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Bioactive compounds in fruits are affected by light quality

Satoru Kondo*

Graduate School of Horticulture, Chiba University, Matsudo 271-8510, Japan

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ABSTRACT

The effects of blue and red light irradiation at night on anthocyanin synthesis and abscisic acid (ABA) metabolism were investigated in grape berries. Endogenous ABA were highest in red light-emitting diode (LED)-treated skin. In contrast, anthocyanin concentrations were highest in blue LED-treated skin, followed by red LED treatment. However, the expressions of *VlMYBA1-2* and UDP-glucose-flavonoid 3-*O*-glucosyltransferase(*VvUFGT*) did not necessarily coincide with anthocyanin concentrations. The results suggest that blue LED irradiation at night may be effective in increasing anthocyanin in grape berries. The effect of UV-C irradiation on jasmonate, polyamines (putrescine) and antioxidant activities was investigated in apple plants. The EC_{50} values of O_2^- -scavenging activity in the UV-C treated leaves were lower than those in the untreated control. The endogenous jasmonic acid and putrescine concentrations in the UV-C treated plants were higher than those in the untreated control. These facts suggest that the increase of jasmonates and polyamines may be associated with UV-C stimulation, and UV-C irradiation may be effective for increasing antioxidant activity in apple plants.

* *Corresponding author*:
Email: s-kondo@faculty.chiba-u.jp (S. Kondo)

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Introduction

Light quality may influence vegetative growth, reproductive growth and pigment production in plants (Kamuro *et al*., 2003). For instance, grape plants cultured under red light-emitting diodes (LEDs) produce the longest shoots with longer internodes, while chlorophyll content is highest in plants cultured under blue LEDs (Poudel *et al*., 2008). The primary pigment in grape skin is anthocyanin and its synthesis in fruit occurs during the maturation process. Anthocyanin synthesis in grapes can be promoted by exogenous ABA treatment (Ovadia *et al*., 2013). Thus, although light quality, anthocyanin synthesis, and ABA synthesis may interact with each other, the effect of light quality on endogenous ABA synthesis remains unclear.

Ultraviolet (UV) has a beneficial effect on stress tolerance. Physiologically active substances such as ethylene, abscisic acid (ABA), and jasmonates play a role in signal transduction substances for stress and induce the production of secondary metabolites through the expression of tolerant genes against stress (Koo *et al*., 2009). It has been reported that polyamine conjugates may function as a radical scavenger in tobacco plants (*Nicotiana tabacum* L.) (Bors *et al*., 1989), and spermidine and jasmonate application reduced chilling injury in mangosteens (*Carcinia mangostana* L.) (Kondo *et al*., 2004). However, the relationship between their physiologically active substances and UV-C in plants is unclear.

In the present study, we investigated the effects of red and blue LED irradiation at night on endogenous ABA, anthocyanin synthesis, their related gene expressions in grape berries. In addition, the effects of UV-C irradiation on polyamines and jasmonates as well as the effects of UV-C on antioxidant activity were also investigated in apple plants.

Materials and methods

Plant materials

Twelve 7-year-old Kyoho grapevines [*Vitislabrusca* x *V. vinifera*] were grown in a greenhouse at Chiba University were used for LED irradiation experiments. Three test groups each were created. Plants in the first group were irradiated with a red LED (peak wavelength 660 nm) for three h before sunrise and three h after sunset from 25 to 99 DAFB. Plants in the second group were irradiated with a blue LED (peak wavelength 450 nm) on the same schedule. The third group consisted of untreated controls. The photosynthetic photon flux (PPF) of the red and blue LEDs was adjusted to 50 μmol/m^2/s at a distance of 10 cm.

'Orin' apple seeds [*Malus sylvestris* (L.) Mill. Var *domestica* (Borkh.) Mansf.] were sown in moist vermiculite and grown in a greenhouse at day/night temperatures of 25°C and 16 °C. Fifty-day-old seedlings were irradiated at a distance of 15 cm by UV-C fluorescent tube (National, GL 15) with a peak emission at 253.7 nm for 40 min. The luminous intensity of the ultraviolet light was measured to be 3W/m^2 and the exposure was 7.2kJ/m^2.

Quantitative analysis of ABA, jasmonate, putrescine, anthocyanin, O_2^- - radical scavenging activity, and ascorbic acid

The extraction and quantification of ABA and JA were performed according to a previously reported method (Kondo *et al*., 2001b) with GC-MS-SIM. Free putrescine was analyzed according to Kondo *et al*. (2001a). Anthocyanin was determined following the method described by Wang *et al*. (2012) with a slight modification. Analyses of the O_2^- - radical scavenging activity and ascorbic acid were performed by the method described previously (Kondo *et al*. 2002).

RNA extraction, cDNA synthesis, and quantitative real-time RT-PCR analysis

Total RNA extraction,cDNA synthesis and quantitative real time RT-PCR analysis for the *VlMYBA1-2* and *VvUFGT* were the same with previous report (Kondo *et al*., 2014).

Statistical analysis

Data are shown as means±SE of three replications, subjected to analysis of variance procedures, and separated by Fisher's least significant difference at $p \leq 0.05$ using SAS statistical analysis package (SAS Institute, Cary, NC, USA).

Results

The effects of blue and red light irradiation on bioactive compound

Endogenous ABA concentrations in red LED-treated skin increased toward 77 DAFB, then decreased toward 99 DAFB (Figure 1). ABA concentrations in red LED-treated skin were significantly higher than those in blue LED-treated or untreated control skin at 53 and 77 DAFB.

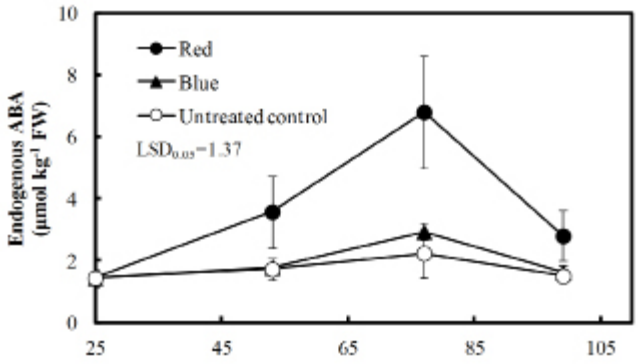

Figure 1 Endogenous ABA concentrations in red or blue LED treated-grape skins from 25 to 99 days after full bloom(DAFB). The samples were collected at 25, 53, 77, and 99 DAFB. Data are the means±SE of three replications of 30 berries.

Twenty-three anthocyanins were detected in the skins (Data not presented). The total anthocyanin concentrations in each treated group increased after 53 DAFB. In general, the concentrations in the blue LED-treated skin were highest, followed by those in red LED-treated skin, and the lowest were those in the untreated control skin. The expressions of *VlMYBA1-2* in red and blue LED-treated skin were significantly higher than those in the untreated control at 53 DAFB (Figure 3). The expression of *VvUFGT* in both the blue and red LED-treated skin increased significantly compared to that in the untreated control at 53 DAFB, but was lower than that in the untreated control at 77 DAFB.

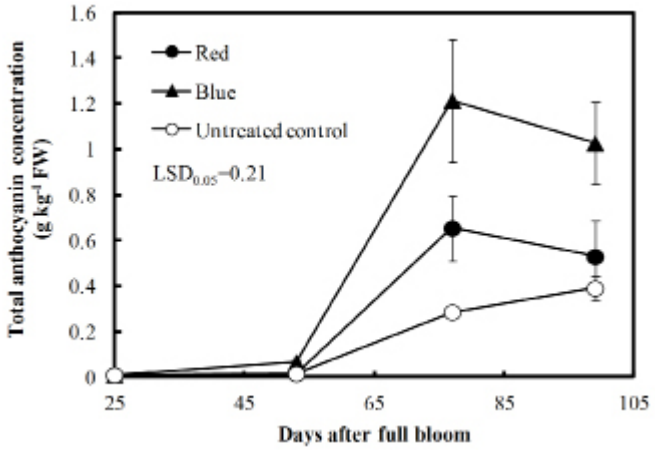

Figure 2 Changes of total anthocyanin concentrations in red or blue LED-treated skin. The samples were collected at 25, 53, 77, and 99 DAFB. Data are the means±SE of three replications of 30 berries.

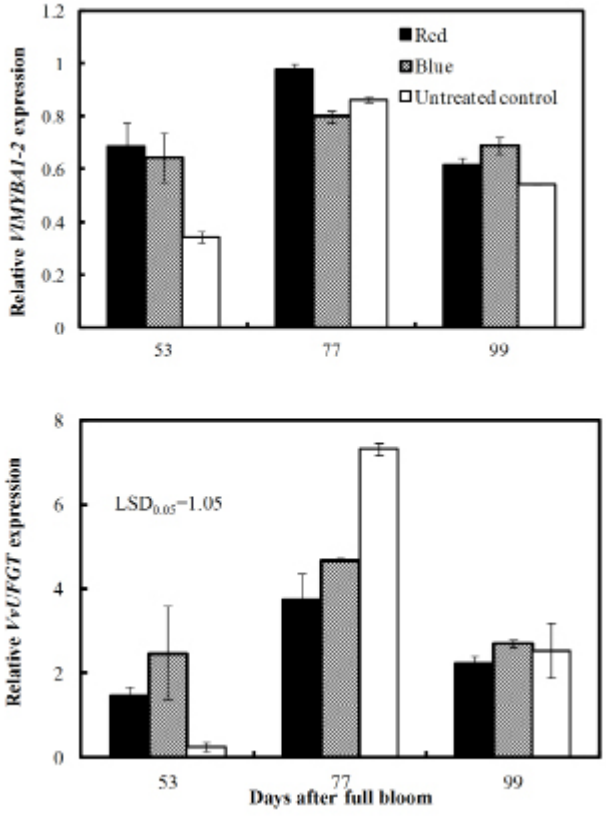

Figure 3 Quantitative real time RT-PCR analysis of *VlMYBA1-2* and *VvUFGT* in grapeskins. The experimental values are plotted compared to the control (*Ubiquitin*) value. Data are the means±SE of three replications of 30 berries.

The effects of UV-C irradiation on bioactive compound

Each $EC_{50}$of O_2- -radical scavenging was decided at the mid-point between zero and full inhibition of diazo dye formation. The $O_2^-EC_{50}$ values of the UV treatment were generally lower than those of the untreated control (Figure 4). The total ascorbic acid concentrations were generally higher than the untreated control (Figure 5). UV treatment significantly increased the JA concentrations, particularly at 7 days after UV treatment (Figure 6). The putrescine concentrations increased sharply after UV treatment (Figure 7).

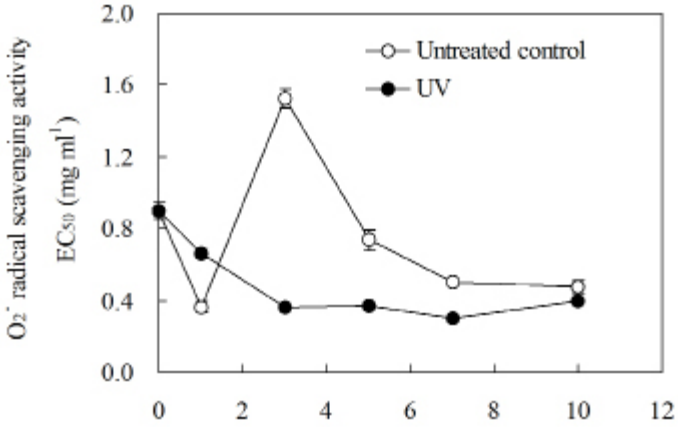

Figure 4 EC_{50} values of O_2- -radical scavenging activity in apple leaves after UV-C treatment. Data are the means of three replications.

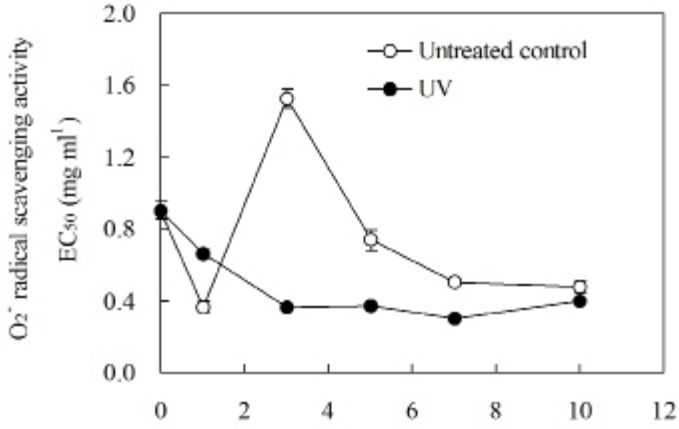

Figure 5 Total ascorbic acid concentrations in apple leaves after UV-C treatment. Data are the means of three replications.

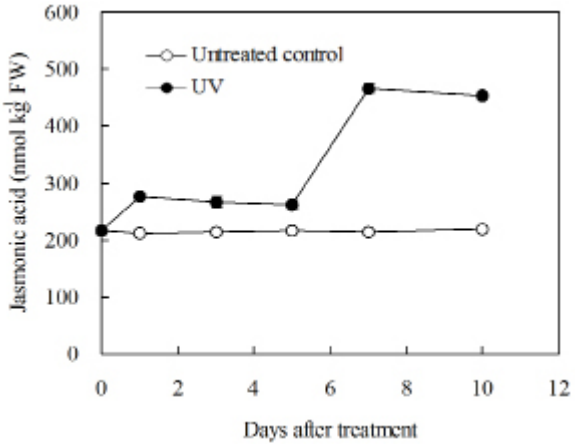

Figure 6 JA concentrations in apple leaves after UV-C treatment. Data are the means of three replications.

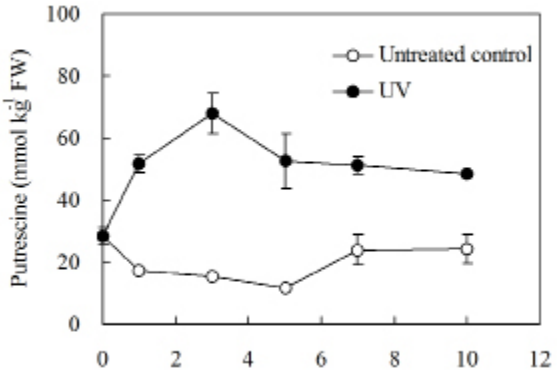

Fig V-C treatment. Data are the means of three replications.

Discussion

The effects of blue and red light irradiation on bioactive compound

Red light is primarily absorbed by phytochromes and blue light is absorbed by cryptochromes and phototropins (Nito *et al*., 2013). The results of the present study, in which whole vines were irradiated by LED, suggest thatthe synthesis of endogenous ABA in the leaves may be associated with the action of phytochromes.

However, changes in the endogenous ABA and anthocyanin concentrations in the skin did not coincide. The present results do not deny the relationship between endogenous ABA and anthocyanin synthesis because both endogenous ABA and anthocyanin concentrations in red LED-treated skin were generally higher than those in the untreated control. However, our results suggest that another factor may influence anthocyanin concentrations in grape berries significantly more than endogenous ABA. This is because ABA concentrations were highest in the red LED-treated skin, which had lower anthocyanin concentrations than the blue LED-treated skin. The present results suggest that the wavelength of blue light may promote anthocyanin synthesis in grape berries. *VlMYBA* and *UFGT* are key genes in anthocyanin synthesis in grape berries (Azuma *et al*., 2011).In the present study, the expressions of *VlMYBA1-2* and *VvUFGT* increased in red and blue LED-treated skin, though the expressions of these genes were not necessarily correlated with anthocyanin concentrations. It has been concluded that final anthocyanin concentrations in grape skin cannot be determined solely by the expression levels of *myb*-related genes (Azuma *et al*., 2011); the results of the present study are consistent with this conclusion. While anthocyanin concentrations were lowest in untreated control skin, the expression of *VvUFGT* was the lowest at 53 DAFB but the highest at 77 DAFB, suggesting that the expression of *VvUFGT* at veraison (53 DAFB) may significantly influence anthocyanin synthesis.

The effects of UV-C irradiation on bioactive compound

From the result of Figure 4, we considered the low O_2^-EC $_{50}$ values in the UV-C treated leaves to imply a high production of reactive oxygen. Our study suggested that suitable UV-C irradiation may supply beneficial effects such as the increase of antioxidant activity and ascorbic acid.

As shown in Figure 6, the JA concentrations in leaves were also increased by UV-C irradiation. On jasmonate signaling for the induction of antioxidants, it has been reported that the accumulation of phenolic compounds in wild type *Nicotiana attenuate*was stimulated by UV-B treatment compared to transgenic jasmonate-deficient plants, and jasmonate treatment increased the expression of *trypsin proteinase inhibitor*(*TPI*),which is a wound response gene(Demkura *et al*., 2010). These results suggest that UV may be associated with the synthesis of the secondary metabolites such as antioxidants through jasmonate production.

It has been suggested that polyamines may maintain membrane integrity by binding to phospholipid components of the membrane under stress conditions (DiTomaso *et al*., 1989). Therefore, polyamines may function as radical scavengers as well as antioxidants. In our study, UV-C irradiation increased the putrescine in contrast to the decrease of O_2^- EC_{50} values. These facts suggest that polyamines may play a role in scavenging reactive oxygen.

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