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Full Length Research Paper

Assessment of microarray analysis of genes affected by salt stress in tomato

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Large-scale gene expression affected by salt stress was analyzed with tomato seedlings (*Lycoperson esculentum* Mill cv. Money Maker) by a cDNA microarray (Tom1). The significantly differentially expressed genes (5% Benjamini-Hochberg false discovery rate) consisted of 1757 sequences in the analyzed tissues (cotyledons + shoot tip). Genes with over 2 fold difference were selected from the list and further categorized into different function and cellular processes. Tomato homologous genes for the chaperone proteins, antioxidant enzymes (catalase and peroxidase), and ion transporters (Na^+-driven multidrug efflux pump, vacuolar ATPase, and others) were induced. The ACC oxidase and ethylene-responsive gene tomato homologs had higher transcript level after salt treatment. Multiple members with different expression patterns were identified for the bZIP, WRKY, and MADS-box transcription regulator. Different genes in the signal transduction pathway, such as the protein kinases (Shaggy kinase, mitogen-activated protein kinase, ethylene receptor neverripe, and others), protein phosphatases, calmodulin, G -protein, and the N - myristoyltransferase were regulated by salt stress. Most of the protease and the inhibitor homologs were suppressed by salt stress. In addition, different isoforms of cytochrome P450, genes for polyamine biosynthesis (putrescine and proline) and detoxification compounds (glutathione and thioredoxin), several key enzyme genes in the metabolic pathways of carbohydrates, amino acids, and fatty acids, were also affected by salt treatment. This study has provided a set of candidate genes, especially those in the regulatory machinery that can be further investigated to define salt stress in tomato and other plant species.

Key words: Antioxidants, cellular metabolism, cell wall, chaperonine, ethylene, protein kinase, tomato, transcription regulator, translation regulator, salt stress.

INTRODUCTION

Salinity is a major problem that plagues more than 35% of the world's arable lands. Extensive research has been conducted to investigate the molecular control for this stress factor and the potential to improve the salt tolerance of important agricultural crops. Mechanism of salt-induced growth inhibition consists of osmotic and ionic stress, as well as the resultant nutrient deficiency.

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Na^+ transport mechanism has been identified to control the salt tolerance in the halophytic species *Thellungiella salsuginea* (Pall.) Schulz (Vera-Estrella et al., 2005). Transgenic tomato (*Lycopersicon esculentum* Mill) plants expressing a cation transport gene *HAL1* and a vacuolar Na^+/H^+ antiporter gene *AtNHX1* exhibited higher capacity for salt exclusion, and increased significantly their salt tolerance (Gisbert et al., 2000; Zhang and Blumwald, 2001). Expression of genes involved in antioxidant and detoxification mechanism (Avsian-Kretchmer et al., 2004; and the references therein), intracellular vesicle traffic-

king system and ion transport (Mazel et al., 2003;and the references therein; Vera-Estrella et al., 2005), accumulation of compatible solutes and polyamines (Ferjani et al., 2003; Pommerrenig et al., 2007), and others, are correlated with salt stress and tolerance/ sensitivity in different plant species. Different salt signal transduction mechanisms have been identified as the Salt Over Sensitive (SOS) pathway, the Calcium Dependent Protein Kinase (CDPK) pathway, the Mitogen Activated Protein (MAP) kinase pathway, and interconnection of different pathways (Ouyang et al., 2007, and references therein) in *Arabidopsis thaliana* (L.) Heynh. Plant hormones, including abscisic acid (ABA) and ethylene, play import-ant roles in mediating different processes at both molecular and cellular levels during stress period (Borsani et al., 2001; Tanaka et al., 2005).

Genetic studies on salt tolerance in tomato dates back to the 1940's, when a different physiological mechanism was observed between the salt sensitive tomato (*L. esculentum* Mill.) and the tolerant wild relatives (Lyon, 1941; Flowers, 2004). Molecular studies have since been conducted on QTL mapping of salt tolerance (Foolad et al., 1999), and cloning salt responsive genes from salt tolerant and sensitive cultivars (Ouyang et al., 2007).

Like many salt sensitive crop species, performance of tomato plant under salt stress is modulated by many physiological and agronomical characteristics through regulation of a complex genetic mechanism (Foolad, 2004). Identification of the regulatory pattern in multiple genes on whole genome scale, or the most available gene sequences, will facilitate the dissection of the inherent molecular control, especially for the multigenic traits such as salt stress. Here we report a microarray (Tom1) analysis of salt affected genes in a tomato cultivar (*L. esculentum* Mill cv. Money Maker) using the available analysis platform of tomato gene chips and sequence annotation. The salt-regulated genes in respective cellular processes were corroborated with published research, and database information.

METHODS AND MATERIALS

Plant material and cultivation conditions

Tomato (*L. esculentum* Mill cv. Money Maker) seeds were surface sterilized in 50% bleach for 10 min. After three washes in sterile water, 10 seeds were inoculated into 250 ml of a medium containing Gamborg basic minerals, MS vitamins and 10 g/L sucrose, pH 5.0. The salt treatment was supplemented with NaCl at 0.4% (75 mM). The culture was incubated at 25^{O}C, 125 rpm shaking, for 17 d when the two cotyledons were fully open, but, no true leaves have emerged. The leaf tissue consisting cotyledons and shoot tips were collected and used for the procedures in this study.

Experimental design and microarray used

The microarry (Tom1) comprising 12,000 elements (http://bti.cornell.edu/CGEP/) were used in this study. Salt treatment and control were compared by cohybridizing differentially labeled mRNA from the two samples on a set of slides. The experiment included three biological replicates. A self-self hybridization was included to monitor the hybridization process. Each biological sample represented a pool of 20 seedlings from two flasks.

RNA extraction and removal of DNA

Total RNA extraction was gotten by using the RNA pure kit (Genhunter, USA). To remove genomic DNA, the total RNA was treated with RNase-free DNase (RQ1; Promega, USA) for 15 min at 37°C, extracted with phenol: chloroform (1:1) and chloroform, precipitated with ethanol, and finally re-suspended in diethyl pyrocarbonate-treated water. Quality and quantity of the RNA extraction was monitored on 2.0% denatured agarose gels and a ND-1000 nanodrop spectrometer (Nanodrop Technologies, USA). The DNA free RNA samples were used for further analysis.

Microarray hybridization, data acquisition and analysis

To prepare the fluorescent probes, total RNA was amplified using the Amino Allyl MessageAmp II aRNA Amplification Kit (Ambion, USA) following the manufacture's instructions. In brief, the first strand cDNA was synthesized from 1,000 ng of total RNA with ArrayScript and then subsequently converted into double stranded cDNA. The amino allyl aRNA was obtained by *in vitro* transcription and amino allyl UTP nucleotide. Five ug of this aRNA was used to couple with the fluorescent cyanine Dye Cy3-dCTP and Cy5-dCTP (Amersham Biosciences, USA). One hundred pmol of fluorescent aRNA probes were fragmented with the fragmentation buffer (Ambion) at 70^{O}C for 15 min, and denatured in a final concentration of 0.075% SDS, 2 X SSC, 5.6% Liquid Block (GE Healthcare, USA) at 65^{O}C for 5 min.

A 70 l of the probe preparation was loaded onto each slide. Hybridization was conducted at 43^{O}C dark for 16 h. After following the protocols at the microarray supplier's website (http://bti.cornell.edu/CGEP), images were obtained on a GenePix 4000B slide scanner and the data acquisition was gotten by using Genepix 6.0 software (Molecular Devices). Each image was examined manually; the bad spots were all flagged. After filtering out all the bad spots and those with signal intensity below 500, the raw data was loaded into GeneSpring (v.7) (Agilent Technologies, Inc., USA) using GenePix-format defaults and normalized with the Lowess curve. The genes were listed by first passing through a t-test with a multiple testing correction using 5% Benjamini-Hochberg false discovery rate, and subsequently according to their fold changes. Detailed information is included in MIAME (Accession number: GSE8883, Link, http://www.ncbi.nlm.nih.gov/geo/info/linking.html). The selected genes were subjected to function annotation using the TOM1 array GO term analysis (http://ted.bti.cornell.edu/cgi-bin/array/TOM1GO.cgi).

Quantitative real-time PCR (qRT-PCR)

Real-time qRT PCR assay was performed following the instruction in the SYBR- green PCR mix/RT kit (Applied Biosystems, USA). The unigenes for RT-PCR were picked randomly from the gene list with different fold change. A reference gene was selected from the gene list with p 1.0, and 1.0 normalized ratio between treatment and control. The DNA sequences of the selected clones were retrieved from the Array manufacture's website using the Array ID. qRT-PCR primers were designed using the Primer Designer 3 software (Applied Biosystems) and synthesized by the Invitrogen Company, USA. The primers for the selected genes and endogenous control (actin, Lemaire-Chamley et al., 2005) were listed in supplemental Table S1.

Supplemental Table S1. Primers used for qRT-PCR amplification.

Primers	Unigene	Array-ID	Ratioz(salt/ctr)	Primers
S2	U319872	1-1-2.1.20.8	0.12 (L)	f- GATGTGGGAGAATTGATTGGAGTAC r- TGTCCGTTGCAAAAGCCTTT
S7	U312983	1-1-7.3.16.19	ND (L)	f- TGTCCATAAAGAACACGGAGATAGC r- TGCATCCTCAGCAAGCATACTG
S10	U337885	1-1-7.4.4.7	0.31 (L)	f- CCCCGAATGCTTATCAAAACTAATA r- AAGGCCAACATCCATCTTTTTATAAAG
S13	U316401	1-1-1.4.2.4	3.40 (L)	f- CAAACATGTGATTGGATAAAGAAACG r- CTGACCAACCAAACTTTCCTGAT
S17	U319230	1-1-3.3.10.10	2.66 (L)	f- TTACCCGGAACTCGTGAATCTC r- CAATAGCGATGTCAGTGTTTTCGT
S18	U319230	1-1-3.3.10.10	2.66 (L)	f- ATTTTACCCGGAACTCGTGAATC r- TAGCGATGTCAGTGTTTTCGTGAT
S19	U319535	1-1-3.3.14.13	2.11 (L)	f-CGCCAGTTTACCATCAGCAA r-ACGTCGATGCCACTGATACAGA
S35	U320587	1-1-7.4.9.3	3.87 (L)	f- ATTACTGTCCTCGTCAGATTCCTACTT r- GGATACCATCACATCGGAGTAACC
actin	U60480			f- TGTCGGTTCAGGGAGCTTAC r- TCCAATTGCAGACCGATGTA

ND: no difference. *Actin* (U60480) was used as the endogenous control (Lemaire-Chamley et al, 2005).

cDNA was synthesized with the Taqman reverse transcriptase (Applied Biosystems), using RNA samples prepared following the same procedure as for microarray hybridization. Briefly, 400 ng of total RNA (genomic DNA free) was reverse transcribed into cDNA using a program of 25^{o}C for 10 min, 48^{o}C for1 h, and 95^{o}C for 5 min. These PCR products were diluted into 200 l of water, and 13 l of the cDNA (corresponding to 28 ng total RNA) were added into the PCR master mix of 30 l containing forward/reverse primers (250 nM each) and 2 X PCR master mix supplied in the SYBR-green PCR mix/RT kit. The PCR amplification was preformed using a program of 40 cycles of 94^{o}C for 30 s, and 60^{o}C for 1 min on a 7000 Real Time PCR System (Applied Biosystems). At the end of the PCR cycles, the data were analyzed with the ABI Prism 7000 SDS software and presented as Ct values. To check the specificity of annealing of the oligonucleotides, dissociation kinetics was performed by the machine at the end of the experiment. In addition, the PCR products were separated on 2.5% agarose gels to validate the specificity of the PCR reaction.

Each measurement had 4 replicates of the cDNA samples. The Ct value of each gene was normalized with the endogenous actin to obtain the value of Ct. Values of Ct relative to control was used to calculate relative gene expression following the procedure described in the Relative Quantification Using the Comparative Ct Method in the User Bulletin #2, ABI Prism 7700 Sequence Detection System (Applied Biosystems).

The fold changes were estimated based on the Relative Quantification (RQ) between salt treatment and control, which was calculated using the equation: $2^{-(CT)}$. The RQmax/min was calculated using the equation: $2^{-(CT \pm T \times VAB(CT\ (target\ gene,\ endogenous\ control))}$. The T value was 1.895, corresponding to one- tail t-test, at 95% confidence for 8 samples (4 treatment and 4 control). VAB (CT (target gene, endogenous control) was calculated using the equation:

$$VAB = \frac{\sqrt{SD(target\ gene)2 + SD\ (actin)2}}{\sqrt{8\ (total\ sample\ number)}}$$

RESULTS AND ANALYSIS

General features of the salt regulated gene expression profile

A total of 1757 genes were regulated, of these, 563 genes were down regulated (1.45 - 20 fold), and 1194 genes were up-regulated (1.24 - 6.72 fold). For the down-regulated genes, the fold difference was 297 unigenes at 1.24 -1.99 fold, 186 unigenes at 2- 2.99 fold, 40 unigenes

at 3 - 3.99 fold, and 37 unigenes at 4 fold and over. For the up-regulated genes, the fold difference was 806 unigenes at 1.24 - 1.99 fold, 301 unigenes at 2 - 2.99 fold, 52 unigenes at 3 - 3.99 fold, and 31 unigenes at 4 fold and over. Generally, majority of the salt-induced or suppressed genes changed below 3 fold, and less num-ber of these salt-affected genes showed changes at a magnitude of above 3 fold.

Function of the differentially expressed genes

To identify the cellular processes that are affected by salt stress as manifested by relative difference (>2 fold, and 5% Benjamini-Hochberg FDR) at transcript level, the selected genes were classified according to their annotated putative functions. The results are presented in Supplemental Table S2.

Chaperones, proteases, and protease inhibitors

Five gene homologs with chaperone function were induced by salt stress, which include the heat shock proteins (U217418, U218323), the heat shock protein binding / unfolded protein binding (U216392), and the DnaJ-like protein (U217905) which plays key role in defense mechanism.

For the enzyme inhibitor genes, only the cystatin, a cysteine proteinase inhibitor gene (U213642), and Ser / Thr specific protein phosphatase 2A B (U214209) were induced in the salt treated leaf. The cyclin-dependent kinase inhibitor (U217044), a negative inhibitor for cell cycle progression, was repressed. The protease inhibitor II (U212877) and a Kunitz trypsin inhibitor homolog (U214150) were also repressed by salt treatment.

Several different proteases were repressed by salt stress, which include the wound-inducible carboxypep-tidase (U214490), patatin-like protein (U217988), and several endopeptidases (U219177, U221963, U216625). The salt-induced proteases include the nClpP4 (U215-977), proteasome-like protein (U215458, U214388), and the subtilisin-like protease (U212604).

Transcription and translation factors

Five genes belonging to translation factors were identified in leaf, including the down regulated (translation initiation factor IF-3 U215731, 5A- 3 U214373) and up-regulated (elongation factor EF-G U219928, novel cap-binding protein nCBP U216381, initiation factor iso4E U217855).

For the transcription regulators, different distinct genes and different isoforms were affected by salt treatment. The MADS-box protein 15 (U215264) was suppressed, but the FBP9, and 5 homologs (U217082, U213167) were induced. Both belonging to MADS-box protein genes, TDR6 was reduced, but TDR4 was induced. The salt- repressed genes include the myb-related transcriptt-ion factors (U217873, U231251); the WRKY transcription factor 30 (U214107) and two bZIP transcription factors (U221674, U214345). The salt induced transcription factor genes include WRKY homolog (U215688, U221423), and a DNA binding / transcription factor (U220740).

Protein kinase and phosphatases, calmodulin and GTPases

The adenylate kinase gene homolog (U214214) was repressed in the salt treated tissues. Another fourteen tomato kinase homologous genes were also found differentially regulated by salt treatment. The down regulated kinase genes include cyclin-dependent protein kinase p34cdc2 (U215180), a positive regulator in cell progression; two catalytic enzymes in carbohydrate metabolism, (U215972, U216479) . The up-regulated genes include the pathogen elicited kinases (U221780, U225888), and members functional in signal transduction pathways including the SHAGGY-like kinase (U213944), and mutagens-activated protein kinase 3 (U215877).

All the identified protein phosphatase genes were repressed by salt stress, which include the protein serine/ (U233076). In addition to the protein phosphorylation, N-Myristoylation is another major mechanism for protein post-translational modification associated with salt stress (Ishitani et al., 2000). The catalytic NMT1 (N-myristoyltransferase 1) homolog (U215619) was induced.

Calmodulin and GTP-binding proteins are signal molecules triggered by abiotic and biotic stresses. Three calcium binding protein genes (U215468, U218586, U212856) were repressed in leaf. Two Rab genes, the Rab11b and Rab11a (U223327, U216446) were repressed, and Rab2 (U215155) was induced by salt treatment.

Transporters

The cyclic nucleotide-gated calmodulin-binding ion channel can also function as the inward rectifier potassium channel, the tomato homologous gene (U216058) was repressed by salt stress. Genes induced included the membrane bound ion/ protein transporters such as vocuolar H+-ATPase (U212892, U216066) and VP45 (U220-750), calcium ATPase (U216601, U216736), a Na+-driven multidrug efflux pump (U218623), a putative inward rectifying potassium channel (U217439), and a mitochondrial half-ABC transporter (U229989). In contrast, the ABC transporter permease protein (U224841), and the membrane spanning protein 70 in the TM4 family (U223245) were repressed.

Oxidative stress protection and detoxification genes

Tremendous evidences have proven that salt can induce oxidative stress (del Río et al., 2006), and the resultant

Supplemental Table S2. Functional annotation (FDR p<1.0) of salt-regulated genes in tomato Seedlings (2 fold difference at 5% Benjamini-Hochberg false discovery rate).

Unigene ID	Annotation	Leaf
U218323	AAB42159 Hsc70	up
U216392	NP_195626 heat shock protein binding / unfolded protein binding [*Arabidopsis thaliana*]	up
U217418	P51819 Heat shock protein 83	up
U217905	AAB36543 DnaJ-like protein [*Phaseolus vulgaris*]	up
U215229	NP_187434 ATP binding / unfolded protein binding [*Arabidopsis thaliana*]	up
U217044	CAD56868 cyclin-dependent kinase inhibitor [*Nicotiana tabacum*]	dn
U212877	CAA64416 proteinase inhibitor II [*Lycopersicon esculentum*]	dn
U214150	AAC63057 Lemir [*Lycopersicon esculentum*]	dn
U214209	AAG29596 Ser/Thr specific protein phosphatase 2A B regulatory subunit beta isoform [*Medicago sativa* subsp. x varia]	up
U213642	AAF23126 cystatin [*Lycopersicon esculentum*]	up
U217988	AAF98368 patatin-like protein 1 [*Nicotiana tabacum*]	dn
U216427	NP_175778 RPT1A; ATPase [*Arabidopsis thaliana*]	dn
U221963	CAA05894 CYP1, cysteine protease [*Lycopersicon esculentum*],	dn
U219177	NP_192795 cysteine-type endopeptidase/ ubiquitin thiolesterase [*Arabidopsis thaliana*]	dn
U214490	AAF44708 wound-inducible carboxypeptidase [*Lycopersicon esculentum*]	dn
U216625	NP_849967 aspartic-type endopeptidase/ pepsin A [*Arabidopsis thaliana*]	dn
U215977	BAA82068 nClpP4 [*Arabidopsis thaliana*]	up
U215458	ABB16998 proteasome-like protein alpha subunit [*Solanum tuberosum*]	up
U214388	ABB16990 proteasome-like protein alpha subunit [*Solanum tuberosum*]	up
U212604	CAA07001 subtilisin-like protease [*Lycopersicon esculentum*]	up
U215731	NP_567851 translation initiation factor IF-3 [*Arabidopsis thaliana*]	dn
U214373	AAG53649 eukaryotic translation initiation factor 5A-3 [Lycopersicon esculentum]	dn
U216381	AAC17220 novel cap-binding protein nCBP [*Arabidopsis thaliana*], eIF4e or eIF(iso)4E	up
U217855	AAU06579 eukaryotic initiation factor iso4E [*Nicotiana tabacum*]	up
U215264	AAQ72500 MADS-box protein 15 [Petunia x hybrida]	dn
U217873	CAA67600 myb-related transcription factor [*Lycopersicon esculentum*]	dn
U214107	AAR92477 putative WRKY transcription factor 30 [*Vitis aestivalis*]	dn
U221674	CAB57979 THY5 protein [*Lycopersicon esculentum*], a bZIP transcription factor	dn
U214345	AAL27150 bZIP transcription factor [*Nicotiana tabacum*]	dn
U231251	BAD62071 putative golden2-like transcription factor [*Oryza sativa* (japonica cultivar-group)], Myb_DNA-binding	dn
U216815	AAT40488 putative DNA-binding protein [*Solanum demissum*]	dn
U217991	NP_201280 DNA binding / transcription factor [*Arabidopsis thaliana*]	dn
U221679	AAX11684 perakine reductase [*Rauvolfia serpentina*]	up
U217507	NP_194105 ATP binding / kinase [*Arabidopsis thaliana*]	up
U231465	NP_568444 ATP binding / kinase [*Arabidopsis thaliana*]	up
U225888	AAP03880 Avr9/Cf-9 induced kinase 1 [*Nicotiana tabacum*]	up
U220541	AAC23542 receptor protein kinase [*Ipomoea trifida*]	up
U228386	AAK60493 putative protein kinase LESK1 [*Lycopersicon esculentum*]	up
U218650	AAP45176 putative receptor protein kinase [Solanum bulbocastanum]	up
U224043	NP_194459 ATP binding / protein kinase [*Arabidopsis thaliana*]	up
U213944	CAA58594 Petunia Shaggy kinase 4 [*Petunia x hybrida*]	up
U215435	XP_483549 receptor protein kinase PERK1-like protein [*Oryza sativa*	up
U216705	BAA94509 protein kinase 1 [Populus nigra]	up
U220563	AAQ82660 Pto-like serine/threonine kinase [*Capsicum chinense*]	up
U215877	AAP20421 mitogen-activated protein kinase 3 [*Lycopersicon esculentum*]	up

Supplemental Table S2. contd.

U217507	NP_194105 ATP binding / kinase/ protein serine/threonine kinase [*Arabidopsis thaliana*]	up
U214553	CAA07470 PP1A protein [*Catharanthus roseus*]	dn
U216477	CAB07803 protein phosphatase type 1 [*Nicotiana tabacum*]	dn
U233076	AAT37528 purple acid phosphatase 3 [Solanum tuberosum]	dn
U221692	AAY97872 ACI14 [*Lycopersicon esculentum*]	dn
U215739	BAE44441 dual specificity protein tyrosine phosphatase 1 [*Solanum tuberosum*]	dn
U218972	NP_851258 PP7; protein serine/threonine phosphatase [*Arabidopsis thaliana*]	dn
U214118	NP_188632 ATFYPP3 (SERINE/THREONINE PROTEIN PHOSPHATASE); protein serine/threonine phosphatase [*Arabidopsis thaliana*]	dn
U214825	AAQ67226 protein phosphatase 2A catalytic subunit [*Lycopersicon esculentum*]	dn
U215619	NP_568846 NMT1 (N- Myristoyltransferase 1)	up
U215468	NP_568509 calcium ion binding [*Arabidopsis thaliana*]	dn
U218586	AAM91235 calmodulin-like protein [*Arabidopsis thaliana*]	dn
U212856	CAA54583 calmodulin [*Zea mays*]	dn
U213548	CAC84563 putative calmodulin [*Solanum commersonii*]	
U223327	AAD48019 Rab GTP-binding protein Rab11b [*Gossypium hirsutum*]	dn
U216446	AAD48018 Rab GTP-binding protein Rab11a [*Gossypium hirsutum*]	dn
U215155	AAL28022 small GTPase Rab2 [*Nicotiana tabacum*]	up
U223245	NP_568465 transporter [*Arabidopsis thaliana*], endomembrane protein 70,TM4 family	dn
U224841	XP_479148 ABC transporter permease protein-like protein [*Oryza sativa* (japonica cultivar-group)]	dn
U216058	AAF33670 cyclic nucleotide-gated calmodulin-binding ion channel [*Nicotiana tabacum*]	dn
U215065	NP_197853 protein translocase/ protein transporter [*Arabidopsis thaliana*]	up
U214263	AAO72684 putative myosin heavy chain [*Oryza sativa* (japonica cultivar-group)]	up
U212892	CAD27443 vacuolar ATPase subunit B [Mesembryanthemum crystallinum]	up
U220750	NP_565150 VPS45 (VACUOLAR PROTEIN SORTING 45)[*Arabidopsis thaliana*]	up
U213031	CAA90564 plastocyanin a [*Populus nigra*]	up
U219576	AAT39305 putative elicitor-responsive Dof protein [*Solanum demissum*]	up
U218623	NP_566730 ALF5, antiporter/ drug transporter/ transporter [*Arabidopsis thaliana*], Na+-driven multidrug efflux pump	up
U221899	BAB09103 endosomal protein-like [*Arabidopsis thaliana*]	up
U216601	NP_851200 ACA8 (AUTOINHIBITED CA2+ -ATPASE, ISOFORM 8) [*Arabidopsis thaliana*]	up
U216736	AAD31896 calcium ATPase [*Mesembryanthemum crystallinum*]	up
U217439	CAA70947 putative inward rectifying potassium channel [*Solanum tuberosum*]	up
U216066	CAA06757 vag2 [*Nicotiana tabacum*], G of vacuolar-type H+-ATPase	up
U229989	CAB97048 mitochondrial half-ABC transporter [*Arabidopsis thaliana*]	up
U220890	NP_193305 glutaredoxin protein [*Arabidopsis thaliana*]	dn
U212712	AAS58496 chloroplast ferredoxin I [*Nicotiana tabacum*]	dn
U217118	BAC53893 cytochrome P450 [*Petunia x hybrida*]	dn
U226337	BAD15331 cytochrome P450 [*Panax ginseng*]	dn
U212897	CAA50312 P450 hydroxylase [*Solanum melongena*]	up
U221410	CAE46765 NADPH thioredoxin reductase [*Oryza sativa* (japonica cultivar-group)]	dn
U213835	AAL54858 tetratricoredoxin [*Nicotiana tabacum*]	dn
U215527	NP_567300 oxidoreductase [*Arabidopsis thaliana*]	dn
U216843	NP_192705 oxidoreductase [*Arabidopsis thaliana*]	dn
U216781	NP_566838 oxidoreductase	dn
U216820	AAQ03092 glutathione peroxidase [Malus x domestica]	dn

Supplemental Table S2. contd.

U218221	AAG34809 glutathione S-transferase GST 19 [*Glycine max*]	dn
U213325	AAA65636 peroxidase (TPX2) (Secretory peroxidases)	dn
U213324	AAA65637 peroxidase (TPX1)	up
U213345	CAC42086 putative peroxidase [*Solanum tuberosum*]	up
U227713	NP_194601 disulfide oxidoreductase [*Arabidopsis thaliana*]	up
U215989	CAC24711 cytochrome P450 [*Solanum tuberosum*]	up
U215883	NP_197391 oxidoreductase	up
U216944	NP_196225 oxidoreductase [*Arabidopsis thaliana*]	up
U214044	BAC23045 monooxygenase [*Solanum tuberosum*]	up
U212687	P30264 Catalase isozyme 1	up
U212747	P32111 Probable glutathione S-transferase (Pathogenesis-related protein 1)	up
U216884	AAG34813 glutathione S-transferase GST 23 [*Glycine max*]	up

accumulation of the endogenous H_2O_2 has been recognized as a signaling molecule to trigger protection and/or defense response (Pitzschke and Heribert, 2006). Consequently, the enzymatic and non- enzymatic antioxidant systems increase, which functions for the amelioration of the oxidative stress-associated damage to different cellular organelles (Dastidar et al., 2006). In the salt treated tomato seedlings, the oxidative stress enzymes, such as catalase and peroxidase (TPX1) (U212687, U213324) were all induced, but the TPX2 (U213325) was suppressed. Maintaining cellular redox homeostasis is essential for normal cellular processes, especially, under stress conditions. Glutathione and thioredoxin comprise the major pool of antioxidants. Salt was previously shown to induce expression of *gpx1* promoter in transgenic tobacco (*Nicotiana tabacum* L.) cultured cells and plants (Avsian-Kretchmer et al., 2004). However, the glutathione peroxidase gene (U216820), and the thioredoxin reductase gene (U221410) homologs were repressed by the salt treatment.

Catalytic genes in different cellular metabolic processes

The genes that encode for catalytically enzyme proteins in different metabolic pathways are grouped into different cellular processes (Table S3)

Cell wall

Plant cell wall contains glycan (cellulose and callose), hemicellulose, pectin, mannans, and various glycoproteins. Polygalacturonase 2A precursor (PG-2A) (Pectinase) (U213213) was induced by salt treatment. Additional eight wall polysaccharide hydrolysis associated genes were also inducible by the stress, which include beta-D-glucan exohydrolase (U215716), the xyloglucan endo-transglucosylase-hydrolase XTH5 (U213444), pectinesterase (U227174), and glucuronosyl transferase (U214-671, U223321).

Hormones

Genes involved in ethylene biosynthesis and responsive mechanisms were induced by salt. The induction of the E8 and several ethylene responsive factor genes (U214-689, U214815, U219296) was significant. *S*-adenosylmethionine synthetase (SAMS, EC 2.5.1.6) catalyses the conversion of methionine to *S*-adenosylmethionine, which is a precursor of ethylene biosynthesis. Two isoforms of SAM (U213593, U212956) were induced by salt treatment.

The jasmonic acid (JA) binds specifically with various components of the signaling network and thus activates response to multiple stress factors in plants (Creelman and Mullet, 1997). However, gene encoding for 12-oxo-phytodienoate reductase 3, which is the key enzyme for JA biosynthesis, was repressed (U214814, U216263), and the same for the dopamine beta- monooxygenase (U238695) which is involved in the metabolism of dopamine.

Carbohydrate metabolism and glycolysis

In the tomato seedling culture system, the carbohydrate was supplied as sucrose in the medium. In the leaf tissues, the TCA cycle enzyme genes of the PDC complex subunits E1 and E3 (U241669, U234441) were induced, but the citrate synthase (U214298) and NAD-dependent isocitrate dehydrogenase (U216212) were reduced. In contrast, a higher transcript level of alcohol dehydrogenase (U241343) in ethanol metabolism was observed.

Fatty acid, lipid and conjugates

In the leaf, genes induced by salt stress include lipid des-

Supplemental Table S3. Catalytic enzymes in different cellular processes regulated by salt in tomato seedlings (2 fold difference at 5% Benjamini-Hochberg false discovery rate).

UnigeneID	Annotation	Leaf
U219946	XP_468492 dTDP-6-deoxy-L-mannose-dehydrogenase -related [*Arabidopsis thaliana*], dTDP-L-rhamnose synthase	dn
U215716	BAA33065 beta-D-glucan exohydrolase [*Nicotiana tabacum*]	up
U214178	callose synthase 1 catalytic subunit [*Arabidopsis thaliana*],	up
U213444	AAS46240 xyloglucan endotransglucosylase-hydrolase XTH5 [*Lycopersicon esculentum*]	up
U227174	NP_197474 pectinesterase family [*Arabidopsis thaliana*],up	up
U213213	CAA32235 Polygalacturonase 2A precursor (PG-2A) (Pectinase),	up
U214671	S39507 glucuronosyl transferase homolog, ripening-related - tomato (fragment)	up
U223321	CAI93191 UDP-glucuronyltransferase-like protein [imported] - *Arabidopsis thaliana*	up
U214814	CAC21424 12-oxophytodienoate reductase 3 [*Lycopersicon esculentum*]	dn
U212956	AAQ14854 S-adenosylmethionine synthase [*Nicotiana tabacum*]	up
U238695	NP_001030764 dopamine beta-monooxygenase [*Arabidopsis thaliana*], biosynthesis of catecholamines and amidated peptides, from tyrosine	dn
U216263	BAD45236 putative 2-oxoglutarate-dependent oxygenase [*Oryza sativa* (japonica cultivar-group)]	dn
U219631	AAD15756 gibberellin 20-oxidase-3; 20ox-3 [*Lycopersicon esculentum*]	dn
U214292	BAD17856 gibberellin 2-oxidase 2 [*Nicotiana tabacum*]	up
U213593	CAA80866 S-adenosyl-L-methionine synthetase [*Lycopersicon esculentum*]	up
U214689	AAM66054 ethylene-responsive protein, putative [*Arabidopsis thaliana*]	up
U214815	AAO34705 ethylene response factor 3 [*Lycopersicon esculentum*]	up
U215649	NP_178471 ST; sulfotransferase [*Arabidopsis thaliana*], ethylene responsive	up
U219296	AAF72100 ELI3 [*Lycopersicon esculentum*]	up
U212799	CAA31789 1-aminocyclopropane-1-carboxylate oxidase homolog	up
U217762	AAB28813 cytochrome c1 precursor [*Solanum tuberosum*]	dn
U241343	CAA37333 alcohol dehydrogenase [*Solanum tuberosum*]	up
U213321	ABA86964 glyceraldehyde-3-phosphate dehydrogenase B subunit [*Glycine max*]	dn
U214848	AAU00726 glucose-6-phosphate isomerase [*Solanum tuberosum*]	dn
U218934	AAC26113 fructose-6-phosphate 2-kinase/fructose-2,6-bisphosphatase [*Solanum tuberosum*]	up
U214149	AAR24912 fructokinase 3 [*Lycopersicon esculentum*]	up
U213281	CAA88840 phosphoglycerate kinase (PGK) [*Nicotiana tabacum*]	up
U214298	P49299 Citrate synthase, glyoxysomal precursor (GCS)	dn
U216212	CAA74776 NAD-dependent isocitrate dehydrogenase [*Nicotiana tabacum*]	dn
U241669	AAC72194 pyruvate dehydrogenase E1 beta subunit isoform 3 [*Zea mays*]	up
U234441	AAG17888 dihydrolipoamide dehydrogenase precursor [*Solanum tuberosum*] (E3)	up
U225510	ABA81853 NADH:ubiquinone oxidoreductase-like [*Solanum tuberosum*]	up
U217048	NP_199142 catalytic/ enoyl-CoA hydratase [*Arabidopsis thaliana*] nFatty acid -oxidation	dn
U214190	CAA64414 lipid desaturase-like protein [*Lycopersicon esculentum*]	up
U237900	AAT67159 digalactosyldiacylglycerol synthase [*Xerophyta humilis*] Chloroplast galactolipid synthesis	up
U214254	XP_480769 + putative [*Arabidopsis thaliana*], phosphlipid synthesis	up
U217151	NP_567912 (S)-coclaurine-N-methyltransferase/ S-adenosylmethionine-dependent methyltransferase/ cyclopropane-fatty-acyl-phospholipid synthase [*Arabidopsis thaliana*]	up
U214254	XP_480769 putative phosphoethanolamine cytidylyltransferase [*Oryza sativa* (japonica cultivar-group)]	up
U213373	AAN14410 bifunctional lysine-ketoglutarate reductase [*Gossypium hirsutum*]	up
U219060	AAA18948 ferredoxin-dependent glutamate synthase	dn
U213411	AAR92031 cystathionine gamma-synthase isoform 1 [*Solanum tuberosum*]	dn
U220820	NP_172142 3-hydroxyisobutyryl-coenzyme A hydrolase [*Arabidopsis thaliana*), valine metabolism	dn
U213163	BAB83948 CIG1 [*Nicotiana tabacum*], Proline dehydrogenase	dn
U214945	AAR18403serine acetyltransferase 4 [*Nicotiana tabacum*],up	up
U213768	1407140B Acetolactate synthase II, chloroplast precursor , leucine, isoleucine, and valine	up
U213635	AAO92255 gamma-aminobutyrate transaminase subunit precursor isozyme 1 [*Lycopersicon esculentum*]	up

Supplemental Table S3 Contd.

U217075	Q93Z70 N-acetyl-glutamate semialdehyde dehydrogenase	up
U218707	2CVO Chain D, Crystal Structure Of Putative N-Acetyl-Gamma-Glutamyl- Phosphate Reductase (Ak071544) From Rice (*Oryza Sativa*)	up
U213123	CAI39242 arginine decarboxylase 1 [Datura stramonium], putrescine biosynthesis, (liu et al., 2006) salt induced in apple	up
U215516	CAD47830 (putrescine) hydroxycinnamoyl transferase [*Nicotiana tabacum*]	up
U214067	CAA07683 geranylgeranyl reductase [*Nicotiana tabacum*]	
U212844	P37273 Phytoene synthase 2, chloroplast precursor, beta-carotene (provitamin A)	up
U222640	CAA42573 phytoene desaturase [*Lycopersicon esculentum*]	up
U217416	NP_180649 dihydroxypolyprenylbenzoate methyltransferase [*Arabidopsis thaliana*], ubiquinone biosynthesis	up
U215516	CAD47830 hydroxycinnamoyl transferase [*Nicotiana tabacum*]	up
U221357	AAP03021 4-coumarate-CoA ligase-like protein [*Arabidopsis thaliana*]	dn
U219914	BAD45907 putative dihydroflavonol-4-reductase DFR1 [*Oryza sativa* (japonica cultivar-group)]	up
U216573	NP_189325 ATP binding / shikimate kinase [*Arabidopsis thaliana*]	up
U220611	P10748 5-enolpyruvylshikimate-3-phosphate synthase) (EPSP synthase)	up
U215754	AAS79603 prephenate dehydratase [Ipomoea trifida], decarboxylation of prephenate to phenylpyruvate	up
U212746	P21568Peptidyl-prolyl cis-trans isomerase (PPIase) (Cyclophilin)	up
U213332	NP_189160 peptidylprolyl isomerase (ROF1) [*Arabidopsis thaliana*],	up
U234166	CAH55766 peptidylprolyl cis-trans isomerase [*Oryza sativa* (indica cultivar-group)],	up
U215175	AAK83088 Pin1-type peptidyl-prolyl cis/trans isomerase [Malus x domestica], (salt induced; Marivet et al. 1994)	dn
U217630	NP_194104 aldose 1-epimerase [*Arabidopsis thaliana*], Carbohydrate transport and metabolism	dn
U215645	P46253 Acyl-[acyl-carrier-protein] desaturase	up
U214488	AAS92256 putative pyridoxine biosynthesis protein isoform B [*Nicotiana tabacum*] (VB6)	up
U324743	protein pirin	dn
U331930	protein pirin	dn

saturase (U214190), which plays a key role in the maintenance of the proper structure and functioning of biological membranes; digalactosyldiacylglycerol synthase (U237900) for the biosynthesis of glycolipid, and S-coclaurine-N-methyltransferase (U217151) and ethanolamine-phosphate cytidylyltransferase (U214254) for the biosynthesis of phosphlipid. The gene for fatty acid -oxidation (U217048) was suppressed.

Amino acid and polyamine metabolism

Methionine supply plays key role in secondary metabolism under stress condition. The cystathionine gamma-synthase isoform 1 (U213411), the first specific enzyme for methionine synthesis, was repressed (Amir et al., 2002).

In addition, the suppressed genes include the proline dehydrogenase cognate (U213163), and the ferredoxin-dependent glutamate synthase (U219060). The induced genes include serine acetyltransferase (U214945), catalyzing the first step of cysteine synthesis; acetolac- tate synthase II, (U213768), the first common enzyme in the biosynthetic pathways leading to leucine, isoleucine, and valine (branched amino acids); gamma-aminobutyrate transaminase (U213635); arginine biosynthesis genes of N-acetyl-glutamate semialdehyde dehydrogenase (U21-7075) and N-acetyl-gamma- glutamyl-phosphate reductase (U218707); and the salt inducible arginine decarboxylase (U215516) in the putrescine biosynthesis (Liu et al., 2006).

The secondary metabolites

Genes associated with the secondary metabolites are mostly induced by salt treatment in leaf. The carotenoid biosynthesis enzyme phytoene synthase homologous gene was induced. A higher transcript level of phytoene desaturase in the same pathway (U222640) was observed, together with two other genes, dihydroxypolyprenylbenzoate methyltransferase (U217416) for in ubiquinone biosynthesis, and hydroxycinnamoyl transferase (U215-516) for the biosynthesis of hydroxycinnamic acid amides, which is associated with plant defense responses in integral cell wall component.

Several genes were identified in the shikimate and the subsequent phenylpropanoids pathway. The induced genes include shikimate kinase (U216573), 5-enolpyruvylshikimate-3-phosphate synthase) (EPSP synthase) (U220611), and prephenate dehydratase (U215754). Giv-

en the enhanced activity of the shikimate pathway with more accumulation of phenylalanine, the suppres-sion of 4-coumarate-- CoA ligase 2 (4CL 2) (U217714) for lignin synthesis will provide more substrates available for flavonoid biosynthesis, which coin-cide with the induction of the putative dihydroflavonol-4-reductase DFR1 (U219-914).

Others

Genes that function at multi-levels, affecting multiple pathways and reactions are also affected by salt stress. Different isoforms of peptidylprolyl cis-trans isomerase were up, or down regulated. Transcript level of genes associated with carbon fixation reaction changed in response to salt stress. However, as the seedlings are very small, no authentic photosynthesis is expected, therefore, such information is not presented here.

QRT-PCR result

Transcriptional regulation revealed by microarrays was confirmed using qRT-PCR (Figure 1). Figure 1 shows that transcript abundance amplified with primers S2, and S10 were higher in the control than in the salt treatment, and S13, S17, S18, S19 an S35 were the opposite. S7 did not have significant difference in both microarray and qRT-PCR. The relative quantification data reflected the same regulatory patterns as the microarray data, showing clear induction or repression in response to salt. Consequently, RT-PCR data confirmed the expression data obtained by microarrays.

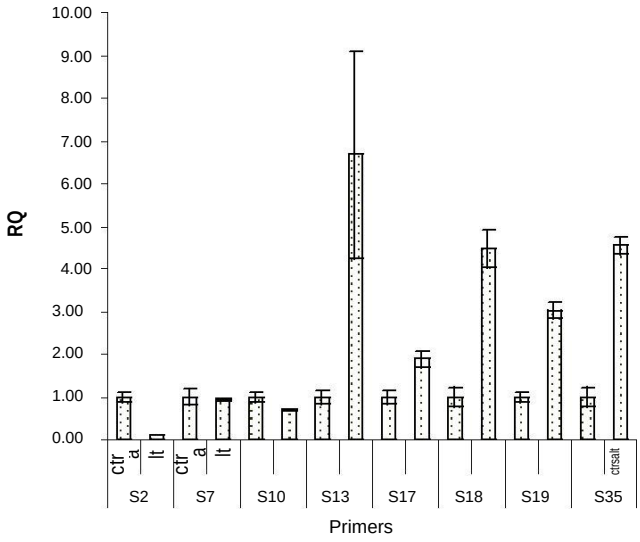

Figure 1. QRT-PCR of tomato leaf tissues under salt treatment.

DISCUSSION

Transcriptional regulation of genes associated with cell production under salt stress.

Plant growth depends on cell production and size enlargement. Salt stress can temporarily inhibit mitotic activity and lead to smaller meristimatic tissues (West et al., 2004). Increased expression of cell cycle regulatory genes (cyclin-dependent kinases, CDK) is directly related with the extent of cell production (Doener et al., 1996). On the other hand, reduction in the expression of these genes was concomitant with increased cell cycle time in the basal meristem of leaves exposed to cold nights (Rymen et al., 2007). Transcript profiling in this study also revealed a salt-inducible suppression of the cell cycle positive regulator gene, the cyclin-dependent protein kinase p34cdc2 (U215180), and enhancement of the inhibitor gene (U217044).

Cell progression is also affected by transcription factors. FHA1 is a transcriptional activator containing Forkhead-associated domains (FHA), which is implicated in the RNA polymerase III function and critically involved in regulation of rRNA processing. Over-expression of the tobacco NtFHA, or the FHA, causes a severe growth defect in yeast by slowing down the G_1-S transition in the cell cycle (Kim et al., 2002). The FHA1 tomato homolog (U235936) was induced by salt stress. The translation initiation factor, eIF5A, is an RNA-binding protein (Liu et al., 1997), and it was hypothesized to regulate the translation of a subset of mRNAs that are needed for G_1/S cell cycle progression. The tomato homologous gene (U214373) was also repressed in the salt treated tissue. Another mechanism of salt stress is causing nuclear deformation and nuclear degradation accompanied by apoptosis-like DNA fragmentation (Katsuhara and Kawasaki, 1996) . Genes associated with programmed cell death, such as programmed cell death 6 protein (U214469), the N-rich protein (U213660), and cystatin (U213642) (Girard et al., 2007; and the references therein) RNA polymerase III function and critically involved in showed increased transcript accumulation after salt treatment.

Ethylene metabolism and the down-stream gene regulation

Salt stress has long been recognized to be associated with ethylene metabolism (Garcia and Einset, 1983; Cao et al., 2007), from seed germination (Khan and Huang, 1988) to general cultivar salt sensitivity (Datta et al., 1997). Stress can enhance expression of 1-aminocyclopropane-l-carboxylate oxidase (ACC) genes in tobacco (Kim et al., 1998) and the tomato seedling tissue in this study.

Tomato contains multiple homologous genes of ethylene receptors that are expressed differentially in fruit ripening and defense response processes. In this study, the salt treated tomato accumulated higher transcript level of

several ethylene-regulated genes, such as the E8 gene, which is transcriptionally activated by ethylene (Deikman et al., 1998); and the cell-wall metabolism genes, including polygalacturonase gene (U213213) (Sitrit and Bennett,1998) and xyloglucan endotransglucosylase-hydrolase XTH5 (U213444) (Ookawara et al., 2005); and genes in the secondary metabolism, such as the phytoene synthetase (U212843,U212844) (Bird et al., 1991), and the anthranilate N-hydroxycinnamoyl/benzoyltrans-ferase (U217416) (Fan et al., 1999). Results from the present study shows that genes for ethylene biosynthesis and subsequent mediated reactions are actively affected by salt stress. This is in accordance with the notion that ethylene is required for salt-stress signaling and adaptation (Ma et al., 2006).

Complexity of the response mechanism to salt stress in the salt sensitive tomato plants

Salt stress includes osmotic imbalance and disturbance of the cytoplasmic K^+/Na^+ homeostasis, which subsequently activate the protection/degrading processes in different plant organelles. For instance, Na^+ competitively binds with K^+ for binding sites of about 50 enzymes and disrupts the corresponding enzymatic processes (Bhandal and Malik, 1988). Like other plant species, tomato plants under salt stress have exhibited changes in multiple genes. Different gene expression, at transcription level, have been attributed to salt stress, such as the wound-related genes (Dombrowski, 2003), genes encod-ing for various soluble sugars and other osmoprotestants (Sacher and Staples, 1985), and antioxidant enzymes (Chen et al, 2004). Controlled ion transport and the resultant homeostasis of K^+/Na^+ (Gisbert et al., 2000, Rubio et al., 2004), different hormones, and other signaling molecules play significant role in salt stress in tomatoes (Ouyang et al., 2007).

In this study, salt-regulated genes have been identified. Molecular chaperones and proteases are the two well-recognized mechanisms for plant cells to control the protein environment under adverse conditions (Sjögren et al., 2004).These proteins can facilitate maintaining correct stereo-structure of structural proteins, activate function of enzymes, and remove degraded polypeptides. In this study, all the identified chaperonine genes were induced under salt treatment. Several distinct protease and protease inhibitor genes were affected by salt stress. The salt induction of the genes for the biosynthesis of polyamines (arginine and putrescine) (Liu et al., 2006) and secondary metabolites (flavonoid compounds and ubiquinone) (Xie et al., 2004; Walia et al., 2005), ion-transport (Sottocornola et al., 2006), antioxidant enzymatic systems, and phospholipids and glycolipids are basically in accord with the reported studies from different plant species.

Some genes function at multi- levels, for instance, the Pin1-type peptidyl-prolyl cis/trans isomerase is intimately involved in diverse biological processes, such as cell-cycle control, transcription and splicing regulation, DNA replication checkpoint control, DNA damage response, and cellular signaling (Wulf et al., 2005). There is evidence that transcripts encoding PPiase are regulated by salt stress in maize and bean plants (Marivet et al., 1994, 1995). The protein pirin is involved in determining the direction of pyruvate metabolism towards either the TCA cycle or the fermentation pathways (Soo et al., 2007). The salt induced down-regulation of such genes could have a widespread effect on the whole genome transcriptional activities.

Changes of the regulatory machinery under salt stress

Gene transcription is regulated by interplay of the positive and negative regulators. In this study, multiple genes belonging to different families of transcription factors were induced/or repressed by salt treatment. In addition, The DNA binding/transcription factor (NP 187854) con-tains a class 2 transcription repressor NC2 in the region of 3-89 (aa), which is a global repressor of transcription by associating with TATA binding protein (TBP) and inhibition of preinitiation complex formation (Goppelt and Meisterernst,1996; Creton et al., 2002). The tomato homologous gene (U220740) was induced in leaf. However, the two genes are not aligned in the NC2 region, whether the tomato homologous gene contains such element, need to be further studied. Endogenous siRNAs as manifested by the posttranscriptional gene silencing (PTGS) plays key role in the regulation of gene expression (Peragine et al., 2004). The SGS3 is required for the production of transacting siRNAs in Arabidopsis. The homologous gene (U226511) was also induced by salt stress.

Protein kinase mediated protein phosphorylation and N-myristoylazation catalized by the N-myristoyl transfer-ase are essential components for signal transduction cascade of salt tolerance in Arabidospsis (Liu et al., 2000, Liu and Zhu, 1998; Ishitani et al., 2000). The salt-induc-tion of the myristoyl transferase homologous gene was significant in the leaf. In the salt treated tomato seedlings, several kinase mediated signal trans-duction pathways are affected as shown by the transcript accumulation of the corresponding genes. Both positive and negative regulators for the mitogen-activated protein kinase (MAPK) signaling pathway were identified. The phosphatase 2Cs (PP2Cs) are negative regulators of MAPK; expression of PP2Cs under salt stress could be part of a negative feedback mechanism (Meskiene et al., 2003).

The calcium/calmodulin-dependent kinases (CRCK), which plays an important role in signal transduction in plants (Yang et al., 2004), and the ATP binding/kinase CRCK3 homolog (U221265) were repressed by salt treatment.

SHAGGY-related protein kinase (ASK) is implicated in the evolutionary adaptation of plants to osmotic stress

(Richard et al., 2005). The Arabidopsis GSK3/SHAGGY-like kinase gene is salt inducible (Piao et al., 1999), and regulate proline accumulation through the brassinosteroid (BR) steroid signaling pathway in salt treated Arabidopsis plants (Li and Nam, 2002). This study identified that the SHAGGY kinase 4 homolog (U213944), together with the N-acetyl-glutamate semialdehyde dehydrogenase (U217075), one of the key enzymes in the proline synthesis pathway, were all induced in the salt treated tomato tissues.

Salt stress and many other biotic and abiotic stresses are coordinately regulated by different interconnected pathways (Ludwig et al., 2005; Ma et al., 2006). In the tomato seedlings under salt stress, the gene transcription and translation regulatory machinery, protein post-translational modification, and signal transduction all exhibited a very complex, and fine-tuned change, which emphasize the importance and intricacy of these control points. The comparative studies on hylophyte and glycophyte have revealed that it is the subtle differences in regulation mechanism that may play the key role in determination of salt tolerance/sensitivity (Taji et al., 2004). Our present results provided a set of candidates of regulatory genes that should be further elaborated to define their roles in salt stress.

Supplemental data

Supplemental Table S1: Primers used for qRT-PCR amplification.

Supplemental Table S2: Functional annotation (FDR p<1.0) of salt- regulated genes in tomato seedlings (2 fold difference, at 5% Benjamini-Hochberg false discovery rate).

Supplemental Table S3: Catalytic enzymes in different cellular processes regulated by salt in tomato seedlings (2 fold difference, at 5% Benjamini-Hochberg false discovery rate)

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