PROTEINS AND PLASTICITY

Phosphorus (P) is an essential element for the growth and development of plants. Soybean (Glycine max) is an important food crop that is grown worldwide. Soybean yield is significantly affected by P deficiency in the soil. To investigate the molecular factors that determine the response and tolerance at low-P in soybean, we conducted a comparative proteomics study of a genotype with low-P tolerance (Liaodou 13, L13) and a genotype with low-P sensitivity (Tiefeng 3, T3) in a paper culture experiment with three P treatments, i.e. P-free (0 mmol·L⁻¹), low-P (0.05 mmol·L⁻¹) and normal-P (0.5 mmol·L⁻¹). A total of 4126 proteins were identified in roots of the two genotypes. Increased numbers of differentially expressed proteins (DEPs) were obtained from low-P to P-free conditions compared to the normal-P treatment. All DEPs obtained in L13 (660) were upregulated in response to P deficiency, while most DEPs detected in T3 (133) were downregulated under P deficiency. Important metabolic pathways such as oxidative phosphorylation, glutathione metabolism and carbon metabolism were suppressed in T3, which could have affected the survival of the plants in P-limited soil. In contrast, L13 increased the metabolic activity in the 2-oxocarboxylic acid metabolism, carbon metabolism, glycolysis, biosynthesis of amino acids, pentose phosphatase, oxidative phosphorylation, other types of O-glycan biosynthesis and riboflavin metabolic pathways in order to maintain normal plant growth under P deficiency. Important metabolic pathways such as oxidative phosphorylation, glutathione metabolism and carbon metabolism were suppressed in T3, which could have affected the survival of the plants in P-limited soil. In contrast, L13 increased the metabolic activity in the 2-oxocarboxylic acid metabolism, carbon metabolism, glycolysis, biosynthesis of amino acids, pentose phosphatase, oxidative phosphorylation, other types of O-glycan biosynthesis and riboflavin metabolic pathways in order to maintain normal plant growth under P deficiency. Three key proteins I1KW20 (prohibitins), I1K3U8 (alpha-amylase inhibitors) and C6SZ93 (alpha-amylase inhibitors) were suggested as potential biomarkers for screening soybean genotypes with low-P tolerance. Overall, this study provides new insights into the response and tolerance to P deficiency in soybean.

Keywords: Differentially expressed proteins; phosphorus efficiency; proteomics; root; soybean.

INTRODUCTION

Soybean (Glycine max) is a worldwide important economic and nutritional crop. Its seeds are enriched with high levels of proteins (40–50%), fats (20–30%) and vital phytochemicals, i.e. anthocyanins, tocopherols, isoflavones and saponins (Maleňič et al. 2012; Yamashita et al. 2020). Additionally, it plays cardinal ecological functions in cropping systems such as improving soil phosphorus (P) availability (Xia et al. 2013), soil carbon sequestration (Cong et al. 2015) and nitrogen fixation (Salvagiotti et al. 2008). It is a native crop of China and has more than 5000 years history (Lee et al. 2011; Sedivy et al. 2017). China is one of the main soybean producers in the world with a total production of 14 million tons in 2018 (Food and Agriculture Organization of the United Nations 2019).
The Northeast area represents the main production areas in China, accounting for 44 % of total soybean production (Liu et al. 2008).

Phosphorus is the second most important macronutrient for plant, participating in various physiological and biochemical processes (Malhotra et al. 2018). Therefore, when P is limited in the soil, it restricts plant growth and productivity. Soil P deficiency is a worldwide problem restricting crop production (Smit et al. 2009; Balemi and Negisho 2012). Because of soil adsorption and fixation, P can be unavailable in soil, resulting in low utilization efficiency by plant. Phosphorus limitation is usually overcome by the application of P-containing fertilizers. However, excess use of P-containing fertilizers engenders environmental pollution (Lu 2003). Hence, it is crucial to enhance crop P-utilization efficiency in order to sustainably obtain stable and high productivity. Soybean production is significantly limited by low-P availability in soils (Yang et al. 2020). In China, the negative balance between production and consumption is compensated by importing yearly approximately up to $10 \times 10^7$ tons of soybean (National Bureau of Statistics of China 2018). Developing low-P-tolerant soybean cultivars is one of the proposed strategies to close the gap between demand and supply.

Acquisition of P from soil is performed by the root; therefore, numerous studies have focused on root physiological and morphological traits under P-limited conditions. Plants with a fine root system characterized by high length, volume, biomass, specific root length are able to explore high soil volume thus can acquire more P (Wang et al. 2010). Also, it has been demonstrated that organic acids and phosphatase enzymes released from roots improve P availability and acquisition by plants (Dinkelaker et al. 1989; Hayes et al. 2000). Inter- and intraspecific variability for P-use efficiency has been reported in plants (Hammond et al. 2004; Kochian et al. 2004), which has facilitated the identification of numerous quantitative trait loci (QTLs) for P-use efficiency (Chen et al. 2009; Li et al. 2016; Yuan et al. 2017; Hacisalihoglu et al. 2018; Bernardino et al. 2019). However, most of the detected QTLs have very low contribution and heritability, making them unsuitable for breeding programs. Recently, Omics tools have been deployed to detect important molecular factors controlling P-use efficiency in plants and major players such as OsPSTOL1, AVP1, PHO1, OsPHT1.6 and microRNA399 are being uncovered (Heuer et al. 2017).

Although studies have been carried out to investigate the molecular responses of soybean to the shortage of P in soil (Wang et al. 2010; Sha et al. 2016; O’Rourke et al. 2020; Yang et al. 2020), transcriptome and metabolome analyses were the focus of these studies. Proteins represent the actual functional molecules in the cell and are highly affected by abiotic stresses (Ahmad et al. 2016; Kosová et al. 2018). In soybean, few are focused on identifying key proteins involved in P-deficiency responses (Vengavasi et al. 2017). Importantly, comparing proteomes of genotypes with contrasting low-P tolerance levels will allow us to pinpoint major proteins and pathways involved in P-deficiency tolerance. In the present study, we explored the proteome differences in roots of low-P-tolerant and low-P-sensitive soybean genotypes under different concentrations of P using the Tandem Mass Tag (TMT)-based comparative proteomics approach.

**Materials and Methods**

**Plant materials and growth conditions**

Seeds of low-P-tolerant soybean genotype Liaodou 13 (L13) and low-P-sensitive genotype Tiefeng 3 (T3) previously studied by Xuxia et al. (2004) and Yang et al. (2020) were obtained from Shenyang Agricultural University. This research project began in 2004 with a screening of 220 soybean genotypes with different P efficiencies based on various P concentrations (Xuxia et al. 2004). From the test, 0.5 mM has been identified as the P treatment for a normal plant growth. Seeds were surface-sterilized with H2O2 and put in paper culture bags (8 * 16 cm, diameter * length). Five seeds were put in each bag in three groups for each genotype, i.e. P-free (0 mmol·L−1, P0), low-P (0.05 mmol·L−1, P1) and normal-P (0.5 mmol·L−1, P2). Seeds with normal-P served as control for comparative proteomics. The seed bags were placed in a light culture room, and the cultivation conditions were 16-h light/8-h dark, 18–28 °C temperature and 72-h light. At this stage, distilled water was used as culture medium. Upon emergence of cotyledonal leaf, seedlings were transferred to nutrient solution with three P levels: (0 mmol·L−1 KH2PO4 with KCl to maintain potassium concentration consistent with normal-P supply, P0), low-P (0.05 mmol·L−1 KH2PO4, P1) and normal-P (0.5 mmol·L−1 KH2PO4, P2). For other nutrients (compounds), concentrations were as follows: 4.5 mmol·L−1 KNO3, 1.2 mmol·L−1 NH4NO3, 3.6 mmol·L−1 CaSO4·2H2O, 0.25 mmol·L−1 MgSO4·7H2O. Trace elements are: 9 μmol·L−1 H2BO3, 0.9 μmol·L−1 MnSO4, 0.9 μmol·L−1 ZnSO4, 1.5 μmol·L−1 CuSO4, 0.18 μmol·L−1 (NH4)2MoO4 and 9 μmol·L−1 Fe-EDTA (pH adjusted to 5.8). Seedlings were supported on a 5-cm-thick styrofoam sheet at a spacing of 3 cm × 3 cm. The cotyledons were removed on third day of transfer to nutrient solution to minimize genotypic variation due to seed P content. Three replications with three seedlings each were maintained for all treatment combinations (Yang et al. 2020).

Nine days after the treatment, neat and consistent seedlings were selected for sampling in each treatment, and the average value of 3 plants per bag was taken as one repetition, and three replications were taken for each treatment. After taking out the whole plant, it was slowly washed with running water on the ground and root system of the plants was separated from the cotyledonal nodes. The roots were stored at –80 °C for proteomics study at: Chaya Biotech, Shanghai, China, following their standards procedures.

**Sample preparation**

The roots were taken from –80 °C and quickly ground to powder in a mortar with liquid nitrogen. Hundred milligram of lyophilized powder was taken into a 1.5-mL centrifuge tube and we added 800 μL sodium dodecyl sulfate + dithiothreitol + Tris–HCl protein lystate (4% SDS, 100 mM Tris–HCl, 100 mM DL-Dithiothreitol, pH = 7.6). It was kept in boiling water bath at 100 °C for 5 min, followed by ultrasonication in ice bath for 10 min, again followed by 100 °C boiling water bath for 5 min. The tubes were centrifuged at 14 000 g for 30 min, and finally the supernatant was collected and filtered with 0.22-μm ultrafiltration tube.

**Protein quantification and sodium dodecyl sulfate–polyacrylamide gel electrophoresis**

Bicinchoninic acid assay was used to optimize the concentration of proteins in each sample. Twenty microgram protein samples were used for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The remaining samples were divided into 300-μg portions and stored in refrigerator at –80 °C. Finally the SDS-PAGE gel was scanned to obtain the gel map.

**Enzymatic hydrolysis**

We took 300 μg of each sample for FASP enzymatic hydrolysis. Then, we added 200 μL of UA buffer (8 M Urea, 150 mM Tris–HCl,
The raw data of mass spectrometry analysis were RAW file, and the built-in software SEQUEST Proteome Discoverer 2.1 (Thermo Scientific) was used for protein annotation and quantitative analysis. The parameters used in this search were as follows: no restrictions on protein molecular weight, two missed cleavage, trypsin digestion, monoisotopic mass values, UniprotKB G. max (soybean) protein database, carbamidomethylation of cysteine as fixed modification, oxidation of methionine, peptide mass tolerance of ±20 ppm, fragment mass tolerance of 0.1 Da and peptide charge +1, +2 and +3. For quadrupole time-of-flight (Q-TOF), peptide mass tolerance and fragment mass tolerance were 20 ppm and 0.05 Da, respectively. Proteome Discoverer 2.1 performed screening with false discovery rate (FDR) ≤ 0.01 based on peptide identification results and quantitative analysis based on peptide peak intensity values. The original protein quantification result was the median of the peptide quantification result and was corrected by the sum of the reported ion peak intensity values of all channels. The final quantitative results were then normalized by the ratio of each label to the average of all channel intensity values and the median of the ratio. Functional classification of the proteins was performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/). The KEGG enrichment pathway with Bonferroni-corrected P-value < 0.05 was considered significant by using the hypergeometric test.

Statistical analysis
All data were expressed as the mean ± standard error of mean. Differences among means of paired of groups were assessed by two-tailed t-test function in R software version 4.0.2. Differentially expressed proteins (DEPs) were identified with fold changes >1.5 or <0.67 and P < 0.05 (Zhang et al. 2017; Yang et al. 2019; Ye et al. 2021). Principal component analysis (PCA) was performed using xstat package version 2020.1.

Results
Overview of proteome profiling in two contrasting soybean genotypes
The present study was performed to explore the variable expression of proteins in two genotypes of soybean under three P treatments: P-free (0 mmol·L−1), low-P (0.05 mmol·L−1), and normal-P (0.5 mmol·L−1). L13 is a low-P-tolerant genotype while T3 is a low-P-sensitive genotype as demonstrated by Xuxia et al. (2004) and Yang et al. (2020). Seedlings grown under normal-P served as control for comparing the low-P and P-free treatments. We employed the TMT proteomics approach, which is an isotope-labelling method providing a relatively small error between groups, to profile the proteome of the different samples. A total of 41 678 peptides, 19 612 unique peptides and 4126 proteins were identified and quantified in a triplicate experiment [see Supporting Information—Table S1]. Sequence coverage of the majority of identified proteins was up to 60% (Fig. 1A). Mass distribution of the identified proteins ranges from 5 to 568 kDa with majority of the proteins having molecular weights between 10 and 70 kDa (Fig. 1B). A PCA was performed based on protein quantification data across the 18 samples. As shown in Fig. 1C, all biological replicates were grouped together in the PCA, showing high degree of reproducibility. It also implies that proteome quantification was reliable. The PCA divided samples
from T3 and L13 based on PC1 while P2 samples were distinct from P0 and P1 samples based on PC2. These results suggest that both genotypes have quite different response to P levels and P deficiencies greatly disturb the proteome in soybean.

Differentially expressed proteins analysis in L13 genotype

In order to identify the DEPs between P treatments in L13, we compared L13_P2 (normal-P) to L13_P1 (low-P) and L13_P2 (normal-P) to L13_P0 (P-free). In L13_P2 vs. L13_P1, only seven DEPs were identified and all of them were upregulated in L13_P1 [see Supporting Information—Table S2], indicating a positive response to low-P level in the soil. These DEPs were predicted to play various functions in response to low-P level [see Supporting Information—Table S2]. Concerning L13_P2 vs. L13_P0, a total of 656 DEPs were identified [see Supporting Information—Table S3], showing that P0 induced a stronger response from soybean root proteome. All the DEPs were upregulated in L13_P0, implying that these proteins positively influence soybean response to P-free conditions. These DEPs were enriched in various KEGG pathways with the most enriched being 2-oxocarboxylic acid metabolism, carbon metabolism, biosynthesis of amino acids, glycolysis, oxidative phosphorylation, pentose phosphate pathway, other types of O-glycan biosynthesis and riboflavin metabolism in the metabolic pathways (Morcuende et al. 2007; Huang et al. 2008; Warren et al. 2011; Schlüter et al. 2013; Müller et al. 2015; Pant et al. 2015; Deng et al. 2018; Kc et al. 2018; Liu et al. 2020), likely to be crucial for tolerating P starvation in the soil (Fig. 2A). A cross-comparison of the two lists of DEPs showed that three core conserved proteins (I1KW20, I1K3U8 and C6SZ93) were constantly upregulated in L13 roots in response to low-P and P-free conditions (Fig. 2B; Table 1).

Differentially expressed proteins analysis in T3 genotype

The pair-wise comparisons T3_P2 (normal-P) to T3_P1 (low-P) and T3_P2 (normal-P) to T3_P0 (P-free) were made in order to identify the DEPs involved in T3 response to P deficiencies in the soils. In T3_P2 vs. T3_P1, 23 DEPs were detected, including 22 downregulated and one upregulated proteins in T3_P1 [see Supporting Information—Table S4]. Out of these 23 DEPs, only four were annotated (lipoxigenase, 14-3-3-like protein, nonspecific lipid-transfer protein, disease resistance protein/leucine-rich repeat (LRR) protein, non-specific lipid-transfer protein and urease), while the remaining DEPs were uncharacterized proteins. Concerning T3_P2 vs. T3_P0, 127 DEPs were identified, all being downregulated in T3_P0 [see Supporting Information—Table S5]. Out of these 23 DEPs, only 22 downregulated and one upregulated proteins in T3_P1 [see Supporting Information—Table S4]. Out of these 23 DEPs, only four were annotated (lipoxigenase, 14-3-3-like protein, non-specific lipid-transfer protein, disease resistance protein/leucine-rich repeat (LRR) protein, non-specific lipid-transfer protein and urease), while the remaining DEPs were uncharacterized proteins. Concerning T3_P2 vs. T3_P0, 127 DEPs were identified, all being downregulated in T3_P0 [see Supporting Information—Table S5]. Majority of these DEPs are involved in oxidative phosphorylation, glutathione metabolism and carbon metabolism metabolic pathways (Fig. 2C). The higher number of DEPs induced by P0
compared to P1 in T3 further confirms that P starvation greatly affects the proteome in soybean root. In addition, the extensive downregulation of the DEPs in both low-P and P-free conditions in T3 illustrates that most of the metabolic processes and pathways are suppressed. A cross-comparison of the DEPs obtained from T3_P2 vs. T3_P1 and T3_P2 vs. T3_P0 revealed 17 core conserved proteins constantly suppressed by P deficiencies in T3 (Fig. 2D; Table 2).

**Comparative analysis of DEPs between the two genotypes**

Collectively, 660 and 133 unique DEPs were detected in L13 and T3 genotypes in response to P deficiencies, respectively. We compared DEPs of the two genotypes in order to identify common and genotype-specific DEPs. The result showed that 71 DEPs were commonly regulated in both genotypes under P deficiencies (Fig. 2E; see Supporting Information—Table S6). However, all these shared DEPs were downregulated in T3 while upregulated in L13. This further underscores that the contrasting low-P tolerance observed between T3 and L13 genotypes is underpinned by their opposing proteome responses to P deficiencies. Furthermore, a high number of DEPs were specific to L13 and may contribute to its tolerance to limited P in the soil.

**Discussion**

Yuxia et al. (2004) by screening a large population of soybean under P-limited conditions identified the genotypes L13 and T3 with...
acids, etc. (Nimir and Guisheng 2018; Meyer et al. 2008) plays a crucial role in carbon metabolism and oxidative phosphorylation pathways. Such opposing behaviours could explain their contrasting tolerance to limited P in the soil.

Phosphorus is a principal component of various cellular molecules, such as ATP, nucleic acids, phospholipids, thus playing a crucial role in carbon metabolism and oxidative phosphorylation pathways (Huang et al. 2019). Carbon metabolism and oxidative phosphorylation pathways are known to generate energy and carbon essential for normal cellular function and for the synthesis of DNA, polyamines, amino acids, etc. (Nimir and Guisheng 2018; Meyer et al. 2019). Also, glutathione is a non-enzymatic antioxidant which enhances plant tolerance to different abiotic stresses such as salinity, drought and nutrient deficiency (Hasanuzzaman et al. 2011; Ramirez et al. 2013; Hasanuzzaman et al. 2017). Since P deficiency results in high accumulation of reactive oxygen species (ROS) in cells, glutathione plays cardinal role in scavenging excess ROS in P-limited environments (Juszczuk et al. 2001; Zhang et al. 2014). In this study, we observed that the low-P-sensitive genotype T3 limited the protein activity in the glutathione metabolism, carbon metabolism and oxidative phosphorylation pathways under P deficiencies conditions which ultimately will affect seedling normal growth and ROS scavenging ability, leading to low-P sensitivity.

Table 2. Core conserved proteins responsive to P deficiencies in T3 genotype.

| Protein ID | Description | Isoforms (>90 % identity) | Fold change (T3_P1/T3_P2) | Fold change (T3_P0/T3_P2) | Regulation |
|------------|-------------|---------------------------|----------------------------|----------------------------|------------|
| K7LN5      | Uncharacterized protein | Protein SRC1 | 0.64 | 0.64 | down/down |
| C6T1W3     | Uncharacterized protein | Protein SRC1 | 0.62 | 0.59 | down/down |
| B3TDK4     | Lipoxynase         | — | 0.61 | 0.52 | down/down |
| Q9MK5K7    | 14-3-3-like protein | — | 0.66 | 0.46 | down/down |
| I1K6M2     | Uncharacterized protein | Protein P21 | 0.59 | 0.51 | down/down |
| A0A0R0FEX5 | Uncharacterized protein | Protein SRC1 | 0.38 | 0.49 | down/down |
| A0A0R4J6B1 | Uncharacterized protein | Glucose and ribitol dehydrogenase | 0.55 | 0.34 | down/down |
| I1LVB0     | Uncharacterized protein | Mirculin protein | 0.60 | 0.61 | down/down |
| K7KM5      | Tyrosinase_Cu-bd domain-containing protein | — | 0.54 | 0.66 | down/down |
| I1M676     | Uncharacterized protein | — | 0.63 | 0.61 | down/down |
| C6T3V8     | Uncharacterized protein | Kunitz trypsin inhibitor 2 | 0.61 | 0.60 | down/down |
| C6TFC1     | Non-specific lipid-transfer protein | — | 0.40 | 0.26 | down/down |
| C6ZS00     | Disease resistance protein/LRR protein-related protein | — | 0.66 | 0.63 | down/down |
| I1K3K3     | Urease | — | 0.63 | 0.40 | down/down |
| A0A0R0J65  | Uncharacterized protein | — | 0.60 | 0.48 | down/down |
| C6T488     | Uncharacterized protein | Trypsin inhibitor A | 0.49 | 0.42 | down/down |
| C6SY13     | Uncharacterized protein | Peroxisomal membrane protein PMP22 | 0.66 | 0.66 | down/down |

In contrast, the low-P-tolerant genotype L13 stimulated the carbon metabolism and oxidative phosphorylation, in addition to glycolysis pathways which may have helped seedlings to keep normal growth and tolerate P stress. Besides, several other important pathways were boosted in L13 under P-stress conditions. For example, the biosynthesis of amino acids pathway representing the building blocks of proteins and fundamental for tissue repair, growth and nutrient absorption (Rai 2002) was upregulated under P-limited conditions. Similarly, proteins from the pentose phosphatase pathway (PPP) were upregulated. In PPP, the irreversible oxidative section occurring in non-photosynthetic cells such as in the root is a major source of the reducing equivalent NADPH for biosynthesis and maintaining the redox potential necessary to protect against oxidative stress (Dennis and Blakely 2000). Hence, by activating this pathway, L13 could control oxidative stress resulting from low-P levels in the soil (Valderrama et al. 2006). Similarly, riboflavin has been described as a potent antioxidant involved in various environmental stresses (Mittler 2002). This could explain the upregulation of the riboflavin metabolism pathway in L13 under P deficiencies conditions.

Identifying potential biomarkers for low-P tolerance is useful for a rapid screening of a large germplasm. In this study, we found three proteins (I1KW20 (prohibitins), I1K3U8 (alpha-amylase inhibitors) and C6SZ93 (alpha-amylase inhibitors)) constantly upregulated in L13 but not affected in T3 by low-P levels (Table 1). Both I1K3U8 and C6SZ93 proteins participate in the carbohydrate metabolism pathway which is essential for plant response to abiotic stress including low-P (Singh and Singh 1968; Rychter and Randall 1994; Rosa et al. 2009). I1KW20 containing protein is useful for a rapid screening of a large germplasm. In this study, we found three proteins (I1KW20 (prohibitins), I1K3U8 (alpha-amylase inhibitors) and C6SZ93 (alpha-amylase inhibitors)) constantly upregulated in L13 but not affected in T3 by low-P levels (Table 1). Both I1K3U8 and C6SZ93 proteins participate in the carbohydrate metabolism pathway which is essential for plant response to abiotic stress including low-P (Singh and Singh 1968; Rychter and Randall 1994; Rosa et al. 2009). I1KW20 containing protein is useful for a rapid screening of a large germplasm. In this study, we found three proteins (I1KW20 (prohibitins), I1K3U8 (alpha-amylase inhibitors) and C6SZ93 (alpha-amylase inhibitors)) constantly upregulated in L13 but not affected in T3 by low-P levels (Table 1). Both I1K3U8 and C6SZ93 proteins participate in the carbohydrate metabolism pathway which is essential for plant response to abiotic stress including low-P (Singh and Singh 1968; Rychter and Randall 1994; Rosa et al. 2009).
contributes to mitochondrial biogenesis which is a specific pathway that supports photosynthetic processes and enables continuous survival during abiotic stress exposure in plants (Ahn et al. 2006; Welchen et al. 2014; Taylor 2018). Uregulation of proteins in these pathways as observed in L13 may have favoured the low-P tolerance.

These three proteins could be tested in a large germplasm to confirm their capacity to discriminate low-P-tolerant and -sensitive soybean genotypes. Such biomarkers could facilitate and accelerate breeding efforts towards low-P tolerance in soybean. An interesting follow-up experiment may be to test T3 mutant plants over-expressing the three putative key adaptive proteins in P-limited conditions. Besides, we found homologues of these proteins in several other plant species (I1KW20: A0A151UI57 (Cajanus cajan), V7BRY5 (Phaseolus vulgaris), AO446DM2C2 (Vigna unguiculata)/C6S293: A0A445DA28 (Arachis hypogaea), A0A1537V18 (Vigna radiata var. radiata), Q36861 (V. unguiculata)/I1K3U8: A0A151QZQ6 (C. cajan), V7CR23 (P. vulgaris), A0A17HD23 (Lupinus angustifolius)), hence, we predict that this work could be translated to other botanical species.

**Supporting Information**

The following additional information is available in the online version—

**Table S1.** List and characteristics of the proteins detected in root samples of two contrasting soybean genotypes (T3 and L13) subjected to three P treatments (P0, P1 and P2).

**Table S2.** Differentially expressed proteins between low-P (P1) and normal-P (P2) treatments in L13.

**Table S3.** Differentially expressed proteins between P-free (P0) and normal-P (P2) treatments in L13.

**Table S4.** Differentially expressed proteins between low-P (P1) and normal-P (P2) treatments in T3.

**Table S5.** Differentially expressed proteins between P-free (P0) and normal-P (P2) treatments in T3.

**Table S6.** Differentially expressed proteins commonly regulated in both genotypes under P deficiencies.

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**Conflict of interest**

None declared.

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**Contributions by the Authors**

H.Z., A.Y., X.A.: conceived and designed the study. H.Z., A.Y., L.K., FX., H.W.: performed the experiment and data collection. H.Z., A.Y.: performed data analysis and drafted the manuscript. X.A.: supervised the study, provided funding and revised the manuscript.

**Data Availability**

All data used in this study are available within the manuscript and its supplementary files.

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