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Integration of proteomic, metabolomic, and ubiquitinomic analyses reveals potential mechanisms underlying low-phosphorus stress adaptation in soybean roots

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Abstract

Low-phosphorus (P) stress is a critical factor limiting soybean growth and yield. Ubiquitination, a post-translational protein modification, is increasingly recognised as a regulator of plant adaptive responses to nutrient limitation, including P deficiency. However, the mechanisms by which ubiquitination mediates soybean tolerance to low P remain underexplored. The present study aimed to elucidate the molecular basis of P efficiency in soybean, focusing on the role of ubiquitination.

A P-efficient soybean genotype, Qiandou 11 (QD11), was hydroponically cultivated under low or normal P levels to investigate P uptake mechanisms. Proteomic, metabolomic, and ubiquitinomic analyses were performed to identify the metabolic pathways and proteins regulating the soybean root system in response to P deficiency.

The results indicated that QD11 rapidly adapted to P deficiency by increasing the levels of small-molecule-size organic acids and enhancing specific root length. A total of 377 differentially accumulated metabolites and 1,059 differentially expressed proteins (DEPs) were identified. The sample with the largest number of DEPs was selected for ubiquitination analysis, revealing 929 differential ubiquitination sites (585 upregulated and 344 downregulated) in 585 proteins. Notably, these proteins were significantly enriched in glycolysis, phenylpropanoid metabolism, and isoflavone biosynthesis pathways. Integrated multi-omics analysis revealed that phosphoenolpyruvate carboxylase and phenylalanine ammonia-lyase are hub proteins involved in carbon allocation during the soybean root response to low-P stress, and their regulation may be mediated by ubiquitination.

These findings elucidate ubiquitin-mediated regulatory mechanisms and key physiological traits associated with low-P tolerance in soybean. This study provides valuable insights for breeding P-efficient soybean varieties.

Keywords Ubiquitination, Phosphorus uptake, Proteomics, Metabolomics, Soybean

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One sentence summary

Ubiquitination mediates the regulation of *PEPC*, *PAL*, and *HCT*, promoting the synthesis of organic acids and anthocyanins, and serving as a key mechanism of low-phosphorus tolerance in soybean root systems.

Introduction

Phosphorus (P) is a fundamental macronutrient that supports essential plant functions, ranging from genetic information transfer and energy metabolism to cell membrane formation. Soil P deficiency represents a major limitation to plant productivity worldwide [1]. Phosphate fertilisers are widely used to correct P deficiency. However, their effectiveness is often restricted by P fixation with iron, aluminium, and calcium ions in the soil, which renders it unavailable to plants [2]. Moreover, excessive P use depletes non-renewable P resources and contributes to environmental pollution [3]. Therefore, revealing the molecular mechanisms underlying efficient P acquisition and utilisation is crucial for sustainable crop production and global food security [4].

In plants, inorganic phosphate (Pi) uptake is primarily mediated by the phosphate transporter 1 (PHT1) family within a coordinated, multi-layered P acquisition system. For example, OsPT1 in rice [5] and PHT1;1 in *Arabidopsis* [6] are high-affinity Pi transporters that are strongly induced under P deficiency, thereby enhancing Pi acquisition. Moreover, certain isoforms, such as LePT1 [7], SbPT9 [8], and TaPT31-7 A [9], are specifically localised in arbuscule-containing cells colonised by arbuscular mycorrhizal fungi (AMF), where they mediate Pi transfer from fungal hyphae to host plants. The extensive extraradical mycelium produced by AMF considerably expands the P foraging capacity of the roots. In addition to symbiotic adaptations, low-P conditions also induce substantial remodelling of root system architecture. This remodelling includes the formation of shallow root systems to explore the topsoil layers, increased root hair density, and lateral root proliferation, all of which amplify the absorptive surface area and improve Pi uptake efficiency [10]. These morphological adjustments are regulated by phosphate starvation-induced (PSI) genes, such as *GmETO1* [11] and *GmRR1* [12] in soybean. These genes modulate root architecture through hormone signalling and metabolic reprogramming.

Beyond morphological adaptations and symbiotic partnerships, plants enhance P mobility and availability through root exudation. Under P stress conditions, soybean demonstrates a pronounced ability to synthesise and secrete organic acids, such as oxalate, citrate, and succinate [13, 14]. These acids chelate metal ions or lower rhizosphere pH, thereby solubilising otherwise fixed P compounds [2, 15]. Some organic acids (e.g., benzoic acid and p-hydroxybenzoic acid [16]) and flavonoids

(e.g., genistein [17]) also act as signalling molecules. These molecules, in turn, recruit phosphate-solubilising microbes (e.g., *Burkholderia* and *Pseudomonas*) that release organic acids and phosphatases to mineralise organic P. This interaction establishes a positive feedback loop that enhances P mobilisation in the rhizosphere [18]. Thus, plants form a robust P acquisition network by employing an integrated strategy combining membrane transport, architectural modifications, chemical exudation, and biological interactions. Notably, this network can be dissected using multi-omics approaches, including proteomics.

Ubiquitination is a common post-translational protein modification in which ubiquitin (Ub) molecules are covalently attached to target proteins, thereby influencing their degradation, subcellular localisation, and interactions. In plants, ubiquitination plays a crucial role in controlling P uptake by regulating the transcription, localisation, and degradation of PHT1 [19, 20]. Under sufficient P conditions, plants phosphorylate PHT1 using casein kinase II (CK2), preventing its localisation to the cell membrane. Meanwhile, the E2 ubiquitin-conjugating enzyme PHO2 mediates the ubiquitination of PHT1, thereby targeting it for degradation by the 26 S proteasome to maintain cellular phosphate homeostasis. However, under P deficiency, CK2 itself is ubiquitinated and degraded, leading to a decrease in ubiquitinated PHT1 levels. This reduction allows root cells to increase P uptake [21]. Moreover, ubiquitination plays a role in the root architecture's response to low-P stress. For example, the E3 ligase Transport Inhibitor Response1 (TIR1) modulates auxin and strigolactone signalling by targeting inhibitory proteins involved in axillary branch formation [22]. The ubiquitination system can further control root architecture by regulating the distribution of the auxin transport protein PIN [23]. Collectively, these findings underscore the essential role of ubiquitination in plant responses to P [21]. However, current knowledge on ubiquitination in P signalling is primarily derived from *Arabidopsis* [21]; therefore, its role in soybean adaptation to low P remains poorly understood [24, 25].

Soybean (*Glycine max* (L.) Merr.) is a P-sensitive crop that is highly susceptible to low-P stress [26], which significantly restricts its growth and potential yield [27]. This sensitivity renders the soybean an ideal model for investigating P-efficient mechanisms. Therefore, based on earlier field and pot trials that screened soybean germplasm [28], we utilised the high-P-efficiency genotype “Qian-dou 11” (QD11) in hydroponic experiments to investigate physiological dynamics, metabolic profiles, and proteomic responses under low-P stress in the present study. We aimed to elucidate the molecular basis of P efficiency in soybean, focusing on ubiquitination-mediated

regulatory mechanisms, and underscore novel strategies for breeding P-efficient varieties.

Materials and methods

Experimental design and plant cultivation

Soybean plants of the cultivar QD11, officially released by the “Institute of Oil Crops, Guizhou Academy of Agricultural Sciences”, were used in this study. Seeds were obtained from the same institute (Guiyang, China), and the cultivar was formally identified and registered with the authorization number “Qian Shen Dou 2016002” by the Guizhou Provincial Crop Variety Approval Committee.

Soybeans were cultivated under hydroponic conditions and subjected to two phosphate treatments: normal P supply (+Pi, 0.5 mM) or low P stress (−Pi, 0.02 mM), with KH_2PO_4 as the P source. The −Pi level was selected to induce phosphate-starvation responses while maintaining plant viability during short-term treatments. The nutrient solution, based on a modified Hoagland formulation [29], was composed of: 1.5 mM KNO_3 , 1.2 mM $\text{Ca}(\text{NO}_3)_2$, 0.4 mM NH_4NO_3 , 0.3 mM K_2SO_4 , 0.5 mM MgSO_4 , 0.025 mM MgCl_2 , 0.0015 mM MnSO_4 , 0.3 mM $(\text{NH}_4)_2\text{SO}_4$, 0.0015 mM ZnSO_4 , 0.0005 mM CuSO_4 , 0.00015 mM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 0.0025 mM $\text{Na}_2\text{B}_4\text{O}_7$, 0.0001 mM CoCl_2 , and 0.04 mM Fe-EDTA. The potassium concentration was balanced between treatments by supplementing with appropriate amounts of KCl. Each plant was grown individually in a separate black plastic hydroponic container (volume: 3.5 L) to ensure biological independence and to prevent root entanglement. To maintain an adequate oxygen supply, the nutrient solution was aerated for 15 min every hour. This ensured adequate dissolved oxygen while reducing potential root damage. The pH was stabilised at 5.9 by adjusting with KOH every 48 h. Nutrient solution was refreshed weekly, and plants were maintained under a 16 h light (26 °C)/8 h dark (20 °C) regime. The plants were first grown under sufficient P supply until the three-leaf stage, after which samples were collected at 0 h (CK), 12 h (H12), 1 day (D1), 3 days (D3), and 7 days (D7) following treatment initiation. For each treatment, triplicate biological replicates were obtained, with each replicate comprising pooled tissues from five uniformly grown plants. The plant was separated into shoot and root at the cotyledonary node. A root portion for multi-omics analysis was flash-frozen in liquid nitrogen and stored at −80 °C, while tissues for physiological assays were kept at 4 °C and processed within 24 h. The remaining intact root was scanned for architecture before being oven-dried with the shoot for biomass and phosphorus determination.

Determination of plant physiological and biochemical indicators

Chlorophyll fluorescence was measured on intact plants using a JUNIOR-PAM-modulated chlorophyll fluorometer (Zequan Technology Co., Ltd., Shanghai, China) on the youngest fully expanded leaf. For root exudate collection from living roots, the roots of two plants per sample were rinsed with deionised water and immersed in 100 mL of 0.5 mM CaCl_2 solution (with two drops of 20 g L^{−1} thymol) under dark conditions for 4 h. Filtered (0.22 µm) exudates were analysed using high-performance liquid chromatography (HPLC; 1260 Infinity; Agilent Technologies, Santa Clara, CA, USA) to determine carboxylate content, as previously described [30]. The carboxylate content was normalized to root dry weight (DW) and expressed as a secretion rate per unit time and per unit root biomass (mg·h^{−1}·g^{−1} DW).

Superoxide dismutase and peroxidase activities were determined using the nitroblue tetrazolium and guaiacol methods, respectively. Malondialdehyde and free proline (Pro) contents were measured using the thiobarbituric acid colourimetric and acid ninhydrin methods, respectively. Further details are described in the Supporting Information (Additional file 3).

Root morphology was recorded using a scanner (Perfection V850 Pro; Epson, Nagano, Japan) and analysed with Win-RHIZO software (Regent Instruments, Quebec, Canada). Tissue samples were dried, sieved, and analysed for P content using the molybdenum blue method. P accumulation was calculated by multiplying the dry weight of the aboveground or belowground parts by their respective P concentrations.

Metabolomic analysis

Samples for metabolomic, proteomic, and ubiquitinomic analyses were stored at −80 °C. For metabolomic analysis, the tissue was freeze-dried, ground, and approximately 200 mg of the resulting powder was extracted with methanol containing 2-chlorophenylalanine (4 ppm) as an internal standard, followed by homogenization and centrifugation. The supernatant was filtered (0.22 µm) and transferred for analysis. For quality control, 20 µL aliquots of each sample were pooled. Metabolites were analysed using an HPLC system (Thermo Ultimate 3000, Thermo Fisher Scientific, Waltham, MA, USA) coupled with a Q Exactive Focus mass spectrometer (Thermo Fisher).

An ACQUITY UPLC® HSS T3 column (2.1 × 150 mm, 1.8 µm; Waters Co., Milford, MA, USA) was used for HPLC analysis. Gradient elution employed formic acid–water/acetonitrile (positive mode) and ammonium formate–water/acetonitrile (negative mode) at 0.25 mL/min. The Orbitrap system (Thermo Fisher) operated in both positive (3.5 kV) and negative (−2.5 kV) ionisation

modes, with standard gas flow and a capillary temperature of 325 °C. Full MS scans (m/z 81–1000; resolution 70,000) and data-dependent HCD MS/MS were acquired with dynamic exclusion. In the quality control samples, 85.8% and 83.8% of metabolic features in the positive and negative ion modes, respectively, exhibited relative standard deviations below 30% (Additional file 2: Figures S1B, S1D). Furthermore, the quality control samples formed a tight cluster in the principal component analysis scores plot (Additional file 2: Figures S1A, S1C). These results indicate good system reproducibility, supporting the suitability of the data for subsequent analysis. Differentially accumulated metabolites (DAMs) were identified with thresholds of variable importance in projection (VIP) score ≥ 1.0 , fold-change ≥ 2.0 , and a Benjamini-Hochberg adjusted p -value (FDR) < 0.01 .

Protein extraction, digestion, and quantitative proteomics

Soybean root samples were ground in liquid nitrogen. The powder was homogenised in extraction buffer (four volumes, w/v; containing 10 mM dithiothreitol, 1% protease inhibitor cocktail, and 50 μ M PR-619) by ultrasonication and centrifuged (12,000 $\times g$, 10 min, 4 °C). The supernatant was precipitated with 20% trichloroacetic acid at 4 °C for 2 h. The precipitate was then collected by centrifugation, rinsed three times with ice-cold acetone (centrifugation at 4,500 $\times g$ for 5 min each time), and finally dissolved in 8 M urea. Protein concentration was determined using the BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). Proteins were reduced with 5 mM dithiothreitol (56 °C, 30 min), alkylated with 11 mM iodoacetamide (25 °C, 15 min, in the dark), diluted with 200 mM triethylammonium bicarbonate (final urea < 2 M), and digested overnight at 37 °C with trypsin (enzyme: substrate = 1:50). For proteomic and ubiquitinomic analyses, three biological replicates per treatment and time point were processed, with duplicate technical runs for each. Quantitative proteomics, ubiquitinomics, and parallel reaction monitoring (PRM) were performed by PTM-Biolabs Cd., Ltd. (Hangzhou, China). Detailed liquid chromatography-tandem mass spectrometry (LC-MS/MS) procedures are provided in Additional file 1: Table S1. The missed cleavage rate was 17.26%, indicating efficient digestion. Peptide lengths were predominantly distributed between 7 and 20 amino acids, consistent with the expected patterns for tryptic digestion and mass spectrometric detection. Over 85% of the quantified proteins were supported by two or more unique peptides, and the Pearson correlation coefficient for protein quantification across all replicate samples exceeded 0.97 (Additional file 2: Figure S2), ensuring the reliability of the quantitative data.

Functional annotation was performed using eggNOG-mapper (v2.0) for Gene Ontology (GO) terms and the

Kyoto Encyclopaedia of Genes and Genomes (KEGG) database for pathway mapping. Proteins with a fold-change ≥ 1.5 and FDR < 0.01 were considered differentially expressed proteins (DEPs). Protein structural domain annotation was performed using the PfamScan tool in the Pfam database. Subcellular localisation was annotated by UniProt.

Ubiquitinated peptide enrichment and quantitative ubiquitinomics

Based on the proteomic profile which showed the maximum number of differentially expressed proteins at D3, root samples collected at this time point were selected for ubiquitinomic analysis. For the enrichment of ubiquitinated peptides, we employed an immunoaffinity-based method targeting the ubiquitin-derived diGly (K- ϵ -GG) remnant motif. Peptides were dissolved in IP buffer (pH 8.0) and incubated with pre-washed K- ϵ -GG motif-specific ubiquitin affinity resin (PTM Biolabs, Hangzhou, China) overnight at 4 °C under gentle rotation. After incubation, resins were washed four times with IP buffer and twice with deionised water. Bound peptides were eluted three times with 0.1% trifluoroacetic acid, and the combined eluates were lyophilised. Desalting was performed using C18 ZipTips (MilliporeSigma, Burlington, MA, USA) according to the manufacturer's instructions. Detailed LC-MS/MS procedures are provided in Additional file 1: Table S1. A site probability threshold of > 0.75 was set to ensure high-confidence localization of the ubiquitin modification. Ubiquitination sites with a fold-change ≥ 1.5 and an FDR < 0.01 were defined as differentially ubiquitinated sites.

PRM analysis

To validate proteomics results, 17 target proteins were selected based on the following criteria: significant differential expression (FDR < 0.01), high fold-change, representation of ubiquitination system components, and coverage of the central metabolic pathways identified in our multi-omics analysis. For each, one or two characteristic peptides (detection frequency $> 80\%$, $p < 0.05$) were chosen for PRM analysis. LC separation was performed using an EASY-nLC 1000U HPLC system (Thermo Fisher). Mobile phase A comprised 0.1% formic acid and 2% acetonitrile; mobile phase B comprised 0.1% formic acid and 90% acetonitrile. The gradient for mobile phase B was: 0–16 min, 6–25%; 16–22 min, 25–35%; 22–26 min, 35–80%; 26–30 min, 80%. The flow rate was 500 nL/min. The separated peptides were ionised using an NSI ion source (2.0 kV) and subsequently analysed using a Q Exactive™ Plus Mass Spectrometer (Thermo Fisher). Full MS scans (m/z 360–1305, resolution 70,000) were acquired, followed by MS/MS with normalised collision energy of 27. Fragment ions were detected in the

Orbitrap at 35,000 resolution. Automatic gain control thresholds were set at 3E6 for MS1 and 1E5 for MS2, with a 1.6 m/z isolation window. Fragment ion spectra were quantified using Skyline (v21.1; Enzyme: Trypsin [KR/P], Max missed cleavage: 0, Peptide length: 7–25, Fixed modification: Carbamidomethyl on Cys; Precursor charges: 2, 3, Ion charges: 1, Ion types: b, y, p; The product ions were set as from ion 2 to the second to last one; Ion match tolerance: 0.02 Da; Peak integration was performed automatically followed by manual validation based on retention time alignment and dot-product (dotp) scores (>0.9) to ensure accuracy).

Data analysis

The effects of low-P stress duration on physiological parameters were assessed using repeated-measures analysis of variance (ANOVA) with time as the within-subject factor, performed with Data Processing System software (v7.05; Zhejiang University, Hangzhou, China). Tukey's HSD post-hoc tests were used for pairwise comparisons ($p < 0.05$). The Circlize package in R was used to generate GO enrichment plots (v0.4.16 [31]). To analyse the protein motif characteristics, the Motif-x algorithm-based MoMo analysis tool was applied with a threshold motif score of >16 . Principal component analysis (PCA), KEGG pathway enrichment, heatmap, Mfuzz, and motif analyses were performed using the Jingjie Bioinformatics platform (<http://www.ptmbiolab.com>).

Results

Physiological response of soybean to low-P stress

To assess the response of QD11 to P deficiency, we examined dynamic changes in the agronomic traits and physiological indices of plants following exposure to the two P treatments. We considered plant P accumulation a sensitive indicator of P status. On day 1 after the induction of low-P stress, marked reductions in P accumulation (Fig. 1A, $p < 0.05$) were observed in QD11 plants subjected to low-P (–Pi) conditions compared to those under the normal (+Pi) condition. Root architecture changed after 3 days of low-P stress. Compared with plants under the normal (+Pi) condition, although the root dry weight was significantly lower under low-P stress (Fig. 1C, $p < 0.05$), the specific root length (SRL; Fig. 1D, $p < 0.05$), root volume, and total root length (both detailed in Additional file 1: Table S2, $p < 0.05$) were significantly increased. Moreover, the secretion of root carboxylic acids, including oxalic, acetic, lactic, and citric acids (Additional file 1: Table S3), was significantly increased (Fig. 1E, $p < 0.05$). These observations suggest that in response to low-P stress, QD11 soybeans allocate large quantities of photosynthetic assimilates to belowground tissues. They also promote P activation and interception by secreting large amounts of carboxylic acids and developing finer and longer roots.

After 3 days of treatment, the levels of malondialdehyde were higher in plants under –Pi conditions than in those under +Pi conditions (Fig. 1F, $p < 0.05$), indicating an elevated level of cell membrane peroxidation in the

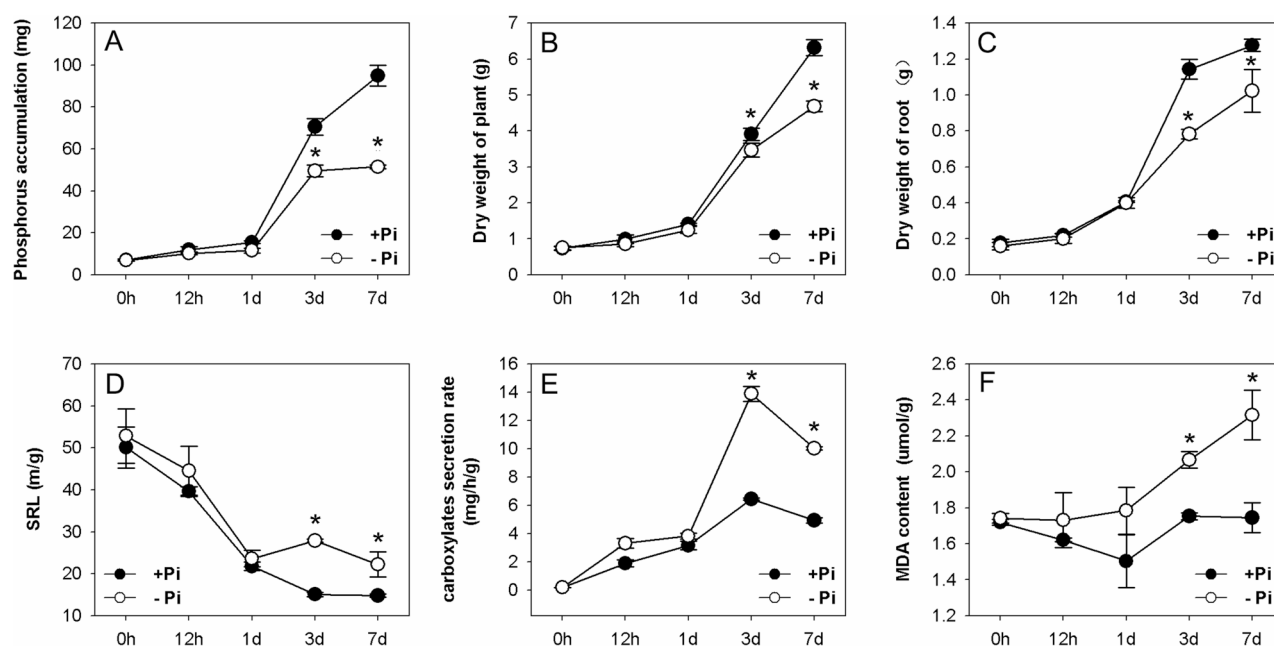


Fig. 1 Dynamic physiological responses of the P-efficient soybean genotype Qindou 11 to low-phosphorus stress. **A** total phosphorus (P) accumulation in plants, **B** total plant dry weight, **C** total root dry weight, **D** specific root length (SRL), **E** secretion rate of carboxylates, and **F** malondialdehyde (MDA) content. +Pi, normal P availability; –Pi, low P availability. * denotes a significant difference between P treatments ($p < 0.05$)

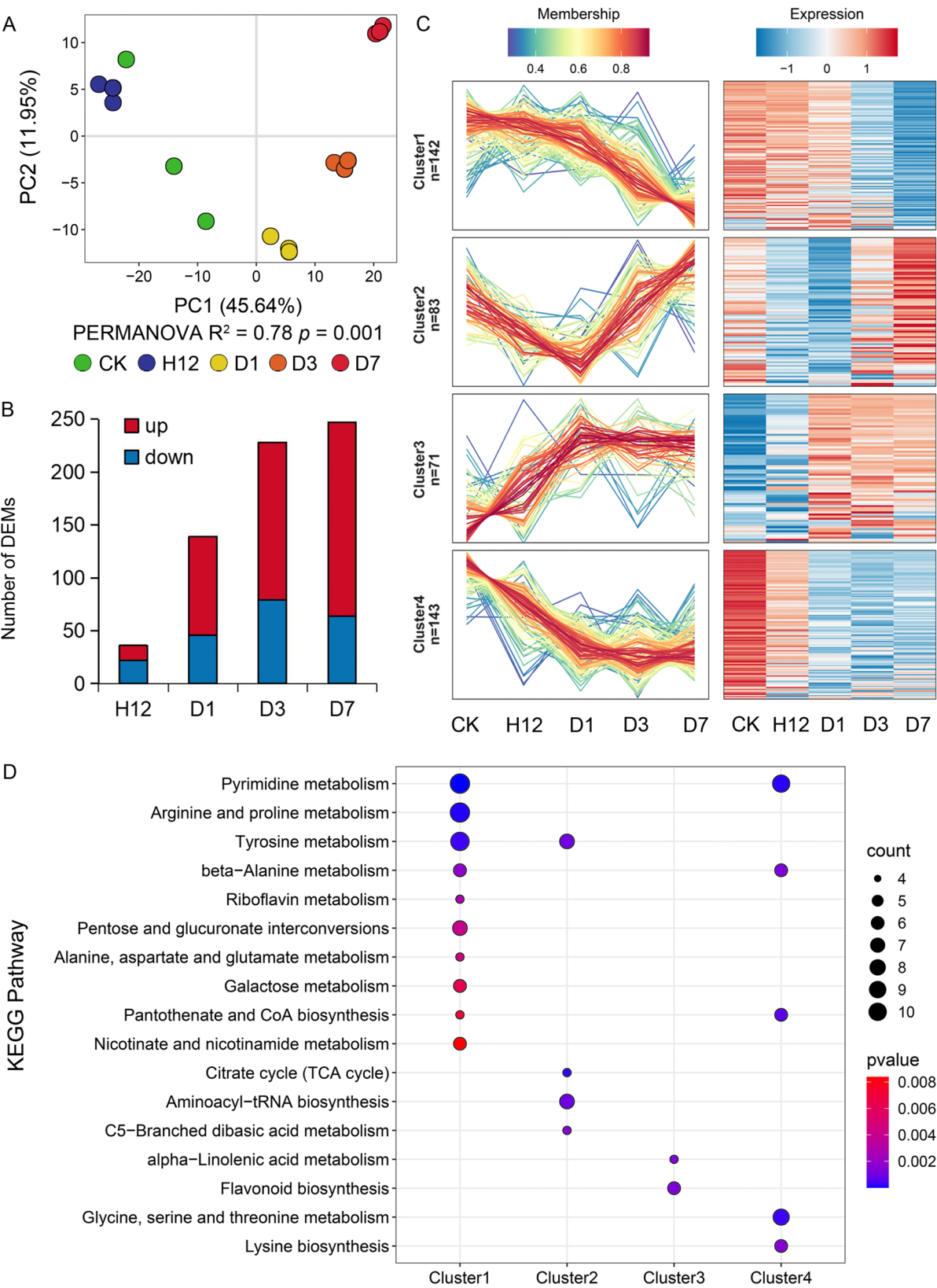


Fig. 2 (See legend on next page.)

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Fig. 2 Metabolomic analysis of soybean roots under low-phosphorus (P) stress. **A** Number of differentially accumulated metabolites (DAMs) among treatments. **B** Principal component analysis (PERMANOVA: $R^2 = 0.78$, $F(4, 10) = 9.0443$, $p = 0.001$). **C** MFuzz analysis based on DAM change patterns. The trend of protein expression in successive treatments is depicted on the left. The horizontal axis is the continuous sample; the vertical axis is the protein expression. DAMs with similar fold-change patterns are grouped into four clusters. Heatmaps of DAMs are plotted on the right side for each cluster. **D** Kyoto Encyclopedia of Genes and Genomes enrichment analysis of clusters from MFuzz

stressed plants. Compared with +Pi plants, -Pi-treated QD11 plants were characterised by the rapid activation of the antioxidant system *in vivo*, exhibiting elevated superoxide dismutase and peroxidase levels after 12 h and 3 days, respectively (Additional file 1: Table S4). Chlorophyll fluorescence parameters were also affected by low-P stress conditions (Additional file 1: Table S5). Specifically, the -Pi group exhibited lower values for Φ_{PSII} , qP , and Fv/Fm than the +Pi group after 12 h of stress. After 1 day of stress exposure, significant reductions were observed in the values of ETR and Fv/Fo , while NPQ was activated to protect the Φ_{PSII} reaction centre.

Temporal changes in soybean root metabolites under low-P stress

A total of 469 distinct metabolites were detected across all 15 samples analyzed. Of these, 377 metabolites were identified as DAMs at one or more time points under low-P stress (Fig. 2A). PERMANOVA revealed a significant effect of time point on the root metabolome under low-P stress ($R^2 = 0.78$, $F(4, 10) = 9.0443$, $p = 0.001$). PCA (with PC1 and PC2 explaining 37.96% and 6.95% of the variance, respectively) revealed that the metabolites of the CK and H12 samples were distributed in the left-hand quadrant of the ordination plot, whereas those of the D1 and D3 samples were distributed in the lower-right-hand quadrant. For D7 samples, the metabolites were tightly clustered in the upper-right-hand corner (Fig. 2B). A similar pattern was observed in the heatmap analysis (Additional file 2: Figure S1). The observed greater dispersion at H12 may reflect initial physiological asynchrony, while the tighter clustering at later time points indicates a more stable and coordinated metabolic adaptation under prolonged low-P stress. The number of DAMs gradually increased with prolonged exposure to low-P stress across the observed time course, reaching the highest count of 247 at D7 (183 upregulated and 64 downregulated).

An MFuzz analysis categorised all the detected metabolites into four clusters (Fig. 2C), among which clusters 1 and 4 contained 142 and 143 metabolites, respectively; their relative abundances declined throughout the 7-day treatment period. These metabolites were primarily enriched in pathways related to pyrimidine, arginine, proline, and tyrosine metabolism (Fig. 2D), indicating that low-P stress significantly alters the metabolism of these key nitrogen-containing compounds. Cluster 2 contained 83 metabolites, whose relative abundances decreased rapidly after 12 h of stress, reaching their

lowest levels on D1. However, these metabolites exhibited a strong resurgence thereafter, eventually exceeding the levels observed in the control plants on D7. These metabolites were mainly enriched in tyrosine metabolism and aminoacyl-tRNA biosynthesis pathways. These results suggest an active modulation of cellular functions in response to stress conditions after 1 day of exposure. Moreover, Cluster 3 contained 71 metabolites, which were enriched in the flavonoid biosynthesis pathway, including key flavonoid intermediates such as naringenin, liquiritigenin and pelargonidin. Notably, compared to CK, the low-P-responsive antioxidant pelargonidin increased by 898- to 950-fold from D1 to D7 ($p < 0.05$).

Temporal proteomic analysis of soybean roots in response to low-P stress

Among the 8,883 proteins identified in total, 7,027 were successfully quantified, which included 1,059 DEPs (Additional file 2: Figure S3). PCA (Fig. 2A) and Pearson's correlation (Additional file 2: Figure S2) analyses highlighted good reproducibility of the samples. The number of DEPs (478), including those that were down-regulated (252), was the highest on D3 (Fig. 3B). This suggests that D3 may represent a critical transitional period in QD11's low-phosphorus stress response, during which early stress signals are integrated to drive the reprogramming of the metabolic system. We subjected these DEPs to GO functional annotation, which revealed changes in biological functions in the plants exposed to low-P conditions. Compared with those in the control plants, the DEPs detected in the plants after 12 h of low-P exposure were mainly enriched in terms related to nitrate transmembrane transporter activity (GO:0015112) and nitrite reductase (NO-forming) activity (GO:0050421) (Fig. 3C; Additional file 1: Table S6). However, after 1 and 3 days of stress, the DEPs were enriched in the cellular carbohydrate biosynthetic process, including the cellular glucan metabolic process, cellulase and xylanase activity, which mediate cell wall assembly (GO:0070726), and the regulation of root morphogenesis (GO:2000067; Fig. 3D, E; Additional file 1: Table S6). After 7 days of exposure, the DEPs were mainly enriched in cellular responses related to nutrient levels (GO:0031669) and the phenylpropanoid catabolic process (GO:0046271, Fig. 3F; Additional file 1: Table S6).

An MFuzz analysis classified 7027 proteins into six clusters (Additional file 2: Figure S4; Additional file 1: Table S7). Proteins in clusters 1 and 3 showed

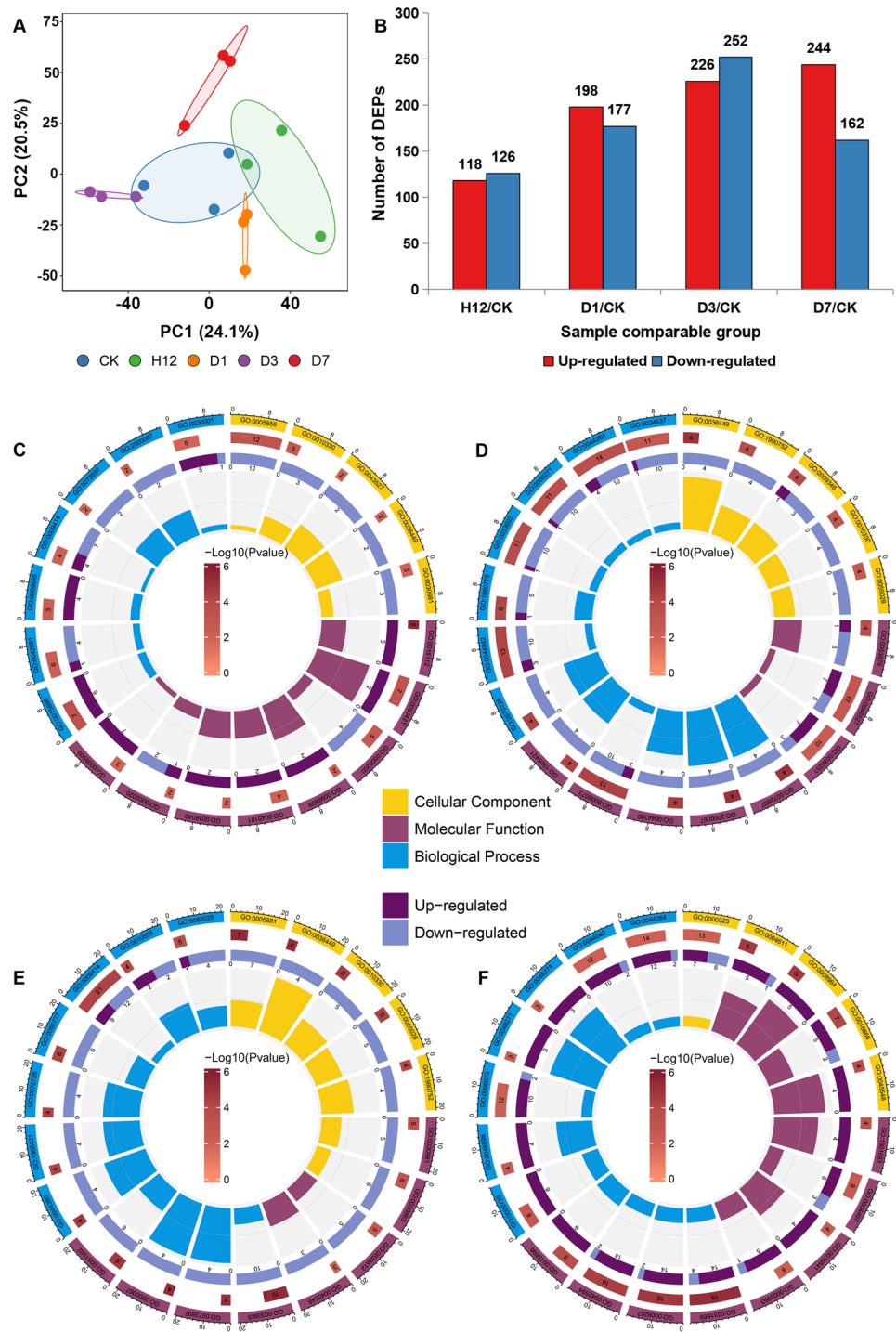


Fig. 3 Proteomic analysis of soybean roots. **A** Principal component analysis. **B** Number of differentially expressed proteins (DEPs) among treatments; Gene Ontology enrichment Circize plots of DEPs after 12 h (**C**), 1 day (**D**), 3 days (**E**), and 7 days (**F**) of low-P exposure. From the outside to the inside, the first circle indicates the name of the enriched pathway and the total number of background genes; the second indicates the number of enriched genes and p-value; and the third represents the number of DEPs enriched in the pathway. The fourth circle presents the enrichment factors for each pathway

similar trends. The relative abundances of these proteins declined during the early stress stages, reaching their lowest levels on D3, but rapidly increased thereafter. These proteins were particularly enriched in

enzymes involved in flavonoid biosynthesis, phenylalanine, and galactose metabolism pathways. Moreover, GO analysis revealed that cluster 1 and 3 proteins were enriched in terms related to root development

and response to gibberellin, aligning with the observed changes in root morphology (Fig. 1D). In contrast, proteins in clusters 2 and 4 exhibited opposite trends, with their levels peaking on D3 and H12, respectively, before subsequently declining (Additional file 2: Figure S4). These proteins were primarily enriched in nitrogen, alanine, aspartate, and glutamate metabolism, as well as alpha-amino acid biosynthetic processes and ATP-binding processes. This pattern was consistent with the metabolomic results (Fig. 2C), further indicating that low-P stress impaired the capacity of root cells for amino acid and protein synthesis. Cluster 5 contained 74 proteins, including components of the proteasomes, Ub family (Additional file 2: Figure S4), and ribosomes. The expression of these proteins declined significantly during the early stress response (H12), with fold-changes (H12/CK) ranging from 0.44 to 0.62 for proteasomal subunits and from 0.36 to 0.75 for ribosomal proteins.

We performed PRM validation for 36 peptides from 17 proteins associated with P uptake, glycolysis, and phenylpropanoid and flavonoid metabolism. This analysis identified 34 peptides with quantitative results, the peptide fragmentation ion peak area distributions of which are presented in Additional file 1: Table S8. Notably, the consistency observed between the PRM results for the candidate peptides and the trend from the label-free quantitative proteomics analysis ($p < 0.05$, $n = 15$) confirmed the reliability of the proteomic data (Additional file 2: Figure S5).

Functional analysis of ubiquitinated differential soybean proteins under low-P stress

We selected the root samples collected on D3 for quantitative ubiquitinomic profiling and quantified 1,772 proteins with 5,610 peptides containing ubiquitinated lysine sites (Kub). Using a threshold of 1.5-fold change and $FDR < 0.01$, we identified 585 upregulated and 344 downregulated ubiquitination sites. Among the identified proteins, the ubiquitinated proteins containing a single ubiquitination site accounted for 67%, and those containing four or more sites accounted for 5%. Ubiquitination-modified differential proteins were primarily enriched in pathways associated with carbohydrate metabolism, energy supply, and phenylpropanoid and flavonoid metabolism (Additional file 2: Figure S6). Proteins enriched in the glycolysis, pyruvate, pentose phosphate, and starch metabolism pathways were characterised by increased ubiquitination at specific Kub sites (Fig. 4A). In contrast, proteins enriched in the phenylpropanoid biosynthesis pathway exhibited downregulation of the Kub site ubiquitination (Fig. 4B). Moreover, Ub-modified DEPs were mainly associated with the cytoplasm (38%), chloroplast (22%), nucleus (19%), and plasma membrane

(12%) (Fig. 4C). Notably, the results of the EuKaryotic Orthologous Group (KOG) functional annotations were consistent with those of the KEGG pathway enrichment analysis (Additional file 1: Table S9).

Protein structural domain enrichment analysis indicated that proteins with upregulated ubiquitination at Kub sites were enriched in domains such as fructose-bisphosphate aldolase class I, pathogenesis-related proteins of the Bet v I family, NOP5NT domain, and glutathione *S*-transferase (Additional file 2: Figure S7A). In contrast, proteins with downregulated Kub site ubiquitination were enriched in domains such as cation transporter/ATPase, N terminus, lipoxygenase, ABC transporter and cytochrome, P450 (Additional file 2: Figure S7B).

We selected the following six motif sequences: A-4 A-1KubE+3 (contributed by 31 ubiquitination sites), E-4KubA+2 (151 sites), KubA+1 A+2 (129 sites), E-3KubQ+1 (111 sites), K-7D-1Kub (76 sites), and R-6KubG+1 (59 sites; Additional file 2: Figure S8A). These sequences comprised alanine (A), glutamate (E), L-glutamine (Q), lysine (K), L-aspartic acid (D), and glycine (G) residues. Our results indicate that E, K, and R were mainly located upstream of the ubiquitination site; A was distributed both upstream and downstream; and Q and G were located downstream. Furthermore, a motif enrichment heatmap revealed that the amino acids A, D, E, K, and R were significantly enriched in the regions surrounding the ubiquitination sites (Additional file 2: Figure S8B). Secondary structure analyses of the identified lysine-ubiquitylated proteins indicated high levels of lysine ubiquitylation in the unstructured regions of the proteins, accounting for 67% of the total ($p = 8.57 \times 10^{-4}$; Fig. 5A). We further observed that 40% of the ubiquitylated lysine was located on the surfaces of the proteins ($p = 1.21 \times 10^{-5}$; Fig. 5B).

The changes observed in the glycolysis pathway in response to low-P stress were categorised into two phases (Fig. 6). In the first phase (0–24 h), we detected elevated levels of sucrose, fructose, UDP-glucose, and glucose 1-phosphate in the root system. However, with prolonged exposure to the low-P stress conditions, the glycolysis pathway entered phase 2 (3–7 days). The first characteristic of this phase was a reduction in the PPi content and glycolytic products. Meanwhile, the expression of PPi-dependent phosphofructokinase (PFP; I1KKN7, I1ND14; Additional file 1: Table S10) was upregulated, whereas that of ATP-dependent 6-phosphofructokinase (PFK; I1MCW1) was downregulated. The second characteristic was a significant increase in the contents of carboxylic acids such as malate and citrate. Correspondingly, we observed upregulation of phosphoenolpyruvate carboxylase (PEPC; I1KDL4, I1KEN4, I1LCU8, Q6Q2Z8, Q8H928) and downregulation of phosphoenolpyruvate

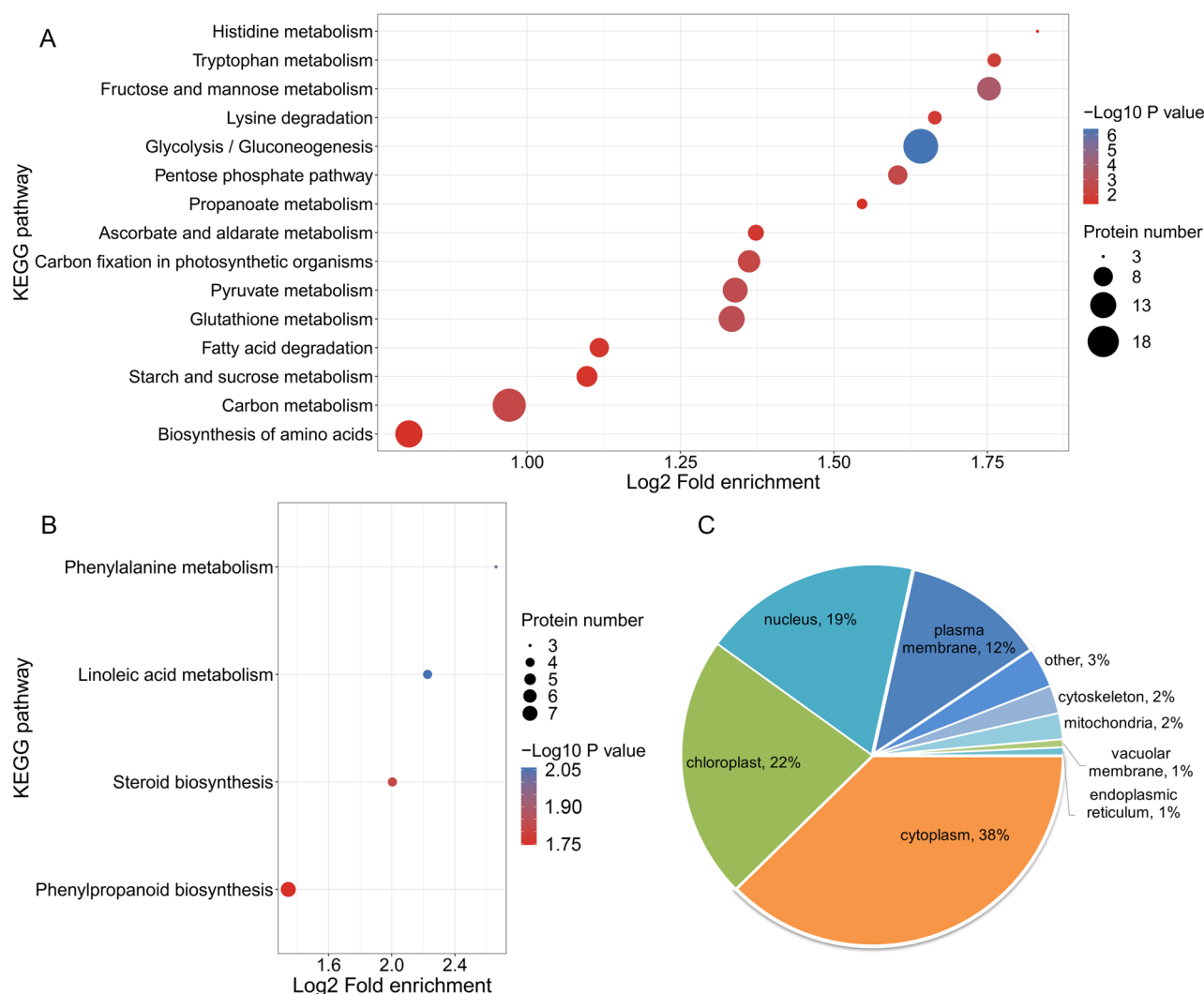


Fig. 4 Kyoto Encyclopedia of Genes and Genomes enrichment analysis of differentially upregulated (**A**) and downregulated (**B**) ubiquitinated proteins, and subcellular structure localisation and distribution of differentially expressed proteins modified by ubiquitination (**C**)

carboxykinase (PEPCK; I1L4W3). In the glycolytic pathway, the protein PEPCK4 exhibited the greatest reduction in ubiquitylation levels (Q6Q2Z8, Lys-629, D3/CK ratio = 0.16, $p < 0.001$; Additional file 1: Table S11). These results suggest that under $-P_i$ conditions, ubiquitination may facilitate the substitution of PEPCK, which uses P_i as a substrate with PEP, an enzyme involved in the synthesis of organic acids rather than their direct release.

Among the identified proteins, phenylalanine ammonia-lyase (PAL; I1L905, D3/CK Ratio: 0.38–0.66, $p < 0.01$; Additional file 1: Table S11) comprised the largest number of ubiquitination sites, with 13 significantly downregulated sites (Fig. 7). In the flavonoid biosynthesis (map00941) and isoflavonoid biosynthesis (map00943) pathways, we detected 6, 1, and 4 downregulated ubiquitination sites in chalcone synthase (CHS; P30081, D3/CK Ratio: 0.09–0.44, $p < 0.05$; Additional file 1: Table S11), isoflavone synthase (IFS; Q9M6D6, D3/CK Ratio: 0.36,

$p < 0.01$), and isoflavone 7-O-glucosyltransferase (IF7GT; I1NID6, D3/CK Ratio: 1.78–2.79, $p < 0.05$), respectively (Fig. 7). The expression of all these proteins was markedly elevated in plants subjected to stress for 7 days, coinciding with substantial anthocyanin synthesis (Fig. 7). The ubiquitination sites of hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase (HCT), cinnamoyl-CoA reductase (CCR), and isoflavone O-methyltransferase (IOMT) were also upregulated; however, their expression declined during the late stage of stress exposure (Fig. 7). These findings suggest that changes in the ubiquitination of key enzymes, such as PAL, CHS, and HCT, may be associated with enhanced carbon allocation towards the anthocyanin synthesis pathway.

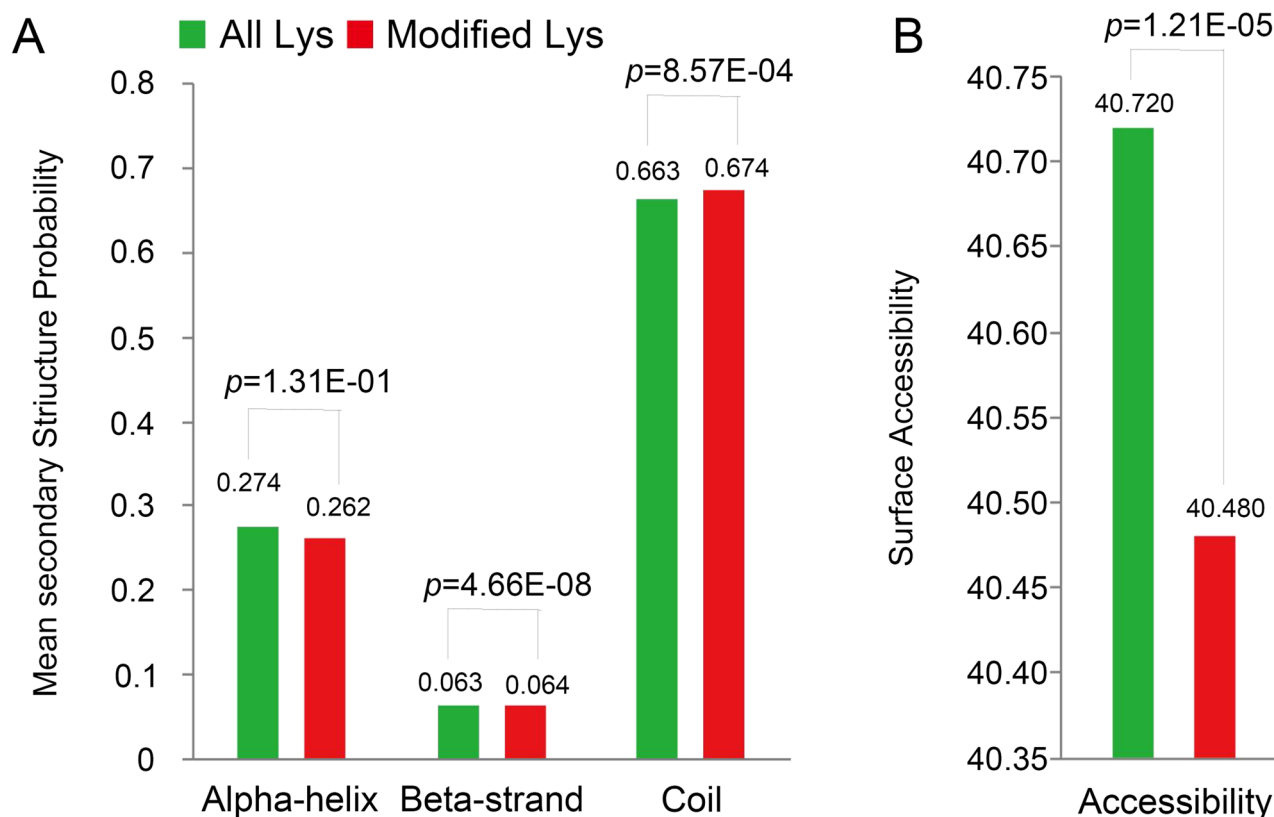


Fig. 5 Secondary structure analysis of ubiquitinated proteins under low-phosphorus stress. **A** Probability of ubiquitination in the α -helix and β -sheet regions; **B** Prediction of ubiquitination site affinity. All lysine sites are presented in green, and ubiquitinated lysine sites are depicted in red

Discussion

In this study, we integrated physiological, metabolomic, proteomic, and ubiquitinomic analyses to identify the potential roles of ubiquitination in regulating soybean root metabolism under P-deficient conditions. Multi-omics analyses revealed that ubiquitination modifications are widely involved in the response to P stress and exhibit significant correlations with the synthesis and accumulation of root secretions. We identified multiple genes that undergo ubiquitination modifications in pathways such as glycolysis, TCA cycle, phenylpropanoid, and flavonoid metabolism. Our findings provide novel mechanistic insights into post-translational regulatory strategies for P-efficient adaptation in crops and offer valuable candidate targets for future molecular breeding efforts.

The ability of plants to acquire P is positively correlated with organic anion secretion [32, 33]. This ability is particularly pronounced in soybean, a representative legume [34–36] (Fig. 1E). In the present study, exposure to low-P stress for 3 days significantly increased the contents of six organic acids in root exudates, including malic acid (+165%, $p<0.001$) and tartaric acid (+145%, $p<0.001$; Additional file 1: Table S3). Metabolomic analyses further confirmed that the tricarboxylic acid cycle in roots responded rapidly to low-P stress (Fig. 6). These results

are consistent with a previous study on other P-efficient soybean cultivars, including Maetsue, Kurotome, and Fukuutaka [14]. Notably, the small organic carboxylic acids secreted by plants help chelate cations, including those of Fe, Al, and Ca, thereby releasing bound soil phosphate in the soil and increasing the available P for root uptake (Wang and Lambers, 2020).

PEPC is a key rate-limiting gene that controls the release of organic acids in plants [37, 38]. In our study, ubiquitinomic analysis revealed a strong reduction in the ubiquitylation levels of PEPC4 on Lys-629 (D3/CK ratio=0.16, $p<0.001$). Correspondingly, proteomic analysis indicated that the abundance of PEPC4 (D3/CK ratio=4.10, $p<0.001$) and MDH (I1MTU1, D3/CK ratio=2.59, $p<0.001$; I1JZP0, D3/CK ratio=1.69, $p<0.001$) was significantly upregulated in D3 (Fig. 6). Under soil sufficient P conditions, PEPC activity is inhibited by the downstream product malate, which is associated with the mono-ubiquitination of the p107 subunit. In contrast, during P depletion, a de-ubiquitinating enzyme converts the PEPC p110 subunit to the p107 subunit [39]. Consequently, PEPC is no longer inhibited by malic acid, facilitating the catalysis of PEP with HCO_3^- to yield large amounts of Pi and oxaloacetate [39]. Notably, Pi can replenish the metabolic P pool, while

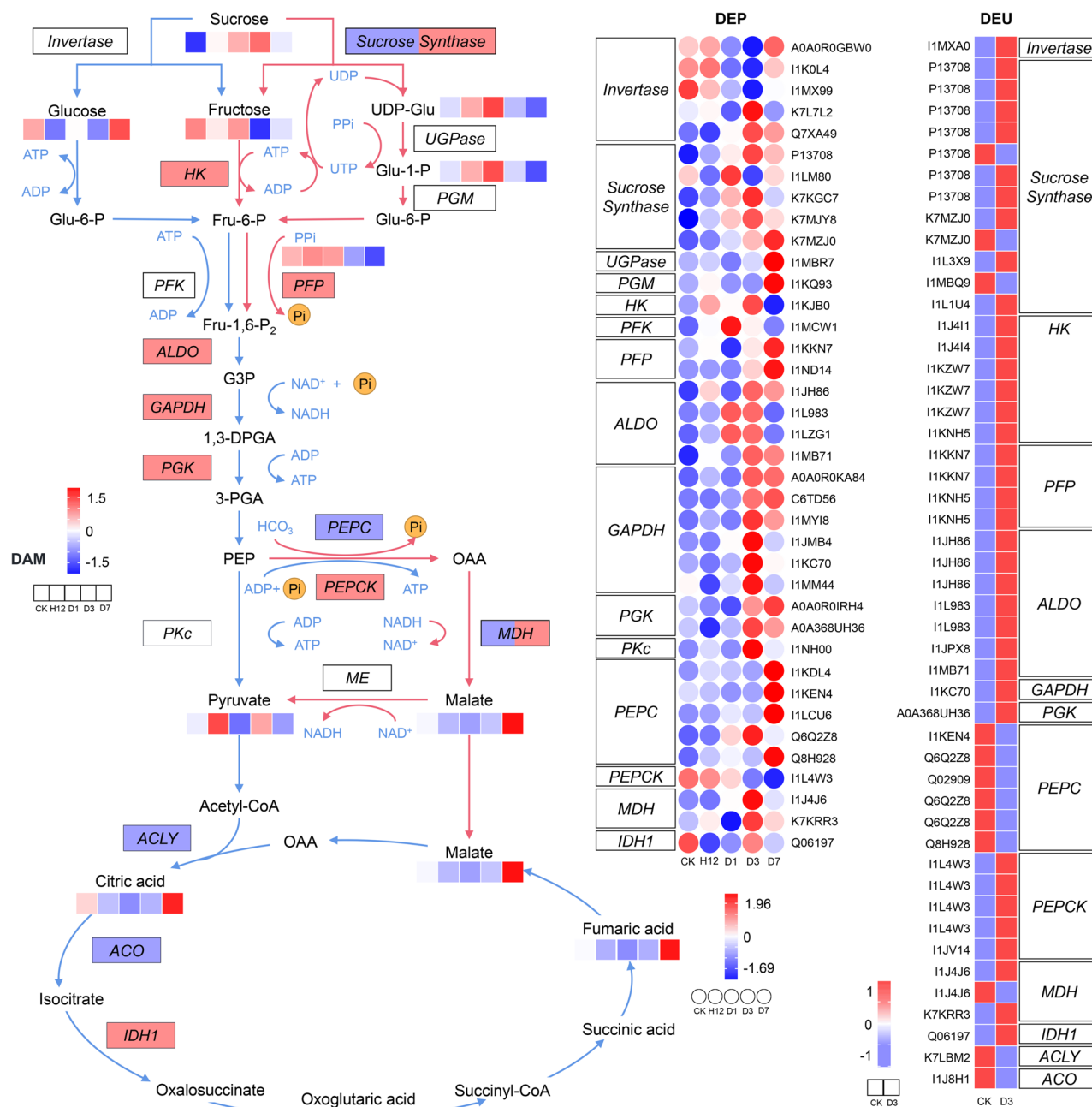


Fig. 6 Differential metabolites, proteins, and ubiquitinated proteins in the glycolysis pathway and TCA cycle. Metabolomic results are presented in the heatmap alongside metabolite names. Proteomic and ubiquitinomic results are depicted in the heatmap alongside the gene ID. The heatmap to the left of the horizontal line presents changes in differential proteins and that to the right shows changes in differential ubiquitin-labelled proteins. High to low expression is indicated by a change in colour from red to blue. PGM, phosphoglucomutase; HK, hexokinase; PFK, 6-phosphofructokinase; PFP, diphosphate-dependent phosphofructokinase; ALDO, fructose-bisphosphate aldolase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PKC, pyruvate kinase; PEPC, phosphoenolpyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; MDH, malate dehydrogenase; IDH1, isocitrate dehydrogenase; ACLY, ATP citrate (pro-S)-lyase; ACO, aconitate hydratase

oxaloacetate is metabolised into carboxylic acids that are subsequently secreted from the cell via aluminium-activated malate transporters (Fig. 6). Overall, these results suggest that ubiquitination may be involved in modulating PEPC activity, thereby influencing the rate of organic carboxylic acid production. Moreover, genome-wide

association studies and comparative transcriptome analyses have demonstrated that PEPC plays a crucial role in regulating root development under low-P stress in soybeans [40], potentially through its involvement in organic acid secretion [41]. In summary, PEPC plays a central role in the secretion of organic acids in plants and may

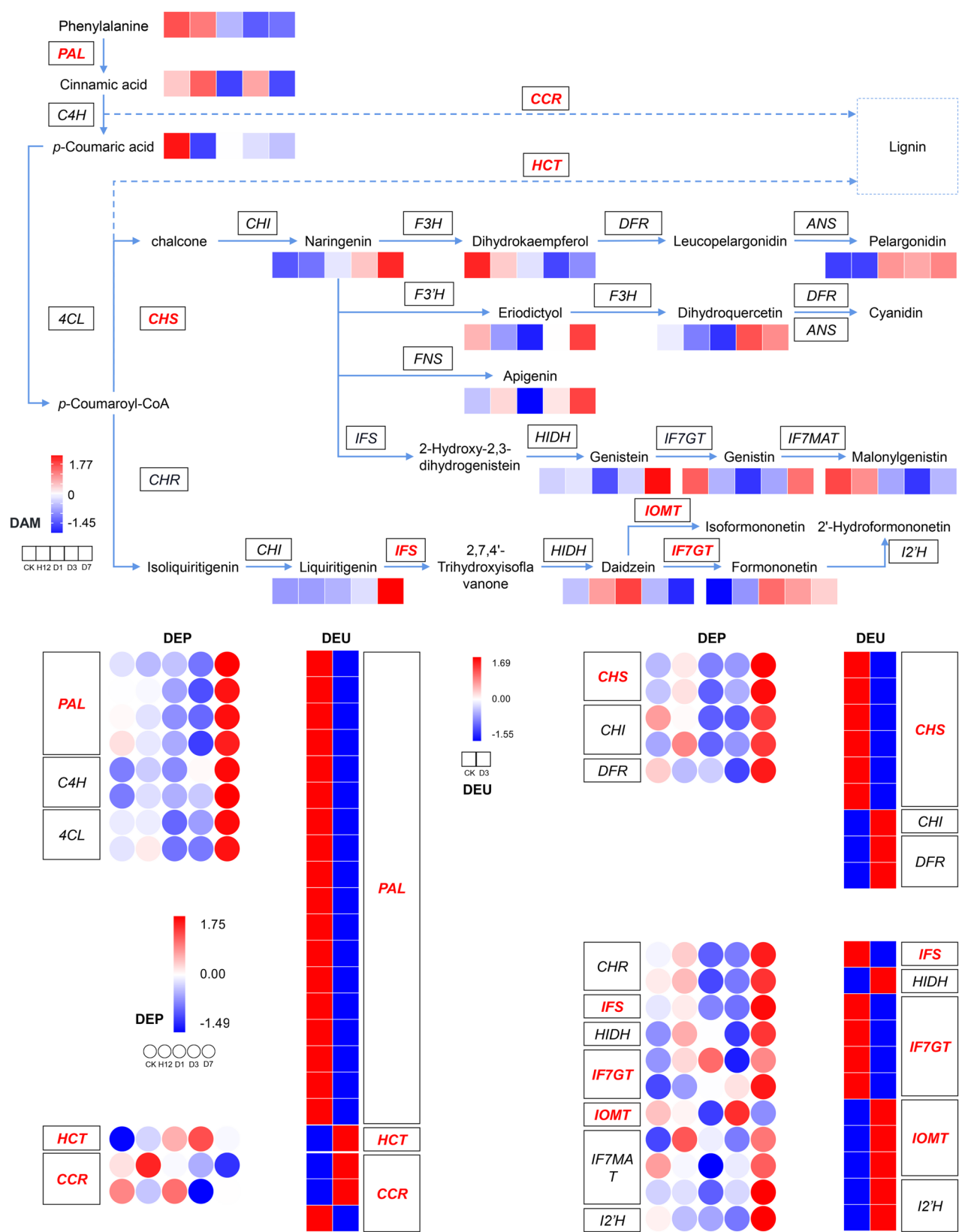


Fig. 7 (See legend on next page.)

(See figure on previous page.)

Fig. 7 Differential metabolites, proteins, and ubiquitinated proteins in the phenylpropanoid, flavonoid, and isoflavonoid pathways. Metabolomic results are presented in the heatmap alongside metabolite names. Proteomic and ubiquitinomic results are depicted in the heatmap alongside the gene ID. The heatmap to the left of the horizontal line shows changes in differential proteins, while that to the right illustrates changes in differential ubiquitin-labelled proteins. High to low expression is indicated by a change in colour from red to blue. PAL, phenylalanine ammonia-lyase; HCT, hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase; CCR, cinnamoyl-CoA reductase; CHS, chalcone synthase; CHI, chalcone isomerase; DFR, dihydroflavonol 4-reductase; CHR, chalcone reductase; IFS, isoflavone synthase; HIDH, 2-hydroxyisoflavanone dehydratase; IF7GT, isoflavone 7-O-glucosyltransferase; IOMT, isoflavone O-methyltransferase; IF7MAT, isoflavone 7-O-glucoside-6"-O-malonyltransferase; I2'H, isoflavone 2'-hydroxylase

also be involved in regulating root architecture. Under low-P stress, the ubiquitination system is likely involved in modulating the catalytic activity of PEPC in soybean roots.

In the present study, both the metabolomic and proteomic data consistently indicated that during the later stages of low-P stress exposure (D3–D7), the phenylpropanoid and flavonoid biosynthesis pathways were activated in the soybean roots (Fig. 2). This activation ultimately led to the substantial accumulation of pelargonidin in the roots (D3/CK ratio = 832.85, $p < 0.001$; D7/CK ratio = 951.09, $p < 0.001$; Fig. 7). These findings align with previous metabolomic studies in cotton [42], tobacco [43], and the high-P-efficiency soybean cultivar YC03-3 [44]. These results suggest that phenylpropanoid and flavonoid biosynthesis contribute to low-P responses in roots, in part through anthocyanin production. Anthocyanins (e.g., pelargonidin) are well-known stress-responsive metabolites and may help mitigate photoinhibition in aboveground tissues under P limitation [45]. However, their role in belowground tissues remains unclear. We observed that low-P stress led to increased levels of malondialdehyde (Fig. 1F) and antioxidant enzymes (Additional file 1: Table S4), indicating aggravated oxidative stress in the roots. Low P availability induced rhizosphere acidification, increasing the solubility and activity of aluminium and heavy metals in the soil [29]. This increased solubility impeded the mobility of P within the plant and continuously triggered the production of reactive oxygen species (ROS). Therefore, we propose that the primary function of anthocyanins in roots is to chelate metal ions, whose availability increases owing to rhizosphere acidification, and to scavenge ROS, thereby maintaining cellular homeostasis.

Another intriguing hypothesis is that anthocyanins may be secreted into the rhizosphere. In our study, proteomics analysis revealed that the expression of the ABC transporters I1LTM3, I1LZP7, and K7MZ73 significantly increased in response to low-P stress (Additional file 2: Figure S7B). Anthocyanins can be secreted into the soil via ABC transporter proteins [46]. If the hypothesis is confirmed, root-secreted anthocyanins may support organic acids in activating soil P [47] and protect these acids from rapid degradation via their antibacterial properties [48]. We found that flavonoids, such as apigenin (D7/CK ratio = 2.02, $p < 0.01$), naringenin (D7/

CK ratio = 1.94, $p < 0.01$), and liquiritigenin (D7/CK ratio = 1.87, $p < 0.01$), also accumulated in the roots under low-P stress conditions (Fig. 7). Flavonoids play various roles in mediating plant–microbe interactions, including promoting rhizobial and arbuscular mycorrhizal colonisation and mediating the assembly and function of inter-root microbiomes [48]. Thus, flavonoid production and secretion may represent a candidate indicator for screening P-efficient plants.

Anthocyanin synthesis is further regulated by ubiquitination. In our study, among all the identified proteins, PAL exhibited the highest number of ubiquitination sites (13), which also exhibited the most substantial decrease in ubiquitination levels (D3/CK ratio: approximately 0.087–0.443). Moreover, under low-P stress, the ubiquitination levels of the key rate-limiting enzymes of anthocyanin synthesis, including PAL, CHS, and DFR, decreased. In contrast, those of HCT and CCR, which are crucial enzymes in lignin synthesis, significantly increased. By further integrating the results of metabolomic and proteomic analyses, we propose that this ubiquitin-mediated regulation is likely associated with the redistribution of carbon flux within the P-efficient soybean roots. Specifically, carbon is redirected towards the phenylpropanoid-flavonoid synthesis pathway, whereas carbon flux towards the lignin synthesis pathway is suppressed (Fig. 7). Notably, the ubiquitination levels of glutathione and the glutathione transferases involved in anthocyanin transport were also significantly upregulated under low-P stress (Additional file 2: Figure S7B). Collectively, these results suggest that a regulatory signalling system may be shared among the synthesis and transport of anthocyanins to ensure rapid post-translational responses to low-P stress.

In general, ubiquitination appears to function as a regulatory mechanism modulating carbon flow in the high-P-efficiency soybean genotype. Through this mechanism, soybeans may achieve their low-P response by adjusting the abundance or activity of certain rate-limiting enzymes. Unlike transcriptional regulation, which requires the *de novo* synthesis of multiple proteins, ubiquitination modifies pre-existing proteins directly. Given that low-P conditions severely limit plant primary metabolism (Additional file 1: Table S5), transcriptional regulation would demand substantial amounts of already scarce energy and photosynthetic products (Fig. 2C). Therefore,

ubiquitination could provide a rapid route to modulate metabolic responses by acting on pre-existing proteins, complementing transcriptional regulation.

Conclusion

This study provides a systematic overview of molecular responses in the P-efficient soybean genotype “QD11” under low-P stress using integrated physiological, metabolomic, proteomic, and ubiquitinomic analyses. Our results indicated that the low-P tolerance phenotype of QD11 is likely associated with its efficient root system architectural remodelling and rhizosphere acidification capacity. Under low-P stress, QD11 optimised its root system architecture by increasing its root volume, and SRL to expand its P absorption capacity. Concurrently, its roots secreted organic acids such as oxalic and citric acids. These compounds can chelate metal ions and may promote P mobilisation, supporting efficient P acquisition. In addition, multi-omics analyses revealed the temporal characteristics of soybean root systems. During the early stages of stress (1–3 days), carbon metabolic flux is primarily directed towards organic acid synthesis to support rhizosphere acidification. During the late stages of stress (3–7 days), the phenylpropanoid and flavonoid metabolic pathways are significantly activated, leading to the substantial accumulation of anthocyanins and other flavonoids. We identified proteins regulated by ubiquitination, providing valuable targets that can be explored as genetic resources for breeding P-efficient soybean varieties through gene editing or molecular marker-assisted selection technologies. Future studies are required to directly verify the functional impact of ubiquitination on these proteins (e.g., through in vitro ubiquitination assays, site-directed mutagenesis, or functional complementation) to fully elucidate the mechanistic links between ubiquitination and low-P adaptation.

Abbreviations

P	Phosphorus
QD11	Qiandou 11
DEP	Differentially Expressed Protein
DEU	Differentially Expressed Ubiquitinated Sites
Pi	Inorganic Phosphate
AMF	Arbuscular Mycorrhizal Fungi
PSI	Phosphate Starvation-Induced
Ub	Ubiquitin
PHT1	Phosphate Transporter 1
CK2	Casein Kinase II
SOD	Superoxide Dismutase
Pro	Proline
DAM	Differentially Accumulated Metabolite
PRM	Parallel Reaction Monitoring
GO	Gene Ontology
KEGG	Kyoto Encyclopaedia of Genes and Genomes
SRL	Specific Root Length
KOG	Eukaryotic Orthologous Group
PFK	6-phosphofructokinase
PPi	Pyrophosphate
PAL	Phenylalanine Ammonia-Lyase
ROS	Reactive Oxygen Species

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-026-08388-y>.

Additional file 1: Table S1 Protocol of proteomics and ubiquitinomics analysis. Table S2 Effects of low phosphorus stress on soybean root architecture. +Pi: normal phosphorus supply; -Pi: low phosphorus stress. Each value is the mean ($\pm$ SE) of three replicates. * indicates the difference between different phosphorus treatments ($p < 0.05$). Table S3 Effects of low phosphorus stress on organic acids in soybean exudates. +Pi: normal phosphorus supply; -Pi: low phosphorus stress. Each value is the mean ($\pm$ SE) of three replicates. * indicates the difference between different phosphorus treatments ($p < 0.05$); ** indicates a significant difference between different phosphorus treatments ($p < 0.01$). Table S4 Effects of low phosphorus stress on SOD and POD activity, and proline content in soybean roots. +Pi: normal phosphorus supply; -Pi: low phosphorus stress. Each value is the mean ($\pm$ SE) of three replicates. * indicates the difference between different phosphorus treatments ($p < 0.05$). Table S5 Effects of low phosphorus stress on chlorophyll fluorescence parameters of soybean. +Pi: normal phosphorus supply; -Pi: low phosphorus stress. Each value is the mean ($\pm$ SE) of three replicates. * indicates the difference between different phosphorus treatments ($p < 0.05$). Table S6 GO enrichment of differentially expressed proteins in soybean root. Table S7 Functon enrichment analysis of proteins cluster by Mfuzz. Table S8 ion peak area of unique peptides. Table S9 COG/KOG functional annotations of the differential ubiquitination-regulated proteins of soybean roots under low-phosphorus stress. Table S10 Detailed information of differentially expressed proteins in glycolysis pathway, TCA cycle, phenylpropanoid, flavonoid, and isoflavonoid pathways. Table S11 Detailed information of differentially expressed ubiquitinated sites (DEU) in glycolysis pathway, TCA cycle, phenylpropanoid, flavonoid, and isoflavonoid pathways.

Additional file 2: Figure S1 Quality control assessment of the metabolomics data. (A) PCA score plot of all samples (positive ion mode). Quality control (QC) samples (red) show tight clustering, indicating analytical stability. (B) Distribution of relative standard deviation (RSD) for metabolic features in QC samples (positive ion mode). (C) PCA score plot of all samples (negative ion mode). (D) RSD distribution of features in QC samples (negative ion mode). (E) Heatmap diagram of differentially expressed metabolites. Figure S2 Pearson's Correlation Coefficient of differentially expressed proteins. Figure S3 The distribution of DEPs shown in an upset chart. Set size denotes the total number of DEPs. Counts of intersection represents the amount of DEPs in the comparisons marked by filled circles (single comparisons) or circles with connection lines (multiple comparisons). Figure S4. Mfuzz analysis based on differentially expressed proteins changing patterns. The left side shows the trend in protein expression in successive treatments. The horizontal axis is the continuous sample; the vertical axis is the protein expression. The fold lines are categorized into four different trends according to the cluster analysis. Heatmaps and enrichment analysis of the GO functions, KEGG pathways and protein structural domains of the DEPs are plotted on the right side for each cluster. Figure S5. Correlation analysis between the parallel reaction monitoring and proteomics results of 15 peptides. Figure S6. KEGG enrichment analysis of the differential ubiquitination-regulated proteins of soybean roots under low-phosphorus stress. Figure S7 Domain enrichment analysis of differentially up-regulated (A) and down-regulated (B) proteins of soybean root ubiquitination under low phosphorus stress. Figure S8. Motif analysis of ubiquitinated peptides under low-phosphorus stress. (A) Central (position 0). K represents ubiquitinated lysines; (B) Heatmap of the amino acid sequence enrichment upstream and downstream of the ubiquitination modification sites.

Additional file 3.

Acknowledgements

Not applicable.

Authors' contributions

LT and YT participated in manuscript writing. JW, GS and FL conducted the soybean cultivation experiments and contributed to data analysis. ZC designed the research, performed data analysis, reviewed the literature, and

contributed to manuscript writing. JH, SY and WZ: funding acquisition. RB writing – review & editing. All authors read and approved the final manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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