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Full Length Article

De novo Assembly and Discovery of Genes in Potato (*Solanum tuberosum*) under Drought Stress and Rehydration

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Abstract

As a non-grain crop, potato represents an important food source worldwide. Tuberization, yield and quality of potato can be seriously affected by drought stress. However, the molecular mechanisms underlying potato leaf's response to drought stress and rehydration remain inadequately studied. Here, we subjected a potato (*Solanum tuberosum* L.) variety, Kexin1, to drought stress treatment (DT), drought stress control (DCK), rehydration treatment (RT), and rehydration control (RCK) at seedling stage. Through transcriptome sequencing analyses, there were 655 differentially expressed genes (DEGs) in DT *vs* DCK, 644 DEGs were identified in RT *vs* RCK, 3443 DEGs were identified in DT *vs* RT. Interestingly, among the 115 shared genes in DT *vs* CK and RT *vs* RCK, 8 genes showed opposite expression trend. The functions of these genes include major facilitator superfamily, sugar transporter, ferritin, and glycoside hydrolase. It is likely that these eight genes play an important role in potato recovery from drought. Based on the differentially expressed genes, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis identified some important pathways, such as 'flavonoid biosynthesis' and 'stilbenoid, diarylheptanoid and gingerol biosynthesis' significantly enriched in DT *vs* CK, 'glutathione metabolism' and 'plant-pathogen interaction' significantly enriched in RT *vs* RCK. In addition, we used quantitative real-time PCR and validated the expression patterns for 10 genes that were identified through RNA-seq as being involved in transcript abundance changes under drought stress and rehydration. The globally sequenced genes showed a significant proportion of the potato transcriptome, and the expression results may shed light to further disseminate the knowledge on the drought tolerance of potato, providing a theoretical framework for the cultivation of new potato varieties.

Keywords: Differential expression genes; Drought; Potato; Rehydration; Transcriptome

Introduction

One of the major abiotic stresses, drought poses a serious threat to the world food security, especially in the situation of climate changes (Yooyongwech *et al*., 2013), leading to limiting plant production. As a non-grain crop, potato represents an important food source worldwide (Gong *et al.*, 2015). The seedling, tuber formation and tuber expansion, yield of potato can be affected seriously by drought stress, even the potato quality (Gong *et al*., 2015), water deficit inhibits potato distribution and acreage, and affects the further development. Many physiological studies on potato, such as the physiological response and growth characteristics of off-season potatoes affected by moderate water deficit (Ierna and Mauromicale, 2006), the relationship between partial root-zone drying and potato tuber yield (Yactayo *et al.*, 2013), the chlorophyll concentration in potato leaves in water-shortage conditions (Ramirez *et al*., 2014), the clonal differences of potato caused by intrinsic water use efficiency (Topbjerg *et al.*, 2014), the chlorophyll concentration related to potato yield under drought stress (Rolando *et al.*, 2015), thus physiological characteristics of potato under drought stress has been studied. But it is also necessary to study potato drought resistance mechanism from the molecular perspectives to breed new drought tolerant potato variety. Now with the genome sequence of potato (Potato Genome Sequencing Consortium *et al*., 2011), 18 gene modules form the potato transcriptome identified by gene expression network analysis (Massa *et al.*, 2011), the molecular mechanisms of potato under drought stress through transcriptome profiling (Gong *et al.*, 2015) were conducted to provide theoretical basis for the mechanism of drought resistance of potato. However, there remain insufficient data on drought, rehydration and biological growth processes at the transcriptomic level. Critically, the key genes and detailed pathways during drought stress need to be researched.

The new genes, metabolic pathways, gene expression patterns, transcriptome map were studied by transcriptome sequencing technologies (Gong *et al.*, 2015). The plant genes expressed under drought stress were identified

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through RNA-Sequencing, for example, cotton (Padmalatha *et al.*, 2012), *Cymbidium hybridum* (Zhao *et al.*, 2014), *Haloxylon ammodendron* (Long *et al.*, 2014), *Sophora moorcroftiana* (Li *et al.*, 2015), *Erigeron breviscapus* (Chen *et al.*, 2015), *Camellia sinensis* (Liu *et al.*, 2016) have been studied. In this paper, for identifying drought-resistance genes, potential dehydration-responsive genes were first identified based on Illumina tag-sequencing, followed by screening DEGs and validated by qRT-PCR. This study will help clarify the molecular mechanism of potato response to drought treatment, in future drought resistance will be improved on genetic level.

Materials and Methods

Treatments

As the experimental material, Kexin1 original species of the potato (*Solanum tuberosum* L.) was used, and 40 pots of potato seedlings growing well at the same status were selected for this test, led in Inner Mongolia Agricultural University of China. Drought stress treatment (DT) with field soil and 40% water content, drought stress control (DCK), with field soil and 70% water content, rehydration treatment (RT), with field pre-soil 40% water content and later restored at 70%, and rehydration control (RCK), with field soil water content with a constant 70% water content, were set in this pot experiment. In DT, using artificial control of water natural drought method to simulate water stress maintained for 14 days at 40% water content. After 14 days of drought stress, RT was rehydrated to the control level (70%) and maintained for 7 days.

The control and treatment groups were set up in ten biological replicates, and were weighed daily in the interval 9:00–10:00 AM and replenished, to make sure the moisture content for each treatment. At the end of DT and RT, the young leaves of the potato were collected from ten individuals in DT, DCK, RT and RCK groups, which were taken as the sequencing materials. All the materials were frozen by taking into liquid nitrogen, and stored in a -80℃ cryogenic refrigerator for sequencing analysis.

Transcriptome

Total RNA was extracted from potato leaf materials, and RNA purity and integrity met the extraction requirement. Q20, Q30, GC content and sequence duplication level of the clean data, which obtained from raw reads, were calculated. FPKM (Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced) was used for estimating gene expression levels commonly (Trapnell *et al.*, 2014).

Differential Expression Analysis

Differentially expressed genes (DEGs) were analyzed from three biological replicates, according to adjusted P-value, which is smaller than 0.05 was assigned as differentially expressed. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways were conducted for enrichment analysis using KOBAS software.

Verification of DEGs

qRT-PCR (Quantitative Real-time PCR) is a method for testing the validity and accuracy of transcriptome sequencing data. According to differential expression analysis, 10 genes were selected for fluorescent qRT-PCR. *ef1α* (the elongation factor 1-α) was used as reference gene (Nicot *et al.*, 2005). The $2^{-(\Delta\Delta Ct)}$ method (Livak and Schmittgen, 2001) was used to calculate the expression level of selected genes.

Results

DEG Analysis of Potato in DT and RT

A total of 52481881, 51325932, 45604570 and 47472519 raw reads were obtained respectively by transcriptome sequencing from cDNA libraries of DT, DCT, RT and RCK. Among these reads, 28.38 G clean bases were obtained by filtering impurities, and 64.49% of them in line with the published potato genome. By sequencing analyzing, DT, DCT, RT and RCK cDNA libraries produced 25640, 26049, 26043, 26056 genes, respectively (Table 1).

Then, data obtained from the twelve samples in DT, DCT, RT and RCK four groups were studied for comparative analysis, and DEGs were identified by corrected p-value <0.05, due to biological duplication. We identified a total of 4742 DEGs (among them 3573 DEGs were expressed solely). In DT *vs* DCK, there were 349 DEGs up-regulated and 306 DEGs down-regulated. In RT *vs* RCK, there were 149 DEGs up-regulated and 495 DEGs down-regulated. In DT *vs* RT, there were 1965 DEGs up-regulated, and 1478 DEGs down-regulated. Up-regulated transcripts accounted for 53, 23 and 57% of the total DEGs in DT *vs* DCK, RT *vs* RCK, DT *vs* RT, respectively (Table 2). From the volcano plot, the differences in gene expression differences were seen, in which DEGs were expressed significantly as red dots (up-regulated) and green dots (down-regulated), blue dots indicated no significant difference in DEGs (Fig. 1).

In order to eliminate the process of biological growth regulation, Venn diagram among DT *vs* DCK, RT *vs* RCK, and CK *vs* RCK was used (Fig. 2). Results showed that in 655 DEGs there were 336 genes individually controlled drought mechanism; In 644 DEGs there were 115 genes individually controlled rehydration mechanism; there were 32 shared genes between DT *vs* DCK and RT *vs* RCK, indicating a linkage between drought treatment and rehydration, among them PGSC0003DMG400008787, PGSC0003DMG400020950, PGSC0003DMG400008345 were up-regulated under drought treatment, but down-regulated under rehydration treatment, these three genes

Table 1: Statistical analyses of cDNA libraries from potato leaves under different treatment conditions

Collection site	DT(Drought stress treatment)	DCK(Drought stress control)	RT(Rehydration treatment)	RCK(Rehydration treatment control)
Raw reads	52481881	51325932	45604570	47472519
Clean reads	49863308	49651786	44071742	45593042
Total mapped	31377909(62.93%)	32523133(65.50%)	28817595(65. 39%)	29233720(64.12%)
Clean bases(G)	7.48	7.45	6.61	6.84
Error rate(%)	0.02	0.01	0.01	0.01
Q30(%)	92.03	94.38	94.25	94.17
GC content(%)	43.07	42.53	42.74	42.78
Gene number	25640	26049	26043	26056

Table 2: The number and regulation pattern of differentially expressed genes

Comparison group	DT *vs*.DCK		RT*vs*.RCK		DT *vs*.RT	
Regulation type	Up-regulated	Down-regulated	Up-regulated	Down-regulated	Up-regulated	Down-regulated
Number/ratio	349(53%)	306(47%)	149(23%)	495(77%)	1965(57%)	1478(43%)
Total	655		644		3443	

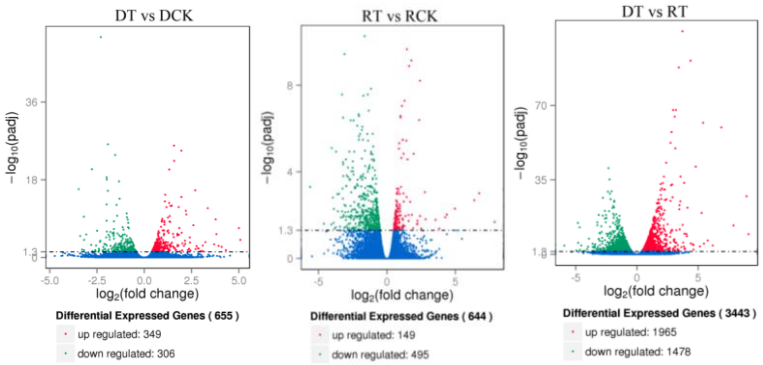

Fig. 1: Volcano plots of differential expressed genes in DT *vs* DCT, DT *vs* RT and RT *vs* RCK

played an important role in the drought to rehydration recovering process (Table 3); In addition, a total of 81 DEGs overlapped in DT *vs* DCK, RT *vs* RCK, and DCK *vs* RCK, showing a linkage among drought treatment, rehydration treatment and biological process, among them PGSC0003DMG401023109, PGSC0003DMG400016638, PGSC0003DMG400011642, PGSC0003DMG400019465 were down-regulated during drought treatment, up-regulated during rehydration, PGSC0003DMG400024171 were up-regulated under drought treatment, but down-regulated under rehydration treatment (Table 3).

KEGG Pathway Analysis of Potato DEGs in DT and RT

In the pathway annotation analysis, KOBASA database was used to compare with our data. According to the KEGG pathway database, we searched the DEGs matched with KEGG, for clarifying the specific pathways of the drought stress-responsive genes. We found that there were 4742 DEGs enriched in 274 pathways (among them 118 pathways were unique). Fig. 3 showed the top 20 obviously enriched pathways in DT *vs* DCK. X-axis and Y-axis are the Rich factor and the KEGG pathway. A low q value is marked in red, and a high q value is marked in blue.

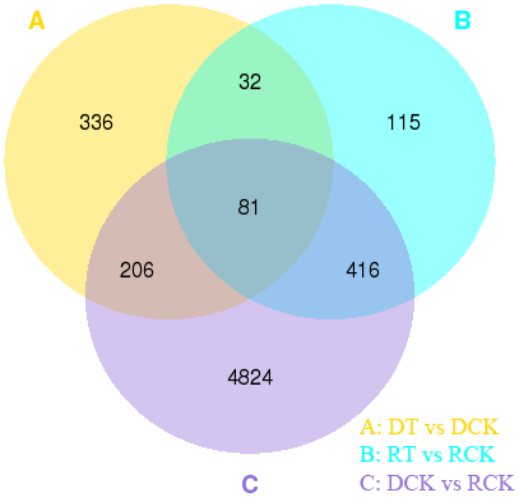

Fig. 2: Venn diagram of relationship between DEG groups

In DT *vs* DCK, the most enriched pathway was 'metabolic pathways', it related to 83 DEGs, which accounted for 4%; the second enriched pathway was 'biosynthesis of secondary metabolites', it related to 51 DEGs, which accounted for 4.7% (Fig. 3).

Table 3: The opposite expression DEGs of DT *vs* DCK and RT *vs* RCK

Gene ID	Regulation type		Function descriptions
	DT	RT	
PGSC0003DMG401023109	Down-regulated	Up-regulated	F-box domain, cyclin-like
PGSC0003DMG400016638	Down-regulated	Up-regulated	/
PGSC0003DMG400011642	Down-regulated	Up-regulated	/
PGSC0003DMG400019465	Down-regulated	Up-regulated	Major facilitator superfamily‖Major facilitator superfamily domain‖Sugar transporter, conserved site‖Major facilitator superfamily domain, general substrate transporter
PGSC0003DMG400008787	Up-regulated	Down-regulated	Ferritin/DPS protein domain‖Ferritin-related‖Ferritin-like diiron domain‖Ferritin/ribonucleotide reductase-like‖Ferritin‖Ferritin, conserved site
PGSC0003DMG400020950	Up-regulated	Down-regulated	Protein of unknown function DUF2838
PGSC0003DMG400024171	Up-regulated	Down-regulated	Zinc finger, CCCH-type
PGSC0003DMG400008345	Up-regulated	Down-regulated	Glycoside hydrolase, family 31‖Glycoside hydrolase, superfamily‖Glycoside hydrolase-type carbohydrate-binding

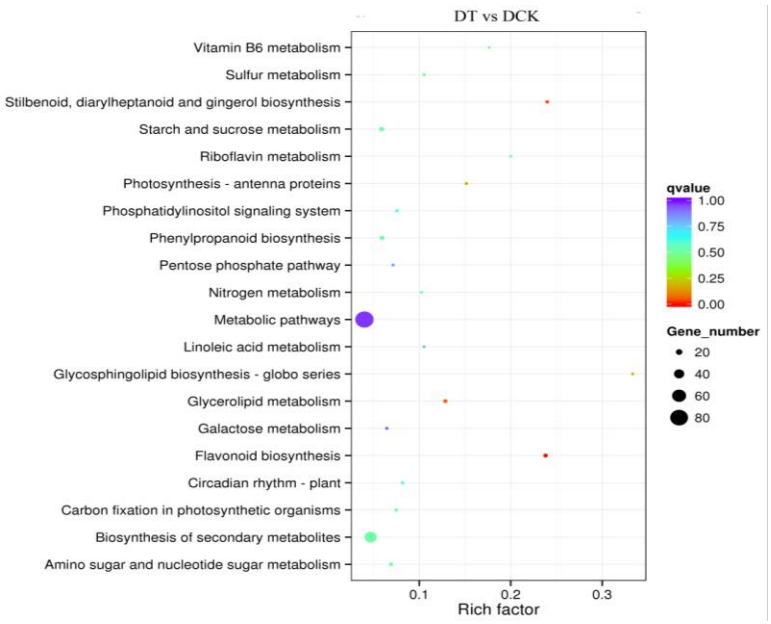

Fig. 3: KEGG enrichments of the annotated DEGs in DT *vs* DCK

Although, 42 DEGs enriched significantly in 'flavonoid biosynthesis', which accounted for 23.8%, 25 DEGs genes enriched significantly in 'stilbenoid, diarylheptanoid and gingerol biosynthesis', which accounted for 24%, showing that drought stress affected potato oxidoreductases and transferases of flavonoid biosynthesis.

In RT *vs* RCK, the pathways 'biosynthesis of secondary metabolites' related to 42 DEGs (Fig. 4), approximately 4% of the DEGs were enriched. Although, only 12 and 17 genes were related respectively to the pathways 'glutathione metabolism' and 'plant-pathogen interaction', 11 and 8% DEGs were enriched significantly, suggesting that rehydration affected potato transferases, oxidoreductases, protein kinases, ion channels, transcription factors and protein processing in endoplasmic reticulum.

In DT *vs* RT, the DEGs were enriched in 'ribosome', 'ribosome biogenesis in eukaryotes', 'glucosinolate biosynthesis', 'lysine biosynthesis', 'vitamin B_6 metabolism', 'glycosphingolipid biosynthesis - globo series'(Fig. 5), where 55%, 46%, 71%, 55%, 41% and 44% DEGs were enriched respectively. Among them, the DEGs of 'ribosome' and 'ribosome biogenesis in eukaryotes' the pathways were enriched significantly, in which 200 and 41 genes were related to these pathways, this indicated that the drought stress to rehydration process strongly affected 'ribosome' and 'ribosome biogenesis in eukaryotes' in potato, the reason maybe the effect of the ribosome-related genes adjusting the transcription of genes.

Further Explanation of the KEEG Pathways

We selected important KEEG pathways according to a corrected p-value <0.05. The stilbenoid, diarylheptanoid and gingerol biosynthesis, flavonoid biosynthesis pathways were enriched significantly in KEEG pathways of DT *vs* DCK comparison group. Flavonoid biosynthesis was one of the secondary metabolic pathways in plants, flavonoid had various functions in growth, development, reproduction, and stress defense (Ma *et al.*, 2014).

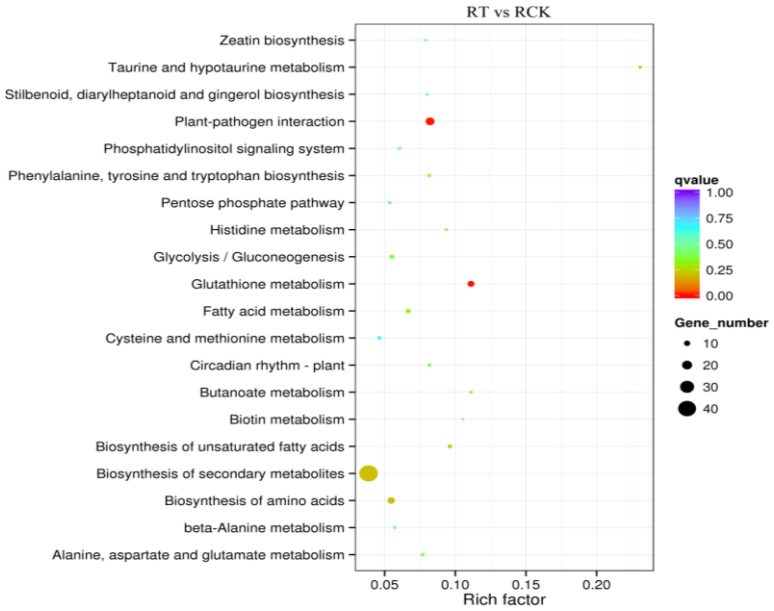

Fig. 4: KEGG enrichments of the annotated DEGs in RT *vs* RCK

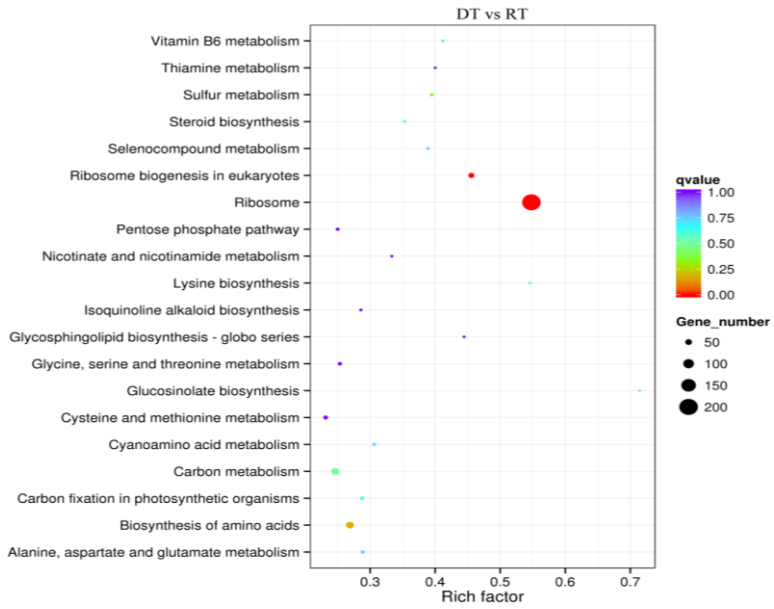

Fig. 5: KEGG enrichments of the annotated DEGs in DT *vs* RT

The key enzymes of stilbenoid, diarylheptanoid and gingerol biosynthesis pathway included in the flavonoid biosynthesis pathway. One of the key enzymes was oxidoreductases, such as cytochrome P450 98A3-like, coumaroylquinate (coumaroylshikimate) 3'-monooxygenase, flavanone 3 beta-hydroxylase, naringenin 3-dioxygenase, flavonoid 3',5'-hydroxylase, flavonoid 3'-monooxygenase, related to Novel00078, PGSC0003DMG400003289, PGSC0003DMG400003563, PGSC0003DMG400000425, PGSC0003DMG400024643 genes. The other key enzymes were transferases, such as flavonoid 3', 5'-methyltransferase-like, caffeoyl-CoA O-methyltransferase, chalcone synthase 2, HQT, shikimate O-hydroxycinnamoyl transferase, related to PGSC0003DMG400006448, PGSC0003DMG400006214, PGSC0003DMG400019110, PGSC0003DMG400011189, PGSC0003DMG400014152 genes (Table 4).

Glutathione metabolism and plant-pathogen interaction pathways were enriched significantly in KEEG pathways of RT *vs* RCK comparison group. In plant cells, glutathione is the major low molecular weight soluble antioxidants, the glutathione biosynthesis pathway consists

Table 4: The key enzymes of flavonoid biosynthesis and stilbenoid, diarylheptanoid and gingerol biosynthesis pathways

Enzyme	Enzyme name	Enzyme type	Gene ID	Regulation type
1.14.13.36	cytochrome P450 98A3-like; coumaroylquinate (coumaroylshikimate) 3'-monooxygenase	Oxidoreductases	Novel00078; PGSC0003DMG400003289	Up/ down regulated
2.1.1.104	flavonoid 3',5'-methyltransferase-like; caffeoyl-CoA O-methyltransferase	Transferases; Transferring one-carbon groups	PGSC0003DMG400006448; PGSC0003DMG400006214	Up/ down regulated
1.14.11.9	flavanone 3 beta-hydroxylase; naringenin 3-dioxygenase	Oxidoreductases	PGSC0003DMG400003563	Up regulated
1.14.13.88	flavonoid 3',5'-hydroxylase	Oxidoreductases	PGSC0003DMG400000425	Up regulated
1.14.13.21	flavonoid 3'-monooxygenase	Oxidoreductases	PGSC0003DMG400024643	Down regulated
2.3.1.74	chalcone synthase 2	Transferases; Acyltransferases	PGSC0003DMG400019110	Up regulated
2.3.1.133	HQT; shikimate O-hydroxycinnamoyltransferase	Transferases; Acyltransferases	PGSC0003DMG400011189; PGSC0003DMG400014152	Down regulated

Table 5: The key enzymes of glutathione metabolism pathway

Enzyme	Enzyme name	Enzyme type	Gene ID	Regulation type
2.5.1.18	probable glutathione S-transferase	Transferases	PGSC0003DMG400011731; PGSC0003DMG400002167; PGSC0003DMG400002168; PGSC0003DMG400002169; PGSC0003DMG400019956; PGSC0003DMG400002163; PGSC0003DMG400011012; PGSC0003DMG400028783; PGSC0003DMG400019728	Down regulated
1.11.1.11	L-ascorbate peroxidase 1, cytosolic	Oxidoreductases	PGSC0003DMG400030056	Down regulated
1.1.1.49	glucose-6-phosphate 1-dehydrogenase, cytoplasmic isoform-like	Oxidoreductases	PGSC0003DMG400020269	Down regulated
1.1.1.44	6-phosphogluconate dehydrogenase, decarboxylating 3-like	Oxidoreductases	PGSC0003DMG400001932	Down regulated

of two ATP-dependent steps, first catalysed by γ-glutamyl cysteine synthetase (γ-ECS) and glutathione synthetase (GS), which not only occurred in the cytosol and chloroplasts of plant cells, but also found in non-photosynthetic and photosynthetic tissues (Foyer, 2001). One of the key enzyme of glutathione metabolism of potato in RT *vs* RCK was glutathione S-transferase, related to PGSC0003DMG400011731, PGSC0003DMG400002167, PGSC0003DMG400002168, GSC0003DMG400002169; PGSC0003DMG400019956, PGSC0003DMG400002163, PGSC0003DMG400011012, PGSC0003DMG400028783, PGSC0003DMG400019728 genes. Other key enzymes were oxidoreductases, such as L-ascorbate peroxidase 1, cytosolic, glucose-6-phosphate 1-dehydrogenase, cytoplasmic isoform-like, 6-phosphogluconate dehydrogenase, decarboxylating 3-like (Table 5).

A plant and its pathogens affect each other in two ways. While the plant develops mechanisms to identify and defend itself from a potential pathogen falling on the plant surface, the pathogen strives to manipulate the biology of the plant to create suitable conditions for its reproduction and growth. The plant and pathogen communication has evolved a suite of genes. The enzymes of plant-pathogen interaction of potato in RT *vs* RCK were protein kinases, ion channels, transferases, transcription factors, protein processing in endoplasmic reticulum, related to PGSC0003DMG400016433, PGSC0003DMG402017989, PGSC0003DMG400009883, PGSC0003DMG400008163, PGSC0003DMG400016313, PGSC0003DMG400019527, PGSC0003DMG400033685, PGSC0003DMG40001333, PGSC0003DMG400011633, PGSC0003DMG400016769, PGSC0003DMG400028904, PGSC0003DMG400020099, PGSC0003DMG400027945, PGSC0003DMG400014405 genes (Table 6).

qRT-PCR Verification

Ten genes were selected to verify data validity by qRT-PCR analysis, according to the target genes of interest from sequencing data. The expression of 10 genes was significantly down-regulated or up-regulated under drought and rehydration treatment, and the expression trend of 10 genes were consistent with the changes in the results of RNA sequence (Fig. 6), therefore supporting the validity of the DEG results.

Discussion

A total number 655, 644, 3443 of DEGs were identified in comparisons DT *vs* DCK, RT *vs* RCK, DT *vs* RT, respectively. These DEGs were involved in 118 KEGG pathways. In DT *vs* DCK comparison group, the significant enriched pathways were the stilbenoid, diarylheptanoid and gingerol biosynthesis and flavonoid biosynthesis pathways, so secondary metabolic played a decisive role on defending drought stress. Flavonoid biosynthesis is was one of the secondary metabolic pathways in plants, flavonoid had various functions in growth, development, reproduction, and stress defense (Ma *et al.*, 2014). Other plant species, such as *Reaumuria soongorica* (Liu *et al.*, 2013a), *Chrysanthemum morifolium* (Xu *et al.*, 2013), and *Triticum aestivum* (Ma *et al.*, 2014) also had the same reaction of flavonoid

Table 6: The key enzymes of plant-pathogen interaction pathways

Enzyme	Enzyme name	Enzyme type	Gene ID	Regulation type
CERK1	chitin elicitor receptor kinase 1	Protein kinases	PGSC0003DMG400016433	Down regulated
CNGCs	cyclic nucleotide-gated ion channel 1-like	Ion channels	PGSC0003DMG402017989	Down regulated
CDPK	calcium-dependent protein kinase 29	Transferases; Protein kinases	PGSC0003DMG400009883	Down regulated
CaMCML			PGSC0003DMG400008163;PGSC0003DMG400016313;PGSC0003DMG400019527;PGSC0003DMG400033685;PGSC0003DMG40001333	Down regulated
WRKY	probable WRKY transcription factor 26; double WRKY type transfactor; WRKY transcription factor 33	Transcription factors	PGSC0003DMG400011633; PGSC0003DMG400016769	Down regulated
SGT1	suppressor of G2 allele of SKP1		PGSC0003DMG400028904	Down regulated
RIN4	RPM1-interacting protein 4		PGSC0003DMG400020099; PGSC0003DMG400027945	Down regulated
HSP90	molecular chaperone HtpG	Protein processing in endoplasmic reticulum	PGSC0003DMG400014405	Up regulated

biosynthesis under drought. But the result was different from *Boehmeria nivea* under drought stress, the most DEGs enriched in the 'ribosome' pathway (Liu *et al.*, 2013b). Chalcone isomerase (CHI) and chalcone synthase (CHS) of chrysanthemum were down-regulated by drought, which were the key enzymes in the flavonoid biosynthesis pathway, and their changes were significant (Xu *et al.*, 2013), but in our study, flavonoid 3',5'-hydroxylase, flavanone 3 beta-hydroxylase, naringenin 3-dioxygenase, and coumaroylquinate (coumaroylshikimate) 3'-monooxygenase flavonoid 3',5'-methyltransferase-like; caffeoyl-CoA O-methyltransferase chalcone synthase 2, flavonoid 3'-monooxygenase, HQT, shikimate O-hydroxycinnamoyl transferase were potato key enzymes in flavonoids biosynthesis. Chalcone isomerase (CHI) was of no change and the trend of chalcone synthase (CHS) in potato under drought was the opposite to chrysanthemum. Among these enzymes, genes of flavonoid 3',5'-hydroxylase and coumaroylquinate (coumaroylshikimate) 3'-monooxygenase belonged to the cytochrome P450 family. Cytochrome P450 is terminal oxidase of mixed function oxidase system in the endoplasmic reticulum membrane, which is widely distributed in organism, the main function is endogenous and exogenous substances catalysis in the body of oxidation reaction. Cytochrome P450 belongs to b-type cytochrome and it is a kind of ferrous porphyrin protein, which is a hemoglobin protein, divided into oxidized and reduced cytochrome P450. Cytochrome P450 has a visible light absorption spectrum the absorption spectrum is changed when combined with other compounds. Stimulating the cytochrome P450-related enzymes of the secondary metabolite biosynthesis by drought in potato could be considered as a protective measure against drought damages through eliminating reactive oxygen to protect plant from drought damage and maintain plant survival under the rapid lack of water condition. Therefore, potato mainly through flavonoid 3', 5'-hydroxylase and coumaroylquinate (coumaroylshikimate) 3'-monooxygenase, removed excess reactive oxygen in the body, to avoid excessive accumulation of reactive oxygen species on causing damage to potato.

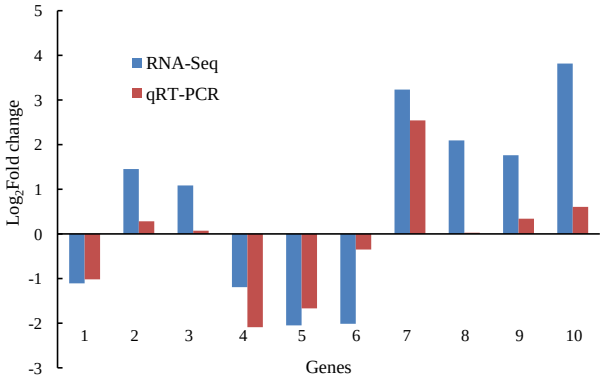

Fig. 6: Verification of differentially expressed genes by qRT-PCR

In drought treatment, compared with drought control, among DEGs, 336 genes individually controlled drought mechanism. In rehydration treatment, among DEGs, 115 genes individually controlled rehydration mechanism. Drought to rehydration, there also existed 113 shared genes, among them, eight genes had opposite expression trend, we thought these eight genes played an important role in potato drought recovery, eight genes had functions of major facilitator superfamily, sugar transporter, ferritin, glycoside hydrolase, they were PGSC0003DMG400008787, PGSC0003DMG400020950, PGSC0003DMG400008345, PGSC0003DMG401023109, PGSC0003DMG400016638, PGSC0003DMG400011642, PGSC0003DMG400019465, PGSC0003DMG400024171.

Based on DEGs detection and KEGG pathways analysis, a new insight into the molecular mechanism of potato under drought stress and rehydration during seedling stage were described in this paper. Our data suggest directions for further study on drought control genes and the identification of enzymes involved in various metabolic pathways under drought stress. More detailed studies on the components involved in these pathways are warranted to further the understanding of the precise regulatory mechanisms governing drought stress response in potato, as well as for generation of new breeds of drought tolerant plants.

Conclusion

From this study, it can be concluded that secondary metabolic of which the stilbenoid, diarylheptanoid and gingerol biosynthesis and flavonoid biosynthesis pathways played a decisive role on potato defending drought stress, especially through the enzymes of flavonoid 3',5'-hydroxylase and coumaroylquinate (coumaroylshikimate) 3'-monooxygenase in flavonoid biosynthesis to avoid drought damage. In the drought to rehydration process, eight DEGs of major facilitator superfamily, sugar transporter, ferritin, glycoside hydrolase were expressed significantly, so these genes played an important role in potato drought recovery.

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