Exploring differentially expressed genes of microspore embryogenesis under heat stress in sweet pepper

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Received 2 July, 2020; Accepted 2 September, 2020

Stress is considered to be the inducer of microspore embryogenesis (ME), and heat stress is indispensable in the ME of sweet pepper. The aim of the study was to explore differentially expressed genes of microspore embryogenesis under heat stress in sweet pepper. The swollen rate of microspore was significantly affected by heat stress, while no green plant could be acquired without heat pretreatment. Anthers with or without heat stress were used for whole transcriptome analysis by RNA sequencing to provide new insights on how cells adapt to stress. A total of 5031 differentially expressed genes were identified, among which 2657 differentially expressed genes were up-regulated and 2374 differentially expressed genes were down-regulated in the early stage of heat stress. KEGG pathway analysis identified "plant hormone signal transduction" (67; 11.20%), followed by starch and sucrose metabolism (63; 10.54%). RNA-Seq data and quantitative real-time polymerase chain reaction showed that 224 genes related to glutathione metabolism, starch and sucrose metabolism, plant hormone signal transduction and phenylpropanoid biosynthesis were the most likely specific genes in ME under heat stress. This research provides new insights into molecular regulation during the early stage of ME in sweet pepper under heat stress.

Key words: Differentially expressed genes, heat stress, microspore embryogenesis, sweet pepper.

INTRODUCTION

Stress is considered as a kind of mutation on microspore embryos: unconstrained microspores form flower powder along the normal binding pathway (Touraev et al., 1997). Among various stress treatments, heat stress is widely used to initiate microspore embryogenesis (ME) in many crops (Asadi et al., 2018; Bhatia et al., 2018; Cimò et al., 2017; Dubas et al., 2014). The anthers of sweet pepper cultured under heat stress can be induced to develop into haploid embryos with complete functions instead of mature pollen (Bárány et al., 2005). Despite extensive advances have been made, compared to cruciferous and cereal species, sweet pepper is still considered recalcitrant to ME and doubled haploid production, which limit the use of this technology in breeding programs (Seguí-Simarro et al., 2011). In the past few years, most molecular biology researchers have focused on the culture system of sweet pepper (Popva et al., 2016; Heidari-Zefreh et al., 2019; Sánchez et al., 2020). We do not know the mechanism of cell fate transformation and the regulation of gene expression that initiates
embryogenesis, and this knowledge gap is particularly
obvious (Tsuwamoto et al., 2007). Many biochemical
and morphological changes are closely related to the forced
manipulation and alteration of gene expression patterns
in embryos (Soriano et al., 2013).

Today, transcriptome analysis is an important means to
study possible mechanisms and identify potential genes.
Using this method, the roles of stress to induce the
embryogenesis and alternation of gene expression have
been examined in several crops (Elhiti et al., 2012; Liu et
al., 2016; Zhang et al., 2019). However, it is still unclear
for the differentially expressed genes in the early stage of
ME under heat stress in sweet pepper.

In our previous work, an efficient ME system for pepper
was established (Cheng et al., 2013). The present study
aimed at exploring differentially expressed genes of
microspore embryogenesis under heat stress in sweet
pepper using high-throughput sequencing technology.
This discovery will provide new insights into the
molecular mechanism of sweet pepper micro-ecosystem.

MATERIALS AND METHODS

Plant material and heat treatment

Sweet pepper (Capsicum annum L.) variety Jinjiao 203, a
responsive genotype in ME, grew up in the greenhouse of Shanxi
Academy of Agricultural Sciences, China. In February 2017, seeds
were sown in the soil, and flower buds were collected from the
donor plants. Anther pretreatment was performed as previously
described by Cheng et al. (2013). Briefly, flower buds were sterilized
immediately after collection. They were dissected and cultured on
pretreatment medium (10 mM CaCl₂, 1 mM MnSO₄·7H₂O, 1 mM
KNO₃, 200 M KH₂PO₄, 1 M KI, 100 mM CuSO₄·5H₂O, 0.37 M
mannitol and 0.5% agar) for embryogenesis induction. Anthers
cultured at 25°C were used as control group and anthers cultured at
34°C as heat treatment group. Each group was repeated four times.
Anther load of each biological replica was isolated from at least five
feed plants and divided into two groups on average. After 7 days of
pre-culture, the microspores of 5 anthers were separated in 1 ml
sterile water, and the microspore expansion rate (the total
expanded microspores and the fraction of the total microspores in 5
microscope fields) of each treatment was analyzed. At the same
time, a small amount of anthers (about 100 mg) were collected into
test tubes by liquid nitrogen quick freezing method, and then stored
at -80°C or RNA isolation. As described by Cheng et al. (2013),
the remaining anthers were isolated and cultured for embryogenesis.
After 2 months of culture, the number of green plants in every 100
anthers was counted.

RNA isolation and cDNA library construction

In order to separate RNA from pretreated anthers, frozen samples
were ground into fine powder in a microcentrifuge tube. Total RNA
was isolated by triazone reagent (Invitrogen, Carlsbad, California,
USA), which produced about 10 micrograms of total RNA per
sample. The nanodrop 2000 spectrophotometer (Thermo) was used
to detect the concentration, and the RNA nano6000 detection kit of
Agilent Bioanalyzer 2100 system (Agilent Technologies, California,
USA) was used to evaluate the integrity. After adjusting their
concentration to 10 nm, appropriate total RNA samples were
collected in the same volume in 4 repeated pretreated anthers.

A total of 1 microgram of ribonucleic acid was used as input
material in each sample to prepare a ribonucleic acid sample.
Following the manufacturer's recommendations, the NEBNext
UltraTM RNA Library Preparation Kit for Illumina (NEB USA) was
used to create a sequencing library and the index code to the
attribute sequence of each sample was added. Briefly, mRNA was
purified from total RNA using magnetic beads and addition of
oligonucleotide poly-T. At high temperature, divalent cations was
used to adhere to the buffer of NEBNext synthesis reaction (5x).
The first cDNA strand was synthesized with 6 polyisilicon random
seeds and mulv arz. Then deoxyribonucleic acid polymerase I and
ribonuclease H were used to synthesize the second strand of
deoxyribonucleic acid. The remaining overhangs were converted to
blunt ends by exonuclease/polymerase activity. After adenylation at
the 3' end of deoxyribonucleic acid fragment, NEBNext adapter with
hairpin loop structure was connected to prepare for hybridization.
To select a cDNA fragment with a length of 240 bp, the library
fragment was purified by AMPure XP system (Beckman Coulter,
Beverly, USA). Then, before polymerase chain reaction, 3 μL·L⁻¹ of
user enzyme (National Biological Laboratory, USA) was used to
treat the cDNA ligated with the adapter selected in size at 37°C for
15 min, and then treated at 95°C for 5 min. Then polymerase chain
reaction was carried out with polymerase, universal primer and
index primer. Finally, the PCR products (Ample XP system) were
purified on Agilent Bioanalyzer 2100 system, and the library quality
was evaluated.

RNA sequencing and transcript analysis

According to the manufacturer's instructions, TruSeq PE Cluster
Suite v4-cBot-HS (Illumia) was used to cluster the index coded
samples on cBot cluster generation system. After the cluster is
created, the preparation order of libraries on the Illumina Hiseq
Xten platform is specified, and generates an end reading pair. The
raw data in Fastq format (raw reading) was first processed by
internal perl script. This step deletes the adapter-containing read,
policy-containing read and low-quality read from the original data to
obtain clean data (clean read). Q20, Q30, GC- content and
sequence iteration level of clean data are also calculated. All
downstream analysis is based on high quality and clean data. The
adapter sequences and low quality sequence reads would be
removed from the dataset. The original sequence was converted to
clean reading after data processing. These clean reads were then
mapped to the reference genome sequence database Zunla-1.
Only completely consistent or inconsistent readings are further
analyzed and annotated according to the reference genome.
Tophat2 tool software is used to map reference genomes. The
Kyoto Encyclopedia of Genes and Genomes predicts metabolic and
cellular pathways. The gene expression level was calculated by
reading millions of Millennium bug (FPKM), and the fragments used
were mapped to reference sequences. The two groups of DEGs
were screened according to gene expression levels using the
DESeq R software package (1.10.1). Binary negative sign
distribution is used to identify differential expression in digital
expression data. More than twice the expression level changes and significantly different expressions (P<0.05) are
considered as the differential expressions among different
treatments.

Quantitative real-time PCR analysis

To verify the expression of SDR, 20 candidate genes identified by
KEGG concentration analysis were randomly selected for real-time
PCR quantitative analysis (QR-PCR). Selected gene names and
primer information are listed in Table 1. Before assembling RNA
sequences, the total RNA (1 μg) of each sample was used as a
Table 1. Primer information for qRT-PCR.

| Gene id            | Forward (5’→3’)          | Reverse (5’→3’)          |
|--------------------|--------------------------|--------------------------|
| Actin_GQ339766.1   | GAAGCACCTCTCAACCCTAAG    | GTACGACCCTAGCATACAAAG    |
| Capana00g002630    | CTTGCTGATTGTGATGCTGAG    | AATCCTTAGTCAGAAGGACTG    |
| Capana01g000883    | AGTCACATTCAGAAAGAGCAG    | CAGATCCAGACGAGTACAG     |
| Capana00g003106    | GGATGTTTATGGGCTACTGTTG   | TGTTGAGAGATGAGACTATCA   |
| Capana01g002457    | TCAATGTTGCTCGGACTCTTC    | CACCCTTCTCATCCTCTCAG    |
| Capana00g001129    | AAAGTTCTCATGGTAACGATGC   | TCTGATACTGAGTACCTCA    |
| Capana01g004182    | AAAGTTCTCATGGTAACGATGC   | TCTGATACTGAGTACCTCA    |
| Capana00g004687    | GGATGTTTATGGGCTACTGTTG   | TGTTGAGAGATGAGACTATCA   |
| Capana01g000278    | ACCTTTTACACATTTTGGGCTG   | TCTGATACTGAGTACCTCA    |
| Capana01g000279    | GGACTACCCTTACAGCAACTTC   | TCTGATACTGAGTACCTCA    |
| Capana01g000500    | AGTCCATATTCAGAAGGCGG     | TCTGATACTGAGTACCTCA    |
| Capana00g004543    | ACCCACCCCTTCGGCTG        | TCTGATACTGAGTACCTCA    |
| Capana01g003124    | CACCCTTCTCATCCTCTCAG     | TCTGATACTGAGTACCTCA    |
| Capana01g003125    | TCTGATACTGAGTACCTCA      | TCTGATACTGAGTACCTCA    |
| Capana01g000731    | AAGGCTTGAGAGGCTGCTTTC    | TCTGATACTGAGTACCTCA    |
| Capana01g003124    | CACCCTTCTCATCCTCTCAG     | TCTGATACTGAGTACCTCA    |
| Capana01g003125    | TCTGATACTGAGTACCTCA      | TCTGATACTGAGTACCTCA    |

template for synthesizing the first strand cDNA. Gene names and information of selected seeds are shown in Table 1. Before compiling the nucleic acid sequence, the whole DNA [1 of each sample (1 microgram)] was used as a model for synthesizing the first cDNA strand. On the Bio-Rad CFX96 instrument, trans start top green qpcr super mix (AQ131) was used to replicate each biological sample for three times, and quantitative reverse transcription polymerase chain reaction analysis was carried out using capsicum actin nany gene as internal control. Quantitative comparison CT method (∆∆CT method) is used to quantify the relative expression of specific genes (Livak and Schmittgen, 2001).

RESULTS

Effects of heat stress on the induction of ME in sweet pepper

In order to check the influence of thermal stress on electromagnetic induction, 7 days 34°C anther pretreatments were processed. In all four biological replicates, the anther volume increased after heat stress compared with the control group (Figure 1A). Moreover, more microspores in anthers were inflated, while fewer expanded microspores and no green plants were observed in the control (Figure 1B). The results indicated that both the swollen rate of microspore and green plants production were significantly affected by the heat stress (34°C, 7 days) in anther pretreatment, which means that heat stress could improve the embryogenesis initiation and possibility of microspore to develop into green plants.

Illumina sequence analysis and verification of selected genes by quantitative reverse transcription polymerase chain reaction

The cDNA library of embryogenic microspores of sweet pepper was sequenced on Illumina Hiseq Xten platform, and paired terminal readings were generated. A total of 143,541,722 and 151,420,406 original readings were obtained from two cDNA libraries composed of control group and thermal pressure group (Table 2). 92.91% of the readings were useful after data quality inspection and screening with quality greater than 30 (NQ30), among which 89.88% (128,990,381) of the control group readings and 82.90% (125,760,669) of the heat stress group readings were located in the pepper genome. About 86.39% of the readings were located on genes, and 13.61% were not located on genes, which indicated that most of the readings were located on reference genes.

In order to confirm the differentially expressed genes confirmed in sequencing and computational analysis, 20 DEGs concentrated in KEGG pathway were randomly selected for quantitative real-time polymerase chain reaction including 2 signal transduction mechanisms protein (Capana00g002630, Capana01g000883), 1 probable glutathione S-transferase (Capana00g003106), 1 agamous-like MADS-box protein (Capana01g002457), 1 peroxidase of carbohydrate transport and metabolism (Capana00g001129), 1 ent-copaly diphosphate synthase...
Figure 1. Comparison of ME of sweet pepper and resulted green plants produced between the control group (25°C, 7 days) and heat stress group (34°C, 7 days). (A) The pretreated anthers (7 days) between two groups. (B) The output of green plants is between two groups. Data represent the mean standard deviation (n=4). Compared with the control group, the single asterisk and double star numbers showed statistical differences (T test, P < 0.05 or 0.01, respectively)

Table 2. Summary of the illumina sequencing.

| Sample | Reads   | Raw reads  | Clean reads | Q20 (%) | Q30 (%) | GC (%) |
|--------|---------|------------|-------------|---------|---------|--------|
| Control |         |            |             |         |         |        |
| Replicate1 | 47539426 | 23769713   | 100         | 93.04   | 42.71   |
| Replicate2 | 52194066 | 26097033   | 100         | 92.91   | 42.80   |
| Replicate3 | 43808230 | 21904115   | 100         | 93.26   | 42.65   |
| Total    | 143541722| 71770861   | 100         | 93.07   | 42.72   |
| Treatment |        |            |             |         |         |        |
| Replicate1 | 50034294 | 25017147   | 100         | 92.99   | 43.01   |
| Replicate2 | 52372404 | 26186202   | 100         | 93.07   | 42.56   |
| Replicate3 | 49013708 | 24506854   | 100         | 93.16   | 42.85   |
| Total    | 151420406| 75710203   | 100         | 93.07   | 42.81   |

of coenzyme transport and metabolism (Capana01g001728), 1 cytokinin dehydrogenase of energy production and conversion (Capana01g004182), 1 GDSL-like Lipase/Acylhydrolase (Capana00g004135), 1 tetrahydrocannabinolic acid synthase (Capana00g004867), 1 thaumatin-like protein
Figure 2. Relative gene expression of 20 randomly selected genes detected by quantitative real-time polymerase chain reaction. Data represent the mean standard deviation (n=3). Compared with the control group, the single asterisk and double star numbers showed statistical differences (T test, p<0.05 or 0.01, respectively).

(Capana01g000278), 1 protein P21 of thaumatin family (Capana01g000279), 1 hydrophobic seed protein (Capana01g000500), 1 probable lipid transfer protein (Capana01g000731), 1 pollen-specific leucine-rich repeat elongation protein 1 (Capana01g003124), 1 C1-like domain (Capana01g003125), 1 serum kinase protein /su-aminotransfer LRR receptor (Capana00g004543), 1 shikimate O-hydroxycinnamoyltransferase (Capana01g004373), and 3 function unknown protein (Capana00g005078, Capana01g0001352, Capana01g001414).

The results of quantitative reverse transcription polymerase chain reaction (Figure 2) on the expression of 20 selected genes showed that compared with the control group, the average expression level of 12 genes in the heat stress group was significantly increased. However, the average gene expression level of heat stress group was significantly down-regulated. The expressions of these genes were consistent in RNA RNA-Seq and qRT-PCR data.
KEGG enrichment analysis on DEGs

In addition to 32832 unchanged genes, 2657 up-regulated gene expression regions and 2374 down-regulated gene expression regions were found in the microenvironment between the control group and the hot group. In order to further identify the possible functional pathways, 598 DEGs were found in 20 KEGG functional pathways by KEGG pathway analysis. Among these pathways, the category of ‘plant hormone signal transduction’ (67; 11.20%) represented the largest group, followed by ‘starch and sucrose metabolism’ (63; 10.54%), ‘biosynthesis of amino acids’ (61; 10.20%), ‘Carbon metabolism’ (57; 9.53%), ‘phenylpropanoid biosynthesis’ (51; 8.53%) and ‘Plant-pathogen interaction’ (50; 8.36%). KEGG enrichment analysis showed that heat stress regulatory genes were enriched in four main pathways (p<0.05), including ‘glutathione metabolism’, ‘starch and sucrose metabolism’, ‘plant hormone signal transduction’ and ‘phenylpropanoid biosynthesis’ (Figure 3). Among the four main pathways, 224 estimated heat stress specific genes have been confirmed, of which 138 genes are up-regulated under heat pressure and 86 genes are down-regulated under heat pressure (Supplementary Table 1).

DISCUSSION

High stress tolerance could be presumed the first prerequisite for successful ME induction, for the reason that ME was induced or at least strongly stimulated by a stress pre-treatment (Zoriniants et al., 2005). One of the most important components of stress resistance is an effective antioxidant system composed of enzymes and low molecular antioxidants, which protects cells from the production of reactive oxygen species (Mittler, 2002). Glutathione was a major antioxidant in all forms of life and an indicator of cellular oxidative stress (Belmonte and Stasolla, 2009). In a reduced form, glutathione was metabolized in multiple ways leading to the biosynthesis of mercapturonate, glutamate, glycine, cysteine and other amino acids (Noctor et al., 2012). It also participates in the regulation of cell cycle, proliferation and programmed cell death as a signal molecule. Our results proved the importance of glutathione metabolism under heat stress in ME of sweet pepper. The key role of glutathione in embryo and meristem development was confirmed by analyzing the phenotype of glutathione-deficient Arabidopsis mutants (Noctor et al., 2012). The published results show that some glutathione response genes have encoded transcription factors and proteins, and are involved in cell division, redox potential (such as thioredoxin, glutamore), auxin biosynthesis, transport and regulation of transcription reaction (Schnaubelt et al., 2013). It revealed that the initial environment of embryogenesis requires a reduced environment (high GSH/GSH + GSSG), which may promote cell proliferation by enhancing nucleotide synthesis and mitotic activity (Stasolla, 2010). More and more recently published data
indicate that ROS accumulation initiates signal transduction leading to microspore reprogramming and embryogenic development (Zur et al., 2019). In this study, starch and sucrose metabolism were detected as the second major pathways in ME of sweet pepper under heat stress. The metabolism of starch and sucrose fuels all aspects of plant growth and development. The kinetics of starch synthesis is related to the differentiation process and the clear change of cell wall structure and organization, which is characterized by the de-esterification of pectin and the increase of RGI and XG components (Bárany et al., 2005; Satpute et al., 2005). The accumulation of plastid starch occurred in differentiated cells, which showed high levels of de-esterified pectin and rich RGI and XG, while the proliferation cells rich in esterified pectin did not show starch deposition (Bárány et al., 2010). Data show that one of the important changes of carbohydrate pathways with specific markers is for the largest number of DEGs. The up-regulated DEGs related to phenylpropanoid biosynthesis in this study might have important function to keep microspore from death and promote embryonic microspore dedifferentiation.

Conclusions

Heat stress is indispensable in the ME of sweet pepper. In this study, transcriptome analysis of the anther of sweet pepper in the early stage of ME showed that the DEGs between heat treatment and control were mainly associated with glutathione metabolism, starch and sucrose metabolism, plant hormone signaling and phenylpropyl ester biosynthesis. Our research provides new insights into molecular regulation during the early stage of ME in sweet pepper under heat stress.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by Basic Research Projects of Application in Shanxi Province (201701D121090) and Shanxi Academy of Agricultural Sciences (YGG17073, YGJPY1903, YGC2019TD02).

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Zur et al., 2019). Phenylpropanoid biosynthesis was induced by several stresses (Dixon and Paiva, 1995), and became the main pathway in ME of sweet pepper under heat stress. In plants, the phenylpropanoid pathway was responsible for the synthesis of secondary metabolites, including lignin monomers, flavonoids, and coumarins, which play essential roles in determining plant structure, biomass recalcitrance, and stress tolerance (Vogt, 2010). It had been the significantly enriched pathways with specific common DEGs mainly including peroxidase, phenylalanine ammonialyase, and β-glucosidase (Zhang et al., 2019).
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Supplementary Table 1. 224 putative heat stress-specific genes.

| Pathway                  | Gene id                  | Expression | Annotation                                                                                     |
|--------------------------|--------------------------|------------|-----------------------------------------------------------------------------------------------|
| Glutathione metabolism   | Capana03g000768          | up         | PREDICTED: glutathione S-transferase U9 [Nicotiana sylvestris]                                   |
|                          | Capana01g003296          | up         | PREDICTED: glutathione transferase GST 23-like [Solanum lycopersicum]                          |
|                          | Capana09g001742          | up         | PREDICTED: glutathione S-transferase U8-like [Solanum tuberosum]                               |
|                          | Capana01g002551          | up         | uncharacterized protein LOC543813 [Solanum lycopersicum]                                       |
|                          | Capana09g001740          | up         | uncharacterized protein LOC543814 [Solanum lycopersicum]                                       |
|                          | Capana09g001741          | up         | PREDICTED: probable glutathione S-transferase [Solanum lycopersicum]                          |
|                          | Capana02g000952          | up         | PREDICTED: probable glutathione S-transferase-like [Solanum tuberosum]                         |
|                          | Capana09g001858          | up         | PREDICTED: probable glutathione S-transferase [Solanum lycopersicum]                          |
|                          | Capana02g000950          | up         | PREDICTED: probable glutathione S-transferase-like [Solanum tuberosum]                         |
|                          | Capana00g002164          | up         | PREDICTED: LOW QUALITY PROTEIN: probable glutathione S-transferase parA-like [Solanum tuberosum] |
|                          | Capana00g003105          | up         | uncharacterized protein LOC543817 [Solanum lycopersicum]                                       |
|                          | Capana00g004478          | up         | PREDICTED: probable glutathione S-transferase [Solanum lycopersicum]                          |
|                          | Capana03g004566          | up         | glutathione S-transferase 12 [Capsicum chinense]                                               |
|                          | Capana07g002010          | up         | glutathione S-transferase/peroxidase [Capsicum chinense]                                      |
|                          | Capana09g001764          | up         | probable glutathione S-transferase [Capsicum annuum]                                            |
|                          | Capana03g004562          | up         | glutathione S-transferase 12 [Capsicum chinense]                                               |
|                          | Capana06g003058          | up         | glutathione S-transferase GST1 [Capsicum chinense]                                             |
|                          | CapsicumannuumL_newGene_14186 | up   | PREDICTED: probable glutathione S-transferase [Solanum lycopersicum]                          |
|                          | Capana10g001792          | up         | PREDICTED: glutathione S-transferase L3-like [Solanum tuberosum]                               |
|                          | Capana08g001515          | up         | PREDICTED: glutathione S-transferase U8-like [Solanum tuberosum]                               |
|                          | Capana09g001763          | up         | probable glutathione S-transferase [Solanum lycopersicum]                                     |
|                          | Capana00g001895          | up         | PREDICTED: glutathione S-transferase U9-like [Nicotiana tomentosiformis]                      |
|                          | Capana09g001861          | up         | PREDICTED: probable glutathione S-transferase [Nicotiana sylvestris]                          |
|                          | Capana08g001520          | up         | unnamed protein product [Coffea canephora]                                                     |
|                          | Capana10g001806          | up         | PREDICTED: glutathione S-transferase L3-like [Solanum tuberosum]                               |
|                          | Capana09g001760          | up         | glutathione S-transferase [Capsicum annuum]                                                    |
|                          | Capana09g001860          | up         | PREDICTED: probable glutathione S-transferase [Solanum lycopersicum]                          |
|                          | Capana12g001177          | up         | PREDICTED: probable glutathione peroxidase 8-like [Solanum tuberosum]                          |
|                          | Capana12g001176          | up         | PREDICTED: probable glutathione peroxidase 8-like [Solanum tuberosum]                          |
|                          | Capana01g000225          | up         | PREDICTED: probable phospholipid hydroperoxide glutathione peroxidase-like [Solanum tuberosum]  |
|                          | Capana09g001761          | down       | glutathione S-transferase [Capsicum annuum]                                                    |
|                          | Capana02g002285          | down       | glutathione S-transferase, partial [Capsicum annuum]                                            |
|                          | Capana00g003106          | down       | PREDICTED: probable glutathione S-transferase parA [Solanum lycopersicum]                     |
|                          | Capana03g003600          | down       | PREDICTED: glutathione S-transferase PARB [Nicotiana sylvestris]                               |
|                          | Capana12g0000354         | down       | PREDICTED: glutathione transferase GST 23-like [Solanum tuberosum]                             |
|                          | Capana09g002045          | down       | uncharacterized protein LOC543815 [Solanum lycopersicum]                                       |
|                          | Capana02g000947          | down       | PREDICTED: probable glutathione S-transferase [Nicotiana sylvestris]                           |
|                          | Capana11g001532          | down       | PREDICTED: glutathione S-transferase U17-like [Nicotiana sylvestris]                           |
|                          | Capana11g001536          | down       | PREDICTED: glutathione S-transferase U17-like [Nicotiana sylvestris]                           |
|                          | CapsicumannuumL_newGene_14187 | down | PREDICTED: probable glutathione S-transferase parC [Nicotiana tomentosiformis]                |
|                          | Capana11g001525          | down       | PREDICTED: glutathione S-transferase U17-like [Solanum tuberosum]                              |
### Supplementary Table 1. Contd.

| Accession           | Change | Description                                                                 |
|---------------------|--------|-----------------------------------------------------------------------------|
| Capana07g002005     | down   | PREDICTED: probable glutathione S-transferase-like [Solanum tuberosum]       |
| Capana02g002271     | down   | PREDICTED: probable glutathione S-transferase parC-like [Solanum tuberosum] |
| **Starch and sucrrose metabolism** |
| Capana08g002622     | down   | PREDICTED: endoglucanase 24-like [Solanum tuberosum]                        |
| Capana07g000730     | down   | PREDICTED: sucrose synthase-like [Solanum tuberosum]                        |
| Capana01g000086     | down   | PREDICTED: beta-amylase 1, chloroplastic-like [Nicotiana tomentosiformis]   |
| Capana03g001539     | down   | PREDICTED: sucrose synthase 6-like [Solanum tuberosum]                      |
| Capana01g000524     | down   | PREDICTED: probable trehalose-phosphate phosphatase F-like isoform X1 [Solanum tuberosum] |
| Capana00g002284     | down   | PREDICTED: alpha,alpha-trehalose-phosphate synthase [UDP-forming] 1-like [Solanum tuberosum] |
| Capana03g001994     | down   | PREDICTED: soluble starch synthase 1, chloroplastic/amyloplastic isoform X1 [Solanum lycopersicum] |
| Capana03g004067     | down   | PREDICTED: probable sucrose-phosphate synthase 2-like [Solanum tuberosum]    |
| Capana08g000100     | down   | PREDICTED: alpha,alpha-trehalose-phosphate synthase [UDP-forming] 6-like [Solanum tuberosum] |
| Capana00g000875     | down   | starch branching enzyme II, SBE-II [Solanum tuberosum]                      |
| Capana02g003365     | down   | granule-bound starch synthase 2, chloroplastic/amyloplastic precursor [Solanum tuberosum] |
| Capana10g000293     | down   | PREDICTED: probable alpha.alpha-trehalose-phosphate synthase [UDP-forming] 7 [Solanum lycopersicum] |
| Capana00g004617     | down   | PREDICTED: glucose-1-phosphate adenylyltransferase small subunit, chloroplastic/amyloplastic-like [Solanum tuberosum] |
| Capana01g002934     | down   | ADP-glucose pyrophosphorylase large subunit [Solanum lycopersicum]          |
| Capana10g000025     | down   | PREDICTED: probable galacturonosyltransferase 12 [Solanum lycopersicum]     |
| Capana03g003674     | down   | PREDICTED: cellulose synthase-like protein D5-like [Solanum tuberosum]      |
| Capana00g004214     | down   | PREDICTED: probable galacturonosyltransferase 12 [Solanum lycopersicum]     |
| Capana04g001640     | up     | PREDICTED: alpha-amylase [Solanum lycopersicum]                             |
| Capana01g001057     | up     | PREDICTED: alpha,alpha-trehalose-phosphate synthase [UDP-forming] 5-like isoform X1 [Solanum tuberosum] |
| Capana01g000074     | up     | PREDICTED: probable trehalase [Nicotiana tomentosiformis]                   |
| Capana03g003656     | up     | sucrose synthase [Solanum tuberosum]                                       |
| Capana04g001118     | up     | PREDICTED: probable alpha-amylase 2 [Nicotiana sylvesteris]                |
| Capana01g003777     | up     | unnamed protein product [Vitis vinifera]                                    |
| Capana09g0000138    | up     | sucrose synthase [Solanum tuberosum]                                       |
| Capana00g011331     | up     | PREDICTED: probable trehalose-phosphate phosphatase J-like [Solanum tuberosum] |
| Capana09g000101     | up     | PREDICTED: UDP-glucuronate 4-epimerase 1-like [Nicotiana sylvesteris]       |
| Capana01g000377     | up     | PREDICTED: UDP-glucuronate 4-epimerase 5-like [Solanum tuberosum]           |
| Capana08g002001     | up     | PREDICTED: beta-xylosidase(alpha-L-arabinofuranosidase 2 [Solanum lycopersicum] |
| Capana00g003684     | up     | PREDICTED: UDP-glucuronate 4-epimerase 1-like [Solanum tuberosum]           |
| Capana01g000079     | up     | PREDICTED: beta-amylase 1, chloroplastic-like [Nicotiana tomentosiformis]   |
| Capana02g002280     | up     | PREDICTED: sucrose synthase 7-like [Nicotiana sylvesteris]                 |
| Capana07g000366     | up     | sucrose-phosphate synthase [Lycium barbarum]                               |
| Capana03g002554     | up     | PREDICTED: trehalose-phosphate phosphatase A-like isoform X1 [Solanum tuberosum] |
| Capana02g001649     | up     | PREDICTED: probable alpha,alpha-trehalose-phosphate synthase [UDP-forming] 7-like [Solanum tuberosum] |
| Capana07g002060     | up     | PREDICTED: alpha,alpha-trehalose-phosphate synthase [UDP-forming] 1-like isoform |
Supplementary Table 1. Contd.

| Genbank ID | Expression | Description                                                                 |
|------------|------------|-----------------------------------------------------------------------------|
| Capana07g000086 | up         | trehalose-6-phosphate synthase [Solanum lycopersicum]                       |
| Capana03g004414 | up         | beta-amylose [Solanum lycopersicum]                                        |
| Capana04g001731 | up         | RecName: Full=Alpha-1,4 glucan phosphorylase L-1 isozyme, chloroplastic/amyloplastic; AltName: Full=Starch phosphorylase L-1; Flags: Precursor [Solanum tuberosum] |
| Capana00g000646 | up         | PREDICTED: probable alpha,alpha-trehalose-phosphate synthase [UDP-forming] 11-like isoform X2 [Solanum tuberosum] |
| Capana12g001615 | up         | PREDICTED: 4-alpha-glucanotransferase, chloroplastic/amyloplastic isoform X1 [Nicotiana sylvestris] |
| Capana11g001362 | up         | PREDICTED: probable alpha,alpha-trehalose-phosphate synthase [UDP-forming] 9 [Nicotiana sylvestris] |
| Capana05g001390 | up         | PREDICTED: probable alpha,alpha-trehalose-phosphate synthase [UDP-forming] 9-like [Solanum tuberosum] |
| Capana10g000752 | up         | PREDICTED: probable trehalose-phosphate phosphatase F-like isoform X1 [Solanum tuberosum] |
| Capana01g000073 | up         | PREDICTED: probable trehalase [Nicotiana tomentosiformis] |
| Capana03g002047 | up         | PREDICTED: probable trehalose-phosphate phosphatase 2 [Solanum lycopersicum] |
| Capana02g000868 | down       | PREDICTED: uncharacterized protein LOC101249042 isoform X2 [Solanum lycopersicum] |
| Capana01g004209 | down       | PREDICTED: beta-amylose 8 isoform X4 [Solanum lycopersicum] |
| Capana06g001686 | down       | PREDICTED: probable trehalose-phosphate phosphatase F-like [Solanum tuberosum] |
| Capana07g001806 | down       | PREDICTED: probable alpha,alpha-trehalose-phosphate synthase [UDP-forming] 7-like [Solanum tuberosum] |
| Capana02g001891 | down       | PREDICTED: glycogen phosphorylase 1-like isoform X1 [Solanum tuberosum] |
| Capana08g000914 | down       | PREDICTED: beta-amylose 2, chloroplastic-like isoform X2 [Phoenix dactylifera] |
| CapsicumannuumL_newGene_14041 | down   | PREDICTED: probable alpha-amylase 2 [Nicotiana sylvestris] |
| Capana08g000917 | down       | PREDICTED: beta-amylose 7-like isoform X1 [Solanum tuberosum] |
| Capana09g001212 | down       | alpha-glucan phosphorylase, H isozyyme [Solanum tuberosum] |
| Capana11g001904 | down       | alpha-1,4 glucan phosphorylase L-2 isozyme, chloroplastic/amyloplastic [Solanum tuberosum] |
| Capana00g001034 | down       | PREDICTED: alpha,alpha-trehalose-phosphate synthase [UDP-forming] 1-like [Solanum tuberosum] |
| Capana02g000320 | down       | PREDICTED: 4-alpha-glucanotransferase DPE2-like [Nicotiana sylvestris] |
| Capana04g000086 | down       | 1,4-alpha-glucan branching enzyme [Solanum tuberosum] |
| Capana12g001588 | down       | PREDICTED: probable trehalose-phosphate phosphatase J [Solanum lycopersicum] |
| Capana02g003313 | down       | PREDICTED: probable galacturonosyltransferase 14-like [Solanum tuberosum] |
| Capana02g000897 | down       | PREDICTED: probable galacturonosyltransferase 13-like isoform X2 [Solanum tuberosum] |
| Capana08g002835 | down       | PREDICTED: probable galacturonosyltransferase 3-like [Solanum tuberosum] |
| Capana10g001175 | down       | PREDICTED: probable galacturonosyltransferase 7 isoform X1 [Solanum lycopersicum] |

**Plant hormone signal transduction**

| Genbank ID | Expression | Description                                                                 |
|------------|------------|-----------------------------------------------------------------------------|
| Capana01g001914 | down       | hypothetical protein JCGZ_23114 [Jatropha curcas] |
| Capana10g002513 | down       | PREDICTED: ABSCISIC ACID-INSENSITIVE 5-like protein 2-like isoform X1 [Solanum tuberosum] |
| CapsicumannuumL_newGene_9344 | down   | PREDICTED: protein TIFY 4A-like [Solanum tuberosum] |
| Capana01g003720 | down       | PREDICTED: jasmonate ZIM-domain protein 3 isoform X1 [Solanum lycopersicum] |
### Supplementary Table 1. Contd.

| Gene Name | Status | Description |
|-----------|--------|-------------|
| Capana09g000285 | down | IAA8 [Solanum lycopersicum] |
| Capana10g002328 | down | PREDICTED: regulatory protein NPR5-like [Solanum tuberosum] |
| Capana06g003038 | down | PREDICTED: protein TRANSPORT INHIBITOR RESPONSE 1-like isoform X1 [Solanum tuberosum] |
| Capana10g001576 | down | PREDICTED: serine/threonine-protein kinase CTR1-like isoform X1 [Solanum tuberosum] |
| Capana12g002655 | down | serine/threonine-protein kinase SAPK1-like [Solanum tuberosum] |
| Capana08g001056 | down | PREDICTED: auxin response factor 9-like isoform X3 [Solanum tuberosum] |
| Capana08g001036 | down | PREDICTED: probable indole-3-acetic acid-amido synthetase GH3.5 [Solanum lycopersicum] |
| Capana10g000405 | down | PREDICTED: probable indole-3-acetic acid-amido synthetase GH3.5 [Solanum lycopersicum] |
| CapsicumannuumL_n ewGene_9164 | down | PREDICTED: protein TIFY 4A-like [Solanum tuberosum] |
| Capana03g002801 | down | leucine zipper transcription factor [Solanum tuberosum] |
| Capana04g000600 | up | CycD3;3 protein [Solanum lycopersicum] |
| CapsicumannuumL_n ewGene_13152 | down | PREDICTED: indole-3-acetic acid-induced protein ARG7-like [Nicotiana sylvestris] |
| Capana09g000028 | up | F-box protein [Capsicum annuum] |
| Capana07g000168 | up | PREDICTED: EIN3-binding F-box protein 1-like [Nicotiana sylvestris] |
| CapsicumannuumL_n ewGene_13200 | up | PREDICTED: histidine-containing phosphotransfer protein 4-like [Nicotiana sylvestris] |
| Capana00g004470 | up | PREDICTED: probable serine/threonine-protein kinase At5g41260 [Solanum lycopersicum] |
| Capana10g002376 | up | PREDICTED: transcription factor HBP-1b(c38)-like [Solanum lycopersicum] |
| Capana06g000155 | up | PREDICTED: auxin transporter-like protein 4 [Nicotiana tomentosiformis] |
| Capana01g000511 | up | PREDICTED: protein phosphatase 2C 37-like [Nicotiana sylvestris] |
| Capana11g002252 | up | PREDICTED: transcription factor HBP-1b(c1)-like [Solanum lycopersicum] |
| Capana09g001771 | up | PREDICTED: auxin transporter-like protein 15A-like [Solanum lycopersicum] |
| Capana03g004568 | up | PREDICTED: gibberellin receptor Gid1B-like [Solanum lycopersicum] |
| Capana09g003488 | up | PREDICTED: histidine-containing phosphotransfer protein 1-like [Solanum lycopersicum] |
| Capana06g000051 | up | PREDICTED: probable indole-3-acetic acid-amido synthetase GH3.1-like [Solanum lycopersicum] |
| Capana02g003021 | up | PREDICTED: probable indole-3-acetic acid-amido synthetase GH3.1-like [Solanum lycopersicum] |
| Capana08g001978 | up | PREDICTED: G-box-binding factor 4-like isoform X2 [Solanum lycopersicum] |
| Capana09g000310 | up | IAA15 [Solanum lycopersicum] |
Supplementary Table 1. Contd.

| Gene ID          | Expression | Description                                                                 |
|------------------|------------|------------------------------------------------------------------------------|
| Capana05g001701  | up         | PREDICTED: ethylene-responsive transcription factor 1B-like [Solanum tuberosum] |
| Capana07g001662  | up         | PREDICTED: probable indole-3-acetic acid-amido synthetase GH3.5-like [Solanum tuberosum] |
| Capana06g003043  | up         | PREDICTED: gibberellin receptor GID1B-like [Solanum tuberosum]                |
| Capana03g003343  | up         | PREDICTED: auxin-induced protein 22D-like [Nicotiana tomentosiformis]        |
| Capana00g000222  | up         | PREDICTED: DELLA protein GAI-like [Solanum lycopersicum]                    |
| Capana02g000676  | up         | PREDICTED: probable indole-3-acetic acid-amido synthetase GH3.1-like [Solanum tuberosum] |
| Capana07g000391  | up         | PREDICTED: auxin-responsive protein IAA20-like [Solanum tuberosum]           |
| Capana03g000749  | up         | PREDICTED: protein TIFY 6B isoform X3 [Solanum lycopersicum]                |
| CapsicumannuumL_newGene_701 | up | PREDICTED: shaggy-related protein kinase eta-like [Solanum tuberosum] |
| Capana03g000391  | up         | PREDICTED: auxin-responsive protein IAA20-like [Solanum tuberosum]           |
| Capana04g000080  | up         | Aux/IAA protein [Solanum tuberosum]                                         |
| Capana08g002278  | up         | auxin and ethylene responsive GH3-like protein [Capsicum chinense]          |
| Capana06g000110  | up         | PREDICTED: auxin-induced protein 22D-like [Solanum tuberosum]                |
| Capana05g000206  | up         | PREDICTED: ethylene-responsive transcription factor 1B-like [Solanum tuberosum] |
| Capana07g000894  | up         | NPR1 [Capsicum annuum]                                                       |
| Capana03g001668  | up         | PREDICTED: uncharacterized protein LOC102598616 [Solanum tuberosum]          |
| Capana04g000996  | up         | PREDICTED: serine/threonine-protein kinase SAPK1 isoform X1 [Nicotiana tomentosiformis] |
| Capana08g002366  | up         | abscisic acid-insensitive 5-like protein [Solanum nigrum]                    |
| Capana04g001165  | up         | PREDICTED: transcription factor TGA1-like [Nicotiana sylvestris]            |
| Capana10g002278  | up         | PREDICTED: two-component response regulator ARR9-like [Solanum tuberosum]    |
| Capana07g000264  | up         | PREDICTED: auxin response factor 1-like [Nicotiana sylvestris]              |
| Capana05g000287  | up         | PREDICTED: serine/threonine-protein kinase SAPK2-like isoform X2 [Nicotiana tomentosiformis] |
| Capana03g001923  | up         | PREDICTED: auxin-induced protein 6B-like [Nicotiana sylvestris]             |
| Capana08g002192  | up         | pathogenesis-related protein PR-1 precursor [Capsicum annuum]               |

**Phenylpropanoid biosynthesis**

| Gene ID          | Expression | Description                                                                 |
|------------------|------------|------------------------------------------------------------------------------|
| Capana03g001810  | up         | RecName: Full=Caffeic acid 3-O-methyltransferase; Short=CAOMT; AltName: Full=S-adenosyl-L-methionine:caffeic acid 3-O-methyltransferase [Capsicum chinense] |
| Capana12g002757  | up         | PREDICTED: 8-hydroxygeraniol dehydrogenase-like [Solanum tuberosum]          |
| Capana03g001805  | up         | RecName: Full=Caffeic acid 3-O-methyltransferase; Short=CAOMT; AltName: Full=S-adenosyl-L-methionine:caffeic acid 3-O-methyltransferase [Capsicum chinense] |
| Capana12g002750  | up         | PREDICTED: probable mannitol dehydrogenase [Nicotiana tomentosiformis]      |
| Capana09g000319  | up         | PREDICTED: aldehyde dehydrogenase family 2 member C4-like [Solanum tuberosum] |
| Capana03g000549  | up         | putative hydroxycinnamoyl transferase [Capsicum annuum]                      |
| Capana02g002632  | down       | PREDICTED: cytochrome P450 84A1-like [Solanum tuberosum]                    |
| Capana12g002758  | down       | sinapyl alcohol dehydrogenase 2 [Nicotiana tabacum]                        |
| Capana10g000692  | down       | PREDICTED: caffeic acid 3-O-methyltransferase-like [Solanum lycopersicum]   |
| Capana02g000251  | down       | PREDICTED: probable cinnamyl alcohol dehydrogenase 6 [Solanum lycopersicum] |
| Capana12g002751  | down       | PREDICTED: 8-hydroxygeraniol dehydrogenase-like [Solanum tuberosum]          |
### Supplementary Table 1. Contd.

| Gene Name                      | Expression | Function/Activity                                                                 |
|-------------------------------|------------|-----------------------------------------------------------------------------------|
| Capana03g000476               | down       | Putative cinnamoyl-CoA reductase [Capsicum annuum]                                 |
| Capana08g002304               | up         | Putative cinnamyl alcohol dehydrogenase [Capsicum annuum]                          |
| Capana06g001112               | up         | Cinnamoyl-CoA reductase [Solanum lycopersicum]                                    |
| Capana00g002455               | up         | PREDICTED: anthocyanidin 3-O-glucosyltransferase 5-like [Solanum tuberosum]       |
| Capana03g000357               | up         | PREDICTED: caffeoylshikimate esterase [Solanum lycopersicum]                      |
| Capana09g000318               | up         | PREDICTED: aldehyde dehydrogenase family 2 member C4-like [Solanum tuberosum]    |
| CapsicumannuumL_newGene_16529 | up         | PREDICTED: 8-hydroxygeraniol dehydrogenase [Solanum lycopersicum]                |
| Capana03g001811               | up         | Caffeic acid O-methyltransferase [Capsicum annuum]                                |
| Capana10g002482               | up         | PREDICTED: cytochrome P450 98A2-like [Solanum tuberosum]                          |
| Capana07g000164               | up         | PREDICTED: shikimate O-hydroxycinnamoyltransferase-like [Nicotiana sylvestris]    |
| Capana08g001159               | up         | Putative p-coumarate 3-hydroxylase [Capsicum annuum]                              |
| Capana03g001807               | up         | RecName: Full=Caffeic acid 3-O-methyltransferase; Short=CAOMT; AltName: Full=S-adenosyl-L-methionine:caffeic acid 3-O-methyltransferase [Capsicum chinense] |
| Capana02g001298               | up         | PREDICTED: probable cinnamyl alcohol dehydrogenase 6 [Nicotiana tomentosiformis] |
| Capana03g002955               | up         | PREDICTED: aldehyde dehydrogenase family 2 member C4 [Nicotiana sylvestris]       |
| Capana06g000179               | up         | PREDICTED: caffeic acid 3-O-methyltransferase-like [Solanum lycopersicum]         |
| Capana12g002756               | up         | PREDICTED: 8-hydroxygeraniol dehydrogenase-like [Nicotiana tomentosiformis]      |
| Capana03g001803               | up         | RecName: Full=Caffeic acid 3-O-methyltransferase; Short=CAOMT; AltName: Full=S-adenosyl-L-methionine:caffeic acid 3-O-methyltransferase [Capsicum chinense] |
| Capana06g000180               | up         | PREDICTED: caffeic acid 3-O-methyltransferase-like [Solanum lycopersicum]         |
| Capana02g002655               | up         | PREDICTED: anthocyanidin 3-O-glucosyltransferase 5-like [Solanum tuberosum]       |
| Capana02g000041               | up         | PREDICTED: anthocyanidin 3-O-glucosyltransferase 5-like [Nicotiana tomentosiformis] |
| Capana09g000321               | up         | PREDICTED: aldehyde dehydrogenase family 2 member C4 [Solanum lycopersicum]       |
| Capana01g002290               | up         | PREDICTED: cinnamoyl-CoA reductase 1 isoform X2 [Solanum tuberosum]               |
| Capana06g001742               | up         | PREDICTED: caffeic acid 3-O-methyltransferase-like [Solanum tuberosum]            |
| Capana09g000320               | up         | PREDICTED: aldehyde dehydrogenase family 2 member C4-like [Solanum tuberosum]     |
| Capana04g002317               | up         | PREDICTED: cytochrome P450 84A1-like [Nicotiana tomentosiformis]                  |
| Capana02g000042               | up         | PREDICTED: anthocyanidin 3-O-glucosyltransferase 5-like [Nicotiana sylvestris]    |
| Capana03g001804               | up         | PREDICTED: caffeic acid 3-O-methyltransferase [Nicotiana sylvestris]              |
| Capana06g000178               | up         | PREDICTED: caffeic acid 3-O-methyltransferase-like [Nicotiana sylvestris]         |
| Capana08g002072               | up         | PREDICTED: acetyl-CoA-benzylalcohol acetyltransferase-like [Nicotiana sylvestris] |
| Capana10g002485               | down       | PREDICTED: cytochrome P450 98A3-like [Solanum tuberosum]                          |
| Capana03g002214               | down       | PREDICTED: acetyl-CoA-benzylalcohol acetyltransferase-like [Nicotiana sylvestris] |
| Capana10g001996               | down       | PREDICTED: cytochrome P450 98A3-like [Nicotiana tomentosiformis]                  |
| Capana11g001541               | down       | Acyltransferase 1 [Capsicum chinense]                                             |
| Capana12g0000381              | down       | PREDICTED: acetyl-CoA-benzylalcohol acetyltransferase-like [Nicotiana tomentosiformis] |
| Capana05g002521               | down       | PREDICTED: BAHD acyltransferase At5g47980-like [Solanum tuberosum]                |
| Capana06g001583               | down       | PREDICTED: deacetylvinindoline O-acetyltransferase-like [Nicotiana sylvestris]    |
| Capana09g002716               | down       | PREDICTED: cytochrome P450 98A2-like [Solanum tuberosum]                          |
| Capana09g000119               | down       | PREDICTED: shikimate O-hydroxycinnamoyltransferase-like [Nicotiana sylvestris]    |
| Capana02g002339               | down       | Acyltransferase [Capsicum frutescens]                                             |
| Capana00g002692               | down       | PREDICTED: deacetylvinindoline O-acetyltransferase-like [Solanum lycopersicum]    |