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Effect of short-term temperature treatment to clusters on anthocyanin and abscisic acid content in the peel of 'Aki Queen' grape

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Summary

The effect of short-term low and high temperature treatment to clusters before and after the onset of color development on anthocyanin and abscisic acid (ABA) content in the peel of 'Aki Queen' (*Vitis labruscana* L.), a tetraploid grape cultivar with red peel was investigated. Grape clusters were exposed to different temperature conditions, either from the beginning of the berry softening to the onset of color development, 47 to 56 days after full bloom (DAFB), or from the onset of color development onwards for 10 days, 56 to 66 DAFB. Low-temperature (2-5 °C lower than control) treatments in both, 47 to 56 DAFB and 56 to 66 DAFB had the tendency to increase anthocyanin concentration and the concentration in the peel was higher than that in the control. The difference in the ABA content after low and high temperature treatment in the 47 to 56 DAFB suggests that temperature from the beginning of berry softening to the onset of color development might affect the ABA content of the peel. On the other hand, treatment from the onset of color development onwards for 10 days might affect the anthocyanin concentration, but the effect on ABA content is relatively low. These results suggest that temperature-dependent accumulation of anthocyanin during maturation correlate with ABA content at the onset of color development in 'Aki Queen' grape.

Key words: ABA; coloration; grapevine; temperature; tetraploid grape cultivar.

Introduction

The color of red and black grape skins is determined by the anthocyanin concentration and composition. In recent years, higher temperatures during grape maturation have caused problems for grape cultivation. Particularly, grape growers in the warm areas have struggled to produce well-colored grapes, and therefore receive a low price for poorly colored grapes.

Accumulation of anthocyanins in grape berries is influenced by various environmental and nutritional factors, such as temperature (Tomana *et al*. 1979, Yamane *et al*. 2006, Koshita *et al*. 2007), light (Matus *et al*. 2009), fruit load (Sato *et al*. 1997, Kitamura *et al*. 2005), and nitrogen fertilization (Kliewer 1977). In addition, the adverse effects of high temperature and the favorable effects of low temperature on grape coloration have often been reported to affect the abscisic acid (ABA) content (Tomana *et al*. 1979, Yamane *et al*. 2006, Koshita *et al*. 2007). Increase in the ABA level of grape skins around veraison followed by a decline (Coombe and Hare 1973), and induction of gene expression involved in anthocyanin biosynthesis and increase of the anthocyanin concentration by exogenous application of ABA to grape clusters (Ban *et al*. 2003, Jeong *et al*. 2004, Koyama *et al*. 2014), are consistent with the role of ABA as a ripening hormone in grape.

Warm growth temperatures adversely affect coloration of tetraploid grape cultivars with red peel. The difficulty to get ideal peel color of 'Aki Queen' grape have been reported (Yamane *et al.* 2006) and to overcome this problem, various studies have investigated the appropriate fruit load (Sato *et al*. 1997, Kitamura *et al*. 2005), the most sensitive period to low temperature (Yamane and Shibayama 2006), and the most suitable stage for girdling treatment (Yamane and Shibayama 2007). Although the most sensitive stage and the optimum duration of chilling treatment for enhancing skin color have been identified (Yamane and Shibayama 2006), the effect of short-term, low-temperature treatment on the levels of endogenous ABA has not been studied.

Here, we studied the effects of short-term low and high cluster temperature before and after the onset of color development on chronological changes in anthocyanin and ABA content to understand the role of endogenous ABA in skin color development in the 'Aki Queen' grapes.

Material and Methods

Plant material: A 23-year-old 'Aki Queen' grapevine grafted on 5BB rootstock and grown in an orchard at the NARO Institute of Fruit Tree Science was used in this study. The vine was trained by long cane pruning and was protected under a rain shelter. Clusters were dipped in a 25 $mg \cdot L^{-1}$ gibberellic acid (GA_3) solution at full bloom, on May 30 to induce seedlessness, and the treatment was repeated 13 d later, on June 12 for berry enlarge-

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ment. The berries were thinned to 30 berries per cluster at the time of cluster thinning. The maturation stage was determined by the softness and color of the berries, and this information was used to determine sampling dates as follows: July 9 (40 DAFB), before the beginning of softening; July 16 (47 DAFB), beginning of softening; July 25 (56 DAFB), at the onset of color development; August 4 (66 DAFB), 10 d after onset of color development; and August 18 (80 DAFB), at harvest time. Three uniformly colored berries were sampled from each cluster and 4 to 5 samples in each treatment were used for analysis.

Temperature treatment: The grapes were subjected to different temperatures at 2 different stages; from the beginning of berry softening (47 DAFB) to the onset of color development (56 DAFB) and from the onset of color development (56 DAFB) onwards, for 10 d (66 DAFB). In each treatment, 5 clusters were used for low-temperature treatments, high-temperature treatments, and control. Clusters for high temperature treatments were maintained by circulating hot (40 °C) water around them through vinyl tubing, and those for low temperature treatments were maintained by circulating chilled (18 °C) water around them through vinyl tubing. All clusters and the vinyl tubes were covered with polypropylene.

Anthocyanin concentration in the berry peel: The anthocyanin in the berry peel were extracted according to the method of SHIRAISHI *et al*. (2007), with slight modification. Anthocyanins were extracted from 1 g fresh weight of peels in 5 ml 50 % (v/v) acetic acid for 24 h at 4 °C in the dark. Anthocyanin concentration was determined as previously described by JEONG *et al*. (2004) by measuring the absorbance of the extract at 530 nm, and total anthocyanin concentration was expressed in milligrams of cyanidin-3-glucoside (Extrasynthese, Genay, France) equivalent per gram of fresh weight.

Analysis of ABA content: TheABAcontent of the berry peels was determined as described by SETHA *et al*. (2004) with some modifications. Briefly, frozen peels were homogenized with a known quantity of hexadeuterated (d_6) ABA in 80 % (v/v) methanol and then filtered. The residue was re-extracted twice with 80 % methanol. The filtrate was reduced to an aqueous residue under vacuum, and the pH was adjusted to 2.5 with 0.1 N HCl. The aqueous residue was extracted 3 times with ethyl acetate, and the extract was evaporated to dryness. The dried extract was dissolved in 80 % methanol, applied to a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) and eluted with 80 % methanol. The eluate was evaporated to dryness, re-dissolved in 25 % (v/v) aqueous acetonitrile containing 25 mM acetic acid, and separated by high-performance liquid chromatography (ODS column: TSK-GEL ODS-100Z [Tosoh, Tokyo Japan] 250 mm × 4.6 mm i.d.; 40 °C; flow rate of 1.3 mL·min^{-1}; solvent A, 25 % (v/v) acetonitrile with 20 mM acetic acid; solvent B, 80 % (v/v) acetonitrile with 20 mM acetic acid; gradient profile 0-20 min, 0 % to 100 % B; 20-30 min, 100 % B; 30-35 min, 100 % to 0 % B). The retention time of the ABA was 10 min. The ABA fraction was dried and methylated with ethereal diazomethane and quantified by gas chromatography-mass spectrometry-selective ion monitoring (GCMS-QP5000, Shimadzu Co. Ltd.), according to the method of KOSHITA *et al*. (1999).

Analysis of total soluble solids (TSS) content and titratable acidity: TSS content was measured on 40, 47, 56, 66, and 80 DAFB with a digital refractometer (PR-100, Atago Co., Tokyo, Japan). The titratable acidity was measured on 80 DAFB by neutralizing the juice with 0.1 N NaOH and expressed as the mass (g) of tartaric acid equivalent per 100 mL of juice.

Results and Discussion

The low temperature fluctuated 2-5 °C lower than that of the control from 47 to 66 DAFB. On the other hand, the high-temperature treatment fluctuated 7-9 °C higher than that of the control (Fig. 1). Both low temperature treatments increase the anthocyanin concentration of the peels (Fig. 2). There was a sharp increase in anthocyanin concentrations immediately after each low-temperature treatment, and consequently, 10 d after the onset of color development (66 DAFB), samples of both low-temperature treatments had same concentrations of anthocyanin. In contrast to the low-temperature treatments, high-temperature treatments did not increase anthocyanin content. At the onset of color development (56 DAFB), the anthocyanin concentration in the peels of the high-temper-

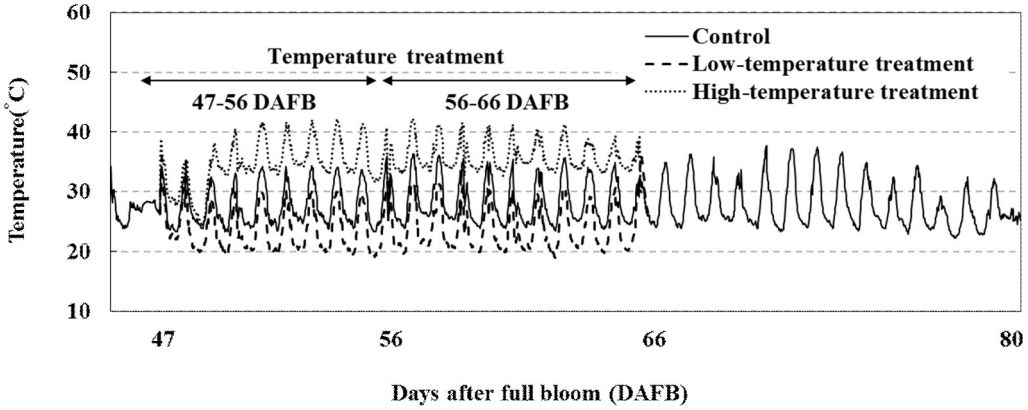

Fig. 1: Temperature conditions maintained around the clusters during berry maturation period. Temperature treatment to the clusters were conducted from the beginning of the berry softening (47 d after full bloom (DAFB)) to the onset of color development (56 DAFB) and from 56 DAFB onwards for 10 d (66 DAFB).

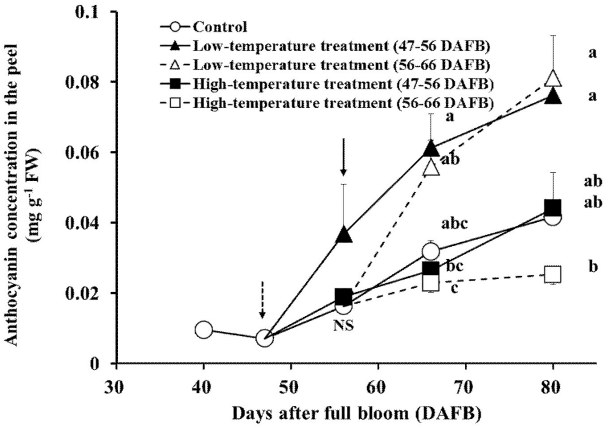

Fig. 2: Effects of temperature on the anthocyanin concentration of the 'Aki Queen' grape berries. Error bars represent standard error of the mean (n = 4 to 5 clusters). The dotted arrow shows the beginning of berry softening (47 DAFB) and solid arrows show the onset of color development (56 DAFB). Different letters indicate significant differences among different treatment at the 5 % level by Tukey-Kramer's HSD multiple-range test.

ature-treated clusters was similar to that in the controls. High-temperature treatment from 56 to 66 DAFB resulted in significantly lower anthocyanin concentration than low-temperature treated clusters at harvest. In control clusters, the ABA content of the peels increased around the beginning of berry softening (47 DAFB), and then gradually decreased toward harvest to about half of that at 47 DAFB (Fig. 3). Low-temperature treatment from 47 to 56 DAFB tended to increase the ABA content of the peels, which was significantly higher than that of the control at 66 DAFB. A significant difference in the ABA content was observed at 56 and 66 DAFB between low-temperature treated clusters from 46 to 56 DAFB and high-temperature treated clusters. Low-temperature treatment from 56 to 66 DAFB did not alter the ABA content in the peels compared to control. High-temperature treatments tended to reduce the ABA content of the peels and the reduction caused by the treatment from 47 to 56 DAFB did not lead to a decline of the anthocyanin content. We assumed that the inhibitive effect of high-temperature treatment from 47 to 56 DAFB on ABA biosynthesis might not affect color development of grape skins under the coloring difficult condition, because the anthocyanin content at 56 DAFB was the same level of the control (Fig. 2). Low-temperature treatment to grape clusters throughout the maturation period enhances the accumulation of anthocyanins in the peel of 'Kyoho' (Tomana *et al*. 1979) and 'Aki Queen' grapes (Koshita *et al*. 2007). A simultaneous increase in anthocyanin and ABA content suggests that ABA plays an important role in anthocyanin biosynthesis. Since exogenously applied ABA increases anthocyanin content (Matsushima *et al*. 1989, Kitamura *et al*. 2007, Koyama *et al*. 2014), and upregulates the gene expression involved in anthocyanin biosynthesis (Ban *et al*. 2003, Jeong *et al*. 2004), ABA is believed to act as a regulator of skin color in the grapes. Although no significant differences were detected in anthocyanin concentration between low-temperature treated and control clusters, a trend of higher anthocyanin concentration during maturation was observed (Fig. 2) and coincidently, significantly higher levels of ABA content than that of the control were observed at 66 DAFB (Fig. 3). These data are consistent with the results of our previous research showing higher ABA content at the onset of color development when grape clusters were chilled before veraison to harvest (Koshita *et al.* 2007). However, we observed a trend of higher anthocyanin content than that of control clusters without any change in the ABA content following low temperature treatment from 56 to 66 DAFB (Fig. 2, 3). These data suggest that ABA content have little influence on anthocyanin contents from the onset of coloring onward for 10 d in 'Aki Queen' grapes. Chronological changes in ABA content in the tetraploid grape with black peel, 'Pione' has been previously reported, and peak ABA content in the peel has been observed at 70 DAFB (Kondo and Kawai 1989). In the present study, peak ABA content in control clusters occurred at the time of berry softening (47 DAFB) (Fig. 3). Further studies should be performed to demonstrate if the peak ABA level occurs earlier in 'Aki Queen' than in 'Pione'. Low-temperature treatment from 47 to 56 DAFB temporarily reduced TSS content (56 DAFB); however, comparable to that of the control at 66 DAFB and harvest (Fig. 4). There was no difference in the titratable acidity of the harvest berries subjected to the different treatments (data not shown). Grape coloration is also affected by the TSS of the berries. Increased fruit load decreases TSS, which leads to poor coloration (Sato *et al.* 1997). In our study, despite the lower TSS content at 56 DAFB in low-temperature-treated grape clusters, the anthocyanin concentration was higher than that of the control or high-temperature-treated clusters (Figs. 2, 4). Since girdling treatment 30 to 35 d after full bloom increased TSS and anthocyanin concentration (Yamane and Shibayama 2007), it is possible that the slightly lower TSS at 56 DAFB does not affect the anthocyanin concentration.

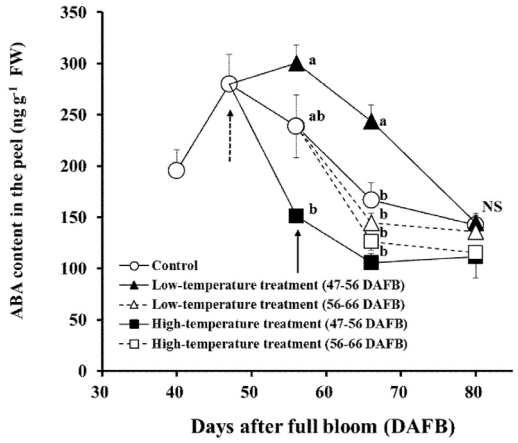

Fig. 3: Effects of the temperature on the ABA contents of the 'Aki Queen' grape berries. Error bars represent standard error of the mean (n = 4 to 5 clusters). The dotted arrow shows the beginning of berry softening (47 DAFB) and solid arrows show the onset of color development (56 DAFB). Different letters indicate significant differences among different treatment at the 5 % level by Tukey-Kramer's HSD multiple-range test.

Since exogenous application of ABA also increases anthocyanin concentration (Matsushima *et al*. 1989, Kitamura *et al*. 2007, Koyama *et al*. 2014), it is possible that

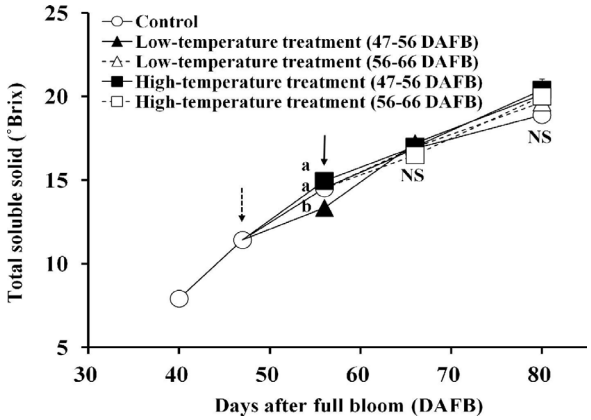

Fig. 4: Effects of the temperature on the TSS content of the 'Aki Queen' grape berries. Error bars represent standard error of the mean (n = 4 to 5 clusters). The dotted arrow shows the beginning of berry softening (47 DAFB) and solid arrows show the onset of color development (56 DAFB). Different letters indicate significant differences among different treatment at the 5 % level by Tukey-Kramer's HSD multiple range test.

ABA promotes anthocyanin biosynthesis by at least two different mechanisms, one associated with endogenous increase in ABA triggered by low temperature and the other independent of endogenous ABA that might be triggered by exogenous application of ABA.

As mentioned above, ABA application promotes expression of genes related to anthocyanin biosynthesis. In general, the action of the plant regulators is dependent on their biosynthesis as well as their perception by a receptor. Recently, the entire signaling process involved in ABA perception has been characterized and putative ABA receptors have been identified in grape (BONEH *et al.* 2012, LI *et al.* 2012). Since ABA, as a ripening regulator, plays an important role in grape maturation, changes in the expression and activity of ABA receptor should be further investigated.

Although it is known that high temperature during berry maturation inhibits coloration of the grape skin, only one report describes that the most sensitive period is from 1 to 3 weeks after the onset of coloration in 'Aki Queen' grapes (YAMANE and SHIBAYAMA 2006). The authors observed that high-temperature treatment within 1 week after the onset of coloration had no effect on the coloration, whereas high-temperature treatment from 1 to 3 weeks after the onset of color development inhibited coloration in 'Aki Queen' grapes. Our results are consistent with these findings (Fig. 2) and highlight that relatively lower temperatures after the onset of coloration also lead to better-colored grapes.

In conclusion, exposure to low temperature 10 d before or after the onset of coloration enhances color development in 'Aki Queen' grapes. The temperature-dependent accumulation of anthocyanin during maturation correlate with ABA content at the onset of color development, but the effect of the ABA content is relatively low after the onset of color development in 'Aki Queen' grapes. The results of this study might help improve our understanding of the relationship between the temperature and coloration of 'Aki Queen' grape during ripening.

Acknowledgements

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References

BAN, T.; IISHIMARU, M.; KOBAYASHI, S.; SHIOZAKI, S.; GOTO-YAMAMOTO, N.; HORIUCHI, S.; 2003: Abscisic acid and 2,4-dichlorophenoxyacetic acid affect the expression of anthocyanin biosynthetic pathway genes in 'Kyoho' grape berries. J. Hortic. Sci. Biotech. **78**, 586-589.

BONEH, U.; BITON, I.; ZHENG, C.; SCHWARTZ, A.; BEN-ARI, G.; 2012: Characterization of potential ABA receptors in *Vitis vinifera*. Plant Cell Rep. **31**, 311-321.

COOMBE, B. G.; HALE, C. R.; 1973: The hormone content of ripening grape berries and the effect of growth substance treatments. Plant Physiol. **51**, 629-634.

JEONG, S. T.; GOTO-YAMAMOTO, N.; KOBAYASHI, S.; ESAKA, M.; 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.

KITAMURA, H.; NAKAYAMA, M.; KONDOU, H.; NISHIKAWA, Y.; KOSHIOKA, M.; Hiratsuka, S.; 2005: Effect of crop load on expression of deep color in 'Aki Queen' grape berry. Hortic. Res. (Japan) **4**, 343-346 (in Japanese).

KITAMURA, H.; NAKAYAMA, M.; KONDO, H.; NISHIKAWA, Y.; KOSHIOKA, M.; HIRATSUKA, S.; 2007: Effect of abscisic acid treatment at variable stages on the expression of proper and deep color in 'Aki Queen' grape berry skins. Hortic. Res. (Japan) **6**, 271-275 (in Japanese).

KLIEWER, W. M.; 1977: Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. Am. J. Enol. Vitic. **57**, 96-103.

KONDO, S.; KAWAI, M.; 1988: Relationship between free and conjugated ABA levels in seeded and gibberellin-treated seedless, maturing 'Pione' grape berries. J. Am. Soc. Hortic. Sci. **123**, 750-754.

KOSHITA, Y.; TAKAHARA, T.; OGATA, T.; GOTO, A.; 1999: Involvement of endogenous plant hormones (IAA, ABA, GAs) in leaves and flower bud formation of satsuma mandarin (*Citrus unshiu* Marc.). Sci. Hortic. **79**, 185-194.

KOSHITA, Y.; ASAKURA, T.; FUKUDA, H.; TSUCHIDA, Y.; 2007: Nighttime temperature treatment of fruit clusters of 'Aki Queen' grapes during maturation and its effect on the skin color and abscisic acid content. Vitis **46**, 208-209.

KOYAMA, R.; DE ASISS, A. M.; YAMAMOTO, L. Y.; BORGES, W. F.; BORGES, R. S.; PRUDÊNCIO, S. H.; ROBERT, S. R.; 2014: Exogenous abscisic acid increase the anthocyanin concentration of berry and juice from 'Isabel' grapes (*Vitis labrusca* L.). HortScience **49**, 460-464.

LI, G.; XIN, H.; ZHENG, X. F.; LI, S.; HU, Z.; 2012: Identification of the abscisic acid receptor *VvPYL1* in *Vitis vinifera*. Plant Biol. **14**, 244-248.

MATSUSHIMA, J.; HIRATSUKA, S.; TANIGUCHI, N.; WADA, R.; SUZAKI, N.; 1989: Anthocyanin accumulation and sugar content in the skin of grape cultivar 'Olympia' treated with ABA. J. Japan. Soc. Hortic. Sci. **58**, 551-555 (in Japanese).

MATUS, J. T.; LOYOLA, R.; VEGA, A.; PEÑA-NERIA, A.; BORDEU, E.; ARCE-JOHNSON, P.; ALCALDE, J. A.; 2009: Post-veraison sunlight exposure induces MYB-mediated transcriptional regulation of anthocyanin and flavonol synthesis in berry skins of *Vitis vinifera*. J. Exp. Bot. **60**, 853-867.

SATO, T.; SASAKI, E.; SATO, Y.; TAKASE, K.; 1997: Optimum fruit bearing and optimum fruit yield of grape 'Aki Queen' and 'Honey Black. Bull. Yamagata Hortic. Exp. Stn. **12**, 1-9 (in Japanese).

SHIRAISHI, M.; YAMADA, M.; MITANI, N.; UENO, T.; 2007: A rapid determination method for anthocyanin profiling in grape genetic resources. J. Japan. Soc. Hortic. Sci. **76**, 28-35.

SETHA, S.; KONDO, S.; HIRAI, N.; OHIGASHI, H.; 2004: Xantoxin, abscisic acid and its metabolite levels associated with apple fruit development. Plant Sci. **166**, 493-499.

Tomana, T.; Utsunomiya, N.; Kataoka, I.; 1979: The effect of environmental temperature on fruit ripening on the tree. II. The effect of temperature around whole vines and clusters on the coloration of 'Kyoho' grapes. J. Japan. Soc. Hortic. Sci. **48**, 261-266 (in Japanese).

Yamane, T.; Jeong, S. T.; Goto-Yamamoto, N.; Koshita, Y.; Kobayashi, S.; 2006: Effects of temperature on anthocyanin biosynthesis in grape berry skins. Am. J. Enol. Vitic. **57**, 54-59.

Yamane, T.; Shibayama, K.; 2006: Effects of changes in sensitivity to temperature on skin coloration in 'Aki Queen' grape berries. J. Japan. Soc. Hortic. Sci. **74**, 458-462.

Yamane, T.; Shibayama, K.; 2007: Effects of treatment date, width of girdling, and berry number of girdled shoot on the coloration of grape berries. Hortic. Res. (Japan) **6**, 233-239 (in Japanese).

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