Pak. J. Bot., 52(2): 489-500, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-2(9)

TRANSCRIPTOMIC ANALYSIS OF *ANABASIS APHYLLA* SEEDLINGS UNDER FREEZING TREATMENT

YA-LING CHANG, MENG-WEN PENG, GUANG-MING CHU* AND MEI WANG*

Department of Forestry, Agricultural College, Shihezi University, Shihezi, Xinjiang 832000, China
**Corresponding author's email: chgmxj@163.com*

Abstract

Anabasis aphylla is a salt and alkali resistant shrub which grows in arid and semi-arid areas. There are few reports about the transcriptome of *A. aphylla*. The objective of this study was to analyze the transcriptome of *A. aphylla* under freezing treatment so as to better understand its molecular mechanism of freezing adaptation. Illumina sequencing was used to obtain large-scale transcriptome sequencing of *A. aphylla* under freezing treatment. Nine genes were then analyzed using real-time quantitative PCR. A total of 7101 differentially expressed genes were obtained, including 2460 up-regulated genes and 4641 down-regulated genes. Gene Ontology annotation analysis indicated many freeze-relevant categories like 'response to freeze and stimulus'. The Kyoto Encyclopedia of Genes and Genomes pathway analysis uncovered some important pathways, such as 'metabolic pathways' and 'plant hormone signal transduction pathway'. The 9 genes analysis showed that the real-time fluorescence quantitative analysis results were consistent with the expression trend of transcriptome analysis genes. The acceptance and conduction of the freeze signal is an important process of freezing adaptation in plants and adaptation to the environment.

Key words: *Anabasis aphylla*; Freeze treatment; Desert plant; Transcription factor.

Introduction

Anabasis aphylla is a chenopodiaceous shrub mainly distributed in Xinjiang, region of northwest China, and has high capabilities to endure alkali salts, resist drought and check sand drift, which is important to exploit and utilize land that has become salinized and alkaline under drought, and thus to improve these environments by introducing plant cover (Chu *et al*., 2014; Wang *et al*., 2015). *A. aphylla* population is a constructive species of desert; it has unique bio-ecological traits and has conducted a wide range of economic, medicinal, and ecological value (Chu *et al*., 2014). At present, there are many studies on *A. aphylla* biological characteristics and medicinal ingredient content, while the transcriptome study has not been reported (Wang *et al*., 2017a; Xi *et al*., 2006). The low temperature is one of the major abiotic adaptation, which limits geographical distribution and reduces productivity of wild plants and crops. Therefore, it is very important to elucidate the physiological and molecular mechanisms of plant adaptation under low temperature. In recent years, the use of omics molecular techniques to explain plant's freeze adaptation has become more widespread. Using transcriptome and Differential Expression Genes (DEGs) data, the abiotic adaptation response of *Arabidopsis thaliana* and other non-model plants has been widely studied (Dugas *et al*., 2011; An *et al*., 2012; Nishiyama *et al*., 2012; Wang *et al*., 2013; Dang *et al*., 2013; Liu *et al*., 2013). However, until now, few genes have been identified, and few molecular researches of this species have been reported, despite the importance of the genes. In this area, snow melt is a vital water source for *A. aphylla* seed germination, and it can regulate plant phenology and ecosystem nutrient cycling. Through a combination of field investigation and observations with a simulation experiment, we found that a large number of *A. aphylla* seeds germinated in snow melt in the field, which was consistent with *Anabasis elatior* germination characteristics (Han *et al*., 2011). With the development of molecular technology, more and more research on plant adaptation to the environment is carried out using transcriptome methods. Considering the large genome size of the plant, the whole genome sequencing of *A. aphylla* is difficult. Therefore, the construction of large Expressed Sequence Tags (EST) collections of this species is the most promising approach for providing functional genomics level information (Wu *et al*., 2015).

Low temperature signal transduction system requires a complex signal system, including Ca^{2+}, ABA, protein phosphorylase, protein kinase, soluble sugar molecules and some transcription factors. In this study, in order to obtain genes related to freeze adaptation, 9 genes were selected from DEGs for further PCR sequencing. This study will be helpful for elucidating the molecular responsive freeze adaptation mechanism of *A. aphylla.*

Materials and Methods

Plant materials and freeze treatments: Seeds of *A. aphylla* (the seed were identified by a plant taxonomist) were obtained from the southern edge of the Gurbantünggüt Desert in Xinjiang. Seeds were sterilized with 10% H_2O_2 for 30 minutes and rinsed with distilled water. For the control treatment, the sterilized seeds were germinated until seedlings were approximately 3 cm length on filter paper soaked in distilled water in petri dishes at room temperature and a 16-h light/8-h dark photoperiod, while freeze treatment seedlings were kept in a -3°C growth chamber for 12h. For each treatment, 3g seedlings were selected as samples and quickly frozen in liquid nitrogen separately, stored at -80°C for transcriptomic analysis. Every treatment had three biological replicates.

RNA extraction and cDNA library preparation: The quality and purity of RNA were assessed by determining the absorbance at 280, 260 and 230 nm using Nano Drop 2000 Spectrophotometer (Thermo Scientific, Wilmington, USA).

RNA was used when the OD260/280 was greater than 1.8. RNA integrity was checked with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) and the value was no less than 7. The extracted total RNA was stored at -80°C for later use. After DNase I digestion, magnetic beads with Oligo (dT) were utilized to isolate mRNA from the total RNA. The mRNA was cleaved into short fragments with divalent cation at elevated temperature. Then, cDNA was synthesized using the m RNA fragments as templates. Short fragments were purified and dissolved with EB buffer for end reparation and single nucleotide a (adenine) addition. The short fragments were ligated to random hexamer adapters. The final products were size selected and enriched by PCR to create the final cDNA library for transcriptome sequencing and DGE sequencing. Agilent 2100 Bioanaylzer (Agilent Technologies, Santa Clara, USA) and Bio-Rad my IQ2 Real-Time PCR System (Bio-Rad Laboratories, Hercules, USA) were used for quantification and quality control of the sample library during the quality control steps (Gao *et al*., 2015).

Transcriptome sequencing and assembly: Raw reads produced from sequencing machines contain low quality reads, which negatively affect subsequent bio-informatics analysis. Therefore, we discarded these reads, including those with adaptors, unknown nucleotides larger than 5% and low quality (< 20% of the bases with a quality score $Q \leq 10$) using an in-house Perl script. The average proportion of clean reads in each sample was 91.5%, on which the following analysis was based. Transcriptome *de novo* assembly was performed using the short-reads assembly program, Trinity (Grabherr *et al*., 2011), which first combined reads with certain lengths of overlap to form longer fragments, known as contigs. These reads were then mapped back to contigs. Using paired end reads, we detected contigs from the same transcript as well as the distances between these contigs. Next, we used Trinity to connect the contigs and obtained sequences that could not be extended on either end, known as unigenes. Finally, we used TGICL (Pertea *et al*., 2003), a software system for rapid clustering of large EST data sets, to assemble all the unigenes from both samples to form a single set of non-redundant unigenes.

Functional annotation: We aligned the unigene sequences to the above-mentioned protein databases by blast (E-value < 0.00001) and to the nucleotide sequence database Nt (E-value < 0.00001) by blastn. We thus obtained proteins with the highest similarity to the given unigenes, as well as the functional annotations. According to the Nr annotation, we obtained the GO functional annotation using the Balst GO program (Conesa *et al*., 2005), and the GO functional classification for all unigenes using the WEGO software (Ye *et al*., 2006) to understand the distribution of gene functions of the species from the macro-level. After aligning the all unigenes to the COG database, the COG functional annotations were obtained. Next, we further examined the complex biological behaviors. To investigate the plant hormone signal transduction pathway annotation of unigenes, we aligned the all unigenes to the KEGG database (Kanehisa *et al*., 2008), to obtain pathway annotations.

Quantitative real-time reverse transcription PCR analysis: Total RNAs was quantified by the Nano Drop ND-2000 (Thermo Scientific) and the RNAs integrity was assessed using agarose gel electrophoresis. While QC of RNA was qualified, in turn to treat RNA of DNase I, then reverse transcription, cDNA dilution, last chose 9 genes and primers for PCR (Table 1) by Applied Biosystems 7900HT PCR. After completion of the reaction the data analysis based on the obtained sample Ct value.

Results and Discussions

Transcriptome sequencing and assemble: Illumina RNA-seq technologies are much simpler and more cost-effective, especially for species without sequenced genome, such as non-model plant species *A. aphylla*. These sequences accelerating gene discovery and permitting expression analysis evolutionary genome dynamics studies. The two libraries (freeze-treated and control samples), were sequenced respectively using Illumina technology. We obtained approximately 25 million raw reads for the freeze-treated sample (CT) and 20 million (Table 2) for the control sample (CK). In order to ensure the quality of information analysis, filtered raw reads to obtain clean reads. The assembly of the clean reads resulted in 160619 transcripts and 129161 unigenes (Table 3). The length statistics of the assembled unigenes and transcripts shows that most of the length is between 200-300bp. (Fig. 1). This is the first report of *A. aphylla* transcriptome data until now.

Table 1. Genes and primers for real-time quantitative PCR.

Gene	Forward primer (5'-3')	Reverse primer (3'-5')
TRINITY_DN58369_c1_g1	GTTGACATGCGAGCTTCGAG	TCACCACCATGCTCTTGAGG
TRINITY_DN68772_c2_g2	GGACGGTTGCGTGCTCTAA	TGTTGATGGTGGTGCTGATG
TRINITY_DN61978_c0_g2	AACAGGTGAGCACCCATCAC	ATGATCAAGCGGCGTTTTCG
TRINITY_DN67515_c0_g1	CGTTGCTAATGGCCAGGTC	TCCACAGTAACCGCATGTCC
TRINITY_DN108295_c0_g1	GATGACGTTGGCGGTGTTAC	GCTTAAGTCCGGTCGTCTCG
TRINITY_DN58157_c0_g1	CATACGGTTCCGTGATCGAG	AAGGACCGAGGTTGTTGTCC
TRINITY_DN59403_c0_g1	TTCCTCTTCCAAGCCTCCTG	CAGCAGCTCGTTGATTGTGA
TRINITY_DN55955_c0_g1	AGCAGTCGTGCAAGGTGGTA	ACAAGAGGCAGCCATGAAGG
TRINITY_DN50697_c0_g1	TCGCCATGTTGGATTGACTC	GCAACAGTCAAGCGGAGAGA
S-18S	ACCATAAACGATGCCGG	CACCACCCATAGAATCAAGA

Table 2. Summary of data output quality.

Sample	Raw_reads	Clean_reads	Clean_bases (Gb)	Error_rate (%)
CT	25686615	25418387	3.81	0.01
CK	20781089	20588970	3.21	0.01

Table 3. Summary of assembly length frequency distribution.

Interval	200-400bp	400-600bp	600-800bp	800-1000bp	1000-1200bp	1200-1400bp	1400-1600bp	1600-1800bp	1800-2000bp	>2000bp	Total
Transcripts	86922	24376	11948	7860	5851	4370	3594	3017	2343	10338	160619
Unigenes	129161	51098	32670	24413	19234	15488	12760	10502	8511	6967	129161

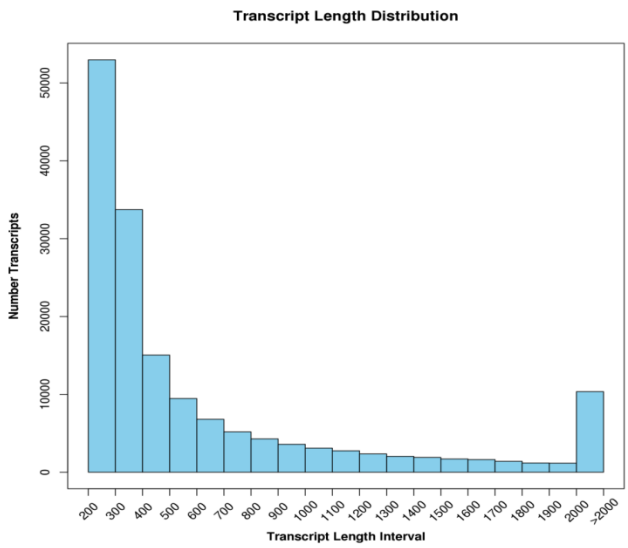

Fig. 1A. Length distribution of the assembled transcript.

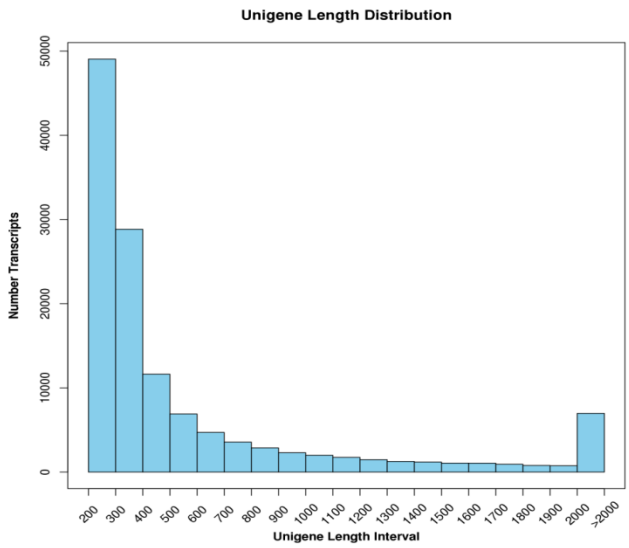

Fig. 1B. Length distribution of the assembled unigene.

Blast analysis: To predict and analyze the function of the assembled transcripts, non-redundant sequences were submitted to a blast search against the following databases: Nr (NCBI non-redundant protein sequences), GO (Gene Ontology), KOG (eukaryotic orthologous groups) and KEGG (Kyoto Encyclopedia of Genes and Genomes). The unigenes were subjected to public databases for similarity searching (Li *et al*., 2015). The unigenes were subjected to public databases for similarity searching. Among these unigenes, 49943 (38.67%), 31520 (24.4%), 29719 (23%) and 91293 (70.68%) unigenes showed identity with sequences in the NCBI Nr, Nt, KOG and GO respectively.

In order to understand the whole unigenes sequence information, all unigenes were compared with NR, KEGG, KOG and other gene databases by blast to get the direction of gene, reading frame and gene function annotation. The results of blast comparison of 79941234 unigenes by using NR and other databases indicated that there were 191731 transcription sequences annotated by NCBI, NR database functions. According to the E-value distribution (Fig. 2A), 31.23% of unigenes sequences have very strong homologous alignment information and there are 68.77% unigenes sequences similarity value in evalue 1.0E 5 to 1.0E60. By the similarity distribution of annotation unigenes (Fig. 2B), the similarity of more than 80% of unigenes accounted for 37.64%, while the specific protein matching similarity of the range from 18% to 80% of unigenes accounted for 62.37%, the similarity of less than 40% of unigenes accounted for 5.85%. According to the species distribution of total homologous unigenes (Fig. 2C), 0.94% of unigenes sequence were highly matched with the grape sequence, and the proportion of the match with the sugar beet accounted for 52%, which accounted for about 27.54% of the match with the spinach and matching with soybean accounted for 1.26%.

To identify the biological pathways in the annotated sequences using the Kyoto Encyclopedia of Genes and Genomes (KEGG), the assembled unigenes were assigned to five specific pathways, including Cellular Processes, Environmental Information Processing, Genetic Information Processing, Metabolism, and Organism Systems (Fig. 3). From the KEGG classification, there are 124 pathways involved in metabolism. In the metabolic pathway, the largest number of genes involved in the pathway is Overview, then nucleotide metabolism and Carbohydrate metabolism; 22 pathways involved in genetic information processing; 35 pathways involved in environmental information processing; 19 pathways involved in cellular processes; 71 pathways involved in organismal systems.

To classify the orthologous gene products, 29719 unigenes were subdivided into 26 eukaryotic Orthologous Groups (KOG) classifications (Fig. 4). Among these classifications, the cluster of 'general function prediction only' represented the largest group, followed by 'Posttranslational modification, protein turnover, chaperones', 'signal transduction', 'translation, ribosomal structure and biogenesis', 'carbohydrate transport and metabolism', 'transcription', 'cell motility'. Two categories involving 'cell motility' and 'unnamed protein' represented the smallest KOG classifications.

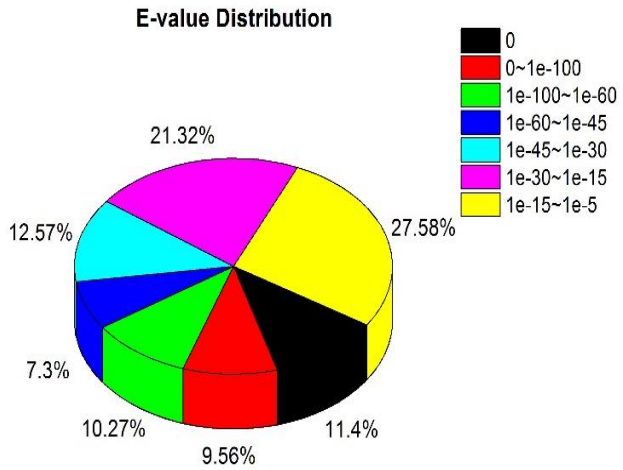

Fig. 2A. E-value distribution of each loquat.

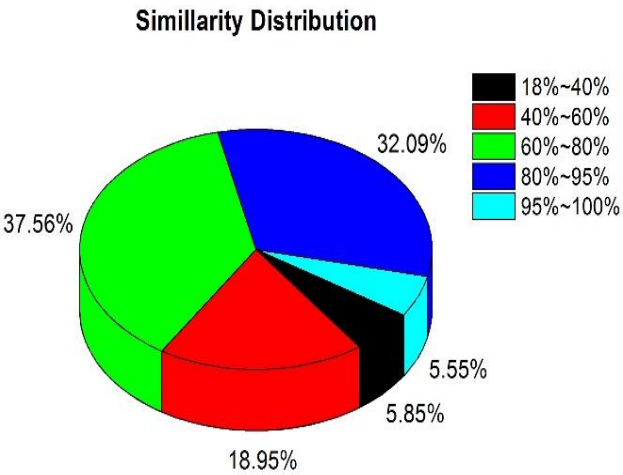

Fig. 2B. Similarity distribution of annotation unigenes.

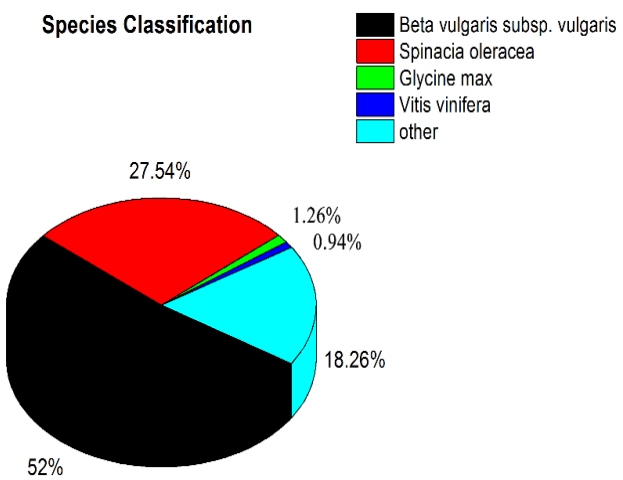

Fig. 2C. Species distribution of total homologous unigenes.

Gene Ontology (GO) assignments were used to classify the functions of the predicted *A. aphylla* genes; 7101 DEGs were classified into three major functional categories (Biological Process, Cellular Component and Molecular Function) and 50 subcategories (Fig. 5). In terms of Biological Processes, 'macromolecule metabolic processes' and 'nitrogen compound metabolic processes' were the top two GO terms. In terms of Molecular Function, the top three GO terms were related to the following categories: 'binding', 'organic cycle compound binding' and 'hetero cycle compound binding'. A detailed analysis of the Cellular Component showed that the most representative categories were 'intracellular part' and 'intracellular'.

Freezing adaptation response: Freezing adaptation can cause excess production of ROS, which are important signal molecules in plants, however, ROS can also cause irreversible damage in plant cells (Asada, 1999). In order to relieve cellular damage caused by ROS, plant have developed ROS-scavenging systems that include enzymatic scavenging systems, such as SOD, ascorbate peroxidase (APX), GSH-Px, SAM, and POD (Wang *et al*., 2017b).

Under freezing treatment (Fig. 6), the abundance of SOD was increased, this was consistent with the response of maize seedlings to freeze treatment in a previous study (Wang *et al*., 2017b). The abundance of MDH and GSH-Px was also increased. The POD isozyme levels were decreased in response to freeze treatment. POD is involved in respiration, photosynthesis, and the oxidation of auxin, its activity changes continuously in the process of plant growth and development (Zhao *et al*., 2013). In the present study, the POD levels were decreased in response to freeze adaptation. In previous studies, the abundance of POD was increased under abiotic adaptation in wheat, barley, and cucumber, but decreased in creeping bent grass (Peng *et al*., 2009; Witzel *et al*., 2009; Du *et al*., 2010; Xu *et al*., 2010). This suggested that the expression mechanism of peroxidase in plants under freezing treatment was complex.

Transcription factor prediction: Transcription factors (TFs) are important upstream regulatory proteins and play significant roles in plant responses to abiotic and biotic adaptation. In this study, 920 TFs were identified and classified into 54 different common families by searching from unique transcripts. The largest gene family was the bHLH family, followed by MYB_related family, ERF family, C2H2 family, and WRKY family (Fig. 7A). BHLH is a kind of important and conservative transcription factor in eukaryotic cells and involvement of plant tolerance to freeze adaptation. However, the research of bHLH transcription factors involved in abiotic adaptation response is still in the initial stage, many studies only indirectly inferred some potential downstream target genes (Wang *et al*., 2016). WRKY, bZIP, AP2/EREBP and MYB four families of transcription factors in plant adaptation resistance research is more in-depth (Umezawa *et al*., 2006). MYB transcription factor is one of the largest members of the plant transcription factor family, it is involved in the regulation of cell differentiation, cell cycle, hormone and environmental factor response, and plays an important regulatory role in plant secondary metabolism and morphogenesis of leaf and other organs (Liu *et al*., 2011).WRKY is not constitutively expressed in plants but is affected by various environmental factors (such as pathogens, elicitors, drought, hypothermia, trauma, mechanical adaptation, abiotic adaptation and biotic adaptation) induced expression, and involved in plant adaptation response (Su *et al*., 2007). WRKY plays a very important role as a transcription factor in many biological processes under normal and multiple adaptation

conditions. WRKY family members are widely involved in plant defense response, including positive regulation of disease resistance and negative regulation of disease resistance. The positive regulation and negative regulation can make the plant broad-spectrum resistant (Eulgem & Somssich, 2007). A total of 920 unigenes were identified to be involved in transcription, including 180 DEGs (35 up-regulated and 145 down-regulated) (Fig. 7B).

The DEGs between CK and CT were analyzed of 129162 unigenes, of which 7101 were detected as significantly different by DEG-seq (Fig. 8). In this study, DEGs with higher expression levels in CT compared with CK were denoted as 'up-regulated', while those with lower expression levels in CT were 'down-regulated'. The expression levels of 2460 of the 7101 genes were up-regulated in CK while the other 4641 genes showed lower expression in CK.

To identify the biological pathways in the annotated sequences using the Kyoto Encyclopedia of Genes and Genomes (KEGG), the DEGs were assigned to five specific pathways, including cellular processes, environmental information processing, genetic information processing, metabolism, and organism systems (Fig. 9). KEGG pathway analysis of the freeze adaptation-responsive genes. To determine whether the freeze adaptation-responsive genes engaged in specific pathways, the DEGs was used as objects to search against the KEGG pathway database. The KEGG pathway analysis indicates all differently expressed genes involved a total of 278 pathways. Top 20 obviously enriched pathways (Table 4) including substance metabolism, energy metabolism and signal transduction. By comparing differently expressed genes were involved in the largest number of is Phenylpropanoid biosynthes pathway, phenylpropanoids are related to plant growth regulation and disease resistence, it has extensive physiological activity. The acceptance and conduction of freeze signal is an important process of freezing adaptation in plants when in the process of adapting to the environment. Among the top 20 pathways, plant hormone single transduction involved in signal exchange directly. The pathway of plant hormone single transduction is an important signal transduction pathway of biotic adaptation (Cheng *et al*., 2015). The plant hormone single transduction system is an intracellular signal transduction pathway, with hormone receptor as the starting point and character production as the end point (Wang & Li, 2001).

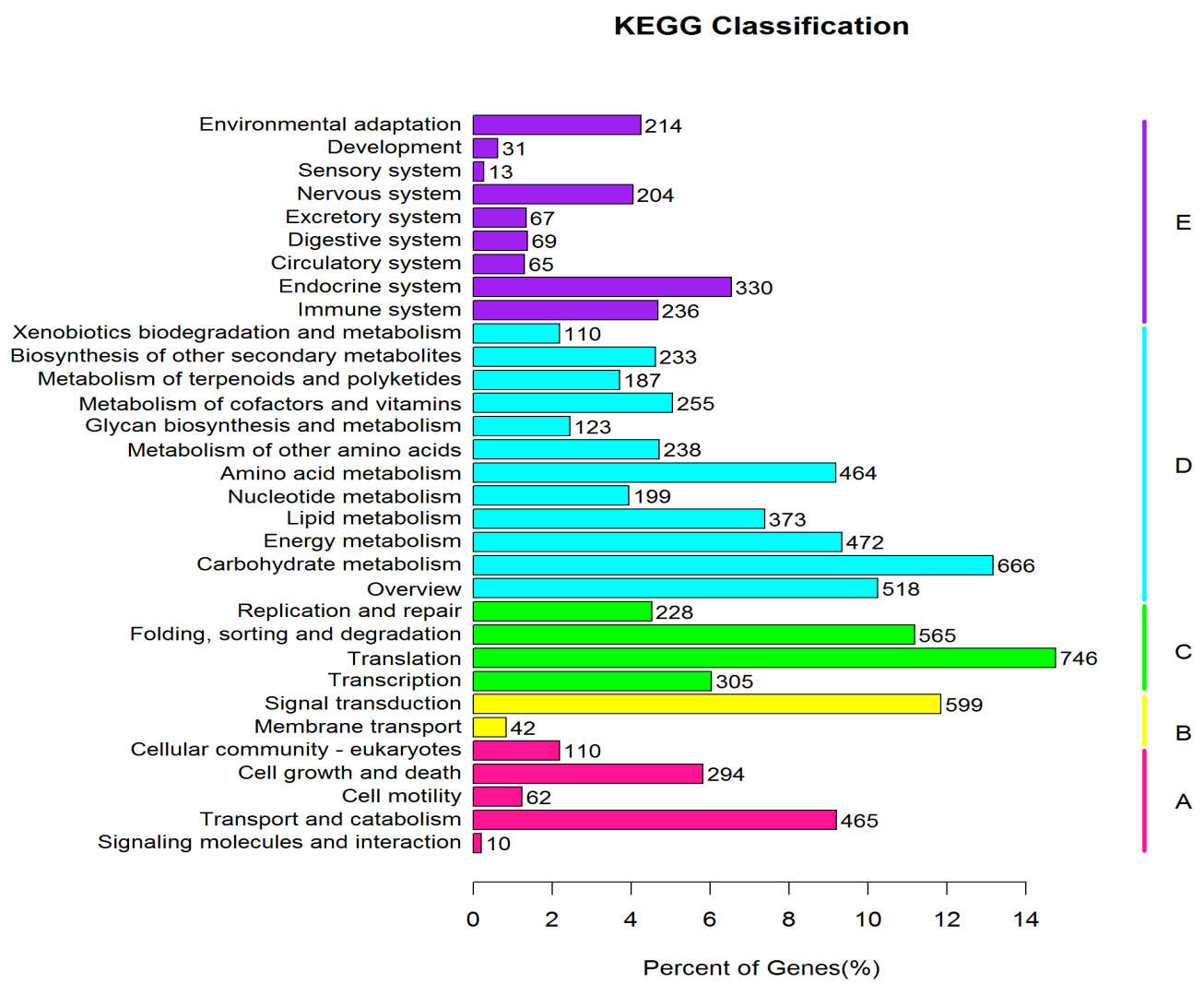

Fig. 3. Histogram presentation of the KEGG classification of the annotated transcripts. The X-axis indicates the percentage. The left Y-axis indicates the KEGG pathway. The right Y-axis indicates the sub-branches (A. cellular processes; B. environmental information processing; C. genetic information processing; D. metabolism; E. organismal systems of unigenes that were assigned to a specific pathway).

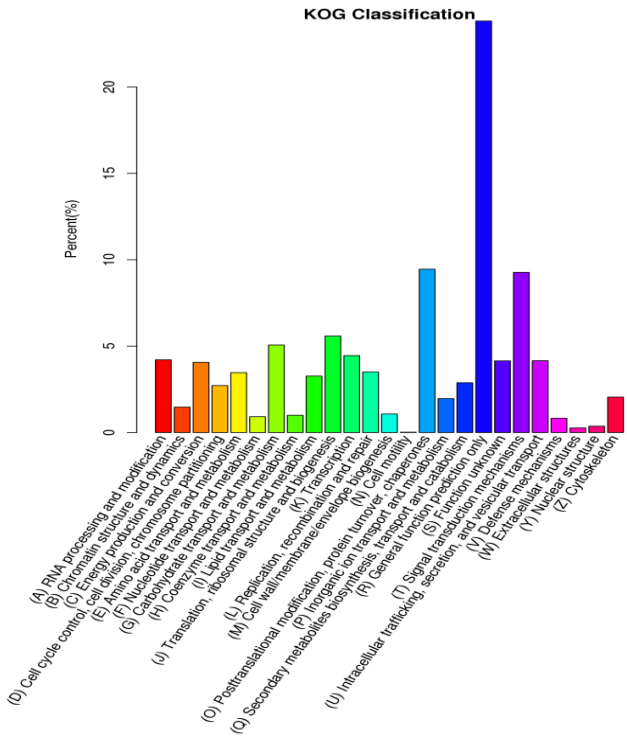

Fig. 4. KOG classification of the putative proteins. All 129162 unigenes were subdivided into 26 eukaryotic Orthologous Group (KOG) classifications. The Y-axis indicates the number of unigenes in a specific functional cluster.

In this study, 56 differential genes were found involved in plant hormone single transduction pathway (Fig. 10). Plant hormones are plant endogenous signal molecules involved in plant organ formation, morphogenesis, environmental response, resistance to pests and diseases and so on. Studies have shown that JAZ can inhibit JA signaling by inhibiting transcription factor function (Yin *et al*., 2011). As an important second messenger, Ca^{2+} is known to play a role in plants freeze adaptation response. The concentration of Ca^{2+} increases rapidly during freeze treatment, followed by a series of signals mediated by a combination of protein Phosphorylation / dephosphorylation cascades (Saijo *et al*., 2000). As a large sub-family of plant kinases, Calcium dependent protein kinase (CDPKs) are implicated as important sensors of Ca^{2+} flux in plants in response to a variety of biotic and abiotic adaptation stimuli (Ludwig *et al*., 2004). It can convert the extracellular signal received by the cell surface receptor into intracellular signals.

Real time quantitative PCR analysis and validation: In this study, total 9 genes were selected and analyzed by real-time quantitative PCR before and after freezing treatment. The results of fluorescence quantitative analysis showed that the real-time fluorescence quantitative analysis results were consistent with the expression trend of transcriptome analysis genes (Fig. 11), indicating the reliability of transcriptome data obtained by Illumina sequencing.

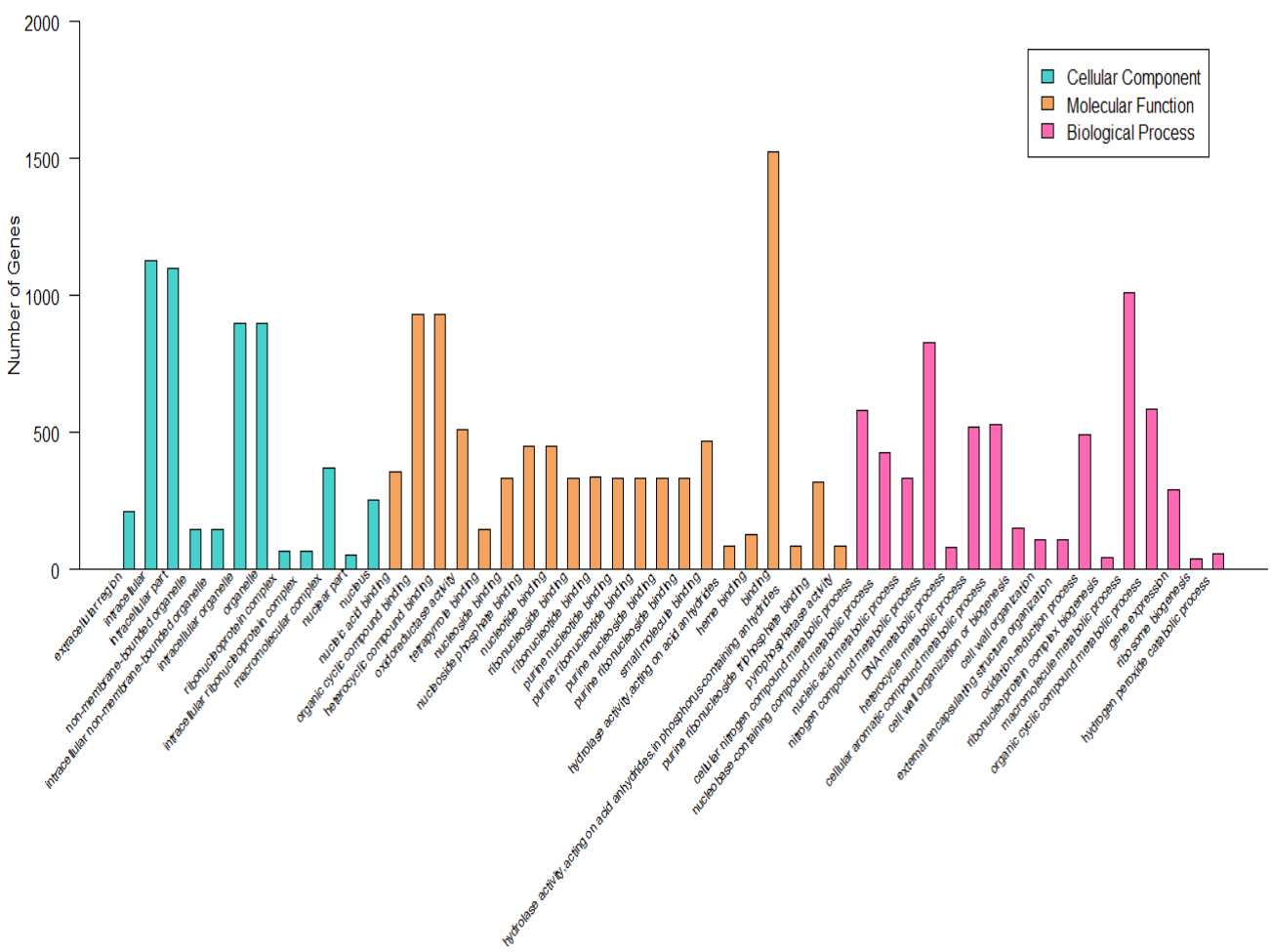

Fig. 5. Go categories of DEGs.

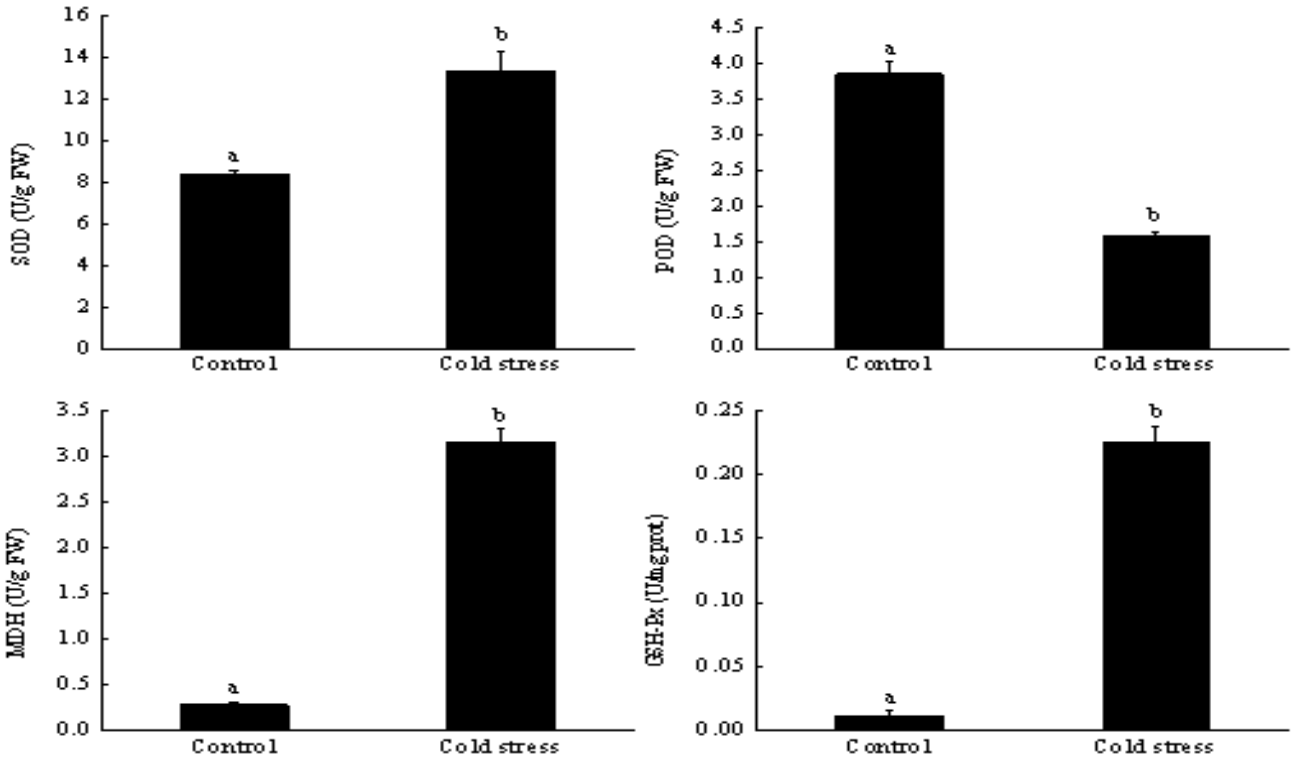

Fig. 6. Activities of superoxide dismutase (SOD), peroxidase (POD), malate dehydrogenase (MDH) and glutathione peroxidase (GSH-Px) in *A. aphylla* under the control treatment and freeze treatment.

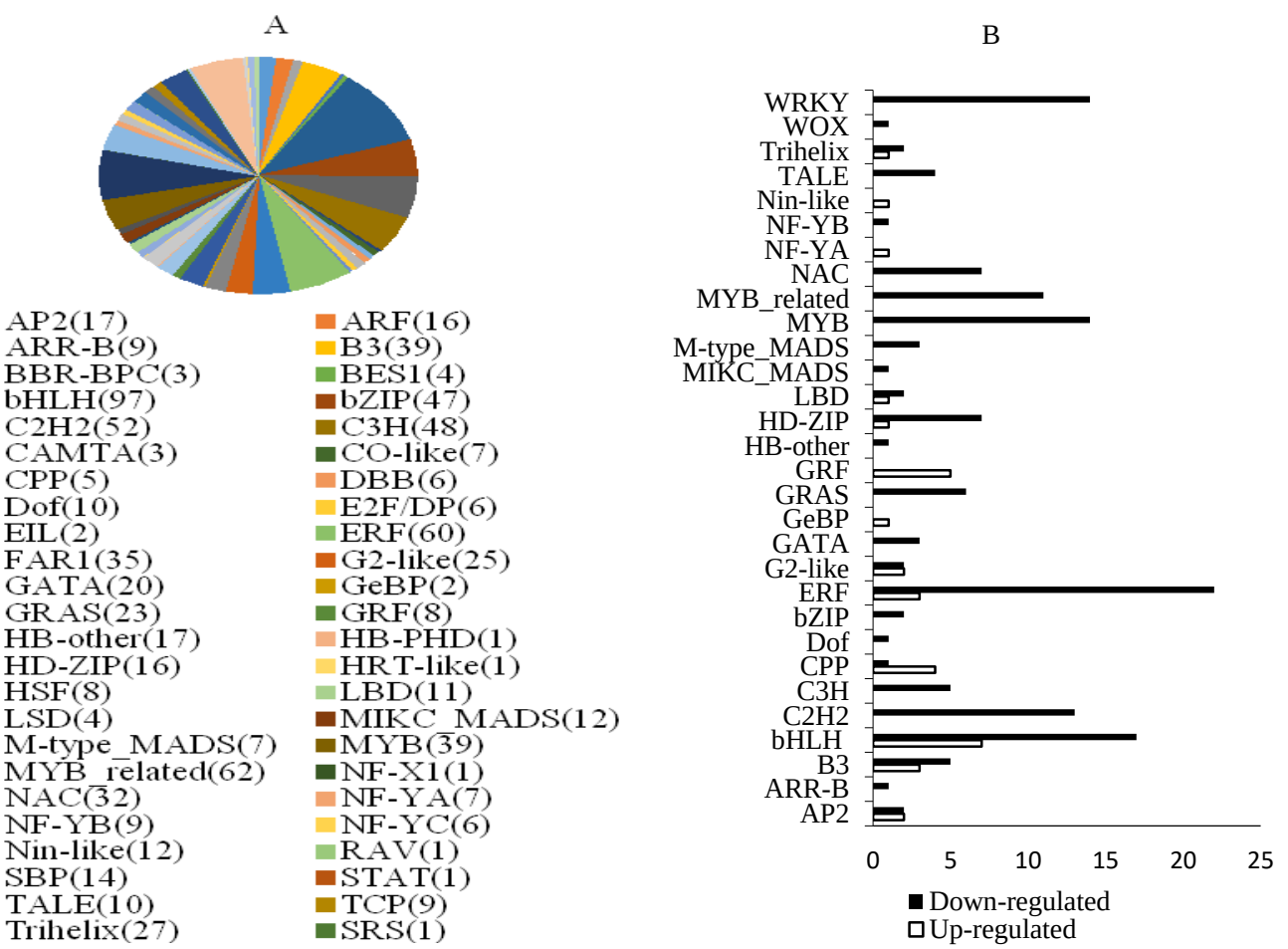

Fig. 7. Number of unique transcripts that were annotated as transcription factors. A. The distribution of transcription factors according to the gene family information. B. DEGs from every gene family involved in transcription.

Table 4. Top 20 pathways with different expression genes.

Pathway	Gene number	Pathway type	Pathway ID
Metabolism	181	Carbohydrate metabolism	ko00500
Environmental Information Processing	140	Signal transduction	ko04075
Metabolism	135	Biosynthesis of other secondary metabolites	ko00940
Metabolism	125	Energy metabolism	ko00195
Metabolism	116	Amino acid metabolism	ko00280
Metabolism	102	Overview	ko01200
Metabolism	101	Lipid metabolism	ko00071
Metabolism	74	Metabolism of other amino acids	ko00480
Cellular Processes	72	Transport and catabolism	ko04144
Genetic Information Processing	69	Folding, sorting and degradation	ko04141
Genetic Information Processing	68	Translation	ko03010
Metabolism	58	Metabolism of terpenoids and polyketides	ko00903
Organismal Systems	58	Endocrine system	ko04915
Organismal Systems	54	Environmental adaptation	ko04626
Cellular Processes	53	Cell growth and death	ko04110
Organismal Systems	51	Immune system	ko04620
Organismal Systems	50	Nervous system	ko04722
Metabolism	48	Xenobiotics biodegradation and metabolism	ko00982
Metabolism	44	Metabolism of cofactors and vitamins	ko00900
Metabolism	24	Nucleotide metabolism	ko00230

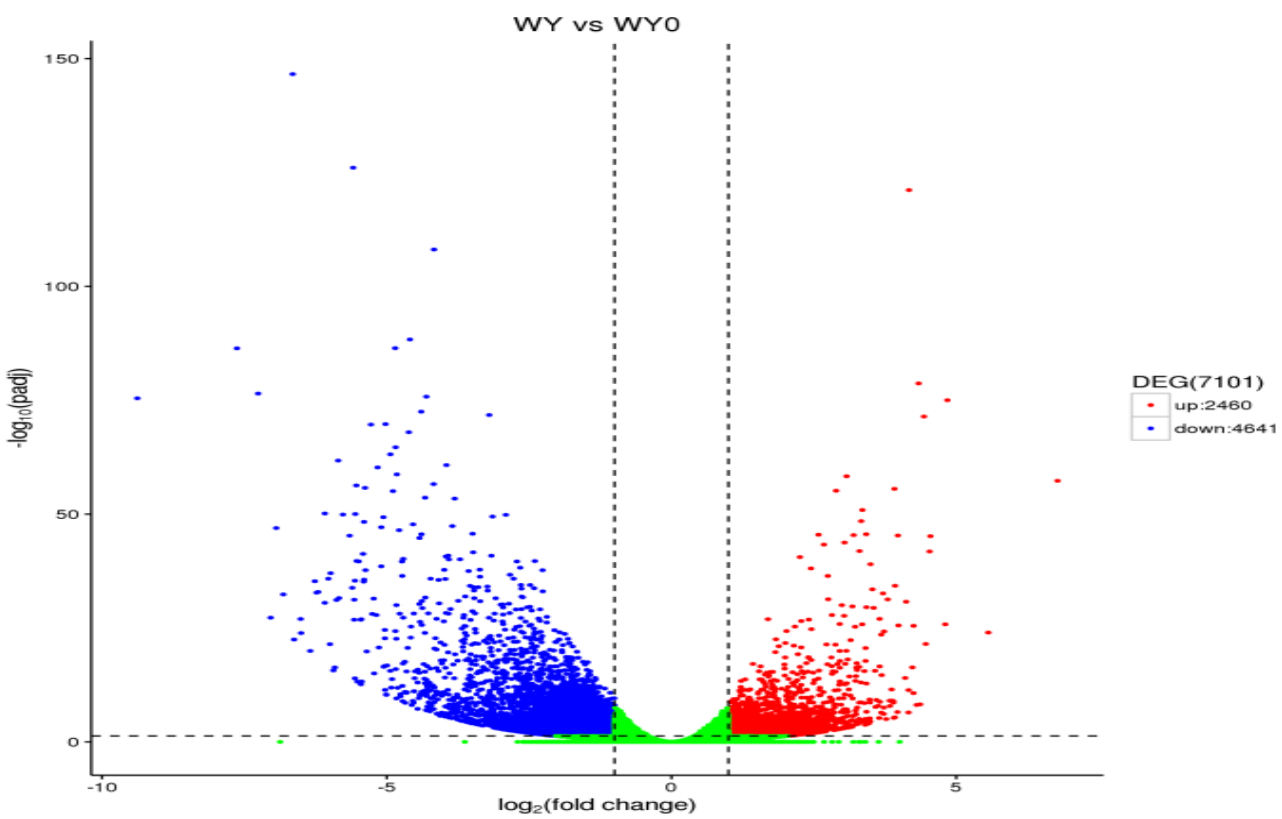

Fig. 8. Volcano plot displaying differential expressed genes between CT and CK sample.

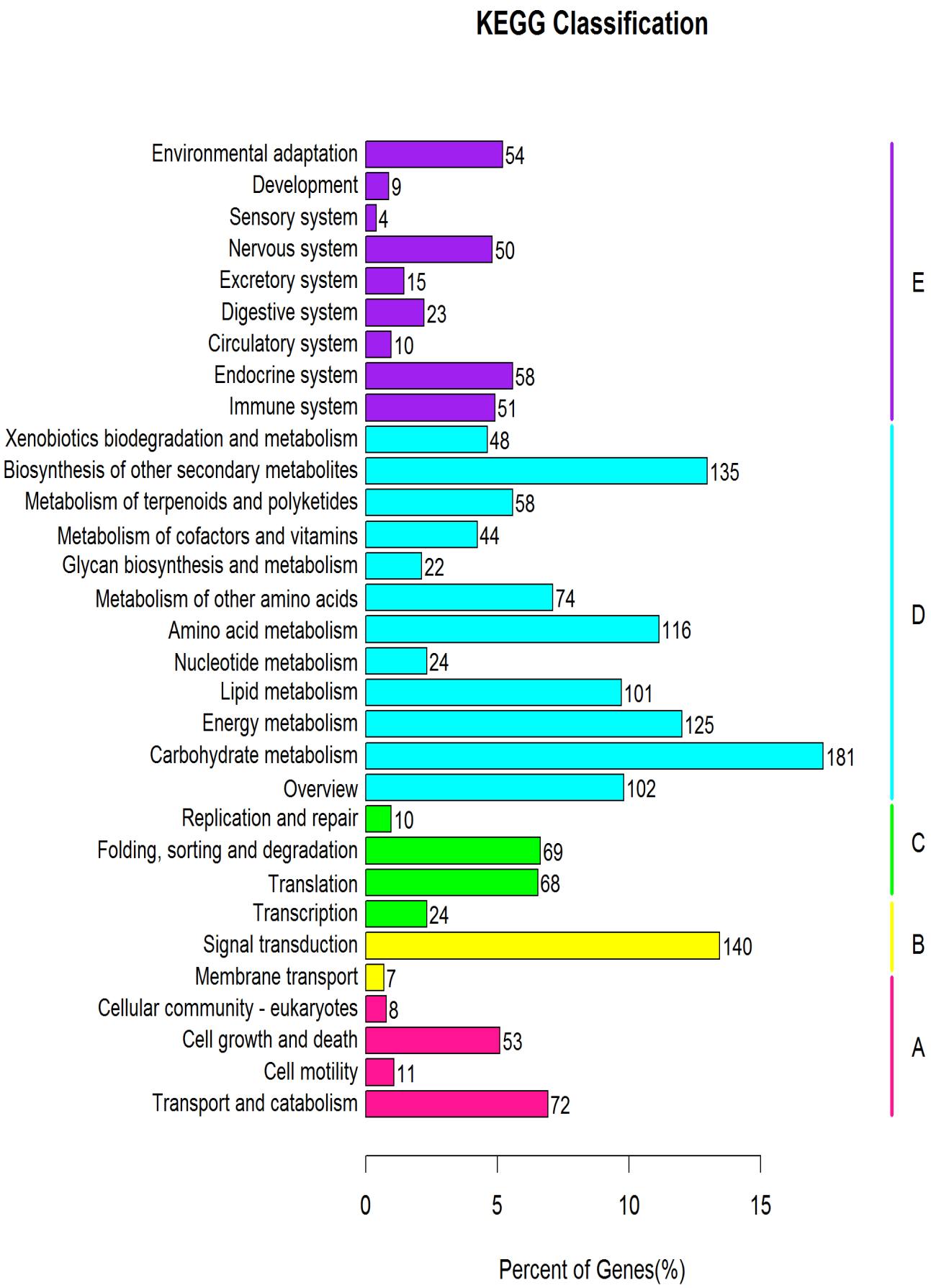

Fig. 9. KEGG classification of DEGs. The X-axis indicates the percentage. The left Y-axis indicates the KEGG pathway. The right Y-axis indicates the sub-branches (A. cellular processes; B. environmental information processing; C. genetic information processing; D. metabolism; E. organismal systems of unigenes that were assigned to a specific pathway).

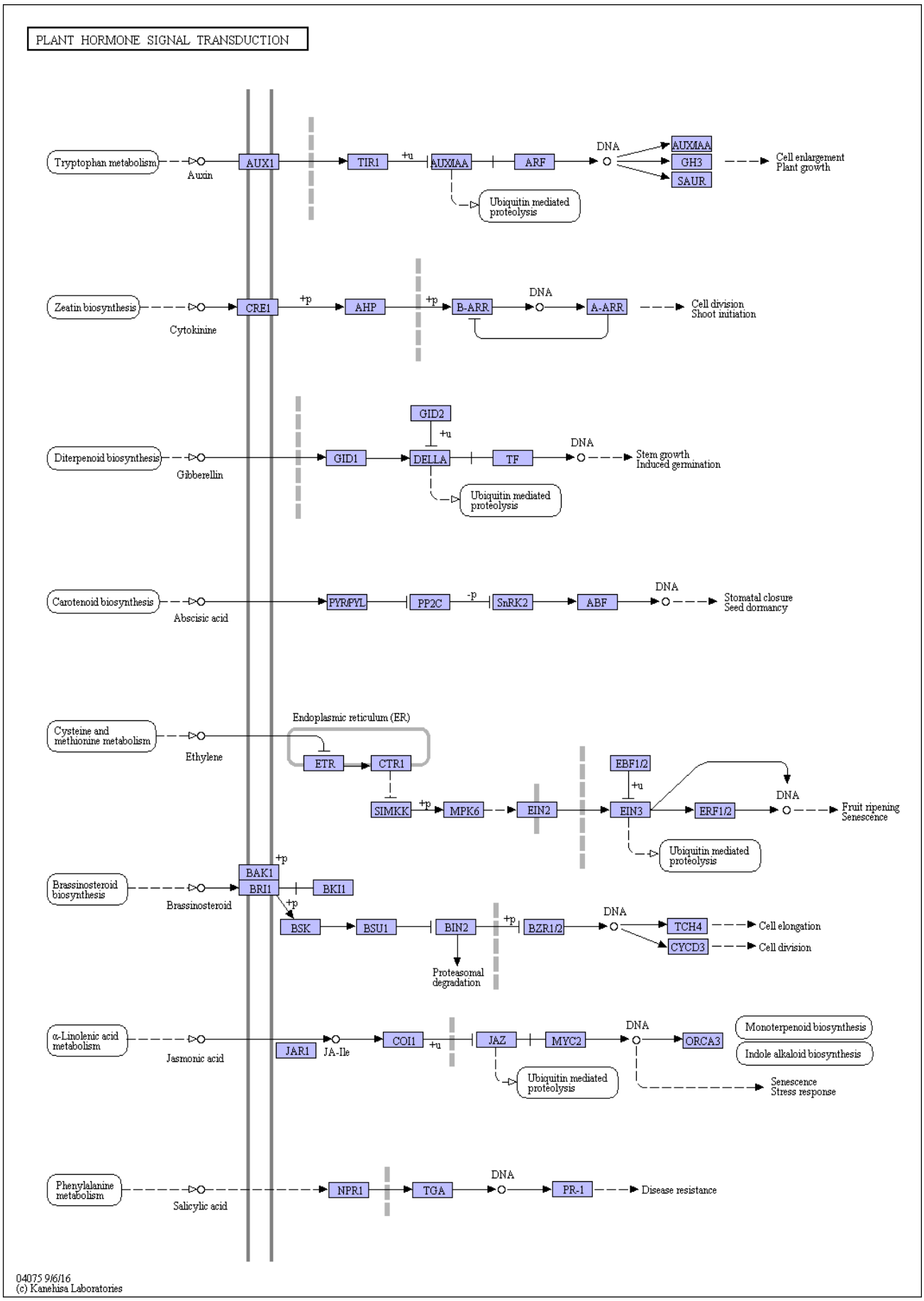

Fig. 10. Plant hormone singnal transduction pathway.

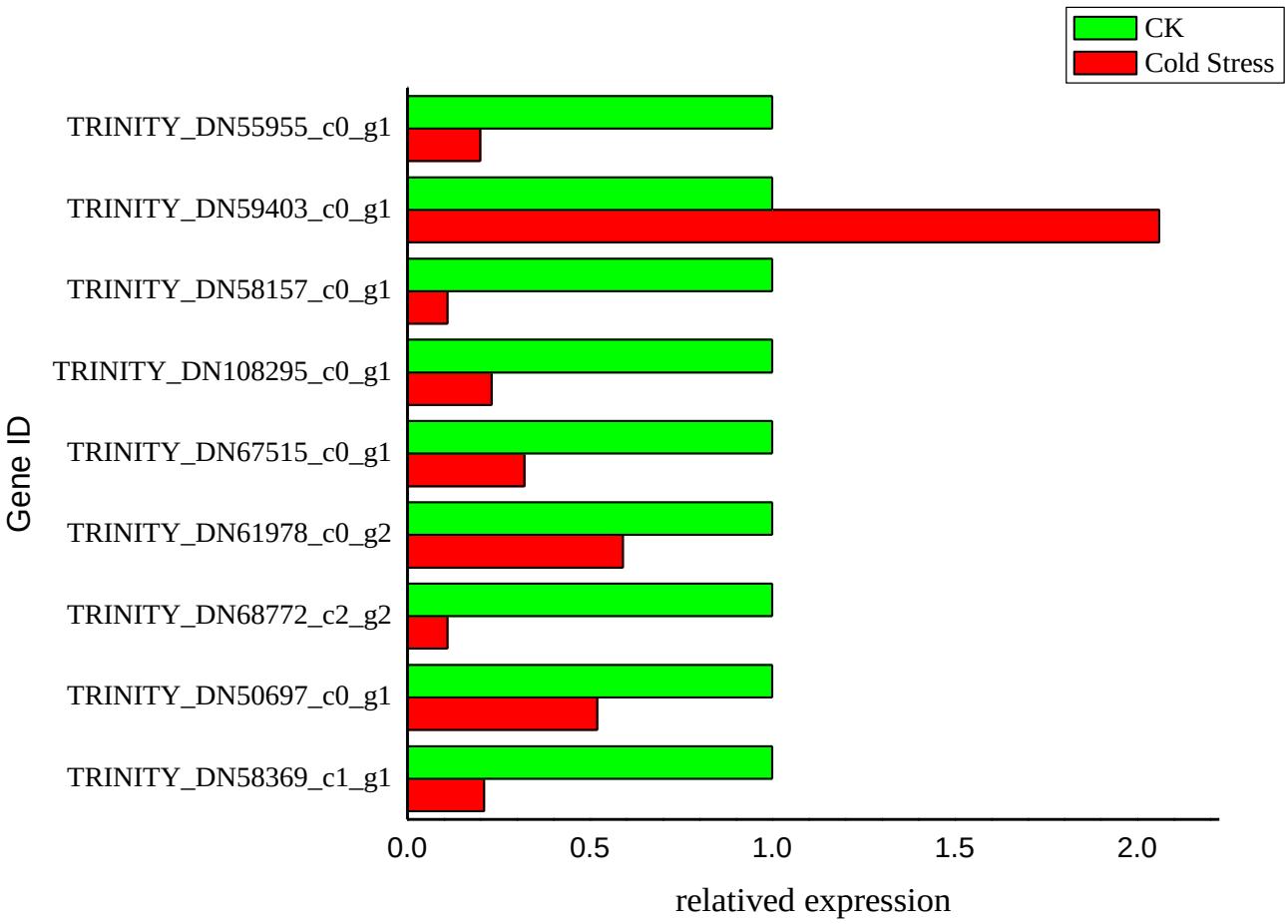

Fig. 11. PCR analysis and validation.

This study analyzed the molecular regulation of *A. aphylla* after freezing by transcriptome sequencing technology, through statistical bio-informatics and molecular biology methods of differential gene expression. The results of this study not only deepened our understanding of *A. aphylla* freeze adaptation reaction mechanism, but also provided research value for other desert plants. There are many aspects of low temperature adaptation and are subject to transcriptional regulation, therefore many transcription factors are selected as plant freeze adaptation in the process of the target tool for the future application of transgenic lay molecular biological basis.

Conclusions

This is the first report of transcriptome sequencing of *A. aphylla* under freezing adaptation using Illumina. A total of 129162 unigenes were assembled, 7101 of which were differently expressed with 2460 unigenes showing up-regulation and 4641 unigenes showing down-regulation. By performing blast analysis of the all unigenes against public databases (Nr, Swiss-Prot, KEGG and COG), we obtained functional annotations and classifications. The large number of transcriptomic sequences and their functional annotations provide sufficient resources for molecular studies of *A. aphylla.* Moreover, information on the KEGG plant hormone signal transduction pathway and transcription factors will facilitate the discovery of other freeze adaptation genes.

Acknowledgements

The research was supported by the Program of the National Natural Science Foundation of China (31570595, 31660194), General Financial Grant of the China Postdoctoral Science Foundation (2017M613253) and Scientific Research Foundation of Shihezi University for Advanced Talents (RCZX201518, RCZX201521).

References

An, D., J. Yang and P. Zhang. 2012. Transcriptome profiling of low temperature treated cassava apical shoots showed dynamic responses of tropical plant to cold stress. *Bmc. Genomics,* 13(1): 64-89.

Asada, K. 1999. The water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Ann. Rev. Plant Physiol. Plant Mol. Biol*., 50(1): 601-639.

Cheng, Z.Y., M. Li, Y. Shi, P.H. He and F.S. Li. 2015. Research of drought-response mechanism in sugarcane by high through-put sequencing-based digital gene expression profiling. *Mol. Plant Breed*., 13: 2018-2028.

Chu, G.M., M. Wang and S.X. Zhang. 2014. Spatial point patters of *Anabasis aphylla* populations in the Proluvial Fan of South Junggar Basin. *Scientia Silvae Sinicae*, 50(4): 8-14.

Conesa, A., S. Götz, J.M. García-Gómez, J. Terol, M. Talón and M. Robles. 2005. Blast2 GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bio. Informatics*, 21(18): 3674-3676.

Dang, Z., L. Zheng, J. Wang, Z. Gao, S. Wu, Z. Qi and Y.C. Wang. 2013. Transcriptomic profiling of the salt-stress

response in the wild recretohalophyte *Reaumuria trigyna*. *BMC Genomics*, 14(1): 29-47.

Du, C.X., H.F. Fan, S.R. Guo, T. Tezuka and J. Li. 2010. Proteomic analysis of cucumber seedling roots subjected to salt stress. *Phytochem*., 71(13): 1450-1459.

Dugas, D.V., M.K. Monaco, A. Olson, R.R. Klein, S. Kumari, D. Ware and P.E. Klein. 2011. Functional annotation of the transcriptome of *Sorghum bicolor* in response to osmotic stress and abscisic acid. *BMC. Genomics*, 12(1): 514-536.

Eulgem, T., I.E. Somssich. 2007. Networks of WRKY transcription factors in defense signaling. *Curr. Opin. in Plant Biol*., 10(4): 366-371.

Han, J., Y. Wei and C. Yan. 2011. The vivipary characteristic of *Anabasis elatior* and its ecological adaptation. *Chinese Acta Ecologica Sinica*, 31(10): 2662-2668.

Gao, F., J. Wang, S. Wei, Z.L. Li, N. Wang, H.Y. Li, J.C. Feng, H.L. Li and Y.J. Zhou. 2015. Transcriptomic analysis of drought stress responses in *Ammopiptanthus mongolicus* leaves using the RNA-Seq technique. *Plos One*, 10(4): e0124382.

Grabherr, M.G., B.J. Haas, M. Yassour, J.Z. Levin and D.A. Thompson. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnol*., 29(7): 644-652.

Kanehisa, M., M. Araki, S. Goto, M. Hattori, M. Hirakawa, M. Itoh, T. Katayama, S. Kawashima and S. Okuda. 2008. KEGG for linking genomes to life and the environment. *Nucl. Acids Res*., 36: D480-D484.

Li, H., W. Yao, Y. Fu, S. Li and Q. Guo. 2015. *De novo* assembly and discovery of genes that are involved in drought tolerance in Tibetan *Sophora moorcroftiana*. *Plos One*, 10(1): e111054.

Liu, S.J., H.J. Wan, Q.J. Ye, R.Q. Wang, M.Y. Ruan, Z.P. Yao and Y.J. Yang. 2011. Progress in the study of plant MYB transcription factors. *Mol. Plant Breed*., 9(1): 1835-1842.

Liu, T., S. Zhu, Q. Tang, Y. Yu and S. Tang. 2013. Identification of drought stress responsive transcription factors in ramie (*Boehmeria nivea* L.Gaud). *BMC. Plant Biol.*, 13(1): 1-9.

Ludwig, A.A., T. Romeis and J.D.G. Jones. 2004. CDPK-mediated signaling pathways: specificity and cross-talk. *J. Exp. Bot*., 55(395): 181-188.

Nishiyama, R., D.T. Le, Y. Watanabe, A. Matsui, M. Tanaka, M. Seki, K. Yamaguchi-Shinozaki and K. Shinozaki. 2012. Transcriptome analyses of a salt-tolerant cytokinin deficient mutant reveal differential regulation of salt stress response by cytokinin deficiency. *Plos One*, 7(2): e32124.

Pertea, G., X. Huang and F. Liang. 2003. TIGR Gene Indices clustering tools (TGICL): a software system for fast clustering of large EST datasets. *Bio. Informatics*, 19(5): 651-652.

Peng. Z.Y., M.C. Wang, F. Li, H.J. Lv, C.L. Li and G.M. Xia. 2009. A proteomic study of the response to salinity and drought stress in an introgression strain of bread wheat. *Mol. Cell Proteomics*, 8: 2676-2686.

Saijo, Y., S. Hata and J. Kyozuka. 2000. Over-expression of a single Ca^{2+} dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *Plant J*., 23(3): 319-327.

Su, Q., Y.H. Shang, M.Y. Du and J.M. Du. 2007. Progress on plant WRKY transcription factor. *Chinese Agri. Sci. Bull*., 23: 94-98.

Umezawa, T., M. Fujita, Y. Fujita, K. Yamaguchi-Shinozaki and K. Shinozaki. 2006. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Curr. Opin. in Biotechnol*., 17(2): 113-122.

Wang, B., W.M. Li. 2001. Advances in signal transduction pathways of plant hormones. *Chinese J. Fujian Agri. Uni.*, 30(4): 433-438.

Wang, T.T., G.M. Chu, P. Jiang, P.X. Niu and M. Wang. 2017a. Effects of sand burial and seed size on seed germination, seedlings emergence and seedling biomass of *Anabasis aphylla*. *Pak. J. Bot*., 49(2): 391-396.

Wang, T.T., C.X. Ye, M. Wang and G.M. Chu. 2017b. Identification of cold-stress responsive proteins in *Anabasis aphylla* seedlings via the iTRAQ proteomics technique. *J. Plant Interact*., 12(1):505-519.

Wang, X.C., Q.Y. Zhao, C.L. Ma, Z.H. Zhang, H.L.Cao, Y.M. Kong, C. Yue, X.Y. Hao, L. Chen and J.Q. Ma. 2013. Global transcriptome profiles of *Camellia sinensis* during cold acclimation. *BMC. Genomics*, 14(1): 415-430.

Wang, X.J. and K.Z. Li. 2015. Progress of plant bHLH transcription factors involved in abiotic stress signaling pathways. *Chinese Bull. of Life Sci*., 27(2): 208-216.

Wang, X.Y., X.H. Shan, Y. Wu, S.Z. Su, S.P. Li, H.K. Liu, J.Y. Han, C.M. Xue and Y.P. Yuan. 2016. iTRAQ-based quantitative proteomic analysis reveals new metabolic pathways responding to chilling stress in maize seedlings. *J. Proteomics*, 146: 14-24.

Witzel, K., A. Weidner, G.K. Surabhi, A. Börner and H.P. Mock. 2009. Salt stressinduced alterations in the root proteome of barley genotypes with contrasting response towards salinity. *J. Exp. Bot*., 60: 3545-3557.

Wu, F.Z., H.X. Wang, G.H. Xu and Z.C. Zhang. 2015. Research Progress on the physiological and molecular mechanisms of woody plants under low temperature stress. *Scientia Silvae Sinicae*, 51(7): 116-128.

Xi, J.B., F.S. Zhang and C.Y. Tian (Eds.). 2006. Halophytes in Xinjiang. Science Press, China.

Xu. C., T. Sibicky and B. Huang. 2010. Protein profile analysis of salt-responsive proteins in leaves and roots in two cultivars of creeping bent grass differing in salinity tolerance. *Plant Cell Reports*, 29: 595-615.

Ye, J., L. Fang, H. Zheng, Y. Zhang, J. Chen, Z.J. Zhang, J. Wang, S.T. Li and R.Q. Li. 2006. WEGO: a web tool for plotting GO annotations. *Nucl. Acids Res*., 34: W293-W297.

Yin, K.Q. 2011. Construction of *Arabidopsis* transcription factors library and its application in plant hormones signal transduction (PhD thesis). University of Beijing, China.

Zhao, Q., H. Zhang, T. Wang, S.X. Chen and S.J. Dai. 2013. Proteomics-based investigation of salt-responsive mechanisms in plant roots. *J. of Proteomics*, 82: 230-253.

(Received for publication 10 July 2019)