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Transcriptome analysis of potato phosphorus-tolerant variety seedlings (Atlantic) revealing the gene expression profile under low phosphorus stress

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Abstract

Phosphorus (Pi) is one of several essential plant macronutrients. Potato is the fourth largest food crop in the world, and potato tuber quality is significantly affected by Pi. The potato variety "Atlantic" has been reported to have a strong tolerance to low Pi conditions; however, the molecular mechanism by which this tolerance occurs remains unclear. In this study, we used 454 GS FLX sequencing technology to investigate the mixed transcriptional profile of this tolerant potato variety after 3, 6, 12, and 24 h of exposure to Pi-deficient conditions. A final Pi concentration of treatment was 10 μM. A total of 29,563 unigenes were assembled after sequencing. Of them, 27,255 (92.2%) were annotated using multiple public protein and nucleotide databases. They were categorized into 33 GO functional groups and 220 KEGG pathways, and 5,361 unigenes were assigned to 24 COG groups. Additionally, 733 simple sequence repeats (SSRs) were identified. Real-time PCR was performed to confirm the validity of the sequencing data. Furthermore, we have found 5 most important genes that are active under Pi deficiency such as ribulose diphosphate carboxylase, ribosomal protein, photosystem I and II reaction center, elongation factor and glycolytic enzymes . Our study is the first comprehensive transcriptome analysis of a Pi-tolerant potato variety under low Pi stress. Our results provide useful information that may facilitate further research of important genes involved in plant adaptation to low Pi conditions.

Keywords: Potato; Transcriptome; 454 GS FLX Sequencing; Pi.
Abbreviations: BLAST_Basic Local Alignment Search Tool; GO_Gene Ontology; COG_Cluster of Orthologous Groups of proteins; KEGG_Kyoto Encyclopaedia of Gene and Genomes; Pi _ Phosphorus.

Introduction

Phosphorus (Pi) is an essential macronutrient involved in many physiological and metabolic processes. Thus, it is a limiting factor for plant growth and development. Although it is abundant in nature, its concentration in most soils rarely exceeds 2 mM to 10 mM because it exists in fixed forms, which restrict its diffusion and mass flow to the rhizosphere (Hammond and White, 2008). However, cellular Pi concentrations are greater than 10 mM; thus, plants waste significant energy acquiring it against a steep concentration gradient. The large input of Pi from fertilizer is not only expensive and unsustainable, but it also causes environmental pollution and destroys the ecological balance. Plants have evolved in numerous ways to accommodate Pi deficiency, including increasing the root/shoot ratio, promoting organic acid synthesis and secretion, and enhancing the expression of acid phosphatases and high-affinity phosphate transporters (Plaxton and Tran, 2011). In recent years, important mutants have been associated with defects in Pi stress responses. The *pho1* mutant lacks the ability to transport Pi from the root to shoot. Therefore, PHO1 is probably involved in Pi transport into the xylem (Hamburger et al., 2002; Wang et al., 2004). Molecular cloning of *PHO2* has suggested that it encodes an E2 conjugase (Aung et al., 2006), and additional studies have shown that micro RNA399 indirectly regulates *PHO2*, participating in a phosphate-signalling pathway with *PHR1* (Bari et al., 2006). PHR1, an MYB transcription factor, can bind to an 8 base pair (bp) sequence (GNATATNC) in the promoters of genes induced by Pi starvation, including micro RNA399 (Bari et al., 2006). This finding indicates that PHR1 has a regulatory role in the Pi-signalling pathway (Vicente et al., 2001). SIZ1 encodes an SUMO E3 ligase, and *siz1* produces more lateral roots, root hairs, and anthocyanins than wild-type plants, suggesting that protein sumoylation affects many genes involved in Pi starvation responses (Miura et al., 2005). These mutants provide vital clues that aid in the understanding of the molecular mechanisms of plant adaptation to Pi deficiency.The Roche 454 Genome Sequence (GS) FLX platform enables high-throughput sequencing to allow for the characterization of the transcriptomes of various tissues. To date, 454 pyrosequencing has been widely used for transcriptome analysis of non-model species (Liu et al., 2012). The 454 GS FLX sequencing technology has increased the availability of expressed sequence tag (EST)-based resources. This technique not only permits the successful identification of a large number of high-abundance transcripts, but it can also uncover many rare transcripts (Liu et al., 2010).Potato (*Solanum tuberosum*) is the fourth most important crop in the world after wheat, rice, and corn. The yield and quality of potato tubers are significantly affected by Pi (Rosen and Bierman, 2008). Unlike other plants, potato does not have a conventional breeding cycle because it reproduces asexually. Several important genes, including *StPT1*, *StPT2*, and *StPT3*, have been shown to be involved in increasing Pi absorption (Leggewie et al., 1997; Gordon et al., 2003). Three purple acid phosphatases from potato have been cloned, and two of the transcripts, *StPAP2* and S*tPAP3*, are the most abundant in Pi-deprived roots. This result suggests that these genes are involved in the response to Pi stress (Zimmermann et al., 2004). Results obtained from potato oligonucleotide arrays have revealed that 1,659 genes are significantly differentially expressed following Pi

withdrawal using a hydroponic method (Hammond et al., 2011). A previous report has suggested that the "Atlantic" potato variety has a strong tolerance to low Pi levels (Yang et al., 2011). After treatment of this potato with 0.06 μm/L Pi for 40 days in Hoagland nutrient solution, its lateral root numbers and the guaiacol peroxidase (POD) and catalase (CAT) activities remained relatively stable compared with controls. To elucidate the molecular mechanism underlying this phenomenon in the "Atlantic" variety, we used 454 GS FLX sequencing technology to investigate the mixed transcriptional profile of seedlings after 3, 6, 12, and 24 h of exposure to Pi-deficient conditions. In this study, 293,206 ESTs were generated by sequencing, and 29,563 unigenes were assembled and classified according to Gene Ontology (GO), Clusters of Orthologous Groups (COG), and the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database. Real-time PCR was used to confirm the sequencing data. The large number of unigenes identified in our study constitutes an important resource for future investigations into the molecular biology and functional genomics of potato.

Results and Discussion

454 sequencing and assembly

A potato seedling cDNA library was constructed using SMART technology from a pool of mRNA extracted from samples exposed to low Pi conditions for 3, 6, 12, and 24 h. The library was sequenced on a 454 GS FLX Titanium platform with a one-quarter plate run after initial quality filtering with default parameters. This run yielded 293,206 high-quality (HQ) sequence reads. An overview of the sequencing and assembly processes are shown in Table 1. After removing sequences shorter than 50 bp, a total of 179,031 reads with an average length of 337 bp were used in the assembly. Overall, 24,494 (82.8% of the total) unigenes had lengths of over 200 bp, which is the widely accepted minimum cutoff for validity (Dassanayake et al., 2009).

Functional annotation

Our annotation approach was based on database sequence similarity searches. The aim of this approach was to find potential genes involved in tolerance to low Pi. Public protein and nucleotide databases (SwissProt, KEGG, TAIR, Nr, Nt, and PGSC) were used to annotate all unigenes. A total of 27,255 unigenes (92.2%) had at least one hit in these databases with an e-value threshold of $\leq 10^{-5}$. However, the remaining non-annotated unigenes (7.8%) may have relevant biological functions that have not yet been investigated. These genes require further investigation to confirm their functions. A total of 29,563 annotated sequences were categorized into 33 functional groups (Fig. 1) by GO analysis. These groups were divided into the categories cellular component, molecular function and biological process, which contained 8, 13 and 12 groups, respectively. The unigene sequences were submitted to COG searches to obtain protein annotations. Of the 29,563 unigenes, 5,361 were assigned to 24 COGs (Fig. 2). The group "translation, ribosomal structure, and biogenesis" (1,535 sequences, 28.63%) was the largest cluster, while "cell motility" and "nuclear structure" contained the fewest genes (2 each). Next, a total of 29,563 annotated sequences were mapped to 220 KEGG pathways. The top 24 KEGG pathways are shown in Supplementary table 2. Of them, energy metabolism (21.65%), carbohydrate metabolism (19.3%) and translation (13.83%) predominated. These results indicate that a variety of metabolic processes and genetic information processing pathways are switched on after the induction of Pi stress. In short, these metabolic pathways provide valuable information about potato gene functions.

Most highly expressed unigenes in potato under low Pi stress

The expression level of a unigene can be estimated based on its abundance after assembly. We found that a total of 40 unigenes had numbers of greater than 120 (Table 3). The ribulose bisphosphate carboxylases were the most highly expressed at 5,781, followed by ribosomal proteins (2,872) and elongation factors (1,623), suggesting that the expression of genes related to photosynthesis and transcriptional processes is altered to enable potato to adapt to low Pi stress. However, a previous report has suggested that Pi-dependent transcriptional changes in photosynthetic genes occur secondarily because their expression levels remain unchanged after the re-addition of Pi (Morcuende et al., 2007). Expression of ribosomal proteins and elongation factors have been shown to decrease after exposure to low Pi conditions for 28 days (Hammond et al., 2011), indicating that a long period of low Pi-induced stress suppresses gene transcription and protein synthesis. Pi-related stress and sugar metabolism are closely related, and many genes involved in glycolysis are up-regulated in response to this type of stress (Muller et al., 2007). A total of 1,536 glycolysis-related genes were identified in this study, in addition to 833 involved in the ubiquitin/26S proteasome pathway. The expression of genes involved in protein degradation is known to increase during Pi starvation in *Arabidopsis* (Wu et al., 2003; Misson et al., 2005), suggesting that the ubiquitin proteasome pathway functions early in the stress response to help plants to adjust their survival strategies. A previous study has revealed that in *Arabidopsis*, the altered expression of a chitinase is the most specific response to low Pi stress and Pi recovery (Woo et al., 2012). In the present study, 280 unigenes were identified for chitinase, demonstrating the abundance of this enzyme during Pi stress. Another study has demonstrated that ferritins respond to abiotic stress (Briat, 1996), but our current study is the first to report that 146 ferritin genes are likely involved in the response to Pi deficiency in potato.

Analysis of genes putatively involved in Pi stress in potato

Modern transcriptional sequencing technologies have revealed that Pi deficiency alters normal gene expression patterns, including the expression of genes that encode proteins involved in Pi acquisition, remobilization, metabolism, and signal transduction (Fang et al., 2009). In this study, we used 454 GS FLX sequencing technology to investigate the mixed transcriptional profile of a Pi deficiency-tolerant potato variety. Although we only sequenced a mixed sample after exposure to Pi deficiency, we were still able to obtain important gene expression information. Our process is similar to that used to elucidate key genes involved in cold tolerance in the physic nut (*Jatropha curcas L.*) by transcriptome sequencing of a mixed sample of untreated and cold-treated seedlings (Wang et al., 2014). Transcriptome analysis of one black pepper root has identified the functions of many important genes involved in biotechnological breeding, the pathogen response and defence processes (Cordo et al., 2012). Taken together, these studies demonstrate that transcriptome sequencing is a rapid and efficient method to facilitate research on gene functions (Wang et al., 2010).To confirm the accuracy of the sequencing data obtained in this study, twelve highly expressed genes during low Pi stress were further examined by quantitative real-time PCR (qRT-PCR) (Fig. 3). The results allowed for the classification of the expression patterns of these 12 unigenes into two groups. The first group included 10 genes that were

Table 1 . Summary of sequencing and assembly results.

Items	Number
Sequencing reads before preprocessing	
Number of high –quality(HQ) reads	293206
Average length of HQ reads	216.85
Total length (bp)	69,573,438
Reads after trimming and preprocessing	
Number of reads used for assembly	179,031
Number of unigenes	29563
Unigenes above 200bp	24,494
Average length of unigene (bp)	337.13
Total length of unigene (bp)	9,966,606

significantly up-regulated within the first 6 h of treatment, and the second included 2 genes related to ubiquitin pathways that were significantly up-regulated after 6 h of treatment. The transcription factors BZIP and AP2/ERF are important in plant responses to Pi deficiency (Morcuende et al., 2007; Woo et al., 2012). Results obtained from ATH1 arrays have demonstrated that *AtBZIP12* is up-regulated by 3.44-fold after exposure to low Pi conditions for 7 days (Morcuende et al., 2007).In the current study, the abundance of unigenes for ATHB-15, a BZIP transcription factor, was 104, and that for the AP2/ERF transcription factor was 90. Because transcripts encoding the two types of transcription factors were highly represented in the assembly data, we speculated that many target genes were regulated by these proteins during Pi stress. OsPTFl overexpression is known to enhance tolerance to low phosphate conditions in rice (Yi et al., 2005), in which a unigene encoding PTF1 has been identified. Therefore, we speculate that this transcription factor has the same function in potato as in rice. This assumption should be confirmed through further experiments. In addition, we identified 1 WRKY, 9 MYB, and 10 ERF transcription factor unigenes in the current study. The involvement of aquaporin in drought and freezing tolerance in tobacco and *Arabidopsis* has been previously studied (Zhang et al., 2008; Peng et al., 2008). A total of 1,187 aquaporins were found in our current study, and their expression continuously increased at all four time points. This is the first report of the involvement of aquaporins in the response to low Pi stress. A similar expression profile had been previously observed for the auxin-induced protein 5NG4. Auxin induces cluster-root formation in white lupin under stress induced by Pi deficiency (Meng et al., 2013). Cytochrome P450-regulated auxin synthesis in *Arabidopsis* has previously been reported (Bak et al., 2001), and 245 unigenes for P450 were found in our study. Thus, we propose that P450 may be involved in Pi deficiency-induced stress by modulating root growth to some extent. Cyclophilin expression in potato has been shown to be induced by abiotic and biotic stresses (Godoy et al., 2000). OsCYP2 participates in auxin signal transduction and the promotion of lateral root formation (Kang et al., 2013). In contrast, plants overexpressing OsCYP2 display increased tolerance to salt stress compared with wild-type seedlings (Ruan et al., 2011). We therefore hypothesize that the 421 cyclophilin unigenes play important roles in stress caused by Pi deficiency. Similar to other abiotic stresses, Pi deficiency elevated the reactive oxygen species (ROS) levels (Torres et al. 2005). Many genes related to ROS (including 386 PODs, 184 CATs, 23 superoxide dismutases, 2 polyphenol oxidases, and 8 glutathione reductases) were found, supporting the credibility of the transcriptome sequencing data. *TFT6* and *TFT7*, two members of tomato 14-3-3, have important roles in the response to Pi deficiency (Xu et al., 2012). In this study, 82 14-3-3 protein unigenes were identified. Sugar transporter

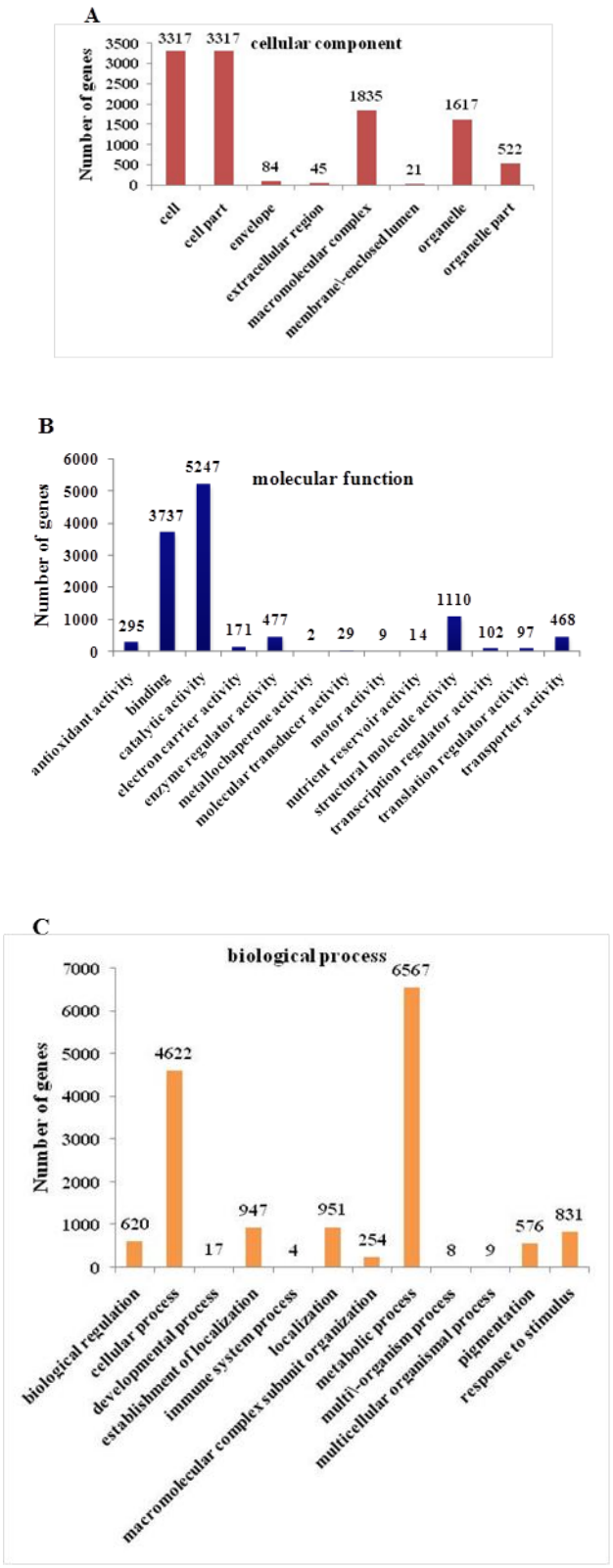

Fig 1. Gene ontology classification of all annotated sequences. Note: A functional annotation and number of putative transcripts in the "cellular component" class;B information regarding putative transcripts in the "molecular function" class; C information regarding putative transcripts in the "biological process" class.

Table 2. Summary of SSRs identified in transcripts.

Repeat composition	Number	Percentage
Dinucleotides	220	
TG/AC	19	8.63%
TC/AG	91	41.40%
TA/AT	36	16.40%
GT/CA	11	5.00%
GA/CT	63	28.63%
Trinuleotides	495	
TTG/AAC	23	4.64%
TTC/AAG	76	15.35%
TTA/AAT	14	2.83%
TGT/ACA	10	2.02%
TGG/ACC	17	3.43%
TGC/ACG	6	1.21%
TGA/ACT	11	2.22%
TCT/AGA	72	14.54%
TCG/AGC	9	1.81%
TCC/AGG	10	2.02%
TCA/AGT	10	2.02%
TAT/ATA	16	3.23%
TAC/ATG	6	1.21%
TAA/ATT	22	4.44%
GTT/CAA	12	2.42%
GTG/CAC	29	5.85%
GTC/CAG	10	2.02%
GTA/CAT	6	1.21%
GGT/CCA	28	5.65%
GGA/CCT	4	0.80%
GCT/CGA	8	1.61%
GCG/CGC	4	0.80%
GCC/CGG	4	0.80%
GAT/CTA	6	1.21%
GAG/CTC	12	2.42%
GAA/CTT	51	10.30%
CTG/GAC	6	1.21%
ATC/TAG	7	1.41%
GGC/CCG	3	0.60%
GCA/CGT	3	0.60%
Tetranucleotides	12	
TTCT/AAGA	1	8.33%
TTAA/AATT	2	16.66%
TCTT/AGAA	1	8.33%
TAAG/ATTC	1	8.33%
GATA/CTAT	1	8.33%
GAAG/CTTC	1	8.33%
CTTT/GAAA	1	8.33%
ATGA/TACT	1	8.33%
AATC/TTAG	1	8.33%
AAAC/TTTG	1	8.33%
GATC/CTAG	1	8.33%
Pentanucleotides	3	
TTGAT/AACTA	1	33.33%
TTCTC/AAGAG	2	66.66%
Hexanucleotides	3	
GAGTCT/CTCAGA	1	33.33%
CTCTAT/GAGATA	1	33.33%
CCTACA/GGATGT	1	33.33%

proteins are known to be strongly induced by Pi deficiency in *Arabidopsis* seedlings (Morcuende et al. 2007). In this study, we found 82 unigenes for sugar transporter proteins, 2 for inorganic phosphate transporters, and 2 for glycerol-3-phosphate transporters, indicating that transporter proteins play a vital role in stress induced by Pi deficiency.

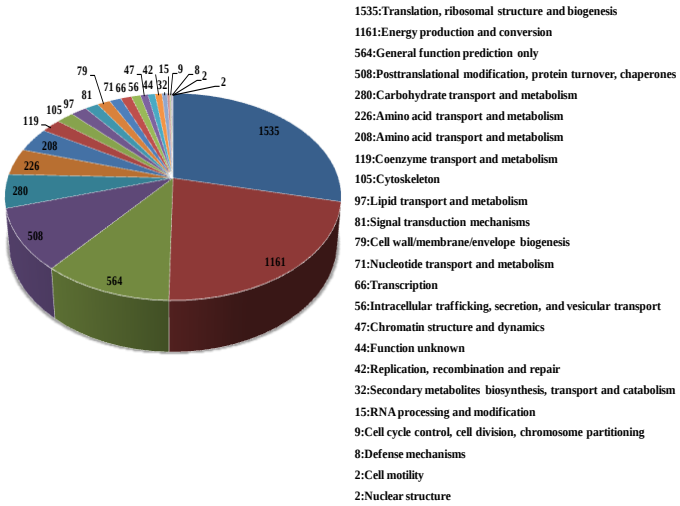

Fig 2. COG functional classification of all annotated sequences. Note: number presents gene number, every part presents cog function class.

Materials and Methods

Plant materials and stress treatment

Seedlings of the Pi deficiency-tolerant potato variety "Atlantic" were collected from tissue grown for 20 days on MS medium containing 3% (w/v) sucrose and 0.6% (w/v) agar. The tissue culture room was maintained at 25 °C under 16 h light (100 umol·m^{-2} s^{-1}/8 h dark cycle). First, seedlings were carefully removed from tissue culture bottles, and agar was washed from the roots with distilled water. Control seedlings were grown hydroponically in nutrient solutions containing 2 mM $Ca(NO_3)_2$, 2 mM NH_4NO_3, 0.75 mM $MgSO_4$, 0.5 mM KOH, 0.25 mM KH_2PO_4, 0.1 mM FeNaEDTA, 30 µM H_3BO_3, 25 µM $CaCl_2$, 10 µM $MnSO_4$, 3 µM $CuSO_4$, 1 µM $ZnSO_4$, and 0.5 µM Na_2MoO_4. Seedlings in the experimental group were grown under similar nutrient solutions, but the KH_2PO_4 was replaced with the same concentration of K_2SO_4 to induce Pi deficiency, at a final Pi concentration of 10 µM.

RNA extraction and cDNA library synthesis

Immediately after treatment, the seedlings were frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Total RNA was extracted from the seedlings with an RNA Isolation Kit (Invitrogen), according to the manufacturer's protocol. Poly(A) RNA was then separated using an Oligotex mRNA Kit (Qiagen). The quality and purity of the poly(A) RNA was analysed with 1.0% agarose gels and a GE GeneQuant 100 Spectrophotometer. cDNA synthesis was performed using a SMART™ PCR cDNA Synthesis Kit (Clontech) with the four treatment samples equally mixed, following the manufacturer's recommendations. The cDNA was amplified using PCR Advantage II Polymerase (Clontech) with the following thermal profile: 1 min at 95 °C, followed by 12 cycles of 95 °C for 15 s, 65 °C for 30 s, and 68 °C for 6 min. Approximately 5 µg of cDNA product were used to construct a 454 library after purification to generate HQ cDNA using a PureLink™ PCR Purification Kit (Invitrogen).

454 sequencing and assembly

Library sequencing was performed on a 454 GS FLX platform (454 Life Sciences, Roche) according to the manufacturer's

Table 3. Unigenes with abundance more than 120 contributing Pi deficiency tolerance in potato (Atlantic).

Gene	Abundance	Biological process
Ribulose diphosphate carboxylase	6034	Carbohydrate Metabolism
Ribosomal protein	2872	Translation
Photosystem I and II reaction cente	1815	Carbohydrate Metabolism
Elongation factor	1623	Transcription
Glycolytic enzymes	1536	EnergyMetabolism
Chlorophyll a/b binding protein	1224	Carbohydrate Metabolism
Aquaporin	1187	Membrane Transport
Pathogenesis-related protein	558	Diseases
Malate dehydrogenase	461	Energy Metabolism
Lipid transfer protein	435	Lipid Metabolism
Auxin-induced protein	426	Signaling Molecules and Interaction
Cyclophilin	421	Signal Transduction
Peroxidase	386	Enzyme Families
Tubulin	379	Cell Motility
Ubiquitin conjugating enzyme E2	356	Folding, Sorting and Degradation
S-adenosylmethionine decarboxylase	326	Biosynthesis of Other Secondary Metabolites
Chitinase	280	Cell Growth and Death
Alpha-1,4-glucan-protein synthase	275	Cell Growth and Death
Adenosylhomocysteinase	272	Amino Acid Metabolism
Ubiquitin ligase E3	255	Folding, Sorting and Degradation
Cytochrome P450	245	Biosynthesis of Other Secondary Metabolites
Glycolic acid oxidase	237	Energy Metabolism
Isoflavone reductase-like protein	227	Metabolism of Terpenoids and Polyketides
26S protease regulatory subunit	222	Folding, Sorting and Degradation
Xyloglucan transferase enzyme	203	Cell Growth and Death
Peptidyl-prolylcis-transisomerase;PPIase	196	Amino Acid Metabolism
Endoglucanase -1,3-β-glucosidase	181	Cell Growth and Death
Mitochondrial outer membrane protein	168	Transport and Catabolism
Glutamic acid decarboxylase	156	Enzyme Families
S-phase kinase-associated protein	150	Cell Growth and Death
Ferritin	146	Metabolism of Cofactors and Vitamins
S-adenosylmethionine	137	Enzyme Families
ADP ribosylation factor	136	Transport and Catabolism
UDP-glucose transferase	127	Energy Metabolism
ATP synthase	127	Energy Metabolism
Thioredoxin	125	Energy Metabolism
Hydroxypyruvate reductase	125	Amino Acid Metabolism
Actin depolymerizing factor	122	Cell Motility
dTDP-glucose 4-6 - dehydratase	121	Enzyme Families
Aspartic protease	121	Enzyme Families

protocol. All raw sequencing data were analysed with GS FLX Software (454 Life Sciences, Roche). In addition, HQ 454 sequences were isolated by removing weak signals, low-quality reads, and adaptor sequences using a series of normalization, correction, and quality filtering algorithms. These HQ reads were assembled using GS De Novo Assembler Software. The assembly was completed using the default parameters.

Sequence annotation

Unigene functional annotation was carried out by performing a BLAST search against a series of protein and nucleotide databases (SwissProt, KEGG, TAIR, Nr, and Nt). Any BLASTX hit with a threshold *e*-value of $\leq 10^{-5}$ was considered a credible match. GO and COG databases were used to categorize the transcripts based on the *Arabidopsis* proteomic sequences. Pathways with unigene involvement were identified using KEGG database. In the pathway databases, the sequences were substituted for enzyme commission numbers.

Gene expression analysis by qRT-PCR

We randomly selected 12 genes to detect expression differences after 3, 6, 12, and 24 h of exposure to Pi-deficient conditions. qRT-PCR was performed with an IQ5 Multi-color Real-Time PCR Detection System (Bio-Rad, USA) using SYBR Premix Ex Taq™ (Applied Biosystems, Warrington, UK). The 20 μL reaction mixture included 10 μL of 2× SYBR Green Master Mix Reagent, 10 ng cDNA template, and 0.3 μM each of gene-specific primers. PCR amplification was performed under the following conditions: 50 °C for 2 min and 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Three independent biological replicates and three technical repeats were included for each sample. The relative expression of candidate genes was normalized using elongation factor 1 alpha (*EF1α*) as an internal control.

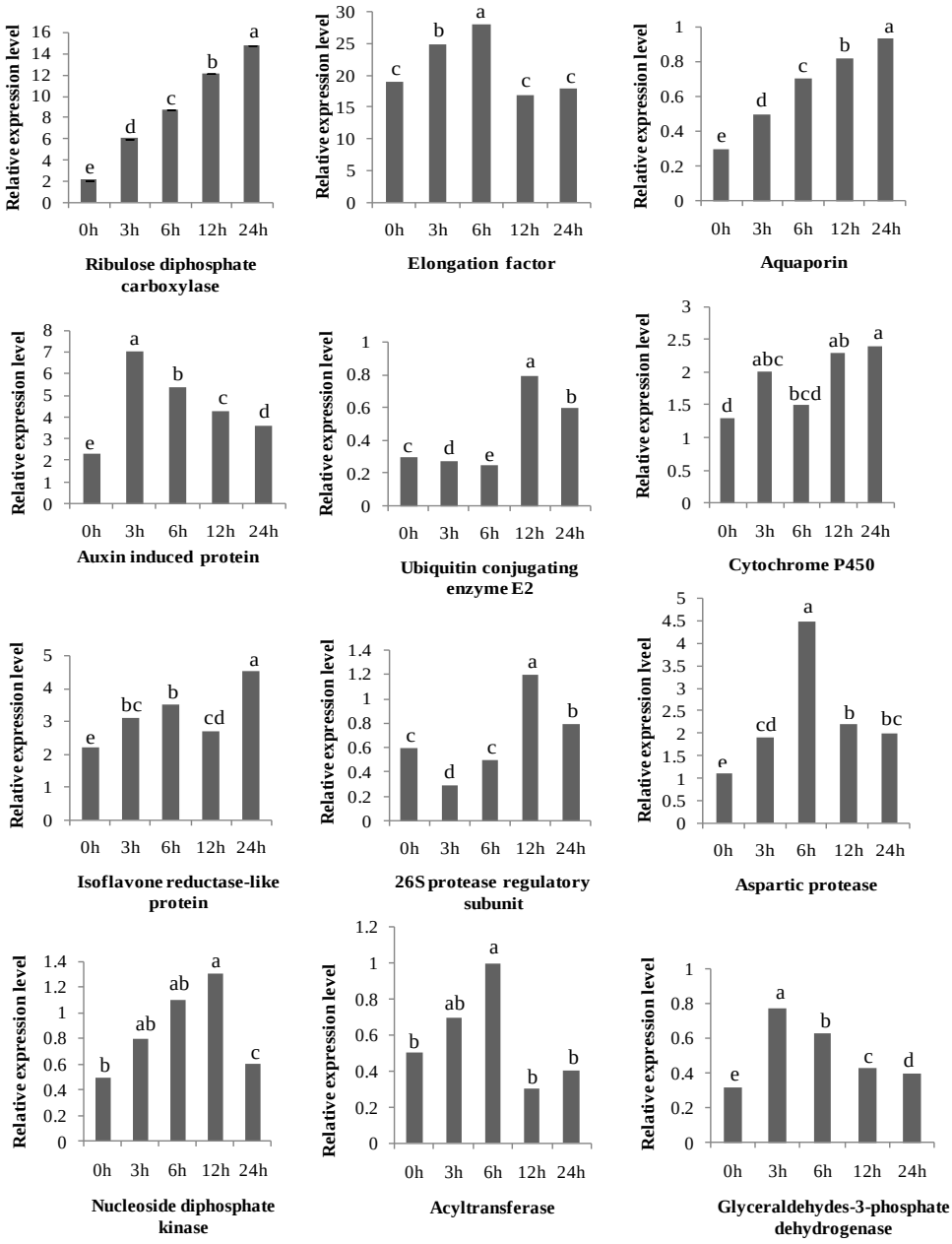

Fig 3. QRT-PCR analysis of 12 unigene expressions under phosphorus deficiency treatment. Note: different letters indicate statistical significance according to t-test ($P<0.05$).

The relative expression of target genes was quantified by comparing the cycle thresholds between the 12 genes and *EF1α* using the $2^{-\Delta\Delta CT}$ method. All primers used in this study are listed in Supplementary table 3.

Conclusion

The potato variety "Atlantic" has a strong tolerance to low Pi conditions. In this study, 454 GS FLX sequencing technology was used to examine the mixed transcriptional profile of "Atlantic" seedlings after 3, 6, 12, and 24 h of exposure to Pi-deficient conditions. A total of 29,563 unigenes were assembled after sequencing and categorized into 33 GO functional groups and 220 KEGG pathways, and 5,361 unigenes were assigned to 24 COG groups. In addition, 733 SSRs were identified. Our results suggest that the BZIP and AP2/ERF transcription factors, aquaporins, P450, cyclophilin, 14-3-3 proteins and genes related to ROS are likely involved in the tolerance of the variety "Atlantic" to low Pi conditions. The aims of this study were to discover the molecular basis of the potato response to stress caused by low Pi and to provide a valuable compilation of gene functions for use in future research of the effects of Pi deficiency in crops.

Competing interests

The authors declare that they have no competing interests.

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