Chrysanthemum × Grandiflora Leaf and Root Transcript Profiling in Response to Salinity Stress

He Liu
Northeast Forestry University
Yu Liu
Northeast Forestry University
Ning Xu
Northeast Forestry University
Ying Sun
Northeast Forestry University
Qiang Li
Northeast Forestry University
Liran Yue
Northeast Forestry University
Yunwei Zhou (yunweizh@jlau.edu.cn)
Jilin Agricultural University
Miao He
Northeast Forestry University

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Abstract

As high soil salinity threatens the growth and development of plants, understanding the mechanism of plants' salt tolerance is critical. As a newly developed species with a strong salt resistance, the *chrysanthemum × grandiflora* possesses multiple genes that control its quantitative salt resistance. Because of this multigene control, we chose to investigate the plant's stress genes' overall responses at the transcriptome level. *C. Grandiflora* were treated with a 200 mM/L NaCl solution for 12 h, and the effect of salt stress on their roots and leaves was analyzed via Illumina RNA sequencing. Most DEGs were enriched in GO annotations of 'biological process,' 'nucleus,' and 'protein binding'. There are many genes in plant hormone signal transduction and phenylpropane biology pathways, and we observed that most of the genes had the same expression patterns in the roots and leaves. Among them, *PAL*, *CYP73A*, and *4CL* in the phenylpropane biosynthetic pathway were upregulated in roots and leaves. In the salicylic acid signal transduction pathway, *TGA7* was upregulated in the roots and leaves, and in the jasmonic acid signal transduction pathway, *TIFY9* was upregulated in the roots and leaves. In the ion transporter gene, we identified that *HKT1* showed identical expression patterns in the roots and leaves. In order to verify the accuracy of the Illumina sequencing data, we selected 16 DEGs for transcription polymerase chain reaction (qRT-PCR) analysis. qRT-PCR and transcriptome sequencing analysis revealed that the transcriptome sequencing results were reliable.

Introduction

Soil salinity is a serious global threat to the environment and to agricultural production. Currently, more than 20% of the world's arable land (1 billion hectares) is affected by salinity, and this number is increasing due to global climate change and poor irrigation and fertilization management. Generally, when the salt content of the soil exceeds 1%, it becomes difficult for plants to grow. Salt stress affects the various stages of plants' seed germination, growth, differentiation, and development. The study of plants' salt tolerance and the development of salt-tolerant plant varieties are critical for environmentally sustainable development (Tattini et al. 2016; Monika et al. 2020). At present, salt tolerance and the potential mechanisms of salt tolerance of a large number of plant varieties have been studied, such as *Arabidopsis* (Lescano et al. 2015; Irani et al. 2016), tobacco (Meng et al. 2020), corn (Dyoni et al. 2020) and tomatoes (Lovelli et al. 2014). Research on soybeans (Silva et al. 2020) and rice (Serrão et al. 2020) have shown that the method of increasing the plants' expression of intrinsic salt tolerance genes can effectively enhance their salt tolerance.

Salt stress has a significant impact on plants' growth and development. First, salt stress reduces plants' water absorption capacity and inhibits their growth. This is called osmotic stress. Osmotic stress is the first type of stress that plants are introduced to upon exposure to salty soil, and it has a direct effect on their growth. If excessive salt enters the transpiration stream of plants, it damages cells in the leaves, thus further affecting plants' growth. This type of stress upon the leaves' cells is called the excess ion effect. Second, when the salt level reaches a threshold value, ion toxicity occurs. If the threshold is exceeded, the plant cannot maintain an ion balance, which can also cause secondary reactions such as

oxidative stress (Hussain et al. 2021). Studies have shown that under salt stress conditions, more transcriptome changes were observed in the root tissue than in the leaf tissue of the chrysanthemum cultivar ‘Jinba’. Its response to stress was mainly manifested in the downregulation of genes in its leaves and roots (Cheng et al. 2018). Under drought conditions and salt stress, transcriptome changes were observed more in the leaf tissue compared with the root tissue of *Chrysopogon zizanioides*. The response to stress in the transcriptome of *C. zizanioides* was mainly manifested in the upregulation of genes in the leaves and roots (Suja et al. 2017). Finally, when comparing root with leaf tissues in *Rosa chinensis* under drought conditions, the transcriptome underwent more changes in the leaf tissues, with a downregulation of genes in the leaves and roots (Ze et al. 2019).

High salinity leads to high osmotic stress, oxidative stress, ion imbalance, Na^+ toxicity, and even death in plants. In order to respond to salt stress conditions, plants have developed a series of morphological, physiological, biochemical, and molecular regulation mechanisms to ensure normal growth and development. These mechanisms include osmotic regulation, ion homeostasis, signal transduction, and the induction of antioxidant enzyme activity. Salt tolerance is a complex trait controlled by genetic factors. The functions of many salt-responsive genes involve the regulation of ion accumulation and rejection, stress signal transduction, transcription regulation, redox reactions, and the accumulation of specific osmotic regulation substances (Saisho et al. 2016). Transgenic plants with different degrees of enhanced salt tolerance can be obtained by controlling the expression of some salt tolerance genes. These genes include HKT (high affinity potassium transporter) (Shohan et al. 2019), AKT (K^+ channel gene) (Xu et al. 2014), NHX (Na^+/ H^+ antiporter gene) (Dong et al. 2021), WRKY transcription factor gene (Liu et al. 2021), NAC transcription factor gene (Wang et al. 2021), bZIP transcription factor gene (Vaishali et al. 2021), and ERF transcription factor gene (WU et al. 2021).

The *C. Grandiflora* is a new ground-planted chrysanthemum species selected after natural hybridization and satellite loading based on the introduction of ground cover chrysanthemums (*chrysanthemum morifolium*). Because of its long flowering period, its copious dense flowers, its bright color, and its high resistance to stress, this new species has become a popular urban green ground cover plant in recent years (Liu et al. 2019). Transcriptome sequencing was used to find *C. Grandiflora* genes that were resistant to abiotic stress, thus providing a physical foundation to improve plants’ stress resistance and to provide new stress-resistant materials, both of which would have a great significance in the study of chrysanthemum germplasm resources. Current studies have shown that when the chrysanthemum is exposed to salt stress, genes encoding proteins related to osmotic regulation, ion transport (Na^+, K^+, and Ca^{2+} transport), ROS clearance, and ABA signaling are all affected in the roots and leaves. Osmotic regulation genes and Ca^{2+} transport genes overlap in the roots and leaves after salt treatment, which may serve as the main regulator of the plant’s salt response. These results indicate that the regulation of the transcriptome plays a key role in the morphological and physiological adaptation of chrysanthemum roots and leaves in response to salt stress (Cheng et al. 2018). In our research, we discovered the key metabolic pathways, a large number of salt-tolerant genes, and the underlying mechanisms of adaptation of *C. Grandiflora* in response to salt stress.

Materials And Methods

Plant materials and experimental design

Chrysanthemum×grandiflora is a new chrysanthemum variety cultivated in Northeast Forestry University. It is a new ground-grown chrysanthemum variety group based on the introduction of *Chrysanthemum morifolium*, after natural hybridization and satellite loading. The stem explants collected from the mother plant were rinsed in tap water for 60 min. The stems were sterilized with 75% ethanol solution for 30 s, rinsed with sterile distilled water for 1 min, and soaked in a 4% sodium hypochlorite solution for 10 min. The stems were then rinsed with sterile, distilled water 5 times, placed on sterile filter paper to absorb the water, and cut open into sections about 2.5 cm long. One or more of the shoots were inoculated in MS medium. When the plant had grown 7–8 cotyledons, the seedlings were transferred to nutrient soil (vermiculite : peat soil = 1:1).

In our previous study, it was found that when *C. Grandiflora* was treated with 200mMol/L Nacl solution concentration, the plants showed a relatively obvious stress response, so we chose this concentration to treat the material. When the plants had grown 9–10 leaves, they were treated with 200 mM NaCl. In the morning of the same day that the plants were treated with 200 mM NaCl, each plant was irrigated with a 60 mL NaCl solution. After 12 h of NaCl treatment, the roots were taken, and the leaf samples were subjected to transcriptome sequencing. Root and leaf samples were collected at 0, 0.5, 3, 6, and 9 d after plant cultivation to determine the leaves' and roots' water, Na^+, K^+, and H_2O_2 content and SOD, POD, and CAT activity.

The plants' growth media contained 30 g/L sucrose and 0.6% (w/v) agar and was adjusted to pH 5.8–6.0 and autoclaved at 121°C for 20 min. The plants were grown in plant culture pots (11 cm high ´ 7 cm diameter, 300 Ml) and placed in an air-conditioned incubator with a temperature of 25°C ± 2°C, a relative humidity of 50%–70% and a G13 fluorescent lamp (Philips, Tianjin, China) light source.

Determination of antioxidant enzyme activity and H_2O_2 content

From the roots and leaves, 0.1 g fresh weight were taken, and the antioxidant enzyme activity was measured on 0, 0.5, 3, 6 and 9 d. The activities of SOD (U/gFW), POD (U/gFW), and CAT (nmol/min/gFW), and the content of H_2O_2 (μmol/gFW) were determined according to the instructions included in the chemical analysis kit (Solebao Technology Co., Ltd., Beijing, China). After a one-way analysis of variance, a Duncan multiple range test in SPSS24 was used to evaluate the mean value of the treatment.

Determination of Na^+ and K^+ content

The collected root and leaf samples were dried at 80°C for 2 d. The samples were then ground into powder, and 1 mMol/L HCl was added to the samples to react for 12 h. The volume of the filtered solution was adjusted to 50 ml, and the content of Na^+ and K^+ was measured using 4210MP-AES (Agilent Technology) (Takahashi et al. 2007).

Transcriptome sequence annotation

Based on the original data, we calculated the proportion of unknown nucleotides, and the base (Q20) base recognition accuracy exceeded 99.0%. In order to annotate the reassembled sequence, after cleaning the original reader and processing the poor quality reader, the assembled transcript was aligned with NCBI (https://www.ncbi.nlm.nih.gov/; June 2021, 1st visit).

Gene expression level analysis

FPKM was used to calculate gene expression levels through density distribution and analysis of the number of selected transcripts, and the expression of all the samples was analyzed. According to the results of FPKM, the correlation between the samples was calculated to determine the stability and reliability of the experimental operation.

Identification and functional annotation of DEGs

The DEGs were mapped to each item in the GO database. The hypergeometric test method was used to detect significantly rich GO annotations. The corrected *P* value was <0.05 [50]. In addition, the hypergeometric test calculated the number of DEGs at different levels to determine the main pathways involved in salt tolerance. When *P* <0.05, the KEGG pathway was significantly enriched (Eden et al. 2009; Mao et al. 2005) .

qRT-PCR analysis

For qRT-PCR analysis in the KEGG enrichment pathway, 16 candidate genes, including 8 upregulated genes and 8 downregulated genes, were randomly selected. The Roche LightCycler96® system was used for qRT-PCR. The differential expression analysis of each candidate gene used the $2^{-\triangle\triangle CT}$ method, and each candidate gene was repeated three times (Livak et al. 2001).

Results

Effects of salt stress on the contents of Na^+ and K^+ in the roots and leaves of *C. Grandiflora* and the activities of antioxidant-related enzymes

After treatment with a 200 mM/L NaCl solution, the growth of *C. Grandiflora* in the control group was significantly better than the growth of those in the treatment group (Figure 1a). As the treatment time increased, the water content of the leaves gradually decreased (Table 1). At the same time, we found that the activity of superoxide dismutase (SOD) in the roots and leaves increased initially and then decreased. The SOD's activity of the roots on the sixth day of treatment was significantly higher than the activity at the other time points, and the activity of the leaves on the sixth day of treatment was significantly higher than the activity at the other time points. Point peroxidase (POD) activity in the roots and leaves (Figure 1b) showed a trend of an initial increase followed by a subsequent decrease. The POD's activity of the roots was significantly higher at Day 6 of treatment than at the other time points, and the activity of the

leaves was significantly higher than at the other time points at Day 3 of treatment (Figure 1c). Catalase (CAT) activity in the roots and leaves showed a trend of an initial increase followed by a subsequent decrease similar to that of POD. CAT's activity in the roots at Day 3 and in the leaves at Day 6 were significantly higher than at the other time points in the treatment (Figure 1d). The H_2O_2 content in both the roots and the leaves increased over the course of treatment with the most significant content change occurring after Day 3 in the roots and after Day 9 in the leaves (Figure 1e). The Na^+ content in both the leaves and the roots increased as well with the greatest Na^+ change occurring in the roots and the leaves at Day 9 of treatment (Figure 1f). The K^+ content showed a downward trend in both the roots and the leaves with the most significant change in K^+ concentration occurring after Day 3 for both the leaves and the roots (Figure 1g). We witnessed that, under salt stress, antioxidant enzyme activity and ion accumulation in the roots and leaves of *C. Grandiflora* began to change after 12 h of treatment. This indicated that after 12 h of treatment with 200 Mm/L NaCl, the expression of salt stress-responsive genes were upregulated. Therefore, we used RNA-seq to analyze the root and leaf samples of *C. Grandiflora* during the 12 h salt stress treatment.

Table 1 Changes in water content of *C. Grandiflora* leaves after 9 d of salt stress. The data in the table are the mean ± standard error.

Salt stress days（d）	Control leaf water content（%）	Treatment group leaf water content（%）
0	93.73 ± 0.33	93.71 ± 0.35
0.5	93.65 ± 0.37	93.42 ± 0.43
3	93.51 ± 0.41	92.76 ± 0.45
6	92.47 ± 0.32	90.47 ± 0.36
9	92.28 ± 0.44	87.58 ± 0.38

Assembly and transcriptome quality assessment

Twelve samples were sequenced on the Illumina sequencing platform. Among them, there were 558,054,470 original fragments. By removing low-quality areas and adapters, 543,328,476 clean readings remained, Q20 > 97.05% (Table 2). Figure 2 shows the transcriptome's quality and single gene length distribution.

Table 2 Statistics of output sequence of *C. Grandiflora*

Samples	Raw_Reads (No.)	Valid_reads (No.)	Raw_Bases (bp)	Valid_Bases (bp)	Q20 (%)	Q30 (%)
SCK_L1	43,153,100	42,326,634	6.47G	5.90G	97.84	93.34
SCK_L2	46,156,038	44,286,876	6.92G	6.13G	97.34	92.45
SCK_L3	41,415,656	39,998,304	6.21G	5.54G	97.43	92.68
S200_L1	49,751,488	49,026,640	7.46G	6.80G	97.41	92.24
S200_L2	41,337,408	39,900,828	6.20G	5.53G	97.49	92.68
S200_L3	43,824,844	42,081,102	6.57G	5.82G	97.31	92.46
SCK_R1	47,160,892	45,560780	7.07G	6.31G	97.35	92.38
SCK_R2	49,676,308	47,754,498	7.45G	6.60G	97.05	91.93
SCK_R3	50,919,822	50,130,238	7.64G	6.95G	97.64	93.02
S200_R1	50,235,962	49,426,242	7.54G	6.83G	97.46	92.76
S200_R2	46,372,458	45,630,372	6.96G	6.30G	97.24	92.39
S200_R3	48,050,494	47,205,962	7.21G	6.50G	97.18	92.38

Transcriptome annotation

Using BLAST to screen data with an *E* value of <1e-5, there were 31,181 (79.57%) single genes matching the known genes in the NR database, and 21,333 (54.44%) single genes matching the Swiss-Prot database (Table 3). The sequence with the highest annotation rate in the NR database (*E* value <1e-45) was 32.70% (Figure 3a). Approximately 70.54% of the single genes were similar to the identified sequence (Figure 3b). Among the following 6 species, about 94.30% of the single gene annotations matched *C. Grandiflora*, including mugwort (79.55%), sunflower (4.97%), thistle (4.75%), lettuce (3.46%), chrysanthemum (1.08%), and grapes (0.42%) (Figure 3c).

Table 3 Annotation results of *C. Grandiflora* in the database

Annotation databases	Number of genes	Percentage (%)
NR	31181	79.57
GO	25624	65.39
KEGG	20601	52.57
eggNOG	27484	70.14
Swiss-prot	21333	54.44
All	39185	100.00

Unigenes' EggNOG functional categories annotation

Comparing the single genes with the eggNOG database, we found that 28,580 single genes in *C. Grandiflora* could be classified into 24 categories according to the prediction function (Figure 4). ‘Unknown function’ is the most annotated category among these eggNOG categories, followed by ‘post-translational modification,’ ‘protein renewal,’ ‘chaperone protein,’ and "nuclear structure" in that order.

Gene expression and differentially expressed gene analysis

According to the hierarchical clustering analysis of the differentially expressed gene expression pattern, we found that there were more downregulated expressed genes in the samples of *C. Grandiflora* (Figure 5). Comparing the treatment group with the control group, we found that there were more DEGs in the leaves (Figure 6a), and the ratio of DEGs in the NR database was 79.57%. A total of 3094 DEGs were obtained from the roots treated for 12 h, and 7880 DEGs were obtained from the leaves treated for 12 h (Figure 6b). In the roots, there were 1297 upregulated genes. Comparatively, in the leaves, there were 3125 upregulated genes.

DEGs GO annotation enrichment analysis

There were a total of 135,710 genes annotated in the GO database in the *C. Grandiflora* library undergoing salt stress for 12 h. Among them, there were 49,890 single genes for biological processes; 45,951 single genes for cell components; and 39,869 single genes for molecular functions, including nuclear, cytoplasmic, and cytoplasmic genes. The number of DEGs enriched in membranes, membrane components, and the cytosol was the largest (Figure 7a). A total of 13,245 genes in the root library were annotated in the GO database. Within these genes, there were 5116 single genes for biological processes; 4159 single genes for cell components; and 3970 single genes for molecular functions, including nuclear, plasma membrane, cytoplasm, and membrane components. The number of DEGs enriched in protein binding was the largest (Figure 7b). There were 33,729 genes in Ye’s library annotated in the GO database. Among them, there were 12,524 single genes for biological processes; 11,271 single genes for cell components; and 9934 single genes for molecular functions, including nuclear, plasma membrane, cytoplasm, and membrane components. The number of DEGs enriched in chloroplasts was the largest (Figure 7c).

Enrichment analysis of the KEGG pathway of DEGs

There were a total of 14,059 genes annotated in 140 KEGG pathways in the library of *C. Grandiflora* undergoing salt stress for 12 h. Among these genes, differences could be seen in ribosomes, protein processing in the endoplasmic reticulum, plant hormone signal transduction, spliceosomes, and plant pathogen interaction pathways. The number of expressed genes was the most enriched (Figure 8a). A total of 1417 genes in the root library were annotated in 120 KEGG pathways. Of these genes, phenylpropane biosynthesis, protein processing in the endoplasmic reticulum, starch and sucrose metabolism, plant hormone signal transduction, and galactose metabolism pathways were abundant in

DEGs. The number of sets was the largest (Figure 8b). There were a total of 3480 genes in Ye's library annotated in 132 KEGG pathways, among which are DEGs in plant pathogen interactions, plant hormone signal transduction, protein processing in the endoplasmic reticulum, starch and sucrose metabolism, and phenylpropane biosynthesis pathways. The number of enrichments was the largest (Figure 8c).

Analysis of DEGs of plant salt stress-related transporters

Salt stress usually induces ion stress in plants, and it is critical for plants to maintain a balance of Na^+ and K^+ in their cells under conditions of salt stress. Ion balance is a way for plant cells and tissues to maintain homeostasis under certain external environmental stimuli. The accumulation of Na^+ and K^+ in plants is a key factor affecting salt tolerance, and salt stress-related transporters are closely related to plants' salt tolerance. In this study, the root transport genes *HKT1*, *AKT1*, *AKT2*, *NHX2*, *NHX3*, *NHX5*, and *CLC-A* were all upregulated, and *CLC-B* genes were downregulated. In the leaves, the salt stress transporter genes *HKT1*, *CHX17*, *CHX18*, *AKT2*, and *NHX3* were upregulated, and *AKT1*, *CLC-C*, and *CLC-D* were downregulated. We found that more ion transporter genes were upregulated in the roots, which mainly affected the response of *C. Grandiflora*' roots to salt stress (Table 4).

Table 4 Differential genes related to salt stress-related transporters

Tissue site	Gene_ID	Name	log2FC	regulation
root	TRINITY_DN116960_c1_g1	*HKT1*	0.97	up
	TRINITY_DN119869_c1_g2	*AKT1*	1.05	up
	TRINITY_DN110456_c0_g2	*AKT2*	1.86	up
	TRINITY_DN109744_c0_g1	*NHX2*	0.56	up
	TRINITY_DN90305_c0_g1	*NHX3*	0.50	up
	TRINITY_DN121777_c2_g1	*NHX5*	0.45	up
	TRINITY_DN109895_c0_g3	*CLC-A*	0.78	up
	TRINITY_DN109895_c0_g1	*CLC-B*	-0.63	down
leaf	TRINITY_DN101309_c3_g1	*HKT1*	3.39	up
	TRINITY_DN124753_c1_g3	*CHX17*	3.82	up
	TRINITY_DN124753_c1_g2	*CHX18*	4.38	up
	TRINITY_DN110456_c0_g2	*AKT2*	3.69	up
	TRINITY_DN90305_c0_g1	*NHX3*	2.10	up
	TRINITY_DN124299_c2_g4	*AKT1*	-1.48	down
	TRINITY_DN115730_c1_g1	*CLC-C*	-1.44	down
	TRINITY_DN108068_c1_g3	*CLC-D*	-1.26	down

Analysis of DEGs in the phenylpropane biosynthetic pathway

Phenylpropane compounds play an important role in plants' growth, development, and response to adversity stress. The phenylpropane biosynthesis pathway is important for plants' secondary biomass metabolism. All substances containing the phenylpropane skeleton are the direct or indirect products of this pathway, and these compounds play a critical role in the growth, development, and resistance of plants. Phenylpropane biosynthesis also has an important physiological significance in plants, which is mainly manifested by changes in enzyme activity and the differentiation of intermediate products, further transformation products, and cells in plants' development. In this study, there were 268 DEGs annotated in the phenylpropane biosynthetic pathway in the *C. Grandiflora* samples. In the phenylpropane synthesis pathway in the roots, all 17 of the DEGs were upregulated, including *PAL* (10), *CYP73A* (5), and *4CL* (2). On the other hand, in the phenylpropane synthesis pathway in the leaves, there were 25 DEGs, of which 24 were upregulated, including *PAL* (16), *CYP73A* (5), and *4CL* (3). We found that there were more upregulated genes in the leaves, indicating that under conditions of salt stress, phenylpropane biosynthesis in *C. Grandiflora* leaves was more active, thereby providing leaves with more salt stress resistance (Table 5, Figure 9).

Table 5 Differentially expressed genes in the phenylpropane biosynthetic pathway

Tissue site	Gene_ID	KOEntry	Name	log2FC	regulation
root	TRINITY_DN110915_c2_g1	K10775	PAL	1.69	up
	TRINITY_DN95790_c1_g4	K10775	PAL1	1.65	up
	TRINITY_DN123821_c2_g5	K10775	PAL	1.49	up
	TRINITY_DN112712_c0_g1	K10775	PAL5	1.32	up
	TRINITY_DN101076_c4_g6	K10775	PAL	1.23	up
	TRINITY_DN112712_c0_g3	K10775	PAL1	1.19	up
	TRINITY_DN105957_c0_g1	K10775	PAL1	1.16	up
	TRINITY_DN94239_c0_g3	K10775	PAL1	1.15	up
	TRINITY_DN112712_c0_g2	K10775	PAL	1.10	up
	TRINITY_DN123821_c2_g1	K10775	PAL	1.79	up
	TRINITY_DN93014_c2_g1	K00487	CYP73A12	1.58	up
	TRINITY_DN115534_c1_g1	K00487	CYP73A1	1.54	up
	TRINITY_DN93497_c3_g1	K00487	CYP73A1	1.29	up
	TRINITY_DN115534_c1_g3	K00487	CYP73A1	1.14	up
	TRINITY_DN115534_c0_g1	K00487	CYP73A13	1.07	up
	TRINITY_DN100929_c1_g1	K01904	4CL2	2.12	up
	TRINITY_DN122193_c0_g2	K01904	4CL	1.40	up
leaf	TRINITY_DN123821_c2_g4	K10775	PAL	4.21	up
	TRINITY_DN112712_c0_g2	K10775	PAL	4.05	up
	TRINITY_DN94239_c0_g3	K10775	PAL1	3.73	up
	TRINITY_DN112712_c0_g3	K10775	PAL1	3.61	up
	TRINITY_DN123821_c2_g5	K10775	PAL	3.41	up
	TRINITY_DN123821_c2_g1	K10775	PAL	3.35	up
	TRINITY_DN110915_c2_g1	K10775	PAL	2.74	up
	TRINITY_DN94239_c0_g4	K10775	PAL	2.63	up
	TRINITY_DN95790_c1_g1	K10775	PAL	2.50	up
	TRINITY_DN94239_c0_g1	K10775	PAL1	2.49	up
	TRINITY_DN123821_c1_g1	K10775	PAL1	2.45	up

TRINITY_DN94239_c0_g5	K10775	PAL	2.32	up
TRINITY_DN105957_c0_g1	K10775	PAL1	2.28	up
TRINITY_DN110915_c2_g3	K10775	PAL	2.20	up
TRINITY_DN123821_c1_g2	K10775	PAL	2.07	up
TRINITY_DN108603_c1_g2	K10775	PAL	1.71	up
TRINITY_DN115534_c0_g1	K00487	CYP73A13	6.47	up
TRINITY_DN93497_c3_g1	K00487	CYP73A1	5.62	up
TRINITY_DN115534_c1_g1	K00487	CYP73A1	5.36	up
TRINITY_DN115534_c1_g3	K00487	CYP73A1	2.63	up
TRINITY_DN93014_c2_g1	K00487	CYP73A12	2.55	up
TRINITY_DN100199_c0_g1	K01904	4CL9	1.39	up
TRINITY_DN100929_c1_g1	K01904	4CL2	6.93	up
TRINITY_DN122193_c0_g2	K01904	4CL	4.78	up

Analysis of DEGs in the plant hormone signal transduction pathway

Phytohormones are key endogenous factors that mediate plants' stress responses, and they play an important role in plants' defense and response to environmental stimuli. In the plant hormone signal transduction pathways of this study, a total of 329 DEGs were found. We focused on the salicylic acid signal transduction pathway and the jasmonic acid signal transduction pathway. The results showed that there were 7 DEGs in the salicylic acid signal transduction pathway in the *C. Grandiflora* samples, of which 6 genes were upregulated and 1 gene was downregulated; there were 13 DEGs in the jasmonic acid signal transduction pathway, of which 11 genes were upregulated and 2 genes were downregulated. In the leaves and roots, both signal transduction pathways revealed DEGs that were annotated. *TGA7* was upregulated and *PR1B1* was downregulated in the salicylic acid signal transduction pathway in the roots. In the jasmonic acid signal transduction pathway, *TIFY9* was upregulated, and *COI2* was downregulated in the roots. *NPR1*, *NPR2*, *NPR3*, *TGAL5*, and *TGA7* in the salicylic acid signal transduction pathway in the leaves were all upregulated; Similarly, *TIFY10B*, *TIFY9*, *TIFY10A*, *TIFY6A*, *TIFY6B*, *MYC2*, *MYC4* and *AIB* were all upregulated in the jasmonic acid signal transduction pathway in the leaves, yet *GH3.5* and *COI* were downregulated. By comparing the expression patterns of DEGs in the roots and leaves, we found that the number of leaf genes in both the salicylic and jasmonic acid signal transduction pathway was relatively larger than root. The differential gene expression patterns of the salicylic and jasmonic acid signal transduction pathways in the leaves and roots were similar. (Table 6, Figure 10).

Table 6 Differentially expressed genes of the plant hormone signal transduction pathway

Tissue site	Hormone signal transduction pathway	Gene_ID	KOEntry	Name	log2FC	regulation
root	Salicylic acid signal transduction pathway	TRINITY_DN104980_c2_g1	K14431	*TGA7*	1.88	up
		TRINITY_DN87692_c0_g1	K13449	*PR1B1*	-4.10	down
	Jasmonic acid signal transduction pathway	TRINITY_DN111903_c2_g1	K13463	*COI2*	-1.48	down
		TRINITY_DN91100_c0_g2	K13464	*TIFY9*	1.05	up
leaf	Salicylic acid signal transduction pathway	TRINITY_DN93638_c0_g1	K14508	*NPR3*	2.14	up
		TRINITY_DN111453_c0_g1	K14508	*NPR2*	1.69	up
		TRINITY_DN107526_c3_g1	K14508	*NPR3*	1.68	up
		TRINITY_DN123194_c1_g2	K14508	*NPR1*	1.11	up
		TRINITY_DN119181_c0_g2	K14431	*TGAL5*	1.31	up
		TRINITY_DN104980_c2_g1	K14431	*TGA7*	6.81	up
	Jasmonic acid signal transduction pathway	TRINITY_DN123106_c1_g2	K14506	*GH3.5*	-1.11	down
		TRINITY_DN111903_c2_g1	K13463	*COI2*	-2.60	down
		TRINITY_DN110816_c0_g1	K13464	*TIFY10B*	4.47	up
		TRINITY_DN91100_c0_g2	K13464	*TIFY9*	3.07	up
		TRINITY_DN92901_c0_g2	K13464	*TIFY10A*	1.91	up
		TRINITY_DN91844_c0_g3	K13464	*TIFY6A*	1.67	up
		TRINITY_DN116306_c1_g1	K13464	*TIFY10A*	1.63	up
		TRINITY_DN113060_c0_g1	K13464	*TIFY6B*	1.37	up
		TRINITY_DN109853_c2_g3	K13422	*MYC2*	1.44	up
		TRINITY_DN111782_c0_g3	K13422	*MYC4*	1.42	up
		TRINITY_DN120917_c6_g4	K13422	*MYC4*	1.11	up
		TRINITY_DN111782_c0_g1	K13422	*AIB*	1.08	up
		TRINITY_DN110997_c0_g2	K13422	*BHLH14*	3.76	up

Differentially expressed transcription factor analysis

As transacting factors, transcription factors can bind to cis-acting element regions upstream of the target gene through the protein structure's specific DNA binding region, thereby activating the expression of the

target gene. This transcription level regulation is the most important way to regulate gene expression. From a protein structure analysis, a transcription factor is composed of a DNA binding region, a transcription regulatory domain, an oligomerization site, and a nuclear localization signal. These functional regions determine a transcription factor's structure and characteristics. According to the characteristics of DNA binding regions, transcription factors can be divided into different families. In this study, a total of 1604 transcription factors from 54 families were annotated. Among them, the transcription factor families bHLH, NAC, MYB, ERF, WRKY, and bZIP had the most annotated transcription factors, which were 171, 139, 101, 88, 82, and 75, respectively (Table 7, Figure 11).

Table 7 Analysis of differentially expressed transcription factors

TF_family	TF_ID	Quantity	Percentage
bHLH	MDP0000254650	171	10.66%
NAC	KN539001.1_FGP003	139	8.67%
MYB	Aan017619	101	6.30%
ERF	GSVIVT01018226001	88	5.49%
WRKY	Aan015851	82	5.11%
bZIP	Do001279.1	75	4.68%
FAR1	KN538871.1_FGP005	74	4.61%
GRAS	MDP0000258655	68	4.23%
C3H	Bostr.18994s0001.1.p	64	4.00%
B3	676753378	58	3.61%
C2H2	MDP0000321222	58	3.61%
G2-like	KHN04400.1	47	2.93%
MYB	Sme2.5_02552.1_g00001.1	44	2.74%
GATA	KN538788.1_FGP023	41	2.56%

Analysis of gene expression with qRT-PCR

We selected 16 candidate DEGs for transcription polymerase chain reaction (qRT-PCR) analysis to verify the accuracy of the Illumina sequencing data. In the two processing parts, qRT-PCR and RNA-seq analysis showed that the single gene expression trend was basically the same (Figure 12). The gene expression results revealed that the transcriptome sequencing data reflected the response of the roots and leaves of the *C. Grandiflora* to salt stress.

Discussion

Salt stress affects the normal growth and development of plants and can even cause plant death with increasing salinity. Plants utilize a series of physiological and biochemical responses when faced with stressful conditions (Hao et al. 2021). Studies have shown that under salt stress, increasing soil salinity leads to the gradual accumulation of salt in plant cells. Excessive salt induces osmotic stress and causes plant cells to lose water. Ion poisoning caused by osmotic stress affects the plant's absorption of other ions and causes nutrient loss. The primary damage inflicted by osmotic stress and ion poisoning can lead to secondary damage. For example, the replacement of Ca^{2+} in the cell membrane by Na^{+} leads to the destruction of the cell membrane system. Damage to the cell membrane then leads to oxidative stress due to the increase in reactive oxygen species, thus leading to metabolic disorders and a decline in photosynthetic efficiency (Gupta et al. 2021). Specifically, the accumulation of salt in plant cells causes plants to produce excessive amounts of active oxygen leading to an excessive membrane peroxidation reaction, which produces harmful substances like malondiene. In order to cope with the increase in active oxygen, the plant's own peroxidation protection enzyme systems will initiate changes (e.g., the activities of SOD, POD, and CAT enzymes increase). When the stress intensity reaches a certain level, however, the plant's enzyme system cannot cope, and the excessive increase of active oxygen causes the plant to enter a state of oxidative stress (Cheng et al. 2018). In this study, under salt stress with the added stress of time under duress, the tissue water content of C. Grandiflora showed a downward trend. This was caused by the excessive salt ion concentration, which disrupted the ion balance and caused a water deficit. The enzyme activities of SOD, POD, and CAT of C. Grandiflora initially increased followed by a subsequent decrease with increasing time and increasing salt concentrations. Adversity stress, but then there is a downward trend, which indicates that With increasing time and salinity, salt stress begins to inhibit enzymes' effectiveness in eliminating excessive reactive oxygen species.

Studies have shown that under salt stress, Na^{+} enters the cell through nonselective cation channels and causes plasma membrane depolarization, which in turn activates the outward K^{+} channel, allowing K^{+} to flow out of the cell (Yao et al. 2021). In this study, with an increase in time under salt stress, the Na^{+} content gradually increased and the K^{+} content gradually decreased in the roots and leaves of *C. Grandiflora*, thus confirming results of previous studies. There are a large number of ion transporter genes that play important roles in the process of ion transport in plants such as *HKT*, *NHX* and *AKT* (Zhang et al. 2019). During the transportation of Na^{+} in plants, *HKT* is located on the plasma membrane, *NHX* is located on the vacuolar membrane (Tada et al. 2019). *HKT* is responsible for the recovery of Na^{+} entering the root xylem into xylem parenchyma cells, thereby reducing the Na^{+} accumulation of xylem. *NHX* is responsible for separating Na^{+} from the vacuoles and reducing its ion toxicity for other organelles (Wu et al. 2019). Our results revealed that there were more upregulated ion transporter genes in the roots, and we concluded that this is one of the important factors that affects *C. Grandiflora* roots' response to salt stress.

According to GO annotations, we found that the number of DEGs enriched in the nucleus, cytoplasm, plasma membrane, membrane components, and the cytosol of the *C. Grandiflora* samples was the largest.The largest number of differentially expressed genes were enriched in the nuclear, plasma membrane, cytoplasm, membrane components, and protein binding in the root library. The largest number of differentially expressed genes were enriched in the leaf library for the nuclear, plasma membrane, cytoplasm, membrane components, and in the chloroplasts. We found that the GO annotations with the highest degree of enrichment of DEGs in the roots and leaves were the same. According to the KEGG annotation, in the samples of *C. Grandiflora* we found that the number of DEGs enriched in the ribosomes and spliceosomes or those genes involved in protein processing in the endoplasmic reticulum, phytohormone signal transduction, and phytopathogen interaction pathways was the largest. We also found the largest number of differentially expressed genes were enriched in the root library for phenylpropane biosynthesis, ER protein processing, starch and sucrose metabolism, plant hormone signaling, and galactose metabolism pathways. And the largest number of differentially expressed genes were enriched in the library of plant pathogen interactions, plant hormone signaling, protein processing in the endoplasmic reticulum, starch and sucrose metabolism, and phenylpropane biosynthesis pathways. Transcriptomic and metabolomic analysis have shown that DEGs and differential metabolites obtained in the phenylpropane biosynthetic pathway are significantly related under salt stress (Cao et al. 2020). Previous studies have found that plant hormones are small chemicals that play a key role in plants' growth and development (Zhu et al. 2019). Stress hormones such as salicylic acid (SA) and jasmonic acid (JA) mediate the balance between salt stress signals and control growth and stress responses. Therefore, we mainly identified the SA and JAs signal transduction pathways in the phenylpropane biosynthesis pathway and the plant hormone signal transduction pathway.

The metabolic pathway of phenylpropane in plants is a very complex metabolic network, which is the main synthesis pathway of important secondary metabolites such as phenols, flavonoids, anthocyanins, and lignins. These secondary metabolites are widely involved in various physiological activities of plants, especially in the response process of plants to biotic and abiotic stresses. In this study, we found that there were 268 DEGs annotated in the phenylpropane biosynthetic pathway in the *C. Grandiflora* samples. Among them, there were 24 DEGs in the phenylpropane synthesis pathway of the leaves, all of which were upregulated, including *PAL*, *CYP73A* and *4CL*. In the roots, there were 17 DEGs in the phenylpropane synthesis pathway, all of which were upregulated including *PAL*, *CYP73A* and *4CL*. *PAL* is the key rate-limiting enzyme that connects plants' primary and secondary metabolism and catalyzes the first reaction of phenylpropane metabolism. It plays an important role in the growth and development of plants. In addition, PAL is closely related to the content of secondary metabolites (such as lignin, phytoalexin, flavonoids, etc.), and it also plays an important role in the response of plants to biotic and abiotic stress (Li et al. 2019). 4-coumarate:coenzyme A ligase (*4CL*) is the last key enzyme for the phenylpropane biosynthesis pathway to shift to the downstream branch pathway. It contains cinnamic acid, 4-coumaric acid, caffeic acid, erucic acid, ferulic acid, and 5-hydroxyferulic acid, which are used as substrates to generate corresponding acyl-CoA esters. Chlorogenic acid is then generated under the action of

hydroxycinnamoyl coenzyme quinic acid hydroxycinnamate acyltransferase (*HQT*) (Praveen et al. 2019). We found that the upregulated expression of genes was closely related to the response of *C. Grandiflora* to salt stress. The upregulated expression of *PAL* in the roots and leaves could affect the content of secondary metabolites. The expression of *4CL* affects chlorogenic acid. The synthesis of chlorogenic acid is closely related to antioxidants. There were more *PAL*, *CYP73A* and *4CL* genes involved in phenylpropane biosynthesis in the leaves of *C. Grandiflora*. Therefore, we speculated that the phenylpropane biosynthesis pathway is an important way for leaves to defend against salt stress.

Plants use complex signaling pathways to respond to stress. In addition to some other small molecules (such as Ca^{2+} and ROS), plant hormones can initiate a specific signal cascade after sensing biological and abiotic stress. Fluctuations in the levels of several major hormones such as ABA, ET, SA, and JA, as an early response to stress, will initiate metabolic processes that lead to changes in the plant's growth patterns. The Nonexpressor of Pathogenesis-Related gene 1 (*NPR1*) is an activator of plant resistance. *NPR1* not only plays a core regulatory role in plant system resistance acquisition and induction of systemic resistance, but it also acts as an important regulatory factor for plants' basic resistance and resistance determined by disease resistance genes (Yuan et al. 2021). Transcription factors TGA (Han et al. 2019), TIFY (Bai et al. 2011), and MYC (Yang et al. 2018) also play an important role in plant resistance. This study found that there were a large number of genes related to salt stress in the signal transduction pathway of plant hormones, and the number of DEGs in the leaves was relatively large. We focused on the salicylic and JAs signal transduction pathways. In the SA signal transduction pathway, *NPR1*, *NPR2*, *NPR3*, *TGAL5* and *TGA7* are upregulated, and *TGA7* is upregulated in the roots and leaves. Expression, the rest is only upregulated in leaves. *TIFY10B*, *TIFY9*, *TIFY10A*, *TIFY6A*, *TIFY6B*, *MYC2*, *MYC4* and *AIB* are upregulated in the JAs signal transduction pathway. Studies have shown that signal recognition is a key step for JA to play a role in plants, and TIFY is a key factor that regulates the signal recognition of JA. When plants activate the JAs signal pathway, *MYC2*, *bHLH* and other transcription factors are inhibited by the TIFY protein through ubiquitination. The first step toward activation of this pathway begins when the content of exogenous or endogenous JA increases. With this increase in JA, the JA receptor *COI1* binds to the TIFY protein to form a *SCFCOI1* complex. The deubiquitinated TIFY protein is degraded by the 26S proteasome, and the *MYC2* and *bHLH* transcription factors are released concurrently, which finally activates the JAs signal pathway. Since *TIFY9* was upregulated in the roots and leaves in this study, by comparing gene expression patterns, we found that there were more upregulated genes in the leaves. Therefore, we speculated that *TIFY9* in *C. Grandiflora* could play a role in both the roots and the leaves. Both the salicylic and the JAs signal transduction pathways are important ways to defend against salt stress in the leaves of *C. Grandiflora*.

Plant transcription factors are members of a vital gene family that are widely present and participate in the regulation of plants' growth and development and resistance to stress. Transcription factors can regulate the expression of stress-related target functional genes by combining with specific action elements in downstream gene promoters, thereby directly or indirectly participating in many biological processes of plants, such as cell morphogenesis, metabolism, physiological balance, and signal

transduction. Studies have shown that transcription factor families such as bHLH, NAC, MYB, ERF, WRKY, and bZIP are all related to plants' stress response. bHLH transcription factors help regulate plants' growth, development, secondary metabolism, and stress response (Zheng et al. 2019). Most members of the NAC transcription factor family are involved in the process of plant development, including floral organ morphogenesis, lateral root development, root tip meristem and branch development, and the lignification and fiber formation of vascular plants. Many NAC transcription factors are also involved in nonbiological process of plants. They play a key role in coercion and defense responses (Wang et al. 2019). MYB transcription factors can regulate cell differentiation, organ formation, leaf morphogenesis, secondary metabolism, and abiotic stress responses (Wang et al. 2021). Most members of the ERF transcription factor family directly or indirectly participate in the response process of abiotic stresses such as ethylene, JA, abscisic acid, SA, drought, salinity, and cold, and the same ERF transcription factor can be induced by multiple stresses. ERF transcription factor involved in the transduction of stress response signals in plants (Long et al. 2019). In this study, the transcription factor families bHLH, NAC, MYB, ERF, WRKY and bZIP had the most annotated transcription factors, so we speculated that these transcription factor families were closely related to the salt tolerance of *C. Grandiflora*.

In summary, transcriptome sequencing showed that salt stress changed the transcription levels of many genes related to many regulatory networks, including osmotic regulation, ion transport, reactive oxygen scavenging systems, and plant hormone signal regulation. Studies have found that under salt stress, there are more DEGs in the roots of the chrysanthemum cv. 'Jinba' than in its leaves, and the number of downregulated genes involved in the salt stress response exceeds the number of upregulated genes. At the same time, the roots of chrysanthemums under salt stress are mainly dominated by the upregulation of genes encoding proteins involved in ion transport, while in the leaves, the response is focused on osmotic regulation and Ca^{2+} transport (Cheng et al. 2018). In our research, we found that there are more DEGs in the leaves of *C. Grandiflora* in response to salt stress than in its roots, and there are more upregulated than downregulated genes in the roots and leaves. Moreover, the root response to salt stress mainly encoded the upregulation of ion transporter genes, while the leaf response focused on the salicylic and JAs signal transduction pathways in phenylpropane biosynthesis and plant hormone signal transduction. We speculated that this may be the result of the differences in the resistance of plant materials. Research into drought and salt stress on *Chrysopogon zizanioides* revealed that more transcriptome changes occurred in the plant's leaf tissues than in its root tissues. The response to stress was mainly manifested in the upregulation of the gene expression in the leaves and roots (Suja et al. 2017). These results are similar to the what we found with the number of genes and their expression patterns in the roots and leaves of *C. Grandiflora*. In order to more clearly show from a molecular perspective the salt tolerance mechanism of *C. Grandiflora*, we created a molecular mechanism diagram of *C. Grandiflora* in response to salt stress (Figure 13).

Conclusions

In summary, the results of transcriptome sequencing showed that salt stress changed the transcription levels of many genes related to many regulatory networks, including osmotic regulation, ion transport, and reactive oxygen scavenging systems. The functional enrichment analysis of candidate genes showed the tissue-specific pattern of the transcriptome under salt treatment. Compared with the root tissues of *C. Grandiflora*, more transcriptome changes were observed in the plants' leaf tissues, and the number of genes involved in the salt stress response was greater than root tissues. The response to salt stress was mainly manifested in the upregulation of gene expression in both the leaves and roots. Root response to salt stress mainly encoded the upregulation of ion transporter genes, and the upregulated expression of ion transporter genes NHX and HKT appeared to be important factors in the roots' response to salt stress. In the leaves, the response was focused on phenylpropane biosynthesis and plant hormone signal transduction. Among the genes that were seen to play important roles in the leaves' response to salt stress, *PAL*, *CYP73A* and *4CL* were upregulated in the phenylpropane biosynthetic pathway; *NPR1*, *NPR2*, *NPR3*, *TGAL5* and *TGA7* were upregulated in the SA signal transduction pathway; and *TIFY10B*, *TIFY9*, *TIFY10A*, *TIFY6A*, *TIFY6B*, MYC2, MYC4, and *AIB* were upregulated in the JAs signal transduction pathway. Furthermore, the upregulated expression of these genes was observed to play an important role in the leaves' defense response to salt stress. It is known that plant salt stress-responsive transcription factors can reduce the damage caused by salt stress by regulating the expression of downstream target genes. Within the several families that differentially express transcription factors in response to salt, we found that the bHLH and NAC families had the largest number of transcription factor coding genes, and these genes were differentially expressed in the roots and leaves under salt stress. This work has greatly enriched the existing sequence resources of *C. Grandiflora* and will provide a large number of salt-tolerant candidate genes for further functional analysis to improve plants' salt tolerance.

Declarations

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Availability of data and material

The datasets supporting the conclusions of this article are included within the article. Sequencing database for *Chrysanthemum×grandiflora* could download from NCBI under the accession number SRR17510868, SRR17510867, SRR17510866, SRR17510865, SRR17510874, SRR17510873, SRR17510876, SRR17510875, SRR17510872, SRR17510871, SRR17510870 and SRR17510869. The data will be shared on reasonable request of the corresponding author.

Competing interests

On behalf of all authors, the corresponding author states that there is no confict of interest.

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Authors' contributions

H.L. designed the research and wrote the manuscript. Y.Z. and M.H. provided guidance on the experiment. Y.L., N.X, Y.S., Q.L. and L.Y. reviewed and revised the manuscript. All authors read and approved the fnal manuscript.

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Authors' information

Affiliations

College of Landscape Architecture, Northeast Forestry University, No. 26 Hexing Road, Harbin, Heilongjiang, China

He Liu

College of Landscape Architecture, Northeast Forestry University, No. 26 Hexing Road, Harbin, Heilongjiang, China

Yu Liu

College of Landscape Architecture, Northeast Forestry University, No. 26 Hexing Road, Harbin, Heilongjiang, China

Ning Xu

College of Landscape Architecture, Northeast Forestry University, No. 26 Hexing Road, Harbin, Heilongjiang, China

Ying Sun

College of Landscape Architecture, Northeast Forestry University, No. 26 Hexing Road, Harbin, Heilongjiang, China

Qiang Li

College of Landscape Architecture, Northeast Forestry University, No. 26 Hexing Road, Harbin, Heilongjiang, China

Liran Yue

College of Horticulture, Jilin Agricultural University, 2888 Xincheng Street, Changchun, Jilin, China

Yunwei Zhou

College of Landscape Architecture, Northeast Forestry University, No. 26 Hexing Road, Harbin, Heilongjiang, China

Miao He

Corresponding author

Correspondence to Yunwei Zhou and Miao He.

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Figures

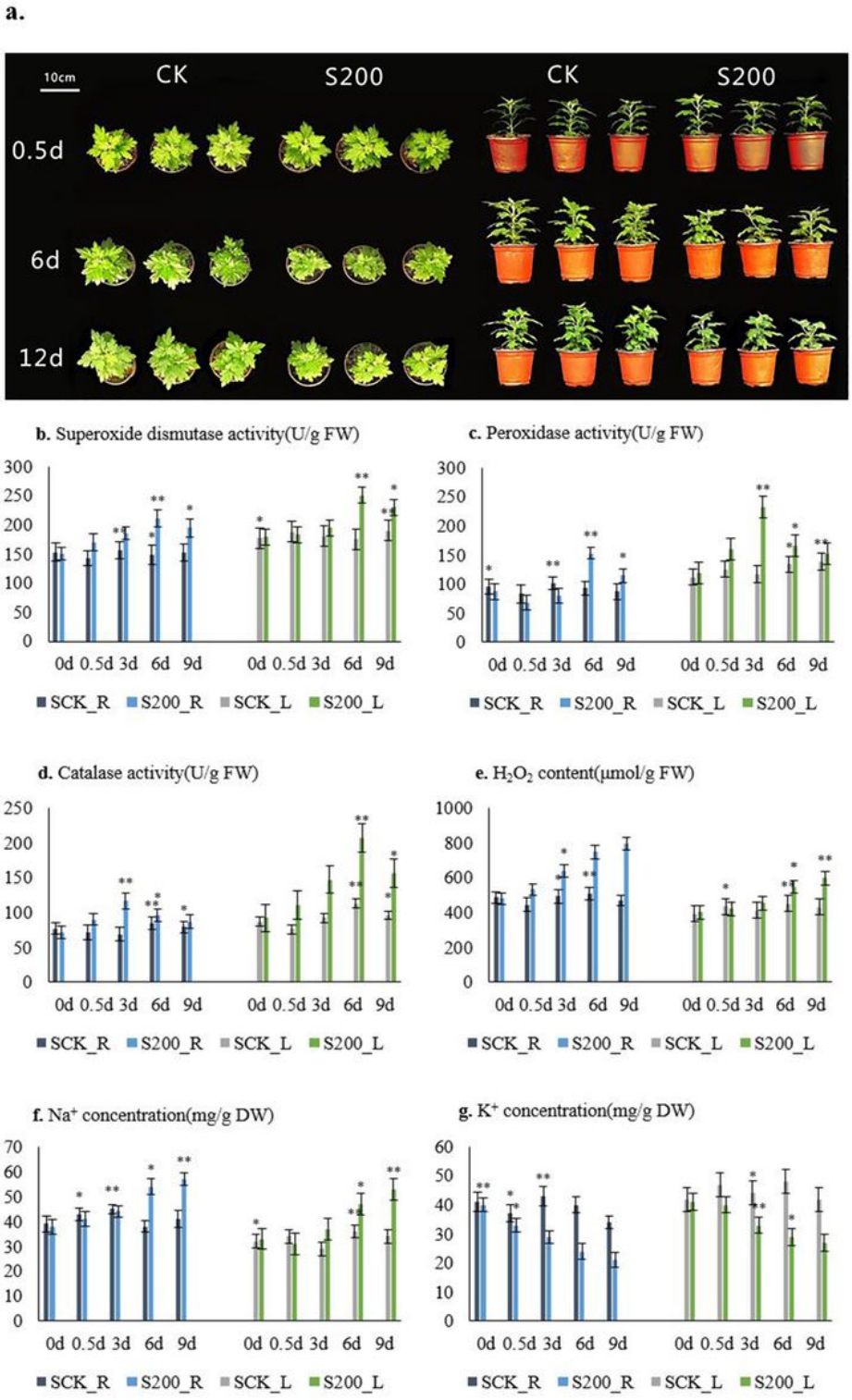

Figure 1

The effect of NaCl treatment on the growth of *C. Grandiflora*. Growth phenotype of *C. Grandiflora* under salt stress (a) antioxidant enzyme activity of roots and leaves, (b) SOD, (c) POD, (d) CAT, (e) H_2O_2 content, (f) Na^+ content, (g) and K^+ content. ** and * represent Duncan's multiple range test ($P < 0.05$) based on statistically significant differences for the specified treatment, where ** represents extremely significant differences, and * represents significant differences.

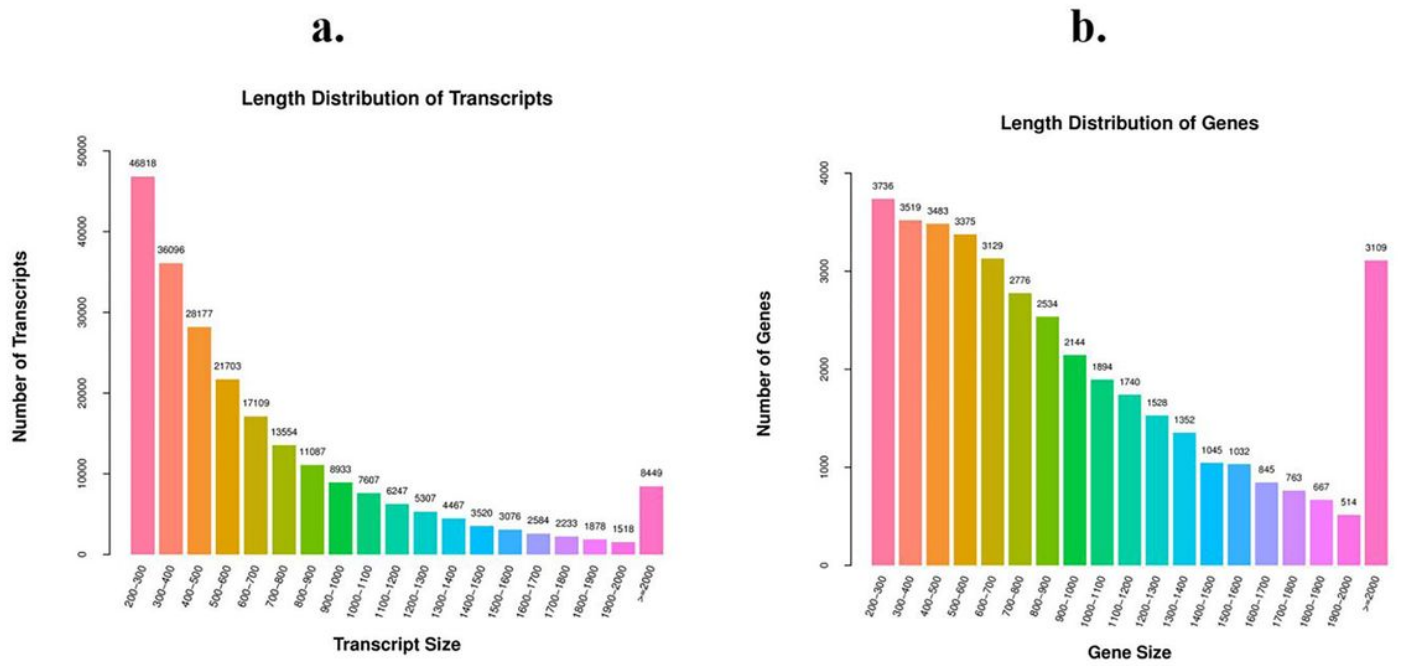

Figure 2

The length distribution of transcript (a) and single genes (b) in *C. Grandiflora*.

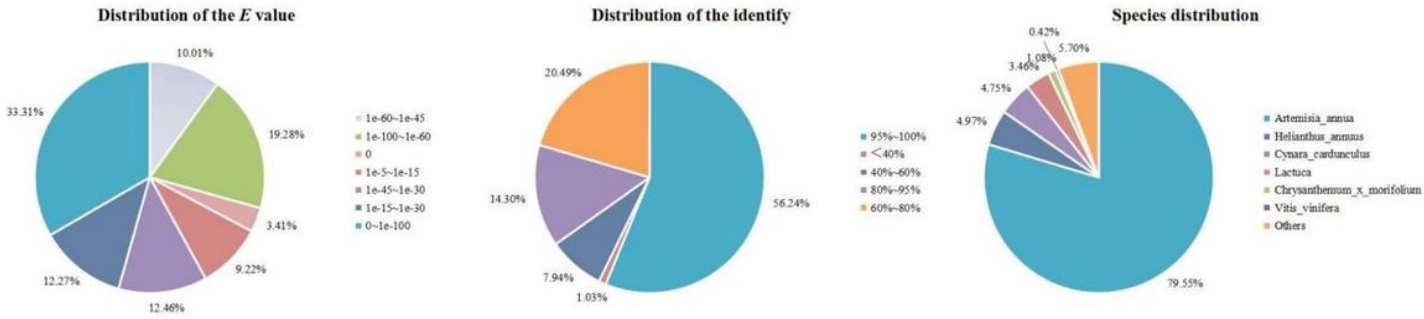

Figure 3

The homology comparison of the single gene in *C. Grandiflora* with the NR database. (a) Distribution of *E* values in the results annotated in the NR database. (b) Single gene distribution identification of *C. Grandiflora*. (c) Distribution of species with homology to *C. Grandiflora*.

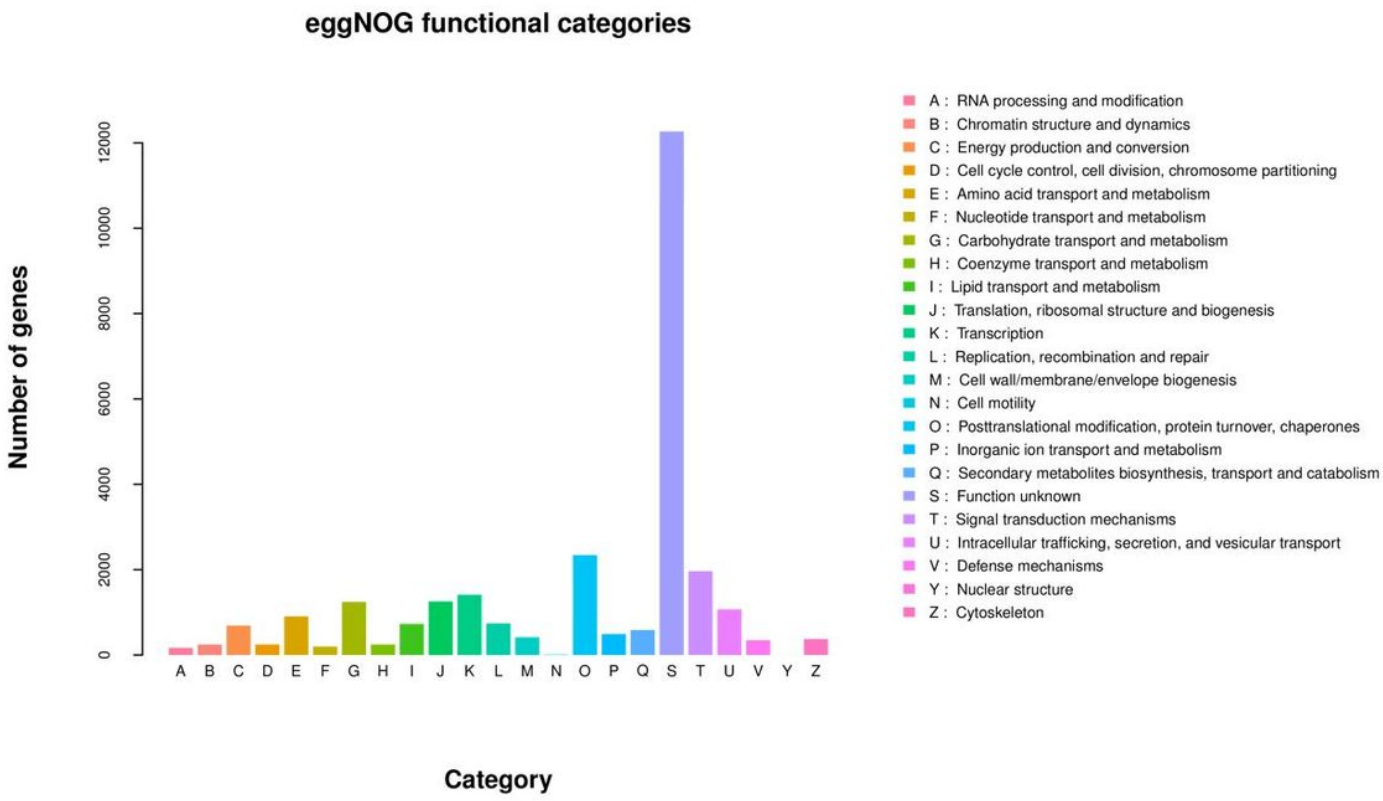

Figure 4

EggNOG functional classification of all single genes in *C. Grandiflora*.

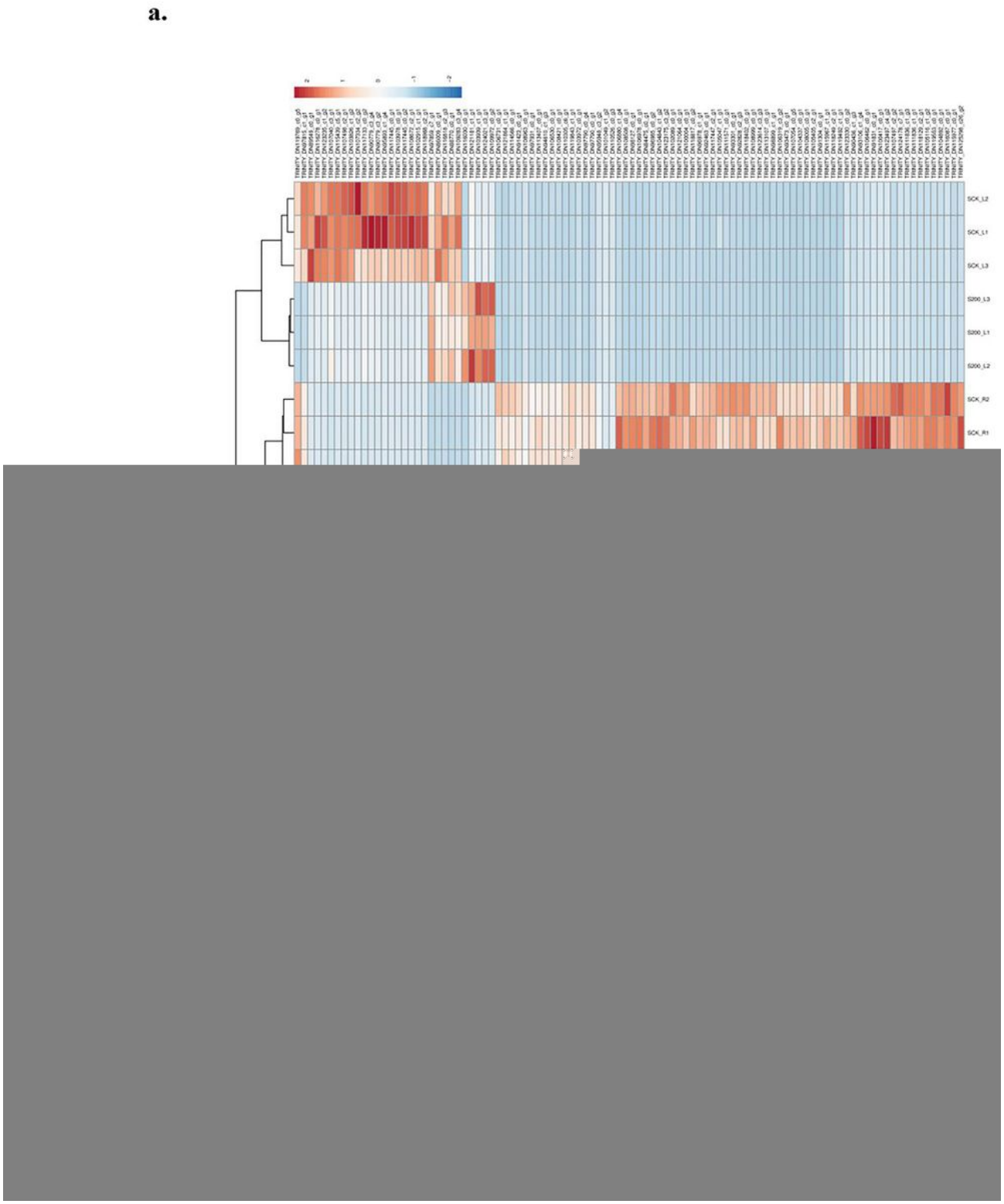

Figure 5

Differentially expressed gene (DEG) expression analysis. (a) A cluster heat map of DEGs in *C. Grandiflora* treated with salt for 12 h. (b) A volcano map of DEGs in S200_R vs SCK_R and (c) a volcano map of DEGs in S200_L vs SCK_L.

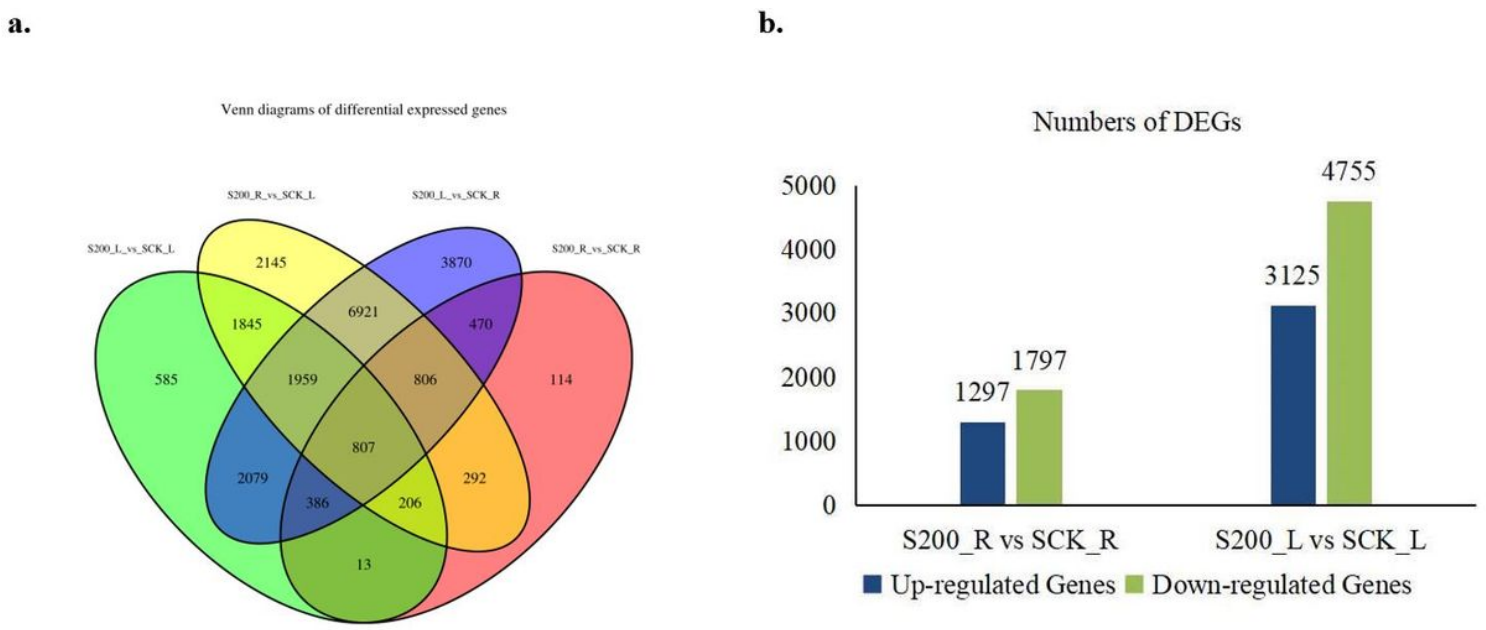

Figure 6

Venn diagram analysis of the number of DEGs after 12 h of treatment. (a) Venn diagram analysis of DEGs annotated in the NR database, and (b) the number of DEGs upregulated and downregulated after 12 h of treatment.

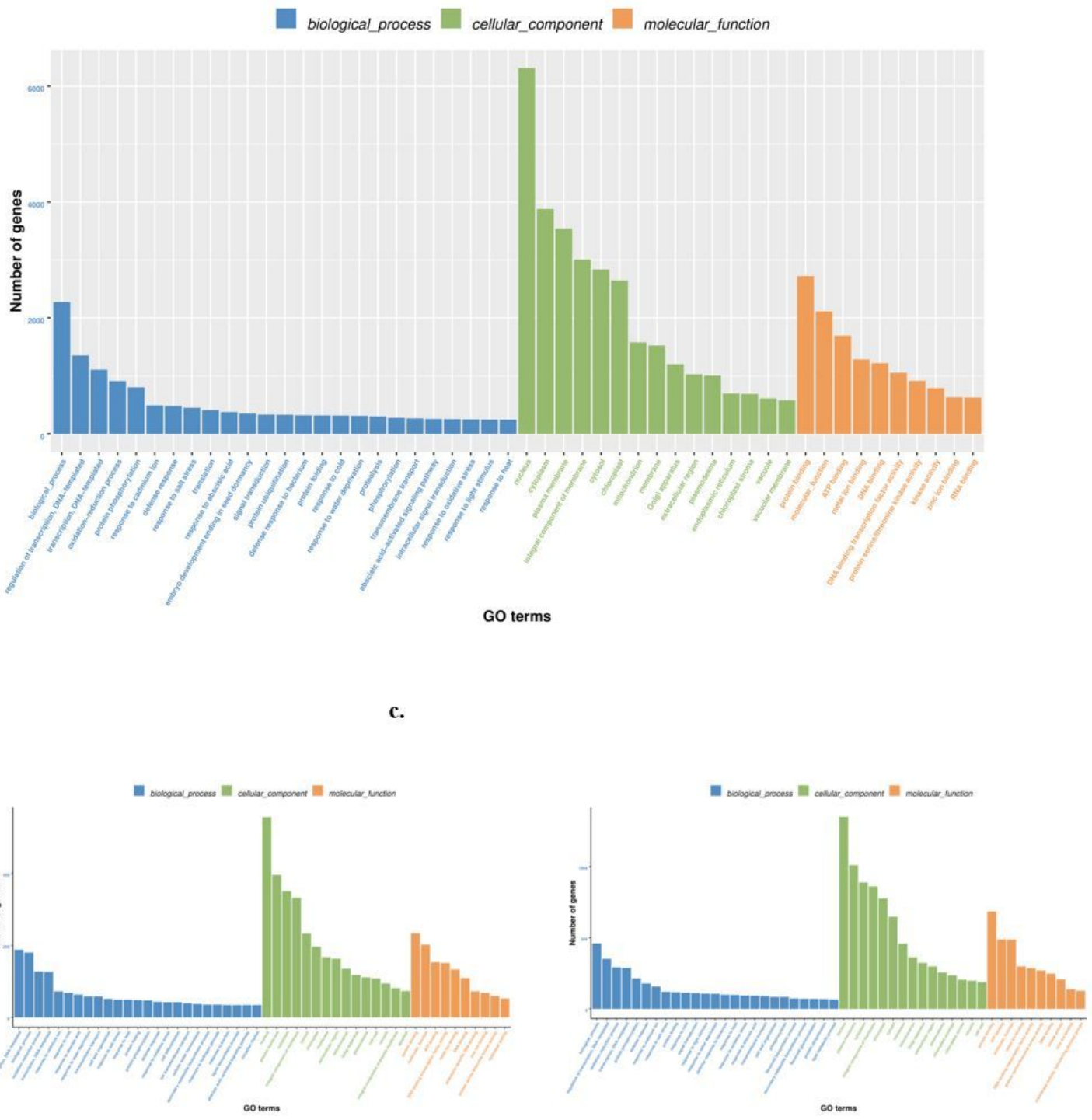

Figure 7

Classification of single genes of GO annotation of *C. Grandiflora*. (a) S200_R vs SCK_R vs S200_L vs SCK_L, (b) S200_R vs SCK_R, and (c) S200_L vs SCK_L.

Figure 8

Enrichment analysis of the KEGG pathway of DEGs in *C. Grandiflora*. (a) S200_R vs SCK_R vs S200_L vs SCK_L, (b) S200_R vs SCK_R, and (c) S200_L vs SCK_L.

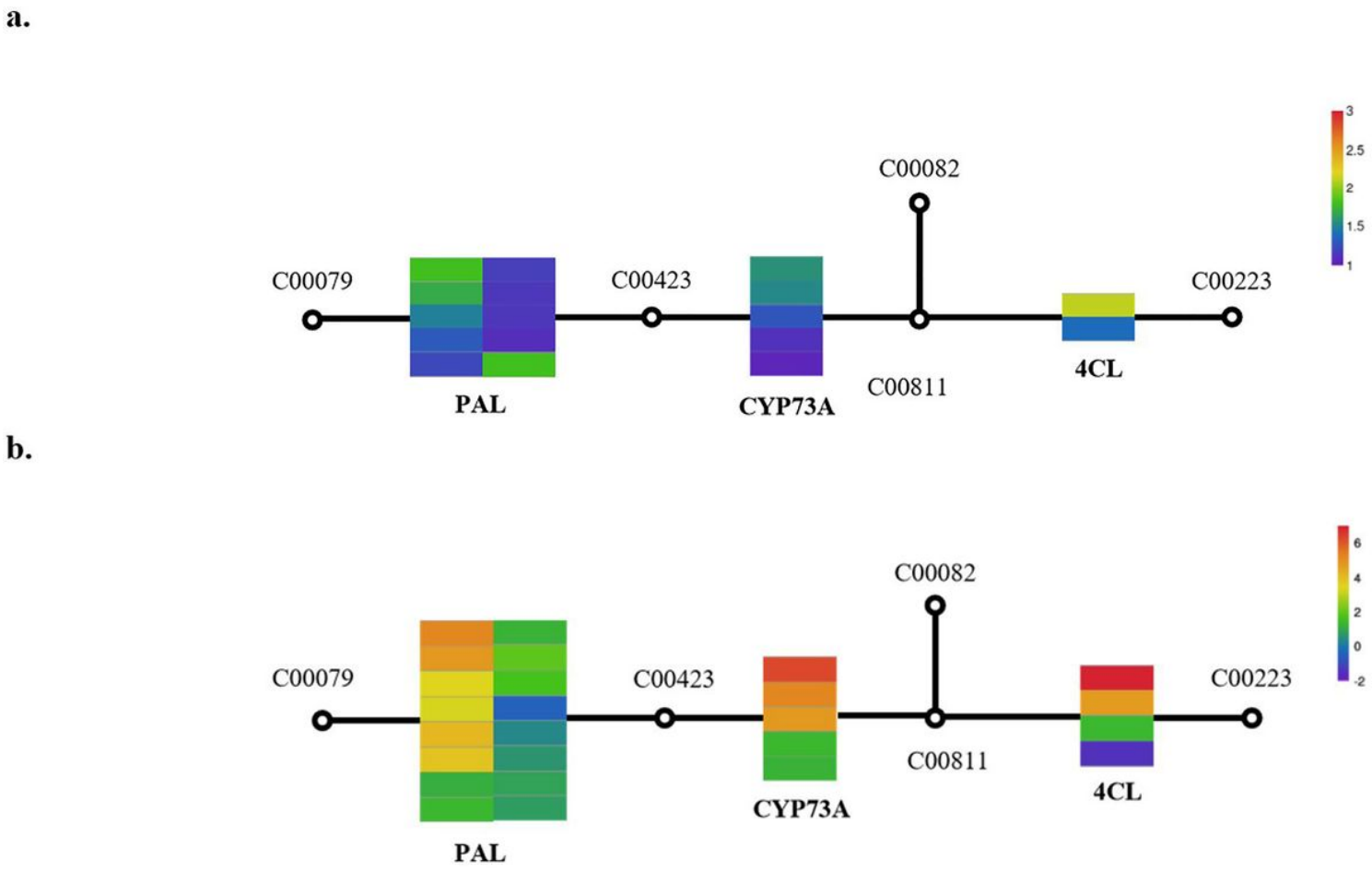

Figure 9

DEGs in the phenylpropane biosynthesis pathway under salt stress. (a) Root sample and (b) leaf sample.

Figure 10

The salicylic acid signal transduction pathway, (a) root sample and (b) leaf sample. The jasmonic acid signal transduction pathway, (c) root sample and (d) leaf sample.

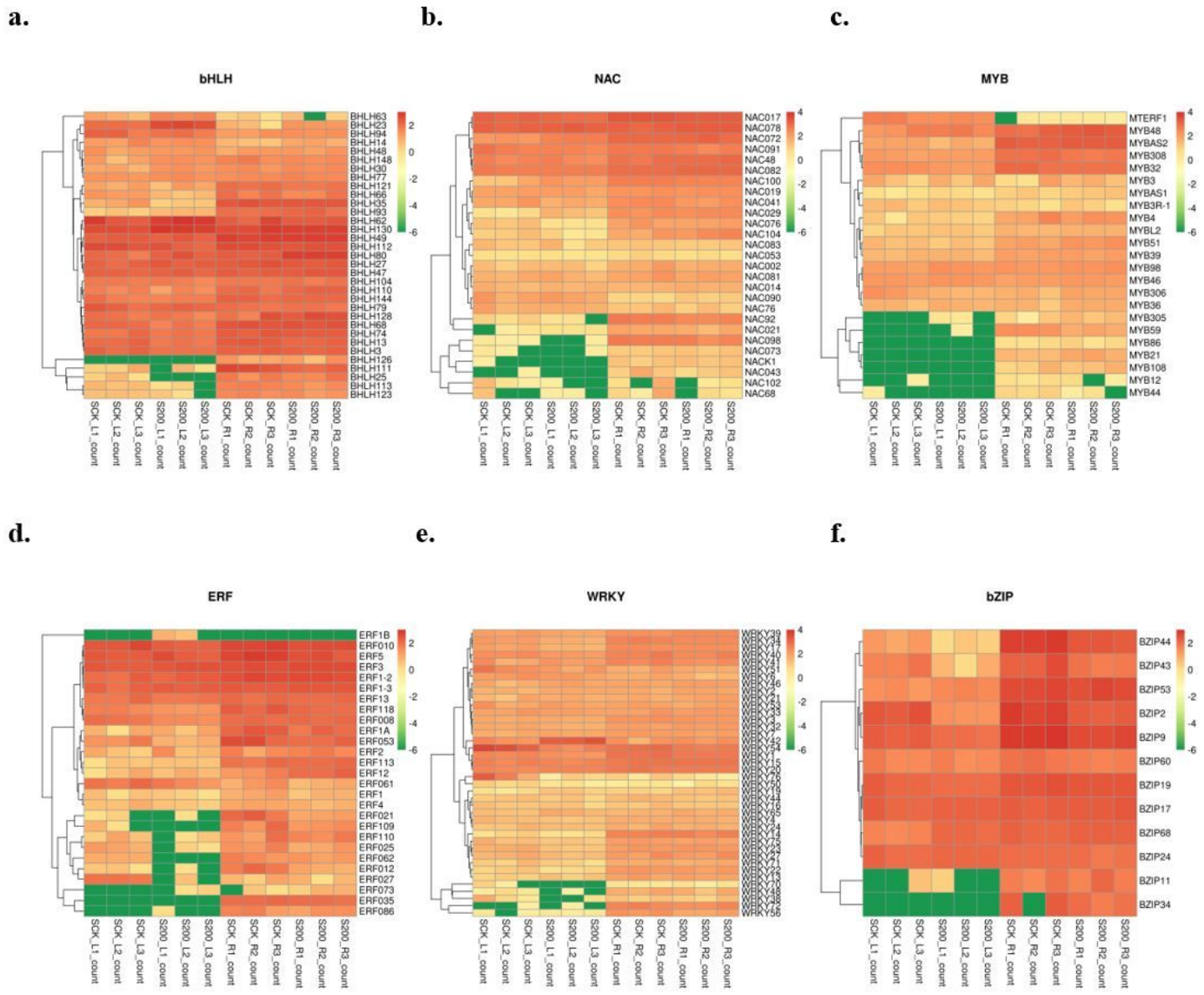

Figure 11

Heat map of transcription factor family expression.

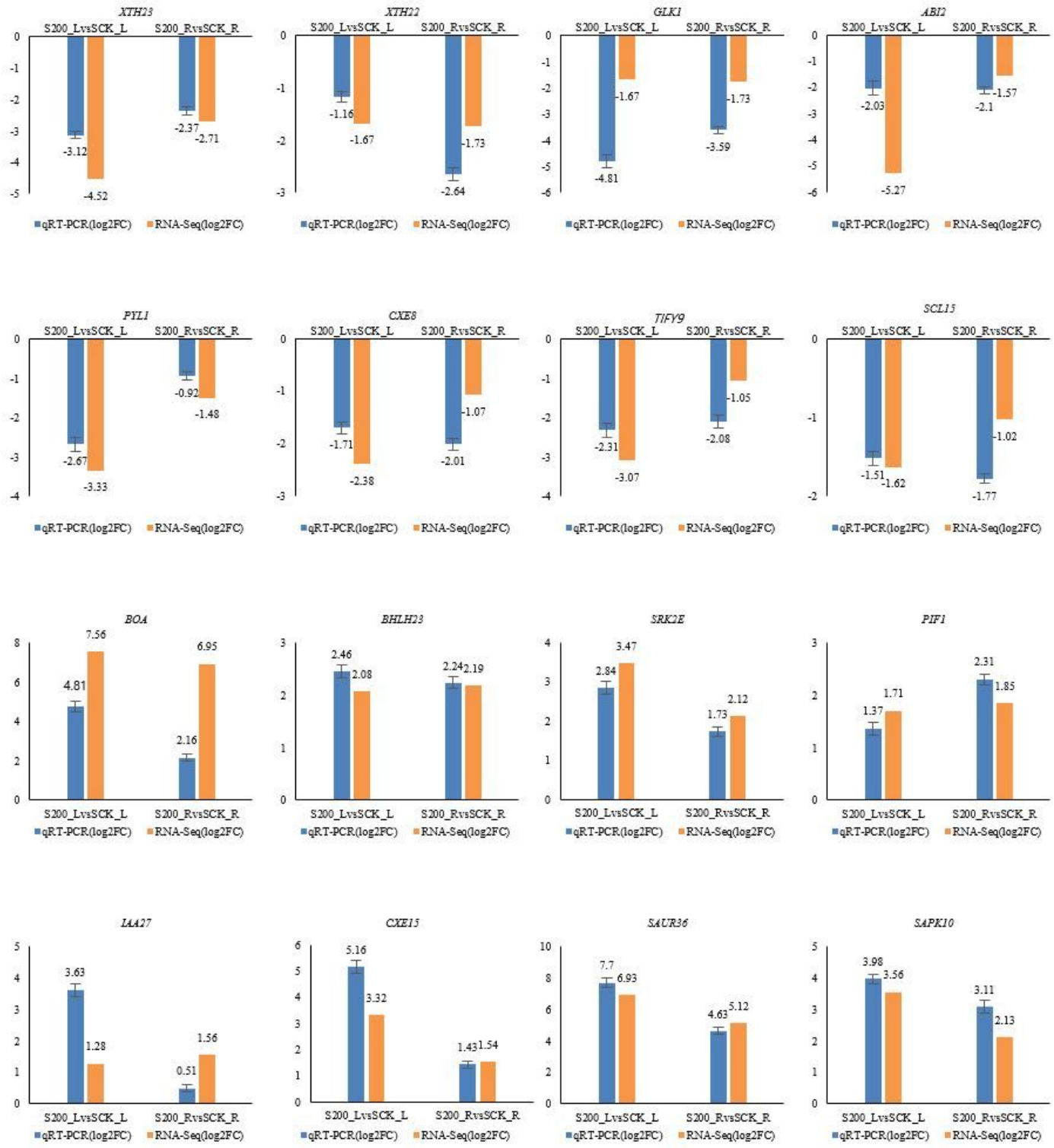

Figure 12

The 16 candidate genes in the *C. Grandiflora* were identified by RNA-seq and qRT-PCR to determine their multiple changes. The *x*-axis represents the treatment site and the control site, and the *y*-axis represents the multiple of change in $\log_2$FC.

Figure 13

Molecular mechanism diagram of salt stress resistance of *C. Grandiflora*. (a) Roots and (b) leaves.