

Effect of Light Quality Selective Plastic Films on Anthocyanin Biosynthesis in *Vitis vinifera* L. cv. Yatomi Rosa

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ABSTRACT

The accumulation and composition of berry anthocyanin was measured in *Vitis vinifera* L. cv. Yatomi Rosa using various light qualities provided by polyethylene films (red, orange, green, blue, and white). The anthocyanin has been enhanced significantly by blue light filter film and suppressed by red, orange, and green films compared to white film (control), which suggests that Yatomi Rosa has adapted photoreceptors to UV-A/blue light. We also observed that Yatomi Rosa mainly accumulated glucosides of peonidin (Pn). Significant differences in composition of anthocyanin were observed in different light filter films. Despite the use of different light quality films, similar patterns were observed for phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), 4-coumarate: coenzyme A ligase (4CL), and chalcone isomerase (CHI) activities, which are components of the anthocyanin biosynthetic pathway. But the activities of PAL and CHI were induced significantly under blue light filter film, which shows that blue light may increase anthocyanin accumulation in *V. vinifera* L. by stimulating PAL and CHI activities. The present study confirmed the importance of blue irradiance spectrum in anthocyanin biosynthesis in Yatomi Rosa. The increase of blue light under enough effective transmission light conditions in greenhouses can improve the color of Yatomi Rosa and may be used as a solution to overcome poor berry colors in hot and humid regions of Southern China.

Keywords: Blue light, Coloration of berry, Polyethylene films, Southern China.

INTRODUCTION

Light is an optical signal that regulates photomorphogenesis, photoperiodic response and plant endogenous biological rhythms. It also simultaneously regulates plant growth and development with sugar and hormones signals interacting in an intricate signaling network (Chory and Wu, 2001; Pierik *et al.*, 2009). Light quality (e.g., the specific wavelength of light) by selective plastic films may serve as a non-chemical growth regulating tool for plant production in greenhouses (Patil *et al.*, 2001; Casierra-Posada *et al.*, 2012).

With the development of rain shelter facility in recent years, *Vitis vinifera* L. has been developing rapidly in the Southern

China for its high quality and long shelf life. But in Southern China, coloration of red *V. vinifera* L. is poor because of high temperature, humidity, and low-light climatic conditions. As we all know, the red, black, and blue-black colours in grapes are produced by a group of anthocyanins, and are important factors for quality, market acceptance, and commercial value. Anthocyanin biosynthesis in berry is controlled genetically, and gene expression of the enzymes in flavonoid pathway associates fundamentally with the pigmentation (Boss *et al.*, 1996). In addition to genetic factors, anthocyanin accumulation is also affected by climate, soil conditions and cultural practices such as fertilizer application. Light quality has been reported to affect flavonoid metabolism in various plants.

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UV-B, UV-A, and blue regions of the spectrum are considered to trigger gene expressions of chalcone synthase (CHS), the first committed step in the flavonoid biosynthetic pathway, mediated by specific photoreceptors in *Arabidopsis* (Fuglevand et al., 1996). Solar UV exclusion does not affect the concentration and composition of proanthocyanidins, whereas this exclusion remarkably decreased flavonols concentration (Koyama et al., 2012). The synthesis of anthocyanins and its regulation in response to light conditions have been studied in several types of grape (Jeong et al., 2004; Matus et al., 2009).

However, few studies about light quality regulation of the biosynthesis of flavonoids, particularly anthocyanin accumulation, in response to light quality during grape berries development have been done. In this study, we examined the effects of red, orange, green, and blue light quality obtained by sunlight filter films on anthocyanin accumulation and on PAL, 4CL, C4H and CHI activities in anthocyanin biosynthetic pathway in *V. vinifera* L. cv Yatomi Rosa from veraison to maturity. The aim of this study was to prove the effects of light quality on berry anthocyanin synthesis, expecting to find out the best suitable spectra for berry coloration, and for suitable plastic film and light-filling technology for grape production in greenhouse.

MATERIALS AND METHODS

Vineyard

This study was conducted in 2011 within a 4 year old commercial *V. vinifera* L. cv Yatomi Rosa vineyard at Zhejiang Academy of Agricultural Sciences (120° 24' E, 30° 26' N), located in Southern China. The soil type was marine-fluvigenic yellow loamy paddy soil, with the mean annual air temperature of 17.8°C and the average annual rainfall of 1,400 mm. Vines were planted in greenhouse at a spacing of 1×2.5 m with 4,000 vines per hectare. The vines

were trained according to the "T" trellis system. Using cane pruning technique, those canes furthest away from the trunk were completely removed in winter, the four near the trunk were shortened to contain 6 to 8 buds, to generate fruitful shoots. Another two nearest canes were shortened to 2 buds to produce vegetative branches. The number of buds per hectare was about 128000. For this experiment, the grapevines of the same age, size, and growth conditions were selected and the goal was to use a zone of uniform vine vigor to investigate specifically the influence of light quality.

Light Quality Treatments

Grape clusters from 30 Yatomi Rosa trees vines were divided randomly into six groups with 5 vines (replicates) in each group and grape clusters were covered with transparent red, orange, green, blue, and white control light filter film bags (treatments) composed of strips of a 0.03 mm-thick polyethylene film on 8 July, 2011. The radiant energy of white filter film (control) were 70% of solar energy measured by Portable Spectroradiometer LI-1800LI-COR, USA. The solar radiation transmissivity of the red, orange, green, and blue filter films was 63.6, 78.2, 58.8, and 57.8% of white film, respectively. The percentum of blue irradiance spectrum in overall radiant energy in red, orange, green, blue, and white light quality selective plastic film was 1.80, 4.63, 10.03, 19.0, and 8.10%, respectively.

Fruit Sampling

Berries used for anthocyanin analysis, were sampled at 47, 61, 75, 88 DAA (Days After Anthesis) from each treatment with 3 biological replicates. The skins of grapes were separated manually from berries and were immediately frozen in liquid nitrogen upon collection and then stored at -70°C until use.

Fruit Weight, Acid, and Total Soluble Solids and $L^*a^*b^*$

The grapes were weighted after each sampling and average berry weight was recorded prior to peeling. Acid was measured using NaOH titration method and SSC (°Brix) were measured using a digital refractometer (PAL- α , ATAGO, Tokyo, Japan). Colour for individual fruit was measured with a Minolta CR200 chromameter and calibrated with a white standard before use. $L^*a^*b^*$ colour space data values were recorded. According to Hunter Lab colour system (Francis, 1980), the hue angle (h) and chrominance (C) were calculated. Hue angle (h) was calculated as $h = \arctan b/a$. Chrominance (C) was calculated as $C = (a^2 + b^2)^{1/2}$.

Analysis for Total Anthocyanin

Determination of total anthocyanin content in grape peel was based on the method described by Pirie and Mullins (1976). Total anthocyanin content was expressed as optical density (OD₅₂₀) per gram of berry peel.

HPLC Analysis of Anthocyanin

Chromatographic analysis was performed on a Waters 1525 HPLC system consisting of an autosampler, a binary pump, a column compartment, a diode array detector and Waters Breeze™ software (Waters Technologies, USA). A Waters SB-C18 column (150×4.6 mm, 5 μm particle size) was used for separation of anthocyanin compounds. Elution was performed using mobile phase A (10% formic acid aqueous solution) and mobile phase B (acetonitrile). The flow rate was 1 mL min⁻¹ with the linear gradient as follows: 0-13 minutes, 0-20% B; 13-20 minutes, 20-30% B; followed by 5 minutes of re-equilibration of the column before the next run. UV-vis spectra were scanned from 220 to 600 nm on a diode array detector and the detection wavelength for the anthocyanins was 520 nm.

Enzyme Extraction and Assay

PAL, C4H, 4CL and CHI enzyme extractions were performed between 0 and 4°C and activity was assayed (Lister and Lancaster, 1996; Hiratsuka *et al.*, 2001; Chen *et al.*, 2006). Protein was assayed, with bovine serum albumin as the standard, by the dye-binding method of Bradford (1976).

Chemicals

Acetonitrile, methanol, ethanol, glacial acetic acid, ascorbic acid, potassium metabisulfite, and potassium hydroxide were purchased from Sigma-Aldrich (St. Louis, USA). Glycosides standard sample of delphinidin-3-O-glucoside (Dp), cyanidin-3-O-glucoside (Cy), petunidi-3-O-glucoside (Pt), pelargonidin-3-O-glucoside (Pg), peonidin-3-O-glucoside (Pn) and malvidin-3-O-glucoside (Mv) were purchased from Polyphenols Lab. (Hanaveien, Norway). All other chemicals and solvents were of analytical reagent grade and purchased in China.

Statistical Analysis

Data were expressed as mean±standard deviation (SD) and evaluated by one-way analysis of variance (ANOVA) followed by the Duncan's Multiple Range Tests. *P*-values of less than 0.05 were regarded as significant. All statistical analyses were carried out by using SAS Statistical Software Version 8.0 (SAS Institute Inc., Cary, NC).

RESULTS

Fruit Weight, Soluble Solid Content, Acid, and Color

Light is one of the most important environmental factors for plant growth. In current research, several light quality



selective plastic film treatments had no effect on berry weight in Yatomi Rosa (Table 1). The significantly higher soluble solid content ($^{\circ}$ Brix) was observed in berries under blue light filter film, which positively affects sugar metabolism of grape via the influence of blue / UV ratio.

L value of berry with red light filter film treatment was the highest. C value of berry in blue filter film and white filter film (control) increased significantly and a significant decrease in h was observed at maturity, which indicated that the fruit colours were better than those of other films.

Total Anthocyanin Content

The anthocyanin accumulating in Yatomi Rosa grapes grown in different light quality selective plastic films are shown in Figure 1. There was a trend toward increasing anthocyanin concentration during berry development. Anthocyanins increased fast and at higher level in blue filter film since veraison, while white film treatment (control) rapidly increased anthocyanin accumulation. At harvest, the anthocyanin concentrations for the two treatments were similar and higher in comparison with the other films. Compared to white film, the use of blue filter film resulted in a rapid increase in anthocyanin accumulation after 61 DAA. The anthocyanin in red, orange, and green film treatments increased quickly before 61 DAA, and then remained at a lower level.

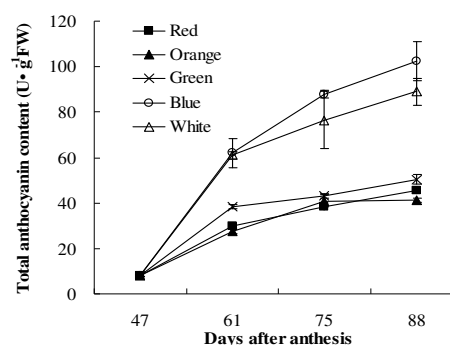


Figure 1. The total anthocyanin changes of skin in different light filter films during berry development. Each value is the mean for three replicates, and vertical bars indicate the standard errors. (■) Red; (▲) Orange; (×) Green; (○) Blue, and (△) White film.

The total anthocyanin in Yatomi Rosa of blue film treatment was 2-fold higher than that observed using red, orange, and green films at maturity. No significant differences in total anthocyanin content were observed among the red, orange, and green film treated berries.

Composition Change of Anthocyanidins

In the present research, attending to the retention time in HPLC and their UV–vis spectral properties, it was possible to identify 8 anthocyanidin compounds in the analyzed extracts of Yatomi Rosa. The most

Table 1. Effect of different light filter film treatments on quality and color of Yatomi Rosa.

Light filter film treatments	Berry weight (g)	Soluble solid content ($^{\circ}$ Brix)	Titrateable acidity (%)	L ^a	C ^b	h ^c
Red	9.37±1.97a	13.46±0.46b	0.27±0.02a	39.99±0.39a	15.82±1.47b	39.14±2.41a
Orange	9.23±0.68a	14.12±0.29b	0.24±0.00a	34.29±1.84b	15.14±1.39b	36.53±5.11a
Green	9.35±1.85a	14.04±0.38b	0.25±0.00a	36.08±1.50b	14.44±1.51b	30.60±6.40a
Blue	9.04±2.18a	15.02±0.33a	0.25±0.00a	31.40±1.73c	22.73±2.17a	18.03±4.26b
White (Control)	9.78±0.84a	13.50±0.23b	0.22±0.01a	29.20±1.36c	21.22±0.73a	17.16±1.91b

^a Luminance ^b chrominance ^c hue angle, ^a Results are mean±standard deviation on fresh weight basis. Values with the same letters are not significantly different ($P < 0.05$) within columns.

prominent compound was peonidin 3-O-glucoside (Pn). The concentrations of anthocyanin peonidin, malvidin and their derivatives of *V. vinifera* Yatomi Rosa grapes by HPLC in blue filter film were higher, compared to white film. Figure 2 shows the increase in Cy, Pt, Pn and Mv contents in the berry peel following treatment using five types of light quality selective plastic films after 47 DAA. The anthocyanin composition of berry peel in blue filter film treatment was higher than white film (control) and rapidly accumulated until maturity. In contrast, the anthocyanin content of red, orange, and green filter films was lower than that in white film and increased slowly, except Mv. Compared with the white film at full maturation, Cy, Pt, Pn, and Mv content of berry in blue film increased by 67.6, 19.8, 21.3, and 10.8%, respectively.

Changes of Phenylpropanoid Metabolizing Enzyme Activity

PAL, C4H, 4CL and CHI play pivotal roles in the biosynthesis of plant secondary compounds and the levels of these enzymes seem to be highly regulated in response to a wide variety of developmental and environmental signals (Hahlbrock and Scheel, 1989; Achnine *et al.*, 2004). PAL is a key enzyme in phenylpropanoid metabolism and catalyzes the conversion of phenylalanine to trans-cinnamic acid, the first step in the biosynthesis of phenylpropanoid. Figure 3-A shows the changes in PAL activity during berry development response to light quality, which initially increased, reached a peak and then followed a downward trend. There were some differences among the different film treatments. Blue film treatment already reached the peak at 61 DAA, but other films

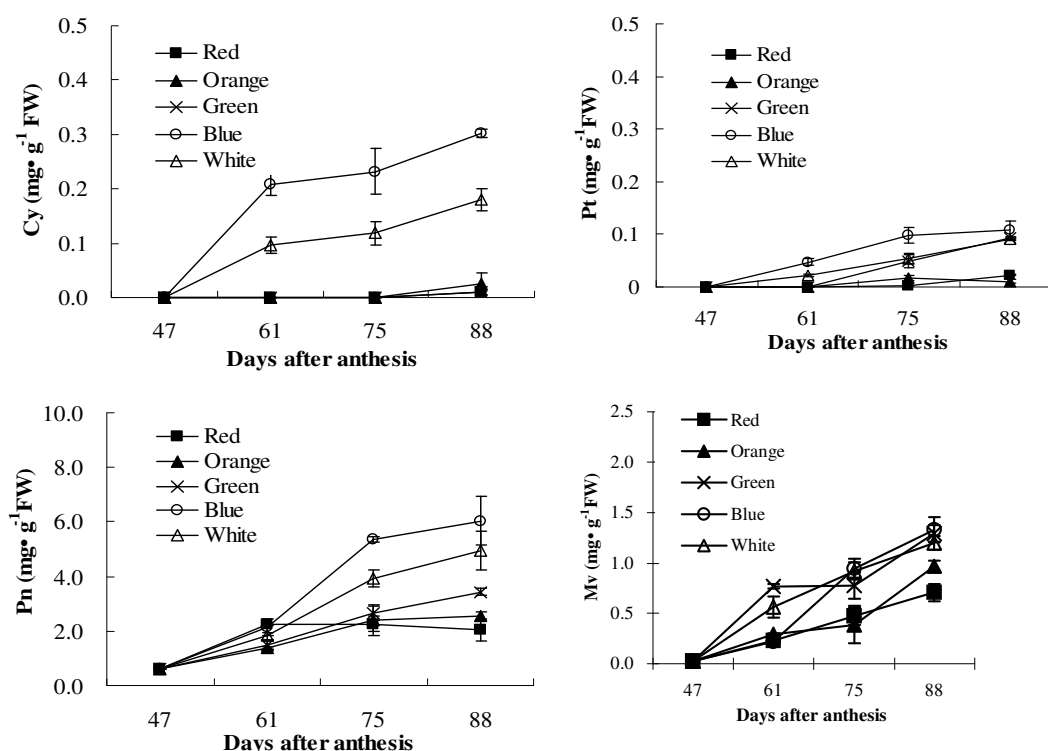


Figure 2. The effect of different light quality on anthocyanin composition changes in skin during berry development. Each value is the mean for three replicates, and vertical bars indicate the standard errors. (■) Red; (▲) Orange; (×) Green; (○) Blue, and (△) White film.

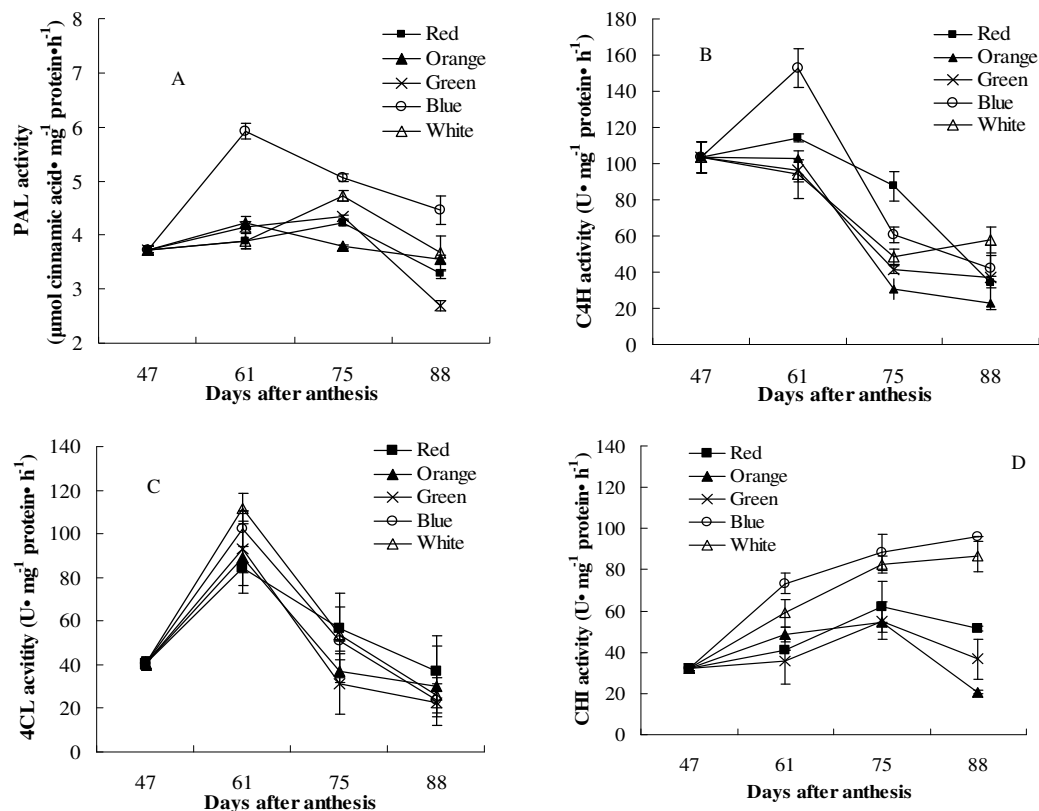


Figure 3. Changes in anthocyanin biosynthesis metabolizing enzyme activities during berry development. (A) PAL activity; (B) C4H activity; (C) 4CL activity, and (D) CHI activity. Each value is the mean for three replicates, and vertical bars indicate the standard errors. (■) Red; (▲) Orange; (×) Green; (○) Blue, and (△) White film.

reached the peak at 75 DAA, except the orange film. Compared with the white film (control), PAL activity in blue film significantly increased during berry development and remained at high activity. The changes in C4H activity in light quality film treatments showed declining trend, except in the blue film treatment (Figure 3-B), which showed an initial increase and then a downward trend, and accumulation peak during berry development at 61 DAA. Changes in 4CL activity of berry in different films increased at first, which was followed by a downward trend and showed one accumulation peak during berry development at 61 DAA (Figure 3-C). The 4CL activity in white and blue films was very high, contrary to that in red, which was lowest at 61 DAA. However, no significant

change in trend in different films was observed from 47 to 88d of berry development. Figure 3-D shows changes in CHI activity in different film treatments. The activity of CHI in blue and white film treatments showed accumulation process from 47 to 88 DAA of berry development, maintaining a higher activity than others. An increase in CHI activity in red, orange, and green films also was observed before 75 DAA, which later declined. Compared to white film, the CHI activity of berry using the blue filter film increased significantly.

DISCUSSION

The anthocyanins are responsible for the red to black colors of fruit. The red color in

apples is produced by a group of anthocyanins, mainly cyanidin-3-galactoside (Ubi *et al.*, 2006). In strawberry, the main component of anthocyanins is pelargonidin 3-glucoside (Lopez da Silva *et al.*, 2007). In *V. vinifera* L. red cultivars, there are only cyanidin, delphinidin, petunidin, peonidin, and malvidin 3-monoglucosides along with the corresponding acetyl, *p*-coumaroyl and caffeoyl derivatives. The anthocyanin accumulation levels in *V. vinifera* Tannat, the main red cultivar in Uruguay, were petunidin 3-glucoside and malvidin 3-glucoside (González-Neves *et al.*, 2004). This research showed that *V. vinifera* Yatomi Rosa primarily accumulated glucosides of Pn, in agreement with red table grape varieties 'Red Globe', 'Flame Seedless', 'Crimson Seedless', and 'Napoleon' (Cantos *et al.*, 2002; Human and Bindon, 2008). This indicates that the anthocyanin biosynthetic pathway in this cultivar is mainly by the F3'H branch toward Pn, rather than the F3'5'H branch toward Mv (Boss *et al.*, 1996). Recent research has shown alteration of anthocyanin composition in response to altered light conditions. Shaded fruit in Shiraz grapes was shown to have an increased proportion of anthocyanins Cy and Pn (Downey *et al.*, 2004; Ristic *et al.*, 2007). However, previous studies have shown that anthocyanin cyanidin-3-glucoside in Crimson Seedless berry skins decreased significantly by shade (Human and Bindon, 2008). In the current study, the alteration in anthocyanin composition was observed in response to altered light conditions by light quality selective plastic films. The biosynthesis of Cy and Pn responded significantly to different filter films and was induced significantly by blue filter film. The proportion of Cy in total anthocyanins in blue filter film was enhanced, whereas it decreased in red, orange, green, and blue filter films. However, the proportion of Pn in total anthocyanins in different films was not altered, probably because the synthesis of

this anthocyanin from Cy in this cultivar is genetically controlled.

Light quality has been reported to affect flavonoid metabolism in various plants. Flavonoids in fruits were found to be particularly sensitive to light environments around clusters, which reflects a possible role of these compounds for photoprotection (Jackson and Lombard, 1993; Downey *et al.*, 2004). Many researches have shown that low light environment or excessive exposure to sunlight could decrease anthocyanin levels in berries. Cluster shading resulted in a substantial decrease in anthocyanins in Pinot noir grape (Cortell and Kennedy, 2006). Red light and far-red light increased the total anthocyanin level in cranberry fruit by 41.5 and 34.7%, respectively (Zhou and Singh, 2004). Besides, UV-A induction of anthocyanin accumulation was also observed in grapes (Kataoka *et al.*, 2003). In the present study, the increasing blue irradiance spectrum in the blue filter film influenced significantly the anthocyanin biosynthesis of Yatomi Rosa with fast accumulation. Three types of photoreceptors developed when plants evolved adaptation to light: (i) Phytochromes for the absorption of red and far-red light (600–750 nm); (ii) UV-A/blue photoreceptors including cryptochromes that mediated their responses to blue light and UV-A (320–500 nm) and phototropin mediating phototropism; (iii) Photoreceptors UVR8 for UV-B (282–320 nm) (Heijde and Ulm, 2012). Different light receptors are initiated by different light qualities, and then affect plant photosynthetic characteristics, growth, yield, quality, adversity, aging, etc. (Patil *et al.*, 2001; Casierra-Posada *et al.*, 2012). Our results showed that anthocyanins of grape berry were induced by blue light; it can be speculated that anthocyanins in Yatomi Rosa evolve adaptation to light by UV-A/blue photoreceptor types.

Numerous studies have demonstrated that the increase of PAL, C4H and 4CL activities result in the accumulation of



phenolic compounds, such as anthocyanin (Sgarbi *et al.*, 2003; Achnine *et al.*, 2004). Phenolic compounds in fruits might improve the quality and nutritional value because of strong antioxidant and antibacterial activities (Yasoubi *et al.*, 2007; Salmanian *et al.*, 2014). It has been reported that the response mediated by light receptor is related to specific light regulatory gene. UV-B increased the accumulation of anthocyanins by stimulating the expression of *CHS*, *DFR*, and *F3H* gene encoding enzymes in the anthocyanin biosynthetic pathway (Fuglevand *et al.*, 1996; Park *et al.*, 2007). *PAL*, *CHS*, *F3H*, *DFR* and *ANS* genes increased during a 24-h exposure to UV-A in turnip (Zhou *et al.*, 2007). Our experiment showed increases in PAL and CHI activities under blue filter film. This shows that blue light increased the anthocyanin accumulation of *V. vinifera* L. by stimulating the increase of PAL and CHI activities.

CONCLUSIONS

The research confirmed the importance of blue irradiance spectrum in anthocyanin biosynthesis of *V. vinifera* L. Yatomi Rosa. It has been shown that anthocyanin of berry evolved adaptation to light by UV-A/blue photoreceptors and anthocyanin synthesis was induced by enhancing PAL and CHI activities. Therefore, increasing blue light under enough effective transmission light conditions improves color of grape in greenhouses, which could be a likely cost-effective and physical-technical manipulation to address the problem of poor berry colors in hot and humid regions.

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اثر ورقه های پلاستیکی انتخاب کیفیت نور روی زیست-ساخت (بیوسنتز) آنتوسیانین در انگور *Vitis vinifera* L. رقم یاتومی روسا

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چکیده

انباشت و ترکیبات آنتوسیانین در میوه انگور *Vitis vinifera* L. رقم یاتومی روسا با استفاده از کیفیت های مختلف نور با کار برد ورقه های پلی اتیلن (قرمز، نارنجی، سبز، آبی، و سفید) اندازه گیری شد. آنتوسیانین در اثر ورقه صافی نور آبی به طور معنی داری زیاد شد ولی مقدارش در مورد ورقه های قرمز، نارنجی، و سبز در مقایسه با ورقه سفید (تیمار شاهد) کاهش یافت و این نشانگر آن است که رقم یاتومی روسا نورپذیرهای (Photoreceptors) سازگار با نور آبی UV-A دارد. همچنین مشاهده شد که این رقم بیشتر گلوکسیدهای (Pn) peonidin را انباشت می کند. نیز، تفاوت های معنی داری در ترکیبات آنتوسیانین در ورقه های صافی نور مختلف مشاهده شد. با وجود کار برد ورقه های مختلف صافی کیفیت نور، طرح های مشاهده شده از فعالیت (C4H) cinnamate-4-hydroxylase، (PAL) phenylalanineammonia-lyase، (4CL) و (CHI) chalcone isomerase (که همگی از اجزای مسیر زیست-ساخت (بیوسنتز) آنتوسیانین هستند) مشابه بودند. اما، فعالیت PAL و CHI زیر ورقه صافی نور آبی به طور معنی داری القا شدند و این نشان می دهد که نور آبی ممکن است با تحریک فعالیت PAL و CHI انباشت آنتوسیانین را در *V. vinifera* L. افزایش دهد. در پژوهش حاضر اهمیت تابش طیف آبی در بیوسنتز آنتوسیانین در رقم یاتومی روسا تایید شد. افزایش نور آبی در شرایط کفایت نور انتقالی و موثر در گلخانه می تواند رنگ انگور یاتومی روسا را بهبود بخشد و راه حلی باشد برای رفع مشکل رنگ میوه در مناطق گرم و مرطوب جنوب چین.