Horticulture, Environment, and Biotechnology
https://doi.org/10.1007/s13580-021-00418-y

RESEARCH REPORT

Identification of hub salt-responsive genes in *Cucumis sativus* using a long non-coding RNA and mRNA interaction network

Marzieh Karimi[1] · Mohammad Hossein Pakdel[2] · Khosro Bali lashaki[3] · Aboozar Soorni[2]

Received: 8 September 2021 / Revised: 29 December 2021 / Accepted: 29 December 2021

Abstract

Cucumber is a commercially important vegetable crop whose growth and productivity are significantly influenced by salinity. Currently, there is little information about salt-related genes and the associated biological pathways involved in salt stress response and tolerance in this crop. Accordingly, this study aimed to unravel the complex molecular mechanism components underlying salinity in cucumber using long non-coding RNA (lncRNA) identification and weighted gene co-expression network analysis (WGCNA). Here, two previously published high-throughput RNA-seq datasets obtained from control and salt-treated tissues of cucumber roots and leaves were employed. First, the potential lncRNAs were identified based on a bioinformatics pipeline. Subsequently, differentially expressed genes (DEGs) and differentially expressed lncRNAs (DE-lncRNAs) were utilized as the input for the WGCNA to identify the clusters of highly interconnected lncRNAs and mRNAs. According to the results, 17 DE-lncRNAs out of 279 and 7 DE-lncRNAs out of 166 putative lncRNAs were detected between salt-treated and control samples in root and leaf tissues, respectively. Using WGCNA, 2226 unique DEGs and 23 DE-lncRNAs were categorized into 10 distinctive co-expression modules, of which, four modules, including blue, brown, yellow, and turquoise, contained the highest number of salt-related genes. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis identified four significantly enriched pathways, including, "plant hormone signal transduction", "starch and sucrose metabolism", "MAPK signaling pathway", and "phenylpropanoid biosynthesis", related to salt stress response in cucumber. The novel hub genes identified in this study could be exploited for further functional studies aiming to introduce salt-tolerant cucumber varieties utilizing molecular engineering approaches.

Keywords Cucumber · lncRNAs · Salt stress · Transcriptome · WGCNA

1 Introduction

All plants are subjected to a multitude of stresses throughout their life cycle. Among various stresses, salinity is the major environmental stress worldwide, which has an impressive detrimental effect on plants and can result in plant death and/or decreases in productivity (Nawaz et al. 2010). Plant responses to environmental stresses occur via a series of physiological, cellular, and molecular mechanisms. For example, by exposing the plant to salinity stress, critical processes such as photosynthesis, protein synthesis, energy, and lipid metabolism are affected (Kosová et al. 2013). Indeed, major plants evolve these mechanisms to exclude salt from their cells or to tolerate its presence within the cells (Parida and Das 2005; Yousefirad et al. 2020). Until now, a large number of studies have been designed to dissect the molecular mechanisms involved in plant response to salt stress (Amirbakhtiar et al. 2019; Chandran et al. 2019).

Communicated by Seon-In Yeom.

✉ Aboozar Soorni
soorni@iut.ac.ir

Marzieh Karimi
mr.karimi83@gmail.com

Mohammad Hossein Pakdel
mhpakdel97@gmail.com

Khosro Bali lashaki
khosrobali1@alumni.ut.ac.ir

[1] Independent Researcher, Isfahan, Iran

[2] Department of Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran

[3] Department of Horticultural Science, Faculty of Agricultural Sciences, University of Guilan, Rasht, Guilan 4199613776, Iran

Published online: 05 July 2022

In this regard, the most relevant genes associated with the biochemical and physiological processes, like signal transduction, ionic homeostasis regulation, and detoxification response, have been unraveled (Krishnamurthy et al. 2017; Nefissi Ouertani et al. 2021; Yang and Guo 2018). Although the results have provided reliable insight into the understanding of salt-responsive mechanisms, there is still more to be deciphered. Accordingly, systems biology approaches introduced to reconstruct the transcriptional networks can be used as complementary analysis for a more detailed investigation of the complex signaling pathways involved in salinity (Liu et al. 2019; Shumayla et al. 2017; Suratanee et al. 2018; Wang et al. 2013). For instance, weighted gene co-expression network analysis (WGCNA) is an improved GCNA-based method that has been successfully utilized in visualizing co-expression networks (Tai et al. 2018). Using this approach, the complicated gene expression profiling datasets could be condensed to confine co-expression modules (Tai et al. 2018; Yuan et al. 2018), resulting in the identification of intra-modular of highly connected genes. Moreover, the relationship between modules and their correlation with special phenotypes or traits could be detected (van Dam et al. 2018). It has been demonstrated that genes in the same module presumably contribute to similar biological processes (Liang et al. 2014).

In addition, the key lncRNAs recognized by identifying lncRNA-mRNA co-expression modules using WGCNA are candidate biomarkers in salt response mechanisms (Eom et al. 2019; Zhang et al. 2019a). The lncRNAs are a class of non-coding functional RNAs with ≥ 200 nucleotides thought to regulate various biological processes by modulating gene expression at the transcriptional, post-transcriptional, translational, and post-translational levels (Sun et al. 2020; Zhang et al. 2019b). In recent studies, the potential regulatory role of lncRNAs in salt stress responses has been illustrated in several species, including bread wheat (Shumayla et al. 2017), cotton (Deng et al. 2018), maize (Huanca-Mamani et al. 2018), poplar (Ma et al. 2019), sorghum (Sun et al. 2020), tea (Wan et al. 2020), and duckweed (Fu et al. 2020). Nonetheless, a limited number of salt stress–related lncRNAs have been functionally investigated. For example, lncRNA npc536 and lncRNA DRIR in *Arabidopsis* (Amor et al. 2009; Qin et al. 2017) and lncRNA973 in cotton (Zhang et al. 2019a) were characterized as positive regulators involved in salt stress tolerance. Therefore, lncRNAs play key roles in controlling molecular mechanisms involved in salt stress responses. Among the economically important crops, cucumber (*Cucumis sativus* L.) is a valuable vegetative species with a significant amount of vitamins and minerals (Mukherjee et al. 2013). Cucumber is also considered a salt-sensitive crop, as a salinity level higher than 1.3 $dS \cdot m^{-1}$ significantly influences growth and productivity (Marium et al. 2019). In recent years, many studies have revealed salt-related genes and pathways in model plants (Chen et al. 2017; Rolly et al. 2020; Sun et al. 2020; Xiong et al. 2017), but few studies have focused on cucumber (Chen et al. 2020; Jiang et al. 2020; Xiong et al. 2017; Zhu et al. 2019a, c; b). Therefore, there is a dire need to identify salt-related genes, unexpected RNA molecules like lncRNAs, signaling pathways, and regulatory networks in this species. Hence, we characterized lncRNAs at the transcriptome level in root and leaf tissues of cucumber under salt stress. Furthermore, WGCNA and functional enrichment analysis were applied on the whole transcriptome sequencing data of *C. sativus* to identify the novel lncRNA-mRNA co-expression network that elucidated the undiscovered parts of salt-related molecular mechanisms in this salt-sensitive plant.

2 Materials and methods

2.1 Transcriptome datasets

Two previously published transcriptome datasets from control and salt-treated roots (Bioproject accession number PRJNA511946) (Zhu et al. 2019c) and leaves (Bioproject accession number PRJNA477930) (Zhu et al. 2019c) of *Cucumis sativus* cv. 'JinYou 1' were downloaded from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA). Each dataset included six samples representing three biological replicates for both the control and salt treatment. After quality control of raw reads by FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and Trimmomatic v0.30 (Bolger et al. 2014) programs, only reads with an average quality of 30 and minimum read length of 50 bp were kept for downstream analyses.

2.2 The lncRNA pipeline

To identify lncRNAs, clean reads were aligned to the reference genome of cucumber (v3, http://cucurbitgenomics.org/organism/20) with STAR v2.7.1 (Dobin and Gingeras 2015). Subsequently, StringTie v2.0.6 (Pertea et al. 2015) and StringTie's merge were used to assemble all transcripts and combine the assembled transcripts, respectively. Then, the unannotated transcripts with the class codes of "u", "x," "i," "o", and "e" were extracted from gffcompare output. Finally, the unannotated transcripts were subjected to some filtering approaches to identify potential lncRNAs, as follows:

1. The unannotated transcripts with < 200 nucleotides and CPM < 1 were excluded.
2. FEELnc (Wucher et al. 2017) with a shuffle mode (-m "shuffle") was used to infer potential lncRNAs.

3. The coding potential of predicted lncRNAs was further evaluated using coding potential calculator (CPC2) software (Kang et al. 2017).
4. tRNAscan-SE 2.0 (Lowe and Chan 2016) and Barrnap 0.9 (https://github.com/tseemann/barrnap) were applied to filter out possible transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs).
5. The remaining transcript sequences were then inputted into CREMA (Simopoulos et al. 2018) to increase the specificity and accuracy of lncRNA prediction.
6. The transcripts with at least one significant (E-value, 1^{e-5}) hit against the UniProt, Pfam, and Rfam databases were excluded.

2.3 Mining of differentially expressed genes (DEGs)

For differential expression analysis, gene read-count data matrices were produced from assembled transcripts with python script prepDE.py. Then, the IDEAMEX website (Jimenez-Jacinto et al. 2019) was used to call all DEGs and differentially expressed lncRNAs (DE-lncRNAs) through DESeq2 (Love et al. 2014) software with ''FDR $\leq$ 0.05, logFC $\geq$ 2 and CPM = 1″ parameters.

2.4 RT-qPCR-based confirmation of cucumber lncRNAs

To validate the expression levels of predicted lncRNAs, nine DE-lncRNAs were evaluated by real-time quantitative PCR (RT-qPCR) analysis. For this purpose, plant materials were prepared according to Zhu et al. (2019c). First, hot water–sterilized seeds of *C. sativus* L. were germinated at 28 °C and after 2 days transplanted to trays containing equal parts peat, perlite, and sand followed by incubation at 28/18 °C (day/night), 60–70% relative humidity, and a 12-h light/dark regime in a growth chamber. At the two-leaf stage, uniform seedlings were transferred to plastic pots containing ½ strength Hoagland nutrient solution, which was continuously aerated. After 1 week, two salt stress treatments were applied using 50 and 100 mM solutions of sodium chloride (NaCl). After 3 days of salinity treatments, leaf and root samples were collected from salt-stressed and control plants in three biological replicates and immediately frozen in liquid nitrogen. To decrease the variance caused by interindividual differences in gene expression, each sample was gathered from at least 10 plants. Total RNA extraction was performed using the DENAzist column RNA isolation kit. One microgram of total RNA per sample was reverse-transcribed into first-strand cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, Co., USA) according to the manufacturer's instruction. The RT-qPCR reaction was conducted in a final volume of 20 µl using SYBR Green PCR Master Mix (BioFACT, Korea) and specific forward and reversed primers (Table 1) on the ABI system (ABI ViiA 7 Real-time PCR). All reactions were executed in three technical replicates for each of the three biological replicates. The RT-qPCR program was performed with a single step of initial denaturation at 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 20 s. In this analysis, *CACS* (accession number (NCBI): GW881874) and *40SRPS8* (gene ID in cucumber: Csa6G382970; Li et al. 2019) housekeeping genes were selected for the normalization of data, and the relative expression levels of lncRNAs were quantified using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Table 1 lncRNAs and primers used for qRT-PCR analysis

lncRNA Name	Length (bp)		Primers
lMSTRG.14303.2	219	F	ATATGGGCAAGGCAG AAATG
		R	ACTTTCGGAACACGA TCCAC
lMSTRG.14505.1	182	F	GCTCGTTTCATTCCATCC AT
		R	GTGGAGCTTTATGGGAGA CG
lMSTRG.8396.2	203	F	CAACCTGCCAAAGAA GGAAG
		R	TGGTTGTGCTTGAGATTT GC
rMSTRG.12032.1	207	F	CTCGACTCTCCGTCCTCA TC
		R	TCAAAATGGCATCTCAAC CA
rMSTRG.7061.1	151	F	ATAAATTCGACCCGTCAT CG
		R	GATCAACCACCTCCA CCAAT
rMSTRG.14093.1	187	F	GGTTTTGGCCTCAATCAA AA
		R	GAATCGTGAAGCGAA TGGTT
rMSTRG.335.1	241	F	GGGCAAAACAAAGCA TCATT
		R	ATGGGAATGGGGAGA AGAAG
rMSTRG.5342.1	172	F	GGCGGTTCCTTATCGATC TT
		R	GAAGCAGGTTAAAGC CCACA
rMSTRG.9138.5	194	F	ATCCAGGATAGGGGA ATTGG
		R	TCGACACTCATTCTGCCT TG

2.5 Coding/non-coding gene co-expression network and functional analysis

To detect similar expression patterns between the lncRNAs and mRNAs, we used the WGCNA (Langfelder and Horvath 2008) R software package. The methods and parameters were adjusted according to a previous studies (Ghorbani et al. 2021; Ahmadi Khoei et al. 2021). In this regard, the normalized read count values were used as the input file for significantly differentially expressed lncRNAs and mRNAs. Based on $\log_2$ (FPKM + 1) values, a similarity matrix was generated by calculating Pearson's correlation between all pairs of genes and then transformed into an adjacency matrix. According to the scale-free topology criterion (Zhang and Horvath 2005), soft power was set to 9. After that, the topological overlap measure (TOM) and corresponding dissimilarity (1-TOM) were calculated using the adjacency matrix. Then, the modules, which are clusters of highly interconnected genes, were identified by hierarchical clustering of 1-TOM and the DynamicTree Cut algorithm (Langfelder and Horvath 2008). Additionally, correlations among gene expression modules and phenotypic traits were investigated. Modules that were significantly correlated with the trait were identified, and genes in significant modules were then exported for further analysis. Finally, to determine the genes that are highly connected in the modules, we selected the 50 top hub genes through the Cyto-Hubba plug-in (Chin et al. 2014) and visualized these genes with Cytoscape (Shannon et al. 2003) as an unsigned network. Accordingly, in this study the biological functions of DEGs were investigated via Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) pathway enrichment analysis on each module (Kanehisa and Goto 2000).

3 Results

3.1 Identification and characterization of lncRNAs

In this study, we obtained the clean reads from two previously published RNA-seq datasets to understand the molecular mechanisms of cucumber lncRNA responses to salt stress in root and leaf tissues. Each dataset included six libraries representing three biological replicates of control and salt-treated samples. All 12 datasets were mapped to the *C. sativus* genome using the STAR program (Dobin and Gingeras 2015) to reconstruct the transcriptome. In this case, more than 92% of reads was mapped to the cucumber reference genome. Subsequently, the transcripts were assembled and annotated using StringTie and then were subjected to a suitable pipeline shown in Fig. 1a to identify confident lncRNA transcripts. Using this comprehensive filtering pipeline, 830 and 698 transcripts, belonging to class codes "u" (intergenic), "o" (generic overlap with known exon), "i" (intronic), and "x" (overlap with a known gene on the opposite strand) with CMP > 1 were identified in root and leaf samples, respectively. Afterward, FEELnc identified 701 and 621 lncRNAs among the remaining unannotated transcripts in roots and leaves, respectively. We further assessed these transcripts for their protein-coding potential using the CPC program and filtering out tRNA and rRNAs, which resulted in 615 and 500 remaining transcripts. After that, transcripts were inputted into CREMA (https://github.com/gbgolding/crema; Simopoulos et al. 2018) for lncRNA prediction. Utilizing CREMA's numerical scoring system for lncRNAs prediction, 398 and 290 transcripts with a prediction score > 0.5 were considered putative lncRNAs in roots and leaves, respectively. In the last filtration step and after removing transcripts that were homologous to protein-coding genes, known protein domains documented in the Pfam database, and housekeeping RNAs such as tRNAs, rRNAs, snRNAs, and snoRNAs in the Rfam database, 279 and 166 transcripts were detected as putative lncRNAs in root and leaf samples, respectively. Among all identified novel lncRNAs, 93 lncRNAs were shared between root and leaf samples. A file containing all the identified lncRNAs sequences, along with their genomic locations, is provided in DataS1.

Gene expression profiling of the salt stress and control *C. sativus* plants allowed us to identify lncRNAs whose expression levels were significantly changed upon salt stress. Out of all identified lncRNAs, 17 and 7 lncRNAs were differentially expressed between salt and control samples in root and leaf tissues, respectively. Of these, three DE-lncRNAs were commonly expressed in both tissues. To investigate the relationship between DE-lncRNAs from different samples, we performed a correlation analysis among the normalized expression values from all the samples and generated heat maps (Fig. 1b). The heat maps indicated that salt treatment samples were clustered together compared to the control samples. As shown in the plot, lncRNAs exhibited a varied expression pattern between the control and salt treatment. The majority of lncRNAs identified in root tissue and all lncRNAs identified in leaf tissue were overexpressed in response to salinity.

3.2 Confirmation of differentially expressed lncRNAs

RT-qPCR analysis was employed to verify the expression patterns of nine lncRNAs detected by RNA-seq analysis (Fig. 2). In this experiment, the RT-qPCR results of all nine examined DE-lncRNAs were consistent with the RNA-seq results, suggesting the reliability of the RNA-seq data. Importantly, to evaluate concordance in gene expression between RNA-seq and qPCR, we focused our analysis on

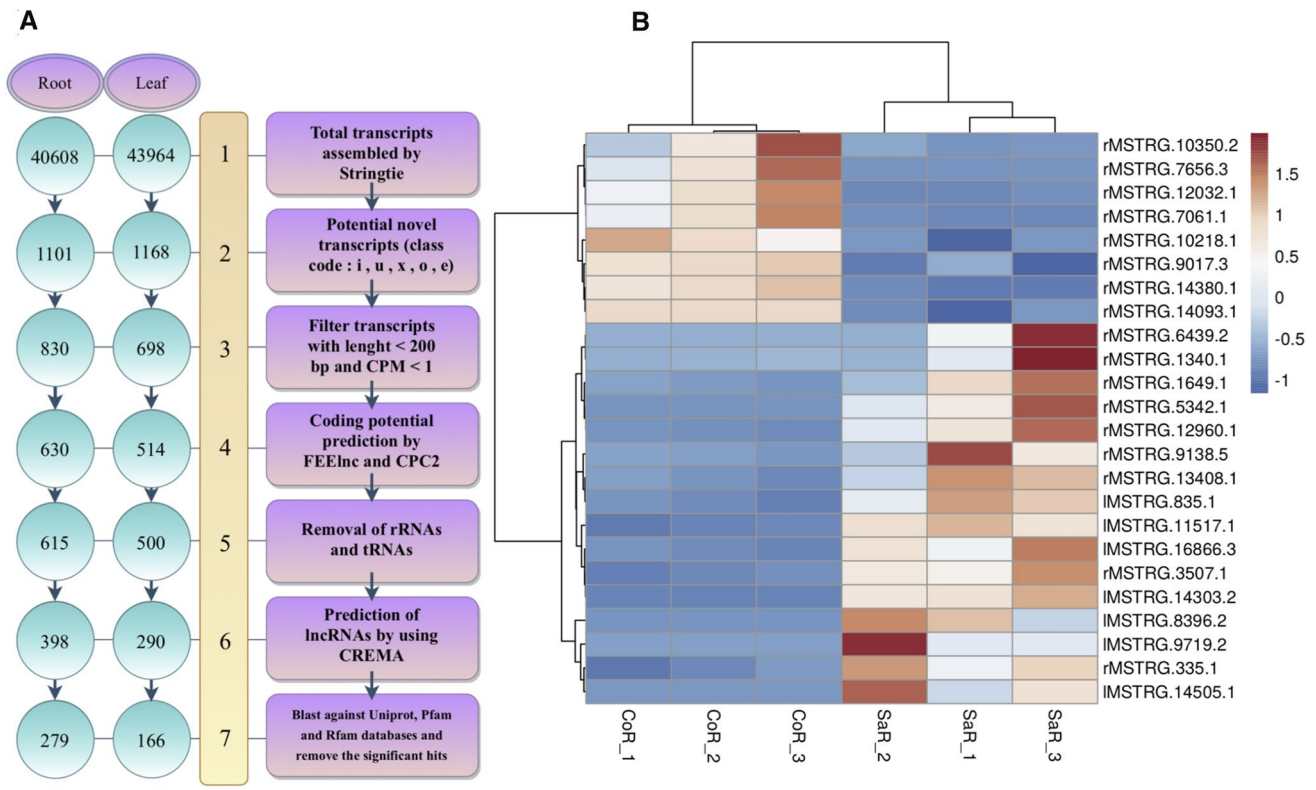

Fig. 1 Identification and characterization of lncRNAs in *C. sativus*. **a** Flow diagram of the bioinformatics pipeline for the identification of lncRNAs. **b** Heatmap shows the expression profile of DE-lncRNAs across all samples. lMSTRG: DE-lncRNAs in leaf samples; rMSTRG: DE-lncRNAs in root samples. SaR represents salt stress sample and replication number. CoR represents control sample and replication number

gene expression correlation between normalized RT-qPCR Cq-values and log-transformed RNA-seq expression values ($R^2 = 0.92$), indicating that our analysis of the RNA-seq data was reliable. In this regard, the expression patterns of all nine lncRNAs in qPCR were similar to the results of transcriptome analysis and there were no dissimilarities between them. For instance, based on RNA-seq and RT-qPCR data analysis, three lncRNAs, lMSTRG.14303.2, lMSTRG.14505.1, and lMSTRG.8396.2, were identified as up-regulated lncRNAs in leaves from salt-treated plants. Additionally, according to RT-qPCR results, these lncRNAs were significantly up-regulated in response to salt stress at two levels of salinity stress treatments (50, 100 mM) in both leaf and root tissues. Based on the two expression profiling approaches, rMSTRG.12032.1, rMSTRG.7061.1, and rMSTRG.14093.1 were significantly down-regulated in roots from salt-stressed plants. The RT-qPCR analysis revealed down-regulation of these lncRNAs in leaves and roots at both salt stress concentrations, except for rMSTRG.7061.1, which was up-regulated in leaves. By contrast, rMSTRG.335.1, rMSTRG.5342.1, and rMSTRG.9138.5 were characterized as overexpressed genes in the salt-treated roots. Furthermore, based on RT-qPCR analysis, an opposite expression pattern for rMSTRG.5342.1 was detected between roots and leaves, which demonstrated the tissue-specific expression pattern of lncRNAs.

3.3 Construction of lncRNA-mRNA co-expression networks by WGNNA

In this study, WGCNA analysis was used to identify the regulatory lncRNA-mRNA co-expression network in *C. sativus* under salt stress. Regarding WGCNA results, 2226 unique DEGs and 23 DE-lncRNAs were categorized into 10 co-expression modules with a distinctive color and ranging in size from 32 to 689 genes per module (Table 2). Four modules were characterized with the highest number of genes responding to salinity stress: turquoise (251 salt-related genes of 689 genes), blue (157 salt-related genes of 425 genes), brown (121 salt-related genes of 348 genes), and yellow (118 salt-related genes of 276 genes). The turquoise and brown modules contained the largest number of DE-lncRNAs (7 DE-lncRNAs), followed by the blue and yellow modules containing 4 and 3 DE-lncRNAs, respectively.

To identify the overall expression level of the modules and their association with salt stress, module eigengene

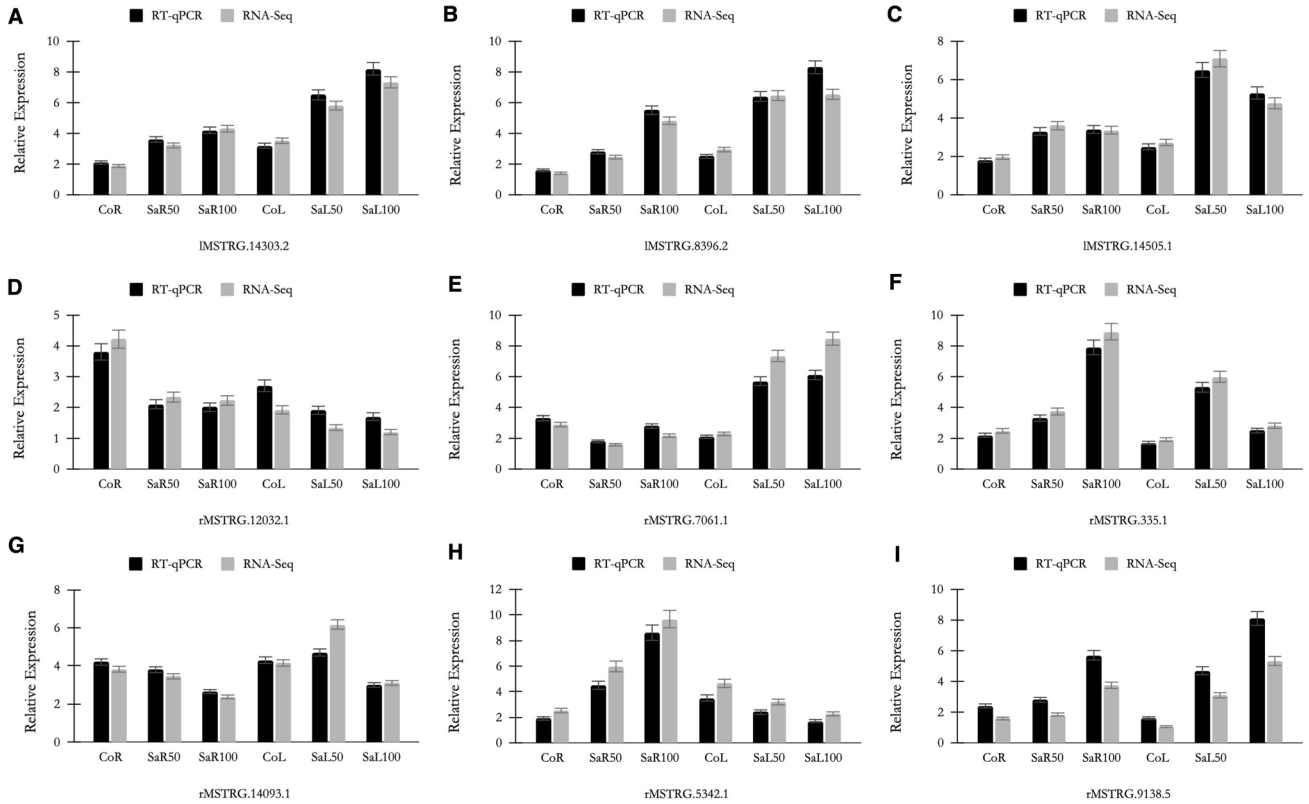

Fig. 2 The relative expression of selected lncRNAs determined by qPCR in root and leaf tissues. Genes: **A** lMSTRG.14303.2, **B** lMSTRG.8396.2, **C** lMSTRG.14505.1, **D** rMSTRG.12032.1r, **E** rMSTRG.7061.1, **F** MSTRG.335.1, **G** rMSTRG.14093.1, **H** rMSTRG.5342.1, and **I** rMSTRG.9138.5. CoR, SaR50, and SaR100 represent control and salt-treated root samples by 50 and 100 mM NaCl, while CoL, SaL50, and SaL100 represent similar leaf samples

Table 2 The number of DEGs and DE-lncRNAs in each module

Module name	Number of mRNAs	Number of DE-lncRNAs
Turquoise	689	7
Blue	425	4
Brown	348	7
Yellow	276	3
Green	168	0
Red	87	1
Black	75	0
Pink	64	1
Magenta	62	0
Purple	32	0

(ME) and Pearson correlation coefficients between the gene expression values and ME values in each module were calculated. As shown in Fig. 3, three modules, including MEbrown (with a correlation coefficient of − 0.98), MEblue (with a correlation coefficient of − 0.93), and MEturquoise (with a correlation coefficient of 0.97), were significantly associated with salt stress. The strong positive correlation in the turquoise module represented the considerable numbers of up-regulated genes that regulate salt stress tolerance in cucumber. Hierarchical clustering of all differentially expressed genes, which was performed based on the TOM dissimilarity measure (1-TOM), is represented as a cluster dendrogram along with assigned module colors in Fig. 4. The dynamic clustering modules with high similarity were combined to create the merged dynamic modules. In this analysis, the correlation between the corresponding modules eigengenes was calculated, and modules with eigengene similarity > 0.8 were combined, which reduced the number of modules from 10 to 6. This reduction facilitated analysis and allowed the modules to be large enough to include the significant Gene Ontology (GO) terms.

3.4 Functional enrichment analysis of the respective co-expressed modules

For further investigation, the blue, brown, turquoise, and yellow modules that were highly correlated with salt stress were selected for functional annotation and biological interpretation. GO and KEGG pathway enrichment analyses

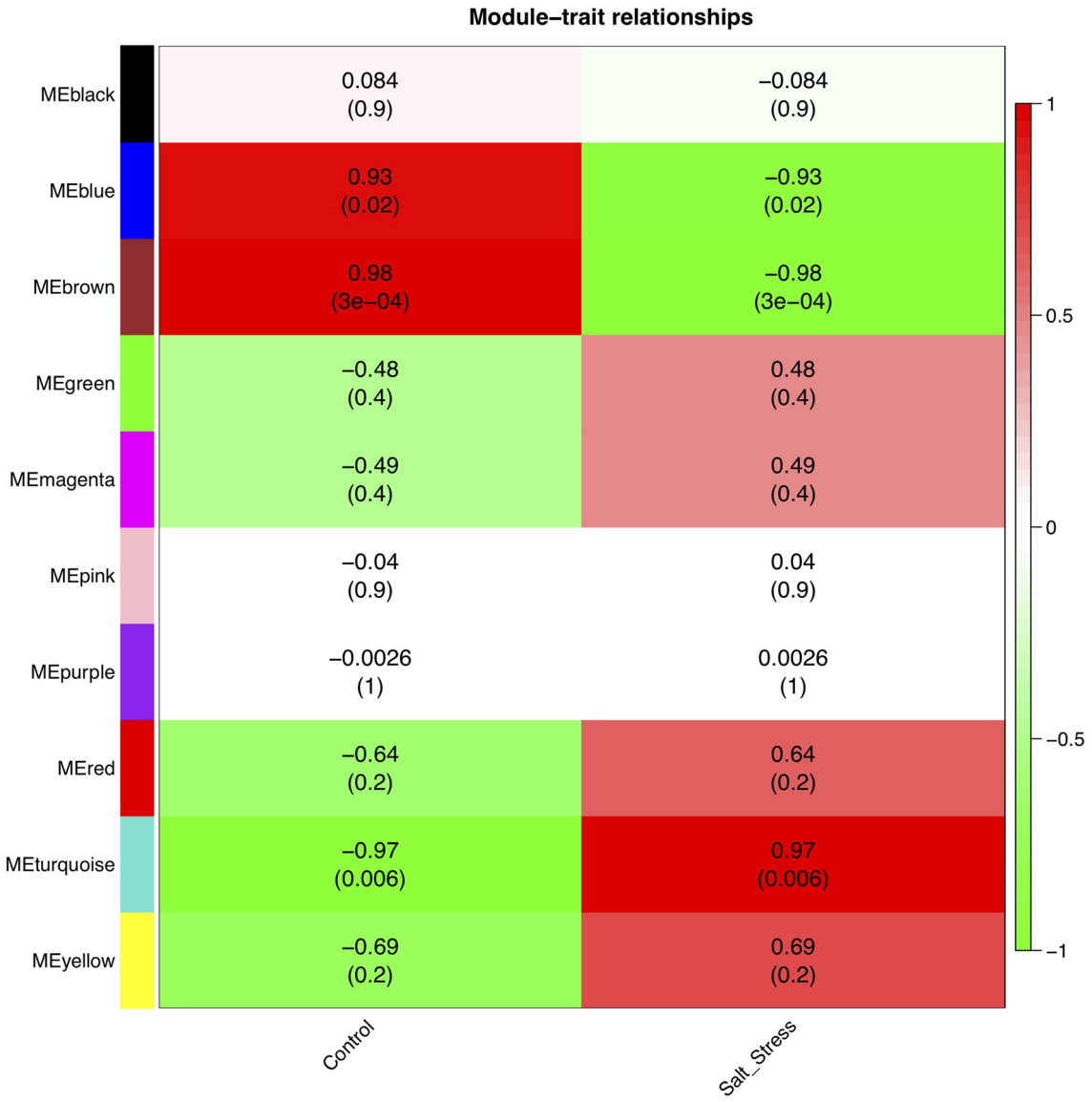

Fig. 3 The module–trait relationships of different co-expression modules under control and salt stress conditions. Red and green represent positive and negative correlations, respectively. The numbers represent the Pearson correlation coefficient values and *P*-values

were performed on each module of DEGs. Furthermore, the eigengene expression profile in these major modules was investigated in control and salt-treated samples. According to the results, the genes in the blue and brown modules genes appeared to be modulated by salinity through down-regulation (Fig. 5a, b), whereas the genes in the turquoise and yellow modules were positively regulated by salt stress (Fig. 5c, d). The top 10 terms in the biological process category of different modules are shown in Fig. 5. The GO analysis demonstrated that modules were not very different in terms of enriched biological process, and genes in the four modules were similarly enriched in the biosynthetic process (GO: 0009058) (as a highly enriched GO term), nucleobase-containing compound metabolic process (GO:0006139), cellular process (GO:0009987), metabolic process (GO:0008152), transport (GO: 0006810), carbohydrate metabolic process (GO: 0005975), and response to stress (GO:0006950). Cellular component organization (GO: 0016043) in the brown module, catabolic process (GO: 0009056) in the turquoise module, and lipid metabolic process (GO:0006629) in the yellow module were recognized as biological processes in which these modules were specifically enriched. Furthermore, the KEGG pathway enrichment analysis of DEGs in four major co-expressed modules (Fig. 6) revealed "plant hormone signal transduction", "starch and sucrose metabolism", "MAPK signaling pathway", and "phenylpropanoid biosynthesis" as significantly enriched pathways, which are considered major pathways involved in salt stress response.

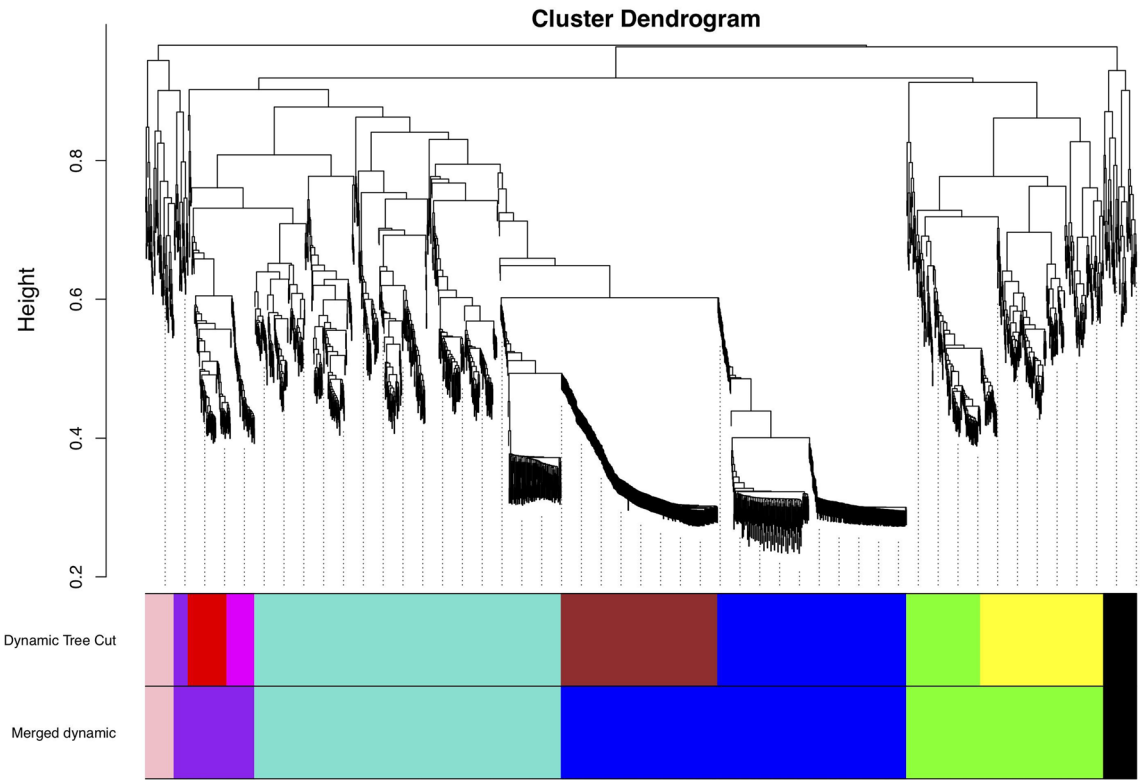

Fig. 4 Hierarchical cluster tree displaying the co-expression modules. The branches constitute 10 unique modules, and each line represents one gene. Original modules and merged modules are depicted as colored bands below the cluster tree

3.5 Construction of co-expression network using hub genes

Since hub genes and lncRNAs are the most important elements of the network in each module, we identified the top 50 genes in the four main modules and visualized them using Cytoscape (Fig. 7). In the blue module, we identified 2 lncRNAs and 48 protein-coding genes, of which, around 19 genes were found to be salt-related genes. In this module, several well-known transcription factors (TFs), including ethylene response factor (ERF: CsaV3_3G021840), Myb-like (CsaV3_2G025610), F-box/kelch-repeat protein (CsaV3_7G005560), basic-leucine zipper transcription factor family protein (bZIP: CsaV3_4G033330), and zinc finger protein CONSTANS-LIKE2 (COL2: CsaV3_4G009980), were found in the list of the protein-coding genes. Additionally, we found DEGs such as S-receptor-like serine/threonine-protein kinase (SRK: CsaV3_3G021760), ureide permease (UPS: CsaV3_5G033300), peptide methionine sulfoxide reductase (MsrA: CsaV3_1G000860), aminotransferase-like protein (CsaV3_3G033240), and auxin-responsive protein (CsaV3_3G028380), which are involved in salt stress response. In the brown module, a single lncRNA and 49 protein-coding genes were identified, of which 11 genes were salt stress–associated genes. Zinc finger protein CONSTANS-LIKE6 (COL6: CsaV3_5G026860) and protein SHI RELATED SEQUENCE1 (SRS1: CsaV3_7G006060) were recognized as TFs in this module. Furthermore, remarkable numbers of DEGs including desumoylating isopeptidase (CsaV3_3G029920), receptor-like kinase (RLK: CsaV3_1G011490), luc7-like protein 3 (CsaV3_5G005710), classical arabinogalactan protein 25 (CsaV3_6G005680), xyloglucan endotransglucosylase/hydrolase (CsaV3_6G038030), spermidine synthase (SPDS: CsaV3_5G000580), alpha/beta hydrolase-like (CsaV3_7G032780), and oxidoreductases, acting on NADH or NADPH (CsaV3_4G005910), were identified, which are known to be involved in salt stress response as well as salt stress tolerance. Similar to the brown module, we identified one lncRNA and 49 protein-coding genes in the yellow module, of which 25 genes were revealed to be related to salinity. In this module, heat shock transcription factor (HSF: CsaV3_2G025510) and MADS-box protein (CsaV3_7G006940) were found as the salt stress–responsive TFs. In addition, the main DEGs were related to the regulation of salt stress response or salt stress tolerance, including sodium/hydrogen exchanger (CsaV3_6G005700), pentatricopeptide repeat (CsaV3_5G029410), trehalose 6-phosphate phosphatase (TPP: CsaV3_3G003030), glutathione *S*-transferase (GST: CsaV3_3G011460),

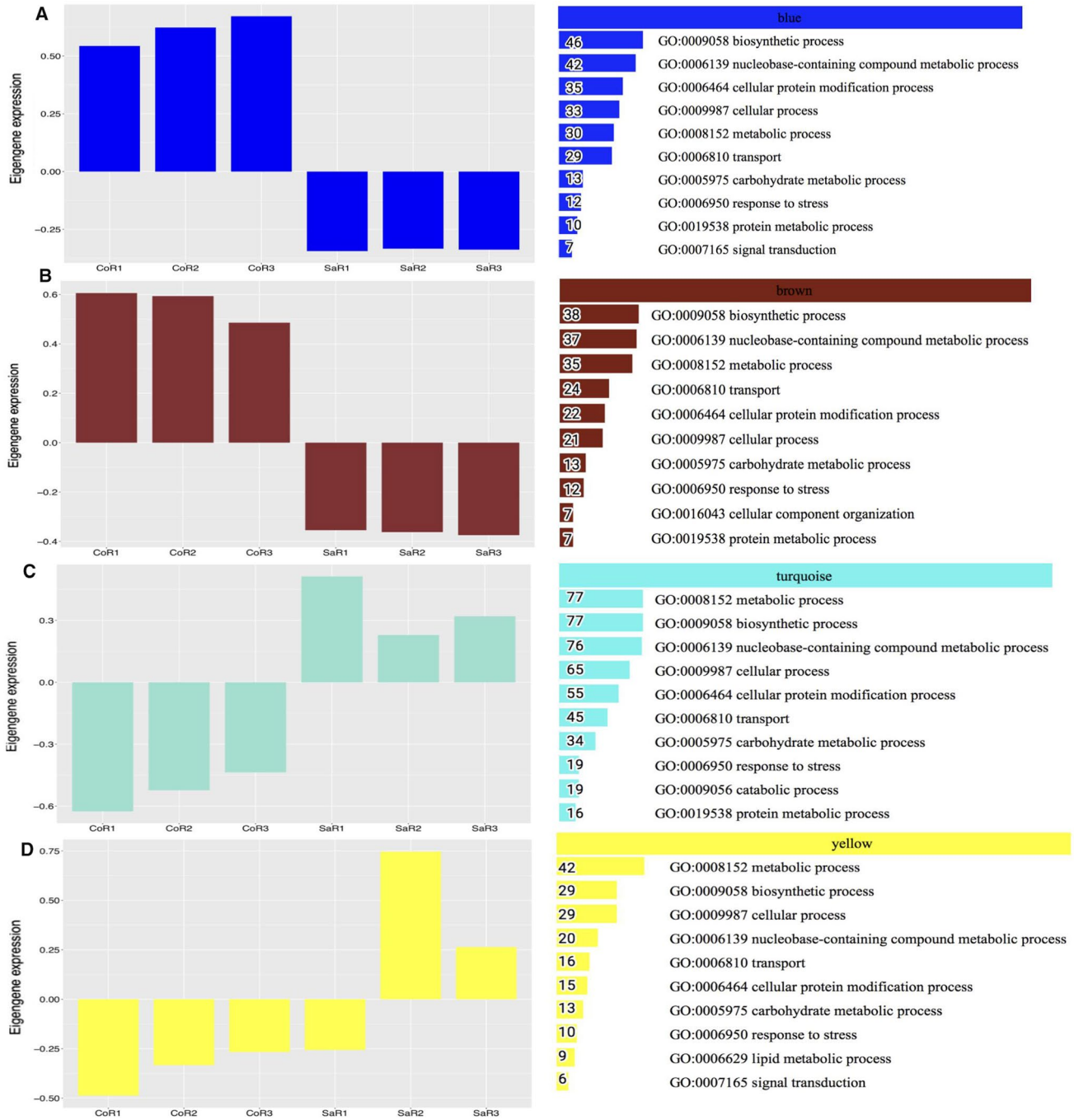

Fig. 5 The Eigengene expression profile and top 10 enriched biological process GO terms of four major modules related to salt stress. **a** Blue module, **b** brown module, **c** turquoise module, and **d** yellow module. The length of the bar plot represents the numbers of enriched DEGs

Beta-expansin (CsaV3_3G007390), peroxidase (PRX: CsaV3_6G019020 and CsaV3_6G046710), methyltransferase (CsaV3_4G027850), cysteine proteinase inhibitor (CsaV3_7G006960), calcium-transporting ATPase (CsaV3_6G044140), and mitogen-activated protein kinase kinase kinase (CsaV3_3G021810). In the turquoise module, out of the 50 protein-coding genes, 15 DEGs were associated with salinity. In this module, three TFs, bHLH35-like (CsaV3_2G008770), DICHOTOMA-like (CsaV3_1G003610), and General transcription factor IIH subunit (CsaV3_4G027560), were identified. In addition, the major salinity-relevant genes were recognized, including far upstream element-binding protein 2 (CsaV3_5G039940), glutamate dehydrogenase (GDH: CsaV3_4G013270), Sulfate transporter 1.1 (CsaV3_7G006330), serine/threonine protein phosphatase 2A regulatory subunit

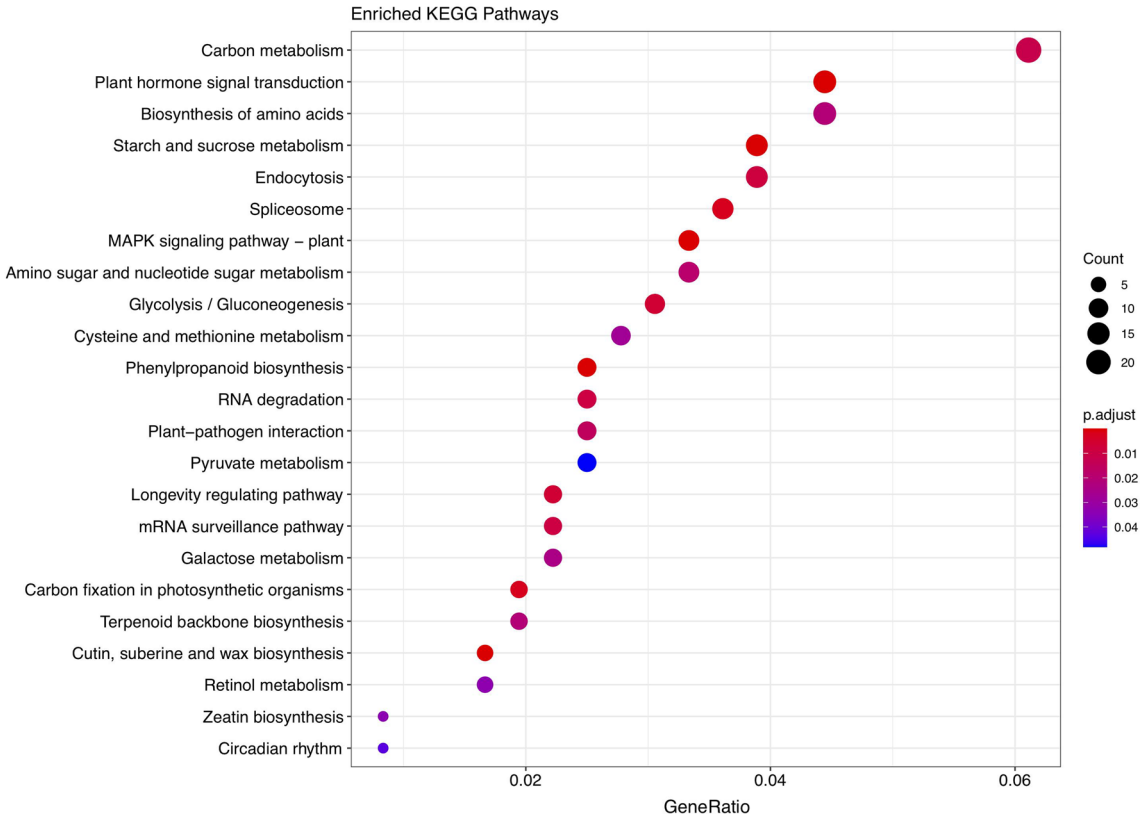

Fig. 6 KEGG pathway enrichment analysis of DEGs in four major modules (blue, brown, turquoise, and yellow). The x-axis represents the ratio number of DEGs, and the y-axis displays the KEGG pathway terms. The size and the color of circles indicate the number of enriched DEGs and the levels of significance, respectively

(CsaV3_6G040010), peroxidase (PRX: CsaV3_1G030120), and cysteine proteinase inhibitor (CsaV3_5G025580).

4 Discussion

In this study, we identified salinity-related lncRNAs and constructed the regulatory lncRNA-mRNA co-expression network using WGCNA in cucumber. Among 10 identified co-expression modules, four modules containing the highest amount of salt-responsive DEGs were selected for further analysis. KEGG pathway enrichment analysis of these major modules revealed DEGs that significantly contributed to the phenylpropanoid pathway, suggesting the determinative role of this pathway in the regulatory response of cucumber to salt stress. The phenylpropanoid pathway is considered a principal gateway for the synthesis of large numbers of secondary metabolites, including flavonoids, monolignols (the main building blocks of lignin), and phenolic compounds. Several studies have demonstrated that the phenylpropanoid pathway encompasses the main precursors for a large variety of phenolic compounds that are involved in plant protective processes against environmental stimuli (Feduraev et al. 2020; Valifard et al. 2015). In the phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL; E.C.4.3.1.5) has been recognized as the first key enzyme in the formation of secondary metabolites. Cell wall composition and phenolic metabolism remodeling is affected by PAL genes under various environmental conditions (Oliveira et al. 2020). The positive correlation between the NaCl concentration and the activity of PAL has been detected in several crops, such as *Triticum aestivum* (Cuong et al. 2020), *Olea europaea* (Rossi et al. 2016), *Carthamus tinctorius* (Dehghan et al. 2014), and *Jatropha curcas* (Gao et al. 2008). In terms of PAL activity alteration, this enzyme has been employed as a biochemical marker for the synthesis of protective and defensive compounds under stress conditions (Nadernejad et al. 2012). Additionally, the role of PAL genes has been revealed in lignin synthesis (Bido et al. 2010; Chun et al. 2019). Lignin was found to synthesize via the phenylpropanoid pathway followed by oxidative radical coupling of lignin monomers and provides mechanical support by surrounding the secondary cell walls of vascular plants (Oliveira et al. 2020). Kelij et al. (2013) reported the PAL-mediated lignin accumulation and lignification under salt stress lead to cell wall rigidity and, therefore, cell growth limitation. The MAPK signaling

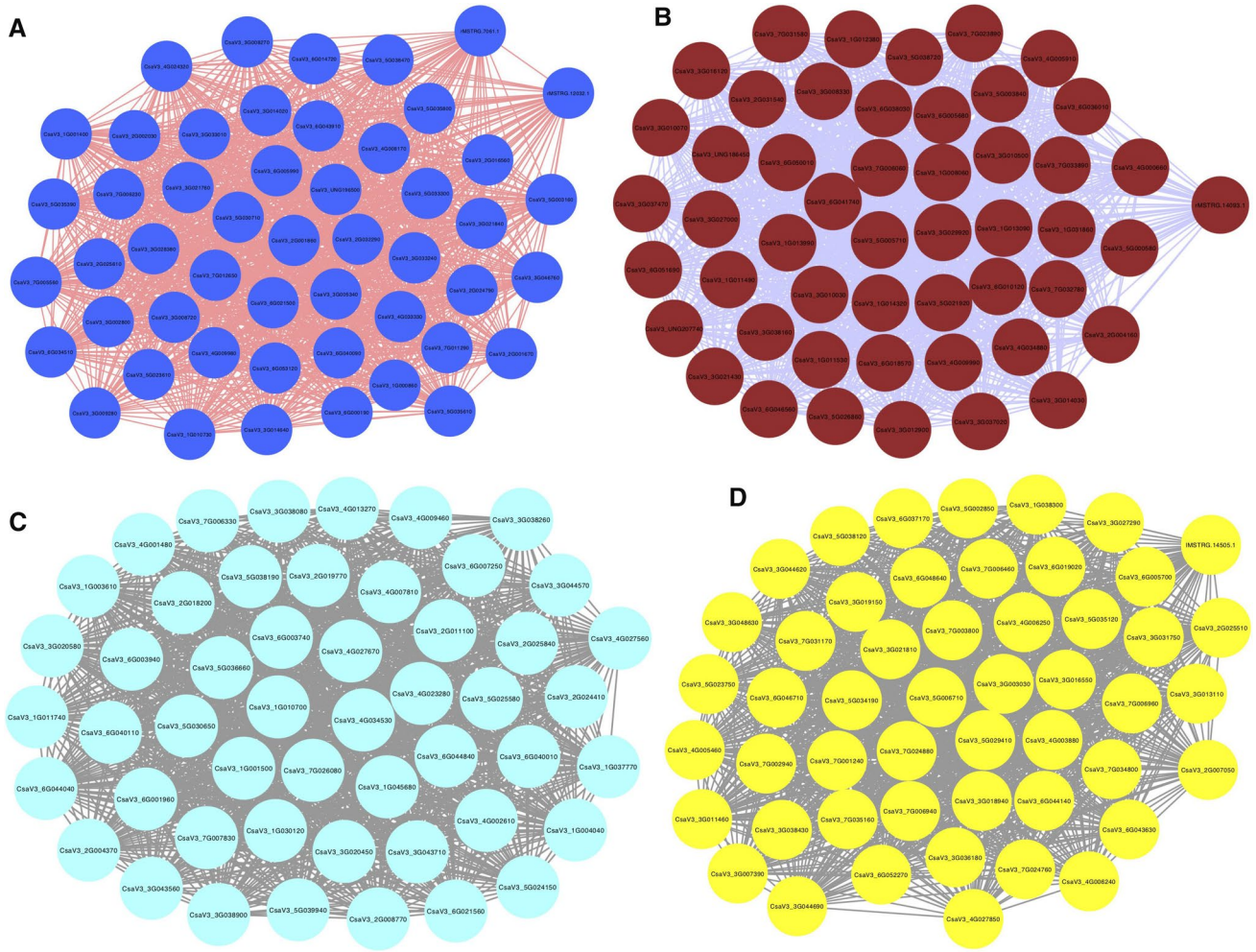

Fig. 7 Co-expression network analysis of 50 hub genes from the four modules. **a** Blue module, **b** brown module, **c** turquoise module, and **d** yellow module

pathway was recognized as another significantly enriched salt-related pathway. This detoxification-signaling pathway includes several sequentially phosphorylating and activating components that are involved in salt-stress signal transduction, antioxidant defense response, and eventually ROS homeostasis regulation (Yang and Guo 2018). Additionally, starch and sugar metabolism were among the enriched pathways, which could indicate the role of relevant DEGs in salt stress response through starch–sugar interconversion. Indeed, this is a common adaptive response of plant species to regulate the sugar levels by cycles of accumulation and degradation of source/sink starch under stress conditions (Dong et al. 2018; Zhu et al. 2019b). Sugar metabolites are considered ROS scavengers, osmoprotectants, and an easy-access source of carbon and energy to mitigate the negative effects of stress (Dong et al. 2018; Zhu et al. 2019b).

Through WGCNA analysis, we identified 50 distinct hub genes as the central and highly connected genes in each of the four major modules. Among hub genes, we identified TFs, key salt-related genes, as well as lncRNAs associated with salt stress in cucumber. In the blue module, TFs such as F-box/kelch-repeat protein, MYB-like, ERF, and bZIP were identified as the paramount regulators in response to salinity. F-box proteins have been identified as key elements in response to salt in several species, including *Oryza sativa* (Jain et al. 2007), *Medicago truncatula* (Song et al. 2015), *T. aestivum* (Zhou et al. 2015), and *Glycine max* (Yu et al. 2020). These studies have also illustrated that F-box proteins alleviate the negative effects of salt stress by regulating phenylpropanoid biosynthesis and ABA-mediated responses (Yu et al. 2020; Zhang et al. 2013). MYB family members are involved in diverse biological processes during growth and development (Zhang et al. 2018). The positive regulatory role of genes including *OsMYB6* in *Oryza sativa* (Tang et al. 2019), *ThMYB13* in *Tamarix hispida* (Zhang et al. 2018), and *AtMYB20* in *Arabidopsis thaliana* (Cui et al.

2013) have demonstrated the pivotal functions of this TF family in improving plant tolerance to salt stress. CRF4-like was another identified salt-responsive TF in the blue module, previously predicted as the target of *csa-miRn6-3p* (Zhu et al. 2019b). CRF4 is a component of the cytokinin signaling pathway that can participate in various stress responses through regulation of gene expression (Rashotte et al. 2006). Even though the role of this gene in modulating salt stress response has not been functionally investigated yet, the positive regulatory role of other CRFs such as tobacco stress-induced gene 1 (Tsi1) and *S. lycopersicum* SlCRF1 have been displayed in response to salinity (Zwack and Rashotte 2015). bZIP TFs are also considered the most important components in signal transduction pathways under various stress conditions like salinity (Zhu et al. 2018). This TF family has been reported as an important regulator in ABA-mediated stress response (Yoon et al. 2020) and flavonoid biosynthesis (Malacarne et al. 2016). It has been demonstrated that TabZIP-overexpressing *Arabidopsis* (Agarwal et al. 2019), OsbZIP71-overexpressing rice (Liu et al. 2014), and CabZIP25-silenced pepper (Gai et al. 2020) could confer salt tolerance in transgenic species. In addition to the salt-associated TFs, two salt-responsive lncRNAs, rMSTRG.12032.1 and rMSTRG.7061.1, as well as several key salt-related protein-coding genes, including SRK, UPS, and MsrA, were identified in the blue module. SRK is considered a major subclass of the receptor protein kinase (RPK) family, which is involved in phosphorylating serine/threonine residues (Afzal et al. 2008) and different signal transduction pathways (Bi et al. 2018). The positive regulatory role of this gene in conferring salt stress tolerance has been confirmed in GsSRK-overexpressing plants of *Arabidopsis* and in OsSRK1-overexpressing plants of *O. sativa* (Lei et al. 2020; Xu et al. 2013). UPS proteins have been found to play a major role in enhancing salt stress tolerance in *Arabidopsis* through transportation of the accumulated allantoin. Allanation is the process of purine degradation, a pathway that is intermediate for recycling the extra nitrogen assimilated in purines. This metabolite was suggested to act as a ROS protectant whose accumulation was essential for the induction of salt stress tolerance (Lescano et al. 2016). Furthermore, allanation is involved in the activation of ABA metabolism in response to abiotic stresses (Irani and Todd 2016). Numerous studies have displayed increased MsrA expression under high salt stress in diverse crops, including *O. sativa* (Zhang et al. 2009), *Solanum lycopersicum* (Dai and Wang 2012), *Hordeum vulgare* (Fatehi et al. 2012), *Nicotiana tabacum* (Liu et al. 2014), and *Glycine max* (Chu et al. 2016). The role of this enzyme has been demonstrated to prevent oxidative damage arising from the ROSs derived from ambient stimuli. Since methionine (Met) has a remarkable reactivity with ROS molecules, its oxidation leads to the conformation of many vital proteins. Hence, MsrA could enzymatically reduce two oxidized forms, including methionine-S-sulfoxide (Met-S-SO) and methionine-R-sulfoxide (Met-R-SO), back to Met in a post-translational modification process (Dai and Wang 2012; Guo et al. 2009).

In the brown module, rMSTRG.14093.1 was recognized as the only salt-associated lncRNA, together with two TFs, COL6 and SRS1, and other neighboring co-expressed mRNAs, such as RLK and SPDS. The SRS transcription family members are involved in various physiological and biochemical processes via their conserved RING-like zinc-finger domain (CX2CX7CX4CX2C2X6C) (Elenbaas et al. 1996). Zhou et al. (2020) analyzed the stress-related cis-elements of the SRS family and determined their possible involvement in the abiotic stress signaling pathway. Moreover, these researchers demonstrated the potential function of *GmSRS18* in the negative regulation of salt-related genes in GmSRS18-overexpressing *Arabidopsis* seedlings. RLK proteins have been indicated to be involved in abiotic stress responses, including salinity through signal transduction (Chen et al. 2013). The extracellular and intracellular kinase domains of these transmembrane proteins play a major role in perceiving and propagating signals, respectively. The role of different RLK proteins, including *OsSIK1*, *OsSIT1*, and *OsSTRK1*, in *O. sativa* has been indicated as a positive regulator in salt stress response. Moreover, it was found that *OsSTRK1* serves as a H_2O_2 homeostasis regulator and protects plants against cellular damage under salinity (Zhou et al. 2018). In other crops, the fundamental roles of RLK proteins in conferring enhanced salt tolerance are consensual (Sun et al. 2018; Wang et al. 2017; Zhao et al. 2013). SPDS proteins were reported to enhance salt tolerance in transgenic plants including *Arabidopsis* (Kasukabe et al. 2004), and *Pyrus communis* (Wen et al. 2008). They were also found to positively regulate the key stress-responsive genes and lead to build-up protection mechanisms under stress conditions (Kasukabe et al. 2004). SPDS is known to be involved in the biosynthesis of two polyamines, spermidine and spermine. Polyamines have emerged as the hub promoting salt stress tolerance through the scavenging of ROS and inducing antioxidant activity (Todorova et al. 2013).

In the yellow module, lMSTRG.14505.1 was detected as the only lncRNA, and its relevance was found with two salt-responsive TFs, namely HSFs and MADS-box protein, and several major salt-associated genes, such as TPP, GST, and PRX. The participation of HSFs was found in various signaling pathways under abiotic stresses (Hwang et al. 2014). These TFs can mediate salt stress tolerance through transcriptional regulation. For instance, PeHSF in *Populus euphratica* regulates the expression of *PeWRKY1* by binding to HSE (Shen et al. 2015). In another study, AtHSFA7b has emerged as the transactivator of regulator genes, which can directly or indirectly induce salinity tolerance through binding to their E-box-like or HSE motifs (Zhang et al. 2019b).

MADS-box genes are known as important components in regulatory networks underlying abiotic stress responses. The positive regulatory roles of MADS-box genes have been recognized in ABA signaling, ROS homeostasis, and detoxification processes mediated by the antioxidant enzymatic activities, such as by PRX and catalase (Castelán-Muñoz et al. 2019). Krasensky et al. (2014) demonstrated that TPP is involved in the final step of trehalose metabolism and its overexpression leads to increased trehalose biosynthesis. This metabolism positively modulates abiotic stress tolerance. Other studies have illustrated the accumulation of trehalose levels under salinity (Garg et al. 2002). Lee et al. (2003) reported that the accumulated trehalose in chloroplasts could protect the thylakoid membranes against salt stress damage. Additionally, Il et al. (2013) reported the enhanced salt stress tolerance as well as photosynthesis rate through the constitutive overexpression of TPP in *S. lycopersicum*. The involvement of the GSTs in abiotic stress response has been illustrated in previous studies (Ding et al. 2017; Kumar and Trivedi 2018; Nianiou-Obeidat et al. 2017). Qi et al. (2010) have shown that the overexpression of GST in transgenic *Arabidopsis* plants positively modulated high metabolic activity maintenance and negatively regulated lipid peroxidation, thereby inducing peroxide scavenging capacity and consequently conferring salt stress tolerance. The prominent role of PRX in antioxidant responses under salt stress has been proved in various studies (Jin et al. 2019), and the association of different members of this ROS-scavenging gene family with salt tolerance induction has been functionally investigated (Su et al. 2020; Wang et al. 2017; Zhang et al. 2017).

In the turquoise module, unlike other modules, no lncRNA was identified. In this module, bHLH35-like was identified as the key salt-responsive TF along with a set of key salt-related genes, including GDH and PRX, which was also recognized in the yellow module. Basic helix-loop-helix (bHLH) TFs have been identified as the second-largest family that modulates gene expression upon different environmental stimuli and promotes tolerance to diverse abiotic stresses (Chen et al. 2018). Jiang et al. (2019) confirmed the role of *OsbHLH035* in conferring salt stress tolerance and recovery of plants after stress through the ABA-independent pathway. GDH is known as a mitochondrial enzyme that plays a major role in regulating glutamate homeostasis (Fontaine et al. 2013). Tercé-Laforgue et al. (2015) demonstrated that the activity of GDH is impacted by a post-transcriptional mechanism under different NaCl concentrations.

According to the achieved results from the study of Zhu et al. (2019a), reduction in root hydraulic conductivity was the primary response of cucumber cv. 'Jinyou 1' due to inhibition of the water uptake by salt-treated roots. This change triggers the reduction of leaf hydraulic conductivity, decreasing the water content in both leaf and root tissues. According to the same study, aquaporin gene family members are considered the main positive regulators of water flow through biological cell membranes, and prominent moderators retain the cellular water homeostasis in cucumber. In this study, this gene family was categorized in the turquoise module containing co-expressed genes that are positively regulated by salt stress. Since co-expressed genes in a module presumably coordinate a common process and share a similar function, the genes in the turquoise module can potentially be involved in saline sensing and initial signaling events in cucumber roots and modulate the alteration of hydraulic conductivity and water content under salinity. A significant decrease of growth variables has been reported in the cultivar 'Jinyou 1' grown under different salt stress treatments (Ahmad et al. 2017; Zhu et al. 2019a). It has been demonstrated that the length of shoots and roots as well as plant fresh/dry weights usually decrease due to disruption of water uptake, ionic imbalance, deficiency in nutrients, and eventually unsettled metabolic activity. Osmolyte accumulation, cell division, and enlargement are the most common mechanisms in response to these changes (Sahin et al. 2018; Shahid et al. 2014; Steffens et al. 2005). Here, the brown module comprised Hydroxyproline *O*-galactosyltransferase (CsaV3_1G008060), Trehalose 6-phosphate phosphatase (CsaV3_6G045560), Sugar transporter ERD6-like (CsaV3_3G014030), and Inositol oxygenase (CsaV3_1G040000) associated with osmolyte production and transduction. Furthermore, in this module and the blue module, phytohormone-associated genes such as Cytokinin dehydrogenase (CsaV3_4G036030), ethylene-responsive transcription factor CRF4-like (CsaV3_3G021840), Gibberellin 20-oxidase, putative (CsaV3_6G005990), and jasmonic acid-amido synthetase JAR1 (CsaV3_3G008270) were identified. It has been indicated that phytohormones regulate osmolyte accumulation under abiotic stress, especially salinity (Sharma and Zheng 2019), elucidating the possible contribution of coding genes and lncRNAs (including rMSTRG.12032.1, rMSTRG.7061.1, and rMSTRG.14093.1) in brown and blue modules in cucumber adaptive responses that affect growth-related attributes under salt stress. Assessment of physiological parameters in the cultivar 'Jinyou 1' revealed the induction of H_2O_2 content in plants treated with 75 mM NaCl (Zhu et al. 2019a), as well as an increase in superoxide dismutase (SOD) and peroxidase (PRX) in plants subjected to 60 mM, followed by a decrease in activity under 120 mM treatment (Ahmad et al. 2017). In addition, the increased amount of malondialdehyde (MDA) content at two levels of salt stress (60 and 120 mM NaCl) has been reported (Ahmad et al. 2017). Since enzymatic antioxidants play a crucial role in protection against cellular damage induced by overproduced ROS under salt stress, the identification

of ROS scavenging–related genes such as PRX, GST, and MADS-box in turquoise and yellow modules suggest regulatory roles of lMSTRG.14505.1 and genes with unknown function in pathways related to ROS-scavenging detoxification processes, affecting the antioxidant enzyme activity values against different salt stress treatments in this sensitive cucumber cultivar.

According to the module–trait relationship and the eigengene expression profile, it was found that the most important salt-related genes identified in two major blue and brown modules were negatively regulated by salinity stress in the cucumber cultivar 'Jinyou 1', while in other salt-tolerant crops, most of these salt-related genes were shown to have a positive regulatory role in response to salinity (Abd-Hamid et al. 2020; Ahmad et al. 2017; Guo et al. 2018; Medici et al. 2014; Tang et al. 2019; Ye et al. 2017). In conclusion, the salt stress sensitivity of cultivar 'Jinyou 1' can be demonstrated by this negative correlation (between module eigengenes and salinity) in opposition to tolerant crops (with a positive relationship). This result is validated by previous studies (Ahmad et al. 2017; Zhu et al. 2019a) that revealed the susceptibility of this cultivar via physiological and morphological characteristics under salinity.

5 Conclusion

The findings of this study unveil the hub genes consisting of TFs (including SRS1, MADS-box protein, and DICHOTOMA-like) as well as protein-coding genes (including SRK, UPS, MsrA, Alpha/beta hydrolase-like, Pentatricopeptide repeat, and Cysteine proteinase inhibitor) associated with salinity response in cucumber. Hub genes, such as dymeclin (CsaV3_3G012900), Pectinesterase (CsaV3_7G029580), U2 snRNP auxiliary factor large subunit (CsaV3_6G003130), MLP-like protein 28 (CsaV3_3G021430), and Magnesium transporter MRS2-like protein (CsaV3_1G014320), were for the first time associated with crop response to salinity. Additionally, lncRNAs were identified as the leading components of regulatory networks underlying salt stress response. These markers can be further used to analyze salt tolerance (and susceptibility) of cucumber cultivars and employed in breeding programs to enhance tolerance and alleviate the detrimental (deleterious) effects of salinity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13580-021-00418-y.

Acknowledgements The authors gratefully acknowledge the Sheikh Bahaei National High Performance Computing Center (SBNHPCC) for providing computing facilities and time. SBNHPCC is supported by scientific and technological department of presidential office and Isfahan University of Technology (IUT).

Author contributions AS and MK contributed to designing the experiment. MHP, KBL and MK collected samples and extracted RNAs. AS and MK analyzed and interpreted the data. AS and MK wrote the paper. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Bi G, Zhou Z, Wang W, Li L, Rao S, Wu Y, Zhang X, Menke FLH, Chen S, Zhou JM (2018) Receptor-like cytoplasmic kinases directly link diverse pattern recognition receptors to the activation of mitogen-activated protein kinase cascades in arabidopsis. Plant Cell 30:1543–1561. https://doi.org/10.1105/tpc.17.00981

Abd-Hamid NA, Ahmad-Fauzi MI, Zainal Z, Ismail I (2020) Diverse and dynamic roles of F-box proteins in plant biology. Planta 251:68. https://doi.org/10.1007/s00425-020-03356-8

Afzal AJ, Wood AJ, Lightfoot DA (2008) Plant receptor-like serine threonine kinases: Roles in signaling and plant defense. Mol Plant-Microbe Interact 21:507–517. https://doi.org/10.1094/MPMI-21-5-0507

Agarwal P, Baranwal VK, Khurana P (2019) Genome-wide analysis of bZIP transcription factors in wheat and functional characterization of a TabZIP under abiotic stress. Sci Rep 9:4608. https://doi.org/10.1038/s41598-019-40659-7

Ahmad H, Hayat S, Ali M, Ghani MI, Zhihui C (2017) Regulation of growth and physiological traits of cucumber (*Cucumis sativus* L.) through various levels of 28-homobrassinolide under salt stress conditions. Can J Plant Sci 98:132–140. https://doi.org/10.1139/cjps-2016-0404

Ahmadi Khoei M, Karimi M, Karamian R, Amini S, Soorni A (2021) Identification of the complex interplay between nematode-related lncRNAs and their target genes in *Glycine max* L. Front Plant Sci 12:779597. https://doi.org/10.3389/fpls.2021.779597

Amirbakhtiar N, Ismaili A, Ghaffari MR, Firouzabadi FN, Shobbar ZS (2019) Transcriptome response of roots to salt stress in a salinity-tolerant bread wheat cultivar. PLoS ONE 14:e0213305. https://doi.org/10.1371/journal.pone.0213305

Bido G de S, Ferrarese M de LL, Marchiosi R, Ferrarese-Filho O (2010) Naringenin inhibits the growth and stimulates the lignification of soybean root. Brazilian Arch Biol Technol 53:533–542

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Castelán-Muñoz N, Herrera J, Cajero-Sánchez W, Arrizubieta M, Trejo C, García-Ponce B, Sánchez M de la P, Álvarez-Buylla ER, Garay-Arroyo A (2019) MADS-box genes are key components of genetic regulatory networks involved in abiotic stress and plastic developmental responses in plants. Front Plant Sci. https://doi.org/10.3389/fpls.2019.00853

Chandran AKN, Kim JW, Yoo YH, Park HL, Kim YJ, Cho MH, Jung KH (2019) Transcriptome analysis of rice-seedling roots under soil–salt stress using RNA-Seq method. Plant Biotechnol Rep 13:567–578. https://doi.org/10.1007/s11816-019-00550-3

Chen C, Chen X, Han J, Lu W, Ren Z (2020) Genome-wide analysis of the WRKY gene family in the cucumber genome and transcriptome-wide identification of WRKY transcription factors that

respond to biotic and abiotic stresses. BMC Plant Biol 20:443. https://doi.org/10.1186/s12870-020-02625-8

Chen HC, Cheng WH, Hong CY, Sen CY, Chang MC (2018) The transcription factor OsbHLH035 mediates seed germination and enables seedling recovery from salt stress through ABA-dependent and ABA-independent pathways, respectively. Rice 11:50. https://doi.org/10.1186/s12284-018-0244-z

Chen J, Zhang J, Hu J, Xiong W, Du C, Lu M (2017) Integrated regulatory network reveals the early salt tolerance mechanism of Populus euphratica. Sci Rep 7:6769. https://doi.org/10.1038/s41598-017-05240-0

Chen LJ, Wuriyanghan H, Zhang YQ, Duan KX, Chen HW, Li QT, Lu X, He SJ, Ma B, Zhang WK, Lin Q, Chen SY, Zhang JS (2013) An S-domain receptor-like kinase, OsSIK2, confers abiotic stress tolerance and delays dark-induced leaf senescence in rice. Plant Physiol 163:1752–1765. https://doi.org/10.1104/pp.113.224881

Chin C-H, Chen S-H, Wu H-H, Ho C-W, Ko M-T, Lin C-Y (2014) cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 8(Suppl 4):S11–S11. https://doi.org/10.1186/1752-0509-8-S4-S11

Chu HD, Nguyen KL, Watanabe Y, Le DT, Tran LSP (2016) Expression analyses of soybean genes encoding methionine-R-sulfoxide reductase under various conditions suggest a possible role in the adaptation to stress. Appl Biol Chem 59:681–687. https://doi.org/10.1007/s13765-016-0213-4

Chun HJ, Baek D, Cho HM, Lee SH, Jin BJ, Yun D-J, Hong Y-S, Kim MC (2019) Lignin biosynthesis genes play critical roles in the adaptation of Arabidopsis plants to high-salt stress. Plant Signal Behav 14:1625697. https://doi.org/10.1080/15592324.2019.1625697

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

Cuong DM, Kwon SJ, Van Nguyen B, Chun SW, Kim JK, Park SU (2020) Effect of salinity stress on phenylpropanoid genes expression and related gene expression in wheat sprout. Agronomy 10:390. https://doi.org/10.3390/agronomy10030390

Dai C, Wang MH (2012) Characterization and functional analysis of methionine sulfoxide reductase A gene family in tomato. Mol Biol Rep 39:6297–6308. https://doi.org/10.1007/s11033-012-1451-0

Dehghan S, Sadeghi M, Oppel AP, Fischer R, Lakes-Harlan R, Kavousi HR, Vilcinskas A, Rahnamaeian M (2014) Differential inductions of phenylalanine ammonia-lyase and chalcone synthase during wounding, salicylic acid treatment, and salinity stress in safflower, Carthamus tinctorius. Biosci Rep 34:273–282. https://doi.org/10.1042/BSR20140026

Deng F, Zhang X, Wang W, Yuan R, Shen F (2018) Identification of Gossypium hirsutum long non-coding RNAs (lncRNAs) under salt stress. BMC Plant Biol 18:23. https://doi.org/10.1186/s12870-018-1238-0

Ding N, Wang A, Zhang X, Wu Y, Wang R, Cui H, Huang R, Luo Y (2017) Identification and analysis of glutathione S-transferase gene family in sweet potato reveal divergent GST-mediated networks in aboveground and underground tissues in response to abiotic stresses. BMC Plant Biol 17:225. https://doi.org/10.1186/s12870-017-1179-z

Dobin A, Gingeras TR (2015) Mapping RNA-seq reads with STAR. Curr Protoc Bioinforma 51:11.14.1–11.14.19. https://doi.org/10.1002/0471250953.bi1114s51

Dong S, Zhang J, Beckles DM (2018) A pivotal role for starch in the reconfiguration of 14C-partitioning and allocation in Arabidopsis thaliana under short-term abiotic stress. Sci Rep 8:9314. https://doi.org/10.1038/s41598-018-27610-y

Elenbaas B, Dobbelstein M, Roth J, Shenk T, Levine AJ (1996) The MDM2 oncoprotein binds specifically to RNA through its ring finger domain. Mol Med 2:439–451. https://doi.org/10.1007/bf03401903

Eom SH, Lee HJ, Lee JH, Wi SH, Kim SK, Hyun TK (2019) Identification and functional prediction of drought-responsive long non-coding RNA in tomato. Agronomy 9:629. https://doi.org/10.3390/agronomy9100629

Fatehi F, Hosseinzadeh A, Alizadeh H, Brimavandi T, Struik PC (2012) The proteome response of salt-resistant and salt-sensitive barley genotypes to long-term salinity stress. Mol Biol Rep 39:6387–6397. https://doi.org/10.1007/s11033-012-1460-z

Feduraev P, Skrypnik L, Riabova A, Pungin A, Tokupova E, Maslennikov P, Chupakhina G (2020) Phenylalanine and tyrosine as exogenous precursors of wheat (*Triticum aestivum* L.) secondary metabolism through PAL-associated pathways. Plants 9:476. https://doi.org/10.3390/plants9040476

Fontaine JX, Tercé-Laforgue T, Bouton S, Pageau K, Lea PJ, Dubois F, Hirel B (2013) Further insights into the isoenzyme composition and activity of glutamate dehydrogenase in Arabidopsis thaliana. Plant Signal Behav 8:e23329. https://doi.org/10.4161/psb.23329

Fu L, Ding Z, Tan D, Han B, Sun X, Zhang J (2020) Genome-wide discovery and functional prediction of salt-responsive lncRNAs in duckweed. BMC Genomics 21:212. https://doi.org/10.1186/s12864-020-6633-x

Gai WX, Ma X, Qiao YM, Shi BH, ul Haq S, Li QH, Wei AM, Liu KK, Gong ZH (2020) Characterization of the bZIP transcription factor family in pepper (*Capsicum annuum* L.): CabZIP25 positively modulates the salt tolerance. Front Plant Sci. https://doi.org/10.3389/fpls.2020.00139

Gao S, Ouyang C, Wang S, Xu Y, Tang L, Chen F (2008) Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. Plant, Soil Environ 54:374–381. https://doi.org/10.17221/410-pse

Garg AK, Kim JK, Owens TG, Ranwala AP, Do Choi Y, Kochian LV, Wu RJ (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc Natl Acad Sci U S A 99:15898–15903. https://doi.org/10.1073/pnas.252637799

Ghorbani F, Abolghasemi R, Haghighi M, Etemadi N, Wang S, Karimi M, Soorni A (2021) Global identification of long non-coding RNAs involved in the induction of spinach flowering. BMC Genomics 22:704. https://doi.org/10.1186/s12864-021-07989-1

Guo X, Wu Y, Wang Y, Chen Y, Chu C (2009) OsMSRA4.1 and OsMSRB1.1, two rice plastidial methionine sulfoxide reductases, are involved in abiotic stress responses. Planta 230:227–238. https://doi.org/10.1007/s00425-009-0934-2

Guo Y, Jiang Q, Hu Z, Sun X, Fan S, Zhang H (2018) Function of the auxin-responsive gene TaSAUR75 under salt and drought stress. Crop J 6:181–190. https://doi.org/10.1016/j.cj.2017.08.005

Huanca-Mamani W, Arias-Carrasco R, Cárdenas-Ninasivincha S, Rojas-Herrera M, Sepúlveda-Hermosilla G, Caris-Maldonado JC, Bastías E, Maracaja-Coutinho V (2018) Long non-coding RNAs responsive to salt and boron stress in the hyper-arid lluteño maize from atacama desert. Genes (basel) 9:170. https://doi.org/10.3390/genes9030170

Hwang SM, Kim DW, Woo MS, Jeong HS, Son YS, Akhter S, Choi GJ, Bahk JD (2014) Functional characterization of ArabidopsisHsfA6a as a heat-shock transcription factor under high salinity and dehydration conditions. Plant Cell Environ 37:1202–1222. https://doi.org/10.1111/pce.12228

Il LJ, Min SR, Lee JH, Lim YH, Kim JK, Bae CH, Liu JR (2013) Overexpression of a trehalose-6-phosphate synthase/phosphatase fusion gene enhances tolerance and photosynthesis during drought and salt stress without growth aberrations in tomato. Plant Cell Tissue Organ Cult 112:257–262. https://doi.org/10.1007/s11240-012-0225-7

Ma J, Bai X, Luo W, Feng Y, Shao X, Bai Q, Sun S, Long Q, Wan D (2019) Genome-wide identification of long noncoding RNAs and their responses to salt stress in two closely related poplars. Front Genet. https://doi.org/10.3389/fgene.2019.00777

Irani S, Todd CD (2016) Ureide metabolism under abiotic stress in Arabidopsis thaliana. J Plant Physiol 199:87–95. https://doi.org/10.1016/j.jplph.2016.05.011

Jain M, Nijhawan A, Arora R, Agarwal P, Ray S, Sharma P, Kapoor S, Tyagi AK, Khurana JP (2007) F-Box proteins in rice. Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. Plant Physiol 143:1467–1483. https://doi.org/10.1104/pp.106.091900

Jiang J, Ren X, Li L, Hou R, Sun W, Jiao C, Yang N, Dong Y (2020) H2S Regulation of metabolism in cucumber in response to salt-stress through transcriptome and proteome analysis. Front Plant Sci. https://doi.org/10.3389/fpls.2020.01283

Jiang L, Tian X, Li S, Fu Y, Xu J, Wang G (2019) The aabhlh35 transcription factor identified from anthurium andraeanum is involved in cold and drought tolerance. Plants 8:216. https://doi.org/10.3390/plants8070216

Jimenez-Jacinto V, Sanchez-Flores A, Vega-Alvarado L (2019) Integrative differential expression analysis for multiple experiments (IDEAMEX): a web server tool for integrated RNA-seq data analysis. Front Genet. https://doi.org/10.3389/fgene.2019.00279

Jin T, Sun Y, Zhao R, Shan Z, Gai J, Li Y (2019) Overexpression of peroxidase gene GsPRX9 confers salt tolerance in soybean. Int J Mol Sci 20:3745. https://doi.org/10.3390/ijms20153745

Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30. https://doi.org/10.1093/nar/28.1.27

Kang YJ, Yang DC, Kong L, Hou M, Meng YQ, Wei L, Gao G (2017) CPC2: A fast and accurate coding potential calculator based on sequence intrinsic features. Nucleic Acids Res 45:W12–W16. https://doi.org/10.1093/nar/gkx428

Kasukabe Y, He L, Nada K, Misawa S, Ihara I, Tachibana S (2004) Overexpression of Spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic Arabidopsis thaliana. Plant Cell Physiol 45:712–722. https://doi.org/10.1093/pcp/pch083

Kelij S, Majd A, Nematzade G, Jonoubi P, Haghighi L (2013) Phenylalanine ammonialyase gene expression and activity in relation to lignin deposition in salt stressed Aeluropus littoralis. Adv Stud Biol 5:403–412

Kosová K, Prášil IT, Vítámvás P (2013) Protein contribution to plant salinity response and tolerance acquisition. Int J Mol Sci 14:6757–6789. https://doi.org/10.3390/ijms14046757

Krasensky J, Broyart C, Rabanal FA, Jonak C (2014) The redox-sensitive chloroplast trehalose-6-phosphate phosphatase AtTPPD regulates salt stress tolerance. Antioxidants Redox Signal 21:1289–1304. https://doi.org/10.1089/ars.2013.5693

Krishnamurthy P, Mohanty B, Wijaya E, Lee D-Y, Lim T-M, Lin Q, Xu J, Loh C-S, Kumar PP (2017) Transcriptomics analysis of salt stress tolerance in the roots of the mangrove Avicennia officinalis. Sci Rep 7:10031. https://doi.org/10.1038/s41598-017-10730-2

Kumar S, Trivedi PK (2018) Glutathione S-transferases: Role in combating abiotic stresses including arsenic detoxification in plants. Front Plant Sci. https://doi.org/10.3389/fpls.2018.00751

Langfelder P, Horvath S (2008) WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics 9:559. https://doi.org/10.1186/1471-2105-9-559

Lee SB, Bin KH, Kwon SJ, Park SC, Jeong MJ, Han SE, Byun MO, Daniell H (2003) Accumulation of trehalose within transgenic chloroplasts confers drought tolerance. Mol Breed 11:1–13. https://doi.org/10.1023/A:1022100404542

Lei L, Zheng H, Bi Y, Yang L, Liu H, Wang J, Sun J, Zhao H, Li X, Li J, Lai Y, Zou D (2020) Identification of a major QTL and candidate gene analysis of salt tolerance at the bud burst stage in rice (*Oryza sativa* L.) using QTL-seq and RNA-Seq. Rice 13:55. https://doi.org/10.1186/s12284-020-00416-1

Lescano CI, Martini C, González CA, Desimone M (2016) Allantoin accumulation mediated by allantoinase downregulation and transport by Ureide Permease 5 confers salt stress tolerance to Arabidopsis plants. Plant Mol Biol 91:581–595. https://doi.org/10.1007/s11103-016-0490-7

Li M, Xing Q, Lihong G, Qing L, Shuzhen L, Chaoxing H, Yansu L, Xianchang Y (2019) Selection of reference genes for quantitative real-time PCR analysis in cucumber (*Cucumis sativus* L.), pumpkin (Cucurbita moschata Duch) and cucumber-pumpkin grafted plants. PeerJ 2019:e6536. https://doi.org/10.7717/peerj.6536

Liang YH, Cai B, Chen F, Wang G, Wang M, Zhong Y, Cheng ZMM (2014) Construction and validation of a gene co-expression network in grapevine (*Vitis vinifera* L.). Hortic Res 1:14040. https://doi.org/10.1038/hortres.2014.40

Liu A, Xiao Z, Li MW, Wong FL, Yung WS, Ku YS, Wang Q, Wang X, Xie M, Yim AKY, Chan TF, Lam HM (2019) Transcriptomic reprogramming in soybean seedlings under salt stress. Plant Cell Environ 42:98–114. https://doi.org/10.1111/pce.13186

Liu C, Mao B, Ou S, Wang W, Liu L, Wu Y, Chu C, Wang X (2014) OsbZIP71, a bZIP transcription factor, confers salinity and drought tolerance in rice. Plant Mol Biol 84:19–36. https://doi.org/10.1007/s11103-013-0115-3

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550. https://doi.org/10.1186/s13059-014-0550-8

Lowe TM, Chan PP (2016) tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44:W54–W57. https://doi.org/10.1093/nar/gkw413

Malacarne G, Coller E, Czemmel S, Vrhovsek U, Engelen K, Goremykin V, Bogs J, Moser C (2016) The grapevine VvibZIPC22 transcription factor is involved in the regulation of flavonoid biosynthesis. J Exp Bot 67:3509–3522. https://doi.org/10.1093/jxb/erw181

Marium A, Kausar A, Ali Shah SM, Ashraf MY, Akhtar N, Akram M, Riaz M (2019) Assessment of cucumber genotypes for salt tolerance based on germination and physiological indices. Dose-Response 17:1559325819889809. https://doi.org/10.1177/1559325819889809

Medici A, Laloi M, Atanassova R (2014) Profiling of sugar transporter genes in grapevine coping with water deficit. FEBS Lett 588:3989–3997. https://doi.org/10.1016/j.febslet.2014.09.016

Mukherjee PK, Nema NK, Maity N, Sarkar BK (2013) Phytochemical and therapeutic potential of cucumber. Fitoterapia 84:227–236. https://doi.org/10.1016/j.fitote.2012.10.003

Nadernejad N, Ahmadimoghadam A, Hosseinifard J, Pourseyedi S (2012) Phenylalanin ammonia-lyase activity, total phenolic and flavonoid content in flowers, leaves, hulls and kernels of three pistachio (*Pistacia vera* L). Cultivars 12:807–814

Nawaz K, Hussain K, Majeed A, Khan F, Afghan S, Ali K (2010) Fatality of salt stress to plants: morphological, physiological and biochemical aspects. Afr J Biotechnol 9:5475–5480. https://doi.org/10.4314/ajb.v9i34

Nefissi Ouertani R, Arasappan D, Abid G, Ben Chikha M, Jardak R, Mahmoudi H, Mejri S, Ghorbel A, Ruhlman TA, Jansen RK (2021) Transcriptomic analysis of salt-stress-responsive genes

in barley roots and leaves. Int J Mol Sci 22:8155. https://doi.org/10.3390/ijms22158155

Nianiou-Obeidat I, Madesis P, Kissoudis C, Voulgari G, Chronopoulou E, Tsaftaris A, Labrou NE (2017) Plant glutathione transferase-mediated stress tolerance: functions and biotechnological applications. Plant Cell Rep 36:791–805. https://doi.org/10.1007/s00299-017-2139-7

Oliveira DM, Mota TR, Salatta FV, Sinzker RC, Končitíková R, Kopečný D, Simister R, Silva M, Goeminne G, Morreel K (2020) Cell wall remodeling under salt stress: insights into changes in polysaccharides, feruloylation, lignification, and phenolic metabolism in maize. Plant Cell Environ 43:2172–2191

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf 60:324–349. https://doi.org/10.1016/j.ecoenv.2004.06.010

Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL (2015) StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol 33:290–295. https://doi.org/10.1038/nbt.3122

Qi YC, Liu WQ, Qiu LY, Zhang SM, Ma L, Zhang H (2010) Overexpression of glutathione S-transferase gene increases salt tolerance of arabidopsis. Russ J Plant Physiol 57:233–240. https://doi.org/10.1134/S102144371002010X

Qin T, Zhao H, Cui P, Albesher N, Xionga L (2017) A nucleus-localized long non-coding rna enhances drought and salt stress tolerance. Plant Physiol 175:1321–1336. https://doi.org/10.1104/pp.17.00574

Rashotte AM, Mason MG, Hutchison CE, Ferreira FJ, Schaller GE, Kieber JJ (2006) A subset of Arabidopsis AP2 transcription factors mediates cytokinin responses in concert with a two-component pathway. Proc Natl Acad Sci USA 103:11081–11085. https://doi.org/10.1073/pnas.0602038103

Rolly NK, Imran QM, Lee IJ, Yun BW (2020) Salinity stress-mediated suppression of expression of salt overly sensitive signaling pathway genes suggests negative regulation by AtbZIP62 transcription factor in Arabidopsis thaliana. Int J Mol Sci 21:1726. https://doi.org/10.3390/ijms21051726

Rossi L, Borghi M, Francini A, Lin X, Xie DY, Sebastiani L (2016) Salt stress induces differential regulation of the phenylpropanoid pathway in Olea europaea cultivars Frantoio (salt-tolerant) and Leccino (salt-sensitive). J Plant Physiol 204:8–15. https://doi.org/10.1016/j.jplph.2016.07.014

Sahin U, Ekinci M, Ors S, Turan M, Yildiz S, Yildirim E (2018) Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (Brassica oleracea var. capitata). Sci Hortic (amsterdam) 240:196–204. https://doi.org/10.1016/j.scienta.2018.06.016

Shahid MA, Balal RM, Pervez MA, Garcia-Sanchez F, Gimeno V, Abbas T, Mattson NS, Riaz A (2014) Treatment with 24-epibrassinolide mitigates NaCl-induced toxicity by enhancing carbohydrate metabolism, osmolyte accumulation, and antioxidant activity in Pisum sativum. Turk J Botany 38:511–525. https://doi.org/10.3906/bot-1304-45

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: A software Environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504. https://doi.org/10.1101/gr.1239303

Sharma A, Zheng B (2019) Molecular responses during plant grafting and its regulation by auxins, cytokinins, and gibberellins. Biomolecules 9:397. https://doi.org/10.3390/biom9090397

Shen Z, Yao J, Sun J et al (2015) Populus euphratica HSF binds the promoter of WRKY1 to enhance salt tolerance. Plant Sci 235:89–100. https://doi.org/10.1016/j.plantsci.2015.03.006

Shumayla SS, Taneja M, Tyagi S, Singh K, Upadhyay SK (2017) Survey of high throughput RNA-seq data reveals potential roles for lncrnas during development and stress response in bread wheat. Front Plant Sci. https://doi.org/10.3389/fpls.2017.01019

Simopoulos CMA, Weretilnyk EA, Golding GB (2018) Prediction of plant lncRNA by ensemble machine learning classifiers. BMC Genomics 19:316. https://doi.org/10.1186/s12864-018-4665-2

Song JB, Wang YX, Li HB, Li BW, Zhou ZS, Gao S, Yang ZM (2015) The F-box family genes as key elements in response to salt, heavy mental, and drought stresses in Medicago truncatula. Funct Integr Genomics 15:495–507. https://doi.org/10.1007/s10142-015-0438-z

Steffens D, Hütsch BW, Eschholz T, Lošák T, Schubert S (2005) Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. Plant Soil Environ 51:545–552. https://doi.org/10.17221/3630-pse

Su P, Yan J, Li W, Wang L, Zhao J, Ma X, Li A, Wang H, Kong L (2020) A member of wheat class III peroxidase gene family, TaPRX-2A, enhanced the tolerance of salt stress. BMC Plant Biol 20:392. https://doi.org/10.1186/s12870-020-02602-1

Sun M, Qian X, Chen C, Cheng S, Jia B, Zhu Y, Sun X (2018) Ectopic expression of GsSRK in Medicago sativa reveals its involvement in plant architecture and salt stress responses. Front Plant Sci. https://doi.org/10.3389/fpls.2018.00226

Sun X, Zheng H, Li J, Liu L, Zhang X, Sui N (2020) Comparative transcriptome analysis reveals new lncRNAs responding to salt stress in sweet sorghum. Front Bioeng Biotechnol. https://doi.org/10.3389/fbioe.2020.00331

Suratanee A, Chokrathok C, Chutimanukul P, Khrueasan N, Buaboocha T, Chadchawan S, Plaimas K (2018) Two-state co-expression network analysis to identify genes related to salt tolerance in Thai rice. Genes (basel) 9:594. https://doi.org/10.3390/genes9120594

Tai Y, Liu C, Yu S, Yang H, Sun J, Guo C, Huang B, Liu Z, Yuan Y, Xia E, Wei C, Wan X (2018) Gene co-expression network analysis reveals coordinated regulation of three characteristic secondary biosynthetic pathways in tea plant (Camellia sinensis). BMC Genomics 19:616. https://doi.org/10.1186/s12864-018-4999-9

Tang Y, Bao X, Zhi Y et al (2019) Overexpression of a myb family gene, Osmyb6, increases drought and salinity stress tolerance in transgenic rice. Front Plant Sci. https://doi.org/10.3389/fpls.2019.00168

Tercé-Laforgue T, Clément G, Marchi L, Restivo FM, Lea PJ, Hirel B (2015) Resolving the role of plant NAD-glutamate dehydrogenase: III. Overexpressing individually or simultaneously the two enzyme subunits under salt stress induces changes in the leaf metabolic profile and increases plant biomass production. Plant Cell Physiol 56:1918–1929. https://doi.org/10.1093/pcp/pcv114

Todorova D, Katerova Z, Sergiev I, Alexieva V (2013) Role of polyamines in alleviating salt stress. In: Ecophysiology and responses of plants under salt stress. Springer, Berlin, pp 355–379

Valifard M, Mohsenzadeh S, Niazi A, Moghadam A (2015) Phenylalanine ammonia lyase isolation and functional analysis of phenylpropanoid pathway under salinity stress in Salvia species. Aust J Crop Sci 9:656–665

van Dam S, Võsa U, van der Graaf A, Franke L, de Magalhães JP (2018) Gene co-expression analysis for functional classification and gene-disease predictions. Brief Bioinform 19:575–592. https://doi.org/10.1093/bib/bbw139

Wan S, Zhang Y, Duan M, Huang L, Wang W, Xu Q, Yang Y, Yu Y (2020) Integrated analysis of long non-coding RNAs (lncRNAs) and mRNAs Reveals the regulatory role of lncRNAs associated with salt resistance in Camellia sinensis. Front Plant Sci. https://doi.org/10.3389/fpls.2020.00218

Wang J, Chen L, Wang Y, Zhang J, Liang Y, Xu D (2013) A Computational systems biology study for understanding salt tolerance mechanism in rice. PLoS ONE 8:e64929. https://doi.org/10.1371/journal.pone.0064929

Wang X, Fang G, Yang J, Li Y (2017) A Thioredoxin-Dependent Glutathione Peroxidase (OsGPX5) Is Required for Rice Normal Development and Salt Stress Tolerance. Plant Mol Biol Report 35:333–342. https://doi.org/10.1007/s11105-017-1026-2

Wen XP, Pang XM, Matsuda N, Kita M, Inoue H, Hao YJ, Honda C, Moriguchi T (2008) Over-expression of the apple spermidine synthase gene in pear confers multiple abiotic stress tolerance by altering polyamine titers. Transgenic Res 17:251–263. https://doi.org/10.1007/s11248-007-9098-7

Wucher V, Legeai F, Hédan B et al (2017) FEELnc: A tool for long non-coding RNA annotation and its application to the dog transcriptome. Nucleic Acids Res 45:e57–e57. https://doi.org/10.1093/nar/gkw1306

Xiong H, Guo H, Xie Y, Zhao L, Gu J, Zhao S, Li J, Liu L (2017) RNAseq analysis reveals pathways and candidate genes associated with salinity tolerance in a spaceflight-induced wheat mutant. Sci Rep 7:2731. https://doi.org/10.1038/s41598-017-03024-0

Xu F, Zhang G, Tong C, Sun X, Corke H, Sun M, Bao J (2013) Association mapping of starch physicochemical properties with starch biosynthesizing genes in waxy rice (Oryza sativa L.). J Agric Food Chem 61:10110–10117. https://doi.org/10.1021/jf4029688

Yang Y, Guo Y (2018) Unraveling salt stress signaling in plants. J Integr Plant Biol 60:796–804. https://doi.org/10.1111/jipb.12689

Ye Y, Ding Y, Jiang Q, Wang F, Sun J, Zhu C (2017) The role of receptor-like protein kinases (RLKs) in abiotic stress response in plants. Plant Cell Rep 36:235–242. https://doi.org/10.1007/s00299-016-2084-x

Yoon Y, Seo DH, Shin H, Kim HJ, Kim CM, Jang G (2020) The role of stress-responsive transcription factors in modulating abiotic stress tolerance in plants. Agronomy 10:788. https://doi.org/10.3390/agronomy10060788

Yousefirad S, Soltanloo H, Ramezanpour SS, Nezhad KZ, Shariati V (2020) The RNA-seq transcriptomic analysis reveals genes mediating salt tolerance through rapid triggering of ion transporters in a mutant barley. PLoS ONE 15:e0229513. https://doi.org/10.1371/journal.pone.0229513

Yu Y, Bai Y, Wang P, Wang Y, Wan H, Liu C, Ni Z (2020) Soybean nuclear factor YA10 positively regulates drought resistance in transgenic Arabidopsis thaliana. Environ Exp Bot 180:104249. https://doi.org/10.1016/j.envexpbot.2020.104249

Yuan H, Zeng X, Yang Q, Xu Q, Wang Y, Jabu D, Sang Z, Tashi N (2018) Gene coexpression network analysis combined with metabonomics reveals the resistance responses to powdery mildew in Tibetan hulless barley. Sci Rep 8:14928. https://doi.org/10.1038/s41598-018-33113-7

Zhang B, Horvath S (2005) A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol. https://doi.org/10.2202/1544-6115.1128

Zhang HH, Xu N, Li X, Jin WW, Tian Q, Gu SY, Sun GY (2017) Over-expression of 2-Cys Prx increased salt tolerance of photosystem II in tobacco. Int J Agric Biol 19:735–745. https://doi.org/10.17957/IJAB/15.0348

Zhang L, Tian LH, Zhao JF, Song Y, Zhang CJ, Guo Y (2009) Identification of an apoplastic protein involved in the initial phase of salt stress response in rice root by two-dimensional electrophoresis. Plant Physiol 149:916–928. https://doi.org/10.1104/pp.108.131144

Zhang T, Zhao Y, Wang Y, Liu Z, Gao C (2018) Comprehensive analysis of myb gene family and their expressions under abiotic stresses and hormone treatments in tamarix hispida. Front Plant Sci. https://doi.org/10.3389/fpls.2018.01303

Zhang X, Dong J, Deng F, Wang W, Cheng Y, Song L, Hu M, Shen J, Xu Q, Shen F (2019a) The long non-coding RNA lncRNA973 is involved in cotton response to salt stress. BMC Plant Biol 19:459. https://doi.org/10.1186/s12870-019-2088-0

Zhang X, Gou M, Liu CJ (2013) Arabidopsis kelch repeat F-Box proteins regulate phenylpropanoid biosynthesis via controlling the turnover of phenylalanine ammonia-lyase. Plant Cell 25:4994–5010. https://doi.org/10.1105/tpc.113.119644

Zhang X, Wang W, Zhu W, Dong J, Cheng Y, Yin Z, Shen F (2019b) Mechanisms and functions of long non-coding RNAs at multiple regulatory levels. Int J Mol Sci 20:5573. https://doi.org/10.3390/ijms20225573

Zhao J, Gao Y, Zhang Z, Chen T, Guo W, Zhang T (2013) A receptor-like kinase gene (GbRLK) from Gossypium barbadense enhances salinity and drought-stress tolerance in Arabidopsis. BMC Plant Biol 13:110. https://doi.org/10.1186/1471-2229-13-110

Zhou M, Han R, Ghnaya T, Lutts S (2018) Salinity influences the interactive effects of cadmium and zinc on ethylene and polyamine synthesis in the halophyte plant species Kosteletzkya pentacarpos. Chemosphere 209:892–900. https://doi.org/10.1016/j.chemosphere.2018.06.143

Zhou SM, Kong XZ, Kang HH, Sun XD, Wang W (2015) The involvement of wheat F-box protein gene TaFBA1 in the oxidative stress tolerance of plants. PLoS ONE 10:e0122117. https://doi.org/10.1371/journal.pone.0122117

Zhou Y, Zhang Y, Wang X, Han X, An Y, Lin S, Shen C, Wen JL, Liu C, Yin W, Xia X (2020) Root-specific NF-Y family transcription factor, PdNF-YB21, positively regulates root growth and drought resistance by abscisic acid-mediated indoylacetic acid transport in Populus. New Phytol 227:407–426. https://doi.org/10.1111/nph.16524

Zhu M, Meng X, Cai J, Li G, Dong T, Li Z (2018) Basic leucine zipper transcription factor SlbZIP1 mediates salt and drought stress tolerance in tomato. BMC Plant Biol 18:83. https://doi.org/10.1186/s12870-018-1299-0

Zhu Y, Yin J, Liang Y, Liu J, Jia J, Huo H, Wu Z, Yang R, Gong H (2019a) Transcriptomic dynamics provide an insight into the mechanism for silicon-mediated alleviation of salt stress in cucumber plants. Ecotoxicol Environ Saf 174:245–254. https://doi.org/10.1016/j.ecoenv.2019.02.075

Zhu YX, Jia JH, Yang L, Xia YC, Zhang HL, Jia JB, Zhou R, Nie PY, Yin JL, Ma DF, Liu LC (2019b) Identification of cucumber circular RNAs responsive to salt stress. BMC Plant Biol 19:164. https://doi.org/10.1186/s12870-019-1712-3

Zhu YX, Yang L, Liu N, Yang J, Zhou XK, Xia YC, He Y, He YQ, Gong HJ, Ma DF, Yin JL (2019c) Genome-wide identification, structure characterization, and expression pattern profiling of aquaporin gene family in cucumber. BMC Plant Biol 19:345. https://doi.org/10.1186/s12870-019-1953-1

Zwack PJ, Rashotte AM (2015) Interactions between cytokinin signalling and abiotic stress responses. J Exp Bot 66:4863–4871. https://doi.org/10.1093/jxb/erv172