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Research article

Effect of varying concentrations of melatonin on anthocyanin and sugar metabolism in grapes (*Vitis labruscana* L.)

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Abstract

Importance of the work: The effects of melatonin on fruit maturation are still unclear and these effects may vary according to treatment concentrations.

Objectives: To investigate the effects of melatonin treatment on anthocyanin, sugar, abscisic acid (ABA) and indole 3-acetic acid (IAA) concentrations and their related genes.

Materials & Methods: In the first and second groups, clusters of 'Kyoho' grape vines were randomly immersed in 100 µM and 1,000 µM melatonin, respectively, with the surfactant Approach BI at 30 d after full bloom. In the third group, clusters of the untreated control were treated with Approach BI only.

Results: Treatment with 100 µM melatonin promoted anthocyanin accumulation and expression of the *VlMYBA1-2* and *VvUF3GT* genes. In addition, it increased the concentrations of sugars, such as fructose, glucose and sucrose, and upregulated the expression of sugar biosynthesis-related genes (*VvSUT11*, *VvSUS4*, *VvAI* and *VvHT1*). Melatonin treatment increased ABA concentrations with the upregulation of *VvNCED1* at 24 d after treatment. IAA concentrations and the expression of *VvGH3-1* decreased in the 100 µM melatonin-treated berries. In contrast, the 1,000 µM melatonin treatment did not influence the anthocyanin or sugar concentrations.

Main finding: Melwatonin may affect anthocyanin and sugar metabolism through ABA and IAA biosynthesis. Treatment with 100 µM melatonin was most effective at increasing the anthocyanin and sugar concentrations in grapes.

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Introduction

Abscisic acid (ABA) is recognized as an important hormone that is related to maturation, such as anthocyanin and sugar accumulation in grapes (*Vitis* spp.) (Pilati et al., 2017). ABA biosynthesis is regulated by 9-cis-epoxycarotenoid dehydrogenase (NCED), which is the main rate-limiting step upstream of ABA biosynthesis. ABA is primarily catabolized to 8′-hydroxy ABA by ABA 8′-hydroxylase, which is encoded by the *CYP707A* gene and then isomerized to phaseic acid (PA) (Saito et al., 2004). It has been shown that auxin treatments, such as 1-naphthaleneacetic acid (NAA) treatment, delays maturation in grapes; however, the effect depends on the timing of treatment (Davies et al., 2022).

Anthocyanins are secondary metabolites synthesized in grape berry skins from veraison to maturation (Boss and Davies, 2009). The major anthocyanins in grapes are delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and malvidin-3-*O*-glucoside (Escribano - Bailón et al., 2006). Several structural genes encoding enzymes in the anthocyanin biosynthetic pathway have been described, including UF3GT, which encodes UDP glucose:flavonoid-3-O-glucosyltransferase (responsible for the glycosylation of anthocyanidin), and MYBA1/2 transcription factors, which activate the expression of *UF3GT* and *OMT* (a structural gene responsible for methylation downstream of anthocyanin glycosylation) (Walker et al., 1999; Kobayashi et al., 2002; Bogs et al., 2006; Cutanda-Perez et al., 2009).

Sugar accumulation is known as an important factor in fruit quality. Sucrose is the primary sugar transported in the phloem of the grapevine and can be hydrolyzed to glucose and fructose toward maturation (Liu et al., 2006). In grape berries, sucrose generally remains at very low concentrations at harvest; consequently, glucose and fructose are recognized as the predominant sugars (Conde et al., 2007). Manning (1998) suggested that total sugar movement into the fruit depended on the rate of sucrose unloading from the phloem and the *sucrose transporter* (*SUT*) gene in strawberry (*Fragaria* × *ananassa* Duch.). *Sucrose synthase* (*SUS*) is a key gene that regulates sucrose metabolism (Geigenberger and Stitt, 1993) and sucrose can be cleaved into glucose and fructose by acid invertase (AI) in vacuoles (Koch, 2004). In an alternative pathway, sucrose is broken down into glucose and fructose by an extracellular invertase in the phloem with glucose and fructose being subsequently transported into vacuoles by hexose transporters (HTs) (Atanassova et al., 2003).

In plants, melatonin (N-acetyl-5-methoxytryptamine) is a biosynthetic compound from tryptophan that comprises steps, such as decarboxylation by tryptophan decarboxylase (Harderland, 2016). Melatonin has been shown to promote ripening in tomato (*Solanum lycopersicum* L.) (Sun et al., 2016). In banana (*Musa acuminata* L.), melatonin was also recognized as an indicator of fruit ripening (Hu et al., 2017). Conversely, the application of melatonin delayed postharvest senescence in strawberry (Liu et al., 2018) and peach fruits (*Prunus persica* L.) (Gao et al., 2016) through the regulation of the antioxidant system. These studies implied that melatonin participated in fruit maturation and senescence. It has been shown that melatonin is associated with leaf senescence, parthenocarpy and plant growth, as well as the phytohormones ABA and indole 3-acetic acid (IAA) in horticultural crops (Wu et al., 2021). However, the effects of melatonin on fruit maturation are still unclear, with such se effects perhaps varying according to the treatment concentrations and kinds of fruit. Thus, the present study investigated the effects of melatonin treatment concentrations on the anthocyanin and sugar concentrations in grapes.

Materials and Methods

Plant materials and treatment

In 2019 and 2020, three 15- and 16-year-old ‘Kyoho’ grape vines (*Vitis lasbruscana* L.) grafted onto a ‘Teleki-Kober 5BB’ rootstock (*V. berlandieri Planch*. × *V. riparia* hybrids) were used at Chiba University (35°N, 140°E), at an altitude of 37 m above mean sea level. The data from 2019 were used in the present study because similar data were recorded in both years. Each vine had three main stems, with one being assigned to each of the three separate treatments. A branch on each main stem had one cluster with 30 berries at each sampling date. In the first and second groups, the clusters (9 clusters per group; 3 replicates of 3 clusters) were randomly immersed in 100 μM or 1,000 μM melatonin mixed with 0.1% surfactant Approach BI (50% polyoxyethylene hexitan fatty acid ester; Kao; Osaka, Japan) on the vine for 3 min at 30 d after full bloom (DAFB) and just before veraison. In the third group, 9 clusters of the untreated control (3 clusters of each replicate at each sampling date) were treated with 0.1% Approach BI only. The clusters

were sampled at 0 d (before treatment) (30 DAFB), 24 d (in veraison) (54 DAFB) and 57 d (after veraison) after treatment (DAT; 87 DAFB), frozen in liquid nitrogen and stored at -80 °C until analysis.

Total soluble solids, titratable acidity and firmness

The grape berries (3 replications of 60 berries from 3 clusters at each sampling date) were used to evaluate TSS, TA and firmness. Juice was squeezed from the pulp of the berries for the measurement of TSS using a refractometer. TA was calculated as the tartalic acid concentration. The firmness of the peeled berry was measured using a Rheo Meter (CR-100; Sun Scientific Co.; Tokyo, Japan). The diameter of the spindle used in this study was 3 mm. Additionally, the firmness was determined in newtons.

Anthocyanin analysis

Anthocyanin was analyzed according to Kondo et al. (2014). First, 0.5 g fresh weight (FW) of skin was extracted (3 replications of 90 berries from 3 clusters at each sampling date) with 5 mL of 2% volume per volume (v/v) formic acid for 1 d at 4 °C in the dark. The extract was determined using high-performance liquid chromatography (HPLC) and an HPLC mass spectrometer (LC-MS; model LCMS-2010 EV; Shimadzu; Kyoto, Japan) with a Mightysil RP-18 ODS column. A column at 40 °C with a flow rate of 1 mL/min and an ultraviolet/visible light (UV/VIS) detector at 525 nm were used.

Abscisic acid and indole 3-acetic acid extraction quantitation

The ABA and IAA concentrations (3 replications of 90 berries from 3 clusters at each sampling date) were analyzed using the method of Durgbanshi et al. (2005), with modification. The skin sample was mixed with 5 mL distilled water and internal standard ((±)-2 cis, 4-trans-d_6 ABA (Shokotsusho Co.; Tokyo, Japan), d_5 IAA (Wako Co.; Tokyo, Japan), homogenized on ice, and centrifuged at 15,000×g at 4 °C for 10 min. The supernatant was transferred to a new tube and the pH was adjusted to 3.0 using 30% acetic acid. The acidified extract water was partitioned twice against 3–5 mL of diethyl ether. Then, the organic layer was transferred to a glass tube and evaporated using a heating block (37 °C). Before LC-MS analysis, the dry residue was redissolved in 1 mL of 100% MeOH and filtered through a membrane (0.45 µm; Sigma-Aldrich Inc.; St. Louis, MO, USA). The LC-MS conditions were: mobile phase of 80% MeOH with 20 mM formic acid; column, Mightysil RP-18 (5 µm, 2 mm internal diameter × 150 mm; Kanto Chemical; Tokyo, Japan); flow rate of 0.3mL/min; auto-sampler temperature at 4 °C; detector pressure at 1.5 kV; nebulizer g as (N2) flow rate of 1.5 L/min; ESI voltage of 4.5 kV; and block heater temperature at 250 °C. The ABA concentrations were determined from the ratio of the peak areas for *m/z* 263 (ABA) / 269 (ABA-d_6). The IAA concentrations were determined from the ratio of the peak areas for m/z 176 (IAA) / 181 (IAA-d_5).

Sugar analysis

Sugar concentrations were detected according to the method of Kondo et al. (2014). Skin samples (1 g FW; 3 replications of 90 berries from 3 clusters at each sampling date) in 5 mL 80% (v/v) ethanol were heated at 60 °C for 10 min and homogenized after cooling. The homogenate was filtered, evaporated and analyzed using HPLC (model L-6200; Hitachi; Tokyo, Japan).

RNA extraction, cDNA synthesis and quantitative real-time reverse transcriptase polymerase chain reaction analysis

Total RNA was extracted using a cetyltrimethylammonium bromide (CTAB)-based method based on Wang et al. (2018). The sample (0.5 g FW; 3 replications of 90 berries from 3 clusters at each sampling date) and was purified using a MicroElute Clean-Up Column (Favorgen Biotech Co.; Tokyo, Japan) and RQ1 RNase-Free DNase (Promega Corp.; Tokyo, Japan). cDNA synthesis followed the instruction manual of ReverTra Ace® qPCR RT Master Mix (Code No. FSQ-201; Toyobo Co., Ltd.; Osaka, Japan). Quantitative PCR was performed on a StepOnePlus™ system (Applied Biosystems Inc.; Foster City, CA, USA). The primers listed in Table 1 were used for quantitative real-time reverse transcriptase polymerase chain reaction analysis.

Statistical analysis

Each result was presented as the mean of three replications ± SD and subjected to analysis of variance procedures, with mean comparison based on the Tukey-Kramer test using the SAS statistical analysis package (version 8.2; SAS Institute; Cary, NC, USA). The tests were considered significant at $p < 0.05$.

Table 1 Real-time polymerase chain reaction forward (F) and reverse (R) primers

Gene	Primer	Sequences (5' -3')	Accession number or Reference
VvNCED1	(F)	TTTGTGCACGACGAGAAGAC	Speirs et al., 2013
	(R)	TCTGCAATCTGACACCAAGC	
VLMYBA1-2	(F)	TGGTCACCACTTGAAAAAGA	Azuma et al., 2012
	(R)	GAATGTGTTAGGGGTTTATT	
VvUFGT	(F)	GGGATGGTAATGGCTGTGG	Jeong et al., 2004
	(R)	ACATGGGTGGAGAGTGAGTT	
VvCYP707A1	(F)	AAGTCCTGAAGCTGCAAGCTG	NM_001281052
	(R)	AAGCTTCCTCAACTTGGCATGG	
VvSUS4	(F)	CAAATGTTGGCAATCCACAG	Da Silva et al., 2013
	(R)	AGATGGACCGTGTCAGGAAC	
VvSUT11	(F)	CTACAGTGGTTGGTTCAGG	Venturini et al., 2013
	(R)	GTTGACTCTGCCATTTCTTC	
VvAI	(F)	GCTGTGCCCAAAAATCTCTC	Venturini et al., 2013
	(R)	CCAAGCAGTCGTAGGGTCTC	
VvHT1	(F)	CGTTGTTCACATCGTCGCTTTATC	Venturini et al., 2013
	(R)	GAGCAGTCCTCCGAATAGCATTG	
VvUbiquitin	(F)	TCTGAGGCTTCGTGGTGGTA	Fujita et al., 2006
	(R)	AGGCGTGCATAACATTTGCG	

Results

Skin coloration and physiological changes

At 24 DAT and 57 DAT, the coloration of the berries treated with 100 µM melatonin was superior to those in the other two groups (Fig. 1). At 57 DAT, the 100 µM melatonin-treated grape berries had significantly more TSS (16.8 °Brix) than the untreated control (16.1°Brix) (data not shown). At 24 DAT, although there were no significant differences in berry weight, berry firmness or TA among all treatments (data not shown).

Abscisic acid and indole 3-acetic acid concentrations and the expressions of related genes

Endogenous ABA accumulation was detected at 30 DAFB before veraison (0 DAT). The ABA concentrations had increased significantly in berries treated with 100 µM melatonin at 24 DAT, accompanied by increased expression levels of *VvNCED1* and *VvCYP707A1* (Fig. 2). Although the ABA concentrations in the berries treated with 1,000 µM melatonin were lower than those in the untreated control at 24 DAT, there were no significant differences in the expression levels of *VvNCED1* and *VvCYP707A1*.

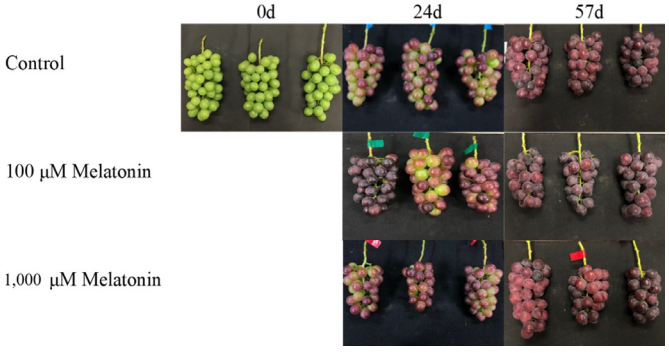

Fig. 1 Effects of melatonin application on coloration in untreated control and in 100 µM and 1,000 µM melatonin-treated grape berries

At 24 DAT, the endogenous IAA concentrations had decreased in berries treated with 100 µM melatonin, although there were no significant differences between the 1,000 µM treatment and the untreated control (Fig. 3). The expression of *VvGH3-1* (IAA amido-transferase enzyme gene; Gretchen Hagen 3) in the 100 µM melatonin-treated berries was lower at 24 DAT than in the other treatments. *VvTAR2* (tryptophan aminotransferase-related gene) expression was lower in the 100 µM melatonin treatment than in the 1,000 µM treatment.

Sugar concentrations and the expression of related genes

The concentrations of total sugar, fructose, glucose and sucrose increased significantly in berries treated with 100 µM melatonin and more than in the untreated control at 24 DAT (Fig. 4). However, at 57 DAT, there were no significant differences among any of the treatments.

The expression levels of *VvAI*, *VvSUS4*, *VvHT1* and *VvSUT11* were upregulated significantly at 24 DAT in the 100 µM melatonin-treated berries (Fig. 5). At 57 DAT, the expression levels of *VvHT1* and *VvAI* were slightly upregulated in the 1,000 µM melatonin-treated berries compared to the untreated control, whereas there were no significant differences in the expression levels of the *VvSUT11* and *VvSUS4* genes.

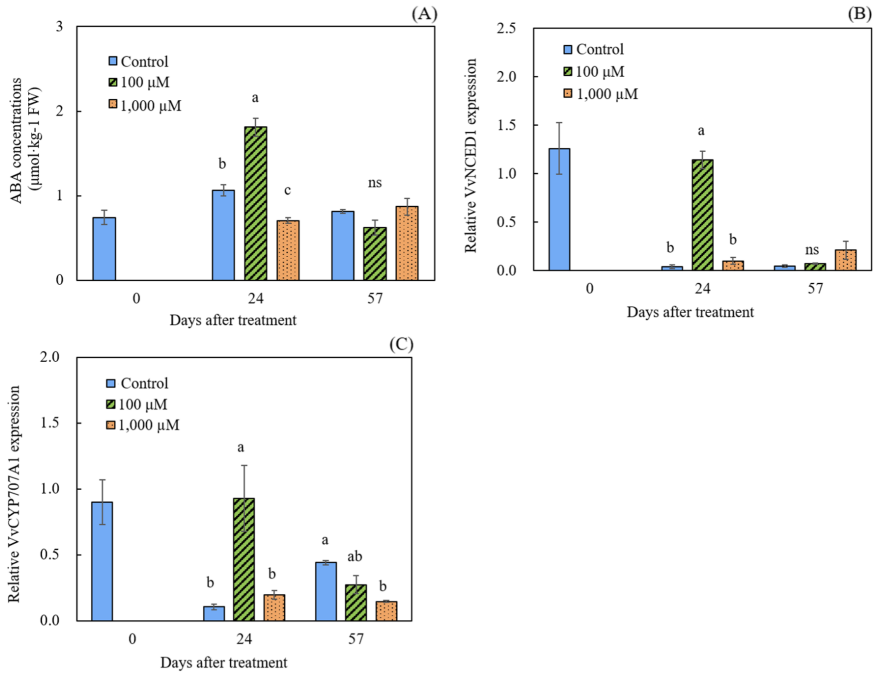

Fig. 2 (A) Effects of melatonin application in untreated control and in 100 μM and 1,000 μM melatonin-treated grape berries on: (A) abscisic acid (ABA) concentrations; (B) normalized expression of ABA biosynthesis-related gene *VvNCED1*; (C) normalized expression of ABA biosynthesis-related gene *VvCYP707A1*, where error bars represent SD; different lowercase letters above bars indicate significant ($p < 0.05$) differences within each time point; ns = non-significant difference; FW = fresh weight

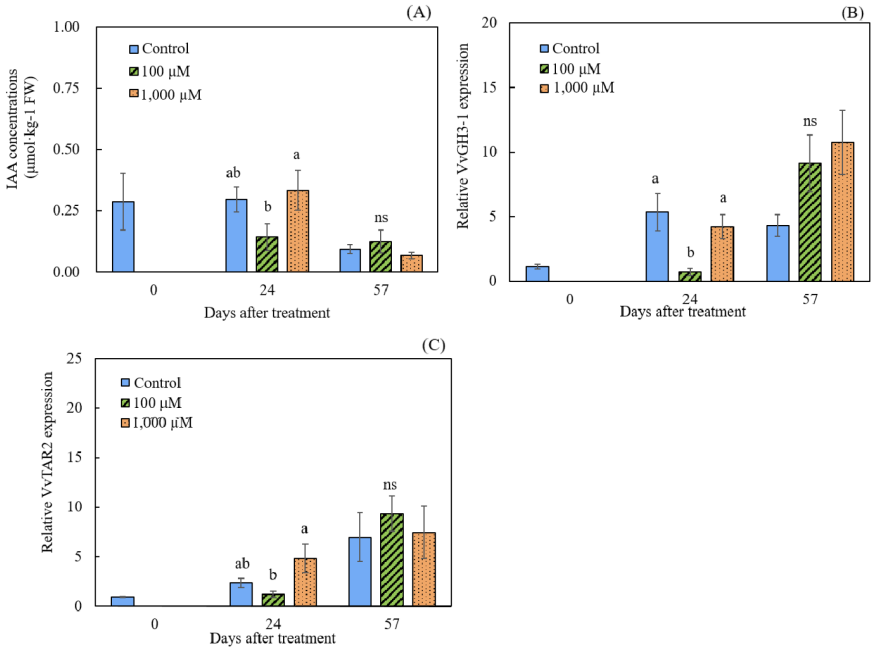

Fig. 3 (A) Effects of melatonin application in untreated control and in 100 μM and 1,000 μM melatonin-treated grape berries on: (A) indole 3-acetic acid (IAA) concentration; and normalized expression of IAA biosynthesis-related genes: (B) *VvGH3-1* and (C) *VvTAR2*, where error bars represent SD; different lowercase letters above bars indicate significant ($p < 0.05$) differences within each time point; ns = no significant difference; FW = fresh weight

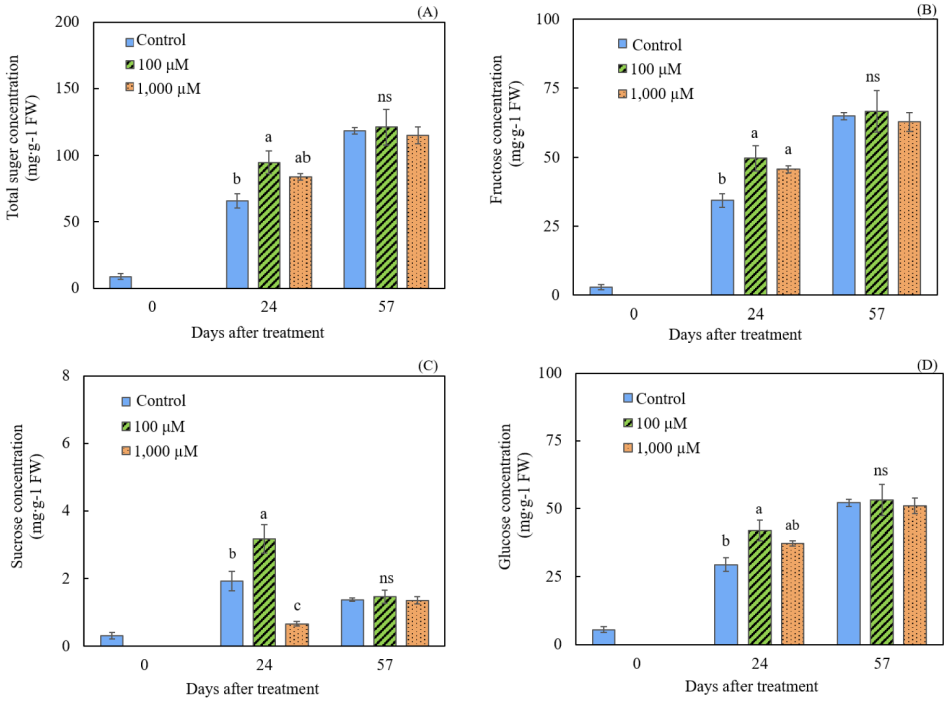

Fig. 4 Effects of melatonin application in untreated control and in 100 μM and 1,000 μM melatonin-treated grape berry on: (A) total sugar; (B) fructose; (C) sucrose; (D); glucose, where error bars represent SD; different lowercase letters above bars indicate significant ($p < 0.05$) differences within each time point; ns = non-significant difference; FW = fresh weight

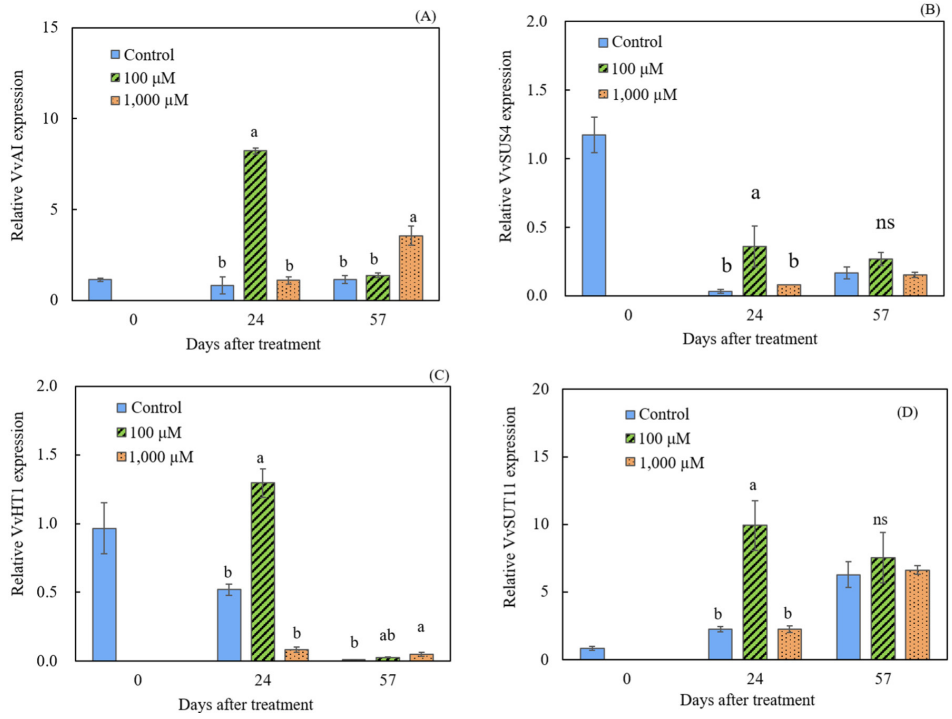

Fig. 5 Normalized expression of sugar biosynthesis-related genes in the untreated control and in 100 μM and 1,000 μM melatonin-treated grape berries: (A) *VvAI*; (B) *VvSUS4*; (C) *VvHT1*; (D) *VvSUT11*, where error bars represent SD; different lowercase letters above bars indicate significant ($p < 0.05$) differences; ns = non-significant difference

Anthocyanin accumulation and expression of related genes

The total anthocyanin concentrations as well as malvidin-derived, cyanidin-derived, petunidin-derived and petunidin-derived anthocyanin concentrations were highest in berries treated with 100 µM melatonin at 24 DAT (Fig. 6). In general, anthocyanin concentrations in berries treated with 1000 µM melatonin did not significantly differ from those in the untreated control. At 24 DAT, the expression levels of *VvUF3GT* and *VlMYBA1-2* were significantly higher in the 100 µM melatonin-treated berries than at the other time points (Fig. 7). From these results, a schematic diagram of the effects of the melatonin treatment on ABA, IAA, anthocyanin and sugar concentrations is shown in Fig. 8. The application of 100µM melatonin induced a decrease in IAA and an increase in ABA. Both a decrease in IAA and an increase in ABA may have had a synergistic effect on the anthocyanin and sugar accumulations.

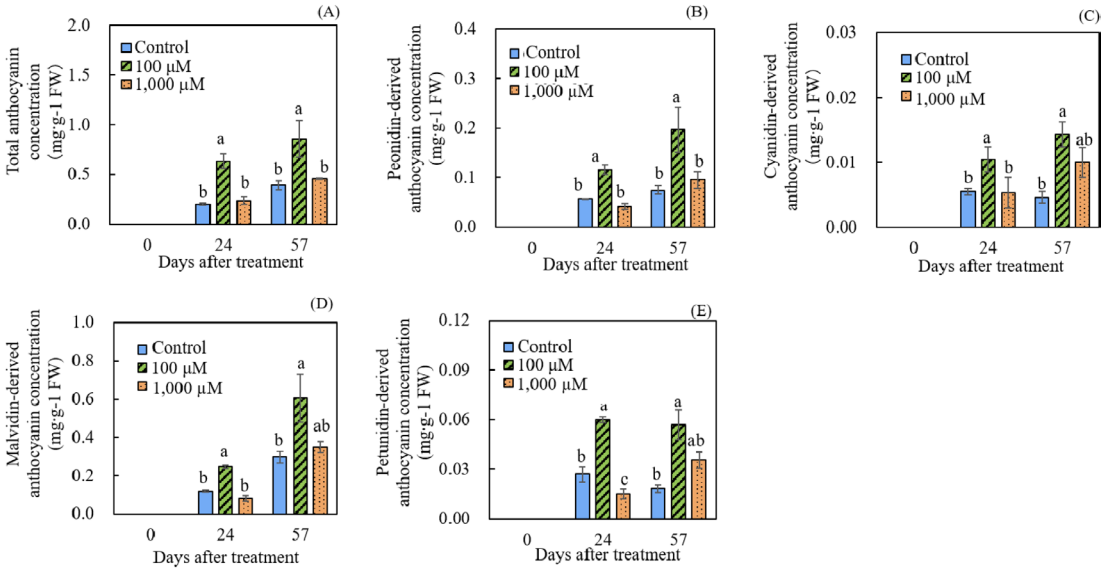

Fig. 6 Effects of melatonin application in untreated control and in 100 µM and 1,000 µM melatonin-treated grape berries on: (A) total anthocyanin; (B) petunidin-derived anthocyanin; (C) cyanidin-derived anthocyanin; (D) malvidin-derived anthocyanin; (E) peonidin-derived anthocyanin, where error bars represent SD; different lowercase letters above bars indicate significant ($p < 0.05$) differences within each time point; FW = fresh weight

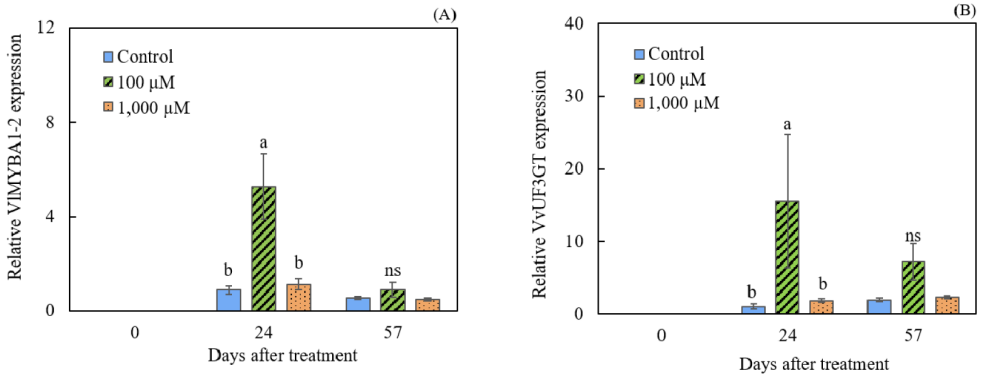

Fig. 7 Normalized expression of anthocyanin biosynthesis-related genes in the untreated control and in 100 µM and 1,000 µM melatonin-treated grape berries: (A) *VlMYBA1-2*; (B) *VvUF3GT*, where error bars represent SD; different lowercase letters above bars indicate significant ($p < 0.05$) differences within each time point; ns = non-significant difference

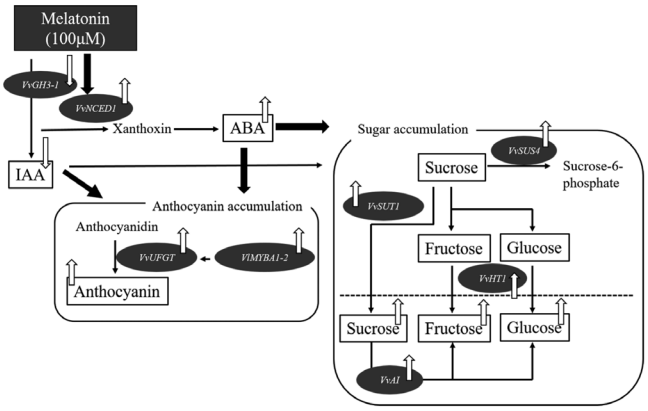

Fig. 8 Schematic diagram of effects of melatonin treatments on abscisic acid (ABA), indole 3-acetic acid (IAA) anthocyanin and sugar concentrations in 'Kyoho' grape berries, where dark arrow indicates factor that each compound has an effect upon and direction of blank arrow indicates increase or decrease

Discussion

ABA is a key hormone that regulates the accumulation of anthocyanin and sugar in grape berries (Murcia et al., 2016; Pilati et al., 2017). Exogenous ABA or nordihydroguaiaretic acid (an inhibitor of ABA biosynthesis) induced or delayed grape maturation, respectively (Jia el al., 2017; Lin et al., 2018). Light irradiation induced maturation concomitant with the increases in ABA and anthocyanin concentrations by upregulating *VvNCED1* and *VvCYP707A1*, respectively, in 'Kyoho' grape berries (Kondo et al., 2014). Melatonin treatment increased ABA, H_2O_2 and ethylene concentrations in the wine grape 'Moldova' (*Vitis teutonica* L.) according to Xu et al. (2018). In the present study, melatonin increased the ABA concentrations with the upregulation of *VvNCED1* and *VvCYP707A1* at 24 DAT. Therefore, melatonin may affect the maturation of grape berries by acting on the levels of ABA through the *VvNCED1* and *VvCYP707A1* genes.

Auxin inhibited anthocyanin biosynthesis genes, such as *VvUF3GT*, and softening-related genes, such as *VvPG* and *VvPE* (Jia et al., 2017). The present study showed that 100 μM melatonin treatment decreased IAA concentrations and *VvGH3-1* gene expression. The expressions of *VvTAR2* and *VvGH3-1* have been correlated with IAA concentrations (Bottcher et al., 2013). These results suggested that the decrease in IAA concentration through the decrease in *VvGH3-1* for the 100 μM melatonin treatment resulted in increases in the anthocyanin and sugar concentrations.

There have been few studies on the effect of melatonin on sugar synthesis. Hu et al. (2017) and Yang et al. (2019) reported that melatonin had a positive effect on soluble sugar content during postharvest ripening in banana and apple (*Malus domestica* cv.) and Xu et al. (2018) found that fructose and glucose were induced by melatonin treatment in the wine grape 'Moldova' (*Vitis teutonica* L.) and the genes encoding putative sucrose transporters, *VvSUC11* and *VvSUC12*, had similar expression patterns in that fruit. Their mRNAs were upregulated with the accumulation of hexose, which was released from the phloem and might be taken up by parenchyma cells prior to hydrolysis derived from sucrose (Manning et al., 2001).

The expression of *VvSUS* has been induced concomitantly with the increase in glucose during maturation (Wang et al., 2017). The high expression level of the *AI* gene may have caused the high soluble sugar content (glucose and fructose) in grapes (Shangguan et al., 2014). *VvHT1* encodes a putative hexose transporter expressed during the maturation of grapes, with both ABA and hexose treatment being positively regulated by this transporter (Atanassova et al., 2003; Conde et al., 2006). The present results suggested that melatonin treatment increased sugar concentrations by regulating the gene expression of *VvSUT11*, *VvSUS4*, *VvAI* and *VvHT1*.

In tomato fruits, melatonin treatment promoted the accumulation of flavonoids, including carotenoid and anthocyanin, and accelerated fruit softening (Sun et al., 2016, 2020). In cabbage (*Brassica oleracea* L.), melatonin treatment enhanced the anthocyanin concentration by activating the expression levels of the transcription factors *MYB*, *bHLH (basic-helix-loop-helix)* and *WD40*, which were responsible for the cooperative regulation of anthocyanin biosynthesis (Zhang et al., 2016). In addition, melatonin treatment increased the anthocyanin accumulation in crabapple leaves (*Malus* cv.), with flavonoid biosynthetic genes and related transcription factors, such as *MYB4*, *MYB10*, *MYB12*, *HLH3*, *chalcone synthase* (*CHS*), *flavonone 3-hydroxylase* (*F3H*) and *UF3GT* (Chen et al., 2019). The concentration of 100 μM melatonin in the present study suggested that melatonin treatment increased anthocyanin accumulation via the gene expression of *VlMYBA1-2* and *VvUF3GT*. However, the 1,000 μM concentration did not significantly affect the anthocyanin concentrations. These results suggested that the effect of melatonin on anthocyanin formation in grape berries depends on the melatonin concentration. In the present study, the 1,000 μM melatonin treatment showed different effects on ABA and IAA concentrations compared to the 100 μM melatonin

treatment. It has been shown that IAA promotes plant growth at low concentrations, whereas conversely, high concentrations of IAA inhibit plant growth (Zafar et al., 2020). Furthermore, auxin application to grapevines prior to veraison delayed berry maturation, including sugar accumulation (Davies et al., 2022). Both melatonin and IAA are synthesized from tryptophan and have similar structures (Harderland, 2016; Zafar et al., 2020; Jahn et al., 2021). Therefore, similarly to IAA, high concentrations of melatonin may affect grape maturation because phytohormones can exhibit promotive effects following suitable concentrations. Specifically, low concentrations of melatonin may be effective at promoting berry maturation in grapes. Thus, the effects of melatonin treatment on maturation in grape berries depend on the concentrations used and are complicated.

Conclusion

The anthocyanin and sugar concentrations in grape berries were improved by the application of 100 μM melatonin but not by applying 1,000 μM. These results suggested that melatonin treatment with suitable concentrations can regulate the maturation in grapes.

Conflict of Interest

The authors declare that there have no conflicts of interest.

References

Atanassova, R., Leterrier, M., Gaillard, C., Agasse, A., Sagot, E., Coutos-Thévenot, P., Delrot, S. 2003. Sugar-regulated expression of a putative hexose transport gene in grape. Plant Physiol. 131: 326–334. doi.org/10.1104/pp.009522

Azuma, A., Yakushiji, H., Koshita, Y., Kobayashi, S. 2012. Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. Planta 236: 1067–1080. doi.org/10.1007/s00425-012-1650-x

Boss, P.K., Davies, C. 2009. Molecular biology of anthocyanin accumulation in grape berries. In: Kalliopi, A.R.A. (Ed.). Grapevine Molecular Physiology and Biotechnology. Springer. Dordrecht, the Netherlands, pp. 263–292.

Bottcher, C., Burbidge, C.A., Boss, P. K., Davies, C. 2013. Interactions between ethylene and auxin are crucial to the control of grape (*Vitis vinifera* L.) berry ripening. BMC Plant Bio. 13: 222. doi.org/10.1186/1471-2229-13-222

Bogs, J., Ebadi, A., McDavid, D., Robinson, S.P. 2006. Identification of the flavonoid hydroxylases from grapevine and their regulation during fruit development. Plant Physiol. 140: 279–291. doi.org/10.1104/pp.105.073262

Chen, L., Tian, J., Wang, S., Song, T., Zhang, J., Yao, Y. 2019. Application of melatonin promotes anthocyanin accumulation in crabapple leaves. Plant Physiol. Bioch. 142: 332–341. doi.org/10.1016/j.plaphy.2019.07.024

Conde, C., Agasse, A., Glissant, D., Tavares, R., Gerós, H., Delrot, S. 2006. Pathways of glucose regulation of monosaccharide transport in grape cells. Plant Physiol. 141: 1563–1577. doi.org/10.1104/pp.106.080804

Conde, C., Silva, P., Fontes, N., et al. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food 1: 1–22.

Cutanda-Perez, M.C., Ageorges, A., Gomez, C., Vialet, S., Terrier, N., Romieu, C., Torregrosa, L. 2009. Ectopic expression of *VlmybA*1 in grapevine activates a narrow set of genes involved in anthocyanin synthesis and transport. Plant Mol. Biol. 69: 633–648. doi.org/10.1007/s11103-008-9446-x

Da Silva, C., Zamperin, G., Ferrarini, A., et al. 2013. The high polyphenol content of grapevine cultivar tannat berries is conferred primarily by genes that are not shared with the reference genome. Plant Cell 25: 4777–4788. doi.org/10.1105/tpc.113.118810

Davies, C., Bottcher, C., Nicholson, E.L., Burbidge, C.A., Boss, P.K. 2022. Timing of auxin treatment affects grape berry growth, ripening timing and synchronicity of sugar accumulation. Aust. J. Grape Wine Res. 28: 232–241. doi.org/10.1111/ajgw.12528

Durgbanshi, A., Arbona, V., Pozo, O., Miersch, O., Sancho, J.V., Gómez-Cadenas, A. 2005. Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. J. Agr. Food Chem. 53: 8437–8442. doi.org/10.1021/jf050884b

Escribano-Bailón, M.T., Alcalde-Eon, C., Muñoz, O., Rivas-Gonzalo, J.C., Santos-Buelga, C. 2006. Anthocyanins in berries of maqui [*Aristotelia chilensis* (Mol.) Stuntz]. Phytochem. Anal. 17: 8–14. doi.org/10.1002/pca.872

Fujita, A., Goto-Yamamoto, N., Aramaki, I., Hashizume, K. 2006. Organ-specific transcription of putative flavonol synthase genes of grapevine and effects of plant hormones and shading on flavonol biosynthesis in grape berry skins. Biosci. Biotech. Biochem. 70: 632–638. doi.org/10.1271/bbb.70.632

Gao, H., Zhang, Z.K., Chai, H.K., Cheng, N., Yang, Y., Wang, D.N., Yang, T., Cao, W. 2016. Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. Postharvest Biol. Technol. 118: 103–110. doi.org/10.1016/j.postharvbio.2016.03.006

Geigenberger, P., Stitt, M. 1993. Sucrose synthase catalyses a readily reversible reaction in vivo in developing potato tubers and other plant tissues. Planta 189: 329–339. doi.org/10.1007/BF00194429

Harderland, R. 2016. Melatonin in plants-diversity of levels and multiplicity of functions. Front. Plant Sci. 7: 198. doi.org/10.3389/fpls.2016.00198

Jahn, L., Hofmann, U., Ludwig-Muller, J. 2021. Indole-3-acetic acid is synthesized by the endophyte *Cyanodermella asteris* via a tryptophan-

dependent and -independent way and mediates the interaction with a non-host plant. Int. J. Mol. Sci. 22: 2651. doi.org/10.3390/ijms22052651

Jeong, S.T., Goto-Yamamoto, N., Kobayashi, S., Esaka, M.J.P.S. 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. Plant Sci. 167: 247–252. doi.org/10.1016/j.plantsci.2004.03.021

Hu, W., Yang, H., Tie, W., et al. 2017. Natural variation in banana varieties highlights the role of melatonin in postharvest ripening and quality. J. Agr. Food Chem. 65: 9987–9994. doi.org/10.1021/acs.jafc.7b03354

Jia, H., Xie, Z., Wang. C., et al. 2017. Abscisic acid, sucrose, and auxin coordinately regulate berry ripening process of the Fujiminori grape. Funct. Integr. Genomics 17: 441–457. doi.org/10.1007/s10142-017-0546-z

Kobayashi, S., Ishimaru, M., Hiraoka, K., Honda, C. 2002. Myb-related genes of the Kyoho grape (*Vitis labruscana*) regulate anthocyanin biosynthesis. Planta 215: 924–933. doi.org/10.1007/s00425-002-0830-5

Koch, K. 2004. Sucrose metabolism: Regulatory mechanisms and pivotal roles in sugar sensing and plant development. Curr. Opin. Plant Biol. 7: 235–246. doi.org/10.1016/j.pbi.2004.03.014

Kondo, S., Tomiyama, H., Rodyoung, A., Okawa, K., Ohara, H., Sugaya, S., Terahara, K., Hirai, N. 2014. Abscisic acid metabolism and anthocyanin synthesis in grape skin are affected by light emitting diode (LED) irradiation at night. J. Plant Physiol. 171: 823–829. doi.org/10.1016/j.jplph.2014.01.001

Lin, H., Wang, S., Saito, T., et al. 2018. Effects of IPT or NDGA application on ABA metabolism and maturation in grape berries. J. Plant Growth Regul. 37: 1210–1221. doi.org/10.1007/s00344-018-9820-0

Liu, H.F., Wu, B.H., Fan, P.G., Li, S.H., Li, L.S. 2006. Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. J. Sci. Food Agr. 86: 1526–1536. doi.org/10.1002/jsfa.2541

Liu, C., Zheng, H., Sheng, K., Liu, W., Zheng, L. 2018. Effects of melatonin treatment on the postharvest quality of strawberry fruit. Postharvest Biol. Technol. 139: 47–55. doi.org/10.1016/j.postharvbio.2018.01.016

Manning, K. 1998. Isolation of a set of ripening-related genes from strawberry: Their identification and possible relationship to fruit quality traits. Planta 205: 622–631. doi.org/10.1007/s00425 0050365

Manning, K., Davies, C., Bowen, H.C., White, P.J. 2001. Functional characterization of two ripening-related sucrose transporters from grape berries. Ann. Bot. 87: 125–129. doi.org/10.1006/anbo.2000.1316

Murcia, G., Pontin, M., Reinoso, H., Baraldi, R., Bertazza, G., Gómez-Talquenca, S., Bottini, R., Piccoli, P.N. 2016. ABA and GA_3 increase carbon allocation in different organs of grapevine plants by inducing accumulation of non-structural carbohydrates in leaves, enhancement of phloem area and expression of sugar transporters. Physiol. Plant. 156: 323-337. doi.org/10.1111/ppl.12390

Pilati, S., Bagagli, G., Sonego, P., et al. 2017. Abscisic acid is a major regulator of grape berry ripening onset: New insights into ABA signaling network. Front. Plant Sci. 8: 1093. doi.org/10.3389/fpls.2017.01093

Saito, S., Hirai, N., Matsumoto, C., Ohigashi, H., Ohta, D., Sakata, K., Mizutani, M. 2004. Arabidopsis *CYP*707As encode (+)-abscisic acid 8′-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. Plant Physiol. 134: 1439–1449. doi.org/10.1104/pp.103.037614

Shangguan, L., Song, C., Leng, X., Kayesh, E., Sun, X., Fang, J. 2014. Mining and comparison of the genes encoding the key enzymes involved in sugar biosynthesis in apple, grape, and sweet orange. Sci. Hortic. 165: 311–318. doi.org/10.1016/j.scienta.2013.11.026

Speirs, J., Binney, A., Collins, M., Edwards, E., Loveys, B. 2013. Expression of ABA synthesis and metabolism genes under different irrigation strategies and atmospheric VPDs is associated with stomatal conductance in grapevine (*Vitis vinifera* L. cv Cabernet Sauvignon). J. Exp. Bot. 64: 1907–1916. doi.org/10.1093/jxb/ert052

Sun, Q., Liu, L., Zhang, L., et al. 2020. Melatonin promotes carotenoid biosynthesis in an ethylene-dependent manner in tomato fruits. Plant Sci. 298: 110580. doi.org/10.1016/j.plantsci.2020.110580

Sun, Q., Zhang, N., Wang, J., et al. 2016. A label-free differential proteomics analysis reveals the effect of melatonin on promoting fruit ripening and anthocyanin accumulation upon postharvest in tomato. J. Pineal Res. 61: 138–153. doi.org/10.1111/jpi.12315

Venturini, L., Ferrarini, A., Zenoni, S., et al. 2013. *De novo* transcriptome characterization of Vitis vinifera cv. Corvina unveils varietal diversity. BMC Genom. 14: 41. doi.org/10.1186/1471-2164-14-41

Walker, R.P., Chen, Z.H., Técsi, L.I., Famiani, F., Lea, P.J., Leegood, R.C. 1999. Phosphoenolpyruvate carboxykinase plays a role in interactions of carbon and nitrogen metabolism during grape seed development. Planta 210: 9–18. doi.org/10.1007/s004250050648

Wang, S., Saito, T., Ohkawa, K., Ohara, H., Suktawee, S., Ikeura, H., Kondo, S. 2018. Abscisic acid is involved in aromatic ester biosynthesis related with ethylene in green apples. J. Plant Physiol. 221: 85–93. doi.org/10.1016/j.jplph.2017.12.007

Wang, X.Q., Zheng, L.L., Lin, H., Yu, F., Sun, L.H., Li, L.M. 2017. Grape hexokinases are involved in the expression regulation of sucrose synthase-and cell wall invertase-encoding genes by glucose and ABA. Plant Mol. Biol. 94: 61–78. doi.org/10.1007/s11103-017-0593-9

Wu, X., Pien, J., Huang, Z., Zheng, X., Tian, Y., Shi, L., Dong, P., Li, Z. 2021. Melatonin: Biosynthesis, content and function in horticultural plants and potential application. Sci. Hort. 288: 110392. doi.org/10.1016/j.scienta.2021.110392

Xu, L., Yue, Q., Xiang, G., Bian, F., Yao, Y. 2018. Melatonin promotes ripening of grape berry via increasing the levels of ABA, H_2O_2, and particularly ethylene. Hortic. Res. 5: 41. doi.org/10.1038/s41438-018-0045-y

Yang, J., Zhang, C., Wang, Z., et al. 2019. Melatonin-mediated sugar accumulation and growth inhibition in apple plants involves down-regulation of fructokinase 2 expression and activity. Front. Plant Sci. 10: 150. doi.org/10.3389/fpls.2019.00150

Zafar, S., Akhtar, M., Perveen, S., Hasnain, Z., Khalil, A. 2020. Attenuating the adverse aspects of water stress on wheat genotypes by foliar spray of melatonin and indole-3-acetic acid. Physiol. Mol. Biol. Plants 26: 1751–1762.

Zhang, N., Sun, Q., Li, H., et al. 2016. Melatonin improved anthocyanin accumulation by regulating gene expressions and resulted in high reactive oxygen species scavenging capacity in cabbage. Front. Plant Sci. 7: 197. doi.org/10.3389/fpls.2016.00197