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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, H_2O_2 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-

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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 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 (5 μg) from each selected gene was treated with DNAse I (Invitrogen) and translated into first strand cDNA, which was synthesized with TransScript One-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 PtACTIN calculated by the $2^{-\Delta\Delta Ct}$ 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 1 - Summary of Illumina transcriptome assembly for coix.

Length Range	Contig		Transcript		Unigene	
200-300	4,437,049	(98.39%)	34,163	(19.66%)	31,108	(33.50%)
300-500	30,027	(0.67%)	30,211	(17.38%)	25,201	(27.14%)
500-1000	22,782	(0.51%)	30,288	(17.43%)	18,433	(19.85%)
1000-2000	12,755	(0.28%)	35,085	(20.19%)	10,795	(11.62%)
2000+	7,239	(0.16%)	44,039	(26.34%)	7,328	(7,89%)
Total Number	4,509,852		173,786		92,865	
Total Length	280,627,656		243,778,545		68,520,280	
N50 Length	49		2,474		1,256	
Mean Length	62.23		1402.75		737.85	

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

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

ferentially 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, 3NAC, 2 MYB, 1 Hsp70. Furthmore, 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:0003824). 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

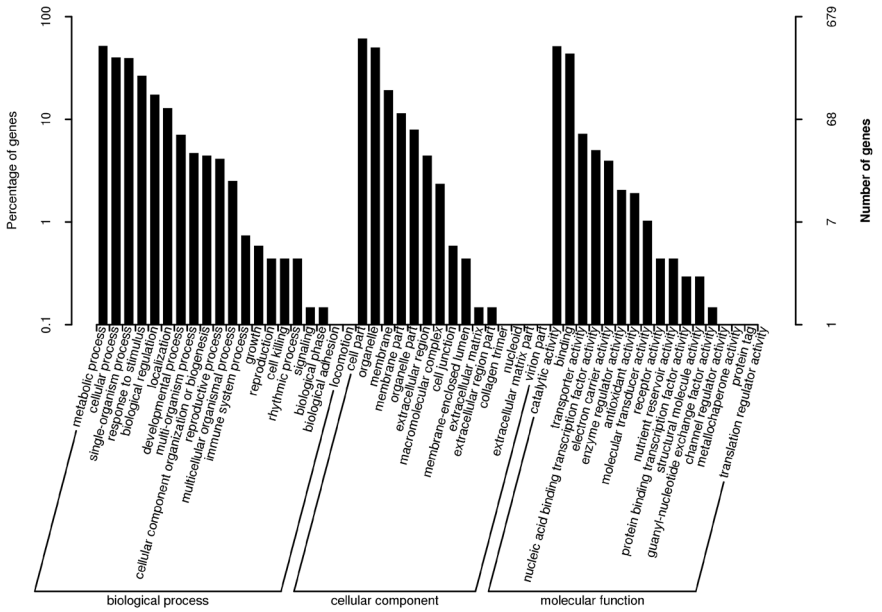

Figure 1- GO classification of differentially expressed genes. The horizontal axis shows secondary nodes of three categories in GO. The vertical axis displays the percentage of annotated genes versus the total gene number. The red, green and blue columns separately display biological process, cellular 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 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

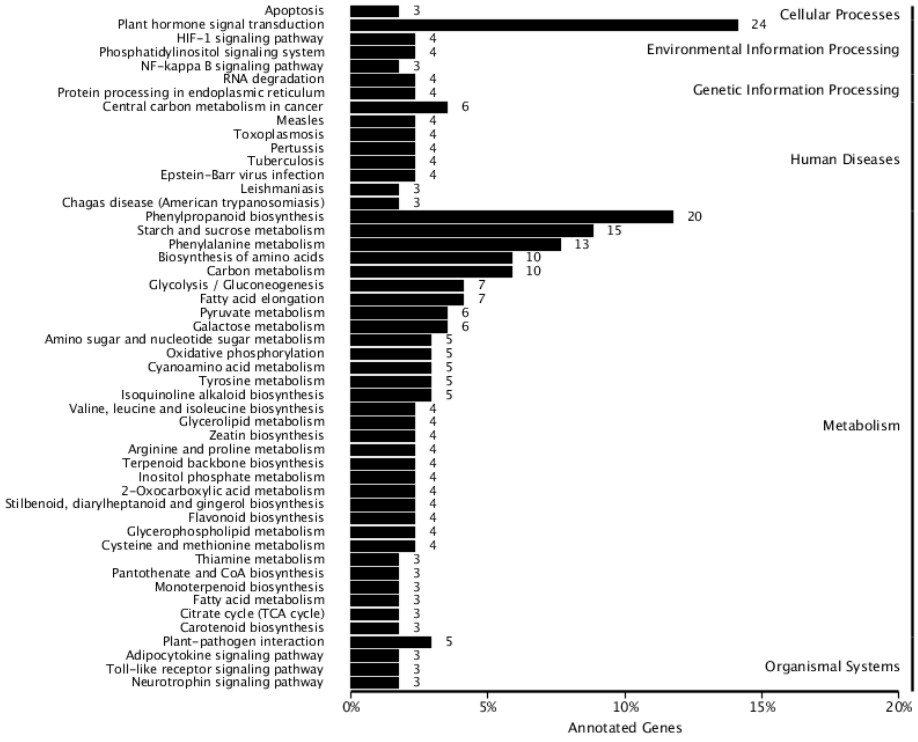

Figure 2 - KEGG categories of differentially expressed genes. The vertical axis lists the names of the metabolic pathways in the KEGG, and the horizontal axis shows the proportion of annotated genes in each pathway versus the total number of annotated genes. The differentially expressed genes are distributed in 50 metabolic pathways, the pathway of metabolism holds the highest all number of genes.

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

Gene ID	Normalized fold expression CK-VS-PEG	Description	Primers
c21891.graph_c0	2.32183	PREDICTED: uncharacterized protein LOC100192073 isoform X1 [Zea mays]	F:TCCCACTAGCGAAAGGAGAA R:TCCCACTAGCGAAAGGAGAA
c20325.graph_c0	1.86275	probable protein phosphatase 2C 9 [Zea mays]	F:CTCAGCAGCAGTCAGGTCAG R: ACGGCAAGCACCATTTCTAC
c18635.graph_c0	4.59796	Hypothetical protein SORBIDRAFT_04g017790 [Sorghum bicolor]	F:GATCGAGTCTGGGCTAATGG R: TTCCATGCTGTGATCGTACC
c23092.graph_c0	1.99897	hypothetical protein SORBIDRAFT_01g030270 [Sorghum bicolor]	F:CATCAGCAAGTCCCACTGAA R: AAAGGCACGATCCAGATGTT
c24857.graph_c0	1.46316	Malate synthase, glyoxysomal GN=LIP OS=Zea mays (Maize) PE=2 SV=1	F: AACACCCCGTTCCATATCCT R: TTCGGCCTCTACTTCTTCCA
c33833.graph_c0	1.4800	hypothetical protein SORBIDRAFT_08g002710 [Sorghum bicolor]	F:AGGGCAAACAAAGGGTAACA R: AATGCCACTTGATCCAAACC
c27827.graph_c0	1.72445	Uncharacterized protein LOC100272911 [Zea mays]	F:GCGGACGACAAGAAGAAGAT R: TGGAGATGATGGGGTTGC
c22563.graph_c3	1.61075	putative AP2/EREBP transcription factor superfamily protein [Zea mays]	F: CACGCTCGCTGGTGTTTAC R: CCCTCCCATCTCTGACCTCT
Actin(Sorghum)			F: GCTACGAGATGCCTGATG R: CCACTGAGGACAACATTACC

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 annotated, which can show the ABA gene was highly expressed under drought stress. These results were consistent with the higher expression level of maize root AOs and NCEDs under drought stress (Nishiyama et al, 2013). NCEDs was a key catalytic enzyme of ABA synthesis from 9'-cis-new xanthine or 9'-cis-purple xanthine xanthine to aldehyde oxidation (Neill et al, 1999; Merlot et al, 1997), while AOs may encode indole acetic aldehyde oxidase and abscisic acid aldehyde oxidase. AOs can enhance the ABA content in plant by encoding abscisic acid aldehyde oxidase (Sekimoto et al, 1997). Taken together, the potential components of the ABA signaling pathway were largely identified during drought stress from the transcriptome results, which indicated that a relationship between the ABA signaling pathway and drought stress in coix.

TFs typically regulating the expression of multiple genes in a metabolic pathway, and TFs analysis has played a crucial important part in stress response research. Many WRKY, MYB, NAM, and NAC transcription factors were found to confer resistance to drought stress in plants, and over expression of these transcription factors enhanced the drought tolerance of plants (Takasaki et al, 2010; Jeong et al, 2010; Hao et al, 2011; Krasensky et al, 2012; Lata et al, 2011). 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 *DREB2A* 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 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

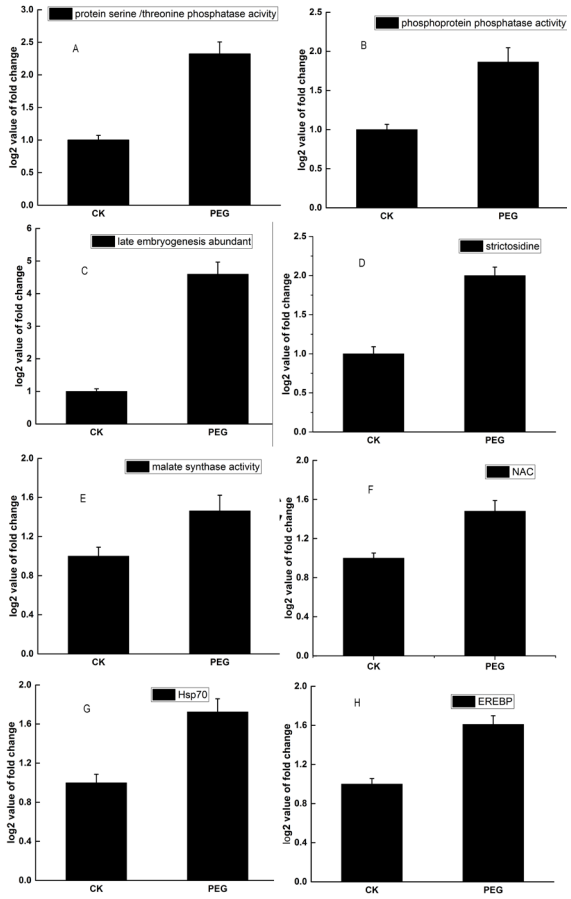

Figure 3 - Confirmation of expression profiles by qRT-PCR with 8 selected differentially expressed genes (DEGs). Relative expression of the 8 genes in coix seedlings was detected by qRT-PCR under PEG stress conditions. Transcript levels were normalized to the expression level of actin. Error bars SE from three independent experiments.

genes in the Coix transcriptome. In our study, one MAPK gene (c33419.graph c0) was detected, which the transcription level was increased under drought stress. Plant MAPK cascades have been implicated in the development and responses to stress, as a major plant transduction components, which regulate numerous processes can relay downstream of receptors or sensors and transduce environmental and developmental signals into adaptive and programmed responses (Xu et al, 2015). Furthermore, some late embryogenesis abundant proteins were also identified. Previous studies have reported that the LEA genes responded to drought in different plant species (Vaseva et al, 2010; Yue et al, 2008). Some researchers have also reported that the LEA genes were upregulated in transgenic plants overexpressing ZmDREB2A (Qin et al, 2007), which were accordance with the results of LEA expression being upregulated in our study. The kinases and functional proteins are important for the acclimatization of plants to drought 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.

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References

Anders S, Huber W, 2010. Differential expression analysis for sequence count data. Genome Biology 11: R106

Arora RK, 1977. Job's-tears (*Coix lacryma-jobi*): aminor food and fodder crop of Northeastern India. Econ Bot 31: 358-366

Chen HJ, Chung CP, Chiang W, Lin YL, 2011. Anti-inflammatory effects and chemical study of a flavonoid-enriched fraction fromadlay bran. Food Chem 126: 1741-1748

Chen HJ, Shih CK, Hsu HY, Chiang W, 2010. Mast cell-dependent allergic responses are inhibited by ethanolic extract of adlay (*Coix lachryma-jobi* L. Var. ma-yuen Stapf) testa. J Agric Food Chem 58: 2596-2601

Christmann A, Grill E, Huang J, 2013. Hydraulic signals in long-distance signaling. Curr Opin Plant Biol 16: 293-300

Rodrigues FA, Fuganti-Pagliarini R, Marcolino-Gomes J, Nakayama TJ, 2015. Daytime soybean transcriptome fluctuations during water deficit stress. BMC Genomics 16: 505

Qin F, Li JS, Li SH, Harold C, 2005. AFLP and RFLP

linkage map in Coix. Genetic Resources and Crop Evolution 52: 209-214
Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, 2011. Full length transcriptome assembly from RNA Seq data without a reference genome. Nature Biotechnol 29: 644-652
Haas BJ, Papanicolaou A,Yassour M, Blood PD, Bowden J, Couqer MB, Eccles D, Li B, 2013. *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nature Protoco 8: 1494-1512
Hao YJ, Wei W, Song QX, Chen HW, Zhang YQ, Wang F, Zou HF, Lei G, Tian AG, Zhang WK, Ma B, Zhang JS, Chen SY, 2011. Soybean NAC transcription factors promote abiotic stress tolerance and lateral root formation in transgenic plants. Plant J 68: 302-313
Huang L, Zhang F, Wang W, Zhou Y, Li Z, 2014. Comparative transcriptome sequencing of tolerant rice introgression line and its parents in response to drought stress. BMC Genomics 15: 1026
Lee HJ, Ryu J, Park SH, Seo EK, Han AR, Lee SK, Kim YS, Hong JH, Seok JH, Lee CJ, 2015. Suppressive effects of coixol, glyceryl trilinoleate and natural products derived from *Coix lachryma-jobi* var. ma-yuen on gene expression, production and secretion of airway MUC5AC mucin. Arch Pharm Res 38: 620-627
Jeong JS, Kim YS, Baek KH, Jung H, Ha SH, Do Choi Y, Kim M, Reuzeau C, Kim JK, 2010. Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions. Plant Physiol 153: 185-197
Jia X, Sun CS, Zuo YC, Li GY, Ren LY, Chen GL, 2016. Integrating transcriptomics and metabolomics to characterise the response of *Astragalus membranaceus* (Bge) to progressive drought stress. BMC Genomics 17: 188
Kim SO, Yun SJ, Jung B, Lee EH, Hahm DH, Shim I, Lee HJ, 2004. Hypolipidemic effects of crude extract of adlay seed (*Coix lachrymajobi* var. mayuen) in obesity rat fed high fat diet: relations of TNF-alpha and leptin mRNA expressions and serum lipid levels. Life Sci 75: 1391-1404
Krasensky J, Jonak C, 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J Exp Bot 63: 1593-1608
Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, Seki M, Shinozaki K, Yamaquchi-Shinozaki K, 2003. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. Plant Physiol 133: 1755-1767
Langmead B, Trapnell C, Pop M, 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10: R25
Lata C, Prasad M, 2011. Role of DREBs in regulation of abiotic stress responses in plants. J Exp Bot 62: 4731-4748
Leng N, Dawson JA, Thomson JA, Ruotti V, Rissman AI, Smits BM, Haaq JD, Gould MN, Stewart RM, Kendziorski C, 2013. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. Bioinformatics 29: 1035-1043
Li B, Dewey CD, 2011. RSEM: accurate transcript quantification from RNA Seq data with or without a reference genome. BMC Bioinformatics 12: 323
Liu X, Zhang X, Rong YZ, Wu JH, Yang YJ, 2015. Rapid Determination of Fat, Protein and Amino Acid Content in Coix Seed Using Near-Infrared Spectroscopy Technique. Food Analytic Methods 8: 1-9
Lu Y, Zhang BY, Jia ZX, Wu WJ, Lu ZQ, 2011. Hepatocellular carcinoma HepG2 cell apoptosis and caspase-8 and Bcl-2 expression induced by injectable seed extract of *Coix lacryma-jobi*. Hepatobiliary Pancreat Dis Int 10: 303-307
Murtagh F, Legendre P, 2014. Ward's hierarchical agglomerative clustering method: which algorithmsImplement Ward's criterion? Journal of Classification 31: 274-295
Naya L, Ladrera R, Ramos J, González EM, Arrese-Igor C, Minchin FR, Becana M, 2007. The response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to the subsequent recovery of plants. Plant Physiol 144: 1104-1114
Pastori GM, Foyer CH, 2002. Common components, networks, and pathways of cross-tolerance to stress. The central role of «redox» and abscisic acid-mediated controls. Plant Physiol 129: 460-468
Peng H, Xiong H, Wang S, Li J, Chen L, Zhao Q, 2011. Soluble starch based biodegradable and microporous microspheres as potential adsorbent for stabilization and controlled release of coix seed oil. Eur Food Res Technol 232: 693-702
Perera IY, Hung CY, Moore CD, Stevenson-Paulik J, Boss WF, 2008. Transgenic Arabidopsis plants expressing the type 1 inositol 5-phosphatase exhibit increased drought tolerance and altered abscisic acid signaling. Plant Cell 20: 2876-2893
Qin F, Kakimoto M, Sakuma Y, Maruyama K, Osakabe Y, Shinozaki K, Yamauchi-Shinozaki K, 2006. Regulation and functional analysis of ZmDREB2A in response to drought and high temperature stress from Zea mays L. Plant Cell Physiol 50: 54-69
Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K, 2006. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. Curr Opin Biotechnol 17: 113-122
Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M,

Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T, 2002. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J 31: 279-292

Seki M, Umezawa T, Urano K. Shinozaki K, 2007. Regulatory metabolic networks in drought stress responses. Curr Opin Plant Biol 10: 296-302

Nishiyama R, Watanabe Y, Fujita Y, Le DT, Kojima M, Werner T, Vankova R, Yamaguchi-Shinozaki K,Shinozaki K, Kakimoto T, Sakakibara H, Schmulling T, Tran LSP, 2011. Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought, salt and abscisic acid responses, and abscisic acid biosynthesis. Plant Cell 23: 2169-2183

Smirnoff N, 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125: 27-58

Song Y, Chen Q, Ci D, Zhang D, 2012. Transcriptome profiling reveals differential transcript abundance in response to chilling stress in *Populus simonii*. Plant Cell Rep 32: 1407-1425

Takasaki H, Maruyama K, Kidokoro S, Ito Y, Fujita Y, Shinozaki K, Nakashima K, 2010. The abiotic stress-responsive NAC-type transcription factor OsNAC5 regulates stress-inducible genes and stress tolerance in rice. Mol Gen Genomics 284: 173-183

Tang S, Dong Y, Liang D, Ye CY, 2015. Analysis of the Drought Stress-Responsive Transcriptome of Black Cottonwood (*Populus trichocarpa*) Using Deep RNA Sequencing. Plant Mol Biol Rep 33: 424-438

Tan Y, Ye QS, Li L, 2001. Recent advances in physiological role of ABA in response of drought. Chinese Bulletin of botany 18: 197-201

Trippi VS, Gidrol X, Pradet A, 1989. Effects of oxidative stress by oxygen and hydrogen peroxide on energymetabolism and senescence in oat leaves. Plant Cell Physiol 30: 157-162

Vaseva II, Grigorova BS, Simova-Stoilova LP, Demirevska KN, Feller U, 2010. Abscisic acid and late embryogenesis abundant protein profile changes in winter wheat under progressive drought stress. Plant Biol 12: 698-707

Wang G, Zhu QG, Meng QW, Wu CA. 2012. Transcript profiling during salt stress of young cotton (*Gossypium hirsutum*) seedlings via Solexa sequencing. Acta Physiol Plant 34:107-115

Wang DJ, Yang CL, Dong L, Zhu JC, Wang JP, Zhang SF, 2015. Comparative Transcriptome Analyses of Drought-Resistant and-Susceptible Brassica napus L. and Development of EST-SSR. J Plant Biol 58: 259-269

Wu TT, Charles AL, Huang TC, 2007. Determination of the contents of the main biochemical compounds of Adlay (*Coxi lachrymal-jobi*). Food Chem 104: 1509-1515

Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li CY, Wei L, 2011. KOBAS 2.0: A web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res 39: 316-322

Xu J, Zhang SQ, 2015. Mitogen-activated protein kinase cascades in signaling plant growth and development. Trends Plant Sci 20: 56-64

Yang YH, Chen XJ, Chen JY, Xu HX, Li J, Zhang ZY, 2011. Identification of novel and conserved microRNAs in Rehmannia glutinosa L. by Solexa sequencing. Plant Mol Biol Rep 29: 986-996

Ye J, Fang L, Zheng H, Zhang Y, Chen J, Zhang Z, Wang J, Li S, Li R, Bolund L, Wang J, 2006. WE GO: a web tool for plotting GO annotations. Nucleic Acids Res 34: 293-297

Yue G, Zhuang Y, Li Z, Sun L, Zhang J, 2008. Differential gene expression analysis of maize leaf at heading stage in response to water-deficit stress. Biosci Rep 28: 125-134

Yu SC, Zhang FL, Yu YJ, Zhang DS, Zhao XY, Wang WH, 2012. Transcriptome profiling of dehydration stress in the Chinese cabbage (*Brassica rapa* L. ssp. pekinensis) by tag sequencing. Plant Mol Biol Rep 30: 17-28

Zhou L, Huang B, Meng X, Wang G, Wang F, Song R, 2010. The amplification and evolution of orthologous 22-kDa a-prolamin tandemly arrayed genes in coix, sorghum and maize genomes. Plant Mol Biol 74: 631-643

Zhu JK, 2002. Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53: 247-273