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METABOLIC PATHWAYS ANALYSIS AND IDENTIFICATION OF HEAT RESPONSE GENES OF PINEAPPLE [*ANANAS COMOSUS* (L.) MERR.] FRUIT AFFECTED BY ELEVATED POSTHARVEST TEMPERATURE

CHUAN-HE LIU[1, 2, 3*], YAN LIU[1, 2, 3], SHI-ZI KUANG[1, 2, 3] AND WEI-QIANG XIAO[1, 2, 3]

[1]*Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, 510640, Guangzhou, P.R. China*
[2]*Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization Ministry of Agriculture, 510640, Guangzhou, P. R. China*
[3]*Guangdong Province Key Laboratory of Tropical & Subtropical Fruit Tree Research, 510640, Guangzhou, P.R. China*
[*]*Corresponding author's email: founderlch@126.com*

Abstract

Pineapple [*Ananas comosus* (L.) Merr.] is an important tropical fruit. The pineapple fruits that are ripened in the high-temperature season have better quality, whereas those ripened in low-temperature season are acidic in taste. Elevated postharvest temperature (EPT) could enhance the quality of winter-harvested pineapple fruit. Based on transcriptome and differentially expressed genes analysis, we explored the GO (Gene Ontology) terms and KEGG (Kyoto Encyclopedia of Genes and Genomes) metabolic pathways associated with the EPT treatment and the differentially expressed heat response genes of pineapple fruit after EPT treatment. GO classifications suggested that DEGs (differentially expressed genes) were predominantly annotated to "response to stimulus", "response to external stimulus" and "aromatic compound biosynthetic process" in the biological process ontology, "hydrolase activity" and "glucosidase activity" in the molecular function ontology, as well as "intrinsic to membrane" and "cell wall" in the cellular component ontology. KEGG metabolic pathways analysis revealed that the DEGs were dominantly enriched to "starch and sucrose metabolism", "biosynthesis of secondary metabolites", "pentose and glucuronate interconversions", "carotenoid biosynthesis", "metabolic pathways" "galactose metabolism" and "plant hormone signal transduction". Nineteen *HSP* (heat shock protein) and *sHSP* (small HSP) DEGs were screened, and most of them were up-regulated by EPT. Most of the transcription factor genes, including *HSF*, *bHLH*, *WRKY*, *MYB*, *AP2/ERF*, *bZIP* and *NAC*, were down-regulated by EPT. The *SOD* (Superoxide dismutase) genes were induced by EPT, while most of the *POD* (Peroxidase) and *CAT* (Catalase) genes were repressed. This work would help to understand the molecular mechanisms for EPT process to improve the quality of pineapple fruits.

Key words: *Ananas comosus* (L.); Gene ontology; Elevated postharvest temperature (EPT); Heat response genes

Introduction

The pineapple [*Ananas comosus* (L.) Merr.] is a popular fruit from tropical and subtropical regions. The pineapple fruits that are ripened in the high-temperature season have a rich flavor with attractive quality, whereas those that are ripened in the low-temperature season are acidic in taste with a slight fragrance. The consumers are willing to pay more for the pineapple fruits ripened in high-temperature season. Prior reports suggest that elevated postharvest temperature (EPT) improves the quality of pineapple fruits ripened in winter and enhance the production of aromatic components when compared with those fruits which are kept at a comparatively lower temperature (Liu & Liu, 2014; 2017). EPT could increase the contents of total sugar and total soluble solid and increase the biosynthesis of ester aromatic components of pineapple fruit (Liu & Liu, 2014; 2017). A few differentially expressed genes (DEGs) have also been identified underlying the EPT process, including those genes which are associated with the coloration, sugar/acid metabolism process, texture softening and aromatic components biosynthesis, which contribute to understand the process for EPT to regulate the quality of pineapple fruits (Liu & Liu, 2017).

Nevertheless, compared to an appropriate temperature, high temperature usually results in the increased synthesis and accumulation of heat shock proteins (HSPs) and small HSPs (sHSPs) (Muthusamy *et al.*, 2017). Transcription factors, which play a key role in the regulation of gene expression under abiotic and biotic stresses in plants, are also affected by high temperature (Sun *et al.*, 2014). To maintain redox homeostasis under high temperature stress, plant cells must elevate their capacity to trigger the gene expression and enhance the activity of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR) (Wang *et al.*, 2014). However, little information is available regarding whether there is a cross-talk among the (*s*)*HSPs*, transcription factor genes and antioxidant enzyme genes and how the cross-talk occurs during the EPT process.

Transcriptomics, dealing with the transcribed regions throughout the genome for various functions of our interest underlying a corresponding biological process, is a sensitive, effective and reliable method to explore the gene expressions (Marioni *et al.*, 2008; Haas & Zody, 2010; Wu *et al.*, 2015; Zhu *et al.*, 2015). RNA-sequencing is a high-throughput screening technology that could be used to isolate and screen the genes which were differentially expressed under specific biological processes and unveil the functional elements in the genome and interpret the phenotypic variation associated with the gene expressions (Garber *et al.*, 2011; Wu *et al.*, 2015). Based on a reference genome sequence database, the identified differentially expressed genes could be annotated with the GO terms and metabolic pathways for further research which contribute to understand the physio-chemical and molecular biology process (Hegedűs *et al.*, 2009; Sun *et al.*, 2014; Wu *et al.*, 2015).

Accordingly, in this work based on transcriptome and differentially expressed genes analysis, the associated metabolic pathways of the DEGs underlying the EPT process for pineapple fruits were investigated. The differentially expressed heat response genes after EPT treatment, including heat shock protein genes, transcription factor genes and antioxidant enzyme genes were explored.

Materials and Methods

Data sets: To understand the associated metabolic pathways and the differentially expressed heat response genes of pineapple fruits affected by EPT, we obtained the RNA-seq data from our previous study (Liu & Liu, 2017) in which the pineapple fruits were exposed to the man-made climate chambers at 30ºC (T) and 15ºC (CK) for 1 d and 2 d with uniformly relative humidity at 60%. The fruits that were kept in the man-made climate chambers for 1 d were considered as T1 and CK1, respectively. And those kept in the man-made climate chambers for 2 d were renamed as T2 and CK2, respectively.

GO and KEGG pathway enrichment analyses of DEGs: To determine the primary biological functions of the DEGs, they were first annotated using the Gene Ontology (GO) database (http://www.geneontology.org/) and Blast2GO (Conesa *et al*., 2005) according to their numerical order in the nr database. After GO annotations were obtained for each of the DEGs, WEGO software (Ye *et al*., 2006) was used to obtain GO functional classifications. KEGG pathway annotation was carried out by Blastall software against the KEGG database (Altschul *et al.*, 1997; Kanehisa *et al.*, 2008).

Heat response DEGs screening and expressions comparisons: A set of differentially expressed heat response genes were screened including *HSPs*, *sHSPs*, transcription factor genes of *HSF*, *bHLH* (*basic helix-loop-helix*), *WRKY*, *MYB*, *AP2/ERF*, *bZIP* (*basic leucine zipper*), *NAC*, and antioxidant enzyme genes of *POD*, *SOD* and *CAT* according to the method described by Audic & Claverie (1997) and determined the threshold p-value, FDR and fold-change ($\log_2{}^{Ratio}$). DEGs were screened according to the standard with an FDR of $\leq$ 0.001 and an absolute value of $\log_2{}^{Ratio} \geq 1$. The fold changes of those screened heat response DEGs which were treated by EPT for 1 d and 2 d were compared.

Results

GO classifications of DEGs: DEGs were explored by determining the comparison groups of T1 and CK1, and T2 and CK2 (Liu & Liu, 2017). The GO annotations of the top DEGs and the number of enriched DEGs are shown in Fig. 1. For the comparison group T1 and CK1, the DEGs were predominantly annotated to "response to stimulus" (GO:0050896), "response to ethylene stimulus" (GO:0009723), "cellular aromatic compound metabolic process" (GO:0006725) , "cell wall polysaccharide metabolic process" (GO:0010383) and "aromatic amino acid family metabolic process" (GO:0009072) in the biological process ontology. Totally 612 (including 205 up-regulated and 407 down-regulated), 44 (including 17 up-regulated and 27 down-regulated), 46 (including 20 up-regulated and 26 down-regulated), 19 (including 6 up-regulated and 13 down-regulated) and 11 (including 2 up-regulated and 9 down-regulated) DEGs were annotated to the 5 former mentioned GO terms. For the molecular function ontology, the DEGs were predominantly annotated to "hydrolase activity, acting on glycosyl bonds" (GO:0016798), "lyase activity" (GO:0016829), "beta-glucosidase activity" (GO:0008422) and "glucosidase activity" (GO:0015926). Totally 21, 10, 2 and 3 up-regulated DEGs were enriched in those molecular function GO terms, respectively. As well, 50, 17, 8 and 13 down-regulated DEGs were enriched. For the cellular component ontology, the DEGs were predominantly annotated to "intrinsic to membrane" (GO:0031224), "cell wall" (GO:0005618) and "anchored to membrane" (GO:0031225) with 279 (including 87 up-regulated and 192 down-regulated), 44 (including 5 up-regulated and 39 down-regulated) and 12 (including 1 up-regulated and 11 down-regulated) enriched DEGs.

In the comparison group T2 and CK2, more GO terms were observed to be annotated by the DEGs. For the biological process ontology, 26 GO terms were annotated predominantly including "response to stimulus" (GO:0050896), "response to abiotic stimulus" (GO:0009628), "carbohydrate metabolic process" (GO:0005975), "response to hormone stimulus" (GO:0009725), "cellular aromatic compound metabolic process" (GO:0006725) and "cell wall organization or biogenesis" (GO:0071554). In all 577 (including 234 up-regulated and 343 down-regulated), 193 (including 81 up-regulated and 112 down-regulated), 104 (including 43 up-regulated and 61 down-regulated), 92 (including 30 up-regulated and 62 down-regulated), 43 (including 22 up-regulated and 21 down-regulated) and 42 (including 17 up-regulated and 25 down-regulated) DEGs were annotated to the 6 former mentioned GO terms, respectively. For the molecular function ontology, 8 GO terms were annotated predominantly including "hydrolase activity, acting on glycosyl bonds" (GO:0016798), "hydrolase activity, hydrolyzing O-glycosyl compounds" (GO:0004553), "glucosidase activity" (GO:0015926), "carboxy-lyase activity" (GO:0016831) and "malate dehydrogenase activity" (GO:0016615). Totally 38, 24, 8, 5 and 8 up-regulated DEGs were enriched in those molecular function GO terms, respectively. As well, 39, 28, 9, 9 and 2 down-regulated DEGs were enriched. For the cellular component ontology, the DEGs were predominantly annotated to "membrane-bounded organelle" (GO:0043227), "intracellular membrane-bounded organelle" (GO:0043231), "intrinsic to membrane" (GO:0031224) and "cell wall" (GO:0005618) with 1360 (including 519 up-regulated and 843 down-regulated), 1358 (including 519 up-regulated and 839 down-regulated), 263 (including 94 up-regulated and 169 down-regulated) and 45 (including 14 up-regulated and 31 down-regulated) enriched DEGs.

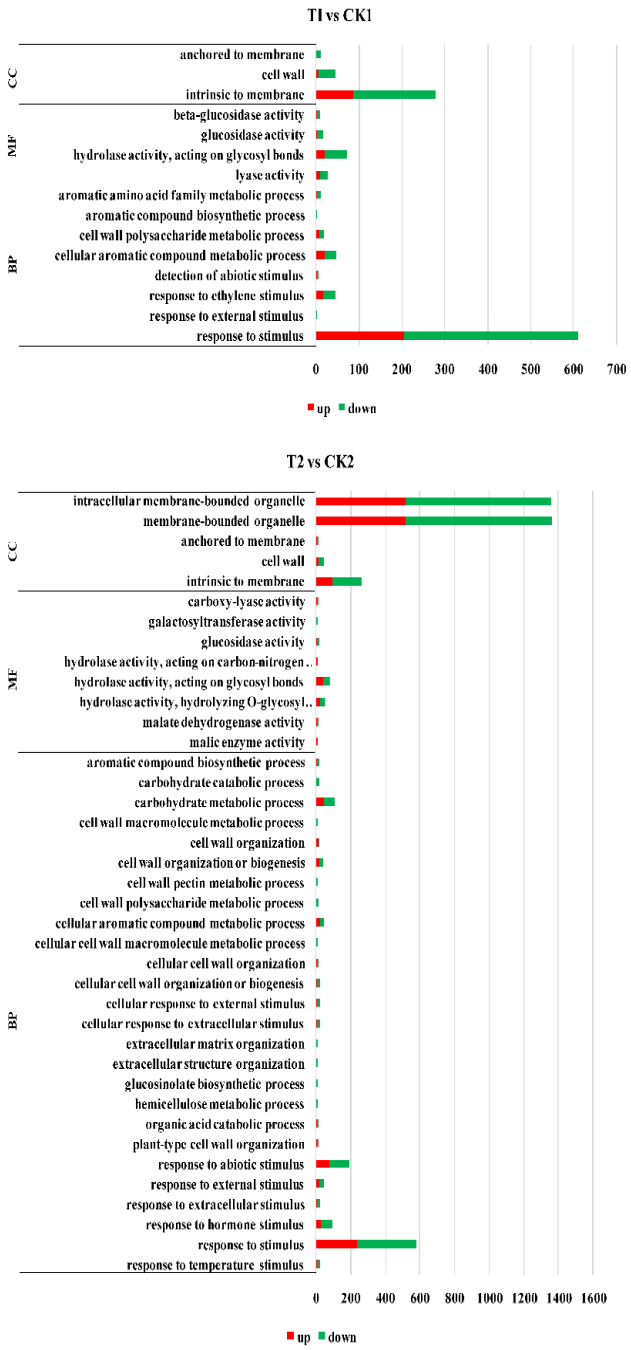

Fig. 1. GO classifications of the DEGs involved in the EPT process
Significantly annotated GO terms for Biological Process (BP), Molecular Function (MF) and Cellular Component (CC) of the up-regulated and down-regulated DEGs involved in EPT process ranked according to the *p*-value (<0.05).

KEGG pathways analysis of DEGs: A KEGG pathway classification was conducted to examine the DEGs further.The KEGG enrichments of the top DEGs and the number of enriched DEGs are shown in Fig. 2. In the comparison group T1 and CK1, 19 KEGG pathways were significantly enriched ($p \leq 0.05$). The important KEGG pathways in which the DEGs were enriched were classified. The top pathways included "starch and sucrose metabolism" (ko00500), "biosynthesis of secondary metabolites" (ko01110), "pentose and glucuronate interconversions" (ko00040), "carotenoid biosynthesis" (ko00906), "metabolic pathways" (ko01100), "galactose metabolism" (ko00052) and "plant hormone signal transduction" (ko04075). Totally 49 (including 10 up-regulated and 39 down-regulated), 182 (including 64 up-regulated and 118 down-regulated), 9 (including 0 up-regulated and 9 down-regulated), 10 (including 6 up-regulated and 4 down-regulated), 344 (including 116 up-regulated and 228 down-regulated), 16 (including 11 up-regulated and 5 down-regulated) and 48 (including 10 up-regulated and 38 down-regulated) DEGs were annotated to the 6 former mentioned KEGG pathways, respectively.

In comparing T2 with CK2, twenty KEGG pathways were significantly enriched ($p \leq 0.05$). The top interesting and important pathways were also included in "metabolic pathways", "biosynthesis of secondary metabolites", "starch and sucrose metabolism", "amino sugar and nucleotide sugar metabolism" (ko00520) and "plant hormone signal transduction". Totally 153, 83, 11, 10 and 11 up-regulated DEGs were enriched in those KEGG pathways, respectively. As well, 221, 66, 36, 14 and 30 down-regulated DEGs were enriched. In addition, the KEGG pathway "plant-pathogen interaction" (ko04626) was observed to be enriched by the DEGs in the comparison of T2 and CK2 with 32 (including 12 up-regulated and 20 down-regulated) enriched DEGs.

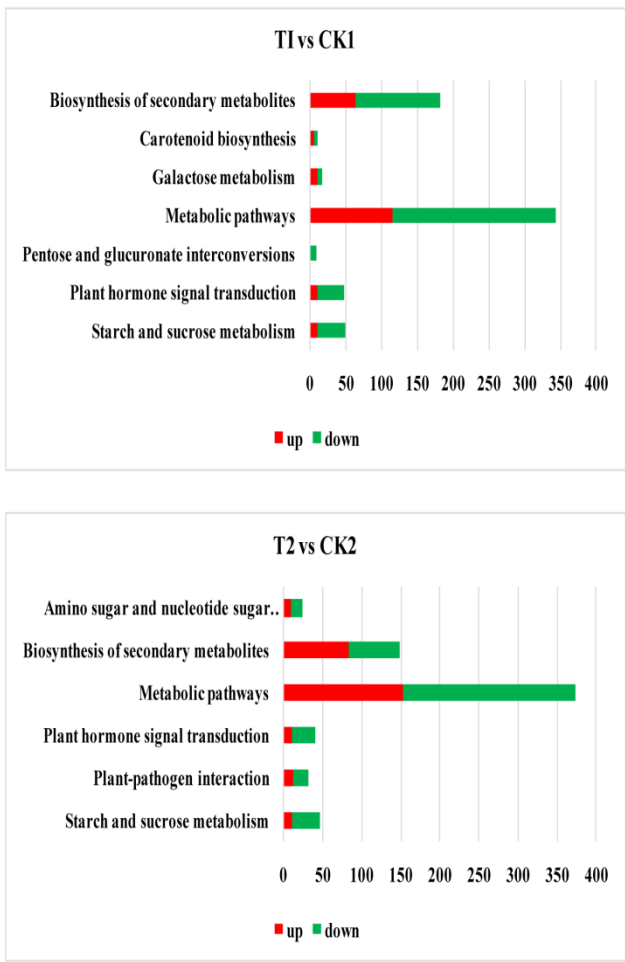

Fig. 2. KEGG pathways of the DEGs involved in the EPT process
Significantly enriched KEGG pathways of the up-regulated and down-regulated DEGs involved in EPT process ranked according to the *p*-value (<0.05).

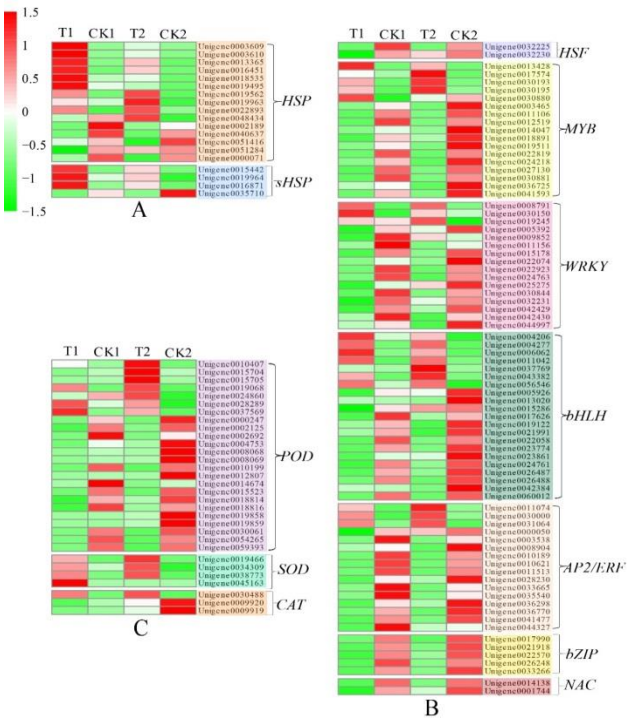

Fig. 3. Screened heat response DEGs as affected by EPT
The heat maps present the screened heat response DEGs of pineapple fruit as affected by EPT-dependent processes, including *HSP* and *sHSP* genes (A), transcription factor genes (B) and antioxidant enzyme genes *POD*, *SOD* and *CAT* (C). Each column represents an experimental treatment (T1, CK1, T2 and CK2) and each row represents a screened DEG. Expression differences of the screened DEGs are shown in different colors. For each DEG, red indicates high expression and green indicates low expression in the four treatments.

HSP and sHSP DEGs affected by EPT: A total of 19 differentially expressed *HSP* and *sHSP* genes were isolated and identified (Fig. 3A). Among them, 13 *HSP* and *sHSP* DEGs were identified in the T1 comparison with CK1. Three *HSP* DEGs (Unigene0002189, Unigene0040637 and Unigene0051284) were down-regulated by EPT, while ten *HSP* and *sHSP* DEGs were found to be up-regulated from 13.85- to 1.11-fold. Similarly, at 2 d, 15 *HSP* and *sHSP* DEGs were identified when comparing T2 with CK2. In addition to three *HSP*s and one *sHSP* that were down-regulated by EPT, eleven *HSP* and *sHSP* DEGs were found to be up-regulated from 11.21- to 1.27-fold.

Transcription factor DEGs affected by EPT: A total of 78 genes encoding transcription factors included *HSF*, *bHLH*, *WRKY*, *MYB*, *AP2/ERF*, *bZIP* and *NAC* were identified (Fig. 3B). At 1 d, the identified genes encoding transcription factors included *HSF* (heat shock transcription factors), *bHLH* (*basic helix-loop-helix*), *WRKY*, *MYB*, *AP2/ERF*, *bZIP* (*basic leucine zipper*) and *NAC*. The *HSF* genes (Unigene0032225 and Unigene0032230) were down-regulated 2.00- and 1.33-fold, respectively, by the EPT treatment. A total of 13 *bHLH* genes were screened as DEGs, four of which were induced to express by EPT, while the other nine were down-regulated from 11.09- to 1.70-fold. Similarly, 13 *WRKY* genes were identified, 2 of which (Unigene0008791 and Unigene0030150) were up-regulated 4.01- and 1.74-fold, respectively, while the other 11 were found to be down-regulated from 3.00- to 1.01-fold. For the *MYB* DEGs identified, 3 (Unigene 0013428, Unigene 0030193 and Unigene 0030880) were up-regulated 2.48-, 2.10-, and 1.10-fold, respectively, while the other 7 were found to be down-regulated from 11.48- to 1.23-fold. A total of 13 *AP2/ERF* genes were identified as DEGs, 2 of which (Unigene0011074 and Unigene0030000) were up-regulated 2.78- and 2.22-fold, respectively, while the other 11 were found to be down-regulated by EPT from 11.36- to 1.18-fold. Four *bZIP* genes were identified and all of them were impressed in expression by EPT from 5.74- to 1.18-fold. Similarly, 2 *NAC* genes, namely, Unigene0014138 and Unigene0001744, were found to be down-regulated 3.00- and 2.74-fold, respectively, by EPT.

Likewise, except for *NAC*, several of the aforementioned transcription factor genes were identified as DEGs at 2 d. Except for a few of the *bHLH*, *WRKY*, *MYB* and *AP2/ERF* that were up-regulated by EPT, most of those transcription factor DEGs were found to be down-regulated.

Antioxidant enzyme DEGs involved in the EPT response: Totally 24 *POD*, 4 *SOD* and 3 *CAT* genes were identified as DEGs (Fig. 3C). At 1 d, a total of 14 *POD* DEGs were identified, 2 of which (Unigene 0019068 and Unigene 0037569) were up-regulated 3.00- and 1.22-fold, respectively, while the other 12 were down-regulated by EPT from 13.08- to 1.72-fold. Two *SOD* genes, namely, Unigene0045163 and Unigene0038773, were isolated as DEGs and were found to be enhanced in expression by 10.86- and 1.07-fold by EPT, respectively. Similarly, two *CAT* genes, namely, Unigene 0030488 and Unigene 0009920, were identified. Unigene 0030488 was up-regulated 1.48-fold, while Unigene 0009920 was down-regulated 4.29-fold by EPT.

Likewise, at 2 d, 21 *POD* genes, 3 *SOD* genes and 2 *CAT* genes were identified as DEGs. Among the *POD* DEGs, 7 were up-regulated from 4.83- to 1.09-fold, while the others were down-regulated from 11.77- to 1.51-fold by EPT. For *SOD*, the 3 DEGs, namely, Unigene 0034309, Unigene 0038773 and Unigene 0019466, were up-regulated 1.46-, 1.45- and 1.11-fold, respectively. For the 2 *CAT* DEGs, Unigene0030488 was up-regulated 1.83-fold, while Unigene0009919 was down-regulated 1.79-fold by EPT.

Discussion

In this study, GO analysis revealed that many DEGs between the EPT treatment and control were annotated to the GO terms such as response to stimulus, response to external stimulus, detection of abiotic stimulus and response to ethylene stimulus as well as the KEGG pathways of metabolic pathways and plant hormone signal transduction. This result suggested that EPT process was a stimulus process compared to an appropriate temperature. Similar results were reported on *Populus euphratica* Oliver (Chen *et al.*, 2015), *Chrysanthemum* (Sun *et al.*, 2015), rice (Wu *et al.*, 2015) and sweet maize (Shi *et al.*, 2017) when subjected to heat stress. A few DEGs were observed to be annotated to GO terms of aromatic compound biosynthetic process, aromatic amino acid family metabolic process and cellular aromatic compound metabolic process, beta-glucosidase activity, glucosidase activity and malate dehydrogenase activity as well as KEGG pathways of starch and sucrose metabolism, biosynthesis of secondary metabolites, pentose and glucuronate interconversions,

carotenoid biosynthesis, metabolic pathways and galactose metabolism which indicated that these genes involved in aroma production and sugar/ acid metabolism were differentially expressed in the EPT process. This result confirmed the physiological-chemical indices on aroma production and sugar/ acid contents (Liu & Liu, 2017). There were also a few DEGs were annotated to hydrolase activity, cell wall organization or biogenesis, carboxy-lyase activity, cell wall and anchored to membrane which suggested that some genes associated with cell wall hydrolyze and metabolism were induced in the EPT process. This result confirmed the decreasing of firmness of pineapple fruit (Liu & Liu, 2017). It should be noted that more DEGs were annotated to these former mentioned cell wall hydrolyze and metabolism associated GO terms and KEGG pathways at 2 d (comparison group of T2 and CK2). At 2 d a lot of DEGs were enriched in the pathway of plant-pathogen interaction, which indicated that EPT process would lead to the emergence of pathogen because of the deceasing of firmness.

A remarkable response of plant subjected to the environment at high temperatures is the raised production and accumulation of HSPs and sHSPs (Schöffl *et al.*, 1998; Perotti *et al.*, 2011; Aghdam *et al.*, 2013; Wu *et al.*, 2015; Zha *et al.*, 2016). In this study, several *HSP* and *sHSP* genes were identified as DEGs. Few *HSP* DEGs were repressed however, most of the *HSP* and all the *sHSP* DEGs were induced in expression by EPT at 1 d and 2 d. This result was similar to those of prior reports on apples (Son *et al.*, 2012), peaches (Spadoni *et al.*, 2014) and oranges (Perotti *et al.*, 2015).

The expression levels of different *HSP* genes are regulated by *HSFs* that can sense abiotic stresses (Aghdam *et al.*, 2013; Sun *et al.*, 2014; Aghdam & Bodbodak, 2014). *HSFs* exert a protective action against stress and play a role in regulating the expressions *HSP*s, and in increasing the resistance to oxidative stress (Aghdam *et al.*, 2013; Aghdam & Bodbodak, 2014), whereas the down-regulation of *HSFs* usually results in decreased resistance to oxidative stress (Spadoni *et al.*, 2015). In this work, the expressions of *HSFs* were observed to be down-regulated by EPT process at the stage of 1 d and 2 d. Similar results were obtained in another study, which reported that the expression levels of several *HSFs* were reduced in *wrky25* mutant *Arabidopsis* when exposed to heat stress (Li *et al.*, 2009). Spadoni *et al.* (2015), however, stated that the expressions of *HSFs* were up-regulated after 1 h to 4 h in apple fruit when exposed to heat treatments. This variation was probably due to the duration of the heat treatment.

Other transcription factor genes, such as *MYB* and *WRKY*, have been identified in different crops. *MYB* was reported to be associated with the tolerance of high temperature through the modulation of amino acid metabolism, and *WRKY* might participate in the *HSP/HSF* signaling pathways concerned the transcriptional reprogramming when the plants were exposed to a heat stress environment (Sun *et al.*, 2014; Thirunavukkarasu *et al.*, 2013). In this work, a few transcription factor genes, including *WRKY* and *MYB*, as well as *AP2/ERF*, *bHLH*, *bZIP* and *NAC*, were identified and were screened as DEGs. Apart from a few that were up-regulated, most of these transcription factor DEGs were impressed by EPT. Similar results were obtained in *Chrysanthemum* which stated that the expression levels and transcript abundances of *MYB*, *WRKY*, *AP2/ERF*, *bHLH* and *bZIP* genes were reduced when exposed to high temperature (Sun *et al.*, 2014).

HSFs depend on the expression of antioxidant genes in *Arabidopsis* (Panchuk *et al.*, 2002) and might take part in HSP biosynthesis and regulation of oxidative stress through antioxidant gene expression (Wang *et al.*, 2014). Heat pretreatment at 38ºC for 10 h induced the expression of *Hsp70* genes of grape berries, and subsequently enhanced the enzyme activities of CAT, SOD and POD, while after exposure to cold stress for 72 h, the expression levels of *Hsp70* were remarkably depressed, and the enzyme activities of CAT and POD were significantly reduced (Zhang *et al.*, 2005). *SOD*, *CAT*, *POD* and *APX* play distinct roles in antioxidant protection against heat stress (Wang *et al.*, 2014; Du *et al.*, 2013). In this work, the expressions of *SOD*, as well as the subsection of *POD* and *CAT* DEGs were increased, while most of the *POD* DEGs were suppressed by the EPT process at 1 d and 2 d. The results obtained suggested that *SOD*, *POD* and *CAT* genes were involved in the EPT process and that there was cross-talk among the *HSPs*, *HSFs* and antioxidant enzyme genes (Zhang *et al.*, 2005). In particular, more down-regulated *POD* DEGs were identified in the comparison of T2 and CK2 at 2 d in this work. With respect to the *SOD* DEGs, the fold change in expression decreased (averaged 1.34 at 2 d) when compared with that at 1 d (up to 10.86 at 1 d). These results revealed that the EPT process decreased the majority of the *POD*, *CAT* genes which inflicted the morphological and physiological damage on pineapple fruit and could be confirmed by the decreased firmness induced by EPT (Liu & Liu, 2017). A similar result was obtained in carambola, where fruit stored at 25ºC was less firm than that stored at 0ºC and 2ºC (Wang *et al.*, 2016). Wang *et al.*, (2015) also reported that SOD, CAT, POD activities were decreased during the sweet cherry fruit softening after hot air treatment.

Conclusion

This work present the GO annotations and KEGG enrichments of the DEGs involved in EPT process. DEGs were predominantly annotated to the terms involved in response to external stimulus, aromatic compound biosynthetic process, carbohydrate metabolic process and response to hormone stimulus. The KEGG metabolic pathways analysis suggested that the DEGs were enriched in starch and sucrose metabolism, plant hormone signal transduction, biosynthesis of secondary metabolites and plant-pathogen interaction. A total of 19 *HSP* and *sHSP* genes were identified and screened as DEGs; most of these were up-regulated by EPT. A total of 78 genes encoding transcription factors included *HSF*, *bHLH*, *WRKY*, *MYB*, *AP2/ERF*, *bZIP* and *NAC* were identified. The overwhelming majority of these transcription factor genes were down-regulated by EPT process at the stage of 1 d and 2 d. Totally 24 *POD*, 4 *SOD* and 3 *CAT* genes were identified as DEGs. Most of the *POD* and *CAT* genes were down-regulated by EPT process, while the *SOD* genes were up-regulated. This work would help to understand the molecular mechanisms for EPT process to improve the quality of pineapple fruits, especially the cross-talk among *HSPs*, transcription factor genes and antioxidant enzyme genes involved in this process.

Acknowledgements

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