

Supplementary Material

Transcriptomic profiling and physiological responses of halophyte *Kochia sieversiana* provide insights into salt tolerance

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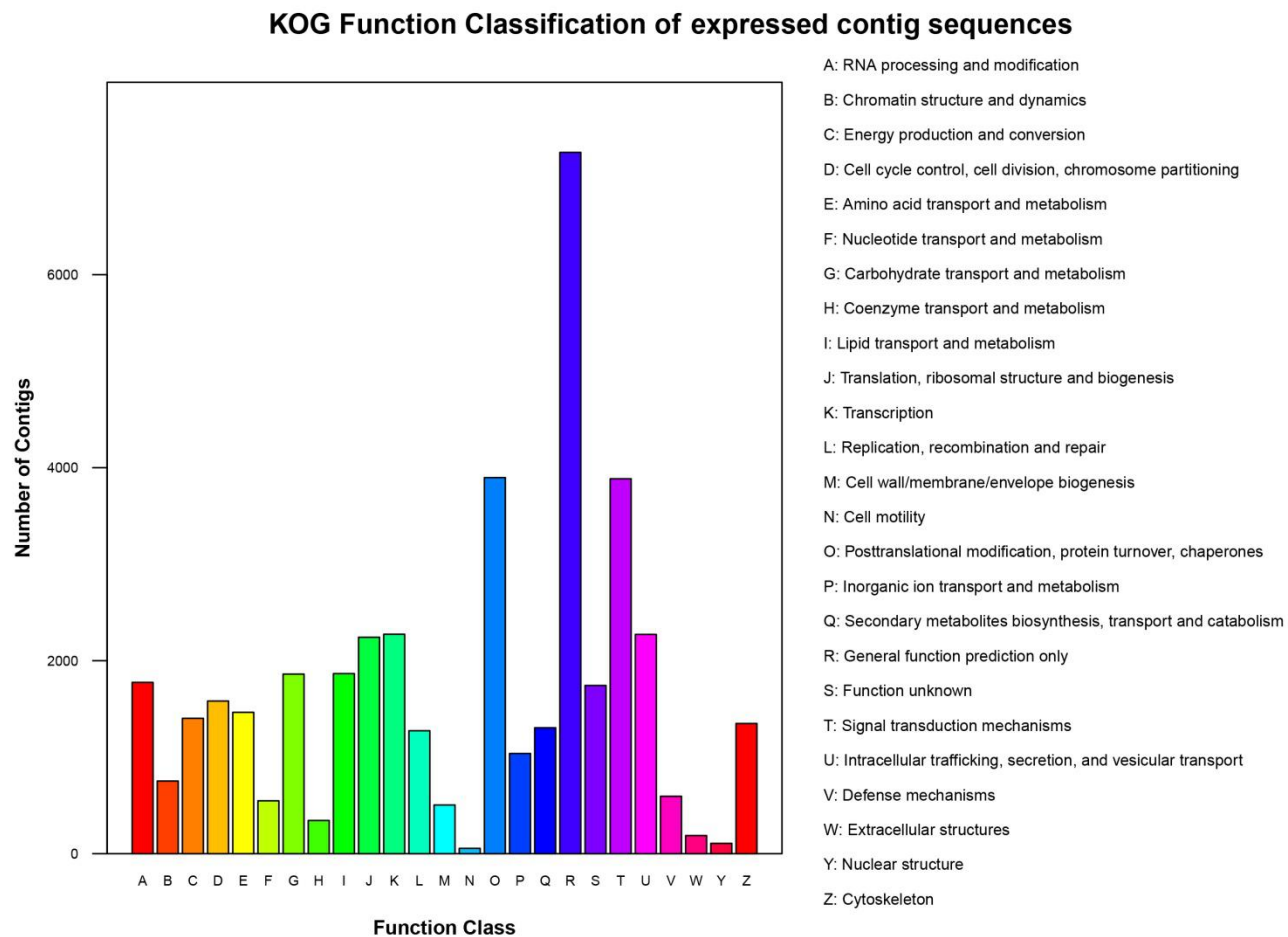
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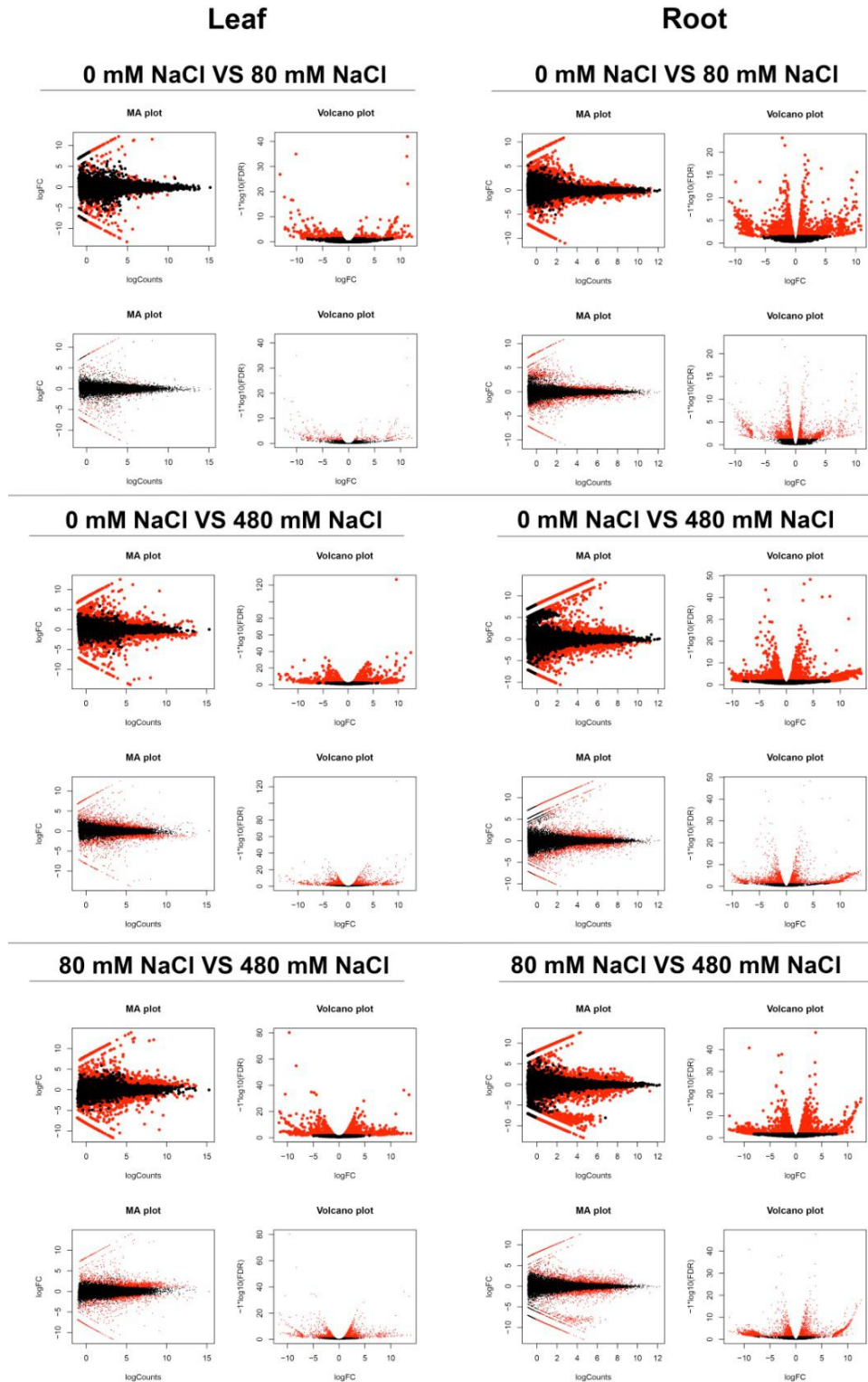
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Supplementary Figures and Tables

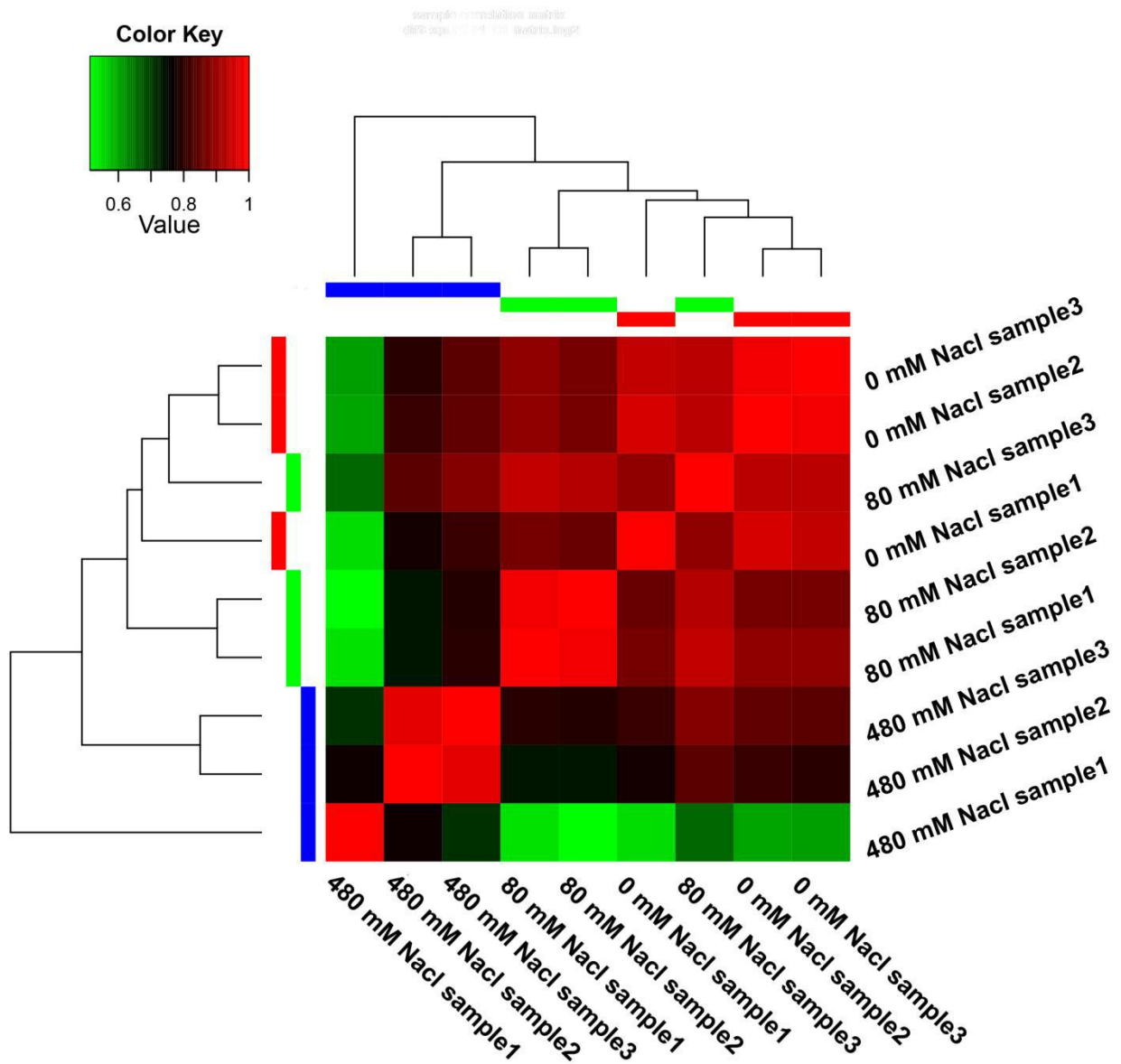


Supplementary Figure 1. COG functional classification of expressed contig sequences of *K. sieversiana*. The 30-d-old seedlings were subjected to 0, 80, and 480mM NaCl for 2 d.

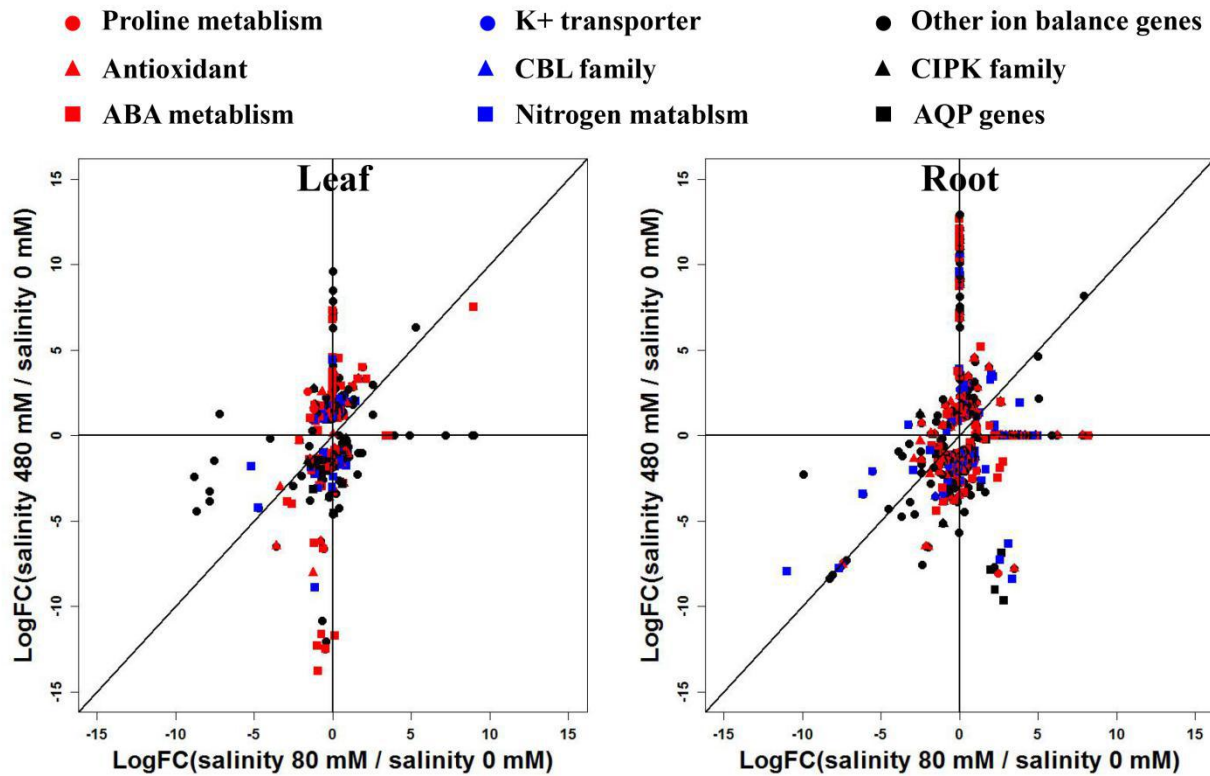


Supplementary Figure 2. MA and volcano plots of responses to different NaCl treatments in the leaf and root of *K. sieversiana*. LogFC represents the $\log_2(\text{Fold change})$, logCounts represents the $\log_2(\text{number of counts})$, and $-1 \times \log_{10}(\text{FDR})$ represents $-1 \times \log_{10}(\text{false discovery rate})$. The 30-d-old seedlings were subjected to 0, 80 and 480 mM NaCl for 2 d.

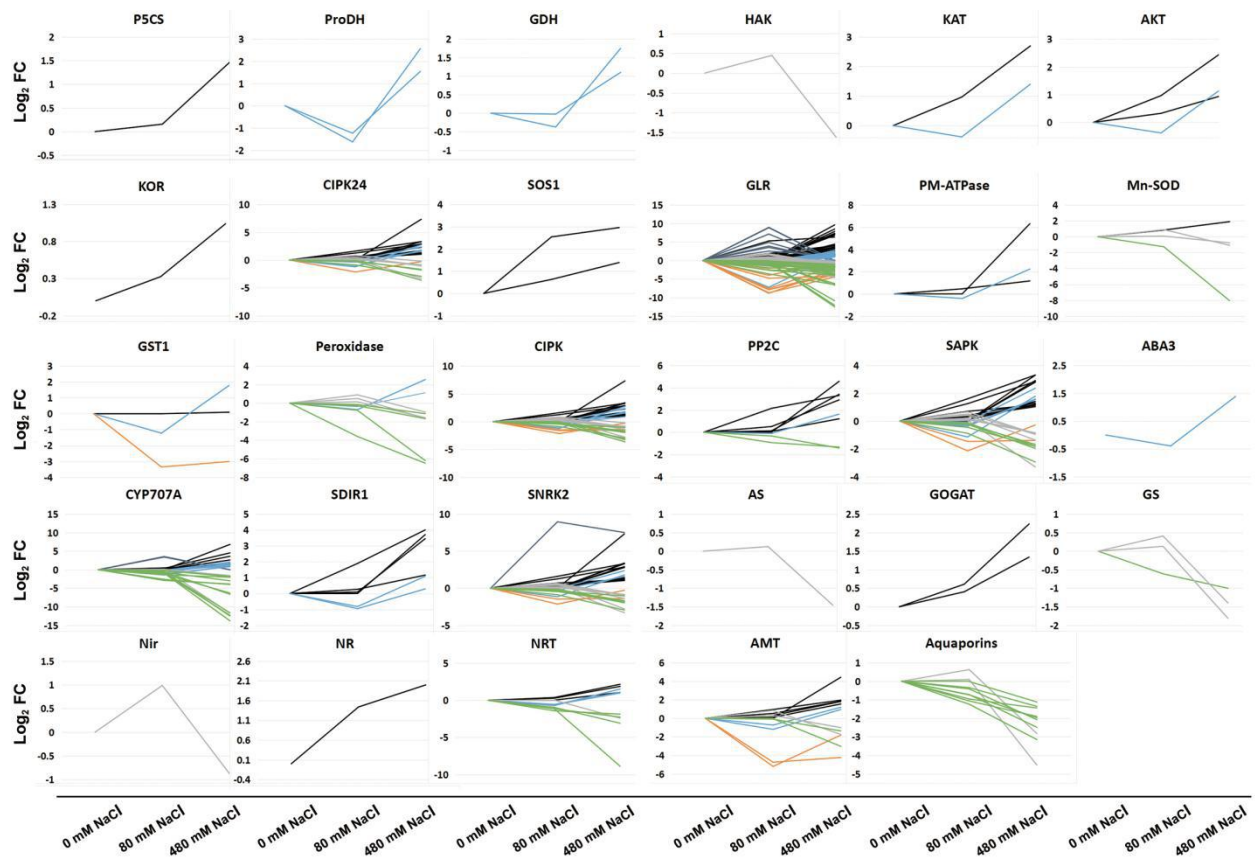
Correlation of DEGs in root



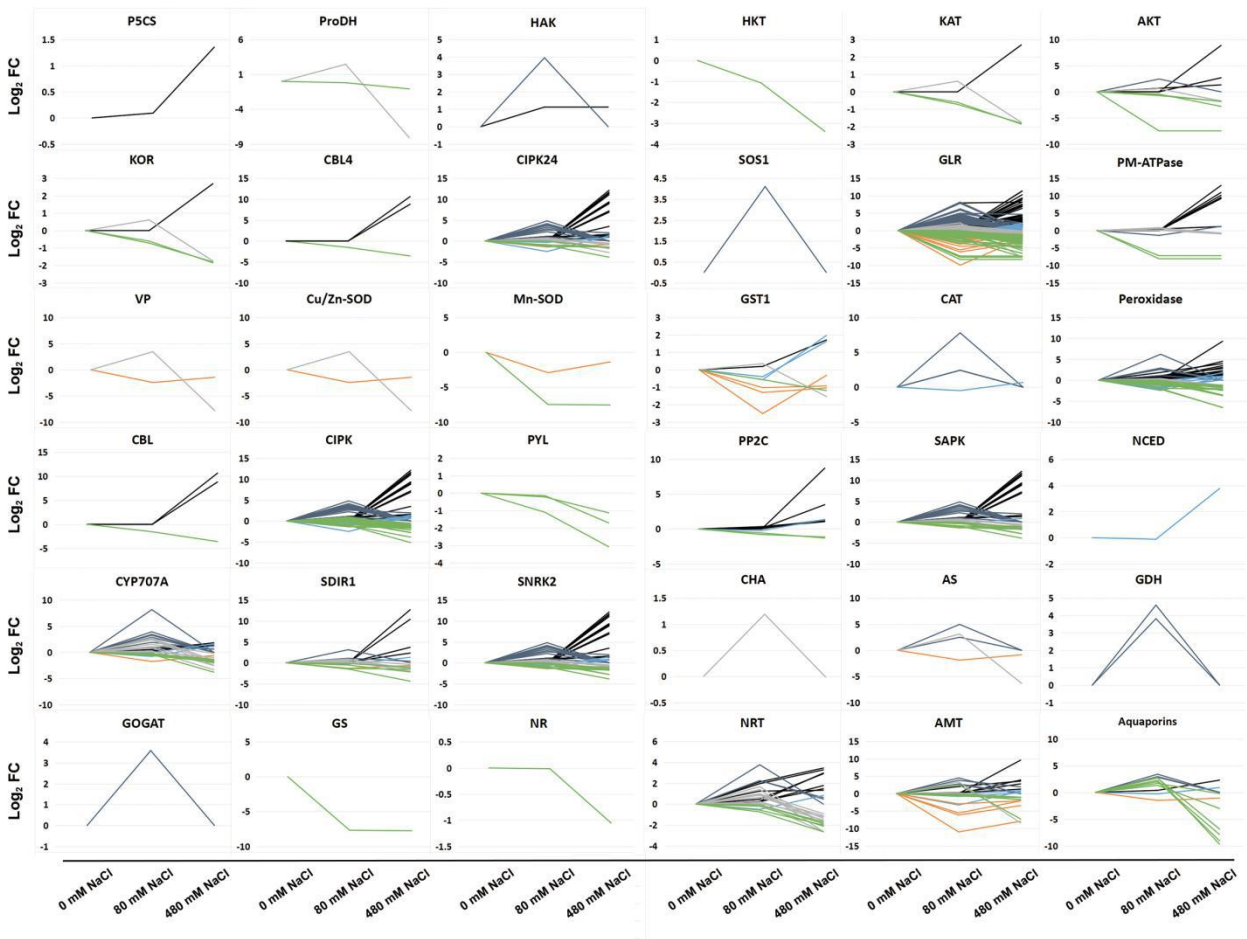
Supplementary Figure 3. Correlations of gene expression levels of nine root samples. The 30-d-old seedlings were subjected to 0, 80, and 480mM NaCl for 2 d.



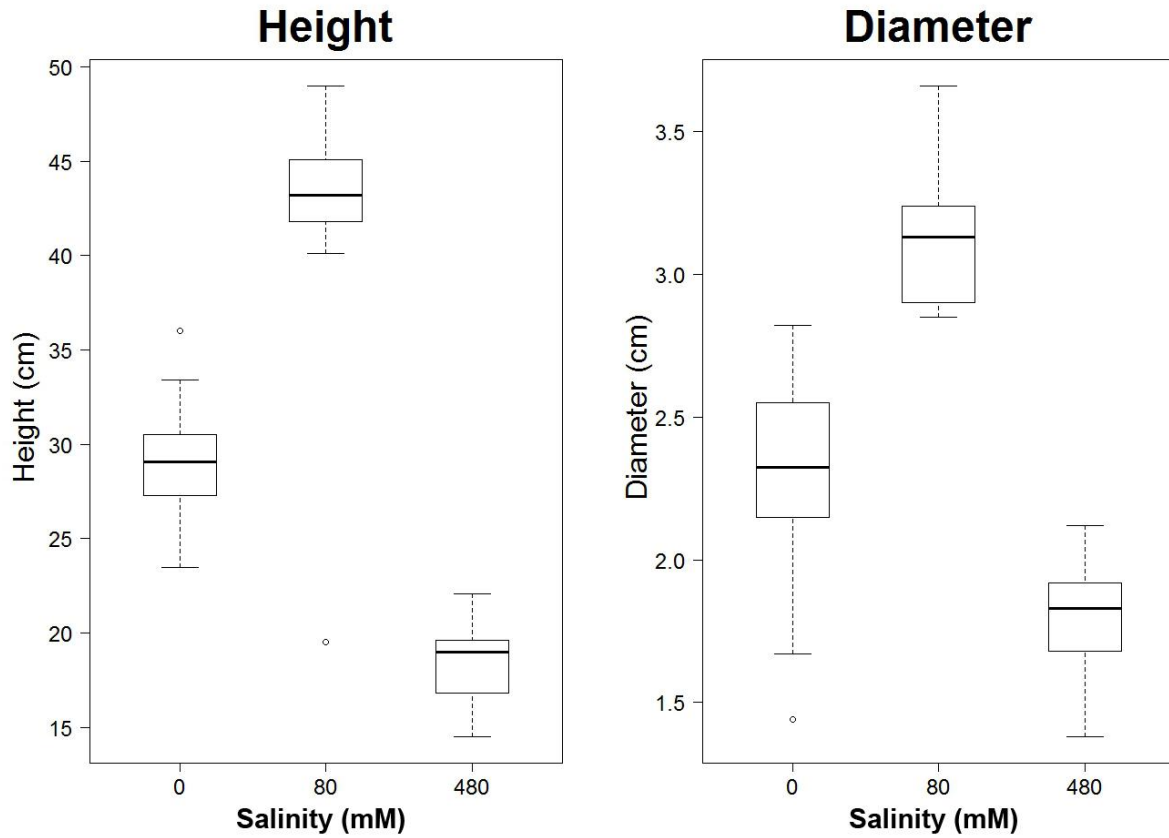
Supplementary Figure 4. Effects of different salinity levels on the expression of salinity-tolerant unigenes in the leaves and roots of *K. sieversiana*. Icons represent differentially expressed salinity tolerance genes. *X*-axis= Log₂ of the ratio of 80-mM salinity to control in gene expression level, and *y*-axis = Log₂ of the ratio of 480-mM salinity to control. The icons close to the *x*-axis indicated unigenes that have small expression differences between 80mM salinity and control, the icons close to the *y*-axis indicate unigenes that have small expression differences between 480mM salinity and control, and the icons that fell on the diagonal *x*=*y* line indicated that 80 and 480mM salinity produced identical effects on the expression of the unigenes. The 30-d-old seedlings were subjected to 0, 80, and 480-mM NaCl for 2 d.



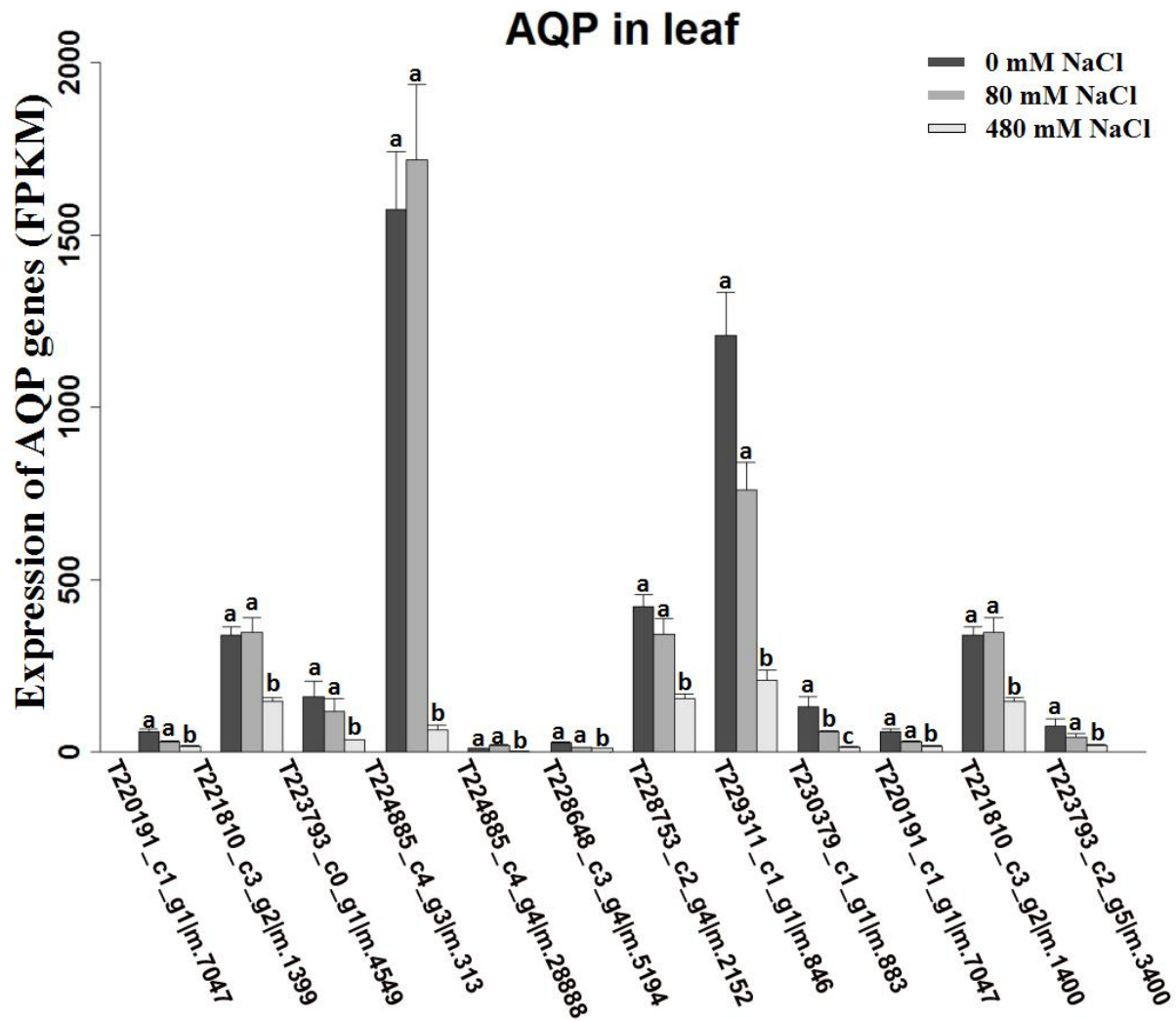
Supplementary Figure 5. Effects of salinity stress on the expression of different members of salinity-tolerant gene families in leaves of *K. sieversiana*. Each curve represents a member of a certain gene family. The 30-d-old seedlings were subjected to 0, 80, and 480mM NaCl for 2 d.



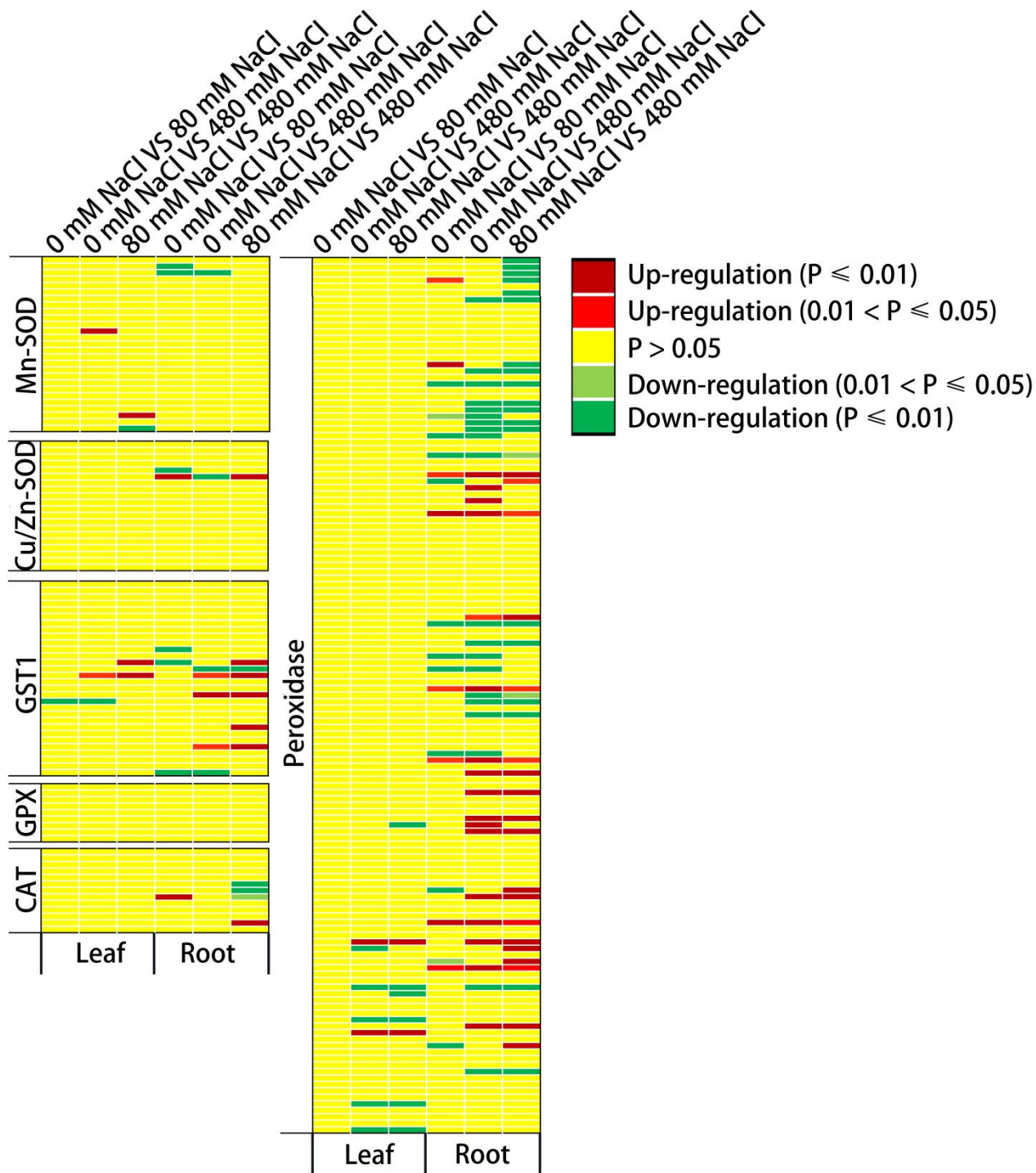
Supplementary Figure 6. Effects of salinity stress on the expression levels of different members of salinity-tolerant gene families in roots of *K. sieversiana*. Each curve represents a member of a certain gene family. The 30-d-old seedlings were subjected to 0, 80 and 480mM NaCl for 2 d.



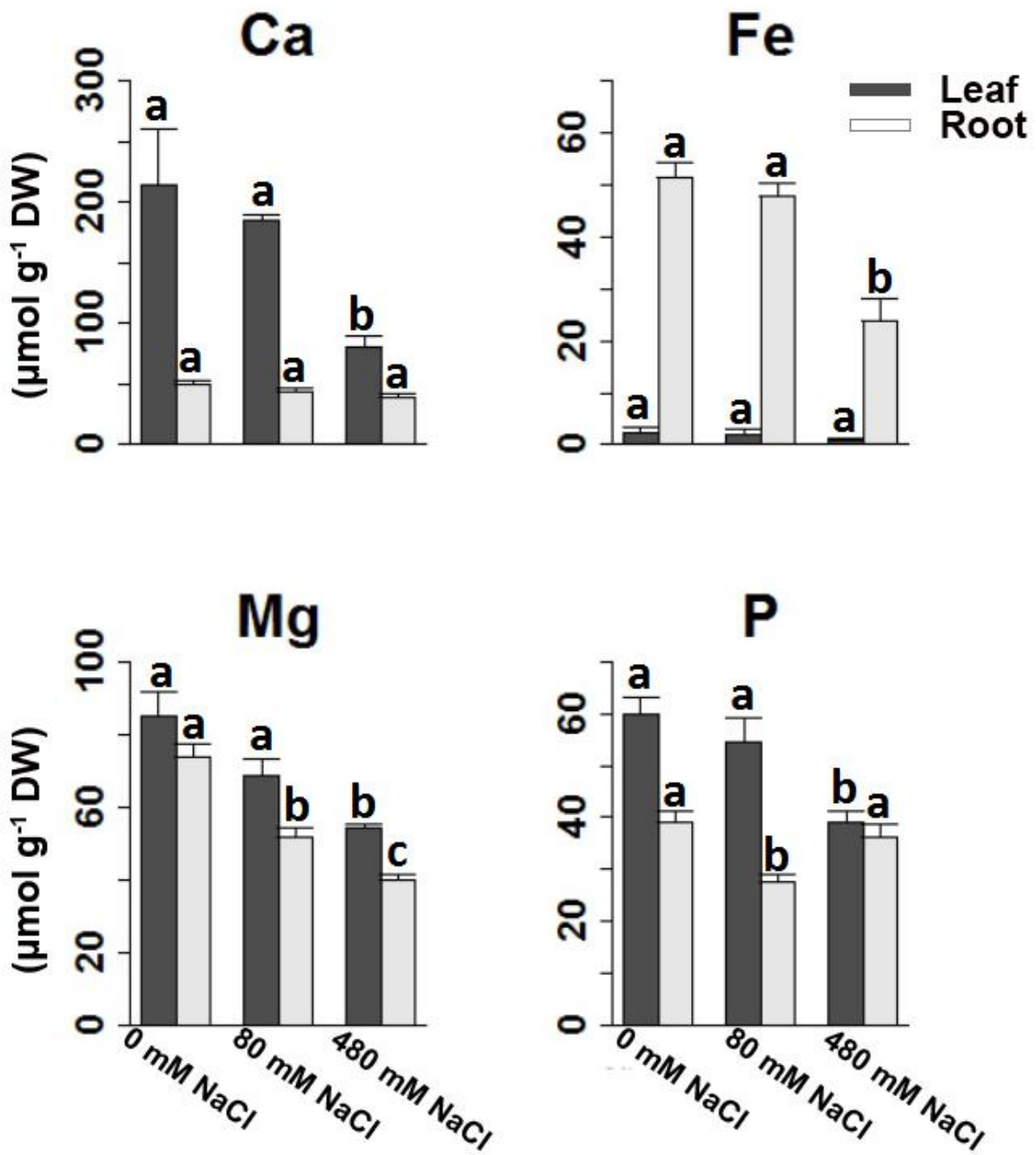
Supplementary Figure 7. Effects of salinity stress on the height (A) and the stem diameter (B) of *K. sieversiana*. Thirty individuals for each treatment were used in the Boxplot. The 30-d-old seedlings were subjected to 0, 80, and 480mM NaCl for 30 d.



Supplementary Figure 8. Expression levels of aquaporin-like unigenes in leaves of *K. sieversiana* under different salinity treatments. The values are means of three replicates (\pm SE). The 30-d-old seedlings were subjected to 0,80, and 480mM NaCl for 2 d. Different letters above the bars indicate significant differences among treatments, according to a least significant difference test ($P \leq 0.05$).



Supplementary Figure 9. Effects of salinity stresses on expression of antioxidative enzyme genes. The 30-d-old seedlings were subjected to 0, 80 and 480mM NaCl for 2 d.



Supplementary Figure 10. Effects of salinity stress on the contents of Ca, Fe, Mg and P in *K. sieversiana*. The 30-d-old seedlings were subjected to 0,80 and 480mM NaCl for 30 d. Different letters above the bars indicate significant differences among treatments according to a least significant difference test ($P \leq 0.05$).

Supplementary Table 1. Primer information for the validation of DEGs.

	F	R
UBQ 10	GAAAGCAGTTGGAAGATGGCAGGAC	CGAAGACGCAGAACCAGGTGGAG
TRINITY_DN223354_c1_g1 m.6046	CGGGCATTCTTCAGTGATAGGGG	TCTCGGCTTTATTTCCAGGGGTT
TRINITY_DN224332_c0_g4 m.1276	TTGGAAGGACTTTACTGTCTCACC	CTATTCCGACTTAACCTTGGACG
TRINITY_DN223233_c1_g1 m.16947	TCACCATTACTTATGCCCTCCTT	TGACTACTGCTGGACCGATACTTC
TRINITY_DN225973_c1_g3 m.8014	CGACGGCTGCGTTGACTACTTTG	GCCCGAGCTTCTTCAGGATTACG
TRINITY_DN198904_c0_g1 m.9620	ACCCAAACAAATAATAATGGCTGAA	GAGGAGTCAAATGATGCGAAGTA
TRINITY_DN227624_c1_g4 m.7191	TGGACTTGGGCTTCAGAATGGTA	TAGCGGCTGTATGACTTGTTGTT
TRINITY_DN219148_c0_g1 m.7706	ATGCTTCTCATTAGTCTTATTCGTGC	GACCTTACTACGCCCTCAACTCC
TRINITY_DN224752_c1_g2 m.7735	AAAGGCAATCTGACTGACAATACA	AACACTTATCAACTGGACCTCCC
TRINITY_DN226384_c0_g1 m.2958	AGTTGTGGGACCCGTTGGTTCAT	AAGCCCGGCTTTGCTCGTTTTCT
TRINITY_DN226441_c0_g1 m.777	GATGAAGGGAATAAGTTGTGGCT	AGGATGACCGTCGTATCGTTGTA

Supplementary Table 2. Overview of unigene annotations.

	Numbers of unigenes	Percentage of unigenes(%)
NR	38,686	82.62
Swiss-prot	29,309	62.59
GO	3,306	7.06
KAAS	6,774	14.47
COG	30,464	65.06
CDD	14,265	30.47

Supplementary Table 3. KEGG pathway enrichment of *K. sieversiana* unigenes.

	Pathway ID		Pathway		DEGs with pathway	All genes with pathway
	ko03010	Genetic Information Processing	Translation	Ribosome	114	240
	ko00195	Metabolism	Energy metabolism	Photosynthesis	32	55
	ko00196	Metabolism	Energy metabolism	Photosynthesis - antenna proteins	9	15
	ko00261	Metabolism	Biosynthesis of other secondary metabolites	Monobactam biosynthesis	6	7
Leaf	ko00860	Metabolism	Metabolism of cofactors and vitamins	Porphyrin and chlorophyll metabolism	16	41
	ko00300	Metabolism	Amino acid metabolism	Lysine biosynthesis	7	11
	ko00260	Metabolism	Amino acid metabolism	Glycine, serine and threonine metabolism	20	64
	ko00710	Metabolism	Energy metabolism	Carbon fixation in photosynthetic organisms	16	48
	ko04110	Cellular Processes	Cell growth and death	Cell cycle	23	85
Root	ko00940	Metabolism	Biosynthesis of other secondary metabolites	Phenylpropanoid biosynthesis	43	119

ko00280	Metabolism	Amino acid metabolism	Valine, leucine and isoleucine degradation	17	51
ko00073	Metabolism	Lipid metabolism	Cutin, suberine and wax biosynthesis	10	21
ko04016	Environmental Information Processing	Signal transduction	MAPK signaling pathway - plant	25	105
ko00250	Metabolism	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	12	37
ko00052	Metabolism	Carbohydrate metabolism	Galactose metabolism	12	38
ko00196	Metabolism	Energy metabolism	Photosynthesis - antenna proteins	7	15
ko00270	Metabolism	Amino acid metabolism	Cysteine and methionine metabolism	18	74
ko00260	Metabolism	Amino acid metabolism	Glycine, serine and threonine metabolism	16	64
ko00710	Metabolism	Energy metabolism	Carbon fixation in photosynthetic organisms	13	48
ko00071	Metabolism	Lipid metabolism	Fatty acid degradation	11	40
ko00910	Metabolism	Energy metabolism	Nitrogen metabolism	8	25
ko00195	Metabolism	Energy metabolism	Photosynthesis	13	55
ko00430	Metabolism	Metabolism of other amino acids	Taurine and hypotaurine metabolism	4	7

ko04075	Environmental Information Processing	Signal transduction	Plant hormone signal transduction	27	159
ko03320	Organismal Systems	Endocrine system	PPAR signaling pathway	8	28
ko00500	Metabolism	Carbohydrate metabolism	Starch and sucrose metabolism	18	94

Supplementary Table 4. Validation of DEGs by real-time quantitative PCR.

ID	R²	P
TRINITY_DN223354_c1_g1 m.6046	0.89	0.017
TRINITY_DN224332_c0_g4 m.1276	0.7	0.118
TRINITY_DN223233_c1_g1 m.16947	0.87	0.024
TRINITY_DN225973_c1_g3 m.8014	0.84	0.036
TRINITY_DN198904_c0_g1 m.9620	0.96	0.002
TRINITY_DN227624_c1_g4 m.7191	0.79	0.062
TRINITY_DN219148_c0_g1 m.7706	0.92	0.008
TRINITY_DN224752_c1_g2 m.7735	0.97	0.002
TRINITY_DN226384_c0_g1 m.2958	0.98	0.001
TRINITY_DN226441_c0_g1 m.777	0.68	0.138

Supplementary Table 5. Effects of salinity stress on expression of ion compartmentation genes. The 30-d-old seedlings were subjected to 0, 80 and 480mM NaCl for 2 d.

Genes ID	Leaf (FPKM)			Root (FPKM)			Genes family
	0 mM NaCl	80 mM NaCl	480 mM NaCl	0 mM NaCl	80 mM NaCl	480 mM NaCl	
TRINITY_DN216785_c2_g2 m.13772	1.75 ± 0.06 ^a	1.60 ± 0.05 ^a	5.52 ± 0.78 ^a	30.08 ± 1.39 ^a	34.92 ± 1.57 ^a	40.84 ± 0.41 ^a	NHX
TRINITY_DN218503_c0_g2 m.12824	1.50 ± 0.08 ^a	1.99 ± 0.22 ^a	4.08 ± 0.33 ^a	13.48 ± 0.43 ^a	15.58 ± 0.84 ^a	15.77 ± 0.37 ^a	NHX
TRINITY_DN226373_c0_g2 m.5681	67.92 ± 6.20 ^a	83.86 ± 15.37 ^a	102.56 ± 7.12 ^a	21.56 ± 1.05 ^a	35.18 ± 3.05 ^a	39.12 ± 1.06 ^a	NHX
TRINITY_DN218402_c0_g2 m.2511	122.03 ± 12.64 ^a	143.31 ± 19.37 ^a	81.01 ± 7.17 ^a	15.44 ± 0.40 ^c	21.11 ± 1.16 ^b	33.30 ± 1.55 ^a	HA
TRINITY_DN228495_c2_g2 m.162	171.02 ± 9.57 ^a	220.40 ± 23.87 ^a	146.49 ± 10.62 ^a	533.17 ± 64.77 ^a	432.29 ± 27.62 ^a	434.73 ± 26.10 ^a	AVP1
TRINITY_DN220826_c3_g1 m.7289	1.92 ± 0.40 ^a	2.11 ± 0.41 ^a	3.82 ± 1.35 ^a	107.98 ± 16.78 ^a	98.05 ± 8.77 ^a	118.90 ± 18.16 ^a	AVP1

