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

Yan Cheng*, Yansheng Jiao, Ruyi Miao, Ruxia Tian, Yanping Liang and Ning Qiao

Horticulture College, Shanxi Agriculture University, No.79 Longcheng Street, Xiaodian District, Taiyuan City, Shangxi Province, China.

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Stress is considered to be the inducer of microspore embyogenesis (ME), and heat stress is indispensible 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 embyogenesis (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

*Corresponding author. E-mail: chengyan820620@163.com.

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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 annumm* 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 decribed by Cheng et al. (2013). Briefly, flower buds were sterilized immediately after collection. They were dissected and cultured on pretreatment medium (10 mM $CaCl_2$, 1 mM $MnSO_4 \cdot 7H_2O$, 1 mM KNO_3, 200 M KH_2PO_4, 1 M KI, 100 nM $CuSO_4 \cdot 5H_2O$, 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 triazole 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 (5×). The first cDNA strand was synthesized with 6 polysilicon 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 $mU \cdot L^{-1}$ 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 adaptor 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 genetic 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')	Rewerse (5'→3')
*Actin*_GQ339766.1	GAAGCACCTCTCAACCCTAAG	GTACGACCACTAGCATACAAGG
Capana00g002630	CTAGGTTTGAGGGTGATAGGC	CTGAATGCAGGCTGGTAGTC
Capana01g000883	AGTCAAAGATGCGTGCTGAG	GACCCTGTATCACTGAGATTGC
Capana00g003106	GGATGTTTATGGGCTACTGTTG	TATCTCAGCTTTCCAGAATCG
Capana01g002457	TCAATGTTGCTCGGACTCTTC	CAGACCAAAACAATTAGAATAG
Capana00g001129	AACTTTCTCATGGTAACGATGC	AATCCTTAGTCGTGAATCGTGG
Capana01g001728	CTTGAAACAGCAAAGACCAGC	CATTGATGGTTGGAACAGCAC
Capana01g004182	AAAGGAATGTGGGCTGTTC	GGGTGAGAGAGTTTATGGGAG
Capana00g004135	TGTGTCTGCATTGTCTCATCC	TTTGAAGCTGGATCTGTTTCAG
Capana00g004867	TGGTTTGTTCAGGTAGGGAAG	ACCAGTTCGACAAGTTCCAG
Capana01g000278	ACCTTTTACACATTTTGGGCTG	GAGGACTATAGAGGCACAAAAC
Capana01g000279	GGACTACCCTTACAGCAACTTC	TCTCAGATCAGTCAAATGGCC
Capana01g000500	AGTCCATATTCAGAAGGCGG	TTGTACTACGTAGACTATCAC
Capana01g000731	AAGTACCAATGAAGAGGGCTG	AAAGCTCAGCGTACCATTAGG
Capana01g003125	GTGCTGATTGTGATTTCGGG	TGGGACTGGATTTGGATTTGG
Capana01g003124	CACCCTTCTCATCCTTCTCAC	CAGATCCACAGGCATTACAGG
Capana00g004543	ACCCACACCCTCTTGCTG	ACACCCAAATTCTCTGTCGAG
Capana01g004373	CGATGTCTGATATCTGTATTAG	CTTTGATAGGATCCGCTACCC
Capana00g005078	TCTGATACTGTGTTCTCTGGTG	GATATCCACCGCTACCTTGTG
Capana01g001352	AGCCCCAAGTTGTATGTGTC	TGGTGAAGTATCCGTTTCTTGG
Capana01g001414	CATCTGAGGCTACTTGGTGTC	GTTCCAGTTCATGCTTCCATTG

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 nanny gene as internal control. Quantitative comparison CT method ($^{\Delta\Delta}$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-copalyl diphosphate synthase

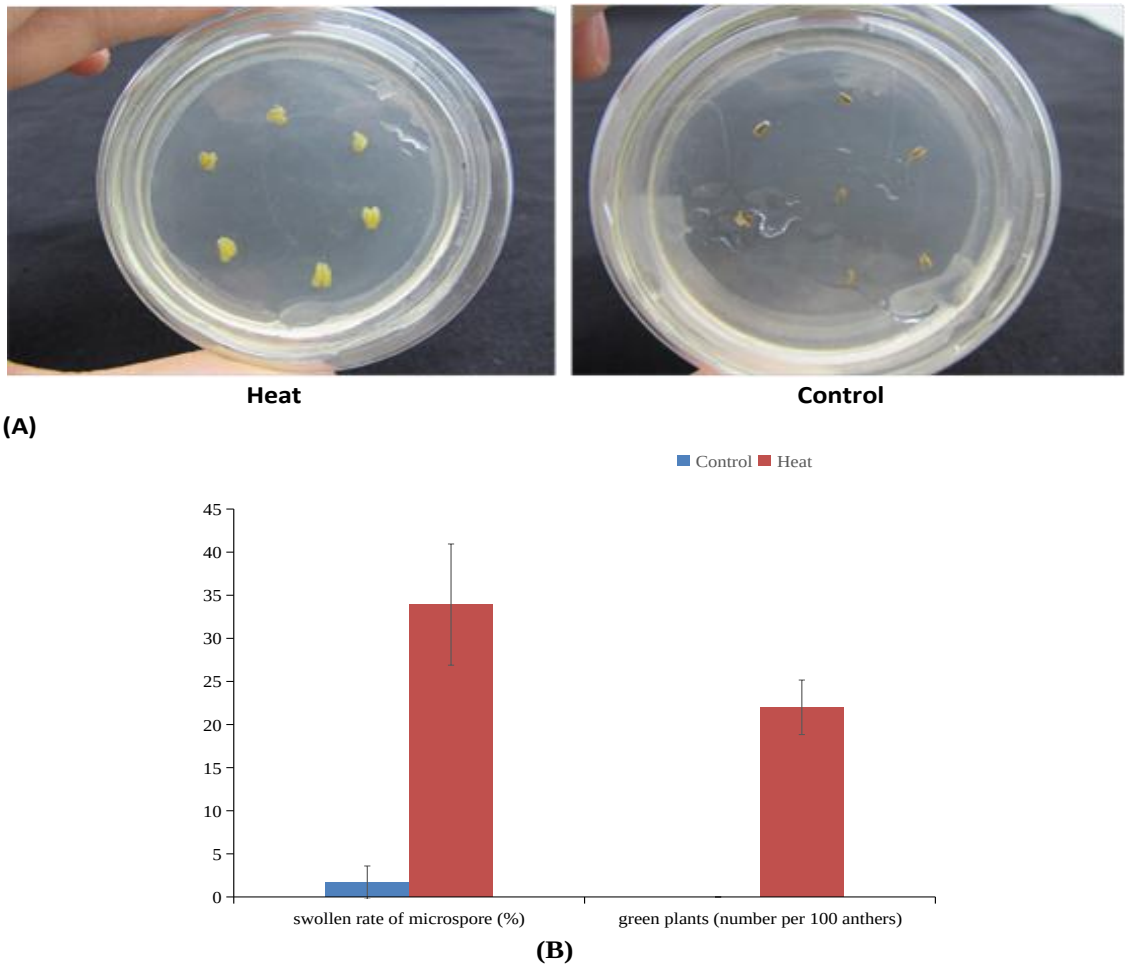

Figure 1. Comparison of ME of sweet pepper and resulted green plants produced between the control group (25℃, 7 days) and heat stress group (34℃, 7 days). (A) The pretreated anthers (7 days) between two groups. (B) The output of green plants is between two groups. Data represent the mean standard deviation (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*, *Capana01g001352, 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.

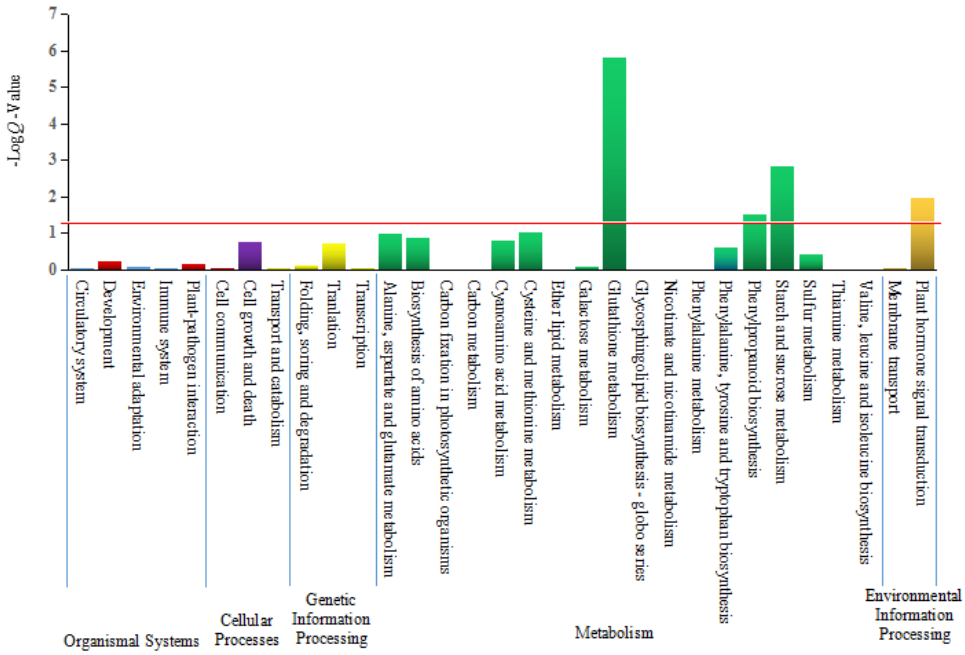

Figure 3. KEGG enrichment analysis identifying three possible main methods. The red line indicates the elevation of p=0.05.

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, glutamoren), 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 (Żur 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 RGII and XG components (Bárány 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 RGII 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 Daisy network is related to the transformation of embryogenesis and development program and cell fate (Corral-Martínez et al., 2019). The results reported in this paper show that many genes related to cell wall, major carbohydrate and starch metabolism are expressed differently during early embryonic development.

It was found that among the 20 KEGG functional pathways, plant hormone signal transduction accounted for the largest number of DEGs. Plant growth regulators are considered as the core signaling molecules for controlling plant growth and development, which respond to environmental stimuli and initiate signaling pathways (Kohli et al., 2013). Moreover, plant growth regulators interfere with the interaction between plant genotypes and environmental factors, and play a very important role in the micro-ecosystem, controlling the differentiation and development of embryos derived from microspheres and the regeneration of haploid/diploid plants (Divi et al., 2010). In the early stage of ME, numerous genes related to axins (Dubas et al., 2014), cytokinins, abscisic acid (Dubas et al., 2013), gibberellins, brassinosteroids, jasmonic acid, salicylic acid, or ethylene may produce translational regulation to adapt the stress. Hormone homeostasis seems to be one of the most important factors that determine the embryogenesis ability of cells, and a more comprehensive method is needed to understand the mechanism controlling this process, so as to break the barrier of self-resistance (Żur et al., 2015).

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, phenylalnine ammonialyase, and β-glucosidase (Zhang et al., 2019). 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 indispensible 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.

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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	PREDICTED: 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]
	Capana12g000354	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.

Capana07g002005	down	PREDICTED: probable glutathione S-transferase-like [Solanum tuberosum]
Capana02g002271	down	PREDICTED: probable glutathione S-transferase parC-like [Solanum tuberosum]
Starch and sucrose 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]
Capana04g000118	up	PREDICTED: probable alpha-amylase 2 [Nicotiana sylvestris]
Capana01g003777	up	unnamed protein product [Vitis vinifera]
Capana09g000138	up	sucrose synthase [Solanum tuberosum]
Capana00g001331	up	PREDICTED: probable trehalose-phosphate phosphatase J-like [Solanum tuberosum]
Capana09g000101	up	PREDICTED: UDP-glucuronate 4-epimerase 1-like [Nicotiana sylvestris]
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 sylvestris]
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.

		X1 [Solanum tuberosum]
Capana07g000086	up	trehalose-6-phosphate synthase [Solanum lycopersicum]
Capana03g004414	up	beta-amylase [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-amylase 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-amylase 2, chloroplastic-like isoform X2 [Phoenix dactylifera]
CapsicumannuumL_newGene_14041	down	PREDICTED: probable alpha-amylase 2 [Nicotiana sylvestris]
Capana08g000917	down	PREDICTED: beta-amylase 7-like isoform X1 [Solanum tuberosum]
Capana09g001212	down	alpha-glucan phosphorylase, H isozyme [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		
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.

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]
Capana10g001370	down	PREDICTED: auxin transporter-like protein 4 [Nicotiana tomentosiformis]
CapsicumannuumL_newGene_9164	down	PREDICTED: protein TIFY 4A-like [Solanum tuberosum]
Capana03g002801	down	PREDICTED: protein phosphatase 2C 37-like [Nicotiana sylvestris]
Capana11g002252	down	PREDICTED: transcription factor HBP-1b(c38)-like [Solanum lycopersicum]
Capana12g000990	down	PREDICTED: protein TIFY 10A-like [Solanum tuberosum]
Capana09g001555	down	PREDICTED: auxin transporter-like protein 4 [Nicotiana tomentosiformis]
Capana01g000511	down	PREDICTED: uncharacterized protein LOC101246270 [Solanum lycopersicum]
Capana06g000011	down	PREDICTED: protein TRANSPORT INHIBITOR RESPONSE 1-like [Solanum tuberosum]
CapsicumannuumL_newGene_13152	down	PREDICTED: indole-3-acetic acid-induced protein ARG7-like [Nicotiana sylvestris]
Capana00g005021	up	leucine zipper transcription factor [Solanum tuberosum]
Capana04g000600	up	CycD3;3 protein [Solanum lycopersicum]
CapsicumannuumL_newGene_11569	up	leucine zipper transcription factor TGA [Capsicum annuum]
Capana09g000028	up	F-box protein [Capsicum annuum]
Capana00g001638	up	PREDICTED: EIN3-binding F-box protein 1-like [Nicotiana sylvestris]
CapsicumannuumL_newGene_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(c1)-like [Solanum tuberosum]
Capana00g001152	up	PREDICTED: ABSCISIC ACID-INSENSITIVE 5-like protein 7-like isoform X1 [Solanum tuberosum]
Capana03g003488	up	PREDICTED: gibberellin receptor GID1B-like [Solanum tuberosum]
Capana06g000051	up	PREDICTED: histidine-containing phosphotransfer protein 1-like [Solanum tuberosum]
Capana06g001979	up	PREDICTED: indole-3-acetic acid-induced protein ARG7-like [Solanum tuberosum]
Capana06g000791	up	PREDICTED: uncharacterized protein LOC101265243 [Solanum lycopersicum]
Capana02g003021	up	PREDICTED: probable indole-3-acetic acid-amido synthetase GH3.1-like [Solanum tuberosum]
Capana08g001978	up	PREDICTED: G-box-binding factor 4-like isoform X2 [Solanum lycopersicum]
Capana03g004568	up	PREDICTED: auxin-responsive protein IAA3-like [Solanum tuberosum]
Capana06g002186	up	PREDICTED: ethylene receptor 2-like [Solanum tuberosum]
Capana09g001771	up	PREDICTED: auxin-induced protein 15A-like [Solanum lycopersicum]
Capana01g003077	up	PREDICTED: histidine-containing phosphotransfer protein 1-like isoform X1 [Solanum tuberosum]
Capana03g000310	up	IAA15 [Solanum lycopersicum]

Supplementary Table 1. Contd.

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]
Capana03g001922	up	Auxin-induced protein X15 [Glycine soja]
Capana12g001341	up	PREDICTED: transcription factor TGA1 [Solanum lycopersicum]
Capana03g000749	up	PREDICTED: protein TIFY 6B isoform X3 [Solanum lycopersicum]
CapsicumannuumL_newGene_701	up	PREDICTED: shaggy-related protein kinase eta-like [Solanum tuberosum]
Capana03g004532	up	PREDICTED: ethylene receptor 2-like isoform X1 [Solanum tuberosum]
Capana04g000808	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		
Capana03g001810	up	RecName: Full=Caffeic acid 3-O-methyltransferase; Short=CAOMT; Short=COMT; AltName: Full=S-adenosysl-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; Short=COMT; AltName: Full=S-adenosysl-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.

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; Short=COMT; AltName: Full=S-adenosysl-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; Short=COMT; AltName: Full=S-adenosysl-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 [Solanum tuberosum]
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]
Capana12g000381	down	PREDICTED: acetyl-CoA-benzylalcohol acetyltransferase-like [Nicotiana tomentosiformis]
Capana05g002521	down	PREDICTED: BAHD acyltransferase At5g47980-like [Solanum tuberosum]
Capana06g001583	down	PREDICTED: deacetylvindoline O-acetyltransferase-like [Nicotiana sylvestris]
Capana00g002716	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: deacetylvindoline O-acetyltransferase-like [Solanum lycopersicum]