J Plant Biotechnol (2022) 49:292-299
DOI:https://doi.org/10.5010/JPB.2022.49.4.292

ISSN 1229-2818 (Print)
ISSN 2384-1397 (Online)

Research Article

Light modulates the transcriptomic accumulation of anthocyanin biosynthetic pathway genes in red and white grapes

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Received: 27 October 2022 / Revised: 1 December 2022 / Accepted: 3 December 2022

Abstract Anthocyanin, an important component in the grape berry skin, strongly affects grape quality. The transcription factors *VvMYBA1* and *VvMYBA2* (*VvMYBA1/2*) control anthocyanin biosynthesis. In addition, cultivation and environmental factors, such as light, influence anthocyanin accumulation. The present study aimed to clarify the effect of shading (reduced light condition) on the transcriptomic regulation of anthocyanin biosynthesis using a red-wine grape cultivar, *Vitis vinifera* 'Pinot Noir', and its white mutant, 'Pinot Blanc', caused by the deletion of the red allele of *VvMYBA1/2*. The grape berry skins were analyzed for anthocyanin content and global gene transcription accumulation. The microarray data were later validated by quantitative real-time PCR. A decisive influence of *VvMYBA1/2* on the expression of an anthocyanin-specific gene, UDP glucose: flavonoid 3-O-glucosyltransferase, was observed as expected. In contrast, upstream genes of the pathway, which are shared by other flavonoids, were also expressed in 'Pinot Blanc', and the mRNA levels of some of these genes decreased in both cultivars on shading. Thus, the involvement of light-sensitive transcription factor(s) other than *VvMYBA1/2* was suggested for the expression control of the upstream genes of the anthocyanin biosynthetic pathway. Furthermore, it was suggested that the effects of these factors are different among isogenes.

Keywords flavonoids, gene expression, shading, *VvMYBA*s

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Introduction

The quality of grape and grape products such as juice and wine largely depends on the amount and composition of flavonoid phenolics, i.e., anthocyanins, proanthocyanidins and flavonols, in the grape berries. Proanthocyanidins, or condensed tannins, contribute to astringency, whereas anthocyanins are responsible for their color. The accumulation of anthocyanins in grape berry skins starts from veraison and increases gradually until just before full maturity. On the other hand, the proanthocyanidins start to accumulate soon after the berry formation and decline during ripening (Downey et al. 2003a). Another flavonoid group, flavonols, is synthesized around flowering and mainly during ripening in the skin (Downey et al. 2003b). These flavonoid compounds are synthesized through the multi-step phenylpropanoid pathway (Fig. 1). The expression of structural genes in the phenylpropanoid pathway is controlled by transcription factors. For example, the expression of the genes specific for anthocyanin biosynthesis such as *UDP-glucose: flavonoid 3-O-glucosyltransferase* (*VvUFGT*) is regulated by the *MYB* transcription factors, i.e. *VvMYBA1* and *VvMYBA2* (*VvMYBA1/2*) (Azuma et al. 2008). In addition, it was also reported that a series of structural genes of the anthocyanin pathway are systematically expressed in red color grapes (Ageorges et al. 2006; Kobayashi et al. 2001; Poudel et al. 2021). Likewise, genes involved in proanthocyanidins biosynthesis are regulated by the transcription factors such as *VvMYB5a*, *VvMYB5b*, *VvMYBPA1*, *VvMYBPA2* and *VvMYBPAR* (Bogs et al. 2007; Deluc et al. 2006, 2008; Koyama et al. 2014; Terrier et al. 2009), while *VvMYBF1* is known for flavonols (Czemmel et al. 2009).

To study the function of *VvMYBA1/2*, a red-wine grape cultivar, 'Pinot Noir' and its white mutant 'Pinot Blanc' are appropriate objects. It was reported that a red allele of

'Pinot Noir' is missing from 'Pinot Blanc' (Vezzulli et al. 2012; Yakushiji et al. 2006). A white allele remains in 'Pinot Blanc', and it consists of *VvMYBA1*, which is not transcribed because of a retrotransposon in its promoter (Kobayashi et al. 2004), and *VvMYBA2*, which is not functional because of a mutation (Walker et al. 2007). Thus, except for the *VvMYBA1/2* region, these two varieties should have the almost same genetic background.

In addition to the genetic control, anthocyanins accumulation in grape berry skins depends on various viticultural factors such as canopy management and irrigation, as well as environmental factors such as temperature and light (Brillante et al. 2018; Goto-Yamamoto et al. 2010; Koyama and Goto-Yamamoto 2008; Mori et al. 2007; Poudel et al. 2009; Yang et al. 2020). Among these factors, light is one of the important abiotic factors that regulate the synthesis of flavonoid compounds in grape berries. It has been demonstrated that a shading condition reduced the amount of anthocyanin and mRNA level of anthocyanin-pathway genes (Jeong et al. 2004). However, its control mechanism is not fully understood. Thus, in order to know if only *VvMYBA1/2* are responsible for the control of anthocyanin biosynthesis under different light regimes, and if some other factors are involved, how they influence the expression of each isogene, we carried out a bunch-shading experiment using 'Pinot Noir' and 'Pinot Blanc' grapes and compared the effects on mRNA levels of related genes. Here, we used very robust and high throughput microarray technology to identify the regulation light induced *MYB* transcription in grape berries. Further, the microarray data were validated by quantitative reverse-transcription real time polymerase chain reaction (qPCR).

Materials and Methods

Plant materials, experimental settings and berry sampling

To determine the influence of light on transcriptomic changes in the skin of grape berries during ripening, the 'Pinot Noir' and 'Pinot Blanc' grapevines cultivated at the National Research Institute of Brewing, Higashi-Hiroshima, Japan were used. Branches facing the same direction from north to south orientation rows were taken for both treatments. A single bunch was taken as a replicate and nine bunches from three grapevines of each cultivar with similar size and berry numbers at veraison were selected and covered with three layers of Victoria lawn. This shading treatment reduced the light intensity during the daytime to 18%-20% (Jeong et al. 2004). The non-shaded bunches of the same grapevines were taken as the control. To confirm the temperature variation between shaded and non-shaded bunches, the daytime temperature was measured at 16:00, and it was revealed that shading had a negligible effect on temperature during the daytime. Three bunches each were sampled at veraison, two weeks after veraison (WAV) and 4 WAV. For each replicate (n = 3), 30 berries were collected randomly. The skins peeled from the 30 berries were immediately frozen in liquid nitrogen and kept at -80°C until use. The frozen skins were crushed for homogenization and used for RNA extraction and anthocyanin quantification. The results of 4 WAV of non-shaded 'Pinot Noir' and 'Pinot Blanc' were used in another publication with a different objective (Poudel et al. 2021), hence those data are not presented in this paper and used for discussion purpose only.

Anthocyanin extraction and quantification using high performance liquid chromatography (HPLC)

Anthocyanin was extracted from 0.2 g of berry skin in 2.5 mL of 2% formic acid in 70% methanol (v/v) solution for 20 min with sonication. A total of 400 μL aliquot was used for HPLC analysis after centrifugation at 15,000 rpm for 10 min and filtration with a 0.45 μM micro-membrane filter (Toyo Roshi Kaisha Ltd., Japan). The anthocyanin quantification method used in this study was similar to those described by Ali and Strommer (2003). A Hewlett Packard Series 1100 HPLC system and a Zorbax SB-C18 (5 μm, 2.1 × 150 mm) column were used to separate and quantify the anthocyanin based on peak area. The total anthocyanin concentration was expressed as a milligram of malvidin-3-glucoside (Extrasynthese, France) equivalents per gram of fresh berry skin weight.

RNA extraction

Total RNA from berry skins for qRT-PCR was isolated according to the protocol reported by Reid et al. (2006) and purified using an RNeasy Plant Mini Kit (Qiagen, USA) following the manufacturer's protocol. The total RNA isolated from 1 g of berry skin was quality assessed and quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA) as well as an RNA Nano chip and RNA 6000 Nano Assay on an Agilent 2100 Bioanalyzer (Agilent Technologies, USA).

Microarray analysis

Microarray and q-PCR analysis methods used in this experiment were identical as those described in our previous study (Poudel et al. 2020). Briefly, for microarray analysis, complementary DNA (cDNA) was synthesized from a total of 10 μg total RNA. The cDNA was cleaned up, quantified, quality assessed and used for hybridization after labelling with Cy3-Random Nonamers (Roche NimbleGen Inc., USA). The hybridization was done with 4 μg Cy3 labelled cDNA to a NimbleGen gene expression 12 × 135 K array (Roche NimbleGen Inc., USA) according to the manufacturer's protocol. The image data were acquired and analysed using NimbleGen MS 200 software. The data analysis was performed using GeneSpring software. To extract the differentially expressed genes, we applied a > 3.5 fold cut off. The signal intensities obtained from different replicates were averaged and the ratio between the average signal intensities of shaded to that of non-shaded was calculated. Additionally, to further extract the genes with significantly differentially expressed, a t-test was applied assuming the equal variance (< 0.05).

Quantitative reverse-transcription real-time PCR (qRT-PCR)

To determine mRNA levels of the anthocyanin pathway and related genes, qRT-PCR was carried out as described in our previous study (Poudel et al. 2020). Briefly, the qRT-PCR mixture was prepared with cDNA, upper and lower primers (Poudel et al. 2021) and SYBR Green Master Mix (Qiagen), and the final volume was adjusted to 20 μL with RNase-free water. The reaction was performed in a StepOnePlus real-time PCR system and StepOne software version 2.1 (Applied Biosystem, USA). The reaction condition was 95°C for 15 min, followed by 40 cycles at 95°C for 15 s, at each annealing temperature for 20 s and at 72°C for 20 s. The reaction was performed in at least three biological replicates and three analytical replicates for each prepared cDNA sample. The average data were normalized to the ubiquitin control gene.

Results and Discussion

Effect of shading on anthocyanin accumulation

The bunch-shading treatment was applied at veraison, the grape berries were sampled at 2 WAV and their anthocyanin compositions were analyzed using 'Pinot Noir'. The shading treatment slightly affected the concentration of anthocyanin at 2 WAV (Table 1). However, the data on anthocyanin content were not different significantly at $P < 0.05$ by *t* test. When we compared the effect of shading at the full ripened stage, i.e., 4 WAV, the shading significantly reduced the total amount of anthocyanin, to nearly half of the control i.e., non-shaded 2.49 mg·g^{-1} fresh weight (Poudel et al. 2021) and shaded 1.19 mg·g^{-1} fresh weight. On the other hand, the composition of anthocyanin was not influenced substantially by shading. Thus, the light was found to be responsible for reducing the amount of anthocyanin rather than altering the composition in this cultivar. Similar results were reported for *V. vinifera* 'Red Globe' (Sun et al. 2020), while the shading treatment reduced the ratio of 3'4'5'-OH anthocyanin to 3'4'-OH anthocyanin of another red-wine cultivar 'Cabernet Sauvignon' to some extent (Koyama and Goto-Yamamoto 2008). The effects of light on the anthocyanin composition are probably different among cultivars.

Table 1 Anthocyanin content (mg·g^{-1} fw) in the berry skin of 'Pinot Noir' with and without shading

Anthocyanins	Non-shaded	Shaded
Delphinidin 3-glucoside	0.02 ± 0.007	0.01 ± 0.001
Cyanidin 3-glucoside	0.01 ± 0.002	0.00 ± 0.000
Petunidin 3-glucoside	0.04 ± 0.010	0.02 ± 0.003
Peonidin 3-glucoside	0.15 ± 0.010	0.14 ± 0.023
Malvidin 3-glucoside	0.58 ± 0.100	0.61 ± 0.095
Total contents	0.80 ± 0.134	0.78 ± 0.116

Microarray analysis and mRNA levels of flavonoid pathway genes

The transcript level of the structural genes and transcriptional factors involved in anthocyanin biosynthesis as revealed by microarray analysis is presented in Table 2. The log2 ratio of non-shaded to that of shaded revealed that majority of the flavonoid/anthocyanin biosynthesis genes were down regulated, and this effect was much pronounced in red grape Pinot Noir compared to that of white grape Pinot Blanc (Table 2). Among the flavonoid biosynthetic pathway genes such as *Chalcone synthase-3* (*CHS3*), *flavonol synthase* (*VvFLS*), *leucoanthocyandin reductase* 1 (*VvLAR1*), *VvUFGT* showed negative values in both the cultivars - Pinot Noir and Pinot Blanc under shaded condition. Likewise, the genes like *phenylalanine ammonia-lyase 1* (*VvPAL*), *chalcone isomerase 1* (*CHI1*), *flavonoid 3' hydroxylase* (*VvF3'H*), *flavonoid-3', 5'-hydroxylase* (*VvF3'5'H*), *Caffeoyl-CoA O-methyltransferase*, *anthocyanin-O-methyltransferase* (*VvOMT*), *glutathione S-transferase*

Table 2 Transcription level of major flavonoid biosynthetic genes as influenced by light condition

Probe ID	Accession no.	Gene name	PN-NS/ PN-S	PB-NS/ PB-S
CHR6_JGVV4_543_T01	GU585850	Phenylalanine ammonia-lyase 1 (*VvPAL*)	-0.18938	0.876675
CHR13_JGVV19_80_T01	BQ796207	Phenylalanine ammonia-lyase 1 (*Manihot esculenta*)	0.014164	0.309994
CHR14_JGVV68_88_T01	AB015872	Chalcone synthase-1 (*CHS1)*	0.273898	0.822124
CHR14_JGVV68_87_T01	AB066275	Chalcone synthase-2 (*CHS2*)	0.177767	1.194718
CHR5_JGVV136_15_T01	AB066274	Chalcone synthase-3 (*CHS3*)	-0.10565	-0.07754
CHR6_JGVV61_85_T01	CB971933	Chalcone synthase	1.044293	0.057527
CHR13_JGVV67_6_T01	X75963	Chalcone isomerase (*CHI1*)	-0.04321	0.468042
CHR18_JGVV1_214_T01	AY257979	Flavonol synthase (*VvFLS*)	-0.61008	-0.56749
CHR17_JGVV0_280_T01	DQ786632	Flavonoid 3'-hydroxylase (*VvF3'H*)	-0.20072	0.137538
CHR6_JGVV9_81_T01	DQ786631	Flavonoid 3',5'-hydroxylase (*VvF3'5'H*)	-0.00846	0.034731
CHR6_JGVV9_83_T01	BM437829	Flavonoid 3',5'-hydroxylase	0.072886	0.144696
CHR4_JGVV23_54_T01	X75965	Flavanone-3-hydroxylase (*F3H1*)	0.222199	-0.20184
CHR18_JGVV1_1071_T01	GU585859	Flavanone-3-hydroxylase 2 (*F3H2*) *V. vinifera* 'Merlot'	0.05915	0.626465
CHR18_JGVV1_928_T01	X75964	Dihydroflavonol reductase (*VvDFR*)	0.239058	0.073193
CHR2_JGVV25_429_T01	X75966	Leucoanthocyanidin dioxygenase (*VvLDOX*)	0.018039	-0.05549
CHR1_JGVV11_360_T01	AJ865335	Leucoanthocyandin reductase (*VvLAR1*)	-0.01258	-0.3349
CHR17_JGVV0_557_T01	AB372550	Leucoanthocyandin reductase (*LAR2*)	0.192885	0.186066
CHRUN_JGVV361_4_T01	DQ129684	Anthocyanidin reductase (*VvANR*)	0.750263	0.362415
CHR16_JGVV39_148_T01	AF000372	UDP glucose:flavonoid 3-O-glucosyltransferase (*VvUFGT*)	-0.2948	-0.55772
CHR3_JGVV63_13_T01	CF214966	Caffeoyl-CoA O-methyltransferase	-0.5509	0.094469
CHR7_JGVV31_32_T01	CB347033	S-adenosylmethionine-dependent methyltransferase (*Arabidopsis thaliana*)	-0.10558	0.031087
CHR1_JGVV10_262_T01	GU237132	Anthocyanin-O-methyltransferase (*VvOMT*)	-0.29061	0.082774
CHR4_JGVV79_54_T01	CF518071	Glutathione S-transferase (*GST4*)	-0.12097	0.01651
CHR12_JGVV28_290_T01	CF517304	Glutathione S-transferase (*GST4*)	0.025776	0.056984
CHR2_JGVV33_33_T01	AB097923	Myb-related transcription factor (*VvMYBA1*)	0.280151	-0.02786
CHR2_JGVV33_30_T01	CB915151	My-related transcription factor (*VvMYBA1*)	0.390959	0.072633
CHR2_JGVV33_31_T01	DQ886419	*MYBA2* red allele	0.216586	0.185839
CHR2_JGVV33_31_T01	DQ886420	*MYBA2* white allele	0.216586	0.185839
CHR15_JGVV46_313_T01	AM259485	*VvMybPA1*	0.071471	0.779112
CHR11_JGVV16_111_T01	EU919682	*VvMybPA2*	-0.0553	0.223363
CHR8_JGVV7_172_T01	AY555190	Myb transcription factor (*MYB5a*)	0.158022	0.43784
CHR6_JGVV4_726_T01	AY899404	*MYB5b*	-0.20858	0.106131

(*GST4*), *VvMYBPA2* and *VvMYB5b* were down regulated in red cultivars and up regulated in white grape cultivar. The genes such as *CHS1*, *CHS2*, *LAR2*, *VvANR*, *MYB5a* had similar pattern in both the grapes. The microarray data were later validated by qPCR. The qPCR data revealed almost similar pattern to that of microarray analysis (Fig. 2). The microarray data revealed that the shading effect was also found to be responsible to reduce the major structural genes specific to anthocyanin biosynthetic pathway (*CHS3*, *VvFLS*, *VvLAR1*, *VvUFGT*). More particularly the gene specific to anthocyanin biosynthesis such as *UFGT* was supressed under shading condition (Table 2). This finding was further supported by qRT-PCR data (Fig. 2). Likewise, anthocyanin biosynthetic specific genes such as *VvOMT*, *GST4* and *Caffeoyl-CoA O-methyltransferase* were downregulated in red grape cultivar Pinot Noir.

The suppression of anthocyanin biosynthesis genes such as *F3H*, *FLS*, *DFR* and *UFGT* in addition to transcription factors *MYB10*, *WD40* and *bHLH* under dark condition have been reported in the leaves of crab apple (Lu et al. 2016). The reduced expression of *DFR*, *LDOX* and *UF3GT* have also been reported in model plant *Arabidopsis thaliana*

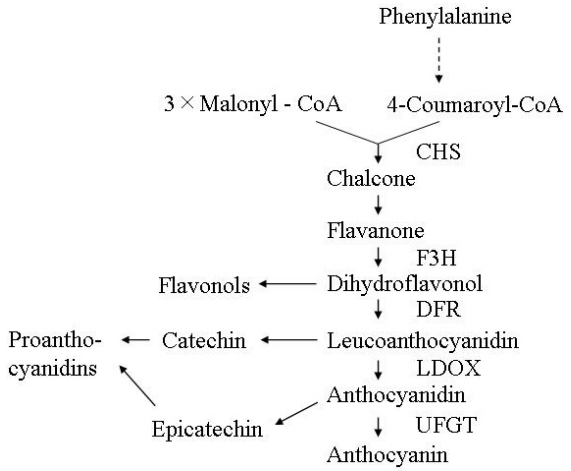

Fig. 1 Simplified scheme of the flavonoid biosynthetic pathway of grapes. *CHS*, chalcone synthase; *F3H*, flavanone-3-hydroxylase; *DFR*, dihydroflavonol 4-reductase; *LDOX*, leucoanthocyanidin dioxygenase; *UFGT*, UDP-glucose flavonoid:3-O-glucosyltransferase

under low light condition (Li et al 2016). Reduced expression of *MYB1* under reduced light condition was reported in eggplant (Jiang et al. 2016), and oranges (Huang et al. 2019). The signal transduction by MYB transcription factors for anthocyanin regulation is quite complex to describe. The G box (CACGTC) within the promoter region of *CsRuby1*, a *R2R3 MYB* transcription factor, was found to regulate anthocyanin in fruit peel of blood orange (Huang et al. 2019). A *MYB* transcription factor, *MYB75* was found to interact with *MAP KINASE4* (*MPK4*) for phosphorylation activity and further playing very crucial role of *MAPK* pathway in light signal transduction in *Arabidopsis thaliana* plant (Li et al. 2016).

The microarray analysis also revealed that the genes such as *LAR2*, *VvANR*, *MYBA2* (both the alleles), *MYBPA1* and *MYB5b* were less influenced (up regulated) under the shaded condition in both the grapes. This finding was in agreement with the qRT-PCR data as well (Fig. 2). Except *MYBA2*, the transcription factors- *MYBPA1*, *MYB5a* and *MYB5b* are mainly involved in procaynidin biosynthesis (Poudel et al. 2020). It has been reported that PA related genes are less affected by shaded condition (Koyama et al. 2012). It is likely that the expression of transcription factors involved in proanthocyanidin biosynthesis are not influenced by light exclusion during the berry ripening period (two weeks after veraision).

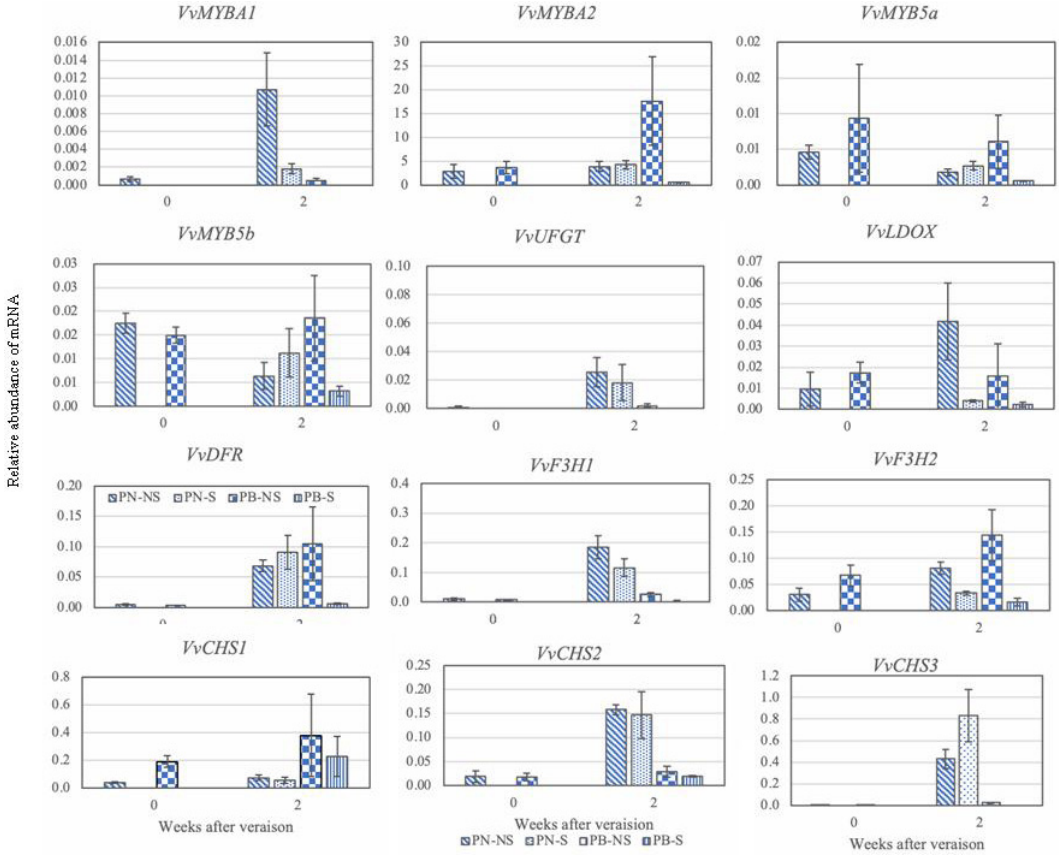

Fig. 2 Abundance of the mRNA of genes related to the anthocyanin biosynthetic pathway in the berry skin of 'Pinot Noir' and 'Pinot Blanc' under shaded and non-shaded conditions. Vertical bars represent mean ± SE. PN, Pinot Noir; PB, Pinot Blanc; S, shaded; NS, Non-shaded

The results of qRT-PCR are shown in Fig. 2. One-way analysis of variance or multiple comparison was not carried out, since many data are not homoscedastic. However, it is obvious that the mRNA level of *VvMYBA1* of 'Pinot Blanc' was almost undetected, and that of 'Pinot Noir' was reduced to a large extent by shading. On the other hand, no clear effects of shading on *VvMYBA2*, *VvMYBA5a* and *VvMYB5b* were observed in both cultivars. The results of *VvMYBA1* and *VvMYB5b* of 'Pinot Noir' are consistent with the study of Koyama and Goto-Yamamoto (2008) using 'Cabernet Sauvignon'. Even though the mRNA level of *VvMYBA2* was reduced by shading in that study, its extent was smaller than that of *VvMYBA1*. Similarly, in the study of *V.* × *labruscana* 'Pione' (Azuma et al. 2012), *VlMybA1-3* was much reduced by a dark condition at 15°C, while the reduction of *VlMYB2* was less extent. Thus, the light sensitivity is possibly different between *VvMYBA1* and *VvMYBA2*.

As for *VvUFGT*, an anthocyanin-specific biosynthetic gene, its mRNA levels were almost negligible in 'Pinot Blanc' and reduced by shading in 'Pinot Noir'. This pattern explains the effect of shading on anthocyanin accumulation well. The difference between 'Pinot Noir' and 'Pinot Blanc' is explained by *VvMYBA1/2* as expected. It should be noticed that *VvMYBA2* of 'Pinot Blanc' is not functional even when it is transcribed.

In contrast, mRNA of upstream genes, i.e., *VvLDOX*, *VvVvDFR*, *VvF3H1/2* and *VvCHS1/2/3*, which are shared by the biosynthesis of other flavonoids, i.e., flavonols and proanthocyanidins (Fig. 2), was detected from both cultivars. Thus, the expression of these genes is probably induced not only by *VvMYBA1/2* but also by other transcription factors. The mRNA level of *VvLDOX* in 'Pinot Blanc' was lower than that in 'Pinot Noir' and reduced by shading in both cultivars, while the regularity of *VvDFR* was not clear. The mRNA levels of both isogenes of *F3H* were reduced by shading, and mRNA levels of *VvF3H1* in 'Pinot Blanc' were consistently lower than that in 'Pinot Noir'. This result suggests that *VvMYBA1/2* influences the expression of *VvF3H1* more strongly than that of *VvF3H2*.

Among three isogenes of *CHS*, mRNA levels of *VvCHS2* in 'Pinot Blanc' were much lower than those of 'Pinot Noir', and those of *VvCHS3* in 'Pinot Blanc' were almost negligible (Fig. 2). No clear influence of shading on these genes was observed. These patterns can be explained by a hypothesis that these two *CHS* isogenes were induced to a large extent by the function of *VvMYBA1/2*. Lower mRNA levels of *VvCHS3* and *VvCHS2* in 'Pinot Blanc' compared with those in 'Pinot Noir' were also observed using a microarray assay (Poudel et al. 2014). In contrast, a certain level of *VvCHS1* mRNA was detected in 'Pinot Blanc', suggesting a strong influence of other transcription factors than *VvMYBA1/2*.

Thus, isogenes of *CHS* and *F3H* were shown to be differently regulated by *VvMYBA1/2* and other transcription factors. Azuma et al. (2012) also reported some isogenes were differentially regulated during light and temperature treatments, which have a synergistic effect on the expression of genes in the pathway using 'Pione'. In addition, the results of 'Pinot Blanc' suggest the involvement of light-sensitive transcription factor(s). A transcription factor for flavonols, *VvMYBF1*, which was reported to be strongly induced by UV light (Czemmel et al. 2009), is a reasonable candidate. *LDOX* and *DFR*, however, are not involved in flavonol biosynthesis (Fig. 1). Thus, the biosynthesis of proanthocyanidins, which are polymers of flavan-3-ols, e.g., catechin and epicatechin, is possibly related to the expression of *VvLDOX* and *VvDFR* in 'Pinot Blanc', even though the proanthocyanidins are mainly synthesized in the earlier stage of berry development and maturation. Otherwise, unknown factor(s) might be involved in the induction of these genes. It was reported that many genes are involved in the regulation of light-induction of anthocyanin in grape berries (Sun et al. 2020). Further research is needed to elucidate the control mechanisms of flavonoid biosynthetic genes under different light conditions. It is also interesting if these control mechanisms other than *VvMYBA1/2* influence the anthocyanin biosynthesis.

Conclusion

Anthocyanin biosynthesis in the grape skin is controlled genetically and influenced by many factors such as light. Comparison of the mRNA levels of anthocyanin biosynthetic pathway genes in 'Pinot Noir' and 'Pinot Blanc' showed the decisive influence of *VvMYBA1* and *VvMYBA2* on the expression of an anthocyanin-specific gene, *VvUFGT*. On the other hand, upstream genes of the pathway, which are shared by other flavonoids, were also expressed in 'Pinot Blanc', a cultivar that lacks functional *VvMYBA* genes, and the influence of light was observed in some genes. Therefore, the involvement of light-sensitive transcription factor(s) other than *VvMYBA1/2* was suggested for the expression of upper-stream genes of the anthocyanin biosynthetic pathway. Also, it was indicated that these transcriptional factors influence the expression differently among isogenes.

Acknowledgement

This research was supported by the grants from Japan Society for the Promotion of Science, Japan, to P. R. Poudel.

Conflict of interests

The authors declare that there is not any conflict of interest.

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