Genetic regulation of salt stress tolerance revealed by RNA-Seq in cotton diploid wild species, *Gossypium davidsonii*

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Table S1. Mapping result of RNA-Seq reads

Sample	Raw reads (M)	Clean reads (M)	Mapped reads (M)	Mapping rate
RC12-1	24.0	23.2	17.1	73.76%
RC12-2	27.1	26.2	19.1	72.89%
RS12-1	64.3	61.5	44.6	72.63%
RS12-2	14.6	13.7	10.2	74.74%
RC24-1	21.2	20.7	15.2	73.18%
RC24-2	32.1	30.5	23.9	78.36%
RS24-1	32.6	31.2	22.5	72.26%
RS24-2	21.8	20.6	14.7	71.14%
RC48-1	36.9	35.7	26.1	72.93%
RC48-2	22.0	21.2	15.3	72.18%
RS48-1	72.8	71.5	52.8	73.92%
RS48-2	19.9	19.5	14.1	72.30%
RC96-1	86.7	84.1	61.2	72.77%
RC96-2	21.1	20.4	14.6	71.61%
RS96-1	137.6	134.9	97.2	72.07%
RS96-2	21.4	20.5	12.5	60.88%
RC144-1	29.5	28.2	20.0	70.94%
RC144-2	33.7	32.1	23.7	73.99%
RS144-1	47.8	46.1	32.9	71.53%
RS144-2	33.5	32.1	23.5	73.18%
LC12-1	37.9	35.7	30.2	84.47%
LC12-2	42.3	40.1	32.9	82.11%
LS12-1	39.3	37.9	29.8	78.55%
LS12-2	23.3	21.4	16.0	75.00%
LC24-1	15.7	14.1	10.7	75.78%
LC24-2	22.2	20.0	15.1	75.58%
LS24-1	32.4	30.6	25.6	83.70%
LS24-2	27.8	26.4	21.9	82.88%
LC48-1	14.1	12.7	9.3	73.41%
LC48-2	35.2	32.9	24.3	74.04%
LS48-1	77.2	73.0	58.6	80.32%
LS48-2	41.5	39.5	32.4	82.00%
LC96-1	21.7	20.1	13.4	66.67%
LC96-2	180.8	176.6	126.6	71.67%
LS96-1	39.1	36.5	21.8	59.74%
LS96-2	43.3	40.4	30.4	75.40%
LC144-1	32.9	29.1	21.2	72.78%
LC144-2	53.3	47.0	34.3	72.86%
LS144-1	42.4	40.7	30.8	75.87%
LS144-2	64.4	61.9	47.6	76.92%

RC and RS: root well-watered and salt treatment, respectively; LC and LS: leaf well-watered and salt treatment, respectively.

Table S2. GO terms related to stimulus or stress.

Term	R12	R24	R48	R96	R144	L12	L24	L48	L96	L144
response to osmotic stress	75	81	31	21	18	11	15	45	60	70
response to oxidative stress	65	63	19	19	15	8	11	38	31	54
response to starvation	31	30	9	13	9	0	0	13	14	19
response to water deprivation	51	51	23	15	16	9	9	32	41	35
cation transport	56	69	34	29	14	6	10	37	53	84
inorganic anion transport	32	28	16	25	21	0	0	10	23	21
monovalent inorganic cation transport	10	16	7	0	0	0	0	11	14	26
di-, tri-valent inorganic cation transport	28	32	16	16	8	6	8	24	30	38
metal ion transport	47	53	24	22	12	6	9	29	41	58
nitrate transport	27	30	14	18	16	0	0	7	14	15
response to abscisic acid stimulus	65	75	29	16	23	9	12	40	51	48
response to ethylene stimulus	36	37	18	14	18	0	5	23	26	30
response to hormone stimulus	125	126	53	41	46	13	16	80	103	101
response to jasmonic acid stimulus	49	58	22	21	24	7	9	29	37	51
response to salicylic acid stimulus	40	49	17	17	20	5	5	26	29	47
response to chitin	55	53	15	24	34	0	0	19	31	36
response to sucrose stimulus	14	27	9	9	11	0	5	18	29	36

The numbers in table indicate the differential expression genes in root (R) and leaf (L) at five time points.

Table S3. Mapman bins of photosynthetic pathway

Bin	Name	Elements	P-value
1	PS	155	4.36E-25
1.1	PS.lightreaction	120	2.52E-24
1.1.1	PS.lightreaction.photosystem II	51	6.97E-14
1.1.1.1	PS.lightreaction.photosystem II.LHC-II	18	1.09E-04
1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits	33	1.26E-10
1.1.2	PS.lightreaction.photosystem I	26	1.56E-05
1.1.2.1	PS.lightreaction.photosystem I.LHC-I	2	1.62E-01
1.1.2.2	PS.lightreaction.photosystem I.PSI polypeptide subunits	24	4.28E-05
1.1.3	PS.lightreaction.cytochrome b6/f	6	1.90E-03
1.1.3.1	PS.lightreaction.cytochrome b6/f.iron sulfur subunit	1	9.80E-01
1.1.3.2	PS.lightreaction.cytochrome b6/f.subunit 4 (PETD)	1	1.91E-01
1.1.3.3	PS.lightreaction.cytochrome b6/f.cytochrome b6 (CYB6)	2	2.54E-02
1.1.4	PS.lightreaction.ATP synthase	9	1.17E-03
1.1.4.1	PS.lightreaction.ATP synthase.alpha subunit	1	7.97E-02
1.1.4.3	PS.lightreaction.ATP synthase.epsilon chain	1	1.67E-01
1.1.4.4	PS.lightreaction.ATP synthase.gamma chain	1	1.74E-01
1.1.4.5	PS.lightreaction.ATP synthase.subunit C	1	9.45E-02
1.1.4.6	PS.lightreaction.ATP synthase.chloroplastic subunit a	2	1.87E-02
1.1.4.7	PS.lightreaction.ATP synthase.delta chain	1	2.34E-01
1.1.4.8	PS.lightreaction.ATP synthase.subunit B (ATPF)	1	8.13E-02
1.1.40	PS.lightreaction.cyclic electron flow-chlororespiration	10	9.60E-02
1.1.5	PS.lightreaction.other electron carrier (ox/red)	11	8.20E-02
1.1.5.2	PS.lightreaction.other electron carrier (ox/red).ferredoxin	7	3.26E-01
1.1.5.3	PS.lightreaction.other electron carrier (ox/red).ferredoxin reductase	3	6.99E-02
1.1.5.4	PS.lightreaction.other electron carrier (ox/red).ferredoxin oxireductase	1	9.80E-01
1.1.6	PS.lightreaction.NADH DH	7	1.16E-02
1.2	PS.photorespiration	9	2.19E-01
1.2.2	PS.photorespiration.glycolate oxydase	3	7.51E-01
1.2.3	PS.photorespiration.aminotransferases peroxisomal	1	9.80E-01
1.2.4	PS.photorespiration.glycine cleavage	2	1.20E-01
1.2.4.4	PS.photorespiration.glycine cleavage.H protein	2	1.20E-01
1.2.6	PS.photorespiration.hydroxypyruvate reductase	1	3.07E-01
1.2.7	PS.photorespiration.glycerate kinase	1	9.80E-01
1.3	PS.calvin cycle	26	1.05E-02
1.3.1	PS.calvin cycle.rubisco large subunit	5	1.27E-04
1.3.11	PS.calvin cycle.RPE	1	9.80E-01
1.3.12	PS.calvin cycle.PRK	1	2.68E-01
1.3.13	PS.calvin cycle.rubisco interacting	2	8.03E-01
1.3.2	PS.calvin cycle.rubisco small subunit	3	9.65E-01
1.3.4	PS.calvin cycle.GAP	5	6.31E-01
1.3.5	PS.calvin cycle.TPI	1	9.80E-01
1.3.6	PS.calvin cycle.aldolase	1	1.33E-01
1.3.7	PS.calvin cycle.FBPase	5	1.66E-01
1.3.8	PS.calvin cycle.transketolase	1	9.80E-01

Table S4. Mapman bins of redox pathway

Bin	Name	Elements	P-value
21	redox	56	1.02E-02
21.1	redox.thioredoxin	16	3.32E-01
21.1.1	redox.thioredoxin.PDIL	1	2.28E-01
21.2	redox.ascorbate and glutathione	13	1.49E-01
21.2.1	redox.ascorbate and glutathione.ascorbate	6	1.23E-01
21.2.1.2	redox.ascorbate and glutathione.ascorbate. GDP-L-galactose-hexose-1-phosphate guanyltransferase	1	2.38E-01
21.2.2	redox.ascorbate and glutathione.glutathione	2	2.99E-01
21.3	redox.heme	1	9.80E-01
21.4	redox.glutaredoxins	19	1.41E-01
21.5	redox.peroxiredoxin	3	8.29E-01
21.5.1	redox.peroxiredoxin.BAS1	1	3.49E-01
21.6	redox.dismutases and catalases	4	1.06E-01

Table S5. Statistics of alternative splicing categories from RNA-Seq data

Category	Event No.	Event ratio (%)	Gene No.	Gene ratio (%)[a]	Events per gene
AA	4146	29.25	3065	45.09	1.35
AD	1809	12.76	1526	22.45	1.19
ES	1093	7.71	888	13.06	1.23
IR	5063	35.73	3492	51.37	1.45
others	2061	14.54	1171	17.23	1.76
total	14172	100	6798	149.19	2.08

[a] Many genes utilize multiple splicing categories, causing percentages to sum to greater than 100.

Table S6. Primers used for qRT-PCR to validate the correlation with RNA-Seq

Gene ID	Transcripts level under salt stress vs. CK	Primers for qRT-PCR(5'-3')
Gorai.005G088800	root up-regulated	F:CCCAAATGGAAGGAAGAA
		R:GCATCTGTGAAGCAAATC
Gorai.010G219200	root up-regulated	F:TTATCTCTTCTGCTCCTT
		R:AGTATCCATAGTTCTTATCAC
Gorai.012G098700	root up-regulated	F:TTCAGGCATCAACATCTA
		R:AGAATCCATAGTCAGCAAT
Gorai.013G186800	root up-regulated	F:ACTTGTCACTTGAATCAC
		R:CTCACTTCATCGTCTCTA
Gorai.011G106000	root up-regulated	F:TAATAACTCGGCTTCTCT
		R:CCACAATTCTCAACAGTA
Gorai.013G002500	root up-regulated	F:CACCGAAGATGAATGAAT
		R:GGATTACACATATTACCAAGA
Gorai.009G240400	root up-regulated	F:GAGTACATCTCCGACTTG
		R:CACAACCTTCACAATCCA
Gorai.009G425300	root up-regulated	F:GGTGTATGATGTTGTTAGG
		R:TCACTATATCCTCCCAAAC
Gorai.009G055400	root up-regulated	F:CTTTCCATATCATTTCCTTATC
		R:CTTGTTGTAGCGAATCTT
Gorai.010G249700	root up-regulated	F:CAACAATCGTGTCTTCAA
		R:CACTATGCCTGTATGCTA
Gorai.010G144000	root up-regulated	F:GTCAACCTTACTCTCACT
		R:CATCATTATCATCACCAAGT
Gorai.013G264800	root no differnce	F:AGTGTCGATATGCTGTAT
		R:AATCATCTTGCTTCTCAC
Gorai.013G260200	root no differnce	F:AGAAATTGGCTCCTGAAA
		R:CGGCGGTAAGTTTATGTA
Gorai.013G229600	root no differnce	F:CTTGAATGTGCTATTGGA
		R:AGAGAATTGATCTGATGGA
Gorai.005G259200	root no differnce	F:TTACAGAAGCACCACTAA
		R:GCCTGAATAGCAACATAC
Gorai.004G144900	root no differnce	F:CTGTCAATGATGAACCTATT
		R:GCTCTGTCTCTTCTGATT
Gorai.007G228300	root no differnce	F:TTATGAAACGGCGAAATGG
		R:TATGGAGGAGGCTCTTCA
Gorai.011G281100	root no differnce	F:ACTATGTTGATCTATTGGTGTT
		R:CACTCCTTCACTTGTAACC
Gorai.001G088900	root no differnce	F:CCAGAGGTATTGAAGAAG
		R:GGAAGTAGAAGAGGAATTG
Gorai.001G177100	root no differnce	F:TACGCTGGACTAACTATT
		R:TAGCCGACCATCTATTAC

Gorai.001G199400	root no differnce	F:AATTAGAACAAGGAGACAATAAG
		R:CAACGCTCATTAACAGATT
Gorai.001G068900	root no differnce	F:TTGCTATGGATGCTTGAT
		R:GGTGCGAGAACATATAACT
Gorai.003G119400	root down-regulated	F:TGAAGGATATGAACATTGC
		R:CTGAGCCGATAATTGAAG
Gorai.010G022800	root down-regulated	F:AGGTTACAGTCGTTGGAT
		R:GTCCCATACTTAGCACTTC
Gorai.004G233300	root down-regulated	F:ATCCATTGAACTTATATGATGAA
		R:TTGCCTTCTTCTCACATT
Gorai.007G007800	root down-regulated	F:GAAGGAAGAGGAGGAGAT
		R:TATCGTAATAGATCACACCTG
Gorai.005G102700	root down-regulated	F:TGACCAAGGAAGAGATAA
		R:CGCATCCATCTTTATTTG
Gorai.008G237900	root down-regulated	F:TCAGCAATCCTACTTCTC
		R:GCAAACATTCACGTTATTC
Gorai.005G088000	root down-regulated	F:TTCATCAGAGTTCTATAAGGT
		R:ATCCACATTCTCCAACTT
Gorai.005G074600	root down-regulated	F:TTTCATGGAAGCCAAAGA
		R:ATCTGTGAGTAGCAATGG
Gorai.005G185500	root down-regulated	F:GAGGTTATGGAGGTTCTAA
		R:GATTGGTAGGACTCTGAA
Gorai.007G027400	root down-regulated	F:ATGCTCTTAAATTAGGTGTATG
		R:AACGAGACCATTGAGAAG
Gorai.011G137300	leaf up-regulated	F:TTGATTCCTTTCCCGATT
		R:TCCTGTTCTTCTGTATGG
Gorai.012G003400	leaf up-regulated	F:GAGACGATGATGAATATAACC
		R:ATGATGGAGAGGACAATG
Gorai.007G318400	leaf up-regulated	F:TTAGTTTGTGTTCTCTTAGC
		R:CACATTCATTTCCTTTGC
Gorai.013G271900	leaf up-regulated	F:GGATTGATGATGCTGTTG
		R:ATGGAATAAGGAGGTATGTG
Gorai.002G250500	leaf up-regulated	F:TGCTTTCAGGCTATAAATC
		R:TTCCATCTCTTCCACTAC
Gorai.001G031100	leaf up-regulated	F:CCTATCATTCCTGTTACTG
		R:TTGTAGATTTCTTCATCCATA
Gorai.004G235900	leaf up-regulated	F:TTCTTGTTGTGAGGTTGT
		R:TTAAGGCTAGTCTTCCATC
Gorai.006G245900	leaf up-regulated	F:CCAGAGTTGTTACAGGTT
		R:TTGTCTTCATAAGTAGGTTCA
Gorai.010G215600	leaf up-regulated	F:ACCTCCATTGATGTATTGT
		R:ACCCATTTCAGTGCTAAT

Gorai.004G068200	leaf up-regulated	F:TTACGAGGCTCATTATCT
		R:TCCATCTGTTCTTGTCTA
Gorai.002G248000	leaf up-regulated	F:CTGCTATAATGGAACATATACTTG
		R:AATGCGTAACGATCTTGT
Gorai.001G002700	leaf no differnce	F:AAGACAAGAACTTCAACTAC
		R:GCAGCAATACGGTAATAG
Gorai.001G084000	leaf no differnce	F:GATTGGCTTATTCTTGTATTG
		R:AACAGGAACCGTATTACT
Gorai.013G090200	leaf no differnce	F:ACGAAGTTGGTGTAGATG
		R:TAACAGTCCGATGTGAAG
Gorai.003G009500	leaf no differnce	F:CAAGCGAGAAGTCAAGATA
		R:CCTCACATAACTCCATAACA
Gorai.009G295900	leaf no differnce	F:TCAGGAATTGGAGTGGAT
		R:TCAATGAACAACTTGGAACT
Gorai.009G288900	leaf no differnce	F:AAGCAGAATCCATTGAAG
		R:AATCTAACTCTTGACTATTACC
Gorai.003G056300	leaf no differnce	F:TCATGTTGCCTGTGTTAT
		R:TTCTATTTCCCTGCCTTC
Gorai.001G089400	leaf no differnce	F:CTGTTGTGTTGATTGACT
		R:TGTGATGAAGCAAGTAAC
Gorai.001G118900	leaf no differnce	F:ACGATGATAGTGACAAGAG
		R:AGTGTAGATTCAAGCAAGT
Gorai.006G025300	leaf no differnce	F:TTGGAACTGAACTTACTC
		R:AACCTGGTCAATATCTTC
Gorai.006G212100	leaf down-regulated	F:GATACATCGAGAATCCATAG
		R:TCCATCACCTTAGTAGAC
Gorai.013G186800	leaf down-regulated	F:AATGGAGAAAGTTTATCAGT
		R:CTTCACCTTCTTCACTATC
Gorai.011G245400	leaf down-regulated	F:GTTGTTGCTTGTTCATAG
		R:CAATACACTTATCACCAATG
Gorai.012G037600	leaf down-regulated	F:ATGACGAATTGGTGAATC
		R:CATACAACGCCATATCTG
Gorai.013G186800	leaf down-regulated	F:AATGGAGAAAGTTTATCAGT
		R:CTTCACCTTCTTCACTATC
Gorai.001G091300	leaf down-regulated	F:TACTCCAGATGATAAATGTG
		R:TTCCCTTACAATGTTCAC
Gorai.013G092300	leaf down-regulated	F:TATGACCAAGAGTGAAGT
		R:CAGCGATAGTAACTCCTA
Gorai.004G140800	leaf down-regulated	F:TCATGTTGCCTGTGTTAT
		R:TTCTATTTCCCTGCCTTC
Gorai.001G180600	leaf down-regulated	F:GGAACAGAATGAGGATAATG
		R:GCACATAAAGTCCCAAAG

Gorai.007G202300	leaf down-regulated	F:CATTCTCTCAGTTCTTCAC R:TATTAATCCAGCGGTCAG
Gorai.009G300600	leaf down-regulated	F:CAAGGCTCTCATTATACG R:TACACAAGACCAACAATT

Table S7. Primers used for validation of alternative splicing

Gene ID	AS pattern	Primers for RT-PCR(5'-3')	Primers for qRT-PCR(5'-3')
Gorai.007G304100	AA	F:TTCTAGCCGCATTGCCTTT	F:AAGGAGAGGAGATATAGAGAA
		R:CGACGAGAGCAAGCGATCA	R:TCGTAATTCAAACAGAAATCC
Gorai.008G004200	ES	F:ATGAGTCGCCTGCCCAGA	F:GTGAGCTATGTTCGATCTTCT
		R:TATCTGGTATCTGTTATTACCGCTT	R:GGTGTTGTCGTCGGAATA
Gorai.008G047400	MEE	F:CTGAAAGCTCTTCTCCCATGC	F:GTGAGCTATGTTCGATCTTCT
		R:TATGAACCACTTTCTGGAGGAGAG	R:GGTGTTGTCGTCGGAATA
Gorai.008G263000	AD	F:TTTGGTAAGCAGTATCTGATTCTCA	F:TCTTCCAATACCGTTCAG
		R:TCACTGTCCCCTCGCCAG	R:CAGATGCTAATTCAAGTGTT
Gorai.002G147600	IR	F:AACACTTCGAATCGTTTTCCA	F:AAGCAGTATCTGATTCTC
		R:AATCTTGGCTTTCACATTATCAA	R:TCAACGGTAATAGACATT

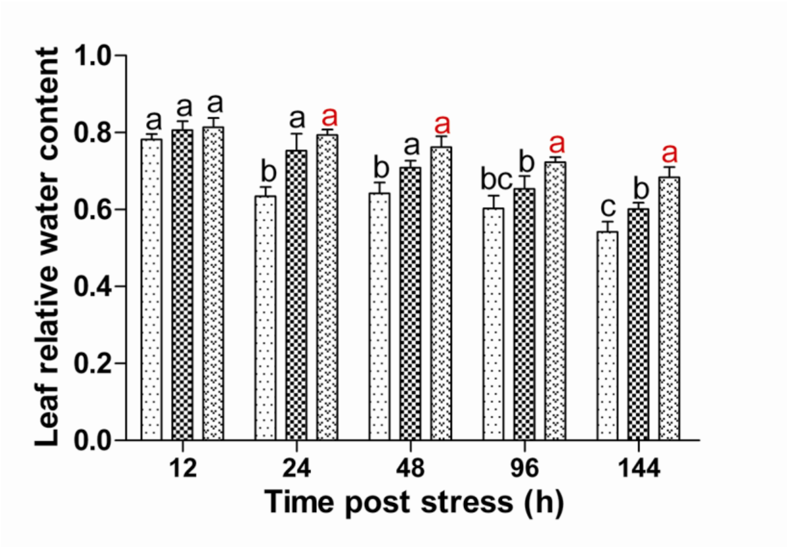

Figure S1. Leaf relative water content in *G. davidsonii* and *G. hirsutum*

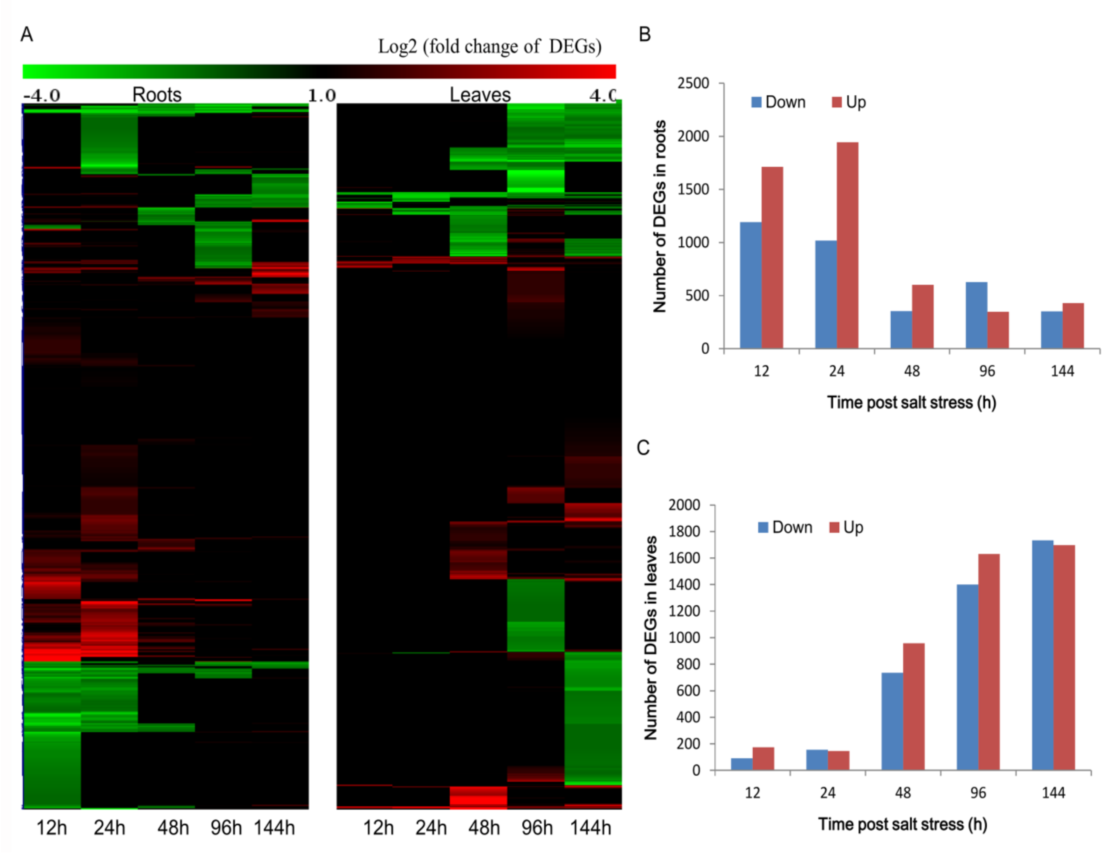

Figure S2. Number of DEGs in roots and leaves.

A, Heat map of total DEGs in response to salt stress in roots and leaves. B, Number of DEGs in roots. C, Number of DEGs in leaves.

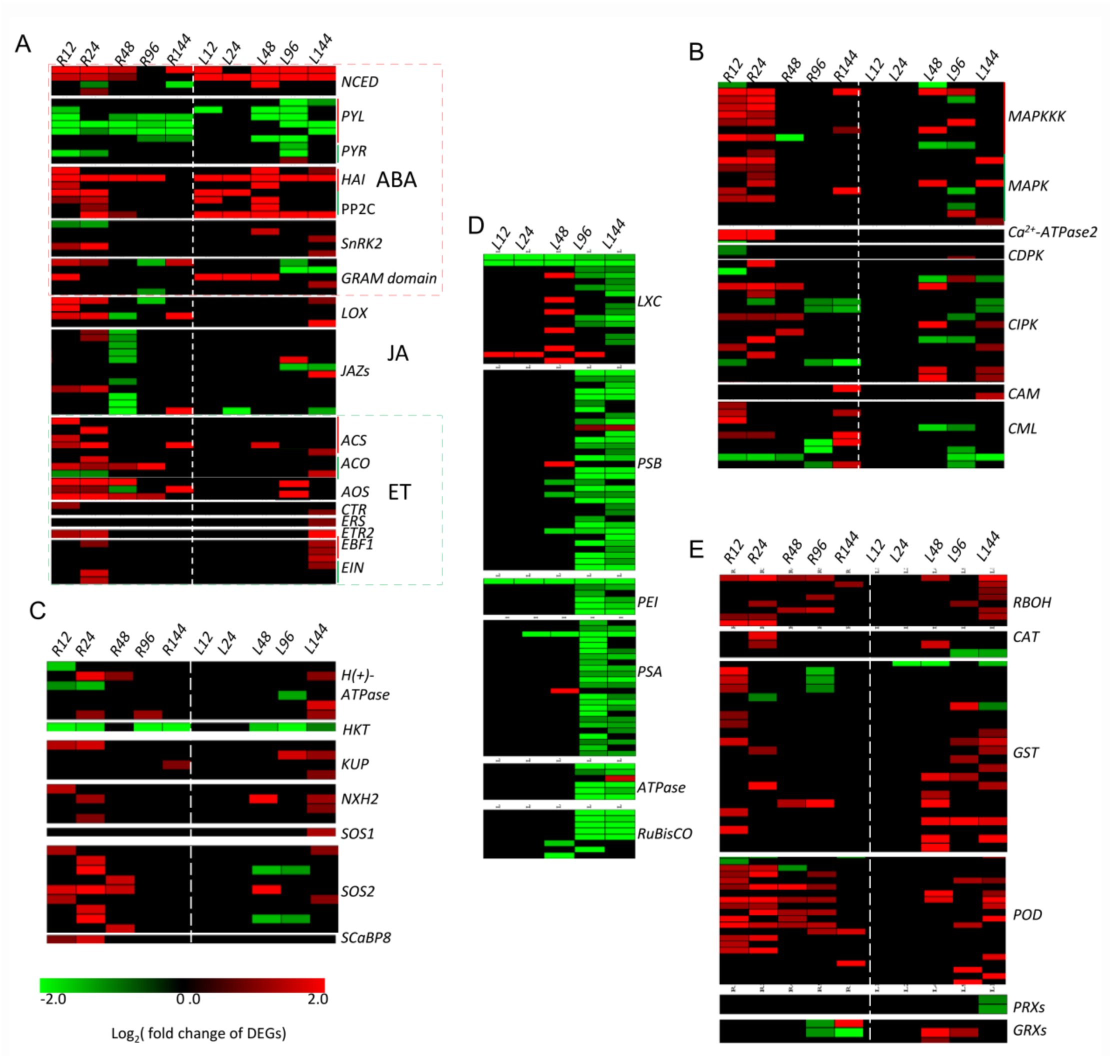

Figure S3. Expression profiling of hormone (A), MAPK cascade and Ca^{2+}-related kinase (B), ion homeostasis (C), Photosynthesis (D) and ROS (E) related genes.

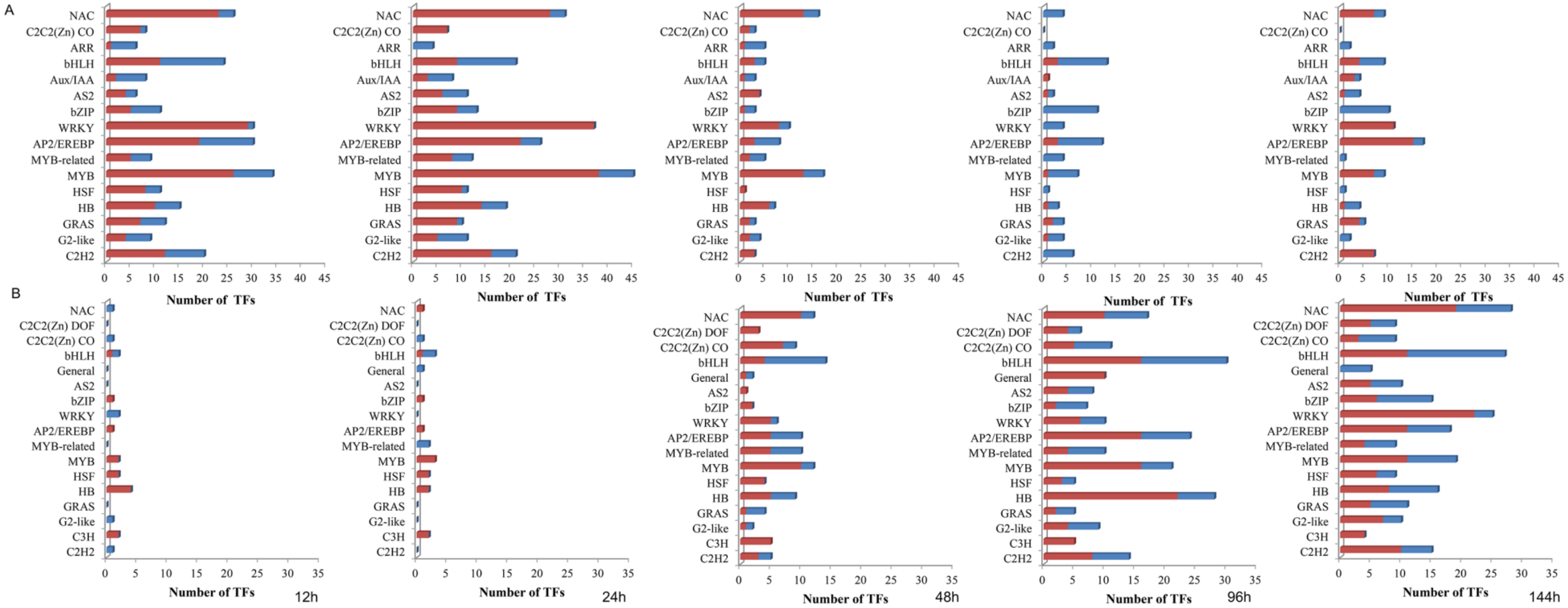

Figure S4. DEGs of transcription factors in response to salt stress. A, TFs of roots; B, TFs of leaves.

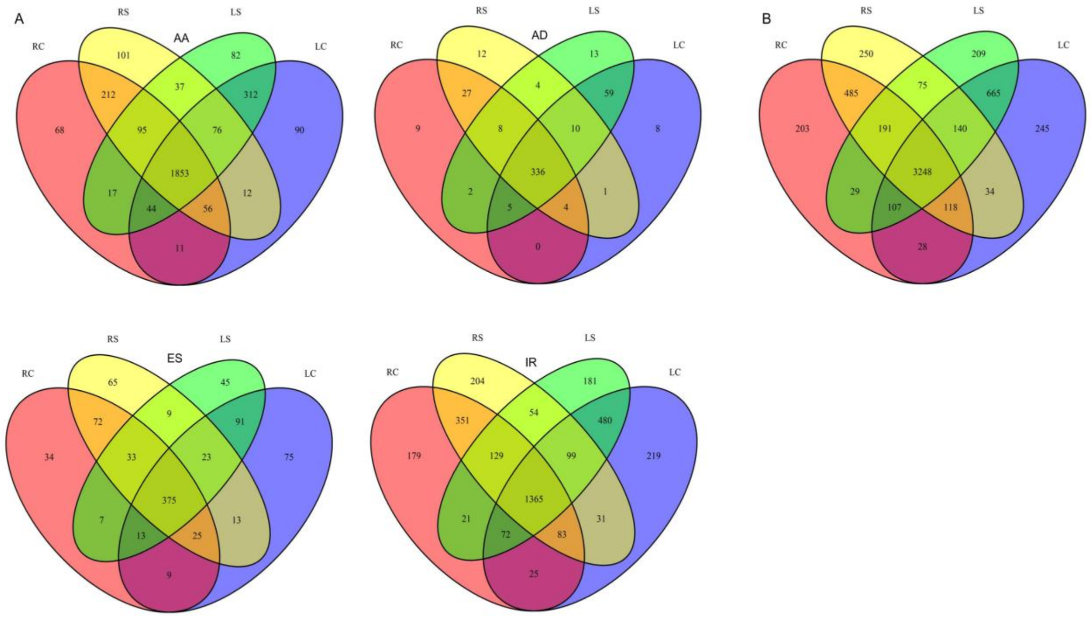

Figure S5. Novel alternative splicing in roots and leaves under salt stress conditions, detected by RNA-Seq.

A, Four main alternative splicing patterns and number of various events in roots and leaves under salt stress and normal conditions. B, Total alternative splicing events in roots and leaves, respectively.