Received: 5 March 2021 | Accepted: 4 May 2021

DOI: 10.1002/agj2.20715

Agronomy Journal

ARTICLE

Agronomic Application of Genetic Resources

Comparative transcriptome analysis of *Rosa chinensis* 'Old Blush' provides insights into the crucial factors and signaling pathways in salt stress response

Ying Bao | **Chao Chen** | **Ling Fu** | **Yuqing Chen**

Dep. of Life Science, Tangshan Normal Univ., Tangshan, Hebei, China

Correspondence
Bao Ying, Dep. of Life Science, Tangshan Normal Univ., Tangshan, Hebei, China.
Email: baoying090924@126.com

Assigned to Associate Editor Mehdi Rahimi.

Abstract

The growth, development, and quality production of *Rosa hybrida* L. are the core issues for being able to have good yields. Inadequate information on genetic background and resistance has hindered the ability to continue improving flower quality, and most cultivars continue to have lower-than-desired levels of resistance. Therefore, this study sequenced the transcription group of the *R. chinensis* Jacq. 'Old Blush' under high salt stress. Transcription group sequencing was carried out on root and leaf materials at different points in time (0, 2, 24, and 48 h) under a high salt stress condition of $NaHCO_3$. The monthly salt stress recorded within 48-h Ca^{2+} sensors was mainly calmodulin and calcium-binding protein. The kinase 5 (RC2G0184500) began to show significant increase from 24- and 48-h process time, respectively, within the leaf blades and the root. There was an increase of superoxide dismutase and L-ascorbate peroxidase from the 2- and 48-h processing times, respectively. Within the blades, MYB4, MYB41, MYB44, MYB20, MYB62, and MYB14 had a log2Foldchange of >4. ERF109 had a log2Foldchange of 7. NAC-like transcription factors had more differential genes in the root than in the leaves, with a log2Foldchange of NAC67, NAC52, NAC16, and NAC2 of >4. The plant hormone signal transduction-related genes involved were abscisic acid, growth hormone, ethylene, erythromycin, jasmine acid, salbonate, and cell division. The EIN4 and EIN3 were only raised in the leaf and the serine/threonine-protein kinase CTR1 was raised in the root, whereas the ethylene pathway was mainly in the leaves. Auxin-responsive protein SAUR72 and SAUR50 were raised in the leaves and the other auxin-responsive protein and SAUR expression in the root were low. The IAA2 was revised upward in the leaf and the IAA11 was revised upward in the root, whereas the rest were revised downward. In conclusion, the study revealed several genes and hormones that are key in salt stress response.

Abbreviations: ABA, abscisic acid; AUX/IAA, auxin/indole-3-acetic acid; DREB, dehydration responsive element binding; GO, Gene Ontology; HSP, heat shock protein; JA, jasmonic acid; log2FC, log2FoldChange; MAPK, mitogen-activated protein kinase; MEKK, mitogen-activated protein kinase kinase; ROS, reactive oxygen species; SOD, superoxide dismutase.

1 | INTRODUCTION

Rosa hybrida L. (often referred to as the "Queen of Flowers") is among the top 10 traditional flowers in China and is the world's leading cut flower as well as an important garden flower (Liorzou et al., 2016). The cultivation area and monthly output value of the *R. hybrida* flower can account for about one-third of the total cut flowers (Qi et al., 2018). The growth, development, and quality production of the flower are therefore the core issues for optimizing the monthly cut flower production for good returns (Koning-Boucoiran et al., 2015). For quality improvement, modern cultivars have been a product of cross breeding between some selected wild species within the *Rosa* genus, repeated hybridization between cultivars, rebreeding and other means (Lim, 2014; Qi et al., 2018). Indeed, narrow information on genetic background and resistance has hindered the need to continue improving the quality of the flower, making most of the varieties to continue having shortcomings in their level of resistance (Bendahmane et al., 2013). In addition, the environmental stress in the saline area seriously restricts the use of this species in local landscaping, and the high salt stress leads to the poor growth of most rose cultivars and the aggravation of pests and diseases, which seriously affects their growth and development of their ornamental value (Cai et al., 2014).

Currently, studies that present the resistance to salt tolerance on *Rosa* flowers, which flower on a monthly basis, lag far behind as compared to studies on other agronomy patterns, such as color, flower type, and flower fragrance despite the impact of excessive salt to the productivity of *R. hybrida* (Liang et al., 2018). With the increasing problem of soil salinization and the continuous improvement of people's demand for the quality of the monthly seasoned flowers like the *R. hybrida*, most of the ongoing studies focuses on the morphological and physiological levels, whereas the research on the level of salinity-resistant molecules in the monthly seasoned flowers are less studied (Salehi et al., 2018). Furthermore, a systematic in-depth study of its high-salt adaptive molecular mechanisms has not been adequately achieved (Ahmad et al., 2015; Zhao et al., 2020). Therefore, understanding and clarifying the molecular regulation mechanism of *Rosa hybrida* salt resistance is very necessary for the smooth progress of breeding work with the main goal of improving salt resistance (Muhammad et al., 2020; Zhang et al., 2013)

Plasma membrane Na+/H+ transporter SOS1, HKT-type protein, and tonoplast Na+/H+ antiporter NHX1 are key Na+ transporters involved in plant salt tolerance. SOS1 gene expression in plants is upregulated in response to salt stress. This upregulation is abated in SOS3 or SOS2 mutant plants, suggesting that it is controlled by the SOS3/SOS2 regulatory pathway (Zhang et al., 2017). A limited number of rose genes related to salt stress response were identified. RcHSP17.8 (cytosolic class I small heat shock protein), present in *Rosa chinensis*, has been isolated and characterized. This gene is induced under salt stress conditions, as well as drought, cold, osmotic, and oxidative stress. *Arabidopsis thaliana* (L.) Heynh. constitutively expressing the RcHSP17.8 shows higher tolerance to salt stress. Small heat shock proteins work as molecular chaperones and play important roles in plant defenses against detrimental conditions, such as high salinity. They are members of the heat shock protein (HSP) family, which works to protect plants against abiotic stresses and maintain protein homeostasis by scavenging cellular reactive oxygen species (ROS) (Mu et al., 2013). RrNHX1 genes are identified with salt resistance in wild *Rosa rugosa*Thunb. (Feng et al., 2015). The Na+/H+ antiporter (NHX) is a transmembrane protein carrier that bars Na+ from the cytosol in return for H+. It is confined on both plasma and vacuolar films. NHX keeps up particle homeostasis by the vehicle of Na+ out the cytosol and into the vacuole. This compartmentalization of Na+ in the vacuole mitigates the cytosol of abundance sodium particles. The action level of Na+/H+ antiporters of salt open minded and salt-delicate plants are unique. The salt touchy cultivar Oryza sativa cv. Kinuhikari overexpressing the AgNHX1 quality from a halophytic plant (*Atriplex gmelini* C.A. Mey.) shows a solid resistance to salt pressure (Ohta et al., 2002).

In *Rosa rugosa*, other important genes related to salt stress tolerance were upregulated by salt exposure. This includes NAC and DREB (dehydration responsive element binding) family genes. *Rosa rugosa* plants irrigated with 25 mM, 50 mM, and 100 mM NaCl did not differ from control plants in measured growth parameters (shoot length and number of leaves). *Rosa chinensis*Jacq. growth is highly susceptible to salinity stress, but this species can have its salt tolerance increased by overexpressing genes related to salt response such as DREB. DREB2A-CA is a member of the

Core Ideas

- In 48 h, the salt stress Ca^{2+} sensors were mainly calmodulin and calcium-binding protein.
- NAC-like transcription factors had more differential genes in the root than in the leaves.
- Auxin-responsive protein SAUR72 and SAUR50 were raised in the leaves.
- Other auxin-responsive protein and SAUR expression in the root were low.
- The IAA2 was revised upward in the leaf.

transcription factor family DREB that play important roles in regulation of stress inducible genes and affect the salt tolerance (Lata & Prasad, 2011). Plants of *Rosa chinensis* overexpressing AtDREB2A-CA under salt stress (NaCl 300 mM) present salinity stress tolerance activated by change in leaf ultrastructure. This observation indicates that AtDREB2ACA could be used to improve salt stress tolerance (Josine et al., 2015). In *Rosa rugosa*, other significant qualities identified with salt pressure resilience were up controlled by salt openness. This incorporates NAC and DREB family qualities. *Rosa rugosa* plants inundated with 25 mM, 50 mM, and 100 mM NaCl did not vary from control plants in estimated development boundaries (shoot length and number of leaves). *Rosa chinensis* development is profoundly helpless to saltiness stress, yet this species can have its salt resistance expanded by overexpressing qualities identified with salt reaction like. DREB2A-CA is an individual from the record factor family DREB that assume significant parts in guideline of pressure inducible qualities and influence the salt resistance (Lata & Prasad, 2011). Plants of *Rosa chinensis* overexpressing AtDREB2A-CA under salt pressure (300 mM NaCl) present saltiness stress resilience actuated by change in leaf ultrastructure. This perception shows that AtDREB2ACA could be used to improve salt pressure resistance (Josine et al., 2015).

The completion of monthly genome-wide sequencing provides a guarantee for increasing monthly salt resistance through molecular breeding (Bourke et al., 2018; Liu et al., 2019). Indeed, a number of studies showed that in alkaline saline soil plants can withstand salt stress while resisting high pH stress. Alkaline salt stress, such as $NaHCO_3$ or Na_2CO_3, significantly does more damage to plants than coercive damage caused by neutral salts such as NaCl or Na_2SO_4 (Ahmad et al., 2015). At the same time, the research on the salt-resistant properties of plants is mainly concentrated in herbs, and mostly salt has been the stress factor, and the research on the anti-salt properties of woody plants under $NaHCO_3$ stress is relatively small (Aghajanzadeh et al., 2018). In this study, *Rosa chinensis* 'Old Blush' was selected as the test material, and the 'Moon Powder', with the characteristics of four seasons of flowering (a single color, easy to insert, simple genetic background, etc.), was used as a model material for the study of the molecular mechanism of the monthly season salt resistance. Therefore, this presented study sequenced the transcription group of the monthly seasoned *R. chinensis* under high salt stress. Transcription group sequencing was carried out on root and leaf materials at different points in time (0, 2, 24, 48 h) in the treatment and control groups under high salt stress. $NaHCO_3$ was used in this study to conduct a coercive treatment test on seedlings planted in the year of the monthly *Rosa chinensis* flower production.

2 | MATERIALS AND METHODS

2.1 | Material preparation and identification

The material used in the experiment was *Rosa chinensis* 'Old Blush'. The seedlings with consistent growth were treated with high salt stress (200 mmol/L $NaHCO_3$), and materials with different processing times (0, 2, 24, 48 h) were sampled and sequenced. The samples were properly labeled. Leave samples at 0 h were labeled LCK; three replicated for LCK were marked as LCK_1, LCK_2, and LCK_3. Leave samples at 2 h were labeled L2; three replicated for LCK were marked as L2_1, L2_2, and L2_3. Leave samples at 24 h were labeled L24; three replicated for L24 were marked as L24_1, L24_2, and L24_3. Leave samples at 48 h were labeled L48; three replicated for L48 were marked L48_1, L48_2, and L48_3. Root samples at 0 h were labeled RCK; three replicated for RCK were marked RCK_1, RCK_2, and RCK_3. Root samples at 2 h were labeled R2; three replicated for R2 were marked R2_1, R2_2, and R2_3. Root samples at 24 h were labeled R24; three replicated for R24 were marked R24_1, R24_2, and R24_3. Root samples at 48 h were labeled R48; three replicated for R48 were marked R48_1, R48_2, and R48_3.

2.2 | Total RNA extraction and RNA-Seq library preparation

The material for RNA-seq are petal discs and root sections. Extraction of the total RNA was accomplished using the hot borate method and treated with RNase-free DNase I (Promega) to remove any contaminating genomic DNA. Three biological repeats were performed for both time points. Strand-specific RNA libraries were constructed and sequenced on a HiSeq 2500 system (Illumina), according to the manufacturer's instructions and sequenced on the Illumina HiSeqTM Xten platform at the Wuhan Igenebook Biotechnology Co., Ltd. (www.igenebook.com).

2.3 | RNA-Seq data processing, assembly, and annotation

The cleaning of the raw data was first done by removing the adaptor-containing sequences, poly-N, and low-quality reads, with those reads shorter than 40 bp being removed with Q-value ≤5. The remaining high-quality, clean reads were used in subsequent analyses. Assembly was then performed with Trinity software with min_kmer_cov set to generate contigs and unigenes. All other parameters were set

TABLE 1 Primers used in transcript abundance

Name	Forward primer (5′−3′)	Reverse primer (5′−3′)
Actin	TCTTTCCCTCTATGCCAGTG	CCAGGTCAAGTCGCAGAAT
RcNAC1	CCACCCCGATGTCATTCCT	CGATTTTGCGTCCTCCTGCTA
RcNAC2	ACTGCCTCGACGACACCTC	CTTTGCCCTCCTTGCTCTT
RcNAC3	AGTTGGAATCAAGAAGGCG	CGACACAGCACCCATTCAT
RcNAC4	CAGACTAATGAACAGGTGCC	GATTGTAAATGCTCGGTGG
RcMYB1	CGAGTTCCTCAAACCCAG	ATCGTAGCCTCCCATAGC
RcMYB2	TTTTGGGCAACAAGTGGTC	AAGCCTCTTCCTAATGTGGGT
RcMYB3	ATCTGCCAAAGACTACTGCT	CCTCCACAACCAAACCATC
RcMYB4	TTGGAAACAGGTGGTCAT	CTCTTGTGCTGCTGGATT
RcMYB5	CAAGCCGAAGACGAAACC	ATTACCCTCGCCCTGATG

to their defaults. To remove the redundancy of the Trinity-assembled contigs, the contigs were again assembled de novo using iAssembler. The final unigenes were then annotated using the National Center for Biotechnology Information nonredundant protein, National Center for Biotechnology Information nonredundant transcript, Swiss-Prot, KEGG (KEGG Ortholog), KOG (eukaryotic ortholog groups), and Gene Ontology (GO) libraries using the BLASTX algorithm with a significance threshold of E-value $\leq 10-5$. The calculation of the unigene expression was performed using the FPKM (fragments per kb per million reads) method. Differentially transcribed genes were analyzed by the edgeR package and defined as genes with a false discovery rate of < 0.001 and at least a twofold difference. Transcription factors were predicted by BLASTX searching of plantTFDB with E-value $\leq 10-5$. KEGG pathway enrichment of differentially transcribed genes was performed using KOBAS. The GO term enrichment was analyzed by the GOseq R package based on Wallenius noncentral hypergeometric distribution.

2.4 | Quantitative RT-PCR

To confirm the RNA-Seq results, the transcript abundance of six selected genes was analyzed using quantitative real team polymerase chain reaction (qRT-PCR). Briefly, the total RNAs of three biological repeats were equivalently mixed for each sample. complementary DNA was generated using Takara Reverse Transcriptase M-MLV, and 1 μL of the first strand of complementary DNA was used as a template in the reaction with the KAPATM SYBRR quantitative PCR kit (Takara), which was run on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). The primers used for determining transcript abundance are listed in Table 1.

2.5 | RT-PCR verification method

From the transcription group data of 0/24 h, the difference in expression was significant for MYB class transcription factor 5 (easy to count, set to MYB1, MYB2, MYB3, MYB4, MYB5) and NAC class transcription factor 4 (easy to count, set to NAC1, NAC2, NAC3, NAC4) for verification analysis. The selected genes are found in Tables 1 and 2. The expression patterns of nine genes (0, 2, 6, 12, 24, 48 h) under salt stress were analyzed in the root system.

2.6 | The statistical method of the data

All experiments were performed with at least three replicants. The significance of differences was determined by ANOVA or Student's *t* test.

3 | RESULTS

3.1 | Sequencing data analysis

Raw data obtained by sequencing 24 transcription groups was obtained between 37,670,038–64,735,678 raw reads, and after filtering out low-quality reads containing adapter sequences, 32,894,172–53,787,036. The GC-rich content of the 24 transcription group data was 45.42%−49.36%, and the Q20 level was greater than 97% (Supplemental Table S1). The clean data of 24 transcription groups can be compared to the monthly reference genome reads between 16,447,086–26,893,518 (ratio = 41.7–93.2%); most transcription group data can be compared to the monthly genome. Among them, the proportion of reads with the only compared position in the monthly reference sequence was 39.18–89.17% (Supplemental Table S2). A total of 39,669 genes were obtained by

TABLE 2 The number of transcription factors upregulated and downregulated in leaves and roots at different processing time

	CK vs. 2 h				CK vs. 24 h				CK vs. 48 h			
	Up		Down		Up		Down		Up		Down	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
MYB	0	5	7	6	15	33	8	10	29	29	12	9
WRKY	1	1	0	3	17	20	1	4	24	22	2	2
NAC	2	1	1	2	7	19	3	5	10	19	6	8
HSF	1	2	2	1	4	4	0	0	3	5	2	3
bHLH	2	2	0	1	12	11	2	9	16	10	8	7
AP2/ERF	2	4	1	1	7	32	3	9	22	35	1	7
AUX/IAA	1	0	0	0	0	4	3	3	0	2	7	6
C2H2	0	0	2	1	3	13	4	4	5	14	11	5
bzip	0	0	0	0	4	7	4	4	4	8	5	3
GRAS	0	1	0	0	9	13	1	6	10	16	0	3

Note. European Society for Clinical Nutrition and Metabolism (ESPEN) guideline on home parenteral nutrition. CK, treatment group.

the pair. (Data upload description: roots transcription group data has been uploaded under SRA accession: PRJNA587482; ID: SUB6482523; and leaves transcription group data has been uploaded under SRA accession: PRJNA689657; ID: SUB8843934.)

3.2 | Genetic analysis of differences over time periods

The number of different expression genes obtained was 14,689 ($p < .05$) in the analysis of gene expression over different time periods. Among them, the number of root differential expression genes was 14,633, and the number of leaf differential genes was 14,442. The three processing time periods were compared with the control, with 2 h and control with the fewest differential genes and 48 h with the largest number of differential genes. The number of different genes in upward expression increased and then reduced over time, whereas the number of downward expression genes increased over time, and the largest increase in the number of expression genes was in processing 24 h (Figure 1a, b). The number of genes expressed differently over the three processing time periods was very small, with only 137 and 173 (leaf, root), and the number of differential genes expressed in both processing 2 h and processing 24 h was also very small, whereas the number of genes expressed at different rates in both processing 2 h and processing 24 h was higher, at 2,115 and 2,928, respectively (Figure 1c, d).

Based on the differential gene and correlation analysis of 24 samples from different processing time periods, it was found that the control group had a higher correlation with processing time of 2 h and a higher correlation with processing time of 48 h (Figure 2). The trend of gene expression of difference between leaf and root was similar, and the control group was more consistent with the treatment of 2-h expression, and the treatment of 24 h was more consistent with the treatment of 48 h expression. The overall differential gene expression was broadly divided into three modes: reduced expression at 2 h, upward expression from 24 h, and maximum expression at 48 h.

3.3 | Differences in GO and KEGG orthology

The result on KEGG orthology and GO differences between LCK vs. L48, RCK vs. R48 are provided in Figures 3 and 4. The result on Pathway classification for differentially expressed genes is provided in Figure 5. There are 239, 476, and 557 GO terms in the blades for the three processing periods, and 226, 572, and 553 GO items in the root.

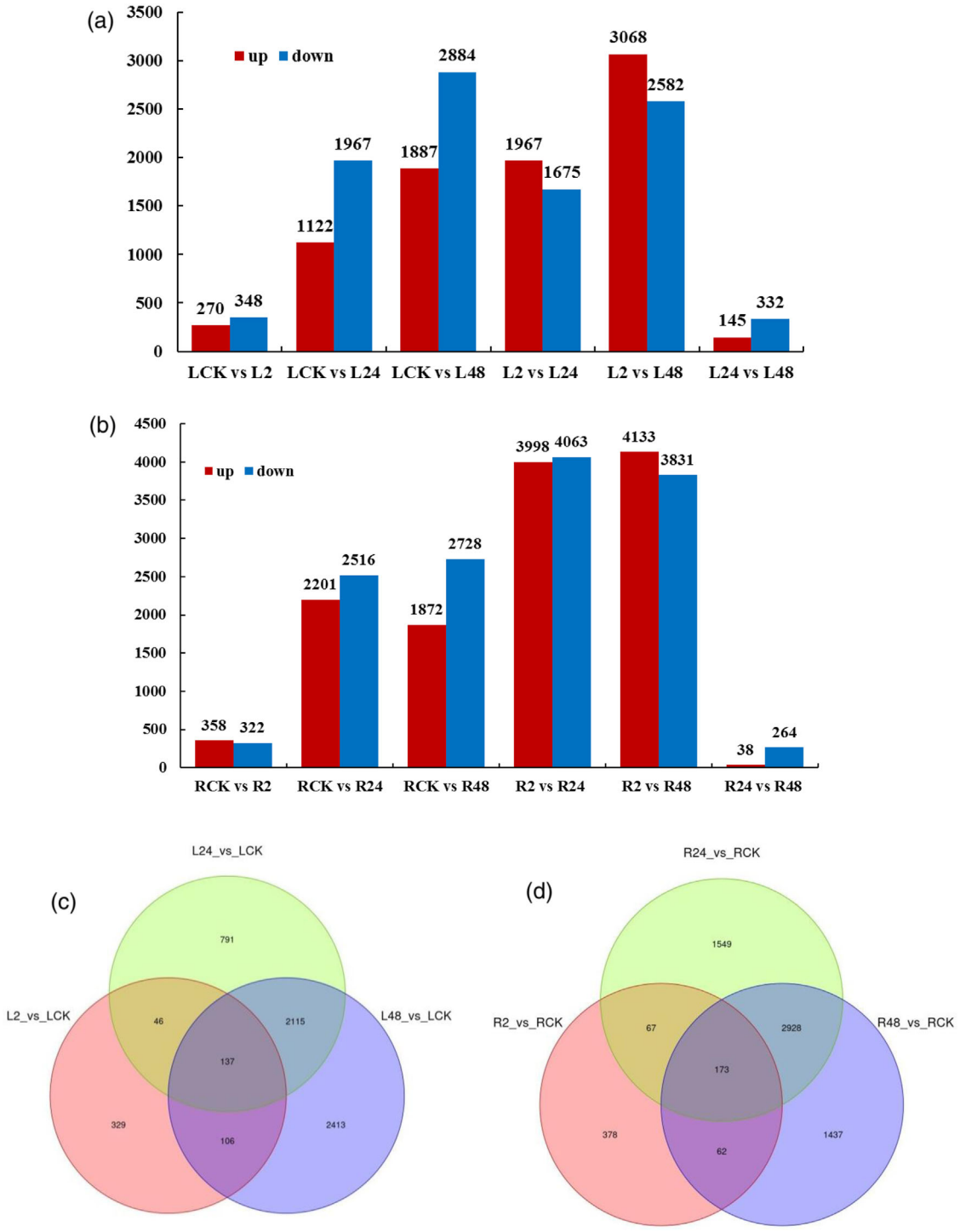

FIGURE 1 The number of up- and downregulated genes in (a) leaf and (b) root for and number of differentially expressed genes in (c) leaf and (d) root

There were differences between the entries for the GO riches of the leaves and roots. The top 20 in the processing of the GO riches in the blades were trehalose biosynthetic process (GO:0005992), protein refolding (GO:0042026), superoxide dismutase (SOD) activity (GO:0004784), starch Catabolic process (GO:0005983), and proline biosynthetic process (GO:0006561), and so on, whereas the root GO rich collection in the top 20 were secondary active sulfate transmembrane transporter activity (GO:0008271), cell wall macromolecule catabolic process (GO:0016998)transferase activity, transferring hexosyl groups(GO:0016758)oxidoreductase activity, acting on CH-OH group of donors(GO:0016614) and response to wounding (GO:0009611). At 24 h, the top 20 GO-rich concentration

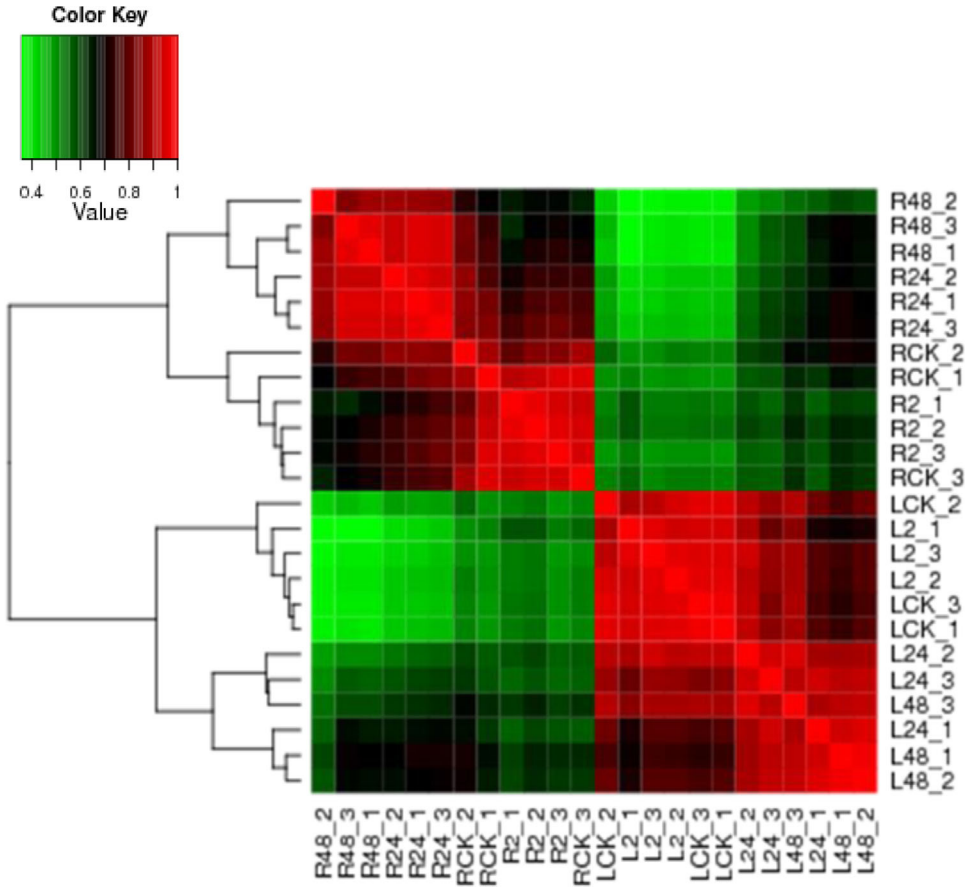

FIGURE 2 Differential gene heat map to express correlation trends in roots and leaves based on different processing time

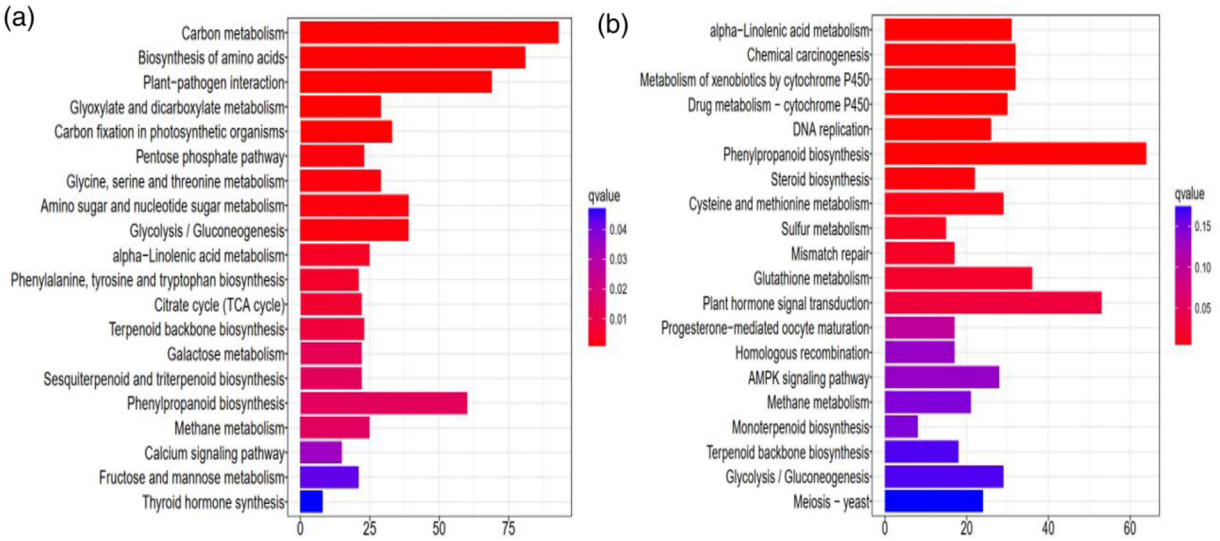

FIGURE 3 KEGG orthology analysis through bar plot for (a) LCK vs. L48 (b) RCK vs. R48

in the blades had response to biotic stimulus (GO:0009607), sequence-specific DNA binding documentation factor activity (GO:0003700), calcium ion binding (GO:0005509), lipid metabolic process (GO:0006629), hydrolase activity (GO:0016787) and response to stress (GO:0006950); 003700), DNA replication (GO:0006260), plant-type cell wall (GO:0009505), peroxidase activity (GO:0004601), and regulation of defense response (GO:0031347). At 48 h, the blade

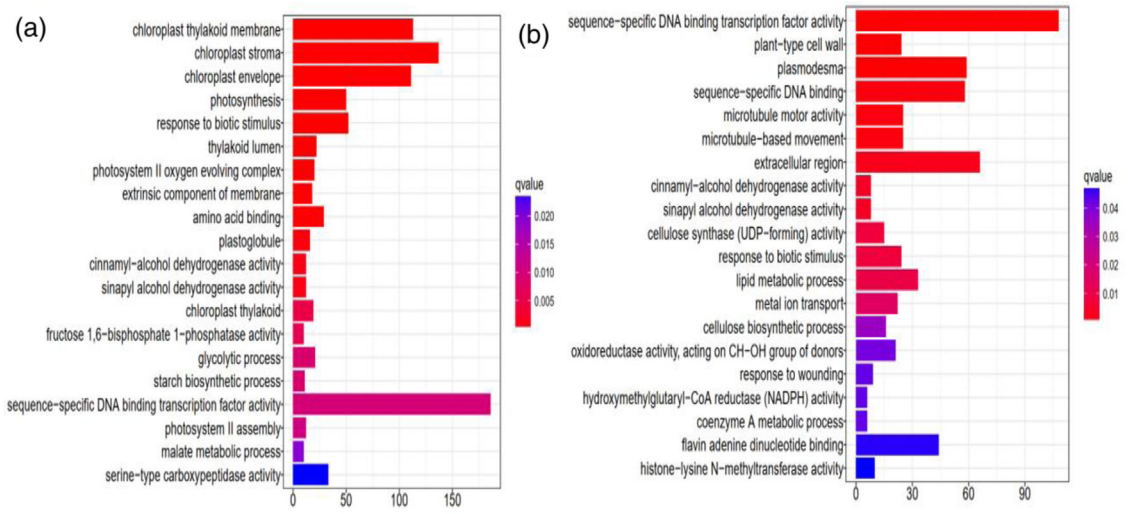

FIGURE 4 Gene Ontology (GE) analysis through bar plot for (a) LCK vs. L48 (b) RCK vs. R48

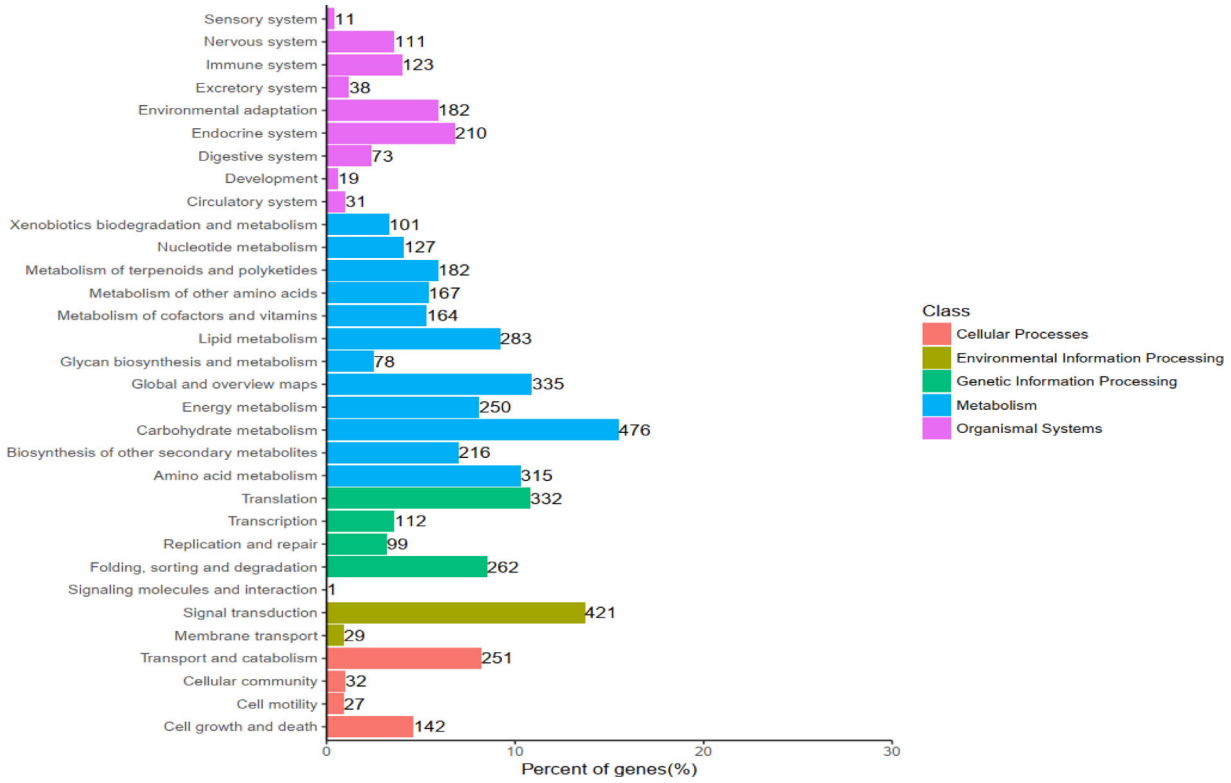

FIGURE 5 Pathway classification for differentially expressed genes

was synthesized in a similar way to the GO entries in the top 20 of the GO-rich collection at 24 h, with different light and effect-related chloroplast stroma (GO:0009570), chloroplast thylakoid (GO:0009535), photosystem II oxygen evolving complex (GO:0009535), and the auxin-activated signaling pathway associated with growth hormone (GO:0009734). At 48 h, the root was comparable to 24-h GO riches, with the same number of GO entries in the top 20 and peroxidase activity (GO:0004601) and regulation of defense response (GO:0031347) not appearing at 48 h, whereas new entries included cellulose synthase (UDP-forming) activity (GO:0016760), hydroxymethylglutaryl-CoA reductase (NADPH) activity (GO:0004420), and metal transport (GO:0030001), and so on.

There were 153, 268, and 277 KEGG pathways in the blades for the three processing periods, and 159, 281, and 279 KEGG pathways within the roots. The KEGG abundance of leaves and roots varied greatly from time to time. The top 10 rich pathways at 2 h within the were flavonoid biosynthesis, porphyrin and chlorophyll metabolism, starch and sucrose metabolism, and phenylalanine, tyrosine, and tryptophan biosynthesis. Within the roots, they included starch and sucrose metabolism, carbohydrate digestion and absorption, isoquinoline alkaloid biosynthesis, tyrosine metabolism, and pentose phosphate pathway. Within the leaf at 24 h, the top 10 rich pathways included biosynthesis of amino acids, terpenoid backbone biosynthesis, alpha-linolenic acid metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, cysteine and methionine metabolism, and protein processing in endoplasmic reticulum. On the other hand, within the roots, they were alpha-linolenic acid metabolism, cysteine and methionine metabolism, glutathione metabolism, and phenylpropanoid biosynthesis. The top 10 rich pathways within the blade at 48 h were carbon metabolism; biosynthesis of amino acids; glyoxylate and dicarboxylate metabolism; carbon fixation in photosynthetic organisms; pentose phosphate pathway; glycine, serine, and threonine metabolism; and amino sugar and nucleotide sugar metabolism. On the roots, they were alpha-linolenic acid metabolism, DNA replication, phenylpropanoid biosynthesis, steroid biosynthesis, cysteine, and methionine metabolism.

3.4 | Signal pathway-related differential genes

Within the blades, most of the Calcium signaling pathway-related genes started at 24 h, with the highest number of upward expressions at 48 h. Among them were calmodulin (RC7G0009300), calcium-binding protein CML45 (RC2G0618500), ADP, ATP carrier protein 1, mitochondrial-like (RC1G0019000), and phosphoinositide polyphase C (RC5G0600100) with log2FoldChange (log2FC) value greater >2. Within the root, the calcium signaling pathway had a smaller number of genes associated with it, mainly at 48 h. Phosphoinositide phospholipase C (RC5G0600100), calmodulin-like protein (RC4G0307100) and ADP, ATP carrier protein 1, and mitochondrial-like (RC1G0019000) had log2FC value of >2. Within the leaf, the mitogen-activated protein kinase (MAPK) signaling pathway-related genes were mostly expressed differently upward from 24 h, with MAPK 5 (RC2G0184500) having log2FC value of >4 and some HSP70s started to show at 24 h. Within the roots, the MAPK signaling pathway was associated with a small number of genes, such as the HSP70 from 2 h. The MAPK 5 (RC2G0184500) having >2 log2FC at 48 h on the upward expression. Within the leaf, most of the plant hormone signal transduction-related genes started at 24 h and at 48 h within the roots. The plant hormones involved were the abscisic acid (ABA), growth hormone, ethylene, erythromycin, jasmine acid (JA), salbonate, and cell division. The coding ausin-responsive protein, auxin-induced protein, auxin transporter-like protein, and auxin response factor gene differences associated with the corresponding signaling pathways of leaf and root growth hormone were expressed in large numbers in a downward expression trend. Most of the genes associated with ethylene response expressed upward. The ethylene-responsive transcription factor 1B was only raised in the root expression at 48 h, and the log2FC was >6. JA-amidosynthetase JAR1 was differentially expressed upward only within the blades. Protein TIFY (repressor of jasmonate responses) was available in both the roots and the leaves. The ABA signaling pathway within the blade began the differential expression from 24 h and from 48 h within the root. The ABA-insensitive 5-like protein in root had log2FC of >4, whereas in the leaf the expression was downward.

3.5 | ROS clears relevant differential gene expression

Peroxisome, ascorbate, and aldarate metabolism-related genes were more differentially expressed in leaves and less in roots. Some of the blades began upward differential expression at 2 h and reduced at 24 h. Genes encoding the SOD (Fe) 3 were only identified to show upward expression in the blade at 2 h. The L-ascorbate oxidase were both down-expressed in both the root and the leaf at 48 h, with the root having log2FC of ←5. (The oxidation of AsA represses responses to high salinity and oxidative stress conditions such as vegetative growth and seed production reductions [AbdElgawad et al., 2016].) The D-galacturonate reductase (involved in ascorbic acid [vitamin C]) biosynthesis was raised in the leaf and lowered in the root. GDP-L-galactose phosphorylase 1, which catalyzes a reaction of the major route to ascorbate biosynsis in plants, was expressed upward in the root at 48 h, with log2FC of >2. It was indicated that salt stress at 2 h induced the ROS removal reaction of peroxidase and astrohetic acid in the leaves, then between 24–48 h gradually increased, whereas for the root it began to respond from 48 h.

3.6 | Differential transcription factor expression

The differentially expressed gene count through volcano for LCK vs. L48 groups and RCK vs. R48 groups are provided in Figure 6. Transcription factors included in

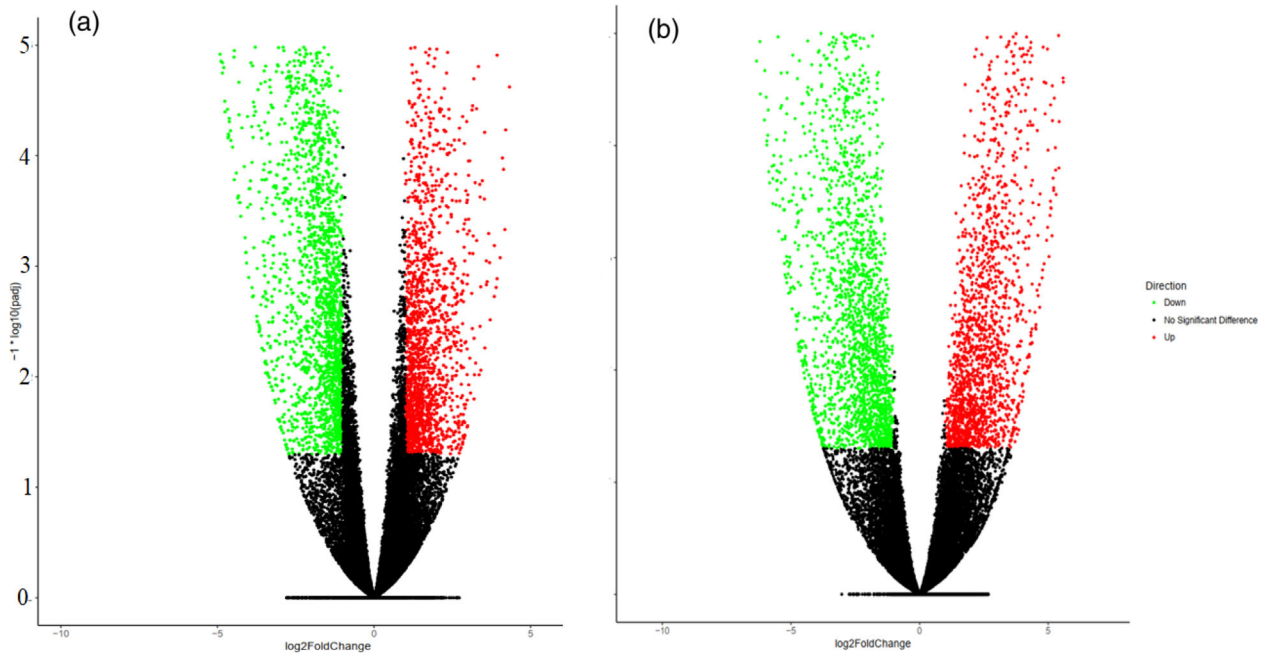

FIGURE 6 Differentially expressed gene count through Volcano for (a) LCK vs. L48 (b) RCK vs. R48

differential genes were MYB, WRKY, NAC, bHLH, AP2/ERF, bzip, and GRAS. MyB, AP2/ERF, and WRKY were among the most varied in the blades and roots. Most transcription factors differed more in the root than in the leaves, especially in the NAC, AP2/ERF, and GRAS categories. Most of the transcription factors in the root of the differential expression of the multiplication was higher than in the leaf. Most transcription factors had a small difference expression at 2 h, and the number increased significantly at 24 h. The bzip class transcription factors had no significant difference expression at 2 h, but there was significant difference in expressions at 24 h (Table 2). Most transcription factors had more upward expression in the leaf and root than downward expression. The auxin/indole-3-acetic acid (AUX/IAA) transcription factors were lower but had upward expression in the root. The C2H2 had lower expression than upward expression in the leaf (Table 1). The largest number of upward expressions in the leaf was in the MYB category, followed by AP2/ERF and WRKY, then AP2/ERF in the root, and lastly MYB and WRKY (Table 3).

Among the blades, the MYB class transcription factor differences expressed the largest number, and the number of upward expressions was higher (Table 2). The number of downward expressions was small, with l log2FC of <3. The MYB4, MYB41, MYB44, MYB20, MYB62 and MYB14 had log2FC of >4. The MYB41 was upwardly expressed in the blades, and the rest were upwardly expressed in the leaves and roots. The WRKY class transcription factors were second only to MYB in the number of differential expressions in the blades, and more than 80% were had upward expressions. The WRKY70, WRKY47, WRKY40, and WRKY71 had log2FC of >4. In addition, the number of bHLH transcription factors expressed differently in the blades was also higher, with log2FC of bHLH162 and bHLH35 being >6.

Among the roots, the AP2/ERF class transcription factor differences were largely expressed, with more than 70% of the expression being upward and the log2FC was >4 (Table 4). For the genes such as ERF5, ERF096, ERF2, ERF020, ERF098, ERF09, ERF022, and ERF109, the log2FC was about 7. NAC-like transcription factors had more differential genes in the root than in the leaves, and the multiples of differential expression were higher than those in the leaves. The log2FC for NAC67, NAC52, NAC16, NAC2 was >4.

3.7 | RT-PCR verification results

The result for RNA sequencing expression analysis and RT-PCR are provided in Figure 7. Based on this result, it is explained that the results of RT-PCR are basically consistent with the results of sequencing expression analysis.

4 | DISCUSSION AND CONCLUSION

Both salt stress and salt shock display an osmotic part and an ionic segment that are liable for the restraint of plant development. The osmotic stress limits water take-up, causing loss of turgor prompting a higher centralization of particles in the cells. The ionic part causes harmfulness in plants and can

TABLE 3 MYB in leaf

No.	ID	CK vs. 2 h	CK vs. 24 h	CK vs. 48 h	Annotation
1	RC7G0579100	NA	−2.266386	NA	transcription factor MYB30-like [*Rosa chinensis*]
2	RC7G0045700	−1.226377	NA	NA	myb-related protein 306-like [*Rosa chinensis*]
3	RC3G0259500	NA	1.1487208	NA	transcription factor MYB3R-2 isoform X1 [*Rosa chinensis*Jacq.]
4	RC2G0625000	−1.566321	−1.898919	NA	transcription factor MYB30-like [*Rosa chinensis*]
5	RC2G0615800	−1.02599	NA	NA	transcription factor MYB106 [*Rosa chinensis*]
6	RC3G0063500	−1.332432	−1.603894	NA	protein REVEILLE 1-like isoform X1 [*Rosa chinensis*]
7	RC2G0560100	NA	6.493386	6.947074	transcription factor MYB4-like [*Rosa chinensis*]
8	RC6G0380400	NA	5.818942	6.393081	putative transcription factor MYB-HB-like family [*Rosa chinensis*]
9	RC2G0561300	NA	3.987836	6.322756	transcription factor MYB41-like [*Rosa chinensis*]
10	RC6G0556000	NA	3.603097	5.500603	transcription factor MYB44-like [*Rosa chinensis*]
11	RC3G0034500	NA	1.621725	5.233227	transcription factor MYB20-like [*Rosa chinensis*]
12	RC7G0082400	NA	4.152233	4.434101	transcription factor MYB62-like [*Rosa chinensis*]
13	RC4G0046100	NA	3.785528	4.241031	transcription factor MYB14-like [*Rosa chinensis*]
14	RC4G0311300	NA	2.8303718	3.673687	transcription factor MYB108 [*Rosa chinensis*]
15	RC5G0053800	NA	1.896429	3.3074724	transcription factor MYB8-like [*Rosa chinensis*]
16	RC2G0560200	NA	NA	3.204665	myb-related protein 308-like [*Rosa chinensis*]
17	RC7G0384200	NA	NA	3.1348273	transcription factor CSA-like isoform X2 [*Rosa chinensis*]
18	RC7G0384300	NA	NA	3.0946064	transcription factor MYB54-like isoform X1 [*Rosa chinensis*]
19	RC0G0132900	NA	2.3325997	2.9945637	myb-related protein 308-like [*Rosa chinensis*]
20	RC5G0056400	NA	NA	2.796574	transcription factor MYB102 [*Rosa chinensis*]
21	RC6G0094600	NA	1.6915917	2.7464021	transcription factor MYB44-like [*Rosa chinensis*]
22	RC5G0225600	NA	NA	2.675067	transcription factor MYB63 [*Rosa chinensis*]
23	RC2G0079200	NA	1.9399311	2.4386356	transcription factor MYBS3-like [*Rosa chinensis*]
24	RC7G0458900	NA	NA	2.3247156	transcription repressor MYB6-like [*Rosa chinensis*]
25	RC6G0024600	NA	NA	2.1401296	myb-related protein 308-like [*Rosa chinensis*]
26	RC5G0590300	NA	1.6184969	1.9297468	transcription factor DIVARICATA-like [*Rosa chinensis*]
27	RC2G0201200	NA	1.145955	1.8719987	transcription factor MYB73 [*Rosa chinensis*]

(Continues)

TABLE 3 (Continued)

No.	ID	CK vs. 2 h	CK vs. 24 h	CK vs. 48 h	Annotation
28	RC0G0047300	NA	NA	1.7363445	transcription factor MYBS3 [*Rosa chinensis*]
29	RC6G0596300	NA	NA	1.4270468	transcription factor MYB8-like [*Rosa chinensis*]
30	RC1G0176800	NA	NA	1.2896645	transcription factor DIVARICATA-like [*Rosa chinensis*]
31	RC0G0132600	NA	NA	1.2894038	transcription repressor MYB6-like [*Rosa chinensis*]
32	RC7G0284500	NA	NA	1.0537836	transcription factor MAMYB [*Rosa chinensis*]
33	RC1G0468300	NA	NA	−1.10638	transcription factor DIVARICATA-like [*Rosa chinensis*]
34	RC1G0394600	−2.272019	−2.728139	−1.190327	protein LHY-like [*Rosa chinensis*]
35	RC4G0444600	NA	NA	−1.290348	transcription repressor MYB6-like [*Rosa chinensis*]
36	RC7G0074400	NA	NA	−1.452184	transcription factor MYBS1 [*Rosa chinensis*]
37	RC3G0322900	NA	NA	−1.832369	transcription factor MYB61-like [*Rosa chinensis*]
38	RC6G0179400	NA	NA	−1.995761	protein RADIALIS-like 3 [*Rosa chinensis*]
39	RC3G0243200	−1.702914	−1.213961	−2.427922	protein REVEILLE 8-like [*Rosa chinensis*]
40	RC1G0332500	NA	NA	−2.854403	transcription factor MYB41 [*Rosa chinensis*]
41	RC7G0249900	NA	NA	−3.020548	transcription factor WER-like [*Rosa chinensis*]
42	RC6G0285000	NA	NA	−3.411642	transcription factor MYB73-like [*Rosa chinensis*]
43	RC7G0006800	−1.195101	−1.842792	−3.685584	protein REVEILLE 7-like isoform X1 [*Rosa chinensis*]

Note. NA, not available; CK, treatment group.

TABLE 4 AP2/ERF in root

No.	ID	CK vs. 2 h	CK vs. 24 h	CK vs. 48 h	Annotation
1	RC2G0064500	NA	−3.118129	NA	ethylene-responsive transcription factor TINY-like [*Rosa chinensis*]
2	RC2G0274000	−1.340057	NA	NA	ethylene-responsive transcription factor RAP2-4-like [*Rosa chinensis*]
4	RC5G0530900	1.0579298	NA	NA	ethylene-responsive transcription factor RAP2-7 isoform X2 [*Rosa chinensis*]
5	RC6G0507700	NA	3.2306804	NA	dehydration-responsive element-binding protein 2D-like [*Rosa chinensis*]
6	RC6G0479700	NA	−1.407206	NA	ethylene-responsive transcription factor ERF003-like [*Rosa chinensis*]
7	RC7G0206900	NA	3.8933261	NA	dehydration-responsive element-binding protein 1E-like [*Rosa chinensis*]
8	RC7G0206600	NA	2.1172964	NA	dehydration-responsive element-binding protein 1E-like [*Rosa chinensis*]
9	RC2G0364800	NA	1.7342325	NA	ethylene-responsive transcription factor ERF110-like isoform X1 [*Rosa chinensis*]
10	RC2G0338100	NA	−1.802108	NA	ethylene-responsive transcription factor ERF054 [*Rosa chinensis*]
11	RC1G0372200	NA	6.9137449	9.5341111	ethylene-responsive transcription factor CRF5-like [*Rosa chinensis*]
12	RC6G0381400	NA	6.8307416	8.0639128	ethylene-responsive transcription factor ERF096-like [*Rosa chinensis*]
13	RC1G0273700	NA	5.0747056	7.7247229	ethylene-responsive transcription factor 2-like [*Rosa chinensis*]
14	RC7G0484200	2.4749585	5.945998	7.6257142	ethylene-responsive transcription factor ERF020-like [*Rosa chinensis*]
15	RC6G0381200	NA	5.6301716	7.2555618	ethylene-responsive transcription factor ERF098-like [*Rosa chinensis*]
16	RC2G0190100	NA	NA	7.025174	ethylene-responsive transcription factor ERF109-like [*Rosa chinensis*]
17	RC6G0116600	NA	5.1148556	6.9598294	ethylene-responsive transcription factor ERF109 [*Rosa chinensis*]
18	RC3G0395900	1.496116	5.743662	6.909615	ethylene-responsive transcription factor 1B-like [*Rosa chinensis*]
19	RC7G0484300	NA	4.3861626	6.8635095	ethylene-responsive transcription factor ERF020-like [*Rosa chinensis*]
20	RC1G0273500	NA	4.5332943	6.8530598	ethylene-responsive transcription factor 2-like [*Rosa chinensis*]
21	RC6G0381500	NA	4.1284062	5.9294796	ethylene-responsive transcription factor ERF098-like [*Rosa chinensis*]
23	RC7G0250400	NA	3.7670404	5.718745	ethylene-responsive transcription factor ERF039-like [*Rosa chinensis*]
24	RC7G0256500	NA	3.7826592	4.808005	LOW QUALITY PROTEIN: ethylene-responsive transcription factor 2 [*Rosa chinensis*]
25	RC5G0632700	NA	3.423058	4.8018572	pathogenesis-related genes transcriptional activator PTI6-like [*Rosa chinensis*]
26	RC4G0042100	NA	NA	4.4454322	ethylene-responsive transcription factor ERF098-like [*Rosa chinensis*]
27	RC1G0273400	NA	3.4346636	4.3080043	ethylene-responsive transcription factor 1-like [*Rosa chinensis*]
28	RC7G0485800	NA	NA	4.1571082	ethylene-responsive transcription factor ERF022-like [*Rosa chinensis*]
29	RC1G0484900	NA	2.0217816	3.7525474	dehydration-responsive element-binding protein 2A-like [*Rosa chinensis*]

(Continues)

TABLE 4 (Continued)

No.	ID	CK vs. 2 h	CK vs. 24 h	CK vs. 48 h	Annotation
30	RC1G0508200	NA	2.4611221	3.5734864	ethylene-responsive transcription factor ERF104-like [*Rosa chinensis*]
31	RC2G0103200	NA	2.9939313	3.5320675	ethylene-responsive transcription factor ERF017 [*Rosa chinensis*]
32	RC7G0256900	NA	3.018862	3.5270022	putative transcription factor RAV family [*Rosa chinensis*]
33	RC3G0393600	NA	3.3042637	3.365743	ethylene-responsive transcription factor RAP2-11-like [*Rosa chinensis*]
34	RC6G0381100	NA	3.1095011	3.3031895	ethylene-responsive transcription factor ERF098-like isoform X3 [*Rosa chinensis*]
35	RC5G0090100	NA	2.2794234	3.2873985	pathogenesis-related genes transcriptional activator PTI5-like [*Rosa chinensis*]
36	RC5G0090300	NA	2.3681794	3.2797074	ethylene-responsive transcription factor ERF091 [*Rosa chinensis*]
37	RC7G0207100	NA	NA	3.152628	dehydration-responsive element-binding protein 1E-like isoform X1 [*Rosa chinensis*]
38	RC1G0508100	NA	3.3327754	3.124107	ethylene-responsive transcription factor 2-like [*Rosa chinensis*]
39	RC1G0534000	NA	NA	2.7278272	dehydration-responsive element-binding protein 1B-like [*Rosa chinensis*]
40	RC1G0423500	1.0075112	1.2801422	2.5624262	ethylene-responsive transcription factor RAP2-7-like isoform X1 [*Rosa chinensis*]
41	RC6G0257700	NA	2.2491186	2.4199494	ethylene-responsive transcription factor ABR1-like [*Rosa chinensis*]
42	RC2G0241000	NA	NA	2.1389708	putative transcription factor AP2-EREBP family [*Rosa chinensis*]
43	RC1G0508300	NA	2.509674	1.8784431	ethylene-responsive transcription factor ERF107-like [*Rosa chinensis*]
45	RC6G0437700	NA	NA	1.5401107	ethylene-responsive transcription factor ERF071 [*Rosa chinensis*]
46	RC6G0581900	NA	−4.17904	−2.445817	AP2-like ethylene-responsive transcription factor ANT [*Rosa chinensis*]
47	RC2G0221800	NA	−1.992294	−2.534393	AP2-like ethylene-responsive transcription factor ANT [*Rosa chinensis*]
48	RC4G0143100	NA	NA	−2.778912	AP2-like ethylene-responsive transcription factor AIL5 [*Rosa chinensis*]
49	RC5G0663500	NA	−2.377109	−3.115232	putative transcription factor AP2-EREBP family [*Rosa chinensis*]
50	RC3G0418300	NA	−3.349891	−3.249107	AP2-like ethylene-responsive transcription factor BBM [*Rosa chinensis*]
51	RC2G0473200	NA	NA	−3.657577	ethylene-responsive transcription factor TINY [*Rosa chinensis*]
52	RC4G0332300	NA	−4.24908	−3.841703	ethylene-responsive transcription factor LEP-like [*Rosa chinensis*]

Note. NA, not available; CK, treatment group.

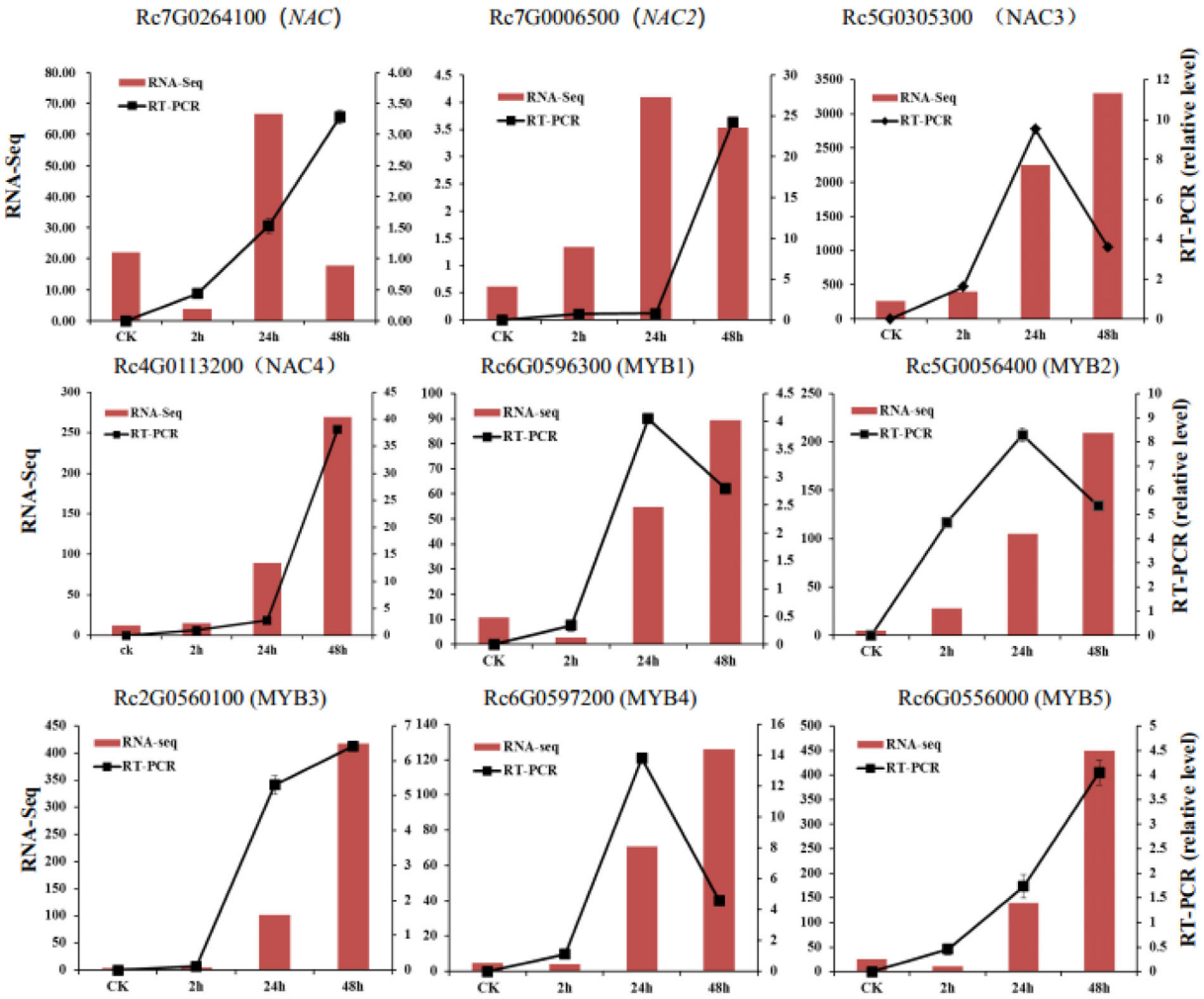

FIGURE 7 RNA sequencing expression analysis and real team polymerase chain reaction (RT-PCR) for different genes (NAC, NAC2, NAC3, NAC4, MYB1, MYB2, MYB3, MYB4, MYB5) at difference processing time durations

prompt cell passing because of unnecessary amassing of particles. The ionic changes happen as a result of solute irregularity, remembering an abatement for the proportion between K+/Na+ and aggregation of Na+ and Cl+ in the cytosol. In addition, salt shock incites osmotic stress (plasmolysis) because of the contrast among outer and inward solutes in the cell cytoplasm. During salt stress, higher convergences of Na+ particles are shipped to the shoot, and a few qualities are actuated in light of osmotic shock (Shavrukov, 2013). At the cell level, an overabundance of the sodium (Na+) and chloride (Cl-) ions incites harmfulness, layer disruption, decrease of leaf surface extension, loss of turgor, lack of hydration, and decrease of root development and stretching. Under salt pressure, creating plants show unnecessary take-up of these particles, advancing injury arrangement, and sudden passing of leaves (Hasegawa, 2013).

Studies showed that a rapid ion of cytosolic Ca^{2+} under sodium stress can also be captured by other Ca^{2+} sensors, including calmodulin, calmodulin-proteins, and calcium-like dependent protein (Cho et al., 2016; Galon et al., 2010). Multiomics techniques revealed that calmodulin exhibits salt-induced expression changes at the transcriptional and translational levels in seedlings or roots, suggesting that these Ca2+ sensors participate in salt stress signaling (El Mahi et al., 2019; Shen et al., 2018; Zhu et al., 2015). In the middle of the month, the calmodulin (RC7G0009300) and calcium-binding protein CML45 (RC2G0618500) in the blades were raised from 12-h processing and the log2FC value was greater than 2, whereas in the root, only calmodulin-like protein 2 (RC4G0307100) was raised from 48-h processing. This was evidence that the calcium signaling pathway responds later in roots than in the blades in the monthly season *Rosa hybrida*. The salt stress measured monthly within 48-h Ca^{2+} sensors was mainly calmodulin and calcium-binding protein.

The MAPK signaling pathway has been confirmed to be involved in various adversity responses of plants, including

salt stress (Kumar et al., 2020). Most of the genes associated with the monthly leaf MAPK signaling pathway were significantly differentially expressed from the 24-h processing time. Root MAPK signaling pathway was among the ranks in the KEGG rich top 20 genes. The involved genes began to show significant differences from the 2-h processing time, with the largest number of differential genes being at 48-h processing time, and the root MAPK signaling pathway was shown to start earlier in the root as compared to the blade. MAPK cascades are an important component of signal transduction in plants operating under diverse environmental stress and are involved in the regulation of salt stress signaling pathway (Wang et al., 2014). Indeed, transcript analysis of *Arabidopsis* MAPK pathway genes under salinity stress revealed that four MAPK kinase kinases (MEKKs) (namely MEKK3, MEKK5, MEKK6, and MEKK7) were induced (Moustafa et al., 2008). In fact, according to the present study, the monthly MAPK 5 (RC2G0184500) begun to show significant increase from 24-h process time within the blades and from 48-h processing time within the root.

Reactive oxygen species, which function as versatile signals, are rapidly induced by a variety of environmental stresses, including high salinity, drought, and heat stress. To reduce the oxidative stress caused by the accumulation of ROS under high salinity, plants rely on the activation of ROS-scavenging machineries. The scavenging of excessive ROS under high salinity may be attributed to nonenzymatic antioxidant metabolites, including ascorbate, glutathione, and tocopherols, and to enzymatic agents, such as catalases, SOD, ascorbate peroxidase, and glutathione reductase (Hanin et al., 2016). Indeed, within the season, there was an increase of from 2-h processing time and an increase in L-ascorbate peroxidase from 48-h processing time within the blade, whereas L-ascorbate oxidase increased within the root form 48-h processing time.

Transcription factor families, such as NAC, ERF/AP2, bZIP, MYB, and WRKY (Sun et al., 2020), have been found to be involved in the salt stress response. A large number of transcription factors responding to salt stress were also found during the month, and most of the transcription factors began to show significant differences in 24 h. This indicated that 24 h may be a key point in turning on transcription regulation under the stress of monthly salt. MYB transcription factors on plant salt stress have been studied (Gao et al., 2017). The abscisic acid biosynthesis and signaling during salt stress and the participation in SOS pathway have also been reported (Fang et al., 2018). In the current study, within the blades and roots, the number of different MYB transcription factors was higher, most of which were expressed in 24-h differentials. The increase in the number of expressions expanded with time. Within the blades, MYB class transcription factor differences were expressed in the largest number. Among them, MYB4, MYB41, MYB44, MYB20, MYB62, and MYB14 had log2FC of >4. Moreover, MYB41 has been associated with osmosis stress (Lippold et al., 2009), whereas MYB4 regulatory genes had been associated with hypothermia, salt, drought, and so on (Vannini et al., 2006). Futher, MYB44, MYB20, and MYB14 had also been reported to be associated with salt stress (Dong etal. et al., 2016; Zhengkun et al., 2019).

The ERF and ERF transcription factors were studied in plant salt stress. ERF transcription factors that increase salt resistance were detected in plants such as tobacco (Zhenjun et al., 2018), apples (Han et al. 2020), *Lotus corniculatus* (Sun et al. 2014) and cotton (Long et al., 2019). ERF transcription factors had the largest number of differential expressions in the root, with more than 70% representing upward expression. ERF109's log2FC was about 7. According to Bahieldin et al. (2018), ERF109 was affected by salt stress in Arabidopsis, and acts as a "master switch" mediator of a cascade of consecutive events across phenylalanine, tyrosine and tryptophan biosynthesis, tryptophan metabolism and plant hormone signal transduction initially by driving expression of ASA1 and YUC2 genes and possibly driving GST, IGPS, and LAX2 genes.

The role of WRKY transcription factors in plant salt stress has been extensively studied (Cai et al., 2017; Yan et al., 2014). WRKY class transcription factors were second only to MYB in the number of differential expressions in blades, and more than 80% had upward expression. WRKY70, WRKY47, WRKY40, WRKY71 had log2FC of >4. WRKY40 has an effect on plant sensitivity to ABA (Chen et al., 2010) and was linked to oxidative stress tolerance (Gong, et al., 2014) with WRKY71 and WRKY47 being resistant (Raineri et al., 2015). Several studies have also captured NAC in plant anti-salt stress (Wang et al., 2019; Yu et al., 2016). NAC-like transcription factors had more differential genes in the root than in the leaves, and the multiples of differential expression were higher than those in the leaves. Log2FoldChange of NAC67, NAC52, NAC16, and NAC2 was >4. Indeed, the overexpression of a NAC 67 transcription factor from finger millet (*Eleusine coracana* L.) was confirmed to confer tolerance against salinity and drought stress in rice (Rahman et al., 2016). Furthermore, the OsNAC52, a rice NAC transcription factor, potentially responds to ABA and confers drought tolerance in transgenic plants (Gao et al., 2010).

Response and adaptation to salt stress require the integration and coordination of multiple phytohormones, including ABA, JA, gibberellic acid, ethylene, and salicylic acid (Zhao et al., 2020). Most of the plant hormone signal transduction-related genes started to differ from with 24 h within the leaves and 48 h within the roots. The plant hormones involved were ABA, growth hormone, ethylene, erythromycin, JA, salbonate, and cell division. Increasing evidence has linked the jasmonate pathway to salt stress responses in plants (Kazan, 2015). Transcriptomic studies revealed that many jasmonate-

biosynthesis genes are upregulated under salt stress and that the JA signaling pathway is involved in the regulation of salt stress-responsive genes (Yu Geng et al., 2013). Jasmonate may act as a positive or negative regulator of salt stress response in a spatially and temporally dependent manner. Within the mid-season, the root jasmonate response was earlier, and protein TIFY (repressor of jasmonate responses) began to significantly increase its expression from 2-h processing time. The JA-amido synthetase was only up at 24-h processing time. Regulatory protein NPR1 and NPR2 (negatively regulators JA-dependent signaling pathway) increased. Specifically, in the root, the NPR1 increased at processing of 48 h to log2FC of >8. This indicated that high salt content may inhibit the monthly JA pathway.

Ethylene is also involved in salt stress tolerance in plants. Mutations in ethylene signaling pathway-associated genes, such as ETR1, EIN4, EIN2, and EIN3, lead to hypersensitivity to high salinity (Peng et al., 2014; Zhang et al., 2012). The ethylene-related genes began to express significant differences from 24-h processing time, and the expression was mostly upward. EIN4 and EIN3 were only raised in the leaf. Serine/threonine-protein kinase CTR1 (acts as a negative regulator in the ethylene response pathway) was raised in the root, whereas the ethylene pathway was mainly in the leaves. Growth hormone signaling pathways are also involved in plant salt stress studies (Lu et al., 2015). In the middle of the month, the growth hormone signaling pathway differential expression gene (auxin-induced protein, auxin-responsive protein, auxin-response factor, etc.) was more, and lowered more. Whereas auxin-responsive protein SAUR72 (auxin transport) and SAUR50 raised in the leaves, the other auxin-responsive protein and SAUR expression in the root were low. SAUR71 plays a role in the regulation of cell expansion, root meristem patterning, and auxin transport. Stresses often regulate auxin signaling by affecting Aux/IAA protein stability. The auxin-responsive protein IAA and Aux/IAA proteins are short-lived transcriptional factors that function as repressors of early auxin response genes at low auxin concentrations. In the current study, the IAA2 was revised upward in the leaf, IAA11 was revised upward in the root, whereas the rest were revised downwards. Among the hormones, ABA is the most involved in the response to diverse abiotic stresses. Osmotic stress imposed on roots results in a very rapid (within several minutes) and massive increase in ABA concentration in both root and leaf tissue. ABA-insensitive 5-like protein (involved in ABA and stress responses) and ABA receptor PYL2-like is required for ABA-mediated responses.

ACKNOWLEDGMENTS

The authors thank Dr. Yu Han (National Engineering Research Center for Floriculture, Beijing Forestry University) for assistance with the experiments and her excellent suggestions. We also thank all the colleagues in our lab for constructive discussion and technical support. This work was supported by grants from Science and Technology Research Project of Higher Education in Hebei Province (BJ2017102) and Doctoral Foundation of Tangshan Normal University (2014A06). The authors also thank Wuhan Igenebook Biotechnology Co., Ltd (Wuhan, China) for technical support.

AUTHOR CONTRIBUTIONS

Ying Bao: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Validation; Visualization; Writing-original draft; Writing-review & editing. Chao Chen: Conceptualization; Data curation; Formal analysis; Investigation; Resources; Visualization. Ling Fu: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Validation; Visualization; Writing-original draft. Yuqing Chen: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing-original draft; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Ying Bao https://orcid.org/0000-0003-2138-3541

REFERENCES

AbdElgawad, H., Zinta, G., Hegab, M. M., Pandey, R., Asard, H., & Abuelsoud, W. (2016). High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. *Frontiers in Plant Science*, *7*, 276. https://doi.org/10.3389/fpls.2016.00276

Aghajanzadeh, T. A., Reich, M., Kopriva, S., & De Kok, L. J. (2018). Impact of chloride (NaCl, KCl) and sulfphate (Na_2SO_4, K_2SO_4) salinity on glucosinolate metabolism in *Brassica rapa*. *Journal of Agronomy and Crop Science*, *204*, 137–146. https://doi.org/10.1111/jac.12243

Ahmad, I., Aslam Khan, M., Qasim, M., Ahmad, R., & Us-samad, T. (2015). Growth, yield and quality of *Rosa hybrida* L. as influenced by NaCl salinity. *Journal of Ornamental Plants*, *3*(3), 143–153.

Bahieldin, A., Atef, A., Edris, S., Gadalla, N. O., Ramadan, A. M., Hassan, S. M., Al Attas, S. G., Al-Kordy, M. A., Al-Hajar, A. S. M., Sabir, J. S. M., Nasr, M. E., Osman, G. H., & El-Domyati, F. M. (2018). Multifunctional activities of ERF109 as affected by salt stress in Arabidopsis. *Scientific Reports*, *8*(1), Article 6403. https://doi.org/10.1038/s41598-018-24452-6

Bendahmane, M., Dubois, A., Raymond, O., & Bris, M. L. E. (2013). Genetics and genomics of flower initiation and development in roses. *Journal of Experimental Botany*, *64*(4), 847–857. https://doi.org/10.1093/jxb/ers387

Bourke, P. M., Gitonga, V. W., Voorrips, R. E., Visser, R. G. F., Krens, F. A., & Maliepaard, C. (2018). Multi-environment QTL analysis of plant and flower morphological traits in tetraploid rose.

Theoretical and Applied Genetics, *131*, 2055–2069. https://doi.org/10.1007/s00122-018-3132-4

Cai, R., Dai, W., Zhang, C., Wang, Y., Wu, M., Zhao, Y., Ma, Q., Xiang, Y., & Cheng, B. (2017). The maize WRKY transcription factor ZmWRKY17 negatively regulates salt stress tolerance in transgenic *Arabidopsis* plants. *Planta*, 2017, *246*(6), 1215–1231. https://doi.org/10.1007/s00425-017-2766-9

Cai, X., Niu, G., Starman, T., & Hall, C. (2014). Response of six garden roses (*Rosa hybrida* L.) to salt stress. *Scientia Horticulturae*, *168*, 27–32. https://doi.org/10.1016/j.scienta.2013.12.032

Chen, H., Lai, Z., Shi, J., Xiao, Y., Chen, Z., & Xu, X. (2010). Roles of *Arabidopsis* WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. *BMC Plant Biology*, *10*, 281. https://doi.org/10.1186/1471-2229-10-281

Cho, K.-.M., Nguyen, H. A. T. K., Kim, S. Y., Shin, J. S., Cho, D. H., Hong, S. B., Shin, J. S., & Ok, S. H. (2016). CML10, a variant of calmodulin, modulates ascorbic acid synthesis. *New Phytologist*, *209*, 664–678. https://doi.org/10.1111/nph.13612

Duan, D., Fisher, S., Merz, P., Bogs, J., Riemann, M., & Nick, P. (2016). An ancestral allele of grapevine transcription factor MYB14 promotes plant defense. *Journal of Experimental Botany*, *67*(6), 1795–1804.

El Mahi, H., Pérez-Hormaeche, J., De Luca, A., Villalta, I., Espartero, J., Gámez-Arjona, F., Fernández, J. L., Bundó, M., Mendoza, I., Mieulet, D., Lalanne, E., Lee, S.-.Y., Yun, D.-.J., Guiderdoni, E., Aguilar, M., Leidi, E. O., Pardo, J. M., & Quintero, F. J. (2019). A critical role of sodium flux via the plasma membrane Na^+/H^+ exchanger SOS1 in the salt tolerance of rice. *Plant Physiology*, *180*, 1046–1065. https://doi.org/10.1104/pp.19.00324

Fang, Q., Wang, Q., Mao, H., Xu, J., Wang, Y., Hu, H., He, S., Tu, J., Cheng, C., Tian, G., Wang, X., Liu, X., Zhang, C., & Luo, K. (2018). AtDIV2, an R-R-type MYB transcription factor of Arabidopsis, negatively regulates salt stress by modulating ABA signaling. *Plant Cell Reports*, *37*, 1499–1511. https://doi.org/10.1007/s00299-018-2321-6

Feng, L., Ding, H., Wang, J. I. A., Wang, M., Xia, W., Zang, S., & Sheng, L. (2015). Molecular cloning and expression analysis of RrNHX1 and RrVHA-c genes related to salt tolerance in *Wild Rosa Rugosa*. *Saudi Journal of Biological Sciences*, *22*, 417–423. https://doi.org/10.1016/j.sjbs.2015.01.008

Galon, Y., Finkler, A., & Fromm, H. (2010). Calcium-regulated transcription in plants. *Molecular Plant*, *3*, 653–669. https://doi.org/10.1093/mp/ssq019

Gao, F., Zhou, J., Deng, R.-Y.U., Zhao, H.-.X., Li, C.-.L., Chen, H., Suzuki, T., Park, S.-U. N., & Wu, Q. I. (2017). Overexpression of a tartary buckwheat R2R3-MYB transcription factor gene, FtMYB9, enhances tolerance to drought and salt stresses in transgenic Arabidopsis. *Journal of Plant Physiology*, *214*, 81–90. https://doi.org/10.1016/j.jplph.2017.04.007

Gao, F., Xiong, A., Peng, R., Jin, X., Xu, J., Zhu, B. O., Chen, J., & Yao, Q. (2010). OsNAC52, a rice NAC transcription factor, potentially responds to ABA and confers drought tolerance in transgenic plants. *Plant Cell, Tissue and Organ Culture*, *100*, 255–262. https://doi.org/10.1007/s11240-009-9640-9

Gong, X.-.Q., Hu, J.-.B., & Liu, J. I.-H. (2014). Cloning and characterization of FcWRKY40, a WRKY transcription factor from *Fortunella crassifolia* linked to oxidative stress tolerance. *Plant Cell, Tissue and Organ Culture*, *119*, 197–210. https://doi.org/10.1007/s11240-014-0526-0

Han, D., Han, J., Yang, G., Wang, S., Xu, T., & Li, W. (2020). An ERF transcription factor gene from *Malus baccata* (L.) Borkh, MbERF11, affects cold and salt stress tolerance in Arabidopsis. *Forests*, *11*(5), 514. https://doi.org/10.3390/f11050514

Hanin, M., Ebel, C., Ngom, M., Laplaze, L., & Masmoudi, K. (2016). New insights on plant salt tolerance mechanisms and their potential use for breeding. *Frontiers in Plant Science*, *7*, Article 1787. https://doi.org/10.3389/fpls.2016.01787

Hasegawa, P. M. (2013). Sodium (Na+) homeostasis and salt tolerance of plants. *Environmental and Experimental Botany*, *92*, 19–23, 2013. https://doi.org/10.1016/j.envexpbot.2013.03.001

Josine, T., Ji, J., Wang, G., Zhao, Q., Yang, H. L., Wang, Y. R., & Wu, W. D. (2015). AtDREB2A-CA gene over-expression in Rosa Chinensis Jacq. affect leaf ultrastructure response to salt stress. *International Journal of Agriculture and Crop Sciences*, *8*(3), 463–476.

Kazan, K. (2015). Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends in Plant Science*, *20*, 219–229. https://doi.org/10.1016/j.tplants.2015.02.001

Koning-Boucoiran, C. F. S., Esselink, G. D., Vukosavljev, M., Van 'T Westende, W. P. C., Gitonga, V. W., Krens, F. A., Voorrips, R. E., Van De Weg, W. E., Schulz, D., Debener, T., Maliepaard, C., Arens, P., & Smulders, M. J. M. (2015). Using RNA-Seq to assemble a rose transcriptome with more than 13,000 full-length expressed genes and to develop the WagRhSNP 68k Axiom SNP array for rose (*Rosa* L.). *Frontiers in Plant Science*, *6*, 249. https://doi.org/10.3389/fpls.2015.00249

Kumar, K., Raina, S. K., & Sultan, S. M. (2020). *Arabidopsis* MAPK signaling pathways and their cross talks in abiotic stress response. *Journal of Plant Biochemistry and Biotechnology*, *29*, 700–714. https://doi.org/10.1007/s13562-020-00596-3

Lata, C., & Prasad, M. (2011). Role of DREBs in regulation of abiotic stress responses in plants. *Journal of Experimental Botany*, *62*, 4731–4748. https://doi.org/10.1093/jxb/err210

Liang, W., Ma, X., Wan, P., & Liu, L. (2018). Plant salt-tolerance mechanism: A review. *Biochemical and Biophysical Research Communications. Volume*, *495*(1), 286–291. https://doi.org/10.1016/j.bbrc.2017.11.043

Lim, T. K. (2014). Rosa hybrida. In T. K. Lim (Ed.), *Edible medicinal and non-medicinal plants* (pp. 668–681. Springer. https://doi.org/10.1007/978-94-017-8748-2_55

Liorzou, M., Pernet, A., Li, S., Chastellier, A., Thouroude, T., Michel, G., Malécot, V., Gaillard, S., Briée, C., Foucher, F., Oghina-Pavie, C., Clotault, J., & Grapin, A. (2016). Nineteenth century French rose (*Rosa* sp.) germplasm shows a shift over time from a European to an Asian genetic background. *Journal of Experimental Botany*, *67*(15), 4711–4725. https://doi.org/10.1093/jxb/erw269

Lippold, F., Sanchez, D. H., Musialak, M., Schlereth, A., Scheible, W.-.R., Hincha, D. K., & Udvardi, M. K. (2009). AtMyb41 regulates transcriptional and metabolic responses to osmotic stress in Arabidopsis. *Plant Physiology*, *149*(4), 1761–1772. https://doi.org/10.1104/pp.108.134874

Liu, X., Li, D., Zhang, S., Xu, Y., & Zhang, Z. (2019). Genome-wide characterization of the rose (*Rosa chinensis*) WRKY family and role of RcWRKY41 in gray mold resistance. *Bmc Plant Biology*, *19*, 522. https://doi.org/10.1186/s12870-019-2139-6

Long, L.u, Yang, W.-.W., Liao, P., Guo, Y.a.-W., Kumar, A., & Gao, W. (2019). Transcriptome analysis reveals differentially expressed ERF

transcription factors associated with salt response in cotton. *Plant Science*, 2019, *281*, 72–81. https://doi.org/10.1016/j.plantsci.2019.01.012

Liu, W., Li, R.-J., Han, T.-T., Cai, W., Fu, Z.-W., & Lu, Y.-T. (2015). Salt stress reduces root meristem size by nitric oxide-mediated modulation of auxin accumulation and signaling in Arabidopsis. *Plant Physiology*, *168*, 343–356. https://doi.org/10.1104/pp.15.00030

Moustafa, K., Lefebvre-De Vos, D., Leprince, A.-S., Savouré, A., & Lauriere, C. (2008). Analysis of the *Arabidopsis* mitogen-activated proteinkinase families: organ specificity and transcriptional regulation upon water stresses. *Scholarly Research Exchange*, *2018*, Article 143656.

Mu, C., Zhang, S., Yu, G., Chen, N.i, Li, X., & Liu, H. (2013). Overexpression of small heat shock protein LimHSP16.45 in *Arabidopsis* enhances tolerance to abiotic stresses. *PLOS ONE*, *8*, e82264, 2013. https://doi.org/10.1371/journal.pone.0082264

Kashif, M. H., Tang, D., Li, Z., Wei, F., Liang, Z., & Chen, P. (2020). Comparative cytological and gene expression analysis reveals potential metabolic pathways and target genes responsive to salt stress in Kenaf (*Hibiscus cannabinus* L.). *Journal of Plant Growth Regulation*, *39*, 1245–1260. https://doi.org/10.1007/s00344-019-10062-7

Ohta, M., Hayashi, Y., Nakashima, A., Hamada, A., Tanaka, A., Nakamura, T., & Hayakawa, T. (2002). Introduction of a Na+/H+ antiporter gene from *Atriplex gmelini* confers salt tolerance to rice. *FEBS Letters*, *532*(3), 279–282. https://doi.org/10.1016/S0014-5793(02)03679-7

Peng, J., Li, Z., Wen, X., Li, W., Shi, H., Yang, L., Zhu, H., & Guo, H. (2014). Salt-induced stabilization of EIN3/EIL1 confers salinity tolerance by deterring ROS accumulation in Arabidopsis. *PLoS Genetics*, *10*, e1004664. https://doi.org/10.1371/journal.pgen.1004664

Qi, W., Chen, X.i, Fang, P., Shi, S., Li, J., Liu, X., Cao, X., Zhao, N.a, Hao, H., Li, Y., Han, Y., & Zhang, Z. (2018). Genomic and transcriptomic sequencing of Rosa hybrida provides microsatellite markers for breeding, flower trait improvement and taxonomy studies. *BMC Plant Biology*, *18*, 119 https://doi.org/10.1186/s12870-018-1322-5

Rahman, H., Ramanathan, V., Nallathambi, J., Duraialagaraja, S., & Muthurajan, R. (2016). Over-expression of a NAC 67 transcription factor from finger millet (*Eleusine coracana* L.) confers tolerance against salinity and drought stress in rice. *BMC Biotechnology*,*16*(Suppl 1), 35. https://doi.org/10.1186/s12896-016-0261-1

Raineri, J., Wang, S., Peleg, Z., Blumwald, E., & Chan, R. L. (2015). The rice transcription factor OsWRKY47 is a positive regulator of the response to water deficit stress. *Plant Molecular Biology*, *88*(4), 401–413. https://doi.org/10.1007/s11103-015-0329-7

Salehi, H., Kafi, M., & Eshghi, S. (2018). Effects of salinity stress on morphological and physiological characteristics of miniature rose (*Rosa chinensis* Jacq. *var. minima Rehd.*). *Iranian Journal of Horticultural Science and Technology*, *19*(1), 41–52.

Shavrukov, Y. (2013). Salt stress or salt shock: Which genes are we studying? *Journal of Experimental Botany*, *64*, 119–127. https://doi.org/10.1093/jxb/ers316

Shen, Q., Yu, J., Fu, L., Wu, L., Dai, F., Jiang, L., Wu, D., & Zhang, G. (2018). Ionomic, metabolomic and proteomic analyses reveal molecular mechanisms of root adaption to salt stress in Tibetan wild barley. *Plant Physiology and Biochemistry*, *123*, 319–330. https://doi.org/10.1016/j.plaphy.2017.12.032

Sun, Y., Zhao, J., & Li, X. (2020). E2 conjugases UBC1 and UBC2 regulate MYB42-mediated SOS pathway in response to salt stress in Arabidopsis. *New Phytologist*, *227*, 455–472.

Sun, Z.-.M., Zhou, M.-.L., Xiao, X.-.G., Tang, Y. I.-X., & Wu, Y.-.M. (2014). Overexpression of a Lotus corniculatus AP2/ERF transcription factor gene, LcERF080, enhances tolerance to salt stress in transgenic Arabidopsis. *Plant Biotechnology Reports*, *8*, 315–324. https://doi.org/10.1007/s11816-014-0323-6

Vannini, C., Iriti, M., Bracale, M., Locatelli, F., Faoro, F., Croce, P., Pirona, R., Di Maro, A., Coraggio, I., & Genga, A. (2006). The ectopic expression of the rice Osmyb4gene in *Arabidopsis* increases tolerance to abiotic, environmental and biotic stress. *Physiological and Molecular Plant Pathology*, *69*(1–3), 26. https://doi.org/10.1016/j.pmpp.2006.12.005

Wang, F., Jing, W., & Zhang, W. (2014). The mitogen-activated protein kinase cascade MKK1-MPK4 mediates salt signaling in rice. *Plant Science*, *227*, 181–189. https://doi.org/10.1016/j.plantsci.2014.08.007

Wang, S., Huang, J., Wang, X., Dang, H., Jiang, T., & Han, Y. (2019). Expression analysis of the NAC transcription factor family of *Populus* in response to salt stress. *Forests*, *10*(8), 688. https://doi.org/10.3390/f10080688

Yan, H., Jia, H., Chen, X., Hao, L., An, H., & Guo, X. (2014). The cotton WRKY transcription factor GhWRKY17 functions in drought and salt stress in transgenic *Nicotiana benthamiana* through ABA signaling and the modulation of reactive oxygen species production. *Plant Cell Physiology*, (12), 2060–2076. https://doi.org/10.1093/pcp/pcu133

Geng, Y., Wu, R., Wee, C. W., Xie, F., Wei, X., Chan, P. M. Y., Tham, C., Duan, L., & Dinneny, J. R. (2013). A spatio-temporal understanding of growth regulation during the salt stress response in Arabidopsis. *Plant Cell*, *25*, 2132–2154. https://doi.org/10.1105/tpc.113.112896

Yu, X., Liu, Y., Wang, S., Tao, Y., Wang, Z., Shu, Y., Peng, H., Mijiti, A., Wang, Z.e, Zhang, H., & Ma, H. (2016). CarNAC4, a NAC-type chickpea transcription factor conferring enhanced drought and salt stress tolerances in Arabidopsis. *Plant Cell Reports*, *35*, 613–627. https://doi.org/10.1007/s00299-015-1907-5

Zhang, H., Han, B., Wang, T., Chen, S., Li, H., Zhang, Y., & Dai, S. (2012). Mechanisms of plant salt response: Insights from proteomics. *Journal of Proteome Research*, *11*, 49–67. https://doi.org/10.1021/pr200861w

Zhang, J.-.L., & Shi, H. (2013). Physiological and molecular mechanisms of plant salt tolerance. *Photosynthesis Research*, *115*, 1–22 https://doi.org/10.1007/s11120-013-9813-6

Zhang, W.-.D., Wang, P., Bao, Z., Ma, Q., Duan, L. I.-J., Bao, A. I.-K. E, Zhang, J.-.L., & Wang, S.-.M. (2017). SOS1, HKT1;5, and NHX1 synergistically modulate Na+ Homeostasis in the halophytic grass *Puccinellia tenuiflora*. *Frontiers in Plant Science*, *8*, 576. https://doi.org/10.3389/fpls.2017.00576

Zhao, C., Zhang, H., Song, C., Zhu, J.-.K., & Shabala, S. (2020). Mechanisms of plant responses and adaptation to soil salinity. *The Innovation*, *1*(1), 100017, https://doi.org/10.1016/j.xinn.2020.100017

Zhengkun, Q., Shuangshuang, Y., Bin, X., Jian, J., Yu, B., Lei, J., Chen, C., Chen, L., Yang, Y., Wang, Y., Tian, S., & Cao, B. (2019). The eggplant transcription factor MYB44 enhances resistance to bacterial wilt by activating the expression of spermidine synthase. *Journal of Experimental Botany*, *70*(19), 5343–5354.

Zhenjun, L., Yongsheng, T., Jing, X., Fu, X., Gao, J., Wang, B., Han, H., Wang, L., Peng, R., & Yaho, Q. (2018). A tomato ERF transcription factor, SlERF84, confers enhanced tolerance to drought and salt stress but negatively regulates immunity against Pseudomonas syringae pv. tomato DC3000. *Plant Physiology and Biochemistry*, *274*, 137–145.683–695.

Zhu, N., Cheng, S., Liu, X., Du, H., Dai, M., Zhou, D.-.X., Yang, W., & Zhao, Y.u (2015). The R2R3-type MYB gene OsMYB91 has a function in coordinating plant growth and salt stress tolerance in rice. *Plant*, *236*, 146–156. https://doi.org/10.1016/j.plantsci.2015.03.023

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Bao, Y., Chen, C., Fu, L., & Chen, Y. Comparative transcriptome analysis of *Rosa chinensis* 'Old Blush' provides insights into the crucial factors and signaling pathways in salt stress response. *Agronomy Journal*, 2021;1–20. https://doi.org/10.1002/agj2.20715