Plant Physiology and Biochemistry 135 (2019) 87–98

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Transcript profiling reveals an important role of cell wall remodeling and hormone signaling under salt stress in garlic

Guang-Long Wang[a,*,1], Xu-Qin Ren[a,1], Jie-Xia Liu[b], Feng Yang[c], Yun-Peng Wang[a], Ai-Sheng Xiong[b]

[a] School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian, 223003, China
[b] State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Horticulture, Nanjing Agricultural University, Nanjing, 210095, China
[c] Institute of Horticulture, Xuzhou Institute of Agricultural Sciences in Jiangsu Xuhuai Area, Xuzhou, 221131, China

ARTICLE INFO

Keywords:
Allium sativum
Cell wall
Hormone signaling
Salt stress
RNA-seq
Transcript profiles

ABSTRACT

Salt stress is one of the environmental factors that evidently limit plant growth and yield. Despite the fact that understanding plant response to salt stress is important to agricultural practice, the molecular mechanisms underlying salt tolerance in garlic remain unclear. In this study, garlic seedlings were exposed to 200 mM NaCl stress for 0, 1, 4, and 12 h, respectively. RNA-seq was applied to analyze the transcriptional response under salinity conditions. A total of 13,114 out of 25,530 differentially expressed unigenes were identified to have pathway annotation, which were mainly involved in purine metabolism, starch and sucrose metabolism, plant hormone signal transduction, flavone and flavonol biosynthesis, isoflavonoid biosynthesis, MAPK signaling pathway, and circadian rhythm. In addition, 272 and 295 differentially expressed genes were identified to be cell wall and hormone signaling-related, respectively, and their interactions under salinity stress were extensively discussed. The results from the current work would provide new resources for the breeding aimed at improving salt tolerance in garlic.

1. Introduction

Salinity stress is becoming one of the most serious environmental factors that adversely affect plant growth and development (Muscolo et al., 2015; Xiong et al., 2017). It is estimated that salinity influences more than about 20% of irrigated land accounting for one-third of worldwide food production (Zhao et al., 2013). Almost every aspect of the plant will experience changes under salt stress. Salinity stress can affect many physiological and cellular processes including photosynthesis, respiration, ion uptake, water absorption, and membrane stability, which all apparently result in yield reduction (Gupta and Huang, 2014; Zhang et al., 2017).

High salt levels generally induce osmotic stress and ionic stress as a consequence of low water availability and solute imbalance (Dang et al., 2013; Passamani et al., 2017). Plants must perceive and react to abiotic stresses timely and in an intricate manner so that they can survive under such conditions. Much effort has been made to unravel the molecular mechanisms modulating plant adaptation and tolerance to salt stress. These mechanisms include reduction of the accumulation of salt absorbed by roots and its segmentation in tissues and cells to avoid excess of toxic substance (Peng et al., 2014). In general, the responses to salinity were often related to alterations in signaling and regulatory pathways modulated by plant hormones, such as ethylene and abscisic acid (Pan et al., 2017; Zhang et al., 2016b). The process also involves complex gene regulation network *via* specific transcription factors, and great changes in the expression of genes essential for cell protection, osmotic adjustment, and acclimation (Campos Juan et al., 2015; Hu et al., 2013). These alterations may be accompanied by variations in ion balance, signaling, buildup of compatible solutes, removal of oxygen free radicals, and growth regulation (Sun et al., 2009; Tang et al., 2015). These responses have been partially described in some plant species through the isolation and identification of genes that are triggered in answer to adverse conditions. However, relevant information about the transcriptomic dynamics during sustained salinity stress

---

[*] Corresponding author.
E-mail address: 11160021@hyit.edu.cn (G.-L. Wang).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.plaphy.2018.11.033
Received 10 September 2018; Received in revised form 7 November 2018; Accepted 28 November 2018

Table 1
Statistics of the garlic unigene library.

Unigene length	Total number	Percentage
200–300	45,069	28.19
300–500	36,243	22.67
500–1000	38,210	23.90
1000–2000	28,720	17.96
2000 +	11,628	7.27
Total number	159,870	
Total length	125,955,119	
Mean length	787	
N50 length	1223	

is still circumscribed. Specifically, the real-time gene expression under salinity stress is not clear.

With the advance of modern technology for identifying genomic sequences, high-throughput sequencing has been routinely applied to obtain transcriptome information (Wang et al., 2009). High-throughput sequencing techniques can detect the gene expression level in different tissues under different conditions. It can also identify novel alternative splice isoforms, new splice sites, new genes, and single nucleotide polymorphisms (Abdelrahman et al., 2017). RNA-seq has been commonly used in plants to observe the responses of genes to different abiotic stresses: salinity stress in upland cotton (*Gossypium hirsutum*) (Guo et al., 2015) roots, cadmium stress in rice (*Oryza sativa*) roots (Tan et al., 2017), and potassium deficiency in soybean (*Glycine max*) roots and shoots (Wang et al., 2012). These transcriptome-wide studies have shed new light on exploring the changes of genes under adverse conditions.

Garlic (*Allium sativum* L.), a member of the Alliaceae family, is widely cultivated in the world. Due to antioxidant, anti-inflammatory, antimicrobial, antifungal, antitumor, and cardioprotective properties, garlic has been used for both culinary and medical purposes for a long time (Diretto et al., 2017). However, the genetic information regarding garlic is limited.

To obtain temporal expression trends of functional genes in response to high salinity, the garlic seedlings were exposed to salt stress and sequenced via a series of transcriptome sequencings. The results from the current work provide a detailed picture of the transcriptomic dynamics under salinity stress. Moreover, we use the data to identify differentially expressed genes that are involved in cell wall biosynthesis and hormone signaling.

2. Materials and methods

2.1. Plant materials and growth conditions

'Siliuban', a garlic cultivar that originated from Cangshan (Shandong Province, China), was used in this study. The cultivar has four to six cloves that were relatively more spicy and larger than that in other cultivars. Garlic cloves were grown in a mixture of organic soil and vermiculite in a greenhouse in Huaiyin Institute of Technology (33°32′N, 119°01′E). Twelve days later, the garlic seedlings were unearthed and allowed to grow for another 10 days. The seedlings were then transferred to 1/2 Hogland nutrient solution for 7 days. Then, the solution was supplemented with 0 or 200 mM NaCl for 0, 1, 4, and 12 h to analyze the responses of garlic cloves to high salinity. Garlic cloves that were about 29 days old from each treatment were harvested and stored at −80 °C until further analysis.

2.2. RNA extraction and sequencing

Total RNA was isolated using the plant RNA extraction kit (Tiangen, Beijing, China) in accordance with the manufacturer's instructions. The RNA samples were exposed to DNaseI (TaKaRa, Dalian, China) for 30 min at 37 °C to eliminate the genomic DNA. Afterwards, magnetic beads with Oligo (dT) are applied to isolate mRNA which was fragmented into short fragments by mixing with the fragmentation buffer. The cDNA was synthesized with the mRNA fragments as templates. Short fragments were purified and resolved with EB buffer for end reparation and single nucleotide A (adenine) addition. After that,

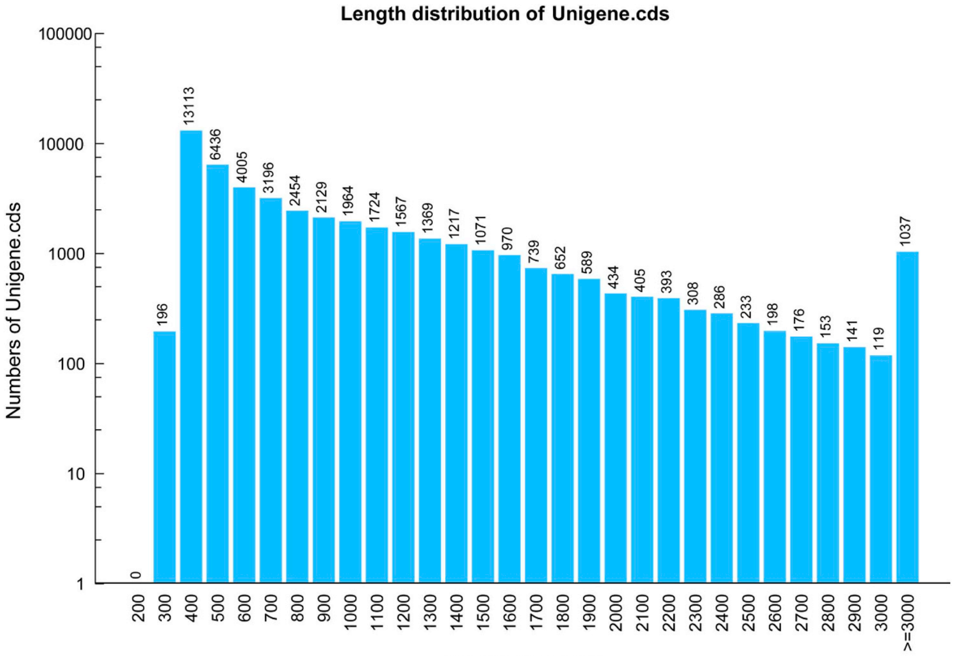

Fig. 1. Length distribution of unigene CDSs in garlic transcriptome.

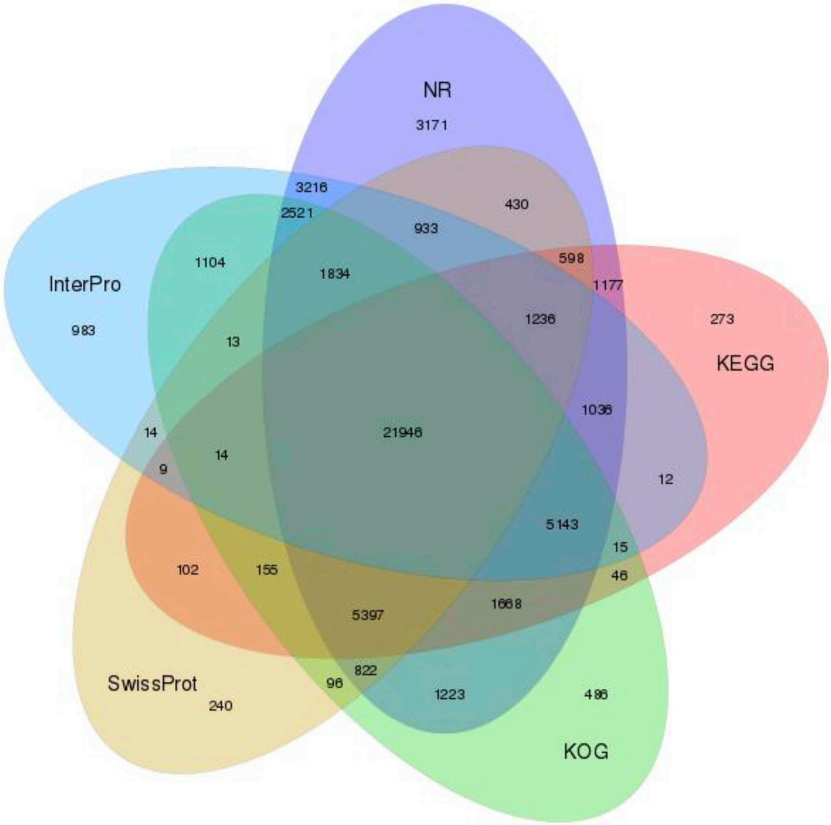

Fig. 2. Venn diagram of number of unigenes annotated in NR, KEGG, KOG, InterPro, and SwissProt databases.

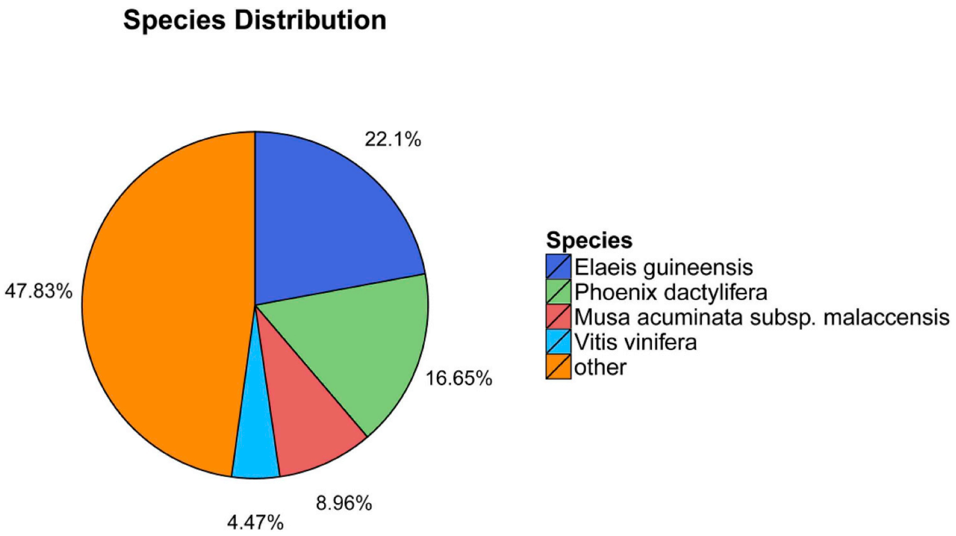

Fig. 3. Species distribution of the top BLAST hits for each unique sequence with a cut-off value of 1E-5.

adapters were ligated to the short fragments. The suitable fragments verified by agarose gel electrophoresis were selected for the PCR amplification. After quantification and qualification, the sample library was sequenced using an Illumina HiSeq™ 4000 platform.

2.3. De novo assembly and functional annotation

To get clean reads, low-quality, adaptor-polluted, and ambiguous base (N) reads were removed using SOAPnuke. The clean reads were gathered and assembled using Trinity and Tgicl to obtain unigenes (Haas et al., 2013). The unigenes were compared with the Nt database using the BLAST software. NR, KOG, KEGG, and SwissProt functional annotation was conducted by Diamond software, whereas Blast2GO and InterProScan5 were employed to perform GO and InterPro annotation, respectively (Buchfink et al., 2015; Conesa et al., 2005; Quevillon et al., 2005). TransDecoder was used to predict the coding sequences (CDSs) in the unigenes.

2.4. Analysis of differentially expressed unigenes

The fragments per kilobase of exon per million mapped fragments (FPKM) method was used to quantify gene expression (Langmead and

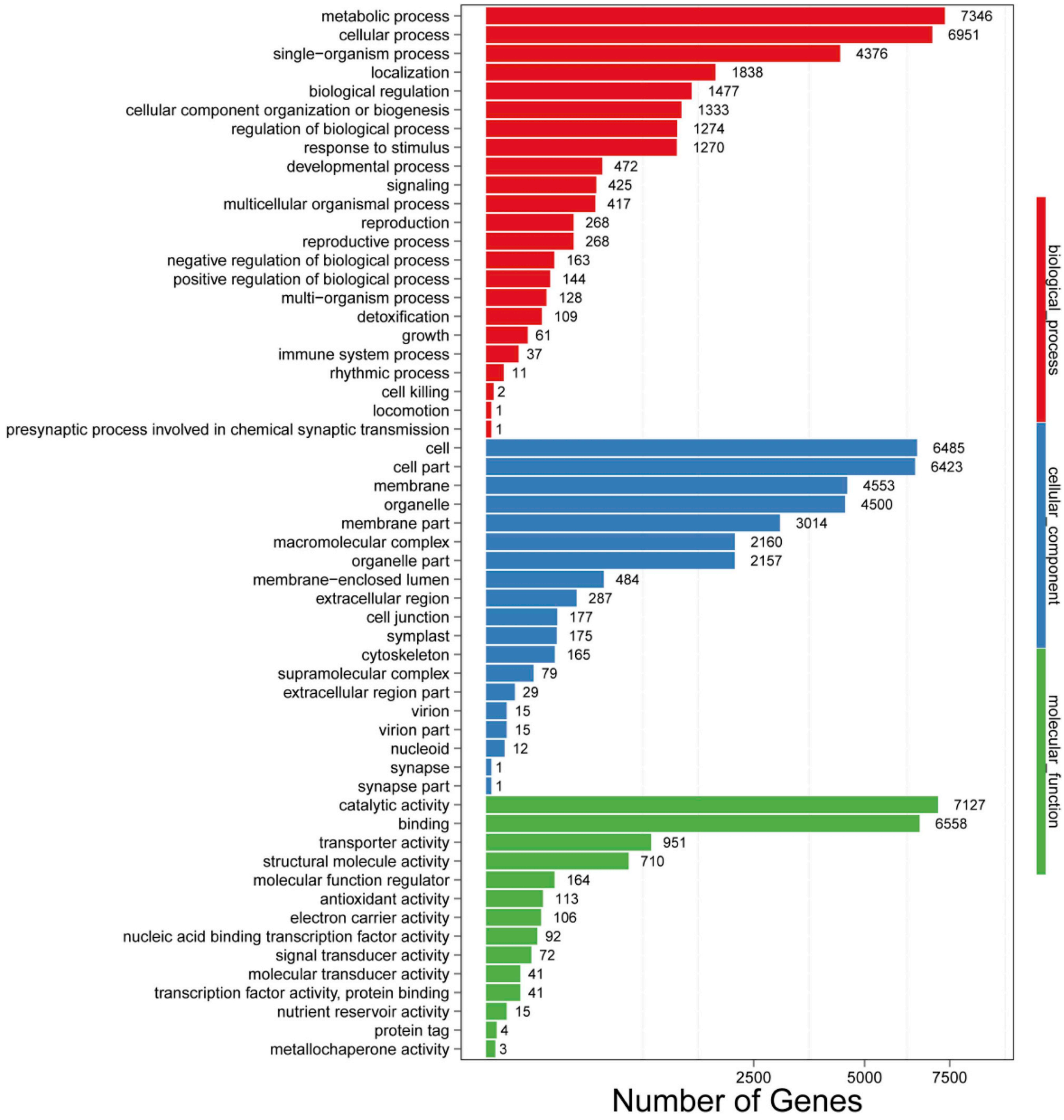

Fig. 4. Gene Ontology analysis of unigenes identified in garlic transcriptome.

Salzberg, 2012; Li and Dewey, 2011). False discovery rate (FDR) was introduced to measure the threshold of P-value in multiple tests. FDR < 0.001 and the absolute value of log2 fold change > 1 were used as the threshold to define the significant differences in gene expression.

2.5. Real-time quantitative PCR (RT-qPCR) validation

For RT-qPCR, total RNA from different samples were used for first-strand cDNA synthesis with a PrimeScript RT reagent kit (TaKaRa, Dalian, China) according to the prescribed protocol. A 1:15 dilution of cDNA was used for PCR reaction. A group of gene-specific primers (Supplementary material 1) were designed for RT-qPCR validation based on the sequence information derived from sequence analysis. Seventeen differentially expressed genes that encode for enzymes or components involved in cell wall biosynthesis and hormone signaling were selected for RT-qPCR analysis. The PCR mix for Real time PCR contained 2 μL of diluted cDNA, 10 μL of SYBR Premix *Ex Taq* (TaKaRa, Dalian, China), and 0.4 μL of each primer in a final volume of 20 μL. Expression was quantified in a CFX96 Real-Time PCR Detection System (Biorad, USA). Amplification of actin gene was used as the internal control for normalization. Three replicates were carried out for each cDNA.

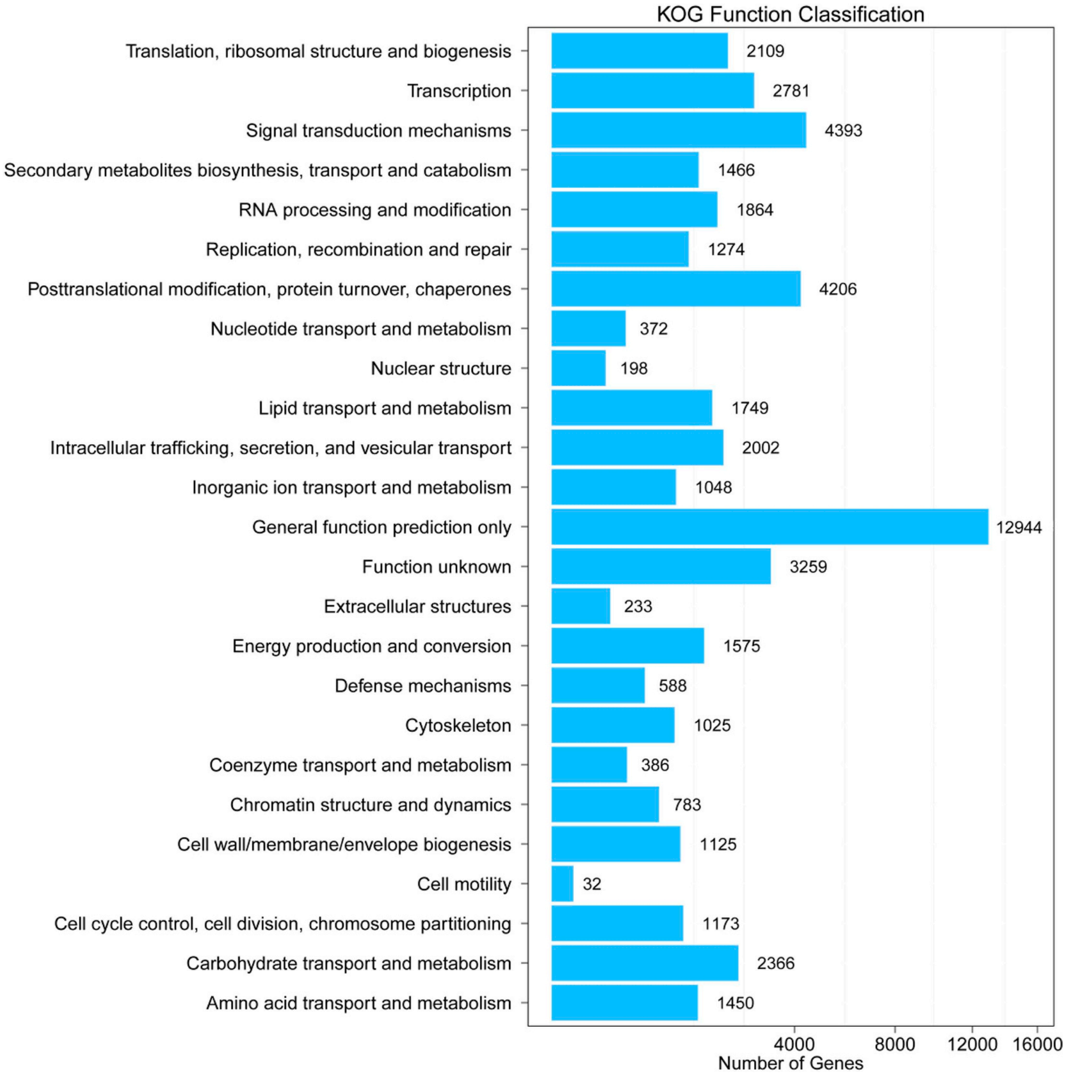

Fig. 5. KOG function classification of unigenes in garlic transcriptome.

3. Results

3.1. Assembly and functional annotation

A total of 159,870 unigenes were obtained with an average length of 787 bp and 39.01% GC content. The detailed unigene indexes are shown in Table 1. The predicted coding sequences (CDSs) from the unigenes were 47,274 in number with 42.57% GC content. The length distribution of the CDSs was listed in Fig. 1.

All unigenes were annotated against the Nt, Nr, KOG, KEGG, SwissProt, GO, and InterPro databases, yielding annotation about 14.07%, 32.75%, 26.57%, 24.29%, 21.17%, 9.20%, and 25.04% of the all unigenes, respectively. A total of 59,697 (37.34%) unigenes were annotated in the seven databases, whereas the remaining unigenes cannot be annotated with known genes (Fig. 2). With respect to species distribution, 22.10% of the unigenes have top hits to genes from *Elaeis guineensis*, followed by *Phoenix dactylifera* (16.65%), *Musa acuminata* (8.96%), and *Vitis vinifera* (4.47%) (Fig. 3).

3.2. Functional classification

To functionally categorize *A. sativum* transcriptome unigenes, GO classification was achieved using Blast2GO. A total of 14,704 unigenes were classified into three main GO categories and 56 subcategories (Fig. 4). “Metabolic process” and “cellular process” were the most highly represented groups within the biological process category, whereas “cell” and “cell part” accounted for the most abundant slims in the cellular component. In the molecular function category, “catalytic activity” and “binding” were found to be the most dominant classes, followed by “transporter activity” and “structural molecule activity” (Fig. 4).

The unigenes were searched against KOG (Clusters of eukaryotic Orthologous Groups), which represents contains protein sequences from seven eukaryote genomes. A total of 42,483 unigenes were grouped into 25 functional categories (Fig. 5). The cluster “General function prediction only” was the largest group, followed by “signal transduction mechanisms”, “posttranslational modification, protein

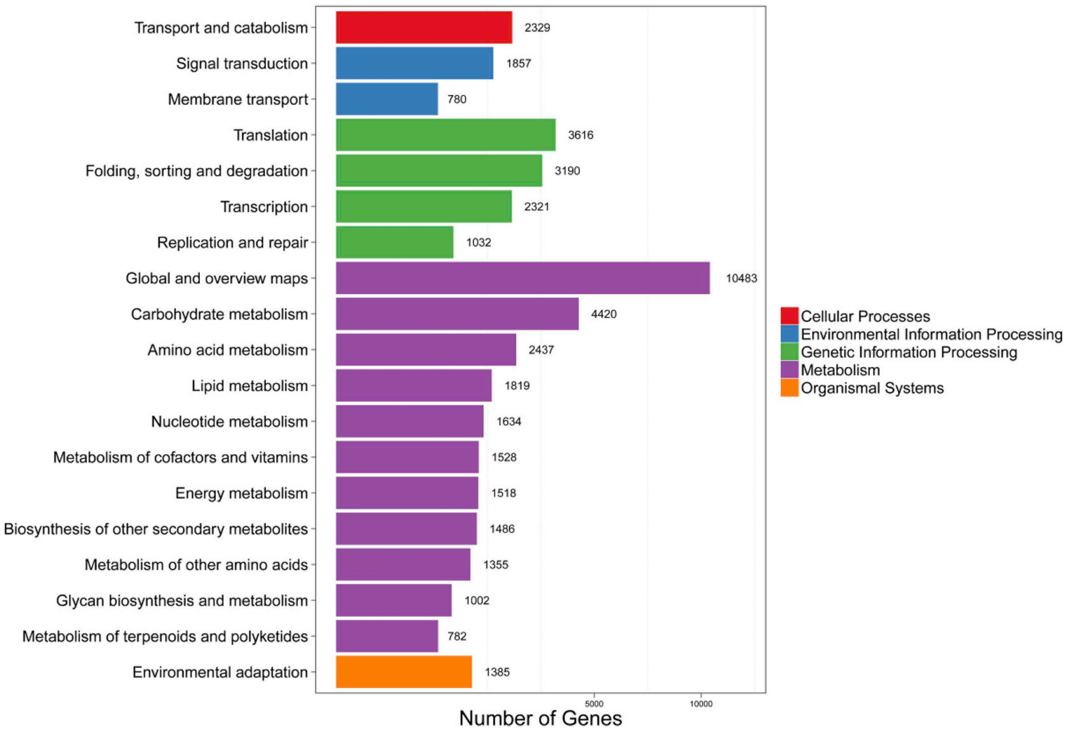

Fig. 6. KEGG classification of unigenes in garlic transcriptome.

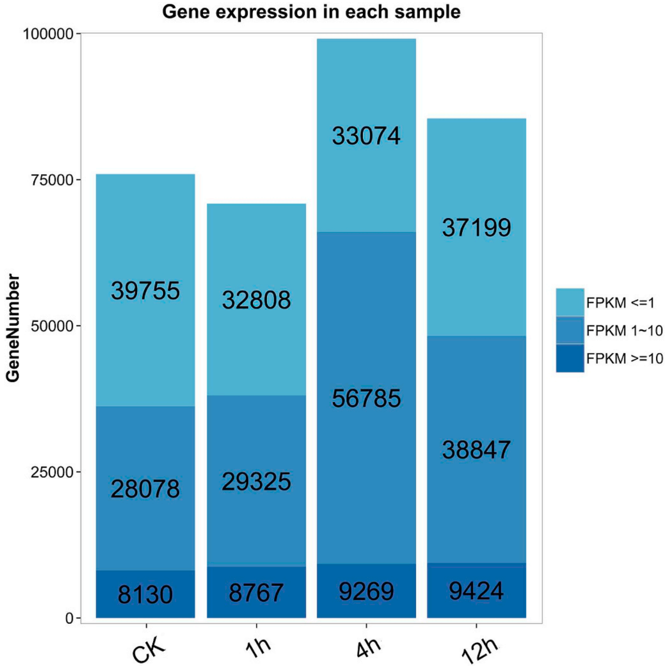

Fig. 7. Gene expression distribution in each garlic sample.

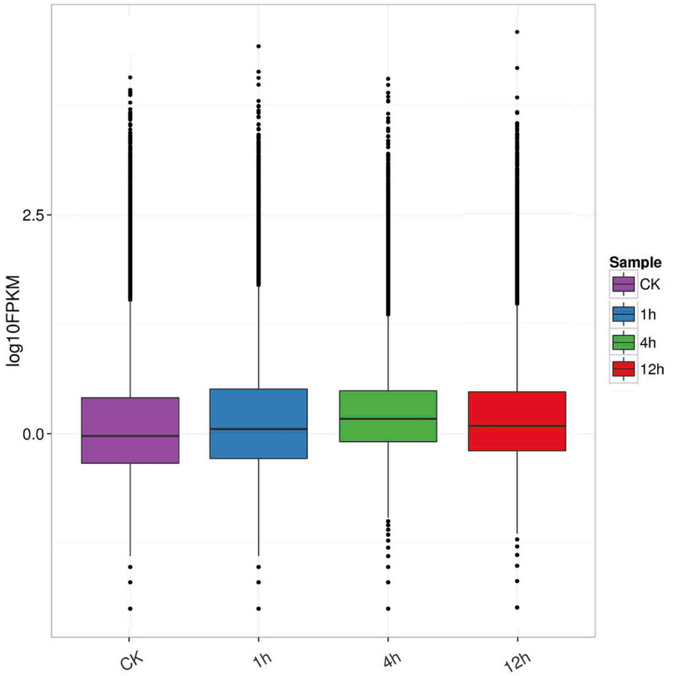

Fig. 8. Expression boxplot in each garlic sample.
Five statistical values in each sample represent maximum, the upper quartile, median, the lower quartile, minimum, respectively.

turnover, chaperones", and "function unknown". In particular, genes involved in secondary metabolite metabolism, cell wall biogenesis were also observed (Fig. 5).

To further characterize the unigenes related to biological functions, all unigenes were annotated with KEGG database. Totally, 38,827 unigenes were assigned to 5 groups. The largest subgroup was "Global and overview maps", followed by "carbohydrate metabolism, which all belonged to the metabolism category (Fig. 6).

3.3. Digital gene expression analysis

FPKM was used to quantify the expression of unigenes. Genes with FPKM in the interval ≤ 1, 1–10, and ≥ 10 were considered to be expressed at low, moderate, and high levels (Fig. 7). The gene number at moderate and high expression levels in treatment for 4 and 12 h was larger than that in treatment for 0 and 1 h, whereas the number of genes lowly expressed at 1 and 4 h was less than that at 0 and 12 h (Fig. 7). As

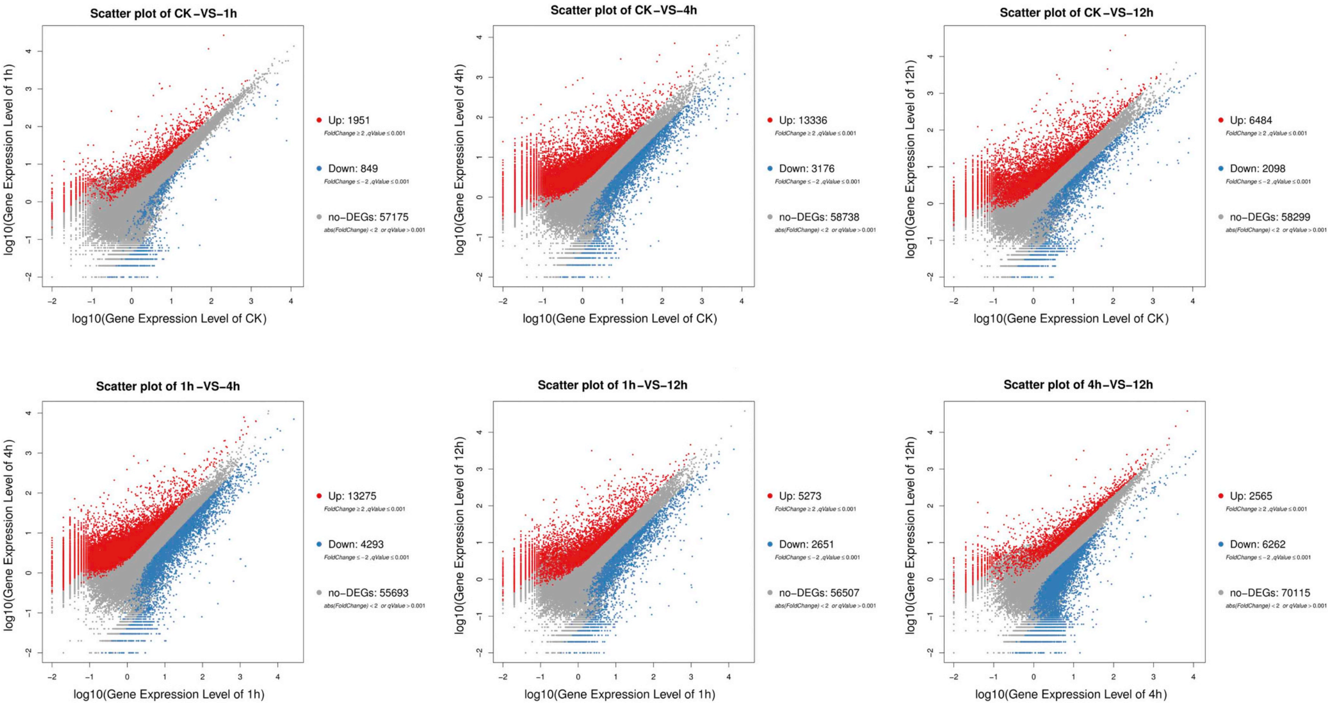

Fig. 9. Distribution of the differentially expressed genes between salt-stressed samples.

Table 2
Comparison of DEGs between salt-treated samples.

Comparison	Up-regulated	Down-regulated
CK VS 1 h	1951	849
CK VS 4 h	13,336	3176
CK VS 12 h	6484	2098
1 h VS 4 h	13,275	4293
1 h VS 12 h	5273	2651
4 h VS 12 h	2565	6262

shown in the boxplot, gene expression distribution and dispersion was observed in each sample (Fig. 8).

3.4. Analysis of differentially expressed unigenes in response to salt stress

To identify differentially expressed unigenes during the process of salt treatment, the expression levels of each gene in the four samples were compared and filtered with $|\log_2 \text{(fold change)}| >= 1$ and FDR < 0.001. There were 2800, 16512, 8582, 17568, 7924, and 8827 up or down-regulated unigenes detected in "CK vs 1 h", "CK vs 4 h", "CK vs 12 h", "1 h vs 4 h", "1 h vs 12 h", and "4 h vs 12 h" comparisons, respectively (Fig. 9; Table 2). A total of 25,530 unigenes were found to be differentially expressed, accounting for 15.97% of the all unigenes. A heat map containing all differentially expressed unigenes was established based on $\log_2$FPKM values (Fig. 10). According to KEGG database, 13114 out of 25,530 unigenes were observed to have pathway annotation. These genes were mainly enriched in purine metabolism, starch and sucrose metabolism, plant hormone signal transduction, and MAPK signaling pathway (Fig. 11).

3.5. Putative genes related to cell wall remodeling and hormone signaling

To further investigate the role of cell wall remodeling and hormone signaling in salt stress in garlic, the candidate genes related to cell wall and hormone signaling were identified according to the KEGG annotation and local TBLAST search. Only the genes with more than twofold change in FPKM during salt stress were selected (Supplementary material 2 & 3). A total of 272 and 295 genes were identified to be cell wall- and hormone signaling-related, respectively. The genes regarding cell wall remodeling are mainly involved in cell wall formation and modification (Supplementary material 2). Transcriptional changes in biosynthesis and degradation of pectins, celluloses, and hemicelluloses, and lignins were shown. The hormone signaling-related genes identified here encode for signal transduction elements and hormone-induced transcription factors (Supplementary material 3).

A total of 17 genes were validated through RT-qPCR analysis (Figs. 12 and 13). The expression profiles of nine genes involved in cell wall remodeling were well consistent with FPKM values from garlic transcriptome (Fig. 12; Supplementary material 2). Unigene38500, a gene involved in callose degradation, showed a consistently increasing expression when exposed to salt stress. The two genes (Unigene43218, and Unigene40028) that took part in cellulose biosynthesis and hydrolysis presented a steady rise in expression during salt stress. Transcription of *beta-D-xylosidase 7* (Unigene41975) increased slightly after treatment for 1 h, followed by a sharp increase at 4 h. The gene (CL3171.Contig2) that encodes for expansin-like protein showed the least expression in the control group, whereas relatively higher expression was observed under salinity condition. *Pectate lyase 8* (CL9524.Contig2) and *polygalacturonase* (CL909.Contig4) were genes that could act on pectin decomposition. The two genes possessed similar expression patterns, and were both highly expressed at 4 h after salt stress. A gene (CL14705.Contig1) encoding cinnamyl alcohol dehydrogenase in lignin biosynthesis pathway showed a similar expression trend to *beta-D-xylosidase 7*, whereas another gene, *cinnamate-4-hydroxylase* (Unigene33848), involved in lignin biosynthesis, exhibited the highest mRNA abundance at 12 h after treatment (Fig. 12). Eight genes involved in auxin (Unigene35702, CL9633.Contig2), ethylene (Unigene27630, CL2924.Contig5), abscisic acid (CL6313.Contig2), brassinosteroid (Unigene41110, Unigene18473), and jasmonate

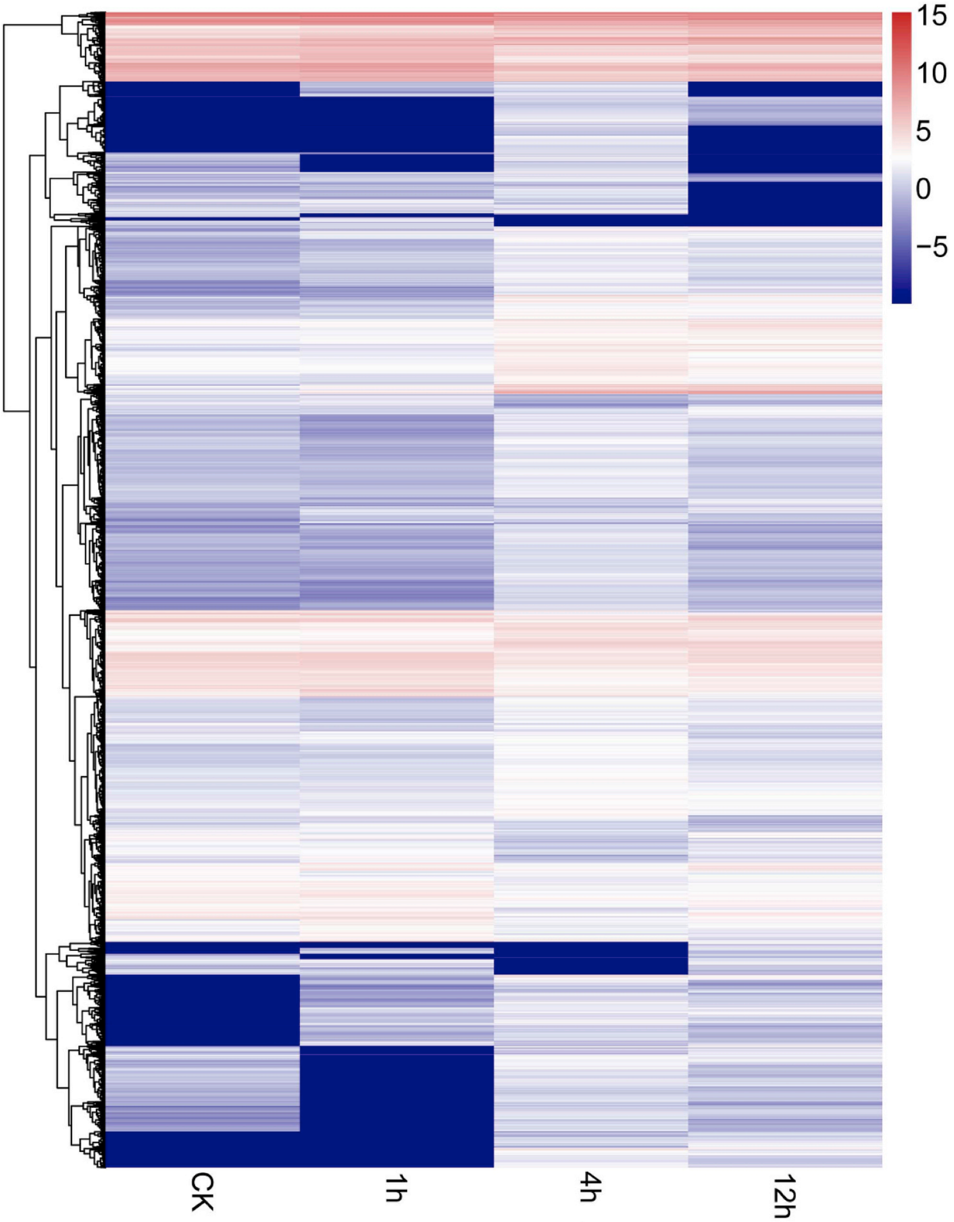

Fig. 10. Heatmap of the relative expression levels of differentially expressed genes in garlic samples under salt stress.
Red represents high expression, whereas blue represents low expression. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(CL8180.Contig1) signaling were also identified and verified to better explore the role of hormone signaling in salt stress. Expression profiling of these genes analyzed by RT-qPCR correlated well with FPKM values in garlic transcriptome (Fig. 13; Supplementary material 3). The two genes encoding auxin-induced protein 22D (AUX22D) and auxin response factor (ARF) showed highly similar expression trends during salt stress. An ethylene-responsive transcription factor (ERF), Unigene27630, was highly expressed at 1 h after treatment, whereas *ethylene receptor 2-like* that encodes an ethylene receptor EIN showed highest expression at the last stage. The *protein phosphatase 2C* gene showed steadily increasing expression during salt stress. The *BES1/BZR1* gene showed the highest mRNA abundance during the later stages of salt treatment, whereas *BRI1* (Unigene18473), a gene encoding brassinosteroid insensitive 1, was highly expressed at 4 h. Unlike *AUX22D*, *ARF*, and *BRI1* genes, the *jasmonate ZIM domain-containing protein* (*JAZ*) gene showed the lowest expression at 4 h after treatment (Fig. 13).

4. Discussion

Given that salinity stress has gradually become a serious threat to agriculture and food safety in the world, illustration of the mechanism underlying salinity tolerance is of vital importance. Salt tolerance is regulated in a complex manner that involves a large number of genes. Knowledge of the plant's reaction to salt stress needs a full-scale assay of stress-responsive genes. Here, RNA-seq, a valuable means for achieving

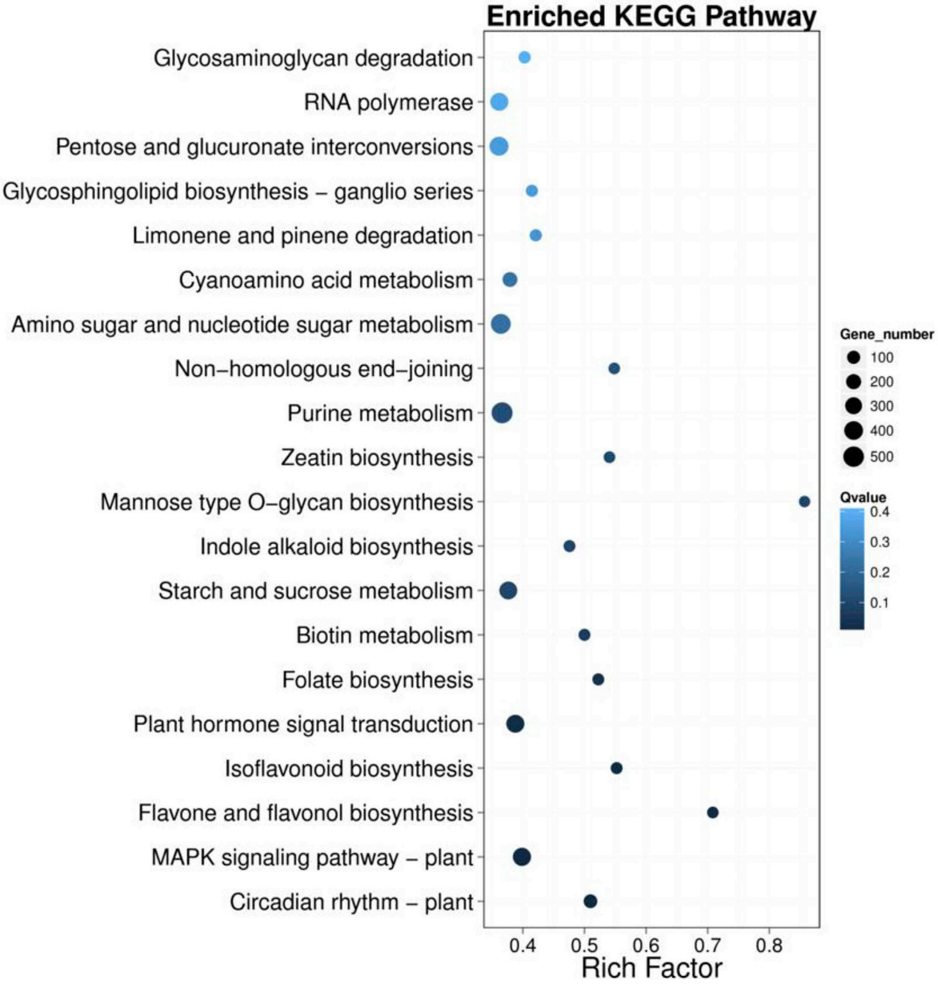

Fig. 11. Pathway enrichment analysis of differentially expressed genes in garlic samples under salt stress.
Top 20 pathway enrichments were displayed.

a nearly complete characterization of transcriptomic events under a specific condition, was applied to elucidate the time course of the response mechanism in garlic under salt stress. The results from the current work would definitely improve the understanding of dynamic changes in gene expression in garlic during salt stress.

Previous studies indicated that salt stress could induce changes at physiological and molecular levels to reduce salt uptake or compartment toxic ions. This may involve a series of activities, such as stomatal movement, sucrose metabolism, flavonol accumulation, hormone signaling, calcium signaling, and so on (Li et al., 2013; Sui et al., 2015). In this study, a large number of salt stress-related differentially expressed genes (DEGs) were identified, providing a basis for further studies aimed at breeding for improved salt tolerance in garlic. A total of 25,530 differentially expressed unigenes were identified. They are mainly involved in purine metabolism, starch and sucrose metabolism, plant hormone signal transduction, flavone and flavonol biosynthesis, isoflavonoid biosynthesis, and MAPK signaling pathway (Fig. 11), which were also extensively reported in other plant species. A previous study revealed a role of circadian rhythm in salt stress tolerance (Park et al., 2016). Here, we found that circadian clock genes were enriched during salt treatment, indicating circadian rhythm may also play an essential role in salt stress in garlic (Fig. 11).

Cell wall is a thick and rigid layer located on the periphery of plant cells, and is a highly active structure competent to undergo rapid and extensive modification when required. It keeps sensing environmental conditions and protecting other parts of the cell from stress (Otulak-Koziel et al., 2018). The cell wall is liable to be affected when exposed to adverse conditions. It is reported that salinity usually results in cell wall structure alterations, which, in general, is a consequence of changes in levels of lignins, pectins, celluloses, and hemicelluloses (de Lima et al., 2014; Janz et al., 2012; Keutgen and Pawelzik, 2007), indicating that cell wall components play essential roles in resistance against salt stress. Li et al. (2014) indicated that alteration in cell wall could lead to cell enlargement to mitigate the salinity-induced ionic toxicity. In addition, cell wall extensibility is a vital factor for maintaining normal turgor pressure under salt stress (Shen et al., 2014). At the molecular level, a large number of cell wall-related genes have been demonstrated to be connected with salt tolerance. Mutants in *PME31*, a gene related to pectin methyl esterification, conferred hypersensitive phenotypes to salt stress in *Arabidopsis* (Yan et al., 2018). Overexpression of *RhEXPA4*, a cell-wall-loosening gene in rose, influenced stomatal development and the arrangement of pavement cells, and therefore enhanced salt tolerance in *Arabidopsis* (Lü et al., 2013). This phenomenon is perhaps due to stress relaxation caused by wall loosening (Cosgrove, 2016). FERONIA, a plasma-membrane-localized receptor kinase, is important to maintain cell wall integrity during salt stress (Feng et al., 2018). In this work, we found that a total of 272 cell wall-related genes differentially expressed during salt stress, further supporting the idea that cell wall-related genes may exert great effects in plant response to salinity.

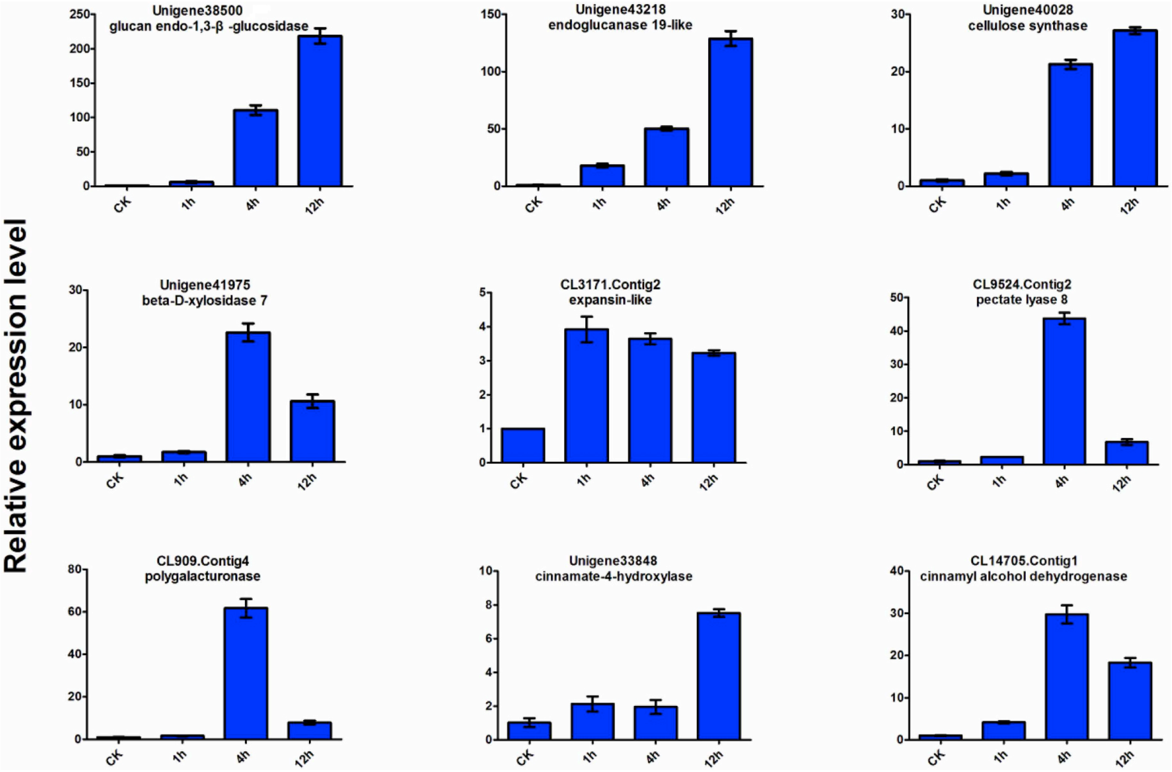

Fig. 12. Expression profiles of nine genes involved in cell wall modification in garlic samples exposed to salt stress.

Enhanced tolerance to salt stress was proved to be associated with abscisic acid, ethylene, auxin, jasmonates, and brassinosteroids in many studies (Dou et al., 2018; Pavlović et al., 2018; Prerostova et al., 2017; Sharma et al., 2013; Zhang et al., 2016a). For instance, increased expression of ERF transcription factor in wheat and tobacco confers improved tolerance to salinity (Rong et al., 2014; Wu et al., 2014), whereas enhanced oxidative stress was detected in the ethylene-insensitve (*ein3-1*) mutant in *Arabidopsis* exposed to salt stress (Asensi-Fabado et al., 2012). In our study, Unigene27630 and CL2924.Contig5, two key genes involved in the ethylene signaling pathway, were strongly induced in response to salt, further inferring that these two genes may play important roles in ethylene-mediated salt tolerance. Improved salt tolerance was observed in tobacco and *Arabidopsis* plants overexpressing a protein phosphatase 2C gene (Hu et al., 2015; Liu et al., 2012). Here, the *PP2C* gene showed a continuously increasing expression under salt stress, indicating that this gene may function in salt stress resistance in ABA-dependent manner.

As mentioned above, cell wall and hormone signaling play vital roles in salt tolerance in plants. How about their connection in plants? Do they interact with each other to coordinately and cooperatively resist against salt stress? It is reported that changes in gibberellin levels or signaling could alter cellulose and lignin accumulation in plant secondary cell walls (Huang et al., 2015; Wang et al., 2017). There are many genes under hormonal regulation that are also involved in secondary cell wall formation and remodeling (Didi et al., 2015). Downregulation of *DIM1*, a gene involved in BR synthesis, led to the reduction of cellulose and lignin content (Hossain et al., 2012), whereas knocking out *ABCG14*, a cytokinin transporter, resulted in decreased lignification (Zhang et al., 2014). Interaction between hormone and cell wall during salt stress was also verified in some studies. Another study revealed that 24-epibrassinolide induced alterations in the root cell walls of cucumber under salinity stress (An et al., 2018). And another study indicated that overexpression of *XTH1*, a cell wall modification gene regulated by hormones including gibberellins, ethylene, auxins, and abscisic acid, led to higher density of cell wall, and thus enhanced tolerance to salt stress (Han et al., 2017). These studies suggested that hormone signaling and the cell wall could work together to take part in salt stress tolerance. Further studies may focus on clarifying the specific molecular mechanisms between hormone signaling and cell wall under abiotic stresses.

5. Conclusion

To our knowledge, this is the first work of global transcriptome analysis and characterization of DEGs in *A. sativum* under salinity stress based on Illumina HiSeq 4000 platform. This analysis identified 159,870 unigenes and 47,274 CDSs. Functional analysis revealed that salinity stress in garlic influences a collection of genes involved in different biological and physiological processes. In addition, the genes involved in hormone signaling and cell wall remodeling provide new resources for the breeding aimed to improve salt tolerance. This study improves our understanding of the salt stress response in garlic at the molecular level. The data can be utilized as a reference for further transcript expression studies in garlic.

CRediT authorship contribution statement

Guang-Long Wang: Investigation, Methodology, Writing – original draft. **Xu-Qin Ren:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Jie-Xia Liu:** Supervision, Writing – review & editing. **Feng Yang:** Resources, Visualization. **Yun-Peng Wang:** Data curation, Formal analysis. **Ai-Sheng Xiong:** Software, Validation.

Acknowledgments

This study was funded by the Natural Science Foundation of Jiangsu Province (BK20170460), Scientific Research Foundation for Doctor from Huaiyin Institute of Technology (Z301B16531), New Century Excellent Talent of the Ministry of Education (NCET-11-0670), and Jiangsu Natural Science Foundation for Distinguished Young Scholars (BK20130027).

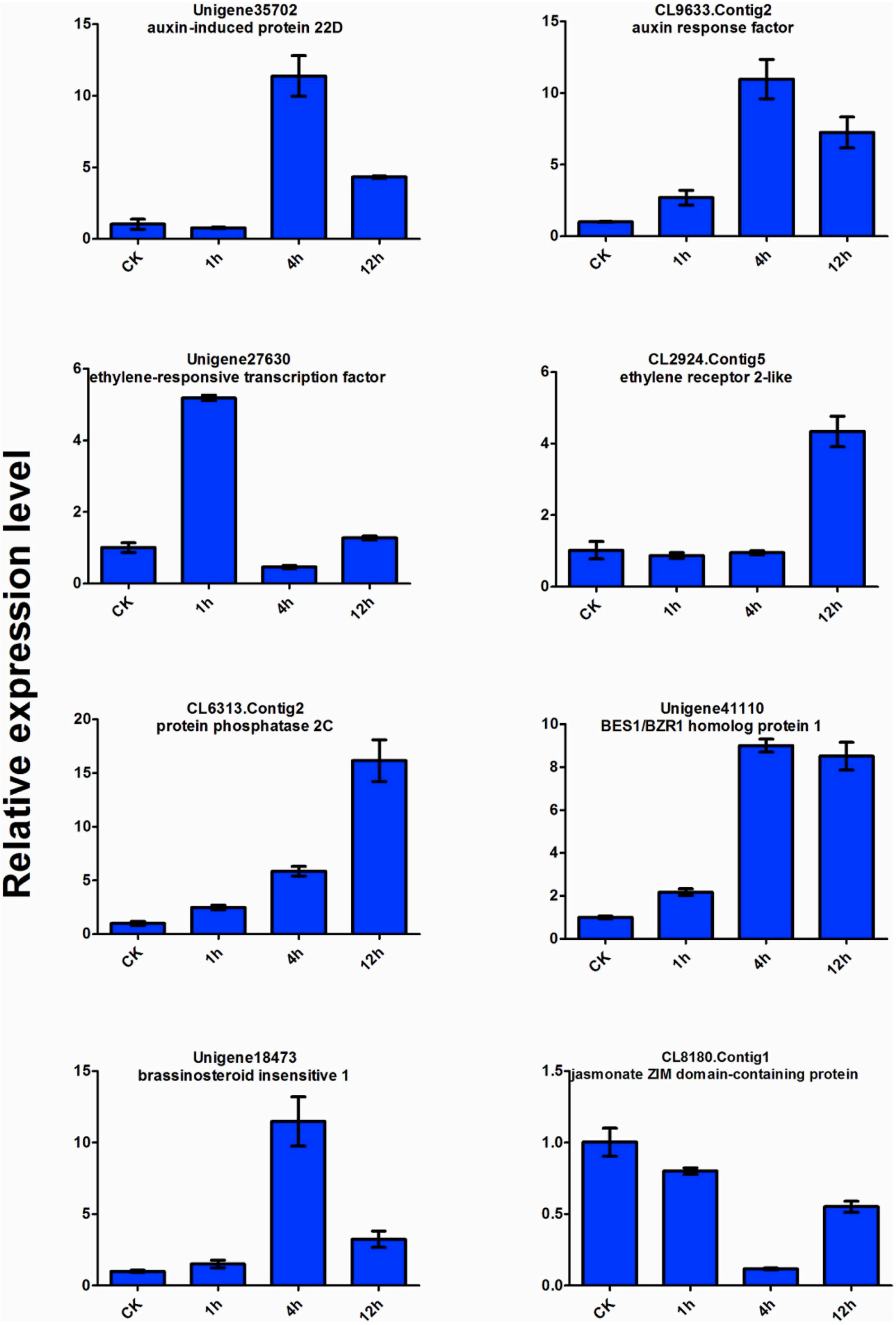

Fig. 13. Expression profiles of eight genes involved in hormone signaling in garlic samples exposed to salt stress.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.11.033.

Conflicts of interest

Authors declare that they have no conflict of interest.

References

Abdelrahman, M., El-Sayed, M., Sato, S., Hirakawa, H., Ito, S.I., Tanaka, K., Mine, Y., Sugiyama, N., Suzuki, Y., Yamauchi, N., Shigyo, M., 2017. RNA-sequencing-based transcriptome and biochemical analyses of steroidal saponin pathway in a complete set of *Allium fistulosum-A. cepa* monosomic addition lines. PLoS One 12, e0181784.

An, Y.H., Zhou, H., Yuan, Y.H., Li, L., Sun, J., Shu, S., Guo, S.R., 2018. 24-Epibrassinolide-induced alterations in the root cell walls of *Cucumis sativus* L. under Ca(NO$_3$)$_2$ stress. Protoplasma 255, 841–850.

Asensi-Fabado, M.A., Cela, J., Müller, M., Arrom, L., Chang, C., Munné-Bosch, S., 2012. Enhanced oxidative stress in the ethylene-insensitive (*ein3-1*) mutant of *Arabidopsis thaliana* exposed to salt stress. J. Plant Physiol. 169, 360–368.

Buchfink, B., Xie, C., Huson, D.H., 2015. Fast and sensitive protein alignment using DIAMOND. Nat. Methods 12, 59.

Campos Juan, F., Cara, B., Pérez-Martín, F., Pineda, B., Egea, I., Flores Francisco, B., Fernandez-Garcia, N., Capel, J., Moreno, V., Angosto, T., Lozano, R., Bolarin Maria, C., 2015. The tomato mutant *ars1* (*altered response to salt stress 1*) identifies an R1-type MYB transcription factor involved in stomatal closure under salt acclimation. Plant Biotechnol. J. 14, 1345–1356.

Conesa, A., Götz, S., García-Gómez, J.M., Terol, J., Talón, M., Robles, M., 2005. Blast2GO:

a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21, 3674–3676.

Cosgrove, D.J., 2016. Plant cell wall extensibility: connecting plant cell growth with cell wall structure, mechanics, and the action of wall-modifying enzymes. J. Exp. Bot. 67, 463–476.

Dang, Z., Zheng, L., Wang, J., Gao, Z., Wu, S., Qi, Z., Wang, Y., 2013. Transcriptomic profiling of the salt-stress response in the wild recretohalophyte *Reaumuria trigyna*. BMC Genomics 14, 29.

de Lima, R.B., dos Santos, T.B., Vieira, L.G.E., de Lourdes Lúcio Ferrarese, M., Ferrarese-Filho, O., Donatti, L., Boeger, M.R.T., de Oliveira Petkowicz, C.L., 2014. Salt stress alters the cell wall polysaccharides and anatomy of coffee (*Coffea arabica* L.) leaf cells. Carbohydr. Polym. 112, 686–694.

Didi, V., Jackson, P., Hejátko, J., 2015. Hormonal regulation of secondary cell wall formation. J. Exp. Bot. 66, 5015–5027.

Diretto, G., Rubio-Moraga, A., Argandoña, J., Castillo, P., Gómez-Gómez, L., Ahrazem, O., 2017. Tissue-specific accumulation of sulfur compounds and saponins in different parts of garlic cloves from purple and white ecotypes. Molecules 22, E1359.

Dou, L., He, K., Higaki, T., Wang, X., Mao, T., 2018. Ethylene signaling modulates cortical microtubule reassembly in response to salt stress. Plant Physiol. 176, 2071–2081.

Feng, W., Kita, D., Peaucelle, A., Cartwright, H.N., Doan, V., Duan, Q., Liu, M.C., Maman, J., Steinhorst, L., Schmitz-Thom, I., Yvon, R., Kudla, J., Wu, H.M., Cheung, A.Y., Dinneny, J.R., 2018. The FERONIA receptor kinase maintains cell-wall integrity during salt stress through Ca^{2+} signaling. Curr. Biol. 28, 666–675 e665.

Guo, J., Shi, G., Guo, X., Zhang, L., Xu, W., Wang, Y., Su, Z., Hua, J., 2015. Transcriptome analysis reveals that distinct metabolic pathways operate in salt-tolerant and salt-sensitive upland cotton varieties subjected to salinity stress. Plant Sci. 238, 33–45.

Gupta, B., Huang, B., 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int. J. Genomics 701596 2014.

Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles, D., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N., Henschel, R., LeDuc, R.D., Friedman, N., Regev, A., 2013. *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat. Protoc. 8, 1494.

Han, Y., Han, S., Ban, Q., He, Y., Jin, M., Rao, J., 2017. Overexpression of persimmon *DkXTH1* enhanced tolerance to abiotic stress and delayed fruit softening in transgenic plants. Plant Cell Rep. 36, 583–596.

Hossain, Z., McGarvey, B., Amyot, L., Gruber, M., Jung, J., Hannoufa, A., 2012. *DIMINUTO 1* affects the lignin profile and secondary cell wall formation in Arabidopsis. Planta 235, 485–498.

Hu, W., Yan, Y., Hou, X., He, Y., Wei, Y., Yang, G., He, G., Peng, M., 2015. *TaPP2C1*, a group F2 protein phosphatase 2C gene, confers resistance to salt stress in transgenic tobacco. PLoS One 10, e0129589.

Hu, Y., Chen, L., Wang, H., Zhang, L., Wang, F., Yu, D., 2013. Arabidopsis transcription factor WRKY8 functions antagonistically with its interacting partner VQ9 to modulate salinity stress tolerance. Plant J. 74, 730–745.

Huang, D., Wang, S., Zhang, B., Shang-Guan, K., Shi, Y., Zhang, D., Liu, X., Wu, K., Xu, Z., Fu, X., Zhou, Y., 2015. A gibberellin-mediated DELLA-NAC signaling cascade regulates cellulose synthesis in rice. Plant Cell 27, 1681–1696.

Janz, D., Lautner, S., Wildhagen, H., Behnke, K., Schnitzler, J.P., Rennenberg, H., Fromm, J., Polle, A., 2012. Salt stress induces the formation of a novel type of 'pressure wood' in two *Populus* species. New Phytol. 194, 129–141.

Keutgen, A.J., Pawelzik, E., 2007. Cultivar-dependent cell wall modification of strawberry fruit under NaCl salinity stress. J. Agric. Food Chem. 55, 7580–7585.

Lü, P., Kang, M., Jiang, X., Dai, F., Gao, J., Zhang, C., 2013. *RhEXPA4*, a rose expansin gene, modulates leaf growth and confers drought and salt tolerance to *Arabidopsis*. Planta 237, 1547–1559.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357.

Li, B., Dewey, C.N., 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinf. 12, 323.

Li, H., Yan, S., Zhao, L., Tan, J., Zhang, Q., Gao, F., Wang, P., Hou, H., Li, L., 2014. Histone acetylation associated up-regulation of the cell wall related genes is involved in salt stress induced maize root swelling. BMC Plant Biol. 14, 105.

Li, X., Kim, Y.B., Kim, Y., Zhao, S., Kim, H.H., Chung, E., Lee, J.H., Park, S.U., 2013. Differential stress-response expression of two flavonol synthase genes and accumulation of flavonols in tartary buckwheat. J. Plant Physiol. 170, 1630–1636.

Liu, X., Zhu, Y., Zhai, H., Cai, H., Ji, W., Luo, X., Li, J., Bai, X., 2012. AtPP2CG1, a protein phosphatase 2C, positively regulates salt tolerance of *Arabidopsis* in abscisic acid-dependent manner. Biochem. Biophys. Res. Commun. 422, 710–715.

Muscolo, A., Junker, A., Klukas, C., Weigelt-Fischer, K., Riewe, D., Altmann, T., 2015. Phenotypic and metabolic responses to drought and salinity of four contrasting lentil accessions. J. Exp. Bot. 66, 5467–5480.

Otulak-Koziel, K., Koziel, E., Lockhart, B.E.L., 2018. Plant cell wall dynamics in compatible and incompatible potato response to infection caused by *potato virus Y* (PVY^{NTN}). Int. J. Mol. Sci. 19, E862.

Pan, Y., Hu, X., Li, C., Xu, X., Su, C., Li, J., Song, H., Zhang, X., Pan, Y., 2017. *SlbZIP38*, a tomato bZIP family gene downregulated by abscisic acid, is a negative regulator of drought and salt stress tolerance. Genes 8, E402.

Park, H.J., Kim, W.Y., Yun, D.J., 2016. A new insight of salt stress signaling in plant. Mol. Cell. 39, 447–459.

Passamani, L.Z., Barbosa, R.R., Reis, R.S., Heringer, A.S., Rangel, P.L., Santa-Catarina, C., Grativol, C., Veiga, C.F.M., Souza-Filho, G.A., Silveira, V., 2017. Salt stress induces changes in the proteomic profile of micropropagated sugarcane shoots. PLoS One 12, e0176076.

Pavlović, I., Pěnčík, A., Novák, O., Vujčić, V., Radić Brkanac, S., Lepeduš, H., Strnad, M., Salopek-Sondi, B., 2018. Short-term salt stress in *Brassica rapa* seedlings causes alterations in auxin metabolism. Plant Physiol. Biochem. 125, 74–84.

Peng, Z., He, S., Gong, W., Sun, J., Pan, Z., Xu, F., Lu, Y., Du, X., 2014. Comprehensive analysis of differentially expressed genes and transcriptional regulation induced by salt stress in two contrasting cotton genotypes. BMC Genomics 15, 760.

Prerostova, S., Dobrev, P.I., Gaudinova, A., Hosek, P., Soudek, P., Knirsch, V., Vankova, R., 2017. Hormonal dynamics during salt stress responses of salt-sensitive *Arabidopsis thaliana* and salt-tolerant *Thellungiella salsuginea*. Plant Sci. 264, 188–198.

Quevillon, E., Silventoinen, V., Pillai, S., Harte, N., Mulder, N., Apweiler, R., Lopez, R., 2005. InterProScan: protein domains identifier. Nucleic Acids Res. 33, W116–W120.

Rong, W., Qi, L., Wang, A., Ye, X., Du, L., Liang, H., Xin, Z., Zhang, Z., 2014. The ERF transcription factor TaERF3 promotes tolerance to salt and drought stresses in wheat. Plant Biotechnol. J. 12, 468–479.

Sharma, I., Ching, E., Saini, S., Bhardwaj, R., Pati, P.K., 2013. Exogenous application of brassinosteroid offers tolerance to salinity by altering stress responses in rice variety Pusa Basmati-1. Plant Physiol. Biochem. 69, 17–26.

Shen, X., Wang, Z., Song, X., Xu, J., Jiang, C., Zhao, Y., Ma, C., Zhang, H., 2014. Transcriptomic profiling revealed an important role of cell wall remodeling and ethylene signaling pathway during salt acclimation in *Arabidopsis*. Plant Mol. Biol. 86, 303–317.

Sui, N., Yang, Z., Liu, M., Wang, B., 2015. Identification and transcriptomic profiling of genes involved in increasing sugar content during salt stress in sweet sorghum leaves. BMC Genomics 16, 534.

Sun, J., Chen, S.L., Dai, S.X., Wang, R.G., Li, N.Y., Shen, X., Zhou, X.Y., Lu, C.F., Zheng, X.J., Hu, Z.M., Zhang, Z.K., Song, J., Xu, Y., 2009. Ion flux profiles and plant ion homeostasis control under salt stress. Plant Signal. Behav. 4, 261–264.

Tan, M., Cheng, D., Yang, Y., Zhang, G., Qin, M., Chen, J., Chen, Y., Jiang, M., 2017. Co-expression network analysis of the transcriptomes of rice roots exposed to various cadmium stresses reveals universal cadmium-responsive genes. BMC Plant Biol. 17, 194.

Tang, X., Mu, X., Shao, H., Wang, H., Brestic, M., 2015. Global plant-responding mechanisms to salt stress: physiological and molecular levels and implications in biotechnology. Crit. Rev. Biotechnol. 35, 425–437.

Wang, C., Chen, H., Hao, Q., Sha, A., Shan, Z., Chen, L., Zhou, R., Zhi, H., Zhou, X., 2012. Transcript profile of the response of two soybean genotypes to potassium deficiency. PLoS One 7, e39856.

Wang, G.L., Que, F., Xu, Z.S., Wang, F., Xiong, A.S., 2017. Exogenous gibberellin enhances secondary xylem development and lignification in carrot taproot. Protoplasma 254, 839–848.

Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. 10, 57.

Wu, D., Ji, J., Wang, G., Guan, C., Jin, C., 2014. LchERF, a novel ethylene-responsive transcription factor from *Lycium chinense*, confers salt tolerance in transgenic tobacco. Plant Cell Rep. 33, 2033–2045.

Xiong, J., Sun, Y., Yang, Q., Tian, H., Zhang, H., Liu, Y., Chen, M., 2017. Proteomic analysis of early salt stress responsive proteins in alfalfa roots and shoots. Proteome Sci. 15, 19.

Yan, J., He, H., Fang, L., Zhang, A., 2018. Pectin methylesterase31 positively regulates salt stress tolerance in Arabidopsis. Biochem. Biophys. Res. Commun. 496, 497–501.

Zhang, J., Yu, H., Zhang, Y., Wang, Y., Li, M., Zhang, J., Duan, L., Zhang, M., Li, Z., 2016a. Increased abscisic acid levels in transgenic maize overexpressing *AtLOS5* mediated root ion fluxes and leaf water status under salt stress. J. Exp. Bot. 67, 1339–1355.

Zhang, J., Yuan, H., Yang, Q., Li, M., Wang, Y., Li, Y., Ma, X., Tan, F., Wu, R., 2017. The genetic architecture of growth traits in *Salix matsudana* under salt stress. Hortic. Res. 4, 17024.

Zhang, K., Novak, O., Wei, Z., Gou, M., Zhang, X., Yu, Y., Yang, H., Cai, Y., Strnad, M., Liu, C.J., 2014. *Arabidopsis* ABCG14 protein controls the acropetal translocation of root-synthesized cytokinins. Nat. Commun. 5, 3274.

Zhang, M., Smith, J.A.C., Harberd, N.P., Jiang, C., 2016b. The regulatory roles of ethylene and reactive oxygen species (ROS) in plant salt stress responses. Plant Mol. Biol. 91, 651–659.

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