Transcriptomic profile analysis of the halophyte *Suaeda rigida* response and tolerance under NaCl stress

Zhan-Jiang Han¹,2,*, Yang Sun¹,2, Min Zhang¹,2 & Jun-Tuan Zhai¹,2

*Suaeda rigida* is a lignified, true haplotype that predominantly grows in the Tarim basin, China. It has significant economic and ecological value. Herein, with aim to determine the genes associated with salt tolerance, transcriptome sequencing was performed on its stem, leaves and root over three set NaCl gradients regimens at treatment intervals of 3 h and 5 days. From our findings, we identified 829,095 unigenes, with 331,394 being successfully matched to at least one annotation database. In roots, under 3 h treatment, no up-regulated DEGs were identified in 100 and 500 mM NaCl treated samples. Under 5 days treatment, 97, 60 and 242 up-regulated DEGs were identified in 100, 300, 500 mM NaCl treated samples, respectively. We identified 50, 22 and 255 down-regulated DEGs in 100, 300, 500 mM NaCl treated samples, respectively. GO biological process enrichment analysis established that down-regulated DEGs were associated with nitrogen compound transport, organic substance transport and intracellular protein transport while the up-regulated genes were enriched in cell wall biogenesis, such as plant-type cell wall biogenesis, cell wall assembly, extracellular matrix organization and plant-type cell wall organization. These findings provide valuable knowledge on genes associated with salt tolerance of *Suaeda rigida*, and can be applied in other downstream haplotype studies.

The growth and development of plants is highly affected by several abiotic stress factors. These factors may include salinity, temperature, drought and heavy metals. Of these many abiotic factors, salinity is a significant environmental factor that affects plant productivity¹. Globally, it is estimated that salinity affects almost 1 billion hectares of land², a situation that has continued to be more critical due to the rising changes in climatic conditions³.

Congruently, plants possess various intricate adaptive responses to these stress factors⁴. In plants with ability to survive high salinity (halophytes), their response and adaptation to high saline levels typically relies on succulence buildup in their cells and tissues³⁵. These high salt tolerance plants include representatives such as *Sonneratia acida*, *Pentatropis sianshoides*, *S. nudiflra*, *S. maritima*, *Limnitzera racemosa*, *Salvadora persica*, *S. salsa* and *S. rigida*³⁶–³⁸. These halophyte plants have the ability to compartmentalize the disproportionate Na⁺ and Cl⁻ ions that are present in the cell vacuole, a mechanism that aids them in reducing the cells’ water potential and thereafter enhance their water absorbance ability from the saline soil, thus providing optimum ion concentration conditions in the cell cytoplasm for enzymatic and other biological activities⁴–⁶.

A previous study had shown that even though the *Arabidopsis thaliana* has a weak salt tolerance, and its response mechanisms is centered on the action of SALT OVERLY SENSITIVE3 [SOS3], a sensor, and SOS2, a protein kinase. These two protein complexes are implicated in detecting and responding to Na⁺ influx³⁷–³⁹, involving activation of SOS1, a plasma membrane-located sodium/proton antiporter. The SOS1 activation mediates the transfer and redistribution of the Na⁺ ions all over the plant³¹–³³. Equally, SOS complex has been found to exist in other plant species, hence it may be a general feature of plants³⁴. Equally, a study on the effect of salinity on *Suaeda fruticosa* linked its salt tolerance to its capability to uptake K⁺ so as to retain a higher K⁺/Na⁺ balance in the shoots³⁵. Further, halophytes have mechanisms capable of regulating several biological pathways like signal transduction, energy metabolism and photosynthesis³⁶. Other studies on plants’ salt and drought tolerance have

¹College of Life Sciences, Tarim University, Alar 843300, Xinjiang, China. ²Xinjiang Production & Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin, Alar 843300, Xinjiang, China. ³email: hanzhanjiang126@126.com
also recognized several inducible stress regulators, with up-regulation of catalase, aquaporins, peroxidase and proline accumulation\(^\text{27}\). Moreover, plant abscisic acid (ABA) signaling pathways have been shown to be stimulated via stress factors\(^\text{28}\). The Sodium and chloride build-up in plant's cytoplasm leads to cell cytotoxicity induced by accumulation of reactive oxygen species (ROS)\(^\text{29}\); a situation that can cause protein and lipid degradation, which may disrupts the cell functions\(^\text{30}\).

_Suaeda rigida_ (HW Kung et GL Chu) is a lignified plant under genus _Polygonaceae_. It is a true haplotype plant that is endemic in the Tarim basin in Xinjiang, North west China. It is a nutrient-rich wild vegetable that is also an excellent local forage resource with important economic and ecological value in the region\(^\text{31}\). Notably, nearly one-third of the cultivated land in Xinjiang province is salinized, and the development and utilization of salt-tolerant plants form a key focal point in plant research in this region. Overall, study of plants’ salt tolerance mechanisms and the mining of related genes have important theoretical and practical significance in the development and utilization of excellent germplasm resources, enhancement of crop stress resistance and improvement of agricultural production. However, presently there is a lack of detailed knowledge on the saline stress and associated signaling pathways of _Suaeda rigida_ plant. Therefore this study was aimed at applying transcriptomic sequencing in identifying and analyzing the putative genes implicated in salt response and tolerance mechanisms of _Suaeda rigida_ plants over a set gradient of NaCl treatment regimens.

### Results

#### Sequencing summary for all the libraries.

A total of 343.88 GB of raw reads were obtained from the 42 libraries of the roots, leafs and stem samples (Supplementary Data 1). The average sequencing error rate was 0.029% while the average GC content was 42.76%. The Q20 and Q30 data ratio of sequencing quality was 97.10% and 92.35%, respectively (Supplementary Data 1). Since _S. rigida_ does not have a reference genome yet, Trinity software was utilized to de novo assemble the obtained clean reads. In total, 2,212 million high-quality clean reads were de novo assembled into 829,095 transcripts with an N50 length 1453 bp and an N90 length of 269 bp (Table 1).

#### Expression analysis, functional annotation, gene ontology assignments and analysis.

After mapping of all the filtered reads to the de novo assembled contigs, we obtained all the expression data for them in all libraries. Thereafter, DESeq was utilized in identifying the differentially expressed genes. Comparison of gene expression analysis for each different experimental condition are as illustrated in Fig. 1.

Gene function annotation was attained by searching the obtained transcripts against the Nr, Nt, KO, Swiss-Prot, Pfam, GO and KOG databases. In sum, 331,394 unigenes successfully matched with at least one database, with 253,490 (58.54%), 241,135 (55.69%), 116,108 (26.81%), 224,239 (51.79%), 219,455 (50.68%), 221,659 (51.19%) and 113,379 (26.18%) annotated transcripts showing a significant hit against the Nr, Nt, KO, Swiss-Prot, Pfam, GO and KOG databases. In sum, 331,394 unigenes successfully matched with at least one data-base, with 253,490 (58.54%), 241,135 (55.69%), 116,108 (26.81%), 224,239 (51.79%), 219,455 (50.68%), 221,659 (51.19%) and 113,379 (26.18%) annotated transcripts showing a significant hit against the Nr, Nt, KO, Swiss-Prot, Pfam, GO and KOG, respectively (Table 2).

GO categorization established that cellular process, metabolic process and single-organism processes were the highly enriched biological process whereas, the genes associated with cell, cell part and organelle as the most significant among the cellular component terms. Three molecular function GO terms; binding, catalytic activity and transporter activity were significantly enriched (Fig. 2).

The KEGG classification analysis revealed that Transport and catabolism, signal transduction, membrane transport, Translation, Carbohydrate metabolism, Energy metabolism and Environmental adaptation were among the few important pathways that were significantly enriched under Cell processes, Environmental information processing, Genetic information Processing, Information processing, Metabolism and Organic Systems of _Suaeda rigida_ plant in response to salt stress (Fig. 3).

#### Differential expression pattern for salt tolerance in root.

Differential gene cluster analysis for all the treatments was conducted (Fig. 4). Generally, roots are the main plant tissue that are directly in contact with high salt concentration in the soil. Thus, gene expression profiles patterns in _Suaeda rigida_ roots were important. Under 3 h of treatment, no up-regulated DEGs were recorded in 100 mM NaCl and 500 mM NaCl treated samples, whereas there were only 26 up-regulated DEGs in 300 mM NaCl treated samples. Meanwhile, there were 3, 37 and 0 down-regulated DEGs in 100 mM NaCl, 300 mM NaCl, 500 mM NaCl treated samples, respectively. However, in the 5 days’ treatment group, 97, 60 and 242 up-regulated DEGs were identified in 100, 300, 500 mM NaCl treated samples, respectively. Further, we identified 50, 22 and 255 down-regulated DEGs in 100, 300, 500 mM NaCl treated samples, respectively. Therefore, based on these findings, we focused on these DEGs in 5 days’ treatment group. Overall, 43 up-regulated DEGs and 24 down-regulated DEGs were present in more than two samples. For the down-regulated genes, GO biological process (BP) enrichment analysis indicated that they were enriched in nitrogen compound transport (GO: 0071705, \(p = 5.61E-06\)), organic substance transport (GO: 0071702, \(p = 0.010173\)) and intracellular protein transport (GO: 0006886, \(p = 0.015322\)) while for the up-

### Table 1. Summary of transcriptome assembly.

| Category | Number | 200–500 bp | 500–1 kbp | 1 k–2 kbp | >2 kbp | Total | Mean length (bp) | N50 (bp) | N90 (bp) |
|----------|--------|------------|-----------|-----------|---------|-------|-----------------|----------|----------|
| Transcripts | 518,152 | 140,336 | 97,377 | 73,230 | 829,095 | 359 | 1,453 | 269 |
| Unigenes | 135,845 | 127,207 | 96,747 | 73,154 | 432,953 | 759 | 1,885 | 531 |
regulated genes, they were found enriched in plant-type cell wall biogenesis related GO entries, such as plant-type cell wall biogenesis (GO: 0009832, p = 0.0088759), cell wall assembly (GO: 0070726, p = 0.0088759), extracellular matrix organization (GO: 0030198, p = 0.0088938) and plant-type cell wall organization (GO: 0009664, p = 0.027692).

qPCR validation. A set of differentially genes were randomly selected for detection and subsequent validation via qPCR, Fig. 5.

**Discussion**

Herein, we conducted a transcriptomic profile analysis of *Suaeda rigida* response to saline stress conditions. In this, sequencing of the leaves, stems and roots of the plant were conducted so as to identify the salt-responsive genes. In spite of the recent development in genome sequence methods, genomic data for various non-model plants is absent. Though transcriptomic mRNA-sequencing, it has been made possible to identify and quantify transcripts even in circumstances where the reference genome sequence is not yet available. Although transcriptome studies have been conducted in closely related species like *Suaeda fruticosa*, such information on *S. rigida* is still lacking.

In this study, we applied transcriptomic sequencing in identifying and analyzing the putative genes related to salt response and tolerance mechanisms in *Suaeda rigida* plants over a set gradient of NaCl treatment regimens. *Suaeda rigida* (HW Kung et GL Chu) is a lignified plant under genus *Polygonaceae*. Hence, it is highly suitable in examining salt tolerant mechanisms in plants. Therefore, *Suaeda rigida* may offer a significant basis for validating salt tolerant associated genes.

|                      | Number of genes | Percentage (%) |
|----------------------|-----------------|----------------|
| Annotated in NR      | 253,490         | 58.54          |
| Annotated in NT      | 241,135         | 55.69          |
| Annotated in KO      | 116,108         | 26.81          |
| Annotated in SwissProt| 224,239        | 51.79          |
| Annotated in PFAM    | 219,455         | 50.68          |
| Annotated in GO      | 221,659         | 51.19          |
| Annotated in KOG     | 113,379         | 26.18          |
| Annotated in all databases | 57,824    | 13.35          |
| Annotated in at least one database | 331,394 | 76.54          |
| Total unigenes       | 432,953         | 100            |
In haplotypes, roots are the main plant tissue that are in direct interaction with high concentration of salt in the soil. Thus, we focused on the gene expression profiles in *Suaeda rigida* roots. From the transcriptome analysis, 28,555 DEGs were identified. A significant majority of these DEGs clustered to four functional groups involving signal transduction, transporters, cell wall metabolism and transcription factors; that might have typical biological functions in *Suaeda rigida* salt-tolerant mechanisms.

**Figure 2.** GO classification. The figure comprises of biological processes, cellular component and molecular function.

**Figure 3.** KEGG classification. The figure is divided into five branches based on the corresponding KEGG metabolic pathways: cell processes (A), environmental information processing (B), genetic information processing (C) information processing, metabolism (D) and organic systems (E).
For plants under abiotic or biotic stress, signal transduction is very crucial for adjustment in such unfavorable conditions. From our study findings, genes associated with signal transduction processes were differentially expressed when under saline stress. Differentially expressed genes associated with control of ABA signaling pathway were highly up-regulated in 500 mM NaCl treated samples (Supplementary Data 2). The genes encoding for probable protein phosphatase 2C 25 (Cluster-47345.139659) and protein phosphatase 2C 22 (Cluster-47345.93406) were up-regulated in response to high salt levels. Phosphatase genes are considered to be important regulators of the ABA signaling in plants. In a study conducted by Liu et al., they established that over-expression of AtPP2CG1 in Arabidopsis thaliana led to improved salt tolerance, while its depletion led to reduced salt tolerance levels. Further, ZmPP2C2 over-expression in tobacco has been established to enhance tolerance to cold stress. Similarly, previously conducted studies on other haplotype such as Suaeda fruticose and Suaeda glauca demonstrated phosphatase 2C family proteins were up-regulated at high salt treatment regimens. Thus, protein phosphatase 2C might have a crucial regulatory function in salt tolerance of Suaeda rigida.

Abscisic acid (ABA) is a key endogenous indicator. Abiotic factors like salinity stimulates ABA biosynthesis, which thereafter initiates the signaling pathways leading to several downstream responses. Cytochrome P450 is elucidated to play a major part in ABA catabolism, while other cytochrome P450s might facilitate growth and stress responses. From our dataset, three unigenes encoding for cytochrome P450 76AD1 (Cluster-47345.95314), cytochrome P450 84A1 (Cluster-47345.105167) and cytochrome P450 CYP73A100 were up-regulated. However, the precise roles of each cytochrome P450 in Suaeda rigida is yet to be known. Notably, these genes might be linked to ABA signal transduction, implying ABA signal transduction may have a significant function and show prompt response of Suaeda rigida in the earlier stage during saline stress.

In plants, Oligopeptide transporters (OPTs) are membrane-confined proteins that have numerous transportation roles. From our study findings, we identified one gene that encodes for an oligopeptide transporter 6 (Cluster-47345.229496), and it was up-regulated, demonstrating that it may be associated with metal resistance.

Figure 4. Differential gene cluster Heatmap.

---

5. Editorial note: The scientific report should be referred to by its DOI: https://doi.org/10.1038/s41598-020-71529-2
transport and homeostasis during salt stress. Further, ABC proteins are actively involved in transportation of various molecules like hormones, secondary metabolites, lipids, metals and modulators of ion channels across membranes. Thus, these ABC transporters augment drought and salt resistance. Herein, three gene associated with ABC transporter families, Cluster-47345.112862, Cluster-47345.140732 and Cluster-47345.152644, were upregulated in the roots of *Suaeda rigida*. Our study findings are consistent with previously reported studies on *Suaeda glauca* and *Suaeda fruticosa*.

As a result of saline stress, normal plant growth and development is highly affected. Cell wall modification is a response measure in plants when under biotic or abiotic stress. Herein, genes related to cell wall responses were enriched. Specifically, plant-type cell wall biogenesis related GO entries, such as plant-type cell wall biogenesis

---

**Figure 5.** Real time-PCR validation of DE transcripts. The figure represents root treatments at three different timepoints (A: R100, B: R300 and C: R500). Bars represent mean ± SE (n = 3).
SLIDINGWINDOW:4:20 MINLEN:60”65. Briefly, clean reads were attained through filtering off reads with
eters “PE-phred33 ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 LEADING:20 TRAILING:20 HEADCROP:10
Trinity66, with min_kmer_cov set to 2 by default and all other parameters set as default. Gene function annota-
tion and sequence duplication level of the clean reads were determined. For the obtained clean data, all read1
residuals and low-quality reads from original raw fastq reads. Simultaneously, Q20, Q30, GC-
wall modification49, were up-regulated.

Several transcription factors (TFs) take part in various significant roles in plant response mechanisms to abiotic
and biotic stress. This is usually achieved through their regulation of genes associated with stress responses,
such as ERFs and WRKY transcription factors50–53. ERFs perform various significant roles in plants’ abiotic stress
tolerance by regulating stress-responsive genes54–57. Three genes, Cluster-47345.149382, Cluster-47345.137633
and Cluster-47345.166382, that encode for ERFs were up-regulated in Suaeda rigida roots under salt stress.
Equally, WRKY transcription factors have been implicated in salt as well as drought tolerance in Gossypium
hirsutum, Arabidopsis thaliana, Jatropha curcas and Jatropha curcas58–61. From our study findings, we identi-
fied two genes, Cluster-47345.231608 and Cluster-47345.62923, encoding WRKYS were up-regulated under
salt stress. Consistent with previously conducted studies in other plants41,62–64, high salinity levels led to up-
regulation of bHLH transcription factor. In our study, four bHLH transcription factors, Cluster-47345.43143,
Cluster-47345.120470, Cluster-47345.67446 and Cluster-47345.180519 were highly upregulated in the Suaeda
rigida roots than in its leaves, implying they might be playing a significant role in plants’ salt tolerance and
regulation in the roots.

In conclusion, 42 Suaeda rigida RNA-seq libraries representing various treated Sodium Chloride (NaCl)
treatment regimens (100 mM NaCl, 300 mM NaCl and 500 mM NaCl), were sequenced in this study. From these
sequenced libraries, a total of 2,212 M high-quality clean reads were assembled into 829,095 transcripts, from
which 28,559 DEGs were identified. These DEGs contained up-regulated and down-regulated genes in Suaeda
rigida. They comprised of genes implicated in four functional groups namely, signal transduction, transporters,
cell wall metabolism and transcription factors; which might have typical biological functions in salt-tolerant
mechanism of haplotype Suaeda rigida. These findings can be further applied in downstream genetic-based
studies that aim to study the salt tolerance mechanisms and regulation in haplotype plants.

Materials and methods
Plant materials and RNA isolation. Suaeda rigida plants were grown at Tarim university, Xinjiang,
China as per a previously published article of a related species29, but with slight adjustments. In duplicates, plants
were exposed to established NaCl treatments as described in Supplementary Data 3. Sodium Chloride (NaCl)
treatment regimens were 100 mM NaCl, 300 mM NaCl and 500 mM NaCl. Control group (CK) was not exposed
to any treatment plan. In duplicates, the leaves (L), stem (S) and roots (R) were collected at set intervals of 3 h
and 5 days post NaCl treatment (Supplementary Table 2). The collected samples were triturated to powder form
in liquid nitrogen using TRizol, total RNA was isolated using Spin Column Plant total RNA Purification Kit
following the manufacturer’s protocol (Sangon Biotech, Shanghai, China). The RNA quality, integrity and con-
centration were evaluated through the NanoPhotometer spectrophotometer (IMPLEN, CA, USA), Agilent Bio-
analyzer 2100 system (Agilent Technologies, CA, USA) and Qubit RNA Assay Kit in Qubit 2.0 Flurometer (Life
Technologies, CA, USA). High quality total RNA samples were utilized for downstream library preparation.

Library preparation for Transcriptome sequencing. From each sample, 1.5 μg RNA was expended for
use in library preparation. NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, USA) was used to generate
the sequencing libraries, as per the manufacturer’s guidelines. Index tags were added to each sample to serve as
identification points. In brief, mRNA was purified from total RNA by means of poly-T oligo-attached magnetic
beads. Fragmentation was done through divalent cations under high temperature in NEBNext First Strand Syn-
thesis Reaction Buffer (5×). First strand cDNA was synthesized using random hexamer primer and M-MuLV
Reverse Transcriptase (RNase H⁻). Next, second strand cDNA synthesis done using DNA Polymerase I and
RNase H. Residual overhangs were converted into blunt ends through exonuclease/polymerase activity. After
adenylation of 3’ DNA fragments ends, NEBNext Adaptor with hairpin loop structure were ligated in prepara-
tion for hybridization. Thereafter, 250 ~ 300 base pairs long cDNA fragments were selected through purification
of the library segments using AMPure XP system (Beckman Coulter, Beverly, USA). Afterwards, 3 μl USER
Enzyme (NEB, USA) was utilized with size-selected, adaptor-ligated cDNA at 37 °C for 15 min followed by
5 min at 95 °C before PCR. Next, PCR was conducted using Phusion High-Fidelity DNA polymerase, universal
primers and Index (X) Primer. Lastly, the obtained PCR products were purified (AMPure XP system) and library
quality evaluated on the Agilent Bioanalyzer 2100 system. The clustering of the index-coded samples was done
on a cBot Cluster Generation System by TruSeq PE Cluster Kit v3-cBot-HS (Illumina), as per the manufacturer’s
guidelines. Thereafter, the prepared libraries were sequenced on an Illumina Hi-seq 4000 platform.

Bioinformatics analysis. Raw fastq reads were initially assessed using trimmomatic v0.38 with param-
eters “PE-phred33 ILLUMINAHelmet:TruSeq3-PE.fa:2:30:10 LEADING:20 TRAILING:20 HEADCROP:10
SLIDINGWINDOW:4:20 MINLEN:60”67. Briefly, clean reads were attained through filtering off reads with
adapters, poly-N residuals and low-quality reads from original raw fastq reads. Simultaneously, Q20, Q30, GC-
content and sequence duplication level of the clean reads were determined. For the obtained clean data, all read1
files and read2 files were pooled into single independent reads and transcriptome assembly performed using
Trinity68, with min_kmer_cov set to 2 by default and all other parameters set as default. Gene function annota-
tion was done using the Nr (NCBI non-redundant protein sequences); Nt (NCBI non-redundant nucleotide
sequences); Pfam (Protein family); KOG/COG (Clusters of Orthologous Groups of proteins); Swiss-Prot; KO
(KEGG Ortholog database)69; GO (Gene Ontology) databases, with an E-value threshold of 1e − 5. Picard—tools
v1.41 and samtools v0.1.18 were used to sort, remove duplicated reads and merge the bam alignment results of each sample. GATK3 software68 was used to perform SNP calling. Raw vcf files were filtered with GATK standard filter method and other parameters (cluster: 3; WindowSize: 35; QD < 2.0 or FS > 60.0 or MQ < 40.0 or SOR > 4.0 or MQRankSum < -12.5 or ReadPosRankSum < -8.0 or DP < 10). SSR of the transcriptome were analyzed by use of the GOSeg R packages71, as per Wallenius non-central hyper-geometric distribution72. DEGs sequences were blast (blastx) to the genome of a closely linked species in the STRING database (https://string-db.org/); so as to obtain the predicted protein interaction (PPI) of them. Cytoscape was used to visualize these DEGs PPI73.

qPCR validation. To validate the RNA-Seq data, some DEG’s were randomly selected from our constructed libraries and their levels of expression under saline conditions was assessed through qPCR analysis. In brief, 3 μg of total RNA was utilized to construct the cDNA. Reverse transcription was conducted using oligo (dT) primer as per the manufacturer’s guidelines (Fermentas, Burlington, Ontario, Canada). Primer 5 software (version 5.2.0) was utilized in designing the specific primers for the selected genes. The qPCR was conducted in a total volume of 20 μL, with each assay comprising 2 μM of the forward and reverse primers, 2 μL cDNA, and 10 μL of 2 × SYBR Green qPCR Mix (Takara, Otsu, Shiga, Japan). Thermal cycling conditions were 35 cycles of fast denaturation at 94 °C for 5 s, followed by annealing and extension at 52–55 °C for 20 s. To test the Amplicon specificity was tested through generation of melting curve by gradually increasing the temperature to 95 °C. To establish the relative fold changes for each test, the 2−ΔΔCT method was applied based on standardization with the reference gene. The qPCR analysis was done in triplicates, and the mean value utilized.

Data availability All genetic data have been submitted to the NCBI Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra) PRJNA644132.

Received: 26 January 2020; Accepted: 17 August 2020

Published online: 16 September 2020

References

1. Zörb, C., Geißl, C. M. & Dietz, K. J. Salinity and crop yield. Plant Biol. 21, 31–38 (2019).
2. Shrivastava, P. & Kumar, R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J. Biol. Sci. 22, 123–131 (2015).
3. Han, J., Shi, J., Zeng, L., Xu, J. & Wu, L. Effects of nitrogen fertilization on the acidity and salinity of greenhouse soils. Environ. Sci. Pollut. Res. Int. 22, 2976–2986 (2015).
4. Zhu, J. K. Cell signaling under salt, water and cold stresses. Curr. Opin. Plant Biol. 4, 401–406 (2001).
5. Guo, J., Li, Y., Han, G., Song, J. & Wang, B. NaCl markedly improved the reproductive capacity of the euhalophyte Suaeda salsa. Funct. Plant Biol. 45, 350 (2018).
6. Aslam, R., Bostan, N., Naibgha, A., Maria, M. & Safdar, W. A critical review on halophytes: Salt tolerant plants. J. Med. Plant Res. 5, 7108–7118 (2011).
7. Song, J. et al. The role of the seed coat in adaptation of dimorphic seeds of the euhalophyte Suaeda salsa to salinity. Plant Species Biol. 32, 107–114 (2017).
8. Guo, J., Suo, S. & Wang, B. Sodium chloride improves seed vigour of the euhalophyte Suaeda salsa. Seed Sci. Res. 25, 335–344 (2015).
9. Han, N. et al. Expression of a Suaeda salsa Vacuolar H +/Ca2+ transporter gene in Arabidopsis contributes to physiological changes in salinity. Plant Mol. Biol. Rep. 30, 470–477 (2012).
10. Liu, Q., Liu, R., Ma, Y. & Song, J. Physiological and molecular evidence for Na+ and Cl− exclusion in the roots of two Suaeda salsa populations. Aquat. Bot. 146, 1–7 (2018).
11. Duan, H. et al. Effect of combined waterlogging and salinity stresses on euhalophyte Suaeda glauca. Plant Physiol. Biochem. 127, 231–237 (2018).
12. Yuan, F., Guo, J., Shabala, S. & Wang, B. Reproductive physiology of halophytes: Current standing. Front. Plant Sci. 9, 1954 (2019).
13. Mirza, H. et al. Potential use of halophytes to remediate saline soils. Biomed Res. Int. 2014, 1–12 (2014).
14. Zhu, J. K. Regulation of ion homeostasis under salt stress. Curr. Opin. Plant Biol. 6, 441–445 (2003).
15. Shao, Q., Han, N., Ding, T., Zhou, F. & Wang, B. ShHTK1 is a potassium transporter of the C3 halophyte Suaeda salsa that is involved in salt tolerance. Funct. Plant Biol. 41, 790 (2014).
16. Zhu, J. K. Plant salt tolerance. Trends Plant Sci. 6, 66–71 (2001).
17. Halfter, U. The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc. Natl. Acad. Sci. 97, 3735–3740 (2000).
18. Cheng, N. H., Pittman, J. K., Zhu, J. K. & Hirschi, K. D. The protein kinase SOS2 activates the Arabidopsis H+/Ca2+ antiporter CAX1 to integrate calcium transport and salt tolerance. J. Biol. Chem. 279, 2922–2926 (2004).
19. Quan, R. et al. SCABP8/CBL10, a putative calcium sensor, interacts with the protein kinase SOS2 to protect Arabidopsis shoots from salt stress. Plant Cell 19, 1415–1431 (2007).
20. Lin, H. et al. Phosphorylation of SOS3-like calcium binding protein8 by SOS2 protein kinase stabilizes their protein complex and regulates salt tolerance in arabidopsis. Plant Cell 21, 1607–1619 (2009).
21. Qiu, Q. S., Guo, Y., Dietrich, M. A., Schumaker, K. S. & Zhu, J. K. Regulation of SOS1, a plasma membrane Na+/H+ exchanger in Arabidopsis thaliana, by SOS2 and SOS3. Proc. Natl. Acad. Sci. U. S. A. 99, 8436–8441 (2002).
22. Quintero, F. J., Ohata, M., Shi, H., Zhu, J. K. & Pardo, J. M. Reconstitution in yeast of the Arabidopsis SOS signaling pathway for Na+ homeostasis. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 9061–9066 (2002).

23. Shi, H., Quintero, F. J., Pardo, J. M. & Zhu, J. K. The putative plasma membrane Na+/H+ antiporter SOS1 controls long-distance Na+ transport in plants. *Plant Cell* **14**, 465–477 (2002).

24. Oh, D. H. et al. Loss of Halophytism by interference with SOS1 expression. *Plant Physiol.* **151**, 210–222 (2009).

25. Diray-Arce, J., Clement, M., Gul, B., Khan, M. A. & Nielsen, B. L. Transcriptome assembly, profiling and differential gene expression analysis of the halophyte *Suaeda fruticosa* provides insights into salt tolerance. *BMC Genom.* **16**, 353 (2015).

26. Liska, A. J., Shevchenko, A., Pick, U. & Katz, A. Enhanced photosynthesis and redox energy production contribute to salinity tolerance in Dunaliella as revealed by homology-based proteomics. *Plant Physiol.* **136**, 2806–2817 (2004).

27. Jithesh, M. N., Prashanth, S. R., Sivaprakash, K. R. & Parida, A. Monitoring expression profiles of antioxidant genes to salinity, iron, oxidative, light and hypersomotic stresses in the highly salt tolerant grey mangrove, *Avicennia marina* (Forsk.) Vierh. by mRNA analysis. *Plant Cell Rep.* **25**, 865–876 (2006).

28. Barrero, J. M. et al. Both abscisic acid (ABA)-dependent and ABA-independent pathways govern the induction of NCE3D, AAO3 and ABA1 in response to salt stress. *Plant Cell Environ.* **29**, 2000–2008 (2006).

29. Hameed, A. et al. Salt tolerance of a cash crop halophyte *Suaeda fruticosa*: Biochemical responses to salt and exogenous chemical treatments. *Acta Physiol. Plant.* **36**, 2331–2340 (2014).

30. Huang, G. T. et al. *Plant Mol. Biol.* Wiley, 539–545 (2013).

31. Singh, A., Jha, S. K., Bagri, J. & Pandey, G. K. ABA inducible rice protein phosphatase 2C confers ABA insensitivity and abiotic stress tolerance in arabidopsis. *PLoS ONE* **10**, e0125168 (2015).

32. Zhang, J. et al. Molecular character of a phosphatase 2C (PP2C) gene relation to stress tolerance in *Arabidopsis thaliana*. *Mol. Biol. Rep.* **40**, 2633–2644 (2013).

33. Liu, X. et al. A PP2C gene, protein phosphatase 2C, positively regulates salt tolerance of Arabidopsis in abscisic acid-dependent manner. *Biochem. Biophys. Res. Commun.* **422**, 710–715 (2012).

34. Hu, X. et al. Enhanced tolerance to low temperature in tobacco by over-expression of a new maize protein phosphatase 2C, *ZmPP2C2*. *J. Plant Physiol.* **167**, 1307–1315 (2010).

35. Jin, H., Dong, D., Yang, Q. & Zhu, D. Salt-responsive transcriptome profiling of sueda glaucia via RNA Sequencing. *PLoS ONE* **11**, e0150504 (2016).

36. Lee, S. C. & Luan, S. ABA signal transduction at the crossroad of biotic and abiotic stress responses. *Plant Cell Environ.* **35**, 53–60 (2012).

37. Kishorio, T. et al. The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: Key enzymes in ABA catabolism. *EMBO J.* **23**, 1647–1656 (2004).

38. Mendoza-Cózatl, D. G. et al. OPT3 is a component of the iron-signaling network between leaves and roots and misregulation of OPT3 leads to an over-accumulation of cadmium in seeds. *Plant J.* **7**, 1455–1469 (2014).

39. Sasaki, A., Yamaji, N., Xia, J. & Ma, J. F. OsYS6 is involved in the detoxification of excess manganese in rice. *Plant Physiol.* **157**, 1832–1840 (2011).

40. Pike, S., Patel, A., Stacey, G. & Gassmann, W. Arabidopsis OPT6 is an oligopeptide transporter with exceptionally broad substrate specificity. *Plant Cell Physiol.* **50**, 1923–1932 (2009).

41. Perlin, M. H., Andrews, J. & Toh, S. S. Essential letters in the fungal alphabet: ABC and MFS transporters and their roles in survival and pathogenicity. *Adv. Genet.* **85**, 201–253 (2014).

42. Hellberg, E., Montanari, F. & Ecker, G. F. The ABC of phytoremediation: *Plant Physiol.* **161**, 474–487 (2013).

43. An, C. et al. Transcriptome profiling, sequence characterization, and SNP-based chromosomal assignment of the EXPANSIN genes in cotton. *Plant Physiol. Genom.* **278**, 539–553 (2007).

44. Shi, H. et al. The cysteine2/histidine2-type transcription factor zinc finger of arabidopsis thaliana modulates biotic and abiotic stress responses by activating salicylic acid-related genes and c-repeat-binding factor genes in arabidopsis. *Plant Physiol.* **165**, 1367–1379 (2014).

45. Jisha, V. et al. Overexpression of an AP2/ERF type transcription factor OsEREBP1 confers biotic and abiotic stress tolerance in rice. *PLoS ONE* **10**, e0127831 (2015).

46. Chai, G. et al. Soybean transcription factor ORFeome associated with drought resistance: A valuable resource to accelerate research on abiotic stress resistance. *BMC Genom.* **16**, 596 (2015).

47. Xu, L. et al. Multiple NUCLEAR FACTOR Y transcription factors respond to abiotic stress in *Brassica napus* L. *PLoS ONE* **9**, e111534 (2014).

48. Liu, D., Ji, L., Wang, G., Guan, C. & Jin, C. LeERF1, a novel ethylene-responsive transcription factor from Lycium chinense, confers salt tolerance in transgenic tobacco. *Plant Cell Rep.* **33**, 2033–2045 (2014).

49. Klav, I. et al. Ethylene response factor SI-ERF3 is responsive to abiotic stresses and mediates salt and cold stress response regulation in tomato. *Sci. World J.* **2014**, 167681 (2014).

50. Pan, Y. et al. An ethylene response factor (ERF5) promoting adaptation to drought and salt tolerance in tomato. *Plant Cell Rep.* **31**, 349–360 (2012).

51. Dong, W. et al. Isolation and characterization of a bread wheat salinity responsive ERF transcription factor. *DNA Cell Biol.* **81**, 474–487 (2015).

52. Scarpeci, T. E., Zanor, M. I., Mueller-Roemer, B. & Valle, E. M. Overexpression of AtWRKY30 enhances abiotic stress tolerance during early growth stages in *Arabidopsis thaliana*. *Plant Mol. Biol.* **83**, 265–277 (2013).

53. Agarwal, P., Dabi, M. & Agarwal, P. K. Molecular cloning and characterization of a group II WRKY transcription factor from *Latropha curcas*, an important biofuel crop. *DNA Cell Biol.* **33**, 503–513 (2014).

54. Ye, S. et al. Constitutive expression of the poplar WRKY transcription factor *Populus* enhances resistance to *Dithiorella gregaria* in transgenic plants. *Tree Physiol.* **34**, 1118–1129 (2014).

55. Yan, H. et al. 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 Physiol.* **55**, 2060–2076 (2014).

56. Sharma, R. et al. De novo assembly and characterization of stress transcriptome in a saline-tolerance variety CS52 of *Brassica juncea*. *PLoS ONE* **10**, e0126783 (2015).

57. Garg, R. et al. Deep transcriptome sequencing of wild halophyte rice, *Porteresia coarctata*, provides novel insights into the salinity and submergence tolerance factors. *DNA Res.* **21**, 69–84 (2014).
64. Hu, L. et al. RNA-seq for gene identification and transcript profiling in relation to root growth of bermudagrass (*Cynodon dactylon*) under salinity stress. *BMC Genom.* **16**, 575 (2015).
65. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
66. Grabherr, M. G. et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* **29**, 644–652 (2011).
67. Minoru, K., Yoko, S., Masayuki, K., Miho, F. & Mao, T. KEGG as a reference resource for gene and protein annotation—PubMed. *Nucleic Acids Res.* **44**, D457–D462 (2016).
68. Van der Auwera, G. A. et al. From fastQ data to high-confidence variant calls: The genome analysis toolkit best practices pipeline. *Curr. Protoc. Bioinform.* **11**, 11.10.1 (2013).
69. Li, B. & Dewey, C. N. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinform.* **12**, 323 (2011).
70. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
71. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria (2018).
72. Young, M. D., Wakefield, M. J., Smyth, G. K. & Oshlack, A. Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biol.* **11**, R14 (2010).
73. Mao, X., Cai, T., Olyarchuk, J. G. & Wei, L. Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. *Bioinformatics* **21**, 3787–3793 (2005).
74. Shannon, P. et al. Cytoscape: A software Environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–2504 (2003).

**Acknowledgements**
This work was financially supported by the National Natural Sciences Foundation of China (31360055), Open Project of Xinjiang Production & Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin (BRZD1902).

**Author contributions**
Z.-J.H. conceived and designed the experiments; Z.-J.H., Y.S. and M.Z. performed the experiments; Z.-J.H., Y.S., M.Z. and J.-T.Z. analyzed the data; Z.-J.H. contributed reagents/materials/analysis tools; Z.-J.H., Y.S., M.Z. and J.-T.Z. wrote the paper.

**Competing interests**
The authors declare no competing interests.

**Additional information**
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-71529-2.

**Correspondence** and requests for materials should be addressed to Z.-J.H.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020