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RESEARCH ARTICLE

# *De novo* characterization of the alligator weed (*Alternanthera philoxeroides*) transcriptome illuminates gene expression under potassium deprivation

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## Abstract

As one of the three macronutrients, potassium participates in many physiological processes in plant life cycle. Recently, potassium-dependent transcriptome analysis has been reported in *Arabidopsis*, rice and soybean. Alligator weed is well known, particularly for its strong ability to accumulate potassium. However, the molecular mechanism that underlies potassium starvation responses has not yet been described. In this study, we used Illumina (Solexa) sequencing technology to analyse the root transcriptome information of alligator weed under low potassium stress. Further analysis suggested that 9253 differentially expressed genes (DEGs) were upregulated, and 2138 DEGs were downregulated after seven days of potassium deficiency. These factors included 121 transcription factors, 108 kinases, 136 transporters and 178 genes that were related to stress. Twelve transcription factors were randomly selected for further analysis. The expression level of each transcription factor was confirmed by quantitative RT-PCR, and the results of this secondary analysis were consistent with the results of Solexa sequencing. Enrichment analysis indicated that 10,993 DEGs were assigned to 54 gene ontology terms and 123 KEGG pathways. Approximately 24% of DEGs belong to the metabolic, ribosome and biosynthesis of secondary metabolite KEGG pathways. Our results provide a comprehensive analysis of the gene regulatory network of alligator weed under low potassium stress, and afford a valuable resource for genetic and genomic research on plant potassium deficiency.

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## Introduction

Potassium is the most important cation in living plant cells, and is essential for plant growth and development. It accounts for 2–10% of plant dry weight and plays vital roles in osmoregulation, signal transduction, plasma membrane potential, and enzyme activation (Clarkson and Hanson 1980). The cytoplasmic $K^+$ concentration in plant cell is ∼100 mM, but the concentration near roots in the soil is very low, varying from 0.1 mM to 1.0 mM (Maathuis 2009). Therefore, most plants need to absorb $K^+$ against the $K^+$ concentration gradient from the soil. This process is implemented by $K^+$ transporters and channels. Most plants have both high-affinity and low-affinity $K^+$ transport systems to adapt to changes in the $K^+$ concentration in the soil. Previous studies have reported various functions of multiple genes encoding $K^+$ transporters and channels (Very and Sentenac 2003). AKT1 ($K^+$ channel) and HAK5 ($K^+$ transporter) control almost all potassium absorption in *Arabidopsis* roots (Pyo *et al.* 2010). AKT2 participates in $K^+$ translocation and loading in different tissues in *Arabidopsis* (Lacombe *et al.* 2000).

In recent years, significant progress toward understanding potassium nutrition in plants has been achieved using *Arabidopsis* as a model plant. The molecular mechanisms of potassium channel regulators have been revealed in *Arabidopsis*. Calcium-mediated CBL–CIPK complexes regulate $K^+$ uptake under potassium-deficiency stress. CBL1 and/or CBL9 carry CIPK23 to the root cell plasma membrane by interaction; CIPK23 then phosphorylates AKT1 to promote $K^+$ uptake (Xu *et al.* 2006). Another CBL4–CIPK6 complex enhances ATK2-mediated $K^+$ currents (Held *et al.* 2011). In addition to $K^+$ transporters, channels and protein kinases, several $H^+$ATPases (Dewitt *et al.* 1996), pyruvate kinases (Armengaud *et al.* 2009) and transcription factors (i.e. REST) (Cheong *et al.* 2005) are involved in potassium-deficiency stress. Experiments have suggested that various plants use different physiological and metabolic regulatory

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networks in adapting to potassium-deficiency stress (Wang *et al.* 2012; Shankar *et al.* 2013).

Alligator weed (*Alternanthera philoxeroides*) is a dicotyledonous perennial herb that originated in South America. This species currently grows worldwide due to its high adaptability to harsh environments (Wang *et al.* 2005). This adaptability is predominantly attributed to genomewide DNA methylation and epigenetic regulation in response to environmental fluctuation (Gao *et al.* 2010). Alligator weed is also particularly popular for its strong potassium accumulation ability (Peng and Hu 1986; Jie and Ni 1987). ApKUPs (ApKUP1, ApKUP2 and ApKUP3) were colonized, and their expression levels were induced by low potassium, abscisic acid (ABA) and polyethylene glycol (PEG) treatment (Song and Su 2013). However, the molecular mechanisms and regulatory networks that underlie the robust low potassium tolerance in alligator weed remain almost unknown. The transcriptome profile of alligator weed under low potassium stress has also not yet studied. To date, only 78 nucleotides and 47 protein sequences of alligator weed could be found in the GenBank database from NCBI.

In this study, we initially expounded the comprehensive transcriptome information of alligator weed root after seven days of low potassium stress. The NGS-based Illumina (Solexa) sequencing platform was used to analyse the *de novo* transcriptome of two alligator weed root cDNA samples: one untreated control (CK) and one subjected to low potassium treatment (LK) for seven days. We also compared the gene expression profiles of LK and CK using a digital gene expression (DGE) system and discovered considerable number of root-specific transcripts in response to low potassium stress. Certain vital enriched networks of the low potassium response were also identified. A total of 12 transcription factors were randomly selected, and the expression level of each transcription factor was confirmed by quantitative RT-PCR. The aim of this study was to discover the molecular basis of potassium accumulation and tolerance in alligator weed, which affords a valuable resource for future research on potassium stress.

## Materials and methods

### *Plant materials and growth conditions*

Naturally grown alligator weed shoots were collected from a test field of the Sichuan Agricultural University (Chengdu, China) and cultured hydroponically in a growth chamber for 10 days (d) to induce root growth. The greenhouse was maintained under a 16 h / 8 h day/night light cycle and a 28°C/25°C day/night temperature cycle. The nutrient solution was refreshed every 2 d. The nutrient solution (pH = 5.8) consisted of 1.427 mM $NH_4NO_3$, 0.323 mM $NaH_2PO_4\cdot 2H_2O$, 0.512 mM $K_2SO_4$, 0.998 mM $CaCl_2$, 1.643 mM $MgSO_4\cdot 7H_2O$, 9.474 $\mu$M $MnCl_2\cdot 4H_2O$, 0.075 $\mu$M $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 18.882 $\mu$M $H_3BO_3$, 0.152 $\mu$M $ZnSO_4\cdot 7H_2O$, 0.155 $\mu$M $CuSO_4\cdot 5H_2O$, 0.031 mM $FeSO_4\cdot 7H_2O$, and 0.031 mM $Na_2EDTA_2H_2O$. After 10 d of culture, the alligator weed plants had grown strong roots. Half of the plants were then transferred to a low potassium nutrient solution (lacking $K_2SO_4$) for 7 d to generate the LK sample. The other half continued to grow in the normal solution for 7 d as sample CK. Two sample roots (CK and LK) were collected, immediately frozen in liquid nitrogen, and then stored at −80°C for further analysis.

### *RNA isolation and Illumina sequencing*

Total RNA was isolated from the roots of alligator weed using TRIzol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. To avoid genomic DNA contamination, the RNA samples were treated with RNase-free DNase I (Takara, Tokyo, Japan). The quality of the RNA was checked using an Agilent 2100 RNA Bioanalyzer (Agilent, Santa Clara, USA) and met the requirements for cDNA library construction. Two alligator weed root cDNA libraries were constructed using the mRNA-seq assay for paired-end transcriptome sequencing, which was performed by the Beijing Genomics Institute according to the manufacturer's instructions (Illumina, San Diego, USA). Sequencing was performed using an Illumina HiSeqTM2000 sequencing system.

### *De novo assembly and functional annotation of unigenes*

The raw reads were filtered to obtain high quality clean reads by removing adaptor sequences, duplicated sequences, reads containing more than 10% 'N' rates (where 'N' represents ambiguous bases in reads), and reads containing more than 10% bases with a Q value $\leq$ 50. SOAP *de novo* software was used to establish transcriptome assembly (http://soap.genomics.org.cn/soapdenovo.html). Clean reads were assembled to create longer contigs by overlapping. The contigs that could not be extended on either end were defined as unique transcripts. They were further used to acquire nonredundant transcripts called unigenes. After assembly, all unigenes were annotated by homology and searched against the NCBI nonredundant (Nr) databases, gene ontology (GO), the clusters of orthologous groups (COGs) proteins, and the kyoto encyclopedia of genes and genomes (KEGG) database with a typical cut-off E-value of $10^{-5}$.

### *Identification and functional annotation of differentially expressed genes*

To identify differentially expressed genes (DEGs) between the two samples, the expression level of each transcript was analysed using the reads per kb per million (RPKM) method (Mortazavi *et al.* 2008). The gene expression levels of the two samples were normalized to the number of transcripts per million clean tags. A rigorous algorithm was used to identify the genes that were differentially expressed between the two samples. The threshold $P$ value was determined by the false discovery rate (FDR) method in multiple analyses. We used an FDR $\leq$ 0.001 and an absolute value of $\log_2$ ratio $\geq$ 1 as the threshold to identify each DEG.

**Table 1.** Unigenes primer sequences of QPCR were listed.

| Gene | Sequence |
|---|---|
| RAV1(Unigene8179_All)F | AGTGCGAGGCTAAGAACT |
| RAV1(Unigene8179_All)R | CCTAGAATACATTGTCCCTC |
| RAV2(CL34.Contig19_All)F | GCTTCAACTCCGTTTTCGG |
| RAV2(CL34.Contig19_All)R | CCCTCCCTTCCATTTCCTTC |
| ERF(CL4914.Contig2_All)F | CCTCAACAATACAATAACCC |
| ERF(CL4914.Contig2_All)R | CTTGTCAAGGTTAAGTTGGC |
| WRKY(CL2606.Contig2_All)F | G GCCATTATGATGGTTTTGTC |
| WRKY(CL2606.Contig2_All)R | CCACAACACACTTCTAAACT |
| GRAS(CL3479.Contig2_All)F | GCGTTGGACCTGAGAATCAT |
| GRAS(CL3479.Contig2_All)R | CATGCTATAATGTTCACAATC |
| BZIP(CL9538.Contig2_All)F | AGTTGGGTCTGATGGCAAGC |
| BZIP(CL9538.Contig2_All)R | GTGTATGGCCATCAAGGG |
| GATA(CL2231.Contig1_All)F | GAGTTTGTGCAATTCGTCGG |
| GATA(CL2231.Contig1_All)R | CAACGGTGGTGATGAAATGATC |
| BTF3(Unigene32381_All)F | AGGTCATCCACTTCGCCAGC |
| BTF3(Unigene32381_All)R | AAGTTGTCGACAAGGTCT |
| DREB(CL1125.Contig7_All)F | GCAGAAGGTTGAGGAAAGTC |
| DREB(CL1125.Contig7_All)R | GGAAGTATAAGCCAGTATCG |
| JMJC(CL179.Contig3_All)F | CAACCCCATATCCAAAACC |
| JMJC(CL179.Contig3_All)R | CCTCCAACGCGTATCATCAC |
| HSP(Unigene23786_All)F | ACACAACAAAGCTCTCACCT |
| HSP(Unigene23786_All)R | TCAAATCCGACCCGAACCA |
| MYB(CL1037.Contig2_All)F | GGAAACAATTATCCTTGAGTTAC |
| MYB(CL1037.Contig2_All)R | AAGTAGGAGAGACATGACTATC |

***Quantitative real-time PCR analysis***

Based on the target gene sequences, 12 gene-specific primer pairs were designed (table 1). Quantitative real-time PCR was performed in a 25 $\mu$L reaction volume. Each pair contained 1 $\mu$L of cDNA, 12.5 $\mu$L of 2×SYBR Green Master Mix and 10 $\mu$M of the forward and reverse primers. The reactions were conducted using the default cycling conditions (95°C for 10 s, 95°C for 15 s, and 50°C for 1 min for 40 cycles). The alligator weed *actin* gene was used as an internal control. Three biological replicates were performed in each experiment. The relative gene expression levels were represented by the $2^{-\Delta\Delta Ct}$ method.

## Results and discussion

***Sequencing and de novo transcriptome assembly***

Two root cDNA libraries (CK and LK) were completed and sequenced using an IlluminaHiSeq 2000 sequencer. In total, 59.40 million raw reads were generated from CK and 55.19 million raw reads were generated from LK. The raw data has been deposited in the Gene Expression Omnibus (GEO) database (accession numbers between GSM1523529 and GSM1523530). Further, 55,234,174 (CK) and 51,512,520 (LK) high quality clean reads were obtained after deleting low quality reads and adapter sequences. These were used for *de novo* assembly. After assembly, 63,281 (CK) and 73,162 (LK) unigenes were acquired, the mean length were 537 and 528 bp, respectively. We obtained 64,949 all-unigenes with an average length of 671 bp and an N50 of 1009 bp by combining CK and LK clean reads (table 2).

***Functional annotation of all assembled unigenes***

All assembled unigenes were first searched against the NCBI Nr protein database using BLASTX with a cut-off E-value of $10^{-5}$. A total of 45,732 unigenes (70.41%) were matched (table 3). For E-value distribution, 77.7% sequences ranged from $E^{-5}$ to $E^{-100}$. The remaining sequences (22.3%) fell between 0 and $E^{-100}$ (figure 1A). The similarity distribution of the unigenes was 38.9% which accounts for the largest proportion (figure 1B). *Vitis vinifera* (23.8%) was the species with the closest match, followed by *Ricinus communis* and *Cucumis sativus* had the least homology with alligator weed (figure 1C).

The GO database was used to classify the functions of the annotated genes. As a result, 33,931 sequences (52.24%) were classified into three groups: molecular function, cellular components and biological processes which include 55 functional groups at the second level (figure 2). To identify the *de novo* assembly transcriptome integrity and phylogenetic classification of alligator weed, all unigene sequences were analysed against the COG database. A total of 18,602 genes were matched and grouped into 25 functional classes (figure 3). The cluster for 'general function prediction only' (5377 sequences, 28.90%) was the largest group, and the least genes were found in the 'extracellular structures'

**Table 3.** Summary of alligator weed transcriptome annotations.

| Total unigenes | 64949 | 100% |
|---|---|---|
| Nr | 45732 | 70.41% |
| SwissProt | 30576 | 47.07% |
| COG | 18602 | 28.64% |
| KEGG | 27700 | 42.64% |
| GO | 33931 | 52.24% |

**Table 2.** Summary of sequencing and assembly results.

| | CK (number) | Mean length (bp) | N50 (bp) | LK (number) | Mean length (bp) | N50 (bp) |
|---|---|---|---|---|---|---|
| Raw reads | 59,408,954 | – | – | 55,195,748 | – | – |
| Clean reads | 55,234,174 | – | – | 51,512,520 | – | – |
| Contigs | 146,038 | 262 | 346 | 167,435 | 261 | 341 |
| Unigenes | 63,281 | 537 | 847 | 73,162 | 528 | 829 |
| All unigenes | Number: 64949 | | | Mean length (bp): 671 | N50 (bp): 1009 | |

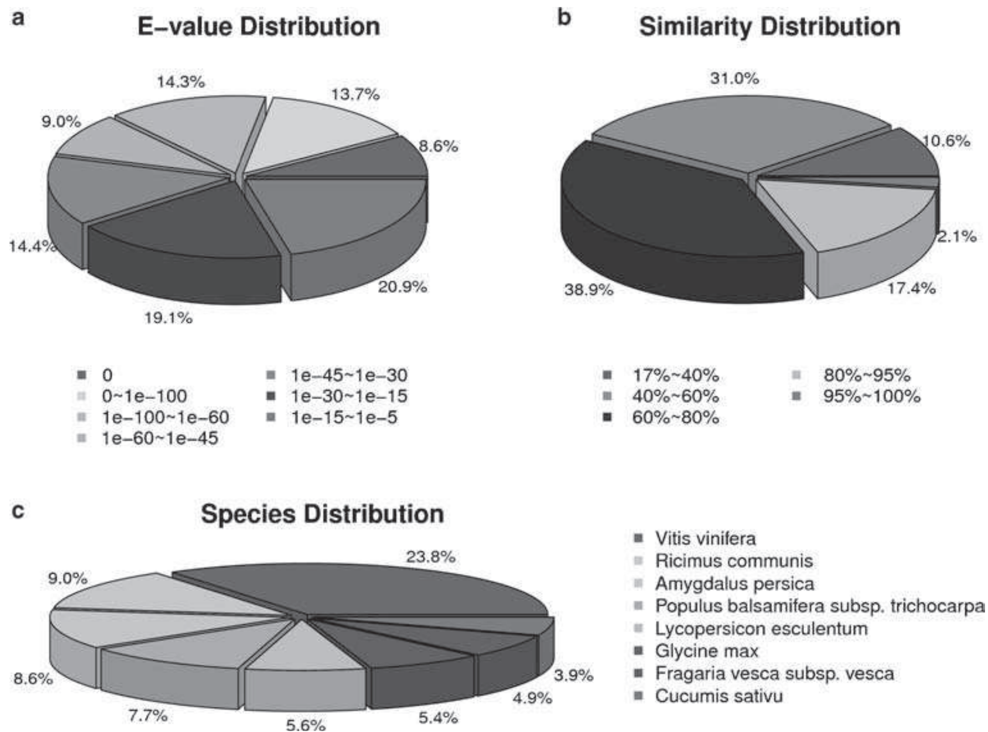


**Figure 1.** Characteristics of homology search of query sequences aligned by BLASTX to the Nr database.

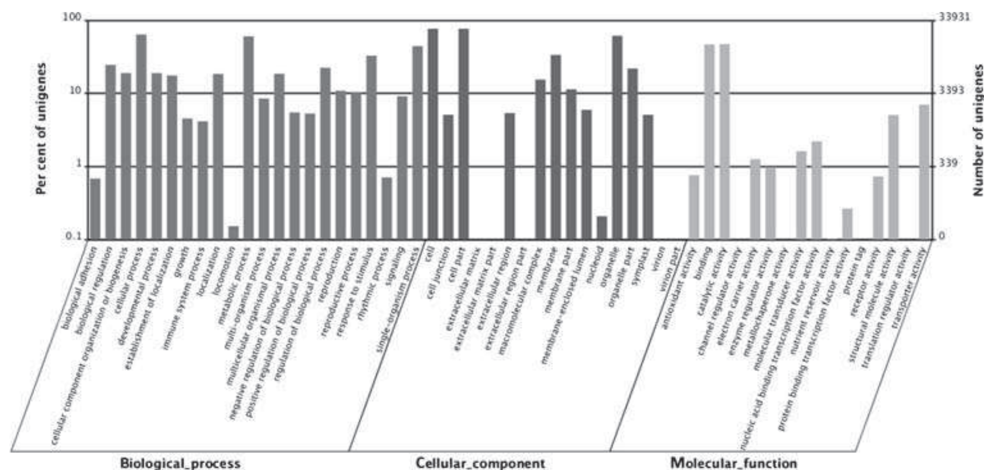


**Figure 2.** GO classification of the assembled transcripts.

(six sequences, 0.03%) and 'nuclear structure' (two sequences, 0.01%) categories.

### *Identification and annotation of DEGs*

The DEGs between the CK and LK samples were evaluated using a rigorous algorithm (CK versus LK, log2 ratio >1, FDR <0.001). The analysis suggested that 9253 DEGs were upregulated and 2138 DEGs were downregulated (figure 4). Among these DEGs, 4149 were not annotated, including 3548 upregulated genes and 691 downregulated genes. Using a hypergeometric test and Bonferroni correction, GO function and KEGG pathway enrichment analyses were performed to characterize all the DEGs. The 10,993 DEGs were assigned to 54 GO terms and 123 KEGG pathways; 26 key pathways were selected and counted carefully (table 4). The three dominant pathways were metabolic pathways (ko01100), ribosome (ko03010), and the biosynthesis of secondary metabolites (ko01110). The numbers and percentage of sequences that belonged to these three pathways were 1192 (10.84%), 913 (8.30%) and 547 (4.97%), respectively.

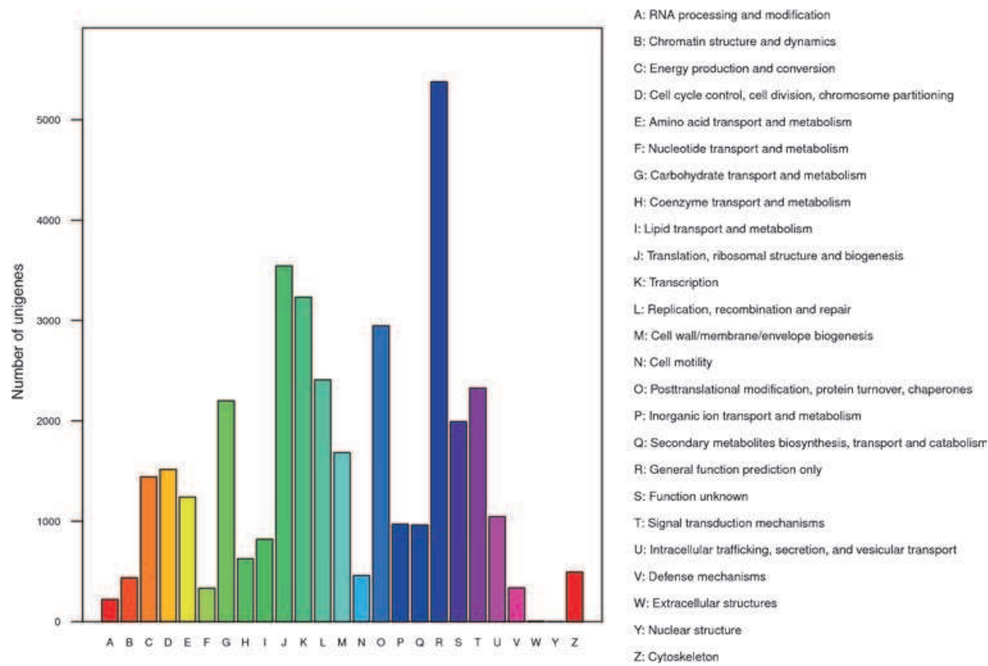


**Figure 3.** Distribution of genes in the transcriptome by COG functional classification.

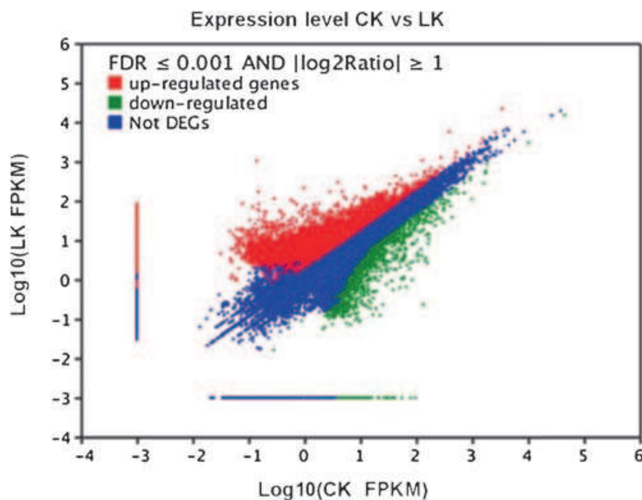


**Figure 4.** Identification of DEGs between the CK and LK samples.

Expression pattern of 13 DEGs involved in pyruvate metabolism pathway were shown, three pyruvate kinases (EC2.7.1.40) were all upregulated (figure 5).

**Table 4.** Unigene numbers listed in pathways.

| KEGG pathway | Unigene number |
|---|---|
| Metabolic pathways | 1192 |
| Ribosome | 913 |
| Biosynthesis of secondary metabolites | 547 |
| RNA transport | 340 |
| Endocytosis | 241 |
| MRNA surveillance pathway | 232 |
| Phagosome | 226 |
| Oxidative phosphorylation | 211 |
| Protein processing in endoplasmic reticulum | 211 |
| Spliceosome | 206 |
| Plant pathogen interaction | 186 |
| Glycerophospholipid metabolism | 183 |
| Ether lipid metabolism | 177 |
| Plant hormone signal transduction | 112 |
| Purine metabolism | 98 |
| Pyrimidine metabolism | 96 |
| Phenylpropanoid biosynthesis | 93 |
| Starch and sucrose metabolism | 87 |
| Glycolysis/gluconeogenesis | 85 |
| Citrate cycle (TCA) | 67 |
| Cysteine and methionine metabolism | 67 |
| Pyruvate metabolism | 64 |
| Amino sugar and nucleotide sugar metabolism | 64 |
| Glyoxylate and dicarboxylate metabolism | 63 |
| Phenylalanine metabolism | 60 |
| RNA degradation | 59 |

### *DEGs encoding transcription factors*

In higher plants, gene expression is regulated by a wide range of transcription factors, which play a crucial role in their response to biotic and abiotic stresses. Our DEG results indicated that 121 putative genes encoded transcription factors, including 98 upregulated and 23 downregulated genes. The AP2/ERF family has the largest number of genes (25), and Kim *et al.* (2012) reported that PAP2.11 (an AP2/ERF transcription factor) could bind to the *AtHAK5* promoter under low-K stress. Thirteen genes encoding WRKY transcription factors were found, of which 12 were upregulated and one was downregulated. The expression level of *GmWRKY50* was upregulated in the low-K-tolerant soybean variety (Wang *et al.* 2012), suggesting that WRKY transcription factors may have positive effects under low-K stress. Another 11 genes were GRAS (GAI, RGA and SCR

**Pyruvate metabolism**

**Figure 5.** Expression pattern of genes involved in pyruvate metabolism. The red and green colour in each column indicate upregulation and downregulation enzymes, respectively. The heat-maps generated were coloured to the corresponding DEGs from the KEGG database (map 00620).

domains) transcription factors. One GRAS transcription factor is reported to function in the auxin pathway (Gao *et al.* 2004). Auxin is also closely associated with root growth in response to low potassium stress (Cao *et al.* 1993). The GRAS transcription factor could be involved in low potassium stress by regulating genes related to auxin. C2H2 and C3H transcription factors were encoded by seven genes each; we also found five RAV, five MYB, five NAC and five Dof transcription factor unigenes. A total of 10 MYB and five Dof transcription factors have been reported to play vital roles in low potassium stress (Zeng *et al.* 2014). The remaining 38 genes are other transcription factors. To further investigate the DGE levels identified by Solexa analysis, 12 transcription factors from the DGE libraries were randomly selected to verify these changes by qRT-PCR. The expression patterns of these 12 genes were similar to the patterns obtained from the Solexa sequencing approach (figure 6).

### *DEGs encoding kinases*

Protein kinases respond to certain stresses and regulate the activity of potassium channels (Jonas and Kaczmarek 1996). In this study, a total of 108 genes that encode kinases were found, including 96 upregulated and 12 downregulated genes. Up to 35 serine/threonine kinases were detected; among these, 30 were upregulated and five were downregulated. Four of these were calcium/calmodulin-dependent protein kinases (CDPKs). In *Arabidopsis*, CDPK3 plays an important role in plant growth development and P and N nutrition (Motiwalla *et al.* 2014). CDPK10 is involved in drought responses by regulating the activity of the target gene HSP1 (Zou *et al.* 2010). In our analysis both *CDPK3* and *CDPK10* have increased expression under LK treatment. Seven CBL-interacting protein kinases were found; six of these kinases were upregulated, and only one was downregulated. A gene that matched *CIPK9* was upregulated 3.48-fold under LK. This result is consistent with the results of previous reports in *Arabidopsis* (Pandey *et al.* 2007; Liu *et al.* 2013). We also found that a *CIPK14* orthologue was induced. *AtCIPK14* is upregulated by salt, ABA responses, and light inhibition (Qin *et al.* 2010). Five MAP kinases and one MAPKK kinase were upregulated. Twelve leucine-rich repeat receptor protein kinases were induced from 2.5-fold to 9.84-fold. Therefore, we hypothesized that 108 kinases might make an important contribution to adaptation to low-K starvation conditions in alligator weed.

### *DEGs encoding transporters and channels*

Potassium deficiency affects the expression of many genes that encode transporters and channels. Our analysis indicated

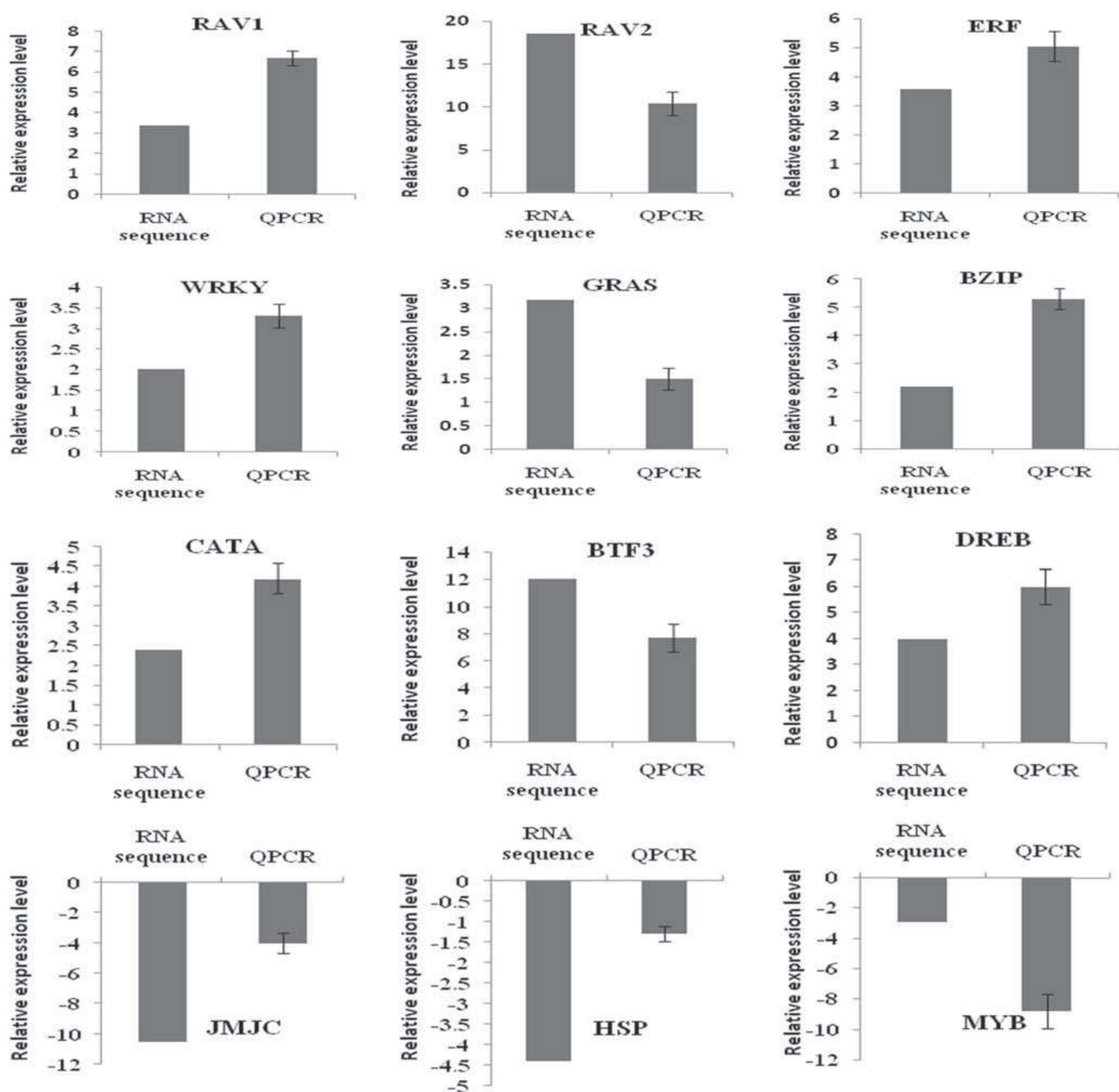


**Figure 6.** Confirmation of Solexa results by qRT-PCR.

**Table 5.** Potassium transporters and channels in response to low potassium treatment.

| Unigene | $Log_2$ ratio (LK/CK) | Annotation |
|---|---|---|
| Unigene31404_All | 4.22 | Potassium transporter 13-like (*Vitis vinifera*) |
| Unigene31901_All | 2.19 | Potassium transporter, putative (*Ricinus communis*) |
| Unigene33818_All | 1.68 | Probable potassium transporter 17-like (*Fragaria vesca* subsp.*vesca*) |
| CL3431.Contig2_All | 1.4 | Potassium transporter 4-like (*Glycine max*) |
| Unigene22592_All | −12.09 | High-affinity potassium transporter (*Oryza sativa Japonica* group) |
| CL5138.Contig1_All | −2.11 | Potassium transporter 5-like (*Fragaria vesca* subsp. *vesca*) |
| CL6427.Contig2_All | 1.48 | Potassium channel SKOR, partial (*Alternanthera philoxeroides*) |
| CL6427.Contig3_All | 1.35 | Potassium channel SKOR, partial (*Alternanthera philoxeroides*) |
| CL1876.Contig2_All | 1.26 | Potassium channel protein Mkt1p (*Mesembryanthemum crystallinum*) |
| CL6571.Contig2_All | 1.25 | Two-pore potassium channel 3-like (*Cucumis sativus*) |

that the expression levels of 136 transporters and 18 channels responded to potassium deficiency. Among these 136 transporters, 103 were upregulated and 33 were downregulated. Up to 22 genes belonged to the ABC transporter family; 14 were sugar transporters, including monosaccharide and hexose transporters; 13 were sulphate transporters; and 10 were phosphate transporters, including inorganic phosphate, phosphate hydrogen and glycerol-3-phosphate transporters. Eight were ammonium transporters and eight were nitrate transporters. We also found six potassium transporters; among them four were upregulated and two were downregulated (table 5). Fourteen genes encoding channels were upregulated, and four genes were downregulated. In particular, four genes encoding potassium channels were all upregulated (table 5). Stelar K+ outward rectifier (SKOR) was reported to mediate $K^+$ secretion from root cortex cells into the xylem participating in $K^+$ translocation (Gaymard *et al.* 1998). Two SKOR channels were upregulated implying that these factors have a significant function in low potassium treatment of alligator weed. The overexpression of a 2-pore potassium channel in tobacco can increase salinity tolerance (Wang *et al.* 2013). A unigene representing a 2-pore potassium channel gene was upregulated, and this channel may therefore function in $K^+$ assimilation and translocation.

### *DEGs related to stress*

Reactive oxygen species are vital signalling molecules that are specific to low potassium stress conditions. Peroxidase, superoxide dismutase, P450, oxidoreductase, glutathione-s-transferase, and catalase were all important players in oxidative stress (Apel and Hirt 2004). Our results suggested that a total of 178 genes associated with oxidative stress were differently expressed under LK. Up to 127 upregulated and 51 downregulated genes were identified. Among these, 38 were cytochrome P450 genes, 31 were peroxidase genes, 18 were oxidoreductase genes, eight were superoxide dismutase genes, six were catalase genes and two were glutathione-s-transferase genes. Simultaneously, 23 heat shock proteins (HSPs), 17 thioredoxins, 11 dehydration proteins and nine NBS-LRR type resistance proteins were identified. Four genes belonged to the glutaredoxin family and four salt-tolerance proteins were upregulated. Three genes were drought-induced proteins, two genes were wound-induced proteins, and one osmotic-induced protein and one BURP domain-containing protein were downregulated.

### *DEGs related to phytohormones and defence*

Previous studies showed that certain phytohormones play vital roles in adapting to low potassium treatment. These phytohormones include ethylene, auxin, gibberellin and jasmonic acid (Armengaud *et al.* 2004; Ma *et al.* 2012). Seventeen auxin-related genes were identified; nine of these were upregulated, and eight were downregulated. A total of seven genes related to ethylene were found and upregulated 2.18-fold to 4.16-fold. Four abscisic acid (ABA)-stress ripening proteins exhibited opposing expression patterns. Eight genes were related to gibberellin and six genes were related to jasmonic acid, which mostly showed downregulation. One cytokinin-related gene was downregulated, and one salicylic acid-related gene was upregulated. After the analysis of DEGs, 28 genes related to plant defence were found, including 19 upregulated cysteine proteases, four upregulated thaumatin-like proteins and four glycosyl hydrolase family genes. One lipoxygenase gene was 3.82-fold upregulated.

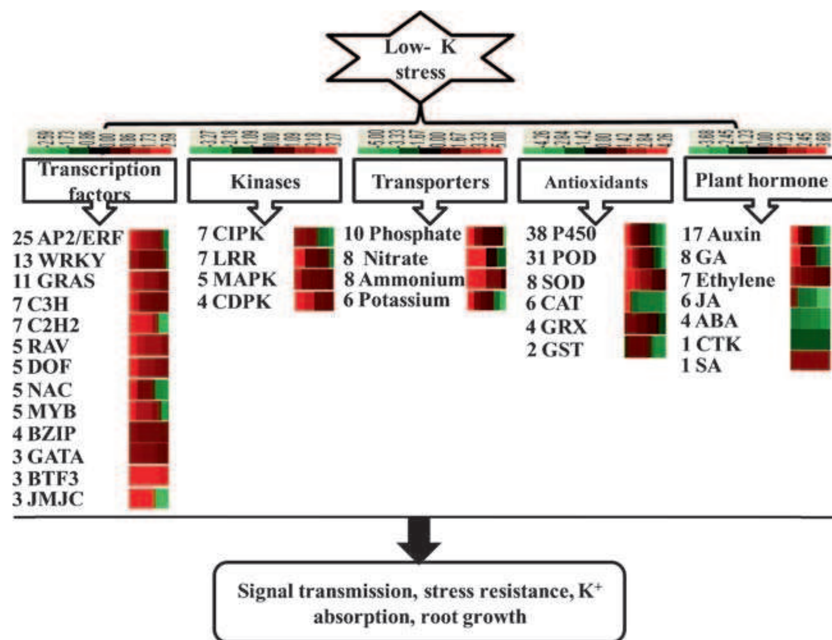


**Figure 7.** Expression pattern of genes involved in low potassium stress.

In brief, this first study on root transcriptome using Solexa sequencing technology in alligator weed after seven days of potassium deficiency reveals upregulation of large number of genes which code for transcription factors, serine/threonine-protein kinases, $K^+$ transporters and $K^+$ channels. Signalling molecules such as $Ca^{2+}$, reactive oxygen species, and phytohormones play vital roles under low potassium conditions. All these acclimation strategies enable alligator weed to survive and grow under low potassium stress (figure 7). Further research should focus on the important genes that were identified. This study establishes a solid foundation for plant potassium deficiency tolerance and the improvement of potassium utilization.

**Acknowledgements**

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