Identification of Genes Associated with Aluminum Toxicity in Tomato Roots Using cDNA Microarrays

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ABSTRACT

cDNA microarrays (Tom 1) were used to analyze changes in transcript accumulation in tomato (*Solanum lycopersicum* 'Money Maker') roots grown in liquid cultures supplemented with 20 μM AlK$(SO_4)_2$. Out of 1,362 genes that had significant changes in transcript accumulation (FDR<0.05), 569 were reduced and 793 were induced. The down-regulated genes included those found in the cell cycle progression (p34cdc2 protein kinase, cyclin, and histone), putrescine synthesis, ABC transporter and germin-like protein. Genes induced by Al included glutamate synthase, pectin modification, histidine synthesis, proline synthesis, metallothionein-like protein, multidrug efflux proteins (MATE), ethylene biosynthesis and detoxification proteins. Different isoforms of V-ATPase, glutathione transferases and others in the multiple gene families were either induced or suppressed. Wide arrays of genes that regulate transcription and translation activities, as well as signal transduction, were also affected.

INTRODUCTION

Aluminum is a non-essential toxic element that prevails in 40% of the world's arable land. Under acidic conditions, the smaller size and higher positive charge of this cation favor its binding to cell plasma membranes over essential ions (Al^{3+}, 0.055 nm; Mg^{2+}, 0.075 nm; Ca^{2+}, 0.105 nm; K^+, 0.133 nm) (Li *et al*. 2002). The fluidity of the lipid bi-layers of the plasma membrane depends on the negative charge on its surface (Vose and Randall 1962). Binding of Al to these membranes causes depolarization of the trans-membrane electrical potentials due to the neutralization of electronegative charges (Papernik and Kochian 1997; Takabatake and Shimmen 1997). Interaction of Al^{3+} with the membrane's lipid components causes it to become rigid (Yamamoto *et al*. 2001), and blocks the movements of Ca^{2+} (Piñeros and Tester 1995) and K^+ (Gassmann and Schroeder 1994). The most noticeable effect of Al toxicity in sensitive plants is reduced root growth due to the loss of cell elasticity (Ma *et al*. 2004) and lower cell numbers due to programmed cell death (Pan *et al*. 2002; Zheng *et al*. 2007).

Metabolic changes induced by Al result in the accumulation of protective secondary metabolites such as proline and polyamines, production of cell wall polymer constituents (callose) and organic chelators such as citrate, malic and *trans*-aconitic acids in *Zea mays* (Suhayda and Haug 1986; Piñeros *et al*. 2005), malic and citric acids in *Secale cereale* L. 'King') and *Triticum aestivum* L. 'Atlas 66' (Li *et al*. 2000), and oxalic acid in *Fagopyrum* sp. (Ma *et al*. 1997).

Al affects the expression of genes that encode for enzymes in antioxidation and detoxification mechanisms such as glutathione *S*-transferase (GST) and peroxidases (Richards *et al*. 1998), transporters (vacuolar H^+-ATPase, Kasai *et al*. 1992, 1993), plasma membrane H^+-ATPase (Matsumoto 1988), citrate-permeable anion channel (Kollmeier *et al*. 2001), malate transporters (Ermolayev *et al*. 2003), cell wall metabolism (cell wall pectin methylesterase, Schmohl *et al*. 2000), pectin acetylesterase (Chandran *et al*.

2008), glucan synthase II for callose synthesis (Widell *et al*. 1994), cell cycles and histone genes (Chandran *et al*. 2008), genes in the phosphoinositide signal transduction pathway (Jones and Kochian 1995), phospholipase C, phospholipase D signalling pathways (Yakimova *et al*. 2007), and phosphoenolpyruvate carboxylase (Ermolayev *et al*. 2003). Exposure of *T. aestivum* and *Saccharum* sp. to toxic levels of soluble Al induced *wali1–wali5*, *wali6* and *wali7* (Snowden and Gardner 1993; Richards *et al*. 1994; Snowden *et al*. 1995) and different homologs of *wali6* and *wali7*, respectively (Drummond *et al*. 2001). Other Al-induced genes in other crops include *Sali3-2* and *Sali5-4* in *Glycine max*, *ZmaI* in *Z. mays*, *Mtn29* in *Medicago truncatula*, *pEARLI8* and *pEARLI1*, *pEARLI2*, *pEARLI4* in *Arabidopsis thaliana*, *pEARLI5* and in *T. aestivum*, phosphatidylserine synthase (Ermolayev *et al*. 2003).

Aluminum resistance can be conferred by a single dominant gene (*TaALMT1*) as in *T. aestivum* (Raman *et al*. 2005), a homologous *ALMT1* gene cluster in *S. cereale* L. (Collins *et al*. 2008) and the *Alp* gene in *Hordeum vulgare* (Tang *et al*. 2000). Transgenic plants expressing S851 encoding for the Δ-8 sphingolipid desaturase (Ryan *et al*. 2007), Arabidopsis blue copper-binding protein (*AtBCB*), *Nicotiana tabacum* GST (*parB*), peroxidase (*NtPox*), and GDP-dissociation inhibitor (*NtGDI1*) (Ezaki *et al*. 2000) had improved tolerance to Al toxicity.

The genomic approach of microarrays and cDNA microarrays enables for large scale transcript profiling and led to the discovery of discrete molecular changes associated with Al toxicity in *Z. mays* roots (Maron *et al*. 2008), in *T. aestivum* near-isogenic lines (Guo *et al*. 2007), and in *Hordeum vulgare* (Furukawa *et al*. 2007). It has also led to the discovery of genes related to tolerance or sensitivity to heavy m*etal*s such as Zn (Chiang *et al*. 2006), and cadmium (Boveta *et al*. 2005) in *A. thaliana*.

The tomato is an important economic crop and a model plant for molecular and genetics studies. When exposed to toxic Al levels, it exhibits similar physiological and cellular changes as observed in other sensitive species. Changes

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Table 1 Distribution of the ratios (Al treated/untreated control) of tomato root culture from Tom 1 microarray analysis.

Ratio (Al/untreated)	<0.6	0.61-0.80	0.81-0.97	1.03-1.30	1.31-2.09	2.13-3.14
Unigene number	13	64	492	570	200	23

such as reduced absorption of different cations (Postma *et al.* 2005), in photosynthetic activities, and lower acid invertase and neutral invertase activity in roots have been reported (Simon *et al.* 1994a, 1994b). Programmed cell death mediated through phospholipase C and phospholipase D signaling pathways is one of the molecular control underlying Al sensitivity in tomato (Yakimova *et al.* 2007). Relevant information on mechanisms involved in tolerance or sensitivity to soluble Al have been reported. The elucidation of connections between different regulatory and metabolic pathways used by *L. esculentum* to defend against the toxic effects of soluble Al is challenging. For this study, cDNA microarray (Tom 1) large scale genomic transcript profiling was used to analyze transcripts in *L. esculentum* roots following prolonged exposure to Al. Genes expressed or suppressed were classified into groups based on their putative functions (Ma 2007). Those observed to behave differently from their counterparts in other plant species could be used to elucidate mechanisms that account for inter-specific differences in tolerance or sensitivity to Al.

MATERIALS AND METHODS

Tomato root culture and Al treatment

Tomato (*Solanum esculentum* Mill 'Money Maker') seeds (Seedman, USA) were surface sterilized by submerging in 50% bleach (sodium hypochloride) for 10 min followed by three washes with sterile distilled water. For seed germination, six clean seeds were placed in a 500 ml flask containing 300 ml of the basal nutrient solution described in Magnavaca *et al.* (1987), pH 4.5, followed by incubation on a shaker (150 rpm) at 25°C for four days. Germinating seeds were then transferred to fresh Modified Magnavaca's solution supplemented with 20 μM AlK(SO$_4$)$_2$ (Fisher Scientific, USA). Seedlings serving as controls were placed in the same solution without Al for 12 days; the solution was changed every four days. At the termination of the experiment, root tips of ~2 cm in length were dissected and soaked in a pre-chilled RNA extraction buffer (Genehunter, USA). Each replicate of treatment or control had 20 flasks, and root tips from the 20 flasks were pooled to make one RNA extraction. Six RNA extraction replicates were preformed for Al treated and control tissues, respectively.

RNA extraction and removal of DNA

Extraction of total RNA was done using Genehunter's RNA pure kit (Genhunter, USA). Genomic DNA was removed by incubation for 30 min with DNase I at 37°C. The RNA samples were purified by extraction in phenol: chloroform (1:1), precipitated with ethanol, and resuspended in diethyl pyrocarbonate- treated water. The quality and quantity of the RNA was monitored in 2.0% denatured agarose gels and a ND-1000 nanodrop spectrometer (Nanodrop Technologies, USA). DNA-free RNA extracts were used for further analysis.

Microarray hybridization, data acquisition and analysis

aRNAs were obtained using the amino allyl MessageAmp II aRNA amplification kit and labeled with cyanine-dye (Cy5/Cy3) (Ambion, USA). Purified targets aRNAs were hybridized with probes on a cDNA microarry (Tom1) (http://bti.cornell.edu/CGEP/). Hybridization was conducted at 43°C in the dark for 16 h (http://bti.cornell.edu/CGEP). Images were captured with a GenePix 4000B slide scanner and data obtained using Genepix 6.0 software (Molecular Devices). Raw data were loaded into GeneSpring (v.7) (Agilent Technologies, Inc., USA) using the GenePix-format defaults and normalized with the Lowess curve. Genes were listed by first passing through a t-test with a multiple testing correction using 5% Benjamini-Hochberg false discovery rate, and subse-

quently according to their fold changes. Detailed information is included in MIAME (Accession number GSE11816, Link, http://www.ncbi.nlm.nih.gov/geo/info/linking.html). Because the identified genes could not be placed into a pathway context using the GO term (http://bti.cornell.edu/CGEP), they were grouped manually based on their putative cellular functions.

QRT-PCR

QRT-PCR was conducted using RNAs extracted for microarray analysis. Six extractions, each having three PCR reaction replicates, were conducted for all Al-treated and control plants. Primer design and QRT-PCR procedures, gene transcript relative quantification were performed as previously described (Zhou *et al.* 2007). After confirming that the RQ value of the Al treated tissues was significantly different from the untreated control, the average RQ values of all measurements (6 extraction × 3 replicates) were used to compare with microarray results.

RESULTS

General features

Analysis of 12,000 unigene probes on the microarray showed that 1362 unigenes passed the Benjamini-Hochberg multiple test (FDR<0.05). Five hundred sixty-nine unigenes had lower transcript levels and 793 had higher transcript levels in Al-treated roots compared to the untreated ones (**Table 1**).

Functional clustering of the Al-regulated genes

Al-regulated genes were clustered according to their putative functions.

Transcriptional and translational regulatory genes

Regulator genes for transcriptional and translational activities were significantly affected by Al treatment (Appendix, **Table 1**). The transcriptional activator FHA1 homolog was suppressed (SGN-U231440) by 1.50-fold while the ethylene-responsive transcriptional coactivator homolog was induced (SGN-U21735) by 1.13-fold. Four MADS box protein genes were induced between 1.18- and 1.41–fold (SGN-U233184, SGN-U220567, SGN-U220213, and SGN-U215264). Other induced genes include two WRKY transcription factors (SGN-U219817 and SGN-U215123) by 1.23- fold, the TDR4 transcription factor (SGN-U213659) by 1.13- to 1.27-fold, and an nonsense-mediated mRNA decay protein (SGN-U220732) by 1.34-fold. Transcript level of Myb gene varied with clones, SGN-U217726 was suppressed by 1.23- fold, but SGN-U218469 induced by 1.3- fold.

Three bZIP transcription factors responded differently: SGN-U214344 was reduced by 1.23-fold, SGN-U213967 and SGN-U217015 induced by 1.42- and 1.59-fold, respectively. The AP2 domain transcriptional factors were either suppressed (SGN-U218022) by 1.27-fold, or induced by 1.85-fold (SGN-U218671). The Dof zinc finger protein, bHLH protein family, CHP-rich zinc finger protein, and DEAD/DEAH box helicase, and others were also affected by Al toxicity. Two eukaryotic translation initiation factors [8 and 11 (SGN-U216323 and SGN-U2163331)] genes were both suppressed by 1.45-fold.

Signal transduction

The receptor-like kinase (SGN-U215435) was repressed by 1.85-fold (Appendix, **Table 2**). A serine/threonine kinase (SGN-U213441) and two shaggy protein kinase 4 (SGN-

U213944 and SGN-U2146560) were induced by 1.57-, 1.27-, and 1.06-fold, respectively. Two isoforms of calcium-dependent protein kinase 2 were affected: one (SGN-U217205) was induced by 1.80-fold and the other repressed (SGN-U228065) by 1.1-fold. Two mitogen-activated protein kinase genes were repressed (SGN-U213820) by 1.25-fold, and (SGN-U216184) 1.11-fold. A rac GTPase activating protein 2 (SGN-U220727) was also repressed by 1.11-fold.

The tomato PP1 (SGN-U215542), PP2C (SGN-U226314), and SGN-U216018 were induced by 1.35-, 1.70-, and 1.14-fold, respectively. Two phosphatase genes were induced (SGN-U213418) by 1.16-fold and (SGN-U218431) by 1.15-fold. In treated roots, two receptor protein kinases were affected differently; one was repressed (SGN-U221567) by1.11- and the other (SGN-U221648) induced by 1.22-fold. Two genes in the ethylene biosynthetic pathways were also induced, by 1.28-fold for SGN-U215359 and 3.13-fold for SGN-U212956.

Transporters

The mitochondrial carrier protein (SGN-U217777) was induced by 1.7-fold (Appendix, **Table 3**). The multidrug efflux tomato protein homolog (SGN-U215960) was induced by 1.26-fold. Two tomato ABC homologs (SGN-U240910 and SGN-U229989) were suppressed by 1.25-fold. The tomato root homolog of multidrug efflux protein (MATE) family function (SGN-U212659) was induced by 1.65-fold. Two V-ATPase homologs (SGN-U213767 and SGN-U212892) were suppressed by 1.25-fold. One of two V-ATPase G isoforms (SGN-U232696) was repressed by 1.1-fold while the other (SGN-U216066) was induced by 1.2-fold.

Oxidative stress and detoxification

In Al-treated tomato roots, the tomato homolog gene for metallothionein-like protein (SGN-U212973) increased 2.8-fold (Appendix, **Table 4**). Four isoforms of GST were differentially regulated, GST 23 (SGN-U216884) and a pathogenic GST gene (SGN-U212747) were suppressed by 1.15-fold whereas GST T2 (SGN-U212756)) and GST 19 (SGN-U218221) were induced by 1.25- and 1.16-fold, respectively. The germin-like protein GLP6 (SGN-U21938) was repressed by 1.12-fold and the detoxification genes encoding for lactoylglutathione lyase (SGN-U218426 and SGN-U220344) were induced by 1.15- and 1.20-fold, respectively.

Secondary metabolites

Two genes for proline synthesis, Δ1-pyrroline-5-carboxylate synthetase P5CS (SGN-U216928 and SGN-U214573), were induced by 1.19- and 1.24-fold, respectively (Appendix, **Table 5**). The arginine decarboxylase 1 (SGN-U213123), a key enzyme for putrecine biosynthesis, was suppressed by 1.49-fold. The two tomato homologs of histidine decarboxylase (SGN-U212591 and SGN-U212595) were induced between 1.85- and 2.24-fold as well as β-carotene hydroxylase for carotenoid accumulation (SGN-U221960) by 1.26-fold, and uracil phosphoribosyltransferase in the pyrimidine salvage pathway (SGN-U2184150) by 1.28-fold.

Cell division, cell wall modification

The tomato homolog p34cdc2 protein kinase (SGN-U215180), cyclin (SGN-U217551) and histone H3 (SGN-U242934) were suppressed by 1.1-fold (Appendix, **Table 6**). The GDP-mannose pyrophosphorylase (SGN-U213366) for cell wall synthesis was also repressed by 1.20-fold. Cell wall alteration genes were either induced or suppressed. The gene for pectinacetylesterase (SGN-U217232) was induced by 1.51-fold and for pectinesterase (SGN-U227174) suppressed by 1.2-fold. Two genes (SGN-U223905 and
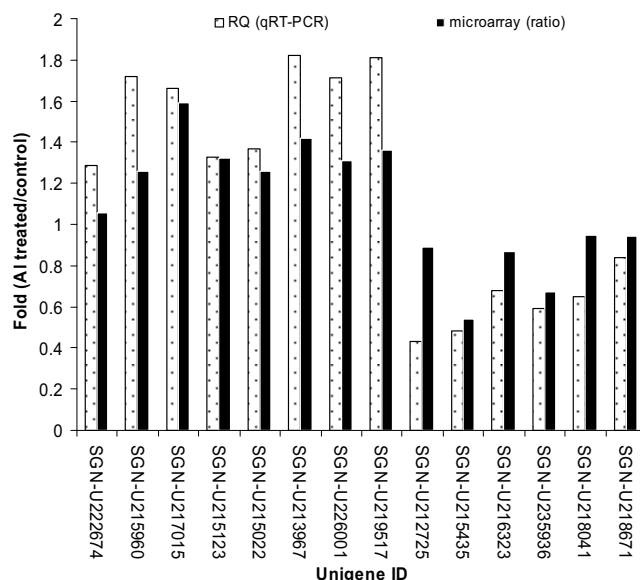
Fig. 1 Comparison of microarray and qRT-PCR results for relative gene transcript abundance in Al treated tomato root tissues. The RD values are the average of 12 qRT-PCR measurements, including two repeats of PCR reaction for each of the six experimental replicates. Fold (Al/Control) indicates the relative abundance of transcript between Al-treated and control root tissues (fold > 1.0 for up-regulated genes, and fold < 1.0 for down-regulated genes).

SGN-U213455) for degrading xyloglucan (hemicellulose) were induced by 1.26- and 1.10-fold, respectively.

QRT-PCR validation of the identified genes from Microarray analysis

Eight up-regulated and six suppressed genes identified by microarray analysis were selected to validate their transcript level using qRT-PCR. The fold values between Al-treated and control root tissues corresponded to both the relative quantification (RQ) in qRT-PCR and the ratio in microarray analysis (**Fig. 1**). A fold level > 1.0 indicates up-regulated genes, and a fold < 1.0 for down-regulated genes. These results indicated that although there were fluctuations between the fold quantities (levels) from qRT-PCR and microarray analysis, the trend remained. The fold (Al treated/control) was > 1.0 for up-regulated genes, and <1.0 for down-regulated genes. The two tests substantiated up-regulated and down-regulated genes. Thus, the microarray results should be considered valid.

DISCUSSION

The most important protection against Al toxicity in higher plants is mechanisms that prevent toxic ions from crossing the plasmalemma and reaching the cytoplasm (Kochian 1995). Al mostly (72–82% of the total cellular Al) binds with small pectic-polysaccharides in the primary cell walls (Chang *et al.* 1999; Zheng *et al.* 2004), particularly to pectin with a low degree of methylation (Blamey 2001). Pectinesterase (PE) catalyses the de-esterification reaction of pectin. Expression of PE can affect the degree of pectin methylation and subsequently the binding of Al to the primary cell wall. Plant PEs are encoded by a family of genes, some isoforms are constitutively expressed throughout the plant while others are differentially expressed in specific tissues and at different developmental stages. In this study, the PE (SGN-U227174) homolog was suppressed. However, in another study (unpublished), eight additional PE unigenes from Al- treated tomato roots were identified. These included the down-regulated SGN-U214672, SGN-U213495, SGN-U213186, SGN-U224340 and SGN-U215316, and up-regulated SGN-U216389 and SGN-U216191.

Pectin actylesterase (EC 3.1.1.6) can specifically hydro-

lyze acetyl ester modifications in homogalacturonan regions of pectin. Chandran *et al.* (2008) suggested that in *Medicago truncatula* 'Jemalong' genotype A17, the hairy roots pectin acetylesterase plays a role in Al resistance. In aluminum-treated *L. esculentum*, pectinacetylesterase (SGN-U217232) was up-regulated by 1.51-fold. In addition, two genes (SGN-U223905 and SGN-U213455) for degrading xyloglucan, a constituent of the secondary cell wall, were enhanced by Al. Based on this analysis, we can conclude that enzymes that modify cell wall structures and components are actively affected by soluble Al. In order to define the function of each individual gene in terms of Al tolerance or sensitivity, more work needs to be done.

Once Al cations cross plasma membranes and enter the cytoplasm, they are excluded by ATPase pumps located in the plasmalemma (Kochian 1995), or are sequestrated into vacuoles (Taylor *et al.* 2000). Plant vacuolar H^+-translocation ATPase (V-ATPase, EC 3.6.1.34) generates an H^+ electro-chemical gradient across the tonoplast. V-ATPase is associated with various membranes of the secretory system such as the endoplasmic reticulum, provacuoles, vacuoles, and root-tip plasma membranes (Herman *et al.* 1994; Robinson *et al.* 1996). Hamilton *et al.* (2001) reported that enhanced expression of vacuolar H^+-ATPase (V-ATPase) was correlated with Al exposures in tolerant *T. aestivum* 'PT741'. In this study, several V-ATPase and interactive proteins (SGN-U232696, SGN-U212892, SGN-U219508, SGN-U213802, and SGN-U213767) were suppressed. Compared to tolerant genotypes, the suppression of V-ATPase and interactive proteins in *L. esculentum* roots could be responsible for its sensitivity to Al cations.

The multidrug efflux (MATE) system plays an important role in the exudation of phytochemicals in root tissues (Paulsen *et al.* 1996; Loyola-Vargas *et al.* 2007) or for the removal of toxins from cells (Morita *et al.* 2000). There are four known families of transporters that contain multidrug efflux systems. They are the major facilitator super family, the small multidrug resistance family, the resistance / nodulation / cell division family and the ATP-binding cassette super family. In this study, the MATE efflux protein (SGN-U215960) was induced by 1.26-fold whereas the ABC transporters (SGN-U229989 and SGN-U240910) were inhibited by 1.25-fold. Metallothionein protects plant cells against metal toxicity. The *wali1* induced by Al in *T. aestivum* is a metallothionein (Snowden and Gardner 1993). The tomato homolog (SGN-U212973) was also induced by Al stress.

The intracellular mechanism for adaptation or tolerance to Al is achieved through alteration of cellular components. It has been suggested that Al^{3+} ions enhance peroxidation of phospholipids which produce oxidative stress in membranes (Cakmak and Horst 1991; Yamamoto *et al.* 1997). Expression of genes for alleviating oxidative stress such as GST is correlated with Al tolerance (Ezaki *et al.* 2000). In this study, the expression of GSH from Al-treated tomato roots was dependent on isoforms, two were reduced (SGN-U216884 and SGN-U212747) while others were enhanced (SGN-U212756 and SGN-U218221). These results suggest that for the multiple gene family of GST, it is necessary to determine the behavior of individual isoform for their role in Al tolerance.

Proline provides cells with multiple protective functions against adverse conditions. Herbaceous plants such as *Pharbitis nil*, *Cassia occidentlis*, *Echinochloa colonum* and *Aeschynomene indica* had increased proline leaf content of when exposed to Al (Peng *et al.* 2006). The Δ1-pyrroline-5-carboxylate synthase (P5CS) is the rate-limiting enzyme in proline biosynthesis in plants. In this study, Al led to higher transcript level of P5CS tomato homologs (SGN-U216928 and SGN-U214573) that could lead to enhanced proline biosynthesis in the treated tomato roots.

Polyamines (putrescine, spermine, and spermidine) are present ubiquitously in all living organisms and participate in a number of physical processes under stressful conditions (Kasukabe *et al.* 2004; Mihoko *et al.* 2006; Pang *et al.*

2007; Kuznetsov and Shevyakova 2007). In higher plants, putrescine is the precursor for the production of spermine and spermidine. Putrescine can be made by the action of ornithine decarboxylase, or through the alternative arginine decarboxylase pathway (ADC). ADC is enhanced under stressful conditions such as lower pH levels (pH< 5.0) (Young and Galston 1983; Ye *et al.* 1997). Increased levels of free putrescine production may enhance the ability of tobacco cells to survive under acidic conditions (Hiatt and Malmberg 1988).

In this analysis, the Al solution was at pH of 4.5, the observed lower transcript level of arginine decarboxylase could be caused mainly by Al inhibition. Whether this change could affect putrescine production needs to be determined. Histamine is a stress regulator, the histidine decarboxylase, the enzyme that catalyzes the formation of histamine from L-histidine was induced by oxidant stress in mammalian cells (Höcker *et al.* 1998), or salt treatment in tomato roots (Ouyang *et al.* 2007). The same gene was also induced in the Al-treated tomato roots.

The association between Al stress and expression of transcriptional factors (bZIP, WRKY, Myb, TDR4) and signal transduction molecules (protein kinases, protein phosphatases) were identified through this study. Several structural genes showed different expression between tomato (Al sensitive) and other Al-tolerant genotypes. Future analysis will be focused on the functional studies of these genes in order to reveal the molecular control of Al toxicity in *L. esculentum*.

ACKNOWLEDGEMENTS

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Appendix Table 1 Al regulated regulatory genes in tomato root culture.

Unigene	Ratio (Al/ctrl)	Annotated genes
SGN-U231440	0.67	transcriptional activator FHA1 (*Nicotiana tabacum*)
SGN-U214011	0.7	DEAD/DEAH box helicase, putative (*Arabidopsis thaliana*)
SGN-U218022	0.79	PHAP2A protein (*Petunia hybrida*)
SGN-U217726	0.81	Myb family transcription factor (*A. thaliana*)
SGN-U214344	0.81	bZIP transcription factor (*N. tabacum*)
SGN-U212597	0.86	transcription factor Myb1 (*M. xiaojinensis*)
SGN-U214618	0.87	DEAD box RNA helicase, putative (*A. thaliana*)
SGN-U224037	0.87	transcription factor TINY, putative (*A. thaliana*)
SGN-U216323	0.87	Eukaryotic translation initiation factor 3 subunit 8 (eIF3 p110) (eIF3c)
SGN-U216333	0.87	Eukaryotic translation initiation factor 3 subunit 11 (eIF-3 p25) (eIF3k)
SGN-U212725	0.89	AAR92477 putative WRKY transcription factor 30 (*Vitis aestivalis*)
SGN-U219135	0.9	transcription factor JERF2 (*Lycopersicon esculentum*)
SGN-U223789	0.91	putative bHLH transcription factor (*A. thaliana*)
SGN-U219135	0.92	transcription factor JERF2 (*L. esculentum*)
SGN-U213317	0.93	transcription factor BTF3-related (*A. thaliana*)
SGN-U213967	1.06	bZIP transcription factor ATB2 (*Glycine max*)
SGN-U220349	1.06	homeobox transcription factor, putative (*A. thaliana*)
SGN-U216297	1.06	AP2 domain transcription factor, putative (*A. thaliana*)
SGN-U225100	1.07	myb family transcription factor (*A. thaliana*)
SGN-U217032	1.09	RING/C3HC4/PHD zinc finger-like protein (*C. melo*)
SGN-U219049	1.1	putative RING-H2 zinc finger protein (*Oryza sativa*)
SGN-U230686	1.1	transducin / WD-40 repeat protein family (*A. thaliana*)
SGN-U219340	1.11	SET-domain transcriptional regulator family (*A. thaliana*)
SGN-U213659	1.12	TDR4 transcription factor (*L. esculentum*)
SGN-U219817	1.13	BAD68776 putative WRKY transcription factor (*O. sativa*)
SGN-U217359	1.13	ethylene-responsive transcriptional coactivator (*L. esculentum*)
SGN-U215416	1.13	transcriptional regulatory protein-related (*A. thaliana*)
SGN-U218469	1.17	Myb-like protein P (*Saussurea medusa*)
SGN-U217064	1.17	CCAAT-binding transcription factor subunit A(CBF-A) (*A. thaliana*)
SGN-U233184	1.18	MADS-box protein 15 (*P. hybrida*)
SGN-U218870	1.2	Dof zinc finger protein (*A. thaliana*)
SGN-U223134	1.22	CHP-rich zinc finger protein, putative (*A. thaliana*)
SGN-U220567	1.22	MADS-box JOINTLESS protein (LeMADS)
SGN-U215022	1.26	bHLH protein family (*A. thaliana*)
SGN-U213659	1.27	TDR4 transcription factor (*L. esculentum*)
SGN-U218469	1.29	Myb-like protein P (*S. medusa*)
SGN-U220213	1.29	MADS box protein TDR3 - tomato (fragment)
SGN-U215123	1.32	WRKY family transcription factor (*A. thaliana*)
SGN-U215777	1.35	probable transcription factor SF3 - common tobacco
SGN-U215264	1.41	MADS-box protein 15 (*P. hybrida*)
SGN-U213967	1.42	bZIP transcription factor ATB2 [*Glycine max*] (SGN-U213967) (10645/7502)
SGN-U217015	1.59	bZIP transcription factor (*N. tabacum*)
SGN-U218671	1.85	AP2 domain transcription factor, putative (*A. thaliana*)

Appendix Table 2 Al regulated genes in signaling transduction in tomato roots.

Unigene	Ratio (Al/ctr)	Annotation
SGN-U215435	0.54	receptor protein kinase PERK1-like protein (*O. sativa*)
SGN-U217507	0.83	leucine-rich repeat transmembrane protein kinase, putative (*A. thaliana*)
SGN-U224686	0.84	protein kinase family (*A. thaliana*)
SGN-U214118	0.85	protein phosphatase (*A. thaliana*)
SGN-U217316	0.86	protein kinase family (*A. thaliana*)
SGN-U213820	0.87	mitogen-activated protein kinase 4 (*Petroselinum crispum*)
SGN-U225194	0.88	protein phosphatase (*Fagus sylvatica*)
SGN-U225548	0.88	protein phosphatase 2C (PP2C), putative (*A. thaliana*)
SGN-U228065	0.90	calcium-dependent protein kinase (*L. esculentum*)
SGN-U221567	0.90	receptor protein kinase-related protein (*A. thaliana*)
SGN-U215442	0.91	protein kinase-related (*A. thaliana*)
SGN-U220727	0.91	rac GTPase activating protein 2 [*Lotus japonicus*]
SGN-U217316	0.92	protein kinase family (*A. thaliana*)
SGN-U215180	0.92	cyclin-dependent protein kinase p34cdc2 (*L. esculentum*)
SGN-U216184	0.95	mitogen-activated protein kinase 1 (*L. esculentum*)
SGN-U214656	1.06	shaggy-related protein kinase etha (EC 2.7.1.-) (*A. thaliana*)
SGN-U222701	1.07	CBL-interacting protein kinase (*Brassica napus*)
SGN-U227773	1.09	MAP3K-like protein kinase [imported] – *A. thaliana*
SGN-U216018	1.14	protein phosphatase-2C; PP2C (*Mesembryanthemum crystallinum*)
SGN-U231291	1.15	leucine-rich repeat transmembrane protein kinase, putative (*A. thaliana*)
SGN-U214714	1.15	CBL-interacting protein kinase 1 (*A. thaliana*)
SGN-U218431	1.15	type 5 serine/threonine phosphatase 55 kDa isoform [*Lycopersicon esculentum*]

Appendix Table 2 (Cont.)

Unigene	Ratio (Al/ctr)	Annotation
SGN-U213418	1.16	phosphoprotein phosphatase (EC 3.1.3.16) 2A regulatory chain - common tobacco
SGN-U218369	1.18	probable protein kinase - tomato
SGN-U221648	1.22	receptor kinase-related (*A. thaliana*)
SGN-U213944	1.27	shaggy protein kinase 4 (EC 2.7.1.-) - garden petunia
SGN-U215359	1.28	1-aminocyclopropane-1-carboxylate oxidase homolog (Protein E8)
SGN-U213441	1.35	Serine/threonine Kinase (*Persea americana*)
SGN-U215542	1.35	Serine/threonine protein phosphatase PP1 isozyme 3
SGN-U213441	1.57	Serine/threonine Kinase (*P. americana*)
SGN-U226314	1.70	protein phosphatase 2C (PP2C), putative (*A. thaliana*)
SGN-U217205	1.73	calcium-dependent protein kinase 2 (*N. tabacum*)
SGN-U212956	3.13	*S*-adenosylmethionine synthetase 3 (AdoMet synthetase 3)

Appendix Table 3 Al regulated transporter genes in tomato roots.

Unigene ID	Ratio (Al/ctrl)	Annotated genes
SGN-U216185	0.80	AAA-type ATPase family
SGN-U212892	0.81	vacuolar ATPase subunit B (*M. crystallinum*)
SGN-U219831	0.85	VAMP (vesicle-associated membrane protein)-associated protein family (*A. thaliana*)
SGN-U219508	0.86	putative vacuolar protein sorting-associated protein (*O. sativa*)
SGN-U240910	0.86	ABC protein
SGN-U229989	0.88	ABC transporter family protein (*A. thaliana*)
SGN-U213767	0.89	putative vacuolar proton ATPase subunit E (*L. esculentum*)
SGN-U216601	0.91	calcium-transporting ATPase (*A. thaliana*)
SGN-U213544	0.91	Calmodulin 2
SGN-U232696	0.93	Vacuolar ATP synthase subunit G 1 (V-ATPase G subunit 1) (Vacuolar proton pump G subunit 1)
SGN-U213802	0.93	proton pump interactor (*A. thaliana*)
SGN-U219955	1.09	Ca2+/H+-exchanging protein - mung bean
SGN-U215960	1.10	MATE efflux protein - related (*A. thaliana*)
SGN-U221263	1.4	major intrinsic protein (MIP) family (*A. thaliana*)
SGN-U216058	1.13	cyclic nucleotide-gated calmodulin-binding ion channel (*N. tabacum*)
SGN-U216066	1.15	Vacuolar ATP synthase subunit G 2 (V-ATPase G subunit 2) (Vacuolar proton pump G subunit 2)
SGN-U215374	1.19	34 kDa outer mitochondrial membrane protein porin (Voltage-dependent anion-selective channel protein) (VDAC) (POM 34)
SGN-U215681	1.20	Mitochondrial import receptor subunit TOM20 (Translocase of outer membrane 20 kDa subunit)
SGN-U216736	1.24	calcium ATPase (*M. crystallinum*)
SGN-U215960	1.26	MATE efflux protein-related (*A. thaliana*)
SGN-U215906	1.31	integral membrane protein-related (*A. thaliana*)
SGN-U212659	1.65	Vacuolar ATP synthase 16 kDa proteolipid subunit
SGN-U217777	1.66	mitochondrial carrier protein family (*A. thaliana*)
SGN-U212973	2.77	Metallothionein-like protein - tomato

Appendix Table 4 Al regulated oxidative-stress related genes in tomato roots.

Unigene ID	Ratio (Al/ctrl)	Annotated genes
SGN-U216884	0.77	glutathione s-transferase GST 23 (*G. max*)
SGN-U212747	0.87	Probable glutathione *S*-transferase (Pathogenesis-related protein 1)
SGN-U219384	0.89	germin-like protein (GLP6) (*A. thaliana*)
SGN-U221959	0.90	glutaredoxin protein family (*A. thaliana*)
SGN-U213345	0.92	putative peroxidase (*Solanum tuberosum*)
SGN-U212963	0.93	glycolate oxidase -related (*A. thaliana*)
SGN-U214598	1.07	thioredoxin peroxidase 1 (*L. esculentum*)
SGN-U214170	1.09	thylakoid-bound ascorbate peroxidase (*N. tabacum*)
SGN-U218426	1.15	glyoxalase family protein (lactoylglutathione lyase family protein) (*A. thaliana*)
SGN-U218221	1.16	glutathione S-transferase GST 19 (*G. max*)
SGN-U218715	1.19	ascorbate oxidase promoter-binding protein AOBP - winter squash
SGN-U220344	1.20	glyoxalase I, putative (lactoylglutathione lyase) (*A. thaliana*)
SGN-U212756	1.26	putative glutathione *S*-transferase T2 (*L. esculentum*)
SGN-U223144	1.44	flavodoxin family (*A. thaliana*)

Appendix Table 5 Al regulated genes in secondary metabolites in tomato roots.

Unigene ID	Ratio (Al/ctr)	Gene annotation
SGN-U213123	0.67	arginine decarboxylase 1 (*Datura stramonium*)
SGN-U214174	1.09	4-coumarate-CoA ligase 1 (4CL 1) (4-coumaroyl-CoA synthase 1)
SGN-U214428	1.09	betaine-aldehyde dehydrogenase (EC 1.2.1.8) [imported] - *Amaranthus hypochondriacus*
SGN-U213921	1.14	prephenate dehydratase family (*A. thaliana*)
SGN-U216928	1.19	Δ-1-pyrroline-5-carboxylate synthetase (P5CS)
SGN-U214573	1.24	Δ-1-pyrroline-5-carboxylate dehydrogenase precursor (*A. thaliana*)
SGN-U221960	1.26	β-carotene hydroxylase (*L. esculentum*)
SGN-U218415	1.28	uracil phosphoribosyltransferase (UMP pyrophosphorylase) (UPRTase)
SGN-U220233	1.29	isochorismatase hydrolase family (*A. thaliana*)
SGN-U212591	1.85	histidine decarboxylase (HDC) (TOM92)
SGN-U212595	2.24	histidine decarboxylase (HDC) (TOM92)

Appendix Table 6 Al regulated genes in cell cycle and cell wall metabolism in tomato roots.

Unigene ID	Ratio (Al/ctr)	Gene annotation
SGN-U227174	0.81	pectinesterase family (*A. thaliana*)
SGN-U213366	0.84	GDP-mannose pyrophosphorylase (*S. tuberosum*)
SGN-U232818	0.89	fiber protein Fb15 (*Gossypium barbadense*)
SGN-U217551	0.89	cyclin family (*A. thaliana*)
SGN-U242934	0.90	histone H3
SGN-U212775	0.95	pectate lyase (*Malus domestica*)
SGN-U215180	0.92	cyclin-dependent protein kinase p34cdc2 (*L. esculentum*)
SGN-U215379	1.09	fiber protein Fb11 (*G. barbadense*)
SGN-U213455	1.10	xyloglucan endo-1,4-β-D-glucanase (EC 3.2.1.-) precursor (clone tXET-B1) - tomato
SGN-U213862	1.10	phytochelatin synthetase-like protein (*A. thaliana*)
SGN-U212922	1.13	P1(p14) protein (*L. esculentum*)
SGN-U212648	1.25	α-tubulin (*N. tabacum*)
SGN-U223905	1.26	probable xylan endohydrolase isoenzyme F9K23.10 [imported] - (*A. thaliana*)
SGN-U212581	1.42	polygalacturonase 2A precursor (PG-2A) (pectinase)
SGN-U217232	1.52	pectinacetylesterase, putative (*A. thaliana*)