

# Response of Chinese fir seedlings to low phosphorus stress and analysis of gene expression differences

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**Abstract** Chinese fir (*Cunninghamia lanceolata*) is an excellent fast-growing timber species occurring in southern China and has significant value in the forestry industry. In order to enhance the phosphorus utilization efficiency in Chinese fir, four clones named X6, S3, S39 and FK were used, and low phosphorus (LP) stress experiments were performed to analyze the response of different clones to phosphorus deficiency. According to the results on seedling height, maximum root length, leaf blade aspect ratio, root ratio, malondialdehyde content, acid phosphates activity, proline content, soluble protein level, and chlorophyll a and b levels of the tested clones, compared to the control groups (CK), the phosphorus high efficiency clone X6 was screen out for transcriptome sequencing experiments. De novo RNA-seq was then used to sequence the root transcriptomes of X6 under LP stress and CK, and we then compared the gene expression differences under the two

conditions. A total of 3416 SDEGs were obtained by comparing the LP and CK groups, among which 1742 were up-regulated and 1682 were down-regulated. All SDEGs obtained from the LP and CK treated samples were subjected to KEGG annotation and classification. Through classification statistical analysis using WEGO software, 607 SDEGs obtained KEGG pathway annotations, which were related to 206 metabolic pathways. In Chinese fir subjected to LP stress, 53 SDEGs related with phosphorus metabolism, and phosphate uptake and transport were obtained from our transcriptome data. Based on the phosphorus metabolism pathway obtained by KEGG classification, combined with previously report on gene annotation related with phosphorus metabolism, the enzymes encoded by SDEG related with phosphorus metabolism and their expression pattern were mapped onto phosphorus metabolism pathway.

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**Keywords** Chinese fir · Low phosphorus stress · Root transcriptomes · SDEG · Phosphorus metabolism

## Introduction

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is a gymnosperm in the cypress family Taxodiaceae. It is an excellent fast-growing timber species occurring in southern of Qinling Mountains in China and has significant value in the forestry industry (Lu et al. 2014; Ma et al. 2016; Duan et al. 2016). Chinese fir has been widely cultivated for over 3000 years and accounts for 20–30% of the total commercial timber production in present-day China (Orwa et al. 2015; Bian et al. 2017).

Phosphorus is one of the most highly demanded nutritious elements for plant growth and development, and is

essential for many key biological processes in plant, including energy metabolism, biosynthesis of nucleic acids and membrane development (Marschner 1995; Simpson et al. 2011; Vance et al. 2003). In the soil of southern China where Chinese firs are grown, however, the available phosphorus is typically deficiency. In order to accelerate growth and maximize production benefits, phosphate fertilizer is generally applied. However, phosphorus is easily fixed in soil by organic compounds, iron, or aluminum oxides into forms that cannot be utilized by plants. Therefore the over-application of phosphate fertilizer can not only satisfy plants demand, but also waste phosphate resource which is expected to be exhausted in the upcoming decades (Miguel et al. 2013). Hence, it is important to increase phosphate usage efficiency and reduce wastage.

To cope with phosphorus deficiency, plants employ tightly controlled strategies to maintain phosphorus metabolism. These strategies include the production of enzymes, such as acid phosphatases; increased synthesis and secretion of organic acids in the roots; enhanced gene expression; development of a larger root system capable and establishment of differential photosynthate distribution between shoots and roots (Vance et al. 2003; Wu et al. 2003). Extensive studies have indicated that different genotypes present with different phosphorus utilization capabilities, and the genes related to phosphorus metabolism are the causes of these variations (Chen et al. 2012; Fort et al. 2015). Previous reports revealed that phosphorus deficiency induced changes of genes expression in many plants (Li et al. 2010; Rouached et al. 2010). A great number of differentially expressed genes were identified (He et al. 2013; Hammond et al. 2011). Transgenic research showed that the expressions of key genes were important for phosphorus absorption and utilization, and the transgenic plants displayed morphological growth advantages in phosphorus deficiency (Yan et al. 2014).

In Chinese fir, however, there were few reports on mechanism of phosphorus utilization. The molecular mechanism underlying phosphorus efficiency by the Chinese fir is still poorly understood. In order to enhance the phosphorus utilization efficiency in Chinese fir, low phosphorus (LP) stress experiments were performed in this study to analyze the response of different clones and screen out the phosphorus high efficiency clone for further transcriptome sequencing experiments. De novo RNA-seq was then used to sequence the root transcriptomes of the clones under LP stress and phosphorus sufficient, and gene expression differences under the two conditions were compared. The phosphorus metabolism-related genes in Chinese fir were screened out and their functions in phosphorus metabolism were determined. Our results undoubtedly are helpful and meaningful for exploring

molecular mechanism of phosphorus utilization in Chinese fir.

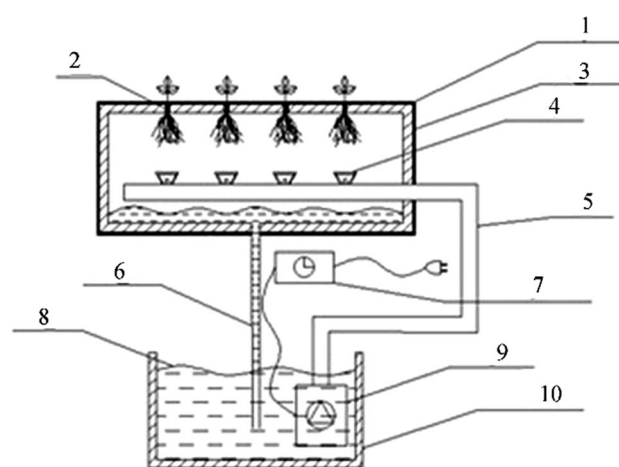
## Materials and methods

### Experimental materials

Four popular, highly adaptive Chinese fir clones were used and were respectively named X6, S3, S39 and FK. In October 2013, stem cuttings of the four clones were collected from the cutting orchard where the substrates is yellow soil in Kaihua Woodland in Zhejiang province (118°42'E, 29°13'N) and propagated in a peat perlite medium in the Germplasm Center of the Zhejiang Forestry College (119°72'E, 30°23'N). In the spring of 2014, disease-free seedlings with complete root systems that appeared to be similar in structure and displayed uniform growth were selected as experimental materials.

### Experimental strategies

All experiments were performed in the Germplasm Center of Zhejiang Agriculture and Forestry University. Aeroponic system was designed for this study (Fig. 1). Hoagland formula was used for major elements except for phosphorus, and Amon formula was used for trace elements (Table 1). Two levels of phosphorus concentration were set, namely, low phosphorus (LP) and phosphorus sufficient (CK). HCl and NaOH were used to adjust the pH value of the nutrient solution to 5.8; and  $\text{KH}_2\text{PO}_4$  was used as solo phosphorus sources. Each group contained five plants, and each experiment was triplicated. The selected



**Fig. 1** Aeroponic system. 1, Black opaque cover; 2, planting device; 3, root chamber; 4, nutrient solution outlet nozzles; 5, nutrient solution transport tubes; 6, nutrient solution recovery tubes; 7, timer; 8, nutrient solution; 9, frequency conversion submersible pump; 10, nutrient solution storage vessel

**Table 1** Nutrient formulas for the mist

	CK (mg/L)	LP (mg/L)
KNO <sub>3</sub>	225	225
Ca(NO <sub>3</sub> ) <sub>2</sub>	410	410
MgSO <sub>4</sub> ·7H <sub>2</sub> O	490	490
KH <sub>2</sub> PO <sub>4</sub>	136	0.278
KCl	0	74.222
H <sub>3</sub> BO <sub>3</sub>	2.86	2.86
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.08	0.08
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.22	0.22
MnCl <sub>2</sub> ·4H <sub>2</sub> O	1.81	1.81
H <sub>2</sub> MoO <sub>4</sub> ·4H <sub>2</sub> O	0.09	0.09
Fe <sub>2</sub> EDTA	20	20

rooted cuttings from the four clones were planted in an aeroponic culture apparatus with the root chamber volume of 60 cm × 50 cm × 50 cm, and the space of seedlings is 10 cm × 10 cm each.

At May 5, the seedlings were cultured in water for 3 days for recovery, after which 1/4 Hoagland nutrient solution was applied for cultivating strong plantlets, and after 1 month, different concentrations of phosphorus solutions were applied for the experimental culture. The nutrient solutions were changed every 3 weeks in each group. The duration of the experiment is 4 months and the seedlings were collected at Oct. 8.

## Experimental methods

### *Measurement of major morphological, physiological and biochemical indexes*

At the end of the LP stress experiment, the heights of the seedlings and the longest root length was measured, the length and width of the leaves were measured and the ratio of leaf length to width was computed, and the aboveground and underground biomass was weighed and the root ratio was calculated. The ethanol-acetone extraction method was used to determine leaf pigment content; the thiobarbituric acid method was used to determine malondialdehyde (MDA) content; coomassie brilliant blue was used to measure soluble protein concentration; the acid phosphates activity was detected by spectrophotometric method; the proline was determined with ninhydrin coloration method. *T* test program in SPSS V. 17 software was used for difference significance analysis.

### *Transcriptome sequencing*

From the results of the phosphorus stress experiment, the phosphorus high efficiency clone was chosen as experimental material (X6 in this study) for transcriptome sequencing. The treated roots from the LP and CK groups were cut and snap frozen in liquid nitrogen, and then stored at - 80 °C after they were kept in liquid nitrogen for 3 h. CTAB + Trizol reagent (Invitrogen, CA, USA) was used to extract RNA. Transcriptome sequencing was done by LC Sciences (USA) using an Illumina platform.

The unigene obtained by splicing was compared with the protein sequences in five open databases (NR, Swiss-Prot, KEGG, KOG and Pfam) and evaluated using a BLAST (Blast Local Alignment Search Tool) algorithm. The threshold was set as  $e$  value  $\leq 1e^{-10}$  to obtain similar sequences, and then the functions were annotated.

### *Differential gene expression analysis*

The transcriptome sequencing results of the LP and CK groups were compared. RPKM (reads per kilobase of exon model per million mapped reads) was used to measure the abundance of gene expression (Mortazavi et al. 2008) and log<sub>2</sub>fold\_change (logarithm of the expression levels of LP and CK groups using base 2). An absolute value  $\geq 1$  and  $p < 0.05$  were considered the thresholds for significantly differentially expressed genes (SDEG) (Benjamini and Yekutieli 2001).

### *Cluster analysis of gene expression patterns and GO classification*

Cluster analysis was conducted on the screened SDEGs using Cluster software (Audic and Claverie 1997) and Euclidean distance as the distance matrix formula was used (Saldanha 2004). The screened SDEGs were aligned with the NR database, and the GO annotations of the genes were obtained by Blast2GO software (Conesa et al. 2005). WEGO software (Ye et al. 2006) was then used to perform functional classification analysis and to reveal the general function distribution characteristics of the SDEGs.

### *Classification of gene expression patterns in the metabolic pathways*

KEGG pathway was used as the unit (<http://www.kegg.jp/kegg/pathway.html>), and the entire gene background was compared using a hypergeometric test to screen the pathways where SDEGs were highly enriched. The main biochemical metabolic and signaling pathways that the SDEGs were involved in were determined.

## Screening of the genes related to phosphorus metabolism in Chinese fir

Phosphorus metabolism pathway was found from the screened KEGG pathways. Genes related to phosphorus metabolism in Chinese fir were then screened, and their expression pattern were mapped.

## Results

### Analysis of major morphological, physiological and biochemical indexes

Compared to CK group (Table 2), the seedling heights were reduced in all four LP groups, and X6 displays the lowest seedling height (3.8 cm) in LP stress. The root lengths were increased in all four LP treated clones. The root length increases of S39 and FK were significant, while those of X6 and S3 were highly significant, and the clone S3 displays the longest root length (50.4 cm) in LP stress. The leaf blade aspect ratios were decreased in all four LP treated groups, but only in Clone S3 was significant, and the values in CK and LP are 23.43 and 18.21, respectively. The root ratios significantly increased in all four clones subjected to LP stress, In Clone S3 and FK, the root ratios were significantly increased, while in X6, and S39 were highly significantly increased, among which the Clone X6 had the largest root ratio of 2.325.

MDA was increased in all four clones subjected to LP stress, with highly significantly increased in FK, and significantly increased in S3, and not significant in X6 and S39. The activities of acid phosphatase were increased in all four LP treated clones, with highly significant in X6, and significant in S39, and not significant in S3 and FK. Proline increased in all four clones subjected to LP stress, with significantly increased in X6, while not significant in S3, S39 and FK. The soluble protein levels were increased in all four clones subjected to LP stress, with significant increase in S39, while not significant in X6, S3 and FK. Both chlorophyll a and chlorophyll b were decreased in all four clones subjected to LP stress. Clone X6 had highly significantly and extremely significantly lower chlorophyll a and b levels than in CK group.

The above results showed that X6 had the largest variation in root ratio, acid phosphates activity, proline content and chlorophyll a and b, while maintaining a relatively high growth rate, among which the root ratio is an important index for phosphorus efficiency. X6 also presented with significant changes in maximum root length. Therefore, X6 was selected as the phosphorus high efficiency clone for further transcriptome sequencing experiments.

### Transcriptome sequencing (mRNA-seq)

RNA samples from Clone X6 subjected to LP stress or CK group were sequenced using high-throughput sequencing by Illumina HiSeq 2000/2005 (LC Sciences, USA). After removing the reads containing adaptors or low quality reads with more than 5% unreadable base information, 55,955,276 reads were acquired from the LP sample, with a total of 6.99 Gbp nucleotides. The Q20 ratio and GC ratio were 95.15 and 42.42%, respectively. From the CK sample, 41,615,988 reads were acquired, with a total of 5.20 Gbp nucleotides. The Q20 ratio and GC ratio were 91.72 and 42.37%, respectively (Table 3). These high quality reads were then assembled using Trinity software, and a total of 32,731 unigenes were obtained.

Chinese fir is a non-model plant with a huge genome, no genome sequencing has been performed and thus no genomic data is currently available. Currently, no genomic data is available for the plants in this family. Thus the information regarding the closest related species and biological information is lacking. In this study, the 32,731 unigenes were BLAST searched against databases including NR, Swiss-Prot and Pfam. Among all the unigenes, 19,836 found a match in the NR database, which accounted for 60.60% of the total unigenes. Of all the unigenes blast searched against the Swiss-Prot database, 13,208 (40.35%) had significant similarities with known protein gene sequences; 16,029 (48.97%) found a match in the Pfam database; 11,720 (35.81%) and 10,825 (32.07%) found a match respectively in GO and KOG databases; and 9379 (28.65%) found a match in KEGG database and obtained 270 metabolism pathways (Table 4).

### GO aggregation analysis of SDEG

A total of 3416 SDEGs were obtained by comparing the LP and CK groups, among which 1724 were up-regulated and 1682 were down-regulated (Fig. 2). These SDEGs were searched against the GO database for annotation. Of these, 1420 had GO annotations and were classified into three ontologies, which were related to biological processes, cellular components and molecular function. These 1420 SDEGs can be categorized into 1409 GO annotations, of which 699 were biological processes and involved 1020 SDEGs, and 189 were cellular components and involved 1165 SDEGs, and 521 were related to molecular functioning and involved 1291 SDEGs. Figure 3 shows the first 10 subcategories that had the most SDEG numbers in the three ontologies. Among the 1020 SDEGs that are related to biological process, most were related to subcategories of proteolysis (84 SDEGs), followed by DNA-dependent transcription (64 SDEGs). Among the 1165 SDEGs that are related to cellular component, most were related to the

**Table 2** Comparison of physiological indexes in different clones

Clone	Treatment	Seedling height (cm)	Longest root length (cm)	Leaf blade aspect ratio	Root/shoot ratio	MDA (mg/g)	Acid phosphatase activity (g min)	Proline (ug/g)	Soluble protein (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
FK	CK	9.2 ± 5.4 a	12.7 ± 4.6 a	20.83 ± 4.098 a	0.186 ± 0.088 a	50 ± 1 A	7.10 ± 4.73 a	81.92 ± 13.60 a	1.62 ± 0.834 a	35.24 ± 8.09 a	12.23 ± 2.82 a
	LP	4.3 ± 2.4 a	35.4 ± 9.4 b	20.41 ± 4.299 a	1.773 ± 0.381 b	146 ± 12 B	7.77 ± 0.39 a	74.91 ± 6.143 a	7.33 ± 5.649 a	25.61 ± 11.6 a	10.00 ± 4.91 a
S39	CK	8.1 ± 4.7 a	26.9 ± 5.2 a	0.455 ± 0.140 a	0.455 ± 0.140 A	57 ± 7 a	3.83 ± 0.52 a	156.5 ± 38.10 a	2.92 ± 1.131 a	16.71 ± 7.53 a	5.23 ± 2.79 a
	LP	4.6 ± 1.1 a	38.0 ± 2.3 b	17.33 ± 3.958 a	1.892 ± 0.199 B	127 ± 44 a	7.867 ± 1.63 b	116.8 ± 10.55 a	5.54 ± 0.770 b	16.50 ± 3.15 a	4.88 ± 1.11 a
S3	CK	11.5 ± 3.1 a	27.3 ± 4.2 A	23.43 ± 4.256 a	0.410 ± 0.043 a	27 ± 4 a	2.57 ± 1.10 a	155.1 ± 6.077 a	9.87 ± 2.131 a	17.59 ± 9.2 a	5.89 ± 2.89 a
	LP	4.4 ± 1.1 b	50.4 ± 9.3 B	18.21 ± 5.374 b	1.844 ± 0.370 b	68 ± 15 b	5.03 ± 1.35 a	108.5 ± 62.42 a	9.47 ± 2.285 a	6.27 ± 5.24 a	2.28 ± 2.41 a
X6	CK	8.6 ± 2.6 A	14.0 ± 4.0 A	25.07 ± 6.327 a	0.231 ± 0.066 A	88 ± 13 a	3.23 ± 0.47 A	74.22 ± 20.54 a	6.90 ± 1.623 a	12.11 ± 1.94 A	3.32 ± 0.58 A
	LP	3.8 ± 1.6 B	35.3 ± 4.1 B	21.52 ± 4.282 a	2.325 ± 0.297 B	234 ± 97 a	10.67 ± 0.77B	126.3 ± 25.11 b	12.87 ± 4.815 a	2.15 ± 0.97 B	0.71 ± 0.36 B

Capital letters indicate highly significant difference, represent 1% significant level; lower case letters indicate very significant difference, represent 5% significant level

subcategory of integral to membrane (366 SDEGs), followed by the nucleus (215 SDEGs) and plasma membrane (190 SDEGs). Among the 1291 SDEGs that are related to molecular function, 366 SDEGs were related to subcategory of ATP binding, followed by protein serine/threonine kinase activity (150 SDEGs) and protein binding (110 SDEGs).

### SDEG metabolic pathway classification

All SDEGs obtained from the LP and CK treated samples were subjected to KEGG annotation and classification. Through classification statistical analysis using WEGO software, 607 SDEGs obtained KEGG pathway annotations, which were related to 206 metabolic pathways. Figure 4 shows the first 20 metabolic pathways that had the most SDEGs, which were related to the phenylpropanoid biosynthesis (58 SDEGs), followed by starch and sucrose metabolism (52 SDEGs), phenylalanine biosynthesis (42 SDEGs), ribosome (31 SDEGs), methane metabolism (30 SDEGs), and flavonoid biosynthesis (25 SDEGs).

### SDEGs related to phosphorus metabolism

Phosphorus is necessary for nucleic acid composition and maintaining cell structure. Through phosphorylation and phosphorylation reaction it involve in materials such as sugar, protein and lipid metabolic regulation and signal transduction. In Chinese fir subjected to LP stress, 31 SDEGs related with phosphorus metabolism, and 22 SDEGs related with phosphate uptake and transport, were obtained from our transcriptome data. In total, these 53 SDEGs encode 27 enzymes and 1 inorganic phosphate transporter related with phosphorus metabolism. These SDEGs are first reported in Chinese fir, and their names and expresses information are shown in Table 5.

### Phosphorous metabolism pathway in Chinese fir

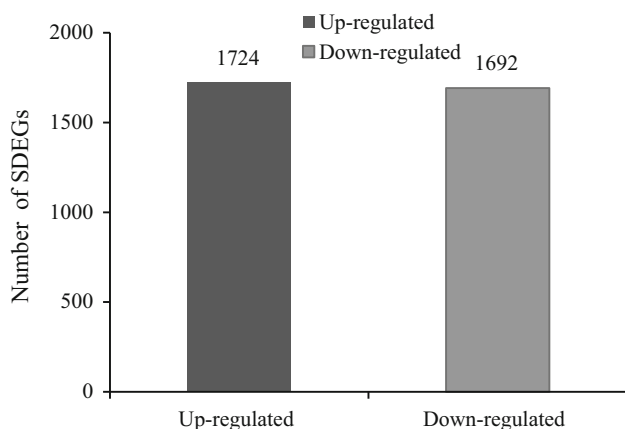
Phosphorus is a fundamental biological component in plants. Phosphorus metabolism is a complicated process and is involved in many major metabolic processes. Based on the phosphorus metabolism pathway we obtained by KEGG classification, combined with previously report on gene annotation related with phosphorus, the enzymes encoded by SDEG related with phosphorus metabolism (the first 32 in Table 5) and their expression pattern were mapped to phosphorus metabolism pathway, including glycolysis/gluconeogenesis, Photosynthesis, Carbon fixation in photosynthetic organisms, citrate cycle (TCA cycle) and phosphonate and phosphinate metabolism (Fig. 5). They are essential metabolisms in plant, and phosphorus is one of the indispensable substances in these processes.

**Table 3** RNAsequence pretreated results

Sample	Raw data read	Valid data read	Valid%	Q20%	Q30%	GC%
LP	56,623,784	55,955,276	98.82	95.15	90.45	42.42
CK	43,558,660	41,615,988	95.54	91.72	84.43	42.37

**Table 4** Blast annotation results

gene_number	swiss-prot	nr	Pfam	KEGG	KOG	GO
32,731	13,208	19,836	16,029	9379	10,825	11,720
100%	40.35%	60.60%	48.97%	28.65%	32.07%	35.81%

**Fig. 2** Statistical analysis of SDEGs between LP and CK

## Discussion

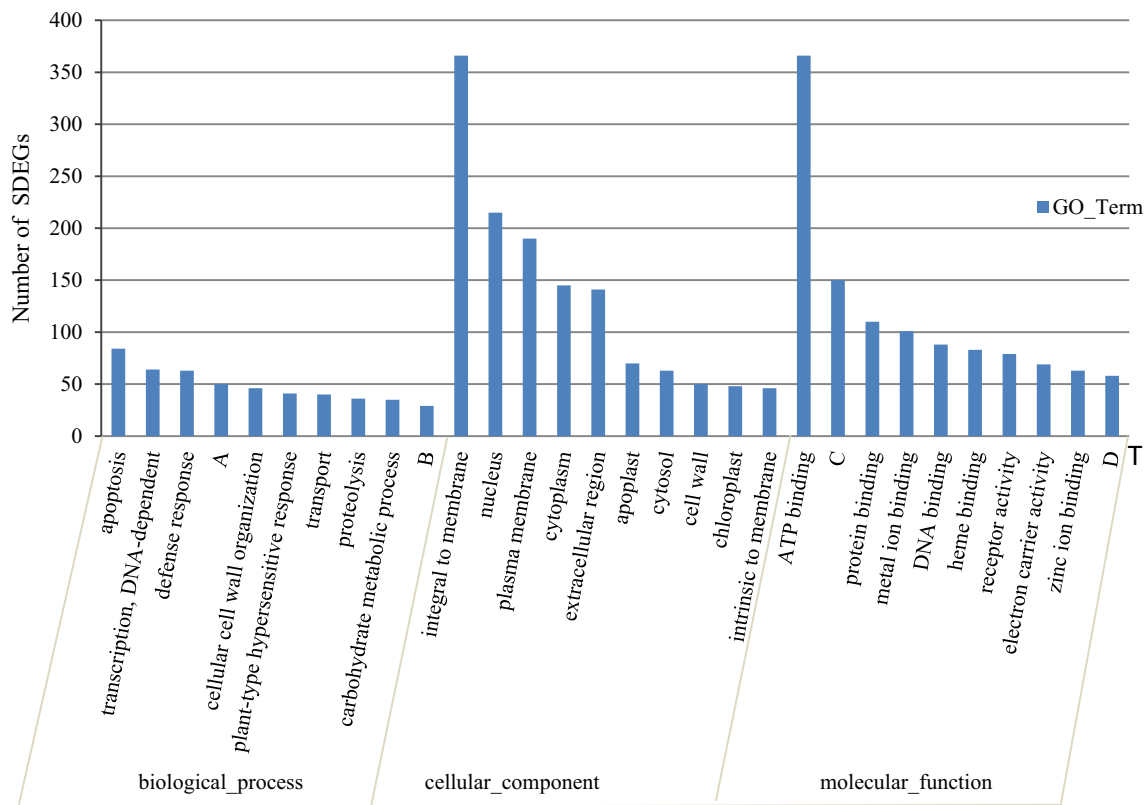
In this study, the transcriptome of the root system of Chinese fir was studied under LP stress and phosphorus sufficient using the Illumina platform. As a result, 3416 SDEGs were obtained by comparing the LP and CK groups. There is no uniform standard available for the time period of LP stress in different woody plant experiments. In our study, only few seedlings under LP stress begun to display symptoms of phosphorus deficiency after the experimental plants had been subjected to LP stress for 3 months. Based on previous research and our own results, we decided to select 4 months as the time period of LP stress treatment. It should be noted that the length of LP stress is the basis for the subsequent screening of SDEGs. If the LP stress period is too short, some genes may have not expressed yet; if the LP stress period is too long, some key early response genes may have lost. Thus, the screened SDEGs in this study might be only part of the genes involved in the Chinese fir response to LP stress. However, according to the currently accepted phosphorus research, most genes that encode the related enzymes were found in our transcriptome data set.

By analyzing the 14 SDEGs involved in glycolysis/gluconeogenesis, we found those 12 SDEGs up-regulated

and only 2 SDEGs down-regulated. The down-regulated SDEGs encode A1E and ACS, respectively. A1E catalyzes the interconversion between  $\alpha$ -D-glucose and  $\beta$ -D-glucose (Li et al. 2013), while ACS is one of the enzymes that catalyzes acetyl CoA synthesis (Dubey et al. 2015), and the decreased expression of A1E and ACS might have high phosphorus use efficiency in phosphorus deficiency. The 9 SDEGs involved in photosynthesis are all down-regulated, indicated that LP stress affects photosynthesis which consistent with previous studies (Fan et al. 2016). The most interesting finding was concerned with Carbon fixation in photosynthetic organisms. Among the 10 SDEGs, three were 9 SDEGs up-regulated and only one was down-regulated. This result reveal that through increase carbon fixed related gene expression, making carbon fixed quantity can maintain basic life activities in LP stressed seedling. We also discovered that the 3 SDEGs involved in TCA cycle and 1 SDEG involved in phosphonate and phosphonate metabolism, respectively, were all up-regulated.

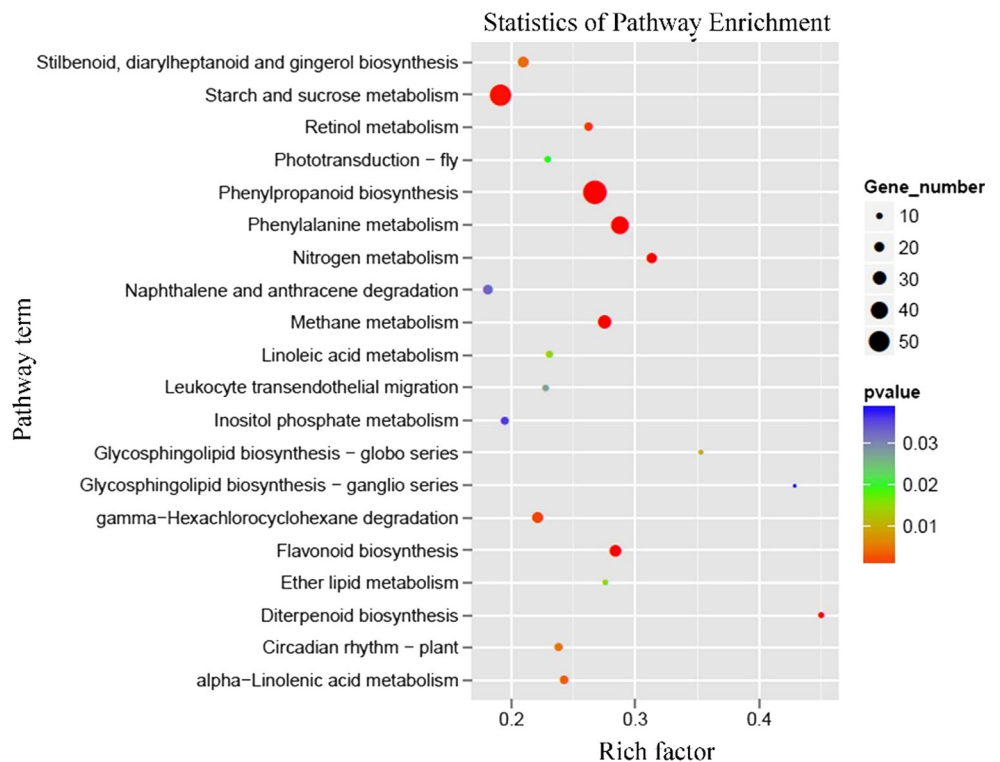
The acid phosphatase (ACP) is a key enzyme for phosphorus uptake and utilization (Bozzo et al. 2003; Gilbert et al. 1999; Richardson et al. 2011; Sharma et al. 2016). In this study, we screened 4 ACP and they all up regulated under LP stress.

Phosphorus transport protein is an essential factor for phosphorus absorption and utilization. Up to now, 4 phosphorus transporter (PHT) families have been identified, including PHT1 (plasmamembrane), PHT2 (plastid inner envelope), PHT3 (mitochondrialinner membrane) and PHT4 (mostly plastid envelope and one Golgi-localized transporter) (Coelho et al. 2010; Guo et al. 2008; Lapis-Gaza et al. 2014; Rausch et al. 2004). There are 9 PHT numbers in Arabidopsis, namely AtPHT1; 1to AtPHT1; 9, among which the AtPHT1; 1 to AtPHT1; 4 play roles in phosphorus uptake from roots surrounding soil (Lapis-Gaza et al. 2014). In our study 18 PHT were screened, and they were significant up regulated in our data. However the type and function of these PHT need to be studied further.



**Fig. 3** GO classification of SDEGs. Note: A, regulation of transcription, DNA-dependent; B, ethylene mediated signaling pathway; C, protein serine/threonine kinase activity; D, sequence-specific DNA binding transcription factor activity

**Fig. 4** KEGG classification of SDEGs



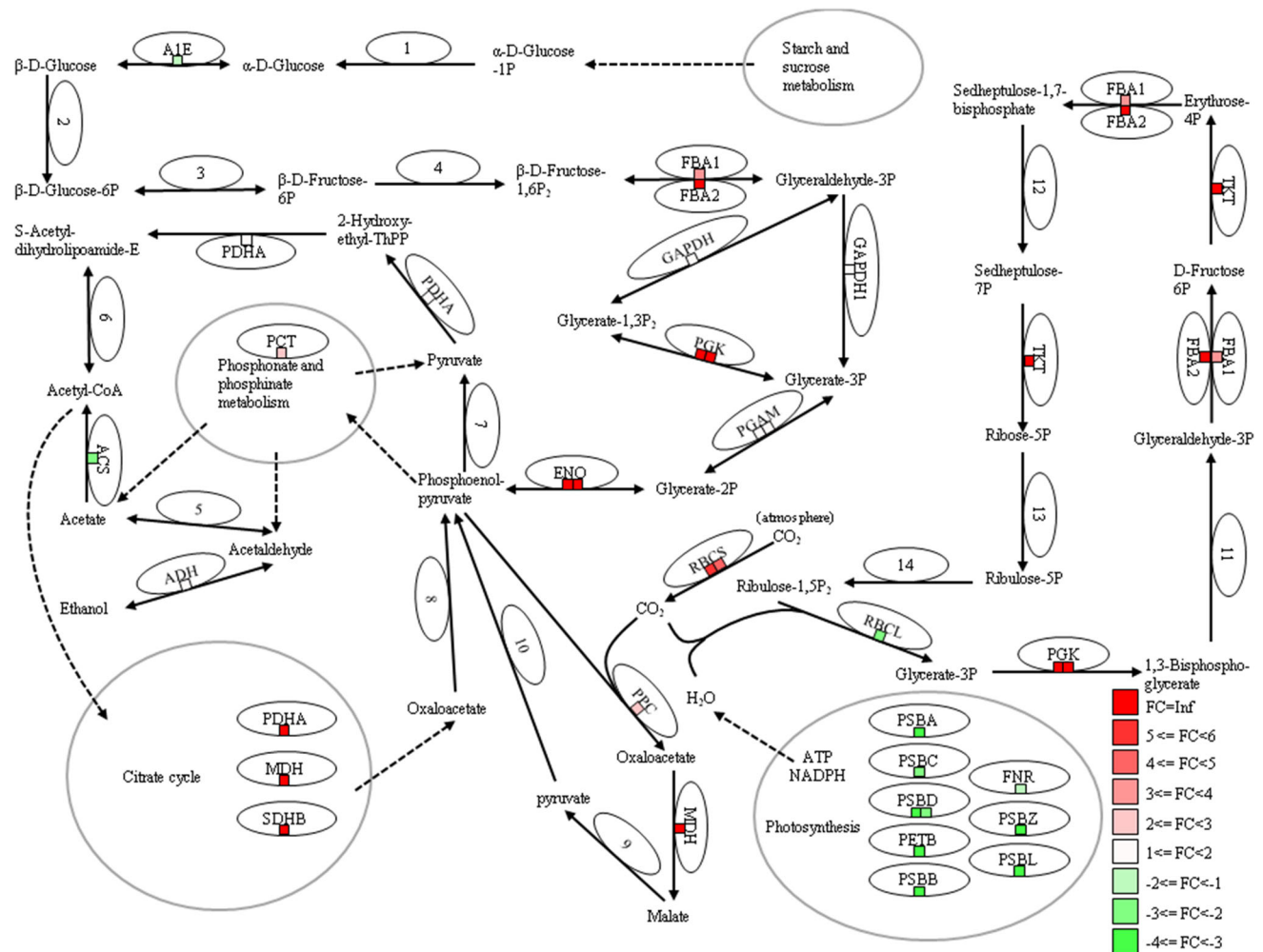
**Table 5** SDEGs involved in phosphorus metabolism

No.	gene_ID	Annotation	Name	log2fold_change	Regulation
1	comp27133_c0	Aldose 1-epimerase	A1E	- 1.73	Down
2	comp50144_c0	Acetyl-CoA synthetase	ACS	- 2.08	Down
3	comp14199_c0	Alcohol dehydrogenase	ADH	1.37	Up
4	comp103197_c0	Enolase	ENO	Inf	Up
5	comp14618_c0	Enolase	ENO	Inf	Up
6	comp9834_c0	Fructose-bisphosphate aldolase, class I	FBA1	3.37	Up
7	comp15959_c0	Fructose-bisphosphate aldolase, class II	FBA2	Inf	Up
8	comp23465_c0	Ferredoxin-NADP <sup>+</sup> reductase	FNR	- 1.39	Down
9	comp20943_c0	Glyceraldehyde 3-phosphate dehydrogenase	GAPDH	1.45	Up
10	comp5803_c0	Glyceraldehyde-3-phosphate dehydrogenase (NADP)	GAPDH1	1.93	Up
11	comp87674_c0	Malate dehydrogenase	MDH	Inf	Up
12	comp23921_c0	Choline-phosphate cytidylyltransferase	PCT	2.69	Up
13	comp16583_c0	Pyruvate dehydrogenase E1 component subunit alpha	PDHA	1.36	Up
14	comp11853_c0	Cytochrome b6	PETB	- 3.39	Down
15	comp24263_c0	Phosphoglycerate mutase	PGAM	1.03	Up
16	comp22056_c0	Phosphoglycerate mutase	PGAM	1.29	Up
17	comp24368_c0	Phosphoglycerate kinase	PGK	Inf	Up
18	comp147930_c0	Phosphoglycerate kinase	PGK	Inf	Up
19	comp14466_c0	Phosphoenolpyruvate carboxylase	PPC	2.70	Up
20	comp24767_c0	Photosystem II PsbA protein	PSBA	- 3.14	Down
21	comp66117_c0	Photosystem II PsbB protein	PSBB	- 3.26	Down
22	comp21571_c0	Photosystem II PsbC protein	PSBC	- 2.86	Down
23	comp11200_c0	Photosystem II PsbD protein	PSBD	- 3.45	Down
24	comp15023_c0	Photosystem II PsbD protein	PSBD	- 2.70	Down
25	comp63310_c0	Photosystem II PsbL protein	PSBL	- 3.50	Down
26	comp54424_c0	Photosystem II PsbZ protein	PSBZ	- 3.35	Down
27	comp23533_c0	Ribulose-bisphosphate carboxylase large chain	RBCL	- 2.69	Down
28	comp20871_c0	Ribulose-bisphosphate carboxylase small chain	RBCS	4.57	Up
29	comp5107_c0	Ribulose-bisphosphate carboxylase small chain	RBCS	5.52	Up
30	comp127366_c0	Succinate dehydrogenase (ubiquinone) cytochrome b subunit	SDHB	Inf	Up
31	comp9052_c0	Transketolase	TKT	Inf	Up
32	comp25863_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	Inf	Up
33	comp6435_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	7.43	Up
34	comp19684_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	5.94	Up
35	comp24269_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	3.66	Up
36	comp23134_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	4.84	Up
37	comp24497_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	1.65	Up
38	comp22135_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	3.58	Up
39	comp21878_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	Inf	Up
40	comp38755_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	Inf	Up
41	comp32218_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	3.17	Up
42	comp12857_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	2.64	Up
43	comp19647_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	8.01	Up
44	comp44357_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	2.76	Up
45	comp66471_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	Inf	Up
46	comp20298_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	1.16	Up
47	comp76223_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	3.79	Up
48	comp132799_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	Inf	Up



**Table 5** continued

No.	gene_ID	Annotation	Name	log2fold_change	Regulation
49	comp196737_c0	MFS transporter, PHS family, inorganic phosphate transporter	PHT	Inf	Up
50	comp16483_c0	Acid phosphatase	ACP	4.57	Up
51	comp26531_c0	Acid phosphatase	ACP	2.06	Up
52	comp26075_c0	Acid phosphatase	ACP	2.36	Up
53	comp12893_c0	Acid phosphatase	ACP	1.84	Up



**Fig. 5** Expression of SDEGs and their functions in phosphorous metabolism. *Note:* the colored small box represent different SDEG. The numbers in the in ellipse means enzymes that absent in our SDGEs data set, and according to previous reports the enzymes are as follows: 1, glucose-1-phosphatase; 2, hexokinase; 3, glucose-6-phosphate isomerase; 4, 6-phosphofructokinase 1; 5, pyruvate kinase; 6, pyruvate dehydrogenase E2 component (dihydrolipoamide

acetyltransferase); 7, aldehyde dehydrogenase ( $\text{NAD}^+$ ); 8, phosphoenolpyruvate carboxykinase (ATP); 9, malate dehydrogenase (oxaloacetate-decarboxylating)( $\text{NADP}^+$ ); 10, pyruvate, orthophosphate dikinase; 11, glyceraldehyde-3-phosphate dehydrogenase ( $\text{NADP}^+$ ); 12, fructose-1,6-bisphosphatase II/sedoheptulose-1,7-bisphosphatase; 13, ribose 5-phosphate isomerase A; 14, phosphoribulokinase

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Quan Lu and Jianhui Li analyzed and interpreted the experiment data, constructed the phosphorus metabolism draft and drafted manuscript. All authors read and approved the final manuscript.

#### Compliance with ethical standards

**Conflict statement** The authors declare that they have no conflict of interest.

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