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Screening and transcriptome analysis of low phosphorus tolerance genes in Pueraria lobata

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Abstract

To explore the effects of low phosphorus stress on the root morphology, growth physiology and molecular mechanisms of *Pueraria lobata*, the present study utilized *P. lobata* from Australia and Hunan and Jiangsu Provinces in China. Three treatment levels of normal phosphorus (0.5 mmol·L^{-1} KH_2PO_4), low phosphorus (0.05 mmol·L^{-1} KH_2PO_4) and extremely low phosphorus (0.005 mmol·L^{-1} KH_2PO_4) were applied by hydroponics. After 20 days of culture, the root index, proline, soluble sugar, and soluble protein contents and malondialdehyde, superoxide dismutase, peroxidase, and catalase activity levels were measured. *P. lobata* from Australia was tolerant to low phosphorus, while *P. lobata* from Jiangsu was sensitive to low phosphorus. Transcriptome sequencing was performed on the roots of *P. lobata* from Australia and *P. lobata* from Jiangsu. Gene function annotation analysis and differential expression gene screening were performed on the transcriptomes. GO enrichment and KEGG pathway enrichment analysis revealed that in the plant hormone signal transduction pathway PR1 and CTR1 were the most differentially expressed genes in Australian *P. lobata*, while ERF1 was the most differentially expressed gene in Jiangsu *P. lobata*. The transcription factor families C2H2, bZIP, MYB, and bHLH were among the most differentially expressed transcription factors under low phosphorus stress in *P. lobata* from Australia, while ERF was the most differentially expressed transcription factor under low phosphorus stress in *P. lobata* from Jiangsu. Through transcriptome sequencing, the differences in gene expression between low-phosphorus-tolerant Australian *P. lobata* and low-phosphorus-sensitive Jiangsu *P. lobata* under low phosphorus stress were preliminarily understood, providing a basis for future research on the mechanism of *P. lobata* stress responses.

1 Introduction

Pueraria lobata is a perennial twining vine in the family Leguminosae. It develops deep roots and can thus improve soil structure and effectively prevent runoff and erosion (Wang et al., 2020). Accordingly, it is an excellent pioneer plant for desertification control of rocky soils. At present, research on *P. lobata* has mainly focused on its nutritional, medicinal and ecological value (Zhou et al., 2023). Phosphorus is one of the macroelements that is essential for plant growth and development. Even when soil is rich in phosphorus, a large amount of phosphorus exists in a form that cannot be directly used by plants, so plants are often deficient in phosphorus (Rouached et al., 2010).

Under low phosphorus stress, the root diameter of plants decreases, and the number of root hairs and root length increase, which is conducive to increasing contact with soil surface area to increase phosphorus absorption (Brown et al., 2013). Phosphorus limitation affects primary and secondary metabolites in plants, including the synthesis of anthocyanins in *Brassica napus* and rice (Hu et al., 2023; He et al., 2021). The malondialdehyde (MDA) content of *Dioscorea zingiberensis* increases under low phosphorus stress, and it physiologically adapts to low phosphorus stress by increasing the content of proline, soluble sugar (SS) and soluble protein (SP) and the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Wang, 2023). In addition, there can be differences in phosphorus utilization efficiency between different genotypes of the same plant species, and the ability to tolerate low phosphorus stress can also differ. For example, Camilo et al. (2021) conducted pot and field experiments to evaluate the contribution of different genotypes of common bean roots to biomass and grain yield under drought and low phosphorus stress. Iqbal et al. (2023) classified the dry matter production and phosphorus use efficiency of 30 cotton genotypes under low phosphorus and normal phosphorus conditions. Reddy et al. (2020) evaluated 12 phosphorus use efficiency traits of 144 different genotypes of mung bean under hydroponic conditions with suitable and low phosphorus levels. Liu et al. (2023) conducted transcriptome analysis on the factors related to phosphorus transport in chrysanthemum, and found that the root development of chrysanthemum with this factor was significantly higher than that of wild type and it was divided into biological processes, cellular components and molecular functions

through gene ontology (GO). However, there is a lack of research on the response and physiological adaptation mechanism of *P. lobata* to low phosphorus stress. Therefore, it is of great practical importance to improve the utilization rate of phosphorus in *P. lobata* and address the problem of soil phosphorus resources by screening for low-phosphorus tolerant *P. lobata* varieties, tapping the full genetic potential of *P. lobata* to absorb and activate soil phosphorus.

In this study, physiological and biochemical changes in *P. lobata* seedlings under low phosphorus levels and the corresponding differential gene expression patterns based on comparative transcriptomics of *P. lobata* roots were explored under hydroponic growth conditions. This work was undertaken to promote the optimal utilization of *P. lobata* germplasm resources and the breeding of low-phosphorus tolerant varieties and to explore and identify the genes regulating phosphorus tolerance, so as to provide an empirical basis for further guiding the agricultural production of *P. lobata*.

2 Materials and methods

2.1 Plant materials

P. lobata seeds from Australia and Jiangsu and Hunan Provinces in China were used as experimental materials. The Australian *P. lobata* seeds were provided by Jiangsu Gangren Boqi Seed Industry Co., Ltd., the Chinese Jiangsu *P. lobata* seeds were provided by Jiangsu Seed Industry Technology Co., Ltd., and the Chinese Hunan *P. lobata* seeds were obtained from a Hunan seed market.

2.2 Experimental design

Uniformly sized and full seeds of *P. lobata* of three different provenances were used as study materials. The seeds were soaked in 3% NaClO for 20 min to disinfect them. The residual NaClO on the seeds was washed away three to four times with sterile distilled water until the seeds were clean. The seeds were soaked in distilled water at room temperature for 6 h and then evenly placed in a Petri dish with two layers of filter paper. The culture conditions followed a 14/10 h light/dark cycle at 25/22°C. Distilled water was added to the Petri dishes once a day to keep the filter paper moist. After about 7 days of cultivation, when two leaves and one heart were grown, seedlings of the same size were selected and moved into a plastic pot (49.5 cm long, 20 cm wide, 15 cm high) for hydroponic cultivation. When transplanting seedlings, ½-strength Hoagland nutrient solution was used and replaced with full-strength Hoagland nutrient solution after 5 days. Low phosphorus treatment was initiated after 2 weeks of full-strength nutrient solution cultivation.

The seedlings of *P. lobata* were maintained in constant temperature culture, and three test levels, normal phosphorus (0.5 mmol·L^{-1} KH_2PO_4), low phosphorus (0.05 mmol·L^{-1} KH_2PO_4), and very low phosphorus (0.005 mmol·L^{-1} KH_2PO_4), were applied. A total of nine treatments were used across the three provenances of *P. lobata* as test materials. Each treatment was repeated three times, with 30 plants per replicate, for a total of 270 pots. The seedlings were fixed with perforated sponge strips, and the nutrient solution was changed every three days, 1 L at a time. When replacing the nutrient solution, the position of each pot of *P. lobata* was randomly moved to reduce the influence of light and other factors. At the same time, potassium chloride was added to the low phosphorus nutrient solution to supplement plants with the missing potassium, and the other nutrient solution components were the same (Table 1). Finally, HCl or NaOH was used to adjust the pH value of the nutrient solution to between 6.0 and 6.2.

Table 1
Conponents and concentration of nutrient solution

Macronutrients		Micronutrients	
Name	Concentration(mmol·L^{-1})	Name	Concentration(μmol·L^{-1})
KNO_3	1.0	H_3BO_3	1.00
$Ca(NO_3)_2$	2.0	$MnSO_4$	1.00
$CaCl_2$	1.5	$CuSO_4$	0.50
$MgSO_4$	0.5	$ZnSO_4$	1.00
KCl	1.8	$(NH_4)_6Mo_7O_{24}$	0.10
		Na_2-EDTA	50.00
		$FeSO_4$	0.05

2.3 Index determination

After 20 days of culture, the agronomic traits and physiological indexes of *P. lobata* were measured.

2.3.1 Determination of agronomic traits

Root length, root surface area, root diameter and other root indexes were measured using a root scanner (Epson Perfection V800 Photo from Beijing Epson Co., Ltd.). The seedlings of *P. lobata* were divided into aboveground and underground parts, and their individual fresh weights were obtained. After being dried at 105°C for 30 min to halt enzymatic activity, the seedlings were dried further at 65°C until the dry weight was recorded. The root phenotype index was based on three replicates, with four plants per replicate.

2.3.2 Determination of physiological indexes

MDA content was determined by the thiobarbituric acid method. SS content was determined by anthrone colorimetry. SP content was determined by the Coomassie brilliant blue G-250 method. CAT activity was determined by the ultraviolet absorption method. POD activity was determined using the guaiacol method. SOD activity was determined by the NBT photoreduction method (Zhang & Qu 2003). The proline content was determined by the acid ninhydrin method.

2.4 Transcriptome sequencing

Based on their agronomic traits and physiological indexes, the roots of *P. lobata* from Australia and Jiangsu were sampled and wrapped in aluminum foil. RNA was isolated from plants by the precipitation extraction: Trizol method for the purpose of validating the transcriptome results by quantitative PCR (qRT-PCR). RNA was obtained from the lysed root cells of *P. lobata*. After adding chloroform and centrifugation, each sample was stratified (acidic conditions separated RNA and DNA, and RNA remained in the supernatant). The supernatant was collected, and then, isopropanol was added to precipitate and reduce the isolated RNA. After labeling, they were immediately frozen in liquid nitrogen and stored at -80°C. Samples were sent to Guangzhou Gideo Biotechnology Co., Ltd. (Guangzhou, China) for high-throughput transcriptome sequencing. Fastp was used to control the quality of raw reads and filter out low-quality raw data. Trinity software was used to assemble reads, and then BUSCO was used to evaluate assembly

integrity. Unigene sequences were aligned with the protein databases Nr, SwissProt, KEGG and COG/KOG (E-value < 0.00001) to predict their functions. In order to explore the changes of differential genes in *P. lobata* roots under low phosphorus stress, Fold Change ≥ 2 and FDR < 0.01 were used as screening criteria to screen differentially expressed genes in *P. lobata* roots under different phosphorus concentrations. The obtained DEGs were subjected to Go gene function annotation analysis and KEGG metabolic pathway enrichment analysis.

2.5 Fluorescence qrt-PCR validation

KH_2PO_4 was used as the phosphorus source to establish 0.5, 0.05, and 0.005 mmol·L^{-1} concentrations of phosphorous, and the roots of *P. lobata* from Australia and Jiangsu were used as experimental materials. RNA was extracted, and five up-regulated genes and five down-regulated genes from the transcriptome analysis were randomly selected for analysis (Table 2). Primers were designed by Shanghai Bioengineering Co., Ltd. (Shanghai, China), and cDNA was synthesized using Thermo Fisher's RevertAid first-strand cDNA synthesis kit (Thermo Fisher, Waltham, MA, USA). Finally, real-time fluorescence quantitative PCR was performed using Kangwei Century's Ultra SYBR Mixture kit (Kangwei Century, Beijing, China). For the control and treatment groups there were three replicates each.

Table 2
Real-time fluorescent quantitative PCR amplification primers

Genes	Forward primer	Reverse primer
Unigene0108615	GTATTTCCCTCAGCAGCCCTCTTG	GCTGGCTCCACTGTCATGTTATCC
Unigene0090905	GATGTTGGTGGCGTTGGAAATGTG	GATTGGGGTGTGCTCTTTGGTTTTG
Unigene0019203	TTCACGCTTGATTCCCGCCTATATG	TGCTCCAGTCGAATTACCACTTGC
Unigene0119001	AGGAAGCAGAGGATGACGAGGAAG	AGCCAAGAGCCAGGAAATAGAACAG
Unigene0019540	CGGTCATTTCACACAAGTCGTTTGG	CTTTACGTCCACCATCAGCATAGGC
Unigene0134233	CGGTGAGATTAAGGTGGTTGAGGTC	ACTGTTCTTTGCGGCGGTGATAG
Unigene0085409	CAACCATGTACCACCACCACCAC	CCATTCTCCATCGTCCGTGTCAATC
Unigene0058619	AGTAGTAAGCCACTTGCAGCAGTTC	AGCACATACCAGAAACGAGAGCATC
Unigene0116632	CTCTGGTCTATGCCGTTATCGTGTG	TTCTGTAGCTCCGCCTCGTCTG
Unigene0068192	TTCTATGCTGCTGGACTCCGTAATG	TGCTGCTTCTCGATTGCTCTTGAC

2.6 Statistical analysis

Microsoft Excel 2013 (Microsoft Corp., Redmond, WA, USA) was used for data collation, SPSS 25.0 software (IBM Corp., Armonk, NY, USA) was used for data analysis, and Origin2021 (OriginLab, Northampton, MA, USA) was used for rendering figures. The effects of phosphorus treatment on the growth characteristics of *P. lobata* from the three different provenances were comprehensively evaluated by interaction effect analysis, low phosphorus tolerance coefficient correlation analysis and membership function analysis.

3 Results

3.1 Two-factor interaction analysis of agronomic traits and physiological indexes of *P. lobata*

There were significant differences in total root length, root tip number, root hair number and SOD activity among the different provenances, different phosphorus concentrations and their different combinations (p<0.05). There were

significant differences in root volume, aboveground dry weight and root-to-shoot ratio between the different provenances and different phosphorus concentrations (p<0.05). There were significant differences in SOD activity between the different provenances of *P. lobata* and the different combinations of provenance and phosphorous concentrations (p<0.05). There were significant differences in root diameter, underground dry weight, CAT activity and Pro, SS, SP and MDA contents among the different provenances (p<0.05). There were significant differences in POD, CAT and Pro between different combinations of provenances and phosphorus concentrations (p<0.05) (Table 3 and 4).

Table 3 Variance analysis on root morphological of Pueraria lobata seedlings under different low phosphorus stresses

Variables	Index	Within different concentration treatment	Within different varieties	Different concentration ×Different varieties
Root length	df	2...	2	4
	F	14.25**	4.361*	5.924**
Root surface area	df	2	2	4
	F	4.076*	0.58	0.398
Root average diameter	df	2	2	4
	F	1.643	7.147**	1.513
Root volume	df	2	2	4
	F	7.06 **	9.718**	1.512
Root Tips	df	2	2	4
	F	9.74 **	18.419**	6.403 **
Root hairs	df	2	2	4
	F	8.842 **	11.672**	2.288*
Shoot dry weight	df	2	2	4
	F	17.03**	2.847*	1.574
Root dry weight	df	2	2	4
	F	2.275	9.507**	0.788
Root-shoot ratio	df	2	2	4
	F	3.818*	4.413*	0.659

Notes: *: Significant difference (p<0.05) ; **: Significant difference (p<0.01).

Table 4 Variance analysis on active material of Pueraria lobata seedlings under different low phosphorus stresses

Variables	Index	Within different concentration treatment	Within different varieties	Different concentration ×Different varieties
SOD	df	2	2	2
	F	3.668*	8.669**	6.154**
POD	df	2	2	2
	F	0.964	3.182	6.259**
CAT	df	2	2	2
	F	2.418	6.372**	3.265
Pro	df	2	2	2
	F	3.207	5.074*	1.046
SS	df	2	2	2
	F	1.851	14.381**	2.093
SP	df	2	2	2
	F	1.828	4.675*	1.08
MDA	df	2	2	2
	F	0.457	7.905**	1.307

Notes: *: Significant difference ($p<0.05$) ; **: Significant difference ($p<0.01$).

As phosphorous concentration decreased, there were different trends in the root indexes of *P. lobata* from each provenance. The total root length, root surface area, root volume, root tip number, root hair number and aboveground dry weight of Australian *P. lobata* increased first and then decreased as phosphorous concentration increased. The total root length, root surface area, root diameter, root volume and aboveground dry weight of *P. lobata* from Hunan first increased and then decreased with phosphorous concentration. In *P. lobata* from Jiangsu, except for the aboveground dry weight and root-to-shoot ratio, the other indicators showed a decreasing trend with phosphorous concentration. The root-to-shoot ratio of three provenances of *P. lobata* first decreased and then increased (Fig.1).

Among the root physiological indexes assayed, except for the content of SP, which decreased with the decrease of phosphorus concentration, the other indexes increased as phosphorous concentration decreased. Among SOD, POD and CAT, the activity levels of *P. lobata* from Hunan was the highest, and the contents of Pro, SS and especially MDA from Jiangsu were the highest (Fig.2).

Fig. 1 Changes of root morphological of *Pueraria lobata* seedlings under different low phosphorus stresses

Note:Different letters on the columns represented significant difference ($p<0.05$).

Fig. 2 Changes of physiological indexes of *Pueraria lobata* seedlings under different low phosphorus stresses

Note:Different letters on the columns represented significant difference ($p<0.05$).

3.2 Correlation of low phosphorus tolerance with agronomic traits and physiological indexes

The correlation analysis of the low phosphorus tolerance coefficients of the traits measured for each *P. lobata* provenance (Fig.3) showed that there were indeed different degrees of correlation between the low phosphorus tolerance coefficients of the different traits. There was a significant positive correlation between shoot dry weight and both root dry weight and root hair number. There was also a significant positive correlation between total root length and root surface area, root volume and root tip number. There was a significant positive correlation between root surface area and root volume. There was a significant positive correlation between MDA content and underground dry weight, root surface area and root volume, which all were negatively correlated with SOD, POD and CAT activities and Pro and SS contents. There was a significant positive correlation between SS content and POD and CAT activities. There was a significant positive correlation between SOD activity and Pro content. Thus, the growth of the aboveground and underground parts of *P. lobata* from the different provenances under low phosphorus stress were related, and the growth of roots plays an important role in the growth of the aboveground parts. Under low phosphorus stress, *P. lobata* can actively regulate the activities of various protective enzymes and promote the growth of roots to resist stress-induced damage. Each single index demonstrates a different trait that plays a different role in the low phosphorus tolerance of *P. lobata*, indicating that the low phosphorus tolerance of *P. lobata* is indeed a complex comprehensive trait, and the direct use of each single index cannot accurately and intuitively be used to evaluate the low phosphorus tolerance of *P. lobata*. Therefore, other statistical methods were further used for analysis.

Fig. 3 Correlation anslysis on low phosphorus tolerance coefficient of trait indexes in *Pueraria lobata* seedings

Notes: *: Significant difference ($p<0.05$).

3.3 Comprehensive evaluation and cluster analysis of low-phosphorus stress tolerance

Membership function analysis can be used to overcome the problem of inaccurate evaluation using a single index and can, for example, to comprehensively evaluate stress tolerance in plants. The comprehensive ranking of the low phosphorus stress tolerance of *P. lobata* from different provenances is shown in Tables 5 and 6. In the comparison of root indexes of *P. lobata* from the same provenance, the total membership function values of *P. lobata* from Australia and Hunan showed an intermediate optimum relative to phosphorous concentration, while *P. lobata* from Jiangsu showed a downward trend as phosphorous concentration decreased. In the comparison of physiological indexes of *P. lobata* from the same provenance, the total membership function values of *P. lobata* from various provenances decreased as phosphorous concentration decreased. In the comparison of root indexes at the same concentration, *P. lobata* from Australia ranked first, and its indexes rose with phosphorous concentration; *P. lobata* from Hunan ranked the lowest in terms of root index, and *P. lobata* from Jiangsu had a root index that declined with phosphorous concentration and then leveled off. Compared with the physiological indexes of the same concentration, *P. lobata* from Australia ranked the lowest, and *P. lobata* from Jiangsu ranked the highest (Table 7 and 8). Thus, the comprehensive analysis showed that *P. lobata* from Australia was low-phosphorous tolerant, and *P. lobata* from Jiangsu was low-phosphorous sensitive.

After the comprehensive score of each index value was subjected to factor analysis, the scores were clustered by the Euclidean distance average linkage method, by which the combinations of phosphorous concentration treatments and provenances could be classified into three groups separated by a Euclidean distance of greater than 5. Among them, the first group includes Jiangsu *P. lobata* under three phosphorous concentrations and Hunan *P. lobata* under normal phosphorus; the second group includes Australian *P. lobata* under normal and low phosphorous treatments; the third group includes Australian *P. lobata* under very low phosphorus treatment and Hunan *P. lobata* under low phosphorus and very low phosphorus treatments (Fig.4).

Table 5 Membership function values on root indexes of Pueraria lobata seedlings under different low phosphorus stresses

Root index	Australia			Hunan			Jiangsu		
	Different concentrations (mmol · L^{-1}) treatment			Different concentrations (mmol · L^{-1})treatment			Different concentrations (mmol · L^{-1}) treatment		
	0.5	0.05	0.005	0.5	0.05	0.005	0.5	0.05	0.005
Root length	0	1	0.005	0.867	1	0	1	0.502	0
Root surface area	0.369	1	0	0.918	1	0	1	0.696	0
Root average diameter	1	0	0.815	0.818	1	0	0.971	1	0
Root volume	0	0.421	1	0.818	1	0	0.971	1	0
Root Tips	0.198	1	0	1	0.436	0	0.789	1	0
Root hairs	0.878	1	0	0	1	0.170	1	0.106	0
Root dry weight	1	0.333	0	0.5	0	0	0.957	1	0
Shoot dry weight	0.419	1	0	0	1	0.068	0.485	1	0
Root-shoot ratio	0.614	0	1	1	0	0.308	0.912	0	1
Total membership function value	4.478	5.754	2.82	5.921	6.436	0.546	8.085	6.304	1
billing	2	1	3	2	1	3	1	2	3

Table 6 Membership function values on physiological indexes of Pueraria lobata seedlings under different low phosphorus stresses

Physiologic index	Australia			Hunan			Jiangsu		
	Different concentrations (mmol · L^{-1}) treatment			Different concentrations (mmol · L^{-1}) treatment			Different concentrations (mmol · L^{-1}) treatment		
	0.5	0.05	0.005	0.5	0.05	0.005	0.5	0.05	0.005
SP	1	0.102	0	0	1	0.278	1	0.905	0
SS	0	0.156	1	0	0.93	1	0	0.375	1
Pro	0	0.564	1	0	0.12	1	0	0.245	1
MDA	0.534	0	1	0.44	0	1	0.207	0	1
SOD	0	0.71	1	0	0.279	1	0	0.202	1
POD	0	0.132	1	0	0.559	1	0	0.598	1
CAT	0	0.380	1	0	0.228	1	0	0.072	1
Total membership function value	1.534	2.044	6	0.44	3.116	6.278	1.207	2.397	6
billing	3	2	1	3	2	1	3	2	1

Table 7 Membership function values on root indexes of Pueraria lobata seedlings

Root index	0.5			0.05			0.005		
	Australia	Hunan	Jiangsu	Australia	Hunan	Jiangsu	Australia	Hunan	Jiangsu
Root length	0	1	0.603	1	0.388	0	1	0.245	0
Root surface area	0	0.087	1	1	0	0.418	0.889	0	1
Root average diameter	1	0	0.451	1	0	0.985	1	0	0.182
Root volume	0.348	0	1	1	0	0.985	1	0	0.182
Root Tips	0.363	1	0	1	0.480	0	1	0.802	0
Root hairs	0.568	0	1	1	0	0.637	0.098	0	1
Root dry weight	0.308	0	1	0.091	0	1	0.778	0	1
Shoot dry weight	1.071	0	1	0.417	0	1	1	0	0.500
Root-shoot ratio	0	0.351	1	0	0.985	1	0.563	0	1
Total membership function value	3.658	2.438	7.054	6.508	1.853	6.025	7.328	1.047	4.864
billing	2	3	1	1	3	2	1	3	2

Table 8 Membership function values on physiological indexes of Pueraria lobata seedlings under different provenance

Root index	0.5			0.05			0.005		
	Australia	Hunan	Jiangsu	Australia	Hunan	Jiangsu	Australia	Hunan	Jiangsu
SP	0.595	1	0	0.060	0	1	1	0	0.739
SS	0.020	0	1	0	0.206	1	0.310	0	1
Pro	0	0.351	1	0.613	0	1	0.195	0	1
MDA	0.512	0	1	0.362	0	1	0.371	0	1
SOD	0	1	0.815	0	1	0.304	0	1	0.278
POD	0	1	0.550	0	1	0.542	0.637	1	0
CAT	0	0.763	1	0	1	0.809	1	0.081	0
Total membership function value	1.127	4.114	5.365	1.035	3.206	5.655	3.513	2.081	4.017
billing	3	2	1	3	2	1	2	3	1

Fig. 4 Clustering analysis on *Pueraria lobata* seedlings under different low phosphorus stresses

3.4 Transcriptome analysis of *P. lobata* under low phosphorus stress

3.4.1 Transcriptome sequencing data quality evaluation

The roots of phosphorus-tolerant Australian *P. lobata* and phosphorus-sensitive Jiangsu *P. lobata* were used as experimental materials, with three replicates in each group. After RNA extraction, transcriptome sequencing was performed. The number of reads obtained after filtering the original sequencing data of *P. lobata* was 6 915 310 149, 6 549 381 188, and 6 325 186 657 for the replicates runs of material originally from Australia and 6 012 734 627, 7 052 479 728, and 6 492 052 540 for the replicate runes of material originally from Jiangsu. After filtering, in each of the transcriptomes, the percentage of Q20 bases was 97.38–97.58 %, the percentage of Q30 bases was 92.53–93.00 %, and the GC content was approximately 44% (Table 9). The above results show that the sequencing results were reliable, indicating their validity for use in subsequent bioinformatics analysis.

Table 9 Base information statistics table

Sample	RawData (bp)	CleanData (bp)	Q20 (%)	Q30 (%)	GC (%)
A-1-1	8059814700	7987685389	97.55%	92.91%	43.98%
A-1-2	6677308500	6605370302	97.54%	92.89%	43.93%
A-1-3	6205357200	6152874757	97.67%	93.21%	43.77%
A-2-1	6156267300	6104528775	97.54%	92.88%	44.04%
A-2-2	6642303600	6574320211	97.54%	92.97%	45.51%
A-2-3	7038328500	6969294577	97.62%	93.13%	45.54%
A-3-1	6919963500	6848259338	97.41%	92.62%	43.91%
A-3-2	6508660500	6457814588	97.19%	92.06%	43.76%
A-3-3	5712375900	5669486046	97.57%	92.91%	44.00%
J-1-1	6371036700	6323727547	97.50%	92.83%	44.41%
J-1-2	5975698800	5928899493	97.56%	92.89%	44.26%
J-1-3	5837791500	5785576840	97.53%	92.93%	44.03%
J-2-1	6958881900	6903477498	97.42%	92.62%	44.24%
J-2-2	7190823000	7136301975	97.44%	92.67%	43.93%
J-2-3	7169801100	7117659712	97.58%	92.97%	43.94%
J-3-1	6145647900	6091563890	97.43%	92.69%	43.92%
J-3-2	6774427200	6707263880	97.32%	92.54%	44.42%
J-3-3	6737523000	6677329850	97.40%	92.67%	44.24%

Note: Sample: sample name; RawData (bp): the total number of bases in the off-machine data (unit bp); CleanData (bp): the total number of high-quality data bases after filtering (unit bp); Q20 (%): the number of bases whose quality value of the sequenced bases reaches the level above Q20 and the percentage of RawData (or CleanData); Q30 (%): the sequencing bases The number of bases with base quality value above Q30 and the percentage of RawData (or CleanData); GC (%) : GC ratio of sequence bases before (after) filtering; A: Australian *Pueraria lobate*; J: Jiangsu *Pueraria lobate*.

3.4.2 Functional annotation of unigenes

Among the 128,744 unigenes identified, 64.97%, 52.71%, 44.24%, and 49.95% returned hits in the Nr, KEGG, KOB and SwissProt databases, respectively (Fig.5).

Fig. 5 Annotation statistical charts of the four major databases

3.4.3 Analysis of differentially expressed genes

In order to explore differential gene expression in *P. lobata* roots under low phosphorus stress, differentially expressed genes in *P. lobata* roots under different phosphorus concentrations were screened (Fig.6). A total of 11,595 differentially expressed genes were detected in comparison A-1_vs_A-2. Among them, 8896 unigenes were up-

regulated, and 2699 unigenes were down-regulated. In comparison A-1_vs_A-3, there were 1488 differentially expressed genes, including 668 up-regulated genes and 820 down-regulated genes. In comparison A-2_vs_A-3, there were as many as 13,451 differentially expressed genes, including 791 up-regulated genes and 12,660 down-regulated genes.

There were 6893 differentially expressed genes in comparison J-1_vs_J-2 among different phosphorus levels in *P. lobata*; among them, 1180 unigenes were up-regulated, and 5013 unigenes were down-regulated. In comparison J-1_vs_J-3, there were 2570 differentially expressed genes, of which 460 were up-regulated and 2110 were down-regulated. In comparison J-2_vs_J-3, there were 4374 differentially expressed genes, of which 1761 were up-regulated and 2613 were down-regulated. Overall, the down-regulated genes outnumbered the up-regulated genes, and the differentially expressed genes in *P. lobata* from Australia outnumbered those in *P. lobata* from Jiangsu.

Fig.6 Bar chart of differentially expressed genes co-existing in different treatment groups

Note: A: Australian *Pueraria lobate*; J: Jiangsu *Pueraria lobate*; 1: Normal phosphorus treatment level; 2: Low phosphorus treatment level; 3: Extremely phosphorus treatment level.

3.4.4 GO enrichment analysis of differentially expressed genes in *P. lobata* under low phosphorus stress

In order to explore the function of low phosphorus stress response genes in *P. lobata* from different provenances, GO functional enrichment analysis was performed on genes that were differentially expressed under different phosphorus concentration stress treatments (Fig.7). The differentially expressed genes between the Australian *P. lobata* and the Jiangsu *P. lobata* accessions under different phosphorus stress treatments were annotated according to three major categorizations, biological processes, cellular components and molecular functions, according to their GO terms, and the most annotated differentially expressed genes among the biological process classification were cellular processes and metabolic processes. The most annotated differentially expressed genes in the cellular component classification were cells and cellular components, and the most annotated differentially expressed genes in the molecular function classification were catalytic activity and binding. In Australian *P. lobata* comparisons A-1_vs_A-3 and A-2_vs_A-3, the metabolic process, cellular process, catalytic activity and binding categories were more often down-regulated than up-regulated, while the rest of the enriched categories were more often up-regulated than down-regulated. Overall, Jiangsu *P. lobata* showed a large proportion of down-regulated differentially expressed genes.

The top 30 most enriched differentially expressed genes in the GO enrichment analysis (Fig.8) were screened. In comparison A-1_vs_A-2, among biological process GO terms, differentially expressed genes were mainly enriched for glucose metabolism and hexose metabolism. Among molecular function GO terms, differentially expressed genes were mainly enriched for the cytoplasm and cytoplasmic. In comparison A-1_vs_A-3, GO terms of differentially express genes were mainly enriched for RNA modification and gene expression. Among cellular component GO terms, differentially expressed genes were mainly enriched for molecular structure activity; among molecular function GO terms, differentially expressed genes were mainly enriched for ribosomes and intracellular nucleoprotein complexes. In comparison A-2_vs_A-3, among biological process GO terms, differentially expressed genes were mainly enriched for glucose metabolism and hexose metabolism. Among molecular function GO terms, differentially expressed genes were mainly enriched for terms related to the cytoplasm. In comparison J-1_vs_J-2, RNA modification and inorganic response were the most enriched GO terms. Among cellular component GO terms, differentially expressed genes were mainly enriched for molecular structure activity; among molecular function GO terms, differentially expressed genes were mainly enriched for ribosomes and non-membrane organelles. In comparison J-1_vs_J-3, among biological process GO terms, differentially expressed genes were mainly enriched for RNA modification, while among cellular component GO terms, differentially expressed genes were mainly enriched for molecular structure activity; among

molecular function GO terms, differentially expressed genes were mainly enriched for ribosomes and ribosomal subunits. In comparison J-2_vs_J-3, among all GO terms, differentially expressed genes were mainly enriched for osmotic stress and RNA modification. Among cellular component GO terms, differentially expressed genes were mainly enriched for molecular structure activity; among molecular function GO terms, differentially expressed genes were mainly enriched for ribosomes and ribosomal subunits.

Fig. 7 GO enrichment analysis of differential genes

Note:A A-1_vs_A-2 B A-1_vs_A-3 C A-2_vs_A-3 D J-1_vs_J-2 E J-1_vs_J-3 F J-2_vs_J-3

Fig. 8 Bar chart of GO enrichment analysis of differentially expressed genes under low phosphorus stress

Note:A A-1_vs_A-2 B A-1_vs_A-3 C A-2_vs_A-3 D J-1_vs_J-2 E J-1_vs_J-3 F J-2_vs_J-3

3.4.5 KEGG analysis of differentially expressed genes in *P. lobata* under low phosphorus stress

In order to further assess which metabolic pathways were affected by low phosphorus stress, KEGG metabolic pathway enrichment analysis was performed on the differentially expressed genes (Table 10). We found enrichment for a plant hormone signaling pathway in the information transduction pathway. In comparison A-1_vs_A-2, a total of 26 unigenes were determined to be involved in hormone regulation. The most up-regulated genes were PR1 and CTR1, and the most down-regulated genes were PR1 and TCH4. In comparison A-1_vs_A-3, a total of five unigenes were determined to be involved in hormone regulation, all of which were down-regulated, with TCH4 being the most down-regulated. In comparison A-2_vs_A-3, a total of 17 unigenes were determined to be involved in hormone regulation, and the most differentially expressed was CTR1 (Fig.9A).

In comparison J-1_vs_J-2, a total of 30 unigenes were determined to be involved in hormone regulation, and the most differentially expressed was ERF1. In comparison J-1_vs_J-3, a total of seven unigenes were determined to be involved in hormone regulation, and the most differentially expressed was ERF1. In comparison J-2_vs_J-3, a total of 17 unigenes were determined to be involved in hormone regulation. The most up-regulated were PR1 and TCH4, and the most down-regulated was ERF1 (Fig.9B).

Table 10 The differentially expressed genes in plant hormone signaling pathways

Gene Name	A-1_vs_A-2		A-1_vs_A-3		A-2_vs_A-3		J-1_vs_J-2		J-1_vs_J-3		J-2_vs_J-3	
	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
PR1	4	4		1	1	4	1	4			3	
JAZ		1							1			
SAUR		2		1			2	1			1	2
ARR-A		1		1								
PP2C	1						1		1			
BIN2	1							2				
TCH4		4		2			1	2			3	
CTR1	4					7				2		
MPK6	2					3		1				
ERF1		2					8		3			3
AHK2_3_4						1	1					
SNRK2						1	2					2
TGA							1					
IAA								2			2	1
GH3							1					

Fig. 9 KEGG enrichment analysis of DEGS in *Pueraria lobate* roots under low phosphorus stress

3.4.6 Differential expression analysis of transcription factor genes under low phosphorus stress

A total of 163 differentially expressed transcription factors in 26 families were screened in the transcripts of comparison A-1_vs_A-2. Among them, the number of differentially expressed genes in the C2H2 family was the largest, followed by the bZIP, ERF, and MYB_related families (Table 11). A total of 53 differentially expressed transcription factors in 16 families were screened in comparison A-1_vs_A-3, among which the number of differentially expressed genes in the ERF family was the largest, followed by the C2H2 and WRKY families (Table 12). A total of 144 differentially expressed transcription factors were screened in comparison A-2_vs_A-3, spanning 22 families. Among them, the number of differentially expressed genes in the bZIP family was the largest, followed by the C2H2 and bHLH families (Table 13).

A total of 128 differentially expressed transcription factors in 24 families were screened in the transcripts of comparison J-1_vs_J-2. Among them, the number of differentially expressed genes in the ERF family was the largest, followed by the MYB_related and C2H2 families (Table 14). A total of 45 differentially expressed transcription factors among 16 families were screened in the transcripts of comparison J-1_vs_J-3. Among them, the number of differentially expressed genes in the ERF family was the largest, followed by the C2H2 and bZIP families (Table 15). A total of 99 differentially expressed transcription factors in 25 families were screened in the transcripts of comparison

J-2_vs_J-3. Among them, the number of differentially expressed genes in the ERF family was the largest, followed by the MYB_ related and bHLH families (Table 16).

Table 11 Distribution of common differentially expressed transcription factors in different libraries under low phosphorus stress of Australian Pueraria lobate A-1

TF family	DEG	Up	Down	TF family	DEG	Up	Down
M-type	1	1	0	WRKY	4	0	4
GRAS	3	0	3	NF-YA	1	0	1
G2-like	1	0	1	C3H	15	9	6
LBD	2	0	2	HB-other	7	7	0
bHLH	15	10	5	NF-YC	2	1	1
Nin-like	1	1	0	GATA	2	2	0
ERF	17	0	17	bZIP	23	18	5
HSF	8	8	0	Whirly	1	1	0
RAV	1	0	1	DBB	2	2	0
MYB	7	5	2	NAC	2	0	2
C2H2	25	20	5	E2F/DP	2	2	0
NF-X1	1	1	0	NF-YB	1	1	0
MYB_related	17	8	9	Trihelix	2	0	2

Table 12 Distribution of common differentially expressed transcription factors in different libraries under low phosphorus stress of Australian Pueraria lobate A-2

TF family	DEG	Up	Down	TF family	DEG	Up	Down
GRAS	2	0	2	MYB	2	0	2
RAV	1	0	1	ERF	11	1	10
C2H2	9	1	8	MYB_related	3	0	3
TALE	1	1	0	YABBY	1	1	0
WRKY	9	0	9	NF-YA	1	0	1
bZIP	1	0	1	C3H	3	2	1
HSF	3	3	0	NAC	3	1	2
bHLH	2	2	0	M-type	1	0	1

Table 13 Distribution of common differentially expressed transcription factors in different libraries under low phosphorus stress of Australian Pueraria lobate A-3

TF family	DEG	Up	Down	TF family	DEG	Up	Down
NF-YB	1	0	1	bHLH	12	1	11
DBB	4	0	4	Nin-like	2	0	2
TALE	1	0	1	HB-other	9	0	9
MYB_related	8	0	8	E2F/DP	3	0	3
ERF	1	1	0	C3H	9	0	9
GATA	7	0	7	M-type	5	0	5
C2H2	27	0	27	MYB	7	0	7
HSF	10	2	8	Trihelix	2	2	0
YABBY	2	1	1	bZIP	28	1	27
LBD	3	3	0	NF-YC	1	0	1
NF-X1	1	0	1	HD-ZIP	1	0	1

Table 14 Distribution of common differentially expressed transcription factors in different libraries under low phosphorus stress of Jiangsu Pueraria lobate J-1

TF family	DEG	Up	Down	TF family	DEG	Up	Down
HB-other	1	0	1	GRAS	3	0	3
M-type	1	0	1	Nin-like	7	0	7
GATA	2	0	2	HSF	4	2	2
G2-like	3	2	1	MIKC	1	1	0
ARF	1	0	1	bHLH	9	5	4
ERF	27	22	5	WOX	1	1	0
Dof	5	5	0	NF-YA	2	1	1
C2H2	12	6	6	B3	1	0	1
NF-YC	1	0	1	LBD	1	1	0
NAC	8	3	5	TCP	1	0	1
MYB_related	17	7	10	C3H	6	0	6
WRKY	7	7	0	bZIP	7	2	5

Table 15 Distribution of common differentially expressed transcription factors in different libraries under low phosphorus stress of Jiangsu Pueraria lobate J-2

TF family	DEG	Up	Down	TF family	DEG	Up	Down
Dof	3	3	0	HSF	2	0	2
ERF	7	4	3	bHLH	3	2	1
ARF	1	1	0	GATA	1	0	1
Nin-like	2	1	1	C2H2	6	0	6
G2-like	1	1	0	WRKY	1	1	0
NAC	4	3	1	Trihelix	2	0	2
bZIP	5	2	3	LBD	2	2	0
MYB_related	4	1	3	M-type	1	0	1

Table 16 Distribution of common differentially expressed transcription factors in different libraries under low phosphorus stress of Jiangsu Pueraria lobate J-3

TF family	DEG	Up	Down	TF family	DEG	Up	Down
bZIP	7	1	6	LBD	3	2	1
MYB_related	10	6	4	WRKY	3	0	3
ERF	20	11	9	Nin-like	4	3	1
Trihelix	3	1	2	HSF	1	0	1
CPP	1	1	0	HB-other	2	1	1
MYB	3	3	0	B3	2	2	0
SRS	3	3	0	SBP	3	3	0
ARF	1	1	0	bHLH	8	4	4
ARR-B	2	1	1	TCP	2	2	0
NAC	5	4	1	G2-like	3	2	1
GATA	1	1	0	GRAS	3	2	1
HD-ZIP	1	1	0	BES1	1	1	0
C2H2	7	4	3				

3.4.7 Quantitative analysis of differentially expressed genes by qRT-PCR fluorescence

In order to verify the accuracy of transcriptomic differential expression results of the identified unigenes under low phosphorus stress, 10 unigenes were randomly selected for quantitative real-time PCR (qRT-PCR) validation. The validation results are shown in Fig. 10. Thus, the expression levels of the 10 validated genes were consistent between the qRT-PCR and RNA-Seq results, indicating that the transcriptomic results of this experiment were reliable.

Fig. 10 Relative expression levels of 10 randomly selected genes validated by qRT-PCR

4 Discussion

Roots are the main organs by which plants absorb nutrients. When the nutrient supply is insufficient, changes are induced in the root morphological structure and the spatial distribution of different root types in order to physiologically adapt to the growth and development needs under the stress environment, mainly by increasing root length and root surface area to improve the absorption of phosphorus (Benjamin et al., 2013). This is consistent with the results of total root length and root surface area increases observed in Australian and Hunan *P. lobata* under low phosphorus stress in this experiment. Australian *P. lobata* exhibited a clear trend of increasing its root length, root surface area, root tip number, and root hair number. Although Hunan *P. lobata* also showed the same trend, it was most obvious in Australian *P. lobata*, indicating its stronger response to low-phosphorous stress. In the present experiment, under extremely low phosphorus stress, each root index was inhibited to varying degrees, indicating that root extension could be promoted within a suitable low phosphorus range, while a low phosphorus concentration would affect plant root growth.

In adverse environments, the content of reactive oxygen species in plants can accumulate in large quantities, which can ultimately cause membrane lipid peroxidation and eventually destroy plant membrane systems(Liu et al., 2016). MDA is one of the products of membrane lipid peroxidation, and thus it can reflect the degree of plant damage to some extent. In the present study, the MDA content of the three accessions of *P. lobata* increased as phosphorous concentration decreased, and the MDA content of *P. lobata* from Jiangsu was the highest; thus, the three accessions of *P. lobata* were damaged to varying degrees under low phosphorus stress. The accumulation of MDA can stimulate the activity of SOD, POD, CAT and other protective enzymes in plants to accelerate MDA clearance (Su et al., 2021). In the present study, the activities of SOD, POD and CAT increased as phosphorous concentration decreased, and MDA content was negatively correlated with SOD, POD and CAT activities; however, CAT activity was positively correlated with SOD and POD activities. Thus, *P. lobata* plants can improve the activity of various protective enzymes to cope with the damage caused by low phosphorus stress through the coordination of various protective enzymes. Pro, SS and SP are important osmotic adjustment substances in plants, and thus the accumulation of these substances occurs to maintain homeostasis when plants face biotic and abiotic stresses (Zhang, 2022). The decrease of MDA context under low phosphorus stress was related to the increases of SS and SP contents (Wang, 2023). In this study, the negative correlation of MDA content with SS and SP contents under low phosphorus stress was consistent with this observation.

The differences in plant phenotypes are mainly owing to differences in gene expression regulation. In the top 30 most enriched GO terms, excluding Australian *P. lobata* under low phosphorus, the down-regulated differentially expressed genes of Australian *P. lobata* in other treatments outnumbered the up-regulated genes. Differentially expressed genes in *P. lobata* were mainly enriched in the GO terms glucose metabolism, hexose metabolism, cytoplasm, gene expression, molecular structure activity and ribosome. Differentially expressed genes in Jiangsu *P. lobata* were mainly enriched for the GO terms inorganic response, gene expression, molecular structure activity, ribosome and response to osmotic stress. Plant hormones induce multiple pathways of gene transcription to synergistically affect plant seed germination, growth and leaf and root development and also play a regulatory role in plant stress (Blázquez et al.,

2020). SAR is an active defense mechanism in plants, and it is triggered by the accumulation of the signal molecule salicylic acid (Kumar, 2014). PR1 is a kind of protein produced by plants damaged by some pathogens or stimulated by some specific compounds, and it is closely related to systemic acquired resistance and allergic necrosis (Edreva, 2004). It accumulates downstream of salicylic acid and is a molecular marker of the SAR resistance mechanism (Wildermuth et al., 2001). In this experiment, the high expression of PR1 under low phosphorus stress may be one of the reasons for its low phosphorus tolerance.

The endogenous hormone ethylene is closely related to root hair development, abiotic stress and pathogen response. Ethylene can not only promote the growth of root hairs, but also promote the formation of ectopic root hairs, which is the first step in the initiation and growth of root hairs (Negi et al., 2008). CTR1 is one of the receptors of ethylene, which binds to ethylene and participates in the regulation of various growth and development processes (Wang et al., 2023).

TCH4 is a member of the XTH family, which is mainly expressed in young leaves, epidermal hairs, lateral root primordia, vascular tissues, flowers and hypocotyls (Xu et al., 1995). Cold stress, heat stress and brassinosteroid and indole-3-acetic acid stimulation can increase the protein expression level of TCH4 (Iliev et al., 2002). In this experiment, the expression of this gene was down-regulated in Australian *P. lobata* and up-regulated in Jiangsu *P. lobata* under low phosphorus stress.

The ethylene response factor ERF1 is a key component of ethylene signal transduction and plays a key role in ethylene-induced root growth inhibition by directly regulating the expression of anthranilic acid synthase α 1 (Mao et al., 2016). In this experiment, the ERF1 gene was highly expressed under low phosphorus stress, which may be owing to its regulation of ethylene expression to inhibit root growth, such that Jiangsu *P. lobata* showed low phosphorus sensitivity.

C2H2 is a zinc finger protein containing a plant-specific QALGGEI sequence and plays an important role in plant growth and development and stress resistance (Kam et al., 2008). The high expression of some genes in the C2H2 zinc finger protein family, the high seed germination rate of transgenic Arabidopsis and gene silencing cotton indicate that they are involved in the salt tolerance of upland *cotton*. (Rehman et al., 2021). In the present experiment, the expression of the C2H2 transcription factor in Australian *P. lobata* was greater than that in Jiangsu *P. lobata* under low phosphorus stress.

The bZIP mutation in rice under drought conditions inhibits auxin signal transduction to promote rice root development and increase the number of thick and long lateral roots (Hasegawa et al., 2021). In this experiment, the expression of bZIP transcription factors in Australian *P. lobata* was significantly higher under low phosphorus stress.

MYB-related (MYB-R) is a subfamily of the MYB transcription factor family that is involved in regulating plant primary and secondary metabolism, defense and stress responses (Chen et al., 2009). MYB transcription factors play a key and diverse role in regulating the development of tea leaves and stems, the biosynthesis of secondary metabolites, and the response to environmental stress (Li et al., 2022). MYB and MYB-related proteins were abundantly expressed in this experiment, but more of these genes were expressed in Australian *P. lobata*.

The bHLH transcription factor is an important transcription factor widely found throughout eukaryotes and plays important regulatory roles in plant responses to stress. The overexpression of MpbHLH transcription factor increased the resistance of transgenic bananas to Fusarium wilt by enhancing the active oxygen scavenging system of transgenic plants (Li et al., 2022). Genes in the bHLH family of transcription factors were highly differentially expressed in this experiment.

Thus, the transcription factors in the C2H2, bZIP, MYB and bHLH families and the corresponding functional genes PR1 and CTR1 were highly expressed under low phosphorus stress, which may explain the observed strong tolerance to low phosphorus in Australian *P. lobata*. The ERF transcription factors and their regulated genes that promote the expression of ethylene and inhibit the growth of plant roots were highly expressed in Jiangsu *P. lobata*, perhaps explaining its low phosphorus sensitivity.

5 Conclusion

By analyzing the root morphological changes and physiological response of the three provenances of *P. lobata* after low phosphorus stress. It is concluded that *P. lobata* has a strong adaptability under low phosphorus stress. Low phosphorus stress can promote the root growth and development of Australian *P. lobata*, which belongs to low phosphorus tolerant type. The root growth and development of Jiangsu *P. lobata* was inhibited under low phosphorus stress, which was a low phosphorus sensitive type. Transcriptome sequencing analysis of the roots of Australia *P. lobata* and Jiangsu *P. lobata* showed that low-phosphorus-tolerant *P. lobata* and low-phosphorus-sensitive *P. lobata* had slightly different ways to respond to low-phosphorus stress, and low-phosphorus-tolerant Australia *P. lobata* responded more actively under low-phosphorus stress. Phosphorus deficiency can promote the expression of genes in plant hormone signal transduction pathway and transcription factors that regulate gene expression, thus affecting root morphological construction and plant stress response. Low phosphorus stress promotes the expression of transcription factors and genes related to plant stress response in Australia *P. lobata*, which is the key to its low phosphorus tolerance. Through genome sequencing, the differences in gene expression between low-phosphorus-tolerant Australia *P. lobata* and low-phosphorus-sensitive Jiangsu *P. lobata* under low phosphorus stress were preliminarily understood. The next step will be to study the regulation and mechanism of genes related to improving plant stress response in *P. lobata* response to low phosphorus.

Declarations

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Authors' Contributions

Shuwei Zhang, Yi Xi, Lili Zhao and Chao Chen conceived and designed the research project. Shuwei Zhang, Li Zhang, Yuyu Liu, and Yanhua Tang conducted the experiments. Shuwei Zhang Yanhua Tang and Yi Xi analyzed the data. Yanhua Tang, Shuwei Zhang and Yi Xi wrote the manuscript. All authors read and approved the manuscript.

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Not applicable.

Declaration of competing interest

None of the authors have conflicts of interest to declare.

Availability of data and materials

The raw datasets generated during the sequencing of current study are available in SRA under the bioproject PRJNA1063191

https://www.ncbi.nlm.nih.gov/sra/PRJNA1063191.

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Figures

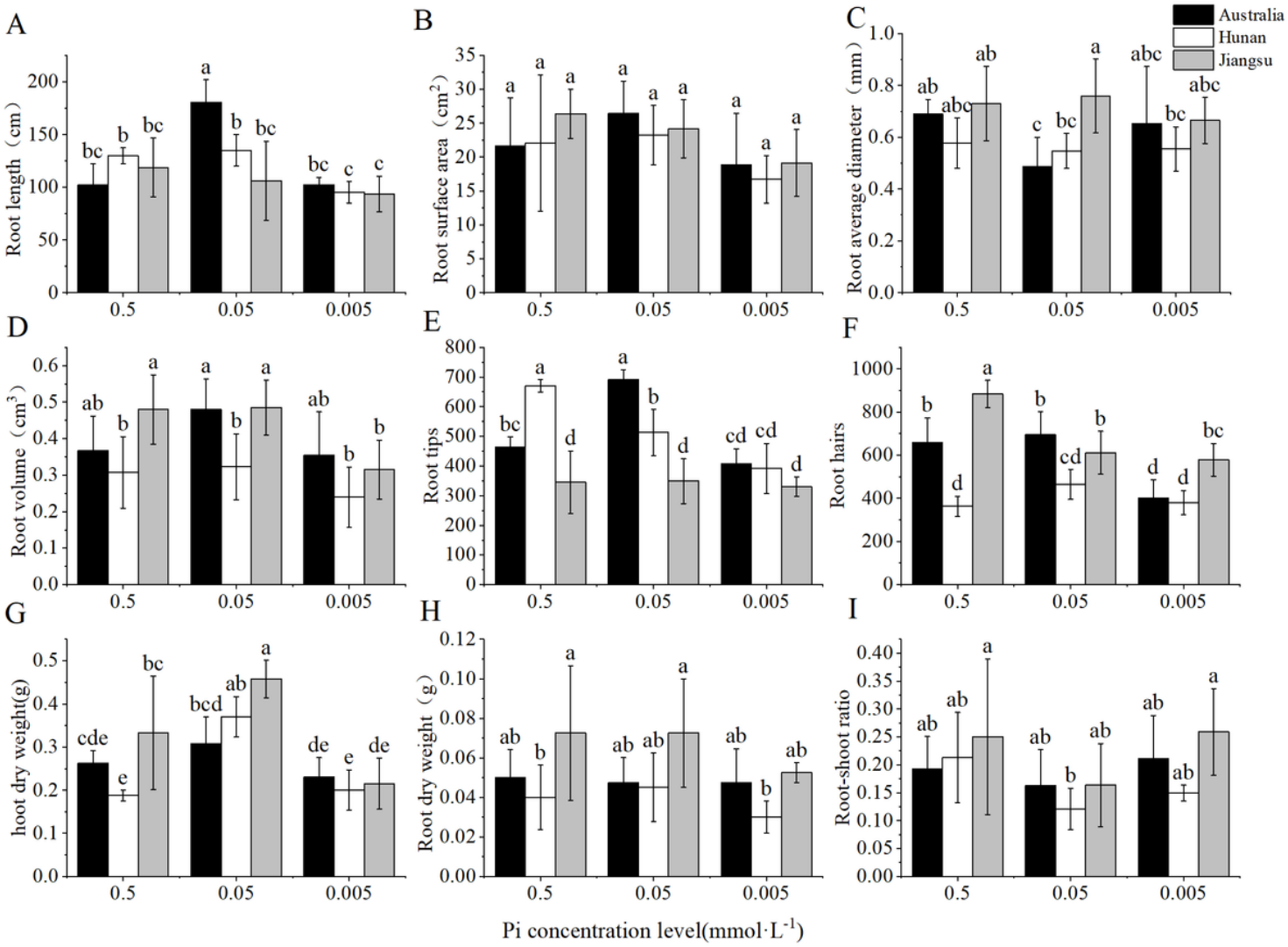

Figure 1

Changes of root morphological of *Pueraria lobata*seedlings under different low phosphorus stresses

Note:Different letters on the columns represented significant difference (*p<0.05*).

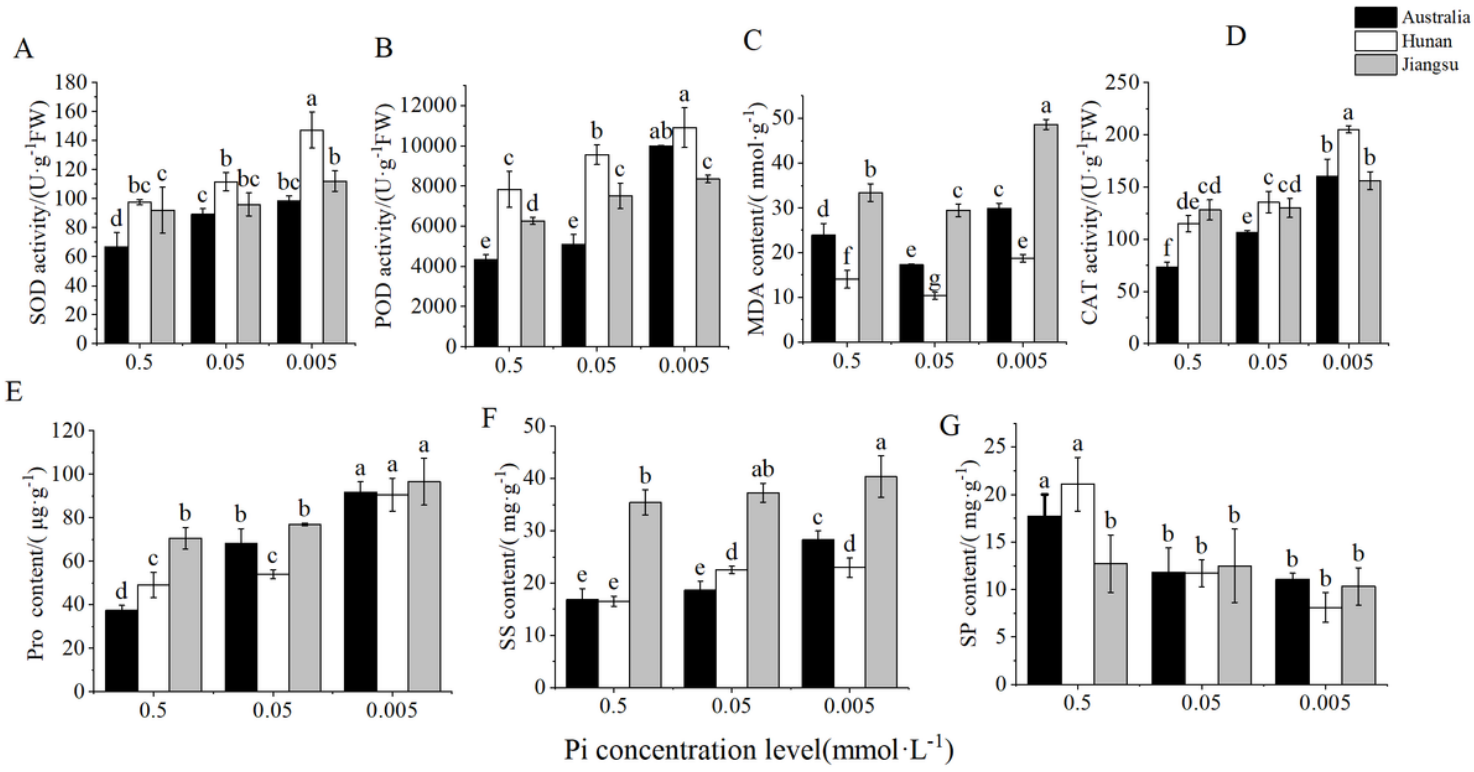

Figure 2

Changes of physiological indexes of *Pueraria lobata* seedlings under different low phosphorus stresses

Note:Different letters on the columns represented significant difference (*p<0.05*).

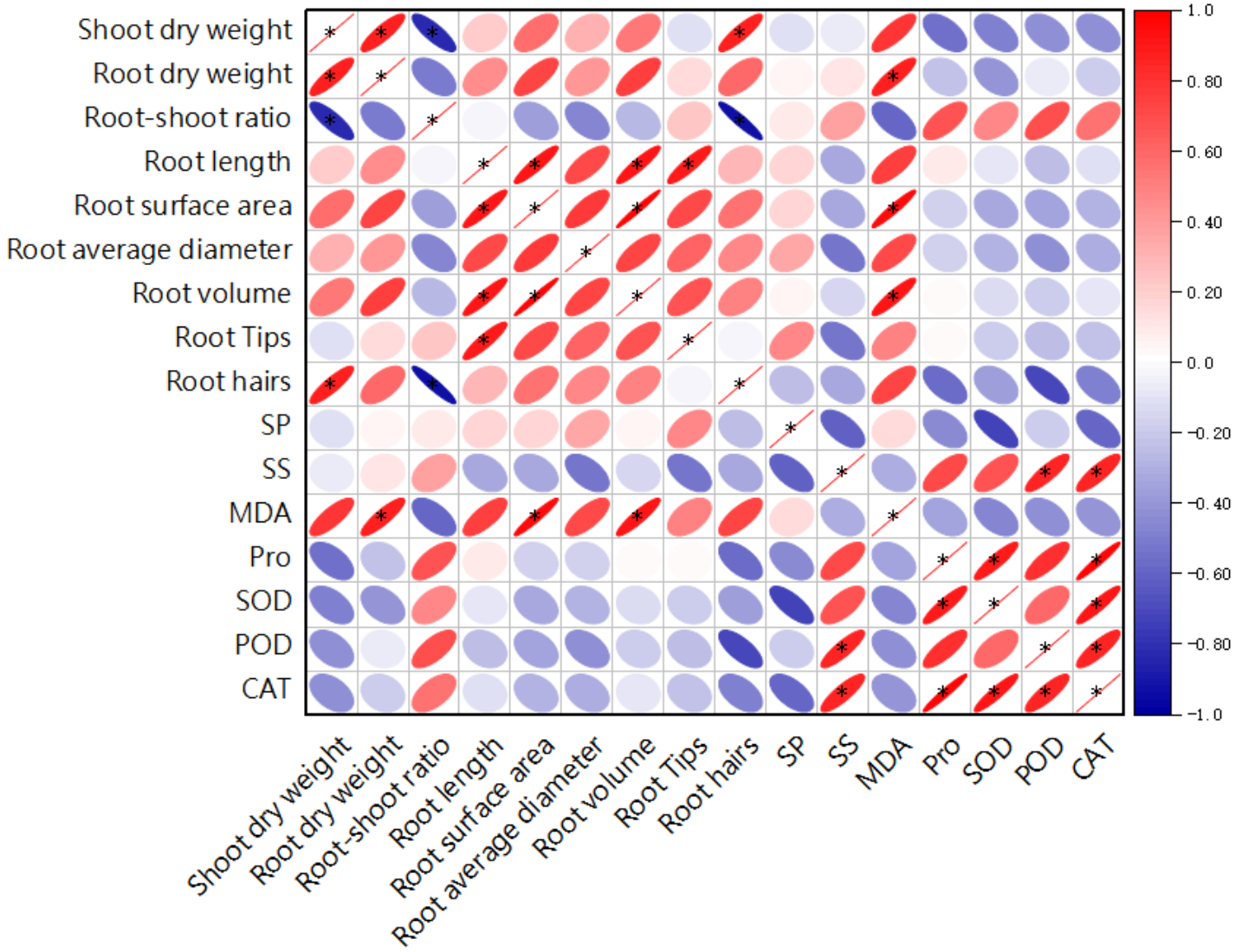

Figure 3

Correlation anslysis on low phosphorus tolerance coefficient of trait indexes in *Pueraria lobata*seedings

Notes: *: Significant difference (*p<0.05*).

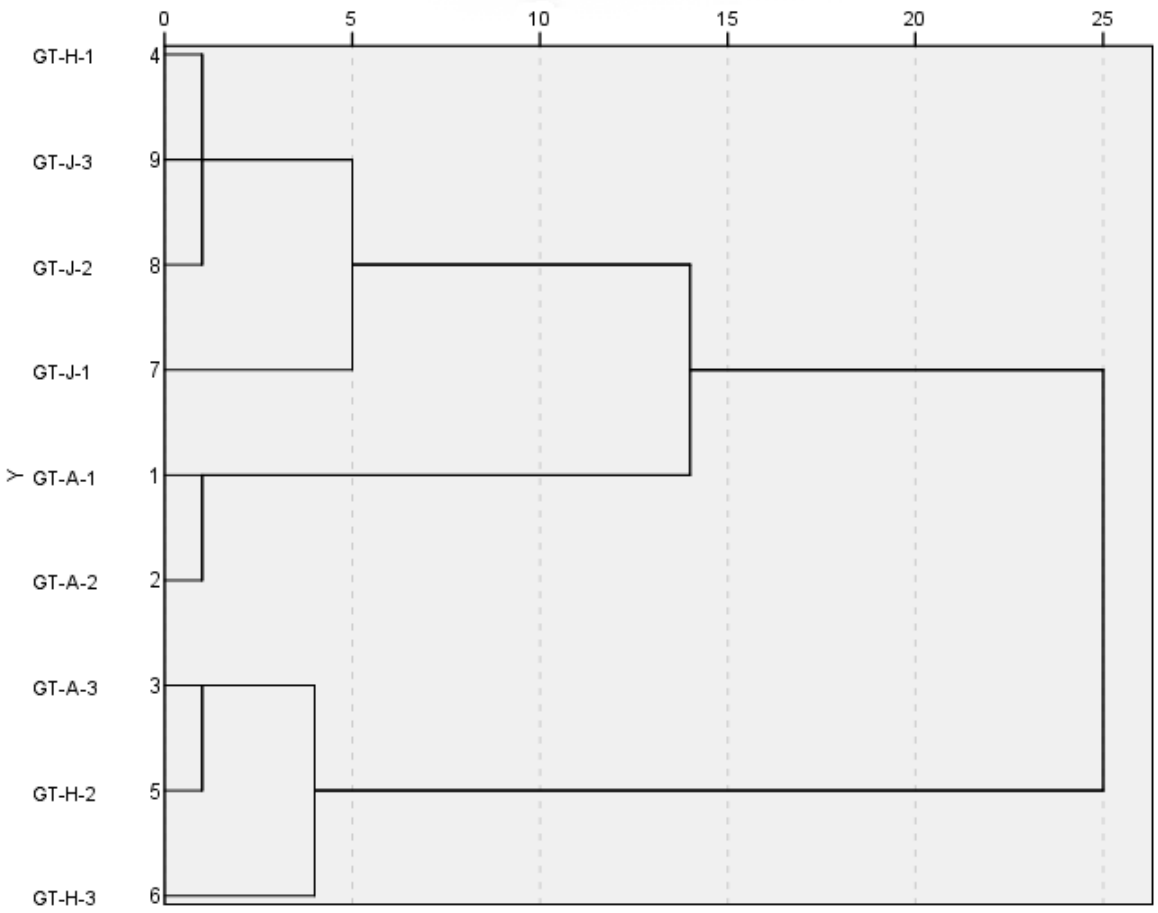

Figure 4

Clustering analysis on *Pueraria lobata* seedlings under different low phosphorus stresses

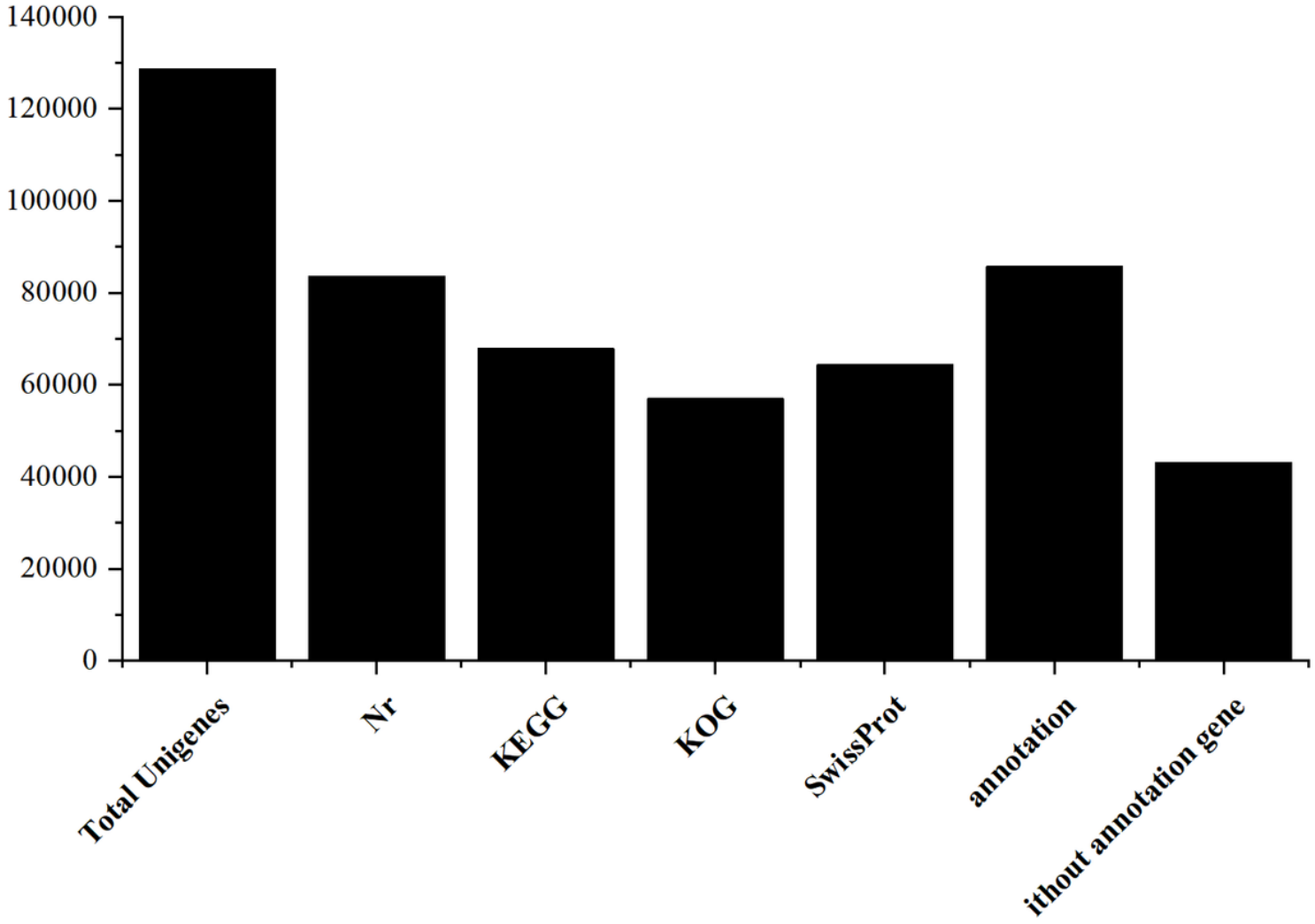

Figure 5

Annotation statistical charts of the four major databases

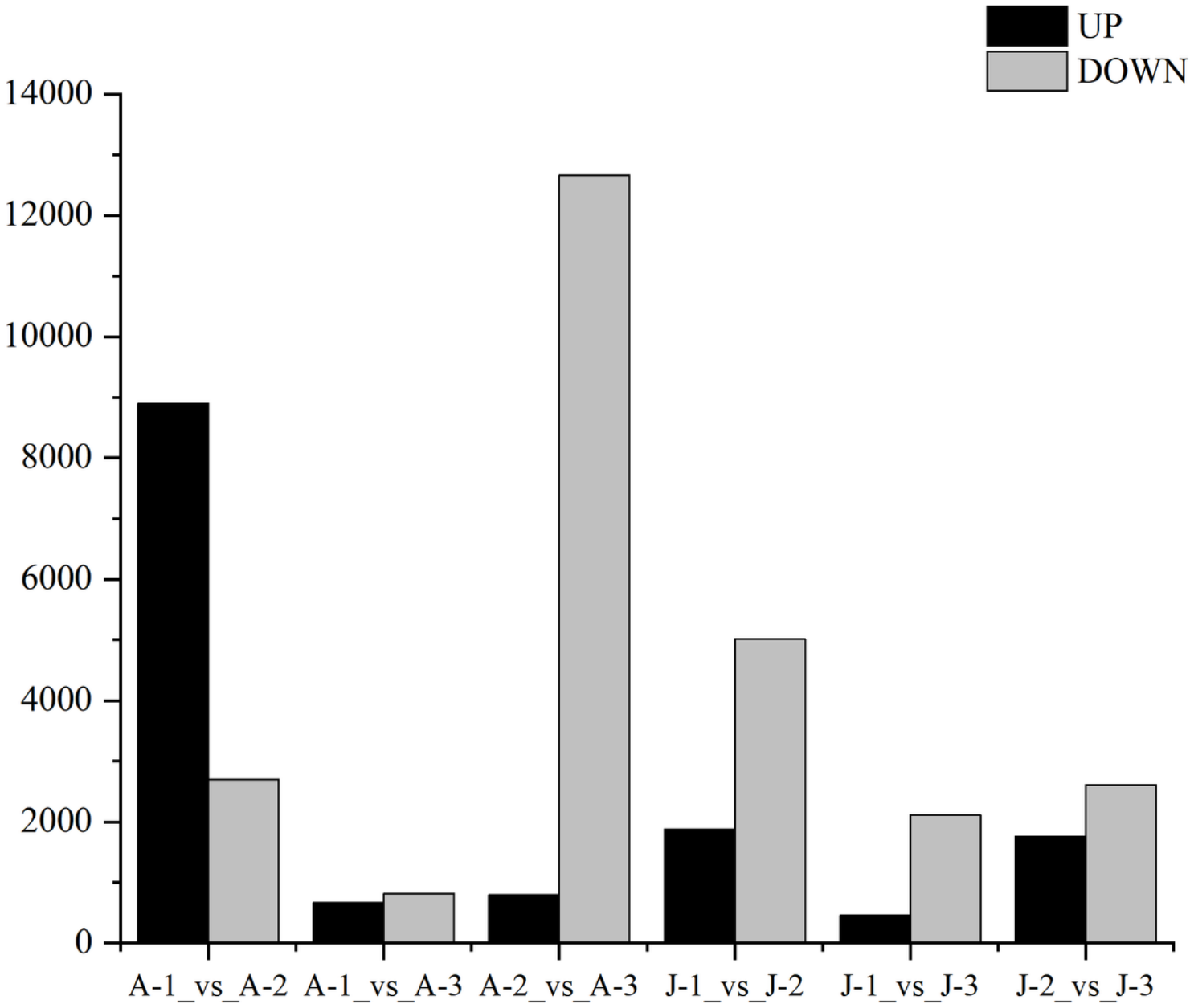

Figure 6

Bar chart of differentially expressed genes co-existing in different treatment groups

Note: A: Australian *Pueraria lobate*; J: Jiangsu *Pueraria lobate*; 1: Normal phosphorus treatment level; 2: Low phosphorus treatment level; 3: Extremely phosphorus treatment level.

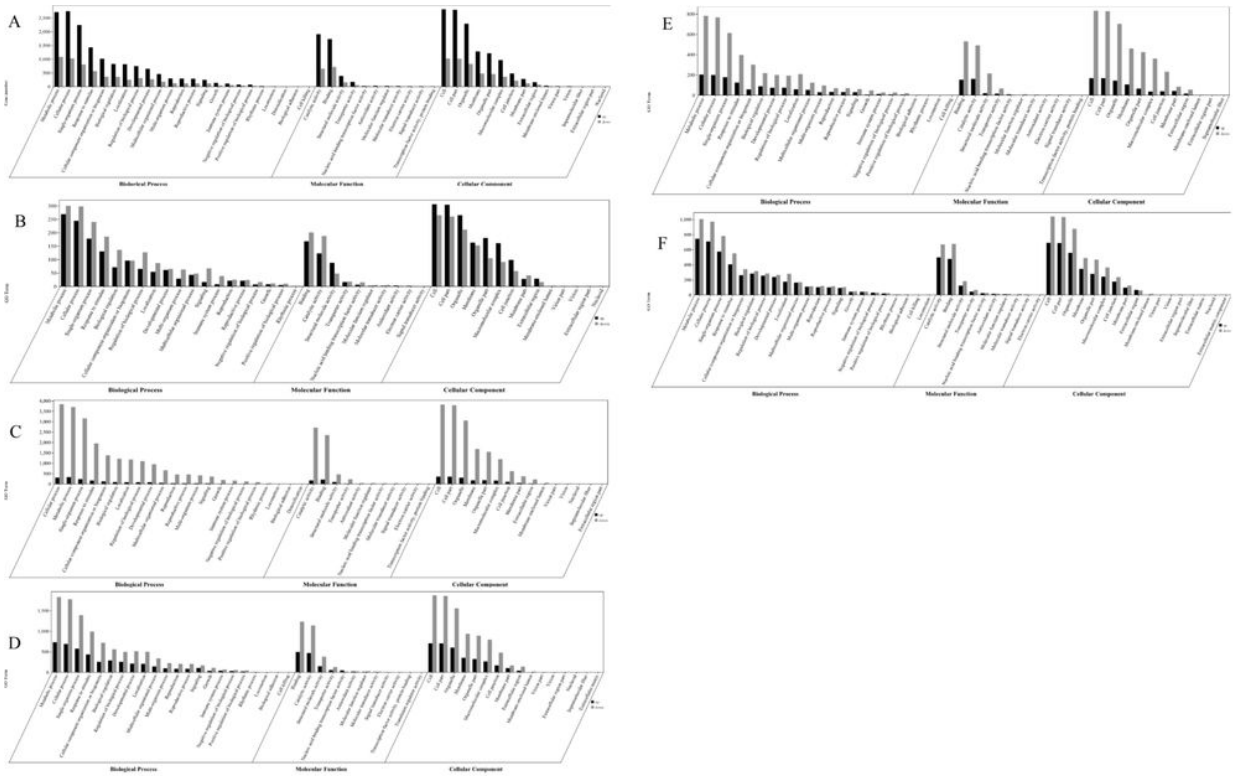

Figure 7

GO enrichment analysis of differential genes

Note:A A-1_vs_A-2 B A-1_vs_A-3 C A-2_vs_A-3 D J-1_vs_J-2 E J-1_vs_J-3 F J-2_vs_J-3

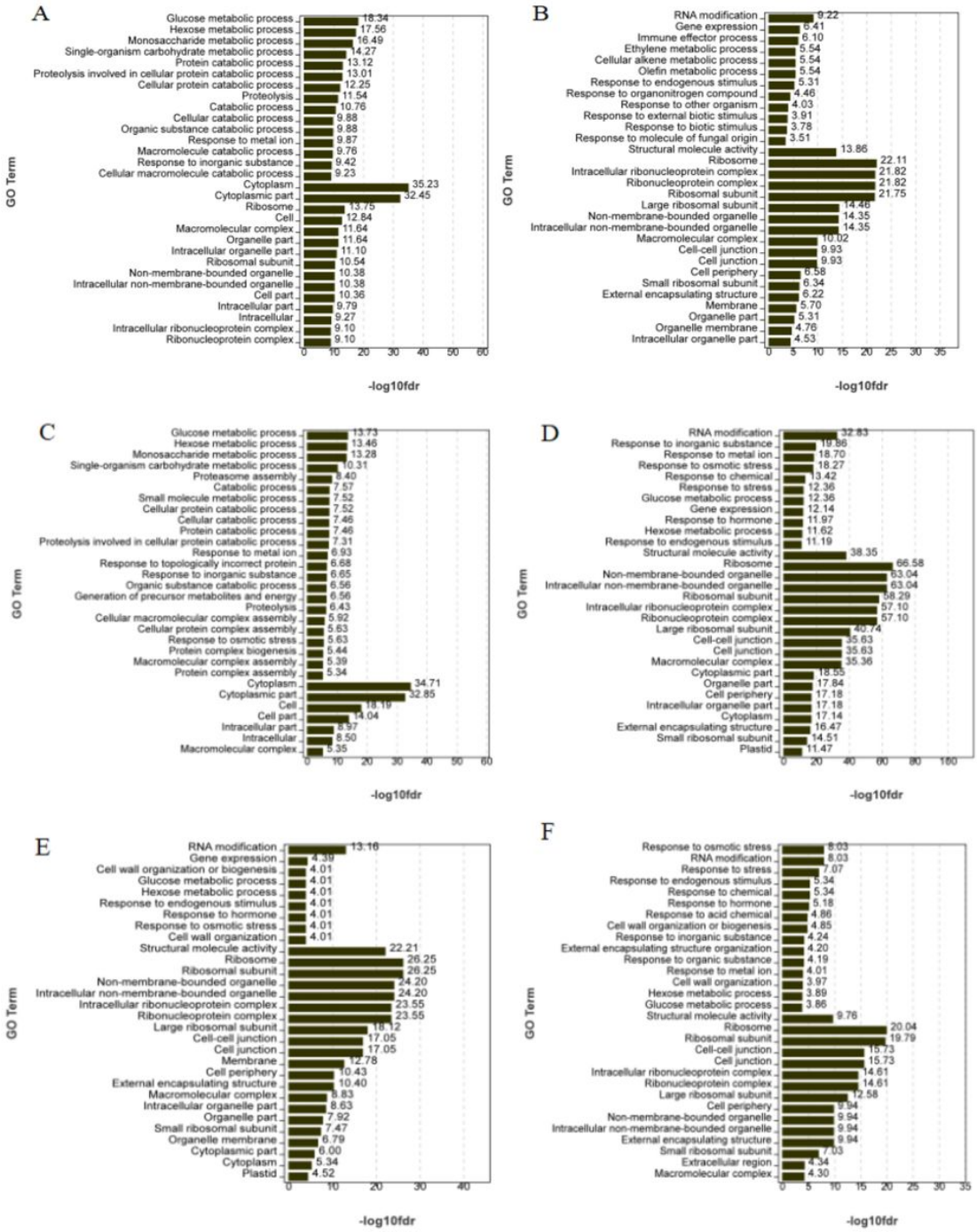

Figure 8

Bar chart of GO enrichment analysis of differentially expressed genes under low phosphorus stress

Note:A A-1_vs_A-2 B A-1_vs_A-3 C A-2_vs_A-3 D J-1_vs_J-2 E J-1_vs_J-3 F J-2_vs_J-3

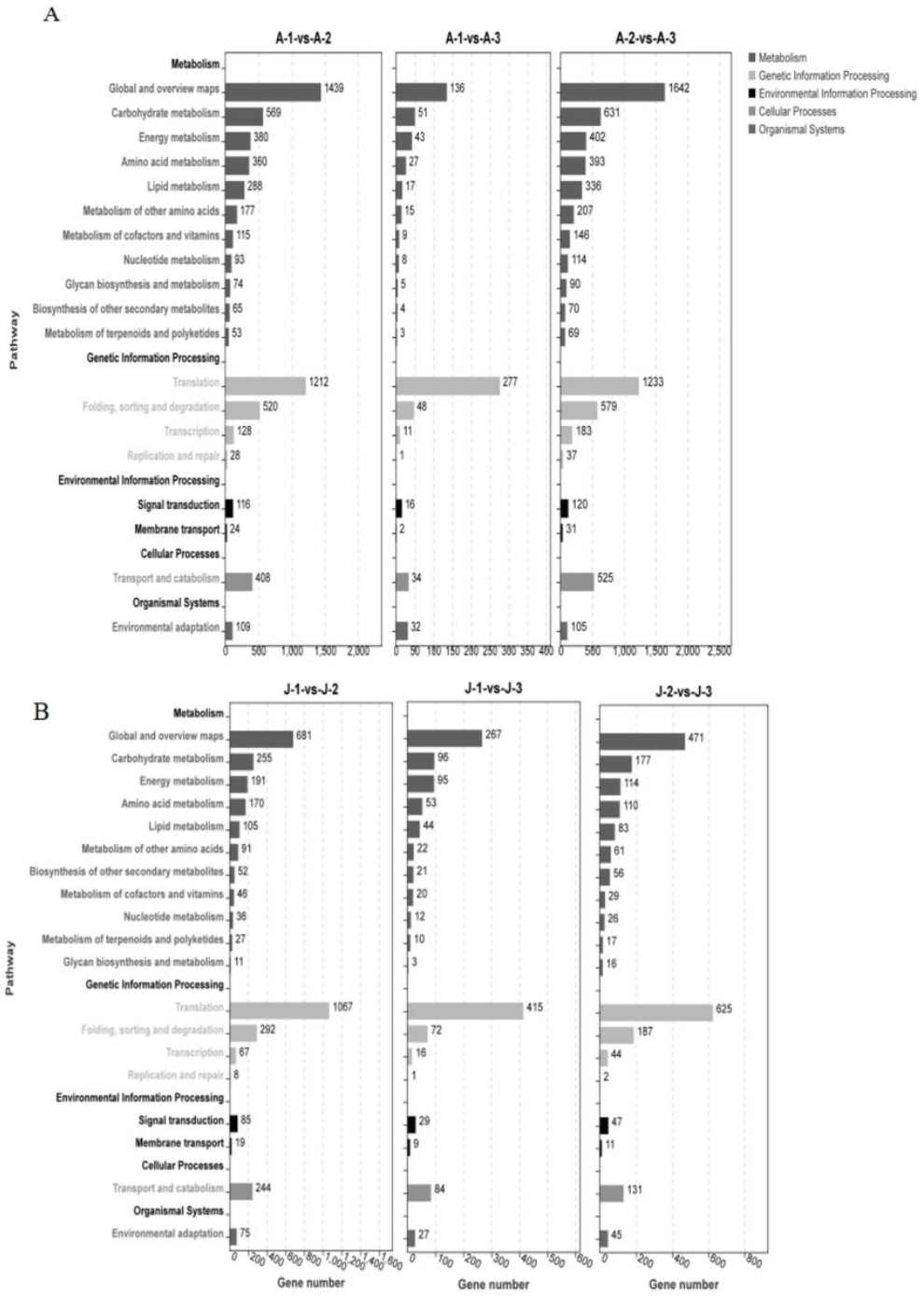

Figure 9

KEGG enrichment analysis of DEGS in *Pueraria lobate* roots under low phosphorus stress

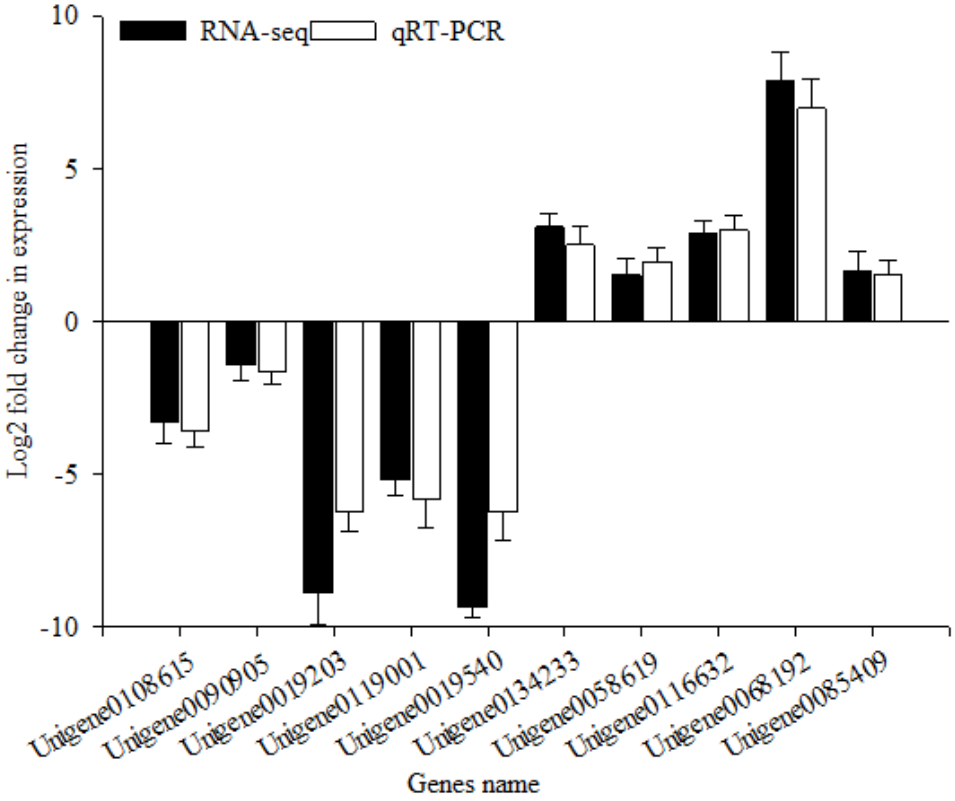

Figure 10

Relative expression levels of 10 randomly selected genes validated by qRT-PCR