S2 at *JXB* online). These alterations are also observed in *Arabidopsis*, white lupin, and rice plants subjected to Pi starvation, thus indicating that their response may be ubiquitous in higher plants. Similarly, transport systems were strongly affected under Pi deficiency. As shown in Table S2 (see Supplementary Table S2 at *JXB* online), alterations in the transcript level of phosphate, sulphate, Fe, and ABC transporters as well as phytosiderophores, sugars, oligo-peptides, and aquaporin encoding genes was identified.

Secondary metabolism

A significant modulation of anthocyanin-related genes occurred under Pi starvation in maize roots, including the induction of *Bronze 2* (Marrs *et al.*, 1995), a gene encoding a transporter of anthocyanins to the vacuole (Fig. 6A). In addition, several putative genes related to the phenylpropanoid pathway and thus lignin biosynthesis were identified as up- or down-regulated (Fig. 6B). Differential changes were identified in the transcript level of genes encoding phenylalanine amonnia-lyase (PAL), 4-coumarate-CoA ligase (4CL), *N*-hydroxycinnamoyl/benzoyl transferase (HCT), caffeoyl-CoA 3-*O*-methyltransferase (CCoAOMT), caffeic acid *O*-methyltransferase (COMT), cinnamoyl-CoA reductase (CCR), cinnamyl alcohol dehydrogenase (CAD), and a member of the laccase gene family. Most of these were up-regulated, although, three HCT, two COMT, one CCR-encoding genes and one laccase-encoding gene were down-regulated (Fig. 6B). In agreement with our results, Uhde-Stone *et al.* (2003) also reported an increase in the transcript level of the gene encoding CCoAOMT and of the laccase in proteoid roots of white lupin under Pi starvation (Uhde-Stone *et al.*, 2003). These results allow us to draw a comprehensive panorama of lignin synthesis modifications in plants under Pi starvation (Fig. 6B).

Pi starvation and other stress responses

Among the responses identified in this work, the up-regulation of Pi-transport and Pi-recycling related genes was the most robust, extensive, and constant adaptation to

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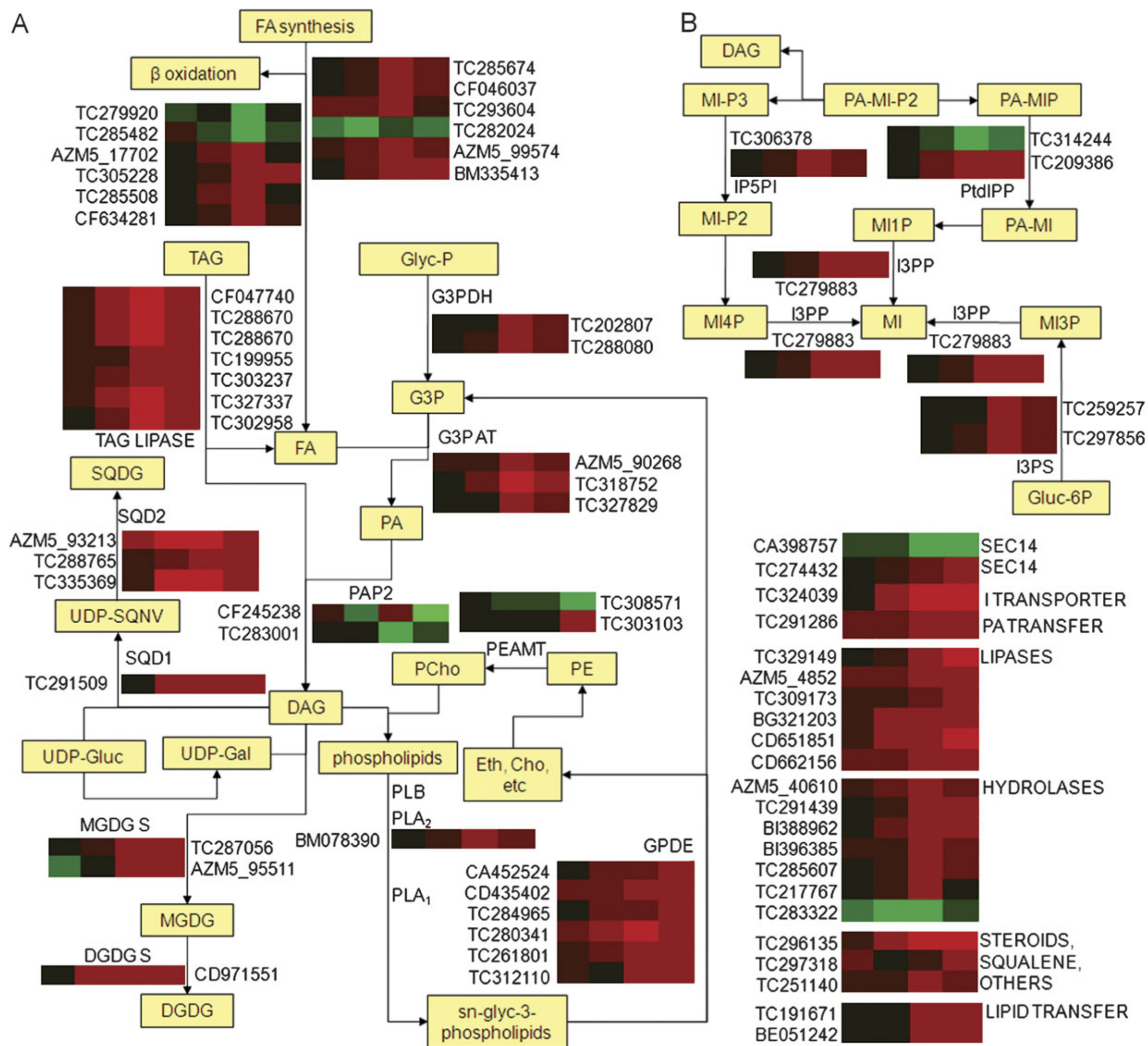

Fig. 5. Effects of Pi-deficiency on transcript accumulation for genes encoding enzymes involved in lipid metabolism (A) and inositol/myoinositol (B) metabolic pathways in maize roots. Gene expression was monitored at 1, 3, 6, and 10 d AOS. Genes encoding represented enzymes were identified and shown as described in Fig. 4. TAG, triacylglycerol. Glyc-P, glycerol-phosphate. G3P, glycerol-3-phosphate. FA, fatty acid. SQDG, sulphoquinovosyl diacylglycerol. PA, phosphatidic acid. UDP-SQNV, UDP-sulphoquinovose. DAG, diacylglycerol. PCho, phosphocholine. PE, phosphoethanolamine. UDP-Gluc, UDP-glucose. UDP-Gal, UDP-galactose. Eth, ethanolamine. Cho, choline. MGDG, monogalactosyldiacylglycerol. DGDG, digalactosyldiacylglycerol. GPDE, glycerophosphodiesterase. G3PDH, glyceraldehyde 3P dehydrogenase. G3PAT, glyceraldehyde 3P acyltransferase. SQD, UDP-sulphoquinovose synthase. PAP, phosphatidic acid phosphatase. PEAMT, PE methyl transferase. MGDG S, MGDG synthase. DGDG S, DGDG synthase. PLB, phospholipase B. PLA, phospholipase A. MI, myo-inositol, MI-P3, MI triphosphate. PA-MI-P2, phosphatidyl MI biphosphate. PA-MIP, phosphatidyl MI phosphate. PA-MI, phosphatidyl MI. MI1P, MI phosphate. MI3P, MI-3-phosphate. MI-P2, MI biphosphate. MI4P, MI-4 phosphate. I3PS, inositol 3P synthase. I3PP, inositol 3P phosphatase. PtdIPP, phosphatidyl inositol 3-phosphatase. IP5PI, inositol-triphosphate 5 phosphatase. SEC14, phosphatidylinositol-transfer protein.

Pi limitation (Fig. 7). Eight putative Pi-transporters, 26 different phosphatases, four ribonucleases, and 12 putative Mt4-like/SPX-domain encoding genes were induced almost throughout the whole experiment. In addition, 18 differentially regulated genes related to biotic and 13 to abiotic stress were identified. Other genes significantly modulated by Pi-starvation were those of the nodulin family (20 genes; see Supplementary Table S2 at *JXB* online). Taken together, these results suggest that intrinsic and common interactions among cell response pathways protect cells against toxic effects produced by different stress conditions.

Signalling/transcription related responsive genes

Of great interest is the finding that a large set of genes related to transcriptional regulation and hormone signalling was identified. The transcriptional regulation of the Pi-starvation responses in maize roots is reflected by the altered expression of 42 TFs (Table 3), of which 28 are induced and 14 repressed. These include bHLH, zinc

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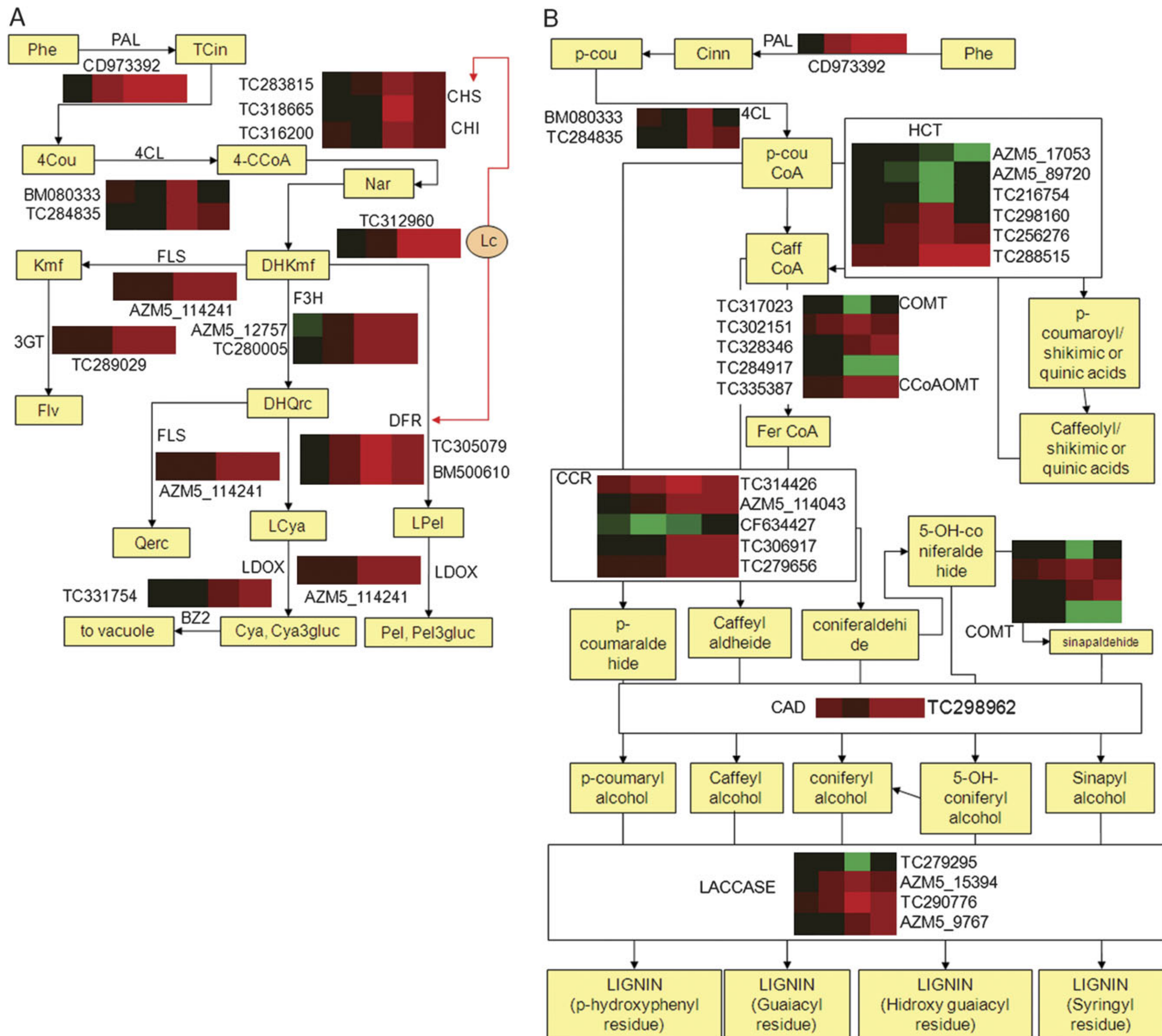

Fig. 6. Transcript accumulation in maize roots for secondary metabolism genes in maize roots under Pi starvation. (A) Genes encoding enzymes involved in anthocyanin synthesis. (B) Transcript profile of the lignin biosynthesis pathway in maize roots. Gene expression was monitored at 1, 3, 6, and 10 d AOS. Genes encoding represented enzymes were identified and shown as described in Fig. 4. TCin, transcinnamate. 4Cou, 4-coumarate. 4-CCoA, 4-coumaroyl CoA. Nar, naringenin. DHKmf, dihydrokaempferol. Kmf, kaempferol. Flv, flavonoids. DHQrc, dihydroquercetin. LCya, leucocyanidin. Qerc, quercetin. Lpel, leucopelargonidin. Cya, cyanidin. Cya3gluc, cyanidin-3-glucoside. Pel, pelargonidin. Pel3gluc, pelargonidin 3-glucoside. PAL, phenylalanine ammonia lyase. 4CL, 4-coumarate CoA ligase. CHS, chalcone synthase. CHI, chalcone isomerase. FLS, flavonol synthase. F3H, flavonoid-3-hydroxylase. 3GT, flavonol glucosyltransferase. LDOX, leucoanthocyanidin dioxygenase. BZ2, glutathione-*S*-transferase encoded by *Bronze2*. DFR, dihidroflavonol-4-reductase. LC, transcriptional activator leaf colour. *p*-cou, *p*-coumaraldehyde; Cinn, cinnamaldehyde; *p*-cou CoA, *p*-coumaroyl-CoA; Caff CoA, caffeoyl-CoA; Fer CoA, feruloyl-CoA; HCT, *N*-hydroxycinnamoyl/benzoyltransferase; COMT, caffeic acid *O*-methyltransferase, CCoAOMT, caffeoyl-CoA 3-*O*-methyltransferase; CCR, cinnamyl-CoA reductase, CAD, cinnamyl alcohol dehydrogenase.

finger, and leucine zipper families. An orthologue of *SCR* and one belonging to the MYB-family were the earliest TFs showing differential expression, starting at day 3 AOS. Likewise, significant up- or down-regulation of calcium, phosphoinositol, G-class and light signalling-proteins was observed. Eleven genes encoding protein kinases and receptor protein kinases were also differentially expressed. Among the hormone-related genes, a set of transcripts related to brassinosteroids, gibberellins, ethylene and cytokinins, abscisic acid and auxins were also up- or down-regulated (Fig. 8).

Discussion

Several reports on the identification of maize Pi uptake/use efficient genotypes were published several years ago (DaSilva and Gabelman, 1992; Hajabbasi and Schumacher, 1994; Gaume *et al.*, 2001; Fan *et al.*, 2003; Zhu and Lynch,

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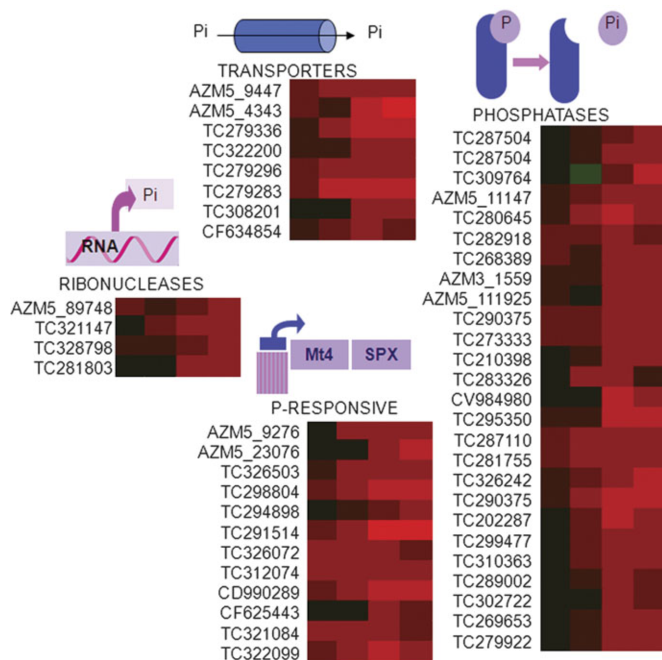

Fig. 7. Transcript accumulation in maize roots for characteristic Pi-deficiency responsive genes in plants. Gene expression was monitored at 1, 3, 6, and 10 d AOS. Genes displayed were identified and represented as described in Fig. 4. Gene identifiers are also shown.

2004; Corrales *et al*., 2007), however, the exploration of maize biodiversity as a potential source of target genes for crop breeding remained to be done. A previous study suggested that L3×228-3 possess an important P-uptake capacity (Corrales *et al*., 2007). The results presented here show that L3×228-3 is able to maintain root mass and increase root length even under low internal P concentrations (Fig. 1). In addition, it is shown that L3×228-3 displays a wide set of biochemical and transcriptome responses to P deprivation in roots.

The robustness of the experimental strategy permitted the expression kinetics of 1179 genes over four time points to be identified. Of these, 774 genes are potentially associated with or have a potential role in responses to P-status. In addition, we have uncovered 407 P-responsive maize genes with no significant hit to any annotated database, whose characterization could lead to the discovery of novel pathways, some of which could be monocotyledonous-specific, involved in mechanisms to cope with P starvation. An overall comparison of the proteome results reported by Li *et al*. (2007) with the results presented here shows that members of around 35% of the gene families identified by Li *et al*. (2007) as phosphate-responsive are also regulated at the transcript level (see Supplementary Table S3 at *JXB* online), however, for the other differentially accumulated proteins no statistically significant difference in transcript level between the controls and Pi-deprived maize plants were determined. Post-transcriptional regulation, as well as technical, genotypical, and experimental variations, may explain those differences. Such contrasts have previously been highlighted when comparing transcript expression levels between different Pi-related microarrays in *Arabidopsis* (Morcuende *et al*., 2007).

The results presented here also show that about 33% of the genes identified as Pi-responsive in maize do not have a significant match with an orthologue in the *Arabidopsis* genome, emphasizing the importance of global gene expression studies directly on crops of economic importance. However, 210 of the maize differentially regulated transcripts matched with 148 *Arabidopsis* orthologues that were also reported as Pi-starvation-regulated (see Supplementary Table S4 at *JXB* online), showing that there are many conserved molecular responses to P availability between maize and *Arabidopsis*.

Pi starvation integrates P, C, and N metabolism in maize roots

Under Pi limitation, L3×228-3 line showed higher Pi-uptake compared with inefficient lines (HS 2841×5046; Corrales *et al*., 2007). The results presented here suggest that the enhanced Pi uptake may be related to the increased transcription of genes encoding Pi transporter, phosphatase and ribonuclease (Fig. 7).

In addition, the identification of altered expression of several sugar-related genes, including those involved in photosynthate partition and glycolysis in maize roots under Pi starvation, supports the evidence of direct cross-talk between sugar metabolism and P stress in plants, as reported by Tesfaye *et al*. (2007) who noted a dark/light-directed expression of several sugar-sensing and metabolism genes in response to Pi stress in proteoid roots of white lupin (Tesfaye *et al*., 2007).

Organic acid synthesis and excretion have been documented in maize as a response to Pi starvation (Gaume *et al*., 2001). A significant induction of several genes encoding PEPCase was observed (see Supplementary Fig. S3 at *JXB* online), whose activity is needed to synthesize malate and citrate. However, no alterations in the transcript level of either malate dehydrogenase or malate/citrate transporters was observed, suggesting that in L3×228-3 roots PEPcase is the limiting step for organic acid synthesis or that the increased PEPcase expression may instead be supporting other C needs in the cell such as providing carbon to increase citrate/malate exudation. In this sense, it is possible that maize roots may be redirecting PEPcase products and other C pathways to provide C skeletons for amino acid synthesis. In fact, six genes involved in Trp, Phe, Pro, Ser, and Cys synthesis were induced (Fig. 4). Besides their structural importance, amino acid synthesis may control either directly or indirectly various aspects of plant growth: Trp is

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a precursor in auxin synthesis (Ljung *et al.*, 2005), Phe is the key substrate for many secondary plant metabolites including anthocyanins and lignin (Fig. 6; Noel *et al.*, 2005), Pro participates directly in cellular osmotic protection (Ashraf and Foolad, 2007), and Ser and Cys synthesis is part of the sulphate assimilation pathway (Kopriva, 2006). Although further research is needed in maize roots, synthesis of such amino acids fits into the framework of Pi responses, given that, under Pi starvation, alterations in root system architecture are probably mediated by auxin (Franco-Zorrilla *et al.*, 2004), a substantial increase of anthocyanins and lignin occurs, and sulpholipid synthesis may demand an increase in sulphate assimilation. The latter observation is further supported by the induction of at least one member of the sulphate transporter gene family (see Supplementary Table S2 at *JXB* online).

The ability of maize roots to preserve N- and C-containing metabolites under Pi starvation is notable. An extensive repression of genes involved in amino acid degradation was found together with modifications in both protein synthesis and degradation (Fig. 4; and see Supplementary Fig. S4 at *JXB* online). These results reflect a contrasting response between maize and *Arabidopsis* roots. In the latter species, modifications in the transcript abundance of genes involved in amino acid metabolism are limited, whereas general decreases in the expression of genes involved in amino acid and protein synthesis in concert with increases in the expression of amino acid and protein degradation genes were reported (see Supplementary Fig. S2 at *JXB* online; Wu *et al.*, 2003; Misson *et al.*, 2005; Morcuende *et al.*, 2007).

It was also found that genes involved in TAG breakdown and β-oxidation are induced upon Pi starvation. Induction of β-oxidation has been observed in sugar-starved detached maize root tips (Dieuaide *et al.*, 1992), presumably to provide C for gluconeogenesis (mediated by the glyoxylate cycle) and also substrates for energy production in mitochondria (Baker *et al.*, 2006). However, in maize roots under Pi starvation, the carbon flow from lipid catabolism towards gluconeogenesis may be attenuated, since the transcript levels of MS, a key enzyme of the glyoxylate cycle are significantly decreased (see Supplementary Fig. S3 at *JXB* online). The apparently contrasting regulation of TAG breakdown and β-oxidation with MS may indicate the turnover of membrane lipids in conjunction with the induction of FA synthesis (Fig. 5), avoiding C loss from the membrane lipid pool or to provide C intermediates to the TCA cycle for energy production during Pi starvation. These observations suggest that Pi starvation in maize induces changes in gene expression that promote flexible use of C skeletons through integrating carbohydrate, glycolysis, and TCA cycle modifications with those found in lipid metabolism. It is also worthwhile mentioning that β-oxidation mediates the activation of signalling molecules including the auxin indole acetic acid, as confirmed by the root phenotype found in mutants with β-oxidation defects (Baker *et al.*, 2006), thus implying that such modifications may produce signals to modify root architecture under Pi starvation.

P recycling

Despite the fact that Pi recycling in maize roots involves the substitution of phospholipids by non-phospholipids, the recycling in maize is distinct from that previously reported in *Arabidopsis* (Misson *et al.*, 2005; Cruz-Ramirez *et al.*, 2006). In maize, phospholipid degradation correlates with the increased transcription of GPDE and PLA coding genes while in *Arabidopsis* it has been shown to be mediated mainly by PLC and D. Moreover, two phosphatidic acid phosphatase 2 encoding genes were repressed, suggesting that the acyl moiety required for glycolipid synthesis is probably produced by TAG degradation (Fig. 5) and not by the direct degradation of phosphatidic acid (PA). In fact, PA synthesis could increase under Pi starvation in maize roots, as a result of the induction of genes encoding G3PDH and G3PAT (Fig. 5). The role of PA as a signalling molecule in root hair elongation and auxin sensitivity has been demonstrated (Li and Xue, 2007), therefore an increase in PA could be influencing changes in maize root architecture in response to Pi-deprivation.

Secondary metabolism

An interesting observation from this work is that anthocyanin accumulation in roots may be co-ordinated through the regulatory leaf colour (*LC*) gene, a TF known to regulate the expression of genes encoding chalcone synthase and dihydroflavonol 4-reductase (Dooner *et al.*, 1991). *LC* was among the most highly induced genes in Pi-deprived maize roots and is an orthologue of the rice transcription factor *OsPTF1*, which was reported to be involved in the tolerance to Pi starvation in rice (Yi *et al.*, 2005). Therefore, it is possible that *LC* might also regulate the expression of other genes involved in Pi-deficiency responses in maize.

The observed changes in lignin biosynthesis (Fig. 6) together with the differential expression of genes encoding other cell wall-degrading proteins such as glucanases, mannosidases, callose synthase, and polygaracturonidases (see Supplementary Table S2 at *JXB* online) suggest a dynamic rearrangement of cell wall structure during Pi starvation in maize roots. In addition, the larger number of Pi-responsive genes of the phenylpropanoid pathway in maize roots indicates that not only lignin synthesis but also the production of a wide range of metabolites derived from this pathway might be significantly altered. Interestingly, *ZRP4*-like genes (encoding *O*-methyltransferases) were found to be either induced or repressed. The

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Table 3. *TF genes responsive to Pi-deficiency in maize roots*

Target accession	Identifier[a]	Annotation	Expression ratio –P to +P				FDR adjusted *P*-value[b]	
			01 AOS	03 AOS	06 AOS	10 AOS	Time×P	P
TC312960	MZ00044487	bHLH anthocyanin Lc regulator	1.00	1.38	7.00	6.31	0.040	0.005
AZM5_85221	MZ00001874	SCARECROW transcription factor family	1.11	2.24	4.92	2.84	0.209	0.017
TC283257	MZ00034417	bHLH ENHANCER OF GLABRA3 like	1.03	0.87	4.35	3.91	0.018	0.006
TC291419	MZ00031274	CCAAT box binding factor HAP2C	1.14	1.28	3.45	2.40	0.021	0.002
TC307172	MZ00030906	Putative AP2/EREBP domain-containing protein	1.44	1.01	3.40	1.84	0.121	0.025
AZM5_55849	MZ00049881	MYB family transcription factor MYB54	1.43	2.35	3.34	2.99	0.626	0.021
CD989927	MZ00009758	Putative SHORT-ROOT transcription factor	0.96	1.28	3.24	1.93	0.045	0.009
TC284463	MZ00006258	Putative heat shock factor RHSF2	1.15	0.90	3.03	2.16	0.143	0.037
TC308677	MZ00019240	Putative zinc finger protein	1.12	1.26	2.96	1.92	0.035	0.003
TC299508	MZ00034215	MADS BOX floral homeotic APETALA 1	1.19	1.26	2.90	2.93	0.193	0.019
TC283462	MZ00018509	Homeodomain leucine zipper protein	1.18	1.45	2.85	1.56	0.199	0.022
TC311393	MZ00042451	Salt tolerance zinc finger protein	0.99	1.22	2.79	1.80	0.057	0.010
AZM5_8982	MZ00002773	Nucleoid DNA-binding protein cnd41-like	1.06	1.45	2.75	1.98	0.164	0.014
TC281091	MZ00016402	Leucine zipper homeobox/lipid-binding START	1.15	0.94	2.56	2.63	0.093	0.031
AZM5_4096	MZ00047215	Typical P-type R2R3 MYB protein	1.16	1.21	2.47	1.86	0.052	0.004
TC267178	MZ00032184	bZIP transcription factor domain	0.96	1.28	2.36	1.76	0.027	0.003
TC334933	MZ00040405	Putative transcription factor OsEIL2 like	0.94	1.16	2.32	3.37	0.012	0.002
CD442176	MZ00023897	DNA-binding protein GBP16	1.08	1.20	2.30	1.87	0.278	0.033
TC282273	MZ00031443	MYB family transcription factor (MYB4)	1.06	0.97	2.19	2.02	0.062	0.019
TC310894	MZ00000937	Putative bHLH084	1.14	1.09	2.13	3.72	0.041	0.007
AZM5_93008	MZ00050691	Putative transcription factor MYB protein	1.22	1.33	2.08	1.30	0.127	0.008
TC292689	MZ00020771	Putative bHLH	1.78	1.98	2.03	1.36	0.824	0.042
TC318137	MZ00041209	LIM domain protein WLIM-1	1.00	1.30	2.02	1.03	0.044	0.022
TC303896	MZ00006412	Similar to SCARECROW gene regulator	1.22	1.50	1.92	2.39	0.100	0.003
AZM5_31785	MZ00002784	Zinc finger putative TRANSPARENT TESTA 1	1.23	0.94	1.74	6.13	0.078	0.049
TC308755	MZ00033781	Zinc finger (C_2H_2 type) family protein	1.13	1.21	1.35	2.14	0.228	0.042
TC305707	MZ00051801	Putative MYB-related protein	0.77	0.85	1.01	2.61	0.034	0.463
TC280230	MZ00042156	Homeobox KNOTTED-1 like 1 (KNAT1) RS1	0.87	0.99	0.65	0.45	0.105	0.032
TC283995	MZ00026870	bZIP RISBZ5	0.81	1.02	0.62	0.45	0.102	0.017
TC309794	MZ00022225	Putative zinc finger protein	0.79	0.76	0.57	2.33	0.004	0.610

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Table 3. (*Continued*)

Target accession	Identifier[a]	Annotation	Expression ratio –P to +P				FDR adjusted *P*-value[b]	
			01 AOS	03 AOS	06 AOS	10 AOS	Time×P	P
AW066019	MZ00035496	Zinc finger embryo defective 2454	0.84	0.98	0.49	0.76	0.125	0.035
AZM5_12653	MZ00022960	Zinc finger Dof-type domain-containing protein	0.80	0.99	0.49	0.98	0.044	0.037
TC329496	MZ00018277	Zinc finger embryo defective 2454	0.72	0.69	0.47	0.60	0.254	0.002
TC318007	MZ00018276	Zinc finger embryo defective 2454	0.83	0.84	0.47	0.76	0.027	0.002
TC291323	MZ00020826	Zinc finger ascorbate oxidase promoter-binding protein	0.96	0.98	0.45	0.90	0.046	0.060
TC279569	MZ00014148	Zinc finger embryo defective 2454	0.73	0.82	0.45	0.60	0.078	0.003
DY685866	MZ00050532	MYB G2-like transcription factor family, GARP	1.02	0.95	0.37	0.65	0.039	0.016
TC279708	MZ00017022	REPRESSOR OF GA1–3, RGA	1.02	0.76	0.37	0.53	0.123	0.025
TC303241	MZ00018657	Auxin response factor 6 ARF6	1.05	0.88	0.33	1.18	0.031	0.110
AZM5_26861	MZ00049753	Putative AP2/EREBP domain containing protein	1.11	0.76	0.31	0.41	0.078	0.019
TC322291	MZ00029091	Zinc finger CONSTANS-like protein	0.90	0.76	0.31	0.51	0.152	0.015
TC288648	MZ00014272	MYB putative LHY protein	0.87	0.93	0.29	0.50	0.097	0.024

[a] Gene identifier.
[b] False discovery rate values from raw *P*-values obtained in both Time×*P* and *P* statistical tests.

development-driven expression of *ZRP4*-like genes occurs preferentially in cell elongation and maturation zones of young roots (Held *et al.*, 1993). This suggests that besides their specific metabolic role these genes may also participate in changes in root architecture, possibly to enhance nutrient conductivity in vascular cylinders.

Signalling and transcription related genes

Research in *Arabidopsis* has shown that the regulation of Pi-starvation responses involves a large set of signalling genes including *PHR1* (Rubio *et al.*, 2001), the *At4*/*Mt4* family (Shin *et al.*, 2006), miR399 (Franco-Zorrilla *et al.*, 2007), and *PHO2* (Bari *et al.*, 2006), but also PHO/SPX/EXS domain-containing proteins (Wykoff and O'Shea, 2001; Giots *et al.*, 2003). The presence of three *PHR1*-like genes in the maize genome and the up-regulation of two *Mt4*-like genes and eight PHO/SPX/EXS domain encoding genes in Pi-deprived maize roots (Fig. 7; Table 1), suggests that some of the mechanisms controlling Pi responses are conserved in maize and *Arabidopsis*. However, although previous studies have suggested that Pi-deficiency responses are well conserved in plants (Franco-Zorrilla *et al.*, 2004), it is striking that only 10 out of 42 maize Pi-responsive TF genes (Table 3) are also differentially regulated in *Arabidopsis*. These results suggest that some of the common responses to Pi-deprivation between *Arabidopsis* and maize are mediated by different TFs that activate a similar set of genes or that the response to Pi deprivation is significantly different between these plants species, as reflected by the higher number of differential genes identified in maize in comparison to *Arabidopsis*.

Changes in root system architecture

The genotype selected for this study showed marked developmental changes in its root architecture under Pi limitation, including an increase in shoot-borne and lateral root length but also changes in root-to-shoot weight ratios indicating its plasticity (Fig. 1). In this context, it is notable that several hormone-related genes were differentially regulated in the root of this maize genotype, including several encoding ARF and AUX/IAA TFs. In addition, orthologues to *SHORT-ROOT* and *SCR* TFs involved in determining meristem identity and thus in root morphology in both dicotyledons and monocotyledons were also differentially regulated (Nakajima and Benfey, 2002; Lim *et al.*, 2005). Similarly, several homologues were found of *ENHANCER OF GLABRA3*, *TRANSPARENT TESTA1*, *NAC*, *AP1*, and *AP2*, all reported as being involved in developmental processes such as lateral root

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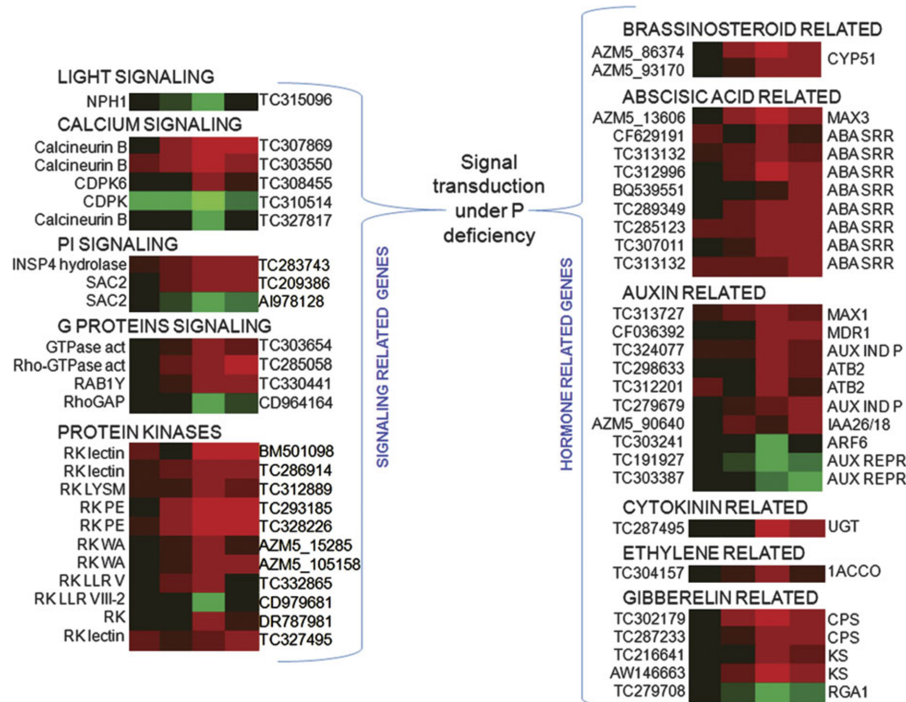

Fig. 8. Transcript profiling of signal transduction and hormone-related genes in maize roots under Pi deficiency. Gene expression was monitored at 1, 3, 6, and 10 d AOS. Genes encoding represented enzymes were identified and represented as described in Fig. 4. NPH1, non-phototropic hypocotyl protein 1. CDPK, calcium-dependent protein kinase. INSP4 hydrolase, diadenosine tetraphosphate hydrolase. SAC2, phosphoinositide phosphatase family protein SAC2. GTPase act, GTPase activating protein. RAB1Y, Ras-related GTP-binding protein. RhoGAP, RhoGAP domain-containing protein. RK lectin, lectin receptor kinase. RK LYSM, peptidoglycan-binding LysM domain-containing protein. RK PE, receptor kinase, proline-, extensin-like. RK WA, wall-associated receptor kinase. RK LLR V, leucine-rich repeat transmembrane protein kinase V. RK LLR VIII-2, leucine-rich repeat transmembrane protein kinase VIII-2. CYP51, cytochrome P450 (obtusifoliol 14-alpha demethylase). MAX, more axillary branches protein. ABA SRR, abscisic acid stress- and ripening-related protein. MDR1, multidrug resistance P-glycoprotein. AUX IND P, putative auxin-induced protein. ATB2, bZIP transcription factor ATB2 protein like. IAA26/18, auxin-responsive AUX/IAA 26/18 protein. ARF6, auxin response factor 6. AUX REPR, auxin-repressed protein. UGT, UDP-glucoronosyl/UDP-glucosyl transferase. 1ACCO, 1-aminocyclopropane carboxylate oxidase. CPS, copalyl diphosphate synthase. KS, kaurene synthase. RGA1, gibberellin response modulator 1.

emergence (Xie *et al.*, 2000, 2002; Hardtke, 2006). All of these genes may be acting as intermediates in the developmental responses to Pi-starvation and the task remains to clarify their roles, considering the remarkable differences between the developmental programs in maize and *Arabidopsis* root systems (Hochholdinger *et al.*, 2004).

Trehalose metabolism plays a key role in plant development (Ramon and Rolland, 2007). In *Arabidopsis*, crucial roles in embryo and vegetative development as well as in floral transition have been reported (Ramon *et al.*, 2007). The maize *RAMOSA3* (*RA3*), gene that encodes a trehalose-6-phosphate phosphatase (TPP) controls inflorescence architecture, and a mutation in this gene presents an altered branching organization (Satoh-Nagasawa *et al.*, 2006). The differential expression in response to Pi-deprivation of three TPP encoding genes, including two related to *RA3*, in maize roots suggests that trehalose-related enzymes may be regulators in root developmental responses, including those triggered by P starvation in maize. Interestingly, three TPP genes are also differentially regulated in *Arabidopsis* roots under Pi-starvation (Misson *et al.*, 2005).

The genes identified here and their proposed role in Pi adaptation, supported by an integral analysis of the data available from existing transcriptome, protein, and metabolite profiling experiments conducted in other species provide an opportunity to identify and functionally characterize different alleles involved in the adaptive response to Pi-deficiency and to identify candidate genes and processes whose manipulation may improve Pi-starvation tolerance in maize but also in other cereal crops. This work also provides the framework to produce Pi-specific maize arrays to study the changes in global gene expression between Pi-efficient and inefficient maize genotypes.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. List of PCR primers used for qRT-PCR and corresponding C_T values.

Table S2. Transcript levels of significant genes in the maize oligonucleotide microarray.

Table S3. Comparison of protein specificity under Pi deficit (Li *et al.*, 2007) with the gene expression changes for the respective gene family members.

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Table S4. Comparison of changes in expression levels of maize transcripts with those found for the corresponding *Arabidopsis* orthologues under Pi limitation.

Fig. S1. Functional classification of genes responsive to Pi-deficiency.

Fig. S2. Comparison of overall metabolic responses to Pi-deficiency reported for *Arabidopsis* with those found in maize roots.

Fig. S3. Effects of Pi-deficiency on the transcript accumulation of genes encoding enzymes involved in primary pathways of Carbon metabolism in maize roots.

Fig. S4. Effects of Pi-deficiency on the transcript accumulation of genes encoding enzymes involved in protein metabolism in maize roots.

Acknowledgements

We would like to thank June Simpson and José Lopez-Bucio for valuable help in reviewing the manuscript. We also thank Enrique Ramirez-Chavez for lipid analyses; Susana ML Fuentes-Guerra and Flor MX Zamudio-Hernandez for qRT-PCR analysis; Liu Jia from TIGR for microarray image analysis; EMBRAPA for maize lines; David Galbraith, Vicki Chandler, and Jack Gardiner at the BIO5 Institute at the University of Arizona who produced the arrays by the Microarray Resources for Maize Research Project supported by NSF DBI 0321663. Cheryl Vanier and J Burgueño for statistical advice. This work was supported in part by grants from SAGARPA (Zea-2006) and HHMI (Grant 55003677) to LH-E.

References

Ashraf M, Foolad MR. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* **59,** 206–216.

Baker A, Graham IA, Holdsworth M, Smith SM, Theodoulou FL. 2006. Chewing the fat: beta-oxidation in signalling and development. *Trends in Plant Science* **11,** 124–132.

Bari R, Datt Pant B, Stitt M, Scheible WR. 2006. PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiology* **141,** 988–999.

Barry D, Miller M. 1989. Phosphorus nutritional requirement of maize seedlings for maximum yield. *Agronomy Journal* **81,** 95–99.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B* **57,** 289–300.

Bucher M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytologist* **173,** 11–26.

Childs KL, Hamilton JP, Zhu W, Ly E, Cheung F, Wu H, Rabinowicz PD, Town CD, Buell CR, Chan AP. 2006. The TIGR Plant Transcript Assemblies database. *Nucleic Acids Research* **35,** D846–D851.

Cornah JE, Germain V, Ward JL, Beale MH, Smith SM. 2004. Lipid utilization, gluconeogenesis, and seedling growth in Arabidopsis mutants lacking the glyoxylate cycle enzyme malate synthase. *Journal of Biological Chemistry* **279,** 42916–42923.

Corrales I, Amenos M, Poschenrieder C, Barcelo J. 2007. Phosphorus efficiency and root exudates in two contrasting tropical maize varieties. *Journal of Plant Nutrition* **30,** 887–900.

Cruz-Ramirez A, Oropeza-Aburto A, Razo-Hernandez F, Ramirez-Chavez E, Herrera-Estrella L. 2006. Phospholipase DZ2 plays an important role in extraplastidic galactolipid biosynthesis and phosphate recycling in Arabidopsis roots. *Proceedings of the National Academy of Sciences, USA* **103,** 6765–6770.

Czechowski T, Bari RP, Stitt M, Scheible WR, Udvardi MK. 2004. Real-time RT-PCR profiling of over 1400 Arabidopsis transcription factors: unprecedented sensitivity reveals novel root- and shoot-specific genes. *The Plant Journal* **38,** 366–379.

DaSilva AE, Gabelman WH. 1992. Screening maize inbred lines for tolerance to low-P stress condition. *Plant and Soil* **146,** 181–187.

Devaiah BN, Karthikeyan AS, Raghothama KG. 2007*a*. WRKY75 transcription factor is a modulator of phosphate acquisition and root development in Arabidopsis. *Plant Physiology* **143,** 1789–1801.

Devaiah BN, Nagarajan VK, Raghothama KG. 2007*b*. Phosphate homeostasis and root development in Arabidopsis is synchronized by the zinc finger transcription factor ZAT6. *Plant Physiology* **145,** 147–159.

Dieuaide M, Brouquisse R, Pradet A, Raymond P. 1992. Increased fatty acid beta-oxidation after glucose starvation in maize root tips. *Plant Physiology* **99,** 595–600.

Dooner HK, Robbins TP, Jorgensen RA. 1991. Genetic and developmental control of anthocyanin biosynthesis. *Annual Review of Genetics* **25,** 173–199.

Duff SMG, Sarath G, Plaxton WC. 1994. The role of acid phosphatase in plant phosphorus metabolism. *Physiologia Plantarum* **90,** 791–800.

Essigmann B, Guler S, Narang RA, Linke D, Benning C. 1998. Phosphate availability affects the thylakoid lipid composition and the expression of SQD1, a gene required for sulfolipid biosynthesis in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **95,** 1950–1955.

Fairhurst T, Lefroy R, Mutert E, Batjes N. 1999. The importance, distribution and causes of phosphorus deficiency as a constraint to crop production in the tropics. *Agroforestry Forum* **9,** 2–8.

Fan M, Zhu J, Richards C, Brown KM, Lynch JP. 2003. Physiological roles for aerenchyma in phosphorus-stressed roots. *Functional Plant Biology* **30,** 493–506.

Fixen P. 2002. Soil test levels in North America. *Better Crops* **86,** 12–15.

Franco-Zorrilla JM, Gonzalez E, Bustos R, Linhares F, Leyva A, Paz-Ares J. 2004. The transcriptional control of plant responses to phosphate limitation. *Journal of Experimental Botany* **55,** 285–293.

Franco-Zorrilla JM, Valli A, Todesco M, Mateos I, Puga MI, Rubio-Somoza I, Leyva A, Weigel D, Garcia JA, Paz-Ares J. 2007. Target mimicry provides a new mechanism for regulation of microRNA activity. *Nature Genetics* **39,** 1033–1037.

Garcion C, Applimath FRI, Métraux JP. 2006. FiRe and microarrays: a fast answer to burning questions. *Trends in Plant Science* **11,** 320–322.

Gaume A, Mächler F, De Leon C, Narro L, Frossard E. 2001. Low-P tolerance by maize (*Zea mays* L.) genotypes: significance of root growth, and organic acids and acid phosphatase root exudation. *Plant and Soil* **228,** 253–264.

Gibson G, Wolfinger R. 2004. Gene expression profiling using mixed models. In: Saxton A, ed. *Genetic analysis of complex traits using SAS*. Cary, NC, USA: SAS Press, 251–278.

Giots F, Donaton MC, Thevelein JM. 2003. Inorganic phosphate is sensed by specific phosphate carriers and acts in concert with glucose as a nutrient signal for activation of the protein kinase A pathway in the yeast *Saccharomyces cerevisiae*. *Molecular Microbiology* **47,** 1163–1181.

Hajabbasi MA, Schumacher TE. 1994. Phosphorus effects on root growth and development in two maize genotypes. *Plant and Soil* **158,** 39–46.

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Hammond JP, Bennett MJ, Bowen HC, Broadley MR, Eastwood DC, May ST, Rahn C, Swarup R, Woolaway KE, White PJ. 2003. Changes in gene expression in Arabidopsis shoots during phosphate starvation and the potential for developing smart plants. *Plant Physiology* **132,** 578–596.

Hammond JP, Broadley MR, White PJ. 2004. Genetic responses to phosphorus deficiency. *Annals of Botany* **94,** 323–332.

Hardtke CS. 2006. Root development: branching into novel spheres. *Current Opinion in Plant Biology* **9,** 66–71.

Hartel H, Dormann P, Benning C. 2000. DGD1-independent biosynthesis of extraplastidic galactolipids after phosphate deprivation in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **97,** 10649–10654.

Held BM, Wang H, John I, Wurtele ES, Colbert JT. 1993. An mRNA putatively coding for an *O*-methyltransferase accumulates preferentially in maize roots and is located predominantly in the region of the endodermis. *Plant Physiology* **102,** 1001–1008.

Hernandez G, Ramirez M, Valdes-Lopez O, *et al*. 2007. Phosphorus stress in common bean: root transcript and metabolic responses. *Plant Physiology* **144,** 752–767.

Hochholdinger F, Woll K, Sauer M, Dembinsky D. 2004. Genetic dissection of root formation in maize (*Zea mays*) reveals root-type specific developmental programmes. *Annals of Botany* **93,** 359–368.

Hodge A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist* **162,** 9–24.

Irizarry R, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4,** 249–264.

Kopriva S. 2006. Regulation of sulfate assimilation in Arabidopsis and beyond. *Annals of Botany* **97,** 479–495.

Leakey AD, Uribelarrea M, Ainsworth EA, Naidu SL, Rogers A, Ort DR, Long SP. 2006. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. *Plant Physiology* **140,** 779–790.

Li G, Xue HW. 2007. Arabidopsis PLDzeta2 regulates vesicle trafficking and is required for auxin response. *The Plant Cell* **19,** 281–295.

Li K, Xu C, Zhang K, Yang A, Zhang J. 2007. Proteomic analysis of roots growth and metabolic changes under phosphorus deficit in maize (*Zea mays* L.) plants. *Proteomics* **7,** 1501–1512.

Lim J, Jung JW, Lim CE, Lee MH, Kim BJ, Kim M, Bruce WB, Benfey PN. 2005. Conservation and diversification of SCARECROW in maize. *Plant Molecular Biology* **59,** 619–630.

Ljung K, Hull AK, Celenza J, Yamada M, Estelle M, Normanly J, Sandberg G. 2005. Sites and regulation of auxin biosynthesis in Arabidopsis roots. *The Plant Cell* **17,** 1090–1104.

López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo M, Simpson J, Herrera-Estrella L. 2002. Phosphate availability alters architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. *Plant Physiology* **129,** 244–256.

Lynch J. 1995. Root architecture and plant productivity. *Plant Physiology* **109,** 7–13.

Marrs KA, Alfenito MR, Lloyd AM, Walbot V. 1995. A glutathione *S*-transferase involved in vacuolar transfer encoded by the maize gene Bronze-2. *Nature* **375,** 397–400.

McLaren JS. 2005. Crop biotechnology provides an opportunity to develop a sustainable future. *Trends in Biotechnology* **23,** 339–342.

Misson J, Raghothama KG, Jain A, *et al*. 2005. A genome-wide transcriptional analysis using *Arabidopsis thaliana* Affymetrix gene chips determined plant responses to phosphate deprivation. *Proceedings of the National Academy of Sciences, USA* **102,** 11934–11939.

Morcuende R, Bari R, Gibon Y, *et al*. 2007. Genome-wide reprogramming of metabolism and regulatory networks of Arabidopsis in response to phosphorus. *Plant, Cell and Environment* **30,** 85–112.

Nakajima K, Benfey PN. 2002. Signaling in and out: control of cell division and differentiation in the shoot and root. *The Plant Cell* **14,** S265–276.

Noel JP, Austin MB, Bomati EK. 2005. Structure–function relationships in plant phenylpropanoid biosynthesis. *Current Opinion in Plant Biology* **8,** 249–253.

Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. 1999. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research* **27,** 29–34.

Pietrini F, Iannelli MA, Massacci A. 2002. Anthocyanin accumulation in the illuminated surface of maize leaves enhances protection from photo-inhibitory risks at low temperature, without further limitation to photosynthesis. *Plant, Cell and Environment* **25,** 1251–1259.

Pingali PL, Pandey S. 2001. Part 1: Meeting world maize needs: technological opportunities and priorities for the public sector. In: Pingali PL, ed. *CIMMYT 1999–2000 world maize facts and trends. meeting world maize needs: technological opportunities and priorities for the public sector*. Mexico, D.F: CIMMYT, 1–24.

Plénet D, Etchebest S, Mollier A, Pellerin S. 2000. Growth analysis of maize field crops under phosphorus deficiency. I. Leaf growth. *Plant and Soil* **223,** 117–130.

Raghothama KG. 1999. Phosphate acquisition. *Annual Review of Plant Physiology and Plant Molecular Biology* **50,** 665–693.

Ramon M, Rolland F. 2007. Plant development: introducing trehalose metabolism. *Trends in Plant Science* **12,** 185–188.

Ramon M, Rolland F, Thevelein JM, Van Dijck P, Leyman B. 2007. ABI4 mediates the effects of exogenous trehalose on Arabidopsis growth and starch breakdown. *Plant Molecular Biology* **63,** 195–206.

Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, Paz-Ares J. 2001. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes and Development* **15,** 2122–2133.

Satoh-Nagasawa N, Nagasawa N, Malcomber S, Sakai H, Jackson D. 2006. A trehalose metabolic enzyme controls inflorescence architecture in maize. *Nature* **441,** 227–230.

Shin H, Shin HS, Chen R, Harrison MJ. 2006. Loss of At4 function impacts phosphate distribution between the roots and the shoots during phosphate starvation. *The Plant Journal* **45,** 712–726.

Tesfaye M, Liu J, Allan DL, Vance CP. 2007. Genomic and genetic control of phosphate stress in legumes. *Plant Physiology* **144,** 594–603.

Theodorou ME, Plaxton WC. 1993. Metabolic adaptations of plant respiration to nutritional phosphate deprivation. *Plant Physiology* **101,** 339–344.

Thimm O, Blasing O, Gibon Y, Nagel A, Meyer S, Kruger P, Selbig J, Muller LA, Rhee SY, Stitt M. 2004. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *The Plant Journal* **37,** 914–939.

Uhde-Stone C, Zinn KE, Ramirez-Yanez M, Li A, Vance CP, Allan DL. 2003. Nylon filter arrays reveal differential gene expression in proteoid roots of white lupin in response to phosphorus deficiency. *Plant Physiology* **131,** 1064–1079.

Usuda H, Shimogawara K. 1991. Phosphate deficiency in maize. II. Enzyme activities. *Plant and Cell Physiology* **32,** 1313–1317.

Wasaki J, Shinano T, Onishi K, *et al*. 2006. Transcriptomic analysis indicates putative metabolic changes caused by manipulation of phosphorus availability in rice leaves. *Journal of Experimental Botany* **57,** 2049–2059.

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Wasaki J, Yonetani R, Kuroda S, *et al*. 2003. Transcriptomic analysis of metabolic changes by phosphorus stress in rice plant roots. *Plant, Cell and Environment* **26,** 1515–1523.

Welti R, Li W, Li M, Sang Y, Biesiada H, Zhou HE, Rajashekar CB, Williams TD, Wang X. 2002. Profiling membrane lipids in plant stress responses. Role of phospholipase D alpha in freezing-induced lipid changes in Arabidopsis. *Journal of Biological Chemistry* **277,** 31994–32002.

Wolfinger RD, Gibson G, Wolfinger ED. 2001. Assessing gene significance from cDNA microarray expression data via mixed models. *Journal of Computational Biology* **8,** 625–637.

Wu P, Ma L, Hou X, Wang M, Wu Y, Liu F, Deng XW. 2003. Phosphate starvation triggers distinct alterations of genome expression in Arabidopsis roots and leaves. *Plant Physiology* **132,** 1260–1271.

Wykoff DD, O'Shea EK. 2001. Phosphate transport and sensing in *Saccharomyces cerevisiae*. *Genetics* **159,** 1491–1499.

Xie Q, Frugis G, Colgan D, Chua NH. 2000. Arabidopsis NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. *Genes and Development* **14,** 3024–3036.

Xie Q, Guo HS, Dallman G, Fang S, Weissman AM, Chua NH. 2002. SINAT5 promotes ubiquitin-related degradation of NAC1 to attenuate auxin signals. *Nature* **419,** 167–170.

Yang YH, Dudoit S, Luu P, Lin DM, Peng V, Ngai J, Speed TP. 2002. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Research* **30,** e15.

Yi K, Wu Z, Zhou J, Du L, Guo L, Wu Y, Wu P. 2005. OsPTF1, a novel transcription factor involved in tolerance to phosphate starvation in rice. *Plant Physiology* **138,** 2087–2096.

Zhu J, Lynch JP. 2004. The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays*) seedlings. *Functional Plant Biology* **31,** 949–958.

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