Vitis 21, 325—332 (1982)

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Effect of abscisic acid and defoliation on anthocyanin accumulation in Kyoho grapes (*Vitis vinifera* L. × *V. labruscana* BAILEY)

by

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Der Einfluß von Abscisinsäure und Entblätterung auf die Anthocyanakkumulation in Trauben der Rebsorte Kyoho (*Vitis vinifera* L. × *V. labruscana* BAILEY)

Zusammenfassung. — Wurden Kyoho-Trauben zu Beginn der Beerenreife mit 1 000 ppm ABS behandelt, so war in den Beerenhäuten verstärkt Anthocyan angereichert, ohne daß Mostgewicht und tritierbare Säure des Beerensaftes irgendwie beeinflußt waren.

Durch Entblätterung der Reben zur Zeit des Weichwerdens der Beeren wurde die Anthocyansynthese vollkommen unterdrückt, und das Mostgewicht des Beerensaftes wie auch die Zuckerkonzentration der Beerenhaut waren gegenüber der Kontrolle bedeutend verringert. Ferner nahm die Konzentration der endogenen ABS in Beerenhaut und -fleisch während der ganzen Reifeperiode nicht zu.

Bei den entblätterten Reben setzte nach der Applikation von ABS die Anthocyanakkumulation in den Beeren wieder ein.

Aus den vorliegenden Ergebnissen wird gefolgert, daß die Anreicherung von ABS in der Beerenhaut einer der Hauptfaktoren ist, die an der Anthocyansynthese beteiligt sind.

Introduction

Skin color is one of the most important parameters for evaluating the quality of red and black grapes.

Color development of a grape berry is influenced by various environmental, physiological and chemical factors: temperature, light, soil moisture, nutrition, leaf area, crop levels, certain chemicals, plant growth substances, etc. (WINKLER *et al.* 1962).

Temperature has most profound effect among the climatic factors. It is well known that in hot regions pigment formation is inhibited in many varieties (KLIEWER 1977, TOMANA *et al.* 1979 a). This is true for Kyoho grape, which, though gaining popularity recently as a high quality table grape in Japan, does not develop its favored deep purple color but is dull red in the southwestern regions with high night temperature during ripening season.

In the previous papers, we studied the influence of controlled temperatures during ripening period on the ripening process and coloration and found that the level of anthocyanin was markedly reduced by such high fruit temperature as 30 °C with concomitant inhibition of abscisic acid (ABA) accumulation in the skin (LEE *et al.* 1979, TOMANA *et al.* 1979 b).

In this paper, we paid attention to the close relationship between anthocyanin development and the level of ABA in the skin and examined whether exogenously applied ABA would improve or enhance coloration of Kyoho grapes under the prevailing conditions in Kyoto (mean daily temperature in July and August is 26.3—27.5 °C).

Furthermore, ABA application was combined with defoliation at ripening stage, the latter being expected to adversely affect sugar content of a berry, thus resulting in poor coloration.

Materials and methods

Plant materials and treatments

Experiment 1. — In 1979, mature vines of cv. Kyoto growing in the experimental farm of Kyoto University were used. Clusters were thinned to one per shoot and the number of berries per cluster was adjusted to 30.

ABA was applied at two different dates: July 20 (at veraison) and August 3 (80 % of berries coloring). ABA solution at 0 or 1000 ppm in 70 % ethanol containing 0.05 % Tween 20 was brushed lightly onto the berries. There were 12 replicates per treatment.

Experiment 2. — 5-year-old pot-grown vines of Kyoho were used. The vines were disbudded to leave a single shoot with one cluster. The number of berries per cluster was adjusted to 20 before treatment. ABA application and total defoliation were conducted on July 19 when soluble solids content in the berry was about 6.5 %. Five treatments were assigned as follows:

1. No defoliation without ABA (control)
2. No defoliation with ABA at 1000 ppm
3. Defoliation without ABA
4. Defoliation with single application of ABA at 1000 ppm (July 19)
5. Defoliation with two applications of ABA at 1000 ppm (July 19 and 26)

ABA was used in the same way as in experiment 1; 0.05 % Tween 20 in 70 % EtOH was applied to treatment 1 and 3. There were 8 replicates per treatment.

Analytical procedures

In both experiment 1 and 2, 20 berries were collected at intervals during ripening period.

For the anthocyanin analysis, 5 discs of the berry skin were extracted in acidified methanol and the absorbance of the extract was measured at 530 nm after appropriate dilution.

Total soluble solids in the juice were measured by a hand refractometer. Total acidity was determined by neutralizing the juice with 0.05n NaOH to the end point of phenolphthalein and expressed as g tartaric acid/100 ml juice. Total sugars in the skin were extracted in 80 % ethanol and measured by anthrone method. These measurements were replicated five times.

For ABA analysis, picked berries were quickly separated into the skin and flesh, then homogenized and extracted in 80 % methanol. The detailed procedure of processing the extract for GLC is shown on page 327.

The fractions of free and bound type ABA were methylated with diazomethane and 2μl of the methylated samples were injected into a Yanaco model G 180EN GLC equipped with a non-radioactive ECD and a glass column (1 m × 3 mm) packed with 10 % SE-30 on 60—80 mesh Chromosorb W (AW, DMCS). The injection port, column and detector temperatures were 265, 210 and 250 °C, respectively. The carrier gas, He, had a flow rate of 40 ml/min. The measurements of ABA were triplicated.

Procedure of processing the extract for GLC

Homogenize materials in 80 % MeOH
|
Extract for 24 h at 0 °C in dark
|
Evaporate the extract to aqueous phase
|
Adjust pH to 3.0
|
Add 200 mg of PVP and filter after 5 min
|
Partition against CH_2Cl_2

Aqueous phase
|
Adjust pH to 10.0
|
Hydrolyse at 60 °C for 1 h
|
Adjust pH to 3.0
|
Partition against CH_2Cl_2
|
Organic phase——— —Bound-ABA

Organic phase
|
Partition against CO_3^{--} buffer pH 10.0
|
Adjust pH to 3.0
|
Partition against CH_2Cl_2
|
Organic phase——— —Free-ABA

Results

Experiment 1. — The anthocyanin concentration in the control berries started to increase gradually after veraison but that of ABA treated berries increased rapidly after the treatment at either date. At the final sample on August 31, anthocyanin was threefold higher in ABA treated berries than in the control (Fig. 1). ABA treatment, however, had no effect on soluble solids and total acids in the juice (Fig. 2 A, B).

Experiment 2. — Total defoliation completely inhibited anthocyanin formation throughout the ripening period (Fig. 3). However, when ABA was applied in combination with defoliation, anthocyanin accumulation proceeded normally and reached the same level as or even higher than the control. Repeated applications were more effective than single ones. Anthocyanin was highest at ABA treatment without defoliation.

Total soluble solids concentration in the juice was greatly reduced by defoliation whether with or without ABA treatment (Fig. 4 A). There was no difference in the total acidity among the treatments (Fig. 4 B). Similar trend to total soluble solids was found with total sugar concentration in the skin (Fig. 5).

Endogenous free ABA in the flesh and skin increased in the control berries after the onset of ripening, while the increase in ABA was completely inhibited with the berries on defoliated shoots (Fig. 6 A). The level of bound ABA in the skin of control berries was kept almost constant during ripening, but it was decreased appreciably by defoliation. Defoliation did not affect bound ABA level in the flesh (Fig. 6 B).

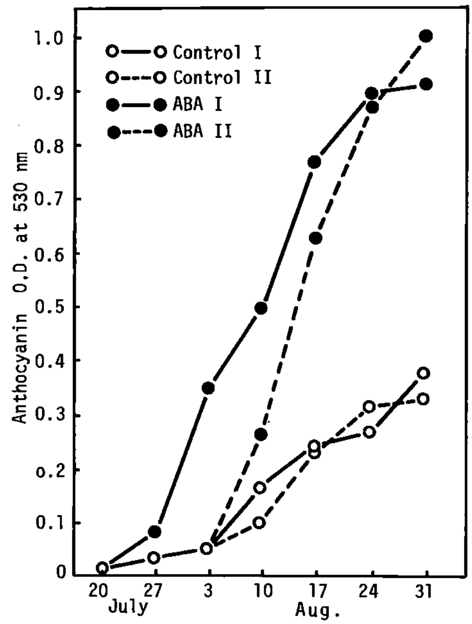

Fig. 1: Effect of ABA on anthocyanin concentration of Kyoho grapes. ABA (1000 ppm) in 70 % EtOH containing 0.05 % Tween 20 was applied on July 20 (ABA I) and August 3 (ABA II). 0.05 % Tween 20 in 70 % EtOH was applied as control on July 20 (Control I) and August 3 (Control II).

Der Einfluß von ABS auf die Anthocyankonzentration von Kyoho-Trauben. 1000 ppm ABS in 70%igem Äthanol mit 0,05 % Tween 20 wurden am 20. Juli (ABA I) bzw. am 3. August (ABA II) appliziert. Als Kontrolle wurde 0,05 % Tween 20 in 70%igem Äthanol am 20. Juli (Control I) bzw. am 3. August (Control II) appliziert.

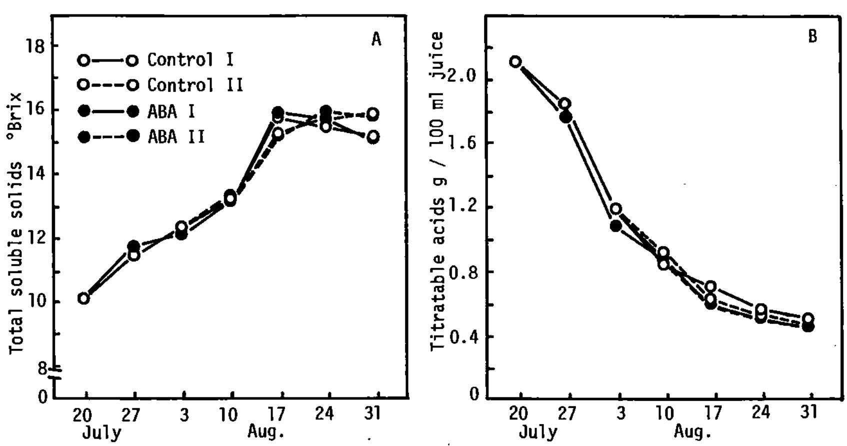

Fig. 2: Effect of ABA on total soluble solids (A) and titratable acids (B) concentration of Kyoho grapes. ABA (1000 ppm) in 70 % EtOH containing 0.05 % Tween 20 was applied on July 20 (ABA I) and August 3 (ABA II). 0.05 % Tween 20 in 70 % EtOH was applied as control on July 20 (Control I) and August 3 (Control II).

Der Einfluß von ABS auf das Mostgewicht (A) und die Konzentration der titrierbaren Säuren (B) von Kyoho-Trauben. 1000 ppm ABS in 70%igem Äthanol mit 0,05 % Tween 20 wurden am 20. Juli (ABA I) bzw. am 3. August (ABA II) appliziert. Als Kontrolle wurde 0,05 % Tween 20 in 70%igem Äthanol am 20. Juli (Control I) bzw. am 3. August (Control II) appliziert.

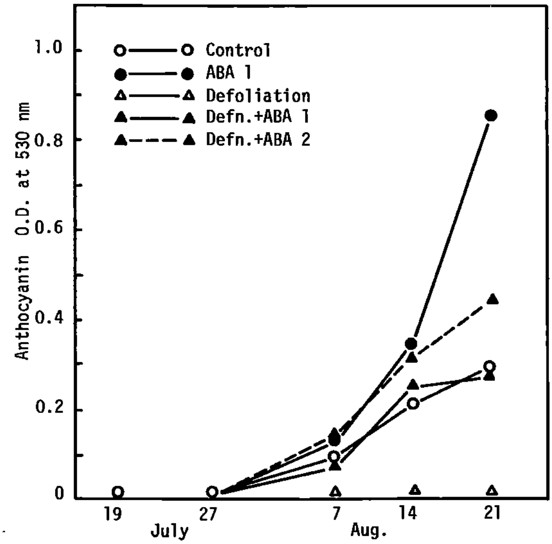

Fig. 3: Effect of ABA and defoliation on anthocyanin concentration of Kyoho grapes. ABA 1 means single application of ABA (on July 19) and ABA 2 means double application (on July 19 and 27). In Control and Defoliation, only 0.05 % Tween 20 in 70 % EtOH was applied on July 19.

Der Einfluß von ABS und Entblätterung auf die Anthocyankonzentration von Kyoho-Trauben. ABA 1 = einmalige Anwendung von ABS (19. Juli), ABA 2 = zweimalige Anwendung von ABS (19. und 27. Juli), Control bzw. Defoliation = 0,05 % Tween 20 in 70%igem Äthanol (19. Juli).

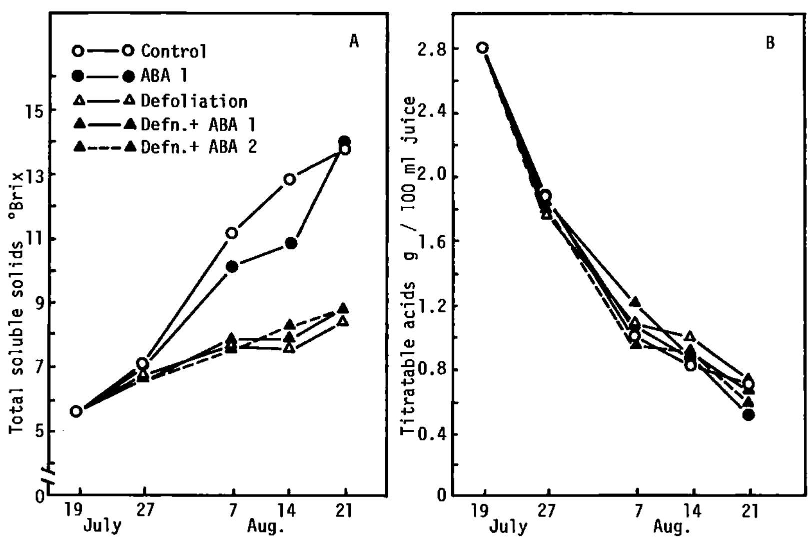

Fig. 4: Effect of ABA and defoliation on total soluble solids (A) and titratable acids (B) concentration of Kyoho grapes. ABA 1 means single application of ABA (on July 19) and ABA 2 means double application (on July 19 and 27). In Control and Defoliation, only 0.05 % Tween 20 in 70 % EtOH was applied on July 19.

Der Einfluß von ABS und Entblätterung auf das Mostgewicht (A) und die Konzentration der titrierbaren Säuren (B) von Kyoho-Trauben. ABA 1 = einmalige Anwendung von ABS (19. Juli), ABA 2 = zweimalige Anwendung von ABS (19. und 27. Juli), Control bzw. Defoliation = 0,05 % Tween 20 in 70%igem Äthanol (19. Juli).

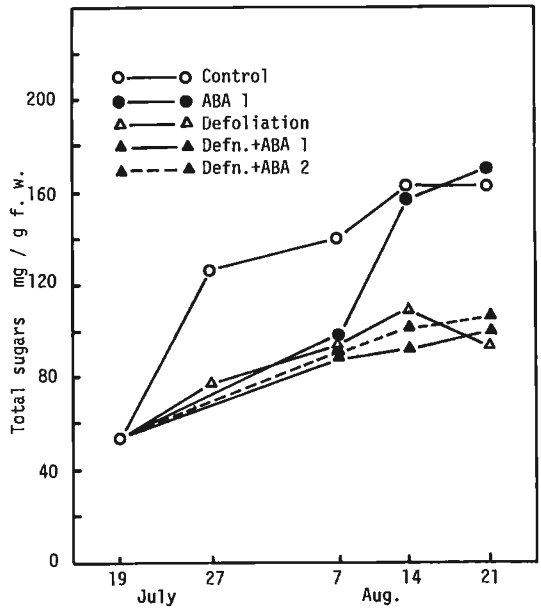

Fig. 5: Effect of ABA and defoliation on total sugar concentration in the skin of Kyoho grapes. ABA 1 means single application of ABA (on July 19) and ABA 2 means double application (on July 19 and 27). In Control and Defoliation, only 0.05 % Tween 20 in 70 % EtOH was applied on July 19.

Der Einfluß von ABS und Entblätterung auf die Konzentration der Gesamtzucker in der Beerenhaut von Kyoho-Trauben. ABA 1 = einmalige Anwendung von ABS (19. Juli), ABA 2 = zweimalige Anwendung von ABS (19. und 27. Juli), Control bzw. Defoliation = 0,05 % Tween 20 in 70%igem Äthanol (19. Juli).

Discussion

Recently, it has been shown that the endogenous ABA level of a grape berry, especially in the skin, rapidly increases at the start of ripening (COOMBE and HALE 1973, COOMBE 1976 a, 1976 b), and that exogenous ABA hastens the onset of ripening when applied just before veraison. Therefore, ABA has been proposed as a possible trigger of ripening process in grapes (COOMBE and HALE 1973, COOMBE 1976 a, DÜRING *et al.* 1978).

In this experiment, we found that ABA applied directly to berries markedly stimulated anthocyanin accumulation in Kyoho grapes without any effect on total soluble solids or titratable acids content (Figs. 1, 2). This suggests a possibility that after veraison anthocyanin synthesis is under the control of ABA accumulating in the skin of Kyoho berries.

In fact, there was no development of anthocyanin when the accumulation of ABA was suppressed by defoliation (Fig. 3). Also, our previous reports on the effect of fruit temperature on coloration of grapes indicated that high fruit temperature inhibits ABA accumulation in the skin and color development (LEE *et al.* 1979, TOMANA *et al.* 1979 b). In both cases, anthocyanin formation proceeded normally when ABA was applied.

On the other hand, PIRIE and MULLINS (1977, 1980) reported that there was a close correlation between the sugar content and level of anthocyanin in the skin (r = 0.96) in

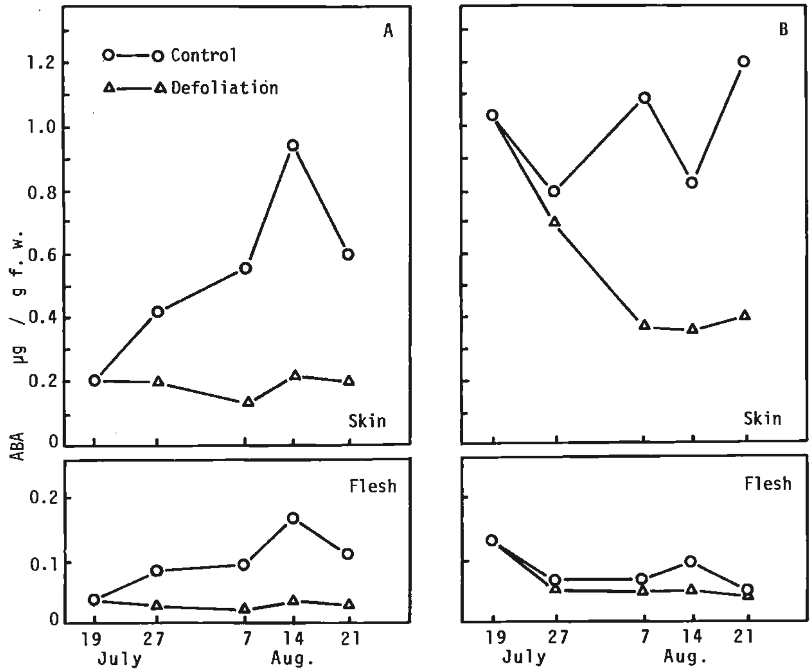

Fig. 6: Effect of defoliation on free (A) and bound ABA (B) concentration in the skin and flesh of Kyoho grapes.

Der Einfluß der Entblätterung auf die Konzentration der freien (A) und gebundenen ABS (B) in Beerenhaut und -fleisch von Kyoho-Trauben.

the first 5 weeks after veraison, and they hypothesized that sugar flux to the grape tissues is one of the factors which govern the rate of phenolics accumulation.

Although the low sugar level in the skin caused by defoliation may be one of the limiting factors for anthocyanin formation, the stimulating effect of ABA on anthocyanin synthesis is, at least, not due to the augment of sugar flux to the skin.

In this respect, it was shown that sucrose and ABA act independently to induce anthocyanin and phenolic accumulation in the excised berries of Cabernet Sauvignon (PIRIE and MULLINS 1976).

So far, we have little information on the mechanism how ABA stimulates anthocyanin production in grape berries, it is, however, assumed that ABA activates the enzymes in anthocyanin (phenolics) biosynthetic system, for instance, L-phenylalanine ammonia-lyase (PAL) which is regarded as a key enzyme of the pathway in several fruits (HYODO 1971, TAN 1979). WALTON and SONDHEIMER (1968) reported that ABA influenced both the development and retention of PAL activity in excised bean axes.

Summary

In Kyoho grapes, ABA treatment at 1000 ppm in the beginning of ripening enhanced anthocyanin accumulation in the skin without any effect on the contents of soluble solids and titratable acids in the juice.

By defoliation at veraison, anthocyanin synthesis was completely inhibited, and the soluble solids content in the juice and sugar levels in the skin became much less than those of the control. Furthermore, endogenous ABA levels in the skin and flesh did not increase throughout ripening period.

On such defoliated vines, the berries recovered anthocyanin accumulation by ABA application.

From these results, it will be assumed that ABA which accumulates in the skin is one of the main factors involved in the anthocyanin synthesis.

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Eingegangen am 1. 2. 1982

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