Acta Physiologiae Plantarum (2018) 40:192
https://doi.org/10.1007/s11738-018-2767-8

ORIGINAL ARTICLE

Anthocyanin accumulation and related gene expression affected by low temperature during strawberry coloration

Yunting Zhang[1] · Yi Liu[1] · Wenjie Hu[1] · Bo Sun[1] · Qing Chen[1] · Haoru Tang[1]

Received: 21 January 2018 / Revised: 6 October 2018 / Accepted: 9 October 2018 / Published online: 22 October 2018

Abstract

Anthocyanins implicated in fruit coloration and prevention of oxidative damage. Previous studies have already expounded that anthocyanin accumulation was generally affected by temperature. The objective of this experiment was to investigate the influences of low temperature (4 °C) on anthocyanin production during strawberry coloration in comparison with the control (25 °C). We measured the anthocyanin content, and transcript abundance of structural genes and *MYBs* involving anthocyanin biosynthesis. Meanwhile, proanthocyanidin content, O_2^- production rate and SOD activity were also monitored in this process. Eventually, the results showed that low temperature induced the anthocyanin and proanthocyanidin accumulation in response to oxidative damage, whereas SOD activity did not increase as expected. Furthermore, *ANS, UFGT* and *MYB10* showed higher expression levels and *MYB1* expression was suppressed in fruits exposed to low temperature, suggesting that the increase of anthocyanin content might be caused by the regulatory genes (*MYB10* and *MYB1*) modulating the *ANS* and *UFGT* structural genes expression.

Keywords Anthocyanins · Gene expression · Low temperature · Strawberry · Oxidative damage

Introduction

Strawberry coloration has been considered as a vital factor in determining the fruit quality and market value. As previously reported, strawberry coloration is attributed to accumulation of anthocyanins that belong to flavonoids, a widely distributed class of phenolic compounds (Crecente-Campo et al. 2012). Anthocyanins are the water-soluble pigments responsible for vivid red, blue, and purple colors of many plants, which are conducive to attract pollinators, disperse seeds, and tolerate various biotic and abiotic stress (Zhang et al. 2014). Moreover, anthocyanins are able to reduce risk of obesity, diabetes, inflammation, cancer, and other chronic diseases in many cell-line studies, animal models and human clinical trials. These health-beneficial effects can be more or less associated with the antioxidant property and free-radical scavenging ability of these compounds (He and Giusti 2010; Pojer et al. 2013).

Anthocyanins consist of an anthocyanidin aglycone bound to one or more glycosyl moieties. At least 23 anthocyanidins and 500 anthocyanins have been characterized. The formation of all these anthocyanin derivatives is primarily based on six common types of anthocyanidins, namely delphinidin (Dp), peonidin (Pn), pelargonidin (Pg), petunidin (Pt), malvidin (Mv), and cyanidin (Cy) (Castañeda-Ovando et al. 2009). Additionally, it has been investigated that Cy as the common aglycone was found in over 82% of examined fruits and berries (Jaakola 2013). However, Pg anthocyanins occupied the most of proportion and Cy derivatives took up a very small percentage in strawberry pigment (da Silva et al. 2007).

The anthocyanin biosynthetic pathway has been almost entirely elucidated, and most of the structural genes encoding the enzymes responsible for each step have been identified including phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate-CoA ligase (C4L), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3′-hydroxylase

Communicated by P. Wojtaszek.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11738-018-2767-8) contains supplementary material, which is available to authorized users.

✉ Haoru Tang
htang@sicau.edu.cn

[1] College of Horticulture, Sichuan Agricultural University, Chengdu 611130, China

(F3′H), flavonoid 3′5′-hydroxylase (F3′5′H), dihydroflavonol 4-reduc-tase (DFR), anthocyanidin synthase (ANS), and UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UFGT), which were coordinately modulated by a stable ternary MBW complex consisting of MYB/bHLH/WD40 transcription factors in most species (Petroni and Tonelli 2011). As is well-known that MYB components from the protein complex are primarily responsible for anthocyanin biosynthesis and they could combine or not with bHLH and WD40 to regulate downstream gene expression in this pathway, like MYBP1 in maize (Grotewold et al. 1994), IbMYB1 in sweet potato (Mano et al. 2007), and PavMYB10.1b in sweet cherry (Jin et al. 2016).

In addition to genetic factor, environmental elements, such as light, temperature, and nutrient depletion, are also able to affect anthocyanin production in plant. It has been documented that red organ coloration is more prominent and remarkable in cooler seasons or regions (Lin-Wang et al. 2011; Man et al. 2015; Mori et al. 2005, 2007; Ubi et al. 2006), which suggests that there is a temperature-specific influence and increases concerns about the effect of ongoing climate warming on red organ coloration (Ibáñez et al. 2010; Sugiura and Yokozawa 2004). Thus, low-temperature stimulation is a potential method to increase anthocyanin content. Basically, this is achieved through a process of regulating the expression of related structural genes and MBW regulatory complex. Increasing evidences have pointed out that MYBs specifically modulate anthocyanin biosynthesis, but the function of bHLH and WD40 in this pathway is intricate. During exposure to low temperature, *BoPAP1* encoding a MYB transcription factor was significantly induced and might play a critical role in activating the anthocyanin structural genes (*C4H, F3H, DFR, ANS* and *UFGT*) for the accumulation of abundant anthocyanins in the purple kale (Zhang et al. 2012). Apple fruit generally requires low temperature to accumulate anthocyanin, which is related to low temperature inducing expression of *MdMYBA* specifically binding to *ANS* (Ban et al. 2007). On the contrary, heat condition could cause a dramatic reduction of both anthocyanin concentration and transcript levels of the structural genes by rapidly decreasing expression of *MYB10* in apple (Lin-Wang et al. 2011). Previously, different results about the impact of temperature on strawberry coloration were displayed. Anthocyanin content increased as the ratio of maximum-to-minimum temperature became higher in ripe strawberries from 'Earliglow' and 'Kent' cultivars (Wang and Zheng 2001), and high air temperature can lead to poor fruit coloration in 'Sachinoka' (Matsushita and Ikeda 2016). However, Ferreyra et al. (2007) concluded that there was no significant difference between summer and winter concerning anthocyanin content in strawberry cultivar "Selva". Therefore, the mechanism by which low-temperature stress affects anthocyanin accumulation in strawberry is not yet clear. Here, anthocyanin contents and the expression levels of structural and regulatory genes were assayed to elucidate the cause of fruit coloration under low temperature.

Materials and methods

Plant materials and treatments

The cultivated octoploid strawberry (*Fragaria* × *ananassa* Duch. cv. Toyonoka) was used in this study. Seedlings in approximately 2-L plastic pots containing the mixture with perlite, garden soil, and nutrient soil (1:2:2, v/v/v) were grown in Sichuan Agricultural University greenhouse. The low-temperature treatment was conducted once the fruit turned white. The potted strawberries with white fruit were divided into two groups and moved to growth chambers set to either a low temperature (4 °C) or control regimen (25 °C) at 75% relative humidity, 100 μmol m^{-2} s^{-1} light intensity for 16 h per day. Fruit samples were harvested at 0, 6, 12, 24, 48, and 72 h after treatment. Subsequently, they were frozen in liquid nitrogen and immediately stored at − 80 °C until needed.

Anthocyanin and proanthocyanidin content determination

Approximately 1.0 g of the fine powder was homogenized in 5 ml 1% (v/v) HCl:MeOH solvent overnight at 4 °C in the dark. After the mixture was centrifuged at 5000×*g* for 20 min, the upper aqueous phase was collected in a brown volumetric flask. Then, the solid layer was repeatedly extracted with the fresh extractant until no more material dissolved. Finally, the supernatant fractions were pooled and set to the final volume of 10 ml. 1 ml of the resulting supernatant was filtered using the Millipore filter with a 0.45 μm nylon membrane. Quantitative determination of anthocyanin content was performed using Agilent 1260 HPLC system (Agilent, USA) installed with ZORBAX SB-C18 column (150 × 4.6 mm, 5 μm) and variable wavelength detectors (VWD). The mobile phases consisted of A (ultra-pure water), B (acetonitrile), C (formic acid). Separation was achieved by a linear gradient elution at the flow rate of 1 ml min^{-1} and column temperature of 30 °C. The injection volume was 10 μl, and gradient elution conditions were as follows: 0 min, 100% A; 13 min, 78% A + 20% B + 2% C; 20 min, 58% A + 40% B + 2% C; 25 min, 100% A. The anthocyanins monitored at 520 nm were quantified by constructing the standard curve with pelargonidin-3-glucoside (Sigma-Aldrich, USA).

The content of proanthocyanidin was colorimetrically analyzed by improved DMAC (4-Dimethylaminocinnamaldehyde) method (Prior et al. 2010). 0.5–1.0 g of the fine

powder from frozen fruit was soaked in 20 ml extraction buffer containing acetone, deionized water, and acetic acid (150:49:1 v/v/v) and then mixed well by shaking for 1 h. After 20 min centrifugation at 10,000×*g* at 12 °C, the upper aqueous phase was used for proanthocyanidin determination at 640 nm.

O_2^- production rate and SOD activity assay

The strawberry powder (~0.5 g) was extracted using 5 ml 50 mM pre-cooling potassium phosphate buffer (PH 7.8) containing 1% (w/v) of polyvinylpolypyrrolidone (PVP), and then the homogenate was spun at 10,000×*g* for 10 min at 4 °C. The supernatant liquor was employed to detect O_2^- (superoxide anion) production rate and SOD (superoxide dismutase) activity. O_2^- production rate was measured by following Cai et al. (2006) method. SOD (EC 1.15.1.1) activity was determined according to the nitroblue tetrazolium (NBT) method (Ali et al. 2013; Beauchamp and Fridovich 1971), and one unit of SOD enzymatic activity (U) was defined as the amount of enzyme that caused a 50% inhibition of NBT photoreduction rate.

Total RNA isolation and first strand cDNA synthesis

The improved CTAB (cetyltrimethylammonium bromide) method as described by Chen et al. (2012) was adopted to extract total RNA from harvested fruit samples. RNA quality and yield were evaluated by spectrophotometric measurement as well as 1% agarose gel electrophoresis analysis. Then 1 μg of total RNA from each sample was reverse-transcribed to cDNA according to the protocol of PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara, Japan). All cDNAs were diluted tenfold prior to use in qRT-PCR (quantitative real-time RT-PCR) reactions.

Quantitative real-time RT-PCR

The 96-well plates were used to perform qRT-PCR reactions on the CFX96 real-time PCR system (Bio-Rad, USA). PCR amplification was done in a 10 μl total volume containing 5 μl SYBR Premix (Takara, Japan), 0.4 μl each primer (0.4 μM), 1 μl diluted cDNA and 3.2 μl RNase-free water. PCR procedure was set with three-step cycling conditions: 3 min pre-denaturation at 95 °C, followed by 40 cycles of 10 s denaturation at 95 °C, 30 s annealing at 60 °C and 15 s polymerization at 72 °C. The specificity of primer amplification was assessed by inserting the melting curve ranging from 65 to 95 °C (increment 0.5 °C/5 s) after the final cycle and the potential reagent contamination was checked by no template added in the mixture. Primers listed in Table 1 were designed using Primer Premier 5.0 software.

Results

Anthocyanin and proanthocyanidin content

Standard curve was obtained using the major anthocyanin in strawberry (pelargonidin-3-glucoside) as reference compound to analyze the samples (Fig. S1). The amount of anthocyanin in control samples at 25 °C showed a stable accumulation and slow increase, while it could be induced quickly by the low temperature (4 °C), and kept a higher level from 12 to 48 h, and subsequently had a sharp decrease showing the value close to the control (Fig. 1a). The change of proanthocyanidin content presented a fluctuant trend in both regimens. Eventually, proanthocyanidin content in low temperature was lower, nevertheless it was very significantly higher than the control samples in the medium-term treatment (Fig. 1b).

Table 1 Primers used for qRT-PCR

Gene	Accession no.	Forward primer (5′–3′)	Reverse primer (5′–3′)	Amplicon size (bp)
ACTIN	LC017712.1	TTCACGAGACCACCTATAACTC	GCTCATCCTATCAGCGATT	122
PAL	AB360393.1	AATGAGAAGAACGCAAACACTT	GATATGACCTGCACTCGATGA	157
CHS	AY997297.1	TGGTGCCGCAGCCATAATTGTTG	GCCCAGGAACATCTTTGAGGAG	170
F3H	AY691918.1	CATAGCGACATTCCAGAACCC	TCTCAGTGTAAGTCATCGGCTC	104
LAR	DQ087253.1	CATTACTTCTGTAGGAACGGTCAA	TCTCAATCGCACGCCTCA	131
DFR	AF029685.1	AACGAAGTGATAAAGCCAACA	GTAAACACCAACCTCCGAAC	89
ANR	DQ664192.1	TCCAAGGCGAAGACCATAC	ACTTAAACAACTGAGACCACCA	161
ANS	AY695818.1	GTGAGGGAGAAATGTAGGGAGGAT	GGAGATGCCGTGGTTGATAAGG	81
UFGT	AY575056.1	CTCAGGATCTGCCAGAGGG	GCAAGCGGTAGCATTAGTC	91
MYB10	EU155162.1	TACCAACAGAACCACCACAG	GCCGATAGTAGCATATCTTCAACCC	169
MYB1	AF401220.1	CTGCCTGGAAGGACAGATAAC	ATTTGGACGAAGAGTAGTGCC	93
MYB5	JQ989280.1	ACTCCTCGGCAATAGATGGTC	GATTGAGTGGCTTGTGGGTTCT	143

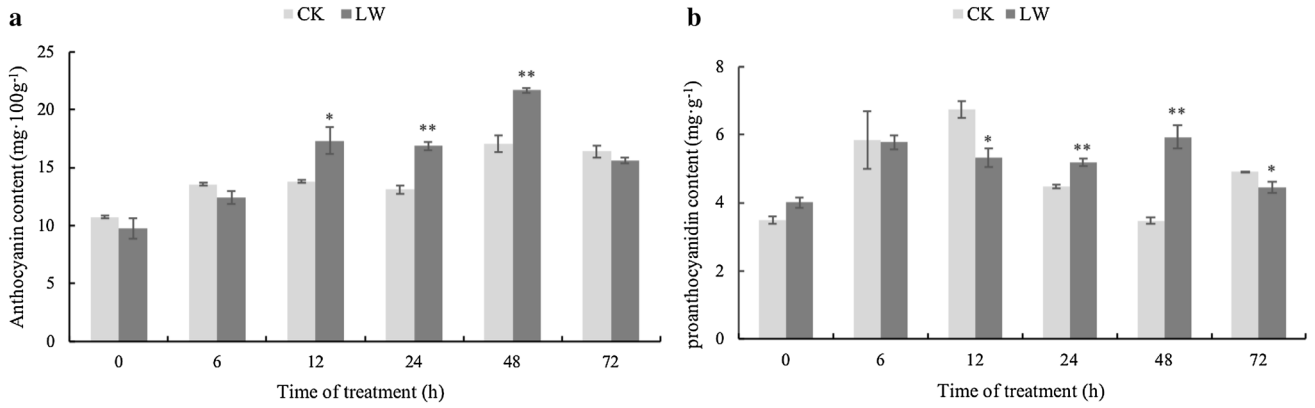

Fig. 1 Anthocyanin (**a**) and proanthocyanidin (**b**) content in strawberry fruits treated at 4 °C and 25 °C. Each value in the histogram represents mean ± standard error from three biological experiments ($n = 3$). Asterisks indicate significant differences based on one-way analysis of variance in SPSS 23.0 followed by the Dunnett t test (*$P < 0.05$; **$P < 0.01$)

O_2^- production rate, SOD enzyme activity

Low temperature stress could cause the accumulation of superoxide anion (O_2^-). As shown in Fig. 2a, the production rate of O_2^- was upregulated by low temperature from 0 to 48 h, though there was no significant difference between two regimes except 24 h. In the late treatment (48–72 h), the production rate of O_2^- was higher in the control samples. In addition, SOD activity of strawberry fruit in 25 °C chamber reached the peak at 24 h and was higher than samples in low temperature (Fig. 2b), which might be the reason why the production rate of O_2^- was remarkably lower in the control samples at that time.

Expression pattern of anthocyanin biosynthetic genes

Expression profiles of genes involving the anthocyanin biosynthetic pathway were investigated in strawberry fruits exposed to two different temperatures through the quantitative real-time RT-PCR technology. Results were illustrated in Fig. 3, which reported that most of genes except *CHS, ANS, UFGT* and *ANR* showed a lower transcript level in the low temperature treatment, but almost had no significant difference compared to control samples at 25 °C. The *ANS* and *UFGT* expressions were remarkably induced by low temperature, and displayed the similar pattern as anthocyanin concentration. The transcript levels of *CHS* and *ANR* in low temperature was higher just during the late course of treatment. In addition, expression pattern of all genes was substantially similar between two regimens during the whole time course of treatment.

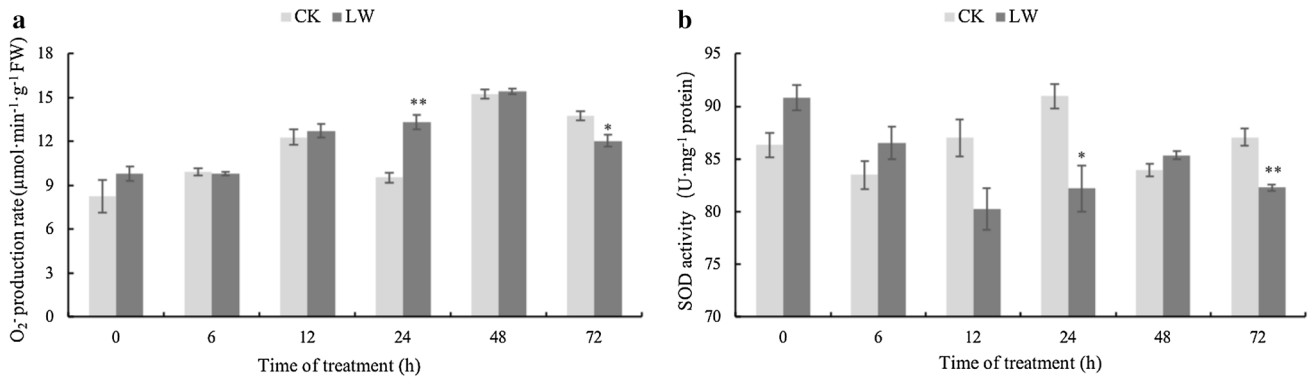

Fig. 2 O_2^- production rate (**a**) and SOD activity (**b**) in strawberry fruits treated at 4 °C and 25 °C. Each value in the histogram represents mean ± standard error from three biological experiments ($n = 3$). Asterisks indicate significant differences based on one-way analysis of variance in SPSS 23.0 followed by the Dunnett t test (*$P < 0.05$; **$P < 0.01$)

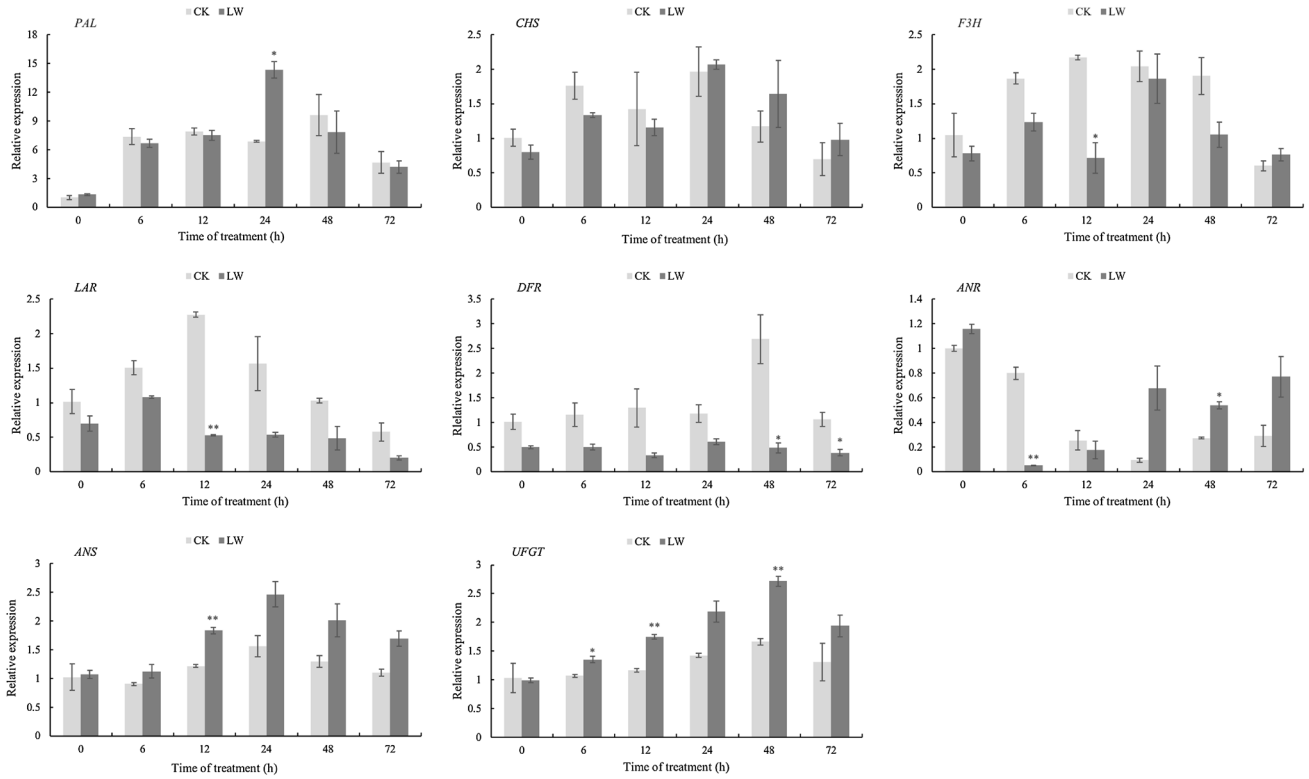

Fig. 3 Expression patterns of structural genes involving anthocyanin biosynthesis in strawberry fruits treated at 4 °C and 25 °C. Each value in the histogram represents mean ± standard error from three biological experiments ($n=3$). Asterisks indicate significant differences based on one-way analysis of variance in SPSS 23.0 followed by the Dunnett t test (*$P<0.05$; **$P<0.01$)

Expression pattern of transcriptional factor *MYB* genes

The MYBs is one of the largest families of transcription factors (TFs) in plant, which have been documented to implicate in the regulation of diverse biological processes. In this work, we only detected the expression profiles of three MYB genes that have been reported they participated in the anthocyanin biosynthesis (Fig. 4). Transcript abundance of *MYB10* had the similar trend in two treatments. Namely, it experienced a decrease and then increase in change, and happened once again during the entire time course. Notwithstanding the higher expression level of *MYB10* in strawberries exposed to low temperature, there was no significant difference in two groups. We can see *MYB1* transcript level had an increase after a slight drop and peaked at 24 h in 4 °C samples; however, the peak value was significantly lower than that in 25 °C. Subsequently, the expression level of *MYB1* began to decline in both regimes, while it rose again at 72 h in low temperature. *MYB5* transcripts had decreased sharply in late period and the value was close to each other under two different temperatures.

Discussion

Strawberry coloration which depends on anthocyanin production and accumulation is one of the most important determinates of fruit quality. As is well-known that anthocyanins have antioxidant capability and have been considered as therapeutic agents due to their beneficial health effects including preventing or lowering the risk of cardiovascular disease, inflammation, diabetes, and cancer (Miguel 2011). However, anthocyanins are difficult to be applied as food dyes because they are extremely unstable and easily degraded in the isolated form (Giusti and Wrolstad 2003). In addition, both external and internal factors can affect anthocyanins biosynthesis and accumulation. Environmental factors, especially temperature, show great influence on these processes. It has been reported that high temperature usually caused inferior accumulation of anthocyanin in comparison to low temperature. With an increase of night temperature, the growth rate of grape berries was accelerated; however, accompanied by the poorer coloration (Mori et al. 2005). After grape berries were exposed to high air temperature (35 °C), the anthocyanin concentration decreased to about half that of control berries (Mori et al. 2007). Red-fleshed fruits of *Malus* crabapple

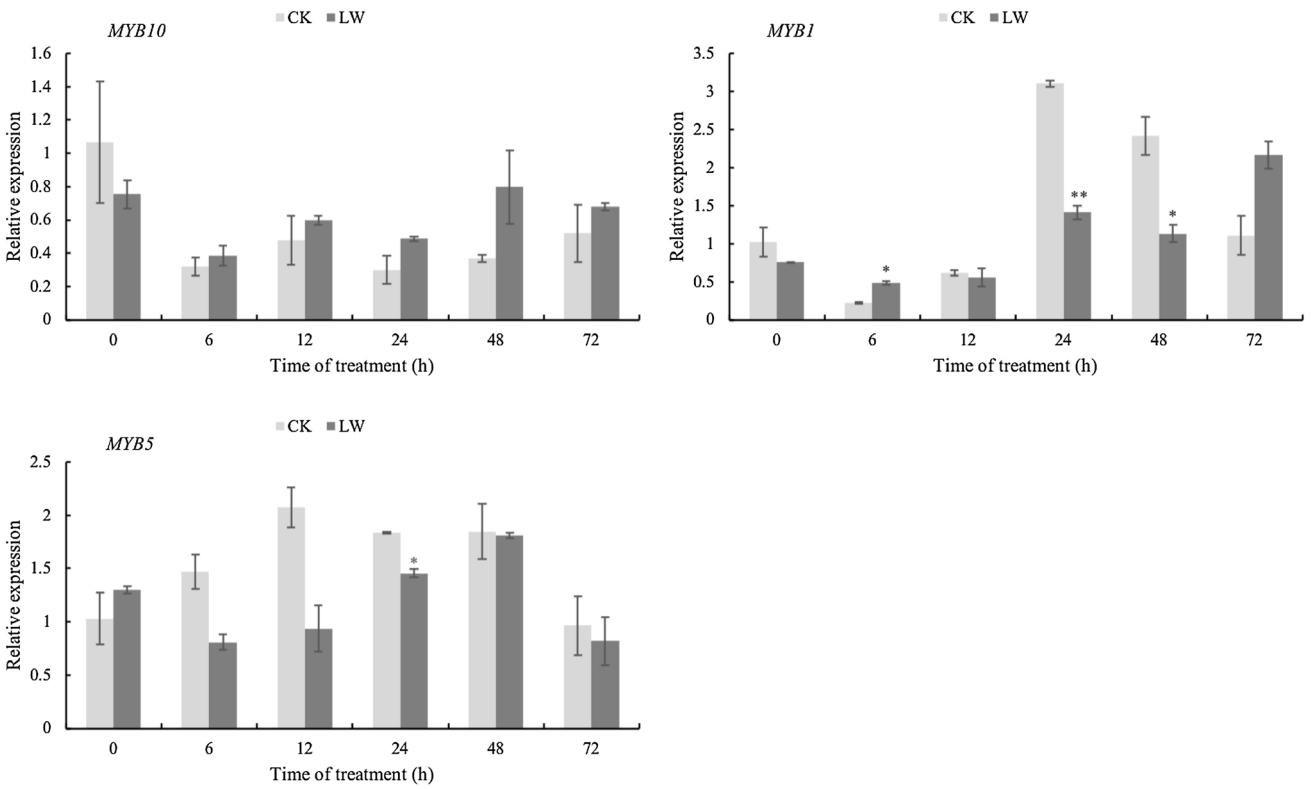

Fig. 4 Expression patterns of *MYB* transcription factors involving anthocyanin biosynthesis in strawberry fruits treated at 4 °C and 25 °C. Each value in the histogram represents mean ± standard error from three biological experiments ($n = 3$). Asterisks indicate significant differences based on one-way analysis of variance in SPSS 23.0 followed by the Dunnett *t* test (*$P < 0.05$; **$P < 0.01$)

lost color gradually during hot summer. Researchers have found that high temperature highly reduced the biosynthetic and accumulated potential of anthocyanins and triggered reactive oxygen species (ROS) generation. Owing to the coupled influence of high temperature and low oxygen, the transcription of specific POD (peroxidase) genes was upregulated. Increased POD and H_2O_2 (hydrogen peroxide) activities gave rise to sequentially-coupled oxidation of anthocyanin pigment and consequently cause color loss in fruit (Rehman et al. 2017). Niu et al. (2017) concluded that the anthocyanin accumulation in plum fruit depended on the counterbalance between its synthesis and degradation at the high temperature. In apples such as 'Akibae', 'Tsugaru', and 'Tsugaru Hime' fruits, anthocyanin production was significantly enhanced under the lower-temperature treatments (15 > 20 °C > 25 °C > 30 °C), whereas the increase of anthocyanin content in 'Akane' fruit was similar at 15, 20 °C and 25 °C treatments, indicating that different cultivars might be have different sensitivity to temperature (Honda et al. 2014). Compared with high temperature, generally, the lower temperature can potentially increase anthocyanin content by maintaining or even increasing expression levels of related genes, and stabilizing physiological attributes (Man et al. 2015; Rehman et al. 2017; Xie et al. 2012). In our study, low temperature (4 °C) could significantly enhance the anthocyanin content in a short term. Meanwhile, the production rate of O_2^- increased rapidly. However, the SOD showed lower activity in comparison to control samples exposed 25 °C during this period. In lettuce, anthocyanin content was increased under low-temperature treatment, while POD activity decreased (Boo et al. 2011). Those suggested that anthocyanin possibly played an important role to scavenge O_2^- in response to low temperature. Therefore, the production rate of O_2^- began to slow down at 48 h during low-temperature treatment in our study.

Several evidences showed that temperature affected anthocyanin production by modulating the genes involved in anthocyanin biosynthetic pathway. Here, we investigated the expression of *PAL, CHS, F3H, DFR, ANS, UFGT, LAR*, and *ANR* as structural genes, and of *MYB10, MYB5* and *MYB1* as regulatory genes. The results showed most of structural genes except *ANS* and *UFGT* had no significant difference between two temperature regimes. The expression level of *ANS* and *UFGT* was higher in low temperature (4 °C) and followed a similar tendency as anthocyanin content. It has been extensively reported that the expression of late-stage genes (*DFR, ANS* and *UFGT*) in anthocyanin biosynthetic pathway at a high level was critical for anthocyanin

accumulation. *UFGT* was the limiting factor of anthocyanin accumulation and pericarp coloration in a given red litchi cultivar (Wei et al. 2011). Besides, absence of *UFGT* leaded to white grape cultivars (Kobayashi et al. 2001). Silencing of *LDOX* in apple and pomegranate caused a shift in the profile of intermediate flavonoids and blocked anthocyanin biosynthesis (Ben-Simhon et al. 2015; Szankowski et al. 2009). Jasmonate-induction of anthocyanin accumulation in Arabidopsis accomplished through significantly up-regulating the 'late' genes *DFR, LDOX*, and *UF3GT* (Shan et al. 2009). This close relationship between the late-stage genes and anthocyanin content was also demonstrated in other strawberry cultivars (Almeida et al. 2007). After the expression level of *DFR* was down-regulated by RNAi technology, the strawberry fruit color became pale. Meanwhile, metabolites were diverted to the quercetin-glycoside biosynthesis pathway when the anthocyanin biosynthesis was hindered (Lin et al. 2013).

In the past decades, numerous studies have indicated that expression of structural genes in anthocyanin biosynthetic pathway is controlled by MYB–bHLH–WD40 ternary complex. The MYB proteins are believed to be key components in the activation of specific gene expression pattern (Jaakola 2013). MYB10 was illustrated to positively regulate anthocyanin synthesis in strawberry through overexpression and suppression assay (Kadomura-Ishikawa et al. 2015a), while FaMYB1 was suggested to negatively modulate anthocyanin biosynthesis in the strawberry fruit using the same verification method (Kadomura-Ishikawa et al. 2015b). Our data showed higher expression of *MYB10* was exerted in low-temperature treatment, but had no significant difference in comparison to control. In addition, *MYB1* expression was diametrically opposite to the anthocyanin content. These results agreed with above reports and also indicated that low temperature might regulate the *MYB10* and *MYB1* expression to affect anthocyanin accumulation. MYB5 was speculated to fine tune both proanthocyanidin biosynthesis during early fruit development and anthocyanin biosynthesis during strawberry ripening through combining with the negative regulator MYB1 (Aharoni et al. 2001; Schaart et al. 2013). In this work, expression level of *MYB5* lacked the remarkable differences between two temperature regimes and therefore could not tell its relationship with anthocyanin accumulation under low temperature.

Taken together, low temperature induced anthocyanin production in strawberry, which contributed to relieve the oxidative damage. The induction of anthocyanin content in strawberry exposed to low temperature might be a result from an increase of *MYB10, ANS* and *UFGT* or a decrease of *MYB1* at the expression level.

Author contribution statement HRT and YTZ conceived this project and designed the experiment. YL and WJH collected samples and measured the physiological indexes. YTZ performed the qRT-PCR, analyzed the data and wrote this paper. BS and QC provided technical support and helped to analyze the data. HRT supervised the analysis and critically revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Aharoni A, De Vos C, Wein M et al (2001) The strawberry *FaMYB1* transcription factor suppresses anthocyanin and flavonol accumulation in transgenic tobacco. Plant J 28:319–332

Ali B, Tao Q, Zhou Y et al (2013) 5-Aminolevolinic acid mitigates the cadmium-induced changes in *Brassica napus* as revealed by the biochemical and ultra-structural evaluation of roots. Ecotoxicol Environ Saf 92:271–280

Almeida JR, D'Amico E, Preuss A et al (2007) Characterization of major enzymes and genes involved in flavonoid and proanthocyanidin biosynthesis during fruit development in strawberry (*Fragaria*×*ananassa*). Arch Biochem Biophys 465:61–71

Ban Y, Honda C, Hatsuyama Y et al (2007) Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin. Plant Cell Physiol 48:958–970

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Ben-Simhon Z, Judeinstein S, Trainin T et al (2015) A "White" anthocyanin-less pomegranate (*Punica granatum* L.) caused by an insertion in the coding region of the Leucoanthocyanidin Dioxygenase (*LDOX; ANS*) gene. PLoS One 10:e0142777

Boo H-O, Heo B-G, Gorinstein S et al (2011) Positive effects of temperature and growth conditions on enzymatic and antioxidant status in lettuce plants. Plant Sci 181:479–484

Cai C, Chen K, Xu W et al (2006) Effect of 1-MCP on postharvest quality of loquat fruit. Postharvest Biol Technol 40:155–162

Castañeda-Ovando A, de Lourdes Pacheco-Hernández M, Páez-Hernández ME et al (2009) Chemical studies of anthocyanins: a review. Food Chem 113:859–871

Chen Q, Yu H, Wang X et al (2012) An alternative cetyltrimethylammonium bromide-based protocol for RNA isolation from blackberry (*Rubus* L.). Genet Mol Res 11:1773–1782

Crecente-Campo J, Nunes-Damaceno M, Romero-Rodríguez M et al (2012) Color, anthocyanin pigment, ascorbic acid and total phenolic compound determination in organic versus conventional strawberries (*Fragaria*×*ananassa* Duch, cv Selva). J Food Compos Anal 28:23–30

da Silva FL, Escribano-Bailón MT, Alonso JJP et al (2007) Anthocyanin pigments in strawberry. LWT Food Sci Technol 40:374–382

Ferreyra RM, Viña SZ, Mugridge A et al (2007) Growth and ripening season effects on antioxidant capacity of strawberry cultivar Selva. Sci Hortic 112:27–32

Giusti MM, Wrolstad RE (2003) Acylated anthocyanins from edible sources and their applications in food systems. Biochem Eng J 14:217–225

Grotewold E, Drummond BJ, Bowen B et al (1994) The myb-homologous P gene controls phlobaphene pigmentation in maize floral

organs by directly activating a flavonoid biosynthetic gene subset. Cell 76:543–553
He J, Giusti MM (2010) Anthocyanins: natural colorants with health-promoting properties. Ann Rev Food Sci Technol 1:163–187
Honda C, Bessho H, Murai M et al (2014) Effect of temperature on anthocyanin synthesis and ethylene production in the fruit of early-and medium-maturing apple cultivars during ripening stages. HortScience 49:1510–1517
Ibáñez I, Primack RB, Miller-Rushing AJ et al (2010) Forecasting phenology under global warming. Philos Trans R Soc Lond B 365:3247–3260
Jaakola L (2013) New insights into the regulation of anthocyanin biosynthesis in fruits. Trends Plant Sci 18:477–483
Jin W, Wang H, Li M et al (2016) The R2R3 MYB transcription factor *PavMYB10. 1* involves in anthocyanin biosynthesis and determines fruit skin colour in sweet cherry (*Prunus avium* L.). Plant Biotechnol J 14:2120–2133
Kadomura-Ishikawa Y, Miyawaki K, Takahashi A et al (2015a) Light and abscisic acid independently regulated *FaMYB10* in *Fragaria*×*ananassa* fruit. Planta 241:953–965
Kadomura-Ishikawa Y, Miyawaki K, Takahashi A et al (2015b) RNAi-mediated silencing and overexpression of the *FaMYB1* gene and its effect on anthocyanin accumulation in strawberry fruit. Biol Plant 59:677–685
Kobayashi S, Ishimaru M, Ding C et al (2001) Comparison of UDP-glucose: flavonoid 3-O-glucosyltransferase (UFGT) gene sequences between white grapes (*Vitis vinifera*) and their sports with red skin. Plant Sci 160:543–550
Lin X, Xiao M, Luo Y et al (2013) The effect of RNAi-induced silencing of *FaDFR* on anthocyanin metabolism in strawberry (*Fragaria*×*ananassa*) fruit. Sci Hortic 160:123–128
Lin-Wang K, Micheletti D, Palmer J et al (2011) High temperature reduces apple fruit colour via modulation of the anthocyanin regulatory complex. Plant Cell Environ 34:1176–1190
Man YP, Wang YC, Li ZZ et al (2015) High-temperature inhibition of biosynthesis and transportation of anthocyanins results in the poor red coloration in red-fleshed *Actinidia chinensis*. Physiol Plant 153:565–583
Mano H, Ogasawara F, Sato K et al (2007) Isolation of a regulatory gene of anthocyanin biosynthesis in tuberous roots of purple-fleshed sweet potato. Plant Physiol 143:1252–1268
Matsushita K, Ikeda T (2016) The effect of high air temperature on anthocyanin concentration and the expressions of its biosynthetic genes in strawberry 'Sachinoka'. Environ Control Biol 54:101–107
Miguel MG (2011) Anthocyanins: antioxidant and/or anti-inflammatory activities. J Appl Pharm Sci 1:7–15
Mori K, Sugaya S, Gemma H (2005) Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. Sci Hortic 105:319–330
Mori K, Goto-Yamamoto N, Kitayama M et al (2007) Loss of anthocyanins in red-wine grape under high temperature. J Exp Bot 58:1935–1945
Niu J, Zhang G, Zhang W et al (2017) Anthocyanin concentration depends on the counterbalance between its synthesis and degradation in plum fruit at high temperature. Sci Rep 7:7684
Petroni K, Tonelli C (2011) Recent advances on the regulation of anthocyanin synthesis in reproductive organs. Plant Sci 181:219–229
Pojer E, Mattivi F, Johnson D et al (2013) The case for anthocyanin consumption to promote human health: a review. Compr Rev Food Sci Food Saf 12:483–508
Prior RL, Fan E, Ji H et al (2010) Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. J Sci Food Agric 90:1473–1478
Rehman RNU, You Y, Zhang L et al (2017) High temperature induced anthocyanin inhibition and active degradation in *Malus profusion*. Front Plant Sci 8:1401
Schaart JG, Dubos C, Romero De La Fuente I et al (2013) Identification and characterization of MYB-bHLH-WD40 regulatory complexes controlling proanthocyanidin biosynthesis in strawberry (*Fragaria*×*ananassa*) fruits. New Phytol 197:454–467
Shan X, Zhang Y, Peng W et al (2009) Molecular mechanism for jasmonate-induction of anthocyanin accumulation in Arabidopsis. J Exp Bot 60:3849–3860
Sugiura T, Yokozawa M (2004) Impact of global warming on environments for apple and satsuma mandarin production estimated from changes on the annual mean temperature. J Jpn Soc Hortic Sci 73:72–78
Szankowski I, Flachowsky H, Li H et al (2009) Shift in polyphenol profile and sublethal phenotype caused by silencing of anthocyanidin synthase in apple (*Malus* sp.). Planta 229:681–692
Ubi BE, Honda C, Bessho H et al (2006) Expression analysis of anthocyanin biosynthetic genes in apple skin: effect of UV-B and temperature. Plant Sci 170:571–578
Wang SY, Zheng W (2001) Effect of plant growth temperature on antioxidant capacity in strawberry. J Agric Food Chem 49:4977–4982
Wei Y, Hu F, Hu G et al (2011) Differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation in the pericarp of *Litchi chinensis* Sonn. PloS One 6:e19455
Xie X, Li S, Zhang R et al (2012) The bHLH transcription factor MdbHLH3 promotes anthocyanin accumulation and fruit colouration in response to low temperature in apples. Plant Cell Environ 35:1884–1897
Zhang B, Hu Z, Zhang Y et al (2012) A putative functional MYB transcription factor induced by low temperature regulates anthocyanin biosynthesis in purple kale (*Brassica Oleracea* var. acephala f. tricolor). Plant Cell Rep 31:281–289
Zhang Y, Butelli E, Martin C (2014) Engineering anthocyanin biosynthesis in plants. Curr Opin Plant Biol 19:81–90