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Comparative Transcriptome Analysis of Seedling Stage of Two Sorghum Cultivars Under Salt Stress

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Abstract

Soil salinity is one of the major abiotic stresses restricting crop production. Mechanisms of salt response have been intensively studied in model plants such as *Arabidopsis* and rice, but are rarely known in sorghum. In this study, we compared the transcriptome profiles between two cultivars with different salt tolerance under salt treatment (0.8% NaCl) for 0, 48, and 72 h. On average, about 243.9 million clean reads, representing 32.4 thousand transcripts and 26.4 thousand unigenes with 829 new genes were detected in each library. Also, over 112,000 single nucleotide polymorphisms were identified, which may supply useful resources for marker development. In total, 5647 differentially expressed genes (DEGs) were identified from all of the comparisons. Functional annotation analysis indicated that expression of genes in transcriptional regulation, signal transduction, and secondary metabolism changed significantly between the two varieties under salt stress, and hundreds of genes involved in the salt stress response were differentially expressed, especially genes encoding receptors like kinases and transcription factors. Besides, qRT-PCR analysis of expression profiles of the selected DEGs was in keeping with the results from RNA-seq analysis. Based on the findings, we proposed several candidate genes that might be used to improve salt tolerance in sorghum. The transcriptional profiles presented here provide further understanding of the salt-tolerance mechanism in sorghum.

Keywords Sorghum · Salt stress · RNA-seq · Time points · Gene expression

Introduction

Salinity is a major environmental factor limiting plant growth and productivity. High salinity leads to ionic stress and water scarcity, which damages cellular ion homeostasis, membrane stability, enzyme activity, as well as biological processes including photosynthesis and respiration (Hasegawa and others 2000; Zhu 2001). A series of developmental processes such as seed germination, and vegetative and reproductive growth are adversely affected under high salt conditions, resulting in the decline of crop yield and quality (Monreal and others 2013). Therefore, it is very important to elucidate salt response mechanisms and discover key genes involved in the salt stress response to achieve efficient crop production. Many physiological and molecular biological studies have investigated mechanisms of salt tolerance in model plants such as *Arabidopsis thaliana* (Shi and others 2000, 2003; Yokoi and others 2002), *Oryza sativa* (Lin and others 2004; Hu and others 2006), *Medicago truncatula* (Zahaf and others 2012) and so on. Other noteworthy reports have provided further understanding of salt-tolerance mechanisms in recent decades (Munns and Tester 2008; Deinlein and others 2014; Munns and Gilliham 2015). However, many indistinct molecular mechanisms remain to be understood due to the complexity of the plant response to salt stress.

As one of the most important cereal crops, sorghum is one of the few resilient crops with good adaptive ability to changing climatic conditions, especially to increasingly serious phenomena such as salinity, drought, and high temperature (Carpita and McCann 2008). In addition, the genome

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of sorghum is small (~ 730 Mb), thus making it an attractive model system in exploring the molecular mechanisms of adaptation to abiotic stresses (Paterson and others 2009; Calvino and Messing 2012; Mace and others 2013). It is, however, difficult to discover salt-tolerance mechanisms in sorghum because of lack of detailed genetic and sequence information. Fortunately, the development of next generation sequencing (NGS) technologies and the release of genome sequences of several sorghum lines (Paterson and others 2009; Mace and others 2013) provide favorable chances for investigating significant mechanisms in sorghum on the transcriptome level. So far, a series of studies about sorghum have been reported and genes were discovered in response to abiotic stress (Sui and others 2015; Surender Reddy and others 2015; Sayyad-Amin and others 2016). However, the integrated dissection of the molecular mechanism in salt response is still largely unknown.

In this study, to get a comprehensive understanding of genes and gene networks in regulating salt stress, we exploited RNA-seq technology to obtain a whole transcriptome and characterize it by employing the sequenced genomes of sorghum, *Arabidopsis*, and rice. We also explored orthologous genes with differential expression levels between two species at different time points after salt treatment.

Materials and Methods

Plant Culture and RNA Isolation

Seeds of sorghum cultivars, 623B and HN (Henong 16), were disinfected with 70% ethanol, soaked, and pre-germinated for 24 h at room temperature (25 °C ± 1 °C). Then the budding and neat seeds were cultured in sterilized quartz sand in a green house. At the three leaves stage, plants were treated with 0.8% NaCl for 0, 48, and 72 h. The NaCl solution was renewed every 24 h. Then the whole plants were collected and frozen in liquid nitrogen and stored at − 80 °C for mRNA isolation. Each treatment was repeated three times.

Total RNAs were extracted using the Trizol plus kit (ThermoFisher, USA), followed by DNA digestion with DNase I. RNA quality and integrity was measured using Nanodrop, Qubit 2.0 and Aglient 2100. Qualified RNA was used for subsequent RNA library construction.

Preparation of mRNA Library and Sequencing

The mRNA library was constructed and sequenced by the BMK cloud company. In brief, the mRNAs with a polyA tail were firstly collected and fragmented using fragmentation buffer. Then first-strand cDNA was synthesized using reverse transcriptase with hexamer primers. Subsequently, the second-strand cDNA was generated using RnaseH and DNA polymerase I, and double-stranded cDNA was purified by AMPure XP beads, and then end repaired. Adapter ligation was carried out after adding "A" tail, and templates with the desired size range were subjected to PCR. Then the mRNA libraries were enriched by PCR and tested with Qubit 2.0 and Agilent 2100, and finally sequenced using Illumina HiSeq 2500 method.

Sequencing Data Quality Control and Transcriptome Alignment

Raw data quality was controlled with criteria of less than 10% low-quality bases (Phred score < 20), followed by adapter sequences and removing of primers. Then the filtered reads (Clean Reads) were mapped to the reference genome using TopHat2 software.

Annotation and Function Analyses of Genes

To annotate the identified genes, the sequences were analyzed with various nucleotide and protein databases, including the non-redundant protein database (nr), SwissPort, Gene Ontology (GO), and Clusters of Orthologous Groups (COG) with criteria of *E* values less than 10^{-5}. The Blast2GO program was used to classify GO terms, and pathway annotation was carried out by search against the KEGG database.

SNP Identification

SNPs were identified between the two cultivars and reference genome using SAMtools. SNP was screened by four factors, TopHat2 score ≥ 50, interval scale between two single mismatched base ≥ 5 bp, Variant Calling score ≥ 20 , and Sequencing Depth Between 5x and 100x.

Differentially Expressed Gene Identification

For the discovery of DEGs, FPKM (Fragments Per Kilobase of transcript per Million fragments mapped) and DESeq (Anders and Huber 2010) were employed to analyze gene expression levels, and the DEGs were screened with Fold Change ≥ 2 and FDR ≤ 0.01.

Results

Statistics of Transcriptome Sequencing Results

The Illumina platform, powerful in quantifying gene expression, is the most common approach to RNA-seq. To investigate molecular mechanisms of sorghum response to salt

Table 1 Statistics of transcriptome sequencing results

Samples	Read number	Base number	Mapped ratio (%)	Uniq mapped ratio (%)	GC content (%)	Q20 (%)
623B-CK	25,820,196	7,732,584,712	80.95	77.80	52.68	93.25
623B-48h	24,506,336	7,327,641,676	80.26	78.43	53.23	92.89
623B-72h	23,416,958	7,009,928,666	79.69	77.28	53.74	92.89
HN-CK	24,448,628	7,317,313,620	76.75	74.49	53.79	92.86
HN-48h	23,884,302	7,153,888,804	77.42	74.14	53.93	92.87
HN-72h	24,241,842	7,246,772,390	76.43	69.73	53.90	92.97
Average	24,386,377	7,298,021,645	78.58	75.31	53.55	92.96

Table 2 Numbers of transcripts, unigenes, and new genes from transcriptome sequencing results

Samples		623B-CK	623B-48h	623B-72h	HN-CK	HN-48h	HN-72h	Average
Transcript number		32,441	33,121	32,484	32,195	32,567	31,984	32,465
Transcript length range	< 300 bp	105	114	101	119	124	121	
	300–500 bp	374	509	367	388	377	363	
	501–1000 bp	3338	3526	3341	3263	3365	3359	
	1001–2,000 bp	14,094	14,350	14,103	14,006	14,188	13,948	
	> 2000 bp	14,530	14,722	14,572	14,419	14,513	14,293	
Mean length (bp)		2101.4	2095.8	2106.3	2103.1	2097.6	2102.1	
Unigene number		26,576	26,663	26,295	26,217	26,443	26,044	26,373
New gene number		850	890	847	795	807	784	829

stress, 6 cDNA libraries from two cultivars, salt-sensitive HN and relatively salt-tolerant 623B were designed (623B-CK, 623B-48h, and 623B-72h; HN-CK, HN-48h, and HN-72h). 623B-CK, 623B-48h, and 623B-72h represented 623B libraries which were treated with 0.8% NaCl for 0, 48, and 72 h, respectively. HN-CK, HN-48h, and HN-72h represented HN libraries which were treated with 0.8% NaCl for 0, 48, and 72 h respectively. Seedling plants with three leaves were used for mRNA isolation, and mRNAs were isolated and subjected to paired-end sequencing using the Illumina HiSeq^{TM}2500 method in the current study.

After quality control (trimming adapters and filtering out low-quality reads), 23,416,958–25,820,196 clean reads (the percentage of Q20 and GC being 92.86–93.25% and 52.68–53.93%) were obtained, which were blasted to reference genome using software TopHat2 (Kim and others 2013). Most reads (76.43–80.95%) could be mapped to the reference genome, among which 69.73–78.43% were unique mapped ones (Table 1).

On average, 32,465 transcripts with 2100 bp in mean length were obtained in every library (Table 2). Among the transcripts, more than 99.6% of them had lengths over 300 bp and about 88% over 1000 bp. The transcripts were mapped to 26,373 unigenes with 829 new genes on average (Table 2). Functions of the new genes from unannotated genomic regions were further systematically explored by searching other databases. Results showed that 466 new genes could be matched with an annotation in GO (Ashburner and others 2000), 104 in COG (Tatusov and others 2000), 23 in KEGG (Kanehisa and others 2004), 398 in Swiss-Prot/UniProt (Apweiler and others 2004), and 755 in NR (Pruitt and others 2005) (Table 3). On average, the length of the 99.74% of annotated new genes (756) was more than 300 bp, and that of 78.50% annotated new genes (595) was over 1000 bp.

Table 3 Annotation summaries of new genes

Annotation database	Annotated number	300 ≤ length < 1000	Length ≥ 1000
GO	466	83	382
COG	104	17	87
KEGG	23	5	18
Swiss-Prot	398	68	329
nr	755	160	593
All	758	161	595

SNP Identification in Sorghum

SNP is a useful molecular marker in the study of plant genetics and plant breeding. In this study, SAMtools (Li and others 2009) were used to screen SNPs based on the comparison of the mapping results of two sorghum cultivars and reference genome. More than 5000 SNPs in each 623B comparison and about 30,000 SNPs in every HN comparison including homozygous and heterozygous were identified. Among the SNPs, more than 90% were genic and about 60% were transitional. Besides, the heterozygosity of 623B sets was 85.74–88.65%, which is about 5 times higher than that of HN (15.65–17.74%), although the SNP number of 623B was much less than that of HN. Detailed information is listed in Supplemental File 1.

Exploration of DEGs in Response to Salt Stress

According to the design, gene responses to salt could be systematically explored in a time course manner, which is applicable to the two cultivars (623B-48h/623B-CK and 623B-72h/623B-CK; HN-48h/HN-CK and HN-72h/HN-CK) as well as genes involved in salt stress regulation (HN-CK/623B-CK, HN-48h/623B-48h, and HN-72h/623B-72h).

To identify genes with a significant change in expression, the FPKM method (Fragments Per Kilobase per Million reads) was used and differentially expressed genes (DEGs) were detected at a threshold of $|\log_2 \text{ratio}| \geq 1$ with p value ≤ 0.05 and FDR ≤ 0.01.

As listed in Table 4, a total of 5647 differentially expressed genes (DEGs) including 375 new genes were identified from all of the seven comparisons. In detail, 604 up-regulated genes and 431 down-regulated genes were detected in the 623B-48h/623B-CK set, whereas in the 623B-72h/623B-CK set, 711 up-regulated genes and 643 down-regulated genes were found. Meanwhile, 477 up-regulated genes and 418 down-regulated genes were identified in HN-48h/HN-CK, whereas in HN-72h/HN-CK, 853 up-regulated genes and 1240 down-regulated genes were picked.

Venn diagram analysis showed that 278 up-regulated and 175 down-regulated genes overlapped between 623B-48h/623B-CK and 623B-72h/623B-CK, whereas 189 common genes were found to be up-regulated and 133 down-regulated between HN-48h/HN-CK and HN-72h/HN-CK (Fig. 1).

Furthermore, we compared the DEGs between HN and B23B under both normal and salt stress conditions (Table 4). In the absence of salt stress, 995 genes were up-regulated and 1119 genes were down-regulated in HN-CK/623B-CK. In contrast, under salt stress condition, 1035 genes were up-regulated and 1133 down-regulated in HN-48h/623B-48h, whereas 900 were up-regulated and 1378 genes were down-regulated in HN-72h/623B-72h. Venn diagram results showed that 305 common genes were up-regulated and 631 were down-regulated in these three comparisons (Fig. 2).

Functional Classification of Differentially Expressed Genes

To describe functional information of the DEGs, GO, COG, and KEGG enrichment analyses were carried out.

Figure 3 shows GO classification results of the DEGs, which were summarized into three categories: cellular component, molecular function, and biological process. Among these categories, "extracellular part and region," "electron carrier activity," "antioxidant activity," "nutrient reservoir activity," and "cell killing" were significantly enriched in all the four comparisons. However, in 623B-48h/623B-CK and 623B-72h/623B-CK, the percent of DEGs involved in "macromolecular complex," "structural molecule activity," and "protein binding transcription factor activity" significantly decreased, whereas DEGs with "nucleoid" or "extracellular matrix/part" function enriched. In HN-48h/HN-CK and HN-72h/HN-CK, genes with "enzyme regulator activity" and "receptor activity" were also enriched, whereas the percent of DEGs involved in "macromolecular complex" and "structural molecule activity" largely decreased only in HN-48h/HN-CK, and DEGs of "nucleoid" just enriched in HN-72h/HN-CK.

Besides, the category "response to salt stress (GO:0009651)" was also significantly enriched among all the comparisons. One hundred and three and 142

Table 4 DEG numbers of different comparisons under salt treatment

DEGs	All DEGs		Up-regulated	Down-regulated
	Annotated	New discovered		
623B-48h/623B-CK	1035	44	604	431
623B-72h/623B-CK	1354	34	711	643
HN-48h/HN-CK	895	24	477	418
HN-72h/HN-CK	2093	37	853	1240
HN-CK/623B-CK	2114	223	995	1119
HN-48h/623B-48h	2168	219	1035	1133
HN-72h/623B-72h	2278	218	900	1378

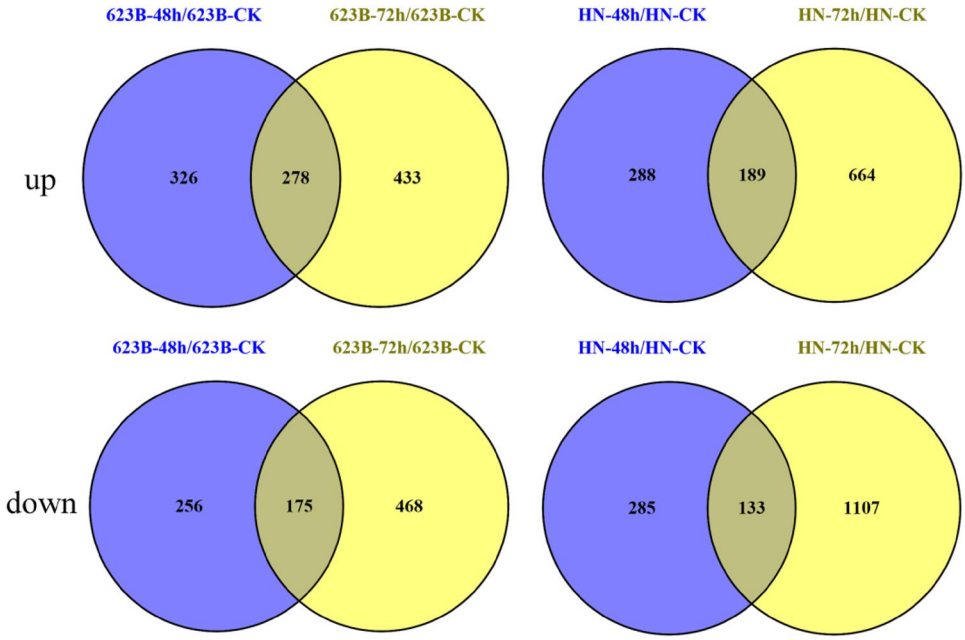

Fig. 1 Venn diagram of the overlapped DEGs in 623B and HN under salt treatment

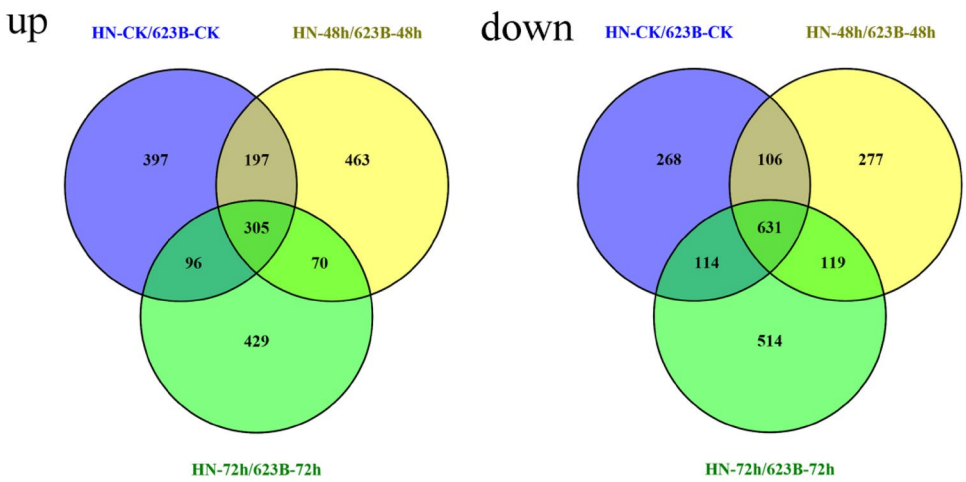

Fig. 2 Venn diagrams of up- and down-regulated genes of different time points between 623B and HN after salt treatment

DEGs with 54 common genes in 623B-48h/623B-CK and 623B-72h/623B-CK, 111 and 215 DEGs with 55 common genes in HN-48h/HN-CK and HN-72h/HN-CK were classified into salt stress response (Fig. 4a, b). In HN-CK/623B-CK, HN-48h/623B-48h, and HN-72h/623B-72h, there were respectively 145, 144, and 159 DEGs which shared 43 genes in the GO term "response to salt stress" (Fig. 4c).

In addition, the DEGs of HN-48h/623B-48h and HN-72h/623B-72h were subjected to the Clusters of Orthologous Groups (COG) classification, 629 and 700 DEGs from which were classified into 22 COG categories, respectively (Fig. 5). Among the COG, the cluster for "general function prediction only" comprises the largest proportion, followed by "secondary metabolites biosynthesis, transport and catabolism," "Signal transduction mechanisms," "transcription," "amino acid transport and metabolism," "carbohydrate transport and metabolism," and "posttranslational modification, protein turnover, chaperones." The categories "cell motility," "extracellular structure," and "nuclear structure" had no corresponding genes.

Furthermore, to identify potential biological pathways represented in the transcriptome, the Kyoto Encyclopedia of Genes and Genomes database (KEGG) was used for further analysis. Results showed that 173 DEGs in HN-72h/623B-72h were assigned to 87 KEGG pathways, and "Flavonoid biosynthesis" [PATHWAY: ko00941], "Biosynthesis of unsaturated fatty acids" [PATHWAY: ko01040], "Photosynthesis" [PATHWAY: ko00195], "Glycolysis/Gluconeogenesis" [PATHWAY: ko00010], and "Nitrogen metabolism" [PATHWAY: ko00910] were significantly enriched (Supplemental File 1). However, in both 623B-72h/623B-CK and HN-72h/HN-CK, KEGGs such as "Protein processing in endoplasmic reticulum," "Plant

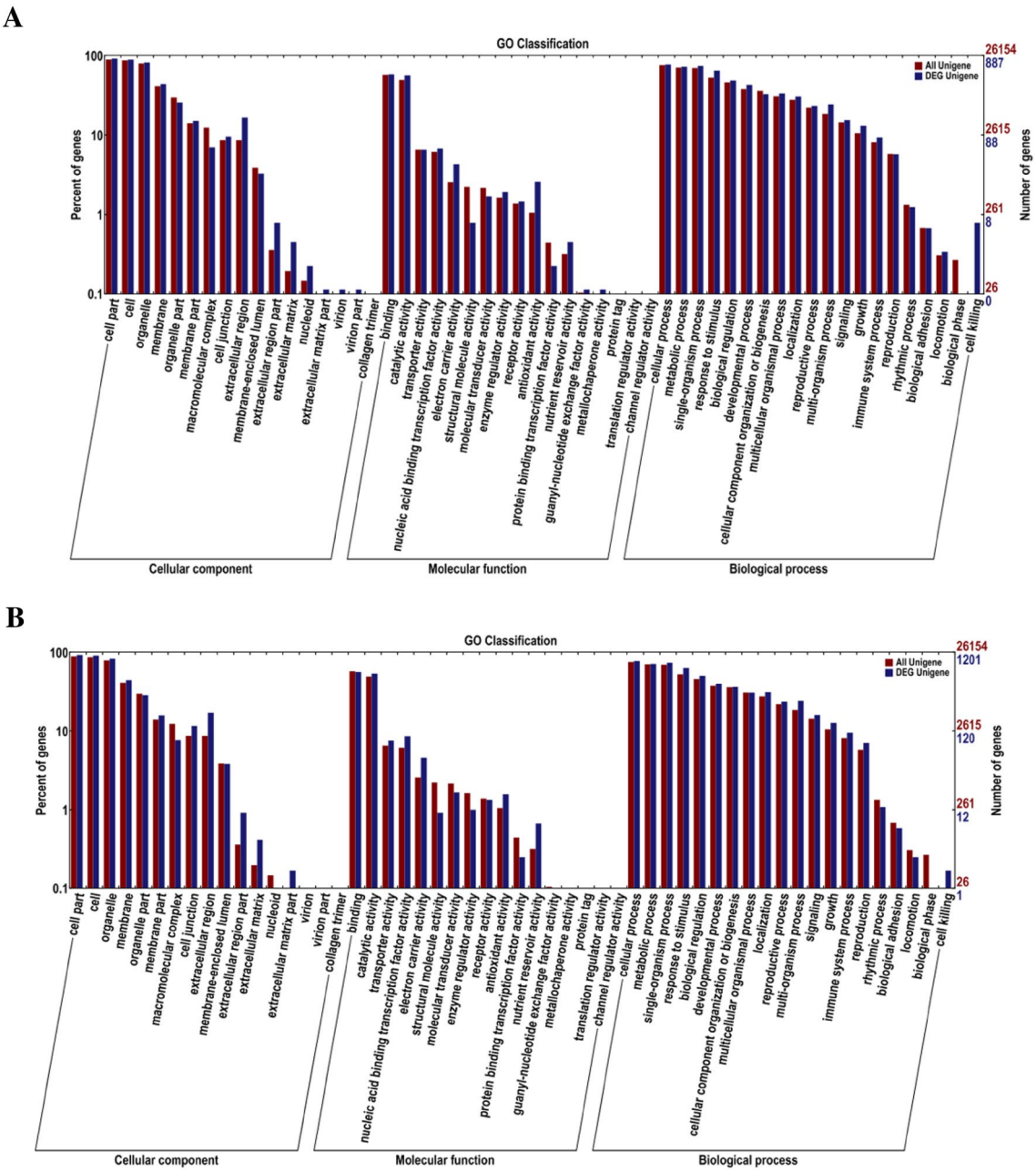

Fig. 3 GO annotation of the DEGs between 623B and HN after salt treatment. **a** and **b** represent GO annotation of the DEGs in 623B-48h/623B-CK and 623B-72h/623B-CK; **c** and **d** represent GO annotation of the DEGs in HN-48h/HN-CK and HN-72h/HN-CK, respectively

hormone signal transduction," and "Starch and sucrose metabolism" were highly enriched (Supplemental File 2 and 3).

Identification of Functional Genes Related to Salt Tolerance

Protein Kinases

Protein kinases play important roles in plant growth and add to tolerance to unfavorable environments (Xiong and others 2002). Among all protein kinases, receptor-like kinases (RLKs) play critical parts in perceiving external signals and activating a series of intracellular reactions under salt and other stress (Lease and others 1998; Ouyang and others 2010; Osakabe and others 2013; Sun and others 2013a, b; Park and others 2014). In our results, 57 and 54 DEGs respectively from 623B-48h/623B-CK and 623B-72h/623B-CK were predicted to encode protein kinases, among which more than 81% were receptor kinases (47 genes in 623B-48h/623B-CK, 82.5%; and 44

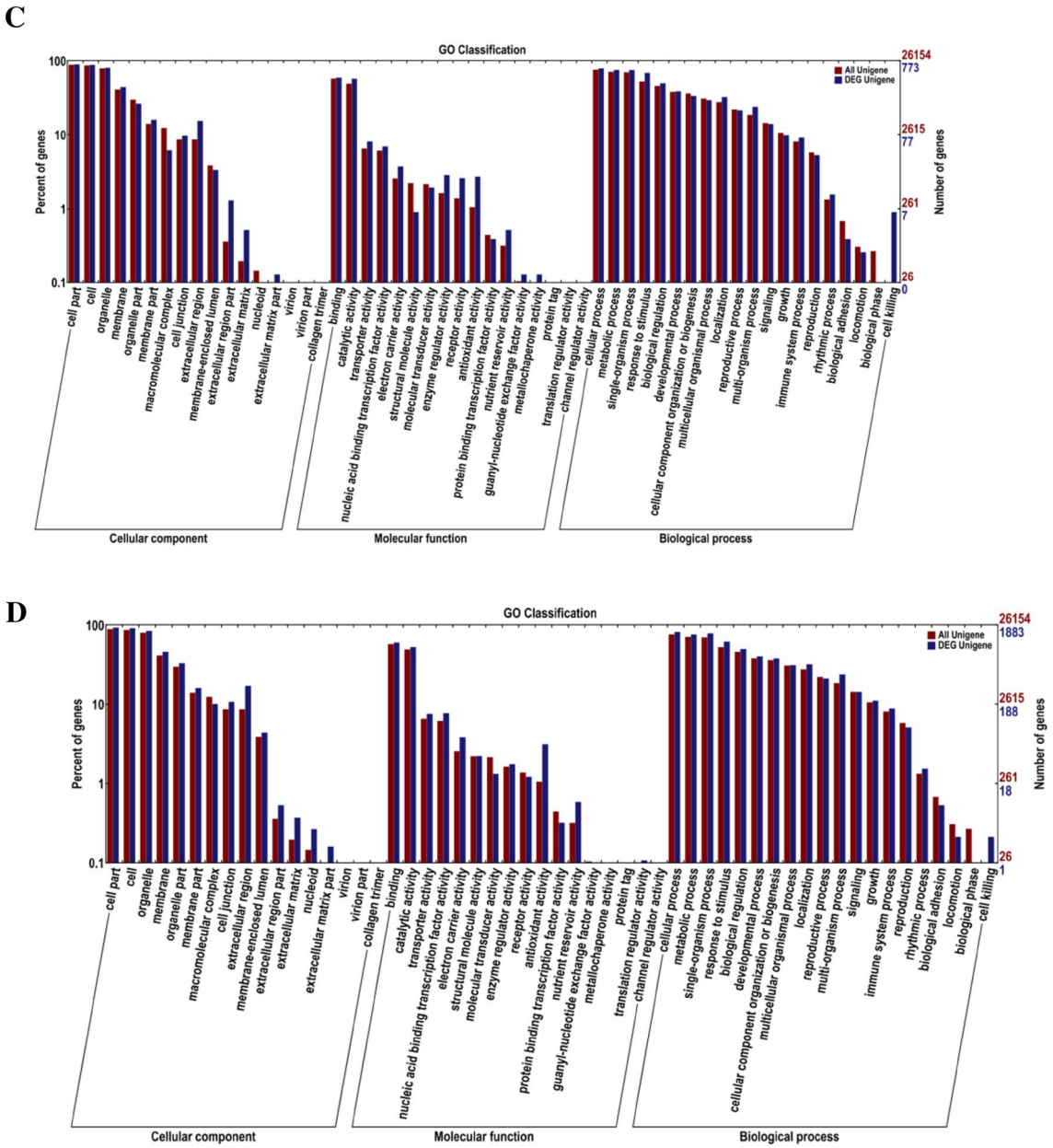

Fig. 3 (continued)

genes in 623B-72h/623B-CK, 81.5%). Besides, there were 19 common protein kinases identified among them, 18 of which were receptor kinases. Meanwhile, there were 40 and 94 protein kinases detected in HN-48h/HN-CK and HN-72h/HN-CK, and more than 80% of them were receptor kinases (34 genes, 85.0% in HN-48h/HN-CK; and 76 genes, 80.9% in HN-72h/HN-CK). In addition, there were 14 receptor kinases among all the 15 common protein kinases between the two comparisons. All of the comparisons shared 3 receptor kinase genes with similar expression levels, two down-regulated genes (*Sobic.002G326400* and *Sobic.006G005500*) and one up-regulated gene *Sobic.008G175000* (Supplemental File 4). Therefore, these genes might play important roles in salt tolerance.

Transcription Factors

Transcription factors (TFs) extensively participated in various biological processes and play significant roles in regulating the expression of specific downstream genes in response to abiotic stress (Golldack and others 2011). In this investigation, classification for differentially expressed transcripts identified 28 transcription factors belonging to 12 families from 623B-48h/623B-CK, and

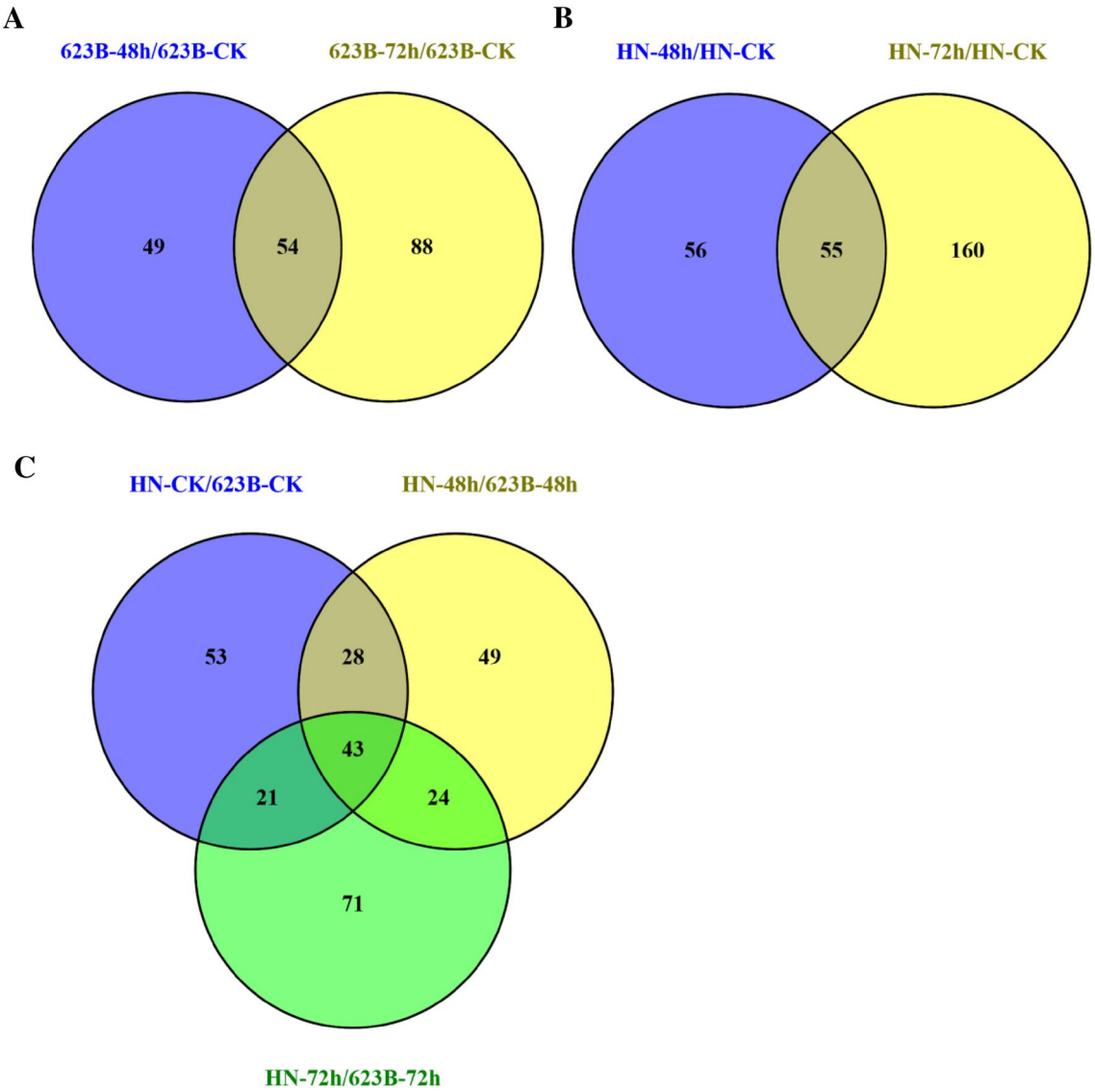

Fig. 4 Venn diagrams of DEGs involved in GO term "response to salt stress" between 623B and HN after salt treatment

ethylene-responsive transcription factors were significantly enriched. In 623B-72h/623B-CK, 67 transcription factors from DEGs were divided into 17 clusters, among which the most distinguished groups are ethylene-responsive transcription factors, MYB and bHLH transcription factors. For all the genes, there were 20 common transcription factors in 623B-48h/623B-CK and 623B-72h/623B-CK. In addition, in HN-48h/HN-CK and HN-72h/HN-CK, there were 32 and 84 differentially expressed transcription factors belonging to more than 7 families, among which ERF, MYB, bHLH, and heat stress transcription factor families were dramatically differentially expressed and they had 16 genes in common (Supplemental File 5). The results indicated that these TFs may elevate salt tolerance in sorghum.

Quantitative Real-Time PCR Validation of DEGs

To validate the transcriptome sequencing results, 9 genes were selected for qRT-PCR analysis, and each gene contained at least one differentially expressed result. Detailed information of these genes and primers are listed in Supplemental File 6 and qRT-PCR results are presented in Fig. 6. In total, 8 of the 9 genes showed different expression patterns between HN and 623B under salt treatment. Expression of *Sobic.001G001200, Sobic.001G044600, Sobic.002G013000*, and *Sobic.003G413600* displayed significant change in HN comparisons, but kept relatively steady in 623B comparisons. Expression of *Sobic.001G066900, Sobic.005G041000*, and *Sobic.001G008600* increased in HN sets but decreased in 623B sets, whereas the expression of *Sobic.002G020000*

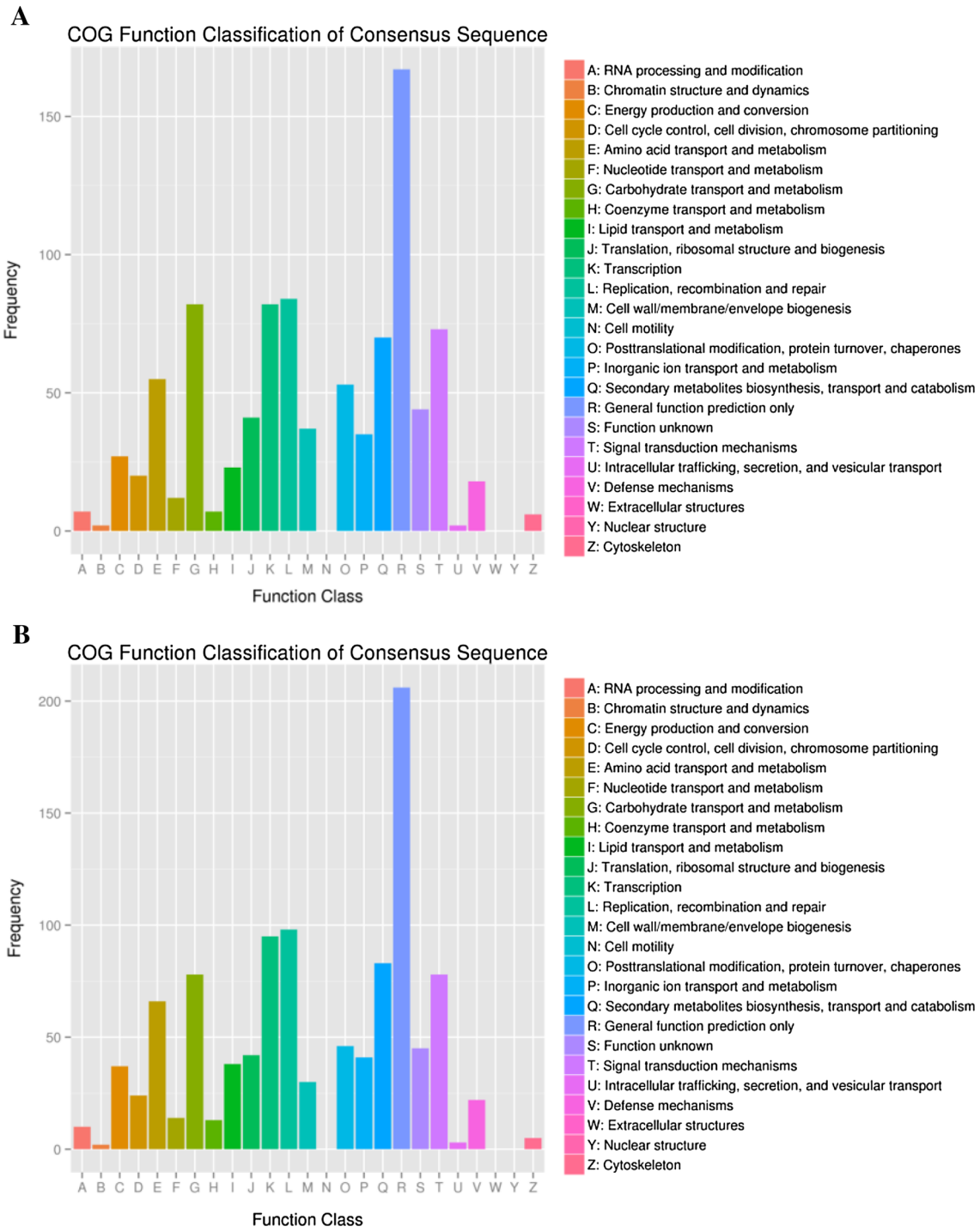

Fig. 5 COG classification results of DEGs between 623B and HN after salt treatment. **a** and **b** represent COG annotation of the DEGs in HN-48h/623B-48h and HN-72h/623B-72h, respectively

displayed opposite results. Only *Sobic.002G012800* showed a similar expression trend between HN and 623B. Overall, 5 and 7 genes showed similar expression patterns in HN and 623B RNA-seq data compared with qRT-PCR results. For example, *Sobic.003G413600*, which may encode an HAK5-like potassium transporter, was down-regulated in HN, but not differentially expressed in 623B, and *Sobic.002G012800*, a NADP-linked oxidoreductase superfamily gene, dramatically increased in both of HN and 623B sets.

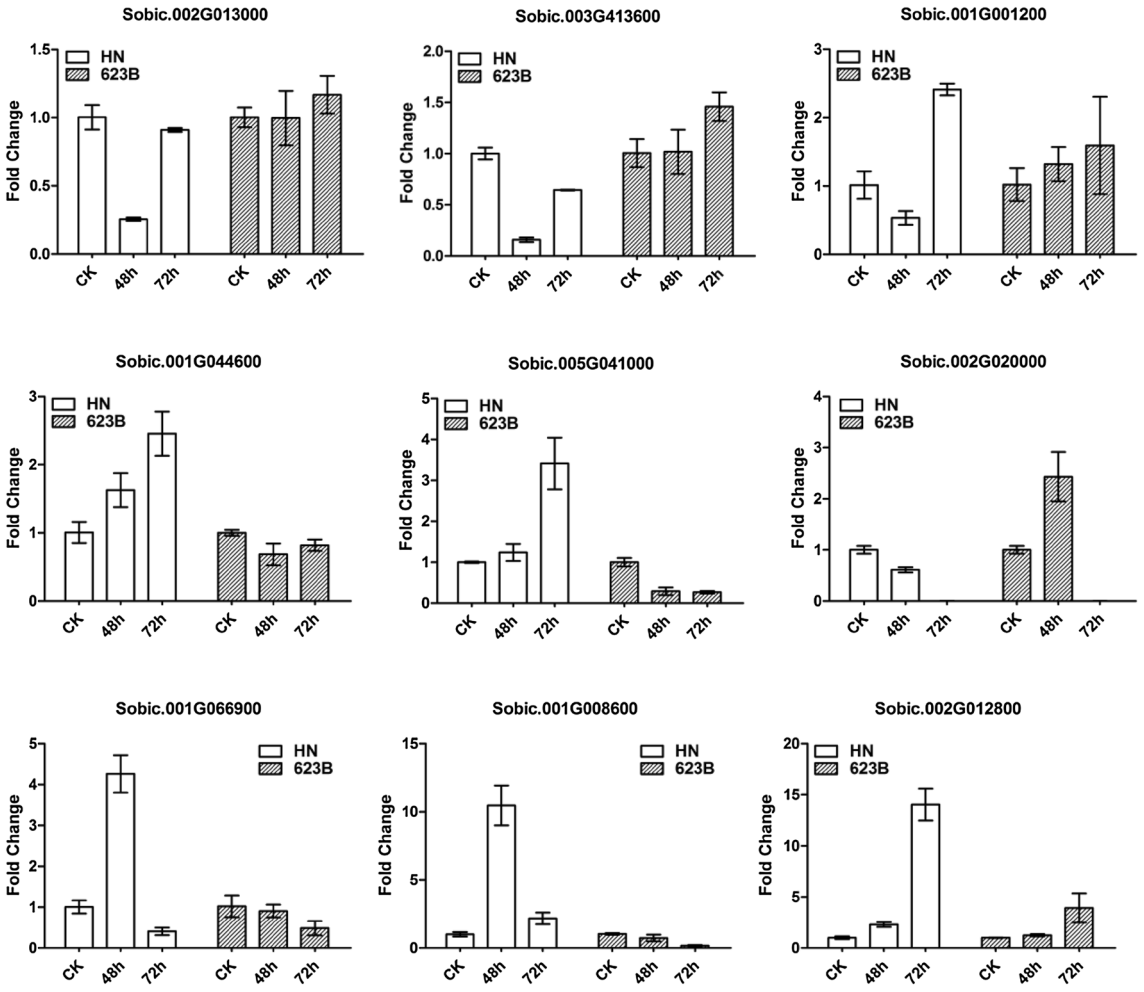

Fig. 6 qRT-PCR results of 9 DEGs in HN and 623B. Error bars represent standard errors from three technical replicates

Discussion

The development of high-throughput sequencing technologies brings great benefits to uncover the molecular basis of stress response, and assists in the understanding of genome-wide expression patterns of genes in sorghum under abiotic stress (Zhang and others 2010; Dugas and others 2011; Gelli and others 2014; Johnson and others 2014; Chopra and others 2015; Yang and others 2015). Expression profiling using RNA-seq is an effective way for discovering genes and pathways associated with the salinity response in sorghum.

Here, we performed transcriptome analysis of two cultivars under salt conditions and compared the expression profiles between control and salt-treated plants. Data of transcriptome sequencing results such as clean reads, mapped reads, as well as transcripts and unigenes were very similar between the two cultivars, which indicated that the sequencing results were credible and suitable for further analysis. In this study, over 110,000 SNPs were identified in total and about 91.8% of them were genic SNPs. Interestingly, the SNP number was 4–5 times more in the salt-sensitive cultivar HN than in the salt-resistant cultivar 623B, whereas the heterozygosity of HN was about 1/5 compared to that of 623B. These results strongly indicated that these SNPs may play important roles in the salt response and could be very valuable markers for salt resistance breeding.

As is well known, a stress signal transduction pathway contains a series of extracellular and intracellular reactions, including outside signal perception, second messenger generation, and protein phosphorylation cascade activation. Then target genes are activated to strengthen the cell resistance or protect cellular activities (Xiong and others 2002). In this study, KEGG pathways such as signal transduction, protein process, and carbohydrate metabolism were highly enriched in both of the two cultivars under salt treatment, which may reflect their similar response to stress. Notably, genes involved in resistance metabolism, photosynthesis, and nutrition processes as well as many newly discovered genes showed significantly different expression profiles between HN and 623B, and genes with "enzyme regulator

activity" and "receptor activity" were enriched in HN rather than 623B. These results may contribute to explaining the reasons why HN is more tolerant than 623B under salt treatment, and exploring the mechanisms of salt resistance.

Previous studies have indicated that SOS1, NHX1, and HKT1 are key Na^+ transporters involved in plant salt tolerance (Apse and others 1999; Shi and others 2000; Platten and others 2006). In our RNA-seq data, the expression of NHX-like and CHX-like genes remained relatively steady after salt treatment, whereas 2 of the 3 HKT1-like genes displayed dramatically higher expression levels in HN than those in 623B (Supplemental File 7). A recent study in *Puccinellia tenuiflora* showed that, under salt stress, expression of *PtSOS1, PtHKT1;5* and *PtNHX1* were significantly increased within 6 h, and then decreased after 24 h (Zhang and others 2017). These results indicated that such genes may act as instantaneous factors in salt response.

At present, some RLKs have been reported to regulate the salt stress response, such as OsSIK1 and OsGIRL1 in rice, as well as GsSRK and GsRLCK in *Glycine soja* (Ouyang and others 2010; Sun and others 2013a, b; Park and others 2014). Transgenic plants with overexpression of OsSIK1, GsSRK, or GsRLCK showed higher tolerance to salt and drought stresses than control plants, whereas *Arabidopsis* plants with overexpressed OsGIRL1 displayed a hypersensitive response to salt stress. In our data, expression of the OsSIK1 ortholog gene *Sobic.001G085700* showed no difference in any of the comparisons. However, *Sobic.004G091300*, an ortholog gene of OsGIRL1, was significantly down-regulated in HN-48h/HN-CK and HN-72h/HN-CK ($|\log_2 FC| = -1.85$ and -3.03). This result was in line with the manifestation of OsGIRL1 and indicated that *Sobic.004G091300* could be a marker gene in salt resistance.

In plants, salt stress signaling always transduces via abscisic acid-dependent and acid-independent pathways, as well as several other factors. *HAB1* and *RD29B* have been reported to function in response to abiotic stress (Dugas and others 2011; Gelli and others 2014). In our results, expressions of their ortholog genes (*Sobic.003G242200* and *Sobic.001G200700*) were dramatically up-regulated after salt treatment, and expression change of the *RD29B*-like gene (*Sobic.001G200700*) even reached more than 24 fold. Moreover, transcription factors including MYB, NAC, and ERF were highly abundant in our results. These transcription factors are relatively widespread, and have significant functions in the abiotic stress response in *Arabidopsis* and rice (Hu and others 2006; Dai and others 2007; Abogadallah and others 2011; Hao and others 2011; Yang and others 2012; Licausi and others 2013; Rong and others 2014). Thus, these transcription factors discovered in this study may be ideal targets for salt-resistant breeding in plants. In addition, RNA-seq data and qRT-PCR results showed that expression of some genes in 623B contrasted with those in HN, demonstrating the different performances between the two cultivars.

In summary, we conducted a comprehensive analysis of sorghum under salt stress using RNA-seq. Hundreds of SNPs and DEGs were identified, and dozens of DEGs related to salt tolerance were discovered. The common DEGs, GO terms, COG classifications, and KEGG pathways demonstrated that both 623B and HN shared similar strategies under salt treatment, whereas the distinct expression pattern and classification of genes indicated the difference between the two cultivars in response to salt stress, which may result in salt sensitivity of 623B and salt tolerance of HN. This transcriptome study develops the molecular basis for understanding the specific salt-tolerance mechanisms in sorghum, and will also provide valuable genetic resources for salt-tolerance breeding in other species.

Availability of Supporting Data

The raw data files for this RNA-seq during our experiment are deposited in NCBI (BioProject: PRJNA395348).

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Author Contributions Conceived and designed the experiments: JC, JC. Performed the experiments: JC, GR, HQ, XX, JC. Analyzed the data: JC, GR, LH, and JC. Contributed reagents/materials/analysis tools: GR, HQ, and XX.

Compliance with Ethical Standards

Conflict of interest The authors declared that they had no conflicting interest.

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