

# Identification of phosphorus stress related proteins in the seedlings of Dongxiang wild rice (*Oryza rufipogon* Griff.) using label-free quantitative proteomic analysis

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## Research article

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# Abstract

**Background:** The lack of available phosphorus (P) in soil is one of the important factors restricting rice growth. Previous studies indicated that Dongxiang wild rice (DXWR, *O. rufipogon* Griff.) was resistant to low-P stress. So far, the research of resistance mechanism in DXWR was very limited.

**Results:** The results showed that 3589 significant differential accumulation proteins were identified between low P and the normal P treated root samples. 60 up-regulated and 15 down-regulated proteins were identified with  $\geq 1.5$ -fold changes as an additional standard. Furthermore, among 75 significantly different expression proteins (SDEPs), 24 proteins also detected in previous transcriptome dataset verified by qRT-PCR, including OsPT2, OsPT8, OsPAP10c, OsPAP10a and OsPHF1. Through comprehensive analysis, it was found that DXWR could increase PAPs, membrane location of PTs, rhizosphere area, alternative splicing and decrease ROS activity to deal with low-P stress. Moreover, among the genes corresponding to 75 SDEPs, 7 uncharacterized genes were located in previous P related QTL intervals, of which two genes (LOC\_Os12g09620 and LOC\_Os03g40670) have been detected at both transcriptome and proteome levels. In addition, the expression patterns of OsPHR1, OsPHR2, OsPHO1 and NAT-OsPHO1 in DXWR were different in cultivated rice, suggesting that the response mechanism of some low-P tolerance in DXWR might be different from that in cultivated rice.

**Conclusions:** This study screened out some candidate genes for low-P resistance and preliminarily verified that there might be a different low-P response mechanism in DXWR than in cultivated rice, which would provide insights in cloning the P-deficiency genes from wild rice, as well as elucidating the molecular mechanism of low-P resistance in DXWR.

## Background

Phosphorus (P) is one of the essential macronutrients in plant growth and development. It is estimated that 43% (about 5.8 billion  $\text{hm}^2$ ) of the world's arable land is deficient in P, and there are 3/4 farmland (about 67 million  $\text{hm}^2$ ) P shortages in China [1] resulted in yield reduction by 5–15% (about 25–75 billion Kg). Although soil available P deficiency can be improved by applying phosphate ( $\text{Pi}$ ) fertilizer, the utilization rate of which plants applied is no more than 20% [2]. This is because most of P in soil is exist in the form of insoluble mineral P or bound organic P which cannot be absorbed by plants. In addition, the main source of  $\text{Pi}$  fertilizer is  $\text{Pi}$  rock, which is a non-renewable resource and is expected to be depleted soon, and the heavy use of  $\text{Pi}$  fertilizer can also cause environmental problem such as eutrophication of water [3].

Plant grows adapting to P deficiency environment covers a series of gene expressions and morpho-physiological events [4], such as regulation of P transporters (PTs), mycorrhizal association, phosphatase secretion, organic acid exudation and alteration in root structure under low-P conditions [5]. Studies have shown that OsPHR2, homologous to AtPHR1 in Arabidopsis, is a major transcriptional regulator of low P response in rice [6, 7] which could active the  $\text{Pi}$  starvation-induced genes through binding to the P1BS (PHR1 Binding Sequence; GNATATNC) motif presented in genes' promoter region, including PHT1

members [8, 9]. There are 13 members of the PHT1 family in rice, and 10 of them have been functionally characterized which have been reported to localize to the plasma membrane. OsPT1 and OsPT8 are constitutively expressed high-affinity Pi transporters in rice, and their expression is not affected by external Pi levels. OsPT2 has been demonstrated to be responsible for root-to-shoot translocation of Pi [10]. OsPT6, OsPT9, OsPT10 and OsPT4 are also involved in Pi absorption and transport. In addition, OsPT4 plays a key role in the development of rice embryos [11], as well as OsPT9 and OsPT10 are functional redundancy [12]. OsPT3 which participates in Pi absorption and transport is only induced to be expressed when the Pi is extremely scarce [13]. In addition, OsPT11 and OsPT13 are involved in the arbuscular mycorrhizal symbiosis process and are induced by mycorrhiza [14]. Therefore, excavating the high efficiency P utilization gene of the crop itself and cultivating new varieties resistant to low P will provide insights in solving the yield problem caused by P deficiency.

Dongxiang wild rice (hereinafter referred to as DXWR) is a common wild rice (*O. rufipogon* Griff.) found in the northernmost distribution latitude to date, which is a unique wild plant resource in Jiangxi Province. Because DXWR grows in the wild for a long time and suffers from various adverse environmental choices, it has more abundant genetic diversity than cultivated rice, and contains a large number of excellent genes that are not or have been lost in cultivated rice, including low-P resistance [15, 16, 17, 18]. Therefore, DXWR is a valuable resource for the excavation and utilization of low-P resistant genes. So far, some QTLs related to low-P stress tolerance have been identified in DXWR [17]. However, how DXWR copes with growth-limiting P deficiency at the gene and protein levels remains unknown, thus limits the full utilization of the excellent low-P resistance gene in DXWR in practical breeding. It is of great significance to study the structure and function of the genes related to low-P resistance of DXWR, to analyze its genetic mechanism and molecular regulation network, and to guide the breeding of low-P resistant rice varieties in order to stabilize rice production.

In order to understand the molecular mechanism related to low-P resistance of DXWR, transcriptome analysis of low-P stress responsiveness in the seedlings of DXWR have been completed [18]. In this study, a proteomic approach involving liquid chromatography and tandem mass spectrometry was conducted to root samples to identify potential unique low-P response genes in DXWR during seedlings. Furthermore, the biological relevance of the differentially abundant proteins in root of DXWR was investigated using various bioinformatics strategies to connect proteins to known biological functions and pathways. Then, combined our previous transcriptome data of DXWR under low-P stress with the results of label free proteomics analysis in this study to excavate the low-P resistance gene in DXWR. The results in this study would provide insights in explaining the molecular mechanism of low-P resistance, as well as cloning and utilizing the P-deficiency genes from wild rice.

## Results

### Label-free quantitative proteomic analysis

After protein extraction, protease solution, LC-MS/MS and label-free data analysis, a total of 4329 protein groups (Additional file 2) and 23,598 peptides (Additional file 3) were identified from six DXWR root

samples (RLP and RCK with three biological repetitions, respectively). Among these proteins, we designated 3589 proteins that were detected in at least two replicates as identified proteins (Figure 1a-c). In addition, clustering analysis of proteins identified in different samples showed that the similarities between the three biological repetitions of the same treatment were high, and between the different treatments were very low (Figure 1d). Based on this, it was indicated that changes in expression of these target proteins could represent a significant effect of biological treatment on samples. Using these data, we selected proteins with  $\geq 1.5$ -fold changes as an additional standard (refer to [19]) and the volcano plot was drawn by using two factors of protein expression fold changes and  $p$  value obtained from  $t$  test to show the significant differential protein accumulation between RLP and RCK (Figure 1e). The results showed that 60 protein groups were up-regulated (Table 1) and 15 down-regulated (Table 2). Those results indicated that P deficiency treatment could affect the accumulation of some gene expression products in DXWR.

### Functional classification by gene ontology (GO)

To gain insight into the functional roles of the proteins significantly different between the RCK and RLP samples, Gene Ontology (GO) annotation and enrichment was conducted and the results were listed in Additional file 4, schematically represented in three ontologies as molecular function, cellular component, and biological process, as in Figure 2a. Enrichment of biological process involved in metabolic process and cellular process were significantly observed. The most significantly enriched molecular function were catalytic activity and binding. A significant enrichment of cellular compartments involved in cell part, cell, membrane, membrane part and organelle part were identified. From the above description, we can give a conjecture that low-P deficiency could affect cell proliferation, enzyme synthesis as well as the ability of cell or membrane to bind to certain stimulus signals.

### Kyoto encyclopedia of genes and genomes (KEGG) pathway mapping

KEGG Pathways analysis were performed on the 75 significantly different expression proteins (SDEPs, 60 up-regulated and 15 down-regulated) identified in this study (Additional file 5). These proteins were involved in 31 metabolic pathways. Four proteins were enriched to the spliceosome pathway, which was one of the top 20 KEGG pathways predicted to be affected by low-P deficiency (Figure 2b), including up-regulated U6 snRNA-associated Sm-like protein 8 (LSm8, *LOC\_Os05g51650*), U1 small nuclear ribonucleoprotein A (U1A, *LOC\_Os05g06280*) and down-regulated splicing factor of arginine/serine-rich (SR, *LOC\_Os01g06290*), pre-mRNA-splicing factor SPF27 (BCAS2, *LOC\_Os01g16010*). Furthermore, two proteins enriched in the pathway of branched-chain amino acids (BCAAs, include valine, leucine and isoleucine) biosynthesis, including up-regulated branched-chain-amino-acid aminotransferase (BCAT, *LOC\_Os03g01600*) and acetolactate synthase small subunit (ALS, *LOC\_Os11g14950*). Two proteins enriched in the pathway of glyoxylate and dicarboxylate metabolism, containing down-regulated ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl, encoded by leucoplast), as well as up-regulated phosphoglycolate phosphatase (PGP, *LOC\_Os09g08660*). In addition, two proteins enriched in amino sugar and nucleotide sugar metabolism, inclusive of up-regulated chitinase (*LOC\_Os01g49320*) and glycosyl hydrolase (*LOC\_Os01g47070*). Two proteins enriched in glutathione pathway, containing up-

regulated GST (*LOC\_Os10g38740* and *LOC\_Os10g38360*). These results indicated that genes involved in these pathways might respond to low-P stress in DXWR.

Protein-protein interactions (PPI) between the identified proteins

Protein is an important component of biological organisms, which do not perform biological function independently, but through the interaction of proteins to regulate physiological and biochemical processes. Therefore, we performed a PPI analysis of the proteins identified by label-free quantitative analysis. As shown (Figure 2c), there was a strong interaction only between a few proteins after screening.

A2X5V0 (Uniprot ID, *LOC\_Os02g33850*, up-regulated) with the function of elongation factor Tu family protein (EF-TU) has 5 mutual proteins, including Q0D840 (*LOC\_Os07g08840*, up-regulated) with function as Thioredoxin, A2YMF8 (*LOC\_Os07g36490*, up-regulated) with the function of RNA recognition, Q5TKF9 (*LOC\_Os05g51650*, up-regulated) with the function of U6 snRNA-associated Sm-like protein LSm8 which involved in RNA splicing, B8AHU1 (*LOC\_Os02g05880*, down-regulated) with function of RNA polymerase which participate in the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates, and B8AC69 (*LOC\_Os01g16010*, down-regulated) with the function of BACS2 protein which is the core component of Prp19 related complex along with involved in important life activities such as splicing of precursor RNA.

Additionally, B8AC69 and A2YMF8 both have 4 mutual proteins involved in splicing of precursor RNA and translation beginnings. What's more, Q0DKM4 (*LOC\_Os05g06280*, up-regulated) and A0A0P0XSK6 (*LOC\_Os10g07229*, up-regulated) have 3 mutual proteins and encode U1 small nuclear ribonucleoprotein A and dehydrogenase, respectively. Q6EP66 (*LOC\_Os09g08660*, up-regulated) and A6MZ96 (*LOC\_Os01g06290*, down-regulated) have 2 mutual proteins and encode Phosphoglycolate phosphatase and splicing factor, respectively. These results suggested that low-P stress induced reduction of transcription-related genes, and the increase of most RNA splicing related genes along with intensification of the gene expression associated with the elongation during translation.

Comparison of abundance expression patterns between proteins and transcripts verified by qRT-PCR

In present study, among 75 SDEPs, there were 24 proteins with fold changes larger than 1.5 both in proteomics and transcriptome level that were associated with P deficiency treatment in DXWR (Table 3). In addition, we also verified its expression at the transcriptome level by qRT-PCR, which showed consistency in transcriptome data and qRT-PCR results, shown in Figure 3. Among these proteins, 21 up-regulated, 2 down-regulated at both transcriptome and proteome level, and only one had the opposite abundance trend (up-regulated in proteome level but down-regulated in transcriptome level). Gene expression abundance was increased at both two levels including *OsPT2* (*LOC\_Os03g05640*), *OsPAP10c* (*LOC\_Os12g44020*), *OsPAP10a* (*LOC\_Os01g56880*), *OsPHF1* (*LOC\_Os07g09000*), as well as a gene encoding glycerophosphoryl diester phosphodiesterase family protein (GDPD, *LOC\_Os03g40670*), three genes encoding glycosyl hydrolase (*LOC\_Os07g23850*, *LOC\_Os05g15770* and *LOC\_Os01g47070*) and one gene encoding glucan endo-1,3-beta-glucosidase precursor (*LOC\_Os07g35560*). Furthermore, a gene

(*LOC\_Os10g35500*) encoding epoxide hydrolase increased by low-P stress at proteomic level, but the corresponding transcription levels decreased, possibly by increasing the stability of the corresponding mRNA and reducing the number of transcriptions to reduce energy consumption.

### Conjoint analysis of proteomic and QTLs related to P deficiency tolerance

As shown in table 4, there are two P-deficiency tolerance related QTLs have been identified in DXWR [17] and 12 QTLs existing in different positions on the chromosome related to P-deficiency stress have been found in *Oryza sativa* based on the Gramene QTL database. Among genes corresponding to 75 SDEPs identified by the proteome in this study, we located 9 genes among these QTL intervals, as shown in Table 5. In the midst of them, two genes (*LOC\_Os12g44020*, *OsPAP10c* and *LOC\_Os04g41970*, *OsGLU3*) have been characterized in previous studies [20, 21], and two of the other seven uncharacterized genes (*LOC\_Os12g09620* and *LOC\_Os03g40670*) have been detected at both transcriptome and proteome levels. Furthermore, the functional expression characteristics of the remaining five genes (*LOC\_Os01g57450*, *LOC\_Os03g29240*, *LOC\_Os03g13540*, *LOC\_Os03g29190* and *LOC\_Os06g07600*) have not been reported yet.

The expression pattern in DXWR of key genes responded to low P in cultivated rice detected by qRT-PCR

Based on previous studies on P response mechanism in cultivated rice, the homologous genes of *PHR1* (*OsPHR2* and *OsPHR1*), *OsPHO2*, *OsPHO1* as well as its NAT genes play an important role in the low-P response process. Therefore, we examined the expression levels of *OsPHR2*, *OsPHO2* as well as three members of *OsPHO1* (*OsPHO1;1*, *OsPHO1;2* and *OsPHO1;3*) and three NATs correspondently, as shown in Figure 4, and found that *OsPHR2* was down-regulated after low-P treatment in DXWR roots, which was contrary to the results of previous studies on cultivated rice [7, 22]. We suspect that maybe *OsPHR1* plays a major role during the P starvation signaling pathway in DXWR. For validation, we examined the expression of *OsPHR1* in DXWR. But the result was consistent with *OsPHR2* and was also down-regulated. Such results may indicate that the transcription levels of *OsPHR1* and *OsPHR2* are inhibited by low-P signals in DXWR. In addition, *OsPHO2* was down-regulated in both DXWR and NP. Among *OsPHO1;1*, *OsPHO1;2* and *OsPHO1;3*, only *OsPHO1;3* expression was up-regulated and the other two were down-regulated in DXWR, but three genes all up-regulated in Nipponbare (NP). In the corresponding NAT, only *OsPHO1;2* NAT expression change trend is consistent with *OsPHO1;2* and the other two NATs change trend is opposite to *OsPHO1;1* and *OsPHO1;3* in DXWR, but all three NATs up-regulated in NP. These results indicate that there may be a difference in the mechanism of resistance to low-P in DXWR compared to cultivated rice.

## Discussion

# Increasing phosphorus uptake in roots of DXWR through PTs and PAPs

Previous study [23] has shown OsPHF1 regulates the plasma membrane localization of low-affinity Pi transporter OsPT2 and the high-affinity Pi transporter OsPT8, of which ortholog in Arabidopsis reported to be only an important factor for the localization of high-affinity Pi transporters to the plasma membrane [24]. Subcellular location experiments show that mutation of OsPHF1 lead to the retention of OsPT2 and OsPT8 in the endoplasmic reticulum, and reduce the accumulation of Pi in shoots due to overexpression of OsPHR2, but OsPHF1 itself is not controlled by OsPHR2 [23, 25]. To the contrary, overexpression of OsPHF1 results in excessive Pi accumulation in leaf and Root.

Furthermore, OsPT2 is the only low affinity transporter in the PHT1 family induced by Pi deprivation under the transcriptional control of OsPHR2, whereas OsPT8 is constitutively expressed high-affinity Pi transporters in rice whose expression is not affected by external Pi levels. Here, in our study, the expression levels of OsPT2, OsPT8 and OsPHF1 were up-regulated, and OsPT2 and OsPT8 may be induced by low-P stress in DXWR, and the up-regulated expression of OsPHF1 increased the plasma membrane localization of these two PTs.

On the other hand, purple acid phosphatases (PAP) is a family of metallophosphoesterases involved in a variety of physiological functions, especially low Pi adaptations in plants [26] and has non-specific acidic phosphatase activity, which can catalyze the hydrolysis of various organic P into Pi under acidic pH conditions, thus providing more Pi for plants [20, 27]. PAP plays critical role in plant's ability to utilize organic P in growth medium.

Together, these results demonstrated that DXWR enhanced its absorption of Pi by increasing rhizosphere Pi content through up-regulating PAPs, while the root membrane localization as well as expression of OsPT2 and OsPT8.

## **Alternative splicing improves the resistance to low-P in DXWR**

Studies have shown that the absence of SR or SPF protein can lead to changes in splice sites [28, 29]. LSm8 is essential for the assembly of the LSM nuclear complex (LSM2-8) and this complex acts in pre-mRNA splicing through U6 snRNA stabilization, thus allowing the formation of the U6 snRNP [30]. The Arabidopsis LSM2-8 complex differentially regulates plant tolerance to abiotic stresses by controlling the constitutive and alternative splicing of specific introns from selected abiotic stress-related pre-mRNAs [31]. In this study, the up-regulation of LSm8 (LOC\_Os05g51650) and U1A (LOC\_Os05g06280) alternates splicing of pre-mRNA, while the down-regulation of SPF (LOC\_Os01g16010) and SR (LOC\_Os01g06290) may change pre-mRNA splicing site, thus improving the tolerance to low-P deficiency of DXWR.

## **To reduce the damage caused by low-P stress in DXWR**

Studies have shown that plants under abiotic stress are coerced to increase the activity of reactive oxygen species (ROS) and antioxidant enzymes [32, 33]. As shown, a key enzyme in the BCAAs biosynthesis pathway, ALS, was down-regulated under stress [32]. In addition, by increasing the concentration of BCAAs to 100 mg/L in the culture medium, ROS was significantly reduced, thereby reducing the level of

antioxidant enzymes in herbicide-stressed plants [33]. In present research, DXWR may be increasing the biosynthesis of BCAAs through up-regulating BCAT (LOC\_Os03g01600) and ALS (LOC\_Os11g14950) to reduce ROS and consequently antioxidant enzyme levels. What's more, Glutathione is a cosubstrate for glutathione-S-transferase (GST), which in rice participate in various functions such as phytohormone homeostasis, hydroxy peroxide detoxification, and apoptosis regulation [34], and also have key roles in response to biotic and abiotic stresses [35]. Two GST proteins (LOC\_Os10g38740 and LOC\_Os10g38360) enriched in glutathione metabolism pathway were up-regulated after P deficiency treatment of DXWR which might be increase the tolerance to cope with low-P stress. Furthermore, Epoxide hydrolase has been reported as an enzyme that reduces the content of epoxides in organisms by means of chemically catalyzed transformation as well as metabolizes endogenous aliphatic and aromatic epoxides [36]. It is a key enzyme involved in metabolism, detoxification and signaling regulation in the organism [37]. An epoxide hydrolase protein (LOC\_Os10g35500) increased in protein level might be increase the tolerance to low-P stress, but the corresponding transcription levels decreased, possibly by increasing the stability of the corresponding mRNA and reducing the number of transcriptions to reduce energy consumption.

## **Improving Pi absorption capacity by changing root structure of DXWR**

Studies have shown that PGP inactivation attenuated triosephosphate isomerase activity, increased triglyceride levels at the expense of the cellular phosphatidylcholine content, and inhibited cell proliferation [38, 39]. These effects were prevented under hypoxic conditions or by blocking phosphoglycolate release from damaged DNA. Moreover, as shown, EF-TU plays an important role in the reproduction, development and response to environmental stress of higher plants [40]. Here, it is likely that phosphoglycolate is reduced by down-regulating *rbcL*, while the synthesis of PGP (LOC\_Os09g08660) increases in this study, thus promoting cell proliferation with increased EF-TU (LOC\_Os02g33850). Furthermore, chitinase leads to the separation of parallel chitin microfibrils connected by  $\beta$ -1,6-branched chain  $\beta$ -1,3- glucans in the cell wall, thus increasing the interval between the insertion of newly synthesized chitin and  $\beta$ -1,3- glucans under swelling in vivo [41]. It was worth noting that the expression of chitinase (LOC\_Os01g49320) and glycosyl hydrolase (LOC\_Os01g47070) increased when the root of DXWR was under low-P stress. These results may suggest that DXWR promotes the elongation of cell wall to increase the absorption area of the root.

## **DXWR may exist a low-P tolerance mechanism different from cultivated rice NP**

PHR1 (Phosphate Starvation Response Regulator 1) is a central regulator of P-deficiency stress response [6]. There are two homologous genes of PHR1 in rice, namely OsPHR1 and OsPHR2. Some studies have shown that OsPHR2 plays a major role in the P starvation signaling pathway [7, 22]. But in this study, OsPHR2 as well as OsPHR1 were down-regulated in DXWR after received low-P stress, which was opposite to that in NP. After Pi enters the column cells of the root, PHOSPHATE1 (PHO1) located in the xylem of the root vascular bundle is responsible for loading Pi into the xylem and then transporting it from the xylem to the shoot [42, 43]. In Arabidopsis, mainly AtPHO1 and AtPHO1;H1 are involved in the P transportation from

root to shoot [44]. There are three homologous genes of AtPHO1 and AtPHO1;H1 in rice, which are OsPHO1;1, OsPHO1;2 and OsPHO1;3, and all three genes have natural reverse transcripts (Cis-Natural Antisense Transcripts, NATs) at the 5' end [22]. Studies among cultivated rice showed that OsPHO1;2 played a main role in the transport and distribution of P, while OsPHO1;2 NAT was induced by P starvation and could activate the expression of OsPHO1;2 [22], which was consistent with our result. But in DXWR, OsPHO1;2 and its NAT both down-regulated by low-P stress in DXWR, whereas OsPHO1;1 down-regulated and its NAT up-regulated, OsPHO1;3 up-regulated and its NAT down-regulated. In addition, OsPHO2 containing a E2 ubiquitin-binding domain, which could degrade OsPHO1 [45], was down-regulated in both DXWR and NP. These differences indicate that DXWR may have a unique resistance to low-P regulation, and these candidate genes screened by transcriptome and proteome in present study may also have unique functions in DXWR differ from cultivated rice. These are all subject to further research.

## Conclusions

In this study, a proteomic approach involving liquid chromatography and tandem mass spectrometry as well as joint analysis with transcriptome dataset were conducted to root samples to identify potential unique low phosphorus response genes in DXWR during seedlings. 75 SDEPs were detected, 24 of which were also detected in previous transcriptome dataset verified by qRT-PCR. Furthermore, it was found that DXWR could increase PAPs, membrane location of PTs, rhizosphere area, alternative splicing and decrease ROS activity to deal with low-P stress. Moreover, among the genes corresponding to 75 SDEPs, 7 uncharacterized genes were located in previous P related QTL intervals, of which two genes (LOC\_Os12g09620 and LOC\_Os03g40670) have been detected at both transcriptome and proteome levels. In addition, the expression patterns of OsPHR1, OsPHR2, OsPHO1 and NAT-OsPHO1 in DXWR were different in cultivated rice, suggesting that the response mechanism of some low-P tolerance in DXWR might be different from that in cultivated rice. These findings would provide insights in cloning the P-deficiency genes from wild rice, as well as elucidating the molecular mechanism of low-P resistance in DXWR.

## Methods

### Plant materials and phosphorus deficiency treatment

In present study, DXWR from Jiangxi academy of agricultural sciences was carried out as experimental material. The DXWR seeds were surface sterilized using mixed solutions of NaClO (0.6%) for 15 min, followed by washing three times with sterile water. The surface sterilized seeds were first soaked in petri dishes containing 20 mL deionized water at room temperature for 3 days. Then the seeds were selected with same growth and planted in the plastic pot in a climate control chamber at day/night 14 h/10 h (30°C/26°C) [18]. Germinated seeds with coleoptiles 10 mm approximately in length, adding Yoshida culture medium (pH5.8) [46]. At the emergence of the third leaf (about 15 days), plants were transferred into either a nutrient solution with the P concentration reduced to 0.016 mM NaH<sub>2</sub>PO<sub>4</sub> or a culture medium with the normal P concentration (0.32 mM NaH<sub>2</sub>PO<sub>4</sub>), which corresponded to the -P treatments (RLP) and

+ P treatments (RCK), respectively. The culture medium was replaced every two days. There were 20 seedlings per treatment with three biological replications. Roots were harvested at 9 days after the experimental treatments started, and the samples were frozen immediately using liquid nitrogen and stored at -80°C for further analyzing.

## Protein extraction and enzymatic hydrolysis

Root proteins of six DXWR samples were extracted using Tris-HCl method connected with trichloroacetic acid (TCA) - acetone precipitation method. Briefly, the sample was ground into powder in liquid nitrogen, and then suspended in extraction buffer (8 M urea, 1% DTT, 0.1 M Tris-HCl, pH 8.8, 1% complete protease inhibitors (Roche, Germany)). Repeated ultrasound and vortex of the sample and then removal of insoluble precipitation by centrifugation at 14,000 × g for 40 min. The supernatant was precipitated overnight with 20% (v/v) TCA, washed three times with cold acetone, and solubilized in extraction buffer. All operations are performed at low temperatures. The protein concentration was measured using the Bradford method. All resulting protein solutions were stored in -80°C for further treatment.

The final concentration of urea in protein solution was adjusted to 2 M with 40 mM NH<sub>4</sub>HCO<sub>3</sub> solution. Trypsin (Promega) was added to each sample and incubated overnight at 37 °C following the instructions of manufacturer.

## Nano-LC MS/ MS analysis

Sample analysis was performed using a Q-Exactive mass spectrometer (Thermo Fisher Scientific, USA) coupled to an Easy nano-LC Biosystem (Thermo Fisher Scientific, USA). Balance chromatographic column with buffer A (0.1% formic acid, 3% acetonitrile and 97% H<sub>2</sub>O). Each sample was automatically injected into the prepacked column (2 cm × 100 μm 3 μm-C<sub>18</sub>), then flowed into an analytical column (10 cm × 75 μm 3 μm-C<sub>18</sub>) at a speed of 250 nL/ min controlled by intelligiflow technology. After that, the sample was separated with a linear gradient of buffer B from 6% (80% acetonitrile, 0.08% formic acid) to 95% over 86 min and then followed by an equilibration of the column at 6% buffer B for 4 min.

The files obtained from nano-LC MS/ MS analysis were converted to RAW format and searched against the Uniport\_Oryza sativa\_168264\_20171201.fasta Database (<http://www.uniprot.org/>) using MaxQuant [47] software (version number 1.5.3.17). The maximum missed cleavages used for the database search were set to 2. The mass tolerance was set to 20 ppm on full scans. For label-free quantitative methods, retention time matching between runs was performed within a time window of 2 min. The peptide false discovery rate (FDR) and protein FDR not exceeding 0.01.

The t-test was used for statistical analysis, the results of which were represented by p values. We used p value ≤ 0.05 as the threshold to judge the significance of protein accumulation difference.

## Gene expression analysis by qRT-PCR

For qRT-PCR analysis, total RNA in roots RLP and RCK samples were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The first cDNA was synthesized

with 2 µg of total RNA, using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO). Then synthesized cDNAs were used as templates for quantitative real-time PCR (qRT-PCR) with THUNDERBIRD™ SYBR® qPCR Mix (TOYOBO) and the LightCycler® 96 instrument (Roche). Rice Ubi5 was used as an internal control. The  $2^{-\Delta\Delta Ct}$  method was used for relative quantification. The statistical significance was evaluated by t-test analysis. The primers used are listed in Additional file 1.

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

### Competing interests

We have no competing interests.

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### Authors' contributions

LXD and XJK conceived and supervised the entire project; DQW, BLWD, DLF and CYL performed the experiments and analyzed the data; DQW wrote the manuscript; BLWD, DLF and CYL reviewed the manuscript. All authors read and approved the final manuscript.

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Not applicable

## Abbreviations

**P**: phosphorus; **DXWR**: Dongxiang wild rice; **SDEPs**: significantly different expression proteins; **PTs**: P transporters; **P1BS**: PHR1 Binding Sequence; **GO**: gene ontology; **KEGG**: kyoto encyclopedia of genes and genomes; **LSm8**: U6 snRNA-associated Sm-like protein 8; **U1A**: U1 small nuclear ribonucleoprotein A; **BCAT**: branched-chain-amino-acid aminotransferase; **ALS**: acetolactate synthase small subunit; **rbcl**: ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit; **PGP**: phosphoglycolate phosphatase; **PPI**: protein-protein interactions; **EF-TU**: elongation factor Tu family protein; **GDPD**: glycerophosphoryl diester phosphodiesterase family protein; **PAP**: purple acid phosphatases; **GST**: glutathione-S-transferase; **PHR1**: Phosphate Starvation Response Regulator 1; **qRT-PCR**: quantitative real-time PCR.

## References

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## Tables

Table 1 Up-regulated proteins identified from label-free quantitative analysis

RAP (Os ID)	MSU (LOC_Os ID)	Description	Gene Name	Ratio[RFP/RCK]	Pvalue
<i>Os03g0603600</i>	<i>LOC_Os03g40670</i>	Glycerophosphoryl diester phosphodiesterase family protein	<i>OSJNBa0004G03.4</i>	16.03000419	0.006552
<i>Os03g0150800</i>	<i>LOC_Os03g05640</i>	Inorganic phosphate transporter 1-2	<i>PTH1-2</i>	3.960889785	0.033385
<i>Os01g0776600</i>	<i>LOC_Os01g56880</i>	Purple acid phosphatase	<i>Os01g0776600</i>	3.622714483	0.02748
<i>Os10g0444700</i>	<i>LOC_Os10g30790</i>	Probable inorganic phosphate transporter 1-8	<i>PHT1-8</i>	3.188357926	0.000168
<i>Os12g0637100</i>	<i>LOC_Os12g44020</i>	Purple acid phosphatase	<i>LOC_Os12g44020</i>	3.158117784	0.001597
<i>Os01g0660200</i>	<i>LOC_Os01g47070</i>	Glycosyl hydrolase	<i>OsJ_02893</i>	2.640670999	0.030145
<i>Os03g0719300</i>	<i>LOC_Os03g51000</i>	3,4-dihydroxy-2-butanone kinase	<i>B1377B10.5</i>	2.626305612	0.0442
<i>Os12g0198000</i>	<i>LOC_Os12g09620</i>	Expressed protein	<i>Os12g0198000</i>	2.624379146	9.82E-05
<i>Os08g0434100</i>	<i>LOC_Os08g33710</i>	Ribonuclease		2.61471967	0.002771
<i>Os06g0291100</i>	<i>LOC_Os06g18790</i>	Anthocyanidin 3-O-glucosyltransferase	<i>B1026E06.27</i>	2.435586728	0.046309
<i>Os02g0704900</i>	<i>LOC_Os02g47600</i>	Soluble inorganic pyrophosphatase	<i>IPP</i>	2.329695866	0.001458
<i>Os07g0129200</i>	<i>LOC_Os07g03710</i>	SCP-like extracellular protein	<i>OsJ_22957</i>	2.29462251	0.001414
<i>Os05g0402900</i>	<i>LOC_Os05g33400</i>	Basic 7S globulin precursor	<i>OsJ_18488</i>	2.229986706	0.045047
<i>Os03g0405900</i>	<i>LOC_Os03g29240</i>	Probable nucleoredoxin 1-2	<i>Os03g0405900</i>	2.22151414	0.0006
<i>Os07g0630400</i>	<i>LOC_Os07g43670</i>	Ribonuclease T2 family domain containing protein	<i>P0011H09.133</i>	2.18513976	0.004417
<i>Os10g0538200</i>	<i>LOC_Os10g39300</i>	Aspartic proteinase nepenthesin, putative, expressed	<i>OsI_34482</i>	2.147759239	0.030829
<i>Os01g0357100</i>	<i>LOC_Os01g25484</i>	Ferredoxin-nitrite reductase	<i>OsI_01871</i>	2.139517104	0.015234
<i>Os11g0256050</i>	<i>LOC_Os11g14950</i>	Acetolactate synthase small subunit	<i>LOC_Os11g14950</i>	2.118353002	0.005821
<i>Os02g0543300</i>	<i>LOC_Os02g33850</i>	Elongation factor Tu family protein, Protein synthesis factor, GTP-binding domain containing protein	<i>OsI_07585</i>	2.087060146	0.000463
<i>Os07g0549800</i>	<i>LOC_Os07g36490</i>	RNA recognition motif containing protein	<i>OsI_26412</i>	2.035407774	0.042675
<i>Os03g0738600</i>	<i>LOC_Os03g52860</i>	Linoleate 9S-lipoxygenase 2	<i>LOX1.1</i>	2.017839574	0.006819
<i>Os10g0191300</i>	<i>LOC_Os10g11500</i>	SCP-like extracellular protein	<i>LOC_Os10g11500</i>	2.010712464	0.0018
<i>Os05g0247100</i>	<i>LOC_Os05g15770</i>	Glycoside Hydrolase Family 18	<i>dip3</i>	2.004889132	0.03089
<i>Os10g0476000</i>	<i>LOC_Os10g33630</i>	Adaptin ear-binding coat-associated protein 2	<i>Os10g0476000</i>	1.997085229	0.009577
<i>Os04g0480900</i>	<i>LOC_Os04g40490</i>	Glycosyl hydrolase family 5 protein	<i>OsI_16340</i>	1.983137882	0.00484
<i>NONE</i>	<i>LOC_Os07g23850</i>	Glycosyl hydrolase	<i>OsI_25770</i>	1.977235788	0.016379
<i>Os03g0238600</i>	<i>LOC_Os03g13540</i>	Purple acid phosphatase	<i>LOC_Os03g13540</i>	1.955506958	0.0009
<i>Os06g0172800</i>	<i>LOC_Os06g07600</i>	Uncharacterized glycosyltransferase	<i>OsJ_20295</i>	1.871718049	0.010126
<i>Os01g0949900</i>	<i>LOC_Os01g72150</i>	Glutathione S-transferase	<i>Os01g0949900</i>	1.826722973	0.002976

<i>Os03g0405500</i>	<i>LOC_Os03g29190</i>	Probable nucleoredoxin 1-1	<i>Os03g0405500</i>	1.822377514	0.017644
<i>Os07g0162700</i>	<i>LOC_Os07g06860</i>	Gibberellin receptor GID1L2	<i>P0428D12.107</i>	1.818251432	0.034418
<i>Os01g0747500</i>	<i>LOC_Os01g54370</i>	Dihydropyrimidinase	<i>OsI_03720</i>	1.785266614	0.046646
<i>Os06g0320000</i>	<i>LOC_Os06g21550</i>	Thioredoxin-like protein Clot	<i>Os06g0320000</i>	1.776329132	0.032953
<i>Os07g0658600</i>	<i>LOC_Os07g46480</i>	Eukaryotic aspartyl protease domain containing protein	<i>OsJ_25435</i>	1.774156573	0.006259
<i>Os07g0539900</i>	<i>LOC_Os07g35560</i>	Glucan endo-1,3-beta- glucosidase precursor	<i>OsJ_24595</i>	1.773462618	0.027652
<i>Os01g0687400</i>	<i>LOC_Os01g49320</i>	Chitinase		1.760766916	0.04272
<i>Os02g0771700</i>	<i>LOC_Os02g53200</i>	Glucan endo-1,3-beta- glucosidase precursor	<i>Os02g0771700</i>	1.735448414	0.022941
<i>Os07g0186000</i>	<i>LOC_Os07g08840</i>	Thioredoxin H1	<i>TRXH</i>	1.701278791	0.011916
<i>Os04g0456700</i>	<i>LOC_Os04g38390</i>	Wound/stress protein, putative, expressed	<i>OSJNBa0036B21.4</i>	1.667066756	0.004973
<i>Os07g0187700</i>	<i>LOC_Os07g09000</i>	WD40 protein, Regulation of the plasma membrane localization of phosphate transporters, Phosphate uptake and translocation	<i>Os07g0187700</i>	1.663966106	0.037637
<i>Os01g0132000</i>	<i>LOC_Os01g04050</i>	BBTI12-Bowman-Birk type bran trypsin inhibitor precursor	<i>Os01g0132000</i>	1.654255218	0.017942
<i>Os07g0683600</i>	<i>LOC_Os07g48460</i>	Stress responsive protein, putative, expressed	<i>OsJ_25614</i>	1.644255664	0.005868
<i>Os03g0106400</i>	<i>LOC_Os03g01600</i>	Branched-chain- amino-acid aminotransferase	<i>LOC_Os03g01600</i>	1.610695927	0.030361
<i>Os10g0498100</i>	<i>LOC_Os10g35500</i>	Epoxide hydrolase	<i>OsJ_32041</i>	1.592639094	0.014471
<i>Os06g0717900</i>	<i>LOC_Os06g50390</i>	Aspartic proteinase nepenthesin II-like	<i>P0541C02.19-1</i>	1.585853919	0.014796
<i>Os07g0634600</i>	<i>LOC_Os07g44070</i>	Pectin acetylesterase	<i>P0455H11.118-1</i>	1.583424242	0.017612
<i>Os09g0261300</i>	<i>LOC_Os09g08660</i>	Phosphoglycolate phosphatase	<i>B1077E10.18-1</i>	1.57844691	0.004606
<i>Os04g0628200</i>	<i>LOC_Os04g53640</i>	Peroxidase	<i>prx56</i>	1.572207357	0.001422
<i>Os10g0527800</i>	<i>LOC_Os10g38360</i>	Glutathione S- transferase	<i>OsI_34399</i>	1.558245126	0.045511
<i>Os05g0154800</i>	<i>LOC_Os05g06280</i>	U1 small nuclear ribonucleoprotein A	<i>Os05g0154800</i>	1.55665498	0.007471
<i>Os03g0661600</i>	<i>LOC_Os03g45960</i>	Similar to Alpha- amylase/trypsin inhibitor (Antifungal protein).	<i>OSJNBb0065L20.2</i>	1.553387114	0.019769
<i>Os03g0214000</i>	<i>LOC_Os03g11530</i>	Purple acid phosphatase	<i>LOC_Os03g11530</i>	1.550828031	0.035302
<i>Os07g0638100</i>	<i>LOC_Os07g44410</i>	WD40-like Beta Propeller Repeat family protein	<i>OJ1340_C08.105</i>	1.550189963	0.002186
<i>Os10g0530900</i>	<i>LOC_Os10g38740</i>	Probable glutathione S-transferase GSTU6	<i>GSTU6</i>	1.530424487	0.014539
<i>Os01g0783500</i>	<i>LOC_Os01g57450</i>	Universal stress protein domain containing protein	<i>Os01g0783500</i>	1.526457541	0.005096
<i>Os10g0159800</i>	<i>LOC_Os10g07229</i>	dehydrogenase	<i>Os10g0159800</i>	1.524049105	0.049332
<i>Os02g0139100</i>	<i>LOC_Os02g04650</i>	Activator of Hsp90 ATPase	<i>Os02g0139100</i>	1.511733905	0.004122
<i>Os06g0266400</i>	<i>LOC_Os06g15600</i>	Similar to chemocyanin Phycocyanin	<i>OsI_22465</i>	1.508518489	0.04803

<i>Os05g0182100</i>	<i>LOC_Os05g08930</i>	chloroplast lumen common family protein	<i>OsI_18722</i>	1.500572906	0.021029
<i>Os05g0594900</i>	<i>LOC_Os05g51650</i>	U6 snRNA-associated Sm-like protein LSm8	<i>Os05g0594900</i>	1.50032122	0.029511

Table 2 Down-regulated proteins identified from label-free quantitative analysis

RAP (Os ID)	MSU (LOC_Os ID)	Description	Gene Name	Ratio[RFP/RCK]	P value
<i>Os02g0822800</i>	<i>LOC_Os02g57690</i>	Acyl-CoA binding protein-like	<i>Os02g0822800</i>	0.657506509	0.019206616
<i>Os03g0219200</i>	<i>LOC_Os03g11960</i>	copper/zinc superoxide dismutase	<i>Os03g0219200</i>	0.637308003	0.049986265
<i>Os08g0374000</i>	<i>LOC_Os08g28670</i>	Bet v I allergen family protein	<i>Os08g0374000</i>	0.636556967	0.001251447
<i>Os06g0104300</i>	<i>LOC_Os06g01490</i>	monocopper oxidase	<i>Os06g0104300</i>	0.636141093	0.039078829
<i>Os01g0155600</i>	<i>LOC_Os01g06290</i>	Splicing factor[arginine/serine-rich		0.635942121	0.032973117
<i>Os08g0441500</i>	<i>LOC_Os08g34280</i>	Cinnamoyl-CoA reductase, Lignin formation	<i>P0528B09.35-1</i>	0.617419753	0.001449421
<i>Os05g0278500</i>	<i>LOC_Os05g19910</i>	Acyl transferase 5	<i>AT5</i>	0.606305592	0.016918153
<i>Os05g0135700</i>	<i>LOC_Os05g04510</i>	S-adenosylmethionine synthase[ Catalyzes the formation of S-adenosylmethionine from methionine and ATP.	<i>sams</i>	0.59241418	0.001074265
<i>Os05g0375400</i>	<i>LOC_Os05g31140</i>	Glucanase	<i>GLU</i>	0.56122895	0.010900711
<i>Os02g0620500</i>	<i>LOC_Os02g40710</i>	Ammonium transporter 1 member 3	<i>AMT1-3</i>	0.473226337	0.03929424
<i>Os01g0717700</i>	<i>LOC_Os01g52010</i>	alliin lyase precursor	<i>Os01g0717700</i>	0.428345703	0.003389562
<i>Os04g0497200</i>	<i>LOC_Os04g41970</i>	Endoglucanase 12	<i>GLU3</i>	0.414420949	0.019558331
<i>Os01g0264900</i>	<i>LOC_Os01g16010</i>	BCAS2 protein, putative, expressed	<i>OsI_01292</i>	0.253067847	0.00757617
	cpDNA	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, RuBisCO	<i>rbcL</i>	0.208373843	0.03601915
<i>Os02g0152700</i>	<i>LOC_Os02g05880</i>	DNA-directed RNA polymerase subunit	<i>OsI_05888</i>	0.069922407	0.045181841

Table 3 Significant differential expression proteins ( $p \leq 0.05$ ) with fold changes both in transcriptome and proteomics level larger than 1.5

RAP (Os ID)	MSU (LOC_Os ID)	RLP/RCK in Transcriptome	RLP/RCK in proteomic	P value	Protein IDs	Annotation
<i>Os03g0603600</i>	<i>LOC_Os03g40670</i>	3.572344	16.03	0.006552	Q6AUZ6	Glycerophosphoryl diester phosphodiesterase family protein, expressed
<i>Os03g0150800</i>	<i>LOC_Os03g05640</i>	4.470767	3.96089	0.033385	Q8GSD9	Low-affinity transporter for inorganic phosphate (Pi)
<i>Os01g0776600</i>	<i>LOC_Os01g56880</i>	1.57171	3.622714	0.02748	A0A0P0V8Z3	Purple acid phosphatase
<i>Os10g0444700</i>	<i>LOC_Os10g30790</i>	2.315579	3.188358	0.000168	Q8H6G8	Probable inorganic phosphate transporter 1-8
<i>Os12g0637100</i>	<i>LOC_Os12g44020</i>	2.109139	3.158118	0.001597	Q2QLL9	Purple acid phosphatase
<i>Os01g0660200</i>	<i>LOC_Os01g47070</i>	4.556587	2.640671	0.030145	A2ZW76	glycosyl hydrolase
<i>Os12g0198000</i>	<i>LOC_Os12g09620</i>	2.500715	2.624379	9.82E-05	Q2QWE5	Expressed protein
<i>Os08g0434100</i>	<i>LOC_Os08g33710</i>	1.591087	2.61472	0.002771	Q9FRU0	Ribonuclease
<i>Os07g0129200</i>	<i>LOC_Os07g03710</i>	1.872105	2.294623	0.001414	B9FVB5	SCP-like extracellular protein, expressed
<i>Os05g0402900</i>	<i>LOC_Os05g33400</i>	3.22764	2.229987	0.045047	B9FPI6	basic 7S globulin precursor, putative, expressed
<i>Os07g0630400</i>	<i>LOC_Os07g43670</i>	1.90585	2.18514	0.004417	Q8H4E4	ribonuclease T2 family domain containing protein, expressed
<i>Os10g0538200</i>	<i>LOC_Os10g39300</i>	7.334062	2.147759	0.030829	A2Z9R9	aspartic proteinase nepenthesin, putative, expressed
<i>Os01g0357100</i>	<i>LOC_Os01g25484</i>	2.89252	2.139517	0.015234	B8A7W8	ferredoxin--nitrite reductase, putative, expressed
<i>Os10g0191300</i>	<i>LOC_Os10g11500</i>	1.800917	2.010712	0.0018	Q8LMW8	SCP-like extracellular protein, expressed
<i>Os05g0247100</i>	<i>LOC_Os05g15770</i>	4.70642	2.004889	0.03089	Q5WMX0	similar to glycosyl hydrolases Family 18
<i>NONE</i>	<i>LOC_Os07g23850</i>	1.549946	1.977236	0.016379	A2YKM4	glycosyl hydrolase
<i>Os07g0539900</i>	<i>LOC_Os07g35560</i>	2.59231	1.773463	0.027652	B9FXQ1	glucan endo-1,3-beta-glucosidase precursor, putative, expressed
<i>Os01g0687400</i>	<i>LOC_Os01g49320</i>	1.944762	1.760767	0.04272	Q7XXQ0	Chitinase
<i>Os07g0187700</i>	<i>LOC_Os07g09000</i>	1.874014	1.663966	0.037637	Q6Z4F3	WD40 protein, Regulation of the plasma membrane localization of phosphate transporters, Phosphate uptake and translocation
<i>Os01g0132000</i>	<i>LOC_Os01g04050</i>	1.955063	1.654255	0.017942	Q9LGB2	BBTI12 - Bowman-Birk type bran trypsin inhibitor precursor, expressed
<i>Os10g0498100</i>	<i>LOC_Os10g35500</i>	0.314414	1.592639	0.014471	A3C655	epoxide hydrolase
<i>Os07g0638100</i>	<i>LOC_Os07g44410</i>	1.589194	1.55019	0.002186	Q8GVH2	WD40-like Beta Propeller Repeat family protein

<i>Os08g0374000</i>	<i>LOC_Os08g28670</i>	0.489589	0.636557	0.001251	Q6ZD29	Bet v I allergen family protein OsBetvI
<i>Os02g0620500</i>	<i>LOC_Os02g40710</i>	0.635764	0.473226	0.039294	Q6K9G3	Ammonium transporter 1 member 3

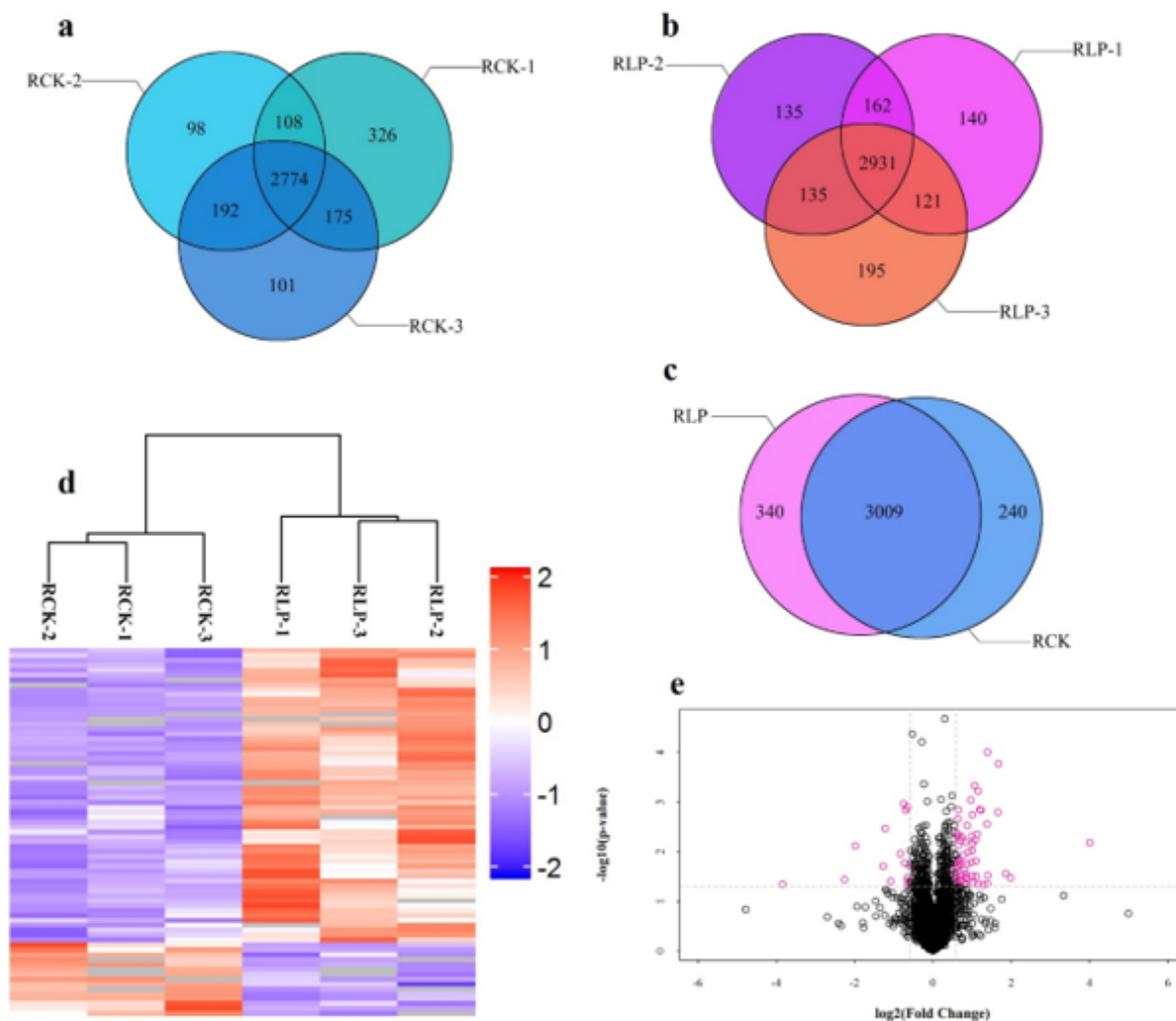
Table 4 Previously identified P-deficiency responses related QTL intervals

QTL ID	Species name	Chromosome	Position
AQBD004	<i>Oryza sativa</i>	1	41,967,890-41,969,197 bp
AQCI001	<i>Oryza sativa</i>	2	8,984,645-18,496,476 bp
AQCI008	<i>Oryza sativa</i>	3	6,753,341-10,322,897 bp
AQCI006	<i>Oryza sativa</i>	4	88,362-4,439,573 bp
AQCI011	<i>Oryza sativa</i>	4	24,690,120-27,908,404 bp
AQCI002	<i>Oryza sativa</i>	6	3,536,009-4,952,592 bp
AQCI009	<i>Oryza sativa</i>	6	1,644,474-4,952,592 bp
AQCI003	<i>Oryza sativa</i>	10	7,639,733-14,271,753 bp
AQBD007	<i>Oryza sativa</i>	12	1,548,040-1,548,464 bp
AQCI012	<i>Oryza sativa</i>	12	3,885,926-27,489,485 bp
AQCI013	<i>Oryza sativa</i>	12	1,548,040-18,867,702 bp
AQAZ001	<i>Oryza sativa</i>	12	13,101,084-15,120,848 bp
qMLR-1	DXWR	1	33,053,493-36,734,272 bp
qTDW-2	DXWR	3	12,407,382-23,822,102 bp

Table 5 Located genes encoded significantly different expression proteins identified from label-free quantitative analysis among previously identified P-deficiency responses related QTL intervals

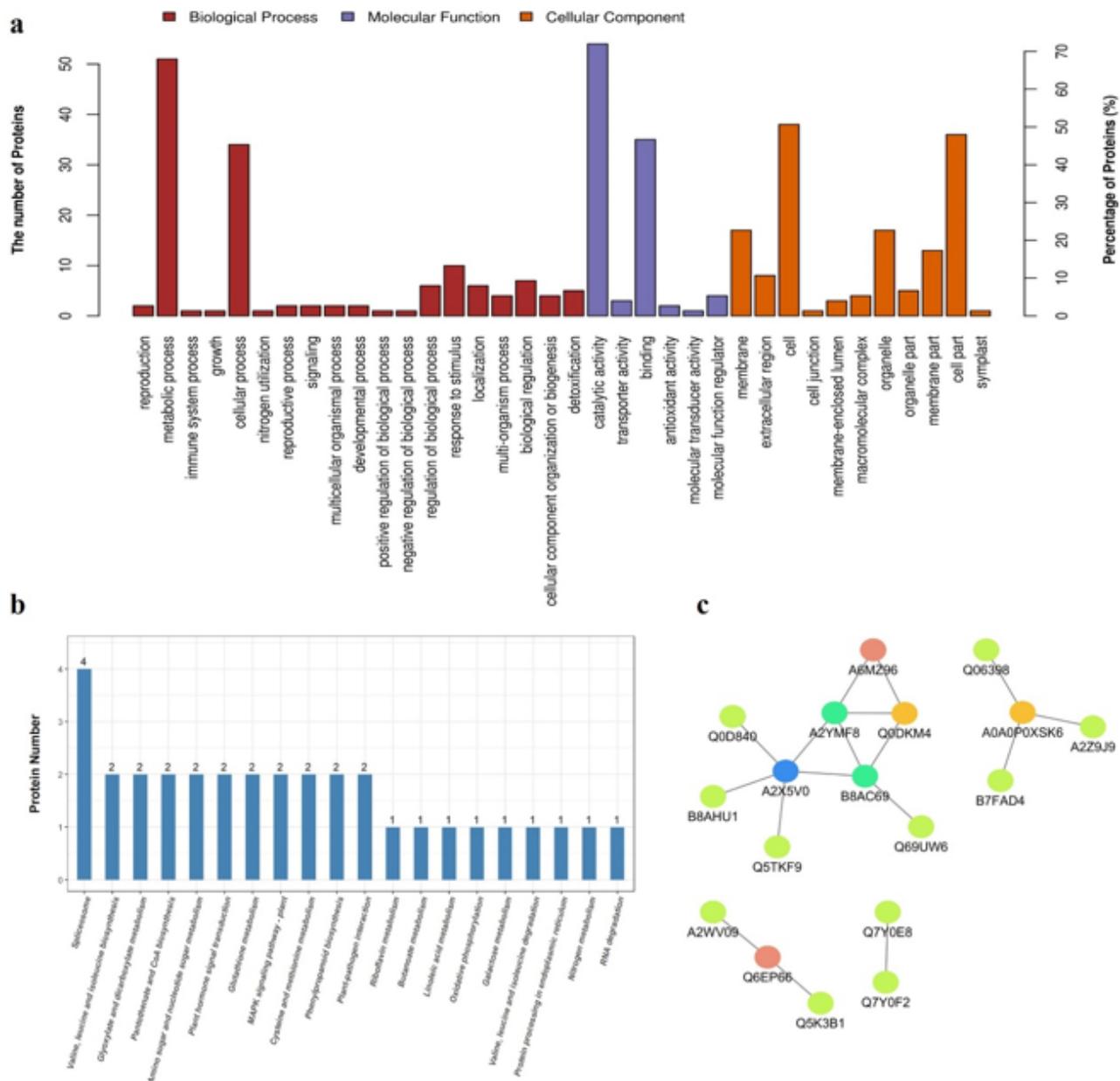
RAP (Os ID)	MSU (LOC_Os ID)	Mapped QTL accession ID	Description	Ratio[RLP/RCK]	P value
<i>Os01g0783500</i>	<i>LOC_Os01g57450</i>	<i>qMLR-1</i>	Universal stress protein domain containing protein	1.52646	0.005096
<i>Os03g0603600</i>	<i>LOC_Os03g40670</i>	<i>qTDW-2</i>	Glycerophosphoryl diester phosphodiesterase family protein	16.03	0.006552
<i>Os03g0405900</i>	<i>LOC_Os03g29240</i>	<i>qTDW-2</i>	Probable nucleoredoxin 1-2	2.22151	0.0006
<i>Os03g0238600</i>	<i>LOC_Os03g13540</i>	AQCI008	Purple acid phosphatase	1.95551	0.0009
<i>Os03g0405500</i>	<i>LOC_Os03g29190</i>	<i>qTDW-2</i>	Probable nucleoredoxin 1-1	1.82238	0.017644
<i>Os06g0172800</i>	<i>LOC_Os06g07600</i>	AQCI002-AQCI009	Uncharacterized glycosyltransferase	1.87172	0.010126
<i>Os12g0637100</i>	<i>LOC_Os12g44020</i>	<i>AQCI012</i>	Purple acid phosphatase	3.15812	0.001597
<i>Os12g0198000</i>	<i>LOC_Os12g09620</i>	<i>AQCI012-AQCI013</i>	Expressed protein	2.62438	9.82E-05
<i>Os04g0497200</i>	<i>LOC_Os04g41970</i>	<i>AQCI011</i>	Endoglucanase 12	0.41442	0.019558331

## Figures



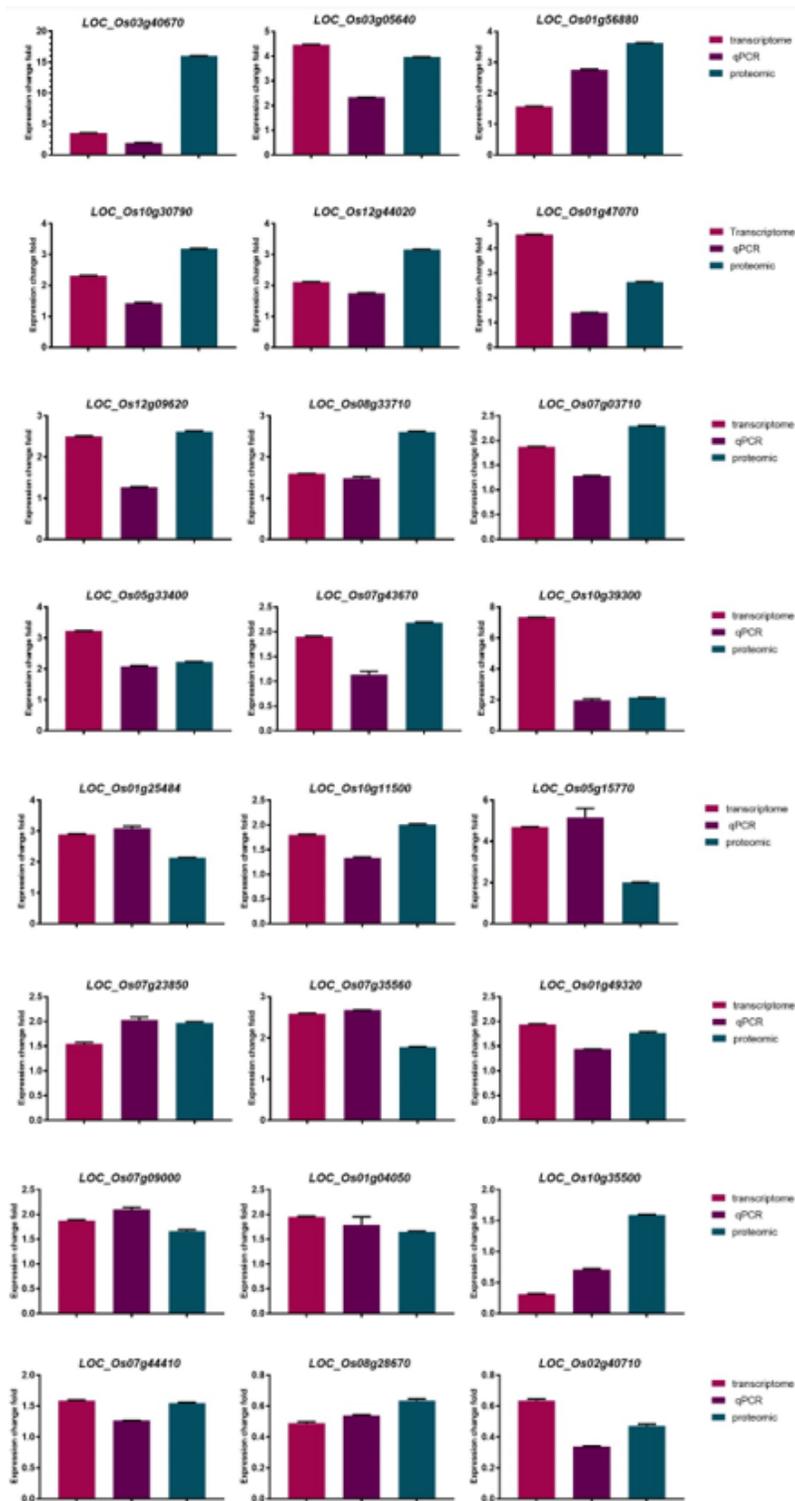
**Figure 1**

Identification and analysis of proteins that differentially accumulated between RCK and RLP. RLP, roots under low phosphorus stress treatment with three biological repetitions; RCK, roots under phosphorus sufficiency stress treatment with three biological repetitions, same to below. (a) proteins identified in three RCK repeated materials. (b) proteins identified in three RLP repeated materials. (c) proteins identified in RCK and RLP. (d) Clustering analysis of proteins identified in RCK and RLP samples. (e) Volcano pot. The gene expression values were transformed to log2 scale. The protein expression fold changes (X-axis) was plotted against the p value obtained from t test log10-value (Y-axis). Small circle represents protein. The red circle represents a protein with a change fold greater than 1.5.



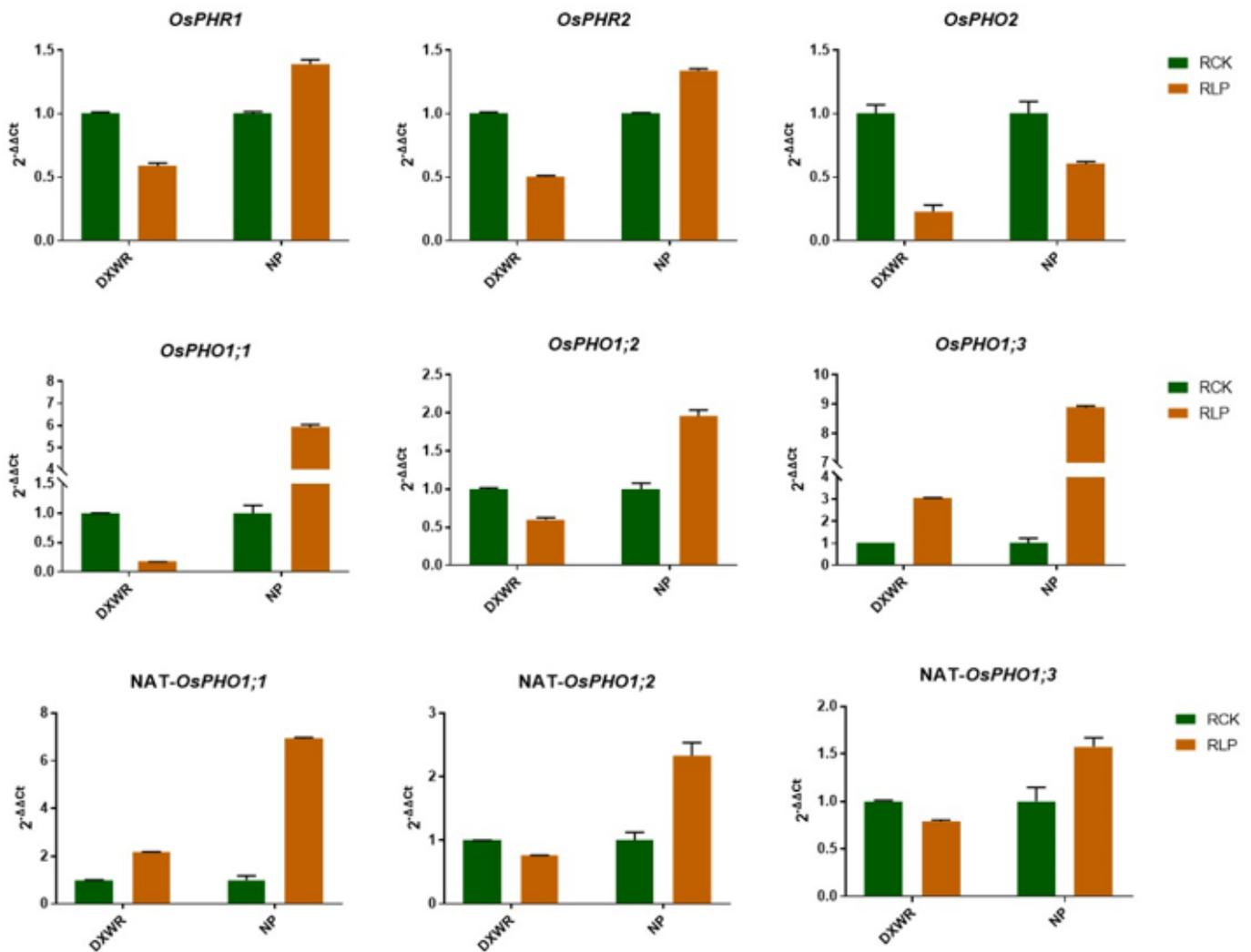
**Figure 2**

Analysis of identified proteins significantly different between the RCK and RLP samples. (a) Gene Ontology (GO) annotation of the proteins significantly different between the RCK and RLP samples. (b) The top 20 KEGG pathway assignments of the proteins significantly different between the RCK and RLP. The represented categories ( $Q \leq 0.05$ ) and the number of proteins predicted to belong to each category are shown. (c) The protein-protein interactions (PPI) between the identified proteins. The sphere represents the protein, and the straight line represents the interaction between the proteins at both ends of the straight line.



**Figure 3**

Quantitative real-time PCR analysis of 24 significant differential expression proteins with fold changes both in transcriptome and proteomics level larger than 1.5 in DXWR. Bars mean SD. Expression change fold refers to the change of the treatment group compared with the control group. Expression change fold > 1, means up-regulated; Expression change fold < 1, means down-regulated.



**Figure 4**

Expression pattern in DXWR and NP of key genes which characterized in cultivated rice participating in the P regulation network detected by qRT-PCR. Error bar means SD.

## Supplementary Files

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