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Comparative transcriptome analysis reveals fruit discoloration mechanisms in postharvest strawberries in response to high ambient temperature

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ABSTRACT

The aim of this study was to examine both physiological and molecular evidences related to fruit discoloration in postharvest strawberries under high ambient temperature. The results showed the total anthocyanin and their main components in the strawberries under 35 °C were greatly increased due to the significant up-regulations of anthocyanin biosynthetic genes and transportation genes. High ambient temperature greatly improved the activities of peroxidase (POD) and enhanced gene expressions of *POD3*, *POD6* and *POD63*. At the same time, high storage temperature activated laccase genes expression including *laccase-9* and *laccase-14*, which was closely related to anthocyanin degradation. Levels of reactive oxygen species (ROS) metabolism were also increased under high ambient temperature at transcript levels. Therefore, we concluded that high ambient temperature could enhance anthocyanin accumulation and degradation at the same time, which maybe the main reasons for the fruits discoloration of postharvest strawberries under high ambient temperature.

1. Introduction

Strawberry (*Fragaria* × *ananassa*) is one of the most popular berries produced in commercial scale worldwide for its attractive color, unique flavor attributes and nutritional benefit. The bright red color of postharvest strawberry significantly influences the choice of consumers and its marketability. Unfortunately, strawberries are perishable commodities that are prone to discoloration due to its relative high metabolic activity, high sensitivity to physical damage and fungal attack, which leads to a short postharvest life (Hashmi, East, Palmer, & Heyes, 2013). It has been reported that the strawberries harvested at fully red stage quickly lose the bright color, which makes the appearance overripe, dull, and less appealing (Kalt, Prange, & Lidster, 1993; Shin, Liu, Nock, Holliday, & Watkins, 2007). In previous research, we observed that high ambient temperature could aggravate the loss of the fresh color in harvested strawberries, as presented with dark red appearance. In China, postharvest strawberries were frequently exposed to high ambient temperature and fruit discolorations were often observed during the fruit transportation and distribution, which caused serious economic loss (Yang et al., 2017).

Anthocyanin is a kind of flavonoid pigment in tolerance response of plant to abiotic stress and their accumulation is also an important biological event during fruit ripening of plum, grape, cherry, strawberry and red orange (Shin et al., 2007; Flores, Blanch, & Castillo, 2015; Martínez-Romero et al., 2017; Carmona, Alquézar, Marques, & Pena, 2017). Anthocyanin synthesis and accumulation during postharvest period can improve the appearance of under ripe strawberry. However, for the full red strawberry, continued color development after harvest could be detrimental to color quality (Kalt et al., 1993). Many evidences indicated anthocyanin accumulations could be promoted when the fruits exposed to temperature stress condition (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2010; Steyn, Wand, Jacobs, Rosecrance, & Roberts, 2010). In our previous research, we also found anthocyanin accumulations might be the most important internal factors leading to postharvest strawberry being dark red and high ambient temperature enhanced these kinds of discoloration (Yang et al., 2017). Although, the effect of high field temperature on the anthocyanin accumulations in the under ripe fruits were studied extensively in recent years, the metabolic mechanism of the anthocyanin accumulations in the postharvest fruits under high storage temperature, especially for postharvest strawberries, remains unconvincing and requires additional studies for clarification.

Oxidation of soluble phenolic compounds plays a role in development of fruit discoloration during storage. The primary enzyme responsible for the browning reaction is polyphenol oxidase (PPO) and peroxidase (POD) (Nadafzadeh, Mehdizadeh, & Soltanikazemi, 2018).

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A recent report described that laccase play an important role in postharvest browning of litchi by involving in the epicatechin mediated anthocyanin degradation (Fang et al., 2015). Then, lipoxygenase (LOX) was reported to be highly associated with browning in fresh-cut pear through catalyzing membrane lipid peroxidation and destroying the structure of the cell membrane (Li, Zhang, & Ge, 2017). However, the metabolic mechanism of fruit discoloration in postharvest strawberries under high ambient temperature is still unclear and need further studies, especially at molecular levels.

Transcriptomic analysis could provide a wealth of data concerning key molecular events during storage period in response to different condition, which will make it easier to investigate the metabolism process of postharvest fruits in more detail (Elsharkawy, Dong, & Xu, 2015; Li et al., 2017). In the present study, transcriptome profiling combined with physiological analysis were performed to investigate the molecular basis of fruit discoloration on postharvest strawberries under high ambient temperature, which sheds fresh light on fruit discoloration during storage period on a genome-wide scale and will facilitate further research on molecular modulation and effective control.

2. Materials and methods

2.1. Materials and treatment

Strawberries (*Fragaria* × *ananassa cv*. Falandi) were obtained from the greenhouse of the experimental station in the Hubei Academy of Agricultural Sciences, Hubei province, China. Strawberries were harvested at commercial maturity and placed in plastic plates, and then transferred immediately to the laboratory. After selected for uniform size and color, free of visible physical injury or disease, the fruits were divided into two groups and stored at 25 °C and 35 °C separately. There are three replicates for each group and every replicate has 30 strawberries. Three samples of every replicate were taken initially and every day during storage. After evaluating fruit color, the sampale were immediately frozen in liquid nitrogen, ground to a fine powder and store at −80 °C for further analysis including anthocyanin and total phenolic, enzyme activities, RNA-seq, and qRT-PCR.

2.2. Evaluation of fruit color of postharvest strawberry

Strawberry external color was measured using a Chroma meter (CR-400; Minolta, Tokyo, Japan) and measurements were taken on opposite sides of each fruit, midway between the pedicel and calyx. The values of L^*, a^* and b^* were measured and the CIRG index of strawberries was calculated with the formula: $CIRG = (180-H)/(L*+C)$, where $C = (a*^2 + b*^2)^{0.5}$and $H = [\arctan(b*/a*)]$.

2.3. Analysis of anthocyanin and total phenolic in strawberry

1.0 g of freeze-dried powder of strawberries was ground to a fine powder in liquid nitrogen, and then soaked in 5 mL of methanol containing 0.1% (v/v) hydrochloric acid overnight at 4 °C. After centrifugation at 10,000 × *g* for 15 min, the supernatant was used to determine the contents of anthocyanin and total phenolic.

Total anthocyanin content was measured by pH differential method using a DU800 spectrophotometer (Beckman Coulter, Fullerton, CA, U.S.) at 510 nm and 700 nm in buffers of pH 1.0 and pH 4.5 (Liu et al., 2016). The results were expressed as pelargonidin-3-O-glucoside equivalent on a fresh weight basis, mg 100 g^{-1} FW. The quantities of the individual anthocyanin were carried out using Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with diode array detector (DAD) and a Hypersil-ODS C_{18} (250 mm × 4.6 mm, 5 μm) at 30 °C and quantified at 520 nm, the quantities of pelargonidin-3-glucoside, pelargonidin-3-(malonyl)-glucoside, cyanidin-3-glucoside, and pelargonidin-3-rutinoside were calculated as pelargonidin-3-glucoside equivalents. The contents of total phenolic were determined according to the method of Folin-Ciocalteu procedure and the results are expressed as Gallic acid equivalent on a fresh weight basis, mg 100 g^{-1} FW (Yang et al., 2017).

2.4. Enzyme activities

For the analysis of enzymes activities, all operations were carries out at 4 °C. To measure phenylalanine ammonia lyase (PAL) and dihydroflavonol 4-reductase (DFR), 1.0 g of strawberry sample was homogenized with 4 mL of pre-chilled 0.2 M $Na_2B_4O_7$-H_2BO_3 buffer (pH 8.8) containing 2.5% polyvingypyrrolidone (PVPP), 0.005 M β-mercaptoethano, 0.001 M ethylene diamine tetraacetic acid (EDTA), 0.001 M dithiothreitol and centrifuged at 12,000*g* for 20 min at 4 °C. The supernatant was used for PAL and DFR enzyme activity assay. PAL activity was assayed according to the method of Assis, Maldonado, Munoz, Escribano, and Merodio (2001) using L-phenylalanine as substrate and monitoring the production of cinnamate at 290 nm. The result was expressed as U/mg protein. The DFR activity was assayed according to the method of Dedaldechamp, Uhel, and Macheix (1995) using dihydroquercetin as substrate and NADPH as reducing agent and the results were expressed as U/mg protein.

For the crude protein extraction of chalcone-flavanone isomerase (CHI), 1.0 g of frozen sample was ground to fine powder in liquid nitrogen and 4 mL of 0.1 M Na_2HPO_4-NaH_2PO_4 (pH7.4, 18 mM β-mercaptoethano, 2% PVPP) was added. The homogenate was centrifuged at 12,000*g* for 20 min at 4 °C. The supernatant was used for CHI enzyme activity assay and the reaction was initiated tetrahydroxychalcone. The results were expressed as U/mg protein.

To determine the POD and PPO, the strawberry samples of 1.0 g were ground with 4 mL of 0.1 M phosphate buffer (pH 6.8) containing 1.5% PVPP. After centrifugation at 12, 000 rpm for 20 min at 4 °C, the supernatant was used to determine POD and PPO activities. The POD activity was assayed using a modification of the method of Wang, Tian, and Xu (2005). The reaction mixture containing 250 μL of 25 mM guaiacol in phosphate buffer (pH 6.8), 19 mL of 30% H_2O_2 and 50 μL of extract was shaken gently at 30 °C. The absorbance at 470 nm was recorded at intervals of 15 s for 3 min and the POD activity was expressed as U/mg protein. The PPO activity was determined by the method of Sulaiman, Soo, Farid, and Silva (2015). The reaction mixture containing 250 μL 20 mM catechol in phosphate buffer and 50 μL of extract was shaken gently for 1.5 min and incubation at 3 °C for 1 min. The absorbance at 420 nm was recorded and the PPO activity was expressed as U/mg protein.

2.5. RNA-seq and data analysis

Samples of strawberries stored for 7d at 25 °C and 35 °C were used as RNA-seq analysis. Total RNA was extracted from frozen strawberries for 7d using the pBIOZOL reagent according to the manufacture's protocol. The integrity of all the total RNAs was checked by 1% agarose gel electrophoresis and the concentration and purity were determined using NanoDropTM. RNA-Seq was performed by technicians at Shenzhen BGI Genomics Technology Co Ltd (Shenzhen, China). 200 ng total RNA sample was purified by oligo (dT) beads, then poly (A)-containing mRNA were fragmented into small pieces with Fragment Buffer. The cDNA library construction using Agilent 2100 Bioanalyzer (Agilent RNA 6000, Nano Kit). Briefly, First-strand cDNA is generated by First Strand Master Mix and Super Script II (Invitrogen) reverses transcription, and then add second strand master mix to synthesize the second-strand cDNA. Double-stranded cDNA was processed with end repair and adapter ligation and then amplified by PCR and purified using the AMPure XP system (Beckman Coulter, CA, USA). cDNA libraries were sequenced on an Illumina Hiseq platform. Two biological replicates were performed.

The raw reads were processed through in-house Perl scripts, and clean reads with high quality were obtained by removing reads

containing adapter and low-quality sequences. All clean reads were aligned to the reference genome of strawberry using TopHat v2.0.12. Gene expression levels were calculated by FPKM (number of fragmentsper kilo base of transcript sequence per million base pairs sequenced), considering the effect of gene length for the reads and sequencing depth simultaneously (Trapnell et al., 2010). Differential gene expression analysis was performed using the DESeq R package. DESeq employs a negative binomial distribution-based model to determine the differential expression of genes (DEGs). The Benjamini and Hochberg approach was employed to adjust the resulting P values to control the false discovery rate (FDR). The FDR and fold change (FC) were used to determine the DEGs. Genes with FDR ≤ 0.001 and |FC| ≥ 1.0 in FPKM were considered as statistically significant DEGs。

2.6. The quantitative real-time PCR (qRT-PCR) analysis

The qRT-PCR was used to assess the results of RNA-seq analysis. Total RNA extraction from these samples used the same procedures described above. Approximately 0.8 μg of total RNA of each sample was subjected to reverse transcription using a PrimeScript™ RT reagent Kit with gDNA Eraser Kit (Takara, Japan) on qTOWER 2.2 (Jena, German). Each reaction contained 5 μL SYBR Premix Ex Taq II, 1 μL of the first-strand cDNA as a template and 4 μM of each primer in a total volume reaction of 10 μL. The amplification program was performed under the following conditions: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Three biological repeats were performed for each sample. The *GAPDH* gene (GenBank accession number: AB363963.1) was used as an internal reference gene for normalization of data. Relative gene expression level was calculated using the $2^{-\triangle\triangle ct}$ method (Zhang et al., 2015).

3. Result

3.1. Fruit color of postharvest strawberries under high ambient temperature

Fruit color is the major quality factor that reflects the fruit ripeness and indicates a fresh-market value of products (Sooriyapathirana et al., 2010). In present study, fruit color of strawberries became dark red and lost glossiness after storage, and high temperature (35 °C) could aggravate this kind of discoloration (Fig. 1A). Then, L^*, a^* and b^* values were further evaluated and the results was shown in Fig. 1B. During the whole storage period, the L^* values of strawberries generally decreased and high ambient temperatures (35 °C) accelerated this kind of decrease. The values of a^* and b^* had similar change tendency with the L^* value, and the fruits stored at 35 °C had much lower values of a^* and b^*. These results indicated high ambient temperature decreased appearance quality of postharvest strawberries.

3.2. Anthocyanin contents in postharvest strawberries under high ambient temperature

The major anthocyanin components in strawberries were pelargonidin 3-glucoside, followed by pelargonidin-3-(malonyl)-glucoside, pelargonidin-3-rutinoside, cyanidin-3-glucoside and minor amounts of other pelargonidin derivatives (Liu et al., 2016). In this study, the levels of anthocyanin and its relative enzymes activities in postharvest strawberries were analyzed. The result was shown in Fig. 2. Total anthocyanin contents were increased during storage and the fruits at 35 °C had much higher anthocyanin levels than the fruits at 25 °C. The concentrations of pelargonidin-3-glucoside, pelargonidin-3-(malonyl)-glucoside, cyanidin-3-glucoside, and pelargonidin-3-rutinoside in fruits had the similar tendency with total anthocyanin levels, especially for pelargonidin 3-O-glucoside. Furthermore, the activities of PAL, CHI and DFR in strawberries at 35 °C were higher than those in the strawberries at 25 °C, especially for the activities of PAL and DFR. Therefore, high ambient temperature enhanced anthocyanin accumulation in postharvest strawberries and increased the enzymes activities involving in anthocyanin synthesis.

3.3. Phenolic and its relative enzymes activities in postharvest strawberries under high ambient temperature

Due to the great effect of phenolic on fruit color, changes of total phenolic and their relative enzymes in strawberries of this study were determined. The results showed the content of total phenolic was increased during storage period and high ambient temperature slightly improved total phenolic levels. POD activity of strawberries under 35 °C were found higher than those of strawberries under 25 °C during the whole period time. However, the fruits under 35 °C showed lower PPO activities than the fruits under 25 °C, especially near the end of storage.

3.4. Gene expresses profile of postharvest strawberries under 25 °C and 35 °C

The gene expression profiling of the strawberries stored at 25 °C and 35 °C for 7d were investigate. In total, 62221815, 62226418, 62220998 and 62221192 total reads were obtained from the strawberries of 25 °C-7d-1, 35 °C-7d-1, 25 °C-7d-2 and 35 °C-7d-2. After removing the low quality reads, more than 96% of the raw reads were clean reads. When mapped these clean reads to the reference strawberry genome, more than 63% were mapped in 25 °C and 35 °C samples. A total of 18952, 18971, 19460 and 18815 genes were expressed in 25 °C-1, 35 °C-1, 25 °C-2 and 35 °C-2 samples based on the strawberry genome respectively.

As shown in Fig. 4A, Venn diagrams of the transcripts illustrates that the commonly expressed and specifically expressed genes between 35 °C and 25 °C were 19573 co-expressed genes, 1134 25 °C-specific and 754 35 °C-specific genes respectively. Hierarchical clustering of differential expression genes that |FC| > 1 between two accessions were produced and shown in Fig. 4B. 1145 genes were identified as DEGs, with 599 up-regulated genes and 546 down-regulated genes by calculating the expression levels (FPKM) for each gene. Sequences with BLAST hits were further classified with GO and KEGG pathway analysis. GO analysis indicated 520 genes were assigned to three main GO categories, including biological process category, cellular component category, and molecular function category, and 58 subcategories shown in Fig. 4C. The DEGs of 35 °C-7 day VS 25 °C-7 day mainly distributed in the subcategories of functional types, including cellular process, metabolic process, catalytic activity, and binding. KEGG pathway analysis revealed that these genes were primarily involved in metabolic pathway, biosynthesis of secondary metabolite, phenylpropanoid biosynthesis, Glutathione metabolism and flavonoid biosynthesis (Fig. 4D).

3.5. Differential expression involved in the anthocyanin biosynthesis under high ambient temperature

Anthocyanin biosynthesis is a dynamic and complex process mainly regulated by transcription factors from the v-myb avian myeloblastosis viral oncogene homolog (MYB), basic helix-loop-helix(bHLH), WD40-repeats protein(WD40), CPC and WRKY families(Bai et al., 2017; Valderrama & Feller, 2014). In this study, 10 regulatory genes coding transcription factors including 2 MYB, 1 CPC, 3 bHLH and 4 WRKY were identified according to annotations (Table 1) and may be involved in the anthocyanin biosynthesis pathway. Among these genes, *MYB39, bHLH66*, *bHLH 128, CPC* and *WRKY44* were up-regulated, and *MYB306, bHLH130*, *WRKY6*, *WRKY14* and *WRKY33* were down-regulated when strawberries stored at 35 °C. However, the regulatory genes related with WD40 were not observed in the DEGs between the strawberries stored at 25 °C and 35 °C.

The anthocyanin pathway is usually divided into early biosynthetic genes including chalcone synthase(*CHS*), *CHI*, flavanone 3-hydroxylase (*F3H*), flavanone-3′-hydroxylase(*F3′H*) and flavanone-3′5′-hydroxylase

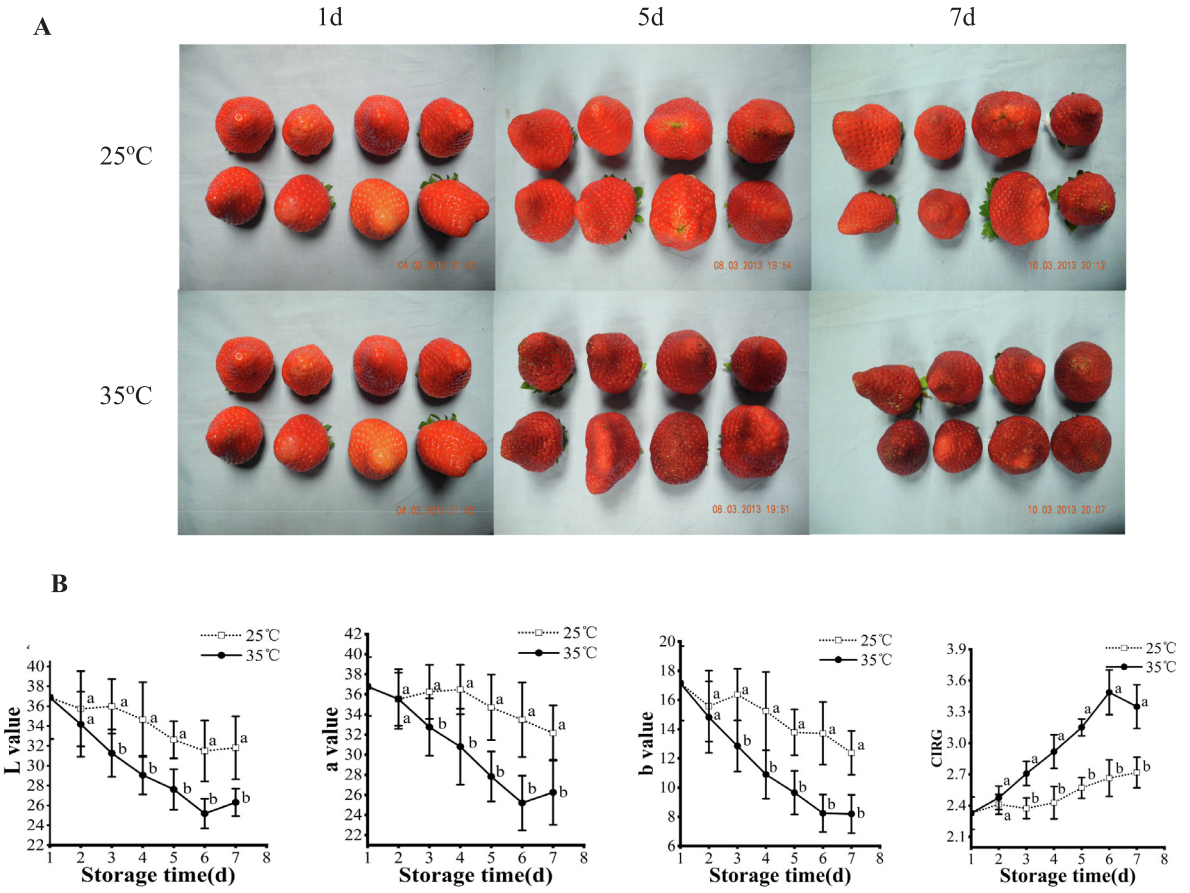

Fig. 1. Fruit appearance (A) and colorimetric parameters (B): L^*, a^*, b^* values and CIRG of postharvest strawberries during storage at 25 °C (·····□·····) and 35 °C (—●—) respectively. Vertical bars represent the standard deviation of three replicates.

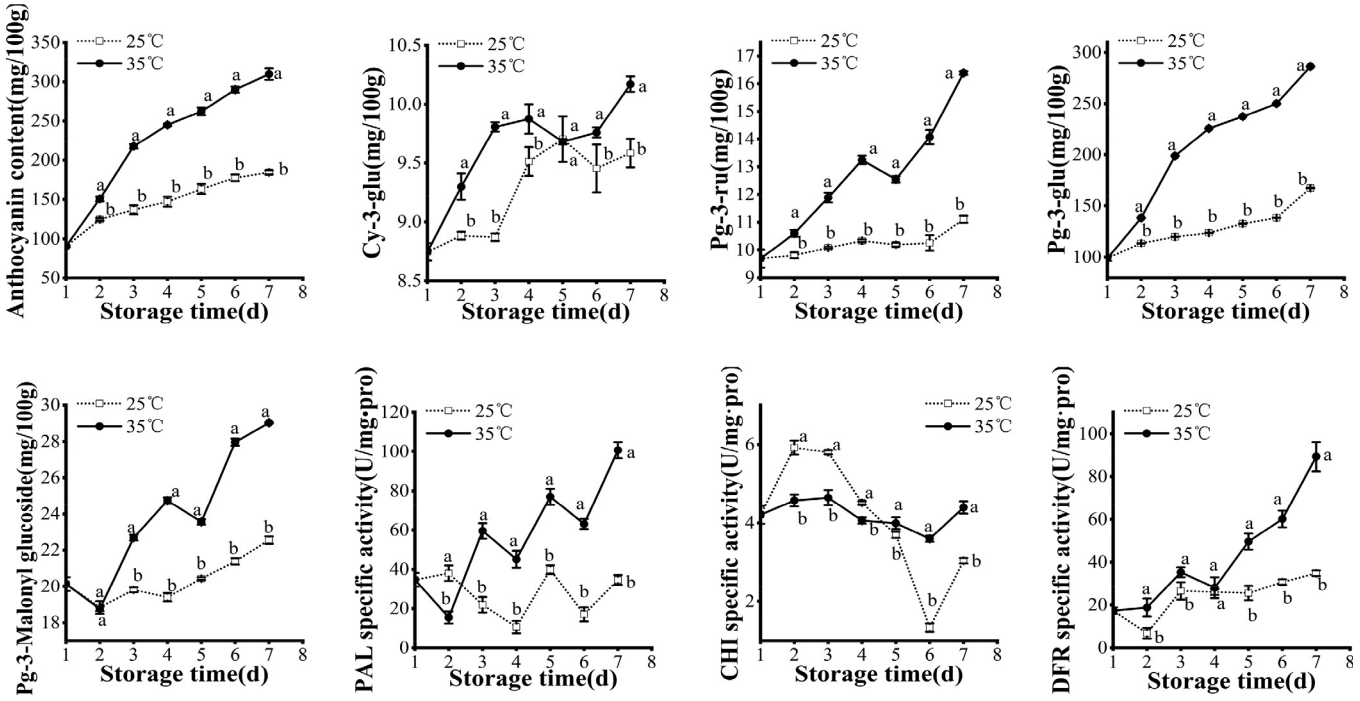

Fig. 2. The levels of anthocyanin and its key enzymes in strawberries stored at 25 °C and 35 °C. Each data point represents mean ± SD (n = 3).

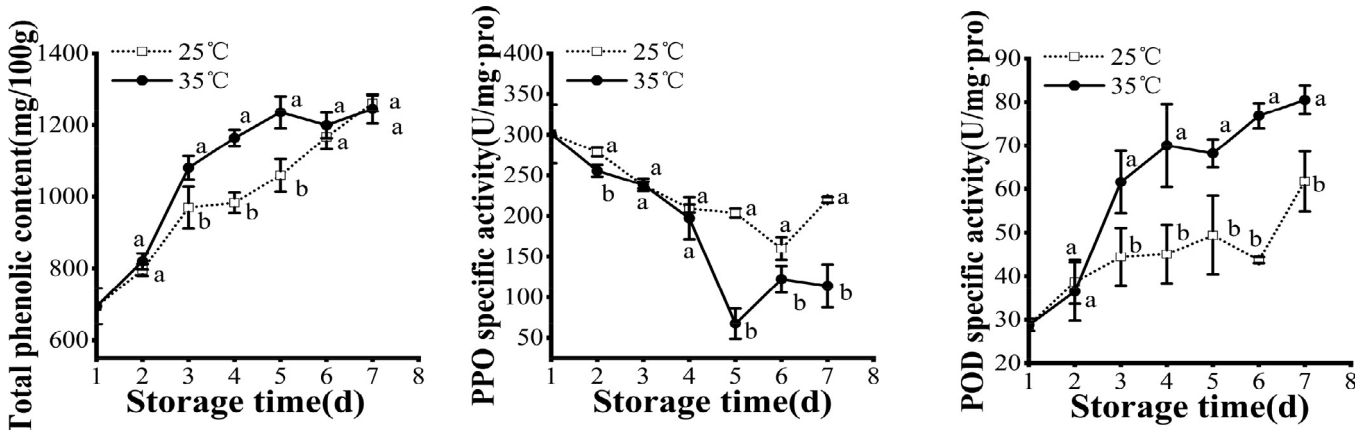

Fig. 3. The levels of phenolic and its key enzymes in strawberries stored at 25 °C and 35 °C. Each data point represents mean ± SD (n = 3).

(*F3′5′H*) and late biosynthetic genes including *DFR*, anthocyanidin synthase(*ANS*)and UDP-glycose:flavonoid 3-O-glycosyltransferase (*UFGT*). In this study, 19 structural genes encoding enzymes of the anthocyanin biosynthesis pathway were identified according to annotations. In the strawberries at 35 °C, most of these genes including *PAL1*, *CHS2*, *CHI*, *DF4R*, *ANS, UA5, 3GT*, *UA3GT5* and *UA3GT2* showed high expressed levels and were significantly up-regulated, which were similar with the changes of anthocyanin contents. However, most of genes encoding UFGT such as *UF3GT3*, *UF3GT6*, and *UF3GT7* were down-regulated in the strawberries at 35 °C.

Anthocyanin is believed to be synthesized at the cytosolic surface of the endoplasmic reticulum (ER) by a multienzyme complex and accumulation in the vacuole. Recent researches showed that glutathione S-transferase (GST), multidrug and toxic compound extrusion (MATE) and multidrug resistance-associated protein(MRP)are closely related to anthocyanin transport from cytoplasm to vacuolar in grapevine and other fruits (Hu et al., 2016). There are 9 DEGs including MATE, GST and MRP families were identified. In the strawberries at 35 °C, 5 MATE-related genes including *MATE DTX1*, *MATE TT12* were identified and greatly up-regulated, which was similar with the tendency of anthocyanin. While the DEGs encoding GST23 and MRP POP1-like were all down-regulated. Therefore, high ambient temperature significantly enhanced the expressions of crucial genes related to anthocyanin biosynthesis and accumulation.

3.6. Genes involved in the ROS and enzymatic browning

Reactive oxygen species (ROS) production-scavenging system was reported to play an important role in fruit browning. In this study, ROS related genes including *Cu/Zn-SOD*, *GRXC1*,*GRXC2*, *GRXC9* and *APX3* were identified and most of them were up-regulated in the strawberries at 35 °C(Table 1), which indicated that high ambient temperature enhanced the ROS metabolism of strawberries.

Oxidation of phenolic compounds is a hallmark of enzymatic browning. The *PPO* and *POD* genes have been associated with the browning of fruit and vegetable tissues, and their encoded enzymes function together to induce browning. In this study, *POD3*, *POD4*, *POD6*, *POD12*, *POD27* and *POD63* were identified. Among these genes in strawberries at 35 °C, *POD3*, *POD6* and *POD63* were up-regulated; *POD4*, *POD12* and *POD27* were down-regulated. While, all the DEPs of *PPO* were inhibited by high ambient temperature with |FC| > 2.0. In addition, two laccase genes including *laccase 9* and *laccase 14* were identified and high ambient temperature stimulated their genes expressions, especially for *laccase 14* with |FC| = 5.2731.

3.7. qRT-PCR validation of gene expression in anthocyanin biosynthesis pathway

To validate the DEGs and trancriptome data from strawberries stored at 25 °C and 35 °C, qRT-PCR were performed. Transcript-specific primers were designed for qRT-PCR analysis of strawberries. Selected key DEGs including anthocyanin biosynthesis and browning related genes were tested for their differential expression in response to high temperature and the results were shown in Fig. 5. qRT-PCR analysis revealed that the key structural genes of anthocyanin biosynthesis including *PAL1*, *CHI 1*, *ANS, LAR, DFR* and *MATE DTX1* were up-regulated. The genes related with enzymatic browning including *POD* and *laccase* were up-regulated, while *PPO* gene was down-regulated for the strawberries at 35 °C compared with the fruits at 25 °C. For all these genes, the qRT-PCR results were consistent with the RNA-seq results.

4. Discussion

The color of fruit products is generally accepted as one of the most relevant quality parameters and plays a decisive role when evaluating the quality of the fruit product at the point of sale. In the present study, high ambient temperature decreased the L^*, a^* and b^* values of the strawberries and enhanced the fruit color being dark red and glossiness losing (Fig. 1). Similar results were observed in strawberries, mangostana, sweetcherry, pineapple and apple (Kalt et al., 1993; Sooriyapathirana et al., 2010; Liu & Liu, 2015). This kind of discoloration decreased the commodity and loss the value of postharvest strawberry. In order to understanding action mode of fruit discoloration of postharvest strawberries under high ambient temperature at a molecular level, we compared the transcriptomes of strawberries stored at 25 °C and 35 °C. The important effects of high storage temperature on physiological process related to fruit color are discussed below.

Anthocyanins play a vital role in protecting plants against various stresses. It was reported that temperature stress enhanced the anthocyanin accumulation in the postharvest fruits of mangosteen, plum, peach, red orange, fresh-cut strawberry, apple and pear (Steyn et al., 2010; Hu et al., 2015). In this study, the accumulations of total and individual anthocyanin including pelargonidin-3-glucoside, pelargonidin-3-(malonyl)-glucoside, cyanidin-3-glucoside, and pelargonidin-3-rutinoside were greatly improved when the strawberries stored under high temperature (Fig. 2). However, strawberries cultivated at high air temperatures have been reported to show poor coloration and anthocyanin accumulation is inhibited during the coloring period (Matsushita, Sakayori, & Ikeda, 2016). Similar phenomenon was also found in the coloring fruits of grapes and apples (Inés de Rosas et al., 2017). But when exposed fruit to high temperature after half ripe stage, the anthocyanin concentration and gene expression in fully ripe fruit were not significantly different from the control. Therefore,

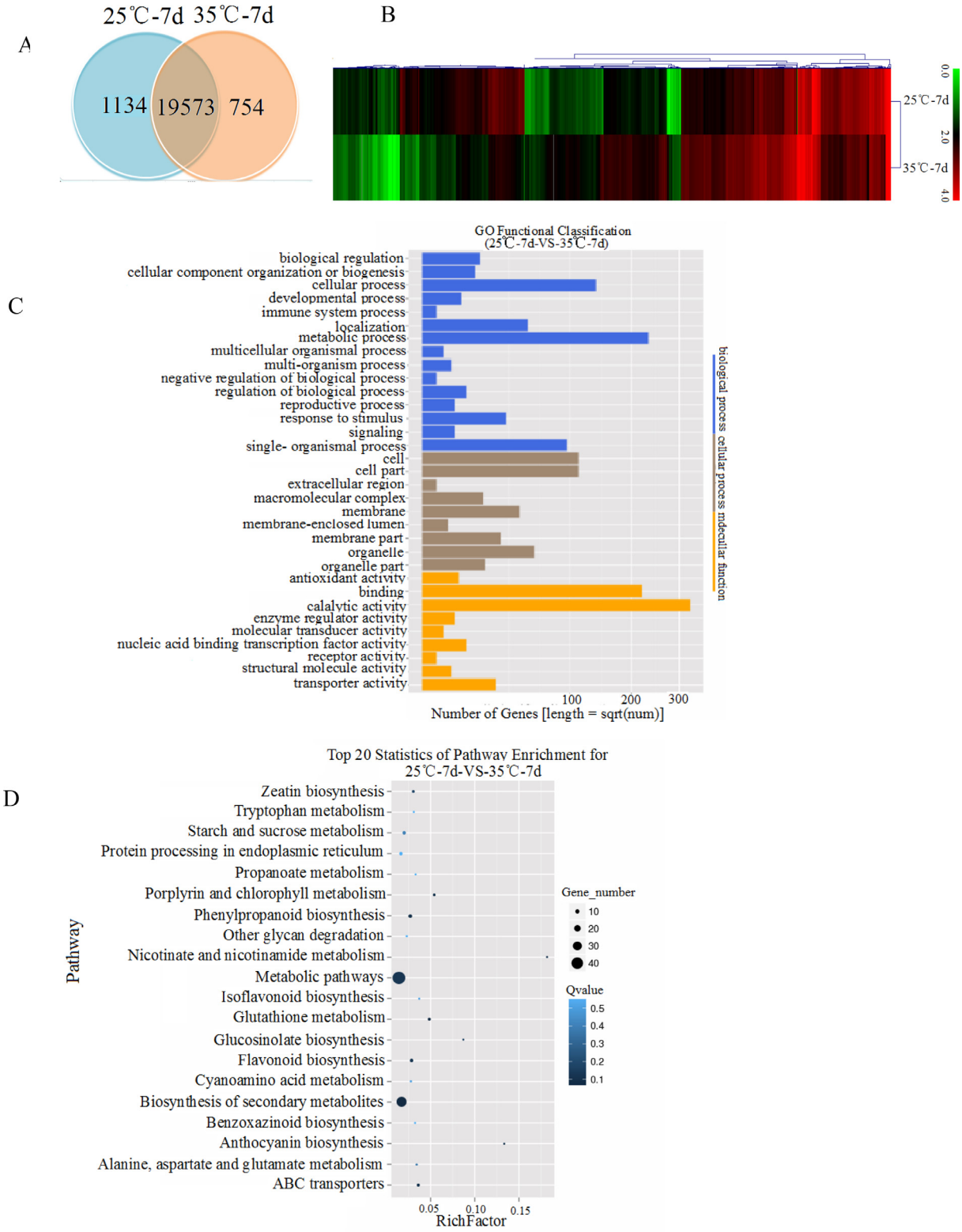

Fig. 4. RNA-seq analysis between two accessions. (A) Comparative study on the number of transcripts between two accessions. (B) differential expression pattern between two accessions (C) GO functional classification of the differentially expressed genes. (D) Significant enrichment analysis of KEGG of differential genes.

anthocyanin biosynthesis under high temperature stress was affected by ripe stage of strawberries.

The anthocyanin biosynthesis is initiated from branches of the flavonoid pathway, starting from the conversion of phenylalanine to cinnamic acid by PAL, and then sequentially catalyzed by C4H, 4CL, CHS, CHI, F3H, F3′H, DFR, LDOX/ANS and UFGT, which comprise the pathways needed for the synthesis of anthocyanins. Temperature stress could regulate the gene expression of the enzymes related to anthocyanin synthesis in fruits of apples, pears and plums at post-transcriptional or post-translational level (Elsharkawy et al., 2015; Li et al., 2017; Niu et al., 2017). In this study, high ambient temperature (35 °C) improved structure gene expressions of anthocyanin including *PAL1*,

Table 1
The gene expression profile of DEGs related to the fruit discoloration in postharvest strawberry under 25 °C and 35 °C.

Annotation	Gene ID	25 °C FPKM	35 °C FPKM	FC
WRKY 6-like	gene19478	3742.78	1850.26	−1.02
WRKY 14-like	gene20152	115.08	16.03	−2.79
WRKY 33-like	gene28174	499.54	195.31	−1.35
WRKY 44-like	gene18152	32.37	119.08	1.86
MYB 39-like	gene04423	14.60	160.23	3.39
MYB 306-like	gene03817	31.64	7.32	−2.11
bHLH 66-like	gene10062	99.05	258.99	1.38
bHLH 128-like	gene22490	33.61	71.56	1.07
bHLH 130-like	gene00545	644.30	263.46	−1.29
CPC-like	gene23919	8.52	79.51	3.12
PAL 1-like	gene09753	1852.74	5231.63	1.50
PAL 1-like	gene23261	16673.54	35598.35	1.09
CHS 2-like	gene26826	4004.99	14205.88	1.83
CHS 2-like	gene26825	3813.35	12896.90	1.76
CHI 1	gene23367	305.53	1165.47	1.93
CHI-like	gene21346	5089.05	10626.50	1.06
DF4R	gene15176	1796.18	5908.21	1.72
DF4R-like	gene03288	17.39	51.69	1.53
ANS-like	gene32347	3345.57	14126.11	2.08
UFGT3	gene22710	6.07	22.94	1.80
UFGT3	gene24224	7482.70	562.93	−3.73
UFGT3	gene24225	1835.97	106.05	−4.10
UFGT6	gene24227	409.90	180.26	−1.18
UFGT6	gene24226	743.09	155.75	−2.25
UFGT7	gene26347	113.22	239.59	1.08
UFGT7	gene20181	453.60	28.61	−3.95
UA5, 3GT	gene26881	87.81	479.99	2.44
UAGT 2	gene12591	7341.02	22360.76	1.61
UAGT 5	gene29533	135.80	317.06	1.22
MATE DTX1-like	gene16070	451.00	1545.21	1.77
MATE TT2-like	gene25149	36.49	81.22	1.14
MATE TT12-like	gene15073	64.18	183.31	1.50
MATE TT12-like	gene13214	294.24	681.44	1.21
MATE TT12-like	gene35021	31.16	65.30	1.05
GST 23-like	gene09260	245.09	113.99	−1.10
GST 23-like	gene08383	206.42	16.39	−3.60
GST 23-like	gene08384	131.49	7.04	−4.09
MRP POP1-like	gene20459	225.74	72.59	−1.63
Cu/Zn-SOD-like	gene16423	431.10	1504.38	1.80
Cu/Zn-SOD-like	gene24452	269.26	625.12	1.21
GRX C1-like	gene32211	105.74	231.64	1.13
GRX C2-like	gene28448	11704.40	4806.32	−1.28
GRX C9-like	gene29769	310.21	874.13	1.49
APX 3-like	gene14579	65.31	136.17	1.05
PPO -like	gene18289	125.55	8.99	−3.70
PPO -like	gene30434	902.21	199.2771	−2.17
POD 3-like	gene14579	65.31	136.17	1.05
POD 4-like	gene28140	282.05	98.63	−1.51
POD 6-like	gene32458	165.10	341.49	1.05
POD 12-like	gene27591	400.89	113.56	−1.81
POD 27-like	gene19544	12387.90	5812.20	−1.09
POD 63-like	gene28350	84.32	178.93	1.08
Laccase 9-like	gene27523	83.31	185.80	1.15
Laccase 14-like	gene12086	9.43	391.44	5.27

CHS2, *CHI*, *DF4R*, *LDOX*, *LAR, UA5, 3GT*, *UA3GT5* and *UA3GT2*, which was consistent with changes of anthocyanin contents in strawberries; however, most of the *UFGT* genes especially for *UFGT3*, *UFGT6* and *UFGT7* were significantly suppressed under high ambient temperature, which was contrary to the changes of anthocyanin's contents under high ambient temperature(Table 1 and Fig. 2).

Early research indicate that UFGT was the final enzyme in the anthocyanin pathway and in charge of transferring the glucosyl moiety from UDP-glucose to the 3-hydroxyl group of anthocyanidins, which was important for anthocyanidin stability and water solubility(Zhao et al., 2012). *FaUFGT6* and *FaUFGT7* mainly converted quercetin to almost equal amounts of quercetin 3-O-glucoside and quercetin 4-O-glucoside in addition to small amounts of the 7-O- and 3-O-isomers, and both of them could not accept anthocyanidins (delphinidin, cyanidin, and pelargonidin) as substrates in strawberries (Sambri et al., 2011). It was found that the partially purified UFGT from grapes were correlated very strongly with the quantitative ability to glucosylaye anthocyanin but not at all with the ability to glucosylate quercetin, which were more appropriately to be considered as UDP-glucose: anthocyanidin 3-O-glucosyltransferase (UAGT) (Ford, Boss, & Høj, 1998). Subsequently, UDP-glucose: anthocyanidin 3-O-glucosyltransferase were identified in clitoria ternatea, grape hyacinth, maize, grape and catalyzes glucosyl transferring from UDP-glucose to anthocyanidins such as delphinidin, pelargonidin, cyanidin (Hiromoto et al., 2015). In this study, four *UAGT* genes including *UA5, 3GT*, *UA3GT5* and *UA3GT2* were identified in postharvest strawberries. High storage temperature improved the expression of these genes, especially for *UA3GT2*, which strongly correlated with anthocyanidin accumulation in strawberry under high temperature. These results indicated UAGT but not UFGT was the crucial enzymes involving anthocyanin glucosides in strawberry and high temperature enhanced the genes expression of *UAGT*.

Recent research indicated that multi-drug and toxic extrusion *MATE TT12* might be involved in transportation of anthocyanin across cell membranes in brown cotton, radish and strawberry (Gao et al., 2016; M'Mbone et al., 2018; Chen et al., 2018). In the present study, six *MATE TT12* genes were identified in the strawberries and high ambient temperature up-regulate their genes expression, which was consistant with the increment of anthocyanidins levels (Table 1). These results indicated *MATE TT12* maybe the important genes involving in the transportation of anthocyanin accumulation of strawberries and high ambient temperature may enhance anthocyanin transportation and accumulation from ER to vacuole, which further deepen the fruit color of postharvest strawberries.

Recent studies have shown that browning development in harvested fruits and vegetables might be attributed to the accumulation of ROS, lipid peroxidation of membrane, and the damage of cellular membrane structure (Lin et al., 2014). In our previous report, we found high ambient temperature enhanced accumulations of ROS and malonaldehyde (MDA) in strawberries (Yang et al., 2017). In this study, the genes of *Cu/Zn-SOD*, *APX* were significant up-regulated (Table 1), which also indicated that high ambient temperature enhance the levels of ROS metabolism in postharvest strawberries.

PPO and POD was the primary enzyme responsible for the browning reaction in fresh fruit and vegetables. However, in this study, the results indicated gene expression and activities of PPO in strawberries under high ambient temperature were greatly inhibited, which indicated PPO was not the critical factor in fruit discoloration of strawberries under high ambient temperature. It was reported that the grape fruit of commercial variety 'Elsanta' showed a good correlation between peroxidase activity and color loss of the samples (Movahed et al., 2016). In the current study, high ambient temperature enhanced gene expressions of *POD3*, *POD6* and *POD63* and increased the POD activity (Table 1 and Fig. 3.). In addition, we found high ambient temperature stimulated genes expression of laccase-related genes (*laccase 9* and *laccase 14*) in postharvest strawberries. Recent years, laccase were found involving in anthocyanin degradation in pericarp tissue skin browning formation of litchi and apples (Fang et al., 2015; Gong et al., 2018). These results indicated high ambient temperature maybe stimulate anthocyanin degradation in strawberries mainly catalyzed by POD and laccase. However, in the current experiment, we also found the FPKM values of the anthocyanin degradation-related genes including *POD3*, *POD6*, *POD63*, *laccase 9* and *laccase 14* were far lower than those of anthocyanin synthesis-related genes(Table 1), which indicated anthocyanin synthesis metabolism was much stronger than degradation metabolism in the strawberries under high ambient temperature. Niu et al. (2017) also found anthocyanin synthesis and degradation co-existed in plums under high temperature stress and anthocyanin content depends on the counterbalance between its synthesis and degradation.

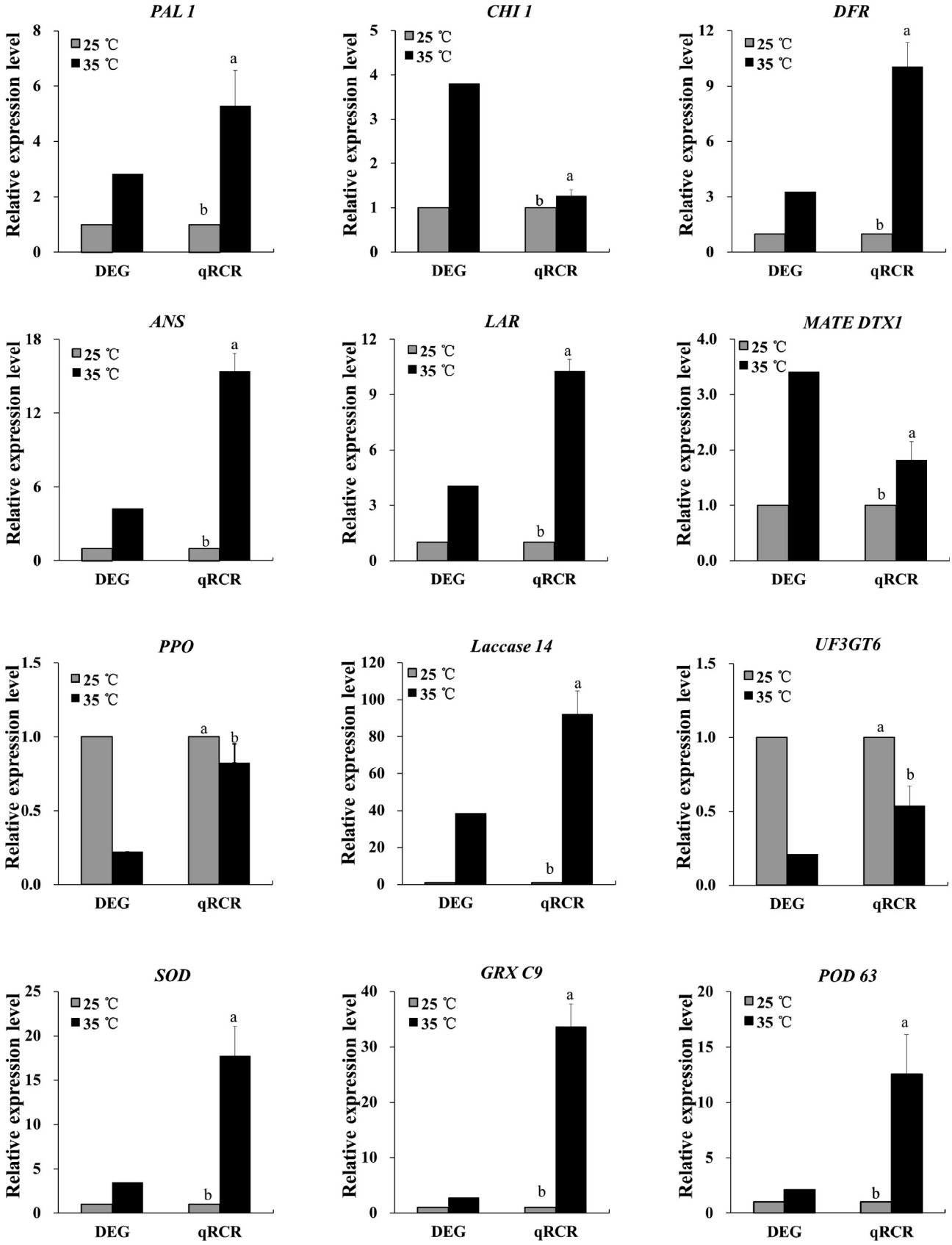

Fig. 5. Verification of qRT-PCR analysis for gene transcripts involving in fruit discoloration of postharvest strawberry under 25 °C and 35 °C. Each data point represents mean ± SD (n = 3).

5. Conclusions

In conclusion, high ambient temperature applied in this study increased anthocyanin content through stimulating the key genes expression involving in anthocyanin biosynthesis and transportation, which deepened the fruit color of postharvest strawberries. Meanwhile, high storage temperature improved the genes expression involving ROS metabolism and triggered the enzymatic degradation of anthocyanin by laccase and POD, which was another important reason for fruit discoloration of postharvest strawberries under high ambient temperature.

Declaration of interests

None.

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