De novo transcriptome sequencing and analysis of genes related to salt stress response in *Glehnia littoralis*

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ABSTRACT

Soil salinity is one of the major environmental stresses affecting plant growth, development, and reproduction. Salt stress also affects the accumulation of some secondary metabolites in plants. *Glehnia littoralis* is an endangered medicinal halophyte that grows in coastal habitats. Peeled and dried *Glehnia littoralis* roots, named Radix Glehniae, have been used traditionally as a Chinese herbal medicine. Although *Glehnia littoralis* has great ecological and commercial value, salt-related mechanisms in *Glehnia littoralis* remain largely unknown. In this study, we analysed the transcriptome of *Glehnia littoralis* in response to salt stress by RNA-sequencing to identify potential salt tolerance gene networks. After *de novo* assembly, we obtained 105,875 unigenes, of which 75,559 were annotated in public databases. We identified 10,335 differentially expressed genes (DEGs; false discovery rate <0.05 and |$\log_2$ fold-change| $\geq$ 1) between NaCl treatment (GL2) and control (GL1), with 5,018 upregulated and 5,317 downregulated DEGs. To further this investigation, we performed Gene Ontology (GO) analysis and the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis. DEGs involved in secondary metabolite biosynthetic pathways, plant signal transduction pathways, and transcription factors in response to salt stress were analysed. In addition, we tested the gene expression of 15 unigenes by quantitative real-time PCR (qRT-PCR) to confirm the RNA-sequencing results. Our findings represent a large-scale assessment of the *Glehnia littoralis* gene resource, and provide useful information for exploring its molecular mechanisms of salt tolerance. Moreover, genes enriched in metabolic pathways could be used to investigate potential biosynthetic pathways of active compounds by *Glehnia littoralis*.

INTRODUCTION

High soil salinity has caused extremely negative effects on global agricultural production and ecological environments. Plant growth, development, metabolism, and protein functions can be retarded and inhibited under salt stress (*Carillo et al., 2011*). High salinity affects plants during two phases, the early and rapid osmotic stress phase and the later and

slower ionic toxicity phase (*Munns & Tester, 2008*). To resist damage caused by salt stress and survive in high-salt conditions, plants have gradually formed a series of complex mechanisms including osmotic regulation, Na^+ exclusion, and Na^+ compartmentalisation (*Deinlein et al., 2014*; *Munns & Tester, 2008*). A variety of signal transduction pathways are involved in plant responses to salt stress, including the phospholipid signaling pathway (*Hong et al., 2016*), salt overly sensitive (SOS) pathway (*Qiu et al., 2002*; *Quan et al., 2007*), abscisic acid (ABA) pathway (*Hauser et al., 2017*), calcium-dependent protein kinase (CDPK) pathway, and mitogen-activated protein kinase (MAPK) cascade pathway (*Zhu, 2016*). These pathways interact to form signal transduction networks in plant responses to salt stress. Numerous breakthroughs have been reported in the study of plant salt tolerance; however, many salt-related functional genes remain to be discovered and identified.

Glehnia littoralis Fr. Schmidt ex Miq. belongs to the Umbelliferae family. *Glehnia littoralis* germplasm resources are distributed in Japan, Russia, China, the Korean Peninsula, the Pacific Rim, and coastal areas of the United States. It is an endangered halophyte shown to exhibit salt tolerance (*Peng et al., 2014*), and has traditional and medicinal uses. Peeled and dried *G. littoralis* roots, named Radix Glehniae, have been used as a traditional Chinese herbal medicine for moistening lungs, removing phlegm, curing respiratory and gastrointestinal disorders, and anti-inflammation. This herbal medicine is also a major tonic component in anti-aging and health promotion prescriptions. Coumarins and polyacetylenes are the primary active constituents of *G. littoralis* (*Yuan et al., 2002*). Although *G. littoralis* has great ecological and commercial value, salt-related mechanisms in *G. littoralis* remain largely unknown. Thus far, most studies of salt tolerance in *G. littoralis* have focused on anatomical and morphological adaptations to high-salinity environments (*Voronkova et al., 2011*); for example, *Glehnia littoralis* leaves exhibit dorsoventral structure and have developed secretory trichomes that remove excess salts. However, the salt tolerance mechanisms and functional gene information of *G. littoralis* have not been explored.

In recent years, *de novo* transcriptome assembly and annotation of non-model plant species, especially those with unknown genomic sequences, have become possible through RNA sequencing (RNA-Seq) technology (*Ward, Ponnala & Weber, 2012*; *Xiao et al., 2013*). Transcriptome analysis has greatly facilitated the discovery of putative functional genes or proteins involved in various plant biological processes (*Cao & Deng, 2017*; *Lu et al., 2017*; *Wang et al., 2017*). Comprehensive transcriptome analysis has been performed on various plant species in response to various abiotic stresses, such as cold, drought, salt, and hormone stresses, and has provided insight into their functional gene expression and regulation, signal transduction networks, and metabolite biosynthetic pathways (*Postnikova, Shao & Nemchinov, 2013*; *Wang et al., 2013*; *Wang et al., 2017*; *Zhang et al., 2014a*; *Zhou et al., 2017*). For example, recently in *Litchi chinensis*, 73,117 unigenes were assembled and 11,741 unigenes were identified as being both chilling and ROS responsive genes (CRRGs) (*Lu et al., 2017*). Some of these CRRGs are involved in flowering, plant hormone signal transduction and plant hormone biosynthesis, and exhibit relationships within genes co-expression networks. *Zhang et al. (2014a)* performed transcriptome analysis of *Populus pruinosa* in response to salt stress by constructing six libraries from control and salt-treatment calli at different time-points. They identified 9,216 differentially expressed genes

(DEGs) and found that during the salt treatment, most of the DEGs were activated early (within 24 h) and stabilized after 48 h.

Halophytes are ideal plants for studying plant salt tolerance genetics using transcriptome sequencing. Comparative transcriptome analyses have previously been reported for a few halophytes, such as *Halogeton glomeratus* (*Yao et al., 2018*; *Wang et al., 2015*), *Iris lactea var. chinensis* (*Gu et al., 2018*), *Kochia sieversiana* (*Zhao et al., 2017*), *Nitraria sibirica* Pall. (*Li et al., 2017a*), *Suaeda maritima* (*Gharat et al., 2016*), *Sporobolus virginicus* (*Yamamoto et al., 2015*), and *Mesembryanthemum crystallinum* (*Oh et al., 2015*). In one such study, *Wang et al. (2015)* compared the transcriptomes of *H. glomeratus* exposed to NaCl for 6, 12, 24, and 72 h and discovered 2,223, 5,643, 7,510 and 10,908 DEGs, respectively. Later, *Yao et al. (2018)* analysed root transcriptomes of *H. glomeratus* under salt stress and identified the core DEGs regulating Na^+ uptake and transport in the roots. *G. littoralis* is a typical halophyte belonging to the family Umbelliferae. To date, no genomic or transcriptome sequencing information about salt stress responses has been reported for any halophytes belonging to Umbelliferara, including *G. littoralis*. As a medicinal halophyte, salt-associated molecular mechanisms in *G. littoralis* are important to a comprehensive understanding of this plant. In the current study, we performed comparative transcriptome sequencing of *G. littoralis* under NaCl treatment, and identified a total of 105,875 unigenes, 71.37% of which were annotated in public databases. These transcriptome analysis results will help to promote genomics and genetics studies on salt-associated mechanisms and on secondary metabolite biosynthesis in *G. littoralis*.

MATERIALS & METHODS

Plant growth and treatment

G. littoralis plant material was collected from Tannanwan Beach, Pingtan, Fujian Province, China (25°26′1.86″N, 119°45′14.4″E) and pot-cultured at the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing, China. *Glehnia littoralis* seedlings were grown in nutrient-enriched sandy soil under a 14-h light (26 °C)/10-h dark (22 °C) photoperiod. After 3 months of pot growth, the seedlings were treated with 200 mM NaCl for 24 h. Seedlings without NaCl treatment (0 h) were used as a control. The shoots and roots were harvested together and immediately frozen in liquid nitrogen and stored at −80 °C for subsequent RNA extraction. The control and salt-treated samples were labelled GL1 and GL2, respectively. Six biological replicates were harvested and mixed together for the RNA extraction of each sample.

RNA extraction and cDNA library preparation

Total RNA of *G. littoralis* was extracted from each sample using Trizol Reagent (TakaRa, Dalian, China) following the manufacturer's instructions. Genomic DNA was removed using DNase I (TaKaRa, Dalian, China). The quality of RNA was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA) and the quantity was determined using a NanoDrop ND-2000 (Thermo Scientific, Wilmington, MA, USA). Only high-quality RNA samples (OD260/280 = 1.8∼2.2, OD260/230 ≥ 2.0, RIN ≥ 6.5, 28S:18S ≥ 1.0, >10μg) were used to construct the sequencing libraries. RNA

purification, reverse transcription, library construction and sequencing were performed at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China) according to the manufacturer's instructions (Illumina, San Diego, CA, USA) as follows. The *G. littoralis* RNA-seq transcriptome libraries were prepared using the Illumina TruSeq^TM RNA sample preparation Kit. Poly(A) mRNA was purified from total RNA using oligo-dT-attached magnetic beads and then randomly fragmented into short fragments (about 200 bp) by metal ions. Taking these short fragments as templates, double-stranded cDNA was synthesized with a SuperScript double-stranded cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) with random primers. Then, the double-stranded cDNA was further end-repaired and A-tailed, and indexed adapters were ligated. After PCR amplification for 15 PCR cycles, cDNA libraries were selected for cDNA target fragments of 200–300 bp on 2% Low Range Ultra Agarose. After quantification with a TBS380 Mini-Fluorometer, two RNA-seq libraries were sequenced in single lane on an Illumina Hiseq 2000 sequencer (Illumina, San Diego, CA, USA) for 2×100 bp paired-end reads.

De novo transcriptome assembly and unigene annotation

After sequencing, the raw paired-end reads were trimmed and quality-controlled (QC) by SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) using default parameters. The reads were filtered as follows: the adapter was removed from the reads; low-quality bases (quality value < 20) at the 3′ end of the sequence were cut; if the quality value of residual sequence was still less than 10, the entire sequence was removed, otherwise the read was retained; reads that contained too many Ns ($\geq 10\%$) were removed; and reads that were less than 20 bp in length after adapter discarding and quality control were removed. Clean data from the two samples (GL1 and GL2) were then used for *de novo* assembly with Trinity (http://trinityrnaseq.sourceforge.net/) (*Grabherr et al., 2011*).

To annotate unigenes, all of the assembled transcripts were searched against public databases including the non-redundant protein database (Nr), euKaryotic Orthologous Groups (KOG), and the Kyoto Encyclopaedia of Genes and Genomes (KEGG), using an *E*-value $\leq 1 \times 10^{-5}$ to obtain optimal functional annotation. The BLAST2GO (http://www.blast2go.com/b2ghome) (*Conesa et al., 2005*) program was used to obtain the Gene Ontology (GO) annotations of unique assembled transcripts for describing biological process, molecular function and cellular component. Metabolic pathway analysis was performed using KEGG (http://www.genome.jp/kegg/).

The original data were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (accession number: SRX547159). This Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under the accession GGSB00000000. The version described in this paper is the first version, GGSB01000000.

Analysis of differentially expressed genes (DEGs)

The RSEM (RNA-Seq by Expectation-Maximization, http://deweylab.biostat.wisc.edu/rsem/) method was used to quantify gene abundances (*Li & Dewey, 2011*). The R

statistical package software edgeR (Empirical Analysis of Digital Gene Expression in R, http://www.bioconductor.org/packages/2.12/bioc/html/edgeR.html) (*Robinson, Mccarthy & Smyth, 2010*) was used for differential expression analysis and identification of DEGs. Read counts were input to the edgeR software and the screening criteria for significant DEGs was set to a false discovery rate (FDR) < 0.05 and |log$_2$ fold-change| ≥ 1. Gene expression levels were reported in fragments per kilobase of exon per million mapped reads (FPKM) (*Mortazavi et al., 2008*). The log$_2$FPKM values of the DEGs (using 0.001 instead of 0) were used to generate heat maps with HemI software (*Deng et al., 2014*). In addition, GO and KEGG enrichment analyses were performed to identify which DEGs were significantly enriched in GO terms and metabolic pathways compared with the whole-transcriptome background with a Bonferroni-corrected *P*-value ≤ 0.05 GO functional enrichment and KEGG pathway analyses of DEGs were carried out by Goatools (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/) (*Xie et al., 2011*). Differentially expressed transcription factors were then identified by alignment to the Plant Transcription Factor Database PlnTFDB (http://plntfdb.bio.uni-potsdam.de/v3.0/) and PlantTFDB (http://planttfdb.cbi.pku.edu.cn).

qRT-PCR analysis of DEGs

Glehnia littoralis total RNA was extracted according to the method described above. Some up- or down-regulated DEGs, which are involved in salt response and secondary metabolite biosynthesis, were selected for quantitative real-time PCR (qRT-PCR) assays. qRT-PCR was performed according to a protocol described previously (*Wang et al., 2017*) on a qTOWER 2.2 Real-Time PCR System (Analytik Jena AG, Jena, Germany) using SYBR Premix Ex Taq^{TM} II (Tli RNaseH Plus) (Takara, Dalian, China). Expression of the *Actin* gene of *Glehnia littoralis* was used as the internal reference. Relative gene expression was analysed using the $2^{-\triangle\triangle CT}$ method (*Livak & Schmittgen, 2001*). All qRT-PCR primers are listed in Table S1.

RESULTS

G. littoralis transcriptome sequencing profile under NaCl treatment

To perform a comprehensive analysis of the *G. littoralis* transcriptome under salt treatment, we constructed two RNA-Seq libraries, representing non-salt treatment (0 h, GL1) and 24 h of 200 mM NaCl treatment (GL2), using the Illumina HiSeq 2000 platform. The GL1 and GL2 libraries produced 5.31 and 5.09 Gb of clean data from the paired-end reads with Q20 percentages of 96.7% and 96.5%, respectively (Table 1). The clean reads from the GL1 and GL2 libraries were used for *de novo* assembly with the Trinity package. The Trinity method determines splice isoforms, distinguishes transcripts from recent duplicates, and identifies allelic variants (*Grabherr et al., 2011*); thus, each isoform corresponded to a unigene in the present dataset. In total, there were 105,875 unigenes (isoforms) of between 351 and 15,667 bp in sequence assembly, with a mean length of 1,314.44 bp (Fig. 1, Table 2).
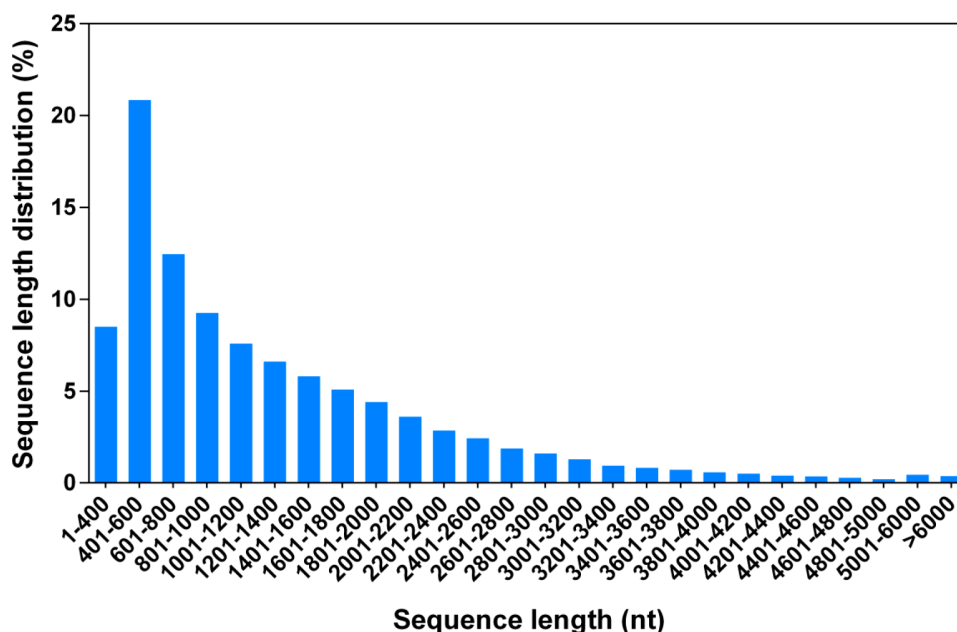
Figure 1 Length distribution of all *G. littoralis* unigenes.

Table 1 Summary of *G. littoralis* RNA sequencing (RNA-Seq).

Samples	Raw reads	Clean reads	Clean bases	Q20 percentage (%)
GL1 (control)	54568070	53715849	5.31 Gbyte	96.70%
GL2 (NaCl)	52469596	51566060	5.09 Gbyte	96.50%

Table 2 Summary of *G. littoralis* transcriptome assembly.

Type	Number
Total genes	53,092
Total unigenes	105,875
Total residues	1.39E+08
Average length	1,314.44
Largest unigene	1,5667
Smallest unigene	351

Sequence annotation and classification

To annotate the total unigenes, sequences were aligned using public databases (Table 3). In total, 75,535 unigenes (71.34%) were matched in the NR database, 25,871 unigenes (24.44%) in the KOG database, 47,043 unigenes (44.43%) in the GO database, and 24,864 (23.48%) in the KEGG database (Table 3). Taken together, 75,559 unigenes (71.37%) were successfully annotated in at least one of the databases (Table 3). In the GO annotation analysis, 47,043 unigenes were found and divided into three groups. The proportions of unigenes aligned in each GO term are shown in Fig. 2. Based on sequence homology with
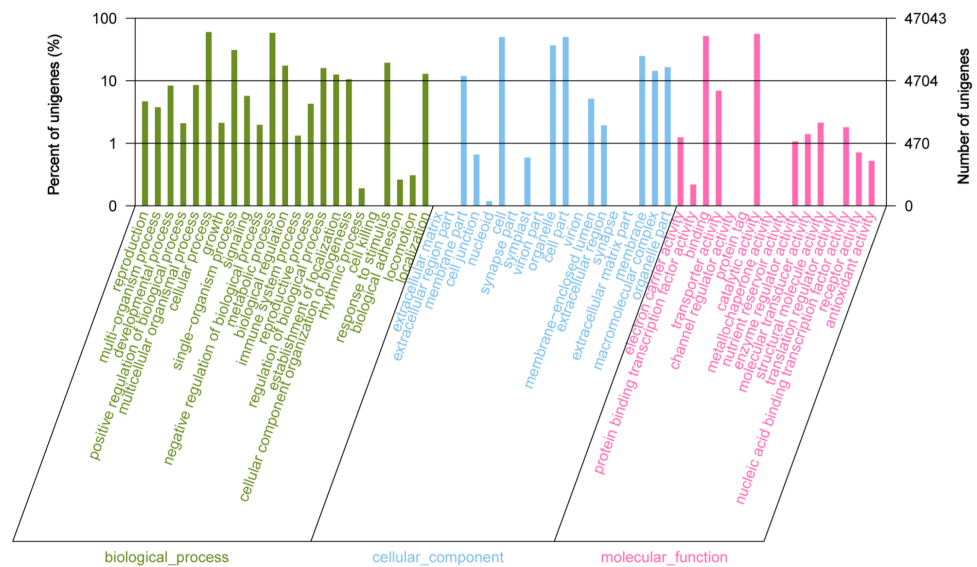
Figure 2 Gene ontology (GO) classification of the unigenes. Based on functional annotation, these unigenes were grouped into three categories: biological process, cellular component, and molecular function.

Table 3 Functional annotations of all unigenes found in the public databases.

Annotation	Number of unigenes	Percentage (%)
Annotated in NR	75,535	71.34356553
Annotated in KOG	25,871	24.43541913
Annotated in GO	47,043	44.4325856
Annotated in KEGG	24,864	23.48429752
Annotated in at least one database	75,559	71.36623377
Total unigenes	105,875	100

genes with known functions, these unigenes could be assigned to one or more ontologies, including 132,455 unigenes assigned to the biological process category, 100,205 unigenes assigned to the cellular component category, and 58,269 unigenes assigned to the molecular function category (Table S2). Some unigenes were assigned to more than one GO term, that is, a unigene may belong to several GO term notes and each GO term may correspond to multiple genes. Cellular process (28,273) and metabolic process (27,405) were highly represented groups within the biological process category. In the cellular component category, cell (23,485), cell part (23,483), and organelle (17,311) were highly represented groups. Moreover, catalytic activity (26,412) and binding (24,277) constituted the largest proportion in the molecular function category (Fig. 2, Table S2).

In addition, 25,871 unigenes had significant matches in the KOG database for functional prediction and classification (Fig. 3, Table 2). Among the 25 KOG categories, the cluster for general function prediction only (5,358) was the largest category, followed by signal transduction mechanisms (3,494), posttranslational modification, protein turnover,

A : RNA processing and modification
B : Chromatin structure and dynamics
C : Energy production and conversion
D : Cell cycle control, cell division, chromosome partitioning
E : Amino acid transport and metabolism
F : Nucleotide transport and metabolism
G : Carbohydrate transport and metabolism
H : Coenzyme transport and metabolism
I : Lipid transport and metabolism
J : Translation, ribosomal structure and biogenesis
K : Transcription
L : Replication, recombination and repair
M : Cell wall/membrane/envelope biogenesis
N : Cell motility
O : Posttranslational modification, protein turnover, chaperones
P : Inorganic ion transport and metabolism
Q : Secondary metabolites biosynthesis, transport and catabolism
R : General function prediction only
S : Function unknown
T : Signal transduction mechanisms
U : Intracellular trafficking, secretion, and vesicular transport
V : Defense mechanisms
W : Extracellular structures
Y : Nuclear structure
Z : Cytoskeleton

Figure 3 **euKaryotic Orthologous Groups (KOG) classification of unigenes.** Functional predictions of unigenes were classified into at least 25 function classes according to the KOG database.

chaperones (2,630), and transcription (1,766). Cell motility (five) and extracellular structures (70) were the smallest categories (Fig. 3, Table S3). We also performed KEGG pathway analysis of the unigenes. In total, 41,556 unigenes participated in 25 predicted metabolic pathways (Fig. 4; Table S4), which were divided into five categories, including metabolism, genetic information processing, environmental information processing, cellular processes, and organismal systems. The largest category was metabolism, including global and overview maps (10,892), followed by carbohydrate metabolism (3,727), amino acid metabolism, (1,987), lipid metabolism (1,931), energy metabolism (1,500), nucleotide metabolism (902), glycan biosynthesis and metabolism (703), metabolism of terpenoids and polyketides (678), metabolism of other amino acids (670), metabolism of cofactors and vitamins (663), biosynthesis of other secondary metabolites (575), and xenobiotics biodegradation and metabolism (539) (Fig. 4, Table S4).

Identification of DEGs under NaCl treatment

The clean reads from GL1 (control) and GL2 (NaCl) were mapped to the assembled transcriptome sequence to acquire read counts data using RSEM. Then, DEGs between two samples were identified using edgeR package with FDR < 0.05 and |$\log_2$ fold-change| ≥ 1. Unigene expression was calculated using the mappable reads, and the results were normalised to FPKM values. For each sample, more than 99% of FPKM values were between 1 and 100 (Fig. 5A). In total, we identified 10,335 DEGs between the GL1 and GL2 libraries; 5,018 DEGs were upregulated and 5,317 DEGs were downregulated (Figs. 5B, 5C). Next, all DEGs were subjected to functional-enrichment GO analysis: 5,055 DEGs were significantly enriched in GO terms (Table S5) and were classified into three main categories, namely biological process, cellular component, and molecular function. In the biological process category, cellular process (2,715) made up the majority, followed by metabolic process (2,659) and single-organism metabolic process (2,421). In the cellular
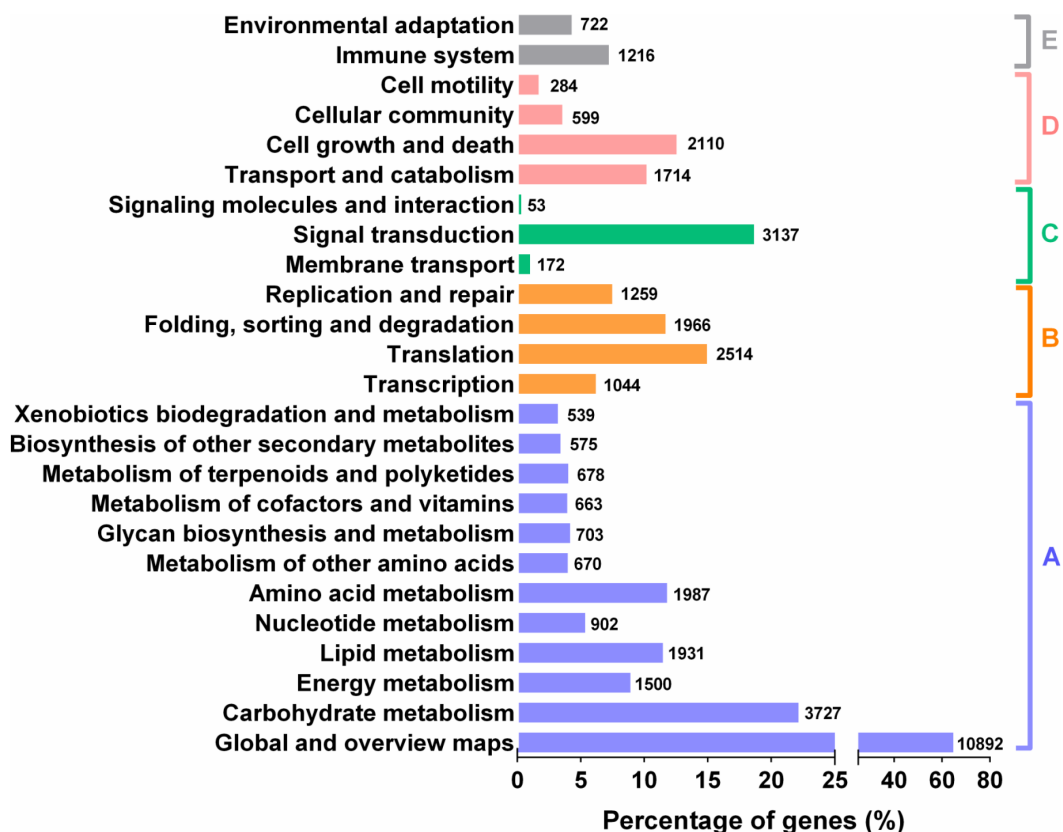
Figure 4 **Kyoto Encyclopaedia of Genes and Genomes (KEGG) metabolism pathway classification of unigenes.** Data were classified into in five main categories: (A) metabolism, (B) genetic information processing, (C) environmental information processing, (D) cellular processes, and (E) organismal systems. Bars represent the number of *G. littoralis* unigene matches in each category.

component category, organelle part (657) and intracellular organelle part (651) were predominant. In the molecular function category, catalytic activity (2,812) and binding (2,641) were prominently represented (Table S6).

We also performed an analysis of KEGG pathway enrichment in DEGs. Of the 10,335 DEGs, 1,188 unigenes were assigned and divided into 178 KEGG pathways (Tables S5, S7). Ribosome (123), lysosome (73), spliceosome (67), endocytosis (53) and plant-pathogen interaction (46) were highly represented pathways under salt stress (Table S7). Additionally, we identified the DEGs involved in the pathways of secondary metabolites biosynthesis and generated a heat map of these DEGs (Fig. 6). Phenylpropanoid biosynthesis (20) represented the largest proportion, followed by tropane piperidine and pyridine alkaloid biosynthesis (11), flavonoid biosynthesis (10), and isoquinoline alkaloid biosynthesis (eight). The annotation details of these DEGs are included in Table S8.

Genes involved in salt response

To identify genes responsible for salt response in *Glehnia littoralis*, we performed a BLAST search against the public databases. We found 1,661 unigenes encoding transcription
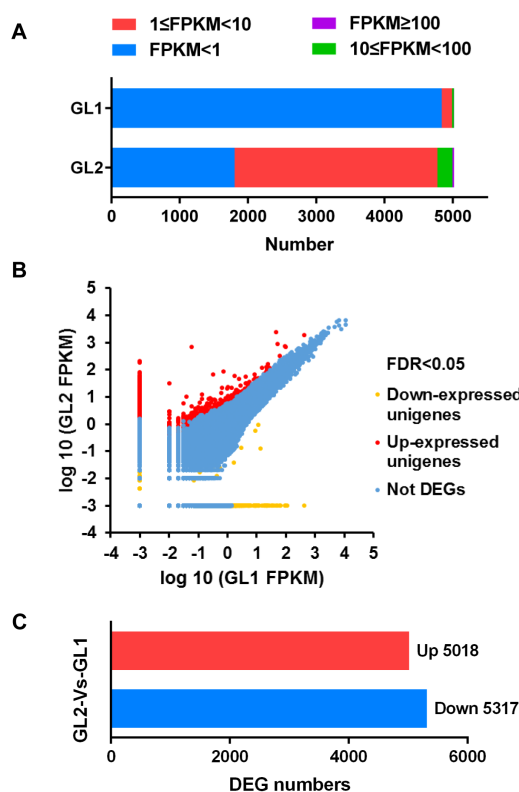
Figure 5 Expression analysis of differentially expressed genes (DEGs) in *Glehnia littoralis* under NaCl treatment. (A) Changes in fragments per kilobase of exon per million mapped reads (FPKM) value of unigenes in the control (GL1) and NaCl (GL2) samples. (B) Scatter plots of gene expression. (C) Number of DEGs up- or downregulated in the samples.

factors (TF); 151 of these were DEGs (Table S5). These DEGs encoding transcription factors were divided into 36 types including MYB, basic leucine zipper (bZIP), ethylene responsive factor (ERF), helix-loop-helix (bHLH), and NAC. Among these, 71 DEGs were upregulated in response to NaCl treatment (Fig. 7). Additionally, unigenes of plant signal transduction pathways were collected from the DEGs according to the KEGG pathway. For example, 38 DEGs related to plant hormone signaling, 30 DEGs related to calcium signaling, and four DEGs related to phospholipase signaling were found to be responsive to NaCl treatment of *Glehnia littoralis* (Table S9). The heat map of these unigenes is displayed in Fig. 8.

Confirmation of DEGs by qRT-PCR

To confirm the accuracy and reliability of the transcriptome analysis data, 15 candidate unigenes associated with salt response and secondary metabolites were randomly selected for qRT-PCR assays (Fig. 9). These unigenes included predicted transcription factors and functional proteins. For example, MYB, bZIP, Whirkly, bHLH, and ERF transcription factors, calcineurin B-like protein (CBL), CBL-interacting protein kinase (CIPK), and phospholipases D and C were tested. Additionally, genes involved in second metabolites
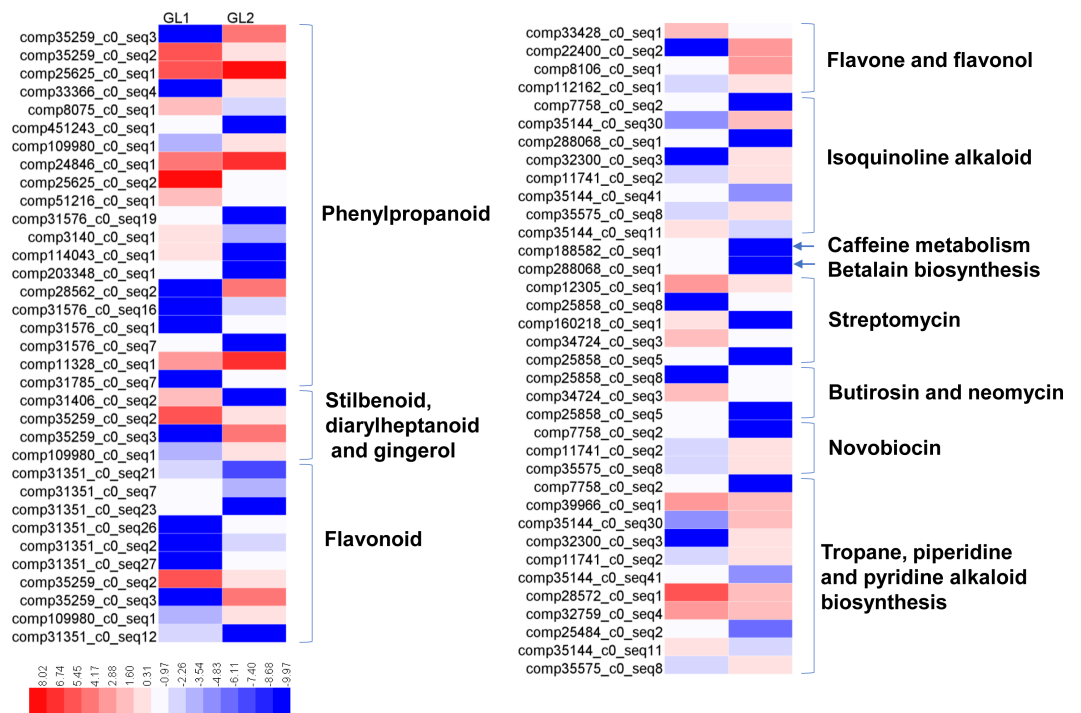
Figure 6 Heat map showing the expression profiles of DEGs in secondary metabolism pathways according to KEGG enrichment. Colour bar presents log2 ratios of FPKM values.

such as hydroxycinnamoyl transferase (HCT) and 4-coumarate-CoA ligase-like (4CL) were tested. As shown in Fig. 9, trends in the expression of these unigenes determined by qRT-PCR were consistent with those shown through RNA-Seq data, although the fold-changes detected by RNA-Seq and qRT-PCR did not match perfectly. Overall, these results suggest that our *Glehnia littoralis* transcriptome analysis using RNA-Seq was reliable.

DISCUSSION

The medicinal plant *Glehnia littoralis* exhibits salt tolerance consistent with its original habitat. At present, little genomic information is available for *Glehnia littoralis*. In this study, we performed *de novo* transcriptome analysis of *Glehnia littoralis* using the Illumina HiSeq 2000 platform to investigate the salt tolerance mechanism and functional genes of *Glehnia littoralis*. Although only two libraries were constructed for *de novo* assembly and analysis with and without NaCl treatment, several plants were pooled for each sequencing treatment to reduce background difference among the samples as much as possible. Certainly, more sequencing replicates could be conducive to statistical screening and some outlier data could be removed by calculating the correlation among samples. Whatever, the expression results of the sequencing should be considered for validation by qRT-PCR and further gene function studies. In this study, our qRT-PCR results also supported the sequencing data. In total, we identified 10,335 DEGs and these DEGs were functionally
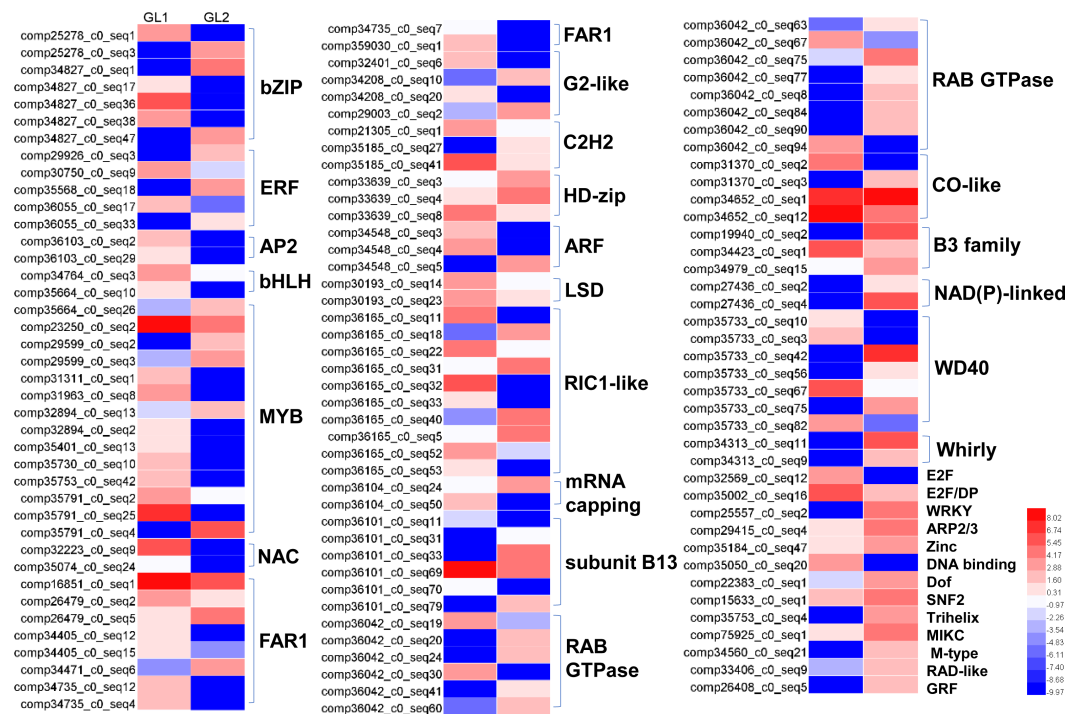
Figure 7 **Heat map showing the expression profiles of DEG transcription factors.** The colour bar presents log2 ratios of FPKM values.

categorised into a variety of physiological and molecular processes, which revealed the conserved mechanisms of salt responsive genes in *Glehnia littoralis*.

Plants subjected to salt stress display complex molecular responses such as stress gene expression, transcriptional regulation, and signal transduction networks (*Deinlein et al., 2014*). Therefore, we also focused on DEGs enriched in KEGG pathways under salt stress, and provided detailed information for genes in *Glehnia littoralis* associated with plant signal transduction pathways such as hormone signaling, calcium signaling, and phospholipase signalling (Fig. 8). ABA is an essential phytohormone that regulates plant stress response to environmental stimuli (*Peleg & Blumwald, 2011*). Drought and salinity cause osmotic stress in plants, and ABA is synthesised rapidly to trigger ABA-inducible gene expression, stomatal closure, and transpiration reduction to defend against water deficiency (*Wilkinson & Davies, 2010*; *Yamaguchishinozaki & Shinozaki, 2006*). In this study, we identified 524 unigenes involved in plant hormone signal transduction pathways. Of them, 34 DEGs including three pyrabactin resistance 1-like (PYL) ABA receptor genes were identified between the control and NaCl treatment samples (Fig. 8, Tables S7, S9). A previous study had shown that overexpression of PYL could enhance drought resistance and drought-induced leaf senescence in both *Arabidopsis* and rice by limiting transpirational water loss and water condition, thus generating an osmotic potential gradient leading water to preferentially flow to developing tissues (*Zhao et al., 2016*).
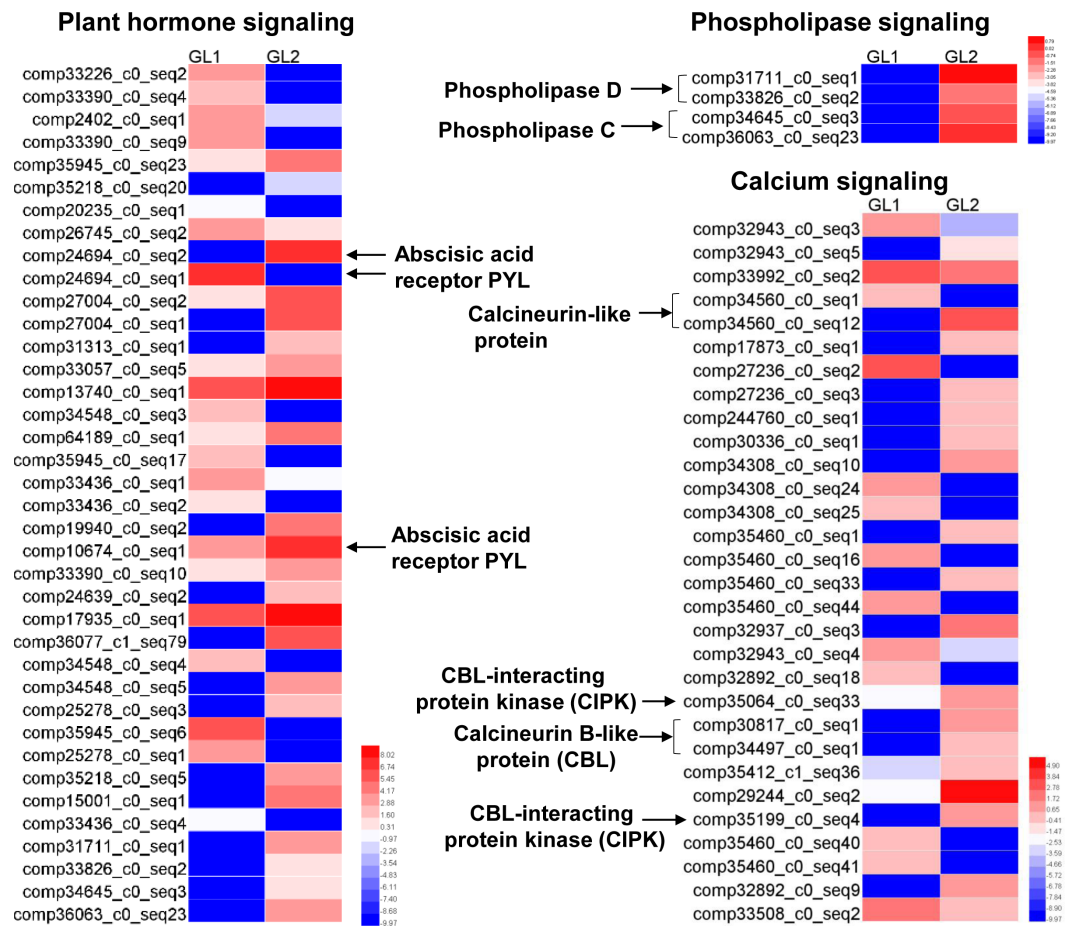
Figure 8 Heat map showing the expression profiles of DEGs in some salt-response signaling pathways. The colour bar represents log2 ratios of FPKM values.

In addition, 30 DEGs were assigned to the calcium signaling pathway in *Glehnia littoralis* (Fig. 8, Tables S7, S9). Ca^{2+} signals are vital transducers and regulators in plant responses to environmental stimuli (*Kudla, Batistic & Hashimoto, 2010*). Typically, the salt-tolerance SOS3-SOS2-SOS1 pathway demonstrates the significance of Ca^{2+} signaling for excluding excess Na$^+$ and maintaining cellular ion homeostasis (*Qiu et al., 2002*). SOS3 is a calcium sensor, belonging to the category of calcineurin B-like proteins (CBLs). SOS3 interacts with SOS2, a CBL-interacting protein kinase (CIPK), and the SOS3-SOS2 complex activates SOS1 (Na$^+$/H$^+$ antiporter) to promote the exclusion of Na$^+$ to extracellular regions (*Qiu et al., 2002*). In Arabidopsis, AtCBL4 and CIPK24 correspond to SOS3 and SOS2, respectively (*Gong et al., 2004*). Furthermore, it has been demonstrated that AtSCABP8 (SOS3-like calcium binding protein8/CBL10) interacts with SOS2 and regulates SOS1 in the shoot response to salt stress (*Quan et al., 2007*). The plant CBL and CIPK gene families encompass several members; different CBL-CIPK complexes regulate different target proteins to respond to environmental stimuli and developmental needs (*Kanwar et al., 2014*; *Liu et al., 2013*; *Pandey et al., 2015*; *Weinl & Kudla, 2009*). In
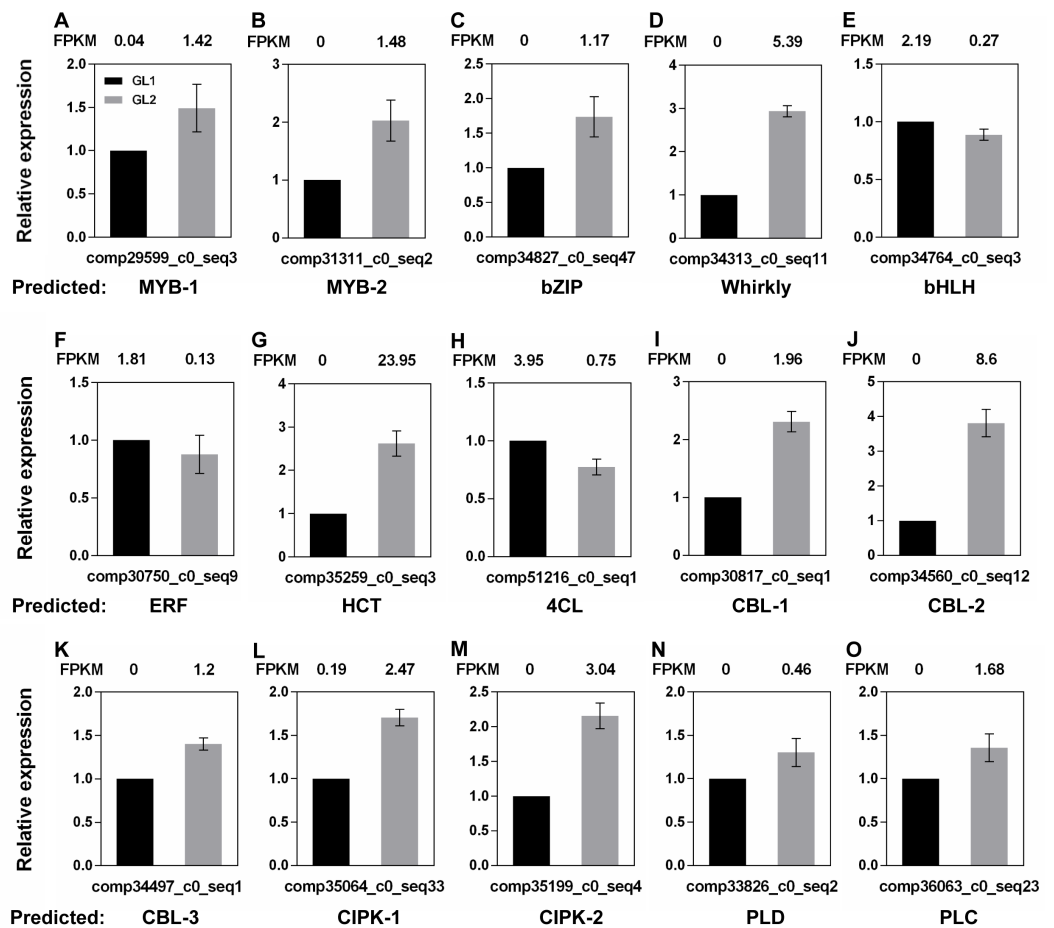
Figure 9 Expression patterns of 15 selected unigenes between *Glehnia littoralis* control and NaCl samples. Expression patterns of 15 selected unigenes between Glehnia littoralis control and NaCl samples. (A) Unigene comp29599_c0_seq3 (MYB-1). (B) Unigene comp31311_c0_seq2 (MYB-2). (C) Unigene comp34827_c0_seq47 (bZIP). (D) Unigene comp34313_c0_seq11 (Whirkly). (E) Unigene comp34764_c0_seq3 (bHLH). (F) Unigene comp30750_c0_seq9 (ERF). (G) Unigene comp35259_c0_seq3 (HCT). (H) Unigene comp51216_c0_seq1 (4CL). (I) Unigene comp30817_c0_seq1 (CBL-1). (J) Unigene comp34560_c0_seq12 (CBL-2). (K) Unigene comp34497_c0_seq1 (CBL-3). (L) Unigene comp35064_c0_seq33 (CIPK-1). (M) Unigene comp35199_c0_seq4 (CIPK-2). (N) Unigene comp33826_c0_seq2 (PLD). (O) Unigene comp36063_c0_seq23 (PLC). Unigene expression was analysed by qRT-PCR using Actin gene as an internal reference. Data represent means ± standard deviation (SD) from three biological replicates (three technical replicates per biological replicate). Repetition of the experiment produced similar results. The FPKM value obtained by RNA sequencing is indicated above each graph (A–O).

the current study, we assessed unigenes related to calcium signaling in *G. littoralis*. Some of these genes, such as CBLs and CIPKs, which are associated with plant salt-tolerance, were significantly upregulated. These data provide valuable genes targets for further research on physiological function in response to salt stress in *G. littoralis*. We also found that phospholipase-related DEGs were upregulated in *G. littoralis* under NaCl treatment (Fig. 8, Table S9). Phospholipases hydrolyse phospholipids. Phospholipase Ds (PLDs) and their hydrolysis product phosphatidic acid (PA), act as essential signal transducers and regulators

involved in hyperosmotic stress responses (*Hong et al., 2016*; *Zhang et al., 2014b*). Previous studies have reported that several PLDs were activated by salt stress (*Wang et al., 2014*). In *Arabidopsis*, a lack of *PLDa1* and *PLDa3* resulted in more sensitivity to salt stress and a lower PA accumulation, and the change of PA level was suggested to affect plant salt response (*Hong et al., 2008*; *Yu et al., 2010*). PA acts as a second messenger by its direct interaction with functional proteins, such as mitogen-activated protein kinase 6 (MPK6) and microtubule-associated protein (MAP65-1), to modulate the catalytic activity of binding proteins in response to salt stress (*Yu et al., 2010*; *Zhang et al., 2012b*). Moreover, a phosphoinositide-specific phospholipase C (OsPLC1)-mediated Ca^{2+} signaling pathway is essential for controlling Na^+ accumulation in the rice response to salt stress (*Li et al., 2017b*).

Plant transcription factors respond to various environmental stimuli such as salt, drought, cold, and hormones and regulate gene expression by binding to core regions of promoters (*Yadav et al., 2011*). Several types of transcription factors associated with salt response have been identified in plants, including the MYB, NAC, bZIP, AP2/ERF, WRKY, and bHLH families (*Cui et al., 2013*; *Hartmann et al., 2015*; *He et al., 2005*; *Jiang, Yang & Deyholos, 2009*; *Tao et al., 2011*; *Yang, Dai & Zhang, 2012*; *Zhang et al., 2009*). In this study, we identified 151 differentially expressed transcription factors in *G. littoralis* under NaCl treatment (Table S5), suggesting their potential function in the regulation of *G. littoralis* salt response. For example, MYB constituted a large proportion of the DEGs encoding transcription factors in this study (Fig. 7). Previous studies have reported that the overexpression of the *OsMYB3R-2*, *LcMYB1*, and *TaMYB56-B* genes confers enhanced salt tolerance in transgenic plants (*Cheng et al., 2013*; *Dai et al., 2007*; *Zhang et al., 2012a*), and *AtMYB20* and *AtMYB73* act as negative regulators to enhance salt resistance (*Cui et al., 2013*; *Kim et al., 2013*). The changes in expression of these transcription factors in *Glehnia littoralis* could indicate their functional mechanism in salt stress.

G. littoralis roots (Radix Glehniae) are rich in polysaccharides, phospho-lipids, coumarins, coumarin glycosides and polyacetylenes (*Tomshich et al., 1997*; *Yuan et al., 2002*). *G. littoralis* grows naturally in coastal regions, and is considered a high-quality medicinal herb in traditional Chinese medicine. Previous studies have shown that salt stress could increase various secondary metabolites in plants, such as flavonoids, jasmonic acid, polyphenol, and polyamine (*Ali, 2003*; *Ksouri et al., 2007*; *Mutlu & Bozcuk, 2007*; *Pedranzani et al., 2003*; *Ramakrishna & Ravishankar, 2011*). Flavonoids were increased in response to salt stress in *Hordeum vulgare* seedlings (*Ali, 2003*). *Ksouri et al. (2007)* reported that polyphenol content and antioxidant activities were related to salinity in halophyte *Cakile maritima*. Free and bound polyamine content have been shown to be induced under salt stress in *Helianthus annuus* roots (*Mutlu & Bozcuk, 2007*). In tomato, endogenous jasmonic acid was increased under salt stress (*Pedranzani et al., 2003*). Based on the habitat of *G. littoralis*, we hypothesize that its active compounds may be associated with environmental salt stress. In a previous study of *Peucedanum praeruptorum* (Umbelliferae), transcriptome sequencing and high-performance liquid chromatography coupled with electrospray-ionization quadrupole time-of-flight mass spectrometry (HPLC-Q-TOF-MS/MS)-based metabolomics datasets were constructed

for investigating the genes involved in coumarin biosynthesis and transport, as well for compound identification (*Zhao et al., 2015*). In total, 40,952 unigenes and 19 coumarin compounds were obtained and a few unigenes were predicted to be related to the formation of the coumarin core compounds in *Peucedanum praeruptorum*. Therefore, the *G. littoralis* transcriptome sequencing dataset could be used in future research to provide essential genes related to metabolism, and investigate biosynthetic pathways of the active compounds of *G. littoralis*.

CONCLUSIONS

In this study, we performed a comprehensive transcriptome analysis of *G. littoralis* in response to salt stress by Illumina 2000 sequencing. A significant number of unigenes, including DEGs, were identified and annotated. The DEGs dataset also provided candidate genes, which may be involved in salt tolerance or secondary metabolism pathways, to be used in subsequent functional analyses. These data will be helpful for future *G. littoralis* genomic studies, and will also be beneficial to studies on other species of Umbelliferae, and on halophytes.

Funding

This work was supported by grants from Public Science and Technology Research Funds Projects of Ocean (201505023) and Jiangsu Key Laboratory for the Research and Utilization of Plant Resources (JSPKLB201812). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Public Science and Technology Research Funds Projects of Ocean: 201505023.
Jiangsu Key Laboratory for the Research and Utilization of Plant Resources: JSPKLB201812.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Li Li analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Mimi Li performed the experiments.
- Xiwu Qi contributed reagents/materials/analysis tools.
- Xingli Tang authored or reviewed drafts of the paper.
- Yifeng Zhou conceived and designed the experiments, authored or reviewed drafts of the paper.

Data Availability

This transcriptome sequencing raw data were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (accession number: SRX547159). This Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under the accession GGSB00000000. The version described in this paper is the first version, GGSB01000000.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.5681#supplemental-information.

REFERENCES

Ali RM. 2003. Response of salt stressed barley seedlings to phenylurea. *Plant Soil and Environment* **49**:158–162.

Cao Z, Deng Z. 2017. De novo assembly, annotation, and characterization of root transcriptomes of three caladium cultivars with a focus on necrotrophic pathogen resistance/defense-related genes. *International Journal of Molecular Sciences* **18**:712 DOI 10.3390/ijms18040712.

Carillo P, Annunziata MG, Pontecorvo G, Fuggi A, Woodrow P. 2011. Salinity stress and salt tolerance. In: Shanker AK, Venkateswarlu B, eds. *Abiotic stress in plants-mechanisms and adaptations.* Rijeka: InTech, 21–38.

Cheng L, Li X, Huang X, Ma T, Liang Y, Ma X, Peng X, Jia J, Chen S, Chen Y. 2013. Overexpression of sheepgrass R1-MYB transcription factor LcMYB1 confers salt tolerance in transgenic *Arabidopsis. Plant Physiology and Biochemistry* **70**:252–260 DOI 10.1016/j.plaphy.2013.05.025.

Conesa A, Gotz S, Garciagomez JM, Terol J, Talon M, Robles M. 2005. Blast2GO, a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **21**:3674–3676 DOI 10.1093/bioinformatics/bti610.

Cui MH, Yoo KS, Hyoung S, Nguyen HTK, Kim YY, Kim HJ, Ok SH, Yoo SD, Shin JS. 2013. An Arabidopsis R2R3-MYB transcription factor, AtMYB20, negatively regulates type 2C serine/threonine protein phosphatases to enhance salt tolerance. *FEBS Letters* **587**:1773–1778 DOI 10.1016/j.febslet.2013.04.028.

Dai X, Xu Y, Ma Q, Xu W, Wang T, Xue Y, Chong K. 2007. Overexpression of an R1R2R3 MYB Gene, OsMYB3R-2, increases tolerance to freezing, drought, and salt stress in transgenic Arabidopsis. *Plant Physiology* **143**:1739–1751 DOI 10.1104/pp.106.094532.

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI. 2014. Plant salt-tolerance mechanisms. *Trends in Plant Science* **19**:371–379 DOI 10.1016/j.tplants.2014.02.001.

Deng W, Wang Y, Liu Z, Cheng H, Xue Y. 2014. HemI: a toolkit for illustrating heatmaps. *PLOS ONE* **9**:e111988 DOI 10.1371/journal.pone.0111988.

Gharat SA, Parmar S, Tambat S, Vasudevan M, Shaw BP. 2016. Transcriptome analysis of the response to NaCl in *Suaeda maritima* provides an insight into salt tolerance mechanisms in halophytes. *PLOS ONE* **11**:e016348.

Gong D, Guo Y, Schumaker KS, Zhu JK. 2004. The SOS3 family of calcium sensors and SOS2 family of protein kinases in *Arabidopsis*. *Plant Physiology* **134**:919–926 DOI 10.1104/pp.103.037440.

Grabherr M, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* **29**:644–652 DOI 10.1038/nbt.1883.

Gu C, Xu S, Wang Z, Liu L, Zhang Y, Deng Y, Huang S. 2018. *De novo* sequencing, assembly, and analysis of *Iris lactea var. chinensis* roots' transcriptome in response to salt stress. *Plant Physiology and Biochemistry* **125**:1–12 DOI 10.1016/j.plaphy.2018.01.019.

Hartmann L, Pedrotti L, Weiste C, Fekete A, Schierstaedt J, Gottler J, Kempa S, Krischke M, Dietrich K, Mueller MJ. 2015. Crosstalk between two bZIP signaling pathways orchestrates salt-induced metabolic reprogramming in Arabidopsis roots. *The Plant Cell* **27**:2244–2260 DOI 10.1105/tpc.15.00163.

Hauser F, Li Z, Waadt R, Schroeder JI. 2017. SnapShot, abscisic acid signaling. *Cell* **171**:1708–1708 DOI 10.1016/j.cell.2017.11.045.

He XJ, Mu RL, Cao WH, Zhang ZG, Zhang JS, Chen SY. 2005. AtNAC2, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development. *Plant Journal* **44**:903–916 DOI 10.1111/j.1365-313X.2005.02575.x.

Hong Y, Pan X, Welti R, Wang X. 2008. Phospholipase Dα3 is involved in the hyperosmotic response in Arabidopsis. *Plant Cell* **20**:803–816 DOI 10.1105/tpc.107.056390.

Hong Y, Zhao J, Guo L, Kim SC, Deng X, Wang G, Zhao G, Li M, Wang X. 2016. Plant phospholipases D and C and their diverse functions in stress responses. *Progress in Lipid Research* **62**:55–74 DOI 10.1016/j.plipres.2016.01.002.

Jiang Y, Yang B, Deyholos MK. 2009. Functional characterization of the Arabidopsis bHLH92 transcription factor in abiotic stress. *Molecular Genetics and Genomics* **282**:503–516 DOI 10.1007/s00438-009-0481-3.

Kanwar P, Sanyal SK, Tokas I, Yadav AK, Pandey A, Kapoor S, Pandey GK. 2014. Comprehensive structural, interaction and expression analysis of CBL and CIPK complement during abiotic stresses and development in rice. *Cell Calcium* **56**:81–95 DOI 10.1016/j.ceca.2014.05.003.

Kim JH, Nguyen NH, Jeong CY, Nguyen NT, Hong S-W, Lee H. 2013. Loss of the R2R3 MYB, AtMyb73, causes hyper-induction of the SOS1 and SOS3 genes in response to high salinity in *Arabidopsis*. *Journal of Plant Physiology* **170**:1461–1465 DOI 10.1016/j.jplph.2013.05.011.

Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C. 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the

halophyte Cakile maritima. *Plant Physiology and Biochemistry* **45**:244–249 DOI 10.1016/j.plaphy.2007.02.001.

Kudla J, Batistic O, Hashimoto K. 2010. Calcium signals, the lead currency of plant information processing. *Plant cell* **22**:541–563 DOI 10.1105/tpc.109.072686.

Li B, Dewey CN. 2011. RSEM, accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12**:323 DOI 10.1186/1471-2105-12-323.

Li H, Tang X, Zhu J, Yang X, Zhang H. 2017a. *De novo* transcriptome characterization, gene expression profiling and ionic responses of *Nitraria sibirica* Pall. under salt stress. *Forests* **8**:211 DOI 10.3390/f8060211.

Li L, Wang F, Yan P, Jing W, Zhang C, Kudla J, Zhang W. 2017b. A phosphoinositide-specific phospholipase C pathway elicits stress-induced Ca^{2+} signals and confers salt tolerance to rice. *New Phytologist* **214**:1172–1187 DOI 10.1111/nph.14426.

Liu LL, Ren HM, Chen LQ, Wang Y, Wu WH. 2013. A protein kinase, calcineurin B-like protein-interacting protein kinase 9, interacts with calcium sensor calcineurin B-like protein 3 and regulates potassium homeostasis under low-potassium stress in Arabidopsis. *Plant Physiology* **161**:266–277 DOI 10.1104/pp.112.206896.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\triangle\triangle CT}$ method. *Methods* **25**:402–408 DOI 10.1006/meth.2001.1262.

Lu X, Li J, Chen H, Hu J, Liu P, Zhou B. 2017. RNA-Seq analysis of apical meristem reveals integrative regulatory network of ROS and chilling potentially related to flowering in Litchi chinensis. *Scientific Reports* **7**:10183 DOI 10.1038/s41598-017-10742-y.

Mortazavi A, Williams BA, Mccue K, Schaeffer L, Wold BJ. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature Methods* **5**:621–628 DOI 10.1038/nmeth.1226.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59**:651–681 DOI 10.1146/annurev.arplant.59.032607.092911.

Mutlu F, Bozcuk S. 2007. Salinity-induced changes of free and bound polyamine levels in sunflower (*Helianthus annuus* L.) roots differing in salt tolerance. *Pakistan Journal of Botany* **39**:1097–1102.

Oh DH, Barkla BJ, Vera-Estrella R, Pantoja O, Lee SY, Bohnert HJ, Dassanayake M. 2015. Cell type-specific responses to salinity—the epidermal bladder cell transcriptome of *Mesembryanthemum crystallinum*. *New Phytologist* **207**:627–644 DOI 10.1111/nph.13414.

Pandey GK, Kanwar P, Singh A, Steinhorst L, Pandey A, Yadav AK, Tokas I, Sanyal SK, Kim BG, Lee SC, Cheong YH, Kudla J, Luan S. 2015. Calcineurin B-Like protein-interacting protein kinase CIPK21 regulates osmotic and salt stress responses in *Arabidopsis*. *Plant Physiology* **169**:780–792 DOI 10.1104/pp.15.00623.

Pedranzani H, Racagni G, Alemano S, Miersch O, Ramírez I, Peñacortés H, Taleisnik E, Machadodomenech E, Abdala G. 2003. Salt tolerant tomato plants show increased levels of jasmonic acid. *Plant Growth Regulation* **41**:149–158 DOI 10.1023/A:1027311319940.

Peleg Z, Blumwald E. 2011. Hormone balance and abiotic stress tolerance in crop plants. *Current Opinion in Plant Biology* **14**:290–295 DOI 10.1016/j.pbi.2011.02.001.

Peng Y, Liu X, Tang X, Xu D, Wu Q, Du F, Zhou Y. 2014. Growth and physiological characteristics of *Glehnia littoralis* in response to salt stress. *Jiangsu Agricultural Sciences* **30**:1273–1278.

Postnikova OA, Shao J, Nemchinov LG. 2013. Analysis of the alfalfa root transcriptome in response to salinity stress. *Plant and Cell Physiology* **54**:1041–1055 DOI 10.1093/pcp/pct056.

Qiu Q, Guo Y, Dietrich MA, Schumaker KS, Zhu J. 2002. Regulation of SOS1, a plasma membrane Na^+/H^+ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. *Proceedings of the National Academy of Sciences of the United States of America* **99**:8436–8441 DOI 10.1073/pnas.122224699.

Quan R, Lin H, Mendoza I, Zhang Y, Cao W, Yang Y, Shang M, Chen S, Pardo JM, Guo Y. 2007. SCABP8/CBL10, a putative calcium sensor, interacts with the protein kinase SOS2 to protect Arabidopsis shoots from salt stress. *The Plant Cell* **19**:1415–1431 DOI 10.1105/tpc.106.042291.

Ramakrishna A, Ravishankar GA. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior* **6**:1720–1731 DOI 10.4161/psb.6.11.17613.

Robinson MD, Mccarthy DJ, Smyth GK. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**:139–140 DOI 10.1093/bioinformatics/btp616.

Tao Z, Kou Y, Liu H, Li X, Xiao J, Wang S. 2011. OsWRKY45 alleles play different roles in abscisic acid signalling and salt stress tolerance but similar roles in drought and cold tolerance in rice. *Journal of Experimental Botany* **62**:4863–4874 DOI 10.1093/jxb/err144.

Tomshich SV, Komandrova NA, Kalmykova EN, Prokof'Eva NG, Momontova VA, Gorovoi PG, Ovodov YS. 1997. Biologically active polysaccharides from medicinal plants of the Far East. *Chemistry of Natural Compounds* **33**:146–149 DOI 10.1007/BF02291530.

Voronkova NM, Burkovskaya EV, Bezdeleva TA, Burundukova OL. 2011. Morphological and biological features of plants related to their adaptation to coastal habitats. *Russian Journal of Ecology* **39**:1–7.

Wang C, Gao C, Wang L, Zheng L, Yang C, Wang Y. 2013. Comprehensive transcriptional profiling of $NaHCO_3$-stressed *Tamarix hispida* roots reveals networks of responsive genes. *Plant Molecular Biology* **84**:145–157.

Wang J, Li B, Meng Y, Ma X, Lai Y, Si E, Yang K, Ren P, Shang X, Wang H. 2015. Transcriptomic profiling of the salt-stress response in the halophyte *Halogeton glomeratus*. *BMC Genomics* **16**:169 DOI 10.1186/s12864-015-1373-z.

Wang R, Xu S, Wang N, Xia B, Jiang Y, Wang R. 2017. Transcriptome analysis of secondary metabolism pathway, transcription factors, and transporters in response to Methyl jasmonate in *Lycoris aurea*. *Frontiers in Plant Science* **7**:1971 DOI 10.3389/fpls.2016.01971.

Wang X, Guo L, Wang G, Li M. 2014. PLD: phospholipase ds in plant signaling. In: Wang X, ed. *Phospholipases in plant signaling. Signaling and communication in plants.* Berlin: Springer Verlag, 3–26.

Ward JA, Ponnala L, Weber CA. 2012. Strategies for transcriptome analysis in nonmodel plants. *American Journal of Botany* **99**:267–276 DOI 10.3732/ajb.1100334.

Weinl S, Kudla J. 2009. The CBL-CIPK Ca^{2+}-decoding signaling network, function and perspectives. *New Phytologist* **184**:517–528 DOI 10.1111/j.1469-8137.2009.02938.x.

Wilkinson S, Davies WJ. 2010. Drought, ozone, ABA and ethylene, new insights from cell to plant to community. *Plant Cell and Environment* **33**:510–525 DOI 10.1111/j.1365-3040.2009.02052.x.

Xiao M, Zhang Y, Chen X, Lee EJ, Barber CJS, Chakrabarty R, Desgagné-Penix I, Haslam TM, Kim YB, Liu E. 2013. Transcriptome analysis based on next-generation sequencing of non-model plants producing specialized metabolites of biotechnological interest. *Journal of Biotechnology* **166**:122–134 DOI 10.1016/j.jbiotec.2013.04.004.

Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li CY, Wei L. 2011. KOBAS 2.0, a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Research* **39**:316–322 DOI 10.1093/nar/gkr483.

Yadav NR, Taunk J, Rani A, Aneja B, Yadav RC. 2011. Role of transcription factors in abiotic stress tolerance in crop plants. In: Narendra T, Sarvajeet SG, eds. *Climate change and plant abiotic stress tolerance.* Weinheinm: Wiley-VCH Verlag GmbH & Co. KGaA, 605–640.

Yamaguchishinozaki K, Shinozaki K. 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Plant Biology* **57**:781–803 DOI 10.1146/annurev.arplant.57.032905.105444.

Yamamoto N, Tomoyuki T, Tanaka K, Ishige T, Terashima S, Endo C, Kurusu T, Yajima S, Yano K, Tada Y. 2015. Comprehensive analysis of transcriptome response to salinity stress in the halophytic turf grass *Sporobolus virginicus. Frontiers in Plant Science* **6**:241 DOI 10.3389/fpls.2015.00241.

Yang A, Dai X, Zhang W. 2012. A R2R3-type MYB gene, OsMYB2, is involved in salt, cold, and dehydration tolerance in rice. *Journal of Experimental Botany* **63**:2541–2556 DOI 10.1093/jxb/err431.

Yao L, Wang J, Li B, Meng Y, Ma X, Si E, Ren P, Yang K, Shang X, Wang H. 2018. Transcriptome sequencing and comparative analysis of differentially-expressed isoforms in the roots of *Halogeton glomeratus* under salt stress. *Gene* **646**:159–168 DOI 10.1016/j.gene.2017.12.058.

Yu L, Nie J, Cao C, Jin Y, Yan M, Wang F, Liu J, Xiao Y, Liang Y, Zhang W. 2010. Phosphatidic acid mediates salt stress response by regulation of MPK6 in *Arabidopsis thaliana. New Phytologist* **188**:762–773 DOI 10.1111/j.1469-8137.2010.03422.x.

Yuan Z, Tezuka Y, Fan W, Kadota S, Li X. 2002. Constituents of the underground parts of *Glehnia littoralis. Chemical & Pharmaceutical Bulletin* **50**:73–77 DOI 10.1248/cpb.50.73.

Zhang G, Chen M, Li L, Xu Z, Chen X, Guo J, Ma Y. 2009. Overexpression of the soybean GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to

salt, drought, and diseases in transgenic tobacco. *Journal of Experimental Botany* **60**:3781–3796 DOI 10.1093/jxb/erp214.

Zhang J, Jiang D, Liu B, Luo W, Lu J, Ma T, Wan D. 2014a. Transcriptome dynamics of a desert poplar (*Populus pruinosa*) in response to continuous salinity stress. *Plant Cell Reports* **33**:1565–1579 DOI 10.1007/s00299-014-1638-z.

Zhang L, Zhao G, Xia C, Jia J, Liu X, Kong X. 2012a. Overexpression of a wheat MYB transcription factor gene, TaMYB56-B, enhances tolerances to freezing and salt stresses in transgenic *Arabidopsis*. *Gene* **505**:100–107 DOI 10.1016/j.gene.2012.05.033.

Zhang Q, Lin F, Mao T, Nie J, Yan M, Yuan M, Zhang W. 2012b. Phosphatidic acid regulates microtubule organization by interacting with MAP65-1 in response to salt stress in Arabidopsis. *The Plant Cell* **24**:4555–4576 DOI 10.1105/tpc.112.104182.

Zhang Q, Qu Y, Jing W, Li L, Zhang W. 2014b. Phospholipase Ds in plant response to hyperosmotic stresses. In: Wang X, ed. *Phospholipases in plant signaling.* Berlin: Springer Verlag, 101–200.

Zhao L, Yang Z, Guo Q, Mao S, Li S, Sun F, Wang H, Yang C. 2017. Transcriptomic profiling and physiological responses of halophyte *Kochia sieversiana* provide insights into salt tolerance. *Frontiers in Plant Science* **8**:1985 DOI 10.3389/fpls.2017.01985.

Zhao Y, Chan Z, Gao J, Xing L, Cao M, Yu C, Hu Y, You J, Shi H, Zhu Y, Gong Y, Mu Z, Wang H, Deng X, Wang P, Bressan RA, Zhu JK. 2016. ABA receptor PYL9 promotes drought resistance and leaf senescence. *Proceedings of the National Academy of Sciences of the United States of America* **113**:1949–1954 DOI 10.1073/pnas.1522840113.

Zhao Y, Liu T, Luo J, Zhang Q, Xu S, Han C, Xu J, Chen M, Chen Y, Kong L. 2015. Integration of a decrescent transcriptome and metabolomics dataset of *Peucedanum praeruptorum* to investigate the CYP450 and MDR genes involved in coumarins biosynthesis and transport. *Frontiers in Plant Science* **6**:996 DOI 10.3389/fpls.2015.00996.

Zhou A, Ma H, Liu E, Jiang T, Feng S, Gong S, Wang J. 2017. Transcriptome sequencing of Dianthus spiculifolius and analysis of the genes involved in responses to combined cold and drought stress. *International Journal of Molecular Sciences* **18**:849 DOI 10.3390/ijms18040849.

Zhu J-K. 2016. Abiotic stress signaling and responses in plants. *Cell* **167**:313–324 DOI 10.1016/j.cell.2016.08.029.