Transcriptomic responses of *Saccharum spontaneum* roots in response to polyethylene glycol – 6000 stimulated drought stress

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Drought is the abiotic factor that adversely affects plant growth, development survival, and crop productivity, posing a substantial threat to sustainable agriculture worldwide, especially in warm and dry areas. However, the extent of damage depends upon the crop growth stage, severity and frequency of the stress. In general, the reproductive growth phase is more sensitive to stresses causing a substantial loss in crop productivity. *Saccharum spontaneum* (L.) is the most variable wild relative of sugarcane with potential for use in sugarcane crop improvement programs. In the present study addresses the transcriptomic analysis of drought stress imposed by polyethylene glycol – 6000 stimulated drought stress. *Saccharum spontaneum* (L.) is the most variable wild relative of sugarcane with potential for use in sugarcane crop improvement programs. In the present study addresses the transcriptomic analysis of drought stress imposed by polyethylene glycol – 6000 stimulated drought stress. *Saccharum spontaneum* (L.) is the most variable wild relative of sugarcane with potential for use in sugarcane crop improvement programs. In the present study addresses the transcriptomic analysis of drought stress imposed by polyethylene glycol – 6000 stimulated drought stress.

KEYWORDS
gene, transcriptome, signal transduction, drought stress, *Saccharum spontaneum*
Introduction

Abiotic stress is a major problem to sustainable agriculture crop productivity around the globe, which can potentially affect the average crop production over 50% (Cui et al., 2017; Naem et al., 2018; Waqas et al., 2019; Verma et al., 2021; Verma et al., 2022). Drought stress is one of the severe abiotic stress that inhibit plant growth, development and yield (Baiazidi-Aghdam et al., 2016). It is well-established that drought stress may impair various morpho-physiological, anatomical, cellular and metabolic processes associated with the regulation of plant development (Yoo et al., 2013; Liu et al., 2013; Cui et al., 2017). It also enhances leaf fall and chlorophyll damage, downregulate photosynthetic efficiency and loss in leaf area expansion/development, resulting in low plant productivity and sometimes total crop failure (Jiang et al., 2016; Kamran et al., 2018; Naem et al., 2018; Verma et al., 2020; Verma et al., 2022). Polyethylene glycon (PEG) having molecular weight of 6000 is a natural polymer that is water soluble and nonionic. PEG 6000 is found to mimic drought stress and results in lowering of plant’s water potential due to osmotic stress (Ahmad et al., 2020).

During adverse environmental conditions, plants produce a large number of reactive oxygen species (ROS) associated with the regulation of various processes, i.e., pathogen defense, programmed cell death (PCD), and stomatal behavior (Liang et al., 2019). It causes oxidative damage to cell membranes, proteins, effective enzymatic activities [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX)], non-enzymatic (phenolic acids, carotenoids, flavonoids, ascorbic acid, proline, etc.), DNA and RNA (Dawood and El-Awadi, 2015; Baiazidi-Aghdam et al., 2016; Cui et al., 2017; Laxa et al., 2019; Verma et al., 2022). ROS plays important roles in maintaining normal plant growth, development and improving stress resistance capacity. The enhanced synthesis of osmolytes, i.e., soluble protein and proline, also plays stress tolerance functions (Sarropoulou et al., 2012; Turk et al., 2014). Cell death (also known as cell injury) is relative to the length or duration of exposure to stress and the severity of the damage caused (Turk et al., 2014; Verma et al., 2020).

Saccharum spontaneum L. is one of the wild species in the Saccharum complex and has a reservoir of genes for various economically important traits, such as tolerance to environmental stresses. Since the beginning of the new century, the world’s major sugarcane-producing countries have attached great importance to the investigation, collection, evaluation, and utilization of S. spontaneum germplasm resources (Zhang et al., 2010; Xu et al., 2017; Li et al., 2018; Xian et al., 2019; Xu et al., 2020; Xu et al., 2022). S. spontaneum has the most widely used wild germplasm for sugarcane breeding programs in China. In China, S. spontaneum grows in the wild and is widespread within the geographical range of northern latitudes (18-33°C) and eastern longitudes (97-122°C). More than 600 S. spontaneum clones were collected and conserved in China (Qi et al., 2022). Analysis of the genetic diversity of S. spontaneum using molecular markers showed significant geographical differences within the collection of S. spontaneum (Fan et al., 2013). It was obtained through interspecific hybridization combined with molecular markers to identify true hybrids and progeny and applied to sugarcane breeding (Yu et al., 2019; Zhang et al., 2019).

Saccharum spontaneum is most important for modern sugarcane breeding, commercial varieties contain about 10% of the chromosomes from S. spontaneum, and effectively used S. spontaneum stress-resistant parents and genes are minimal. At present, different drought stress resistance-related genes of S. spontaneum have been successfully cloned and preliminary functional analysis carried out, including SsPOD-1a, SsPOD-1b, SsSOD-1a, SsDREB2-a, SsDREB2-f, SsDREB2-2, SsGST, SsCAT-1c, SsCAT-1d, SsO-AAT-2, SsREMO-1, ScPP2C, PIN gene family and NAC transcription factors (Xu et al., 2017; Liu et al., 2018; Yang et al., 2019; Huang et al., 2021; Qingqing et al., 2022). Assessing these efficient stress tolerance genes has provided new candidate gene resources for accelerating the genetic improvement of sugarcane. Still, limited reports were available on acquiring or utilizing significant stress tolerance genes in S. spontaneum for better crop improvement.

In the present study, based on the previous research (Wu et al., 2018a; Wu et al., 2018b), the stress-resistant genes were identified from the root system of S. spontaneum for further analysis of the regulation of molecular mechanisms in response to polyethylene glycol-6000 (PEG-6000; w/v 25%) stimulated drought stress and provide advanced approaches to improve the stress tolerance capacity by genetic engineering technologies.

Materials and methods

Plant material and experimental layout

The plant material of S. spontaneum GX83-10, the wild-type stress-resistant core germplasm, was collected in the Germplasm Resource Garden. The experiment was carried out in the greenhouse of Guangxi Sugarcane Genetic Improvement Key Laboratory, Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, China. The pre-cultured seedlings with consistent rooting and growth were selected and transplanted (n = 50) in hydroponic culture with Hoagland nutrient solution (10 L). The nutrient solution was changed in a week at the seedling stage and every three days during elongation phase. The timing was set to oxygenate the nutrient solution once every hour during the experiment.

Each plant maintained 3 reproductive tiller stems during experimental duration, and juvenile tillers were removed regularly. The experiment was designed with five treatment

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levels, such as 0 h (CK), 2 h (T2), 4 h (T4), 8 h (T8), and 24 h (T24) under drought stress. Polyethylene glycol 6000 (PEG-6000 w/v 25%) was used to stimulate drought stress treatment in *S. spontaneum* stems with five visible nodes. At the beginning of the experiment, root samples were collected at 0 h (T0, CK) treatment. PEG-6000 (25%) of the same volume was used to replace Hogland nutrient solution simultaneously, and root samples were collected 2, 4, 8 and 24 h after treatment. Three plants (n = 3) with consistent plant growth and proper root development were selected for transcriptomic analysis of each treatment. During sampling, ten white segments of root tips of each plant with a length of about 3-4 cm were cut and mixed. After naming, root samples were kept frozen under liquid N2 and stored at -80°C.

**Isolation, library construction and sequencing of RNA**

Total RNA of CK, T2, T4, T8 and T24 was extracted with TRIzol reagent, respectively (Wu et al., 2018a). After total RNA extraction, eukaryotic mRNA was enriched by Oligo(dT) beads, while prokaryotic mRNA was enriched for removing rRNA by Ribozero™ Magnetic Kit (Epipcent). The enriched mRNA was fragmented into short fragments using fragmentation buffer and reverse transcribed into cDNA with random primers. Second-strand of cDNA was synthesized by DNA polymerase-I, RNase H, dNTP and buffer. Then the cDNA fragments were purified with a QiaQuick PCR extraction kit, end-repaired, poly(A) added, and ligated to Illumina sequencing adapters. The ligation product size was selected by agarose gel electrophoresis, PCR amplified and sequenced using Illumina HiSeq™ 4000 by Gene Denovo Biotechnology Co. Guangzhou, China (Supplementary Figure S1). All sequencing reads were uploaded to the National Center for Biotechnology Information (NCBI) [SRA accession number – PRJNA835339].

**Transcriptome and reference transcriptome assembly**

Filtering raw reads, *de novo* assembly was performed with short reads assembling program Trinity (Grabherr et al., 2011). The high-quality rRNA clean reads were removed and mapped to the reference transcriptome by default parameters of the short reads alignment tool Bowtie2 (Li et al., 2009) [Figures S2 and S3].

**Analysis of unigene expression and basic annotation**

The unigene expression was calculated and normalized to reads per kilobase per million (RPKM) (Mortazavi et al., 2008). The unigenes were annotated using the BLASTx program ([http://www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) with an E-value threshold of 1e-5 to NCBI non-redundant protein sequence database (Nr) ([http://www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)), Swiss-Prot ([http://www.expasy.ch/sprot](http://www.expasy.ch/sprot)), Kyoto Encyclopedia of Genes and Genomes (KEGG) ([http://www.genome.jp/kegg](http://www.genome.jp/kegg)), and COG/KOG ([http://www.ncbi.nlm.nih.gov/COG](http://www.ncbi.nlm.nih.gov/COG)). Protein functional annotations could be obtained according to the best alignment results.

**Identification and analysis of differentially expressed genes**

The edgeR package ([http://www.r-project.org/](http://www.r-project.org/)) was used to identify DEGs across samples or groups. Identified genes with fold change ≥2 and false discovery rate (FDR) ≤0.05 as significant DEGs. DEGs were subjected to enrichment analysis of GO functions and KEGG pathways (Xu et al., 2022). Gene expression pattern analysis was used to cluster genes of similar expression patterns for multiple samples (@ 3 times). To examine the expression pattern of DEGs, each sample was normalized to 0, log2 (v1/v0), log2 (v2/v0), and clustered by Short Time-series Expression Miner software (STEM) (Ernst and Bar-Joseph, 2006). The clustered profiles with p-value (≤0.05) were considered significant profiles. The DEGs in all or each profile were subjected to Gene Ontology (GO) and KEGG pathway enrichment analysis. The hypothesis of the p-value calculation and FDR correction showed that GO terms or pathways with Q value ≤ 0.05 were enriched GO terms or pathways (Benjamini and Hochberg, 1995).

**Quantitative real-time PCR (qRT-PCR) assay**

Up-regulated genes were randomly selected for verification according to Wu et al. (2018a) and Xu et al. (2022). The validated samples, such as A1 (0 h), A2 (2 h), A3 (4 h), A4 (8 h) and A5 (24 h), treated with PEG-6000 (25%) for 0, 2, 4, 8 and 24 h, respectively. Unigene0049197 (alpha-tubulin) was used as a control gene. Special primers were designed for 13 different genes of expression validation of up-regulated genes using Premier 5.0 software (Supplementary Table S1).

**Results**

The total RNA quality was quantified by Agilent 2100 system (Figure S4). The results of total RNA extraction showed quality requirements for library construction (Table S2). A total of 57,036,239 bases were assembled in the experiment, and 62,988 genes were obtained (Table S3). The maximum length of the
assembly (15429 nt), minimum (201 nt), average (905 nt) and N50 (1533 nt); the N50 length was larger than the average length (Table S3). Figure 1A showed the number of Unigenes of 1500 – 1599 nt was 1141, smaller than the number of genes of average length (1806). The unigenes have coverage distribution on reads of 1-1000 and above in S. spontaneum. Unigenes covering 11-100 reads were at 23216, followed by unigenes covering more than 1000 reads with 6431 (Figure 1B). Comprehensive analysis shows that the quality of the completed assembly was good.

Analysis of gene annotation results

Functional annotation analysis of all 62,988 unigenes was found. The unigenes were matched as 39,411 (62.62%) in Nr database, 28,397 (45.08%) Swissprot database, 23,451 (36.65%) KOG database and 15,311 (24.31%) in the KEGG database. A total of 40,784 (64.75%) genes were successfully annotated, while 22,204 (35.25%) genes failed to be annotation (Table 1). The large number of unigenes were annotated with the Nr database, followed
by Swissprot, KOG and KEGG (Figure 2A). Interestingly, KOG-Nr and Swissprot-Nr found the largest number of co-annotated unigenes. The Nr database revealed that 39,441 unigenes annotated sequences aligned with different plant species to known nucleotide sequences similar to other plants. Annotation results showed as 30.73, 23.44, 15.71, 3.15, 1.88, 1.62, 1.55, 1.46, 1.17 and 1.14%, matching with Zea mays, Setaria italica, Oryza sativa Japonica, Sorghum bicolor, Brachypodium distachyon, Oryza brachyantha, Aegilops tauschii, Saccharum hybrid cultivar R570, Triticum urartu, Oryza sativa Indica, respectively (Figure 2B). The results have high homology with Zea mays, Setaria italica and Oryza sativa Japonica genomes. These results support the genomic similarity of S. spontaneum tissues with the other plants, such as Z. mays, O. sativa and S. bicolor.

**Gene ontology (GO) and KOG analysis**

GO annotation found that all unigenes were enriched in 50 Ontology, 59093 unigenes (38.01%) in biological processes, 32282 (20.77%) in molecular functions, and 64072 (41.22%) in cellular components (Figure 3A). Most annotated classes in the biological process were included in "metabolic process" 15269 (9.82%), "cellular process" 13925 (8.96%), and "single-organism process" 9880 (6.36%). In the molecular function, most annotated unigenes were categorized as "binding" 15050 (9.68%), "catalytic activity" 13616 (8.76%), and "biological regulation" 4316 (2.78%). In the cellular component, most of the annotated unigenes were classified as "cell" 17120 (11.01%), "cell part" 17117 (11.01%), and "organelle" 15196 (9.78%). KOG classified all unigenes, and 39,559 unigenes were found to be aligned to the database and distributed into 25 categories (Figure 3B). KOG category as general function prediction was the largest group, i.e., 7,153 (18.08%), followed by post-translational modification, protein turnover, chaperones 4,602 (11.63%), and signal transduction mechanisms 4,576 (11.57%).

**KEGG analysis**

Our data revealed that 9,056 unigenes were enriched in 130 pathways, and 4,818 (53.20%) unigenes annotated to ribosome of translation, carbon metabolism of global and overview, biosynthesis of amino acids of global and overview, plant-pathogen interaction of environmental adaptation, oxidative phosphorylation of energy metabolism, protein processing in the endoplasmic reticulum of folding, sorting and degradation, spliceosome of transcription, RNA transport of translation, purine metabolism of nucleotide metabolism and phenylpropanoid biosynthesis of other secondary metabolites. Ribosome 1,099 (12.14%) was the largest group, followed by carbon metabolism 652 (7.20%) and biosynthesis of amino acids 498 (5.50%), shown in Table 2.

**Changes in the expression and differentially expressed genes**

The maximum number of genes was expressed in the T4 group, the minimum in T0 (CK), and the coverage of the total expressed genes of the samples were 99.16% (Table 3). Figure S5 represents the log10FPKM value of each treatment is more significant than 2.5, and each sample's corresponding gene expression levels were not much different. Each gene expression's abundance value is higher than (T1) CK and T8 was highest.

The PCA two-dimensional coordinate diagram shows that the PCI explained 69.3% of the overall gene expression variance in all samples, and PCI and PC2 explain 91.6% of the overall variance (Figure 4). As shown in Figure S6, the CK and treated plants are classified into two groups. The treated groups, such as T2, T4 and T8, belong to one subgroup, and T24 is a separate subgroup. The gene expression relationship between T4 and T8 treatments was more similar. The differentially expressed genes was compared among the samples FDR ≤ 0.05, and the difference fold was more than two times. () The number of upregulated (red dots), downregulated (green dots), and no difference (black dots) genes are shown in Figure S7. In terms of up-regulated genes, with the increase in processing time, the number of up-regulated genes among samples showed an increasing trend; group CK-vs-T24 showed the highest (19408) number of up-regulated genes (Figure 5). The number of down-regulated genes was comparatively low among T2, T4 and T8 as compared to T24. The number of down-regulated genes were high, especially T4-vs-T24 (16596) and T8-vs-T24 (16414) group, respectively. The down-regulated genes between CK and each treatment initially decreased and then increased, among which CK-vs-T24 showed that the number of down-regulated genes reached 16,512. The total number of DEGs, CK-vs-T24, monitored the highest number of genes (35920) (Figure S7).

**Gene ontology (GO) and differentially expressed genes (DEGs)**

The DEGs were significantly enriched to 367 GO IDs, including 149 in a cellular component, 113 in molecular

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**TABLE 1** Annotation statistics of four databases.

| Total Unigenes | Nr    | Swissprot | KOG   | KEGG   | annotation genes | without annotation genes |
|----------------|-------|-----------|-------|--------|------------------|-------------------------|
| 62988          | 39441 | 28397     | 23451 | 15311  | 40784            | 22204                   |

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function, and 105 in the biological process. In the cellular component, GO:0016020 (membrane) occupied the first place in the five gene sets, and GO:0044425 (membrane part), GO:0030529 (intracellular ribonucleoprotein complex) also appeared in the top three positions of the five gene sets. In molecular function, GO:0003824 (catalytic activity) occupied the first number in five gene sets, with gene coverage greater than 60%. GO:0016491 (oxidoreductase activity) was significantly enriched in eight gene sets, and the gene coverage in each gene set of about 10%. GO:0005198 structural molecule activity was found in seven gene sets, and the gene coverage was smaller than GO:0016491 (oxidoreductase activity). In the biological process, the top three enriched GO IDs were found in three and mostly in only
one gene set. The more coverage rates in one gene set were GO:0044699 (single-organism process), greater than 50%, followed by GO:0044710 (single-organism metabolic process) and GO:0044763 (single-organism cellular function), greater than 30% (Figure 7; Table 4).

**KEGG analysis of differentially expressed genes**

The KEGG analysis results of all DEGs showed that the fifty-five significant pathways (42.31%) were enriched, and a total of
9056 genes distributed in ten gene sets (Figure 8; Table 5). ko03010 (Ribosome) was significantly enriched in eight gene sets and the pathway with the most significant number of genes in each gene set. ko00940 (Phenylpropanoid biosynthesis) and ko00190 (Oxidative phosphorylation) were found in seven and six gene sets, respectively. It can also be drawn from Table 5 that the three gene sets were significantly enriched, i.e., CK-vs-T24 (6024), T4-vs-T24 (4862) and T2-vs-T24 (4750). T4-vs-T24 (33 pathways) and low T2-vs-T8 (1 pathway) were enriched considerably.

**Trend analysis of expressed genes**

All expressed genes were analyzed by Short Time-series Expression Miner software and corrected by FDR. With Q value ≤ 0.05 as the threshold, a total of 53683 trend genes, 20 trend lines, and 6 significant trend lines were observed (Figure S8). In trend profile 18, a total of 9,548 genes, the gene expression trend showed a significant upward trend within 0-4 h in response to PEG-6000 (25%) stressed condition (Figure S9B). In trend profile 0, a total of 7,207 genes were found, and the trend was different to profile 19. The gene expression showed a continuous downregulated pattern from 0 - 24 h (Figure S9C). A total of 5,195 genes were found in profile 5; the gene expression pattern showed a significant decrease from 0-2 h followed by an increase in 2-4 h, it remained stable at 4-8 h, and decreased again after 8 h (Figure S9D). In profile 16 (4140 genes) was similar to profile18 with significantly increasing trend within 0-4 h, slight reduction between 4 and 8 h, and downregulated after 8 h (Figure S9E). A total of 2,477 genes were observed in profile 2 and similar to profile 5, with significant decrease from 0-2 h, relatively stable in the following 2-4 h, increase in 4-8 h and decline again after 8 h (Figure S9F).

**Trend genes GO enrichment analysis**

The results of GO enrichment analysis of all trend genes showed a total of 2,986 GO IDs, with 20 significantly enriched, including 13 in a cellular component, 7 in molecular functions, and 0 in the biological process (Figure 9; Table 6). The GO enrichment analysis of 6 significant trends showed the cellular component, profiles 0

| Pathway ID | KEGG_B_class | Pathway | Unigene Count | Unigene Ratio (%) |
|------------|--------------|---------|---------------|-------------------|
| ko03010    | Translation  | Ribosome| 1099          | 12.14             |
| ko01200    | Global and Overview | Carbon metabolism | 652              | 7.20              |
| ko01230    | Global and Overview | Biosynthesis of amino acids | 498              | 5.50              |
| ko04626    | Environmental adaptation | Plant-pathogen interaction | 482              | 5.32              |
| ko00190    | Energy metabolism | Oxidative phosphorylation | 444              | 4.90              |
| ko04141    | Folding, sorting, and degradation | Protein processing in endoplasmic reticulum | 402              | 4.44              |
| ko03040    | Spliceosome | Spliceosome | 330              | 3.64              |
| ko03013    | Translation | RNA transport | 314              | 3.47              |
| ko00230    | Nucleotide metabolism | Purine metabolism | 300              | 3.31              |
| ko00940    | Biosynthesis of other secondary metabolites | Phenylpropanoid biosynthesis | 297              | 3.28              |

**TABLE 3** Genes expression statistics of each sample (total reference genes 62988).

| Sample | genes Num | Ratio (%) | All samples genes | Ratio (%) |
|--------|-----------|-----------|-------------------|-----------|
| CK     | 55691     | 88.42     | 62461             | 99.16     |
| T2     | 57455     | 91.22     |                   |           |
| T4     | 56237     | 89.28     |                   |           |
| T8     | 59319     | 94.18     |                   |           |
| T24    | 58583     | 93.01     |                   |           |
FIGURE 4
PCA two-dimensional coordinate diagram of six samples.

FIGURE 5
Statistics of differential genes between samples.
and 19 were enriched with the most GO IDs, both of which were 22, while profile 2 was 0. GO:0044425 (membrane part) and GO:0031224 (an intrinsic membrane component) were enriched in 3 trend sets. In molecular function, profile 18 was found to be enriched to the most GO IDs (32), and GO:0016491 (oxidoreductase activity) and GO:0005198 (structural molecule activity) were found in 2 trend sets. GO: 0005488 (binding) and GO: 0003824 (catalytic activity) were found in profiles 18 and 19 with different genes. In biological process, profiles 5, 18, and 19 were enriched with more GO IDs, i.e., 194, 87 and 73, respectively. GO:0008152 (metabolic process) and GO:0071704 (organic substance

FIGURE 6
Heatmap of 12 genes expression.

FIGURE 7
Significant GO enrichment analysis of genes in roots tips of S. spontaneum plants in response to drought stress. The number of upregulated genes identified in each comparison of specific time intervals.
TABLE 4 Significant GO and top three enriched genes in different gene sets.

| Gene set | Cellular component | Molecular function | Biological process |
|----------|--------------------|--------------------|-------------------|
| CK-vs-T2  | GO:0044391,GO:0005840,GO:0030312,GO:0005492,GO:0025973,GO:0003700,GO:0005776,GO:0011100,GO:00016202 | GO:0046906,GO:0005198,GO:0016491,GO:0016757,GO:0016769,GO:0008417,GO:0016705 | GO:0045229,GO:0071554,GO:0071555,GO:0071669,GO:0010398,GO:000272,GO:0016052 |  |
|          | 1. GO:0081620 membrane 1659(25.5%) | 1. GO:0016491 oxidoreductase activity 8559(9.3%) | 1. GO:0006811 ion transport 413(5.48%) |  |
|          | 2. GO:0044425 membrane part 1410(19.34%) | 2. GO:0016682 GO:0031127 GO:0004533 | 2. GO:0045229 external encapsulating structure organization 225(2.99%) |  |
|          | 3. GO:0031224 intrinsic component of membrane 1300(17.84%) | 3. GO:0005198 structural molecule activity 495(5.8%) | 3. GO:0071554 cell wall organization or biogenesis 156(2.07%) |  |
| CK-vs-T4  | GO:0044391,GO:0005840,GO:0030312,GO:0005492,GO:0025973,GO:0003700,GO:0005776,GO:0011100,GO:00016202 | GO:0046906,GO:0005198,GO:0016757,GO:0016769,GO:0008417,GO:0016705 | GO:0045229,GO:0071554,GO:0071555,GO:0009808 |  |
|          | 1. GO:0044425 membrane part 1495(18.81%) | 1. GO:0003824 catalytic activity 626(62.67%) | 1. GO:0005397 polysaccharide metabolic process 300(3.59%) |  |
|          | 2. GO:0031224 intrinsic component of membrane 1365(17.17%) | 2. GO:0016491 oxidoreductase activity 897(9.33%) | 2. GO:0045229 external encapsulating structure organization 236(2.82%) |  |
|          | 3. GO:0030529 intracellular ribonucleoprotein complex 607(7.64%) | 3. GO:0005198 structural molecule activity 494(5.14%) | 3. GO:0071554 cell wall organization or biogenesis 182(2.05%) |  |
| CK-vs-T3  | GO:0030529,GO:001990904,GO:0005840,GO:0044391,GO:0005395,GO:0015934,GO:00071944,GO:00044425,GO:0005576 | GO:0016491,GO:0016757,GO:0016682 | GO:0000197,GO:0016757,GO:0016682 |  |
|          | 1. GO:0030529 intracellular ribonucleoprotein complex 777(9.24%) | 1. GO:0016491 oxidoreductase activity 943(9.41%) | 1. GO:0010467 gene expression 981(11.06%) |  |
|          | 2. GO:001990904 ribonucleoprotein complex 774(9.24%) | 2. GO:0005198 structural molecule activity 8258(9.24%) | 2. GO:0045229 external encapsulating structure organization 252(2.84%) |  |
|          | 3. GO:0034232 intracellular non-membrane-bounded organelle 641(7.65%) | 3. GO:0016491 transferase activity, transferring glycosyl groups 387(3.86%) | 3. GO:0071554 cell wall organization or biogenesis 182(2.05%) |  |
| CK-vs-T3a | GO:0030529,GO:001990904,GO:0005840,GO:0044391,GO:0005395,GO:0015934,GO:00071944,GO:00044425,GO:0005576,GO:0004322,GO:0032991 | GO:0016491,GO:0016757,GO:0016682 | GO:0000197,GO:0016757,GO:0016682 |  |
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|          | 3. GO:0044425 intracellular non-membrane-bounded organelle 641(7.65%) | 3. GO:0016491 transferase activity, transferring glycosyl groups 387(3.86%) | 3. GO:0071554 cell wall organization or biogenesis 182(2.05%) |  |

(Continued)
| Gene set | Cellular component | Molecular function | Biological process |
|----------|-------------------|-------------------|-------------------|
| T8-vs-T24 (41) | GO:003529,GO:01990904,GO:0005840,GO:00032991,GO:0005576,GO:0044391,GO:00199904,GO:0000785,GO:0000109,GO:0071944,GO:0003312,GO:00015935,GO:1990391 | 1. GO:0005198 structural molecule activity 454(6.37%) 2. GO:0004518 nuclease activity 106(1.49%) 3. GO:0016682 oxidoreductase activity, acting on diphenols and related substances as donors, oxygen as acceptor 23(0.32%) | 1. GO:0006807 nitrogen compound metabolic process 1591(25.01%) 2. GO:0006725 cellular aromatic compound metabolic process 1415(22.24%) 3. GO:0034641 cellular nitrogen compound metabolic process 1407(22.12%) |
| T8-vs-T4 (52) | GO:000586,GO:001620,GO:0015630,GO:0000793,GO:0044328,GO:00043232,GO:0000312,GO:0005739,GO:0004425,GO:0003124,GO:0005737,GO:0004422,GO:0004446,GO:0071944 | 1. GO:000691 oxidoreductase activity 400(10.8%) 2. GO:0005198 structural molecule activity 259(7.8%) 3. GO:004696 tetrapyrrole binding 136(3.67%) | 1. GO:0010038 response to metal ion 97(1.12%) |
| T8-vs-T4 (28) | GO:0016402,GO:0005576,GO:0044430,GO:0015630,GO:0005875,GO:0008586,GO:0003312,GO:0005739,GO:0004425 | 1. GO:000691 oxidoreductase activity 400(10.8%) 2. GO:0005198 structural molecule activity 259(7.8%) 3. GO:004696 tetrapyrrole binding 136(3.67%) | 1. GO:0010038 response to metal ion 97(1.12%) |
| T8-vs-T24 (31.84%) | | | 2. GO:004741 single-organism metabolic process 2760(31.83%) 3. GO:00032991 macromolecular complex 556(18.12%) 3. GO:003529 intracellular ribonucleoprotein complex 341(11.11%) 3. GO:19990904 ribonucleoprotein complex 341(11.11%) 3. GO:000586,GO:001620,GO:0015630,GO:0000793,GO:0044328,GO:00043232,GO:0000312,GO:0005739,GO:0004425,GO:0003124,GO:0005737,GO:0004422,GO:0004446,GO:0071944 | 1. GO:0010038 response to metal ion 97(1.12%) | 2. GO:004741 single-organism metabolic process 2760(31.83%) 3. GO:00032991 macromolecular complex 556(18.12%) 3. GO:003529 intracellular ribonucleoprotein complex 341(11.11%) 3. GO:19990904 ribonucleoprotein complex 341(11.11%) |
metabolic process) have a large number of genes, which are enriched in profiles 18 and 19 (Figure 10; Table 7).

**Trend genes KEGG enrichment analysis**

KEGG enrichment analysis was performed on all trend genes, and a total of 19 KEGG B class pathways and 130 KEGG pathways were observed (Figure 11). Pathway-related genes account for more percentage in the distribution of each trend gene set, with carbon metabolism (25.14%), translation (21.35%), and global and overview accounting for 17.36% (Figure 12A). More genes were significantly enriched in the pathways such as the ribosome (ko030101042; 13.19%), carbon metabolism (ko01200596; 7.55%), biosynthesis of amino acids (ko01230454; 5.75%), as shown in Figure 12B. KEGG analysis of 6 significant trends showed 42 effective pathways were enriched, and 5,630 DEGs were distributed in 6 trend sets (Table 8). The most important pathways and DEGs were enriched in profile 19. Ribosome (ko03010) pathway was found most enriched genes in profiles 0, 2 and 19.

**Gene validation**

Up-regulated differentially expressed genes, such as ABA response component binding factor, phosphatidylinositol phosphate 5-kinase, snRK2, glutamine synthetase, 90 kDa β-heat shock protein, pyrroline-5-carboxylate reductase, glutathione synthetase, serine/threonine-protein kinase, glutathione reductase, calmodulin, phosphoinositide 4-kinase A, and ETR were selected to assess the transcriptomic
| Gene set | Significant pathway/total pathway | DEGs/All genes with pathway annotation | Significant Pathway ID (Pathway, DEGs genes, Q value) |
|----------|----------------------------------|--------------------------------------|-------------------------------------------------|
| CK-vs-T2 | 8/128                            | 3405/9056                            | ko03010(Ribosome, 594 (17.44%), 0.000000)     |
|          |                                  |                                      | ko00940(Phenylpropanoid biosynthesis,180 (5.29%), 0.000000) |
|          |                                  |                                      | ko00480(Glutathione metabolism,125 (3.61%), 0.000030) |
|          |                                  |                                      | ko04075(Plant hormone signal transduction,131 (3.85%), 0.019840) |
|          |                                  |                                      | ko09941(Franoloid biosynthesis,34 (1%), 0.022968) |
|          |                                  |                                      | ko00502(Galactose metabolism),152 (4.16%), 0.035416) |
|          |                                  |                                      | ko00500(Starch and sucrose metabolism,125 (3.67%), 0.047087) |
|          |                                  |                                      | ko01190(Oxidative phosphorylation,195 (5.73%), 0.048035) |
| CK-vs-T4 | 8/127                            | 3652/9056                            | ko00940(Phenylpropanoid biosynthesis,201 (5.5%), 0.000000) |
|          |                                  |                                      | ko03010(Ribosome,591 (16.18%), 0.000000)        |
|          |                                  |                                      | ko00480(Glutathione metabolism,141 (3.86%), 0.000000) |
|          |                                  |                                      | ko04075(Plant hormone signal transduction,148 (4.05%), 0.000216) |
|          |                                  |                                      | ko00508(Starch and sucrose metabolism,144 (3.94%), 0.000338) |
|          |                                  |                                      | ko01196(Photosynthesis - antenna proteins,17 (0.47%), 0.009984) |
|          |                                  |                                      | ko00941(Franoloid biosynthesis,36 (0.99%), 0.011822) |
|          |                                  |                                      | ko0051(Other types of O-glycan biosynthesis, 12 (0.33%), 0.031494) |
| CK-vs-T8 | 3/128                            | 4330/9056                            | ko03010(Ribosome,800 (18.48%), 0.000000)       |
|          |                                  |                                      | ko00940(Phenylpropanoid biosynthesis,19 (4.57%), 0.0000000) |
|          |                                  |                                      | ko00480(Glutathione metabolism,146 (3.37%), 0.000058) |
| CK-vs- T24 | 23/130                         | 6024/9056                            | ko03010(Ribosome,944 (15.67%), 0.000000)       |
|          |                                  |                                      | ko00940(Phenylpropanoid biosynthesis,255 (4.23%), 0.0000000) |
|          |                                  |                                      | ko00190(Oxidative phosphorylation,352 (5.84%), 0.000000) |
|          |                                  |                                      | ko01200(Carbon metabolism,493 (8.18%), 0.000000)  |
|          |                                  |                                      | ko00280(Va1ene, leucine and isoleucine degradation,122 (2.03%), 0.000271) |
|          |                                  |                                      | ko00640(Propanate metabolism,80 (1.33%), 0.000796)  |
|          |                                  |                                      | ko04145(Phagosome,151 (2.51%), 0.001541)        |
|          |                                  |                                      | ko00620(Pyruvate metabolism,155 (2.57%), 0.002733)  |
|          |                                  |                                      | ko00711(Fatty acid degradation,96 (1.59%), 0.006303) |
|          |                                  |                                      | ko01230(Biosynthesis of amino acids,365 (6.06%), 0.006303) |
|          |                                  |                                      | ko00910(Glycolysis/Gluconeogenesis,221 (3.67%), 0.007751) |
|          |                                  |                                      | ko00330(Arginine and proline metabolism,88 (1.46%), 0.007751) |
|          |                                  |                                      | ko00250(Acine, aspartate and glutamate metabolism,123 (2.04%), 0.001146) |
|          |                                  |                                      | ko00950(Quinoline alkaloid biosynthesis,41 (0.68%), 0.018554) |
|          |                                  |                                      | ko00270(Cysteine and methionine metabolism,154 (2.56%), 0.002575) |
| T2-vs- T8 | 6/126                            | 1820/9056                            | ko01212(Fatty acid metabolism,139 (2.31%), 0.038864) |
|          |                                  |                                      | ko00380(Tryptophan metabolism,60 (1%), 0.041102) |
|          |                                  |                                      | ko03010(Ribosome,283 (15.55%), 0.000888)        |
|          |                                  |                                      | ko00190(Oxidative phosphorylation,125 (6.87%), 0.001171) |
|          |                                  |                                      | ko00630(Oxidative phosphorylation,125 (6.87%), 0.001171) |
|          |                                  |                                      | ko03440(Homologous recombination,38 (2.09%), 0.022462) |
|          |                                  |                                      | ko00910(Nitrogen metabolism,24 (1.32%), 0.034340) |
|          |                                  |                                      | ko01196(Photosynthesis - antenna proteins,11 (0.6%), 0.034340) |
| T2-vs-T24 | 1/127                            | 3225/9056                            | ko03010(Ribosome,582 (18.05%), 0.000000)       |
|          |                                  |                                      | ko03019(Ribosome,729 (15.35%), 0.000000)        |
|          |                                  |                                      | ko01200(Carbon metabolism,419 (8.82%), 0.000000)  |
|          |                                  |                                      | ko00250(Acine, aspartate and glutamate metabolism,121 (2.53%), 0.000000) |
|          |                                  |                                      | ko00940(Phenylpropanoid biosynthesis,1 (4.19%), 0.000000) |
|          |                                  |                                      | ko02820(Va1ene, leucine and isoleucine degradation,108 (2.27%), 0.000000) |
TABLE 5 Continued

| Gene set | Significant pathway/total pathway | DEGs/All genes with pathway annotation | Significant Pathway ID (Pathway, DEGs genes, Q value) |
|----------|----------------------------------|---------------------------------------|-----------------------------------------------------|
| ko00710  | (Carbon fixation in photosynthetic organisms, 124 (2.61%), 0.000094) | ko00190 (Oxidative phosphorylation, 278 (5.85%), 0.000112) | ko00640 (Propionate metabolism, 70 (1.47%), 0.000145) |
| ko01212  | (Fatty acid metabolism, 125 (2.63%), 0.000256) | ko009051 (Fructose and mannose metabolism, 49 (1.87%), 0.000452) | ko01010 (Steroid biosynthesis, 53 (1.12%), 0.000596) |
| ko00190  | (Glycolysis/Gluconeogenesis, 1863 (9.2%), 0.000989) | ko00303 (Pentose phosphate pathway, 87 (1.83%), 0.001539) | ko00330 (Arginine and proline metabolism, 76 (1.6%), 0.001539) |
| ko00410  | (beta-Alanine metabolism, 63 (1.33%), 0.001472) | ko01040 (Biosynthesis of unsaturated fatty acids, 66 (1.39%), 0.001472) | ko00711 (Fatty acid degradation, 80 (1.68%), 0.005390) |
| ko00360  | (Phenylalanine metabolism, 61 (1.28%), 0.006538) | ko00520 (Amino sugar and nucleotide sugar metabolism, 147 (3.0%), 0.0066) | T4-vs-T8 11/127 2073/9056 ko03010 (Ribosome, 369 (17.8%), 0.000000) |
| ko01230  | (Biosynthesis of amino acids, 295 (6.21%), 0.006617) | ko00250 (Alanine, aspartate and glutamate metabolism, 55 (2.65%), 0.008438) | ko01200 (Carbon metabolism, 204 (9.84%), 0.000012) |
| ko00950  | (Serine and threonine metabolism, 36 (1.76%), 0.006675) | ko00480 (Glutathione metabolism, 144 (3.03%), 0.008974) | ko0190 (Oxidative phosphorylation, 142 (6.85%), 0.000175) |
| ko00350  | (Tyrosine metabolism, 66 (1.91%), 0.001054) | ko00902 (Citrate cycle (TCA cycle), 66 (3.18%), 0.000188) | ko00200 (Citrate cycle (TCA cycle), 110 (2.32%), 0.017396) |
| ko00415  | (Phagosome, 119 (2.51%), 0.018060) | ko00630 (Glyoxylate and dicarboxylate metabolism, 119 (2.51%), 0.028353) | ko00630 (Glyoxylate and dicarboxylate metabolism, 119 (2.51%), 0.028353) |
| ko00061  | (Fatty acid biosynthesis, 63 (1.39%), 0.028353) | ko00220 (Arginine biosynthesis, 50 (1.05%), 0.039841) | ko00220 (Arginine biosynthesis, 50 (1.05%), 0.039841) |
| ko00220  | (Arginine biosynthesis, 50 (1.05%), 0.039841) | ko01212 (Fatty acid metabolism, 134 (2.76%), 0.000003) | ko00220 (Arginine biosynthesis, 50 (1.05%), 0.039841) |
| ko00330  | (Arginine and proline metabolism, 83 (1.71%), 0.000020) | ko00620 (Pyruvate metabolism, 135 (2.78%), 0.000243) | ko00620 (Pyruvate metabolism, 135 (2.78%), 0.000243) |
| ko00640  | (Propionate metabolism, 72 (1.48%), 0.000061) | ko00520 (Biosynthesis of amino acids, 307 (6.31%), 0.001169) | ko00520 (Biosynthesis of amino acids, 307 (6.31%), 0.001169) |
| ko00100  | (Steroid biosynthesis, 52 (1.07%), 0.002437) | ko01010 (Steroid biosynthesis, 52 (1.07%), 0.002437) | ko00520 (Biosynthesis of unsaturated fatty acids, 67 (1.38%), 0.003973) |

(Continued)
sequencing results with qRT-PCR. In particular, the ABA response component binding factor and snRK2 are associated with ABA metabolism. The results showed that most genes were significantly up-regulated within 8 h during drought stress. Between 8 and 24 h, 9 genes were found up-regulated and 3 down-regulated (Figure 13). The 12 genes were clustered into two categories, among which Unigene0036685, Unigene0033103 and Unigene0042703 belong to one category (Figure 6).

**Table 5 Continued**

| Gene set | Significant pathway/total pathway | DEGs/All genes with pathway annotation | Significant Pathway ID (Pathway, DEGs genes, Q value) |
|----------|----------------------------------|---------------------------------------|--------------------------------------------------|
| T8-vs-T24 | 28/127                           | 46629/9056                            | ko04145(Phagosome,124(2.55%),0.006474)             |
|          |                                  |                                       | ko00400(Phenylalanine, tyrosine and tryptophan biosynthesis,53 (1.99%),0.007411) |
|          |                                  |                                       | ko00564(Glycerophospholipid metabolism,119(2.45%),0.009043) |
|          |                                  |                                       | ko00565(Ether lipid metabolism,370(7.6%),0.014621) |
|          |                                  |                                       | ko00351(Fructose and mannose metabolism,84(1.73%),0.015415) |
|          |                                  |                                       | ko00270(Cysteine and methionine metabolism,129(2.65%),0.017672) |
|          |                                  |                                       | ko00592(alpha-Linolenic acid metabolism,64(1.32%),0.022818) |
|          |                                  |                                       | ko00903(Limonene and pinene degradation,11(0.23%),0.031665) |
|          |                                  |                                       | ko00601(Sphingolipid metabolism,47(0.97%),0.031781) |
|          |                                  |                                       | ko00040(Pentose and glucuronic acid interconversions,53 (1.99%),0.032424) |
|          |                                  |                                       | ko01210(2-Oxocarboxylic acid metabolism,91(1.87%),0.032487) |
|          |                                  |                                       | ko00380(Tryptophan metabolism,51 (1.05%),0.033757) |
|          |                                  |                                       | ko00410(beta-Alanine metabolism,60 (1.23%),0.033757) |
|          |                                  |                                       | ko00561(Glycerolipid metabolism,72 (1.48%),0.042839) |
|          |                                  |                                       | ko00430(Taurine and hypotaurine metabolism,22 (0.45%),0.046042) |
|          |                                  |                                       | ko00071(Fatty acid degradation,94(2.02%),0.000000) |
|          |                                  |                                       | ko00280(Valine, leucine and isoleucine degradation,112 (2.4%),0.000000) |
|          |                                  |                                       | ko01200(Carbon metabolism,408(8.75%),0.000000) |
|          |                                  |                                       | ko00250(Alanine, aspartate and glutamate metabolism,115 (2.47%),0.000001) |
|          |                                  |                                       | ko01212(Fatty acid metabolism,130 (2.79%),0.000003) |
|          |                                  |                                       | ko00640(Propanoate metabolism,71 (1.52%),0.000028) |
|          |                                  |                                       | ko00650(Butanoate metabolism,52 (1.12%),0.000073) |
|          |                                  |                                       | ko00010(Glyceraldehyde/3-phosphoglycerate,187 (4.03%),0.000214) |
|          |                                  |                                       | ko00061(Fatty acid biosynthesis,69 (1.48%),0.000479) |
|          |                                  |                                       | ko00101(S teroid biosynthesis,52 (1.12%),0.000958) |
|          |                                  |                                       | ko00620(Pyruvate metabolism,128 (2.75%),0.001224) |
|          |                                  |                                       | ko00940(Phenylpropanoid biosynthesis,184 (3.95%),0.001491) |
|          |                                  |                                       | ko00400(Phenylalanine, tyrosine and tryptophan biosynthesis,53 (1.14%),0.003082) |
|          |                                  |                                       | ko00790(Folate biosynthesis,20 (0.43%),0.003608) |
|          |                                  |                                       | ko01230(Biosynthesis of amino acids,292 (6.26%),0.004906) |
|          |                                  |                                       | ko01220(Degradation of aromatic compounds,18(0.39%),0.009123) |
|          |                                  |                                       | ko00020(Citrate cycle (TCA cycle),110(2.36%),0.010722) |
|          |                                  |                                       | ko00520(Amino sugar and nucleotide sugar metabolism,143 (3.07%),0.014530) |
|          |                                  |                                       | ko00710(Carbon fixation in photosynthetic organisms,112 (2.4%),0.014530) |
|          |                                  |                                       | ko01040(Biosynthesis of unsaturated fatty acids,63(1.35%),0.014791) |
|          |                                  |                                       | ko00656(Ether lipid metabolism,36(0.77%),0.014894) |
|          |                                  |                                       | ko00330(Arginine and proline metabolism,71 (1.52%),0.014992) |
|          |                                  |                                       | ko00270(Cysteine and methionine metabolism,124(2.66%),0.023659) |
|          |                                  |                                       | ko00410(beta-Alanine metabolism,59(1.27%),0.024742) |
|          |                                  |                                       | ko00564(Glycerophospholipid metabolism,112(2.4%),0.038677) |
|          |                                  |                                       | ko00551(Glycosaminoglycan degradation,180(3.9%),0.038118) |
|          |                                  |                                       | ko00190(Phosphorylation,253 (5.43%),0.046100) |
|          |                                  |                                       | ko00310(Phosphorylation,49 (1.05%),0.047302) |

**Discussion**

Drought stress is a major limiting factor for proper plant growth and development, especially crop production (Liu et al., 2015; Verma et al., 2021; Verma et al., 2022). It affects various physiological and biochemical activities, i.e., photosynthetic CO₂ assimilation rate, chlorophyll synthesis, and nutritional metabolism (Li et al., 2011; Osakabe et al., 2014). Plants
Gene ontology numbers indicated in significant GO IDs in all trends.

- Ribosome (GO:0005840)
- Intracellular ribonucleoprotein complex (GO:0030529)
- Ribonucleoprotein complex (GO:1990904)
- Ribosomal subunit (GO:0044391)
- External encapsulating structure (GO:0030312)
- Non-membrane-bounded organelle (GO:0043228)
- Intracellular non-membrane-bounded organelle (GO:0043232)
- Extracellular region (GO:0005576)
- Microtubule cytoskeleton (GO:0015630)
- Mitochondrion (GO:0005739)
- Cell periphery (GO:0071944)
- Small ribosomal subunit (GO:0015935)
- Cytoskeleton (GO:0005856)
- Catalytic activity (GO:0003824)
- Structural molecule activity (GO:0005198)
- Oxidoreductase activity (GO:0016491)
- Tetrapyrrrole binding (GO:0046906)
- Transferase activity (GO:0016757)
- Hydrolase activity (GO:0016798)
- Oxidoreductase activity, acting on the aldehyde or oxo group of donors (GO:0016903)
rapidly accumulate small molecules that help adjust osmotic response to water deficient conditions (Verma et al., 2021). Reduction in cells’ water potential enhances cells’ efficiency in absorbing and retaining water. It also maintains the swelling pressure of cells and ensures the proper functioning of plant physiological processes (Blum, 2017). Plants have a physiological adaptive strategy to respond to variations in soil moisture (%) and resistance to drought-stress conditions (Farooq et al., 2009). Osmotic adjustment is an essential physiological adaptive strategy of plants subjected to stress (Verma et al., 2022).

Overproduction of ROS accumulation may cause oxidative injury at the cellular level, damage cell membranes, and lead to enzyme inactivation, degradation of protein, and cell ion disturbances (Tarchoune et al., 2010). Plants can eliminate the excess production of ROS in cells via enzymatic and non-enzymatic antioxidative systems (Rao and Chaitanya, 2016; Li et al., 2022).

The transcriptomic sequencing approaches are widely applied to assess plant metabolic pathways, identify new transcripts, enhance genome annotation, and screen for specific functional genes during stress conditions (Zhang et al., 2018; Felix, 2019). In the present study, there were significant changes in S. spontaneum transcript levels during drought stress. Pathway annotation revealed that the DEGs were mainly enriched in plant hormone signal transduction, alanine, aspartate, glutamate metabolism, protein processing in the endoplasmic reticulum, nitrogen metabolism, ABC transporters, plant-pathogen interaction, and pyrimidine metabolism. These findings verified the transcriptomic analysis results of sugarcane during osmotic stress (Santana et al., 2017). Most of the genes were significantly upregulated at 8 h after drought stress, suggesting that stress stimulated S. spontaneum sterols to protect cells and balance normal photosynthetic responses. Similarly, the transcriptome responses reported that the phosphoesterase gene was upregulated and significantly impacted sugarcane stressed plants. Plant responses begin with stress situations at the cellular level via activation of signal transduction pathways (Wang et al., 2020; Li et al., 2022).

The perception of water stress can trigger the activation of abscisic acid (ABA)-dependent and ABA-independent regulatory systems that govern drought-inducible gene expression (Shinozaki and Yamaguchi-Shinozaki, 2007). Genes in response to the drought-stress can be differentiated into two groups based on their functions (Shinozaki and Yamaguchi-Shinozaki, 1997; Yamaguchi-Shinozaki and Shinozaki, 2006). The first group of gene products are involved in protecting cells and regulating the signal transduction pathways of stress responses. LEA genes have been reported to protect cell structures from the effects of water loss (Bray, 1993). The LEA gene products are proposed to be located in the cytoplasm and have several features, such as hydrophilism, biased in amino acid composition, and lacking of Cys and Trp. They mainly function in the sequestration of ions, protection of other proteins or membranes, and renaturation of unfolded proteins (Bray, 1993).

Genes encoding heat shock proteins (HSPs) have also been identified to contribute drought-stress tolerance plants (Shinozaki and Yamaguchi-Shinozaki, 2007). In different plant organs of the Indian sugarcane variety (CoS 767), genes encoding HSPs are shown to be up-regulated in response to drought stress (Gupta et al., 2010). In addition, genes encoding
ribosomal proteins and putative disease-resistance proteins are also identified to be up-regulated in sugarcane plant organs subjected to drought stress conditions (Gupta et al., 2010). Like ribosomal protein and disease resistance protein, it is difficult to identify the regulation of genes encoding the major water channel proteins – aquaporins – in plant leaves during stress. Aquaporins are water transporter proteins essential in adjusting or maintaining the water status during adverse environmental variables (Luu and Maurel, 2005). Other gene products associated with signal transduction, such as lipid-transfer proteins and LHCB proteins, have also been reported to be induced by drought stress in commercial sugarcane varieties (Rodrigues et al., 2009; Rodrigues et al., 2011).

Our study agreed on the expression changes of some known gene families in S. spontaneum in response to polyethylene glycol-6000 stimulated drought stress. A total of 57,036,239
bases were assembled in the experiment, and 62,988 genes were obtained (Table S3). The unigenes have coverage distribution on reads of 1–1000 and above in *S. spontaneum*. Unigenes covering 11–100 reads were at 23216, followed by unigenes covering more than 1000 reads at 6431. Comprehensive analysis shows that the quality of the completed assembly was good. These possible novel genes were identified and required more experiments to explore. Further, their functions in water resistance in *S. spontaneum* are still unknown and need to be studied.

**Conclusion**

In conclusion, we performed the combination of transcriptomic sequencing and differential expression analyses to identify pathways and genes altered under drought stress. Several physiological traits showed various trends, and transcripts in various metabolic processes reacted rapidly and strongly in response to drought stress. Genes in pathways of phenylpropanoid biosynthesis and biosynthesis of amino acids

| Trend set | Significant GO N. and top three enriched genes (description, gene ratio, qvalue) | Biological Process |
|-----------|---------------------------------------------------------------------------------|--------------------|
| Profile 0 | 22 1.GO:0044425 membrane part 645 (20.02%) 0.000684 2.GO:0032991 macromolecular complex 615(19.09%) 0.000000 3.GO:0031224 intrinsic component of membrane 592(18.38%)0.000131 | 16 1.GO:0016491 oxidoreductase activity 353(9.79%) 0.015005 2.GO:0005198 structural molecule activity 343(9.52%) 0.000000 3.GO:0046906 tetrapyrrole binding 208(5.77%) 0.000000 | 42 1.GO:0010467 gene expression 392(12.07%) 0.000939 2.GO:0045229 external encapsulating structure organization 139(4.28%) 0.000000 3.GO:0005976 polysaccharide metabolic process 123(3.79%) 0.02604 |
| Profile 2 | 0 | 3 1.GO:0034062 RNA polymerase activity 16(1.34%) 0.013665 2.GO:0016682 oxidoreductase activity, acting on diphenols and related substances as donors, oxygen as acceptor 9(0.75%) 0.013665 3.GO:0016679 oxidoreductase activity, acting on diphenols and related substances as donors 9(0.75%) 0.013665 | 3 1.GO:0006968 phenylpropanoid metabolic process 151(4.68%) 0.047329 2.GO:0009808 lignin metabolic process 7 (0.68%) 0.047329 3.GO:0006796 phosphate-containing compound metabolic process 214(20.84%) 0.047329 |
| Profile 5 | 17 1.GO:0044425 membrane part 462 (20.5%) 0.00033 2.GO:0031224 intrinsic component of membrane 424(18.81%) 0.01134 3.GO:0044422 organelle part 415 (18.41%) 0.011410 | 17 1.GO:0097159 organic cyclic compound binding 1251(51.48%) 0.006976 2.GO:1901363 heterocyclic compound binding 1148(47.24%) 0.031124 3.GO:0036094 small molecule binding 785(32.3%) 0.000318 | 42 1.GO:0044763 single-organism cellular process 901(40.44%) 0.008002 2.GO:0044260 cellular macromolecule metabolic process 943(42.32%) 0.008138 3.GO:0019538 protein metabolic process 603 (27.06%) 0.01916 |
| Profile 16 | 22 1.GO:0033224 intrinsic component of membrane 287(20.08%) 0.000948 2.GO:0044425 membrane part 303 (21.2%) 0.015800 | 9 1.GO:0055215 transporter activity 108(6.75%) 0.037856 2.GO:0022857 transmembrane transporter activity 103(6.44%) 0.03675 3.GO:0036094 small molecule binding 785(32.3%) 0.000318 | 42 1.GO:0065007 biological regulation 366 (25.81%) 0.000113 2.GO:0057089 regulation of biological process 333(23.48%) 0.03428 3.GO:0044255 cellular lipid metabolic process 92(6.49%) 0.03773 |
| Profile 19 | 22 1.GO:0007537 cytoplasm 622(30.88%) 0.000000 2.GO:0044444 cytoplasmic part 603 (29.94%) 0.000000 3.GO:0032991 macromolecular complex 384(19.07%) 0.000000 | 15 1.GO:0003824 catalytic activity 1616(64.28%) 0.037703 2.GO:0016491 oxidoreductase activity 272(10.82%) 0.000266 3.GO:005198 structural molecule activity 1726(64.84%) 0.000000 | 42 1.GO:0008152 metabolic process 1830(81.59%) 0.000042 2.GO:0009987 cellular process 1730(77.13%) 0.000000 3.GO:0071704 organic substance metabolic process 1510 (67.32%) 0.000000 |

| Trend set | Significant GO N. and top three enriched genes (description, gene ratio, qvalue) | Biological Process |
|-----------|---------------------------------------------------------------------------------|--------------------|
| Profile 18 | 10 1.GO:0005623 cell1924(92.72%) 0.008543 2.GO:0044464 cell part 1924(92.72%) 0.000854 3.GO:0005622 intracellular 1894 (91.28%) 0.008543 | 32 1.GO:0005488 binding1926(72.65%) 0.000000 2.GO:0097159 organic cyclic compoundbinding 1454(54.85%) 0.000000 3.GO:1901363 heterocyclic compoundbinding 1246(47%) 0.021553 | 42 1.GO:0008152 metabolic process 1830(81.59%) 0.000042 2.GO:0009987 cellular process 1730(77.13%) 0.000000 3.GO:0071704 organic substance metabolic process 1510 (67.32%) 0.000000 |
| Profile 19 | 22 1.GO:00003291 macromolecular complex 384(19.07%) 0.000000 | 15 1.GO:0003824 catalytic activity 1616(64.28%) 0.037703 2.GO:0016491 oxidoreductase activity 272(10.82%) 0.000266 3.GO:005198 structural molecule activity 1726(64.84%) 0.000000 | 42 1.GO:0008152 metabolic process 1925(81.16%) 0.000042 2.GO:0009987 cellular process 1720(77.13%) 0.000000 3.GO:0071704 organic substance metabolic process 1568(66.1%) 0.000047 3.GO:006807 nitrogen compound metabolic process 623(26.26%) 0.002907 |

**TABLE 7** Significant GO Enrichment in six significant trends.
FIGURE 11
Pathway enrichment analysis rich factor heat map. NA, Not applicable.
FIGURE 12
Distribution pathway (A) in all profiles and pathway enrichment (B) of all genes (top 20).
**TABLE 8** Significant KEGG ID in six significant trends.

| Trend set | Significant pathway/Total pathway | DEGs/All genes with pathway annotation | Significant ko ID (Pathway, DEGs N., Q value) |
|-----------|-----------------------------------|----------------------------------------|---------------------------------------------|
| Profile0  | 4/120                             | 1394/9056                              | ko03010 (Ribosome, 393 (28.19%), 0.000000) |
|           |                                   |                                        | ko00940 (Phenylpropanoid biosynthesis, 121 (8.68%), 0.000000) |
|           |                                   |                                        | ko00460 (Cyanobacterial metabolism, 31 (2.22%), 0.000267) |
|           |                                   |                                        | ko00941 (Flavonoid biosynthesis, 20 (1.43%), 0.007527) |
| Profile2  | 4/99                              | 408/9056                               | ko03010 (RNA polymerase, 14 (3.43%), 0.000509) |
|           |                                   |                                        | ko04075 (Plant hormone signal transduction, 28 (6.86%), 0.002605) |
|           |                                   |                                        | ko00230 (Purine metabolism, 28 (6.86%), 0.006498) |
|           |                                   |                                        | ko03010 (Ribosome, 70 (17.16%), 0.036715) |
| Profile5  | 12/115                            | 875/9056                               | ko00510 (N-Glycan biosynthesis, 24 (2.74%), 0.000000) |
|           |                                   |                                        | ko03440 (Homologous recombination, 29 (3.31%), 0.0000054) |
|           |                                   |                                        | ko00563 (Glycosylphosphatidylinositol(GPI)-anchor biosynthesis, 12 (1.37%), 0.000166) |
|           |                                   |                                        | ko04626 (Plant-pathogen interaction, 76 (8.69%), 0.000266) |
|           |                                   |                                        | ko03430 (Mismatch repair, 19 (2.17%), 0.029908) |
|           |                                   |                                        | ko00562 (Inositol phosphate metabolism, 19 (2.17%), 0.029908) |
|           |                                   |                                        | ko04141 (Protein processing in endoplasmic reticulum, 37 (4.31%), 0.029908) |
|           |                                   |                                        | ko03410 (Base excision repair, 14 (1.6%), 0.029908) |
|           |                                   |                                        | ko03450 (Non-homologous end-joining, 5 (0.57%), 0.029908) |
|           |                                   |                                        | ko04070 (Phosphatidylinositol signaling system, 22 (2.51%), 0.029943) |
|           |                                   |                                        | ko06040 (Phenylalanine, tyrosine and tryptophan biosynthesis, 15 (1.69%), 0.029908) |

(Continued)
were greatly activated, and they may play important roles in stress tolerance in roots of *S. spontaneum*. Genes associated with ABA synthesis; ABA and solute transport were up-regulated during early drought stress in plant root tips. The differently expressed genes of 56237 (T4), 59319 (T8), and 58583 (T24), among which CK obtained the most significant number of expressed genes (35920) as compared to T24, with a total of 53683 trend genes. Our research is the first to explore the molecular mechanism and provide valuable information on differentially expressed metabolic pathways and genes of *S. spontaneum* in response to drought stress. This transcriptomic data will also stand as an excellent resource to explore drought stressed genes and assist in sustainable sugarcane improvement in years to come.

**Data availability statement**

The original contributions presented in the study are publicly available. This data can be found at NCBI, accession number PRJNA835339.

**Author contributions**

K-CW, C-MH, KV, and LX conduct the experiment. Z-ND, TP, and H-QC made statistical analyses. H-RH, H-QC, H-BL, and S-LJ participated in the analytical analysis. K-CW, C-MH, and KV wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.992755/full#supplementary-material

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