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# Preharvest and postharvest UV radiation affected flavonoid metabolism and antioxidant capacity differently in developing blueberries (*Vaccinium corymbosum* L.)

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ABSTRACT

Flavonoids can protect plants against UV but the mechanism by which specific flavonoids during fruit development is unclear, especially in blueberries on living plants. We analyzed the gene expression and metabolite profiles of flavonols, proanthocyanidins (PAs), and anthocyanins under preharvest UV-B/-C and postharvest UV-A/-B/-C irradiation in developing blueberries. Both pre- and postharvest UV irradiation significantly increased flavonol accumulation during early fruit development, while increased anthocyanin and PA contents during late fruit development. However, PAs decreased during postharvest but increased during preharvest UV irradiation in green fruit. The antioxidant capacity increased by postharvest UV irradiation, while hardly affected by preharvest UV irradiation. Overall, the gene expression changes paralleled the flavonoid contents after UV irradiation. Notably, *VcMYBPA1* was closely related with *VcLAR* and *VcANR* under pre- and postharvest UV irradiation, which could relate to PA biosynthesis. During natural fruit maturation and UV conditions, the elevated PA content exhibited higher potential antioxidant activity. Our results show that UV resistance is greater in living plants than detached fruits, the former showing a systemic and moderate response and the latter a non-systemic but strong response. These results might contribute to the development of pre- and postharvest technologies to promote healthier fruit consumption.

## 1. Introduction

Recently, blueberries (*Vaccinium corymbosum*) have gained increasing attention, mainly due to increased public awareness of their nutritional value and the recognition of their health care function (Yousef et al., 2013). Blueberries are known to have high antioxidant function to a variety of oxygen free radicals, mainly because they are rich in polyphenolics; in particular, flavonoids which play the crucial role (Granato, Santos, Maciel, & Nunes, 2016; Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015). Consumption of blueberries may ameliorate an array of human ailments (e.g. neurodegenerative, cardiovascular, and inflammation disorders, and various cancers) and enhance cognitive and behavioral functions (e.g. improve memory, reverse behavioral deficits related to stroke and aging in older adults) (Manganaris, Goulas, Vicente, & Terry, 2014; Rodriguez-Mateos, Heiss, Borges, & Crozier, 2014; Shukitt-Hale et al., 2015).

In plants, flavonoids are the products of phenylpropanoid and flavonoid pathways. Flavonols, proanthocyanidins (PAs) and anthocyanins are the major flavonoid substances found in blueberries. They share common steps in the upstream pathway, which involves some key structural genes and forms dihydroflavonol. This intermediate is subsequently turned into flavonol by flavonol synthase (FLS) or convert to eucoanthocyanidin via the dihydroflavonol reductase (DFR). Leucoanthocyanidin can be used for PA biosynthesis by

*Abbreviations:* UV, ultraviolet; PAs, proanthocyanidins; TFs, transcription factors; CHS, chalcone synthase; F3H, flavanone 3-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; ANS, anthocyanidin synthase; UFGT, UDP-Glc: flavonoid-3-O-glycosyltransferases; *VcGAPDH*, Glyceraldehyde 3-phosphate dehydrogenase; qRT-PCR, quantitative real-time reverse transcription; HPLC, high-performance liquid chromatograph; WAFB, week after flower bloom; DBH, days before harvest; DPPH, 2,2-Di-(4-*tert*-octylphenyl)-1-picrylhydrazyl; SOD, superoxide dismutase; NBT, nitrotetrazolium blue chloride; MDA, malondialdehyde; TCA, trichloroacetic acid; FW, fresh weight

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leucoanthocyanidin reductase (LAR), producing catechin-type flavan-3-ols, or be used to form anthocyanidin by anthocyanidin synthase (ANS), then turned into PA biosynthetic branch by anthocyanidin reductase (ANR), producing epicatechin-type flavan-3-ols, or glycosylated by UDP-Glc: flavonoid-3-*O*-glycosyltransferases (UFGT) to form anthocyanin, leading to the anthocyanin biosynthetic branch. These biosynthetic enzymes are regulated by transcription factors (TFs), such as MYB (Deluc et al., 2006; Zifkin et al., 2011). Grapevine (*Vitis vinifera*) has been used most extensively to study on MYB in fruit about the flavonoid biosynthesis. The *VvMYBF1* was shown as a specific flavonol synthesis regulator (Czemmel et al., 2009), *VvMYBPA1* and *VvMYBPA2* specifically regulate proanthocyanidin synthesis (Terrier et al., 2009), and *VvMYBA1* controls anthocyanin accumulation in grapevine (Kobayashi, Ishimaru, Hiraoka, & Honda, 2002). However, knowledge of the regulation of flavonoid synthesis in blueberries by MYB TFs is currently limited. *VcMYBPA1* might encodes an R2R3-MYB TF of PA synthesis (Zifkin et al., 2011). Additionally, another R2R3-MYB TF VcMYB9, identified from transcriptome data of blueberry fruit, may be involved in anthocyanin biosynthesis (Yang et al., 2018).

The effect of UV radiation promoted flavonoid accumulation in pear (Zhang, Qian, Yu, & Teng, 2013), strawberries (Xie et al., 2015), apple (Ubi et al., 2006), grape (Zhang et al., 2012), and blueberry (Nguyen, Kim, Yoo, Lim, & Lee, 2014) etc, indicated that the different effect dependent on not only the plant species and development stage, but also the UV type and the irradiation dose (UV-C, < 280 nm; UV-B, 280–320 nm; and UV-A, 320–400 nm) (Inostroza-Blancheteau et al., 2014; Xie et al., 2015; Yang et al., 2018), while the mechanism remain unclear. Moreover, UV irradiation can increase the antioxidant capacity and enzyme activities, delay senescence, and reduce the decay of blueberry fruit postharvest, which had certain correlation with the increasing flavonoid accumulation (Nguyen et al., 2014; Wang, Chen, & Wang, 2009). Similarly, the activation of antioxidant defense systems and secondary metabolism pathways seems to play a critical role in grapevine responses against UV-B radiation (Carbonell-Bejerano, 2014). Furthermore, flavonoid contents and the related gens expression, as well as the TFs are sensitive to the UV irradiation. A transcriptomic analysis revealed that the light-induced regulatory gene *VvMYBF1* was highly correlated with *VvFLS1* expression and flavonol biosynthesis after UV irradiation in *V. vinifera* berries (Czemmel et al., 2017). Mellway, Tran, Prouse, Campbell, and Constabel (2009) found that the increase of PA production and the up-regulated expression of PA biosynthetic genes in poplar responded to UV-B irradiation were related to the activation of *MYB134* and *MYB115*. The significant increase in flavan-3-ol (PA monomer) production by UV-A/B/C radiation in grapes could be involved in the up-regulation of the structural genes and TFs in PA biosynthetic branch (Zhang, Che, Pan, Li, & Duan, 2013). Zhou showed that *CHS*, *F3H*, *DFR* and *ANS* expression were upregulated by UV-A in swollen hypocotyls of turnip, accompanied by increased anthocyanin contents (Zhou et al., 2007). Recently, Nguyen, Lim, Lee, and Lee (2017) found a close correlation between *VcR2R3MYB* and *VcF3H* or *VcUFGT*, which were correlated with the anthocyanin synthesis in harvested blueberry peels induced by UV-B irradiation. Our previous research showed that *VcMYB* had a positive correlation with the anthocyanin biosynthesis, and coincided with downstream biosynthetic genes in response to UV radiation. However, the mechanisms between preharvest and postharvest UV irradiation may differ (Yang et al., 2018). Thus, identifying genes activated by UV is a useful way to elucidate the underlying mechanisms of flavonoid biosynthesis.

Notably, compared with postharvest UV irradiation focused on preventing decay and improving fruit quality in picked-up commodities, preharvest UV application has been paid much less attention. The mechanism underlying the antioxidant capacity and flavonoid metabolism of growing plants in response to UV is not well understood. UV-C irradiation delayed the ripening and inhibited *Penicillium digitatum* growth of tomatoes on the vine (Obande, Tucker, & Shama, 2011). Xie et al. (2015) found that the contents of kaempferol-3-glucuronide and ellagic acid were increased significantly in strawberry fruits harvested from the plants treated by UV-C, while the antioxidant capacity were not significantly effected. Zhang et al. (2013) suggested 10 days before harvest (DBH) fruit had a higher anthocyanin content than other stages after UV irradiation in red Chinese sand pear. Different types UV radiation up-regulated the *VcUFGT* and *VcANS* and anthocyanin accumulation in blueberries were shown in our previous report (Yang et al., 2018), which is highly dependent on the developmental stages of the fruits. Questions remain, however, such as besides anthocyanin, how is the other flavonoids (flavonol, PA) and biosynthetic pathway genes affected and regulated by preharvest and postharvest UV treatment in developing blueberries? And which specific flavonoid protect fruits against UV radiation during different fruit development stages? Since strong correlation between polyphenols and antioxidant activity (Kai, Fuse, Kunitake, Morishita, & Matsuno, 2014; Wang et al., 2009), while which individual flavonoid exhibited higher antioxidant activity in fruits treated by UV irradiation? Usually, antioxidant activities are highly dependent on species and cultivars, as well as the environment, preharvest practices, and postharvest storage. Does preharvest UV irradiation significantly affect the antioxidant capacity of blueberries like postharvest UV treatment? If so, what is about the underlying mechanism? All of these question are remained to be resolved.

Therefore, the present study examined the effects and mechanisms of preharvest UV-B and -C irradiation and postharvest UV-A, -B and -C irradiation on developing blueberry fruit by analyzing gene expression and metabolic profiles for three major flavonoids (flavonols, PAs and anthocyanins) as well as antioxidant capacities (DPPH, SOD and MDA). We aimed to identify the crucial flavonoid compounds and related synthesis genes in the response to UV treatment at different stages. The results provide a substantial insight into the potential response mechanisms on the flavonoid biosynthesis of detached berries and growing plants after UV irradiation in developing blueberry fruit.

## 2. Materials and methods

### 2.1. Plant materials and treatment

Half-highbush blueberry (*V. corymbosum* L. cv. Northland) plants were used in this study. The potted blueberry plants were planted in an experimental nursery and three experiments were carried out, as reported in our previous research (Yang et al., 2018). Berry development was divided into eight stages from 1 week after flower bloom (WAFB) (S1) to 8 WAFB (S8) which classified into three periods, S1–S4 (green), S5–S7 (turning, from green to purple and pink), and S8 (blue), according to the growth characteristics (Fig. 1A).

In experiment 1, the characteristics of the flavonoid synthesis pathway in field-growing blueberries were analyzed during fruit development with two repetitions in 2014 and 2015. About 0.30 kg berries were sampled randomly from multiple blueberry plants and evenly divided into three parts as three replicates once a week from S1 to S8 (Fig. 1A).

In experiment 2, the effects of postharvest UV-A/-B/-C treatment on blueberry fruit were investigated in 2014 according to our previous report (Yang et al., 2018). Approximately 2.0 kg fruit were harvested randomly from multiple blueberry plants at S3 to S8 (Fig. 1A), and taken to the laboratory immediately in an ice box. The harvested berries were evenly divided into four groups for treatment with 2.76 kJ $m^{-2}$ UV-A, B and C irradiation (10 min) or without UV irradiation in the dark at 25 °C. Three replicates for every treatment. After being incubated at room temperature for 24 h, the testing berries were frozen and stored at −80 °C.

The experiment 3 was conducted in 2015 aiming to investigate the preharvest UV on blueberries using the treatment devices showed in our previous report (Yang et al., 2018). UV-B and UV-C were choose considering the results of experiment 2, in which they had more obvious effect on inducing the individual flavonoid synthesis than UV-A.

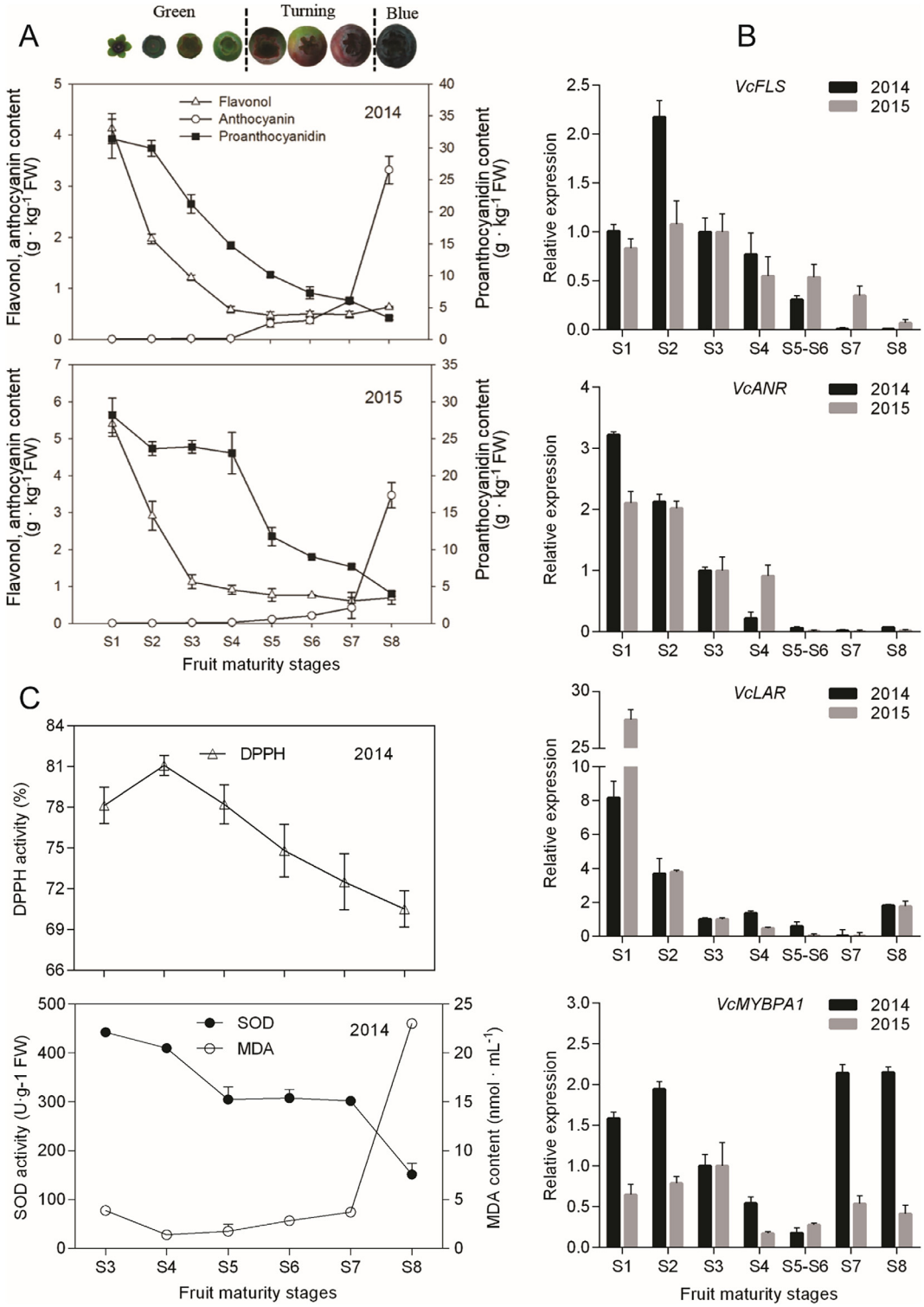


**Fig. 1.** Flavonoid metabolism and antioxidant capacity in developing blueberry fruit under natural condition. (A) Flavonoid composition and contents in blueberry during two years (B) Expression levels of flavonoid biosynthesis genes in blueberry in 2014 and 2015 (C) antioxidant activities of blueberry in 2014. The error bars indicate the standard deviation of three replicates, data are the mean ± SE of three replications. Note: samples of anthocyanin content in Fig. 1A is the same with our previous research (Yang et al., 2018), but are re-determined in this study.

Besides, there already have much UV-A (only a small proportion UV-B and no UV-C reaches the earth surface) radiation from sunlight in field growing condition. Potted blueberries were irradiated by UV (lasting 15 min for the dose of 4.14 kJ $m^{-2}$, this dose was adopted by preliminary trials (Fig. S2) and had no obvious impact on the health and growth of plant) in the morning once a week from S1 to S8 for 8 times. Each treatment was carried out in triplicate with 20 blueberry plants per replicate. Approximately 0.10 kg berries were sampled per replicate 6 h after UV treatment.

### 2.2. Extraction and assay of individual flavonoids.

Total flavonols contents were estimated using HPLC according to Downey, Harvey, and Robinson (2003) with some modifications. The Agilent 1260 HPLC system were used, equipped with the Zorbax SB C-18 column (4.6 × 250 mm), DAD detector (Agilent G1314D). The mobile phase were solvent A (0.2% acetic acid) and solvent B (methanol) (gradient of B mobile phase: 0 min, 24%; 10 min, 28%; 10.1 min, 100%; 13 min, 100%; 13.1 min 24%). The flow rate was 3 mL/min. The concentration of flavonol glycosides in each sample (25 μL injection) were determined by the integrated absorbance at 353 nm, and expressed as quercetin-3-O-glucoside equivalents.

The PAs were extracted and measured according to Pang, Peel, Wright, Wang, and Dixon (2007) with some modification. The acetone extracting solution (70% acetone: 29.5% $H_2O$: 0.5% acetic acid) were used with the solid-liquid ratio of 1:5 (g:mL). Pooled supernatants of thrice extraction were then extracted with 4 mL of chloroform, and the aqueous supernatant re-extracted with 1 mL of hexane. Finally, the lower layer of 1 mL liquid is left as the PAs extract. PAs were assayed in butanol-HCl reaction with catechin standard. In brief, 50 μL samples were mixed with 950 μL of butanol-HCl reagent (95% butanol:5% conc. HCl) in cuvette, which absorption were determined at 550 nm by ultraviolet spectrophotometer, and denoted as OD1. After being boiled for 1 h and cooling to 25 °C, the samples were treated as above, and the absorption of supernatants were determined at 550 nm and the OD denoted as OD2. The PA concentrations were valued as OD2-OD1.

Anthocyanins determined by HPLC-DAD analysis according to our previous research (Yang et al., 2018). 25 μL supernatant extracted by HCl-methanol (pH 2.5) were injected onto a Zorbax SB C-18 column (4.6 × 250 mm) of Agilent 1260 HPLC system. The elution system consisted of mobile phase (A) of 5% formic acid water (v/v) and mobile phase (B) of acetonitrile with the 35 °C column temperature and 1 mL·min$^{-1}$ flow rate. The gradient of solvent B was: 0 min, 10%; 10 min, 20%; 15 min, 20%; 25 min, 50%; 30 min, 60%. The anthocyanin were determined at 535 nm and expressed as cyanidin-3-*O*-glucoside equivalent. Each sample was performed in triplicate.

### 2.3. Determination of antioxidant capacity

The scavenging capacity of the 2,2-Di-(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) radicals was analyzed by a spectrophotometric method according to Nguyen et al. (2014) 24 mg of DPPH was dissolved in 100 mL of 80% ethanol (v/v). 10 mL DPPH solution mixing with 45 mL of 80% ethanol (v/v) was used as control solution. 0.5 g frozen blueberry powder was homogenized with 1.5 mL 80% acetone (v/v), and 0.15 mL of this homogenate was reacted with 2.85 mL DPPH solution for 40 min in darkness. The absorbance was measured at 515 nm.

The superoxide dismutase (SOD) activities were determined according to Jiang, Jahangir, Jiang, Lu, and Ying (2010) with modifications. Blueberry tissues (2.0 g) were homogenized with K-phosphate buffer (6 mL, 50 mM, pH 7.3) containing EDTA (1 mM) and DTT (2 mM). After centrifugation (5000*g*) for 15 min at 4 °C, the supernatant was used for SOD assays, which activity was assayed at 560 nm by its ability to inhibit photochemical reduction of nitrotetrazolium blue chloride (NBT). The amount of enzyme producing a 50% inhibition of NBT reduction among the assay was defined as one unit of SOD (U).

The content of Malondialdehyde (MDA) was measured according to Dhindsa, Plumbdhindsa, and ThorpeH (1981). After being homogenized (4.5 mL of 10% trichloroacetic acid (TCA)) and centrifuged, 1.0 mL supernatant was mixed with 1.0 mL TCA (0.6%), and then heated for 30 min at 95 °C and quickly cooled on ice to room temperature. After centrifugation again, the absorbency of supernatant was measured at 532 nm.

### 2.4. Total RNA isolation, cDNA synthesis, and analysis of transcript level by RT-PCR.

According to our previous report (Yang et al., 2018), the total RNA isolation and cDNA synthesis in blueberry were carried out. The flavonoid biosynthetic genes of *VcFLS*, *VcLAR*, *VcANR* and the TF *VcMYBPA1*were analysis by qRT-PCR. 20 μL reaction mix contained 12 μL SYBR, 0.4 μL forward/reverse specific primer and 2 μL cDNA template. *VcGAPDH* (Glyceraldehyde 3-phosphate dehydrogenase) was used as a house-keeping gene (Zifkin et al., 2011). Amplifx 1.4.5 software was used for the design the primers of *VcFLS*, the primer sequences of flavonoid biosynthetic genes as shown in Table S1, were referred from Zifkin et al. (2011). Each sample was performed in triplicates.

### 2.5. Statistical analysis

Completely randomized design were used in triplicate both in measurements and analysis in this study. The means expressed by standard errors. SPSS 13.0 (SPSS Inc., USA) was used for statistical evaluation and the Duncan test was used for significance of differences ($p < 0.05$). The figures were generated using Graph Pad Prism 6 (version 6.0.2), Sigma Plot 12.5 (SPSS, Chicago, IL, USA), Adobe Illustrator CS5 (version 15.0.0) and MeV (version 4.8.1).

## 3. Results

### 3.1. Flavonoid biosynthesis in blueberries under natural condition

#### 3.1.1. Quantification of flavonoid composition

Flavonol, PA, and anthocyanin profiles were assayed at each development stage in 2014 and 2015. In these two years, the accumulation patterns of the three individual flavonoids showed similarly tendencies (Fig. 1A). No significant differences were found during the fruit development stages (except the flavonol content in turning fruits (S5–S7) ($p = 0.013$)) (Table S2). The flavonol contents were high in the early development stage (S1) and quickly dropped with fruit development to the S4 stage, and then remained low from the S5 to S8 stages. During fruit development, the PA contents gradually declined with fruit maturation. Conversely, the total anthocyanin content was very low in green fruits (S1–S4), increased slowly in the turning stages (S5 to S7), and then quickly increased to a peak in mature fruit (S8). Flavonols and PAs were mainly biosynthesized at the early development stage, while anthocyanins mainly accumulated in the late fruit development stages, and these patterns were obviously developmental stage-dependent. Furthermore, the PA content was always higher than the flavonol and anthocyanin contents throughout fruit development (Fig. 1A); thus, PAs were the main flavonoids in 'Northland' blueberries.

#### 3.1.2. Expression of flavonoid pathway genes

To correlate end-product accumulation with gene expression profiles, *VcANS* and *VcUFGT* expression increase significantly as the fruit ripening, mostly paralleling the appearance of anthocyanins, increasing abundant only in late fruit development from S5 to S8. But *VcANS* gene, which encodes the enzyme required for both PA and anthocyanin biosynthesis, was also high during early development (S1–S3) (Yang et al., 2018). The other flavonoid biosynthesis-related genes *VcFLS*, *VcLAR*, *VcANR*, and the TF gene *VcMYBPA1* were analyzed throughout the development stages by qRT-PCR in this study (Fig. 1B). The results from two consecutive years (2014 and 2015) were used to ensure reliability. *VcFLS*, which encodes flavonol synthase, was highly expressed in the early development stages (S1–S3), and then gradually decreased to a minimum at the mature fruit stage (S8), which was consistent with the flavonol accumulation profile ($r = 0.621^{**}$) (Table S3, Fig. 1). The PA-specific *VcLAR* and *VcANR* genes also exhibited high expression during early fruit development, which was consistent with the change of PA

contents ($r = 0.590^{*}$, $r = 0.912^{**}$) (Table S3, Fig. 1). Additionally, *VcLAR* expression was slightly higher in mature fruit (S8). *VcMYBPA1* expression was also high in very young fruit, similarly to *VcLAR* and *VcANR*, supporting it functions in PA biosynthesis. While, following the minimal levels in the middle berry development stages (S4–S6), *VcMYBPA1* expression increased higher in mature fruit (S7–S8). In general, the accumulation patterns of the three individual flavonoids were consistent with the expression profiles of the flavonoid biosynthesis-related gene.

#### 3.1.3. Antioxidant capacity of blueberries

As shown in Fig. 1C, DPPH and SOD activities were high in green berries (S3–S4), and then gradually declined with fruit ripening, and were lowest in S8 fruits. However, the MDA content remained at a low level during fruit development, except in blue berries where it was high (S8). The changes in DPPH clearance ability, SOD activity and MDA content indicate the antioxidant capacity decreased gradually as the fruit matured, which was consistent with the accumulation of PAs and flavonols (Fig. 1).

### 3.2. Postharvest UV irradiation affects developing blueberry fruit

#### 3.2.1. Flavonoid contents and composition

In green blueberries (S3, S4), the accumulation of flavonols was significantly induced by UV-A/B/C radiation, with an increase of about two times in S4 fruit irradiated by UV-C (Fig. 2A). Conversely, PA accumulation was inhibited by all the UV light, especially in S3 fruit, which showed significant decreases of 9.3%, 25.2%, and 19.4% irradiated by UV-A, -B and -C, respectively. Anthocyanin accumulation showed no significant response to UV irradiation in green fruit, while significantly increased in S4 berries by UV-B and UV-C. In turning and mature berries (S6, S8), the flavonol accumulation was not sensitive to UV irradiation. While, PAs were induced by all the UV radiation, especially at S6, with increases of 30%, 50% and 56%, respectively. Anthocyanins were also significantly activated by these UV treatments, especially by UV-C, and were increased by 261.8% and 23.7% times in S6 and S8 fruit, respectively (Fig. 2A).

In general, postharvest UV radiation changed the contents and composition of flavonoids during the development of blueberry fruit, which was clearly developmental stage- and UV wavelength-dependent. In particular, UV-C had the most obvious effects.

#### 3.2.2. Expression of flavonoid biosynthesis-related genes

In S3 fruit, the structural gene *VcFLS* was dramatically up-regulated 20-fold by all the UV treatment (Fig. 2B), and the transcripts of *VcLAR*, *VcANR* and *VcMYBPA1* were significantly down-regulated. In S4 berries, the expression of *VcFLS* was strongly up-regulated. *VcLAR* was also significantly up-regulated by UV-B and UV-C, reaching 3-fold and 20-fold of their control levels, respectively. Conversely, *VcANR* was significantly suppressed by all the three UV treatments. *VcMYBPA1* was significantly upregulated by UV-B and UV-C while significantly down-regulated by UV-A. In turning berries (S6), the *VcFLS*, *VcLAR*, *VcANR* and *VcMYBPA1* were significantly upregulated by all the UV treatment. In blue fruits (S8), *VcLAR* was strongly activated by UV-B and -C, and *VcANR* and *VcMYBPA1* were strongly activated by UV-C but not by UV-A and UV-B, while the *VcFLS* was not activated by UV radiation (Fig. 2B).

Generally, the results showed that the expression of these genes depended on the blueberry development stage and the type of UV irradiation. Moreover, the changes of *VcFLS* expression were strongly positively correlated with those of flavonol biosynthesis under postharvest UV irradiation ($r = 0.822^{**}$). Similar response patterns were also found between *VcLAR* ($r = 0.611^{*}$)/*VcANR* ($r = 0.815^{**}$) and PA biosynthesis, and the TF *VcMYBPA1* had a close connection with *VcLAR* ($r = 0.740^{**}$) and *VcANR* ($r = 0.626^{*}$), which are related to PA biosynthesis (Table S4). In addition, significantly correlation were found between the expression of *VcANS* and *VcUFGT* and anthocyanin accumulation after postharvest UV radiation in our previous research (Yang et al., 2018).

#### 3.2.3. Antioxidant capacity

In S3 berries, the DPPH and SOD activities, and MDA content were not obviously changed by the three kinds of UV radiation (Fig. 2C). From the S4 to S6 stage, the MDA content still showed no significant activation effect, but the DPPH and SOD activities were activated by – all the UV irradiation, and were especially significantly elevated by UV-C. In S8 fruit, the MDA content was decreased 42%, 18% and 11% after UV-A, UV-B, and UV-C treatment, while the DPPH and SOD activities were significantly increased 33% and 54% by UV-C, respectively, but less increased by UV-B and UV-A. These results showed that the three kinds of UV irradiation obviously improved the antioxidant capacity of blueberries at different fruit development stages (except S3), especially at the S8 stage, and UV-C exerted the most obvious effect.

### 3.3. Preharvest UV irradiation affects developing blueberry fruit

#### 3.3.1. Flavonoid contents and composition

Preharvest UV-B and -C radiation increased the flavonol content in green berries (S3, S4), especially UV-B, which increased the flavonol content by 25.6% and 51.6% at S3 and S4, respectively (Fig. 3A). By contrast, the PA content was significantly increased by UV-B radiation, but not by UV-C. However, the anthocyanin content was not significantly changed by these two UV radiation. At the turning stage (S5–S7), the accumulation of flavonols and PAs decreased both by UV-B and UV-C treatment, but they were only significantly increased by UV-C in S7 fruit. Conversely, the anthocyanin content was greatly increased by UV-B and UV-C in the turning stage, and was significantly increased 57.4% and 113.4%, in S5–S6 fruit, respectively. In S8 fruit, the flavonol contents were not significantly changed by UV irradiation. However, the PA content was significantly increased 50.3%, and 23.1% by UV-B and UV-C irradiation, respectively. The anthocyanin content was also increased of 49.7% and 26.3% by UV-B and -C radiation, respectively. Taken together, these results showed the effects of preharvest UV-B and UV-C irradiation on the three kinds of flavonoids in blueberry were also obviously developmental stage-dependent (Fig. 3A).

#### 3.3.2. Expression of flavonoid biosynthesis-related genes

In green berries (S3, S4), *VcFLS* and *VcLAR* were significantly up-regulated by preharvest UV-B irradiation, especially in S4 fruit, with increases of about 15-fold and 5-fold, respectively (Fig. 3B). Additionally, UV-C irradiation significantly increased *VcFLS* expression in S3 and *VcLAR* expression in S4 fruit, but the increases were smaller than that by UV-B. However, *VcANR* and the TF *VcMYBPA1* was very significantly up-regulated by preharvest UV-C irradiation, which had stronger effects than UV-B. In turning berries (S5–S7), *VcFLS* was inhibited by UV-B and UV-C. However, UV irradiation had not shown substantive effect on *VcLAR* and *VcANR* expression, due to their very low expression, were, except that UV-C significantly upregulated *VcLAR* 5-fold in S7 berries. Similarly, *VcMYBPA1* expression level had no substantive increase by UV radiation in turning berries even though it had about a 1-fold increase under UV-B in S5–S6 fruit. In mature fruit (S8), *VcFLS* was significantly upregulated 1-fold by UV-C, but not by UV-B. *VcLAR* and *VcANR* was significantly upregulated by both UV-B and UV-C treatment. However, the TF *VcMYBPA1* was not activated by UV radiation (Fig. 3B).

Almost all of the flavonoid-related genes in this study showed transcriptional activation by UV-B and UV-C in different blueberry development stages. However, the flavonol-specific gene *VcFLS* and the PA-specific genes *VcLAR* and *VcANR* were mainly activated by UV in green fruit, which roughly paralleled the biosynthesis of the related compounds (Table S5). Besides, *VcANS* and *VcUFGT* (the anthocyanin-specific genes) were mainly activated by UV in turning to mature fruit

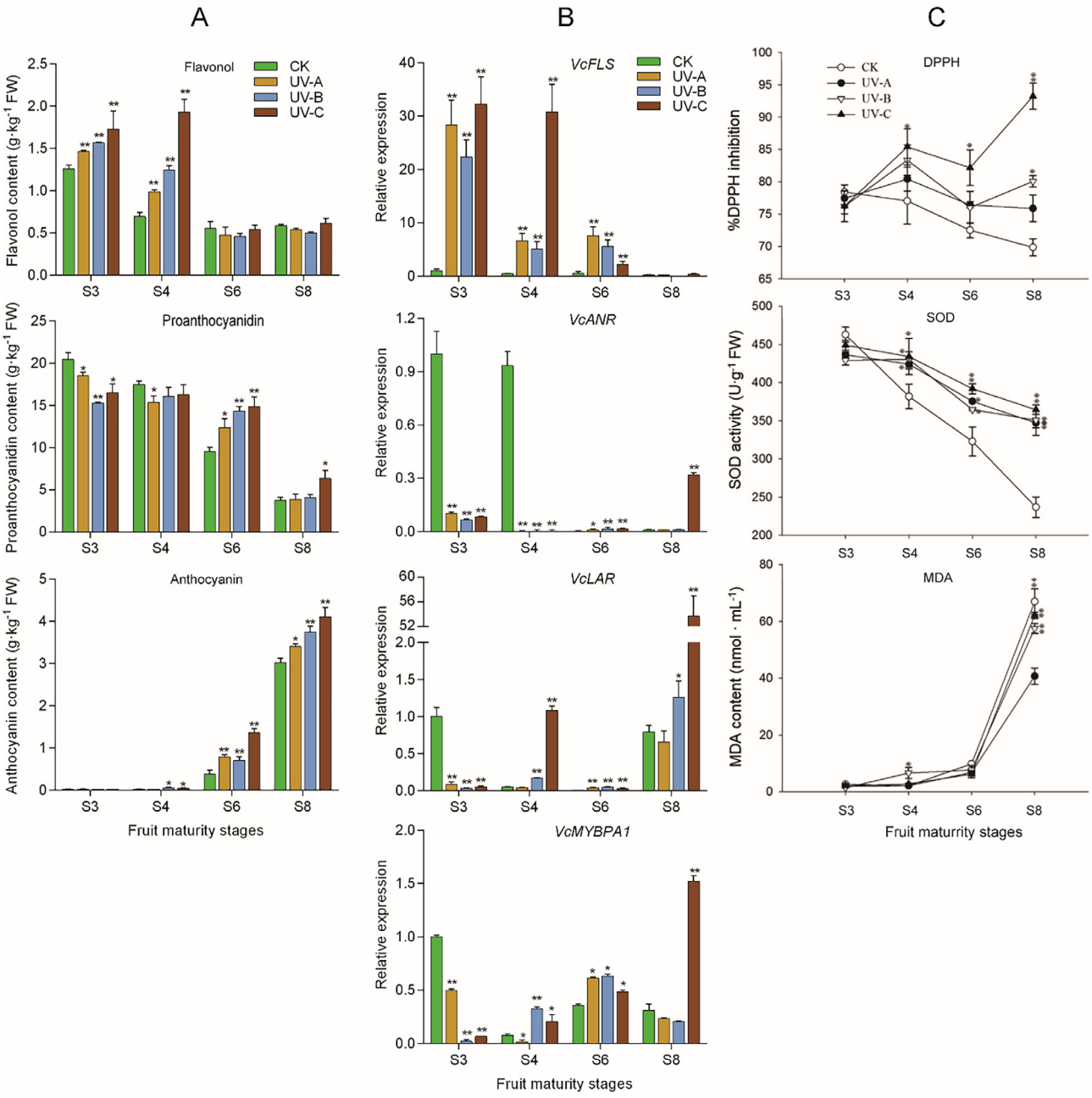


**Fig. 2.** Flavonoid metabolism and antioxidant capacity in developing blueberry treated by postharvest UV radiation (2.76 kJ $m^{-2}$). (A) Flavonoid composition and contents in blueberry (B) Flavonoid biosynthesis-related genes expression level in blueberry (C) Antioxidant activities of blueberry. The error bars indicate the standard deviation of three replicates, data are means ± SE (n = 3). *, $P < 0.05$; **, $P < 0.01$. Note: samples of anthocyanin content in Fig. 2A is the same with our previous research (Yang et al., 2018), but are re-determined in this study.

(Yang et al., 2018).

### 3.3.3. Antioxidant capacity

As shown in Fig. 3C, there had no obviously significant effect on the change of the fruit antioxidant capacity in different development stage of blueberry after preharvest UV-B and UV-C radiation. Among them, the increases of DPPH scavenging capacity and SOD activity induced by UV irradiation through the fruit development were not significantly. From the young fruit to middle development fruit, the MDA contents were hardly affected by UV irradiation. Till mature stage, the MDA contents were very significantly increased by 42% after the UV-C irradiation, while only increased by 5% after UV-B irradiation. Overall, the antioxidant capacity change in blueberry was not very sensitive to the preharvest UV irradiation during the fruit development, especially for the green fruit.

## 4. Discussion

Studies indicated that UV can affect the synthesis and accumulation of flavonoid compounds, which happens might via both the regulation of structural and regulatory genes, followed by accumulation of products (Inostroza-Blancheteau et al., 2014; Mellway et al., 2009; Nguyen et al., 2017; Yang et al., 2018; Zhang et al., 2013). However, there have no comprehensive investigation on the effects of different UV radiation on the biosynthesis and regulation of specific flavonoid products (including flavonols, PAs and anthocyanins) in developing blueberries. And which specific flavonoid protect fruits against UV radiation during different fruit development is unclear, especially in berries on living

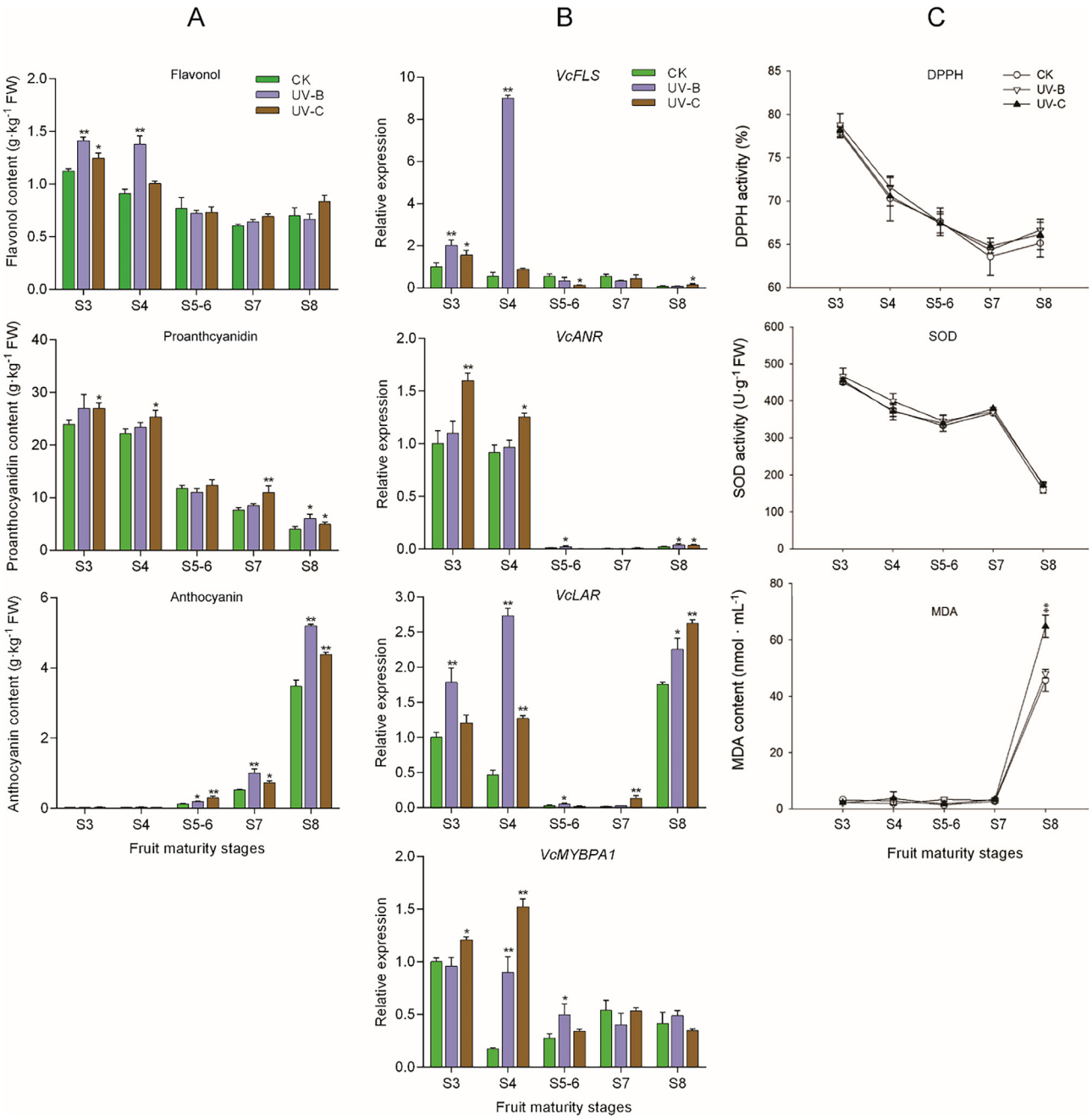


**Fig. 3.** Flavonoid metabolism and antioxidant capacity in developing blueberry treated by preharvest UV irradiation (dose of 4. 14 kJ $m^{-2}$). (A) Flavonoid composition and contents in blueberry (B) Flavonoid biosynthesis-related genes expression level in blueberry (C) Antioxidant activities of blueberry. The error bars indicate the standard deviation of three replicates, data are means ± SE (n = 3). *, $P < 0.05$; **, $P < 0.01$. Note: samples of anthocyanin content in Fig. 3A the same with our previous research (Yang et al., 2018), but are re-determined in this study.

plants. Therefore, this study represents the first attempt to characterize the gene expression and metabolite profiles of flavonoid biosynthesis in developing blueberries under two types of preharvest and three types of postharvest UV irradiation, compared with natural conditions.

In natural conditions, flavonols and PAs showed similar accumulation patterns, which coincided with *VcANR* and *VcLAR* expression and were most concentrated in young fruit. Additionally, the *VcMYBPA1* might be related to the regulation of PA synthesis. By contrast, anthocyanins and the transcripts of *VcANS* and *VcUFGT* accumulated late in the maturation process (Figs. 1 and 2). These result were similar with that in the research of Zifkin et al. (2011). In addition, we found *VcFLS* expression was coincide with flavonol synthesis. Significant positive correlations were observed only between flavonols and *VcFLS*, between PAs and *VcLAR/VcANR* (Table S3), as well as between anthocyanins and *VcUFGT/VcANS* (Yang et al., 2018). This may suggest that each flavonoid synthetic pathway has specific crucial biosynthesis genes during fruit development under natural conditions (Fig. 5).

As in natural conditions, significant positive correlations were observed under both postharvest and preharvest UV irradiation between flavonol content and *VcFLS* expression, PA content and *VcANR* expression in this study, as well as between anthocyanin content and *VcANS/VcUFGT* expression found in our previous research (Yang et al., 2018). However, *VcLAR* expression was significantly positively correlated with PA content only under postharvest UV irradiation (Tables S3 and S4). Additionally, under the different treatments, PA biosynthesis had a closer correlation with *VcANR* than *VcLAR* in blueberry (Tables

S3–S5). This may be because the extension units in the PA polymerization process are mainly formed from epicatechin monomers catalyzed by *VcANR* in blueberry fruit (Zifkin et al., 2011). Regarding the TF *VcMYBPA1*, although its expression had no significant correlation with PA content, it was closely related with *VcLAR* and *VcANR* under postharvest and preharvest UV irradiation (Tables S4 and S5). Hence, we speculate that *VcMYBPA1* induced after UV irradiation might participate in regulating the expression of *VcLAR* and *VcANR*, which cause the accumulation of PAs. In blueberry leaves, *VcMYBPA1* is upregulated by UV-B radiation and may be crucial for phenolic compound biosynthesis through the activation of common biosynthesis pathways of structural genes (Inostroza-Blancheteau et al., 2014). Furthermore, in gene sequence, *VcMYBPA1* was 99% similar to the *VvMYBPA1* gene (Zifkin et al., 2011), confirming that *VcMYBPA1* participates in PA synthesis. Moreover, our recent study showed that *VcMYB9* (Yang et al., 2018) expression was significantly positively correlated with anthocyanin accumulation, and it was also significantly positively correlated with *VcANS* and *VcUFGT* both in the pre- and postharvest UV treatments. Another regulatory gene, *VcMYB21*, had high expression in blueberry peel and was strongly activated after UV-B irradiation, which was consistent with the increase of anthocyanins (Nguyen et al., 2017). This reveals the potential regulatory mechanism during UV radiation by which *VcMYB21*, *VcMYB9* and *VcMYBPA1* participate in the flavonoid biosynthetic pathway in blueberry.

Some similarities were found between preharvest and postharvest UV treatment in the induction of the flavonoid pathway in developing blueberries, with consistent and obvious developmental stage-dependence. In early development stages, the flavonol branches were significantly upregulated, and the anthocyanin biosynthesis branches were mostly unchanged. After turning stages (S5–S8), flavonol biosynthesis was stable at a low level, while PA and anthocyanin biosynthesis were significantly activated (Figs. 4 and 5). Some transcriptomic and metabolomic analysis results for grapes irradiated with UV showed that plants could activate the synthesis of a great number secondary metabolites as part of the complex mechanism on antioxidant defense, which results in changes in berry composition (Czemmel et al., 2017; Loyola et al., 2016; Matus, 2016). These changes vary with the fruit development stage and UV type. It was found that the flavonol content in grape berry peel was higher under solar UV light, while anthocyanin and flavanol (PA monomer) concentration had no obvious activating effects (Carbonell-Bejerano, 2014). Flavonols in grapes at veraison stages were highly induced by UV-B, but the total anthocyanin and PA amounts did not change significantly (Matus, 2016). Our UV treatment mainly induced flavonol accumulation during early fruit development, but increased anthocyanin and PA contents in late fruit development (Fig. 5).

However, there were still many differences in flavonoid biosynthesis in developing blueberries between pre- and postharvest UV radiation. First, the PA content in green berries was increased during preharvest but decreased during postharvest UV radiation (Figs. 4 and 5). Likewise, in our previous study, sugar contents significantly increased during preharvest but were largely reduced during postharvest UV radiation in mature fruit (Yang et al., 2018). In addition, the antioxidant capacities in blueberries were markedly increased by postharvest UV irradiation but barely affected by preharvest UV radiation (Figs. 2 and 3). Wang et al. (2009) showed that postharvest UV-C significantly promoted the hydroxyl radical scavenging capacity (HOSC), oxygen radical absorbance capacity (ORAC), and DPPH activities in blueberries, as in strawberries (Erkan, Wang, & Wang, 2008), mangoes (Gonzálezaguilar, Villegasochoa, Martíneztéllez, Gardea, & Ayalazavala, 2010), and grapes (Sheng et al., 2018). However, as in our study, preharvest UV-C treatment had no obvious effect on antioxidant capacity in strawberry fruit (Xie et al., 2015). This might suggest that living plants deal with environmental UV irradiation to some extent through a series of integral physiological and biochemical regulatory pathways that reduce its effects on fruit, but which differ from those in detached fruit. Moreover, under postharvest UV radiation, flavonoid biosynthesis and the antioxidant capacity in blueberries were more increased by UV-C radiation, and the effect roughly weakened with increasing UV wavelength (UV-C > UV-B > UV-A). Similar phenomena were observed in grapes (Zhang et al., 2012, 2013) and blueberries (Nguyen et al., 2014). Plants have evolved a variety of methods to deal with the extra UV-A and UV-B radiation (Matus, 2016). Among them the epidermal tissues could protect the plants from UV damage by producing different protective products such as flavonoids and hydroxycinnamic acids. However, higher photon energy UV-C, unlike UV-A and UV-B, has more significant effects on plants (Matus, 2016; Yang et al., 2018). In the preharvest UV treatment, UV-B and UV-C affected the gene expression and metabolic profiles of the flavonoid pathway in developing fruits differently (Fig. 3), which suggests that living plants can sustain such radiation doses from high-energy UV-C. All of these different responses of flavonoid biosynthesis and the antioxidant system shows that the resistance to UV irradiation in living plants is much greater than in detached fruits. This finding was also demonstrated by our preliminary UV treatment in which the optimal dosage was

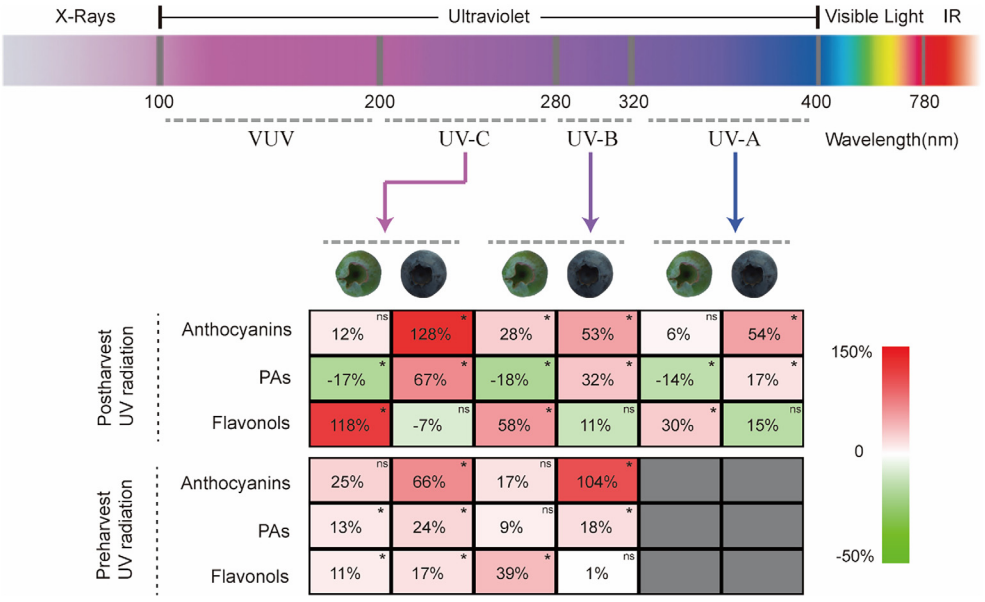


**Fig. 4.** Flavonoid metabolites induced in blueberry fruits in response to postharvest and preharvest ultraviolet radiation. Green and blue berries represent early (S3, S4) and late (S6, S8) period fruits, respectively. The number in each square represents the average change rate (%) in the indicated metabolites in the two fruit periods under UV radiation, with the strength of the change represented by the color in the square. Green indicates downregulation and red indicates upregulation by UV treatment. A "*" in the upper right corner of the square indicates that the change induced by UV was significant while "ns" indicates no significant change. The gray squares indicate that there was no UV-A treatment of preharvest fruits. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

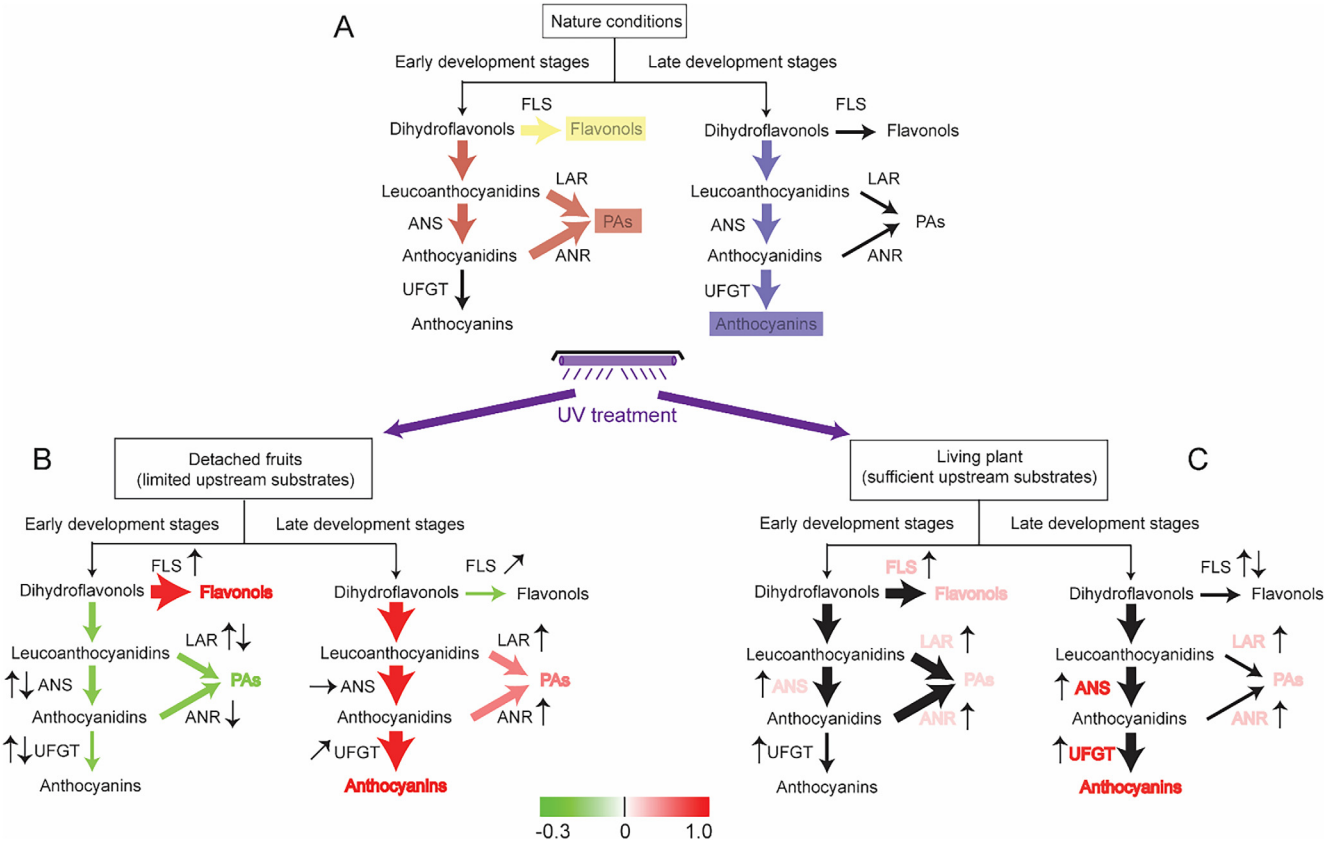


**Fig. 5.** A hypothetical scheme for the potential mechanism of the flavonoid metabolic pathway response to postharvest (B) and preharvest (C) UV irradiation, compared with natural conditions (A), at the early (S1–S4) and late (S5–S8) developmental stages of 'Northland' blueberries. The width of the arrows on the assembly line indicates the flow rate of each metabolite. The arrows shown beside the gene names indicate the trend in each gene's transcript level. Arrows pointing upward indicate a large increase, arrows pointing rightward indicate no obvious change, arrows pointing diagonally upward indicate some increase, arrows pointing downward indicate a sharp decrease, and arrows pointing upward and downward indicate both an increase and a decrease occurs. In (A), yellow represents the flavonols pathway branch, reddish brown represents the PA pathway branch, and blue represents anthocyanin pathway branch. In (B) and (C), green indicates metabolite downregulation, red indicates metabolite upregulation, and black indicates no significant change under UV treatment. The color shade represents the average change multiple in the metabolites induced by UV-B and UV-C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.14 kJ $m^{-2}$ in the preharvest and 2.76 kJ $m^{-2}$ in the postharvest period (Figs. S1 and S2). In response to the highest-energy UV-C and higher-energy UV-B irradiation, with increasing irradiation dose, the flavonoid accumulation in young fruit on living plants increased gradually. In detached fruit, the induction effect on all the researched flavonoids showed an increasing followed by a downward trend.

Correlation analysis between individual flavonoids and antioxidant activity under UV radiation showed that PAs significant correlated with the antioxidant activity determined by DPPH and SOD analysis, except with SOD ($r = 0.443$) under preharvest UV radiation (Tables S4 and S5). Under natural conditions, the change pattern of DPPH and SOD activity also strongly paralleled PA contents ($r = 0.720^{**}$, $r = 0.805^{**}$), which decreased gradually with the ripening of fruit (Table S3). Zhu, Liu, Tan, and Wang (2013) investigated the effect of harvest stage of blueberry leaves on their constituents and antioxidant activity, and showed that leaves had their highest antioxidant capacity in November, which highly correlated with their highest PA content. In jujube (*Zizi-phus mauritiana* Lam.), the total phenolic content gradually decreased together with antioxidant activity during fruit ripening, which showed that condensed tannins (PAs) contributed the highest antioxidant activity in both jujube cultivars (Suzie et al., 2014). Therefore, the antioxidant activity in blueberries may be more affected by PAs.

Obviously, the flavonoid content was more increased by postharvest than preharvest UV treatment, which may reflect different potential mechanisms including a systemic and moderate response in living plants and a non-systemic but strong response in detached fruit (Figs. 4 and 5). Importantly, the key factors for final product accumulation are not only gene expression, but also substrate flow. In detached fruits, changes in the flavonoid composition mostly be derived from metabolism within the fruits themselves, which have limited upstream substrates. Thus, flavonoid biosynthesis in the fruit was more affected by the substrate flow than gene expression levels (Fig. 5). The decrease in PA contents in young fruit under UV irradiation might imply that the precursors used for PA biosynthesis might flow into other flavonoid biosynthesis branches, such as flavonols. The flavonol content was not increased despite a certain increase in *VcFLS* expression, possibly because more of the assimilated carbon flowed into biosynthetic pathways for anthocyanins and PAs rather than flavonols. The regulation of the flavonoid profile and composition seems to be intimately connected to the universal phenomenon of overall homeostasis (Das, Geul, Choi, Yoo, & Park, 2014). Zhang et al. (2013) showed that flavan-3-ols were induced by UV-A, -B and -C in green and veraison-stage grapes, but decreased in the ripening stage, mainly due to a lack of upstream substrates for anthocyanin production. Conversely, living plants can produce carbon resources through photosynthesis and coordinate the allocation of these resources from photosynthetic organs to fruit, which provides sufficient upstream substrates during UV radiation exposure. Thus, the key synthetic gene expression levels determine the accumulation of end products (Fig. 5).

## 5. Conclusion

In the present study, both detached fruits and living plants can protect themselves against UV radiation by regulating the synthesis of flavonols at the early fruit development stage and promoting the accumulation of anthocyanins and PAs at the late fruit development stage in blueberry. But PAs decreased during postharvest but increased during preharvest UV irradiation in green fruit. Antioxidant capacities increased response to postharvest UV but barely to preharvest UV irradiation. *VcMYBPA1* was closely related with *VcLAR* and *VcANR* under UV irradiation. Besides, PAs exhibited higher potential antioxidant activity and *VcMYBPA1* was closely related with *VcLAR* and *VcANR*, which could be related to PAs biosynthesis under UV. These results show that UV resistance is greater in living plants than detached fruits, the former showing a systemic and moderate response and the latter a non-systemic but strong response. Our findings could help reveal the molecular mechanism of flavonoid biosynthesis under different types of UV radiation and contribute to the development of preharvest and postharvest technologies to promote healthier fruit consumption.

## 6. Authors contribution statement

Junfeng Yang and Zhixia Hou conceived and designed research, analyzed data, as well as wrote the manuscript. Junfeng Yang, Wenjun Shi, Binbin Li, and Yongchao Bai conducted experiments. All authors read and approved the manuscript.

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## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125248.

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