

Supplementary Material

Comparative transcriptome analysis reveals unique genetic adaptations conferring salt tolerance in a xerohalophyte

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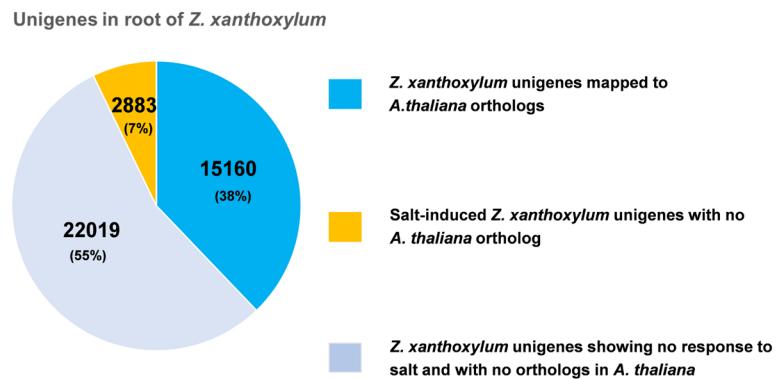


Fig. S1. The overview of the unigenes in root of *Z. xanthoxylum*.

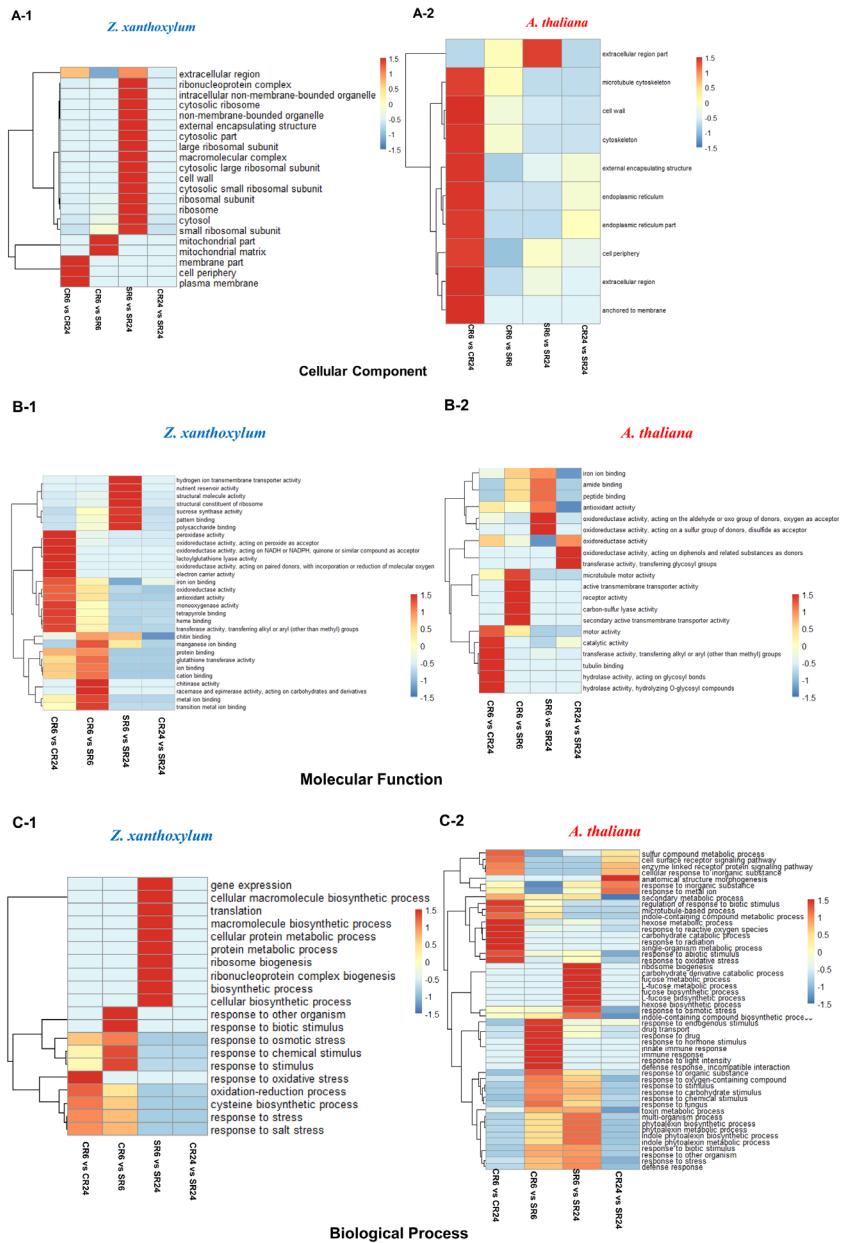


Fig. S2. Gene ontology (GO) classification of the DEGs from CR6 vs CR24, CR6 vs SR6, SR6 vs SR24, CR24 vs SR24 in *Z. xanthoxylum* (A-1, B-1, C-1) and *A. thaliana* (A-2, B-2, C-2). A-1, A-2 show DEGs classified by cellular component, B-1, B-2 show DEGs classified by molecular function, and C-1, C-2 show DEGs classified by biological process. To examine whether cluster size affected enrichment, cluster size was checked for correlation with $-\log_{10}(P\text{-value})$. Red and blue colors delineate the significantly enriched pathways ($P\text{-value} < 0.05$). C and S denote control treatment and salt treatment with 50 mM NaCl, respectively; 6 and 24 denote 6 h and 24 h of treatment, respectively; and R denotes roots.

Table S1. Genes and primers used for the real-time qPCR validation

Gene ID	Forward primer (5'→3')	Reverse primer (5'→3')
<i>Z. xanthoxylum</i>		
(30 unigenes)		
<i>CL4246.Contig5_All</i>	TCGTGGTAGAAGTGCACCAAG	GAGTTGGAGGCGCAGTAGA
<i>Unigene17402_All</i>	GTTTGTCCCTAGAGGGCGTG	CTCCGACCGACAAACAAAG
<i>Unigene22003_All</i>	ACATTATCCCAGCTCGCCTC	GGACAAAGCCTGCATGAAC
<i>Unigene22615_All</i>	CGACGGGTGAAATCTCGTTC	CTTGTCTTGCACACTCGCT
<i>Unigene23616_All</i>	TCGTGGTAGAAGTGCACCAAG	GAGTTGGAGGCCAGTAGA
<i>Unigene61098_All</i>	AGTGGTGGGCAAGGTTGAG	GTCCGATTAGTGGGACGATG
<i>CL8091.Contig1_All</i>	GGACCCCGTGGTTACATTCT	CTGCTCCGATCTTCTCCTC
<i>Unigene10187_All</i>	CACCATGGCTATGCGAACTC	GAGGGAGCAACCCTCAAAC
<i>Unigene5528_All</i>	TACGGTCCAACCCCTGAAC	TGCTCTGCAAGGACCTAAG
<i>CL5577.Contig2_All</i>	CATAGCTCCAACCCCGAC	TCAAAGCAGCCGAATTGAC
<i>Unigene5077_All</i>	CTCAGGTGCATCGATCTGGT	GATGCCATCTCGAAACCAAC
<i>Unigene19871_All</i>	CGCTAACCGTCACGCAGTC	GCGCGAAGATAGGAGAAG
<i>Unigene16368_All</i>	TTGCCTCCTGGATTCGTTTC	AGGCAAAGGTTGGAAAATGC
<i>CL2554.Contig3_All</i>	GTCTGAACCATGGGACTTGC	GCCCTATTGTCCTCATTCCAG
<i>CL6534.Contig1_All</i>	ATGACCTTGGGAACTTCCTG	AGTAGTAACTCGGTTGGTCG
<i>Unigene20426_All</i>	TCTGGCTTGGAACCTTGAC	GTTAGCACCCATGAGCTTGAG
<i>Unigene57160_All</i>	CGGCATGTTCTATGTTGC	TCGGCTCTTCCATATCTGT
<i>Unigene14772_All</i>	CCCAGCCAGGAAACAAGTAG	TGGTGGTGCTTGTGTTGG
<i>Unigene9789_All</i>	AGCCTCTTGTGTTCTGCT	TGACAACGGCTGGAAGATGA
<i>CL9880.Contig2_All</i>	TGCAGTAGCTAAAAGGGTG	TTGGCTTAGCATGGTTGTG
<i>Unigene22440_All</i>	CTACAAAGCCGACTACGC	CCCACGGTGTCTCCAATAAC
<i>Unigene11542_All</i>	GGAGCCATCACCCTCTTAGC	CAACAGCCACACGAAAGTC
<i>Unigene9141_All</i>	GGCACAAAGTTCCATTCA	CGATAACGCAGTAGTTGAGCAC
<i>Unigene474_All</i>	TGTTGCTGGAGTTAAGTGG	CACCTCGTAAGTTGATGAAG
<i>CL2295.Contig1_All</i>	ACGGAAGCAATGCAGACAG	CTCGCATTCCACCAAGTAGTG
<i>Unigene34184_All</i>	TCAATGGTTGTGATGGCTCAG	CATCTGATTGCTCCTGTTG
<i>Unigene6677_All</i>	GGCTTCACTGAGTCCGTTG	TGTATGGCACCAAGATAGTGAG
<i>Unigene20998_All</i>	TGAGATGGTGGGAGAAGAGTG	CCTCCACAAGCAGGTGAGTAG
<i>Unigene19759_All</i>	AATCTGATTCACCTGCTGTCC	TCTTCTTCTCGCCATTCTG
<i>Unigene59673_All</i>	AAGATTGTTGGGTGTCCCTGTAG	TTTCCCTGCTTGCTGTTGTTG
<i>EU019550.1 (ZxACTIN)</i>	TTTCCAGCCATCCCTTGTT	TGCAGTGATCTCCTGCTCATAC
<i>A. thaliana</i>		

(16 genes)

<i>AT4G29340</i>	CTTGGCACCCACGGGATTAT	TATGCCTCCTGCTCCCTTCT
<i>AT1G08900</i>	TCATCCTTGGCATTGGCCT	TACCGGCGGTGATTTACA
<i>AT5G48175</i>	TCTTGGGCTGTTCTGGTC	TCGTTATTGCATCTCCGGCT
<i>AT3G52480</i>	CCAACTGCAACCCTACTCGT	GATACTCCGACGACGGAAACC
<i>AT1G66800</i>	ACATCGTCCACTGCTGCTAC	TGACTATGTCGTACAAAGAACAA
<i>AT4G35190</i>	GAAGAAAGTGCAACAGCCGC	AGGGCAATCTCAGTCTGCAT
<i>AT5G56080</i>	ATCATCCGTCTGACGACGTG	CTGCCTCGAGCTCCATTGA
<i>AT5G21960</i>	CAACAGCCGTGACAGGATCT	GGGGCCACGAAGACAGTAAA
<i>AT4G23210</i>	GACGGCAAGGATCTTGGGA	ATTCTGGGCCATGTAACCG
<i>AT3G26200</i>	TTCACTGACTTCTTCCCCGC	AAAGGCTTGTGCAACTCCG
<i>AT1G24145</i>	GGACTTGGTCACTGATCGCA	AGGCAACATATGTCACGCCA
<i>AT2G21210</i>	TGCAGAAGAGGGAGATTCTGG	AGTGCAGGGAATAGTGAGGC
<i>AT2G17040</i>	TGGAGCAAAGGGCAGAGATG	TACTTGAGGAGCACGGAACG
<i>AT5G01740</i>	AAGGCGACACTTCTACGGTG	GCTTGCTCAAACCTGAACG
<i>AT4G19690</i>	TTCACTCGGTGGTCATTGGA	CAGCCTGGAGGATACAACCG
<i>AT1G01190</i>	GAGGCTCTCGTGTGAGATGG	AACATCCATGCACTGACAGTTT
<i>AT3G18780 (AtACTIN2)</i>	GCACCGCCAGAGAGAAAATAC	CACCACCACGAACCAGATAAGA

Table S2. Summary of RNA-Seq reads from *Z. xanthoxylum* and *A. thaliana*

Samples	Total Clean Reads	Mapped to Genome	Uniquely Mapped	Unknown Reads
		(%)	to a single gene (%)	(%)
ZxC6R	7312773	/	45.30	44.48
ZxC24R	7744887	/	62.87	23.55
ZxS6R	7886181	/	64.67	22.09
ZxS24R	8036394	/	63.57	23.19
AtC6R	11824288	82.98	93.73	/
AtC24R	12174231	80.03	90.27	/
AtS6R	11809609	82.43	92.93	/
AtS24R	12172394	82.92	94.33	/

Z. xanthoxylum has no reference genome.

“/” denotes no data.

Table S3. Differentially expressed genes involved in ion transport

Putative ortholog	Description	<i>Z. xanthoxylum</i> gene ID	<i>A. thaliana</i> gene ID	Fold change			
				6 h	24 h	<i>Z. xanthoxylum</i>	<i>A. thaliana</i>
Na⁺							
<i>NHX4</i>	Sodium hydrogen exchanger 4	Unigene3545_All	AT3G06370	-1.2	-0.2	0.3	1.4
<i>SOS1</i>	Salt overly sensitive 1	Unigene9810_All	AT2G01980	7.8	2.1	0.3	0.9
<i>NCX4</i>	Cation calcium exchanger 4	CL3586.Contig1_All	AT1G54115	7.6	0.2	-0.6	0.3
<i>CAX 11</i>	Cation exchanger 11	Unigene11542_All	AT1G08960	2.6	0.1	1.1	1.5
<i>HKT1;1</i>	Sodium transporter	Unigene11474_All	AT4G10310	3.2	1.2	-0.8	1.1
K⁺							
<i>KUP5</i>	K ⁺ uptake permease 5	Unigene18548_All	AT4G33530	1.3	-0.4	-0.7	0.2
<i>KUP6</i>	K ⁺ uptake permease 6	CL6543.Contig3_All	AT1G70300	1.4	-0.1	-0.4	0.0
<i>KUP7</i>	K ⁺ uptake permease 7	CL11345.Contig1_All	AT5G09400	7.5	-0.1	5.6	0.2
<i>KOC6</i>	Ca ²⁺ activated outward rectifying K ⁺ channel 6	Unigene7773_All	AT4G18160	-4.3	0.3	1.5	0.9
Na⁺, Ca²⁺							
<i>CNGC1</i>	Cyclic nucleotide gated channel 1	Unigene24769_All	AT5G53130	-7.8	0.7	0.9	-0.3
<i>CNGC9</i>	Cyclic nucleotide gated channel 9	CL3101.Contig1_All	AT4G30560	1.2	0.1	-0.4	0.9
<i>CNGC10</i>	Cyclic nucleotide gated channel 10	CL7772.Contig1_All	AT1G01340	1.5	0.4	1.7	-0.6
<i>CNGC17</i>	Cyclic nucleotide gated channel 17	Unigene14905_All	AT4G30360	2.0	0.3	0.7	-0.1
NO₃⁻							
<i>NRT1.2</i>	Nitrate transporter1.2	Unigene6678_All	AT1G69850	2.6	-0.1	0.9	-0.2
<i>NRT1.5</i>	Nitrate transporter1.5	CL10526.Contig1_All	AT1G32450	8.6	-0.7	0.5	1.7
<i>NRT1.8</i>	Nitrate transporter1.8	CL10526.Contig2_All	AT4G21680	2.3	3.4	-1.8	1.1
NH₄⁺							

<i>AMTI;1</i>	Ammonium transporter 1;1	CL9675.Contig2_All	AT4G13510	1.9	0.5	-0.3	-0.2
PO₄³⁻							
<i>PHT1;1</i>	Phosphate transporter 1;1	Unigene33655_All	AT5G43350	5.6	0.4	-0.5	0.7
<i>PHT1;2</i>	Phosphate transporter 1;2	Unigene7644_All	AT5G43370	10.7	1.2	0.7	1.7
<i>PHT1;3</i>	Phosphate transporter 1;3	CL1807.Contig4_All	AT5G43360	3.4	-7.9	0.7	/
Mg²⁺							
<i>MGT2</i>	Magnesium transporter2	CL13362.Contig1_All	AT1G16010	1.1	0.4	0.6	0.8
Zn²⁺, Fe²⁺, Mn²⁺							
<i>ZIP1</i>	Zinc transporter 1	CL6755.Contig2_All	AT3G12750	3.9	0.4	-2.1	0.8
<i>ZIP4</i>	Zinc transporter 2	CL11057.Contig1_All	AT1G10970	2.6	0.4	-0.5	-0.1
<i>ZIP6</i>	Zinc transporter 6	CL11327.Contig1_All	AT2G30080	4.1	-0.2	0.1	-0.4
<i>ZIP8</i>	Zinc transporter 8	Unigene9141_All	AT5G45105	3.7	0.7	-1.3	1.5
Fe							
<i>FRO2</i>	Ferric reduction oxidase 2	Unigene16933_All	AT1G01580	8.4	/	/	/
Cu²⁺							
<i>COPT1</i>	Copper transporter 1	Unigene13736_All	AT5G59030	2.8	/	0.1	/
<i>COPT5</i>	Copper transporter 5	Unigene21186_All	AT5G20650	1.5	0.2	1.1	0.2
BO³⁻							
<i>BOR2</i>	Borate transport 2	CL13290.Contig2_All	AT3G62270	2.0	-0.6	0.7	0.8
SO₄²⁻							
<i>SULTR1;3</i>	Sulfate transporter 1;3	CL7856.Contig1_All	AT1G22150	2.9	-5.0	-2.1	-0.5
<i>SULTR2;2</i>	Sulfate transporter 2;2	Unigene17610_All	AT1G77990	1.0	-2.5	-1.0	0.9
<i>SULTR4;2</i>	Sulfate transporter 4;2	CL12276.Contig2_All	AT3G12520	1.3	1.1	-0.5	0.0
Cl⁻							
<i>CLC-C</i>	Chloride channel C	Unigene8212_All	AT5G49890	-7.9	-0.2	-3.1	1.1
V-H⁺-ATPase							

<i>AVA-P1</i>	Vacuolar H ⁺ -pumping ATPase 1	CL5557.Contig4_All	AT4G34720	3.5	0.1	0.1	0.3
<i>AVA-P3</i>	Vacuolar H ⁺ -pumping ATPase 3	CL5557.Contig6_All	AT4G38920	1.1	-0.1	0.2	0.4
<i>AVA-P4</i>	Vacuolar H ⁺ -pumping ATPase 4	Unigene40214_All	AT1G75630	8.3	-0.4	-0.1	0.0
PM-H⁺-ATPases							
<i>AHA2</i>	Plasma membrane proton ATPase 2	CL12845.Contig4_All	AT4G30190	10.5	-0.7	-1.3	0.7

“/” indicates not found

Table S4. Differentially expressed genes involved in ROS scavenging

Putative ortholog	Description	<i>Z. xanthoxylum</i> gene ID	<i>A. thaliana</i> gene ID	Fold change			
				6 h		24 h	
		<i>Z. xanthoxylum</i>	<i>A. thaliana</i>	<i>Z. xanthoxylum</i>	<i>A. thaliana</i>	<i>Z. xanthoxylum</i>	<i>A. thaliana</i>
GRX							
<i>GRXC2</i>	Glutaredoxin family	CL7620.Contig1_All	AT5G40370	1.6	-0.4	0.0	0.0
<i>GRXC5</i>	Glutaredoxin family	CL11701.Contig4_All	AT4G28730	2.0	1.0	0.4	0.3
APX							
<i>LAC5</i>	Laccase family	CL11251.Contig1_All	AT2G40370	2.5	-0.3	0.0	0.2
<i>LAC10</i>	Laccase family	Unigene12459_All	AT5G01190	1.7	-1.2	0.7	1.4
<i>LAC17</i>	Laccase family	CL1617.Contig1_All	AT5G60020	8.4	-0.2	0.3	1.5
<i>SAPX</i>	Stromal ascorbate peroxidase	Unigene15139_All	AT4G08390	3.6	-0.1	0.0	0.9
<i>SKS6</i>	Oxidoreductase activity	Unigene4123_All	AT1G41830	2.1	-0.7	0.2	1.4
<i>SKS15</i>	Oxidoreductase activity	CL9831.Contig1_All	AT4G37160	1.6	0.3	0.0	3.3
MDAR							
<i>NAD(P)-linked oxidoreductase</i>	NAD(P)-linked oxidoreductase	CL9269.Contig2_All	AT1G60690	4.3	0.3	0.6	1.5
DHAR							
<i>DHAR2</i>	Dehydroascorbatedehydrogenase	Unigene19705_All	AT1G75270	2.7	0.1	0.0	0.5
<i>G6PD5</i>	Glucose-6-phosphate dehydrogenase	Unigene966_All	AT3G27300	-1.3	0.0	0.6	1.1
ALDH							
<i>ALDH3</i>	Aldehyde dehydrogenase	CL7443.Contig1_All	AT4G34240	2.9	0.5	0.3	0.2
<i>ALDH7B4</i>	Aldehyde dehydrogenase	Unigene474_All	AT1G54100	2.1	0.7	0.0	-1.0
GSH							
<i>GSH2</i>	Glutathione synthetase2	Unigene5627_All	AT5G27380	2.0	0.2	0.0	0.1

GST							
<i>GSTU1</i>	The tau class of glutathione s-transferase	CL7539.Contig3_All	AT2G29450	4.1	1.0	0.9	-0.1
<i>GSTU7</i>	The tau class of glutathione s-transferase	CL11683.Contig1_All	AT2G29420	4.0	0.7	0.0	-1.0
<i>GSTU10</i>	The tau class of glutathione s-transferase	Unigene60082_All	AT1G74590	4.1	2.7	0.0	-0.4
<i>GSTU19</i>	The tau class of glutathione s-transferase	CL5645.Contig2_All	AT1G78380	4.3	0.0	0.0	-1.0
<i>GSTU22</i>	The tau class of glutathione s-transferase	Unigene7560_All	AT1G78340	4.0	2.7	0.0	-0.5
<i>GSTU25</i>	The tau class of glutathione s-transferase	Unigene1141_All	AT1G17180	8.8	2.9	0.0	-1.6
<i>GSTI6</i>	Glutathione s-transferase	CL8381.Contig2_All	AT2G02930	1.6	2.7	0.0	-0.2
<i>GST</i>	Glutathione s-transferase	CL10064.Contig2_All	AT1G77290	1.3	/	0.5	/
SOD							
<i>SOD1</i>	Manganese superoxide dismutase	Unigene67157_All	AT3G10920	7.7	0.0	/	0.4
CAT							
<i>CAT1</i>	Catalyzes the reduction of hydrogen peroxide	CL8063.Contig1_All	AT1G20630	4.2	0.3	0.0	-0.1
Peroxidase superfamily							
<i>POD</i>	Peroxidase superfamily protein	Unigene16399_All	AT1G71695	2.1	-0.5	0.1	-1.6
<i>POD</i>	Peroxidase superfamily protein	CL7046.Contig3_All	AT2G39040	8.9	-0.8	/	9.9
<i>POD</i>	Peroxidase superfamily protein	CL9262.Contig2_All	AT5G42180	8.1	-0.5	0.3	1.3
<i>POD</i>	Peroxidase superfamily protein	CL1944.Contig2_All	AT5G66390	8.1	0.7	0.1	0.6
<i>POD</i>	Peroxidase superfamily protein	CL7488.Contig2_All	AT4G11290	7.6	0.4	0.4	0.4
<i>POD</i>	Peroxidase superfamily protein	CL12271.Contig2_All	AT2G43480	3.5	-1.1	0.0	2.3
<i>POD</i>	Peroxidase superfamily protein	Unigene6677_All	AT5G06730	3.4	1.3	0.0	0.5
<i>POD</i>	Peroxidase superfamily protein	CL10576.Contig1_All	AT5G64100	2.5	-0.3	0.0	-1.5
<i>POD</i>	Peroxidase superfamily protein	Unigene59673_All	AT1G44970	2.5	-1.6	0.0	0.8

“/” indicates not found

Table S5. Differentially expressed genes involved in ABA and GA biosynthesis and response to auxin

Putative ortholog	Description	<i>Z. xanthoxylum</i> gene ID	<i>A. thaliana</i> gene ID	Fold change			
				6 h	<i>Z. xanthoxylum</i>	<i>A. thaliana</i>	24 h
ABA							
<i>ABA1</i>	Biosynthesis of abscisic acid	CL3945.Contig1_All	AT5G67030	0.9	0.6	-0.6	-0.2
<i>NCED3</i>	Biosynthesis of abscisic acid	CL86.Contig2_All	AT3G14440	-6.1	4.6	7.1	0.7
<i>ABA2</i>	The conversion of xanthoxin to ABA-aldehyde during ABA biosynthesis	CL6088.Contig2_All	AT1G52340	4.3	-0.2	-2.1	-0.4
<i>ABF2</i>	Abscisic acid responsive elements-binding factor	CL1445.Contig3_All	AT1G45249	2.3	0.0	-0.1	0.3
GA							
<i>GA2</i>	gibberellin biosynthetic	Unigene15467_All	AT1G79460	5.3	-0.3	/	0.6
<i>GA3</i>	gibberellin biosynthetic	Unigene13918_All	AT5G25900	4.6	0.3	6.5	1.2
<i>GA2ox2</i>	Negative regulated gibberellin biosynthetic process,	CL10145.Contig3_All	AT1G30040	-6.1	1.0	-0.1	0.1
AUXIN/IAA							
<i>ILR1</i>	IAA-leucine resistant	CL2976.Contig1_All	AT3G02875	4.6	0.6	/	0.0
<i>ILL3</i>	IAA-leucine resistant	Unigene22539_All	AT5G54140	2.4	0.0	-1.0	0.2
<i>AFB2</i>	Auxin signaling F-box 2	Unigene2651_All	AT3G26810	2.1	0.0	0.4	0.4
<i>ARF7</i>	Auxin response factor	CL163.Contig6_All	AT5G20730	4.6	0.2	4.6	-0.3
<i>TIR1</i>	mediates auxin-regulated transcription	CL1890.Contig1_All	AT3G62980	4.6	0.1	-0.1	-0.3
<i>SAUR16</i>	Small auxin upregulated RNA	Unigene21036_All	AT4G38860	6.5	0.1	7.6	/
<i>SAUR31</i>	Small auxin upregulated RNA	CL124.Contig1_All	AT4G00880	1.5	0.9	-1.0	0.5
<i>SAUR32</i>	Small auxin upregulated RNA	Unigene21000_All	AT2G46690	6.8	-0.7	1.5	1.6
<i>SAUR35</i>	Small auxin upregulated RNA	Unigene22027_All	AT4G12410	6.0	/	-5.3	0.9
<i>SAUR59</i>	Small auxin upregulated RNA	Unigene19731_All	AT3G60690	2.5	0.4	-0.3	0.0

<i>SAUR77</i>	Small auxin upregulated RNA	Unigene16411_All	AT1G17345	3.5	0.1	1.4	1.5
<i>Auxin-responsive</i>	Auxin-responsive	Unigene888_All	AT2G04850	7.1	-0.4	1.4	0.6
<i>Auxin-responsive</i>	Auxin-responsive	CL13269.Contig1_All	AT5G47530	1.7	-0.2	-1.0	1.9
<i>GH3.11</i>	indole-3-acetic acid-amidosynthetase	CL3620.Contig2_All	AT2G46370	6.8	0.7	-0.7	0.7
<i>GH3.17</i>	indole-3-acetic acid-amidosynthetase	Unigene2674_All	AT1G28130	1.7	0.1	-0.7	1.2
<i>GH3.5</i>	indole-3-acetic acid-amidosynthetase	Unigene62182_All	AT4G27260	6.8	-0.7	-0.1	-0.6

“/” indicates not found