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Transcriptomic analysis of rice responses to low phosphorus stress

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Phosphorus is one of the essential macronutrients required for plant growth and development. The plants increase the absorption of phosphorus through an expansion of the root system in the soil when they are in low-phosphorus conditions. At the same time, the changes in biochemical metabolic pathways and the increase of secretion of phosphatase and organic acids activate the insoluble phosphate fixed in the soil. The expression profile in response to low phosphorus was investigated for rice at 6, 24 and 72 h after low-phosphorus stress compared with normal phosphorus conditions as a control with DNA chip. A total of 1207 differentially expressed genes were found in our study; 795 and 450 genes exhibited alterations in their RNA expression in response to inorganic phosphate (Pi) starvation in at least one of the three time points in roots and shoots. Thirty-eight genes overlapped in shoots and roots. The functional classification of these genes indicated their involvement in various metabolic pathways, ion transport, signal transduction, transcriptional regulation, and other processes related to growth and development. A large number of transposable elements changed the expression in rice after low-phosphorus stress. The results may provide useful information about molecular processes associated with Pi deficiency and facilitate the identification of key molecular determinants for improving Pi use by crop species.

phosphorous, rice, DNA chip, expression profile

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Phosphorus is one of the essential macronutrients required for plant growth and development. In general, 60%–70% of applied phosphorus fertilizer is transformed into inorganic phosphorus and about 8%–15% of applied fertilize is transformed into organic phosphorus in the soil [1]. Applied phosphate fertilizer in rice fields is transformed mainly into inorganic phosphorus Fe-P, Al-P, O-P. Al-P is gradually transformed to Fe-P with the extension of fertilized time. Phosphorus absorbed by rice mostly comes from A1-P and Fe-P in the soil [2]. The available phosphorus concentration is very low, usually 10 μmol/L, because of the fixation of phosphorus although the total phosphorus content in the soil is rich [3,4]. Reports indicate that the efficiency of phosphorus fertilizer is 11.6% and no more than 25% for other reasons [5].

When available phosphorus that can be directly absorbed by plants is lacking in the surrounding environment, plants expand the surface of roots by altering the root architecture and by increasing lateral roots and the number and length of root hairs, to strengthen the absorption of phosphorus. Some species, such as white lupine, can format proteoid root (clustered roots) and some species can format mycorrhizas. In their symbiotic relationship, mycorrhizal fungi provide phosphorus to plants and plants provide carbon sources to mycorrhizal fungi. Some plants maintain the dynamic balance of intracellular phosphorus through the regulation of metabolism and active insoluble phosphate fixed in the soil by secretion of phosphatase and organic acids [3,6,7].

Details of signal transduction and the regulation mechanism of plant adaptation to low-phosphorus conditions have not yet been explained completely. PHR1, a Myb transcription factor, was identified in *Arabidopsis* through a

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map-based cloning method. PHR1 can regulate the expression of phosphate starvation-induced genes under conditions of low phosphorus. *PHR1* was expressed in inorganic phosphate (Pi)-sufficient conditions and was only weakly responsive to Pi starvation [8]. PHR1 bound as a dimer to an imperfect palindromic sequence GNATATNC. *PHO3*, *PSR1* and *PDR2* genes play important roles in the phosphate (Pi) signaling pathway because mutations in the *PHO3*, *PSR1* and *PDR2* genes impair Pi starvation signaling [7,9,10]. MicroRNA399 controls Pi homeostasis by regulating the expression of UBC24 encoding a ubiquitin-conjugating E2 enzyme in *Arabidopsis* [11]. Expression of miR399 was strongly reduced in Pi-deprived *Arabidopsis phr1* mutants, and a subset of Pi-responsive genes repressed in Pi-deprived *phr1* mutants was up-regulated in Pi-replete *pho2* mutants. This places miR399 and PHO2 in a branch of the Pi-signaling network downstream of PHR1 [12]. AtSIZ1 is a plant small ubiquitin-like modifier (SUMO) E3 ligase and is a focal controller of Pi starvation-dependent responses. AtSIZ1 influences a greater number of processes considered as adaptation responses to low Pi availability, including root architecture changes. The MYB transcription factor, PHR1, is a sumoylation target of AtSIZ1. AtSIZ1 affects several Pi starvation responses that are not controlled by PHR1, indicating that sumoylation is a more encompassing process in the control of adaptation to Pi deficiency [12,13]. The SPX (SYG/PHO81/XPR1) domain is a conservative domain found in the N-termini of yeast SYG1 and PHO81 and human XPR1 proteins. SPX domain genes have recently been shown to be involved in the phosphate (Pi) signaling pathway in *Arabidopsis* and rice [14,15].

Studies of functional genomics have made great progress in recent years. Several successful studies of gene expression profiles in response to plant defenses against pathogens [16], abiotic stresses [17,18], low N [19,20] and low phosphorus [21–25] have been reported. Here, we applied a microarray of 60000 oligos (70 mer) representing all putative genes of the rice genome purchased from the Beijing Genomic Institute (BGI) to analyze rice Zhongzao 18 genome expression profiles at 6, 24 and 72 h after low-phosphorus stress with normal phosphorus as the control. We hope to further reveal the biologic mechanisms of plant adaptation to low phosphorus.

1 Materials and methods

1.1 Plant cultivation

Seeds of the rice Zhongzao 18 (*Oryza sativa* ssp. *Indica*) were germinated and grown hydroponically in the nutrient solution made up of 1.44 mmol/L NH_4NO_3, 0.3 mmol/L NaH_2PO_4, 0.5 μmol/L $(NH_4)_6Mo_7O_{24}$, 15 μmol/L H_3BO_3, 8 μmol/L $MnCl_2$, 0.12 μmol/L $CuSO_4$, 0.12 μmol/L $ZnSO_4$, 29 μmol/L $FeCl_3$, and 40.5 μmol/L citric acid, pH 5.5 [26]. The culture nutrient solution was refreshed every 3 d. The seedlings were transferred into either a nutrient solution with the phosphorus concentration reduced to 0.01 mmol/L NaH_2PO_4 or a nutrient solution with the normal Pi concentration, designated −P treatments and +P treatments, respectively, at the emergence of the fourth leaf. The planting and harvesting were conducted three times with an interval of 5 days for three biologic repeats.

1.2 Sample collection and RNA preparation

Rice Zhongzao 18 roots and shoots were harvested at 6, 24 and 72 h after the experimental treatments started; the samples were then frozen immediately using liquid nitrogen and stored at −80°C until analyzed. Tissues were ground to fine powder in liquid nitrogen. Total RNA was extracted with TriZol regent (GIBCO/BRL) according to the manufacturer's instructions. Then mRNA was isolated from the total RNA using the oligo $dT_{(25)}$ magnetic Dynal beads (Oslo, Norway).

1.3 Fluorescent probe preparation, hybridization, and washing

The microarray of 60000 oligos (70 mer) representing all putative genes of the rice genome purchased from the BGI was applied. About 2.0 μg mRNA was reverse transcribed in the presence of aa-dUTP (Amersham Pharmacia). The reverse transcription reaction was conducted in a volume of 40 μL, containing 1× first-strand buffer (Life Technologies, Grand Island, NY, USA), 400 U Superscript II reverse transcriptase, 10 mmol/L DTT, 4 μg anchored oligo (dT) 20 mer, 40 U RNAsin (Promega, Madison, WI), 200 μmol/L aa-dUTP, 500 μmol/L each dATP, dCTP and dGTP, and 200 μmol/L dTTP. After incubation at 42°C for 1 h, another 400 U Superscript II reverse transcriptase was added and incubated for another 2 h at 42°C. The reaction was stopped by incubating at 94°C for 3 min. Two units RNase H was added to the reaction mixture and incubated for 10 min at 37°C, followed by addition of 1.0 μg RNase A and incubating for 10 min at 37°C. The reaction mixture was purified with a Microcon YM-30 filter (Millipore). The revere transcription products for −P and +P treatments were labeled with Cy3 or Cy5 fluorescent dyes, respectively, and incubated at room temperature in the dark for 1.5 h. The Cy3- and Cy5-labeled samples were combined and purified with a Microcon YM-30. The volume of the probe was adjusted to 23 μL with filtrated de-ionized water, and then 3.0 μL 20× SSPE, 1.0 μL blocking solution, 2.0 μL poly dA (5 μg/μL), and 46 μL hybridization solution were added. The probe was denatured at 90°C for 3 min and cooled to 4°C.

For the protocol steps of prehybridization, hybridization, and washing, see http://redb.ncpgr.cn/protocols/.

We performed forward and reverse labeling for each time

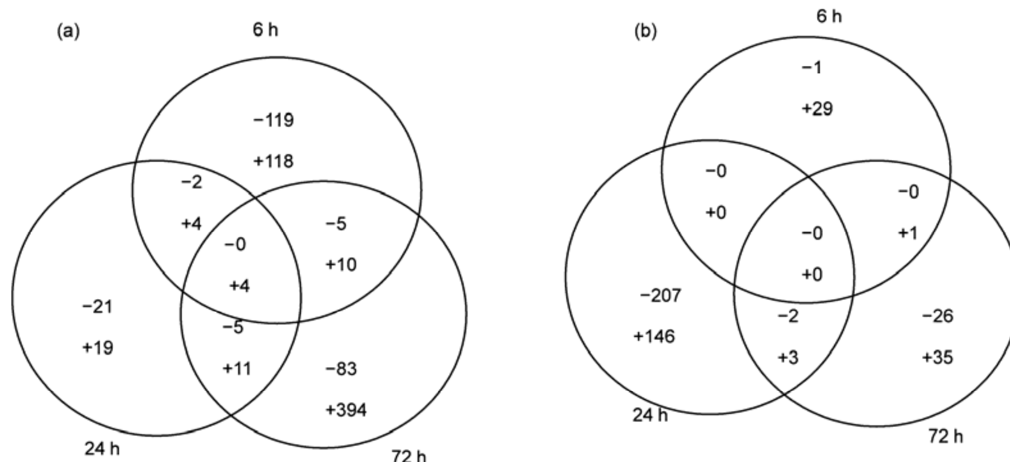

Figure 1 The number of differentially expressed genes in rice Zhongzao 18 roots and shoots under Pi-deficiency. +, Up-regulated; –, down-regulated.

point. In the forward hybridization, the mRNA from low phosphorus-stressed tissue was labeled with Cy3 and the control tissue with Cy5; these labeled probes were mixed in equal amounts and hybridized with the microarray. In the reverse hybridization, the mRNA from stressed tissue was labeled with Cy5 and the control tissue with Cy3.

1.4 Data processing and analysis

The slides were scanned using a GMS418 Array Scanner (Genetic MicroSystems, Worburn, MA) by two separate laser channels for Cy3 and Cy5 emissions. The images were analyzed using the ImaGene 4.2 software (BioDiscovery, Los Angeles, CA), by which the scanning parameters were selected to make the sums of signal intensity equal among the slides. The signal of each spot was subjected to spot filtering. First, spots flagged Bad by ImaGene 4.2 software were excluded from the analysis. Second, only those spots with fluorescent intensity levels at least two times above the local background were selected for further analysis. The signal was normalized by setting the total signals of Cy3 and Cy5 equal.

A cutoff value of 1.5-fold change was adopted to discriminate expression of genes that were differentially altered in response to Pi deficiency [20]. Each of the hybridizations was performed with two technical repeats using independent samples from three different plantings. When the normalized genes expression ratio of Cy5/Cy3 in the five or six hybridization experiments was >1.5, the genes were considered to have significant changes in their expression. We used the average ratio of stress/control in six experiments.

2 Results

2.1 Validation of the microarray data

Planting and harvesting were conducted three times for three biologic repeats. Two technical repeats were performed in every biologic repeat. Correlation coefficients between the data of dye swap ranged from 0.891 to 0.989 with an average of 0.972.

To investigate the correspondence between the signal intensity in the microarray and Northern hybridization using rRNA as the reference, 6 gene sequences were selected for RNA gel-blot analysis. The RNA gel-blot results are generally consistent with the results from microarray data. This indicated the reliability of the microarray hybridization/data analysis system.

2.2 General features of the low-phosphorus stress-responsive expression profile

A total of 1207 genes exhibited alterations in expression in response to low phosphorus stress at at least one of the three time points in rice Zhongzao 18 roots and shoots. There were 450 and 795 differentially expressed genes in rice Zhongzao 18 shoots and roots, respectively and 38 overlapping genes in shoots and roots. This indicated that distinct strategies were used by those two plant organs in response to Pi starvation.

Among the up-regulated genes, 136, 38 and 419 genes showed an elevated level of expression at 6, 24 and 72 h, respectively, under Pi starvation in rice Zhongzao 18 roots. Among the down-regulated genes, 126, 28 and 93 genes showed decreased expression at 6, 24 and 72 h, respectively (Figure 1(a)). In rice Zhongzao 18 shoots, 30, 149 and 39 genes were up-regulated at 6, 24 and 72 h, respectively, whereas 1, 209 and 28 genes were down-regulated at 6, 24 and 72 h, respectively (Figure 1(b)).

2.3 Clustering analysis and functional classification of the differentially expressed genes

Clustering analysis of 1207 differentially expressed genes in rice Zhongzao 18 roots and shoots was performed using the

R-gplots package (Figure 2). The largest number of differentially expressed genes emerged in rice shoots after 24 h of Pi starvation and the least number of differentially expressed genes emerged in rice roots after 24 h of Pi starvation. The differentially expressed genes were significantly different in different organizations at different low phosphorus stress time points in expression patterns. This indicated that the roots and shoots possessed different strategies under low phosphorus stress conditions at different stress time points. Most of the up-regulated genes at 6 h under low phosphorus stress conditions in rice roots were transporter genes and abiotic stress-related genes, and obvious changes in gene expression were not observed in shoots. A large number of transposable elements (including retrotransposons) increased their expression and some ion transporter genes decreased their expression at 24 h under low phosphorus stress conditions in rice shoots. Phosphatase genes and protease genes increased their expression and a large number of photosynthesis-related genes decreased their expression at 72 h under low phosphorus stress conditions in rice roots.

Functional classification of 1207 differentially expressed genes in rice roots and shoots under low phosphorus stress conditions was performed by a homology search in the Gene Ontology and Non-redundancy (NR) databases using the BLAST program (BLASTx, $E\leqslant 10^{-5}$, or BLASTn, scores$\geqslant$100), referring to the KEGG database and gene function annotation provided by the BGI. Expressed protein genes, hypothetical protein genes, and some genes that have no gene function annotation were classified into unknown functional genes. Other differentially expressed genes included those involved in phosphate transport, transport except Pi transport, phosphatase, enzyme except phosphatase, primary metabolism, secondary metabolism, protein degradation, protein synthesis, stress, signaling, lipids, transcription, e-transport, and others (Table 1).

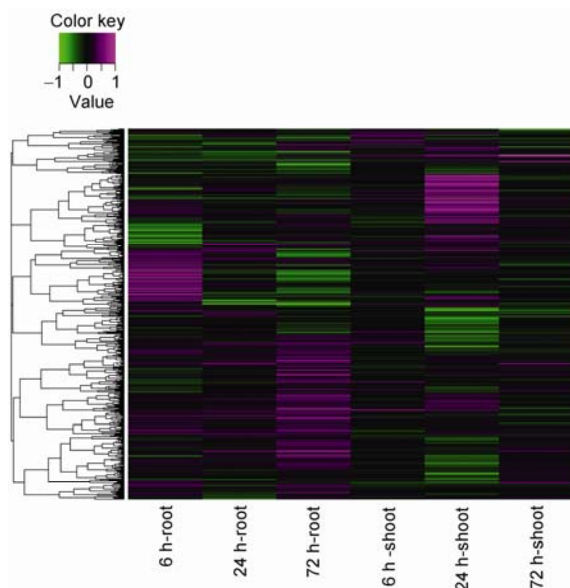

Figure 2 Clustering analysis of the differentially expressed genes. The ratio of stress/control was transferred into log 2 as the end. Red indicates up-regulated genes and green indicates down-regulated genes.

2.4 Energy metabolism-related genes changed their expression

Glycolysis is the metabolic pathway that converts glucose into pyruvate in the cytoplasm. Glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and pyruvate kinase are three key enzymes in glycolysis. Two glyceraldehyde-3-phosphate dehydrogenase genes (*Os054468_01* and *Os055294_01*) and one phosphoglycerate kinase gene (*Os052194_01*) were up-regulated at 6 h and then down-regulated at 72 h in rice roots under low P stress conditions. Two pyruvate kinase genes (*Os054518_01* and *Os054538_01*) were down-regulated at 72 h in rice roots. Two phosphoglycerate kinase genes (*Os036473_01* and *Os008567_01*) were down-regulated at 24 h in rice shoots under low phosphorus stress conditions.

Malic acid dehydrogenase, an important enzyme in the TCA cycle, catalyzes malic acid to generate oxalacetic acid (OAA). One malic acid dehydrogenase gene (*Os022060_01*) was up-regulated at 6 h under low phosphorus stress conditions in rice roots. The increased expression of the malic acid dehydrogenase gene can speed up the process of the TCA cycle to produce more organic acids secreted into the apoplast of rice roots and is conducive to the absorption of phosphorus.

The length and density of lateral roots, the ratio of roots to shoots, and the transportation of hexose from the leaf to the root increased under low phosphorus stress in plants [27]. The strengthened glycolysis provided more material and energy for growth in roots during early stages of treatment of Pi deficiency. With a further lack of phosphorus, glycolysis decreased in rice roots and shoots.

2.5 Phosphorus absorption, reuse-related genes changed their expression

Two high-affinity phosphate transporter genes (*Os022542_01* and *Os054748_01*), 13 phosphatase genes, and 4 RNase genes (*Os008274_01*, *Os008629_01*, *Os009320_01* and *Os009047_01*) increased their expression under low phosphorus stress in rice roots. Increased expression of these genes helped to enhance the absorption and reuse of phosphorus in rice. Three phosphatase genes (*Os052288_01*, *Os017246_01* and *Os002210_01*) were up-regulated and 4 phosphatase genes, (*Os034593_01*, *Os001369_01*, *Os013210_01* and *Os004533_01*) were down-regulated in rice shoots. Phosphate transporter genes and RNase genes did not change their expression in rice

Table 1 Functional category of the differentially expressed genes with known functions

	Shoots		Roots	
	Number	Percentage (%)	Number	Percentage (%)
Phosphate transport	0	0	2	0.467
Transport except Pi transport	13	6.16	21	4.88
Phosphatase	8	3.79	15	3.49
Enzyme except phosphatase	26	12.32	47	10.93
Primary metabolism	40	18.96	93	21.63
Secondary metabolism	10	4.74	8	1.86
Protein degradation	10	4.74	21	4.88
Protein synthesis	7	3.32	19	4.42
Stress	9	4.27	30	6.98
Signaling	20	9.48	19	4.42
Lipids	2	0.95	12	2.79
Transcription	20	9.48	38	8.84
e-transport	16	7.58	25	5.81
Others	30	14.22	80	18.6

shoots.

2.6 Oxidative stress-related genes changed their expression

Peroxidase is involved in the metabolism of reactive oxygen species in the body of a wide variety of animals, plants, and microorganisms and its physiologic functions are associated with a variety of environmental stresses [28–30]. Seven peroxidase genes, (*Os005447_01*, *Os014950_01*, *Os024326_01*, *Os056794_01*, *Os057353_02*, *Os021248_01* and *Os025712 _01*) were up-regulated in rice roots under low phosphorus stress. Metallothionein, a kind of small molecular weight, Cys-rich metal-binding protein, chelates heavy metals through its large number of Cys residues to remove reactive oxygen species, so that plants can avoid the oxidative damage [31,32]. Four metallothionein genes *(Os002159_01*, *Os003266_01*, *Os051892_01* and *Os005955_01*) were up-regulated at 6 h in rice roots under low phosphorus stress. Three cytochrome P450 genes (*Os022841_01*, *Os036691_01* and *Os041263_01*) and three glutathione S-transfer protein genes (*Os010350_01*, *Os011436_01* and *Os029667_01*) increased their expression also in rice roots under low phosphorus stress.

One metallothionein gene (*Os000446_01*) and one peroxidase gene (*Os004183_01*) were up-regulated and another peroxidase gene (*Os004260_01*) was down-regulated in rice shoots under low phosphorus stress.

These gene products are considered associated with oxidative stress in plants. The changes in the expression of these genes under low phosphorus stress in rice indicated that stress response mechanisms were turned on *in vivo* through direct or indirect mechanisms.

2.7 Transport-related genes changed their expression

Two cation transporter genes (*Os053767_01* and O*s057042_02*), two monosaccharide transporter genes (*Os014078_01* and *Os053987_01*), and one lipid-transfer protein gene (*Os023181_02*) were up-regulated in rice roots under low phosphorus stress conditions. Major facilitator superfamily (MFS) protein and ABC transporters, considered two major drug efflux pumps, play an important role in resistance in the body of the fungi [33]. Three MFS protein genes and five ABC transporters genes were up-regulated in rice roots under low phosphorus stress conditions. One toxin transporter gene (*Os024302_01*) was up-regulated also in rice roots. One ABC transporter gene (*Os030003_01*), one Sugar transporter gene (*Os056984_02*), and one cation transporter gene (*Os006082_01*) were down-regulated in rice shoots under low phosphorus stress conditions.

2.8 Signal transduction-related genes changed their expression

Protein kinase plays an important role in the PHO regulation system in the yeast [5]. Protein kinase also plays an important role in signal transduction under low temperatures, drought, resistance, and injury [34–36]. In our study, 8 protein kinase genes and 4 receptor-like protein kinase genes were up-regulated at 72 h in rice roots under low phosphorus stress. In rice shoots, three protein kinase genes, one MAPK kinase gene, and one receptor-like protein kinase gene were down-regulated, and one protein kinase gene was up-regulated.

Auxin treatment promoted root hair density and length, which is similar to the symptoms under low phosphorus stress in plants. Therefore, auxin has an important role in signal transduction in these conditions [37]. One auxin efflux carrier gene was down-regulated in rice roots under low phosphorus stress. The increase of auxin content in rice roots because of the decreased expression of the gene may be beneficial to the absorption of phosphorus.

2.9 Transposable elements changed their expression

The transposable element is a mobile DNA sequence common in bacterium, animal, and plant cells. The transposable element plays an important role in the evolution of plants, and its expression is controlled strictly under normal growth

conditions. However, under tissue culture, γ-ray treatment, pathogen infection, and other conditions, the transposable element is activated [38–40]. At present, it has not been reported that transposon is activated under the conditions of nutritional stress. Eight transposable elements were down-regulated and 16 transposable elements were up-regulated in rice roots under low phosphorus stress conditions. Twenty-three transposable elements were down-regulated, and 91 transposable elements were up-regulated in rice shoots under low phosphorus stress conditions. The changes of expression of transposable elements indicated that the dynamic equilibrium of transposition and transposition suppression was disrupted under the conditions of low phosphorus. The change of expression of transposable elements may be a nonspecific response to low phosphorus stress. Some stress response mechanisms are activated when plants was under low phosphorus stress, thus leading to the activation of expression of transposable genes.

2.10 Thionin genes were up-regulated in rice shoots

Thionine, a thiophene-type small molecule peptide with an aromatic ring structure, was first found in wheat flour. Then, a similar protein was found in other cereal crops and dicot plants. Thionine possesses a planar ring structure with a positive charge, so it strongly affects the transcription of DNA. Thionine was considered as a defense protein because it has an important role in disease resistance of bacteria and fungi in plants [41]. Three thionine genes (*Os050997_01*, *Os051941_01*, and *Os051942_01*) were up-regulated in rice shoots under low phosphorus stress, whereas thionine genes did not change their expression in rice roots.

3 Discussion

Several key enzyme genes in glycolysis were up-regulated in rice roots during the early stages of Pi-deficiency treatments. The increased glycolysis provides the necessary carbon and energy to lateral roots and root hairs for growth and development. With further consumption of phosphorus in rice, glycolysis-related genes were down-regulated in rice roots and shoots during the mid- and late-stages of Pi-deficiency treatments. To overcome the stress condition, plants reduce a variety of metabolic processes and slow down the growth rate. At the same time, the increased expression of phosphate transporter genes prompts the absorption of phosphate in rice roots under low phosphorus stress. The increased expression of phosphatase genes and RNase genes helped to reuse the internal phosphorus in rice. Phospholipid degradation genes increased their expression and glycolipid synthesis genes increased their expression also. Glycolipids replace phospholipids to perform biologic functions. The above response deals with low phosphorus stress in rice roots.

The phosphorus content changed little within 72 h in shoots under low phosphorus stress, but phosphorus content decreased rapidly at 24 h in roots [23]. In our study, the genes directly involved in phosphorus absorption and use did not change significantly in transcription in rice shoots, which may be related to the inadequate low phosphorus treatment. Severe phosphorus starvation did not take shape in rice shoots at 72 h under low phosphorus stress.

Photosynthesis is performed in plant chloroplasts and cannot be performed in roots because roots have no chloroplasts. In our study, the roots of rice were shaded during the growth process, but 31 photosynthesis-related genes were up-regulated at 6 h and then down-regulated at 72 h in rice roots under conditions of low phosphorus. The decreased expression of photosynthesis-related genes in roots has been reported, which may be related to saving energy [20,42]. At present, we do not know the biologic significance of the increased expression of photosynthesis-related genes in roots.

General features of oxidative stress (peroxidase, glutathione S-transfer protein, metallothionein) are also strongly induced in rice roots under low phosphorus stress conditions. This finding suggests that stress response mechanisms are turned on through direct or indirect mechanisms after low phosphorus stress in plants. Oxidative stress makes cells produce a large number of harmful substances, which must be promptly removed *in vitro*. Hazardous substances can be removed by the ABC transporter, MFS, or toxin transporter. Therefore, increased expression of these genes may be a better way to remove harmful substances in cells. These genes were down-regulated in rice shoots.

Transposons, a large class of genes in the plant genome, are inhibited usually due to the role of methylation under normal growth conditions but expressions of transposon genes are activated because of the demethylation under some stress conditions [43]. The expression of many transposon-related genes possesses the specificity of the organ and developmental stage and is induced under specific stress conditions [44]. For example, the expression of transposon Tam-3 genes was regulated by temperature [45]. However, it has not been reported that the transposon gene was activated under low-phosphorus stress at present. A large number of transposons genes changed their expression under low-phosphorus stress conditions in rice in our study, which indicated transposons may play a certain role in adapting to nutrient stress in plants.

Thionine has been considered as a defense protein in disease resistance of bacteria and fungi in plants. Three thionine genes were up-regulated in rice shoots under low phosphorus stress conditions. When rice is under low-phosphorus stress conditions, some stress responses, such as the disease resistance and oxidative stress responses, may be switched on. It is suggested that some cross-signaling in

transduction occurs in response to different stress conditions.

Here, we analyzed the mechanisms of adaptation to low phosphorus stress at the transcriptional level in rice. With the development of proteomics and metabonomics, researching the changes of proteome and metabonomes under different phosphate nutrition conditions in rice, combined with the gene expression profiles, the molecular mechanisms of adaptation to low phosphorus stress in plants can be examined more comprehensively.

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1 Ryan J, Hasan H M, Baasifi M. Availability and transformation of applied phosphorous in calcareous Lebanese soils. Soil Sci Soc Am J, 1985, 49: 1215–1220
2 Xiang W, Huang M, Li X. Progress on fractioning of soil phosphorous and availability of various phosphorous fractions to crops in soil. Plant Nutri Fertilizer Sci, 2004, 10: 663–670
3 Raghothama K G. Phosphate transport and signaling. Curr Opin Plant Biol, 2000, 3: 182–187
4 Vance C P, Uhde-Stone C, Allan D L. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol, 2003, 157: 423–447
5 Zhang F, Wang J, Zhang W, et al. Nutrient use efficiencies of major cereal crops in china and measures for improvement. Acta Pedol Sin, 2008, 45: 915–924
6 Rausch C, Bucher M. Molecular mechanisms of phosphate transport in plants. Planta, 2002, 216: 23–27
7 Ticconi CA, Delatorre C A, Lahner B, et al. *Arabidopsis* pdr2 reveals a phosphate-sensitive checkpoint in root development. Plant J, 2004, 37: 801–814
8 Rubio V, Linhares F, Solano R, et al. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes Dev, 2001, 15: 2122–2133
9 Zakhleniuk O V, Raines C A, Lloyd J C. Pho3: A phosphorus-deficient mutant of *Arabidopsis thaliana* (L.) Heynh. Planta, 2001, 212: 529–534
10 Chen D, Delatorre C A, Bakker A, et al. Conditional identification of phosphate starvation-response mutants in *Arabidopsis thaliana*. Planta, 2000, 211: 13–22
11 Aung K, Lin S, Wu C, et al. *Pho2*, a phosphate overaccumulator, is caused by a nonsense mutation in a microRNA399 target gene. Plant Physiol, 2006, 141: 1000–1011
12 Bari R, Pant B D, Stitt M, et al. *PHO2*, microRNA399, and *PHR1* define a phosphate-signaling pathway in plants. Plant Physiol, 2006, 141: 988-999
13 Miura K, Rus A, Sharkhuu A, et al. The *Arabidopsis* SUMO E3 ligase SIZ1 controls phosphate deficiency responses. Proc Natl Acad Sci USA, 2005, 102: 7760–7765
14 Duan K, Yi K, Dang L, et al. Characterization of a sub-family of *Arabidopsis* genes with the SPX domain reveals their diverse functions in plant tolerance to phosphorus starvation. Plant J, 2008, 54: 965–975
15 Wang C, Ying S, Huang H, et al. Involvement of *OsSPX* in phosphate homeostasis in rice. Plant J, 2009, 57: 895–904
16 Maleck K, Levine A, Eulgem T, et al. The transcriptome of *Arabidosis thaliana* during systemic acquired resistance. Nat Genet, 2000, 26: 403–410
17 Kawasaki S, Borchert C, Deyholos M, et al. Gene expression profiles during the initial phase of salt stress in rice. Plant Cell, 2001, 13: 889–905
18 Seki M, Narusaka M, Abe H, et al. Monitoring the expression pattern of 1,300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell, 2001, 13: 61–72
19 Wang R, Guegler K, Labrie S T, et al. Genomic analysis of a nutrient response in *Arabidopsis* reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. Plant Cell, 2000, 12: 1491–1509
20 Lian X, Wang S, Zhang J, et al. Expression profiles of 10,422 genes at early stage of low nitrogen stress in rice assayed using a cDNA microarray. Plant Mol Biol, 2006, 60: 617–631
21 Wasaki J, Yonetani R, Kuroda S, et al. Transcriptomic analysis of metabolic changes by phosphorus stress in rice plant roots. Plant Cell Environ, 2003, 26: 1515–1523
22 Hammond J P, Bennett M J, Bowen H C, et al. Changes in gene expression in *Arabidopsis* shoots during phosphate starvation and the potential for developing smart plants. Plant Physiol, 2003, 132: 578–596
23 Wu P, Ma L, Hou X, et al. Phosphate starvation triggers distinct alterations of genome expression in *Arabidopsis* roots and leaves. Plant Physiol, 2003, 132: 1260–1271
24 Misson J, Raghothama K G, Jain A, et al. A genome-wide transcriptional analysis using *Arabidopsis* thaliana Affymetrix gene chips determined plant responses to phosphate deprivation. Proc Natl Acad Sci USA, 2005, 102: 11934–11939
25 Wang X, Yi K, Tao Y, et al. Cytokinin represses phosphate-starvation response through increasing of intracellular phosphate level. Plant Cell Environ, 2006, 29: 1924–1935
26 Yoshida S, Forno D A, Cook J H, Gomez K A. laboratory manual for physiological studies of rice. Manila: International Rice Research Institute, 1976. 61–67
27 Cogliatti D H, Clarkson D T. Physiological changes in, and phosphate uptake by potato plants during development of, and recovery from phosphate deficiency. Physiol Plant, 1983, 58: 287–294
28 Chittoor J M, Leach J E, White F F. Differential induction of peroxidase gene family during induction of rice by *Xanthomonas oryzae* pv. *oryzae*. Mol Plant-Microbe Interact, 1997, 10: 861–871
29 Betella M A, Quesada M A, Konowicz A K. Characterization and *in situ* localization of a salt-induced tomato peroxidase mRNA. Plant Mol Biol, 1994, 25: 105–114
30 Miller R, Zilinskas B A. Molecular cloning and characterization of a gene edcoding pea cytosolic ascorbate peroxidase. J Biol Chem, 1992, 267: 21802–21807
31 Roosens N H, Bemard C, Leplae R, Verbruggen N. Evidence for copper homeostasis function metallothionein (MT3) in the hyperaccumulator *Thlaspi caerulescens*. FEBS Lett, 2004, 5: 9–16
32 Mir G, Domenech J, Huguet G, et al. A plant type 2 metallothionein (MT) from cork tissue responds to oxidative stress. J Exp Bot, 2004, 55: 2483–2493
33 Prasad R, Dewerqifosse P, Goffeau A, et al. Molecular cloning and characterization of a novel gene of *Candida albicans*, CDR1, conferring multiple resistance to drugs and antifungals. Curr Genet, 1995, 27: 320–329
34 Martín M L, Busconi L. A rice membrane-bound calcium-dependent protein kinase is activated in response to low temperature. Plant Physiol, 2001, 125: 1442–1449
35 Romeis T, Piedras P, Jonathan D G J. Resistance gene-dependent activation of a calcium-dependent protein kinase in the plant defense response. Plant Cell, 2001, 12: 803–816
36 Chico J M, Raíces M, Téllez-Ińón M T, Ulloa R M. A calcium-dependent protein kinase is systemically induced upon wounding in tomato plants. Plant Physiol, 2002, 128: 256–270
37 Lopez-Bucio J, Hernandez-Abreu E, Sanchez-Calderon L. Phosphate availability alters architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. Plant Physiol, 2002, 129: 244–256
38 Jiang N, Bao Z, Zhang X, et al. An active DNA transposon family in rice. Nature, 2003, 421: 163–167
39 Nakazaki T, Okumoto Y, Horibata A, et al. Mobilization of a transposon in rice genome. Nature, 2003, 421: 170–172
40 He Z, Dong H, Dong J, et al. The rice Rim2 transcript accumulates in response to *Magnaporthe grisea* and its predicted protein produce

shares similarity with TNP2-like proteins encoded by CACTA transposons. Mol Gen Genet, 2000, 264: 2–10

41 Loeza-Angeles H, Sagrero-Cisneros E, Lara-Zarate L, et al. Thionin Thi2.1 from *Arabidopsis thaliana* expressed in endothelial cells shows antibacterial, antifungal and cytotoxic activity. Biotechnol Lett, 2008, 30: 1713–1719

42 Himanen K, Vuylsteke M, Vanneste S, et al. Transcript profiling of early lateral root initiation. Proc Natl Acad Sci USA, 2004, 101: 5146–5155

43 Chinnusamy V, Zhu J. Epigenetic regulation of stress responses in plants. Curr Opin Plant Biol, 2009, 12: 133–139

44 Jiao Y, Deng X. A genome-wide transcriptional activity survey of rice transposable element-related genes. Genome Biol 2007, 8: R28

45 Hashida S, Uchiyama T, Martin C, et al. Temperature-dependent change in methylation of the *Antirrhinum* transposon Tam3 is controlled by the activity of its transposase. Plant Cell, 2006, 18: 104–118