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Regular Article

Effects of exogenous methyl jasmonate and light condition on grape berry coloration and endogenous abscisic acid content

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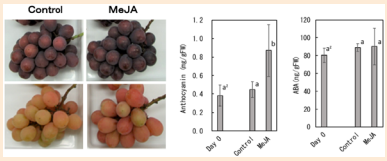
Supplementary material

Suitable postharvest treatment methods were investigated to improve the color of grape berries. Culture solutions containing jasmonic acid (JA), methyl jasmonate (MeJA), and prohydrojasmon (PDJ) enhanced the skin coloration of grape berries ('Pione') harvested at the initial stage of coloration. MeJA vapor treatment under sealed conditions increased anthocyanin accumulation in grape berries ('AkiQueen' and 'Pione') harvested at the early stage of skin coloration. Furthermore, promoting skin coloration by MeJA vapor treatment was as effective in mature clusters as it was in detached berries. These effects were confirmed in light conditions but not in constant darkness. Our results showed that postharvest MeJA vapor treatment improved skin coloration in grapes. In addition, postharvest treatment with MeJA was found to have no effect on the endogenous abscisic acid content of grape berry skins. Therefore, we suggest that MeJA vapor treatment can be a useful and labor-saving method for the horticultural industry.



Keywords: anthocyanin, grape berry, jasmonic acid, methyl jasmonate, postharvest treatment, abscisic acid.

Introduction

Skin coloration is an important indicator of grape berry quality. Skin coloration is mainly related to the content and composition of anthocyanins, which the fruit synthesizes during maturation. Azuma *et al.*[1)] reported that the accumulation of anthocyanins is influenced by both low temperature and light, because several flavonoid biosynthesis-related genes are upregulated under such conditions. Grape berries often fail to achieve satisfactory color at high temperatures,[2)] and this is becoming increasingly problematic in the current global warming scenario.

Plant hormones such as ethylene, abscisic acid (ABA), and jasmonic acid (JA) are closely associated with the maturation process, including during anthocyanin synthesis in fruits such as apples and sweet cherries.[3,4)] The concentration of endogenous ABA increases at the onset of ripening, and it corresponds to the increased coloration of grape berries.[5–9)] Furthermore, the exogenous application of ABA has been shown to increase the accumulation of anthocyanins in the berry skin of some grape varieties ('Kyoho,' 'Olympia,' 'Aki Queen,' and 'Pione').[10–13)] Therefore, ABA is considered to promote anthocyanin synthesis in grape berry skins. However, in Japan, table grapes are usually cultivated in fruit bags, and it is necessary to remove and reattach the bags several times during cultivation to apply ABA for the promotion of coloration, which is a labor-intensive process.

Methyl jasmonate (MeJA), an analog of JA, has been shown to enhance the red coloration of apples both preharvest and postharvest.[3,14,15)] Especially in wine grapes, preharvest application of MeJA has been shown to increase the content of polyphenols, including anthocyanins, in berries.[16,17)] Furthermore, MeJA has been reported to promote anthocyanin accumulation in detached *Petunia* flowers.[18)] Mizuno *et al.*[19)] reported that postharvest treatment with MeJA was effective in reducing nonuniform coloration in early harvested flower buds of doubled-flowered *Eustoma*. Prohydrojasmon (PDJ), a synthetic analog of JA, was developed as a plant growth regulator; however, similar to ABA application, the bags should be removed to apply PDJ during the cultivation of grape berries and then reattached. Postharvest treatment does not require the removal and reattachment of the

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fruit bag from grape clusters, which saves labor. In this study, we investigated whether the effect of JA and its analogs was equivalent to or higher than that of ABA in improving the coloration of grape berries. We investigated suitable postharvest treatment methods to improve the color of grape berries of 'Aki Queen' and 'Pione,' which are varieties known for poor coloration in Japan. Although postharvest MeJA treatment of grape berries was attempted in a previous study,[20] such a treatment has not yet been tested in these varieties. Furthermore, the effect of postharvest MeJA treatment on the endogenous ABA content in the grape berry skin was investigated.

The analysis provided new information about the mechanisms by which postharvest MeJA treatment promotes anthocyanin accumulation in grape berries. The findings will improve our understanding of the effects of JA and its analogs and provide an effective alternative technique for promoting grape coloration.

Materials and methods

1. Plant material

Preharvest and postharvest experiments were conducted using berries or clusters of grapes from mature vines of 'Aki Queen' (*Vitis labrusca* L.×*V. vinifera* L.) and 'Pione' (*V. labruscana* Baily) planted in 2008 in an open field at the NARO Institute of Fruit Tree and Tea Science, National Agricultural Research Organization, Tsukuba, Ibaraki, Japan. The vines had been grafted onto Tereki 5BB rootstocks.

Each grape cluster was treated with a 25 mg L^{-1} solution of gibberellic acid (Kyowa Hakko Bio Co., Ltd., Tokyo, Japan) at full bloom (FB) and 10–15 day after FB to produce seedless fruit. At the time of cluster thinning, grape berries were thinned to 30–40 berries per cluster before wrapping each cluster in a fruit bag.

2. Experiment I: Anthocyanin and ABA contents of grape berry skins after postharvest treatment with JA and its analogs

The detached grape berries used for the test were cultured following the procedure of Gao-Takai *et al.*,[21] with some modifications. The 'Aki Queen' berries were harvested in the initial stage of coloration (collected on July 22, 2010) and cultured on 24-well plates with solutions of 50 and 500 μM jasmonic acid (JA) and 760 μM ABA. In addition, the 'Pione' berries harvested in the initial stage of coloration (collected on August 2, 2011) were cultured on 24-well plates with solutions of 100 and 500 μM jasmonic acid (JA), MeJA, and 500 μM ABA. In the same way, the 'Pione' berries harvested in the initial stage of coloration (collected on August 6, 2015) were cultured on 24-well plates with solutions of 500 μM JA, MeJA, prohydrojasmon (PDJ), or ABA. All culture solutions contained 0.1% Tween 20 and 0.5% ethanol (EtOH). The plates were placed in an incubator (Growth Cabinet MLR-350T, SANYO Electric Co., Ltd., Osaka, Japan) at 23°C with a 16/8 hr light/dark photoperiod. JA and MeJA were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). PDJ and ABA were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). One plate was used for each solution (*i.e.*, one plate per treatment), and 2.3 mL of the solution was added to each well of each plate, which contained absorbent cotton. In the control plate, water containing 0.1% Tween 20 and 0.5% EtOH was added to the wells. The berries were placed in individual wells, and 10 to 13 berries were used for each treatment. To prevent the berries from desiccating, the plates were placed in transparent plastic boxes (4 L), sealed with plastic lids, and placed in the incubator. The boxes were irradiated with a light intensity of 170 μmol m^{-2} s^{-1} during the lighting periods. After 8 day ('Pione') or 11 day ('Aki Queen') of incubation, skin samples of the berries were collected and stored at −80°C for the determination of anthocyanin and ABA contents.

3. Experiment II: Anthocyanin and ABA contents of grape berry skins after postharvest treatment with MeJA vapor

'Aki Queen' and 'Pione' berries were harvested at the beginning of the skin coloration stage (collected on July 27, 2016) and cultured on 24-well plates with water instead of the solutions described in Experiment I. They were then placed in 4 L transparent plastic boxes and sealed with plastic lids. The boxes were then placed in the incubator (23°C; 16/8 hr light/dark or constant darkness). MeJA vapor treatment was performed as described by Mizuno *et al.*,[19] with some modifications. Vapor was prepared by placing MeJA/EtOH (1:9) onto a filter paper in a Petri dish in the boxes. A final MeJA concentration of 9 μM/L of air was calculated based on the assumption that MeJA evaporated completely. The MeJA concentration of 9 μM/L of air was determined to be effective, according to the results of Mizuno *et al.*[19] Concentrations of 9 μM/L were effective without negative effects in our preparatory experiment. The control was exposed to the same volume of 100% EtOH. After 2 day of exposure, MeJA/EtOH vapor was removed from the box, and the berries were further cultured for 7 day under the same light and temperature conditions. Subsequently, samples of the berry skins were collected and stored at −80°C for the determination of anthocyanin and ABA contents.

Furthermore, the detached 'Pione' berries at the maturity stage (collected on September 8, 2016) were cultured, and MeJA or EtOH vapor treatment was performed as described above (23°C, 16:8 L:D). Additionally, for comparison, some grape berries were left untreated, without exposure to MeJA or EtOH. After 4 day of exposure, MeJA/EtOH vapor was removed from the box, and the berries were further cultured for 1 day under the same light and temperature conditions. Subsequently, samples of the berry skins were collected and stored at −80°C for measuring the anthocyanin and ABA contents.

4. Experiment III: Anthocyanin and ABA contents of the berry skins of mature grape clusters after postharvest treatment with MeJA vapor

Clusters of mature 'Pione' grapes were harvested on September 7, 2017, and stored at room temperature (22–24°C) for 1 day before initiating postharvest treatment with MeJA vapor. Clus-

ters with insufficient coloring were selected, and treatment was conducted in translucent white plastic boxes (22.5 L), which were sealed with plastic lids and kept at an external temperature of 20°C. The boxes were under continuous light irradiation with a light intensity of 117 μmol m^{-2} s^{-1}. The temperature inside the boxes was 21.5°C. MeJA vapor treatment was performed as follows. Vapor was prepared by placing MeJA/EtOH (1:9) onto a filter paper in a Petri dish, which was then placed in each box. The MeJA vapor was at a final concentration of 9 μM/L of air, which was calculated based on the assumption that MeJA evaporated completely. The treatment consisted of placing three clusters in one box; a total of nine clusters were treated in three boxes per treatment. The control was exposed to the same volume of 100% EtOH. After 3 day of exposure, the juice was extracted from the grape berries (three berries per cluster), and the Brix value was determined using a digital refractometer (IPR-101α; AS ONE Corporation, Osaka, Japan). Three berries per cluster were randomly collected, and the skins were stored at −80°C for measuring the anthocyanin and ABA contents.

The following year (2018), mature grape clusters of two varieties, 'Aki Queen' and 'Pione,' were subjected to postharvest treatment. 'Aki Queen' clusters were harvested on August 21, 2018, and stored at room temperature for 2 day before treatment. 'Pione' clusters were harvested on August 29, 2018, and were also stored at room temperature for 2 day before treatment. Then, MeJA vapor treatment was performed as described above. The treatment was carried out by placing three or four clusters in one box, and 10 clusters were treated in three boxes per treatment. After 4 day of exposure, the Brix value of the grape berry juice was determined as described above, and samples of the berry skins (three berries per cluster) were collected and stored at −80°C for the determination of anthocyanin and ABA contents.

5. *Measurement of anthocyanin and ABA contents in the skins of grape berries*

Anthocyanin was extracted following the procedure of Kitamura *et al.*,[11] with slight modifications. Approximately 0.5 g of the berry skin was immersed in 5 mL of 50% acetic acid for 96 hr at 2°C. The absorbance of the extract was measured at a wavelength of 520 nm.[22] The total anthocyanin concentration was expressed as milligrams of cyanidin-3-glucoside equivalents per gram of fresh weight.

The extraction and purification of ABA from the grape skins were conducted according to the procedures of Tuan *et al.*,[23] with slight modifications. Frozen samples (approximately 0.5 g) were homogenized in liquid nitrogen with a mortar and pestle, mixed with a solution of methanol/water/formic acid (15:4:1) containing an internal standard (10 ng of [$^2H_6$] ABA, OlchemIm Ltd., Olomouc, Czechia), and then extracted at −20°C overnight. The extract was purified following the method described by Dobrev & Kamínek.[24] The acidic fraction that contained ABA was evaporated to dryness. The dried samples were then dissolved in methanol. Purified samples were analyzed as described by Endo *et al.*[25] Samples were dissolved in methanol and methylated with trimethylsilyldiazomethane (GL Sciences, Tokyo, Japan) at room temperature for 20 min. The samples were analyzed with a mass spectrometer connected to a gas chromatograph (model JMS-Q1000 GCMkII; JEOL Ltd., Tokyo, Japan). The analytical conditions were as follows: GC column, DB-1 capillary column (30 m×0.25 mm internal diameter, 0.25 μm film thickness, Agilent J&W); injection temperature, 250°C; carrier gas, helium at 1 mL/min; ionization, EI (70 eV); source temperature, 250°C; column temperature program, 80°C for 1 min, increased to 245°C at 30°C/min and 280°C at 5°C/min; interface temperature, 250°C; and splitless injection. The endogenous ABA levels were calculated as the ratio of the peak areas of the prominent ions (*i.e.*, m/s 190 for the endogenous ABA and m/s 194 for the internal standard).

6. *Statistical analysis*

To assess differences among the treatments, mean comparisons were performed using Tukey's test with the level of significance at 5%. These analyses were performed using JMP software v.13

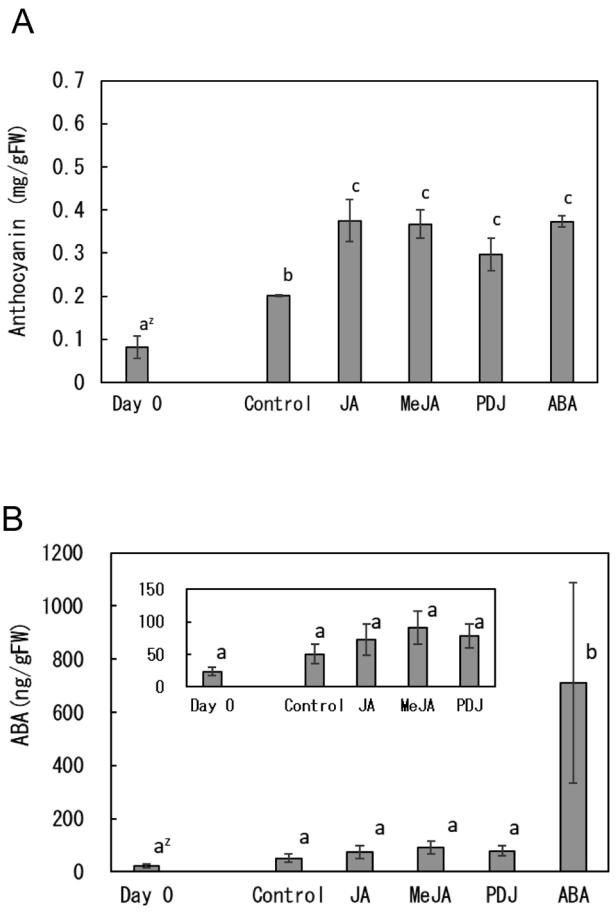

Fig. 1. Effects of 500 μM JA, MeJA, and PDJ treatment on anthocyanin (A) and ABA contents (B, insets show different scales of graphs) in the skins of 'Pione' grape berries harvested at the beginning of coloration. [z]Different letters indicate significant differences at the 5% level, according to the Tukey–Kramer honestly significant difference (HSD) test. Vertical bars represent SD (n=3).

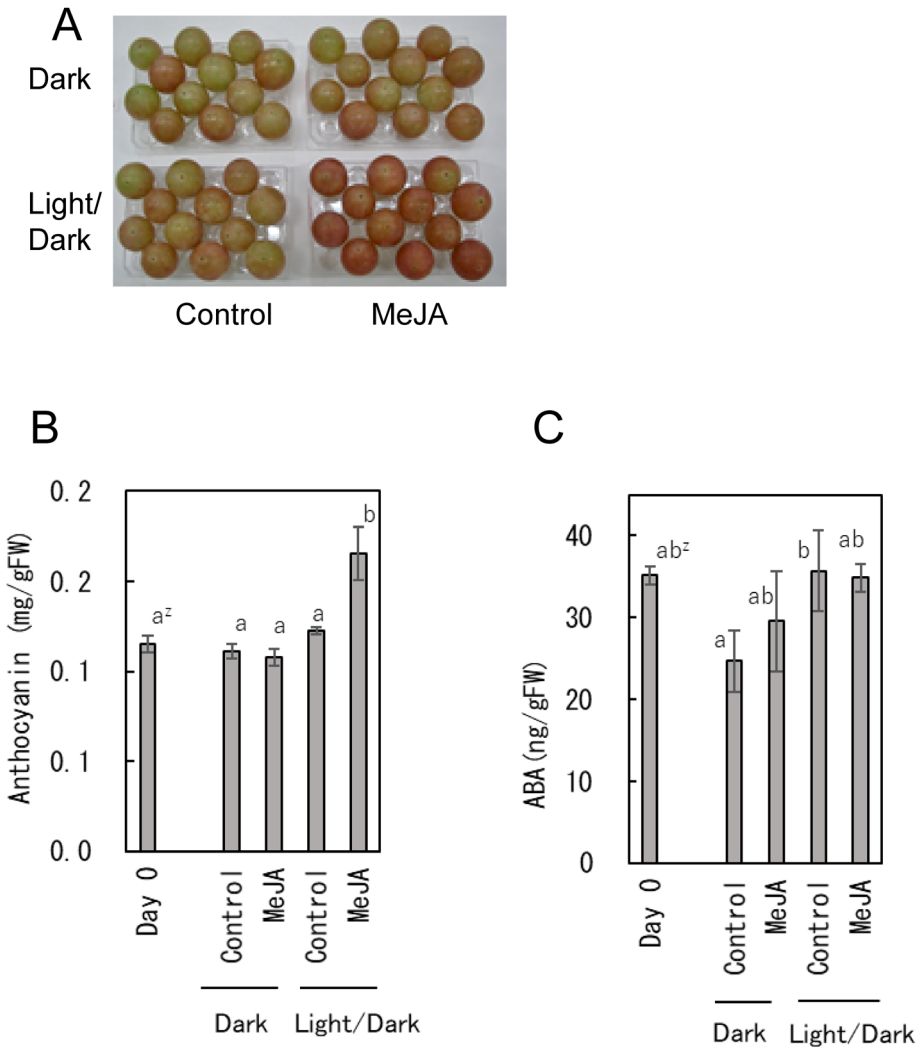

Fig. 2. Effects of MeJA vapor treatment (for 2 day under either light/dark cycles or constant darkness) on the coloration (A), anthocyanin content (B), and ABA content (C) in the skins of detached 'Aki Queen' berries harvested at the early coloration stage. Vertical bars represent SD (n=3). [z]Different letters indicate significant differences at the 5% level, according to the Tukey–Kramer HSD test.

(SAS Institute Inc., Cary, NC, USA).

Results

1. *Effects of postharvest treatment with JA and its analogs on the anthocyanin and ABA contents of grape berry skins* (*Experiment I*)

The anthocyanin content in the skins of 'Aki Queen' grape berries, though not significant, was inclined to increase in berries treated with 500 μM JA (Supplemental Fig. S1). However, treatment with 50 μM JA did not affect the anthocyanin content. Furthermore, in the culture of 'Pione' grape berries, 500 μM JA and MeJA were more effective than 100 μM (Supplemental Fig. S2). ABA was treated at a concentration of 760 μM for 'Aki Queen' and 500 μM for 'Pione,' both of which tended to increase the anthocyanin content (Supplemental Figs. S1, S2). The skins of 'Pione' grape berries treated with solutions of 500 μM JA, MeJA, or PDJ had a substantial increase in coloration and anthocyanin content, either to the same degree or even more substantially than the skins of berries treated with 500 μM ABA (Fig. 1A). In contrast, the ABA content in the skins of berries treated with JA, MeJA, or PDJ was not significantly increased as compared with that in the skins of control berries (Fig. 1B).

2. *Effects of postharvest treatment with MeJA vapor on the anthocyanin and ABA contents of grape berry skins* (*Experiment II*)

The analysis of detached 'Aki Queen' grape berries harvested at the early skin coloration stage showed that MeJA vapor treatment enhanced the coloration and the accumulation of anthocyanin in the berry skins under repeated light/dark cycles, but not under constant darkness (Fig. 2A, B). Similar to the results for 'Aki Queen' berries, the MeJA vapor treatment of 'Pione' berries enhanced the coloration and the anthocyanin accumulation in berry skins under light/dark cycles, but not under constant darkness (Fig. 3A, B). The ABA content in the skins of 'Aki

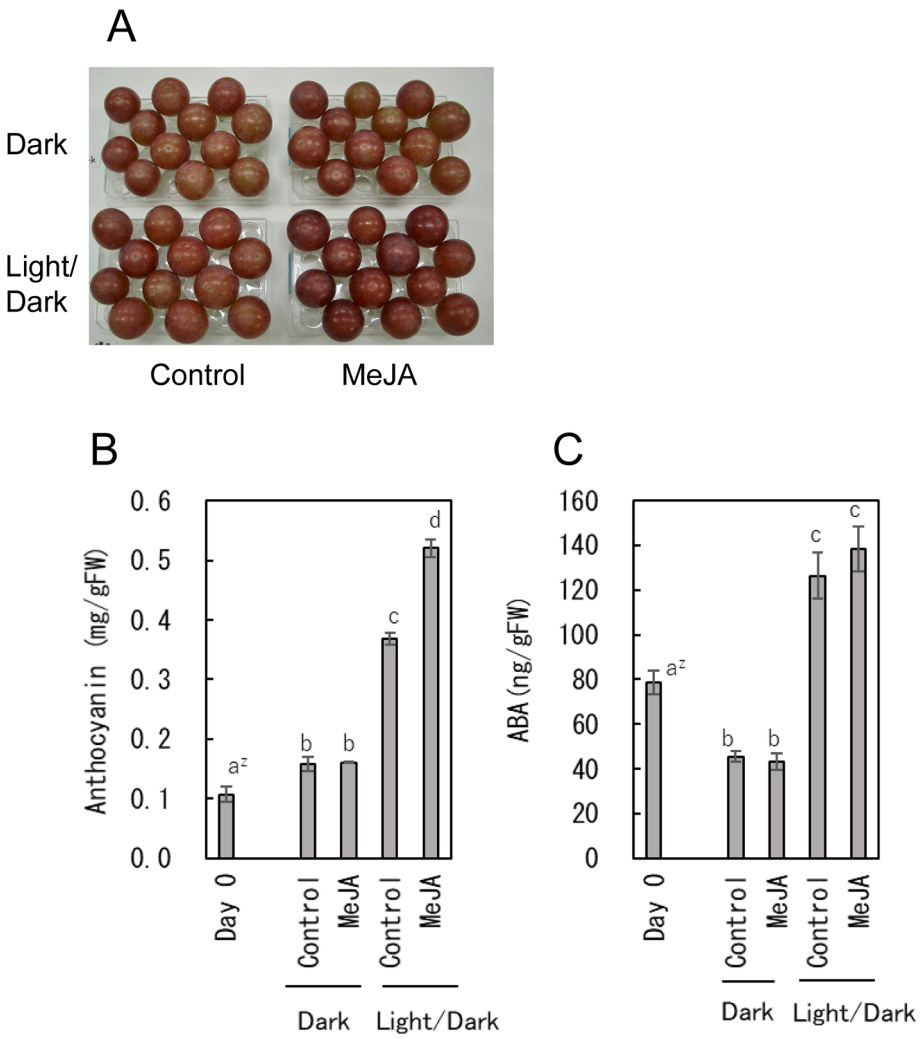

Fig. 3. Effects of MeJA vapor treatment (for 2 day under either light/dark cycles or constant darkness) on the coloration (A), anthocyanin content (B), and ABA content (C) in the skins of detached 'Pione' berries harvested at the early coloration stage. Vertical bars represent SD ($n=3$). [Z]Different letters indicate significant differences at the 5% level, according to the Tukey–Kramer HSD test.

Queen' berries did not differ between incubation under constant darkness and light/dark cycles, and the MeJA vapor treatment had no more effect on the ABA content than did the control treatment (Fig. 2C). Conversely, in 'Pione' berry skin samples, the ABA content was significantly increased by incubation under light/dark cycles, as compared with that under constant darkness. However, MeJA vapor treatment did not affect the ABA content as compared with the control treatment (Fig. 3C).

Moreover, detached 'Pione' berries harvested at the maturity stage were treated with MeJA vapor in the same manner as the berries harvested at the early coloration stage. The skin of MeJA vapor-treated berries had enhanced coloration (Fig. 4A) and a higher accumulation of anthocyanins than did the skin of non-treated and EtOH-treated berries (Fig. 4A, B). The anthocyanin content did not differ between non-treated and EtOH-treated berries (Fig. 4B). The ABA content was decreased by EtOH or MeJA treatment (Fig. 4C).

3. *Effects of postharvest treatment with MeJA vapor on the anthocyanin and ABA contents of the berry skins of mature grape clusters* (*Experiment III*)

Harvested mature 'Pione' grape clusters were treated with MeJA vapor in sealed containers under continuous light. The experiment was conducted twice, once in 2017 and once in 2018. The results in 2017 are shown in Fig. 5. MeJA vapor treatment enhanced skin coloration (Fig. 5A), and the anthocyanin content in the skin was significantly increased as compared with that of the control (Fig. 5B). However, the ABA content of berry skins treated with MeJA vapor was not significantly different from that of control berry skins (Fig. 5C), but both the MeJA-treated and control berries showed an increase in ABA contents as compared with those at the start of the experiment (Fig. 5C). In the 2018 experiment, the skin coloration was substantially enhanced in MeJA vapor treated berries, and the anthocyanin content of their skins was significantly increased more than twice as much as in the control berries (Fig. 6B). These results were the same as

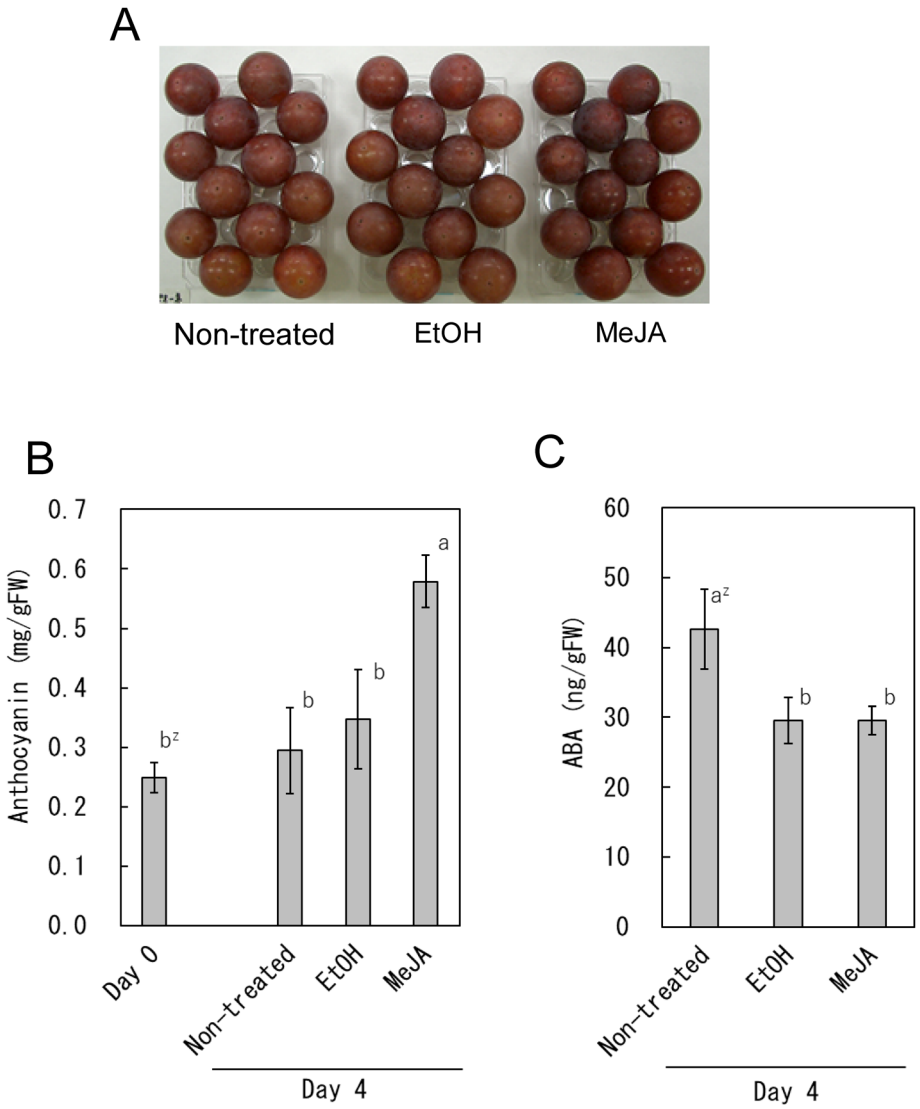

Fig. 4. Effects of MeJA vapor treatment on skin coloration (A), anthocyanin content (B), and ABA content (C) of detached berries of mature 'Pione' grape for 4 days in repeated conditions of light and dark. Vertical bars represent SD (n=3). ^{z} Different letters indicate significant differences at the 5% level, according to the Tukey–Kramer HSD test.

those from 2017. However, there was no variation in the ABA contents between differently treated berry skins included at the start of the experiment (Fig. 6C).

Harvested mature 'Aki Queen' grape clusters were treated with MeJA vapor in the same manner as the 'Pione' clusters in 2018. The skin coloration was substantially enhanced in MeJA vapor treated berries (Fig. 7A), and the anthocyanin content of their skins was significantly elevated more than twice as much as in the control berries (Fig. 7B). However, the ABA contents did not vary between the skins of MeJA vapor treated and control berries (Fig. 7C).

MeJA vapor on the berry skin color was shown to be effective not only on detached berries but also on mature grape clusters. In addition, MeJA vapor treatment did not affect the Brix value of the grapes (Supplemental Figs. S3–S5).

Discussion

The effect of JA, MeJA, and PDJ treatment on the development of skin coloration in detached 'Pione' grape berries was the same as that of ABA under light/dark cycles (Fig. 1A). This result suggested that JA may be involved in skin coloration in grape berries. Since preharvest treatment requires additional effort to attach and remove the fruit bag, for convenience, we examined postharvest treatment. Furthermore, in order to avoid spraying, we experimented with vapor treatment using MeJA, which is highly volatile and easy to apply, due to its liquid rather than viscous state.

MeJA is a naturally occurring methyl ester of JA that is used to impart fragrance to food products. Additionally, it is applied to enhance the coloration of fruits and flowers.[3,15,16,18] In red wine grapes, preharvest treatment with MeJA and benzothiadia-

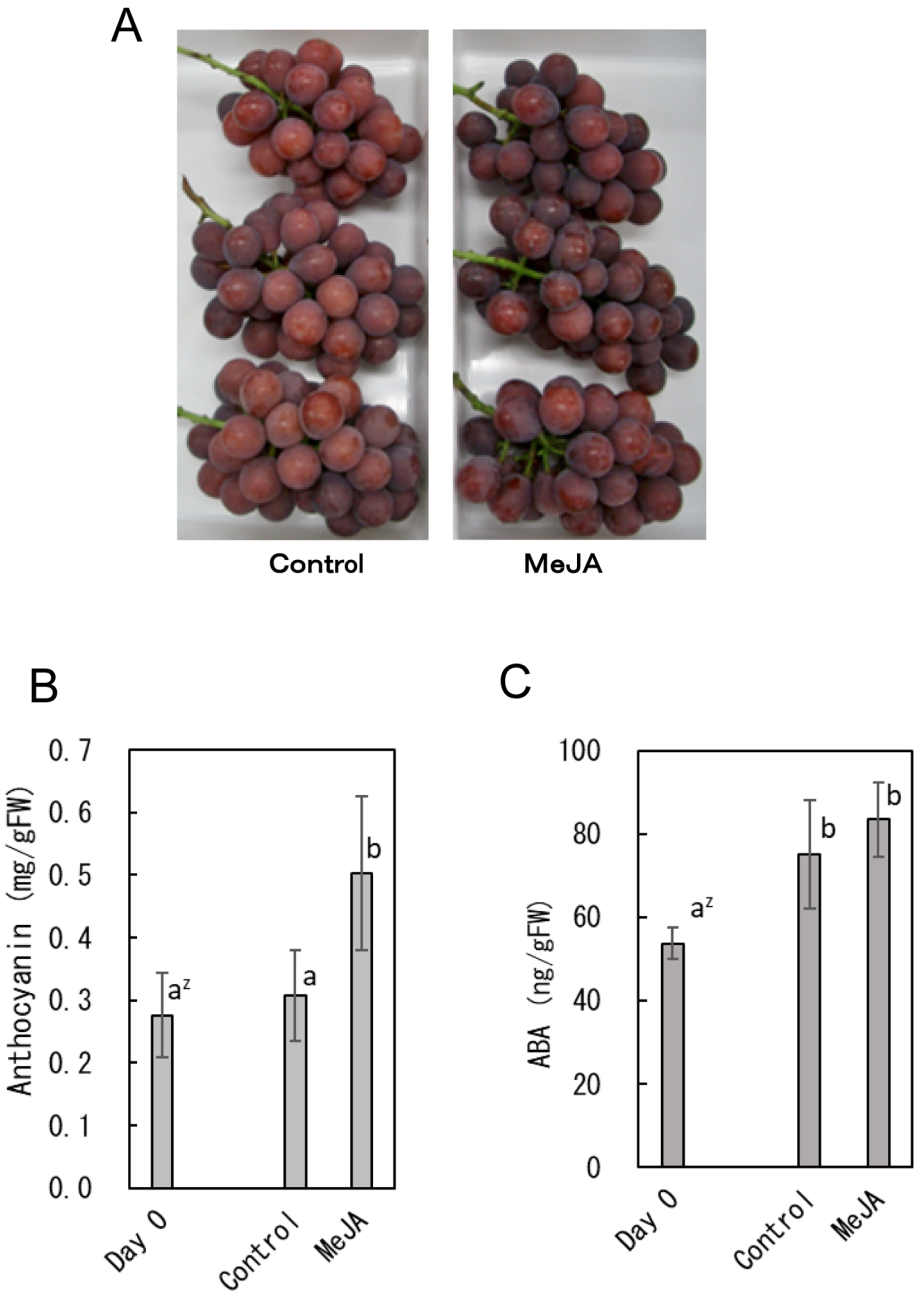

Fig. 5. Effects of postharvest MeJA vapor treatment (for 3 day under continuous light) on the skin coloration (A), anthocyanin content (B), and ABA content (C) of clusters of mature 'Pione' grape berries. This experiment was conducted in 2017. Vertical bars represent SD (n=10). [z]Different letters indicate significant differences at the 5% level, according to the Tukey–Kramer HSD test.

zole increased the anthocyanin, flavonol, and proanthocyanin contents in the skin of grape berries.[16] In the 'Fuji' apple, postharvest treatment with MeJA enhanced the production of anthocyanin cyanidin glucoside content.[15] In 'Tsugaru' apple fruit discs, MeJA stimulated anthocyanin formation.[3] Additionally, MeJA induced anthocyanin accumulation in detached petunia flowers.[18] However, most of these treatments were performed postharvest in the laboratory. The above-mentioned experiment on red wine grapes was performed in an open field[16]; however, the treatment concentration of MeJA was very high (10 mM), and the treatments were applied three times. Mizuno *et al.*[19] reported that postharvest treatment with MeJA vapor effectively reduced nonuniform coloration of early harvested flower buds of doubled-flowered *Eustoma* as it promoted anthocyanin synthesis. Furthermore, postharvest Chinese bayberry fruits exposed to MeJA vapor in a container exhibited higher levels of total anthocyanins than did the control samples.[26]

In our study, to confirm the effect of MeJA vapor treatment on 'Aki Queen' and 'Pione' grapes, experiments were conducted on detached grape berries at the early skin coloration stage that

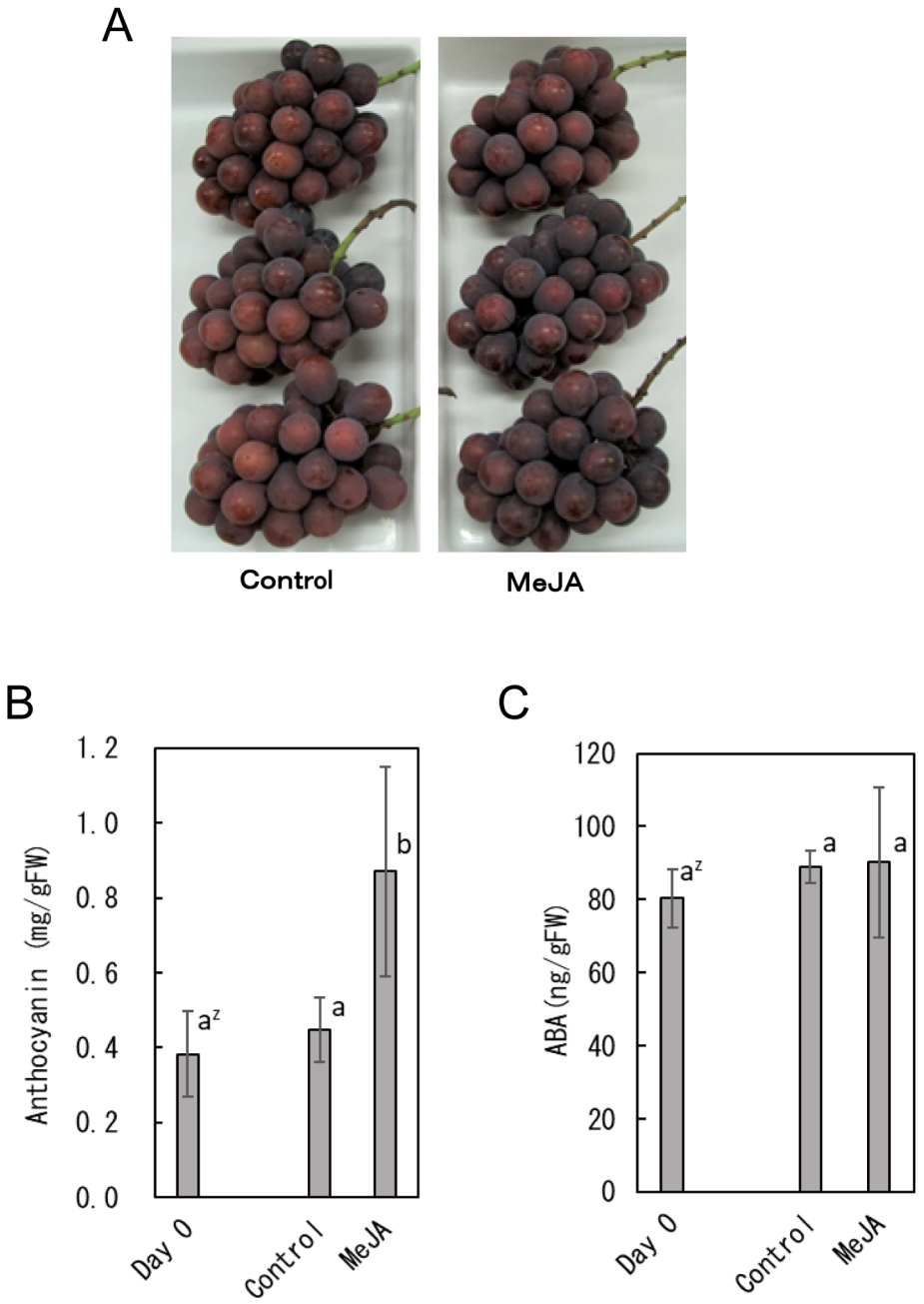

Fig. 6. Effects of postharvest MeJA vapor treatment (for 4 day under continuous light) on the anthocyanin (A) and ABA (B) contents of clusters of mature 'Pione' grape berries. This experiment was conducted in 2018. Vertical bars represent SD (n=10). [z]Different letters indicate significant differences at the 5% level, according to the Tukey–Kramer HSD test.

revealed the enhancement of coloration and the accumulation of anthocyanin in berry skins under light/dark cycles. However, these findings were not observed when conditions of constant darkness were applied (Fig. 2A, B; Fig. 3A, B). Thus, MeJA vapor treatment promoted the coloration of detached grape berries, and the results suggested that light was necessary for this process. It was observed that irradiating with light promoted the coloration of the skin in 'Pione' berries, to some extent, and MeJA vapor treatment further enhanced the coloration (Fig. 3A, B). In experiments with mature 'Pione' berries under light/dark cycles, the anthocyanin accumulation increased in the skin of berries treated with MeJA vapor as compared with that in the skin of non-treated and EtOH-treated berries. Thus, we demonstrated that MeJA vapor treatment was effective in mature grape berries. Flores *et al.*[20] reported that postharvest treatment with MeJA vapor increased the anthocyanin content in grapes (*V. vinifera*); however, the names of the grape cultivars were not provided, and there was no description of the light conditions during treatment. Furthermore, the anthocyanin levels in the grape berries of that experiment were measured after treatment in a

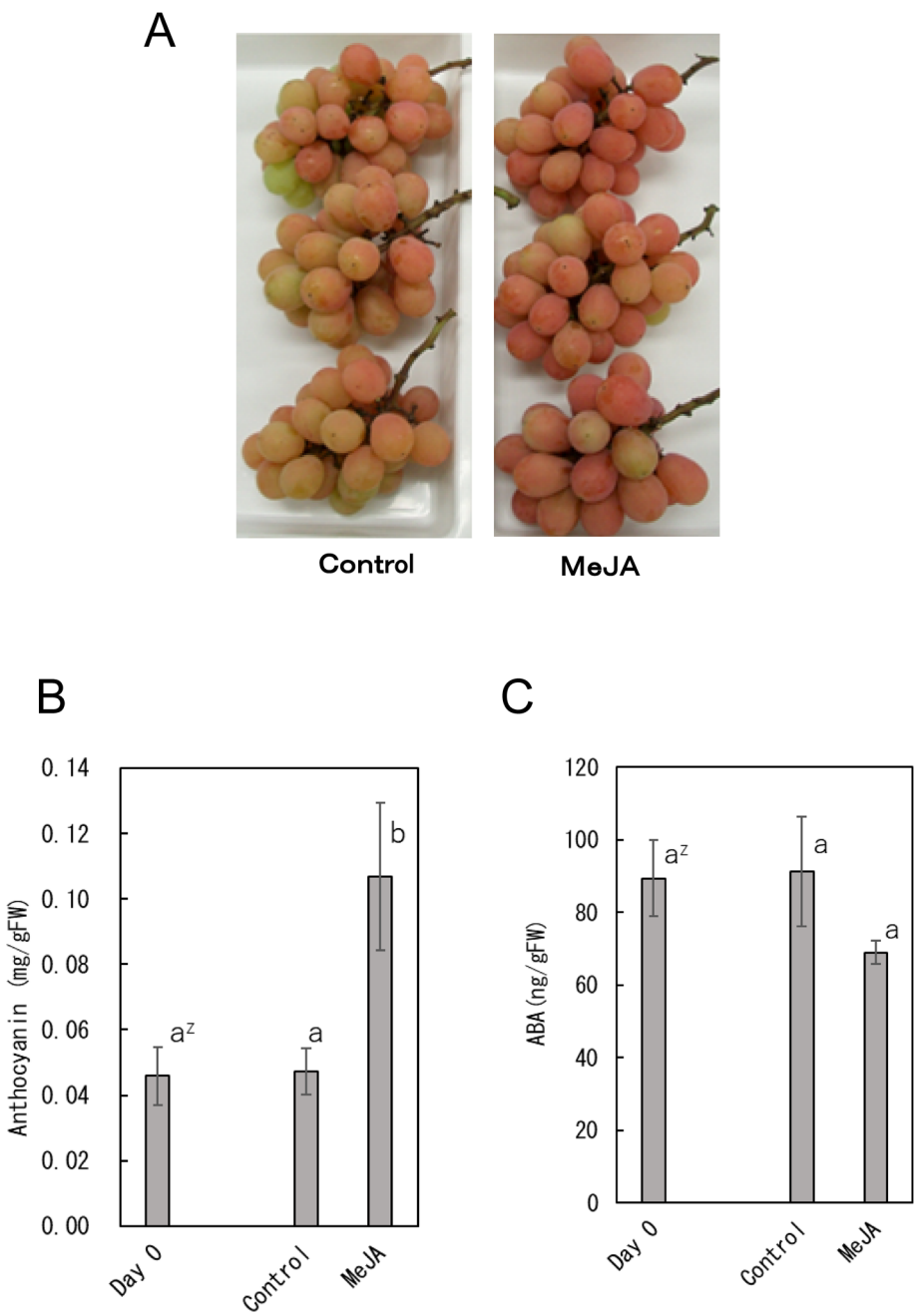

Fig. 7. Effects of postharvest MeJA vapor treatment (for 4 day under continuous light) on the skin coloration (A), anthocyanin content (B), and ABA content (C) of clusters of mature 'Aki Queen' grape berries. This experiment was conducted in 2018. Vertical bars represent SD ($n=10$). [z]Different letters indicate significant differences at the 5% level, according to the Tukey–Kramer HSD test.

container at 25°C for 24 hr, followed by storage for 5 and 7 day; consequently, the total anthocyanin content was up to 1.4 times higher in the MeJA-treated grapes than in the control grapes. Moreover, after treatment under constant darkness, the anthocyanin content might have increased during the subsequent storage period. In our experiments, we treated the clusters of table grape varieties 'Pione' and 'Aki Queen' for 3 or 4 day; however, anthocyanin accumulation was promoted by MeJA treatments during light exposure (Figs. 5B, 6B, 7B). Treatment during light irradiation promoted anthocyanin accumulation to approximately twice as much as that of the control and improved coloration within a relatively short time (Figs. 5B, 6B, 7B). In Japan, the target varieties 'Pione' and 'Aki Queen' often develop poor coloration. According to Azuma *et al.*,[27] the skin color of grape berries, mainly in red- to purple-skinned accessions, can be improved by combining postharvest light irradiation with an appropriate temperature, and anthocyanin biosynthesis-related genes have elevated expression levels under such conditions.

Based on our study, it was suggested that the phenomenon of light-induced postharvest coloration promotion can be further enhanced by MeJA treatment.

The effect of MeJA vapor on promoting berry skin color was shown to be effective not only in detached berries but also in mature grape clusters. In addition, MeJA vapor treatment did not affect the Brix value of the grapes (Supplemental Figs. S3–S5). Postharvest treatment with MeJA vapor improved skin coloration in grape berry clusters to the same extent as in detached individual berries. Further research is necessary to develop effective techniques for practical application. That should be investigated in the next-stage study.

In addition, the regulation of anthocyanin biosynthesis is one of the known functions of JA; however, the mechanism underlying the promotion of grape skin coloration by MeJA treatment remains unclear. Exogenous ABA treatment of grape clusters is known to promote berry skin coloration.[10–12,28–30] Many studies indicate that ABA triggers the ripening of grapes, which coincides with the enhancement of berry skin color.[5–9] Therefore, ABA has been considered to play an important role in the skin coloration of grape berries. However, in this study, we found that JA, MeJA, and PDJ might promote anthocyanin synthesis without ABA involvement (Fig. 1B). We observed that MeJA vapor treatment enhanced coloration and anthocyanin accumulation in berry skins; however, the ABA content of berry skins was not significantly different from that of the control (Figs. 2C, 3C, 4C, 5C, 6C, 7C). Thus, we found that treatment with JA and its analogs promoted the skin coloration of grape berries; however, it did not cause an increase in ABA content. These results showed that JA and its analogs might be involved in the mechanism of skin coloration development in grape berries independent of ABA. Thus, it was assumed that an increase in the ABA content did not contribute to the enhancement of coloration and anthocyanin accumulation in MeJA vapor treated berry skins. In a previous study using discs of grape berries, exogenous treatment with MeJA and ABA solutions either had no effect or reduced each other's levels.[31] These results were consistent with those of this study. Grape berry coloration is generally promoted at low temperatures, and Azuma *et al.*[1] showed that the ABA content of 'Pione' berries harvested at the early skin coloration stage was higher at a low temperature (15°C) than at a high temperature (35°C). In contrast, it was reported that the accumulation of anthocyanins in the berry skins of 'Ruby Roman' grapes was promoted at a low temperature (15°C or 18°C) and suppressed at a high temperature (30°C or 35°C); however, the concentration of ABA and its derivatives increased at a high temperature.[21,32] This result indicates that the promotion of anthocyanin accumulation and the tendency toward increased ABA do not always overlap. Therefore, it was speculated that the regulation of anthocyanin biosynthesis at different temperatures might have different mechanisms in 'Ruby Roman' berries than merely the ABA-mediated regulation.[32] Our results also suggested that the anthocyanin accumulation promoted by MeJA in 'Pione' and 'Aki Queen' berry skins was not accompanied by an increase in ABA content, indicating the existence of a mechanism that regulates anthocyanin biosynthesis without ABA involvement (Figs. 2C, 3C, 4C, 5C, 6C, 7C).

The results of our study showed that postharvest MeJA vapor treatment improved the skin color in grapes. Therefore, we suggest that this treatment can be a useful method for the horticultural industry. Additionally, in this study, we targeted the grape varieties 'Pione' and 'Aki Queen,' which are known to be often color deficient in Japan, and we showed that coloration could be improved in these varieties *via* the above-mentioned method. MeJA vapor treatment is performed postharvest as a labor-saving method that does not require the reattachment of fruit bags following treatment. Moreover, this study clarified that the underlying mechanism for promoting the coloration of 'Pione' and 'Aki Queen' grape berries by MeJA might be not mediated by ABA.

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Electronic supplementary materials

The online version of this article contains supplementary materials (Supplemental Figs. S1–S5), which is available at https://www.jstage.jst.go.jp/browse/jpestics/.

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