

## Transcriptomic analysis of tomato lines reveals putative stress-specific biomarkers

Monther SADDER<sup>1,2,\*</sup>, Abdullah ALSADON<sup>1</sup>, Mahmoud WAHB-ALLAH<sup>2,3</sup>

<sup>1</sup>Department of Plant Production, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia

<sup>2</sup>Department of Horticulture and Crop Science, Faculty of Agriculture, The University of Jordan, Amman, Jordan

<sup>3</sup>Vegetable Crops Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

Received: 04.12.2013 • Accepted: 31.03.2014 • Published Online: 15.08.2014 • Printed: 12.09.2014

**Abstract:** Different abiotic stresses recruit dedicated signaling and regulatory genes in plants. Genome-wide stress-specific biomarkers were investigated in tomatoes. Three major abiotic stresses were compared: drought, heat, and salinity. For each stress type, 2 different tomato lines were included: susceptible and tolerant. Gene expression was examined by hybridizing to an available tomato microarray. Several stress responsive genes were upregulated in tolerant as well as in susceptible lines for each stress. Comparative analysis of gene expression in response to stress (drought, heat, or salinity) resolved a number of common biomarkers, while other groups of putative biomarkers were associated with each abiotic stress. MYB transcription factors, SAUR family proteins, and NAC domain proteins were among the highly upregulated genes under drought, while both proteinase inhibitors and heat shock proteins were prominent in the heat-tolerant line. For salinity stress, the expression of phosphate starvation-induced proteins was observed. Putative abiotic stress biomarkers can be utilized in breeding programs to improve the selection process and to aid in gene stacking.

**Key words:** Biomarkers, drought, heat, microarray, salinity, tomato

### 1. Introduction

Drought, heat, and salinity are major abiotic stress factors affecting plant growth and productivity. A profound understanding of physiology, genetics, and molecular biology is important for breeding tolerant plants (Foolad, 2004). In the last decade, several DNA markers have been developed for tomato and related crops (Areshchenkova and Ganai, 1999; Poysa et al., 2003). Biomarkers, which are mainly applied to human studies, can potentially be deployed for crop plants. Current applications of biomarkers include cancer research and diagnostics, personalized medicine, and drug response (Rolan et al., 2003). Genome-wide biomarkers can facilitate tomato research, particularly for genetic analysis. In addition, they can be used in breeding to improve important traits such as yield, fruit quality, and resistance to biotic stresses and tolerance to abiotic stresses.

In tomatoes, both stress-specific responsive genes (Sun et al., 2010) and general responsive genes (Orellana et al., 2010) were identified. However, there is cross-talk between plant signaling pathways under different abiotic stresses (Knight and Knight, 2001; Albacete et al., 2010). Calcium-signaling genes have been reported to be upregulated in response to both cold and salinity stresses (Mahajan and

Tuteja, 2005). The exposure of drought-stressed plants to heat was shown to induce unique metabolic responses (Krasensky and Jonak, 2012). This cross-talk between various stresses occurs at an upper regulatory level, such as transcription factors. Such factors activate a wider network of genes and could have deleterious effects on total plant performance (Wang et al., 2003). Therefore, it is vital to study the unique molecular mechanisms underlying signaling components for each abiotic stress.

Expression profiling is an important tool to study plant responses to abiotic stresses, such as transcriptional characterization of tomato roots under iron deficiency stress (Zamboni et al., 2012). In some cases, the transcriptional changes can lead to successful adaptation and tolerance. However, if plants fail to adapt to the stressful environment, they are considered sensitive to that condition. Therefore, expression profiling can define both tolerant and sensitive plant responses (Rai et al., 2010). These profiles can lead to specific regulators to elevate stress tolerance and can be used as tools to study regulatory genes (Hazen et al., 2003).

It is possible to detect differences in steady-state transcript accumulation derived from diverse conditions by comparing cDNAs derived from multiple types, or

\* Correspondence: msadder@ksu.edu.sa

from a single type under different conditions. Such differentially expressed products can be identified and sequenced (Liu and Baird, 2003). However, understanding the components and targets of abiotic stress networks needs a holistic approach (Zhu, 2002; Chinnusamy et al., 2005; Munns and Tester, 2008; Amtmann, 2009). The use of DNA microarrays can provide insights into tissue-, developmental-, and environmental stimuli-specific genes. Microarray profiling was found to be useful for analyzing gene expression patterns under stress conditions (Cushman and Bohnert, 2000; Kawasaki et al., 2001; Ma et al., 2006). The objective of this study was to analyze genome-wide biomarkers related to abiotic stresses (drought, heat, and salinity) in tomatoes utilizing 2 lines (susceptible and tolerant) per stress.

## 2. Materials and methods

### 2.1. Plant material and stress treatments

Three abiotic stresses were investigated (drought, heat, and salinity). For each stress, 2 extreme tomato lines were included. Drought stress was applied to drought-tolerant line EC520061 and drought-susceptible line CO-3 (Rai et al., 2010). Heat stress was applied to heat-tolerant line PS-1 and heat-susceptible line H-24 (Rai et al., 2010), while salinity stress was applied to salinity-tolerant line L56 and salinity-susceptible line L46 (Alsadon et al., 2013). Plants were grown under optimal conditions for tomato plants in a greenhouse. Drought stress was applied by withholding water for 7 days, while heat stress was applied by subjecting the plants to 40 °C for 60 min in a growth chamber before sample collection (Rai et al., 2010). Salinity stress (9.6 dS m<sup>-1</sup>) was applied 5 days after transplanting through a drip irrigation system (Alsadon et al., 2013).

### 2.2. Labeling and hybridization

Leaf samples were collected at the flowering stage (75 days). Each line under each stress was represented by 3 biological replicates, each representing a different sample. Total RNA was isolated using a dedicated kit (QIAGEN, USA) and antisense RNA was synthesized and labeled with the GeneChip 3' IVT Express Kit (Affymetrix, USA). Labeled samples were hybridized to Affymetrix GeneChip tomato genome arrays, processed, and scanned; CEL files were generated by the Affymetrix Expression Console.

### 2.3. Data analysis

Data normalization and statistical analysis were performed with ArrayStar 5 software (DNASTAR, USA). Data were normalized using robust multiarray analysis with quantile normalization and were log-transformed. For statistical comparisons of relative expression between pairs of lines, Student's t-test was employed with the FDR < 0.05 (Benjamini-Hochberg) multiple testing correction algorithm. Heat maps were generated by

hierarchical clustering using Euclidean distance metrics and gene expression overlaps were presented as Venn diagrams. Enrichment of gene ontology (GO) annotation was determined by  $P < 0.05$  using the hypergeometric probability distribution. The tomato Affymetrix array was annotated using Blast2Go ([www.blast2go.com](http://www.blast2go.com)).

### 2.4. Real-time PCR

A group of probe sets were tested to verify expression using quantitative PCR (qPCR) with 3 replicates. Corresponding genes were retrieved from the tomato genome (<http://solgenomics.net/>) and primers were designed to span an intron when possible (Table 1). First-strand cDNA was generated by reverse transcriptase (Promega, USA) and expression was amplified with SYBR Green mix (QIAGEN). Amplification data were collected with an ABI 7500 thermal cycler (ABI, USA). Actin was used a reference gene and fold-change in gene expression was determined from  $C_T$  values using the  $2^{-\Delta\Delta C_T}$  method (Livak and Schmittgen, 2001).

## 3. Results

### 3.1. Tomato under drought stress

When comparing differentially expressed genes under drought stress, prominent genes could be identified in the drought-tolerant line as compared to the susceptible line (Table 2). The upregulated genes in the drought-tolerant line were related to energy, plant hormones, and cation transporters. The number of genes upregulated 2-, 3-, 4-, and 5-fold in the drought-tolerant line compared to the susceptible line were 3010, 1680, 1035, and 734 genes, respectively. On the other hand, 1974, 1172, 784, and 586 genes were upregulated in the drought-susceptible line by 2-, 3-, 4-, and 5-fold, respectively.

When the 3010 upregulated genes (2-fold) were compared between the drought-tolerant line and other tolerant lines (heat and salinity), 147 and 82 genes were found to be shared with the heat-tolerant and salinity-tolerant lines, respectively (Figure 1). The unique 2777 genes associated with drought tolerance were GO-enriched (Table 3). We found genes related to regulation of biosynthetic processes as well as transferase activities and cation binding, such as magnesium and calcium. Some putative drought-associated genes covered up to 85.7% of all array genes with the similar GO term.

### 3.2. Tomato under heat stress

Comparing differentially expressed genes under heat stress revealed unique genes in the heat-tolerant line compared to the susceptible line (Table 2). Upregulated genes in the heat-tolerant line were related to protease inhibitors and transcription factors. Numbers of genes upregulated 2-, 3-, 4-, and 5-fold in the heat-tolerant line compared to the susceptible line were 389, 88, 41, and 30, respectively. In

**Table 1.** Real-time PCR primers for a group of tomato probes available in the Affymetrix array.

| Probe set ID       | Gene                         | Primer      | Sequence (5' to 3')          | Tm   | Product (bp) |
|--------------------|------------------------------|-------------|------------------------------|------|--------------|
| Les.3673.1.S1      | Beta-1,3-glucanase           | 02B_F       | AATAGAAAGGATGGAAAACCAAGTGAGC | 59.3 | 171          |
|                    |                              | 02B_R       | TGATATCAAGGAACACAAAAGAGGCC   | 58.6 |              |
| Les.3779.1.S1      | Class ii chitinase           | 06B_F       | GGGAAGTGGATTTTATGGCAGAGG     | 58.8 | 162          |
|                    |                              | 06 B_R      | GCGGTCATCCAGAACCATATTGC      | 59.2 |              |
| Les.3583.1.A1      | Pathogenesis-related protein | 08 B_F      | AAGCAAATGAACTTTGTTGAAGGTGG   | 58.7 | 178          |
|                    |                              | 08 B_R      | CACAACCTCCATTATCATTAGCTTCAAA | 58   |              |
| Les.3460.1.S1      | Cell wall invertase          | 09 B_F      | CAAGGTTCATGTGTTCCGATGC       | 59.4 | 162          |
|                    |                              | 09 B_R      | CCAGCACCAAAACTTCCACTATCG     | 59.8 |              |
| Les.3652.1.S1      | Endo-1,3-beta-D-glucosidase  | 12 B_F      | CGACTCTGCTGGTGATACTTATATTGGC | 59.5 | 179          |
|                    |                              | 12S_R       | GGCTTGGAGAGTTGGTTGATGAGG     | 59.8 |              |
| Les.3940.2.A1      | Na                           | 01S_F       | AAGGAATTGACTCTAACTTGATGTGCG  | 59.2 | 196          |
|                    |                              | 01S_R       | CCAAGATGTTATCAAAAAGACGAACTCG | 59.5 |              |
| Les.2173.1.A1      | Proteinase inhibitor i       | 07S_F       | CATGGCACGAAAAGAAAGTGATGG     | 59.4 | 162          |
|                    |                              | 07S_R       | TCATTTATGGATGGATTTTCCTTCCC   | 59.3 |              |
| Les.2964.3.A1      | Na                           | 13S_F       | CCGCCGAACTTCGCTTACC          | 58.4 | 155          |
|                    |                              | 13S_R       | CCTTGTTTTCTGCATGGTACTCGG     | 58.7 |              |
| LesAffx.62070.1.S1 | Pectate lyase                | 16S_F       | TCACTGGGAAATGTATGCCATTGG     | 59.5 | 162          |
|                    |                              | 16S_R       | TCACCTTCTGATCTCCAGTCCAGC     | 59.6 |              |
| Les.3620.1.S1      | AG1 transcription factor     | 26S_F       | ATCCAAAAGAATGAGCTGTTGTTTGC   | 59.8 | 170          |
|                    |                              | 26S_R       | CATGATAGTTTGATGAACTCCCTGGC   | 58.9 |              |
|                    | Actin7                       | S.lactin7_F | AGGATCCATCCTTGCATCACTTAGC    | 58.7 | 166          |
|                    |                              | S.lactin7_R | TAATTGCCCTTCTTTCATAGCCCC     | 58.6 |              |

**Table 2.** Upper 60 upregulated genes in tolerant lines as compared to susceptible lines.

| Drought-tolerant over susceptible |       | Heat-tolerant over susceptible    |      | Salinity-tolerant over susceptible |      |
|-----------------------------------|-------|-----------------------------------|------|------------------------------------|------|
| Gene                              | Fold  | Gene                              | Fold | Gene                               | Fold |
| Photosystem II subunit n          | 144.7 | Kunitz-type protease inhibitor    | 43.3 | Protein                            | 75.6 |
| Ribosomal protein s3              | 130.3 | Proteinase inhibitor ii           | 35.1 | Beta-1,3-glucanase                 | 31.2 |
| NADH-oxidoreductase               | 113.6 | Cysteine protease inhibitor       | 13.7 | Class ii chitinase                 | 13.5 |
| ATP synthase cf0 subunit iv       | 108.9 | Carboxypeptidase inhibitor        | 13.1 | Pathogenesis-related protein       | 12.4 |
| NADH-oxidoreductase               | 91.7  | Osmotin-like protein              | 7.7  | Pathogenesis-related protein       | 11.6 |
| Hypothetical protein              | 90.5  | Carbonic anhydrase                | 7.5  | Cell wall invertase                | 9.8  |
| Protein                           | 85.1  | Beta-d-glucan glucanohydrolase    | 7.0  | Plant cell wall protein sltfr88    | 8.8  |
| NADH dehydrogenase subunit 4      | 84.7  | Extensin                          | 6.7  | Lignin-forming peroxidase          | 8.5  |
| Histone h3                        | 80.8  | Arginase                          | 6.7  | Endo-1,3-beta-d-glucosidase        | 7.5  |
| Asr2, fruit-ripening protein      | 79.5  | Pathogenesis-related protein      | 6.2  | Protein-binding structural         | 7.1  |
| Gibberellin-induced protein       | 72.1  | Arginase 2                        | 6.2  | Glycine-rich protein               | 6.9  |
| Ribosomal protein s7              | 71.6  | Osmotin-like protein              | 5.0  | Protein kinase chloroplast         | 6.5  |
| ATP synthase cf0 subunit i        | 62.9  | Subtilisin-like protease          | 4.7  | Cytochrome p450                    | 6.5  |
| Transglucosylase                  | 60.7  | Cathepsin d inhibitor protein     | 4.5  | NAC domain protein                 | 6.5  |
| NADH-oxidoreductase               | 56.4  | Cysteine proteinase               | 4.2  | Lipase class 3 family protein      | 6.3  |
| EF-hand-containing                | 55.6  | Wound-induced protein win2        | 4.0  | Mads-box protein 9                 | 5.6  |
| Protein                           | 55.5  | Protein                           | 4.0  | Flavonol synthase flavanone 3      | 5.1  |
| Histone 2                         | 53.7  | Ferric-chelate reductase          | 3.6  | Nonspecific lipid transfer protein | 5.1  |
| Protein                           | 47.9  | Asparagine synthetase             | 3.3  | Pathogenesis-related protein 10    | 4.9  |
| Prosystemin                       | 45.9  | Wcrk1 (wcrk thio redoxin 1)       | 3.2  | F-box and wd40 domain              | 4.6  |
| Copia-like polyprotein            | 43.3  | Proteinase inhibitor i            | 3.2  | Phospholipase pldb1                | 4.6  |
| NADH dehydrogenase subunit d      | 42.8  | Adipocyte membrane-associated     | 3.2  | Protein                            | 4.3  |
| 40s ribosomal protein             | 42.3  | Type-a response regulator         | 3.1  | Calmodulin-binding                 | 4.2  |
| Protein phosphatase 2c abi2       | 40.6  | Short-chain alcohol               | 3.1  | Pre-rRNA-processing protein        | 4.1  |
| Ribosomal protein l22             | 40.2  | Alpha-l-arabinofuranosidase       | 3.0  | Calmodulin-binding                 | 4.0  |
| Cytochrome b6 f complex           | 39.7  | Subtilisin-like protease          | 3.0  | Arginase                           | 3.8  |
| ATP-dependent protease            | 37.5  | Xylem serine proteinase 1         | 2.9  | Respiratory burst oxidase          | 3.8  |
| Protein                           | 36.7  | Wound stress protein              | 2.9  | Tas14 peptide (aa 1-130)           | 3.7  |
| Sinapyl alcohol dehydrogenase     | 34.7  | Brassinosteroid-regulated protein | 2.8  | At1g68530 t26j14_10                | 3.7  |
| Protein                           | 34.6  | Katanin p60 ATPase-containing     | 2.8  | Class ii chitinase                 | 3.6  |
| Alpha beta fold family protein    | 34.6  | Endo-1,4-beta-glucanase           | 2.8  | Endomembrane-associated            | 3.5  |
| Rna polymerase beta subunit       | 33.8  | Beta-galactosidase                | 2.8  | Cinnamoyl reductase                | 3.5  |
| Auxin-responsive protein          | 32.8  | Hyoscyamine 6 beta-hydroxylase    | 2.8  | Cucumber peeling, dicyanin         | 3.5  |
| Ribosomal protein s12             | 32.6  | Subtilisin-like protease          | 2.7  | P-enolpyruvate carboxykinase       | 3.4  |
| Gdsl-motif lipase hydrolase       | 31.5  | Thio redoxin h                    | 2.7  | Protein                            | 3.4  |
| Caffeic acid o-methyltransferase  | 31.4  | AP2 erf transcription factor      | 2.7  | Calcium-dependent protein kinase   | 3.3  |
| Protein                           | 30.1  | Lipoxygenase                      | 2.6  | Protein                            | 3.3  |
| Ubiquitin-conjugating enzyme      | 30.0  | 6-Deoxocastasterone oxidase       | 2.6  | Calmodulin-like protein 15         | 3.2  |
| Protein                           | 28.6  | F-box family protein              | 2.6  | Euful fruitfull-like mads-box      | 3.2  |
| Cytochrome b6 f complex           | 28.5  | Phloem protein                    | 2.6  | Tyramine n-feruloyltransferase     | 3.2  |
| C-4 sterol methyl oxidase         | 27.6  | Alpha-expansin 4                  | 2.6  | Nitrate transporter                | 3.2  |
| Protein                           | 25.9  | Mucin-like protein                | 2.6  | Phosphatidic acid                  | 3.2  |
| Auxin-responsive protein          | 25.0  | Acyl:coa ligase                   | 2.5  | Longevity assurance                | 3.2  |
| Prosystemin                       | 25.0  | Alpha-expansin 13                 | 2.5  | Snak2_soltu ame                    | 3.1  |
| Myo-inositol-1-P- synthase        | 24.8  | Cytochrome p450                   | 2.4  | Tcp family transcription factor    | 3.1  |
| Cytochrome f                      | 24.3  | Serine carboxypeptidase cp-mii    | 2.4  | Protein                            | 3.1  |
| 40s ribosomal protein s9          | 24.1  | Beta-glucuronidase                | 2.4  | Pr protein                         | 3.0  |
| Endotransglucosylase-hydrolase    | 24.0  | Pyruvate decarboxylase            | 2.4  | Phytophthora-inhibited protease 1  | 3.0  |
| Arabinogalactan protein           | 23.5  | Class i chitinase                 | 2.4  | S locus glycoprotein like protein  | 3.0  |
| Ethylene-responsive helicase      | 23.0  | Hero resistance protein 1         | 2.4  | Cer1 protein                       | 3.0  |
| At5g25460 f18g18_200              | 22.9  | Protein                           | 2.3  | Unknown [glycine max]              | 2.9  |
| Strictosidine synthase            | 22.6  | Elf4-like protein                 | 2.3  | Cinnamoyl- reductase-like protein  | 2.9  |
| Ribosomal protein l2              | 22.3  | 21 kDa protein                    | 2.3  | Cytochrome p450                    | 2.9  |
| Nf-yb13 transcription factor      | 22.1  | ATP binding                       | 2.3  | Gamma-aminobutyrate isozyme 1      | 2.9  |
| Ribosomal protein s3              | 21.9  | Purine permease                   | 2.3  | Anthranilate n-benzoyltransferase  | 2.9  |
| Ubiquitin fusion protein          | 21.7  | Senescence-associated protein     | 2.3  | Cysteine protease tdi-65           | 2.9  |
| Protein                           | 21.2  | Protein                           | 2.3  | Rpm1-interacting protein 4         | 2.8  |
| Ca2+-transporting ATPase          | 20.8  | Protein kinase family protein     | 2.3  | AP2 erf transcription factor       | 2.8  |
| Protein                           | 20.6  | Peroxidase 12                     | 2.3  | Lysine-ketoglutarate reductase     | 2.8  |
| Vacuolar ATPase subunit h         | 20.3  | Cathepsin b-cysteine proteinase   | 2.3  | Protein                            | 2.8  |

**Table 3.** Enriched GO terms of genes at least 2-fold upregulated in the drought-tolerant line compared to the drought-susceptible line.

| GO term   | GO ID   | P-value  | Number of genes | Percentage array |
|---|---------|----------|-----------------|------------------|
| <b>Biological process</b>                                       |         |          |                 |                  |
| Cellular response to auxin stimulus                             | 71365   | 5.86E-04 | 16              | 72.7%            |
| Regulation of macromolecule biosynthetic process                | 10556   | 5.99E-04 | 63              | 42.9%            |
| Regulation of biosynthetic process                              | 9889    | 6.32E-04 | 63              | 42.9%            |
| Auxin-mediated signaling pathway                                | 9734    | 6.35E-04 | 16              | 72.7%            |
| Regulation of cellular metabolic process                        | 31323   | 6.61E-04 | 65              | 42.8%            |
| Regulation of cellular biosynthetic process                     | 31326   | 6.70E-04 | 63              | 42.9%            |
| Regulation of RNA biosynthetic process                          | 2001141 | 6.75E-04 | 62              | 43.7%            |
| Response to auxin stimulus                                      | 9733    | 6.92E-04 | 16              | 72.7%            |
| Regulation of cellular macromolecule biosynthetic process       | 2000112 | 7.11E-04 | 63              | 42.9%            |
| Nucleic acid metabolic process                                  | 90304   | 7.36E-04 | 53              | 44.9%            |
| Regulation of RNA metabolic process                             | 51252   | 7.50E-04 | 62              | 43.7%            |
| Regulation of nucleobase-containing compound metabolic process  | 19219   | 7.54E-04 | 65              | 44.8%            |
| Regulation of macromolecule metabolic process                   | 60255   | 7.62E-04 | 68              | 41.7%            |
| Regulation of transcription, DNA-dependent                      | 6355    | 8.44E-04 | 62              | 43.7%            |
| Biotin biosynthetic process                                     | 9102    | 8.69E-04 | 12              | 85.7%            |
| Biotin metabolic process  | 6768    | 1.02E-03 | 12              | 85.7%            |
| Regulation of gene expression                                   | 10468   | 1.10E-03 | 63              | 42.0%            |
| Cellular amide metabolic process                                | 43603   | 1.22E-03 | 12              | 85.7%            |
| Regulation of metabolic process                                 | 19222   | 1.27E-03 | 71              | 40.6%            |
| Regulation of cellular process                                  | 50794   | 1.31E-03 | 73              | 40.3%            |
| Regulation of nitrogen compound metabolic process               | 51171   | 1.51E-03 | 65              | 44.8%            |
| Amide biosynthetic process                                      | 43604   | 1.53E-03 | 12              | 85.7%            |
| Regulation of primary metabolic process                         | 80090   | 1.61E-03 | 69              | 42.9%            |
| Water-soluble vitamin biosynthetic process                      | 42364   | 2.40E-03 | 12              | 75.0%            |
| Regulation of biological process                                | 50789   | 2.43E-03 | 79              | 38.7%            |
| Vitamin biosynthetic process                                    | 9110    | 2.49E-03 | 12              | 75.0%            |
| Vitamin metabolic process                                       | 6766    | 2.59E-03 | 12              | 75.0%            |
| Cellular nitrogen compound metabolic process                    | 34641   | 2.68E-03 | 95              | 37.3%            |
| Water-soluble vitamin metabolic process                         | 6767    | 2.70E-03 | 12              | 75.0%            |
| Biological regulation   | 65007   | 3.12E-03 | 80              | 38.3%            |
| RNA metabolic process   | 16070   | 3.80E-03 | 44              | 43.6%            |
| Nucleobase-containing compound metabolic process                | 6139    | 3.96E-03 | 60              | 40.3%            |
| <b>Cellular component</b>                                       |         |          |                 |                  |
| Nucleus   | 5634    | 7.96E-05 | 77              | 44.0%            |
| Cytoplasmic part  | 44444   | 1.06E-03 | 16              | 12.2%            |
| <b>Molecular function</b>                                       |         |          |                 |                  |
| Magnesium ion binding   | 287     | 2.82E-04 | 18              | 78.3%            |
| Radical SAM enzyme activity                                     | 70283   | 7.08E-04 | 12              | 85.7%            |
| 4 Iron, 4 sulfur cluster binding                                | 51539   | 8.26E-04 | 12              | 85.7%            |
| 8-Amino-7-oxononanoate synthase activity                        | 8710    | 9.91E-04 | 12              | 85.7%            |
| Biotin synthase activity  | 4076    | 1.24E-03 | 12              | 85.7%            |
| Adenosylmethionine-8-amino-7-oxononanoate transaminase activity | 4015    | 1.66E-03 | 12              | 85.7%            |
| Sulfurtransferase activity                                      | 16783   | 1.81E-03 | 12              | 80.0%            |
| Transferase activity, transferring sulfur-containing groups     | 16782   | 2.01E-03 | 12              | 80.0%            |
| Cyclo-ligase activity   | 16882   | 2.26E-03 | 12              | 80.0%            |
| Dethiobiotin synthase activity                                  | 4141    | 2.48E-03 | 12              | 85.7%            |
| Protein dimerization activity                                   | 46983   | 3.28E-03 | 21              | 58.3%            |
| Transaminase activity   | 8483    | 3.47E-03 | 15              | 68.2%            |
| Calcium ion binding   | 5509    | 3.92E-03 | 1               | 2.6%             |
| Sequence-specific DNA binding                                   | 43565   | 4.58E-03 | 31              | 49.2%            |
| DNA binding   | 3677    | 8.58E-03 | 57              | 40.4%            |
| Methyltransferase activity                                      | 8168    | 1.20E-02 | 14              | 63.6%            |
| Transferase activity, transferring nitrogenous groups           | 16769   | 1.64E-02 | 17              | 56.7%            |
| Transferase activity, transferring one-carbon groups            | 16741   | 1.67E-02 | 15              | 60.0%            |
| Polygalacturonase activity                                      | 4650    | 2.14E-02 | 7               | 87.5%            |
| RNA binding   | 3723    | 2.57E-02 | 1               | 3.3%             |
| Metal cluster binding   | 51540   | 2.84E-02 | 14              | 58.3%            |
| Iron-sulfur cluster binding                                     | 51536   | 2.98E-02 | 14              | 58.3%            |
| Hydrolase activity  | 16787   | 3.75E-02 | 41              | 19.1%            |
| 2 Iron, 2 sulfur cluster binding                                | 51537   | 4.28E-02 | 12              | 60.0%            |

the heat-susceptible line, 549, 178, 81, and 44 genes were upregulated 2-, 3-, 4-, and 5-fold, respectively.

When the 389 upregulated genes (2-fold) were compared between the heat-tolerant line and other tolerant lines, 147 and 30 genes were found to be shared with the drought-tolerant and salinity-tolerant lines, respectively (Figure 1). The distinctive 208 genes associated with heat tolerance were GO-enriched (Table 4). We found genes related to negative regulation of catalytic activity, steroid biosynthetic processes, and the regulation of hormone levels as well as genes related to enzymatic activities like peptidase regulator activity, catalytic activity, and hydrolase activity. Some heat-associated genes demonstrated up to 100% coverage of all array genes with a similar GO term.

### 3.3. Tomato under salinity stress

Under salinity stress, comparing differentially expressed genes showed that prominent genes that could be identified in the salinity-tolerant line as compared to the susceptible line (Table 2). The upregulated genes in the salinity-tolerant line were related to transcription factors and calmodulins. The numbers of genes upregulated 2-, 3-, 4-, and 5-fold in the salinity-tolerant line compared with the susceptible line were 356, 92, 42, and 27, respectively. On the other hand, 365, 82, 34, and 17 genes were upregulated in the salinity-susceptible line by 2-, 3-, 4-, and 5-fold, respectively.

When the 356 upregulated genes (2-fold) were compared between the salinity-tolerant line and other tolerant lines, 82 and 30 genes were found to be shared with the drought- and heat-tolerant lines, respectively (Figure 1). The unique 240 genes associated with drought tolerance were likewise GO-enriched (Table 5). We found genes related to response to stimulus, response to stress, and defense responses as well as catalytic activity and beta-D-glucosidase activity. Certain salinity-associated genes showed up to 60% coverage of all array genes with a similar GO term.

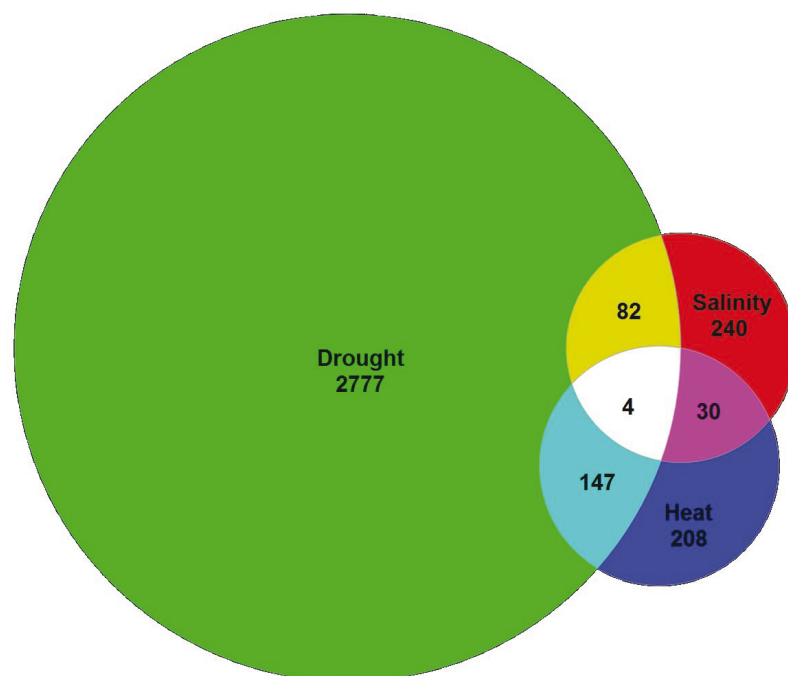
The qPCR performed for selected tomato probes revealed similar trends in fold increase for probes upregulated in the salinity-tolerant line (Figure 2a) and in the salinity-susceptible line (Figure 2b). However, some probes showed different fold-levels, e.g., probe Les.3940.2.A1 showed 20- and 5-fold increases in array and qPCR, respectively, while probe LesAffyx.62070.1.S1 showed 5- and 15-fold increases in array and qPCR, respectively (Figure 2b).

### 3.4. Putative stress-specific biomarkers

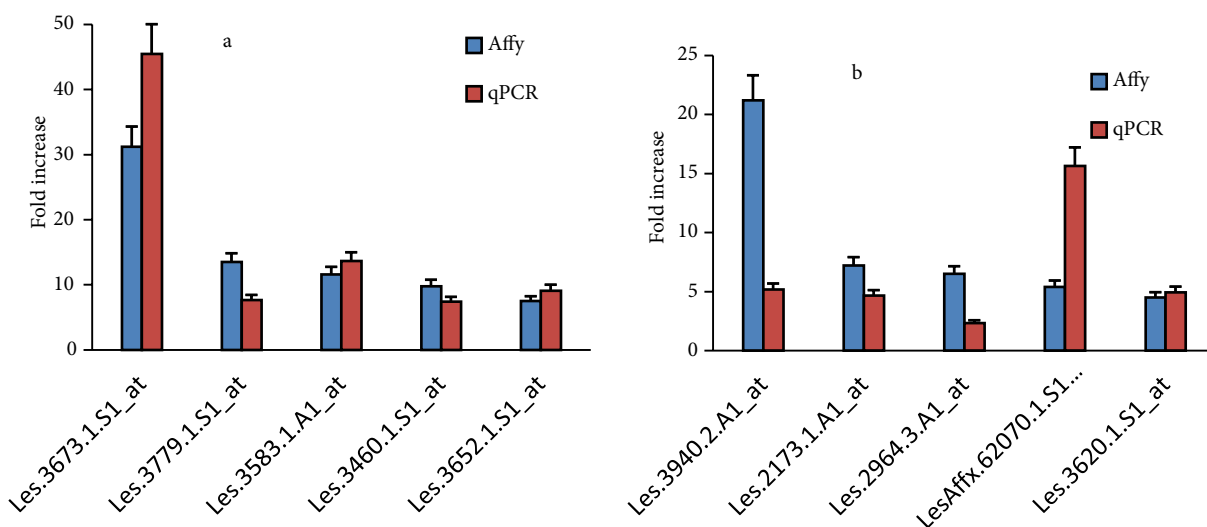
To identify important and unique abiotic responsive genes, another comparative analysis was performed utilizing the expression data of all stresses in tomatoes. Two heat maps were generated to determine important responsive gene clusters for each stress.

**Table 4.** Enriched GO terms of genes at least 2-fold upregulated in the heat-tolerant line compared to the heat-susceptible line.

| GO Term   | GO ID | P-value  | Number of genes | Percentage array |
|---|-------|----------|-----------------|------------------|
| <b>Biological process</b>   |       |          |                 |                  |
| Negative regulation of catalytic activity                             | 43086 | 2.75E-02 | 3               | 37.5%            |
| Negative regulation of molecular function                             | 44092 | 3.00E-02 | 3               | 37.5%            |
| Steroid biosynthetic process  | 6694  | 3.23E-02 | 2               | 100.0%           |
| Steroid metabolic process   | 8202  | 3.59E-02 | 2               | 100.0%           |
| Regulation of hormone levels  | 10817 | 4.03E-02 | 2               | 100.0%           |
| Brassinosteroid biosynthetic process                                  | 16132 | 4.61E-02 | 2               | 100.0%           |
| <b>Molecular function</b>   |       |          |                 |                  |
| Molecular_function  | 3674  | 1.04E-01 | 35              | 3.1%             |
| Enzyme regulator activity   | 30234 | 1.32E-02 | 5               | 17.9%            |
| Peptidase regulator activity  | 61134 | 3.45E-03 | 5               | 29.4%            |
| Peptidase inhibitor activity  | 30414 | 2.07E-03 | 5               | 29.4%            |
| Endopeptidase inhibitor activity                                      | 4866  | 2.59E-03 | 5               | 29.4%            |
| Endopeptidase regulator activity                                      | 61135 | 5.17E-03 | 5               | 29.4%            |
| Enzyme inhibitor activity   | 4857  | 6.86E-03 | 5               | 20.8%            |
| Catalytic activity  | 3824  | 1.83E-01 | 24              | 3.3%             |
| Hydrolase activity  | 16787 | 3.86E-03 | 16              | 7.4%             |
| Hydrolase activity, acting on glycosyl bonds                          | 16798 | 2.27E-03 | 9               | 11.7%            |
| Hydrolase activity, hydrolyzing O-glycosyl compounds                  | 4553  | 1.95E-03 | 9               | 11.7%            |
| Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds | 16810 | 3.58E-02 | 3               | 33.3%            |
| Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds | 16813 | 2.60E-03 | 3               | 75.0%            |



**Figure 1.** Venn diagram of genes upregulated at least 2-fold for tolerant lines compared to susceptible lines, each under its corresponding abiotic stress (drought, heat, and salinity).



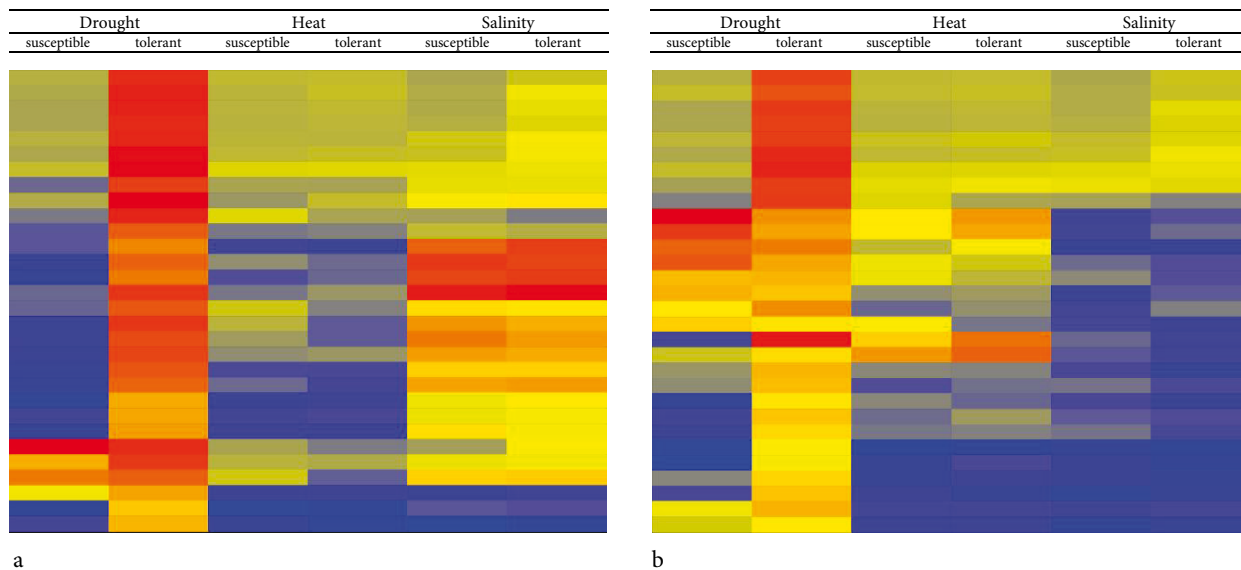
**Figure 2.** Comparison between Affymetrix (Affy) microarray and qPCR data, showing fold increase in upregulated genes in L56 (a) and in L46 (b).

The data showed more upregulated drought stress-responsive genes in the drought-tolerant line (under drought stress) than either the heat-tolerant line (Figure 3a) or the salinity-tolerant line (Figure 3b). On the other hand, another comparative heat map showed upregulated heat stress-responsive genes in both heat-tolerant and susceptible lines (under heat stress) as

compared to the drought-tolerant line (Figure 4a) and the salinity-tolerant line (Figure 4b). A third pair of heat maps showed upregulated salinity stress-responsive genes in both salinity-tolerant and salinity-susceptible lines (under salinity stress) as compared to the drought-tolerant line (Figure 5a) and the heat-tolerant line (Figure 5b).

**Table 5.** Enriched GO terms of genes at least 2-fold upregulated in the salinity-tolerant line compared to the salinity-susceptible line.

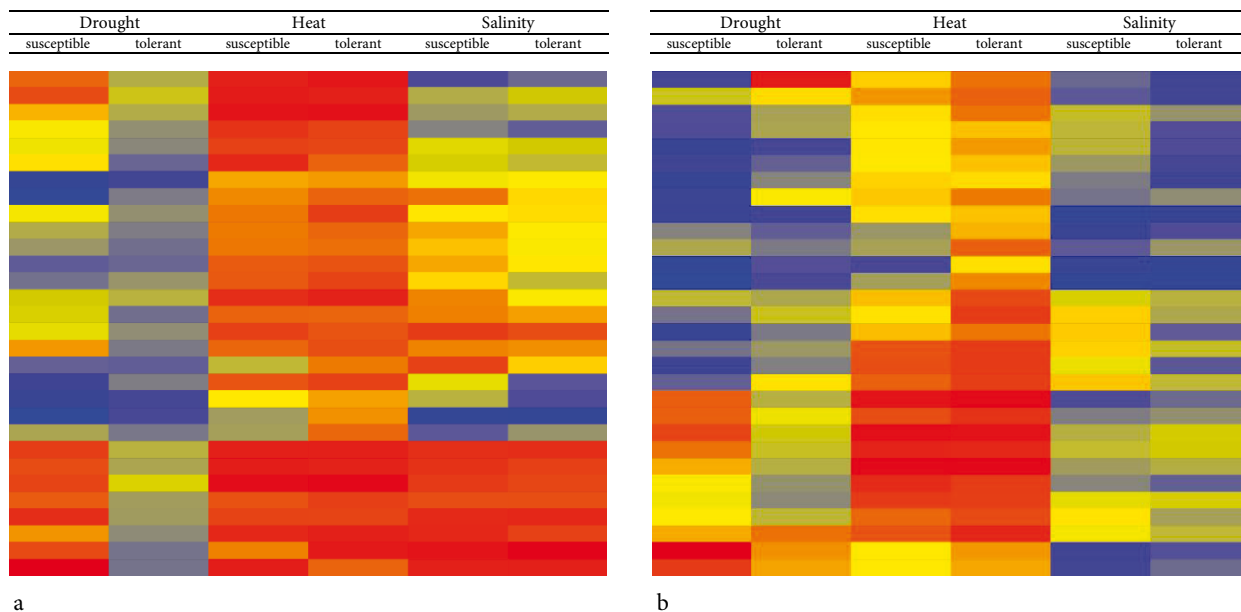
| GO Term                                     | GO ID | P-value  | Number of genes | Percentage array |
|---|-------|----------|-----------------|------------------|
| <b>Biological process</b>                   |       |          |                 |                  |
| Biological_process                          | 8150  | 1.62E-04 | 49              | 4.7%             |
| Response to stimulus                        | 50896 | 1.24E-03 | 15              | 8.9%             |
| Response to biotic stimulus                 | 9607  | 5.43E-05 | 7               | 36.8%            |
| Response to stress                          | 6950  | 7.42E-05 | 14              | 12.5%            |
| Defense response                            | 6952  | 6.28E-06 | 10              | 27.0%            |
| Metabolic process                           | 8152  | 7.18E-03 | 36              | 4.5%             |
| Primary metabolic process                   | 44238 | 1.38E-02 | 28              | 4.8%             |
| Carbohydrate metabolic process              | 5975  | 4.46E-02 | 11              | 7.6%             |
| <b>Cellular component</b>                   |       |          |                 |                  |
| Cellular_component                          | 5575  | 3.13E-02 | 26              | 4.4%             |
| Extracellular region part                   | 44421 | 1.63E-02 | 3               | 60.0%            |
| Extracellular region                        | 5576  | 1.87E-02 | 8               | 10.1%            |
| <b>Molecular function</b>                   |       |          |                 |                  |
| Molecular_function                          | 3674  | 3.19E-02 | 45              | 4.0%             |
| Catalytic activity                          | 3824  | 2.84E-02 | 34              | 4.7%             |
| Glucan endo-1,3-beta-D-glucosidase activity | 42973 | 3.80E-02 | 3               | 50.0%            |

**Figure 3.** Heat map of the first 30 upregulated genes based on fold change of the drought-tolerant line over the heat-tolerant line (a) or over the salinity-tolerant line (b).

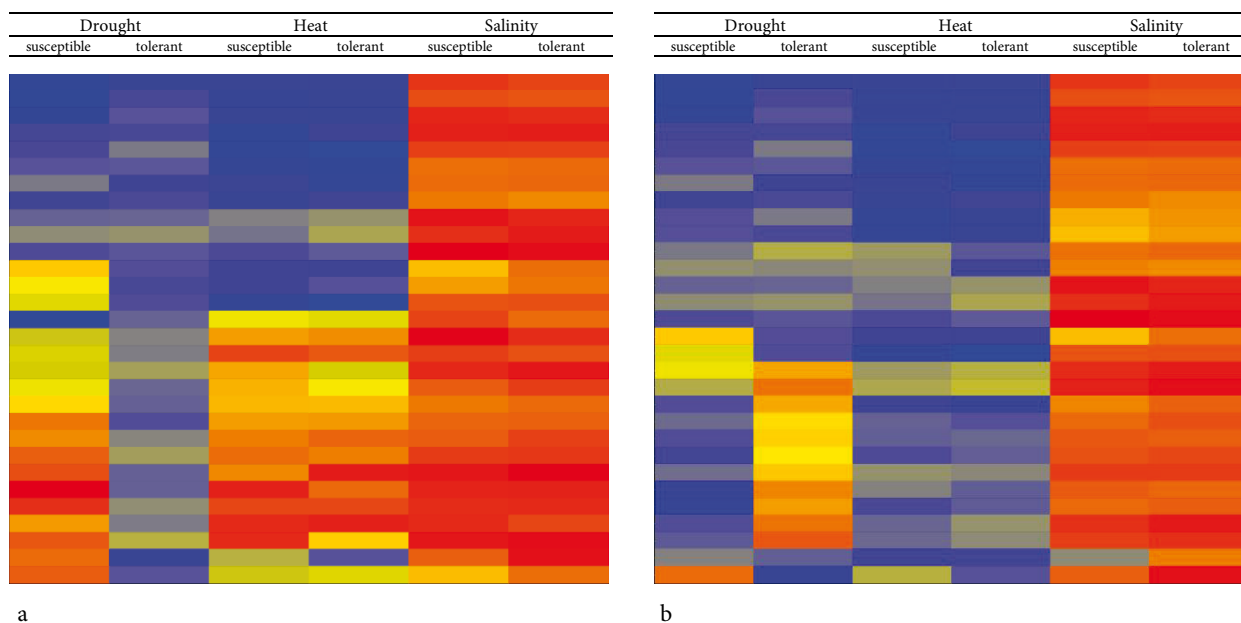
There were 3010 upregulated genes (2-fold) in the drought-tolerant line compared to the susceptible line. This group overlapped with the other 2 groups generated by comparing the drought-tolerant line to the other 2 tolerant lines (heat and salinity). The Venn diagram revealed 1214 common genes associated with drought

tolerance (Figure 6a). Additionally, 2 other similar comparisons were performed for heat and salinity stresses. The Venn diagrams revealed 95 common genes associated with heat tolerance (Figure 6b) and 82 common genes associated with salinity tolerance (Figure 6c).





**Figure 4.** Heat map of the first 30 upregulated genes based on fold change of the heat-tolerant line over the drought-tolerant line (a) or over the salinity-tolerant line (b).

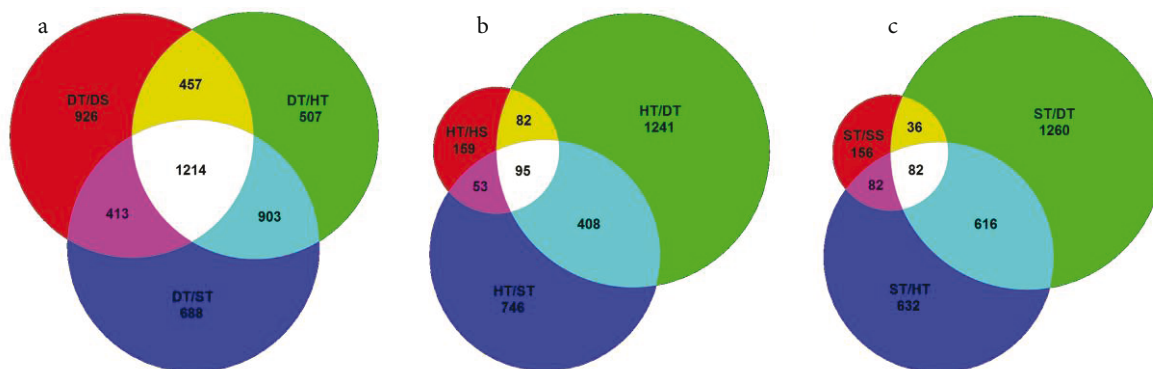


**Figure 5.** Heat map of the first 30 upregulated genes based on fold change of the salinity-tolerant line over the drought-tolerant line (a) or over the heat-tolerant line (b).

#### 4. Discussion

Distinguished upregulated genes were revealed in the drought-tolerant line under drought stress compared to the heat-tolerant line under heat stress (Figure 3a). This included important stress-responsive genes such as MYB transcription factors (Seo et al., 2009; Zhang L et

al., 2012). It also included SAUR family proteins, which are known for rapid induction by transient changes in environmental factors (Kant et al., 2009; Kodaira et al., 2011) and NAC domain proteins (Table 6). NAC domain (NAM, ATAF1, ATAF2, and CUC2) proteins are plant-specific transcription factors, which have crucial roles in



**Figure 6.** Venn diagrams of genes upregulated at least 2-fold for each tolerant line: (a) drought-tolerant line, (b) heat-tolerant line, (c) salinity-tolerant line. Each is compared to the 3 other lines. DT: Drought-tolerant, DS: drought-susceptible, HT: heat-tolerant, HS: heat-susceptible, ST: salinity-tolerant, SS: salinity-susceptible.

plant development, abiotic stress responses, defense, and leaf senescence (Chen et al., 2011). Moreover, some genes were upregulated under control conditions (no stress) in the drought-tolerant line (data not shown). Even though they were expressed at lower levels than under drought treatment, this expression represents an example of priming in the absence of any stress stimuli. The situation was more prominent when comparing the drought-tolerant line under drought stress to the salinity-tolerant line under salinity stress (Figure 3b). Upregulated genes in this comparison included SAUR family proteins and SNF4, which is an important stress signaling molecule in *Arabidopsis* (Halford et al., 2003) (Table 6).

Heat implies the deployment of an unusual set of plant genes to cope with the stress. Upregulated genes in the heat-tolerant line as compared to the drought-tolerant line under drought stress revealed both proteinase inhibitors and heat shock proteins (Table 7). Nonetheless, some genes were also upregulated in other lines (Figure 4a). Probe set LesAffx.286.2.S1 covering the MIP TIP subfamily, which is similar to aquaporin, was overexpressed in all lines under all conditions except in the drought-tolerant line. The MIP genes are known to respond to salinity stress (Zhu et al., 2005). The second set involved comparison of the heat-tolerant line under heat stress over the salinity-tolerant line under salinity stress (Figure 4b). This comparison is similar to the drought stress set, where comparing the heat-tolerant line under heat stress to the salinity-tolerant line under salinity stress revealed a prominent gene expression profile. Both proteinase inhibitors and heat shock proteins were among the upregulated genes (Table 7), which is similar to the earlier comparison (heat versus drought).

In the case of salinity stress, heat maps revealed a clustering of special stress-specific biomarkers (Figure 5). Upregulated genes in the salinity-tolerant line under salinity treatment as compared to the drought-tolerant line under drought condition revealed 2 major clusters (Figure

5a). The first was upregulated merely under salinity stress. This included Psi14A and Psi14B, which are phosphate starvation-induced proteins (Table 8). The second group was upregulated moderately in all other lines except the drought-tolerant. This included both the cathepsin D inhibitor protein and the trypsin proteinase inhibitor precursor. Expression of defense-related genes such as cathepsin D inhibitor and other wound-signaling genes (Herbers et al., 1994) were found to increase in response to 5 days of continual exposure of tomato plants to high salinity stress at 200 mM NaCl (Dombrowski, 2003). Abiotic stress can cause upregulation of several proteolytic enzymes in plants, which cleave defective and denatured proteins. In addition, these enzymes are crucial for the processing and activation of newly synthesized proteins (Mosolov and Valueva, 2011). The serine proteinase inhibitor (22 KDa) was found to accumulate after salinity stress in *Brassica napus* (L.) leaves (Reviron et al., 1992). Furthermore, the special trypsin proteinase inhibitor of a salinity-tolerant hybrid (wheat × *Agropyron*) was found to enhance salinity tolerance in transgenic *Arabidopsis* (Shan et al., 2008).

Our microarray transcriptome profiling revealed more salinity responsive genes with high expression in the tolerant tomato line than in the susceptible one, which agrees with the findings of Sun et al. (2010). In plants, salinity-tolerant lines were found to be primed for some highly influential responsive genes, which are constitutively overexpressed even under unstressed conditions (Taji et al., 2004). The salinity-tolerant line showed 3 probe sets similarly upregulated in PI365967 (Sun et al., 2010), namely cell wall peroxidase, TSI-1 protein, and flavonol synthase. The first 2 genes are grouped under defense, while the third is grouped under oxidoreductase. The probe set Les.3673.1.S1 (beta-1,3-glucanase) showed a 31.2-fold increase in the salinity-tolerant line. Several investigations reported beta-1,3-glucanase-related proteins to be

**Table 6.** The most upregulated probes in the drought-tolerant line over the salinity-tolerant line and over the heat-tolerant line, along with their annotation and fold increase.

| Drought-tolerant line over heat tolerant line |  |      | Drought-tolerant line over salinity tolerant line |   |       |
|---|--|------|---|---|-------|
| Probe set ID                                  | Gene title   | Fold | Probe set ID                                      | Gene title  | Fold  |
| LesAffx.18735.1.A1                            | Ribosomal protein s3   | 85.2 | Les.3983.1.S1                                     | Flower-specific gamma-thionin-like protein/acidic protein precursor | 380.8 |
| Les.3593.1.S1                                 | TAS14 peptide (AA 1-130)   | 79.8 | LesAffx.37707.1.A1                                | PREDICTED: hypothetical protein [ <i>Vitis vinifera</i> ]           | 87.7  |
| LesAffx.44224.1.S1                            | NADH-plastoquinone oxidoreductase subunit 1                        | 78.1 | Les.12.1.S1                                       | SNF4 protein  | 60.0  |
| LesAffx.3499.1.S1                             | ATP-dependent protease subunit                                     | 65.7 | Les.4317.1.S1                                     | Asparagine synthetase   | 50.2  |
| Les.4930.1.A1                                 | Asr2, fruit-ripening protein                                       | 62.8 | LesAffx.38821.1.S1                                | Cytochrome p450   | 42.4  |
| LesAffx.37707.1.A1                            | PREDICTED: hypothetical protein [ <i>Vitis vinifera</i> ]          | 52.5 | Les.2975.2.S1                                     | Aconitate hydratase, metallothionein II-like protein                | 40.3  |
| LesAffx.44474.1.A1                            | NADH dehydrogenase subunit 4                                       | 49.5 | LesAffx.64980.1.S1                                | Saur family protein   | 38.9  |
| LesAffx.66461.1.S1                            | Copia-like polyprotein   | 48.1 | Les.5028.1.S1                                     | Alpha beta fold family protein                                      | 28.6  |
| LesAffx.70834.1.S1                            | ATP synthase cf0 subunit iv  | 46.6 | Les.2934.3.A1                                     | Sinapyl alcohol dehydrogenase                                       | 27    |
| LesAffx.38821.1.S1                            | Cytochrome p450  | 44.0 | Les.5150.1.S1                                     | Amp-binding protein   | 25.7  |
| LesAffx.18338.1.S1                            | Photosystem ii subunit n   | 40.6 | LesAffx.68556.1.S1                                | 26s proteasome non-ATPase   | 24.8  |
| Les.986.1.S1                                  | ATP synthase cf0 subunit i   | 39.9 | LesAffx.34986.1.S1                                | Caffeic acid o-methyltransferase                                    | 23.9  |
| Les.462.1.S1                                  | Udp-glucose:protein transglucosylase, hypothetical LOC543664       | 38.8 | Les.5781.1.A1                                     | Histone 2   | 23.1  |
| LesAffx.66270.1.S1                            | Cbl-interacting serine threonine-protein                           | 36.5 | LesAffx.32198.1.S1                                | Jasmonate o-  | 22.6  |
| LesAffx.44224.1.A1                            | NADH -plastoquinone oxidoreductase subunit 1                       | 35.8 | Les.2084.1.S1                                     | NAC domain protein  | 21.9  |
| LesAffx.11323.1.S1                            | Cytochrome b6 f complex subunit iv                                 | 33.5 | LesAffx.59375.1.A1                                | Slt1 protein  | 21.8  |
| Les.5017.1.S1                                 | Myb transcription factor   | 31.7 | Les.462.1.S1                                      | Udp-glucose:protein transglucosylase, hypothetical LOC543664        | 21.7  |
| LesAffx.64980.1.S1                            | Saur family protein  | 31.2 | LesAffx.46519.1.S1                                | Seven-transmembrane-domain protein 1                                | 21.6  |
| LesAffx.33796.2.S1                            | Ribosomal protein s7   | 30.8 | Les.3365.3.S1                                     | Protein, dehydroascorbate reductase                                 | 21.2  |
| Les.5028.1.S1                                 | Alpha beta fold family protein                                     | 28.5 | Les.3365.2.S1                                     | Dehydroascorbate reductase  | 20.9  |
| Les.4149.3.S1                                 | EF-hand containing   | 27.8 | LesAffx.57775.2.A1                                | Protein phosphatase 2c  | 20.8  |
| Les.122.1.S1                                  | Class ii chitinase   | 27.6 | Les.1665.1.S1                                     | Lactoylglutathione lyase  | 19.2  |
| LesAffx.44202.1.S1                            | RNA polymerase beta subunit  | 27.3 | LesAffx.66461.1.S1                                | Copia-like polyprotein  | 19.1  |
| LesAffx.44474.1.S1                            | NADH dehydrogenase subunit d                                       | 26.0 | Les.4930.1.A1                                     | Asr2, fruit-ripening protein  | 18.9  |
| LesAffx.2632.2.S1                             | Homeobox protein   | 24.9 | LesAffx.67395.1.S1                                | Gibberellin receptor  | 16.8  |
| Les.4356.2.S1                                 | Pyruvate orthophosphate dikinase, cytosolic ascorbate peroxidase 2 | 24.5 | Les.502.1.S1                                      | Citrate synthase  | 16.6  |
| Les.2084.1.S1                                 | NAC domain protein   | 23.1 | LesAffx.40008.1.S1                                | 3 Exoribonuclease family domain 1-containing protein                | 16.6  |
| Les.5024.1.S1                                 | Fruitfull-like mads-box  | 21.8 | LesAffx.70769.1.S1                                | Aspartate aminotransferase  | 16.3  |
| Les.4461.1.S1                                 | Euful fruitfull-like mads-box, TDR4 transcription factor           | 21.5 | LesAffx.2632.2.S1                                 | Homeobox protein  | 15.6  |
| LesAffx.3499.2.S1                             | Ribosomal protein s12  | 21.4 | LesAffx.49191.1.A1                                | Uvb-resistance protein  | 15.6  |

**Table 7.** The most upregulated probes in the heat-tolerant line over the drought-tolerant line and over the salinity-tolerant line, along with their annotation and fold increase.

| Heat-tolerant line over drought tolerant line |  |       | Heat-tolerant line over salinity tolerant line |  |       |
|---|--|-------|--|--|-------|
| Probe set ID                                  | Gene title   | Fold  | Probe set ID                                   | Gene title   | Fold  |
| Les.3035.1.A1                                 | Cathepsin D inhibitor protein  | 194.2 | Les.269.1.S1                                   | Heat shock protein   | 224.2 |
| LesAffx.23349.1.S1                            | Germin-like protein  | 134.8 | Les.5150.1.s1                                  | Amp-binding protein  | 129.3 |
| Les.3739.1.S1                                 | Small heat shock protein   | 94.5  | Les.3677.1.s1                                  | Chloroplast small heat shock protein                                   | 127.7 |
| Les.3090.1.S1                                 | Histone h3   | 90.5  | Les.3983.1.s1                                  | Flower-specific gamma-thionin-like protein/acidic protein precursor    | 111.4 |
| Les.269.1.S1                                  | Heat shock protein   | 90.2  | Les.3739.1.s1                                  | Small heat shock protein   | 95.5  |
| Les.3011.1.S1                                 | Light dependent NADH:protochlorophyllide oxidoreductase 2  | 84.7  | Lesaffx.3918.1.S1                              | Ascorbate peroxidase   | 83.4  |
| Les.3991.1.S1                                 | Beta-xylosidase alpha-l-arabinosidase, LEXYL2 protein  | 76.9  | Lesaffx.5691.1.S1                              | Pathogenesis-related protein 1   | 53.0  |
| Les.3726.1.S1                                 | Ripening regulated protein DDTRF8  | 76.0  | Les.3581.1.S1                                  | Class II small heat shock protein Le-HSP17.6                           | 51.8  |
| Les.4150.1.S1                                 | Mitochondrial heat shock 22 kd, mitochondrial small heat shock protein                                   | 75.3  | Les.2173.1.a1                                  | Proteinase inhibitor i   | 51.2  |
| Les.4868.1.S1                                 | Ribulose biphosphate carboxylase activase  | 73.0  | Lesaffx.69215.1.s1                             | Leucine rich repeat protein  | 46.1  |
| Les.3677.1.S1                                 | Chloroplast small heat shock protein   | 70.5  | Les.4317.1.s1                                  | Asparagine synthetase  | 45.5  |
| Les.5850.1.S1                                 | Protochlorophyllide reductase precursor  | 70.3  | Les.513.1.s1                                   | Subtilisin-like protease   | 36.5  |
| Les.3700.1.S1                                 | Nonsymbiotic hemoglobin class 1  | 68.3  | Les.4820.1.s1                                  | Cysteine protease inhibitor, multicystatin                             | 34.8  |
| Les.5075.1.S1                                 | Ccaat-binding transcription factor subunit   | 65.2  | Lesaffx.63231.1.s1                             | Aspartic proteinase nepenthesin-1                                      | 34.4  |
| LesAffx.286.2.S1                              | Mip tip subfamily, similar to aquaporin  | 59.2  | Les.5442.1.s1                                  | Protein  | 32.6  |
| Les.4426.1.A1                                 | Metallothionein-like protein   | 58.4  | Les.2733.1.S1                                  | Wound/stress protein   | 32.1  |
| Les.4442.1.S1                                 | Histone h2   | 55.3  | Lesaffx.44139.1.s1                             | Lipid transfer protein   | 28.3  |
| Les.3581.1.S1                                 | Class II small heat shock protein Le-HSP17.6   | 55.0  | Les.3726.1.S1                                  | Ripening regulated protein DDTRF8                                      | 27.5  |
| Les.3740.1.S1                                 | Kunitz-type protease inhibitor precursor, inhibitor of yeast proteinase A; cathepsin D inhibitor protein | 53.7  | Les.4705.1.S1                                  | Phosphosulfolactate synthase-related protein                           | 26.6  |
| Les.3578.1.S1                                 | Cytosolic class II small heat shock protein HCT2   | 50.3  | Lesaffx.10596.1.S1                             | Heat shock protein 18  | 25.3  |
| Les.4857.2.S1                                 | Mutt domain  | 50.2  | Les.3578.1.s1                                  | Cytosolic class ii small heat shock protein hct2                       | 24.9  |
| Les.2476.1.S1                                 | Wound induced protein  | 49.0  | Les.2626.1.s1                                  | Wound stress protein   | 24.2  |
| Les.3209.1.S1                                 | Histone h4   | 47.5  | Les.2001.1.s1                                  | Hypothetical protein loc778362   | 24.1  |
| Les.22.1.S1                                   | 12-oxophytodienoate reductase  | 46.5  | Les.4150.1.s1                                  | Mitochondrial heat shock 22 kd, mitochondrial small heat shock protein | 23.4  |
| Les.4287.1.S1                                 | Pectin methyltransferase inhibitor protein 1   | 44.2  | Les.4307.1.s1                                  | Osmotin-like protein   | 22.3  |
| Les.3687.1.S1                                 | N-hydroxycinnamoyl-coa:tyramine N-hydroxycinnamoyl transferase THT7-1                                    | 43.2  | Lesaffx.69957.1.S1                             | Small heat-shock   | 21.9  |
| Les.3234.1.A1                                 | Ferredoxin precursor   | 42.0  | Les.228.1.s1                                   | Hypothetical loc543672   | 21.7  |
| Les.513.1.S1                                  | Subtilisin-like protease   | 41.7  | Les.5850.1.s1                                  | Protochlorophyllide reductase precursor                                | 21.7  |
| Les.1900.1.S1                                 | Sn-2 [Capsicum annum]  | 39.4  | Lesaffx.1276.2.S1                              | Pectate lyase  | 20.1  |
| LesAffx.5691.1.S1                             | Pathogenesis-related protein 1   | 38.5  | Les.4457.1.s1                                  | Epidermal germacrene c synthase, sesquiterpene synthase 1              | 19.4  |

**Table 8.** The most upregulated probes in the salinity-tolerant line over the drought-tolerant line and over the heat-tolerant line, along with their annotation and fold increase.

| Salinity-tolerant line over drought tolerant line |   |       | Salinity-tolerant line over heat tolerant line |   |       |
|---|---|-------|--|---|-------|
| Probe set ID                                      | Gene title  | Fold  | Probe set ID                                   | Gene title  | Fold  |
| Les.3408.1.S1                                     | PR protein  | 520.0 | Les.4024.1.S1                                  | Psi14a protein  | 429.9 |
| Les.3035.1.A1                                     | Cathepsin D inhibitor protein   | 272.2 | Les.3408.1.S1                                  | PR protein  | 259.0 |
| Les.4487.1.S1                                     | Retrotransposon protein   | 257.5 | Les.4487.1.s1                                  | Retrotransposon protein                                     | 244.3 |
| LesAffx.71662.1.S1                                | Senescence-associated protein   | 158.1 | Les.2672.1.s1                                  | Psi14b protein  | 204.7 |
| Les.2672.1.S1                                     | Psi14B protein  | 126.9 | Les.2672.1.S1                                  | Phosphatase, psi14a protein; psi14b protein                 | 185.3 |
| LesAffx.70764.1.S1                                | Ribulose- -bisphosphate carboxylase oxygenase large subunit                       | 106.8 | Lesaffx.3499.1.s1                              | ATP-dependent protease subunit                              | 158.6 |
| Les.3756.1.S1                                     | Trypsin proteinase inhibitor precursor  | 104.2 | Les.4693.1.s1                                  | Pathogenesis-related protein p4                             | 114.8 |
| LesAffx.29730.2.S1                                | ATPase f1 alpha subunit   | 95.1  | Lesaffx.33796.2.S1                             | Ribosomal protein s7  | 108.4 |
| Les.4426.1.A1                                     | Metallothionein-like protein  | 92.2  | Lesaffx.3499.2.A1                              | Ribosomal protein s12                                       | 101.9 |
| Les.22.1.S1                                       | 12-Oxophytodienoate reductase   | 90.2  | Lesaffx.71662.1.s1                             | Senescence-associated protein                               | 92.6  |
| Les.4024.1.S1                                     | Psi14a protein  | 82.4  | Les.3673.1.s1                                  | Beta-1,3-glucanase  | 89.4  |
| Les.3635.1.S1                                     | Xylem serine proteinase 1, subtilisin-like protease                               | 81.8  | Lesaffx.44474.1.a1                             | NADH dehydrogenase subunit 4                                | 88.2  |
| LesAffx.23349.1.S1                                | Germin-like protein   | 76.5  | Lesaffx.70764.1.s1                             | Ribulose- -bisphosphate carboxylase oxygenase large subunit | 80.7  |
| Les.4693.1.S1                                     | Pathogenesis-related protein P4   | 74.0  | Lesaffx.59441.1.S1                             | Ids4-like protein   | 78.8  |
| Les.2672.1.S1                                     | Phosphatase, psi14a protein; psi14b protein                                       | 68.6  | Lesaffx.51226.1.a1                             | Cytochrome f  | 78.3  |
| Les.2672.2.S1                                     | Psi14b protein  | 60.6  | Les.2672.2.s1                                  | Psi14b protein  | 77.5  |
| Les.4868.1.S1                                     | Ribulose bisphosphate carboxylase activase  | 58.5  | Lesaffx.44224.1.a1                             | NADH -plastoquinone oxidoreductase subunit 1                | 76.7  |
| Les.5567.1.S1                                     | Protein   | 52.3  | Les.2474.1.s1                                  | Cell elongation protein                                     | 76.1  |
| Les.3011.1.S1                                     | Light dependent NADH:protochlorophyllide oxidoreductase 2                         | 52.0  | Lesaffx.18735.1.S1                             | Ribosomal protein s3  | 68.1  |
| Les.3673.1.S1                                     | Beta-1,3-glucanase  | 51.4  | Lesaffx.70834.1.s1                             | ATP synthase cf0 subunit iv                                 | 67.4  |
| LesAffx.59441.1.S1                                | Ids4-like protein   | 49.4  | Les.3635.1.s1                                  | Xylem serine proteinase 1, subtilisin-like protease         | 65.1  |
| Les.3687.1.S1                                     | N-hydroxycinnamoyl-coa:tyramine N-hydroxycinnamoyl transferase THT7-1             | 49.3  | Lesaffx.8748.1.A1                              | TPSI1 protein   | 63.9  |
| LesAffx.71664.1.S1                                | ORF137 [ <i>Pinus koraiensis</i> ]  | 41.8  | Les.3683.1.S1                                  | Osmotin-like protein, PR-5x                                 | 61.2  |
| Les.4791.1.S1                                     | Ptac16 (plastid transcriptionally active 16) binding catalytic                    | 41.1  | Les.3981.1.s1                                  | Glucosyltransferase-like protein                            | 61.2  |
| LesAffx.837.1.S1                                  | WRKY transcription  | 40.5  | Lesaffx.71664.1.s1                             | Orf137 [ <i>Pinus koraiensis</i> ]                          | 57.9  |
| Les.3234.1.A1                                     | Ferredoxin precursor  | 40.2  | Les.4298.1.s1                                  | Photosystem i assembly protein ycf3                         | 57.1  |
| Les.5914.1.S1                                     | IDS4-like protein   | 37.5  | Les.218.3.S1                                   | Pectin methylesterase                                       | 52.6  |
| Les.4392.1.A1                                     | M030rath ame: full=uncharacterized mitochondrial protein g00030 ame: full=orf107a | 36.7  | Les.4399.2.s1                                  | Ribosomal protein l2  | 47.7  |
| Les.218.3.S1                                      | Pectin methylesterase   | 33.8  | Lesaffx.29730.2.s1                             | Atpase f1 alpha subunit                                     | 47.3  |
| LesAffx.64823.1.S1                                | Zinc finger (c3hc4-type ring finger) family protein                               | 31.8  | Les.2219.1.a1                                  | Conserved hypothetical protein [ <i>Ricinus communis</i> ]  | 47.0  |

important in response to salinity stress in sorghum (Swami et al., 2011), in grapes (Daldoul et al., 2008), and even in bacteria (Tamoi et al., 2007). On the other hand, the NAC domain proteins (Les.2569.1.S1) were upregulated in the salinity-tolerant line compared to susceptible line by 6.5-fold. Our findings are in agreement with those of Ouyang et al. (2007), who also found that expression of NAC domain proteins was linked with salinity stress based on suppression subtractive hybridization and microarray analysis. Another important stress biomarker is the APETALA2/ethylene-responsive element-binding protein (AP2/EREBP) transcription factor, which is an important responsive gene for both biotic and abiotic stresses, and it has cis-acting elements (Park et al., 2001; Zhang et al., 2005). The AP2 transcription factor (LesAffx.70768.1.S1) was likewise upregulated in the salinity-tolerant line compared to the susceptible line by 5.3-fold.

It is worth noting that exposure to salinity stress for the plant material described herein differs from that recorded by Sun et al. (2010). In our case, tissues were sampled from plants that were grown in a greenhouse for several weeks of continuous salinity stress (9.6 dS m<sup>-1</sup>, ca. 100 mM NaCl). This situation mimics an actual commercial production scheme for tomatoes. In contrast, the plant materials described by Sun et al. (2010) were artificially shocked for a short period (5 h) with 200 mM NaCl. This is a very high salt concentration, which is unusual for growing tomatoes, even for salinity-tolerant cultivars or hybrids. Therefore, screening under greenhouse production conditions would probably lead to a selection of robust lines carrying putative responsive genes and can lead to a reliable breeding program. Upregulated genes in susceptible lines could be byproducts of stress damage; however, others may be involved in tolerance against the stress. Although these lines were considered “susceptible” based on agronomical and biochemical analyses, they still have some putative stress-tolerance biomarkers. Such biomarkers can be utilized in breeding to integrate them into tolerant lines.

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It is important to understand the agronomical and physiological changes associated with both the susceptibility and tolerance for any plant stress. These phenotypic responses are governed by spectacular biochemical and molecular changes. This study and similar reports (Amtmann, 2009; Rai et al., 2010; Sun et al., 2010) emphasize the importance of holistic approaches to study the hidden regulators that may vary along lines. On the other hand, environmental interactions with any investigated line can lead to deviated outcomes. Therefore, it is important to investigate responsive genes with overlapping expressions along different stresses. Some plant responses are very similar across abiotic stresses, while others are unique for each one (Grover et al., 1999; Hazen et al., 2003; Nakashima and Yamaguchi-Shinozaki, 2009). In fact, some unique stress-specific responsive genes were successfully illustrated in a major network governing plant abiotic stresses (Zhang H et al., 2012).

In conclusion, tomato differential expression profile is an invaluable ‘omics’ tool, by which groups of putative stress-specific biomarkers can be disclosed. Such precious candidate genes can be integrated into available breeding lines. However, it is important to be cautious when selecting putative biomarkers from susceptible lines, because some of these stress-upregulated genes could be related to cell damage/degradation or signaling rather than to stress alleviation.

## Acknowledgments

The research team greatly appreciates the National Plan for Science and Technology for financial support of this project (Number 10-BIO970-02). Thanks are also due to the head of the Plant Production Department, College of Food and Agricultural Sciences, and the director of the plant biotechnology laboratory for providing office and laboratory space for researchers. The authors wish to thank the College of Food and Agricultural Sciences Research Center and the Deanship of Scientific Research, King Saud University, Saudi Arabia, for supporting this work.

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