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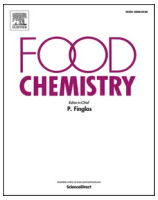

Unravelling effects of red/far-red light on nutritional quality and the role and mechanism in regulating lycopene synthesis in postharvest cherry tomatoes

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ABSTRACT

The main goal of this study was to explore the role of red/far-red light in the preservation of postharvest quality in cherry tomato fruits and the mechanism of red/far-red light in regulation of lycopene synthesis. Results showed that red/far-red light irradiation inhibited weight loss and promoted colour change during storage, and it also increased the content of lycopene and β-carotene compared to control. Gene *PSY*, *ZDS* and *LCY-b* were overexpressed in fruits treated with red/far-red light during 33 days' storage compared to control. The analysis of genes involved in red/far-red light absorbance (*PHYA* and *PHYB*) and mediation (*HY5* and *PIF3*), and fruit ripening (*ACS2* and *RIN*) suggests that red/far-red light promote lycopene accumulation through phytochrome-mediated signalling pathway to induce *HY5*. Elevated HY5 could either directly bind to *PSY* or promote the expression of *ACS2* to induce *RIN* through MADS-loop to enhanced lycopene content.

1. Introduction

Cherry tomato (L. lycopersicum var. cerasiforme) is highly appreciated worldwide due to its good flavour, small size and health benefits, which are contributed by its nutritional compounds including carotenoids, ascorbic acid, total phenolics and flavonoids (D'Aquino et al., 2016; Fagundes et al., 2015; Panjai et al., 2019). Carotenoids are the primary components in cherry tomato fruits, and they play important parts in reducing the incidence of some disease including cancer, heart disease and some chronic diseases (Liu et al., 2009; Rao and Rao, 2007). The main carotenoids in ripe red tomatoes are lycopene (around 90%), β-carotene (5–10%) and lutein (1–5%) (Liu et al., 2011; Schofield and Paliyath, 2005). Ascorbic acid is one of the most important antioxidants in cherry tomatoes, and it can provide protection against cardiovascular and normal cold (Ma et al., 2014). Phenolic compounds, including total phenolics and flavonoids, are important secondary metabolites in plant and play crucial parts in plant physiological and morphological processes (Balasundram et al., 2006; Bravo, 1998). The antioxidative activities of phenolics help to reduce the incidence of neurodegenerative diseases, cardiovascular diseases, and type II diabetes in human (Aggarwal and Shishodia, 2004; Rahman et al., 2006; Raiola et al., 2014).

The biosynthetic pathway of carotenoid has been studied extensively for many years, and it starts with the conversion of geranyl geranyl pyrophosphate to phytoene catalysed by phytoene synthase (PSY), which is the first and key-limiting step (Pandurangaiah et al., 2016; Xie et al., 2019). Phytoene is then desaturated into lycopene undergoing four desaturation reactions catalysed by enzymes of phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS), followed by isomerization reaction catalysed by carotenoid isomerase (CRTISO) (Pandurangaiah et al., 2016; Xie et al., 2019). Lycopene can be converted into β-carotene by the action of chloroplast lycopene beta cyclase (LCY-b), or converted into α-carotene, the precursor of lutein, by the action of lycopene epsilon cyclase (LCY-e) (Pandurangaiah et al., 2016).

Light plays an important part in the biosynthesis of carotenoid (Xie et al., 2019). Red and far-red light can regulate carotenoid biosynthesis through phytochrome-mediated signalling pathways (Bou-Torrent et al., 2015; Toledo-Ortiz et al., 2010). Both red and far-red light are absorbed by phytochromes (PHYs), a family of plant photoreceptors (Hasan et al., 2017; Xie et al., 2019). These receptors mediate many developmental processes of plant, such as seed germination, chloroplast development, photoperiodic flowering, and anthocyanin biosynthesis, and they also modulate the expression of light-responsive gene expression, including PSY (Alba et al., 2000; Li et al., 2011; Schofield and Paliyath, 2005;

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Toledo-Ortiz et al., 2010). Five types of phytochromes, designated phytochrome A (PHYA) to PHYE, have been founded in Arabidopsis thaliana (Li et al., 2011). Among all these phytochromes, PHYA is the primary photoreceptor responsible for perceiving and mediating responses to far-red light, whereas PHYB is the predominant phytochrome that regulates responses to red light (Li et al., 2011). Under light conditions, these phytochromes can interact with downstream signalling intermediates (such as PIFs and HY5), which control the expression of target genes (Li et al., 2011; Toledo-Ortiz et al., 2010). Phytochrome-interacting factors (PIFs) can directly interact with phytochromes, and they are negative regulators of chlorophyll and carotenoid biosynthesis (Toledo-Ortiz et al., 2010). Only 2 members (PIF1 and PIF3) in PIF family were reported to interact with both PHYA and PHYB (Leivar and Monte, 2014). And PIF3 is the foundation member of the PIF subset (Li et al., 2011). Long hypocotyl 5 (HY5) is a potent PIF antagonist, and it has been reported that it can promote the accumulation of photosynthetic pigment in response to light (Toledo-Ortiz et al., 2014). However, all interactions identified in this phytochrome signalling pathway are protein–protein interactions (Li et al., 2011), it is not clear that if the gene expressions of these transcription factors will be affected by red/far-red light irradiation.

The biosynthesis of lycopene in cherry tomatoes is also related to ripening of fruits, which is regulated by hormones (Alba et al., 2000; Schofield and Paliyath, 2005). The hormone ethylene is essential for the ripening of climacteric fruit, and in tomato fruits, the transcription factor ripening inhibitor (RIN) also play a crucial role in ripening regulation (Gao et al., 2019; Yu et al., 2019). Ethylene can control fruit ripening by regulating RIN through a model called MADS-loop (Gao et al., 2019). In this loop, ethylene transcription factor ethylene response 3 (EIN3) binds to the promoter of RIN, and RIN binds to the promoter of ethylene biosynthesis genes, 1-aminocyclopropane-1-carboxylate synthase 2 (ACS2) and 1-aminocyclopropane-1-carboxylic acid oxidase 1 (ACO1), which completes a positive feedback loop (Gao et al., 2019; Lü et al., 2018). With this loop, a small amount of ethylene could induce the expression of downstream ripening genes, including PSY (Gao et al., 2019). However, the mechanism of this induction and interaction between phytochrome-mediated signalling pathway and MADS-loop in the regulation of lycopene synthesis is still not clear.

There is limited research about the role of red/far-red light in preserving postharvest quality in cherry tomatoes. Therefore, the initial aim of this study was to assess the effect of red/far-red light on red index, weight loss and the content of ascorbic acid, lycopene, β-carotene, lutein, total phenolics and flavonoids. Then, to explore the role and mechanism of red/far-red light in regulation of lycopene biosynthesis, genes involved in carotenoids synthesis, red/far-red light receptor genes, red/far-red light transcriptional factors (TFs) genes and ripening transcriptional factors genes were investigated. Our hypothesis was that these genes might be involved in the regulation of carotenoid biosynthesis under red/far-red light irradiation, and there might be connection between phytochrome-mediated signalling pathway and MADS-loop in this process.

2. Materials and methods

2.1. Plant materials and light treatment

Mature-green cherry tomatoes, cultivar 'Piccolo', were harvested manually from a commercial glasshouse in Worcestershire, UK. Fruits were selected for uniform size and shape without mechanical injuries, and transported in a foam box to the laboratory. The fruits were then hand washed with tap water and air-dried at room temperature. Afterwards, the fruits were evenly placed onto plastic trays without touching each other.

Cherry tomato fruits were divided randomly into two groups. The first group was irradiated with red/far-red light (ratio 0.89) at an intensity of 10.1 μmol m-2 s-1 for 33 days at 15 °C (85% relative humidity) in cabinet (970, Sanyo). The red/far-red light was provided by red light filter covering on white tubes (TL-D Eco 32 W/840, Philips), and the lamp tubes were installed parallel to each other on the top of the cabinet. The spectral distribution of the red/far-red light through the red filter was provided in supplementary material, and the wavelength was between 607 and 718 nm. The ratio of red and far-red light was 0.89, calculated using the area under the graphed line between 600 and 700 nm for red light, and between 700 and 750 nm for far-red light (measured with ImageJ software). The light intensity and spectrum were measured at the three-quarter fruit height level using a light spectroradiometer (StellarNet Inc, USA) at the beginning of the experiment. The second group of fruits was stored at 15 °C (85% relative humidity) in the dark cabinet (970, Sanyo) and considered as the control. There were 50 fruits for each replicate and three replicates for treatment and control. Three fruits were taken from each replicate at the beginning of storage (day 1), and six fruits were taken at day 5, 10, 15, 20, 25, 28, 33. The fruits were frozen immediately with liquid nitrogen. Half of the samples were then freeze dried and ground into powder for analysis of lycopene, β-carotene, lutein, ascorbic acid, total phenolics and flavonoids content. And the rest of samples were ground in liquid nitrogen and stored at −80 °C for molecular analysis. The extra five fruits at each replicate of treatment and control were used to measure the weight loss, appearance and red index.

2.2. Weight loss, appearance and red index

Weight loss: The five fruits were weighed together at the beginning of storage and at day 5, 10, 15, 20, 25. The weight loss was calculated and expressed as percent loss from initial weight.

Appearance: Three deepest red fruits in treatment and control were selected at day 1, 5 and 10 for the recording of appearance. All 15 fruits were measured at day 15, 20 and 25.

Red index was evaluated using a subjective scale of visual red percentage on fruit skin according to the method of Kou et al. (2016): 0 = mature green (0% red), 1 = turning (1–25% red), 2 = pink (26–50% red), 3 = light red (51–75% red), 4 = red (>75% red). Red index (%) was determined using the following formula: $\sum$ (red scale (0–4) × fruit number within each class)/ (highest score × the fruit sum) × 100.

2.3. HPLC analysis of carotenoids

Extraction of carotenoid was carried out by the method of Moo-Huchin et al. (2014) with modifications. 0.5 g freeze-dried sample was mixed with 6 mL of 0.1% butylated hydroxytoluene (BHT) in ethanol and put in 85 °C water bath for 5 min. After adding 0.5 mL of 80% KOH, tubes were put in 85 °C water bath again for 10 min. These tubes were then immediately put in ice and added with 3 mL of cold HPLC water. Afterwards, 3 mL of hexane was added and centrifuged, and then the supernatant was collected. The hexane extraction was repeated for another twice to get a final volume of approximately 9 mL. The extracts were put in evaporator until dry, and then re-suspended with 0.25 mL of methanol and 0.25 mL of 1,2-dichloroethane.

Carotenoids were determined with an Agilent 1100 series HPLC. 20 uL of sample was injected into chromatographic column C8 (4 um particle size, 4.6 mm × 250 mm) at a flow rate of 1 mL/min with mobile phase of methanol and HPLC water. Analytes were identified and calculated by comparison of their retention time with those of authentic standards (lycopene, β-carotene, and lutein). Results were represented as mg per 100 g dry mass.

2.4. Ascorbic acid analysis

The ascorbic acid content was determined by 2,6-dichloroinphenolindopehnol titration method described previously by Moo-Huchin et al. (2014) with slight modifications. 0.15 g freeze-dried sample was homogenized in 5 mL of 2% oxalic acid solution and filtered. 1 mL of

filtrated solution was diluted by 5 mL oxalic acid solution and then titrated with 0.01% of 2, 6-dichlorophenolindophenol solution. The final point was considered when the solution showed a very faint beige/pink colour for 30 s. The ascorbic acid concentration was calculated according to the titration volume of 2, 6-dichloroinphenolindophenol, and expressed as mg per g dry mass.

2.5. Total phenolics and flavonoids analysis

Extraction of total phenolics and flavonoids was carried out with freeze-dried sample according to the method described by Moo-Huchin et al. (2014).

The total phenolics content was measured using Folin-Ciocalteau's phenol reagent according to the method described previously by Yu et al. (2012) with some modifications. 1 mL of filtrated solution or standard solution of gallic acid was mixed with 3 mL of distilled water and 1 mL of Folin-Ciocalteu's phenol reagent. After 8 min, 2 mL of 7.5% Na2CO3 was added to the mixture followed by the addition of 3 mL of distilled water, and then thoroughly mixed. After incubation for 30 min at 25 °C, the absorbance was read at 765 nm using a spectrophotometer against blank which contained all reagents except fruit extraction. Gallic acid was used as a standard, and total phenolic content was expressed as mg gallic acid equivalents per g dry mass.

The flavonoids content was determined using the colorimetric assay described previously by Toor and Savage (2006) with some modifications. 1 mL of extraction or standard solution of quercetin was diluted by 1 mL of distilled water. 0.4 mL of 5% NaNO2 was then added to the solution. After 5 min, 0.4 mL of 10% AlCl3 was added, and the mixture was allowed to react for 6 min before adding 2 mL of 4% NaOH. The solution was then immediately diluted to a final volume of 5 mL with distilled water and thoroughly stirred. The absorbance was read at 510 nm using a spectrophotometer against blank which contained all reagents except fruit extraction. Results was expressed as mg quercetin equivalents per g dry mass.

2.6. RNA extraction, cDNA synthesis and quantitative RT-PCR analysis

Total RNA was isolated using Monarch Total RNA Miniprep Kit (New England BioLabs Inc.) according to the manufacturer's protocol, and the genomic DNA removal step was included in the kit. The quantity and quality of RNA were assessed with Nanodrop spectrophotometer (Thermo Fisher Scientific) by measuring the absorbance ratio of A260/A280 and by electrophoresis on a 2% agarose gel. First-strand cDNA was synthesized from 1 μg of total RNA using UltraScript 2.0 cDNA Synthesis Kit (PCR Biosystems Ltd., London, UK).

For RT-qPCR analysis, 10 μL of PowerUp SYBR green PCR master Mix (Applied Biosystems), 7 μL of molecular H2O, 1 μL of gene specific forward primer, 1 μL of gene specific reverse primer and 1 μL of cDNA were mixed in a final volume of 20 μL. The primers used are shown in Table 1. Quantification was performed with the QuantStudio Design and Analysis Software (version 1.5.0). Fruit collected at day 1 was used as calibration sample, and actin was used as internal reference gene. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.7. Statistics analysis

SPSS Version 25.0 was used for data statistical analysis. The significant difference was analysed by means of one-way ANOVA and Tukey's honestly significant difference (HSD) at a significant level of 0.05.

3. Results

3.1. Appearance, red index, weight loss and nutritional quality changes

Postharvest changes on appearance, red index and weight loss of cherry tomato fruits were shown in Fig. 1. The red index increased significantly in both treated and untreated fruits during 25 days of storage, and red/far-red light showed significantly higher red index than control in all days analysed except at day 1 and 25 ($P < 0.01$). At day 10, a near 3-fold increase was noted in red/far-red light treatment, illustrating the quick ripening process at this day (Fig. 1 C). The increase of red index was well correlated with appearance of cherry tomatoes shown in Fig. 1 B. Fruits treated with red/far-red light turned into pink stage at day 5, whereas fruits in control were at turning stage. At day 20, most fruits in red/far-red light treatment were at red stage, while in control, some fruits were still orange.

The weight loss increased gradually during storage in both treated and untreated fruits, while red/far-red light diminished the increase, showing significant lower weight loss than control at day 15 and 25 ($P < 0.05$) (Fig. 1 D).

The content of lycopene and ß-carotene in red/far-red light treatment increased significantly at day 10 ($P < 0.05$), and then continued accumulating till the end of storage, corresponding to 31 ~ and 5.4 ~ fold the value at day 1, respectively ($P < 0.05$), while in control, the content increased gradually to its maximum by the end of storage (Fig. 2 A, B). Red/far-red light treatment exhibited higher lycopene content than control after day 5, and a significant difference was found between treated and untreated fruits at day 28 ($P < 0.05$) (Fig. 2 A). ß-carotene content in red/far-red light treatment was significantly higher than that in control after day 5 ($P < 0.05$) (Fig. 2 B).

Lutein content in red/far-red light treatment increased 1.3 ~ fold and reached to its maximum level after 10 days of storage, while in control, the highest content was detected at day 5 (1.1 ~ fold the initial value). The content was then decreased in both treated and untreated fruits by the end of storage. Red/far-red light treated fruits showed higher lutein content than control, although no obvious differences was observed during storage (Fig. 2 C).

The content of ascorbic acid increased 1.9 and 1.6 ~ fold, respectively, in red/far-red light treated and untreated fruits after 28 days of

Table 1
Genes and primers sequence used for RT-qPCR expression analysis.

Gene symbol	Name	Primer name	Sequence (5́→3́)
Actin1	Actin	Actin-F	TGTCCCTATCTACGAGGGTTATGC
		Actin-R	AGTTAAATCACGACCAGCAAGAT
PSY2	Phytoene synthase	PSY-F	GATGAGGCAGAGAAAGGCGT
		PSY-R	GCGGTACAAGACCAAAGATGC
ZDS2	Zeta carotene	ZDS-F	TCGGAGTGCCTGTTGTTACC
		ZDS-R	GCTCCAAGTCCTGCAACTCT
LCYb2	Chloroplast lycopene beta cyclase	LCYb-F	AAGAACGAATGGTGGCTCGT
		LCYb-R	ACCACCGATTCCAACGACTC
LCYe2	Chloroplast lycopene epsilon cyclase	LCYe-F	GCCGTGCCTATGGAAGAGTT
		LCYe-R	AAAACACCTGCCTCCACACA
PHYA2	Phytochrome	PHYA-F	CTGGTTTTCCTGGGGCTCTT
		PHYA-R	CCTGAACCAGAACAGCCAGT
PHYB2	Phytochrome	PHYB-F	CCACAGTTCAGCTCGGTTCA
		PHYB-R	TTTTCAGCCAACTGCGATGC
HY52	Elongated hypocotyl 5	HY5-F	AGCGACGAGTTCTATTGCCG
		HY5-R	TCCGGCACTCTTCTGATCTC
PIF32	Phytochrome-interacting factor 3	PIF3-F	AAGGGGTTCCGGTGGAGATA
		PIF3-R	TGTCTGATTCTGTGGGCAGC
ACS23	1-aminocyclopropane-1-carboxylate synthase 2	ACS2-F	TGGATGATGGAACGGTTGATATTGC
		ACS2-R	CCATTGTTGCTTCTGTTCCATCGAAC
RIN3	Ripening inhibitor	RIN-F	TAGTCGTGGCAAGCTTTATGAAT
		RIN-R	TCTTGGTAGTTGTTCTGTGAATCTG

1 Su et al. (2015), 2 Xie et al. (2019), 3 Yu et al. (2019).

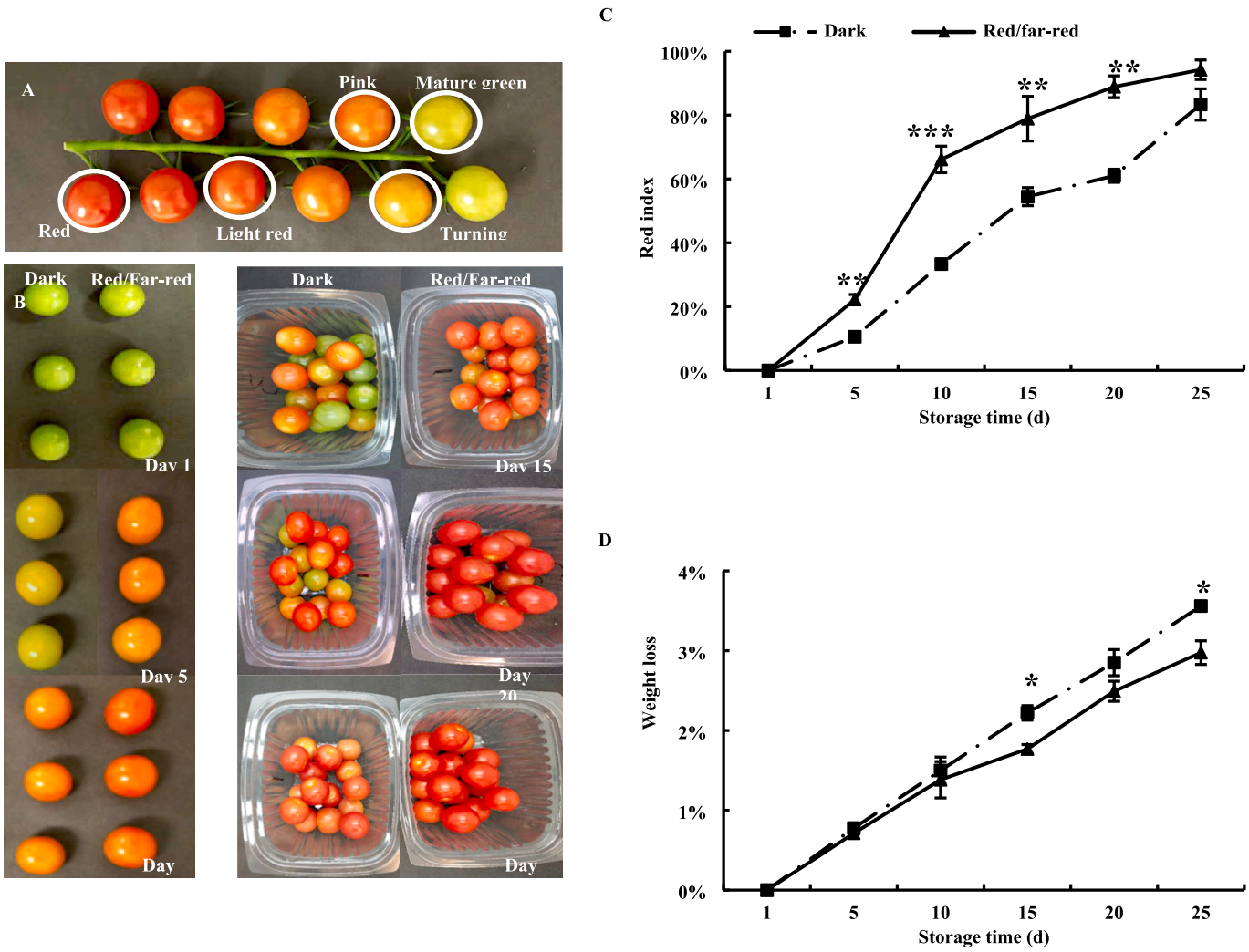

Fig. 1. (A) Classification of cherry tomato fruit ripening stages. Effects of red/far-red light on appearance (B), red index (C) and weight loss (D) of cherry tomatoes during storage. Values are means ± SEs for triplicate samples. Asterisks *, ** and *** indicate significant differences ($P < 0.05$, $P < 0.01$, $P < 0.001$, respectively) between control and treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

storage ($P < 0.05$), and then decreased to their baseline at day 33. Ascorbic acid content was higher in red/far-red light treatment than in control at all days analysed except day 15 and 25, although no significant effect was observed after day 10 (Fig. 2 D). After 33 days of storage, the content of total phenolics increased in both treated and untreated fruits, while a higher increase was found in red/far-red light treatment, with 1.2 ~ fold the value of control (Fig. 2 E). The content of flavonoids decreased significantly after 33 days of storage ($P < 0.05$). No significant difference was found between treated and untreated fruits, but red/far-red light treatment showed higher flavonoids content at all days analysed except day 5 and 28 (Fig. 2 F).

3.2. Transcript levels of genes involved in carotenoids synthesis

Since the red/far-red light treatment enhanced the content of carotenoids (lycopene, ß-carotene and lutein) compared to control, a transcriptional analysis of genes involved in carotenoids synthesis, red/far-red light absorbance and mediation, and ripening was investigated to unravel the mechanism of this phenomenon.

PSY and ZDS are two key genes in lycopene synthesis pathway. The transcription of PSY and ZDS in red/far-red light treatment showed a similar trend during postharvest storage, registering three peaks of expression at day 10, 20 and 28 (Fig. 3 A, B). In these days, the accumulation of PSY mRNA in treated fruits was 29.8~ ($P < 0.05$), 3.3~ ($P < 0.05$) and 1.3~ fold greater than that in control (Fig. 3 A). Expression of ZDS was higher in red/far-red light treatment than in control during storage, being noticeable the difference found at day 10, 15, 20 and 28 ($P < 0.05$) (Fig. 3 B).

LCY-b and LCY-e are genes mediating lycopene metabolism. The expression of LCY-b in both treated and untreated fruits increased significantly to their maximum after 5 days of storage (5.8~ and 2.3~ fold the initial value, respectively) ($P < 0.05$), and this rise was followed by a slight decline. The red/far-red light treatment showed significant higher expression of LCY-b gene than control at all days analysed except day 1, 15 and 25 ($P < 0.05$) (Fig. 3 C).

The transcript of LCY-e also showed a significant increase in treated fruits at day 5 ($P < 0.05$), and the level was 3.2~ fold than that in control ($P < 0.05$). However, the rapid increase expression was followed by a near 3~ fold decline at day 10, and the expression in both treated and untreated fruits continued decreasing to lower than initial value from day 15 (Fig. 3 D).

3.3. Transcript levels of genes involved in red/far-red light absorbance, mediation and ripening

The expression of red/far-red light receptor genes (PHYA and PHYB) in treated fruits increased significantly to their maximum after 10 days of storage (3.5~ and 8.0~ fold the initial value, respectively) ($P < 0.05$),

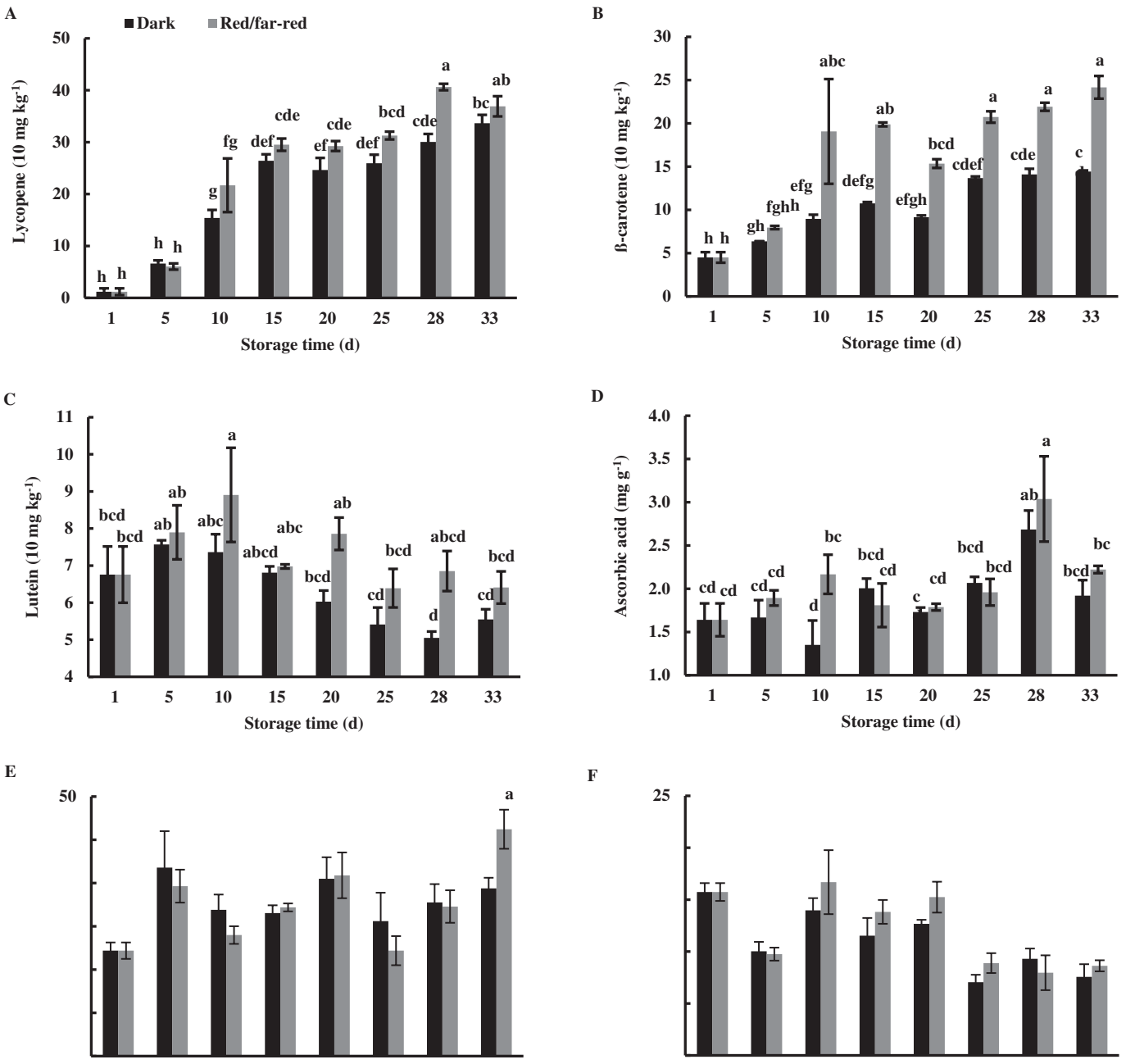

Fig. 2. Effects of red/far-red light irradiation on the content of lycopene (A), β-carotene (B), lutein (C), ascorbic acid (D), total phenolics (E) and flavonoids (F) in cherry tomatoes during 33 days' storage. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

whereas in control, the highest levels were shown at the end of storage (Fig. 4 A, B). PHYA showed higher transcription level in red/far-red light treatment than in control during 28 days of storage, but a significant difference was only found at day 10 ($P < 0.05$) (Fig. 4 A). Although PHYB expression level was higher in red/far-red light treatment than in control at day 5, 10 and 20, no obvious difference was found between treated and untreated fruits (Fig. 4 B).

The expression of light signalling component HY5 in red/far-red light treatment showed a similar trend as PSY during postharvest storage, registering three peaks of expression at day 10, 20 and 28, whereas the expression in control diminished from day 5. HY5 level was higher in treated fruits than in untreated fruits during storage, with significant difference being noticed at day 10 and 20 ($P < 0.05$) (Fig. 4 C). The transcription level of another light signalling component PIF3 was higher in red/far-red light treatment than in control during the first 28 days, but no obvious difference was found during storage (Fig. 4 C, D).

The expression of ethylene biosynthesis gene ACS2 in red/far-red light treatment increased 19.2~ fold the initial value after 10 days of storage, and 29.3~ fold after 20 days ($P < 0.05$),

and the transcription was higher than that in control during the first 25 days, although no obvious difference was found. The transcription trend of ACS2 in red/far-red light treatment was quite similar with that of HY5 during the first 25 days of storage (Fig. 4 E).

The expression of ripening transcription factor gene RIN in red/far-red light treatment showed a similar trend as PSY and ZDS during postharvest storage, registering three peaks of expression at day 10, 20 and 28. In these days, the accumulation of RIN mRNA was 13.7~, 2.0~ and 1.2~ fold greater than that in control. Expression of RIN was higher in treated fruits than in untreated fruits during 28 days of storage, with a significant difference being noticed at day 10 ($P < 0.05$) (Fig. 4 F).

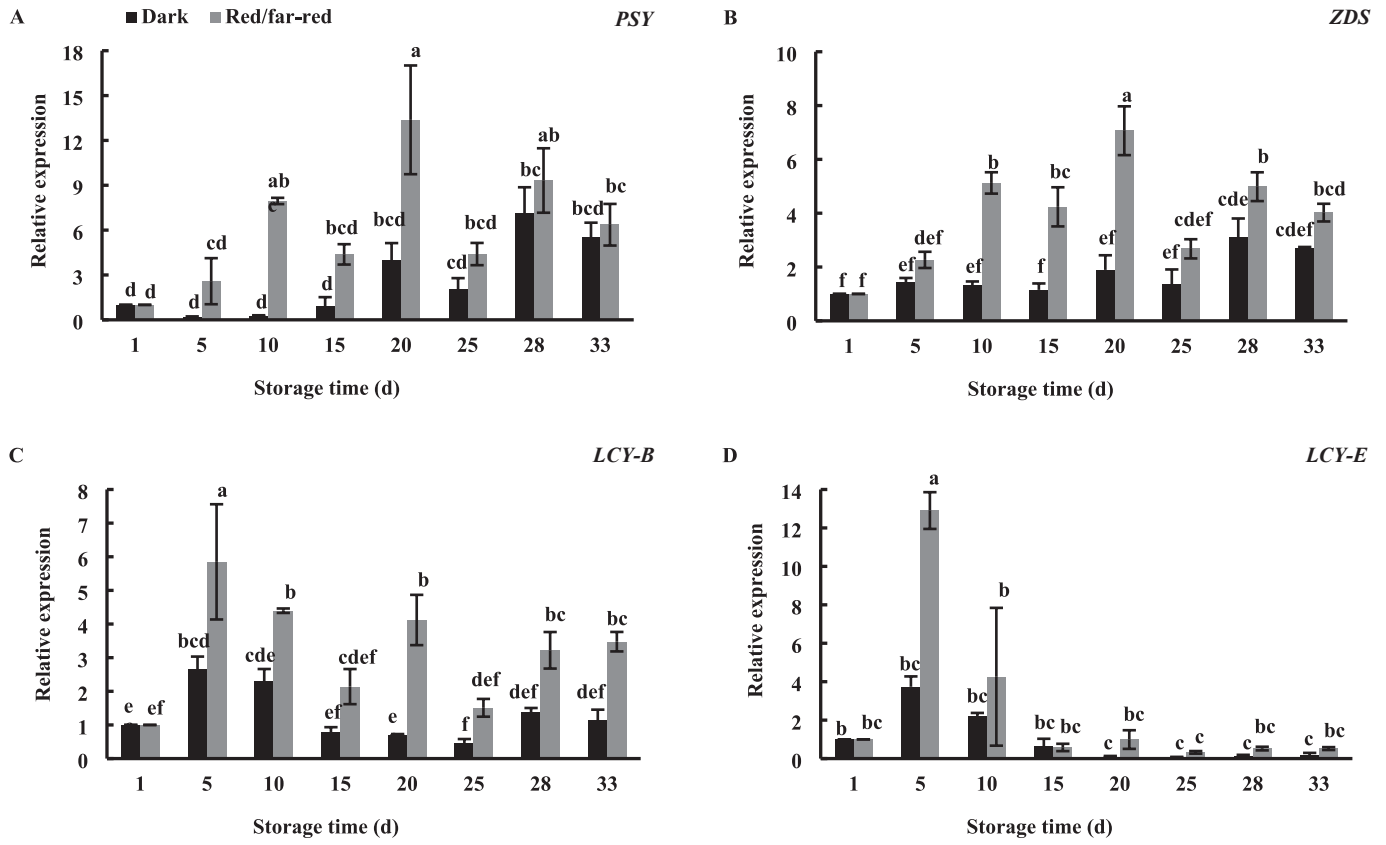

Fig. 3. Effects of red/far-red light on expression of carotenoid biosynthesis genes in cherry tomatoes during 33 days' storage. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

4. Discussion

Cherry tomato has been recognized as a model of climacteric fruit due to its wide consumption and distinct colour changes during ripening (Yokotani et al., 2004). In cherry tomatoes, fruit colour is one of the most important criteria that reflects the ripening process and contributes to marketing value (Liu et al., 2009; Panjai et al., 2019). Red/far-red light induced the ripening process of cherry tomatoes by showing higher red index and quicker colour turning than control during storage. The same results were found by Xie et al. (2019) and Liu et al. (2009), who reported that red light induced colour changing and ripening in tomatoes. Weight loss can lead to shrivelled appearance of fruit skin and shortened shelf life (Hasan et al., 2017). In this study, a steady weight loss caused by water vapour accumulation was shown in both treated and untreated fruits during storage, but the loss of weight was inhibited by red/far-red light irradiation. This could be explained by the evidence that red light aids in moisture retention in fruits (Hasan et al., 2017).

In ripe red tomatoes, the major carotenoids are lycopene, ß-carotene and lutein (Schofield and Paliyath, 2005). The content of lycopene and ß-carotene increased significantly during storage, while lutein content increased during the first 10 days, but decreased afterwards, which were in accordance with the results found by Ballester et al. (2010) and Xie et al. (2019). Red/far-red light induced the accumulation of lycopene and ß-carotene when compared to darkness. The similar results were found by Xie et al. (2019) and Schofield and Paliyath (2005), who reported that the content of lycopene and ß-carotene was higher in red light treated tomato fruits than in darkness.

Ascorbic acid content in cherry tomatoes varies according to fruit ripen stages, storage time and environmental conditions (Raiola et al., 2014). In this study, results showed that ascorbic acid content accumulated during the first 28 days' storage and then decreased. Elwan et al. (2015) reported similar result that ascorbic acid content could be reduced by prolongation of storage period in sugar snap peas. The reduction may be caused by oxidizing enzymes, such as ascorbic acid oxidase, which convert ascorbic acid to dehydroascorbic acid (Elwan et al., 2015). Red/far-red light treatment showed higher ascorbic acid content than control at most days analysed during storage, although no significant difference was found except day 10. We are not aware any research highlighting this finding.

Total phenolics content in red/far-red light treatment increased significantly after 20 days of storage, which was consistent with the finding of Panjai et al. (2019), who reported that total phenolics content of tomatoes exposed to red light increased greatly after 20 days of storage. Red/far-red light treatment showed higher content of flavonoids than control at most days analysed during storage, although no significant difference was found. Panjai et al. (2019) reported a similar result that red light treated tomatoes had higher content of flavonoids than untreated tomatoes.

The biosynthesis of carotenoid is a complex process that regulated by light, hormones, transcriptional and post-translational mechanisms (Alba et al., 2000; Schofield and Paliyath, 2005). The mechanism of red/far-red light in regulating carotenoid biosynthesis has been investigated by studying the transcriptional changes of genes involved in carotenoid synthesis, red/far-red light absorbance and mediation, and ripening.

PSY and ZDS are the rate-limiting enzymes in lycopene synthesis pathway (Xie et al., 2019). The expression of PSY and ZDS in red/far-red light treatment showed a similar trend during storage, and the expression of them was higher in light treatment than in control, resulting in higher lycopene content in red/far-red light treatment during storage. Xie et al. (2019) found similar results that expression of PSY and ZDS in tomato fruits was induced by red light when compared to darkness. Lycopene can be converted to ß-carotene by the action of LCY-b or to the precursor of lutein by the action of LCY-e (Pandurangaiah et al., 2016; Xie et al., 2019). In this study, red/far-red light treatment showed higher

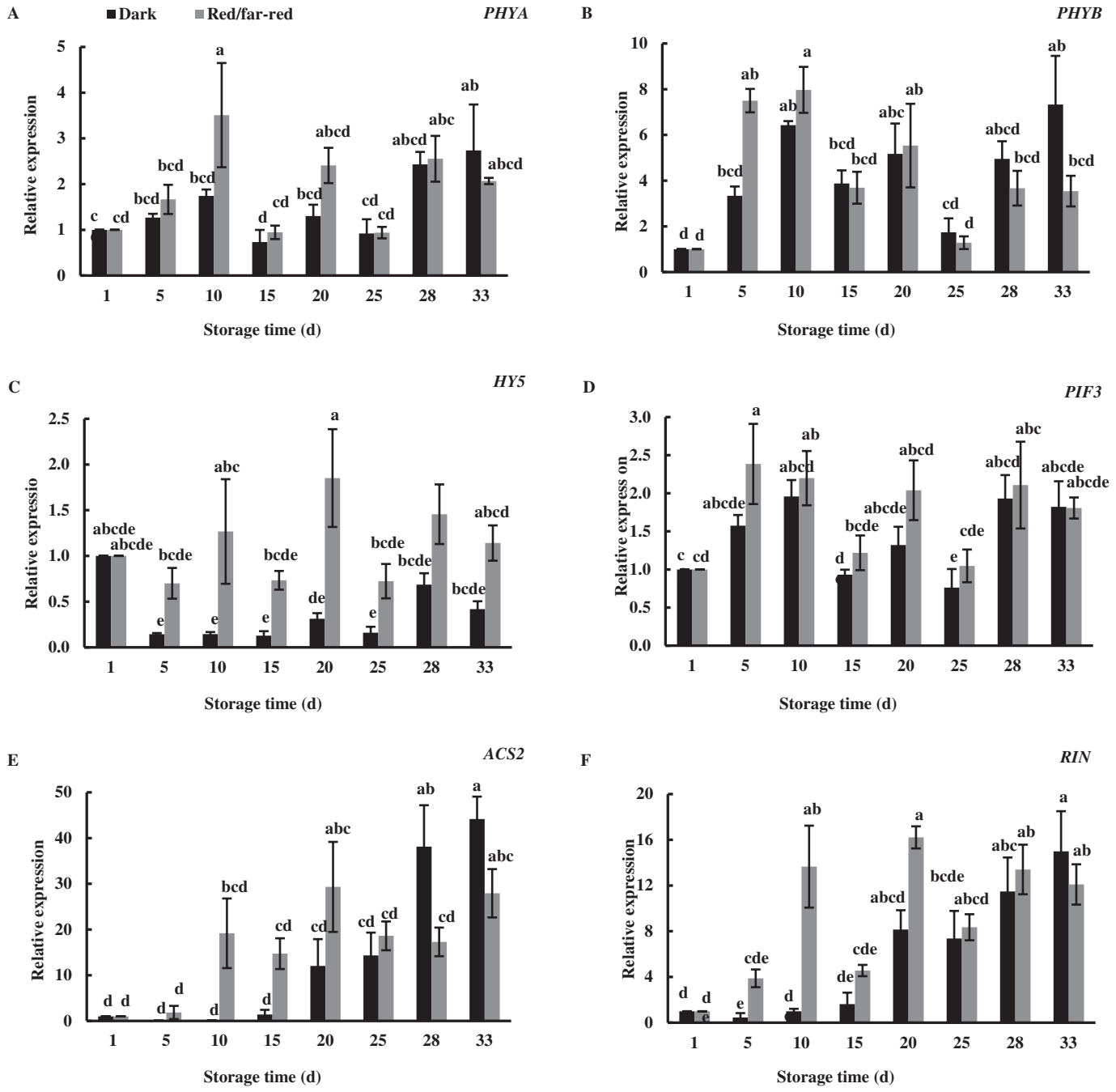

Fig. 4. Effects of red/far-red light on expression of light receptor genes (PHYA (A) and PHYB (B)), light interaction transcription factor genes (HY5 (C) and PIF3 (D)), ethylene biosynthesis gene (ACS2 (E)), and ripening transcription factor gene (RIN (F)) in cherry tomatoes during 33 days' storage. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

expression of LCY-b gene than control during storage, leading to significant higher ß-carotene content. The transcription of LCY-e was increased at day 5, and then decreased till the end of storage, leading to a similar change of lutein content.

The production of PSY is the first and key-limiting step in lycopene synthesis, and regulation of PSY expression is crucial to control biosynthesis of carotenoid (Schofield and Paliyath, 2005; Xie et al., 2019). PHYB and PHYA can perceive red and far-red light, respectively, and transduce light signals to downstream signalling intermediates, which control the expression of target genes, including PSY in tomato fruits (Fig. 5) (Li et al., 2011; Toledo-Ortiz et al., 2010). In this study, no obvious differences in PHYA and PHYB expression level were found between red/far-red light treatment and control, which means that the transcription levels of them are less important in the phytochrome-mediated light signalling pathway that modulate carotenoid biosynthesis. Phytochromes are synthesized in their inactive Pr form (red light-absorbing from), and under red light, the Pr from can be converted to the active Pfr form (far-red light-absorbing from) (Li et al., 2011; Quail, 1997). The Pfr form can be converted back to the Pr from by much faster upon absorption of far-red light (Li et al., 2011; Quail, 1997). Under the irradiation of red/far-red light in this study, both active Pfr form and inactive Pr form might be existing, and the photoactivated phytochromes can then interact with downstream transcription factors.

HY5 and PIF1 have been reported to interact with phytochromes, and they are also direct regulators of PSY expression which can bind to the same G-box motifs in PSY promoter to regulate the biosynthesis of

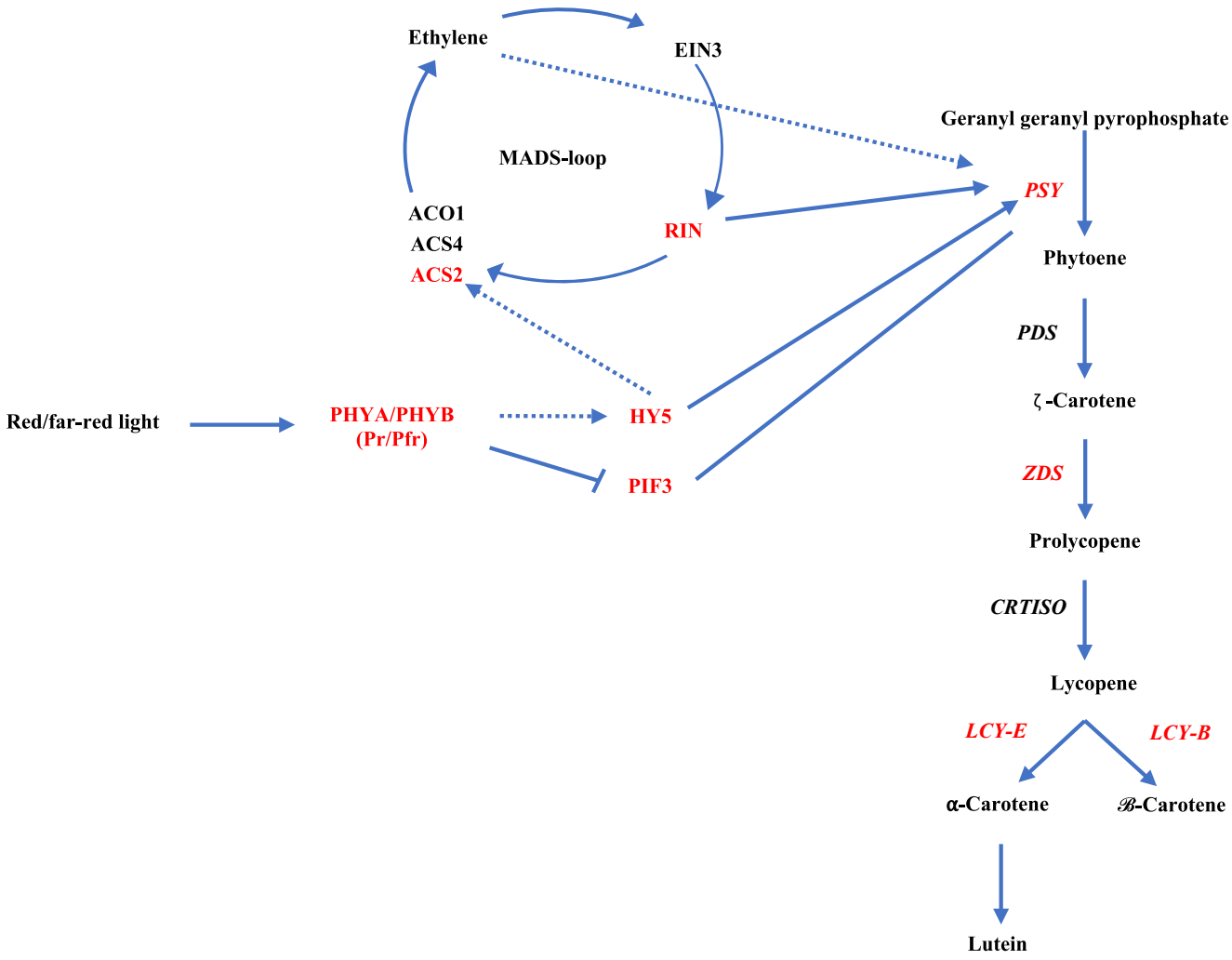

Fig. 5. Hypothetical model of red/far-red light mediated carotenoid biosynthesis. Under red/far-red light irradiation, photoactivated PHYA and PHYB interacted directly with negative transcription factor PIF3, resulting in the degradation of PIF3, and also induced the expression of HY5. Elevated HY5 could either bind directly to PSY and promote its expression or elevate expression of ACS2 to induce RIN through MADS-loop. RIN could then induce the expression of PSY to promote biosynthesis of lycopene. Arrow, positive regulation; bar, negative regulation; solid line, direct regulation; dotted line, indirect regulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lycopene (Bou-Torrent et al., 2015; Toledo-Ortiz et al., 2010; Toledo-Ortiz et al., 2014; Xie et al., 2019). In this study, red/far-red light induced the expression of HY5 during storage when compared to control, whereas the expression of PIF3 was not significantly affected by red/far-red light, which suggests that PHYA and PHYB mainly interact with PIF3 at the post-translational level. Altogether, we can conclude that photo-activated phytochromes induced the expression of HY5 and the degradation of PIF3, and the increase of positive regulator HY5 and the decrease of negative regulator PIF3 induced the expression of PSY (Fig. 5).

As a climacteric fruit, cherry tomato's ripening is a complex process that causes great changes in physiological and biochemical properties, including accumulation of lycopene (Dhakal and Baek, 2014; Ioannidi et al., 2009). The hormone ethylene and RIN are key components in ripening model of cherry tomato, and a small amount of ethylene can induce fruit ripening and the expression of downstream ripening genes, including PSY (Gao et al., 2019). It has been reported that down-regulation of ethylene biosynthesis gene ACS or ACO caused to reduced lycopene content (Yokotani et al., 2004). RIN has also been reported to interact the promoters of genes belonging to lycopene synthesis (Su et al., 2015; Martel et al., 2011). Our results agree with that, because the expression of both ACS2 and RIN was induced by red/far-red light irradiation during storage. The transcription of RIN showed a similar trend as that of PSY in treated fruits during storage, which indicated the possibility that RIN directly interacted with PSY. This could be supported by the findings of Gao et al. (2019) and Su et al. (2015), who reported that RIN could directly bind to the promoter of ripening genes of tomato, including PSY. From all of these, we concluded that MADS-loop might be one way that ethylene and RIN got involved in the regulation of carotenoid biosynthesis, which could be supported by the finding of Gao et al. (2019). Furthermore, Ito et al. (2017) demonstrated RIN independent induction of ACS2 and PSY expression in RIN-knockout mutation tomato, which meant that there might be another way that ethylene interacted with PSY. However, further research is needed to find out the interaction.

It has been reported that phytochrome-regulated carotenoid synthesis is related to ethylene production in red light irradiation (Alba et al., 2000). This meant that there might be connection between phytochrome-mediated light signalling pathway and ethylene in MADS-loop in the regulation of carotenoid biosynthesis. Acting downstream of PHYA and PHYB, transcription factor HY5 directly or indirectly regulates a large number of genes (Li et al., 2011). Ge et al. (2020) reported that HY5 activated the expression of ACS2/6/11 to induce the ethylene production under UV-B irradiation. In this study, the expression pattern

of ACS2 in red/far-red light treatment was similar with that of HY5 during storage. All these results indicated that HY5 might interact with ACS2 to regulate the biosynthesis of ethylene. Overall, we concluded that red/far-red light induced expression of HY5 through phytochrome-mediated signalling pathway. Elevated HY5 could either directly induce PSY or elevate expression of ACS2 to induce RIN through MADS-loop, and RIN then induced the expression of PSY to promote biosynthesis of lycopene.

5. Conclusion

In this study, red/far-red light irradiation preserved postharvest quality of cherry tomatoes by inhibiting weight loss and inducing the synthesis of lycopene and ß-carotene. The content of lutein, ascorbic acid and flavonoids was higher in red/far-red light treatment than in control at all days analysed during storage, but no significant difference was found at most days. Gene expression analysis showed that red/far-red light induced the synthesis of lycopene by elevating the expression of PSY and ZDS in tomato fruits, which was modulated by phytochrome-mediated signalling pathway and MADS-loop. Under red/far-red light irradiation, photoactivated PHYA and PHYB induced the expression of HY5. Elevated HY5 could either directly bind to PSY to induce its expression or promote the expression of ACS2 to induce RIN through MADS-loop, leading to increased expression of PSY during the storage time. Overall results showed that red/far-red light can be used as an effective method to preserve nutritional quality of cherry tomatoes and improve carotenoids content by regulating relative gene expression.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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