

Comparative transcriptome analysis of *Saposhnikovia divaricata* to reveal drought and rehydration adaption strategies

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Abstract

Background

Water scarcity has become one of the most prevalent environmental factors adversely affecting plant growth and development. Different species have developed multiple ways of drought resistance. *Saposhnikovia divaricata* is a commonly used traditional herb in East Asia. However, limited information is available on the drought response of this herb and further clarification of underlying molecular mechanism remains a challenge.

Methods and Results

In this study, a comparative transcriptome analysis was firstly conducted to identify the major pathways and candidate genes involved in the drought adaptive response of *S. divaricata*. The seedlings of *S. divaricata* were subjected to progressive drought by withholding water for 16 days followed by 8 days of rehydration. Transcriptome analysis identified a total of 89,784 annotated unigenes. The number of DEGs gradually increased with the deepening of drought and decreased after rehydration. GO and KEGG enrichment analysis suggested genes related to oxidoreductase activity, carbohydrate metabolism, plant hormone signaling pathway and secondary metabolism were important in drought response of *S. divaricata*. Specific genes involved in ROS removal system (*POD*, *Cu/Zn-SOD*, *APX*), abscisic acid and jasmonic acid signaling pathway (*PYL4*, *PP2Cs*, *JAR1*, *JAZ*) and phenylpropanoid biosynthesis (*4CL*, *CCR*, *CAD*) underwent dynamic alterations under drought and rehydration. Finally, the expression pattern of 12 selected DEGs from the transcriptomic profiling were validated by RT-qPCR.

Conclusions

Our study laid a foundation for understanding the stress response of *S. divaricata* and other Apiaceae family plant at molecular level.

Introduction

Water scarcity has become one of the most recurrent and prevalent environmental factors adversely affecting plant growth and development. Plants respond to water deficit by combining a complex regulatory network that induce various morphological, physiological and metabolic changes [1]. With the progression of drought, accumulation of reactive oxygen species (ROS) causes cellular damage, thus affecting plant growth [2]. ROS scavenging system consists of antioxidant enzymes and non-enzymatic antioxidants coordinate together enabling plant to overcome oxidative damages [3]. In addition, plant respond to drought stress by adjusting certain endogenous hormone levels. Under drought stress, abscisic acid (ABA) is a fundamental regulator of plant morpho-physiological responses [4]. Exogenous application of jasmonic acid (JA) alleviated drought stress by improving antioxidative enzyme activities and modifying osmolyte concentration in different species [5–7]. On the other hand, plants subjected to water deficit conditions often produce a variety of secondary metabolites [8] including flavonoids,

phenylpropanoids, alkaloids and terpenoids, which probably functioning as antioxidant, osmoprotectants and plant hormone precursors. In general, plant respond to drought stress through activation of osmotic adjustment and antioxidant system, fluctuation of plant hormone contents and variation of metabolic pathways. Both drought intensity and frequency may affect the plant response.

Saposhnikovia divaricata (Turcz.) Schischk is a perennial herb belonging to Apiaceae family and widely distributed in East Asia. As a commonly used traditional medicine, the dried root of *S. divaricata* have long been studied, exhibiting diverse pharmacological functions such as antipyretic, analgesic, anti-inflammatory and anti-arthritic effects [9]. At present, given the increasing demand and lack of wild resources, *S. divaricata* is cultivated on large scale in northeast China. Water management in cultivation has become a key problem. Our previous research have revealed that water deficit affected the growth of *Astragalus membranaceus* [10, 11] and *Glycyrrhiza uralensis* [12], meanwhile enhanced the drought related gene expression and metabolic regulation. In this study, a comparative transcriptomic analysis of *S. divaricata* under progressive drought and rehydration was firstly conducted to identify the major pathways and candidate genes involved in the adaptive response of *S. divaricata*.

Materials And Methods

Plant Material and drought treatment

Greenhouse pot experiment was conducted at a day/night temperature of $25 \pm 5^{\circ}\text{C}/16 \pm 3^{\circ}\text{C}$ with 50-70% relative humidity under 14/10 h light/dark cycle from March to July 2018. Seeds of *S. divaricata* were planted in 112 plastic pots (18 cm in diameter at the top and 25.5 cm deep). Three months later, seedlings were randomly divided into two groups making sure there are 6 seedlings in each pot. The control group (CK) was well watered while the treatment group was subjected to drought stress by withholding water for 16 days (DS) and then rewatered for 8 days (RW). On day 0, 4, 8, 12 and 16 of drought treatment and day 4 and 8 of subsequent rehydration, six pots of plants were randomly selected. The roots and fully expanded leaves were collected from each group for physiological measurements. The root samples of CK, DS8, DS12, DS16 and RW8 were collected and used for RNA sequencing. All the samples were immediately used for measurements or frozen in liquid nitrogen and stored at -80°C for further processing.

Physiological measurements

Soil water content was calculated as $\text{SWC} (\%) = (\text{FW}-\text{DW})/\text{DW} \times 100$. Fresh soil was weighed as FW. Then, fresh soil was oven-dried at 105°C to measure the dry weight as DW. The leaf relative water content was determined as $\text{LRWC} (\%) = (\text{FW}-\text{DW})/(\text{TW}-\text{DW}) \times 100$. After determining the fresh weight (FW), the petioles were submerged in water for 48 h to measure the saturated weight (TW) and then dried at 80°C to determine the dry weight (DW). Each treatment was repeated for six times.

Superoxide dismutase (SOD) activity was determined as described by Dhindsa et al [13]. Peroxidase (POD) activity was assayed based on the method of Hemeda and Klein [14]. Ascorbate peroxidase (APX)

activity was determined according to the methods of Nakano and Asada [15]. Each treatment was repeated for three times.

RNA isolation, cDNA library construction and sequencing

Total RNA was extracted with TRIzol reagent (Invitrogen, CA, USA) following the manufacturer's protocol and genomic DNA was removed with DNase I (Takara, Dalian, China). 2100 Bioanalyzer (Agilent, CA, USA) and NanoDrop-2000 (Thermo Scientific, MA, USA) were used to ensure the samples were qualified for transcriptome sequencing. TruSeq™ RNA sample preparation kit (Illumina, CA, USA) was used for RNA-seq library construction. Illumina sequencing was conducted on an Illumina HiSeq™ 4000 platform. These experiments were completed by Shanghai Majorbio Bio-pharm Biotechnology Co. (<http://www.majorbio.com>, Shanghai, China).

De novo transcriptome assembly and functional gene annotation

Trinity software (<https://github.com/trinityrnaseq/trinityrnaseq/wiki>) was used for clean read assembly [16]. Using BLASTx tool with an E-value less than 10^{-5} , the sequences of all assembled unigenes were functionally annotated to Clusters of Orthologous Groups (COG) database, Gene Ontology (GO) database, Kyoto Encyclopedia of Genes and Genomes (KEGG) database, Swiss-Prot database, Protein family (Pfam) database and non-redundant (NR) database.

Screening and analysis of differentially expressed genes

The abundance of all genes was normalized and calculated using uniquely mapped reads by the FPKM method (fragments per kilobase per million reads) [17]. A set of differentially expressed genes (DEGs) between the treatment group and the control group were gained by differential expression analysis using DESeq2 [18]. The screening criteria of DEGs were corrected P value < 0.05 and $|\log_2 \text{fold change}| \geq 1$. Using the DEGs detected at different stages of drought stress and rehydration, GO and KEGG enrichment was implemented by Goatools and KOBAS platform.

Validation of transcriptome sequencing using real-time quantitative PCR (RT-qPCR)

The expression of 12 DEGs was validated by RT-qPCR analysis. First-strand cDNA was synthesized by *TransScript®* Uni All-in-one First-Strand cDNA Synthesis SuperMix for qPCR kit (Transgen Biotech, Beijing, China). The primers were designed using the Primer Express 3.0.1 and listed in Supplementary Table S1.. RT-qPCR was completed by *TransStart®* Tip Green qPCR SuperMix Kit (Transgen Biotech, Beijing, China) and run on ABI7500 Real-Time PCR System (Thermo Fisher Scientific, USA). *EF 1- α* was used as internal control [19]. The thermal cycling conditions were 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C, 34 s at 60 °C. The relative gene expression was calculated by $2^{-\Delta\Delta Ct}$ method and three biological replicates were conducted for all reactions.

Results

Effects of drought and rehydration on relative water content

At the first 8 days of drought, SWC had no significant change. SWC dropped sharply to 13.2% and 9.5% on day 12 and day 16, respectively. After rewatering, SWC in the treatment was restored to the control level (Fig. 1a). LRWC maintained at a higher level from 0 to 12 days. LRWC decreased significantly from 90.5% to 42.6% on day 16 of drought, which was 53% lower than that of the control. Similarly, the LRWC reverted to the control level after rehydration (Fig. 1a).

Effects of drought and rehydration on the antioxidant enzyme activities

The activities of SOD, POD and APX in the root increased as the drought intensified. SOD and APX activities showed a similar trend and reached the highest level after 16 days of drought which increased by 57% and 111%, respectively (Fig. 1bd). In contrast, POD activity in the roots reached its maximum value on day 12. After rewatering, the activities of all three enzymes decreased to the normal level (Fig. 1c).

Sequencing and de novo transcriptome assembly

The root samples of *S. divaricata* from CK, DS8, DS12, DS16 and RW8 were collected and used for RNA sequencing. A total of 116.66 GB clean data were obtained from 15 samples after removal of sequence adapter-containing reads, ploy-N-containing reads and other low quality reads. On average, clean data was greater than 7.07 GB for each sample and Q30 value was more than 92% (Supplementary Table S2). The filtered clean reads were assembled with Trinity program producing 167447 unigenes with mean size of 807.49 bp and N50 length of 1253 bp (Supplementary Table S3). The mapped ratio was 76.80% to 79.08% and the unigenes ranged from 201 to 14,945 bp in length. These data suggested that the sequencing results were reliable to be used for subsequent analysis.

Functional annotation and differential expression analysis

A total of 89,784 unigenes (53.62%) were matched to at least one database, of which 74621(83.11%) unigenes were annotated in NCBI_nr database, 66915(74.53%) in Swiss-Prot database, 58956(65.66%) in Pfam database, 14261(15.88%) in COG database, 59608(66.39%) in GO database and 44508(49.57%) in KEGG pathway database (Fig. 2a). Blasting into NCBI_nr database revealed that species most contributed to the annotation was *Daucus carota* (57.24%), followed by *Quercus suber* (4.39%) and *Helianthus annuus* (3.01%) (Fig. 2b).

To identify DEGs in response to progressive drought and rehydration, differential expression analysis was conducted between the group of DS8, DS12, DS16, RW8 and CK (Fig. 2cd). Generally, the number of DEGs gradually increased with the deepening of drought and decreased after rehydration in comparison with the control. Venn diagrams showed that 145 DEGs were co-expressed in all four groups, with the highest overlap between DS12 and DS16, indicating similar expression patterns in DS12 and DS16 samples. Downregulated genes played a dominant role in moderate and severe treatment, while most of the DEGs

upregulated during mild drought and after rewatering (Fig. 2d). For example, downregulated genes increased from 1831 to 5771 in DS12 and DS16, and then dropped to 676 in RW8.

GO and KEGG pathway enrichment analysis of differentially expressed genes

The up and downregulated DEGs of *S. divaricata* roots were further subjected to GO enrichment analysis to identify the major biological processes involved following the criteria of false discovery rate (FDR) <0.01 (Table 1). Compared with the control, 117 upregulated DEGs at DS8 were correlated to oxidoreductase activity. Polysaccharide metabolism and *O*-glycosyl hydrolysis were enriched in the upregulated DEGs at DS8. However, the DEGs clustered to these GO terms were all downregulated at DS12 and DS16. Interestingly, zinc ion binding and transition metal ion binding function were enriched in the upregulated DEGs at DS16. After rehydration, DNA-binding, protein kinase activity, carbohydrate metabolism and adenyl nucleotide binding function were enriched in a considerable number of upregulated DEGs suggesting the gradual completion of damage repair and cell recovery.

Table 1 GO analysis of the significantly up- and downregulated differentially expressed genes (FDR<0.01)

Group	Up-regulated DEGs			
	GO ID	Up-description	Adjusted <i>P</i> value	DEGs number
DS8	GO:0016491	oxidoreductase activity	0.0005968	117
	GO:0003700	DNA-binding transcription factor activity	0.0010228	36
	GO:0005976	polysaccharide metabolic process	0.0003828	36
	GO:0004553	hydrolase activity, hydrolyzing <i>O</i> -glycosyl compounds	0.0038832	31
DS12	GO:0070300	phosphatidic acid binding	0.0019530	3
DS16	GO:0008270	zinc ion binding	0.0014370	163
	GO:0046914	transition metal ion binding	0.0056387	214
RW8	GO:0003700	DNA-binding transcription factor activity	0.0005065	39
	GO:0140110	transcription regulator activity	0.0004755	42
	GO:0004672	protein kinase activity	0.0004939	99
	GO:0005975	carbohydrate metabolic process	0.0006494	67
	GO:0030554	adenyl nucleotide binding	0.0035831	177
	GO:0016772	transferase activity, transferring phosphorus-containing groups	0.0067178	119
Group	Down-regulated DEGs			
	GO ID	Down-description	Adjusted <i>P</i> value	DEGs number
DS8	GO:0003700	DNA-binding transcription factor activity	0.0037171	23
	GO:0140110	transcription regulator activity	0.0056822	23
DS12	GO:0016798	hydrolase activity, acting on glycosyl bonds	0.0003845	84
	GO:0016705	oxidoreductase activity, acting on paired donors	0.0003845	55
	GO:0003700	DNA-binding transcription factor activity	0.0003845	60
	GO:0005976	polysaccharide metabolic process	0.0003845	61
	GO:0006629	lipid metabolic process	0.0003845	81
	GO:0032774	RNA biosynthetic process	0.0003845	94

DS16	GO:0016491	oxidoreductase activity	0.0028047	501
	GO:0016705	oxidoreductase activity, acting on paired donors	0.0002062	161
	GO:0003700	DNA-binding transcription factor activity	0.0002097	192
	GO:0016773	phosphotransferase activity	0.0014546	381
	GO:0005506	iron ion binding	0.0003592	102
	GO:0016798	hydrolase activity, acting on glycosyl bonds	0.0002096	184
	GO:0005976	polysaccharide metabolic process	0.0002062	147
	GO:0006629	lipid metabolic process	0.0002108	189
	GO:0032774	RNA biosynthetic process	0.0002174	285
RW8	GO:0005215	transporter activity	0.0007451	58

KEGG pathway enrichment analysis of DEGs showed that pathways involved in drought response can be mainly divided into eight physiological categories, such as signal transduction, metabolism of terpenoids and steroids, lipid metabolism, biosynthesis of other secondary metabolites, carbohydrate metabolism, energy metabolism, replication and repair and environmental adaption (Fig. 3). Most of the pathways were enriched in upregulated DEGs at DS8 and RW8, whereas enriched in downregulated DEGs at DS12 and DS16. The highest enrichment factors among all treatment groups were plant hormone related signaling pathway. Linolenic acid metabolism, phenylpropanoid biosynthesis and glutathione metabolism pathway in the root were mostly enriched in upregulated DEGs, indicating the activation of these processes under mild stress. Starch and sucrose metabolism was enriched in upregulated DEGs at DS16 indicating carbohydrates were indispensable for plant to maintain survival under critical conditions.

DEGs involved in antioxidant system, plant hormone signal transduction and secondary metabolism

To further explore the molecular mechanism, a series of candidate genes were screened, including DEGs relevant to antioxidant system and plant hormone signal transduction, as well as DEGs concerning secondary metabolism.

27 genes annotated to antioxidant enzyme system were selected, of which 20 genes were related to POD (Fig. 4a). Most genes encoding POD were upregulated at DS8 and downregulated at DS12 and DS16 with the opposite for SOD, APX and glutathione peroxidase, which were mainly induced by moderate and severe drought. Glutathione S-transferase is the main detoxifying system for plants under extreme conditions. In this study, two glutathione S-transferase genes (DN103932_c0_g1, DN95127_c1_g5) were

significantly upregulated under severe drought and the other two genes (DN91685_c0_g1, DN105946_c0_g2) mainly responded to mild stress and rehydration.

Absciscic acid participates in various physiological responses including drought induced stomatal closure and seedling growth inhibition. Absciscic acid receptor genes (*PYL4*) first upregulated at DS8 and then downregulated at DS16, while its co-receptor protein phosphatase 2C genes (*PP2C*) showed a reverse trend which in turn released protein kinases SnRK2 from inhibition (Fig. 4b). Jasmonic acid signaling pathway genes were mostly induced by drought suggesting their involvement in drought response of *S. divaricata*. Jasmonic acid-amido synthetase genes *JAR1* (DN108879_c2_g1, DN108238_c1_g1) significantly upregulated with the intensification of drought catalyzing the addition of L-isoleucine to jasmonate (Fig. 4b). Additionally, the expression of genes encoding jasmonate ZIM-domain protein (*JAZ*) and transcriptional activator (*MYC2*) also varied within a wide limit under drought and rehydration.

It has been reported that *S. divaricata* root contained high content of phenylpropanoids [8]. A total of 103 DEGs were enriched in phenylpropanoid biosynthetic pathway according to KEGG analysis. *PAL*, *4CL*, *CCR* and *CAD* gene families were upregulated with the increase of drought. *HCT*, *COMT* and *F5H* mainly downregulated under moderate and severe drought and returned to the control level after rehydration (Fig. 5). Peroxidases were found to participate in the oxidative polymerization of phenylpropanoids [20]. Most peroxidase genes were induced by mild stress while a few were induced by moderate and severe drought.

Validation of drought-responsive genes by RT-qPCR

To ensure the reliability of sequencing data, the expression patterns of 12 genes identified as associated with water deficit responses were evaluated by RT-qPCR (Supplementary Table S1). These genes demonstrate different functions and participate in various pathways, including 4 genes involved in plant hormone signal transduction, 4 genes involved in antioxidant system and 4 genes involved in secondary metabolism. As shown in Supplementary Fig. S1, the RT-qPCR results of 12 genes were highly consistent with the results of RNA-seq, which confirmed the reliability of the RNA-sequencing.

Discussion

Drought tolerance is a complex process of polygenic regulation in plant. In this study, a comparative transcriptome analysis was conducted to investigate the molecular mechanism underlying stress response of *S. divaricata*, a species of Apiaceae family without reference genome [21].

The activity of antioxidant systems has been used as one of the effective indicators of plant drought resistance [2]. In this study, the activities of SOD, POD and APX increased as the drought intensified suggesting that these enzymes work together to remove ROS which is consistent with our previous research [22] and similar results can also be found in other species of Apiaceae family such as *Bupleurum chinense* [23], *Ferula assafoetida* [24] and celery [25]. Furthermore, we identified and selected 27 DEGs associated with ROS scavenging system. Among them, genes encoding POD were found to be the most abundant. According to KEGG pathway enrichment analysis, POD also participated in the

phenylpropanoid metabolic pathway, catalyzing the final stage of lignin synthesis [26]. Most *POD* genes were upregulated after eight days of drought, while *Cu/Zn-SOD* and *APX* genes were mainly induced by moderate drought. It suggests that the enzymes play major roles in scavenging ROS are different at different stages of drought. In accordance with these observations, physiological response of *S. divaricata* root showed that POD activity reached its maximum value at DS12, while SOD and APX activities peaked at DS16.

Under limited water conditions, the increase of endogenous ABA level transcriptionally regulates downstream genes protecting plants from dehydration damage [4]. Signaling of the ABA is mediated by PYR/PYL/RCAR receptors, which bind to and inhibit PP2Cs releasing protein kinases SnRK2s from inhibition [27]. Overexpressing *PtPYRL 1* and *PtPYRL5* in transgenic poplar plants significantly enhanced the osmotic stress resistance by positively regulating ABA signaling [28]. In our study, genes encoding PYLs significantly upregulated at DS8 and RW8, while genes encoding PP2Cs and ABF showed a reverse trend which upregulated at DS12 and DS16. *SnRK2* genes were both up and downregulated under drought and rehydration. These results indicated that ABA signaling pathway was closely related to the drought accommodation of *S. divaricata*. JA and its derivatives are significant in plant adaptive response to abiotic stress [29]. As for JA signaling, *JAR1* genes were upregulated with the progression of drought suggesting the gradual accumulation of JA-Ile. The expression of *JAZ*, a repressor of JA, was significantly downregulated at DS12 and DS16. Transcriptional activator *MYC2* genes were more highly induced at the early stage. These findings were confirmed by previous studies which have shown that ABA and JA signaling pathways were both involved in priming-induced stress tolerance [30]. Instead of acting independently, it is more likely that plant hormones coordinate together contributing to better accommodation to environmental stress, which needs to be further examined.

Plant secondary metabolism is the result of long-term evolutionary interaction between plant and environment and plays a critical role in plant environmental adaption, especially for perennial herbs. Drought could improve the accumulation of secondary metabolites in plant [7]. The main chemical components of *S. divaricata* root are phenylpropanoids, terpenoids and flavonoids [9]. KEGG pathway analysis showed a total of 103, 43 and 21 DEGs were enriched in phenylpropanoids, terpenoids and flavonoids biosynthesis, respectively. In *S. divaricata* root under drought, phenylpropanoids biosynthesis was enriched at DS8 and RW8 in the upregulated DEGs, whereas downregulation played a dominant role at DS12 and DS16. *PAL* and *4CL* are two key genes in the early stages of lignin and flavonoid biosynthesis. CCR and CAD consecutively convert acyl-CoA to corresponding alcohols. In this study, two *PAL*, three *4CL*, two *CCR*, and three *CAD* genes were found and most of them showed a drought induced pattern, upregulating with the deepening of drought. Plenty of peroxidase genes likely generate different transcripts for oxidative polymerization of different monolignol units. Most peroxidase genes were induced by mild stress and the other few were induced by moderate and severe drought. Taken together, it can be inferred that phenylpropanoids took part in the drought defense of *S. divaricata* root.

Conclusions

We reported the characterization of drought and rehydration responsive genes of *S. divaricata* based on comparative transcriptomic profiling. A total of 89,784 unigenes were annotated to at least one database. The number of DEGs gradually increased with the deepening of drought and decreased after rehydration. *S. divaricata* reply to drought and rehydration through a diversified and integrated network consist of genes relevant to ROS scavenging, plant hormone signaling pathway and phenylpropanoid biosynthesis. These results would provide useful information and serve as a transcriptomic database for stress resistance of *S. divaricata* and other Apiaceae family plant.

Declarations

Author contributions GC conceived the idea and supervised the whole procedures. MJ prepared the plant material and performed the biological experiments. The bioinformatics analysis and data organization was done by YS. YS and HG contributed to manuscript editing.

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Data availability The raw sequence data was deposited in NCBI database under SRA accession number PRJNA613937 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA613937/>).

Conflict of interest All authors declared no conflict of interest.

Ethical approval This article does not contain any endangered or protected species.

Informed consent Not applicable.

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Figures

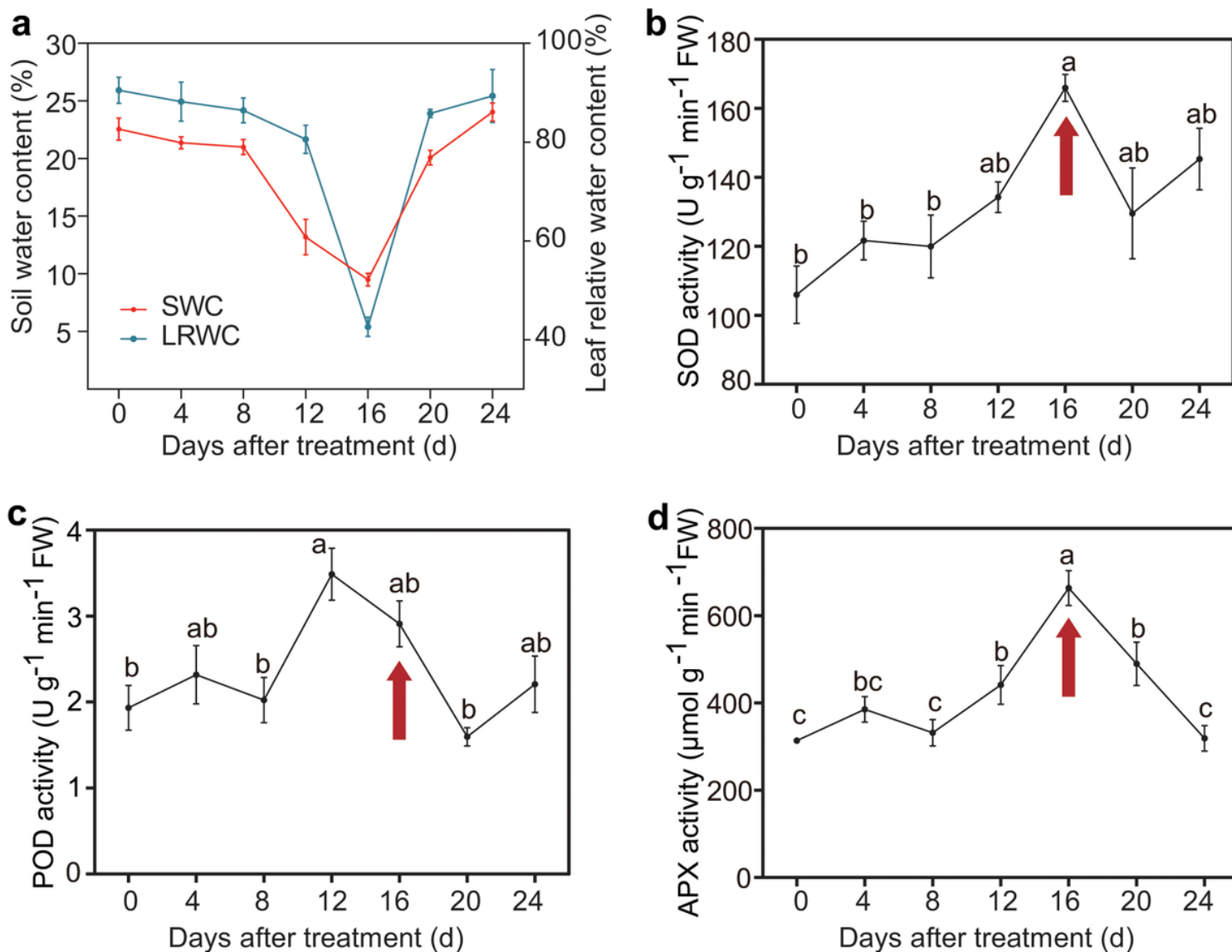


Figure 1

Effects of drought stress and rehydration on relative water content and antioxidant enzyme activities of *Saposhnikovia divaricata*. **a** soil relative water content and leaf relative water content; **b** SOD activity; **c** POD activity; **d** APX activity. Red arrows indicate the beginning of rehydration and different letters indicate significant difference at $P < 0.05$.

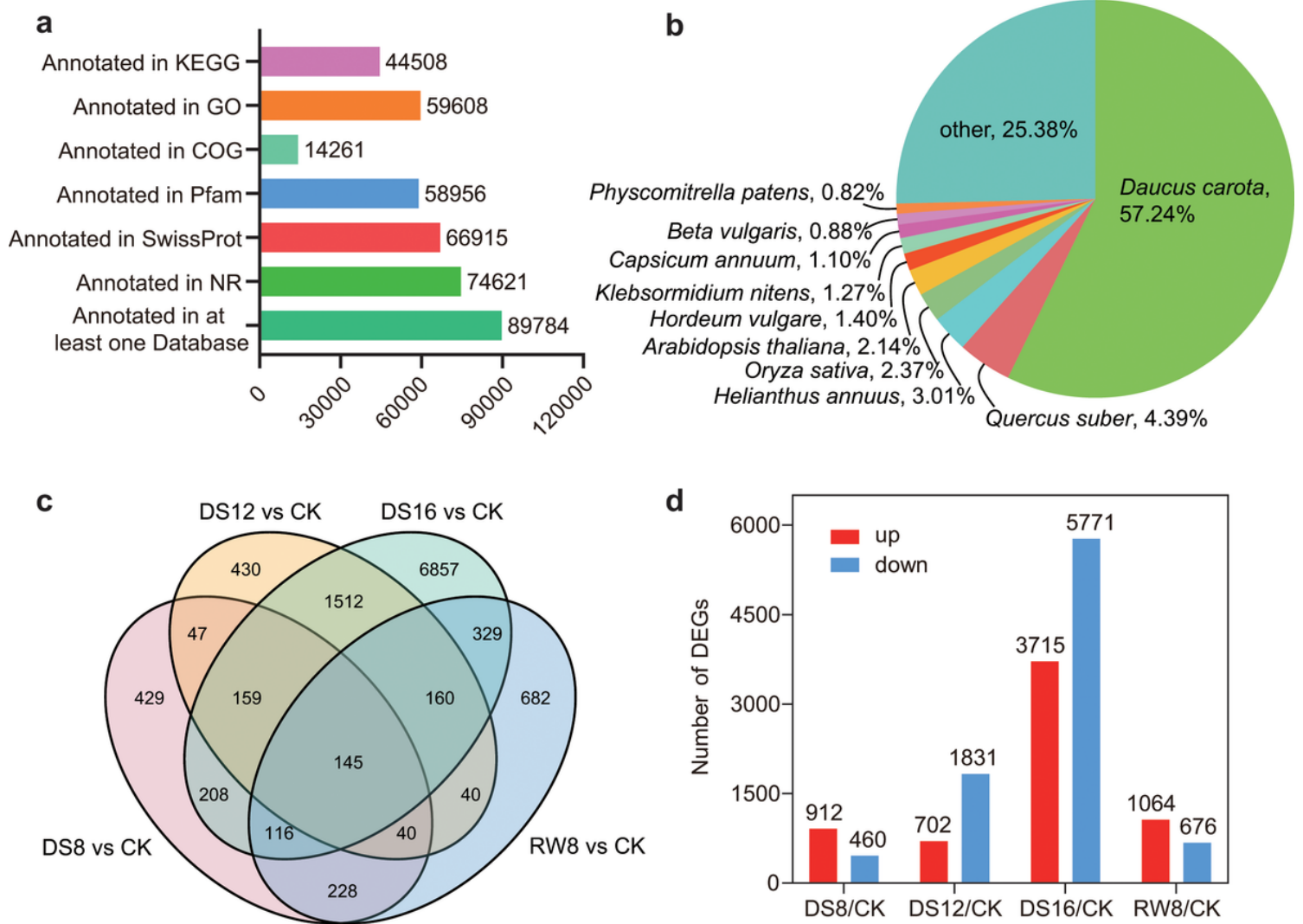


Figure 2

Functional annotation and differential expression analysis for *Saposhnikovia divaricata* transcriptome. **a** unigenes annotated in different databases; **b** unigenes distributed into different species; **c** Venn diagram of differentially expressed genes (DEGs) under progressive drought stress (DS) and rehydration (RW); **d** up and down-regulated DEGs in different group.

physiological category	KEGG pathway	Up-regulation				Down-regulation			
		DS8	DS12	DS16	RW8	DS8	DS12	DS16	RW8
signal transduction	Plant hormone signal transduction	11	0	0	18	10	42	97	11
	MAPK signaling pathway - plant	0	0	0	14	7	0	37	8
metabolism of terpenoids and steroids	Monoterpenoid biosynthesis	0	0	0	0	0	5	13	0
	Sesquiterpenoid and triterpenoid biosynthesis	5	0	0	0	0	0	13	0
	Diterpenoid biosynthesis	0	0	0	0	0	0	9	0
	Zeatin biosynthesis	0	0	0	0	0	5	10	0
	Steroid biosynthesis	0	0	0	0	0	8	23	0
	Brassinosteroid biosynthesis	0	0	0	0	0	0	8	0
	Carotenoid biosynthesis	0	0	7	0	7	0	0	6
lipid metabolism	Fatty acid biosynthesis	0	0	0	0	0	0	27	0
	Biosynthesis of unsaturated fatty acids	0	0	0	0	0	29	44	0
	Cutin, suberine and wax biosynthesis	0	0	0	0	0	0	9	0
	Linolenic acid metabolism	10	0	14	0	0	0	0	0
biosynthesis of other secondary metabolites	Indole alkaloid biosynthesis	0	0	0	0	0	0	8	0
	Phenylpropanoid biosynthesis	22	0	19	0	0	16	62	8
	Stilbenoid, diarylheptanoid and gingerol biosynthesis	0	0	0	0	0	0	14	0
	Flavonoid biosynthesis	0	0	0	0	0	0	16	0
carbohydrate metabolism	Pentose and glucuronate interconversions	10	0	0	0	0	20	37	0
	Amino sugar and nucleotide sugar metabolism	0	0	0	0	0	17	45	0
	O-glycan biosynthesis	0	0	0	0	0	0	0	3
	starch and sucrose metabolism	0	0	28	14	0	0	0	9
Energy metabolism	Nitrogen metabolism	7	0	0	0	0	0	0	0
	Sulfur metabolism	0	0	0	0	0	9	0	0
	Tyrosine metabolism	8	0	0	0	0	0	0	0
Environmental adaption	Plant-pathogen interaction	18	0	0	20	0	0	0	0
	Circadian rhythm - plant	0	5	16	0	5	0	0	0
	Glutathione metabolism	11	0	0	0	0	0	0	0
	Biotin metabolism	0	0	0	0	0	0	11	0
Replication and repair	DNA replication	10	0	0	0	0	18	0	0
	mismatch repair	0	0	0	0	0	8	0	0

Figure 3

Heatmap diagram of KEGG enrichment of differentially expressed genes (DEGs) in response to drought (DS) and rehydration (RW) in *Saposhnikovia divaricata* (FDR<0.05). The red shading indicate the upregulated DEGs while the blue shading represent the downregulated DEGs and the color depth reflects the number of DEGs enriched in each biological process.

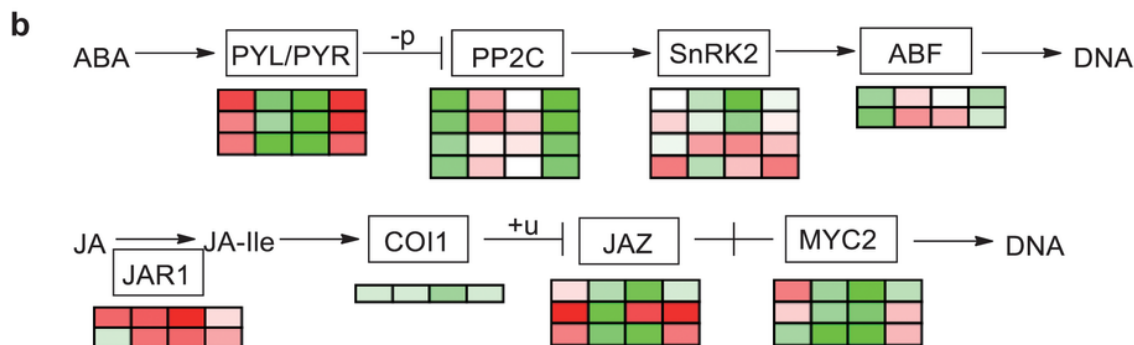
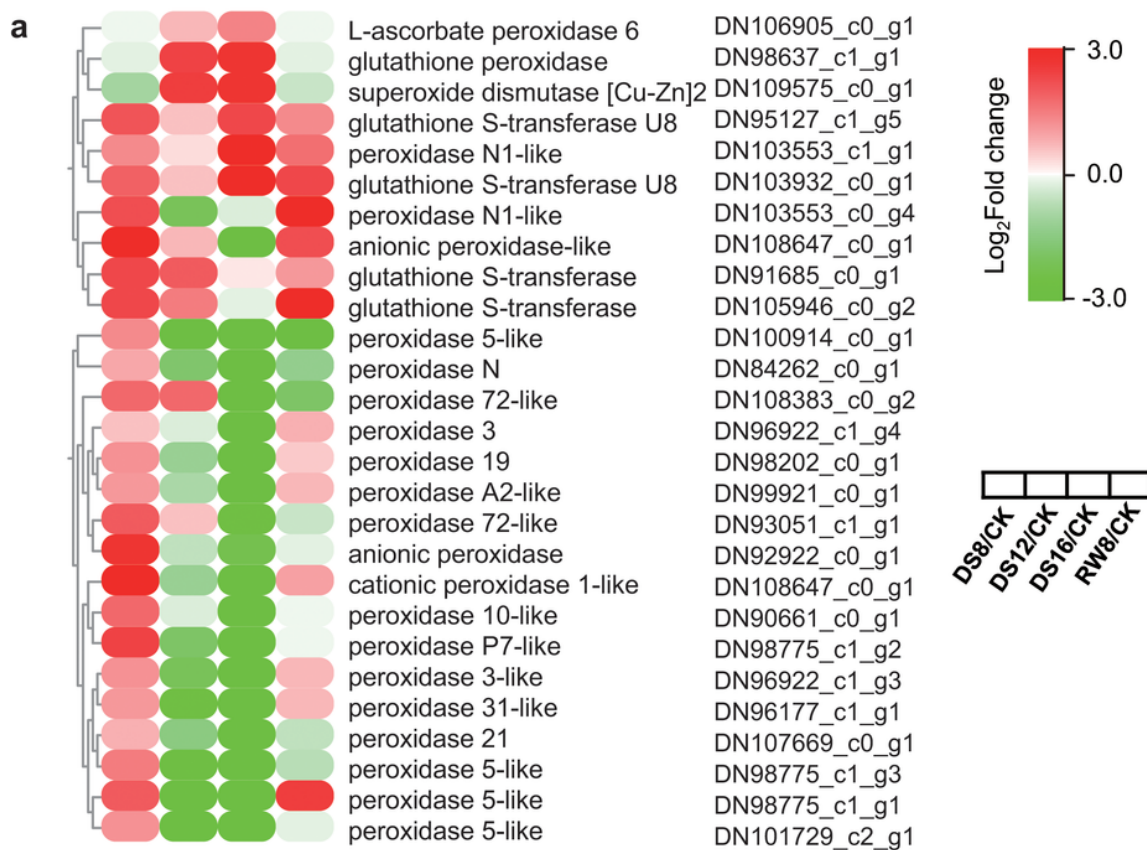


Figure 4

Response of selected genes involved in antioxidant system, plant hormone signal transduction and terpene metabolic processes during drought and rehydration of *Saposhnikovia divaricata*. **a** selected antioxidant enzyme related genes; **b** plant hormone signal transduction related genes.

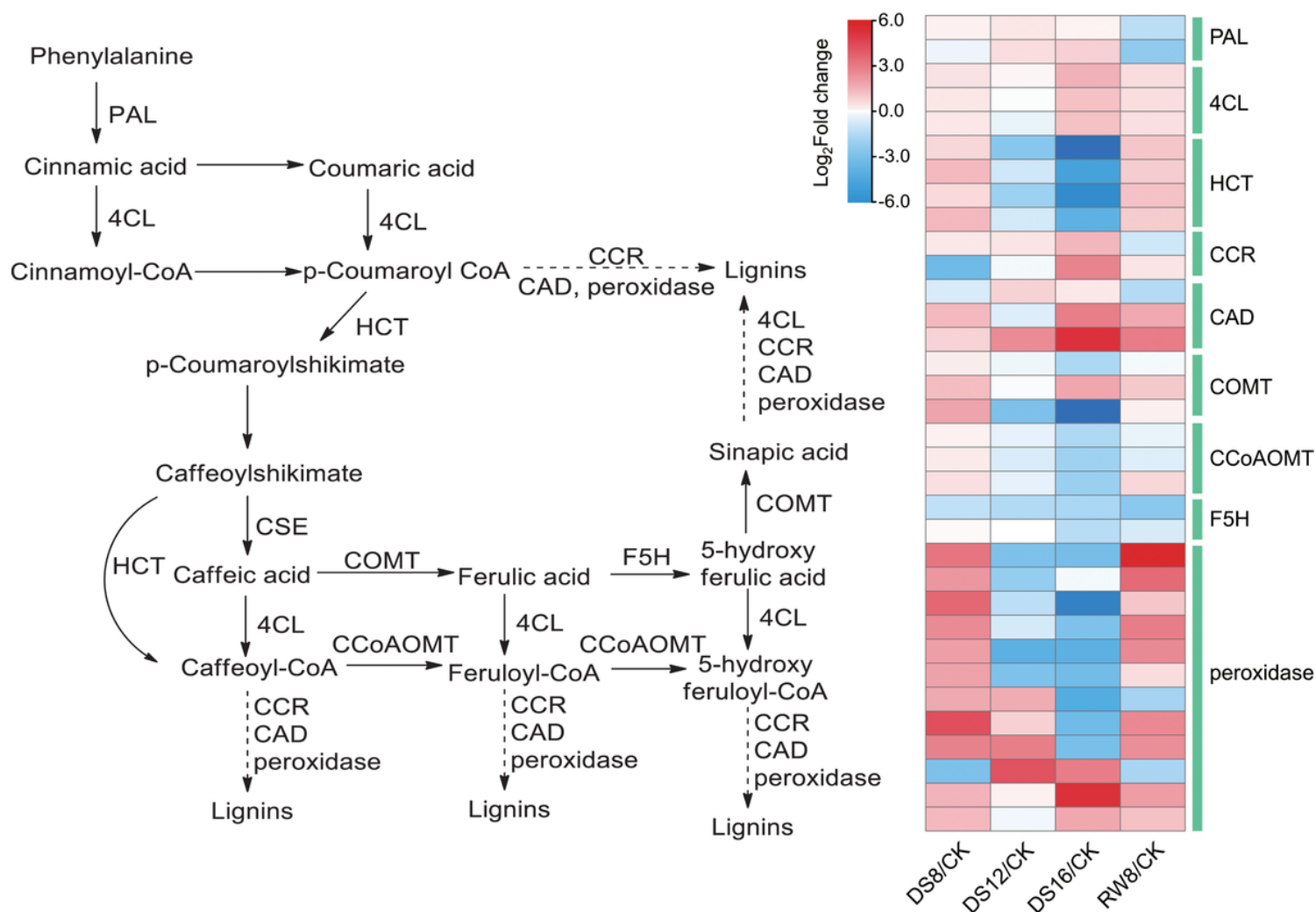


Figure 5

Analysis of genes involved in phenylpropanoid biosynthesis of *Saposhnikovia divaricata* under drought and rehydration. Solid arrow indicates known step and dashed arrows indicate multiple reaction steps. PAL, phenylalanine ammonia-lyase; 4CL, 4-coumarate-CoA ligase; HCT, hydroxycinnamoyltransferase; CCR, cinnamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase; CSE, caffeoylshikimate esterase; COMT, caffeate *O*-methyltransferase; CCoAOMT, caffeoyl-CoA *O*-methyltransferase; F5H, ferulate 5-hydroxylase.

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