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Original Article

Bagging Affecting Sugar and Anthocyanin Metabolism in the Ripening Period of Grape Berries

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Abstract

Grapevine is one of the most important fruit-bearing plants worldwide, for which bagging treatments can effectively improve fruit quality. However, the low-light conditions caused by bagging can delay grape berry maturation. Here, we analyzed glucose, fructose, and anthocyanin contents and the expression of sugar and anthocyanin-metabolism pathway genes in the grape berries of two cultivars, 'Shenhua' and 'Shenfeng', under different bagging treatments. Color development was incomplete in bagged grape berries and their soluble sugar contents were lower than those detected in un-bagged fruits. However, fruit color and SSC could be rapidly restored to normal levels after removing bags. Light affects the accumulation of sugar in grape berries, especially near the maturation period, as well as the contents and compositions of anthocyanins in the skin of grape berries. Although light helps in the accumulation of anthocyanins, significant differences were detected in anthocyanin composition between the two grapevine varieties. In addition, the expressions of myofibroblastic regulatory genes in the anthocyanin pathway were affected by light, and the light-responsive elements elongated hypocotyl 5 and constitutive photomorphogenic 1 acted synergistically to control grape berry coloration. Overall, these results provide a theoretical basis for the maturation mechanism in grape berries.

Keywords: fructose; glucose; grapevine; light stress

Abbreviations: ANS: Anthocyanidin synthase; CHI: Chalcone isomerase; CHS: Chalcone synthase; CIRG: Color index of red grape; COP: Constitutive photomorphogenic; DFR: Dihydroflavonol 4-reductase; DAA: Day after anthesis; F3H: Flavanone-3-hydroxylase; HPLC: High-performance liquid chromatography; HY: Hypocotyl ; MYB: Myofibroblastic; TA: Titratable acidity; SSC: Total soluble solid content

Introduction

Grapevine is grown extensively throughout the world. In 2014, worldwide vineyard area and grape berry production were 7,940,150 ha and 100,186,579 t, respectively (Food and Agriculture Organization, 2016). Grape berries are rich in many nutrients and are very appreciated by consumers. However, during the processes of fruit enlargement and maturation, producers apply pesticides to control pests and diseases, which inevitably affect food safety. Although bagging treatment can effectively reduce pesticide residues on the surface of fruit, it may cause low-light stress. Light is an essential environmental factor for plant growth and development, and thus the appearance and flavor quality of fruit could be affected by bagging treatment. Grape berry organoleptic quality depends largely on both the content and composition of sugars, acids, and anthocyanins, which are all influenced by light.

Sugar accumulation is a key factor determining fruit quality in grape berries. Sucrose metabolism is important for sugar accumulation, as this is the main form of sugar transported in grapevine phloem, and it is rapidly metabolized and converted into other sugars, organic acids, and structural substances at the young fruit stage (Shiraishi, 1993; Davies and Robinson, 1996). Low-light environments may influence the transport and transformation of carbohydrates through the micro-domain environment of fruit, leading to changes in specific sugars (Dokoozlian and Kliewer, 1996; Wang *et al*., 2009). Tartaric and malic acids typically account for more than 90% of total organic acids in grape berries (Lamikanra *et al*., 1995; Esteban *et al*., 1999): enhancing the flavor and contributing to the mouth-feel of table grape berries.

The color of plant flowers and fruit, which is determined by different pigments, is an important visual

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property. Based on their chemical composition, pigments can be divided into flavonoids, carotenoids, chlorophylls, and betalains. Anthocyanins, which are flavonoid compounds, primarily determine organ color in plants (Grotewold, 2006; Tanaka *et al*., 2008). The synthesis and metabolic pathway of anthocyanins in plants have been the focus of considerable research activity (Petroni and Tonelli, 2011) and, to date, six anthocyanins have been characterized, namely, pelargonidin, cyanidin, delphinidin, peonidin, malvidin, and petunidin. Anthocyanins can be combined with glucose, galactose, and arabinose to generate single or double anthocyanin elements (Figueiredo *et al*., 1999; Tanaka *et al*., 2008). In the past few decades, the biosynthesis of anthocyanins has been characterized in model plants, and it has been determined that the biosynthetic process is catalysed in a stepwise manner by chalcone synthase (CHS): chalcone isomerase (CHI): flavanone-3-hydroxylase (F3H): dihydroflavonol 4-reductase (DFR): and anthocyanidin synthase (ANS): which are all under the regulation of several myofibroblastic (MYB) transcription factors (He *et al*., 2010; Petroni and Tonelli, 2011).

Previous studies have shown that light can increase anthocyanin concentrations, particularly in fruit skin (Feng *et al*., 2014). Cryptochromes, which respond to light, regulate photomorphogenic development by suppressing constitutive photomorphogenic 1 (COP1) activity (Wang *et al*., 2001; Yang *et al*., 2001; Liu *et al*., 2011). Downstream of the photoreceptors, the RING-finger-type protein COP1 acts as a ubiquitin E3 ligase responsible for targeting several photomorphogenesis-promoting transcription factors, including elongated hypocotyl 5 (HY5) (Osterlund *et al*., 2000): which is a basic leucine zipper (bZIP) transcription factor that binds directly to the promoters of light-inducible genes, such as anthocyanin structural genes, promoting their expression and photomorphogenic development (Smith *et al*., 2007; Zhang *et al*., 2011).

To our knowledge, few studies have investigated the effects of light on the gene expression of sugar and anthocyanin biosynthetic pathways in grape berries. In the present study, we examined sugar and anthocyanins accumulation and the patterns of gene expression in the sugar and anthocyanin biosynthetic pathways of grape berries grown under different bagging treatments. Furthermore, we investigated the roles of genes *HY5* and *COP1* in regulating anthocyanin biosynthesis. Taken together, our results provide insight into the regulatory mechanisms of sugar and anthocyanin biosynthesis under low-light stress.

Materials and Methods

Plant materials and treatments

Seven-year-old vines of two red table grape (*Vitis vinifera* × *V. labrusca* L.) varieties, 'Shenhua' and 'Shenfeng', were grown in a greenhouse (4 m height): to protect them from rain, in Shanghai, China (30° 89 N, 121° 39 E). The grapevines were spaced 1.5 m within each row, and rows were spaced 3 m and set with a north-south orientation within the greenhouse. The entire vineyard was managed using the same fertilization, irrigation, pruning, and disease control procedures.

Thirty clusters of similar size fruit per variety were selected for experimental manipulation. Bagging treatments, which were performed at 45 days after anthesis (45 DAA, June 30th, 2017), comprised no bag (N) or the use of a white bag (W) or a shading light bag (S). The light permeability of each treatment is shown in Table 1. Grape berry samples were taken at 58 DAA (veraison stage): 72 DAA and 85 DAA (trans-chromic stage): and 91/93 DAA ('Shenhua'/'Shengfeng', mature stage). Grape berry maturity was determined based on the change in seed color from green to dark brown. At 85 DAA, the white and shading bags were removed (treatments WT and ST, respectively).

The juice of twelve grape berries for each treatment was extracted in a juicer and used to determine total soluble solid content (SSC) contents and titratable acidity (TAA). While SSC was determined using a hand refractometer (Master-M; Atago, Tokyo, Japan) and expressed as °Brix values, TAA was measured by titration with 0.1 N NaOH and expressed as a percentage (g tartaric acid/100 mL juice).

The remaining berries within each treatment were peeled with scalpel and forceps, and the cleaned skin tissues were immediately frozen in liquid nitrogen (N_2) and stored at -80 °C for further analyses.

Determination of fruit color

Fruit color was determined from fruits at the equatorial part of the grapevine using a hand-held C410 chroma meter (Konika-Minolta, Tokyo, Japan): the light source of which was set to D65 at an angle of 10°. The Commission Internationale de l'Eclairage (CIE) color indexes L* (brightness): a* (red-green color component): and b* (blue-yellow color component) were determined and used for calculating the color index of red grape (CIRG) based on the following equation:

CIRG = (180-H)/(L* + C*): with $C^* = \sqrt{a^{*2} + b^{*2}}$ and $H = \arctan b^*/a^*$.

Sugar analysis

The extraction of soluble sugars was performed according to the methods of Lu *et al*. (2011) with minor modifications. Briefly, soluble sugars were extracted from 3 g of frozen grape berry powder homogenized in 6 mL ethanol/water (4:1 v/v) at 35 °C for 20 min. The homogenate was centrifuged at 6500 ×*g* for 15 min, and the residues were re-extracted using the same procedure. The supernatants from the two extractions were mixed and brought up to 15 mL with distilled water. Thereafter, 1 mL of each extract was evaporated under vacuum at 35 °C, re-dissolved in 1 mL MilliQ water (MilliporeSigma, Burlington, MA, USA): and filtered through a 0.45-μm Millipore filter. The content of soluble sugars was determined by high-performance liquid chromatography (HPLC) using the Waters E2695 system (Waters, Milford, MA, USA): as described by Ding *et al*. (2002). The sugar contents were quantified using the following equations obtained from calibration curves: Glucose concentration (mg/g) = $5 \times 10^{-6} \times$ Area + 0.0601, with $r^2 = 0.9999$; Fructose concentration (mg/g) = $5 \times 10^{-6} \times$ Area - 0.0615, with $r^2 = 0.9942$.

Anthocyanin analysis

The extraction and measuring of anthocyanins was performed according to Xi *et al*. (2016). The HPLC protocol described by Ding *et al*. (2002) and the E2695 instrument (Waters) equipped with a 2998 photodiode array detector (PAD) were used. Anthocyanins were identified according to their retention time and to the molecular and ion fragment weights of their standards, as well as by comparison to previously published data (Liang *et al*., 2011, 2012; Xi *et al*., 2016, 2018). Total anthocyanin content was quantified using cyanidin 3-*O*-glucoside chloride (Sigma-Aldrich, St. Louis, MO, USA) as a standard and the following equation: concentration (mg/g) = $8 \times 10^{-5} \times$ Area + 4.2211, with $r^2 = 0.9918$.

Gene expression analyses

Total RNA was extracted from the skin of grape berries using an E.Z.N.A. Plant RNA Kit (Omega Bio-tek, Doraville, GA, USA). First-strand cDNA was synthesized using a Takara PrimeScript RT reagent Kit with gDNA Eraser (Takara, Dalian, China). Quantitative real-time PCR was performed using the LightCycler 480 System (Roche, Mannheim, Germany) and SYBR Premix Ex Taq II (Tli RNaseH Plus; Takara): according to the methods of Xi *et al*. (2016, 2018). Gene transcripts were quantified upon normalization to genes *VvEF1r* and *VvGAPDH* (Guillaumie *et al*., 2013) by using the comparative cycle threshold method ($2^{-\Delta ct}$). The normalized expression of genes was calculated using geNorm software (https://genorm.cmgg.be) following a method derived from the algorithms outlined by Vandesompele *et al*. (2002). All primer sequences were obtained from the literature and are listed in Table S1.

Statistical analysis

For each sampling period, the data from the three independent replicates of each treatment were expressed as the means ± standard deviation (SD): and analysed using SPSS v18.0 (SPSS Inc., Chicago, IL, USA).

Results

Color development of grape berries under different treatments

During the growing period, the color development of grape berries under treatment N was faster and more extensive than that under treatments W and S, as expected (Fig. S1 and S2). The bags used in W and S treatments were removed at 85 DAA. The skin color of 'Shenhua' and 'Shenfeng' grapes at maturity are shown in Fig. 1 and 2, respectively. Although the skin color of grape berries in the bagging treatments was less red and black than that in the N treatment, which tended to become more red and black 6 to 8 days after removing the bags.

A higher value of CIGR indicates a darker skin and, in 'Shenhua' at 91 DAA, the CIGR value of the S treatment was the lowest and that of the N treatment was the highest. However, no significant changes were detected among the CIGR values obtained for 'Shenfeng' grape berries subject to the different treatments at 93 DAA (Table 2).

Quality of grape berries under the different treatments

The fruit quality data for 'Shenhua' and 'Shenfeng' grape berries at maturity are shown in Table 3. The single fruit weight of 'Shenhua' and 'Shenfeng' grape berries showed no significant differences among the five treatments. In 'Shenhua', the SSC value under the S treatment was the lowest. Although there were no significant differences in SSC values under the WT and W treatments, values under the ST treatment were significantly higher than under the S treatment. In 'Shenfeng', the SSC value was highest under the N treatment, although this value differs significantly from that under the other four treatments. Furthermore, there were no significant differences in the TAA of 'Shenhua' grape berries among the five treatments. In 'Shenfeng', the TAA under W and S treatments was significantly higher than that under the other three treatments. The single fruit weights, SSC, and TAA of grape berries during the growth period are shown in Table S2.

Table 1. Light permeability in the different bagging treatments

	Photosynthetic active radiation (μmol/m²/s)		Light permeability (%)
	Before bagging	After bagging	
No bag	-	-	100
White bag	1091	265	24.3
Shading bag	1108	0	0

Table 2. Values of the CIGR index obtained for grape berries skin under the different treatments

Treatment[x]	CIGR	
	'Shenhua'(91 DAA)	'Shenfeng'(93 DAA)
N	5.09 ± 0.66a	5.28 ± 0.92a
W	4.14 ± 0.55b	4.85 ± 0.77a
S	3.92 ± 0.47b	4.62 ± 0.66a
WT	4.82 ± 0.54ab	4.92 ± 0.87a
ST	4.76 ± 0.37ab	5.30 ± 0.50a

[x]N: no bag treatment; W: white bag treatment; S: shading bag treatment; WT: white bag removal treatment; ST: shading bag removal treatment
Different lower case letters indicate significant differences ($p < 0.05$)

Fig. 1. Grains (A) and spikes (B) of 'Shenhua' grape berries at 91 DAA. N: no bag treatment; W: white bag treatment; S: shading bag treatment; WT: white bag removal treatment; S: shading bag removal treatment (Bar=1 cm)

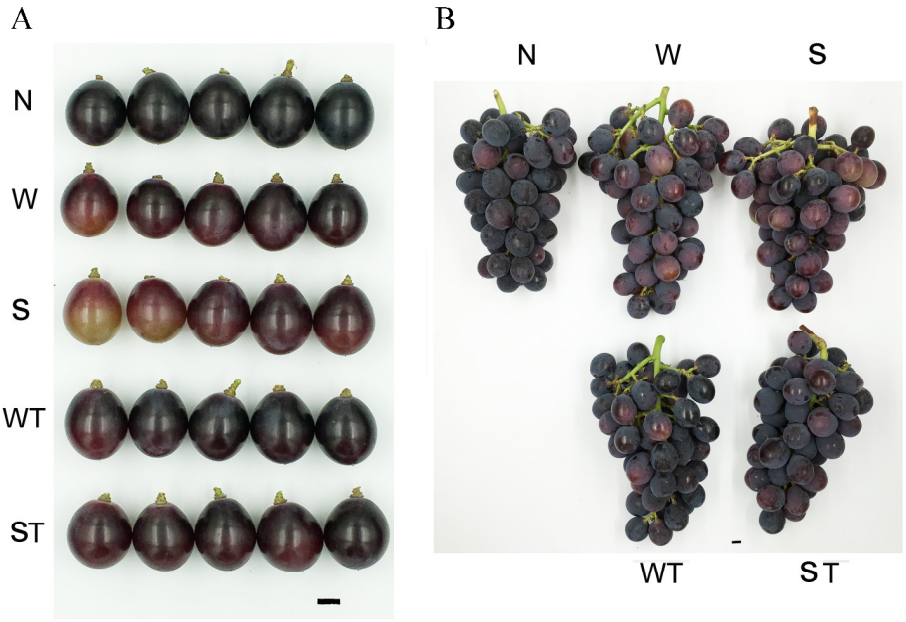

Fig. 2. Grains (A) and spikes (B) of 'Shenfeng' grape berries at 93 DAA. N: no bag treatment; W: white bag treatment; S: shading bag treatment; WT: white bag removal treatment; S: shading bag removal treatment (Bar=1 cm)

For both 'Shenhua' and 'Shenfeng' grape berries, there were no significant differences in single fruit weight among the different treatments from 58 DAA to 85 DAA. However, the SSC of grape berries from both varieties was significantly higher under treatment N from 58 DAA to 85 DAA than under the other treatments. Notably, in grape berries of both varieties, the TAA of the fruits subject to bagging treatments was sometimes higher than that of fruits under the N treatment.

The sugar and anthocyanins of grape berries under different treatments

Fructose, glucose, and anthocyanin contents measured in grape berries subject to the N, W, S, WT, and ST treatments are displayed in Fig. 3A, B, D, and E. No significant changes were detected in the sugar (fructose and glucose) contents of both 'Shenhua' and 'Shenfeng' grape berries among the different treatments from 58 DAA to 72 DAA. However, the sugar contents of grape berries under

the N treatment were higher than those of grape berries under the W and S treatments at 85 DAA. In 'Shenhua' at 91 DAA, sugar contents were significantly higher in grape berries under the WT treatment than in grape berries under the W, S, and ST treatments, whereas in 'Shenfeng' at 93 DAA, the sugar contents of grape berries under treatments W and S were significantly lower than those of grape berries under the N treatment. Although the anthocyanin contents of 'Shenhua' and 'Shenfeng' grape berries showed no changes at 58 DAA, which of grape berries under treatment N was significantly higher than that of grape berries under treatments W and S from 72 DAA to 91/93 DAA. Furthermore, we found that the anthocyanin contents under treatments WT and ST where higher than that under the W and S treatments, respectively. In both 'Shenhua' and 'Shenfeng' cultivars, the anthocyanin concentrations in grape berry skin throughout the ripening period were considerably lower under bagging treatments than under the N treatment (Fig. 3C,F). In 'Shenhua' grape berry skin, the amount of anthocyanin at maturity was higher under treatment N [4 mg/g fresh weight (FW)] than under treatments W (1.2 mg/g FW) and S (0.4 mg/g FW). The amounts of anthocyanin under treatments WT (3.0 mg/g FW) and ST (1.6 mg/g FW) were higher than under treatments W and S, respectively.

The amounts of cyanidin, delphinidin, malvidin, peonidin, and petunidin derivatives were significantly higher under treatment N than under the other four treatments. Similarly, the amounts of these five anthocyanin derivatives under treatments WT and ST were higher than those under treatments W and S, respectively (Fig. 4A). At maturity, the amount of anthocyanin in 'Shenfeng' grape berries under treatment N (10 mg/g FW) was higher than that under treatments W (3.5 mg/g FW) and S (1.4 mg/g FW). In addition, the amounts of anthocyanin under treatments WT (4.4 mg/g FW) and ST (4.5 mg/g FW) were higher than those under treatments W and S, respectively. Moreover, the amounts of cyanidin, delphinidin, malvidin, and peonidin derivatives under treatment N were significantly higher than those under the other four treatments, and the amounts of the derivatives of these four anthocyanins were higher under treatment ST than those under treatment S (Fig. 4B). In 'Shenhua' grape berries at 91 DAA, the percentages of cyanidin, petunidin, and peonidin under treatments W and S were higher than those under treatment N, but the percentages of delphinidin and malvidin under treatments W and S were lower than those under treatment N (Table 4). In 'Shenfeng' grape berries at 93 DAA, the percentages of malvidin and peonidin under treatments W and S were higher than those under treatment N, but the percentages of cyaniding, delphinidin, and petunidin under treatments W and S were higher than those under treatment N.

Table 3. Quality of grape berries under the different treatments at 91/93 DAA

Variety	Treatment[x]	Single fruit weight (g)	SSC (%)	TAA (%)
'Shenhua' (91 DAA)	N	13.60 ± 0.69a	17.53 ± 0.06a	1.35 ± 0.04a
	W	13.93 ± 0.50a	16.10 ± 0.10bc	1.33 ± 0.11a
	S	13.60 ± 0.53a	15.83 ± 0.06c	1.43 ± 0.10a
	WT	13.87 ± 0.42a	16.67 ± 0.06b	1.36 ± 0.10a
	ST	13.53 ± 0.64a	16.23 ± 0.38b	1.50 ± 0.04a
'Shenfeng' (93 DAA)	N	10.73 ± 0.81a	19.63 ± 0.06a	1.25 ± 0.06b
	W	12.07 ± 0.12a	16.50 ± 0.10b	1.40 ± 0.04a
	S	11.33 ± 0.50a	16.93 ± 0.32b	1.38 ± 0.02a
	WT	12.20 ± 0.20a	16.90 ± 0.30b	1.30 ± 0.04b
	ST	11.33 ± 0.81a	17.10 ± 0.10b	1.28 ± 0.08b

[x]N: no bag treatment; W: white bag treatment; S: shading bag treatment; WT: white bag removal treatment; ST: shading bag removal treatment
Different lower case letters indicate significant differences ($p < 0.05$)

Table 4. Percentages of the five anthocyanin derivatives in grape berries under the different treatments at 91/93 DAA

Variety	Derivative[x]	Treatment[y]				
		N	W	S	WT	ST
'Shenhua' (91 DAA)	CY	14.15	11.51	9.45	11.69	11.29
	DP	7.845	7.71	7.35	5.95	7.26
	MV	64.02	63.87	61.86	69.75	66.53
	PN	9.83	10.14	12.38	8.06	8.77
	PT	4.16	6.76	8.98	4.55	6.21
'Shenfeng' (93 DAA)	CY	15.13	12.37	9.03	12.33	14.24
	DP	6.89	5.24	3.60	4.22	6.34
	MV	72.83	76.79	82.79	80.05	74.43
	PN	4.91	5.60	4.58	3.40	5.00
	PT	0.24	0	0	0	0

[x] CY: cyanidin derivatives; DP: delphinidin derivatives; MV: malvidin derivatives; PN: peonidin derivatives; PT: petunidin derivatives.
[y] N: no bag treatment; W: white bag treatment; S: shading bag treatment; WT: white bag removal treatment; ST: shading bag removal treatment.

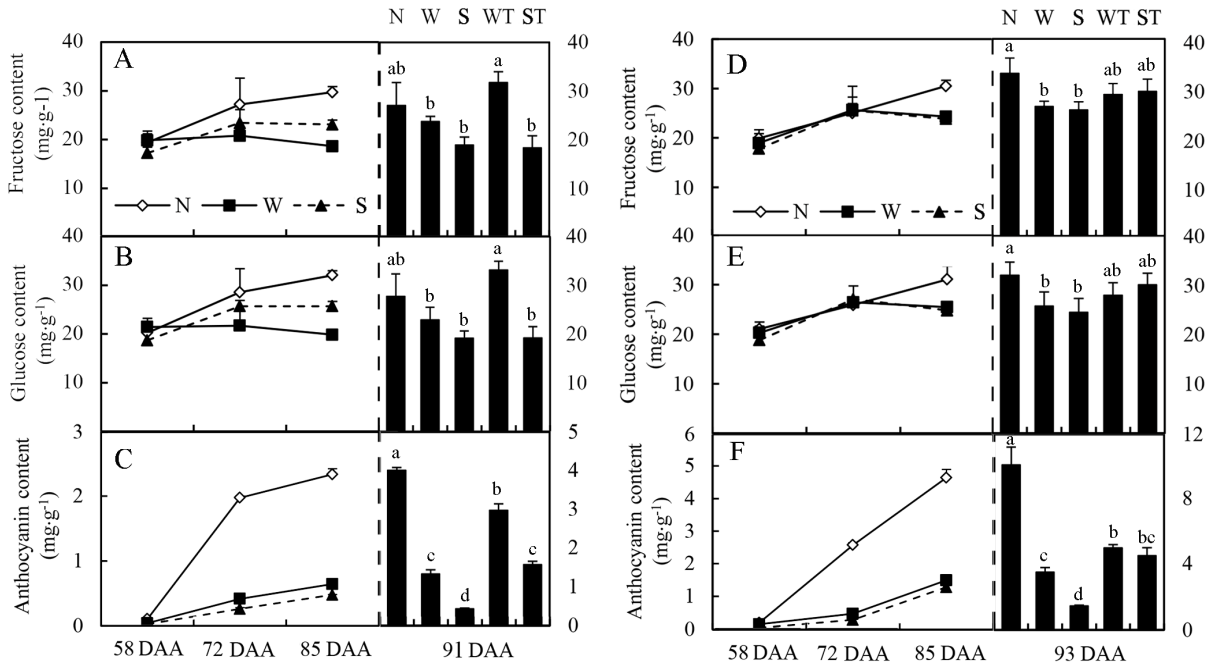

Fig. 3. Fructose, glucose, and anthocyanin contents of 'Shenhua' (A, B, C, respectively) and 'Shenfeng' (D, E, F, respectively) grape berries under the different treatments. N: no bag treatment; W: white bag treatment; S: shading bag treatment; WT: white bag removal treatment; S: shading bag removal treatment. Data are means ± SD of three biological replicates. Different letters denote a statistically significant difference at $p < 0.05$

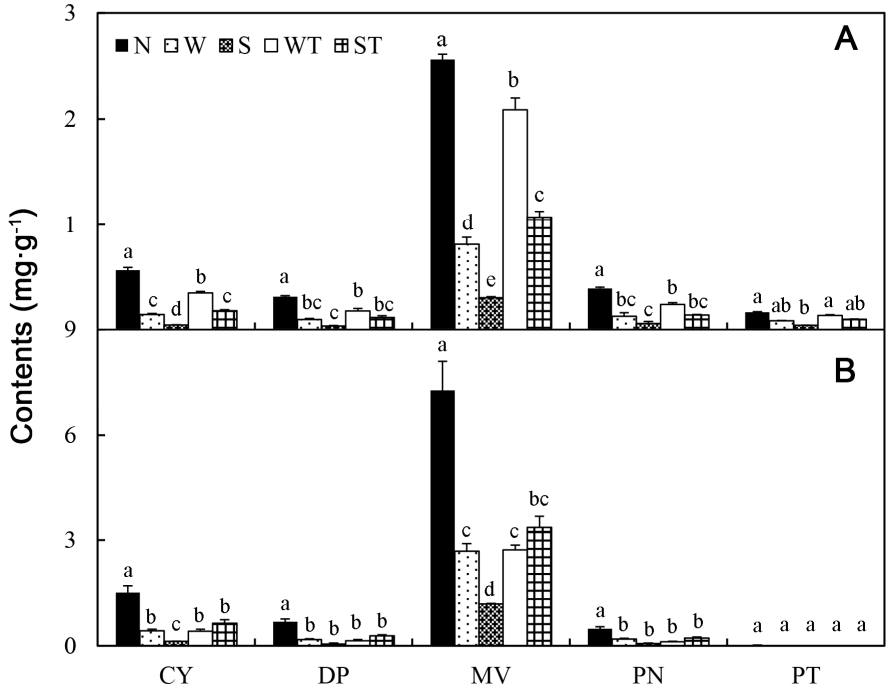

Fig. 4. The contents of the five anthocyanin derivatives in 'Shenhua' (A) and 'Shenfeng' (B) grape berries at 91 DAA and 93 DAA, respectively, under the different treatments in grapevine. CY: cyanidin derivatives; DP: delphinidin derivatives; MV: malvidin derivatives; PN: peonidin derivatives; PT: petunidin derivatives. N: no bag treatment; W: white bag treatment; S: shading bag treatment; WT: white bag removal treatment; ST: shading bag removal treatment. Data are means ± SD of three biological replicates. Different letters denote a statistically significant difference in anthocyanin derivative contents at $p < 0.05$.

The gene expression of grape berries under different treatments

During the growth period, we detected changes in selected sugar metabolism-related genes in grape berry skin (*VvAIN1/2*, *VvNI*, *VvSPS*, and *VvSS*) among the different treatments (Fig. 5). In 'Shenhua', the expression of *VvAIN1/2* under treatment S was higher than that under the other treatments from 58 DAA to 72 DAA. Similarly, in 'Shenfeng', the expression of *VvAIN2* under treatment S was higher than that under the other treatments from 58 DAA to 93 DAA. In 'Shenhua', at 85 DAA, the expression of *VvNI* under treatment N was significantly higher than that under the other treatments, whereas in 'Shenhua', at 93 DAA, the expression of *VvNI* under treatment N was significantly higher than that under the other treatments. In 'Shenhua', at 91 DAA, the expressions of *VvSPS* and *VvSS* under treatments N, WT, and ST was higher than those under treatments W and S, whereas in 'Shenfeng', at 93 DAA, the expression of the same two genes was lower under treatment S than under the other treatments.

In 'Shenhua', at 58 DAA, the expressions of *VvFLS4* and *VvMYBF1* under treatment N were significantly higher than those under treatments W and S (Fig. 6). The expressions of *VvCHS3*, *VvCHI1*, *VvCHI2*, *VvMYBPA1*, and *VvMYB5a* under treatment N were significantly higher than those under treatments W and S at 72 DAA, whereas the expression of *VvMYBPA1* under treatment N was significantly higher than that under the other treatments at 85 DAA. The expressions of *VvCHS2*, *VvF3H1*, *VvDFR*, *VvGST*, and *VvMYBF1* under treatment N were higher than those under the other treatments at 91 DAA.

In 'Shenfeng', the expressions of *VvCHS2*, *VvCHS3*, *VvCHI2*, *VvF3'5'H*, *VvF3H1*, *VvF3H2*, *VvMATE*, *VvDFR*, *VvOMT*, *VvFLS4*, *VvMYBPA1*, *VvMYBA1*, *VvMYBF1*, *VvMYB4*, and *VvMYB5b* under treatment N were significantly higher than those under treatments W and S at 58 DAA (Fig. 7). The expressions of *VvF3H2*, *VvMATE*, *VvGST*, *VvFLS4*, *VvMYBF1*, *VvMYB5a*, and *VvMYB5b* under treatment N were significantly higher than those under treatments W and S at 72 DAA. The expressions of *VvCHS2*, *VvF3'5'H*, *VvF3H1*, *VvF3H2*, *VvGST*, *VvUFGT*, *VvFLS4*, *VvMBYPA1*, and *VvMYB5b* under treatment N were significantly higher than those under the other treatments at 85 DAA. The expressions of *VvCHS2*, *VvF3H1*, *VvDFR*, and *VvFLS4* under treatments N, WT, and ST were higher than those under treatments W and S at 93 DAA.

The expression of *VvHY5* was lower under bagging treatments from 58 DAA-85 DAA in 'Shenhua' and from 58 DAA-72 DAA in 'Shenfeng' than under the N treatment, which was opposite to the expression pattern of *COP1*. The expressions of *VvHY5* and *VvCOP1* also showed opposite trends in 'Shenhua' and 'Shenfeng' at 91/93 DAA (Fig. 8).

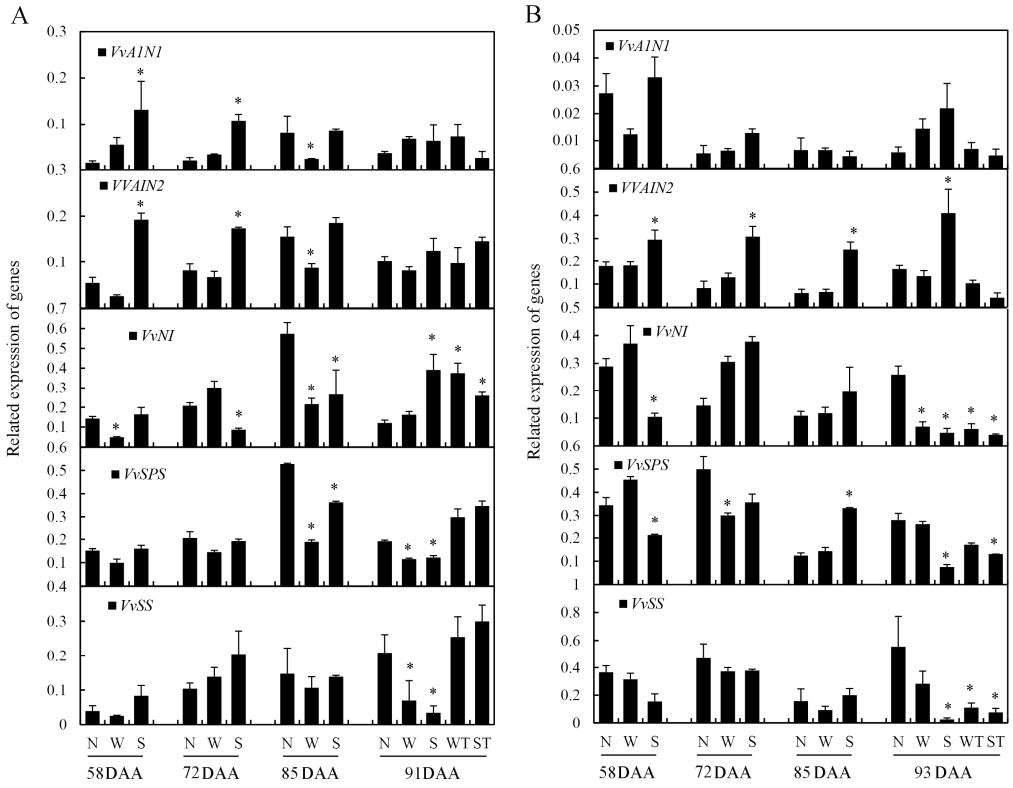

Fig. 5. Expression profiles of sugar metabolism-related genes in 'Shenhua' (A) and 'Shenfeng' (B) grape berries under the different treatments. N: no baag treatment; W: white bag treatment; S: shading bag treatment; WT: white bag removal treatment; S: shading bag removal treatment. Data are means ± SD of three biological replicates. Asterisks (*) denote a statistically significant difference at $p < 0.05$ between bagged/bag-removed treatments and treatment N

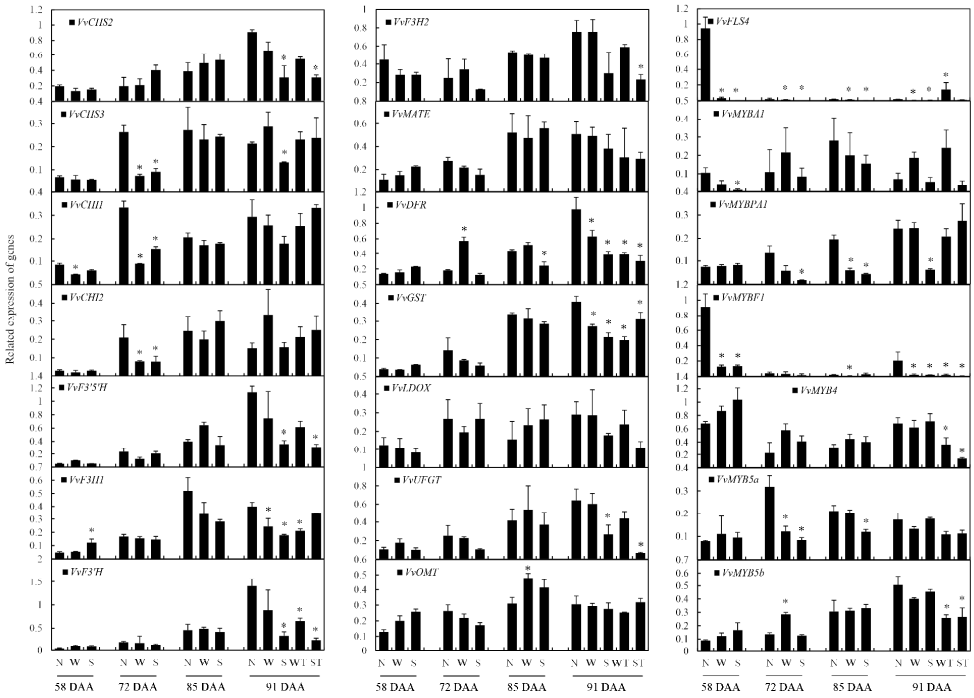

Fig. 6. Expression profiles of anthocyanin metabolism-related genes in 'Shenhua' grape berries under the different treatments. N: no bag treatment; W: white bag treatment; S: shading bag treatment. WT: white bag removal treatment; S: shading bag removal treatment. Data are means ± SD of three biological replicates. Asterisks (*) denote a statistically significant difference at $p < 0.05$ between bagged/bag-removed treatments and treatment N

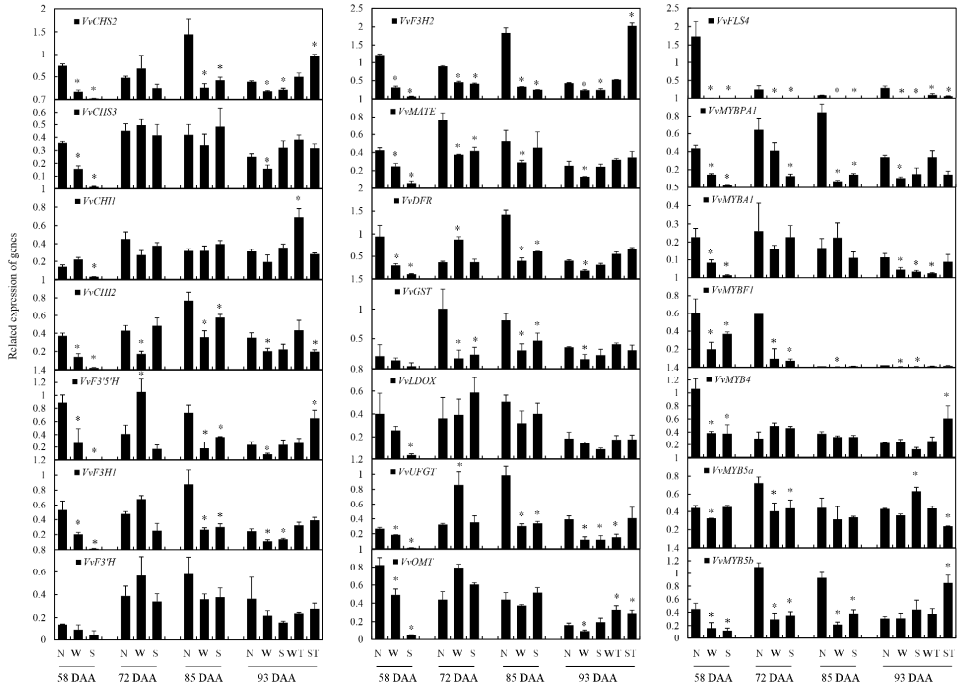

Fig. 7. Expression profiles of anthocyanin metabolism-related genes in 'Shenfeng' grape berries under the different treatments. N: no bag treatment; W: white bag treatment; S: shading bag treatment; WT: white bag removal treatment; S: shading bag removal treatment. Data are means ± SD of three biological replicates. Asterisks (*) denote a statistically significant difference at $p < 0.05$ between bagged/bag-removed treatments and treatment N

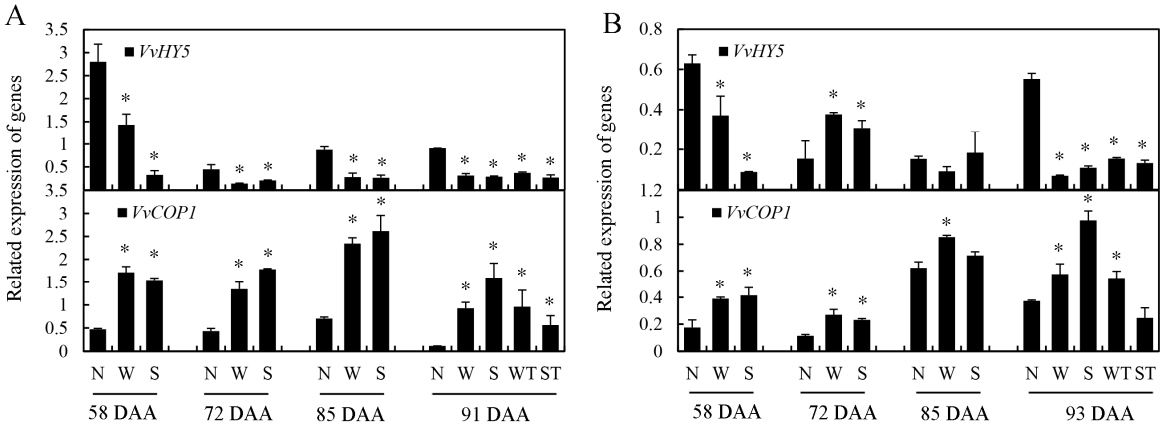

Fig. 8. Expression profiles of *HY5* and *COP1* genes in 'Shenhua' (A) and 'Shenfeng' (B) grape berries under the different treatments. N: no bag treatment; W: white bag treatment; S: shading bag treatment. WT: white bag removal treatment; ST: shading bag removal treatment. Asterisks (*) denote a statistically significant difference at $p < 0.05$ between bagged/bag-removed treatments and treatment N

Discussion

The ripening of 'Shenhua' and 'Shenfeng' grape berries in China occurs mainly in August, which results in a short and specific commercialization time with negative implications for producers. In general, different management practices are used to prolong the grape ripening period, such as early sealing of facilities, treatment with growth regulators, and fruit bagging. Fruit bagging may, however, impose low-light stress on grapevines (Hong *et al*., 2015). In the present study, we used two types of bags to examine the effects of fruit bagging: one fabricated with material designed to prevent the transmission of all light, and the other consisting of a material that allowed transmission of approximately 30% of incident light. We found that SSC contents of grape berries were lower under light stress than under normal conditions and that pigment development tended to be delayed under low-light stress conditions. These observations indicated that low light and light exclusion treatments would affect the ripening of grape berries, which is consistent with the findings of previous studies (Keller *et al*., 1998; Fang *et al*., 2015; Karanjalker *et al*., 2018). The maturation of grape berries during the growth stage can thus be delayed by bagging treatments. We also found that delayed ripening is associated with changes in sugar and anthocyanin contents. Thus, we carried out in-depth analyses of the contents of sugars and anthocyanins and related gene expression, in order to provide a theoretical basis for explaining the effects of light stress on the ripening of grape berries.

We found that bag removal treatments performed 6 to 8 days before fruit ripening can effectively control fruit ripening, which highlights two scientific considerations. Firstly, fruit ripening is closely related to light, which has similarly been reported in apple and other fruit trees (Smart *et al*. 1988; Keller *et al*. 1998; Merzlyak and Chivkunova, 2000). Secondly, cultivation measures such as bagging and bag removal can, to a certain extent, enable producers to control the ripening period of grapes, determine the period when grapes are sold, and improve fruit quality (Sharma *et al*., 2014a, 2014b, 2018).

The accumulation of sucrose in grape berries is mainly dependent on leaf metabolism, phloem transport, and berry metabolism (Coombe, 1992; Lecourieux *et al*., 2013). Sucrose can be broken down into glucose and fructose by acidic invertase (AI) and neutral invertase (NI): and into guanosine diphosphate glucose. Fructose in turn can be catalyzed by sucrose synthase (SS): and guanosine diphosphate glucose and fructose-6-phosphate are catalyzed by sucrose phosphate synthase (SPS) to generate sucrose-6-phosphate. Thus, glucose and fructose are the main sugar components in grape berries (Coombe, 1989; Liu *et al*., 2006). In the present study, we found that the accumulation of glucose and fructose during the pre-growth stages of 'Shenhua' and 'Shenfeng' grape berries was not significantly different among the different experimental treatments. During the maturation period, sugar accumulation in 'Shenhua' grape berries was still not significantly related to light stress treatment, whereas bagging treatments significantly affected the accumulation of glucose and fructose in 'Shenfeng' grape berries. The different responses of these two varieties can be related to their specific characteristics.

Under the light-exclusion treatment, expression of the *AIN1/2* genes in 'Shenhua' and 'Shenfeng' grape berries was relatively high at some time points, which indicated that the fruit increased sugar storage by increasing enzyme activity. The skin of green grape berries contains photosynthetic products to support the fruit's own growth and development (Yen and Koch, 1990). However, the expression of *NI* and *SPS* at 85 DAA and the expression of *SPS* and *SS* at 91 DAA in 'Shenhua' indicated that the expression of sugar metabolism-related genes is not directly related to light stress.

In this study, we used two types of bags with different degrees of light transmission. We found that grape berries from both varieties developed color regardless of whether they were exposed to weak light or no light at all, contrasting

with previous observations on eggplants (Jiang *et al*., 2016): which are entirely dependent on light reception for the development of fruit color. Zheng *et al*. (2013) studied two red Chinese *Vitis vinifera* grape cultivars, namely 'Jingxiu' and 'Jingyan', and found that these were sunlight-dependent and sunlight-independent, respectively. Light exclusion suppresses anthocyanins in the skin of 'Jingxiu' grape berries, but does not change the proportion of the various anthocyanins in the skin of 'Jingyan' grape berries. Based on the results of the present study, we hypothesize that 'Shenhua' and 'Shenfeng' are sunlight-independent varieties that can accumulate sufficient anthocyanins to produce red skin coloration even in the absence of light. However, we observed inconsistencies in the expression of structural genes in the anthocyanin biosynthesis pathway of these two cultivars under the different treatments. This indicates that the expression of structural genes might be light independent, which would be consistent with the findings of Zheng *et al*. (2013). In addition, the expressions of *VvCHS2*, *VvF3H1*, and *VvDFR* at 91/93 DAA were higher under treatment N than under treatments W, S, WT, and ST, although no significant differences were detected between the W/S and WT/ST treatments. In several other fruits, including apple and pear, it has been demonstrated that structural genes involved in anthocyanin biosynthesis are not expressed in darkness, but their expression can be enhanced by sunlight (Kim *et al*., 2003; Zhang *et al*., 2011; Zheng *et al*., 2013).

There are five major types of anthocyanin derivatives in grape berry skin (Xi *et al*., 2016, 2018): and the data obtained here (Table 4) revealed that anthocyanin derivatives composition varied greatly among the grape berries from the two varieties. These derivatives may be positively correlated with light in one variety, but be negatively correlated with light in another. Thus, the relationships between light and individual anthocyanin derivatives were worthy of further analyses.

The expressions of the regulatory genes *VvMYBF1*, *VvMYB5a*, and *VvMYBPA1* at 58 DAA, 72 DAA, and 85 DAA, respectively, were higher under treatment N than under the other treatments. Thus, before the maturation period, the expression of these genes might be light related. In this regard, we examined the relationship among light, anthocyanin biosynthesis, and the transcript levels of *MYB* genes. It has been shown that *MYB* genes determine the light-induced phenotype of petunia (Albert *et al*., 2009): whereas in maize, these genes play an important role in the regulation of anthocyanin biosynthesis in response to different light qualities (Procissi *et al*., 1997; Piazza *et al*., 2002) and sunlight (Takos *et al*., 2006; Feng *et al*., 2014). These responses probably involve light-responsive genes, such as *HY5* and *COP1*, and the flavonoid biosynthesis pathway. *HY5* and *COP1* are downstream signal elements of photoreceptors and are widely involved in photo-morphogenesis and in the regulation of anthocyanin synthesis (Ang *et al*., 1998; Chattopadhyay *et al*., 1998). Under light conditions, *HY5* can activate the expression of genes related to the anthocyanin synthesis pathway, particularly *CHS*, *DFR*, and leucoanthocyanidin dioxygenase (*LDOX*) (Schulze-lefert *et al*., 1989; Gollop *et al*., 2001, 2002). Conversely, under dark conditions, *VvCOP1* can ubiquitinate and degrade the HY5 protein, thereby inhibiting the synthesis of anthocyanins (Lau and Deng, 2012).In the present study, the expressions of *VvHY5* and *VvCOP1* showed opposite patterns, being higher and lower under the N treatment than under the other treatments, respectively. Accordingly, *VvHY5* seems to be a light-responsive factor that plays a positive role in the accumulation of anthocyanins in grape berry skin, whereas *VvCOP1* has the opposite effect.

Conclusions

Bagging significantly affects the metabolism of sugars and anthocyanins in 'Shenhua' and 'Shenfeng' grape berries. Bagged berries contained lower contents of soluble sugars than un-bagged berries; however, the reduced contents could be rapidly restored to normal levels following bag removal. The skin of bagging grape berries were poorly colored and had lower contents of anthocyanins than that of un-bagging grape berries. Furthermore, anthocyanin anabolism was affected by the expression of *VvMYB* genes and by the light-response factors *VvHY5* and *VvCOP1*.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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Supplementary files

Table S1. Primers used for the quantification of gene expression levels by qRT-PCR

Gene name	Primer	Sequence (5′→3′)	Reference
CHS1	F	CAGGCAGACTACCCGGATT	Wang et al., 2013
	R	ACAGACGTTGGGGTTCTCC	
CHS2	F	GAAGATGGGAATGGCTGCTG	Jeong et al., 2004
	R	AAGGCACAGGGACACAAAAG	
CHS3	F	TCGGCTGAGGAAGGGCTGAA	Jeong et al., 2004
	R	GGCAAGTAAAGTGGAAACAG	
CHI1	F	CAGGCAACTCCATTCTTTTC	Jeong et al., 2004
	R	TTCTCTATCACTGCATTCCC	
F3H1	F	CCAATCATAGCAGACTGTCC	Jeong et al., 2004
	R	TCAGAGGATACACGGTTGCC	
F3H2	F	CTGTGGTGAACTCCGACTGC	Jeong et al., 2004
	R	CAAATGTTATGGGCTCCTCC	
F3'H	F	GCCTCCGTTGCTGCTCAGTT	Jeong et al., 2006
	R	GAGAAGAGGTGGACGGAGCAAATC	
F3'5'H	F	AAACCGCTCAGACCAAAACC	Jeong et al., 2006
	R	ACTAAGCCACAGGAAACTAA	
DFR	F	GAAACCTGTAGATGGCAGGA	Jeong et al., 2004
	R	GGCCAAATCAAACTACCAGA	
LDOX	F	AGGGAAGGGAAAACAAGTAG	Jeong et al., 2004
	R	ACTCTTTGGGGATTGACTGG	
UFGT	F	GGGATGGTAATGGCTGTGG	Jeong et al., 2004
	R	ACATGGGTGGAGAGTGAGTT	
OMT	F	GTTCAACTTCATGAGATGGA	Azuma et al., 2009
	R	GGAGAACTACCTCAACTACCA	
GST	F	ACTTGGTGAAGGAAGCAGGA	Terrier et al., 2005
	R	CAGCGAGCTCCATGACTTTT	
MATE	F	GCAAACAACAGAGAGGATGC	Cutanda-Perez et al., 2009
	R	AGACCTCGACAATGATCTTAC	
MYB5a	F	GTGCAGCAGCCATCTAATGTG	Matus et al., 2009
	R	GCAGCAGGTTCCCAGACAGT	
MYB5b	F	GGTGTTCTTTAATTTGGCTTCA	Deluc et al., 2008
	R	CACAACAACACAACCACATACA	
MYBPA1	F	CATGCACGTGCTCACCTT	Azuma et al., 2012
	R	CCGCACGTATCGCTATTATAAG	
MYBA1	F	TAGTCACCACTTCAAAAAGG	Jeong et al., 2004
	R	GAATGTGTTTGGGGTTTATC	
MYB4	F	ACCGGACGTTACAACCATATC	Matus et al., 2008
	R	TCCGTAACTGGGTTTTTCTCA	
COP1	F	AGGAGGTTTCAACGGGTGC	This study
	R	TAGGGCAGAGCGAGTCTTTATC	
HY5	F	CCGGCTGACAAAGAGAACAA	This study
	R	CTTCCTTTCCCTTGCTTGCT	
VvAIN1	F	CCATCTCCATCCCATCGTAACC	Zhu et al., 2017
	R	GGCTATCCAAGTTTCCAACCAACC	
VvAIN2	F	GAGCACAGTTCCAGTAATCAAAGG	Zhu et al., 2017
	R	GTGAGGCGTAGTTTTAGGACTCC	
VvNI	F	GGCTTGGGAAGAGGACTATG	Zhu et al., 2017
	R	GTTGCCTAAACGACGGTAAAT	
VvSPS	F	ACGCTGGGCTGCTTCTAC	Zhu et al., 2017
	R	AGGGGATCAATTCTGGTTTC	
VvSS	F	CTGGGGTTTATGGGTTCTG	Zhu et al., 2017
	R	AATGCCTCTGCCTTTTAGC	
VvEF1r	F	CAAGAGAAACCATCCCTAGCTG	Guillaumie et al., 2013
	R	TCAATCTGTCTAGGAAAGGAAG	
VvGAPDH	F	TTCCGTGTTCCTACTGTTG	Guillaumie et al., 2013
	R	CCTCTGACTCCTCCTTGAT	

Supplementary files

Table S2. The fruit quality of grapevine under different treatments

Variety	Sampling time	Treatments	Single fruit weight (g)	TSS (%)	TAA (%)
'Shenhua'	58 DAA	N	11.53 ± 0.61a	13.70 ± 0.10a	3.70 ± 0.11a
		W	11.40 ± 1.00a	11.73 ± 0.12b	3.98 ± 0.27a
		S	11.73 ± 0.23a	11.73 ± 0.12b	3.90 ± 0.08a
	72 DAA	N	13.53 ± 0.50a	16.53 ± 0.06a	1.73 ± 0.07b
		W	12.93 ± 0.42a	16.00 ± 0.17b	1.85 ± 0.19ab
		S	11.67 ± 0.58a	13.67 ± 0.06c	2.05 ± 0.11a
	85 DAA	N	12.00 ± 0.40a	16.97 ± 0.06a	1.41 ± 0.02a
		W	12.07 ± 0.70a	16.23 ± 0.06b	1.39 ± 0.04a
		S	12.67 ± 0.31a	15.23 ± 0.21c	1.38 ± 0.11a
	91 DAA	N	13.60 ± 0.69a	17.53 ± 0.06a	1.35 ± 0.04a
		W	13.93 ± 0.50a	16.10 ± 0.10bc	1.33 ± 0.11a
		S	13.60 ± 0.53a	15.83 ± 0.06c	1.43 ± 0.10a
		WT	13.87 ± 0.42a	16.67 ± 0.06b	1.36 ± 0.10a
		ST	13.53 ± 0.64a	16.23 ± 0.38b	1.50 ± 0.04a
'Shenfeng'	58 DAA	N	9.67 ± 0.12a	12.17 ± 0.06a	4.95 ± 0.13a
		W	8.13 ± 0.42a	11.43 ± 0.06b	4.75 ± 0.04a
		S	9.60 ± 0.20a	11.43 ± 0.06b	4.03 ± 0.04b
	72 DAA	N	10.33 ± 0.70a	17.97 ± 0.06a	2.25 ± 0.20a
		W	10.27 ± 0.12a	16.43 ± 0.06b	2.23 ± 0.04a
		S	10.33 ± 0.31a	15.60 ± 0.17c	2.15 ± 0.04a
	85 DAA	N	10.80 ± 0.35a	18.10 ± 0.03a	1.71 ± 0.23a
		W	11.80 ± 0.72a	16.43 ± 0.06b	1.51 ± 0.11b
		S	11.33 ± 0.23a	16.17 ± 0.06c	1.50 ± 0.10b
	93 DAA	N	10.73 ± 0.81a	19.63 ± 0.06a	1.25 ± 0.06b
		W	12.07 ± 0.12a	16.50 ± 0.10b	1.40 ± 0.04a
		S	11.33 ± 0.50a	16.93 ± 0.32b	1.38 ± 0.02a
		WT	12.20 ± 0.20a	16.90 ± 0.30b	1.30 ± 0.04b
		ST	11.33 ± 0.81a	17.10 ± 0.10b	1.28 ± 0.08b

Different lower case letters indicate significant differences ($P < 0.05$)

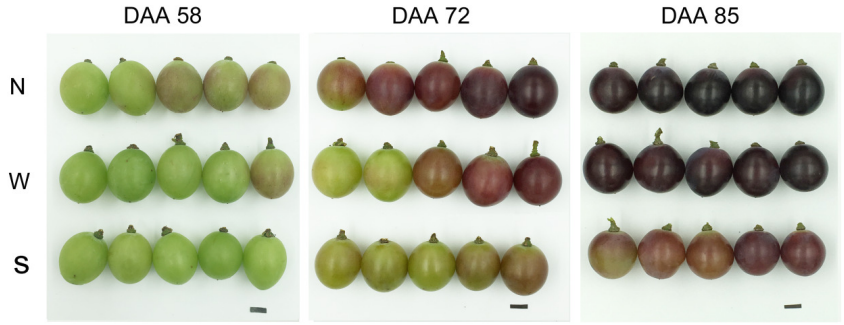

Fig. S1. Fruit grains of 'Shenhua' from 58 DAA to 85 DAA. N: no bag treatment; W: white bag treatment; S: shading bag treatment (Bar=1 cm)

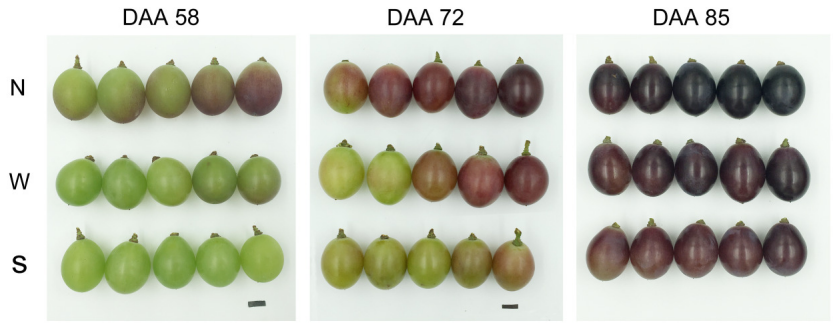

Fig. S2. Fruit grains of 'Shenfeng' from 58 DAA to 85 DAA. N: no bag treatment; W: white bag treatment; S: shading bag treatment (Bar=1cm)