Suppression of red color development associated with anthocyanin accumulation in the epicarp of grape (*Vitis labrusca* × *vinifera* cv. Ruby Roman) berries caused by air temperature in daylight periods higher than 33˚C during maturation

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Abstract

The effect of high air temperature on the anthocyanin accumulation in the epicarp of 'Ruby Roman' grape (*Vitis labruscana* × *vinifera)* berries was examined. Seventeen types of anthocyanin were detected from the epicarp tissue of the matured berries using HPLC. Since a strong positive correlation ($P < 0.005$, $R^2 = 0.937$) was confirmed between the degree of color density evaluated using a color chart and the total content of anthocyanins quantified separately, it seems possible to trace anthocyanin accumulation using the color chart on each berry during maturation at different air temperatures. Four conditions were designed for cooling the fruit clusters with a spot-cooling system, which can keep air temperature 4–5˚C lower for 60–70 days after the full–bloom, i.e. consecutive (0:00–24:00) cooling, daytime (6:00–18:00) cooling, nighttime (18:00–6:00) cooling and no–cooling (control). The degree of color density showed statistically higher ($P < 0.05$) values in the consecutive–cooling treatment than that of the control through the post–cooling duration (0–15 days after cooling). To clarify the effect of temperature zones with or without daylight on the anthocyanin accumulation in the epicarp, percentage of exposure time to each temperature zone of < 21, 21–24, 24–27, 27–30, 30–33, 33–36 and 36˚C ≤ were calculated for both daylight and dark periods, separately. Correlations of the Δ time % (the amount of difference in percentage of exposure time from the control) with the Δ color degree (the amount of difference in degree of color density from the control) in the daylight period were positive at temperature zones of < 30˚C, but turned to negative at those of 30˚C ≤. The fact that correlation coefficient was statistically significant ($P < 0.05$) at the temperature zones of 27–30˚C (positive) and 33–36˚C (negative) in the daylight period shows that the threshold where the anthocyanin accumulation starts to be suppressed exists within 30–33˚C.

45 Keywords

46 Color chart; degree of color density; fruit maturation; HPLC; spot–cooling system.

47

1. Introduction

The 'Ruby Roman' grape (*Vitis labruscana* × *vinifera*) is a brand–new tetraploid cultivar bred and registered in 2007 by Ishikawa prefecture, Japan, known as a progeny of 'Fujiminori' which was open-pollinated in 1994 (Shima et al., 2006). The berries of the cultivar have red color on the epicarp and are consumed mainly as sophisticated fresh fruits. Thus, the epicarp color of the fruit is one of the cultivar's most important qualities. Accumulation of red color in the epicarp commonly starts during the veraison period, 40–50 days after the full–bloom, when the young hard fruits turn soft and begin to enlarge. On the production of grapes with red or black epicarp color, it has been pointed out that high air temperature during the veraison or later causes insufficient color development of the epicarp (Winkler, 1962), which is related to the suppression of anthocyanin accumulation (Kliewer, 1970; Mori et al., 2004; Yamane et al., 2006). In this case, the accumulation of anthocyanins was strongly affected by the local temperature of atmosphere around berries (Tomana et al., 1979; Koshita et al., 2007) and cultivars (Kliewer and Torres, 1972). Recently, Gao-Takai et al. (2019) clearly demonstrated that the accumulation and component of anthocyanins, and gene expression related to the anthocyanin biosynthesis in the epicarp of grape berries depended on the temperature. Shinomiya et al. (2015) reported that color development of the epicarp of 'Kyoho' (*V. labrusca* × *vinifera*) became poor when the average daily air temperature exceeded 27˚C. However, details on the relation of high temperature, such as threshold of temperature, duration of exposure time, with or without light, and so on, had not yet been clarified.

With anthocyanin analysis using chemical methods, plant tissues must be homogenized during the extraction procedure. This fact indicates that the process of anthocyanin accumulation into the epicarp is untraceable using the same berries attached on a vine by utilizing chemical methods. Matsuda et al. (2020a) showed that a

74 positive linear correlation was observed between the degree of color density, evaluated
75 using a color chart created for the 'Ruby Roman' berries specifically, and the content of
76 anthocyanins, quantified as cyanidin 3–glucoside equivalent using colorimetric
77 determination. The color chart seems viable for tracing anthocyanin accumulation
78 using the same berries on a vine without sampling. Furthermore, since there are
79 different kinds of anthocyanin molecules contained in the epicarp tissue of grape
80 berries (Li et al., 2013), colorimetric quantification might be too rough. So, we tried to
81 quantify anthocyanins separately using HPLC in this study, and compared the degree
82 of color density with the total content of anthocyanins, so that we can confirm if degree
83 of color density is viable for tracing anthocyanin accumulation in the epicarp of the
84 berries maturing in different conditions.
85 Another problem in investigating the effect of air temperature on anthocyanin
86 accumulation is that it is very difficult to control air temperature around the berries
87 accurately on the vines grown in a plastic greenhouse. To resolve this matter we
88 employed a spot–cooling system which can keep air temperature around the berries at
89 about 4–5˚C lower than the outside air temperature. Tarara et al. (2008) also tried to
90 control air temperature around berry clusters of ‘Merlot’ grape using a forced convection
91 system to produce a dynamic range of berry temperatures under field conditions in both
92 sun-exposed and shaded fruit. We arranged three treatments on air temperature
93 associated with day/night cooling, and compared the color development on cooled
94 berries with that of the control (without cooling). Furthermore, the amount of
95 difference in both exposure time to a specific air temperature zone and the degree of
96 color density against those of the control were calculated in each treatment, and then
97 correlation of these two factors was used to evaluate the effect of air temperature on
98 the accumulation of red color (anthocyanins) in the epicarp.
99

2. Materials and Methods

2.1. Quantification of anthocyanins using HPLC

The berries of 'Ruby Roman' grape categorized into 1–10 degrees of color density using a specific color chart (Matsuda *et al*., 2020a) were randomly collected in 2014 from 8–year–old vines which had been grown in a rain–protected plastic greenhouse without side covers at Ishikawa Agriculture and Forestry Research Center, Kahoku, Ishikawa, Japan. In this case, three berries with the same degree were used for anthocyanin analyses, respectively. An epicarp disk (8 mm in diameter) of the berry was punched out at around the top (style side) of the berry using a cork borer, homogenized using mortar and pestle with 1 mL of 50%(v/v) acetic acid, incubated for 12 h at 4˚C in the dark, centrifuged at 10,000 × *g*, and then supernatant was filtrated through a polyvinylidene difluoride filter (0.45 μm in pore size, EMD Millipore, Darmstadt, Germany). Anthocyanins were analyzed using a High Performance Liquid Chromatography (HPLC) system (Prominence LC20A instrument, Shimadzu, Kyoto, Japan) as was described by Katayama–Ikegami et al. (2016). The conditions for HPLC were as follows: mobile phase, linear gradient analysis of 1.5%(v/v) phosphoric acid (solvent A) and 1.5%(v/v) phosphoric acid, 20%(v/v) acetic acid and 25%(v/v) acetonitrile (solvent B); pump, LC–20AD (Shimadzu); column, Inertsil® ODS–2 analytical (250 × 6.0 mm, 5 μm in silica gel particle size; GL Sciences, Tokyo, Japan); temperature, 35˚C (CTO–20A column oven; Shimadzu); detector, UV–VIS (SPD–20AV, Shimadzu); absorbance of wave length, 520 nm (A_{520}); flow rate, 0.8 mL min^{-1}; sample volume, 10 μL. The gradients of solvent A/B were as follows: 0–40 min, linear gradient from 75/25 to 15/85; 40–45 min, 75/25. Depending on the peak area monitored at A_{520}, each anthocyanin of mono glucoside was quantified using an external standard curve for cyanidin 3–glucoside (Cy3G), peonidin 3–glucoside (Pn3G), delphinidin 3–glucoside (Dp3G), petunidin 3–glucoside (Pt3G) and malvidin

3–glucoside (Mv3G), respectively. The other anthocyanins of cyanidin 3,5–diglucoside (Cy3,5G), delphinidin 3–(*p*–coumaroyl glucoside)–5–glucoside (Dp3*p*G5G), cyanidin 3–(*p*–coumaroyl glucoside)–5–glucoside (Cy3*p*G5G), petunidin 3–(*p*–coumaroyl glucoside)–5–glucoside (Pt3*p*G5G), delphinidin 3–*p*–coumaroyl glucoside (Dp3*p*G), peonidin 3–(*p*–coumaroyl glucoside)–5–glucoside (Pn3*p*G5G), malvidin 3–(*p*–coumaroyl glucoside)–5–glucoside (Mv3*p*G5G), cyanidin 3–*p*–coumaroyl glucoside (Cy3*p*G), petunidin 3–*p*–coumaroyl glucoside (Pt3*p*G), peonidin 3–*p*–coumaroyl glucoside (Pn3*p*G), malvidin 3–*p*–coumaroyl glucoside (Mv3*p*G), and malvidin 3–acetylglucoside (Mv3aG) were identified by comparing the retention time and elution order with those described by Shiraishi et al. (2007), then quantified as mg of mono glucoside equivalent which have the same aglycone (anthocyanidin) using the standard curves for Cy3G, Pn3G, Dp3G, Pt3G and Mv3G, since no authentic sample was available. Total content of anthocyanins was calculated by adding all of them.

2.2. Procedures on fruit growing

Experiments were performed in 2016 using two vines of 10‑year–old ‘Ruby Roman’ grown in a rain–protected plastic greenhouse without side covers at Ishikawa Agriculture and Forestry Research Center. In this case, the roof of the house was covered in April 2, and the date of the full‑bloom and the onset of veraison was May 20 and June 30, respectively. To induce artificial parthenocarpy and make berries seedless, all the fruit clusters at the full–bloom were dipped in a solution containing 25 ppm of gibberellic acid (GA_3) supplemented with 5 ppm of forchlorfenuron (1–(2–chloro–4–pyridyl)–3–phenylurea, CPPU), then dipped again in the same GA_3 solution without CPPU eleven days after the full–bloom. A few days after the second GA_3 treatment, some fruit clusters were thinned to bring the leaf/cluster ratio closer to 90, i.e. 3 shoots/cluster. This shoots/cluster ratio followed the guideline for growers of

152 ‘Ruby Roman’ vines, which was set by Ishikawa prefecture government since high
153 quality berries must be produced. In addition, some berries in a cluster were also
154 thinned to set the number of berries as 25/cluster. Then, all the fruit clusters were
155 covered with a BIKOO® perforated plastic bag (30 × 40 cm, Nidaiki Corp., Komaki,
156 Japan) with numerous 0.1 mm pores, attached with a paper umbrella for shading on the
157 top of each fruit cluster. This transparent bag can help picking off the cracked-berries
158 from fruit cluster and confirming color development on the epicarp of berries.
159 Perforation is for preventing temperature rise in the bag. In this case, 13% of light
160 intensity was reduced by the bag which was determined using an illuminometer
161 (RTR-574, T&D, Matsumoto, Japan). Other cultivation managements like fertilization,
162 sprinkling and pest control followed the practice. The above–mentioned procedures are
163 very common in the commercial production of ‘Ruby Roman’ berries.
164
165 *2.3. Utilization of a spot–cooling system to decrease air temperature around fruit*
166 *clusters*
167 The spot–cooling system (utility model registration number 3204876, Japan Patent
168 Office) employed in this study is composed of three units: a spot–cooling apparatus
169 (SUASP1DS, Daikin, Osaka, Japan) equipped with 2.4 kW cooler, which can decrease
170 the blowing air temperature 9.4˚C lower than that of the suction air at a flow rate of 6.6
171 m^3 min^{-1}; clear plastic bags covering the fruit cluster but not closed; thermal insulated
172 pipes and tubes connecting the apparatus and bags (Fig. 1). In this case, 60 fruit bunches
173 in maximum can be cooled down to 4–5˚C lower than the outside temperature by the
174 system, simultaneously.
175 Four experimental groups were designed on cooling the fruit clusters: consecutive
176 (0:00–24:00) cooling; daytime (6:00–18:00) cooling; nighttime (18:00–6:00) cooling;
177 no–cooling (control). Duration of the cooling treatment was from July 19 to July 29

178 (from 60 to 70 days after the full–bloom), since color development on the berries was
179 the most sensitive to air temperature during this period (Matsuda et al., 2020a). Eleven
180 fruit clusters were used for each treatment. The air temperature was monitored at the
181 center of the fruit cluster in bags using a thermometer (RTR‑502, T&D, Matsumoto,
182 Japan) and recorded every 15 min. The surface color of the berries was classified into
183 1–10 degrees of color density using the color chart every 5 days from July 19 to August
184 13 (60, 65, 70, 75, 80 and 85 days after the full–bloom).
185
186 *2.4. Statistical analyses of data*
187 The content of anthocyanins and the degree of color density in the epicarp were
188 represented as average ± SE (n = 3 and n = 11, respectively). Differences in the degree
189 of color density among cooling treatments were analyzed statistically using analysis of
190 variance (ANOVA) followed by Tukey's multiple range test.
191
192 **3. Results**
193 *3.1. Anthocyanin content in epicarp related to the degree of color density determined*
194 *using the color chart*
195 An example high performance liquid chromatogram of the anthocyanin analysis
196 was shown in Fig. 2. This sample was extracted from epicarp of matured berries,
197 whose color was classified into the degree 9 in density using the color chart. Seventeen
198 anthocyanin types were confirmed, which was the maximum number in the 'Ruby
199 Roman' berries examined. They were composed of 4 cyanidin types, 3 peonidin types,
200 3 delphinidin types, 3 petunidin types and 4 malvidin types (Table 1). The total
201 anthocyanin content per epicarp area was calculated as 33.92 μg cm^{-2}. Among
202 anthocyanins, the greatest content (6.87 μg cm^{-2}) of Cy3G occupied 20.3% of the total,
203 the second greatest (5.72 μg cm^{-2}) of Cy3*p*G occupied 16.9%, and the third greatest

(3.26 μg cm^{-2}) of Pn3G occupied 9.6%. Similarly, the total anthocyanin content in epicarp of the berries whose color was classified into 1–10 degrees in density was determined by the same manner with three replications, respectively. In this case, correspondence of the color degree to the fruit developmental stage of berries is as follows: degree 1, second stage; degrees 2–7, third stage; degrees 8–10, harvest stage. Of course, the detected types of anthocyanins were smaller than 17 in berries with poor color density (Suppl. 1). Then, the total anthocyanin content was compared with the degree of color density. As a result, a strong positive correlation ($P < 0.005$, $R^2 = 0.937$) was confirmed between both factors (Fig. 3). The results show that the anthocyanin accumulation in epicarp of a berry can be monitored using the color chart exactly.

3.2. Effect of cooling berries on red color development (anthocyanin accumulation) in the epicarp

Effects of the cooling treatments, i.e. consecutive–cooling, daytime–cooling and nighttime–cooling, on red color development in the epicarp were shown in Fig. 4. In all cooling treatments, the degree of color density increased gradually along with the maturing of berries through and after the cooling period. Furthermore, the degree of color density in consecutive–cooling treatment was statistically greater ($P < 0.05$) than that of the control (without cooling) from 0 through 15 days after the cooling period. The curves of both the daytime–cooling and the nighttime–cooling treatments existed between the curves of the consecutive–cooling treatment and the control, but not significantly different from the control.

During the cooling period (July 19–29), sunrise and sunset were at around 5:00 and 19:15, respectively. To evaluate the effect of the air temperature in more detail on the red color development with or without daylight, we employed a concept of temperature

zone composed of 7 zones (< 21, 21–24, 24–27, 27–30, 30–33, 33–36 and 36˚C ≤) of air temperature with 3˚C interval basically in this experiment. Then, percentage of exposure time when the monitoring fruit cluster had been exposed to each temperature zone to total time during the experiment were calculated for daylight (5:00–19:15) and dark (19:15–5:00) period, separately (Fig. 5). It must be noticed that daylight and dark periods did not correspond to the periods of daytime– and nighttime–cooling, respectively. Furthermore, the amount of difference in the percentage of exposure time to each temperature zone from that of the control (Δ time %) were calculated in each cooling treatment. Similarly, the amount of difference in average degree of color density (mean value from 0 through 15 days after the cooling period) in each treatment from that of the control (Δ color degree) were calculated. To clarify the effect of the temperature zones on the red color development (anthocyanin accumulation) in the epicarp, correlations of the Δ time % with the Δ color degree at each temperature zone were shown in Fig. 6. During the daylight period, the relationship between the Δ time % and the Δ color degree was linearly positive at the temperature zones less than 30˚C, but turned negative at the temperature zones greater than 30˚C. In this case, correlation coefficient was statistically significant ($P < 0.05$) at temperature zones of 27–30˚C and 33–36˚C. By contrast, no certain direction was confirmed between these two factors during the dark period.

4. Discussion

The color density of the epicarp in red/black–type grape berries would be affected mainly by the component, kinds and the amount, of anthocyanins and pH in the tissues. The anthocyanin analyses using HPLC in this study revealed that the major kinds were Cy3G and Cy3*p*G, the cyanidin type both with red color, the sum of which occupied 37.2% of the total anthocyanin content. When compared with the other cultivars, the

anthocyanin component in the matured ‘Ruby Roman’ epicarp (Table. 1) was similar to that of ‘Steuben’ with the high content of Cy3G, Cy3*p*G, Dp3G and Dp3*p*G, but containing some Pn3G and Pn3*p*G in contrast to ‘Steuben’ (Shiraishi et al., 2007). Furthermore, the ‘Ruby Roman’ epicarp also contained a certain level of Pt3G, Pt3*p*G, Mv3G and Mv3*p*G. Thus, the red color of ‘Ruby Roman’ berries seems to be due to this specific composition of anthocyanins accumulated in the epicarp.

On the anthocyanin content related to the degree of color density in the ‘Ruby Roman’ epicarp, the correlation coefficient (R^2 = 0.937) in the present study was higher than that (R^2 = 0.745) in the previous study (Matsuda et al., 2020a) where the anthocyanin content was quantified by colorimetric method. Since anthocyanins were quantified more precisely using HPLC, which showed a strong correlation with the degree of color density (Fig. 3), it seems that monitoring the accumulation of anthocyanins in the ‘Ruby Roman’ epicarp using the color chart is quite exact. Indeed, the color chart has also been employed for estimating anthocyanin content in the epicarp of apples (Marsh et al., 1996), raspberries (Stavang et al., 2015) and grapes such as ‘Kyoho’, ‘Pione’ and ‘Suzuka’ (Sugiura et al., 2018; Yamazaki and Suzuki, 1980), even if correlation coefficients were smaller than that of the present study.

It is well known that light intensity play an important role on the anthocyanin accumulation in the epicarp of grape berries. Matsuda et al. (2020b) demonstrated utilizing *in vitro*-cultured ‘Ruby Roman’ berries that the anthocyanin accumulation occurred only when artificial light with luminous intensity of 5,000 lx or more was irradiated from fluorescent tube. In the present study, the light intensity in the green house was kept at more than 5,000 lx from 1h after sunrise to 1h before sunset during the experiment (Suppl. 2). However, we will need to have deeper discussion about the relationship between light intensity and the anthocyanin accumulation.

281 The roles of temperature on the anthocyanin accumulation in grape berries have
282 been discussed in relation to day/night temperature. The discussions were categorized
283 into 4 groups as the accumulation was accelerated by: lower temperature in daytime
284 (Kliewer, 1970); lower temperature in nighttime (Koshita et al., 2007); lower daily
285 average temperature (Shinomiya et al., 2015; Sugiura et al., 2018); the difference
286 between day/night average temperature (Tomana et al., 1979). In the present study, the
287 degree of color density showed statistically higher values in the consecutive–cooling
288 treatment than that of the control through the post–cooling duration (0–15 days after
289 cooling), but the effect of daytime– or nighttime–cooling alone was not clear (Fig. 4).
290 However, it was clearly demonstrated that the relationship between the Δ time % and
291 the Δ color degree was positive at less than 30˚C, but negative at greater than 30˚C
292 during the daylight period. Thus, our results indicate that high air temperature (30˚C ≤)
293 during the daylight period would suppress the anthocyanin accumulation in the epicarp
294 of 'Ruby Roman' berries.
295 The concept of temperature zone was employed in this study, since we thought
296 that anthocyanin biosynthesis related to some enzymatic reactions might have the
297 optimum temperature. Another possibility of concerning air temperature is based on
298 the concept of accumulated temperature during the treatments, which was not a divided
299 section like a zone but whole. From this point of view, the average air temperature was
300 calculated from the temperature data (570 and 390 data for daylight and dark period,
301 respectively) determined every 15 min for each cooling treatment, and correlation
302 between the average values and those of the Δ color degree was examined (Table 2).
303 As a result, the correlation coefficient was statistically significant ($P < 0.05$) only in
304 the case of daylight period. This fact clearly indicates the involvement of daylight on
305 the anthocyanin accumulation in the grape epicarp, which was mentioned previously
306 by many researchers (Jeong et al., 2004; Pereira et al., 2006; Matus et al., 2009;

307 Azuma et al., 2012; Koyama et al., 2012). In this case, it was also demonstrated that
308 effect of the difference in the average air temperature between daylight and dark period
309 on the anthocyanin accumulation was not significant.

310 On the critical temperature which suppresses anthocyanin accumulation, it has
311 been pointed out that high temperature during daytime inhibits anthocyanin
312 accumulation in maturing berries (Kobayashi et al., 1967; Kliewer, 1970; Mori et al.,
313 2004). However, since these studies were performed at a constant daytime temperature
314 of 30˚C or less set by using a controlled climate room, they couldn't clarify the
315 threshold temperature. The facts that the correlation between the Δ time % and the Δ
316 color degree turned from positive to negative at 30˚C, and furthermore the correlation
317 coefficient was statistically significant ($P < 0.05$) at the temperature zones of 27–30˚C
318 (positive) and 33–36˚C (negative) indicate that the critical point (threshold) where the
319 anthocyanin accumulation starts to be suppressed may exist within 30–33˚C (the
320 transitional temperature zone). It is concluded that the anthocyanin accumulation
321 would be suppressed strongly by air temperature more than 33˚C during the daylight
322 period. Thus, it is suggested that keeping the air temperature around the fruit clusters in
323 veraison or later at < 30˚C during daylight period is indispensable for fully developing
324 red color on the epicarp of 'Ruby Roman' berries.

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Legends of Figure

Fig. 1. A picture on cooling fruit clusters of 'Ruby Roman' berries using a spot-cooling system. Cold-air flow was supplied from a cooler via the thermal insulated main pipe (mp), the branch pipes (bp) and the tubes (t) into the clear plastic bags (pb). Length of the branch pipes and the loci where they connected with the main pipe are adjustable.

Fig. 2. An example high performance liquid chromatogram of anthocyanins detected from the epicarp of matured 'Ruby Roman' grape berries. Cy3,5G: cyanidin 3,5–diglucoside, Dp3G: delphinidin 3–glucoside, Cy3G: cyanidin 3–glucoside, Pt3G: petunidin 3–glucoside, Pn3G: peonidin 3–glucoside, Mv3G: malvidin 3–glucoside, Dp3*p*G5G: delphinidin 3–(*p*–coumarylglucoside)–5–glucoside, Cy3*p*G5G: cyanidin 3–(*p*–coumarylglucoside)–5–glucoside, Pt3*p*G5G: petunidin 3–(*p*–coumarylglucoside)–5–glucoside, Dp3*p*G: delphinidin 3–*p*–coumarylglucoside, Mv3aG: malvidin 3–acetylglucoside, Pn3*p*G5G: peonidin 3–(*p*–coumarylglucoside)–5–glucoside, Mv3*p*G5G: malvidin 3–(*p*–coumarylglucoside)–5–glucoside, Cy3*p*G: cyanidin 3–*p*–coumaroylglucoside, Pt3*p*G: petunidin 3–*p*–coumaroylglucoside, Pn3*p*G: peonidin 3–*p*–coumaroylglucoside, Mv3*p*G: malvidin 3–*p*–coumaroylglucoside.

Fig. 3. Relationship between the degree of color density and the total anthocyanin content. Values are average ± SE ($n = 3$). ***$P < 0.005$.

Fig. 4. Effects of the cooling treatments on red color development in the epicarp of maturing berries. Treatments were as follows: consecutive–cooling (open circle),

445 daytime–cooling (open triangle), nighttime–cooling (closed circle) and no–cooling
446 (control) (closed triangle). Cooling treatments were made from 60 to 70 days after
447 the full–bloom. The values represent average ± SE (n = 11). Where no error bar is
448 visible SE was smaller than the symbols. The difference between the values labeled
449 with different alphabets was statistically significant ($P < 0.05$, Tukey's multiple
450 range test).
451
452 Fig. 5. Percentage of exposure time to each temperature zone associated with the
453 cooling treatments in daylight and dark period, respectively.
454
455 Fig. 6. Correlations between the Δ time % and the Δ color degree in each temperature
456 zone in daylight and dark period, respectively. The Δ time % and the Δ color degree
457 mean the amount of difference in percentage of exposure time and in degree of
458 color density from those of the control, respectively.
459

'Ruby Roman' grape
(*Vitis labruscana* × *vinifera*)

HPLC
17 types of anthocyanin
Total content

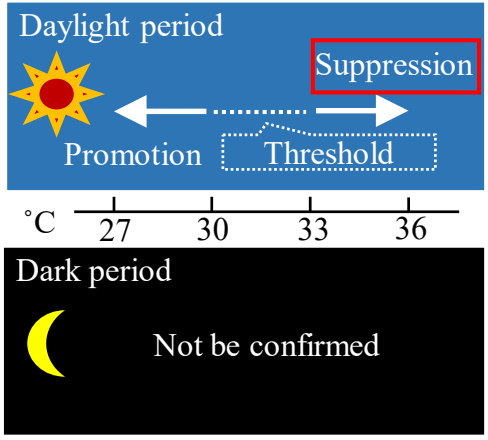
×

color chart

strong positive correlation
($P < 0.005$, $R^2 = 0.937$)

Correlations of the Δ time % (difference exposure time from the control) with the Δ color degree

Anthocyanin accumulation

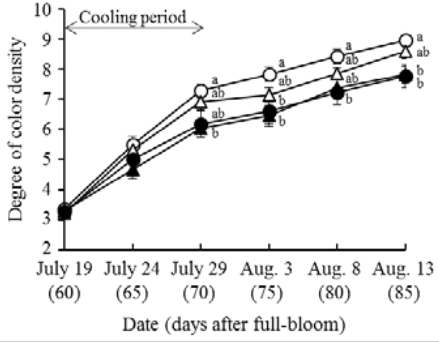


In greenhouse

Spot-cooling system

Spot-cooling system can keep air temperature 4–5˚C lower for 60–70 days after the full–bloom

- Consecutive (0:00–24:00) cooling (○)
- Daytime (6:00–18:00) cooling (△)
- Nighttime (18:00–6:00) cooling (●)
- No–cooling (control) (▲)



Highlights

Seventeen types of anthocyanin were detected from the epicarp of matured berries

Degree of the epicarp color density was positively correlated with total anthocyanins

Fruit clusters after veraison were grown at four different air temperature conditions

Anthocyanin accumulation was suppressed strongly by 33˚C < during daylight period

Anthocyanin accumulation starts to be suppressed at a temperature within 30–33˚C

Table 1. Component of anthocyanins detected from the epicarp tissue of matured 'Ruby Roman' berries.

Anthocyanins	Content (μg·cm^{-2})	(%)
Cy3G	6.871 ± 0.742[z]	20.3
Cy3*p*G	5.723 ± 0.278	16.9
Cy3,5G	0.700 ± 0.112	2.1
Cy3*p*G5G	0.558 ± 0.015	1.6
Pn3G	3.261 ± 0.242	9.6
Pn3*p*G	2.784 ± 0.081	8.2
Pn3*p*G5G	1.093 ± 0.022	3.2
Dp3G	2.676 ± 0.245	7.9
Dp3*p*G	2.185 ± 0.181	6.4
Dp3*p*G5G	0.519 ± 0.051	1.5
Pt3G	1.670 ± 0.170	4.9
Pt3*p*G	1.239 ± 0.085	3.7
Pt3*p*G5G	0.795 ± 0.028	2.3
Mv3G	2.119 ± 0.154	6.3
Mv3*p*G	1.157 ± 0.050	3.4
Mv3aG	0.232 ± 0.005	0.7
Mv3*p*G5G	0.335 ± 0.019	1.0
Total	33.917	100.0

[z]Average ± SE (n = 3).

Table 2. Correlation between the average air temperature during daylight and/or dark period, the difference, and the Δ color degree.

Cooling treatment	Average air temperature (˚C)			Difference (˚C) [A – B]
	Whole day	Daylight period [A] (5:00-19:15)	Dark period [B] (19:15-5:00)	
Consecutive	23.8	25.6	21.2	4.4
Daytime	26.1	26.7	25.2	1.5
Nighttime	25.6	29.0	20.6	8.4
No-cooling (Cont.)	28.6	30.6	25.6	5.0
r value vs. Δ color degree	– 0.765	– 0.956 *	– 0.212	– 0.566

'Δ color degree': see Fig. 6.
'*r* value' means Pearson's correlation coefficient.
*$P < 0.05$.

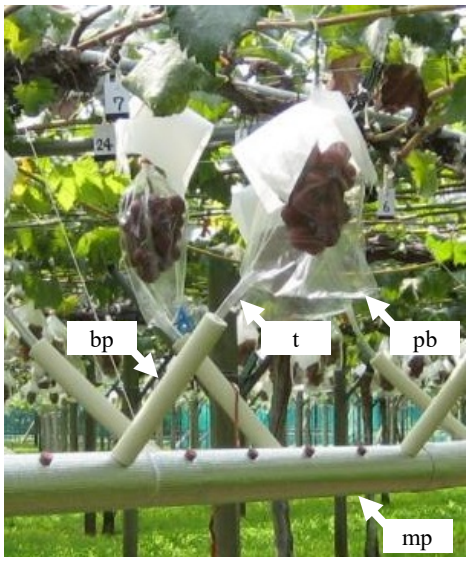


Fig. 1

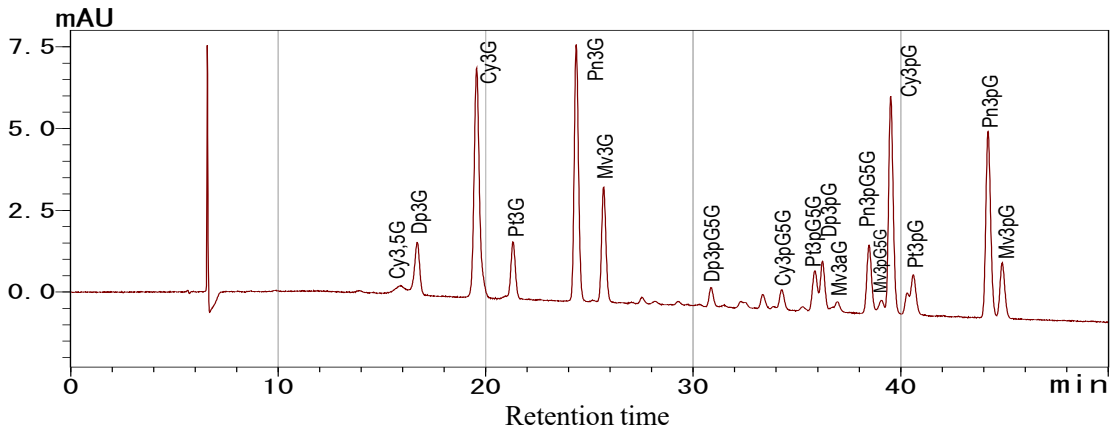

mAU
7.5
5.0
2.5
0.0
Cy3,5G
Dp3G
Cy3G
Pt3G
Pn3G
Mv3G
Dp3pG5G
Cy3pG5G
Pt3pG5G
Dp3pG
Mv3aG
Pn3pG5G
Mv3pG5G
Cy3pG
Pt3pG
Pn3pG
Mv3pG
0
10
20
30
40
min
Retention time

Fig. 2

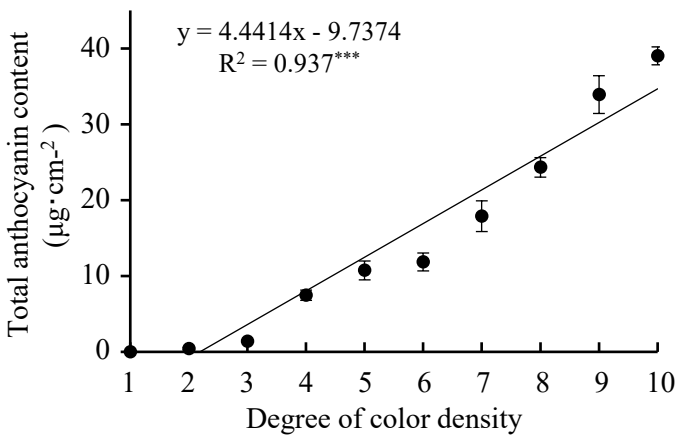

y = 4.4414x - 9.7374
R2 = 0.937***
Total anthocyanin content
(μg·cm-2)
0
10
20
30
40
1
2
3
4
5
6
7
8
9
10
Degree of color density

Fig. 3

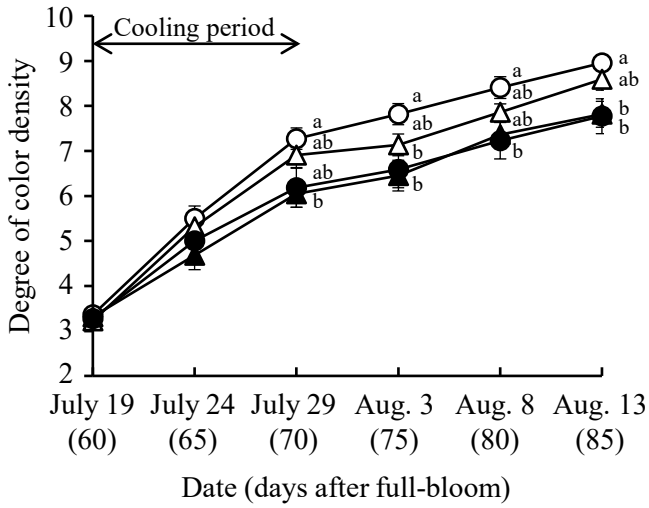

Cooling period
Degree of color density
10
9
8
7
6
5
4
3
2
a
ab
b
July 19 (60)
July 24 (65)
July 29 (70)
Aug. 3 (75)
Aug. 8 (80)
Aug. 13 (85)
Date (days after full-bloom)

Fig. 4

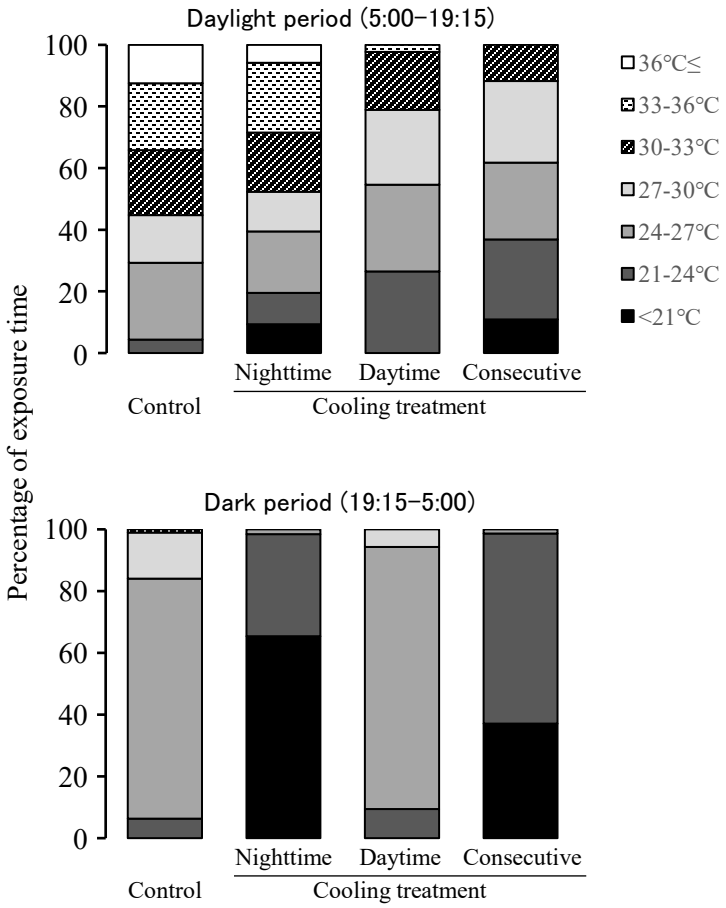

Daylight period (5:00–19:15)
Percentage of exposure time
100
80
60
40
20
0
Control
Nighttime
Daytime
Consecutive
Cooling treatment
36℃≤
33-36℃
30-33℃
27-30℃
24-27℃
21-24℃
<21℃
Dark period (19:15–5:00)
100
80
60
40
20
0
Control
Nighttime
Daytime
Consecutive
Cooling treatment

Fig. 5

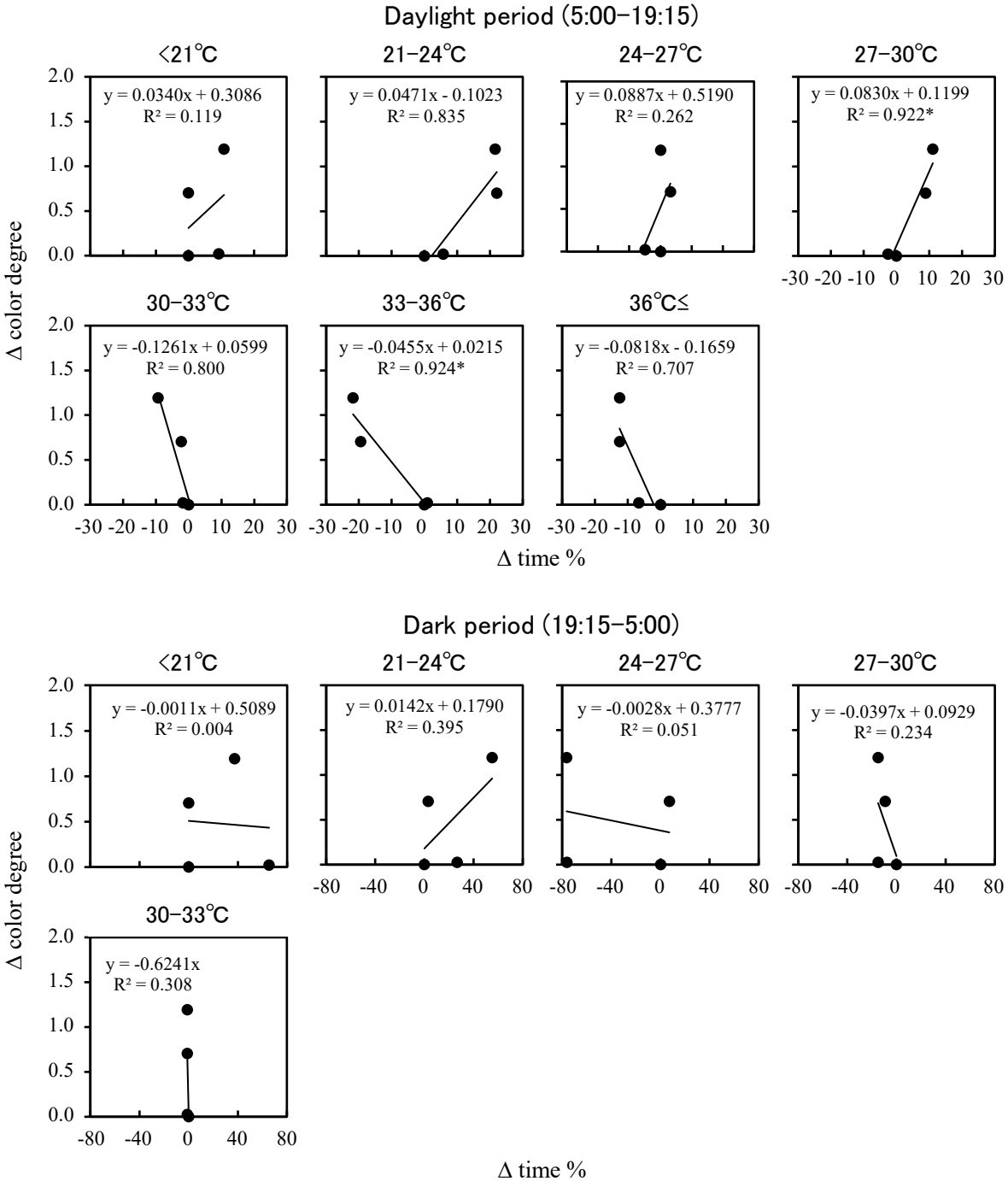

Daylight period (5:00–19:15)
<21℃
y = 0.0340x + 0.3086
R² = 0.119
21–24℃
y = 0.0471x - 0.1023
R² = 0.835
24–27℃
y = 0.0887x + 0.5190
R² = 0.262
27–30℃
y = 0.0830x + 0.1199
R² = 0.922*
30–33℃
y = -0.1261x + 0.0599
R² = 0.800
33–36℃
y = -0.0455x + 0.0215
R² = 0.924*
36℃≤
y = -0.0818x - 0.1659
R² = 0.707
Δ color degree
Δ time %
Dark period (19:15–5:00)
<21℃
y = -0.0011x + 0.5089
R² = 0.004
21–24℃
y = 0.0142x + 0.1790
R² = 0.395
24–27℃
y = -0.0028x + 0.3777
R² = 0.051
27–30℃
y = -0.0397x + 0.0929
R² = 0.234
30–33℃
y = -0.6241x
R² = 0.308
Δ color degree
Δ time %

Fig. 6