Genetics and Molecular Research

Transcriptome analysis of peach (*Prunus persica* L. Batsch) during the late stage of fruit ripening

H.F. Pan[1], Y. Sheng[1], Z.H. Gao[1], H.L. Chen[1], Y.J. Qi[1], X.K. Yi[1], G.H. Qin[1] and J.Y. Zhang[1]

Horticulture Research Institute, Anhui Academy of Agricultural Science, Hefei, Anhui, China

Corresponding author: J.Y. Zhang
E-mail: zjy660@126.com

Genet. Mol. Res. 15 (4): gmr15049335
Received September 21, 2016
Accepted September 21, 2016
Published December 23, 2016
DOI http://dx.doi.org/10.4238/gmr15049335

ABSTRACT. Fruit ripening is a complex developmental process, the details of which remain largely unknown in fleshy fruits. In this paper, the fruit flesh of two peach varieties, "Zhongyou9" (a nectarine; *Prunus persica* L. Batsch) and its mutant "Hongyu", was analyzed by RNA-seq technology during two stages of ripening at 20-day intervals. One hundred and eighty significant upregulated and two hundred and thirty-five downregulated genes were identified in the experiment. Many of these genes were related to plant hormones, chlorophyll breakdown, accumulation of aroma and flavor volatiles, and stress. To the best of our knowledge, this is the first transcriptome analysis of peach ripening, and our data will be useful for further studies of the molecular basis of fruit ripening.

Key words: Peach; *Prunus persica* L. Batsch; RNA-seq; Transcriptome; Ripening

INTRODUCTION

Fruit ripening, a complex developmental process found in most angiosperms, is controlled by genetic and epigenetic regulators. Although strong regulator similarities have been demonstrated between dry and fleshy fruits, most studies have been limited to dry fruits and have used *Arabidopsis* as a model (Gapper et al., 2013). There are two groups of fleshy fruits, climacteric and nonclimacteric fruits. Climacteric fruits, such as tomatoes, apples, peaches, and bananas, show a rise in respiration during the ripening stage and increased ethylene production, and exogenous ethylene can be used to control fruit ripening post-harvest. Nonclimacteric fruits, such as grapes, strawberries, and citrus, do not ripen post-harvest (Seymour et al., 2013).

Previous studies have related fruit ripening to cell wall metabolism, cell turgor variation, ethylene synthesis, pigment accumulation, and fruit plastid development (Gapper et al., 2013). Abscisic acid was reported to interact with ethylene, cell wall, and auxin-related genes in peach (Soto et al., 2013). Jasmonates (JAs) can slow down peach ripening and are associated with the regulation of ethylene and auxin-related gene expression (Soto et al., 2012). Cell wall softening is a sign of fruit ripening, and many proteins that are associated with cell wall re-organization, such as endo-polygalacturonase, pectin methylesterase, endo-1,4-beta-glucanase, endo-1,4-beta-mannanase, alpha-arabinosidase, and beta-galactosidase, have been related to peach ripening (Brummell et al., 2004). Genes related to aroma formation, including lipoxygenase (LOX), hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH), alcohol acyltransferase (AAT), and fatty acid desaturase (FAD), show significant changes during peach ripening (Zhang et al., 2010). Ravaglia et al. (2013) reported that R2R3 MYB transcription factors may regulate flavonoid biosynthesis in nectarines (*Prunus persica*).

Although many genes related to fruit ripening have been discovered, the molecular basis of their action remains largely unknown. As the costs of high-throughput sequencing decline rapidly, many fleshy fruit species have had their genomes sequenced, and these species can now be used as models to study development and ripening on a genome-wide scale. RNA-seq is a revolutionary tool that allows not only transcriptome profiling, but also refinement of gene structures, and the detection of alternative splicing and gene fusion events (Wang et al., 2009).

Several types of fleshy fruits have been subjected to transcriptome analysis during ripening, including sweet orange (Yu et al., 2012), pear (Huang et al., 2014), and mango (Dautt-Castro et al., 2015). Peach (*P. persica*) is an important fruit-producing plant whose genome (Verde et al., 2013) and transcriptome (Wang et al., 2013) have been sequenced. The transcriptome analysis of peach during ripening was limited, however, to a microarray method. In the present study, RNA-seq was used to study the transcriptome profile of two nectarines (*P. persica* L. Batsch) in different stages of ripening.

MATERIAL AND METHODS

Sample preparation and total RNA extraction

Peach fruit flesh was collected from the varieties “Hongyu” (HY) and “Zhongyou9” (ZY9) during the late ripening stage on June 17, 2014 and July 9, 2014 (20 days apart), at the experimental base of the Department of Horticulture, Anhui Academy of Agricultural Sciences. Three fruits from the same peach tree were pooled together, and total RNA was

extracted using an Invitrogen Trizol kit (Thermo Scientific, USA). The yield of RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific), and the integrity was evaluated using 2% agarose gel electrophoresis and ethidium bromide staining.

Library construction and sequencing

Total RNA was subjected to DNase I treatment, and oligo (dT)-labeled magnetic beads were used to isolate mRNAs. The mRNAs were fragmented using fragmentation buffer and cDNAs were synthesized using random hexamer primers. Short fragments were purified and resolved with EB buffer for end repair and single nucleotide A (adenine) introduction. Adapters and barcode sequences were linked to the short fragments. For library size selection, 2% agarose gel electrophoresis was used, and fragments 200-500 bp in length were selected for PCR amplification to generate the sequencing libraries. For library quality control (QC), an Agilent 2100 Bioanaylzer and an ABI StepOnePlus Real-Time PCR System were used to quantify and qualify the constructed libraries, respectively. The libraries that passed QC were sequenced using an Illumina HiSeq 2000 in the form of 90-bp paired-end reads according to the manufacturer instructions. The original image data were converted into sequence data via base calling and saved as FASTQ files, which were referred to as raw data or raw reads. The raw reads were quality controlled and "dirty" sequences were removed using filters that eliminated: 1) reads with only adapters; 2) reads with a high content of unknown bases (more than 10%); and 3) reads with low quality (sequencing quality was no more than 10). The remaining reads were defined as clean reads for further bioinformatic analysis.

Bioinformatic analysis

To map the clean reads to the peach reference genome and transcripts, and to quality-check the alignment, SOAPaligner/SOAP2 (Li et al., 2009) was used with default parameters and no more than five mismatches allowed. The alignment QC procedures: 1) evaluated the alignment rates of clean reads mapped to the reference genome and genes; 2) examined the distribution of reads located on the genes to evaluate fragmentation randomness, i.e., to normalize gene locus positions with a 100-bp window (Wang et al., 2009); and 3) evaluated gene coverage. To identify differentially expressed genes (DEGs), the reads per kilobase of transcript per million mapped reads (RPKM) value was used to calculate relative gene expression, which was compared among samples (Mortazavi et al., 2008). The DEGs between two samples were detected using FDR ≤ 0.001 and the absolute value of Log2 ratio ≥ 1 as the threshold, according to a method previously used to define the significance of digital gene expression profiles (Audic and Claverie, 1997). To further evaluate DEG function, the Cluster and Java Treeview software was used for cluster analysis of gene expression patterns, and Gene Ontology (GO) and KEGG pathway enrichment analysis was performed to discover enriched GO terms and pathways. To refine the gene structures, Cufflink was used (Roberts et al., 2011) to assemble transcripts to find new transcripts and to extend the 5' or 3' ends of genes. The new transcripts should be at least 200 bp away from the annotated gene, with a length greater than 180 bp and a sequencing depth no less than 2. The Coding Potential Calculator was used to assess protein-coding potential. To identify alternative splicing events, TopHat was used. SOAPsnp was used to detect SNPs and SOAPfuse was used to detect gene fusions.

Quantitative real-time reverse transcription PCR (qRT-PCR)

Quantification was performed with a two-step reaction process: reverse transcription (RT) and PCR. Each RT reaction used 0.5 µg RNA, 2 µL PrimerScript Buffer, 0.5 µL of oligo dT, 0.5 µL random 6-mers, and 0.5 µL PrimerScript RT Enzyme Mix I (TaKaRa, Japan), in a total volume of 10 µL. Reactions were performed in a GeneAmp® PCR System 9700 (Applied Biosystems, USA) for 15 min at 37°C, followed by heat inactivation of the RT reaction for 5 s at 85°C. The 10 µL RT reaction mix was then diluted 10X in nuclease-free water and held at -20°C.

Real-time PCR was performed using a LightCycler® 480 II Real-time PCR Instrument (Roche, Switzerland) with a 10 µL PCR mixture that included 1 µL of cDNA, 5 µL 2X LightCycler® 480 SYBR Green I Master (Roche), 0.2 µL forward primer, 0.2 µL reverse primer, and 3.6 µL nuclease-free water. Reactions were incubated in a 384-well optical plate (Roche) at 95°C for 10 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. Each sample was run in triplicate for analysis. At the end of the PCR cycles, melting curve analysis was performed to validate the specific generation of the expected PCR product. The primer sequences were designed in the laboratory and synthesized by Generay Biotech (Generay, China), based on the mRNA sequences obtained from the NCBI database.

The expression levels of the mRNAs were normalized to that of 18S rRNA (three technical repeats were used) and were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

RESULTS

RNA-seq data statistics

RNA-seq produced 48-Mb reads that were 90 bp in length, representing more than 4 Gb of clean RNA-seq data. More than 80% of the clean reads were mapped to a gene or the genome. Approximately 19,000 expressed genes were detected in each sample. The data are shown in Table 1.

Table 1. Basic statistics of the four RNA-seq peach samples.

Sample name	Clean reads (n)	Clean data (bp)	Genome map rate	Gene map rate	Expressed genes
HY-1	48,290,784	4,346,170,560	84.40%	81.76%	19,323
HY-2	48,191,506	4,337,235,540	83.87%	83.34%	19,339
ZY9-1	48,240,156	4,341,614,040	84.67%	82.76%	19,278
ZY9-2	48,248,980	4,342,408,200	85.14%	84.73%	18,945

HY-1, Hongyu stage 1; HY-2, Hongyu stage 2; ZY-1, Zhongyou9 stage 1; ZY-2, Zhongyou9 stage 2.

DEGs

Relative gene expression levels are listed in **Table S1**. The DEGs of HY-2 *vs* HY-1 and of ZY9-2 *vs* ZY9-1 were identified and are shown in Figure 1 (all genes are listed in **Table S2**).

The DEGs common to both ZY9 and HY during fruit ripening, comprising 180 significantly upregulated and 235 downregulated genes, are listed in **Table S3**. To further annotate and evaluate their function in fruit development and ripening, they were selected according to the following criteria: 1) the genes should show a four-fold change between ripening groups and immature groups; and 2) the regulation model should be the same in both

HY and ZY9 groups, or both HY-2 *vs* HY-1 and ZY9-2 *vs* ZY9-1 should be upregulated or downregulated simultaneously.

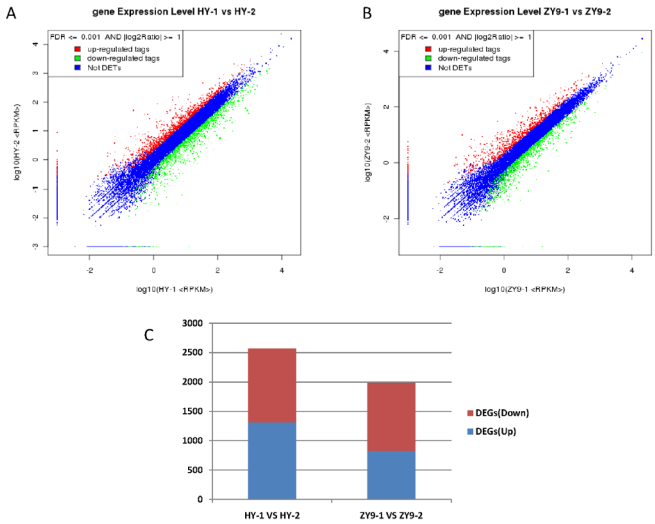

Figure 1. Differentially expressed genes during ripening. HY-1, Hongyu stage 1; HY-2, Hongyu stage 2; ZY-1, Zhongyou9 stage 1; ZY-2, Zhongyou9 stage 2. Scatter diagram of upregulated and downregulated DEGs of HY-1 *vs* HY-2 (**A**), and ZY9-1 *vs* ZY9-2 (**B**); histogram (**C**) of DEGs.

To further dissect the common molecular basis of ripening in these two peach species, enriched GOs of common DEGs were identified (Figure 2). The top 20 enriched pathways of the common DEGs are shown in Figure 3 (all results are listed in **Table S4**).

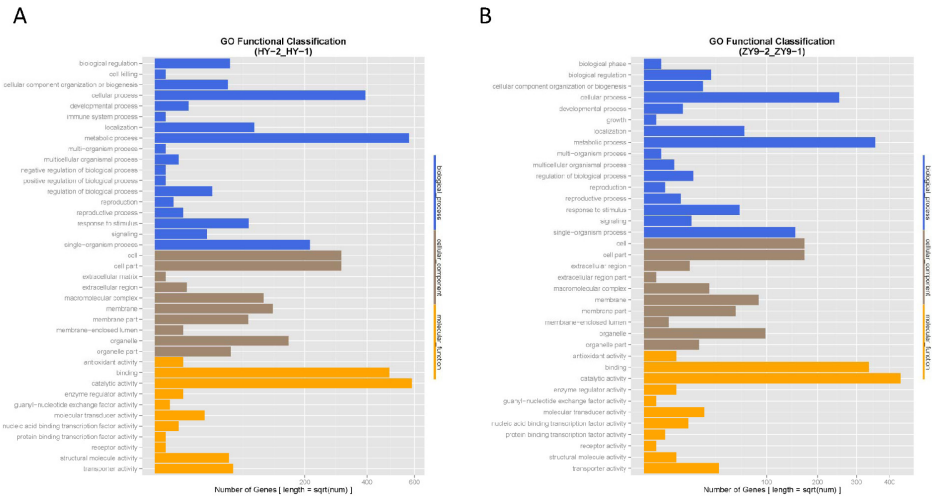

Figure 2. Gene Ontology enrichment of DEGs during ripening. **A.** Downregulated DEGs, **B.** upregulated DEGs.

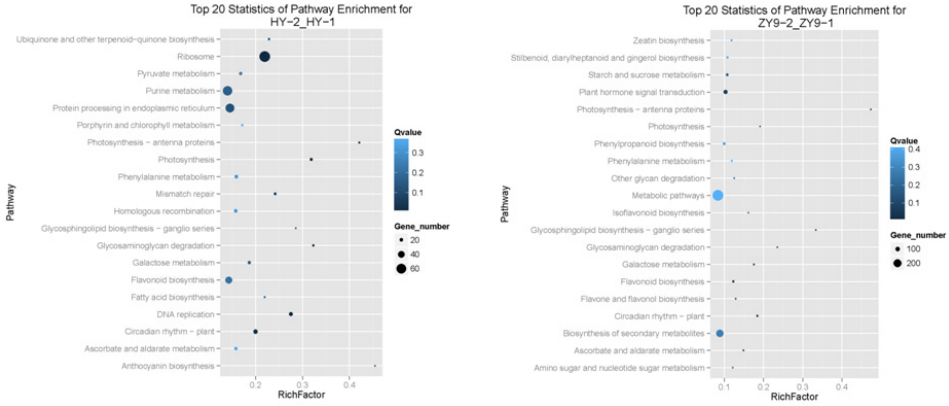

Figure 3. Enriched pathways of ripening-related DEGs. **A.** Downregulated DEGs, **B.** upregulated DEGs.

To further analyze the molecular function of the DEGs, the Genome Database for Rosaceae (GDR) website (http://www.rosaceae.org/) was used for Interpro, GO, and KEGG annotation. Subsequently, PlantTFDB 3.0 was also used to identify transcription factor annotations of these DEGs. Manual curation of selected DEGs was also used according to the value of their gene expression. Genes with a relative expression level of no more than 4 were filtered out, or RPKM, Log2 ratio of different groups was between -2 to 2. Last, PubMed-based data mining was performed using GDR and/or PlantTFDB version 3.0 (http://planttfdb.cbi.pku.edu.cn) annotation with "fruit ripening" as the keyword to determine if these DEGs had previously been reported or were possible novel ripening-related genes. All significant ripening-related genes are listed in **Table S5**.

qRT-PCR

To evaluate the RNA-seq performance, five upregulated and downregulated genes were randomly selected and random primers were generated, as listed in **Table S6**. As shown in Figure 4, the qRT-PCR results agreed well with the RNA-seq results.

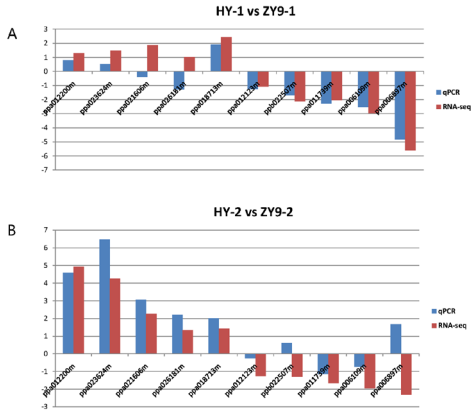

Figure 4. Correlation of qRT-PCR results with the RNA-seq results. **A.** HY-2 *vs* HY-1, **B.** ZY9-2 *vs* ZY9-1.

Alternative splicing events

As shown in Figure 5, major splicing events were found, such as intron retention and genes that had undergone exon skipping during the early stage of ripening. Fewer genes had undergone alternative splicing during ripening, especially in ZY9. All alternative splicing events are listed in **Table S7**.

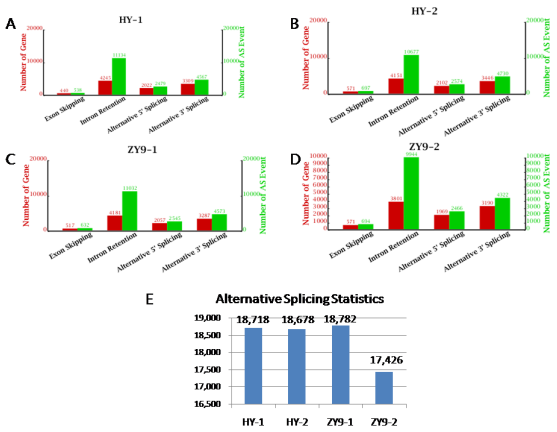

Figure 5. Alternative splicing events during peach ripening. Number of genes and alternative splicing (AS) events in HY-1 (**A**), HY-2 (**B**), ZY9-1 (**C**), and ZY9-2 (**D**). **E.** Statistics of all AS events.

SNPs

SNP analysis was performed on all four samples. As shown in Figure 6, the RNA SNP types were mainly A/G transitions or C/T transversions; however, no gene-disrupting functional SNPs were found.

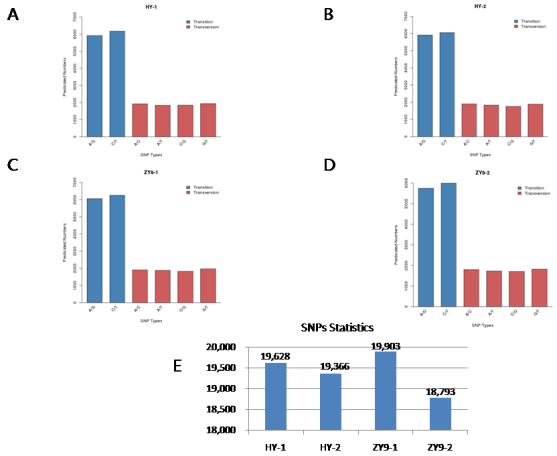

Figure 6. SNP types identified during peach ripening. SNP types in HY-1 (**A**), HY-2 (**B**), ZY9-1 (**C**), and ZY9-2 (**D**). **E.** Statistics of all SNPs.

New transcript detection

As shown in Figure 7, new transcripts from all four samples were detected. All new transcripts are listed in **Table S8**. New transcripts represent those not annotated as genes in the published genome or those that were not present in the previously published transcriptome data.

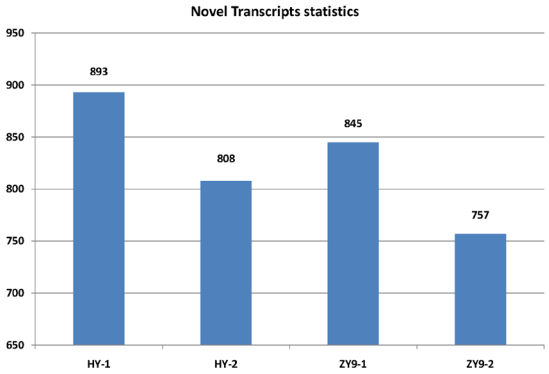

Figure 7. New transcripts detected in four samples.

Gene structure refinement

As shown in Figure 8, the structures of many genes were refined compared with the data from the published genome. All genes with refined structures are listed in **Table S9**.

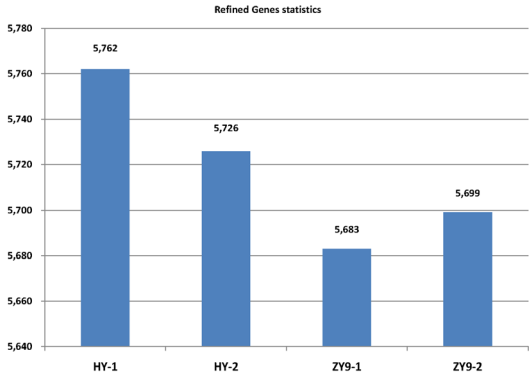

Figure 8. Statistics of genes with refined structures.

Gene fusion

No gene fusion events were identified.

DISCUSSION

The various DEGs were grouped into the following categories according to their different functional roles during fruit ripening: plant hormone metabolism, cell wall

metabolism, chlorophyll breakdown, accumulation of aroma and flavor volatiles, transcription factors, and stress-related genes. These genes are all listed in **Table S10**.

Plant hormones

Plant hormones play important roles in fruit development and ripening. Gibberellin is a plant hormone that is used widely to delay the progress of fruit ripening (Dostal and Leopold, 1967). In our experiment, *ppa020764m* and *ppa026043m*, two putative gibberellin-regulated protein genes, were significantly downregulated.

Cell wall metabolism

Ziosi et al. (2008) performed a microarray-based transcriptome study of peach that aimed to identify DEGs using JAs to delay peach fruit ripening. The DEGs responding to JAs were cell wall-related genes and stress-related genes. Some ripening-related genes, such as pectate lyase (PL) and polygalacturonase (PG), were downregulated. This is consistent with our data, in which *ppa006857m*, encoding a putative glycoside hydrolase from family 28 that might be involved in the PG pathway, was upregulated. Interestingly, *ppa021513m*, encoding a putative glycoside hydrolase from family 38, was found downregulated significantly, especially in HY. Furthermore, *ppa007756m*, a putative alpha-1,4-glucan-protein synthase gene related to cell wall organization, was upregulated, and *ppa006779m*, a putative pectinesterase gene that is related to cell wall modification, was downregulated. A putative cellulose synthase gene, *ppa001952m*, was upregulated.

Accumulation of aroma and flavor volatiles

In our study, *ppa003798m*, encoding a putative FAD-linked oxidase, was significantly downregulated. *Ppa007757m*, a putative ADH super-family gene, was also downregulated. This was not consistent with a previous study that showed FAD genes (*PpFAD1* and *PpFAD2*) were upregulated (Zhang et al., 2010). Further study is necessary to determine the exact function of *ppa007757m*.

Transcription factors (TFs)

Multiple studies suggest that TFs play important roles during fruit ripening. Pirona et al. (2013) reported that *ppa008301m*, a TF of the NAC family with conserved N-terminal DNA-binding domains and variable C-terminal domains, might control the maturity date in peaches. *Ppa021989m*, encoding a putative TF of the NAC family, was significantly downregulated.

MYB factors represent a family of proteins with a conserved MYB DNA-binding domain. *Ppa001765m* and *ppa010846m*, which are putative TFs encoding genes from the MYB family, were found to be upregulated, while *ppa006715m* was downregulated. This phenomenon is consistent with previous studies. Interesting, *ppa005162m*, a putative UFGT (UDP-glucose-flavonoid-3-O-glucosyltransferase) gene, was found to be upregulated. Further functional studies are necessary to dissect the regulatory mechanism of MYB and UFGT genes in ripening.

CO (CONSTANS) TFs, which contain both zinc finger regions and CCT (CO, CO-like, TOC1) domains, play important roles in the regulation of flowering by photoperiod in

Arabidopsis (Griffiths et al., 2003). *Ppa005713m*, encoding a putative transcription factor from the CO-like family, was found to be downregulated. *Ppa007290m*, encoding a possible TF that not only contains a CCT domain, but also was annotated to have protein binding activity according to GDR's GO search, was found to be significantly upregulated. Further studies are necessary to better understand how the CO gene is involved in ripening.

The Sporocyteless/Nozzle (SPL/NZZ) TF family, which is made up of transcriptional repressors, regulates floral organ differentiation (Wei et al., 2015). In our study, *ppa017436m*, encoding a putative TF from the NZZ/SPL family, was significantly downregulated.

The GRAS transcription factor family, which contains both conserved residues in the C terminus and a variable N terminus with homopolymeric stretches of certain amino acids, was found to be a target of RIN (ripening-inhibitor), one of the earliest-acting ripening regulators (Fujisawa et al., 2012). *Ppa020043m*, encoding a putative transcription factor from the GRAS family, was found to be downregulated.

Golden 2-like, also known as GLK, is a TF family that regulates chloroplast development in fruit, such as tomato (Nadakuduti et al., 2014). *Ppa008837m*, encoding a putative TF from the G2-like family, was significantly downregulated. These results are consistent with our previous observation that chlorophyll is broken down during ripening.

The ERF (ethylene response factor) TF family, also known as AP2 (APETALA2), plays important roles, not only in plant development, but also in stress responses (Nakano et al., 2006). Chung et al. (2010) found that *SlAP2a*, a tomato AP2/ERF gene, is a negative regulator of fruit ripening. In the present study, *ppa026499m*, encoding a putative transcription factor of the ERF family, was upregulated, especially in ZY9.

The B3 TF family, which contains an approximately 110-amino-acid region called the B3 domain, includes the auxin response factor (ARF), the LAV family, and the RAV and REM families (Swaminathan et al., 2008). The role of the B3 family in ripening is poorly understood. *Ppa021307m*, a putative TF from the B3 family containing an AP2 domain, was downregulated. Further studies are needed to determine the function of B3 family TFs in peach.

Two genes with zinc finger domains were identified and both showed altered expression during ripening: *ppa012724m* was upregulated, while *ppa022195m* was downregulated. All of these TF genes require further study.

Stress-related genes

Plants may accumulate stress-related proteins during ripening. The NBS-LRR (nucleotide-binding site-leucine-rich repeat) gene family is the largest class of disease resistance genes (R genes) (Vanloon et al., 2006). Two putative leucine-rich repeats containing DEGs, *ppa019742m* and *ppa005241m*, were found to be downregulated. Falara et al. (2011) used a microarray platform to study the DEGs in peach under long cold storage, and they found that beta-D-xylosidase and pathogenesis-related-4B (PR-4B) precursor genes were significantly upregulated. Heat-shock proteins are upregulated during post-harvest heat treatment (Zhang et al., 2011). *Ppa008260m*, a putative heat-shock protein-encoding gene, was upregulated. Bet VI belongs to a pathogenesis-related family and is induced by stress conditions (Bufe et al., 1996). In this study, *ppb010518m*, a putative BetvI domain gene, was upregulated.

A previous study (Itzhaki et al., 1994) reported that ethylene-responsive enhancer elements could downregulate GST expression. In addition, a recent study (Agudelo-Romero et al., 2015) reported that GST was upregulated during *Botrytis cinerea* infection

in *Vitis vinifera*. *Ppa011307m* and *ppa011383m*, two putative GST genes, were found to be upregulated. *Ppa012062m* and *ppa010869m*, which encode putative plant disease resistance response proteins, were found to be downregulated. Further studies are necessary to determine the function of these R genes during peach ripening.

The heme protein is a newly reported plant protein whose function is poorly understood. Lee et al. (2012) determined that disrupting the binding of AtHBP5 (heme protein 5) to HY1 (heme oxygenase 1) might lead to oxidative stress in *Arabidopsis*. *Ppa010934m*, encoding a putative SOUL/heme protein, was upregulated significantly. *Ppa010413m*, encoding a putative heme peroxidase, was found to be upregulated.

Dehydrin genes play important roles in peach in response to cold and water deficit stress (Arora and Wisniewski, 1994). *Ppa010975m*, a possible dehydrin gene, was found to be downregulated.

Other ripening-related genes

Fruit morphogenesis is an important process during fruit ripening, involving the enlargement and differentiation of distinct cell types. Fernandez et al. (2007) found that *ATHB13*, encoding a BURP domain protein, plays an important role in grape berry morphogenesis. In the present study, *ppa014595m*, encoding a putative BURP domain containing protein, was downregulated. This is consistent with a previous study where *ATHB13* expression in a grape mutant was affected.

Two putative GDSL lipase-like protein genes, *ppa021518m* and *ppa021185m*, were found to be upregulated. The function of GDSL lipase-like protein remains to be fully determined. Kikuta et al. (2012) reported that *TcGLIP* encodes a GDSL lipase-like protein that regulates pyrethrins, which are widely used natural insecticides, biosynthesized in *Tanacetum cinerariifolium* via an ester-forming reaction. Petit et al. (2014) reported that a GDSL lipase-like protein plays an important role in cuticle formation and in fruit brightness in tomato.

ACKNOWLEDGMENTS

We thank the Anhui Provincial Fruit “115” Innovation Team, and the Key Laboratory of Genetic Improvement and Ecophysiology of Horticultural Crops, Anhui Province, Institute of Horticulture, Anhui Academy of Agricultural Science. Research supported by the Anhui Fruit-tree Technology System and the Anhui Academy of Agricultural Sciences’ discipline construction projects (#14A0306).

REFERENCES

Agudelo-Romero P, Erban A, Rego C, Carbonell-Bejerano P, et al. (2015). Transcriptome and metabolome reprogramming in *Vitis vinifera* cv. Trincadeira berries upon infection with *Botrytis cinerea*. *J. Exp. Bot.* 66: 1769-1785. http://dx.doi.org/10.1093/jxb/eru517

Arora R and Wisniewski ME (1994). Cold acclimation in genetically related (sibling) deciduous and evergreen peach (*Prunus persica* [L.] Batsch). II. A 60-kilodalton bark protein in cold-acclimated tissues of peach is heat stable and related to the dehydrin family of proteins. *Plant Physiol.* 105: 95-101. http://dx.doi.org/10.1104/pp.105.1.95

Audic S and Claverie JM (1997). The significance of digital gene expression profiles. *Genome Res.* 7: 986-995.

Brummell DA, Dal Cin V, Crisosto CH and Labavitch JM (2004). Cell wall metabolism during maturation, ripening and senescence of peach fruit. *J. Exp. Bot.* 55: 2029-2039. http://dx.doi.org/10.1093/jxb/erh227

Bufe A, Spangfort MD, Kahlert H, Schlaak M, et al. (1996). The major birch pollen allergen, Bet v 1, shows ribonuclease activity. *Planta* 199: 413-415. http://dx.doi.org/10.1007/BF00195733

Chung MY, Vrebalov J, Alba R, Lee J, et al. (2010). A tomato (*Solanum lycopersicum*) APETALA2/ERF gene, SlAP2a, is a negative regulator of fruit ripening. *Plant J.* 64: 936-947. http://dx.doi.org/10.1111/j.1365-313X.2010.04384.x

Dautt-Castro M, Ochoa-Leyva A, Contreras-Vergara CA, Pacheco-Sanchez MA, et al. (2015). Mango (*Mangifera indica* L.) cv. Kent fruit mesocarp *de novo* transcriptome assembly identifies gene families important for ripening. *Front. Plant Sci.* 6: 62. http://dx.doi.org/10.3389/fpls.2015.00062

Dostal HC and Leopold AC (1967). Gibberellin delays ripening of tomatoes. *Science* 158: 1579-1580. http://dx.doi.org/10.1126/science.158.3808.1579

Falara V, Manganaris GA, Ziliotto F, Manganaris A, et al. (2011). A ß-D: -xylosidase and a PR-4B precursor identified as genes accounting for differences in peach cold storage tolerance. *Funct. Integr. Genomics* 11: 357-368. http://dx.doi.org/10.1007/s10142-010-0204-1

Fernandez L, Torregrosa L, Terrier N, Sreekantan L, et al. (2007). Identification of genes associated with flesh morphogenesis during grapevine fruit development. *Plant Mol. Biol.* 63: 307-323. http://dx.doi.org/10.1007/s11103-006-9090-2

Fujisawa M, Shima Y, Higuchi N, Nakano T, et al. (2012). Direct targets of the tomato-ripening regulator RIN identified by transcriptome and chromatin immunoprecipitation analyses. *Planta* 235: 1107-1122. http://dx.doi.org/10.1007/s00425-011-1561-2

Gapper NE, McQuinn RP and Giovannoni JJ (2013). Molecular and genetic regulation of fruit ripening. *Plant Mol. Biol.* 82: 575-591. http://dx.doi.org/10.1007/s11103-013-0050-3

Griffiths S, Dunford RP, Coupland G and Laurie DA (2003). The evolution of CONSTANS-like gene families in barley, rice, and *Arabidopsis*. *Plant Physiol.* 131: 1855-1867. http://dx.doi.org/10.1104/pp.102.016188

Huang G, Li T, Li X, Tan D, et al. (2014). Comparative transcriptome analysis of climacteric fruit of Chinese pear (*Pyrus ussuriensis*) reveals new insights into fruit ripening. *PLoS One* 9: e107562. http://dx.doi.org/10.1371/journal.pone.0107562

Itzhaki H, Maxson JM and Woodson WR (1994). An ethylene-responsive enhancer element is involved in the senescence-related expression of the carnation glutathione-S-transferase (GST1) gene. *Proc. Natl. Acad. Sci. USA* 91: 8925-8929. http://dx.doi.org/10.1073/pnas.91.19.8925

Kikuta Y, Ueda H, Takahashi M, Mitsumori T, et al. (2012). Identification and characterization of a GDSL lipase-like protein that catalyzes the ester-forming reaction for pyrethrin biosynthesis in *Tanacetum cinerariifolium* - a new target for plant protection. *Plant J.* 71: 183-193. http://dx.doi.org/10.1111/j.1365-313X.2012.04980.x

Lee HJ, Mochizuki N, Masuda T and Buckhout TJ (2012). Disrupting the bimolecular binding of the haem-binding protein 5 (AtHBP5) to haem oxygenase 1 (HY1) leads to oxidative stress in *Arabidopsis*. *J. Exp. Bot.* 63: 5967-5978. http://dx.doi.org/10.1093/jxb/ers242

Li R, Li Y, Fang X, Yang H, et al. (2009). SNP detection for massively parallel whole-genome resequencing. *Genome Res.* 19: 1124-1132. http://dx.doi.org/10.1101/gr.088013.108

Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-DDCt} method. *Methods* 25: 402-408. http://dx.doi.org/10.1006/meth.2001.1262

Mortazavi A, Williams BA, McCue K, Schaeffer L, et al. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods* 5: 621-628. http://dx.doi.org/10.1038/nmeth.1226

Moore S, Vrebalov J, Payton P and Giovannoni J (2002). Use of genomics tools to isolate key ripening genes and analyse fruit maturation in tomato. *J. Exp. Bot.* 53: 2023-2030. http://dx.doi.org/10.1093/jxb/erf057

Nadakuduti SS, Holdsworth WL, Klein CL and Barry CS (2014). KNOX genes influence a gradient of fruit chloroplast development through regulation of GOLDEN2-LIKE expression in tomato. *Plant J.* 78: 1022-1033. http://dx.doi.org/10.1111/tpj.12529

Nakano T, Suzuki K, Fujimura T and Shinshi H (2006). Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice. *Plant Physiol.* 140: 411-432. http://dx.doi.org/10.1104/pp.105.073783

Petit J, Bres C, Just D, Garcia V, et al. (2014). Analyses of tomato fruit brightness mutants uncover both cutin-deficient and cutin-abundant mutants and a new hypomorphic allele of GDSL lipase. *Plant Physiol.* 164: 888-906. http://dx.doi.org/10.1104/pp.113.232645

Pirona R, Eduardo I, Pacheco I, Da Silva Linge C, et al. (2013). Fine mapping and identification of a candidate gene for a major locus controlling maturity date in peach. *BMC Plant Biol.* 13: 166. http://dx.doi.org/10.1186/1471-2229-13-166

Ravaglia D, Espley RV, Henry-Kirk RA, Andreotti C, et al. (2013). Transcriptional regulation of flavonoid biosynthesis in nectarine (*Prunus persica*) by a set of R2R3 MYB transcription factors. *BMC Plant Biol.* 13: 68. http://dx.doi.org/10.1186/1471-2229-13-68

Roberts A, Pimentel H, Trapnell C and Pachter L (2011). Identification of novel transcripts in annotated genomes using RNA-Seq. *Bioinformatics* 27: 2325-2329. http://dx.doi.org/10.1093/bioinformatics/btr355

Seymour GB, Østergaard L, Chapman NH, Knapp S, et al. (2013). Fruit development and ripening. *Annu. Rev. Plant Biol.* 64: 219-241. http://dx.doi.org/10.1146/annurev-arplant-050312-120057

Soto A, Ruiz KB, Ravaglia D, Costa G, et al. (2013). ABA may promote or delay peach fruit ripening through modulation of ripening- and hormone-related gene expression depending on the developmental stage. *Plant Physiol. Biochem.* 64: 11-24. http://dx.doi.org/10.1016/j.plaphy.2012.12.011

Soto A, Ruiz KB, Ziosi V, Costa G, et al. (2012). Ethylene and auxin biosynthesis and signaling are impaired by methyl jasmonate leading to a transient slowing down of ripening in peach fruit. *J. Plant Physiol.* 169: 1858-1865. http://dx.doi.org/10.1016/j.jplph.2012.07.007

Swaminathan K, Peterson K and Jack T (2008). The plant B3 superfamily. *Trends Plant Sci.* 13: 647-655. http://dx.doi.org/10.1016/j.tplants.2008.09.006

van Loon LC, Rep M and Pieterse CM (2006). Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* 44: 135-162. http://dx.doi.org/10.1146/annurev.phyto.44.070505.143425

Verde I, Abbott AG, Scalabrin S, Jung S, et al.; International Peach Genome Initiative (2013). The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. *Nat. Genet.* 45: 487-494. http://dx.doi.org/10.1038/ng.2586

Wang L, Zhao S, Gu C, Zhou Y, et al. (2013). Deep RNA-Seq uncovers the peach transcriptome landscape. *Plant Mol. Biol.* 83: 365-377. http://dx.doi.org/10.1007/s11103-013-0093-5

Wang Z, Gerstein M and Snyder M (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 10: 57-63. http://dx.doi.org/10.1038/nrg2484

Wei B, Zhang J, Pang C, Yu H, et al. (2015). The molecular mechanism of sporocyteless/nozzle in controlling *Arabidopsis* ovule development. *Cell Res.* 25: 121-134. http://dx.doi.org/10.1038/cr.2014.145

Yu K, Xu Q, Da X, Guo F, et al. (2012). Transcriptome changes during fruit development and ripening of sweet orange (*Citrus sinensis*). *BMC Genomics* 13: 10. http://dx.doi.org/10.1186/1471-2164-13-10

Zhang B, Shen JY, Wei WW, Xi WP, et al. (2010). Expression of genes associated with aroma formation derived from the fatty acid pathway during peach fruit ripening. *J. Agric. Food Chem.* 58: 6157-6165. http://dx.doi.org/10.1021/jf100172e

Zhang L, Yu Z, Jiang L, Jiang J, et al. (2011). Effect of post-harvest heat treatment on proteome change of peach fruit during ripening. *J. Proteomics* 74: 1135-1149. http://dx.doi.org/10.1016/j.jprot.2011.04.012

Ziosi V, Bonghi C, Bregoli AM, Trainotti L, et al. (2008). Jasmonate-induced transcriptional changes suggest a negative interference with the ripening syndrome in peach fruit. *J. Exp. Bot.* 59: 563-573. http://dx.doi.org/10.1093/jxb/erm331

Supplementary material

Table S1. Genes and expression level of four RNA-seq peach samples.

Table S2. Differential expression genes of HY-2 Vs HY-1 and ZY9-2 Vs ZY9-1.

Table S3. Up and down regulated common differential expression genes during fruit ripening between ZY9 and HY.

Table S4. Pathways of the common DEGs.

Table S5. Significant Differential Expression Genes during fruit ripening.

Table S6. Genes and primers for qRT-PCR.

Table S7. All alternative splicing events during ripening.

Table S8. New transcripts.

Table S9. The genes with refined structures.

Table S10. The related genes in different functions.