Differential gene expression analysis of the Coix transcriptome under PEG stress

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Abstract

Drought stress severely affects plant growth and crop yield. *Coix lachryma-jobi* L. (Coix) commonly known as Job’s tears, is a member of the grass family in the tribe Maydeae. To understand the transcriptome dynamics and explore the important drought resistant genes during drought stress in coix seedlings, in this study, YiLiao 5, with good resistant drought was taken as the experimental material. The results showed 92,865 unigenes were detected and the average gene length was 737.85 bp, the N50 was 1,26bp. A comparison of the treatment and the control samples revealed that 1,128 differentially expressed genes (DEGs) were expressed, including 662 and 466 genes that were up-and down-regulated, respectively. According to the Gene Ontology (GO) database, among biological processes the metabolic process group was the largest group (14,908 genes, 24.28%) and contained high frequency of differentially expressed genes (352 genes, 24.22%). The DEGs are involved in 170 metabolic pathways. The plant hormone signal transduction and starch and sucrose metabolism were relatively obvious. Some DEGs and proteins were found, such as response to abscisic acid genes, 9-cis-epoxycarotenoid dioxygenase, some Transcription factors (TFs), protein serine/threonine phosphatase, late embryogenesis abundant protein (LEA). Eight genes analyzed by Quantitative Real-time PCR (qRT-PCR) confirmed the transcriptome results. Overall, this study had done the transcriptome sequencing and established a genomics database of Coix for the first time. Meanwhile the results also provided a molecular basis and theoretical resource for mechanistic studies on drought resistance in Coix.

Keywords: drought stress, coix, transcriptome, differential gene expression analysis, Gene Ontology, Kyoto Encyclopedia of Genes and Genomes

Introduction

Among various environmental stresses, drought is one of the most important factors limiting the productivity and distribution of plants (Pastori et al, 2002). Drought stress not only intricates plant morphological, physiological, and biochemical changes but also impacts a network of plant gene expression mechanisms (Ashraf and Harris, 2013). Previous studies revealed the effects of different drought stresses on plants, for examples, when plants are subjected to drought stress, various active oxygen species are generated, such as superoxide, H2O2 and hydroxyl radicals, which cause oxidative damage in plants (Smirnoff, 1993; Trippi et al, 1989). Organic acids have also been implicated in the biochemical response to drought stress. For instance, malic acid increased in abundance under mild periods of water stress (Seki et al, 2007). Meanwhile, drought stress can also impact a network of plant gene expression, which genes can be involved in different levels of metabolism, signal transduction, osmotic regulation, stress response and gene regulation (Seki et al, 2002; Rabbani et al, 2003; Umezawa et al, 2006). Therefore, drought has a crucial impact on plant growth and development.

Coix commonly known as Job’s tears, is a member of the grass family in the tribe Maydeae. It has been cultivated in China for thousands of years (Arora, 1977; Feng et al, 2005), which is an annual crop that has long been consumed as both an herbal medicine and a nourishing food. According Conventional wisdom may suggest that seeds of Coix contain anti-inflammatory, stomachic, diuretic, and antispastic activities in vivo (Kim et al, 1977; Wu et al, 2007). Modern scientific studies demonstrate that there are many kinds of pharmacological and physiological effects on Coix seed, including antitumor (Lu et al, 2011), anti-inflammatory (Chen et al, 2011), antiallergic (Chen et al, 2010). Coix is distributed widely in China, Thailand, Burma, and Japan, as well as is planted in most of provinces of China. Coix is a nourishing food including nutrients with 16.2% proteins, 4.65% lipids, 79.17% carbohydrates, and a small quantity of vitamin B1 (Kim et al, 2007). Especially, the high protein nutrient of Job’s tears is more and more concerned by people, especially in Japan. While, Coix is a crop of being keen on water and tolerance to humidity, which habits and characteristics are similar to rice. Therefore, drought is more sensitive than other cereal crops for Coix. Despite its high-
er the value of medicine and food, very little is known about the genetic and molecular function of this species, especially the mechanisms to better understand how Coix adapts to drought conditions.

Over the past several years, high throughput sequence analysis and dramatically improved the speed and efficiency of gene mining, which is an efficient and powerful method for transcriptome analysis. This approach has been widely applied to characterize transcriptomes of non-model plants under adverse stress, such as rice, black cottonwood, soybean and rape (Huang et al., 2014; Tang et al., 2015; Rodrigues et al., 2015; Wang et al., 2015). However, the approach has not been applied to Coix under drought stress up to now. In this study, we applied Illumina sequencing technology to characterize transcriptome of Coix under drought stress. The unigenes being generated by de novo assembly were annotated functionally and analyzed according to their gene GO and KEGG metabolic pathways etc. These results will provide a foundation for the research of gene expression, genomics and functional genomics in Coix. In addition, our analysis can also provide an insight into the transcriptional responses of Coix to drought stress and is expected to serve as valuable transcriptome resources for gramineous plants. Meanwhile, such types of databases can provide an useful data for drought resistance study in Coix.

Materials and Methods

Plant material, growth condition, and stress treatment

The experiment was conducted in plant culture room in Agricultural Department of Heilongjiang Bayi Agricultural University. Yiliao 5, with good resistant drought was obtained from Coix Research Center of Heilongjiang Bayi Agricultural University, Heilongjiang Province, China. Coix seeds were sterilized with 10% NaClO solution for 30 min, rinsed thoroughly three times with distilled water and allowed to germinate in the dark. After 96 h, germinated seedlings were retransferred to Hoagland’s nutrient solution, which seedlings were grown in a greenhouse at 25-30°C with a 13 h/11 h (day/night) photoperiod, and 60-70% humidity. The nutrient solution was renewed every other day, and the pH was maintained at 5.8. When the seedlings grew up to two leaves one, seedlings were grown in a greenhouse at 25-30°C with a 13 h/11 h (day/night) photoperiod, and 60-70% humidity. The nutrient solution was renewed every other day, and the pH was maintained at 5.8. When the seedlings grew up to two leaves one, seedlings were transferred to 25% PEG 6000 solution (-0.8 Mpa) by immersing the roots in the solution. A batch of 30 plants was divided into two groups for drought stress experiments: The Normal group (CK) consisting of 15 plants that were immersed into Hoagland’s nutrient solution, and a PEG-treated drought group (PEG) consisting of 15 plants that were immersed into 25% PEG solution. The leaves were collected when the seedlings from PEG present a certain appearance of wilting, which were frozen in liquid nitrogen, and stored at –80°C for later use.

Extraction and testing of RNA and cDNA library construction, sequencing

Mixed samples of 5 leaves from the above two groups (CK and PEG) were used for RNA extraction (Invitrogen Trizol Reagent of RNA extraction kit 15596018). A cDNA library was constructed using a NEB kit for library preparation and sequenced using an Illumina HiSeq2500 (Beijing Biomarker Technologies Co.)

Transcriptome assembly and functional annotation of genes

Sample sequences were assembled to indirectly deepen the sequencing results and obtain a more complete transcriptome dataset. High-quality sequencing data was assembled using Trinity software (Grabherr et al., 2011). The transcript sequences were then recognized in each fragment collection using a De Bruijin graph-based method and sequencing reads data (Grabherr et al., 2011; Haas et al., 2013).

Unigene sequences were compared in the following databases using BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain annotation information for unigenes. Based on BLAST parameters, those unigenes with an E-value less than 10^{-5} were selected. Sequences of selected unigenes were aligned within databases (Nr, COG, Swiss-Prot, KEGG, GO etc).

Calculating unigenes expression level and detecting differentially expressed genes

Sequencing reads of each sample were compared with the unigene database using the Bowtie method (Langmead et al., 2009), and then processed with RSEM (LI et al., 2011) to estimate the expression levels with the FPKM value.

Differentially expressed gene sets for the two samples were acquired by differential expression analysis using EBSeq (Leng et al., 2013). During this screening process, FDR < 0.01 and Fold Change (FC) ≥2 were used as screening criteria. The selected differentially expressed genes were then hierarchically clustered (Murtagh et al., 2014).

Enrichment analysis of differentially expressed genes

The differentially expressed genes after being screened were mainly analyzed by GO function and KEGG pathway enrichment analysis. We extracted GO annotations of differential expression genes, mapped GO function to the corresponding secondary features based on Unigene’s GO annotation (Ye et al., 2006) and then drew the histogram, The KEGG Pathway enrichment analysis was implemented via KOBAS2.0 (http://kobas.cbi.pku.edu.cn/home.do; Xie et al., 2011).

Quantitative real time PCR verification

Some specific genes, selected according to RNA-Seq data were selected to analyze the differential expression via the qRT-PCR (Quantitative Real-time PCR confirmation).
Primers were designed using Gene Runner software (Hastings Software, New York, USA). The sequences and the annealing temperature (TA) of each primer were shown in the Table 5. Total RNA (6 μg) from each selected gene was treated with DNAse I (Invitrogen) and translated into first strand cDNA, which was synthesized with TranscriptOne-Step gDNA Removal and cDNA Synthesis SuperMix (TransGene Biotech) and then stored at -20°C for subsequent analysis. Each PCR reaction contained 10 μL mixture, consisting of 1 μl cDNA, 5μl of SYBR Green Premix Ex Taq II, 0.2 μl of ROX Reference Dye II, and 1μl of the forward and reverse primers. All qRT-PCRs were performed in three technical replicates in 7500 Real-Time PCR System and performed in two steps: pre-denaturation for 30 min at 95°C and 40 cycles of denaturation for 10 s at 95°C, and annealing /extension for 30 s at 60°C.

After amplification, the PCR products were sequenced to check the specificity of the primer sets (Table 6). Outliers were manually discarded and the housekeeping gene Actin was used as internal standard to calculate the relative expression level, which were standardized to the transcript levels for PtAC-TIN calculated by the 2−ΔΔCt method.

Results

Sequencing analysis and assembly

To investigate the transcriptomic responses to drought stress in Coix, the cDNA samples from mRNA of PEG and CK were sequenced using Illumina deep-sequencing platform. The sequencing results showed that the clean datas of CK and PEG were more than 4 Gb and GC content maintained at between 50.71 - 50.81%, the percentage of all CycleQ20 being 99.00%, the base percentage of Q30 being not less than 85.46%. And then sequences were assembled using the Trinity method (Grabherr et al, 2011), which transcriptome data were shown in Table 1. These data showed the accuracy of sequencing and transcriptome analysis were higher and can be for subsequent analysis.

Annotation and analysis of differentially expressed genes

All assembled unigenes were annotated in COG, GO, KEGG, KOG, Pfam, Swiss-Prot, nr data bases. Around 38.56% unigenes (35,813) were successfully annotated and detailed functional annotations of coix transcriptome were shown in the supplementary data (Supplementary Table 1). Because of the lack of genome and EST information for Coix, 61.44% (57,052) of the contigs did not match any known genes in these databases. As shown in Table 2, a total of 1128 differentially expressed genes were acquired (FDR < 0.01 and Fold Change ≥ 2), including 662 up-regulated and 466 down-regulated genes.

GO was an international standardized gene functional classification system, offering a dynamic updated controlled vocabulary and a strictly defined concept, which can comprehensively describe properties of genes (Wang DJ et al, 2015). The GO database contained three main categories of annotation: molecular function, cellular component, and biological process (Figure 1). In the «biological process» group, the number of genes involved in metabolic processes was the greatest. With regard to the «cellular component» class, the majority of genes were assigned to cell part, organelle membrane, organelle part, membrane part, which can response to drought stimuli. Considering the «molecular function» class of the regulated genes, categories were nucleic acid binding transcription factor, protein binding transcription factor, antioxidant, electron factor, transporter etc., which some genes were related with drought stress.

To further analyse the unigenes, we searched the annotated sequences for the genes involved in COG classifications. All-unigenes were aligned to COG database to predict and classify possible functions, COG function classification of consensus sequences were shown in the supplementary data (Supplementary Figure 1). As can be seen from the Supplementary Figure 1, general function prediction alone was the largest class group, which number of differentially expressed genes was 65, which represents 18.20% of all genes. The number of differentially expressed genes involved in signal transduction mechanisms represented 10.64%; in carbohydrate transport and metabolism, transcription, separately represented 8.68% and 8.4%. Because signal transduction mechanisms and transcription in plants significantly affected drought defense mechanism, which were crucial class groups among the COG categories.

As shown in Figure 2, the KEGG annotation results of differentially expressed genes were classified according to KEGG pathway classification. The dif-

<table>
<thead>
<tr>
<th>Length Range</th>
<th>Contig</th>
<th>Transcript</th>
<th>Unigene</th>
</tr>
</thead>
<tbody>
<tr>
<td>200-300</td>
<td>4,437,049 (98.39%)</td>
<td>34,163 (19.66%)</td>
<td>31,108 (33.50%)</td>
</tr>
<tr>
<td>300-500</td>
<td>30,027 (0.67%)</td>
<td>30,211 (17.38%)</td>
<td>25,201 (27.14%)</td>
</tr>
<tr>
<td>500-1000</td>
<td>22,782 (0.51%)</td>
<td>30,288 (17.43%)</td>
<td>18,433 (19.85%)</td>
</tr>
<tr>
<td>1000-2000</td>
<td>12,755 (0.28%)</td>
<td>35,085 (20.19%)</td>
<td>10,795 (11.62%)</td>
</tr>
<tr>
<td>2000+</td>
<td>7,239 (0.16%)</td>
<td>44,039 (26.34%)</td>
<td>7,328 (7.89%)</td>
</tr>
<tr>
<td>Total Number</td>
<td>4,509,852</td>
<td>173,786</td>
<td>92,865</td>
</tr>
<tr>
<td>Total Length</td>
<td>260,627,656</td>
<td>243,778,545</td>
<td>68,520,280</td>
</tr>
<tr>
<td>N50 Length</td>
<td>49</td>
<td>2,474</td>
<td>1,256</td>
</tr>
<tr>
<td>Mean Length</td>
<td>62.23</td>
<td>1402.75</td>
<td>737.85</td>
</tr>
</tbody>
</table>
Differentially expressed genes were distributed across 50 metabolic pathways. The most enriched metabolic pathways of differentially expressed genes were «Phenylpropanoid biosynthesis» (267 DEGs), «Plant hormone signal transduction» (264 DEGs), «Starch and sucrose metabolism» (261 DEGs), «AMPK signaling pathway» (118 DEGs), «MAPK signaling pathway» (74 DEGs), «Calcium signaling pathway» (57 DEGs). KEGG enrichment analysis of differentially expressed genes was showed in Supplementary Figure 2, and 20 selected metabolic pathways were analyzed, which can indicate that five metabolic pathways were the higher concentration. These five pathways i.e., plant hormone signal transduction, phenylpropanoid biosynthesis, fatty acid elongation, phenylalanine metabolism, starch and sucrose metabolism, and plant hormone signal transduction (ko04075) showed the highest enrichment scores. Among these pathways, four pathways i.e., plant hormone signal transduction, phenylpropanoid biosynthesis, and phenylalanine metabolism are related with ABA metabolism. These annotations were a valuable resource for functions and pathways in drought-tolerant Coix research, which some DEGs may be all related to the drought defense mechanism in coix.

Annotation and analysis of functional genes in coix under drought stress

In the coix transcriptome, many gene sequences that encoded coix enzymes and target proteins related to drought were annotated. In this present study, 66 unigenes that encoded target proteins of plant endogenous hormone were annotated, including 3 abscisic aldehyde oxidase genes (GO:0010293), 15 response to abscisic acid genes (GO:0009737), 7 9-cis-epoxycarotenoid dioxygenase (GO:0045549) genes, 4 ABA responsive element binding factors. There were 57 genes that encoded transcription factors, including 34 sequence-specific DNA binding transcription factor activity (GO:0003700), 6 AP2, 7 Bzip, 4 EREBP, 4 WRKY, 3 NAC, 2 MYB, 1 Hsp70. Furthermore, 5 osmoprotectant synthases genes were annotated, including 4 sugar transmembrane transporter genes (GO:0051119), 1 galactose transmembrane transporter activity (GO:0005354). In this present study, some protein kinase genes were also annotated, including 11 protein kinase genes (GO:0004672), 26 protein serine/threonine kinase genes (GO:0004674), and 9 protein kinase binding genes (GO:003824). Additionally, 11 response to abscisic acid genes, 2 9-cis-epoxycarotenoid dioxygenase, 2 ABA responsive element binding factor; 2 sugar transmembrane transporter genes, 1 galactose

Table 2 - Number of differentially expressed genes and gene annotation.

<table>
<thead>
<tr>
<th>DEGs number</th>
<th>Annotated</th>
<th>COG</th>
<th>GO</th>
<th>KEGG</th>
<th>KOG</th>
<th>Pfam</th>
<th>Swiss-Prot</th>
<th>Nr</th>
</tr>
</thead>
<tbody>
<tr>
<td>All DEG 1128</td>
<td>874</td>
<td>232</td>
<td>679</td>
<td>244</td>
<td>355</td>
<td>630</td>
<td>588</td>
<td>872</td>
</tr>
<tr>
<td>Up Regulated 662</td>
<td>523</td>
<td>134</td>
<td>345</td>
<td>121</td>
<td>231</td>
<td>325</td>
<td>378</td>
<td>512</td>
</tr>
<tr>
<td>Down Regulated 466</td>
<td>351</td>
<td>89</td>
<td>198</td>
<td>38</td>
<td>156</td>
<td>190</td>
<td>202</td>
<td>321</td>
</tr>
</tbody>
</table>

Figure 1: GO classification of differentially expressed genes. The horizontal axis shows secondary nodes of three categories in GO. The vertical axis displays the percentage of annotated genes versus the total gene number. The red, green and blue columns separately display biological process, cellular component and molecular function and The light color columns display annotation information of the total genes and the deep color columns represent annotation information of the differentially expressed genes.
transmembrane transporter activity, 1 L-proline biosynthetic process and 1 delta-1-pyrroline-5-carboxylate synthetase, 1 protein kinase genes, 1 protein serine/threonine kinase genes, 6 protein kinase binding genes, were up-regulated in the drought group compared with the CK group. Detailed KEGG enrichment analysis of differentially expressed genes was shown in Supplementary Figure 2.

Quantitative Real-time PCR confirmation
To confirm the accuracy and reproducibility of the Illumina RNA-Seq results, some transcripts related to drought were selected for examination by real-time RT-PCR (qRT-PCR). Information for these genes and their gene-specific primers were showed in Table 3. The qPCR results for 8 selected contigs showed general agreement with their transcript-abundance changes as determined by RNA-seq, which suggested the reliability of the transcriptomic profiling data. For example, under drought stress, c21891.graph c0, which showed strong homology to protein serine/threonine phosphatase activity, was upregulated 2.32183-fold in Coix leaf (Figure 3A). c20325.graph c0, a homolog of phosphoprotein phosphatase activity, was upregulated 1.48000-fold in Coix leaf (Figure 3B). c18635.graph c0, a homolog of late embryogenesis abundant protein, was upregulated 4.59796-fold in Coix leaf (Figure 3C) and NAC, Hsp70 and EREBP transcription factors were also upregulated 1.48000, 1.72445, and 1.61075-fold respectively in Coix leaf under drought stress (Figure 3D,G,H).

Discussion
Coix is a crop of food and medicine, which receives people’s attention nowadays. In previous studies, people have mainly focused on the research of food efficacy in coix (Peng et al, 2011; Liu et al, 2015). The study of molecular and genetic in Coix is relative less (Zhou et al, 2010; Panuganti et al, 2015). Especially, the genomes and transcriptomes of Coix is still blank up to now. Transcriptome analysis using sequencing technology has been proven to be an useful method for identifying stress-inducible gene (Wang et al, 2012; Yang et al, 2011b; Yu et al, 2012). These studies greatly enhanced the efficiency and quantity of gene annotation and improved research into plant resistance to adversity stresses.

In this study, transcriptomes sequencing analysis of PEG and CK were conducted using the Illumina platform. Our study primarily focused on the identification and analysis of drought stress-responsive genes from Yiliao 5 seedlings, aiming to investigate the common regulatory mechanisms and important functional genes after PEG treatment. After drought stress, Yiliao 5 should acquire some differentially expressed genes, which were distributed across 50 metabolic pathways; the bigger category of these pathways is metabolism.
pathways was “Phenylpropanoid biosynthesis” and “Plant hormone signal transduction”. In this study, many genes involved in hormone pathways were differently expressed in response to abiotic stress. Previous studies on plants have suggested that phytohormones were involved in stress adaptation (Nishiyama et al., 2011; Christmann et al., 2013; Perera et al., 2008). In our study, plant hormone signal transduction pathway, for instance, ABA signal transduction pathway was a significant different involving in the response to drought stress. Drought stress can prompt the resynthesis and redistribution of ABA and other endogenous hormones in plant. As a messenger between cells, ABA can affect the expression of related enzymes and drought resistance gene by inducing the expression of ABA gene (Tan et al., 2001). In the coix transcriptome analysis, 66 were related to plant endogenous hormone targets. There were 14 response to abscisic acid genes, 3 abscisic aldehyde oxidase and 2 9-cis-epoxycarotenoid dioxygenase were detected, such as protein kinase genes, protein kinase, MAPK, LEA, HSP70 gene families displayed differential induction by drought stress. Osmotic phosphatases mediating osmotic homeostasis or stress responses, which was in accordance with the previous study (Hao et al., 2011; Krasensky et al., 2012). In this study, we analyzed the gene expression of a few important transcript factors (AP2, NAC, bZIP, MYB, NAC, EREBP, and WRKY) that were known to be involved in drought tolerance in Coix. We found that the AP2 and EREBP genes exhibited similar changes in expression in response to drought stress, which expression of the genes were up-regulated under drought stresses. Increased expression of the DRE-B2A genes has also been found to play an important role in the plant response and adaptation to abiotic stress (Qin et al., 2006; Qin et al., 2007), which was in accordance with our study. However, some TFs (e.g. NAC, MYB, WRKY, and bZIP) responded differently under drought stress condition, which the up-regulated and down regulated members were existing. The transcription factors can interact synergistically to control water loss, and then can help to survive under dehydration or osmotic stress conditions, which was in accordance with the previous study (Xu et al., 2013). These results indicated that some AP2, EREBP, NAC, and bZIP transcription factors might function as positive regulators of drought tolerance in Coix.

In addition to TFs, a number of additional functional protein such as kinases, LEA, which were also important for the acclimatization of plants to environmental stress. In this study, members of the protein kinase, MAPK, LEA, HSP70 gene families displayed differential induction by drought stress. Osmotic stresses can activate several protein kinases and phosphatases mediating osmotic homeostasis or detoxification responses (Zhu, 2002). In this study, some genes that encoded protein kinase genes were detected, such as protein kinase genes, protein kinase binding genes, protein serine/threonine kinase

### Table 3 - List of the expression profiles selected for confirmation by qRT-PCR.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Normalized fold expression</th>
<th>Description</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>c21891.graph_c0</td>
<td>2.32183</td>
<td>Predicted: uncharacterized protein</td>
<td>F: TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Zea mays) isofrom X1</td>
<td>R:TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td>c20325.graph_c0</td>
<td>1.86275</td>
<td>Probable protein phosphatase 2C 9 (Zea mays)</td>
<td>F: TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R:TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td>c18635.graph_c0</td>
<td>4.59796</td>
<td>Hypothetical protein SORBiDRAFT_04g17790</td>
<td>F: TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Sorghum bicolore]</td>
<td>R:TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td>c23092.graph_c0</td>
<td>1.99897</td>
<td>Hypothetical protein SORBiDRAFT_01g020270 (Sorghum bicolore)</td>
<td>F: TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R:TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td>c24857.graph_c0</td>
<td>1.46316</td>
<td>Malate synthase, glycoxyxomal SN=LP OS=Zea mays (Maize) PE=2 SV=1</td>
<td>F: AACACCCCTGCACARCECT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: TGGGCTCTGACTTCCCTCA</td>
</tr>
<tr>
<td>c33833.graph_c0</td>
<td>1.4800</td>
<td>Hypothetical protein SORBiDRAFT_01g002710 (Sorghum bicolore)</td>
<td>F: TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td>c27827.graph_c0</td>
<td>1.72445</td>
<td>Uncharacterized protein LDC10027291 (Zea mays)</td>
<td>F: TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: TCCCACTAGGGAAAGAGAGAA</td>
</tr>
<tr>
<td>c22563.graph_c3</td>
<td>1.61075</td>
<td>Putative AP2/EREBP transcription factor superfamily protein (Zea mays)</td>
<td>F: TCCCACTAGGGAAAGAGAGAA</td>
</tr>
</tbody>
</table>

Actin(Zea mays)
transcriptome analysis of Coix under PEG stress

Proline has been widely considered to be a key drought-inducible metabolite because it played an osmoprotective role in plants (Xin et al., 2016), which could be involved in the synthesis and transport of osmotic regulation substances. In our study, we found that proline was one of the most significantly changed metabolites under drought stress. Especially, the key enzyme 1-pyrroline-5-carboxylate synthetase (P5CS) (c9063.graph c0) in Proline synthesis from glutamate was significantly up-regulated under drought stress compared to the control. Additionally, responses of carbon metabolism or starch metabolism to drought stress have been found in many plants, such as alfalfa (Naya et al., 2007) and maize, while soluble sugars often accumulate and perform functions in osmotic adjustment (Chaves et al., 2009). In our study, some sugar transmembrane transporter proteins and sugar: hydrogen symporter proteins were found to be differentially expressed under drought stress. The expression data suggested that sugar or carbon metabolism may be influenced by drought stress in coix. In general, the large number of accumulation of proline and soluble sugar will play an important role under drought stress. To validate the transcription results, the 8 differential expression genes were corroborated using qRT-PCR. The results of qRT-PCR were consistent with RNA-Seq datas, which further confirmed the reliability of RNA-Seq results.

Acknowledgements

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transcriptome analysis of Coix under PEG stress


