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TRANSCRIPTOME PROFILING OF *CANNABIS SATIVA* L. RESPONSE TO LOW PHOSPHORUS STRESS

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Abstract

The regulation of differential gene expression in *Cannabis sativa* L. was investigated under low-phosphorus stress treatment for 75 days. The results of transcriptional sequencing showed that 1,242 differentially expressed genes (697 upregulated and 545 downregulated) were identified 75 days after low-P treatment. The results of GO functional enrichment analysis showed that the differentially expressed genes were mainly concentrated in biological processes, cellular components, and molecular functions. Results of the enrichment analysis of the KEGG pathway, which comprised three functional classes of function, showed that these differentially expressed genes were involved in cellular processes, environmental information processing, genetic information processing, metabolism, and organic systems. A total of 1,356 expression genes from 60 transcription factor families were annotated, most of which belonged to MYB, AP2-EREBP, BHLH, NAC, MADS, ABI3VP1, C3H, FAR1, WRKY, and GRAS. A total of 143 genes directly related to P and 25 genes related to auxin were identified. These differentially expressed genes revealed the transcriptional regulation pathway involved in low-phosphorus tolerance in *C. sativa* and provide a basis for the cloning and functional verification of genes related to low-phosphorus tolerance in this species.

Key words: *Cannabis sativa* L.; Transcriptome; Differentially expressed genes; Low phosphorus stress; Response; Screening.

Introduction

Phosphorus (P) is easily fixed by organic matter and minerals, and crop plants cannot directly absorb fixed P. The phenomenon of P deficiency in cropland is widespread, and P deficiency has become the main factor restricting crop growth (Carstensen *et al*., 2018). Plants absorb P primarily in the form of phosphoric acid, which contains $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}, and plants most easily absorb $H_2PO_4^-$ (Vance, 2001). Fertilisation is the main artificial means of alleviating soil P deficiency. However, large-scale application of P fertiliser increases production costs, accelerates the depletion of P resources, and causes environmental pollution. The study of low-P stress responses in crop plants and the regulation mechanisms of gene expression is an important scientific and technological issue in the field of crop biology, which has important ecological and social significance. *Cannabis sativa* L. is a very old crop with a long history of cultivation in China and has important applications in agriculture, industry, and medicine (Wang *et al*., 2019; Zhang *et al*., 2018). P is an essential element for the growth and development of *C. sativa*. Scientific research on P absorption and utilisation by *C. sativa* and improvement of the species in terms of adaptability to low P stress are of great significance for realising "double reduction" of agricultural fertiliser in China, protecting the environment, avoiding environmental pollution caused by excessive application of chemical fertiliser, realising the goals of water quality ultimately, achieving the broader goals of green and sustainable development.

P is a vital nutrient for plant growth (Li *et al*., 2017; Patrick *et al*., 2021). P deficiency limits the growth, development and productivity of rice (Prathap *et al*., 2023). P is a component of many important organic compounds in crops and participates in various metabolic processes, namely photosynthesis, respiration, membrane lipid synthesis, and nucleic acid synthesis (Theodorou & Plaxton, 1993). When crop plants are subjected to P stress, their growth rates and root-to-shoot ratios change significantly (Cakmak *et al*., 1994; Eircsson *et al*., 1996). At present, the ways in which crops adapt to low P fall into two categories: P uptake and transport in low P environments. Low P stress is first signalled through the root tips (Doerner, 2008). The regulatory pathways of root morphological remodelling under low-P conditions are diverse, and many biological compounds have been found to be involved in this function (Abel *et al*., 2002). In particular, some Transcription factors (TFs) were identified that have a big role in low P conditions, such as MYB TF PHR1 (Rubio *et al*., 2001), WRKY6 (Chen *et al*., 2009) and WRKY75 (Devaiah *et al*., 2007) of *Arabidopsis thaliana* L., as well as *Oryza sativa* L. bHLH TF OsPTF1 (Yi *et al*., 2005) and *Nicotiana tabacum* L. bZIP TF Phi-2 (Toshio & Toshiyuki, 2002). There are approximately 1,800 TFs in *A. thaliana*, including 72 WRKY TFs, 133 MYB TFs, and more than 600 zinc-finger protein genes. Under low-P conditions, approximately 30 TF genes in *A. thaliana* were up-regulated or down-regulated (Hammond *et al*., 2003; Misson *et al*., 2005). Many P transporters in plants are divided into the PHT1, PHT2, and PHT3 families. P transporters are categorized based on the different kinetic characteristics of binding P; that is, high- and low-affinity P transporter proteins, which are active in environments of low P and sufficient P, respectively (Chen *et al*., 2008). Members of the PHT1 family are located on the plasma membrane of cells and play roles in P absorption and distribution. The number of PHT1 family members differs between plant species (Karandashov & Bucher, 2005; Shin *et al*., 2004). In low-P environments, At4 (Shin *et al*., 2006), AtSPS (Duan *et al*., 2008) and others participate in P transport and distribution.

A published sequence map of the *C. sativa* genome has greatly advanced the study of this species (Laverty *et al*., 2019). At present, the molecular mechanisms and genes involved in the regulation of low-P stress in *C. sativa* remain unclear. The expression of specific responsive genes determines the morphological and physiological changes in *C. sativa* under P stress; thus, the study of differential gene expression is helpful in revealing the internal molecular response mechanism of *C. sativa* under P stress, with the goal of advancing molecular-assisted breeding. In this study, the differentially expressed genes (DEGs) of the *C. sativa* variety Longdama No.5 were identified after low P treatment, and the molecular and signalling pathways related to P stress were uncovered, laying a basis for the cloning and functional verification of genes related to low P tolerance in *C. sativa*.

Material and Methods

Plant materials: Seeds of *C. sativa* variety Longdama No.5 were provided by the Institute of Industrial Crops, Heilongjiang Academy of Agricultural Sciences.

Growth conditions and stress treatment: The culture substrate was sand. Soak sand in tap water, washed off the soil to clear bottom, after which the sand was soaked in 0.5% HCl for 24 h and then rinsed with neutral tap water. The sand was then packed into a plastic bucket with the following dimensions: bottom diameter 22.0 cm, top diameter 28.5 cm, height 27.0 cm. Each basin contained 13.0 kg of quartz sand, 4.0-5.0 cm above the basin, for the watering of nutrient solution and water.

Longdama No.5 seeds were disinfected and sowed in the prepared sand (4 seeds sown in 637.6 cm^2 area in each pot). Seeds were sown on May 1, 2020 and distilled water was added to prior to the emergence of the seedlings. Three days after seeding, 1 L of water-nutrient solution was added once to each pot twice each day (from 8:00 a.m. to 9:00 a.m. in the morning and 5:00–6:00 in the afternoon). Each pot was washed with 5 L of distilled water every 5 d to remove the accumulated salt in the sand. Over the course of 75 d in the greenhouse, the indoor temperature was between 26°C and 20°C, the relative humidity was maintained over 60%, and the light was 14 h/d. On May 6, the seedlings were treated with nutrient solutions of different concentrations of P. Hoagland medium consisted of 2 mmol/L KCl, 1 mmol/L KH_2PO_4, 5 mmol/L KNO_3, 4 mmol/L $Ca(NO_3)_2$, 1 mmol/L NH_4NO_3, 2 mmol/L $MgSO_4$, 0.1 mmol/L $FeSO_4$, 0.1 mmol/L EDTA-2NA, 0.13 mmol/L $MnSO_4$, 0.1 mmol/L H_3BO_3, 0.03 mmol/L $ZnSO_4$, 1 μM Na_2MoO_4 and 0.1 μM $CuSO_4$. Two P levels of KH_2PO_4 (0.1 mmol/L and 1 mmol/L) and KCl (2.9 mmol/L and 2 mmol/L) in Hoagland medium, named LP and CK, were used to balance the concentration of K. Each treatment was repeated three times.

Whole seedlings were sampled at the flowering stage and stored at -80°C after freezing with liquid nitrogen, to be utilized for RNA extraction for and sequencing analysis.

Transcriptome sequencing: Whole plant RNA extracted from CK and LP were named CK-1, CK-2, CK-3, LP-1, LP-2 and LP-3, respectively. Quality and quantity analyses, database construction, and Illumina sequencing of the total RNA were performed using BGI (Shenzhen, China).

Results and Analysis

RNA-seq sequencing results: An Illumina sequencing library was constructed by comparing the total RNA of samples treated with low-P for 75 d with the corresponding time. Each library averaged 43.82 M million raw sequencing readings, of which more than 97.92% were filtered. When the filtered readings were compared with the genome sequence of *C. sativa*, at least 82.63% of the filtered readings corresponded to the reference genome sequence in the database (Table 1).

In this study, the threshold of the difference in multiple DEGs was set as ≥2.0. A Q-value threshold of <0.05 and an absolute value of Log_2 ratio ≥1 was used to screen DEGs with low P content. In total, 1,242 low-P response genes were identified after 75 d of low-P treatment.

Gene ontology (GO) functional enrichment: GO was divided into biological processes, cell components, and molecular functions. Functional classification was performed based on the results of differential gene tests. Each major category had a hierarchy of subcategories. The following (Fig. 1) shows the GO annotation classification results of the DEGs.

KEGG pathway enrichment: Based on the official KEGG classification and annotation results, DEGs were classified into biological pathways. Generally, the function of Qvalue≤0.05 was considered as significant enrichment.

Genes involved in the KEGG metabolic pathways were divided into five branches: cellular processes, environmental information processing, genetic information processing, metabolism, and organic systems. Each branch was then classified and counted. The KEGG pathway annotation classification results for the DEGs are shown in (Fig. 2).

Differential expression transcription factor analysis: Over a long period of evolution, *C. sativa* has developed a complex and effective mechanism of adaptation and resistance to various biological and abiotic stresses. Transcriptional regulation of gene expression plays a significant role in the stress response of cannabis plants. After TF annotation of DEGs for 75 d under low P stress, 1,356 DEGs from 60 TFs families were annotated (Table 2), most of which belonged to MYB, AP2-EREBP, bHLH, NAC, MADS, ABI3VP1, C3H, FAR1, WRKY, and GRAS.

Functional analysis of DEGs: In this study, 143 genes directly related to P were screened out from the DEGs in the low-P transcriptomic data of *C. sativa* (Table 3), among which 55 genes were up-regulated (2.00-9.24 fold up-regulated) and 88 genes were down-regulated (2.01-10.26 fold down-regulated). These genes may play a significant role in the P stress response.

In the difference comparison groups, 25 genes were annotated as auxin-related genes (Table 4). These genes regulate the growth of C. *sativa* plants under low P stress; thus, low P levels may induce the differential expression of these genes, regulate hormone synthesis, and affect the growth and development of *C. sativa*.

Table 1. Major characteristic of six libraries.

Sample	Total raw reads (M)	Total clean reads(M)	Clean reads ratio (%)	Ratio of mapped reads to total clean reads (%)	Uniquely mapping (%)
CK-1	43.82	42.99	98.11	82.68	63.49
CK-2	43.82	43.04	98.21	82.63	63.42
CK-3	43.82	42.91	97.92	82.77	63.02
LP-1	43.82	42.83	97.73	83.41	65.16
LP-2	43.82	43.08	98.30	83.04	64.83
LP-3	43.82	42.94	97.99	83.94	65.42

Table 2. Main TFs under low P stress.

TFs	Number	TFs	Number	TFs	Number
MYB	178	HSF	19	CPP	6
AP2-EREBP	114	SBP	18	Sigma70-like	6
bHLH	107	C2C2-GATA	18	CSD	5
NAC	84	bZIP	17	SRS	5
MADS	69	OFP	16	EIL	4
ABI3VP1	66	Alfin-like	13	GeBP	4
C3H	59	zf-HD	12	TIG	4
FAR1	52	Tify	11	VOZ	4
WRKY	49	LIM	11	BBR/BPC	3
GRAS	44	PLATZ	11	HRT	3
C2H2	43	C2C2-CO-like	10	NOZZLE	3
LOB	39	C2C2-YABBY	10	TAZ	3
Trihelix	29	GRF	9	PBF-2-like	2
G2-like	28	HB	8	S1Fa-like	2
C2C2-Dof	27	RWP-RK	8	SAP	2
mTERF	24	ARR-B	7	ULT	2
FHA	23	BES1	7	VARL	2
ARF	21	BSD	7	CAMTA	1
TCP	19	E2F-DP	7	DBP	1

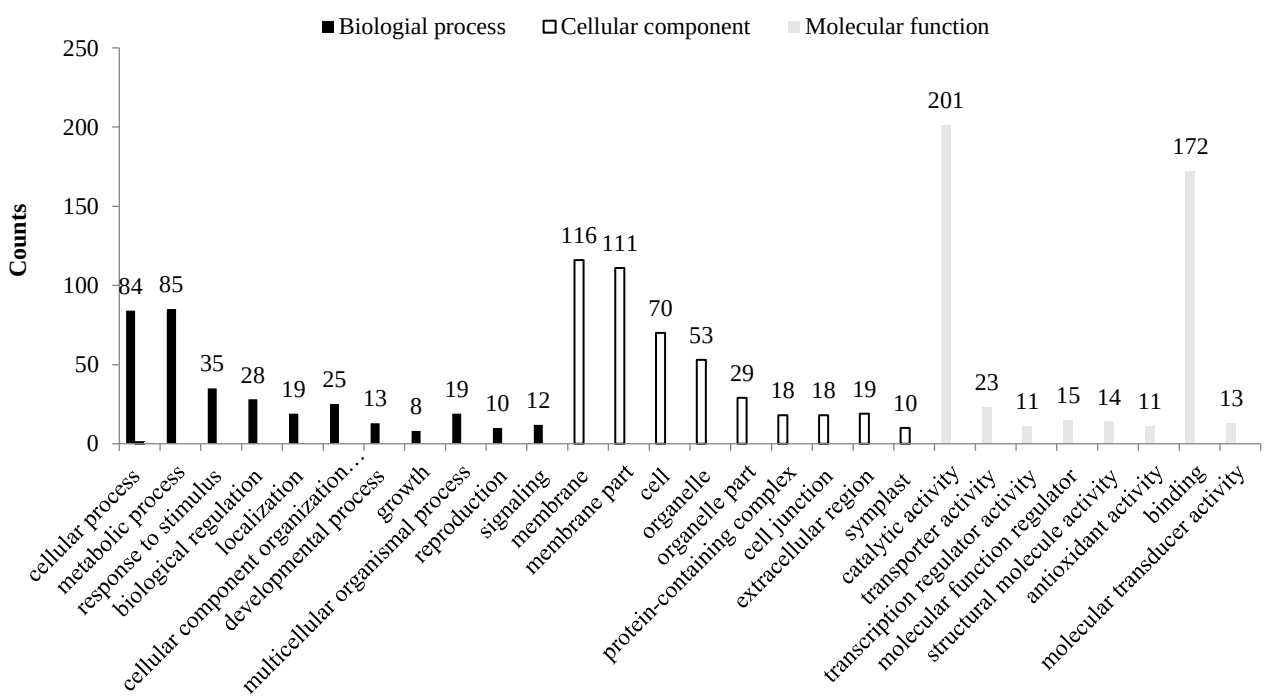

Fig. 1. GO enrichment of the DEGs under low P treatment after 75 days in *C. sativa* L.

Table 3. Screening of 143 genes directly related to low P treatment in *C. sativa* L.

Gene ID	LP/CK log2 values log2 FC	Annotation
Cs115704090	9.24	Transferase activity, transferring phosphorus-containing groups
Cs115706090	9.01	
Cs115716746	9.01	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115706092	9.00	Phosphorus metabolic process
Cs115698909	8.91	
Cs115713886	8.38	Transferase activity, transferring phosphorus-containing groups
Cs115704891	8.03	
Cs115694875	7.94	
Cs115694876	7.00	Phosphorus metabolic process
Cs115704458	6.83	transferase activity, transferring phosphorus-containing groups
Cs115704502	6.31	
Cs115698747	5.84	
Cs115696874	4.84	
Cs115717222	4.53	
Cs115724568	4.31	Phosphorus metabolic process
Cs115714881	4.17	Transferase activity, transferring phosphorus-containing groups
Cs115717424	3.93	Transferase activity, transferring phosphorus-containing groups
Cs115700924	3.91	
Cs115718016	3.73	
Cs115725707	3.57	
Cs115715243	3.56	
Cs115698016	3.43	Phosphorus metabolic process
Cs115717072	3.38	Transferase activity, transferring phosphorus-containing groups
Cs115702895	3.38	
Cs115698306	3.03	
Cs115702351	2.98	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115709783	2.90	Transferase activity, transferring phosphorus-containing groups
Cs115723758	2.89	
Cs115712424	2.86	Transferase complex, transferring phosphorus-containing groups
Cs115707636	2.67	Transferase activity, transferring phosphorus-containing groups
Cs115709650	2.55	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115700857	2.53	
Cs115712166	2.49	Transferase activity, transferring phosphorus-containing groups
Cs115724123	2.48	
Cs115704842	2.45	Phosphorus metabolic process
Cs115704817	2.40	Transferase activity, transferring phosphorus-containing groups
Cs115718571	2.38	
Cs115705484	2.30	
Cs115720952	2.34	
Cs115698864	2.22	
Cs115699542	2.22	
Cs115705501	2.19	
Cs115697461	2.19	
Cs115710531	2.18	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115706576	2.18	Transferase activity, transferring phosphorus-containing groups
Cs115704759	2.13	
Cs115695532	2.13	

Table 3. (Cont'd.).

Gene ID	LP/CK log2 values log2 FC	Annotation
Cs115724778	2.10	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115697891	2.09	Transferase activity, transferring phosphorus-containing groups
Cs115716260	2.06	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115705023	2.06	Transferase activity, transferring phosphorus-containing groups
Cs115710492	2.04	
Cs115723355	2.03	
Cs115714544	2.02	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115709286	2.00	Transferase activity, transferring phosphorus-containing groups
Cs115708323	-10.26	Phosphorus metabolic process
Cs115723863	-8.53	Transferase activity, transferring phosphorus-containing groups
Cs115709767	-8.49	Transferase complex, transferring phosphorus-containing groups
Cs115714715	-7.53	Transferase activity, transferring phosphorus-containing groups
Cs115716820	-7.23	
Cs115702681	-6.85	
Cs115715604	-6.37	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115714782	-6.00	Transferase activity, transferring phosphorus-containing groups Phosphorus metabolic process
Cs115705847	-5.83	
Cs115713883	-5.77	
Cs115696485	-5.77	
Cs115711449	-5.65	Transferase activity, transferring phosphorus-containing groups
Cs115713880	-5.64	
Cs115723435	-5.55	
Cs115717484	-5.30	
Cs115713879	-5.17	
Cs115708008	-5.05	
Cs115697923	-4.78	
Cs115700275	-4.75	
Cs115695347	-4.69	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115716855	-4.66	Transferase activity, transferring phosphorus-containing groups
Cs115723017	-4.20	
Cs115719690	-4.15	
Cs115717936	-3.95	
Cs115721224	-3.86	
Cs115705352	-3.80	
Cs115703641	-3.72	
Cs115695328	-3.63	
Cs115702369	-3.62	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115695531	-3.59	Transferase activity, transferring phosphorus-containing groups
Cs115719899	-3.52	
Cs115695801	-3.52	
Cs115723844	-3.51	
Cs115720554	-3.42	
Cs115697714	-3.31	
Cs115699457	-3.28	
Cs115701084	-3.14	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115719710	-3.14	

Table 3. (Cont'd.).

Gene ID	LP/CK log2 values log2 FC	Annotation
Cs115713888	-3.13	Transferase activity, transferring phosphorus-containing groups
Cs115722745	-3.12	
Cs115716856	-3.10	
Cs115717483	-3.08	
Cs115700858	-3.07	
Cs115704873	-2.99	
Cs115694758	-2.97	
Cs115716853	-2.94	
Cs115708143	-2.94	
Cs115707679	-2.91	
Cs115705341	-2.90	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115702574	-2.90	
Cs115694938	-2.88	Transferase activity, transferring phosphorus-containing groups
Cs115702823	-2.85	
Cs115707631	-2.85	
Cs115696624	-2.81	
Cs115724184	-2.79	Phosphorus metabolic process
Cs115720422	-2.76	Transferase activity, transferring phosphorus-containing groups
Cs115724602	-2.74	
Cs115710421	-2.70	
Cs115725660	-2.68	
Cs115711057	-2.67	
Cs115709169	-2.62	
Cs115724117	-2.62	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115720879	-2.58	Transferase activity, transferring phosphorus-containing groups
Cs115713528	-2.55	
Cs115721787	-2.53	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115696830	-2.47	Transferase activity, transferring phosphorus-containing groups
Cs115697794	-2.44	
Cs115713994	-2.39	
Cs115711049	-2.39	
Cs115714749	-2.37	
Cs115696785	-2.36	
Cs115707346	-2.36	
Cs115704132	-2.34	
Cs115706267	-2.30	
Cs115696906	-2.26	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115702829	-2.25	Transferase activity, transferring phosphorus-containing groups
Cs115707474	-2.25	
Cs115717997	-2.22	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115712371	-2.18	Transferase activity, transferring phosphorus-containing groups
Cs115707472	-2.17	
Cs115702328	-2.15	
Cs115722026	-2.14	
Cs115708176	-2.14	Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides
Cs115707098	-2.13	Transferase activity, transferring phosphorus-containing groups
Cs115718803	-2.11	
Cs115702983	-2.07	
Cs115719493	-2.03	
Cs115720841	-2.01	

Table 4. Screening of 25 auxin related genes under low P treatment in *C. sativa* L.

Gene ID	LP/CK log2 values log2 FC	Annotation	Gene ID	LP/CK log2 values log2 FC	Annotation
Cs115725651	4.86	Response to auxin	*Cs115721011*	2.78	Response to auxin
Cs115695781	4.74		*Cs115714004*	2.40	
Cs115721015	4.07		*Cs115721441*	2.32	
Cs115710200	3.95		*Cs115725165*	2.30	
Cs115700775	3.59		*Cs115725551*	2.23	
Cs115695450	3.56		*Cs115724786*	2.22	
Cs115720602	3.36		*Cs115721008*	2.20	
Cs115725166	3.29		*Cs115695779*	2.18	
Cs115721439	3.20		*Cs115721440*	2.12	
Cs115721009	3.19		*Cs115695783*	2.11	
Cs115708265	3.11		*Cs115704933*	-3.89	
Cs115721014	2.90		*Cs115695780*	-3.83	
Cs115709564	2.81	Cellular response to auxin stimulus			

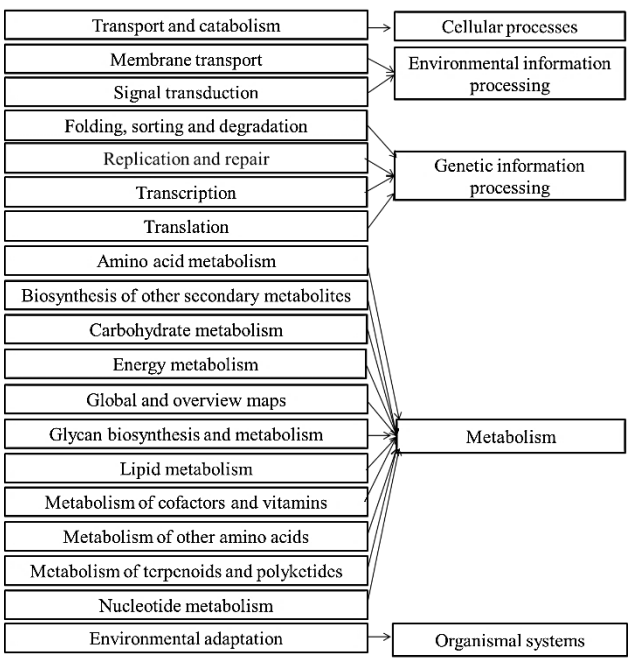

Fig. 2. KEGG pathway enrichment of the DEGs under low P treatment after 75days in *C. sativa* L.

Discussion

Under low P stress, *C. sativa* plants form a complex gene expression regulation network to resist environmental damage. Transcriptome high-throughput sequencing technology can not only provide comprehensive and rapid transcriptional sequence information of *C. sativa* but also provide functional analysis of genes using a variety of biological databases to uncover the molecular mechanisms of plant responses to stress. In our study, the Illumina HiSeq 4000 sequencing platform was used for transcriptome sequencing of *C. sativa* plants treated with low P stress, and 1,242 DEGs were identified. GO enrichment analysis revealed that these DEGs were related to biological processes, cell components, and molecular functions. KEGG pathway enrichment analysis showed that these DEGs were involved in cellular processes, environmental genetic information processing, information processing, metabolism, and organic systems pathways, suggesting that these functions and the response to low-P stress played a positive role, as this annotated information also provided data resources for further mining of resistance genes of *C. sativa*.

TFs are the main regulatory factors of many stress-response genes and play significant roles in plant responses to stress. In our study, several TFs, namely ERF, bHLH, WRKY, and MYB, whose expression levels changed significantly under low P stress, have been shown to be key regulatory factors involved in various abiotic stresses and are thought to be widely involved in the synthesis of secondary metabolites in plants (Ali *et al*., 2018). ZmGPX-PDE1 and ZmGPX-PDE5 were transcriptionally regulated by ZmPHR1, a well-described phosphate starvation-responsive TF of the MYB family (Wang *et al*., 2021). A total of 1,356 TFs were significantly differentially expressed under low P stress (Table 2). It was speculated that these TFs responded to the low P levels in *C. sativa.*

When P starvation occurs, the TF OsPTF1 gene, a bHLH TF that can tolerate low P levels in *O. sativa* roots, is induced and participates in the response to low P (Yi *et al*., 2005). In our study, the expression of bHLH TFs differed under low P stress, indicating that bHLH TFs may induce and regulate the expression of stress-related genes, which is of great significance in the stress-resistant response of the cannabis plants. In previous studies, functional genomics was applied to determine the overall gene expression of *Glycine max* L. under P deficiency stress, and it was found that most of the differentially expressed TFs induced by P deficiency in *G. max*. roots belonged to the MYB family (Hernández *et al*., 2007). As well, 81 WRKY TFs have been identified in *Solanum tuberosum* L., which show different expression patterns during various abiotic stress responses (Huang *et al*., 2012). They studied the possible role of *GmWRKY46* in the P starvation stress tolerance of soybean (Li *et al*., 2021). The results of the present study also showed that MYB and WRKY TFs were differentially expressed under stress induction and that these TFs may regulate the gene expression of *C. sativa* in response to low P stress.

Plant hormones are involved in stress physiology and biochemical reactions. When plant hormones sense biological or abiotic stress, they trigger specific signalling pathways that affect plant metabolism, ultimately leading

to changes in growth patterns to adapt to stress. IAA is believed to play a significant role in mediating plant defence responses to abiotic stress (Verma *et al*., 2016). IAA plays a significant role in the response to low P in plants. Studies have found that by regulating the expression of the auxin carrier gene OsAUX1, IAA promotes the growth of *Oryza sativa* L. root hairs to obtain more P in response to low P (Giri *et al*., 2018). In the present study, 25 auxin-related genes were identified. Low P significantly promoted the expression of IAA-related DEGs in *C. sativa*, suggesting that IAA signalling plays a significant role in the response of cannabis plants to low P stress, laying a basis for uncovering the molecular mechanism of auxin response to low P stress in this species.

Conclusions

By analysing the transcriptome data of *C. sativa* plants under low P treatment for 75 d, 143 genes were found to be directly related to levels of P. Low levels of P directly affected the expression of these genes, as evident in signal transduction and adjustment of P levels, as well as the physiological process in the plants. Twenty-five auxin related genes were identified. Low P levels induce the expression of these genes and regulate auxin synthesis, thus affecting the growth and development of the cannabis plants. The differential genes identified above preliminarily uncovered the transcriptional regulatory pathways involved in low P tolerance in *C. sativa*, which may lay the basis for the cloning and functional validation of low P tolerance-related genes in this species.

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