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Differential Effects of Abscisic Acid, 6-Benzyladenine and 5-Aminolevulinic Acid on Berry Coloration and Internal Quality During Ripening of 'Kyoho' and 'Summer Black' Grapes

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Highlights

What are the main findings?

- Selected ABA treatments showed more pronounced effects on berry coloration and anthocyanin accumulation.
- Selected 6-BA and ALA treatments produced stronger responses in several internal quality parameters, with cultivar-dependent differences.
- The observed functional differentiation among PGR treatment regimes provides new insights into the coordination of external coloration and internal quality during grape ripening.

What are the implications of the main findings?

- This study provides a physiological basis for precision PGR management by matching specific regulators with targeted quality objectives.
- Combined or sequential application of different PGRs warrants further investigation as a potential strategy for coordinating external coloration and internal quality.

Abstract

Table grape quality is determined not only by berry skin coloration but also by internal quality parameters such as sugar–acid balance. However, these quality parameters are regulated by partially distinct physiological processes and may respond differently to external regulatory treatments during berry ripening. In this study, the effects of three widely used PGRs—abscisic acid (ABA), 6-benzyladenine (6-BA), and 5-aminolevulinic acid (ALA)—on berry coloration, internal quality, anthocyanin accumulation, and the expression of anthocyanin biosynthesis-related genes were comparatively evaluated in two table grape cultivars, 'Kyoho' and 'Summer Black'. All three PGR treatments promoted berry coloration and enhanced anthocyanin accumulation, although the magnitude and timing of their effects differed. The selected ABA treatments showed more pronounced effects on berry coloration and anthocyanin accumulation, whereas selected 6-BA and ALA treatments produced stronger responses in several internal quality parameters. These responses also varied between cultivars. Overall, the selected PGR treatment regimes produced distinct response patterns in berry coloration and internal quality during grape ripening, with responses depending on cultivar and treatment concentration. The observed differential response patterns provide insights into the differential effects of PGRs on berry coloration and internal quality and highlight the potential value of cultivar- and treatment-specific PGR strategies for table grape quality management.

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Keywords: table grape; plant growth regulators; ABA; 6-BA; ALA; berry coloration; internal quality; sugar-acid balance

1. Introduction

Table grape (*Vitis vinifera* L.) is one of the most economically important fruit crops worldwide, and fruit quality is the primary determinant of its commercial value and consumer acceptance. Unlike wine grapes, the market competitiveness of table grapes depends largely on their fresh appearance and eating quality. Among numerous quality parameters, berry skin coloration and sugar–acid balance are considered the most important indicators of visual appearance and fresh quality, respectively [1,2]. Although these quality traits develop simultaneously during berry ripening, they are regulated by distinct physiological processes and do not always progress synchronously. Environmental conditions, particularly temperature and light, can also affect berry coloration and sugar–acid accumulation during ripening [3,4]. As a result, berries may exhibit satisfactory coloration while failing to achieve optimal internal quality, or vice versa. Therefore, improving the coordination between external coloration and internal quality has become an important objective in modern table grape production.

Berry skin color in red and black grapes is primarily determined by the accumulation of anthocyanins, which are synthesized through the flavonoid biosynthetic pathway [5]. This process is regulated by a series of structural genes, including *CHS*, *CHI*, *F3H*, *DFR*, *LDOX*, and *UFGT*, together with transcription factors of the MYB–bHLH–WD40 (MBW) regulatory complex [6–9]. Meanwhile, internal quality development during berry ripening is accompanied by substantial metabolic changes, particularly an increase in soluble sugars and a decrease in organic acids. In table grapes, sweetness and acidity strongly influence consumer preference. Total soluble solids (TSS) and titratable acidity (TA) are commonly used to evaluate sugar and acid level. Glucose and fructose are the predominant sugars, whereas organic acids mainly include tartaric and malic acids in grapes. Sugar accumulation is regulated by coordinated processes of synthesis, transport, and cleavage, involving enzymes such as sucrose synthase (SS), sucrose phosphate synthase (SPS), and invertases [10]. Organic acid metabolism is closely linked to respiratory pathways and involves key enzymes such as malate dehydrogenase (MDH), phosphoenolpyruvate carboxylase (PEPC), and isocitrate dehydrogenase (IDH) [11,12]. Thus, berry coloration and internal quality development involve interconnected but partially distinct physiological and metabolic processes, which may contribute to differences in their temporal responses during berry ripening.

Plant growth regulators (PGRs) are widely applied in fruit production to modulate fruit development and ripening. Abscisic acid (ABA) is a key regulator of grape berry ripening, and its exogenous application before véraison, has been shown to promote anthocyanin accumulation and advance coloration in several grape cultivars, including 'Aki Queen', 'Crimson Seedless', 'Pinot Noir' and 'Pionnier' [13–16]. In addition, 6-benzyladenine (6-BA), a synthetic cytokinin, has been reported to influence sugar accumulation and fruit quality in different grape cultivars, including 'Sahebi', 'Crimson Seedless', and 'Jumeigui' [17–19]. More recently, 5-aminolevulinic acid (ALA), a precursor of tetrapyrrole biosynthesis, has attracted increasing attention as a potential plant growth regulator because of its diverse effects on plant growth, development, and fruit ripening. Exogenous ALA has been reported to promote seed germination, root and shoot growth, photosynthetic activity, and biomass accumulation, as well as to enhance plant tolerance to various environmental stresses [20–23]. During fruit development and ripening, ALA can also regulate photosyn-

thesis, pigment biosynthesis, antioxidant metabolism, and the accumulation of sugars and organic acids, thereby promoting fruit coloration and improving internal quality in several fruit crops, including grape [24–27]. However, compared with ABA and 6-BA, studies on exogenous ALA in grape remain relatively limited and have mainly focused on a small number of cultivars, such as 'Delaware', and 'Crimson Seedless' [27,28]. Although these PGRs have individually been reported to improve berry coloration or fruit quality, previous studies have generally focused on the physiological effects of a single regulator. Consequently, whether different PGRs differentially regulate visual coloration and internal quality development through distinct physiological processes during berry ripening remains poorly understood.

Moreover, the effectiveness of PGR applications may vary with cultivar, developmental stage and growing environment. Therefore, responses observed in one cultivar or production region may not necessarily be directly transferable to other grape-growing conditions, highlighting the need to evaluate PGR effects under specific production environments. 'Kyoho' and 'Summer Black' are commercially important table grape cultivars widely grown for fresh consumption in China. Although they share part of their genetic background, 'Kyoho' is a tetraploid (2n = 4x) interspecific hybrid derived from 'Ishihara Wase' × 'Centennial', whereas 'Summer Black' is a triploid (2n = 3x) seedless cultivar derived from 'Kyoho' × 'Thompson Seedless'. Their partially shared pedigree but different ploidy and genetic constitution provide a useful basis for examining cultivar-dependent responses to exogenous PGRs.

In Jiangsu and other regions of eastern China, grape production commonly uses protected cultivation, under which high temperatures during berry ripening may adversely affect berry coloration. Therefore, improving berry appearance and internal quality under local production conditions is important for maintaining fruit quality and marketability. Evaluating PGR-mediated regulation of berry ripening under such conditions may provide useful information for improving the quality and marketability of table grapes. In the present study, these two cultivars were selected to comparatively investigate the effects of ABA, 6-BA and ALA on berry coloration, fruit quality, anthocyanin accumulation and the expression of anthocyanin biosynthesis-related genes during berry ripening. We hypothesized that ABA, 6-BA and ALA would produce different response patterns in visual coloration and internal quality development, and that these responses would vary between cultivars under the tested treatment concentrations. The findings of this study provide insights into the differential regulation of visual coloration and internal quality by exogenous PGRs during grape berry ripening and may help inform strategies for better coordinating external appearance with eating quality through differential PGR application.

2. Materials and Methods

2.1. Plant Material and Treatments

Three-year-old grapevines of 'Kyoho' and 'Summer Black' (*V. vinifera* × *V. labrusca*) were used as experimental materials. The vines were grown under a rain-shelter cultivation system with a large H-shaped trellis in the germplasm orchard of Jiangsu Longcheng Agricultural Development Co., Ltd. (Xuyi, Jiangsu, China), with an intelligent integrated water and fertilizer management system. The vines were cultivated in loam soil with a pH of 5.8, containing 200 mg·kg^{-1} available nitrogen, 29.1 mg·kg^{-1} available phosphorus, and 51.4 mg·kg^{-1} available potassium. Both 'Kyoho' and 'Summer Black' were grafted onto SO4 rootstock. Vines with uniform growth and similar vigor were selected. The selected vines were randomly assigned to the different treatment groups to minimize positional and growth-related variation among treatments. All vines were grown under the same soil and cultivation conditions and received routine fertilization, irrigation, and pest and disease

management, which were kept consistent among all treatments throughout the experiment. Fruit load, seedlessness management, and fruit thinning were conducted according to the standard management practices for commercial table grape production in the orchard. Thus, all experimental treatments were conducted under the same routine orchard management conditions. Temperature and relative humidity were not continuously monitored during the experiment, while direct rainfall was excluded by the rain-shelter system.

Due to differences in phenological development, 'Kyoho' and 'Summer Black' were treated on July 5 and June 15, respectively, approximately two weeks after the fruit enlargement stage, when the berries were still green and had not yet entered the coloration stage. The concentrations of ABA, 6-BA, and ALA used in the experiment are shown in Table S1 and were selected based on relevant published studies and preliminary experiments. ABA, 6-BA, and ALA were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China), with purities of ≥98%, ≥98%, and ≥99%, respectively. The required amounts of the respective PGRs were dissolved in water and diluted to the designated concentrations, with 0.1% (v/v) Tween-80 added as a surfactant. A single grapevine was used as the experimental unit, with three independent vines serving as biological replicates for each treatment (n = 3). The PGR solutions were uniformly sprayed onto the fruit surfaces of the experimental vines using the same type and size of sprayer for all treatments, ensuring consistent and adequate coverage of the berries.

The same concentration series of ABA, 6-BA, and ALA was initially applied to both cultivars for preliminary screening. Based on the coloration patterns observed throughout the developmental stages shown in Figure S1, T1 (900 mg L^{-1} ABA), T4 (300 mg L^{-1} 6-BA), and T9 (50 mg L^{-1} ALA) were selected for further physiological and molecular analyses in 'Kyoho', whereas T2 (600 mg L^{-1} ABA), T5 (200 mg L^{-1} 6-BA), and T9 (50 mg L^{-1} ALA) were selected for 'Summer Black'. Berry samples were collected at 4-day intervals for 'Summer Black' and 5-day intervals for 'Kyoho'. At each sampling stage, three clusters were selected from each experimental vine, and ten berries were collected from each cluster for subsequent analyses. Sampling was conducted from June to September until total soluble solids and titratable acidity stabilized and seeds were fully browned.

2.2. Determination of Firmness and Fruit Skin Color

Ten berries were collected from each biological replicate for measurement. The individual vine was considered the biological replicate (n = 3), whereas berries collected from the same vine were treated as subsamples. Skin color was measured using a colorimeter (CR-400, Konica Minolta, Tokyo, Japan). Fruit skin color was measured at two symmetrical positions on the equatorial surface of each berry using a colorimeter (CR-400, Konica Minolta, Tokyo, Japan). The L*, a*, and b* values were recorded, and the average value was calculated. The chromaticity index for red grapes (CIRG) was calculated as follows: CIRG = (180 − H)/(L* + C), where H = arctan (b*/a*) represents the hue angle and C = $[(a^*)^2 + (b^*)^2]^{0.5}$ represents chroma. Berry firmness was measured using an ITALY-type fruit firmness tester, with each measurement repeated five times. Skin color and firmness were measured at each sampling stage, covering the progressive development and ripening of the berries from fruit enlargement to full ripening, as described in Section 2.1.

2.3. Determination of the Total Soluble Solids, Titratable Acidity, and Anthocyanin

Total soluble solids (TSS) were measured using a digital refractometer (PAL-BX/ACID 1, ATAGO, Tokyo, Japan). Titratable acidity (TA) was measured by neutralization with 0.1 N NaOH using phenolphthalein as the indicator and expressed as a percentage of citric acid equivalents (% TA). Total anthocyanin content was measured using the pH differential method [24]. Briefly, 0.3 g of grape skin was extracted with 3 mL of extraction solution

(methanol: formic acid: ethanol = 70:2:28, $v/v/v$) for 30 min, followed by shaking at 250 rpm for 2 h at 30 °C. After centrifugation, absorbance was measured at 510 and 700 nm under pH 1.0 and pH 4.5 conditions using a UV–visible spectrophotometer (BioMate 3S, Thermo Scientific, Waltham, MA, USA). Anthocyanin concentration was calculated using the standard formula:

$$\text{Total anthocyanin content}\left(\text{mg L}^{-1}\right) = \frac{\text{A} \times \text{Mw} \times \text{Df} \times 1000}{\varepsilon \times l}$$

$$\text{A} = \left(\text{A}_{510} - \text{A}_{700}\right)_{\text{pH1.0}} - \left(\text{A}_{510} - \text{A}_{700}\right)_{\text{pH4.5}}$$

Mw is the molecular weight. Df is the dilution factor. ε is the extinction coefficient. l is the optical path (1.0 cm).

2.4. Determination of Anthocyanin Compositions

Based on the coloration results, the selected concentrations of ABA, 6-BA, and ALA were selected for further analysis of the anthocyanin composition. Three developmental stages were defined: pre-véraison, (no coloration), véraison, and fully ripen. For each cultivar, samples collected on July 10 ('Kyoho') and June 15 ('Summer Black') were used as controls.

Anthocyanin profiles were analyzed using a liquid chromatography–mass spectrometry (LC–MS) system (G2-XS QTof, Waters, Milford, MA, USA). Briefly, 0.3 g of grape skin was homogenized in 3 mL of extraction solution (water: ethanol: hydrochloric acid = 30:70:1, $v/v/v$) for 20 min. The extract was freeze-dried and then re-dissolved in ultrapure water to a final volume of 5 mL. The reconstituted solution was filtered through a 0.45 μm organic-phase membrane filter prior to LC–MS analysis. A 2 μL aliquot was injected onto an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm; Waters, Milford, MA, USA). The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile), at a flow rate of 0.4 mL min^{-1}. The gradient elution program was as follows: 5% B for 2 min, 5–95% B over 15 min, and 95% B for 2 min.

Mass spectrometric analysis was performed using an electrospray ionization source in both positive- and negative-ion modes with MSE acquisition over an m/z range of 50–1200. Leucine-enkephalin was used as the lock-mass reference for mass correction, with m/z values of 556.2771 and 554.2615 in positive- and negative-ion modes, respectively. Data acquisition and processing were performed using MassLynx 4.1 (Waters, Milford, MA, USA). Anthocyanin compounds were qualitatively identified by comparison with the grape anthocyanin HPLC-UV-MS database established by the Mass Spectrometry Testing Center, College of Life Sciences, Nanjing Agricultural University. Daidzein was used as an internal standard for the relative quantification of anthocyanin glycosides. The relative content of each anthocyanin glycoside was calculated based on its peak area relative to that of the internal standard.

2.5. Determination of Soluble Sugar Contents

Glucose, fructose, and sucrose contents were determined using high-performance liquid chromatography (HPLC). Briefly, 1 g of grape flesh was ground in liquid nitrogen and transferred to a 10 mL centrifuge tube. Five milliliters of 80% (v/v) ethanol were added, and the mixture was extracted at 35 °C for 10 min. After centrifugation at 12,000 rpm for 10 min at 4 °C, the supernatant was collected. The extraction was repeated with another 5 mL of 80% (v/v) ethanol, and the two supernatants were combined and concentrated. The resulting extract was then adjusted to a final volume of 5 mL with ultrapure water. The extracts were freeze-dried at −80 °C and re-dissolved in ultrapure water. The solution was filtered through a 0.22 μm membrane before analysis.

Chromatographic analysis was performed using an HPLC system equipped with an evaporative light scattering detector (ELSD). Separation was carried out on a Prevail Carbohydrate ES column (100 mm × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile and water (80:20, *v*/*v*), with a flow rate of 1.0 mL min^{-1} and a column temperature of 50 °C. The injection volume was 20 μL. Standard curves were established using glucose, fructose, and sucrose standards. Quantification was performed using the external standard method.

2.6. Determination of Organic Acid Contents

The contents of tartaric acid, malic acid, and citric acid were determined using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA). The same extracts prepared according to the procedure described in Section 2.5 were used for subsequent organic acid analysis.

Separation was performed using a Synergi Fusion column at 30 °C. The mobile phase consisted of solvent A (25 mM KH_2PO_4 containing 0.1 mM EDTA, pH 2.5) and solvent B (methanol). A gradient elution program was applied: solvent A decreased from 100% to 90%, while solvent B increased from 0% to 10% over 10 min. The system was then returned to initial conditions within 20 min for column re-equilibration. The flow rate was maintained at 1.0 mL min^{-1}. Organic acids were identified and quantified based on retention times and standard curves according to previously described methods.

2.7. RNA Extraction and Quantitative Real-Time PCR (qRT-PCR) Experiments

Total RNA was extracted using the RNAprep pure Plant kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions. The concentration of extracted RNA was determined using a NanoPhotometer® spectrophotometer (Implen, Westlake Village, CA, USA). Thereafter, 1 μg of total RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, Dalian, China). A PrimeScript™ RT reagent kit (TakaRa, Dalian, Japan) was used for reverse transcription. The real-time quantitative PCR (qRT-PCR) was performed using SYBR® Premix Ex Taq TM Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. The amplification program was as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s, followed by a final melting curve analysis. The relative expression of genes was calculated by the $2^{-\Delta\Delta CT}$ method, with Actin used as the reference gene. The primers used are listed in Table S2.

2.8. Statistical Analysis

All data are presented as the mean ± standard error (SE) of three biological replicates (n = 3). Statistical analyses were performed using SPSS 22 software (SPSS Inc., Chicago, IL, USA). For each grape cultivar and sampling stage, differences among PGR treatments were analyzed separately using one-way analysis of variance (ANOVA), with PGR treatment considered as the experimental factor. Duncan's multiple range test was subsequently performed for multiple comparisons at $p < 0.05$. Cultivar and sampling stage were not included as factors in the same ANOVA model, as the two cultivars differed in their developmental timing and were sampled at different intervals. Pearson's correlation analysis was conducted to evaluate the relationships between metabolite contents and gene expression levels. Figures and heat maps were generated using SigmaPlot 14.0 software (Systat Software Inc., San Jose, CA, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

3. Results

3.1. Effects of PGR Treatments on Berry Coloration, Anthocyanin Accumulation, and Firmness

Berry development and coloration patterns of 'Kyoho' and 'Summer Black' grapes after véraison, are shown in Figure S1. Compared with the control (CK), all plant growth regulator (PGR) treatments significantly enhanced berry coloration in both cultivars.

In 'Kyoho', the most effective concentrations were T1 (900 mg L^{-1} ABA), T4 (300 mg L^{-1} 6-BA), and T9 (50 mg L^{-1} ALA) (Figure 1A). The color index of red grape (CIRG) increased progressively during berry development, with no significant differences among treatments at the full ripeness stage. However, berries treated with T1 exhibited an earlier increase in CIRG, which became evident on July 15 (Figure 1B). Consistently, anthocyanin content in the berry skin was significantly higher in all treated groups than in CK, with T1 maintaining the highest levels throughout development (Figure 1C). Berry firmness was significantly reduced by all PGR treatments compared with the control. T4 and T9 showed a stronger effect on firmness reduction than T1, whereas no significant differences were observed among treatments at later stages (Figure S1C).

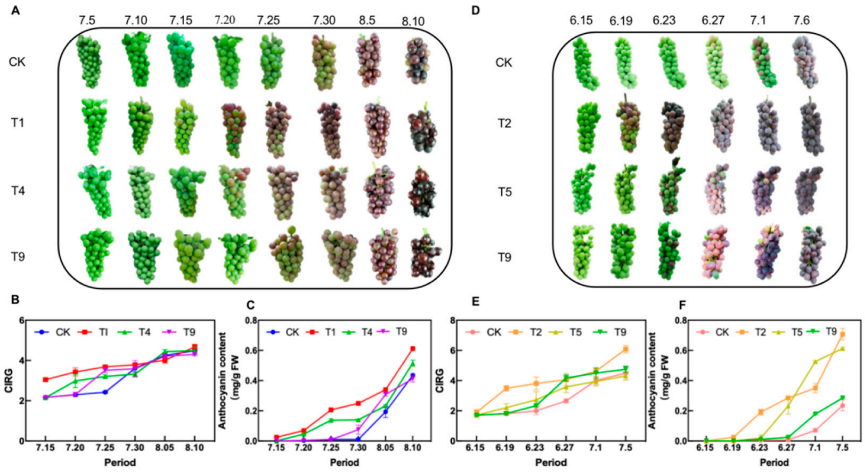


Figure 1. Effects of different PGR treatments on coloration of 'Kyoho' (**A**–**C**) and 'Summer Black' (**D**–**F**) berries. 'Period' refers to the fruit developmental stages represented by the sampling dates shown in the figures. Values are mean ± SE (n = 3). CK: control group; T1: 900 mg L^{-1} ABA; T2: 600 mg L^{-1} ABA; T4: 300 mg L^{-1} 6-BA; T5: 200 mg L^{-1} 6-BA; T9: 50 mg L^{-1} ALA.

In 'Summer Black', T2 (600 mg L^{-1} ABA), T5 (200 mg L^{-1} 6-BA), and T9 (50 mg L^{-1} ALA) were identified as the selected effective treatments. All treatments significantly increased CIRG values compared to CK, with T2 showing the greatest effect (Figure 1E). Similarly, anthocyanin content at the full ripeness stage was highest under T2 (Figure 1F). Firmness was significantly reduced only under T5 at the full ripeness stage, while other treatments showed no significant differences compared to CK (Figure S1D).

These results indicate that, among the selected treatment regimes, ABA showed a more pronounced effect on the early progression of berry coloration, whereas 6-BA and ALA also promoted pigmentation but with weaker or more delayed effects under the tested conditions.

3.2. Effects of PGR Treatments on Anthocyanin Composition in Grape Skins

Five major anthocyanidin classes, including delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv), were detected in both cultivars at the full ripeness stage. The relative proportions of individual anthocyanidins differed markedly among PGR treatments (Tables S3 and S4).

At the full ripeness stage in 'Kyoho', T1 and T4 markedly increased total anthocyanin accumulation but exhibited distinct compositional profiles. T1 preferentially enhanced malvidin-derived anthocyanins, whereas T4 increased delphinidin- and cyanidin-derived anthocyanins. T9 mainly promoted peonidin accumulation (Figure 2A). A total of 21 anthocyanin compounds were identified in 'Kyoho', with malvidin 3-O-coumaroylglucoside-5-O-glucoside being the dominant component. At the véraison stage, PGR treatments accelerated berry coloration mainly through enhanced accumulation of malvidin- and peonidin-derived anthocyanins, and this promotive effect remained evident at the full ripeness stage (Figure 2C).

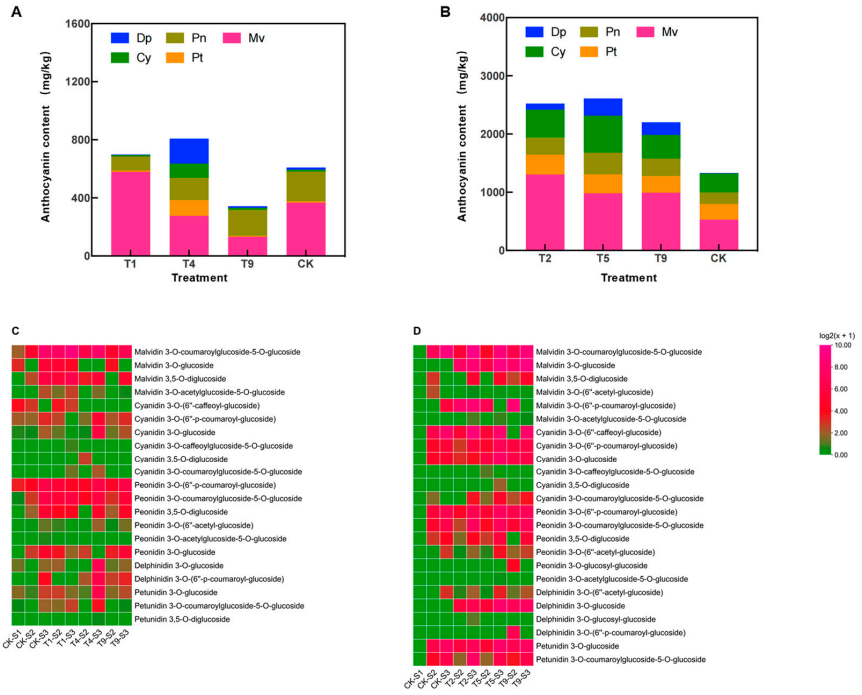


Figure 2. Effects of different PGR treatments on anthocyanin composition in grape skins of 'Kyoho' (**A**,**C**) and 'Summer Black' (**B**,**D**). Anthocyanin composition was analyzed at three developmental stages: pre-véraison, (S1), véraison (S2), and fully ripen (S3). CK: control group; T1: 900 mg L^{-1} ABA; T2: 600 mg L^{-1} ABA; T4: 300 mg L^{-1} 6-BA; T5: 200 mg L^{-1} 6-BA; T9: 50 mg L^{-1} ALA.

In 'Summer Black', all PGR treatments increased the contents of the five anthocyanidins, with T2 showing the strongest promotion of malvidin accumulation, T5 enhancing delphinidin and cyanidin, and T9 increasing delphinidin content (Figure 2B). A total of 24 individual anthocyanin compounds were identified in 'Summer Black', with malvidin 3-O-coumaroylglucoside-5-O-glucoside being the most abundant component, accounting for approximately 30% of total anthocyanins (Table S4). At the véraison stage, T2 and T5 accelerated berry coloration mainly through enhanced accumulation of malvidin-, cyanidin-, and delphinidin-derived anthocyanins, whereas T9 mainly promoted the accumulation of malvidin- and delphinidin-derived anthocyanins. At the full ripeness stage, all treatments resulted in further accumulation of multiple anthocyanin components, with treatment-specific differences in individual anthocyanin compounds (Figure 2D).

These results suggest that, in addition to increasing total anthocyanin content, the selected PGR treatment regimes were associated with treatment-specific changes in anthocyanin composition, indicating that PGR treatments affected not only the extent but also the composition of berry pigmentation.

3.3. Expression of Anthocyanin Biosynthesis and Transport-Related Genes

The transcript levels of genes involved in anthocyanin biosynthesis and transport were analyzed by qRT-PCR in both cultivars. In 'Kyoho', T1 treatment significantly upregulated the expression of *F3′5′H*, *UFGT*, and *GST* during the early coloration stage, while *PAL* and *C4H* showed marked increases at the full ripeness stage. T4 treatment mainly enhanced the expression of *UFGT* and *GST* during the early coloration stage, followed by a general upregulation of most genes at the full ripeness stage. Under T9 treatment, early coloration was associated with increased expression of *F3H*, *DFR*, *UFGT*, and *GST*, with their expression remaining relatively high at the full ripeness stage (Figure 3A and Table S5).

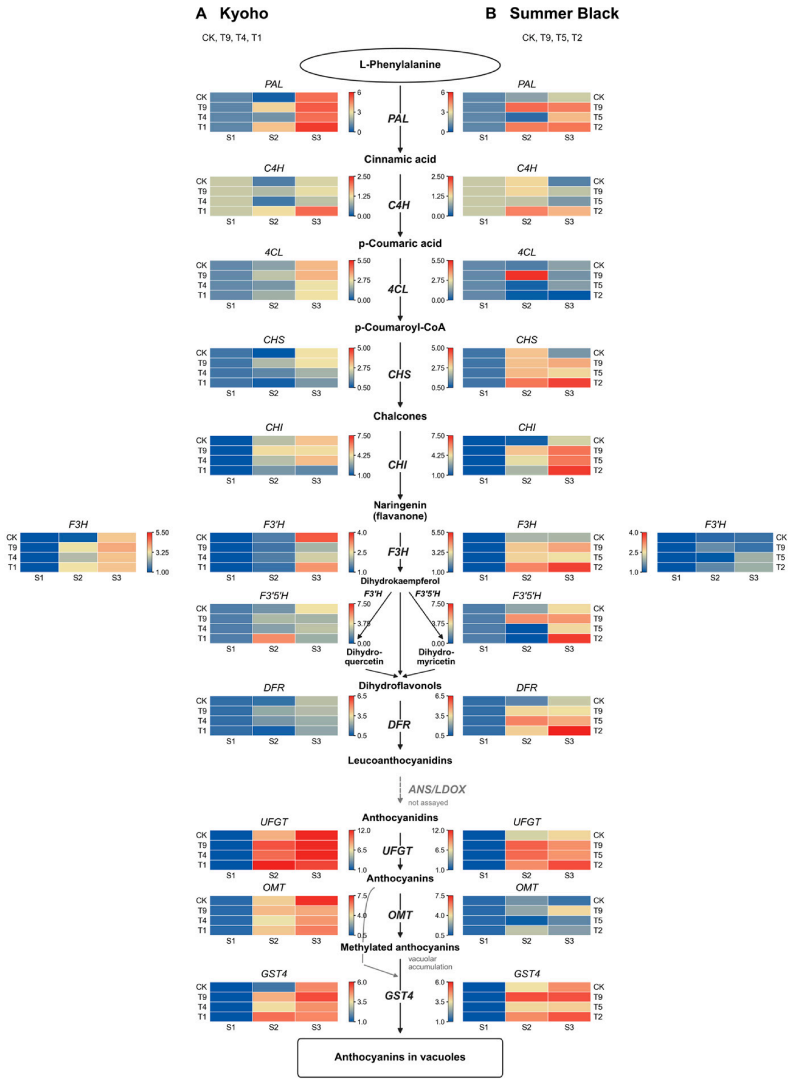


Figure 3. Relative expression of anthocyanin biosynthesis and transport-related genes in 'Kyoho' (**A**) and 'Summer Black' (**B**) under different PGR treatments. Heatmaps display log2-transformed relative expression levels. Sampling stages: pre-véraison, (S1), véraison (S2), and fully ripen (S3). CK: control group; T1: 900 mg L^{-1} ABA; T2: 600 mg L^{-1} ABA; T4: 300 mg L^{-1} 6-BA; T5: 200 mg L^{-1} 6-BA; T9: 50 mg L^{-1} ALA.

In 'Summer Black', most anthocyanin-related genes were upregulated by PGR treatments compared with the control. T2 treatment induced a broad increase in gene expression during the early coloration stage, particularly *PAL*, *4CL*, *UFGT*, and *GST*. T5 treatment mainly increased *DFR* and *UFGT* expression during the early coloration stage and enhanced *CHI* and *OMT* expression at the full ripeness stage. T9 treatment significantly increased

the expression of *PAL*, *CHI*, *F3′5′H*, *UFGT*, and *GST* during the early coloration stage, with *CHI*, *UFGT*, and *GST* remaining highly expressed at the full ripeness stage (Figure 3B and Table S6).

These results indicate that the differences in berry coloration associated with PGR treatments were accompanied by differential transcriptional activation of genes involved in the anthocyanin biosynthetic and transport pathways, with cultivar-, treatment-, and developmental stage-dependent patterns.

3.4. Effects of PGR Treatments on TSS, TA, and TSS/TA Ratio

Total soluble solids (TSS) increased, while titratable acidity (TA) decreased during berry development, leading to a rising TSS/TA ratio (Figure 4). In 'Kyoho', T1 and T4 significantly increased TSS at the onset of véraison,, with T4 resulting in the highest TSS at the full ripeness stage. TA was significantly reduced by T1 at véraison,, but no significant differences among treatments were observed at the full ripeness stage. All PGR treatments increased the TSS/TA ratio, particularly during the early developmental stages (Figure 4A–C).

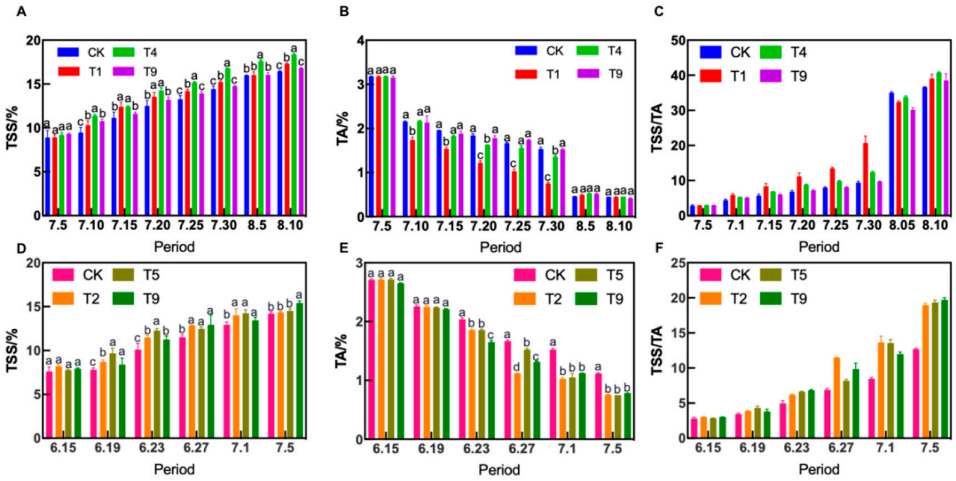


Figure 4. Effects of different PGRs on the TSS, TA and TSS/TA of 'Kyoho' (**A**–**C**) and 'Summer Black' (**D**–**F**) fruits. 'Period' refers to the fruit developmental stages represented by the sampling dates shown in the figures. Values are mean ± SE (n = 3). Different letters indicate significant differences among treatments according to Duncan's multiple range test ($p < 0.05$). CK: control group; T1: 900 mg L^{-1} ABA; T2: 600 mg L^{-1} ABA; T4: 300 mg L^{-1}; 6-BA; T5: 200 mg L^{-1} 6-BA; T9: 50 mg L^{-1} ALA.

In 'Summer Black', T2 and T5 significantly increased TSS during early coloration, whereas T9 showed a more pronounced effect at the full ripeness stage. All PGR treatments significantly reduced TA after June 23, although no significant differences were detected among treatments at the full ripeness stage. The TSS/TA ratio was highest under T2 during the early developmental stages, while differences among treatments diminished at the full ripeness stage (Figure 4D–F). These results indicate that the tested PGR treatment regimes were associated with temporal changes in TSS and TA during berry development, with responses varying between cultivars and developmental stages.

3.5. Effects of PGR Treatments on Soluble Sugar and Organic Acid Contents

In both cultivars, fructose and glucose were the predominant soluble sugars, occurring at an approximately 1:1 ratio, and their concentrations increased progressively during berry development (Figure 5).

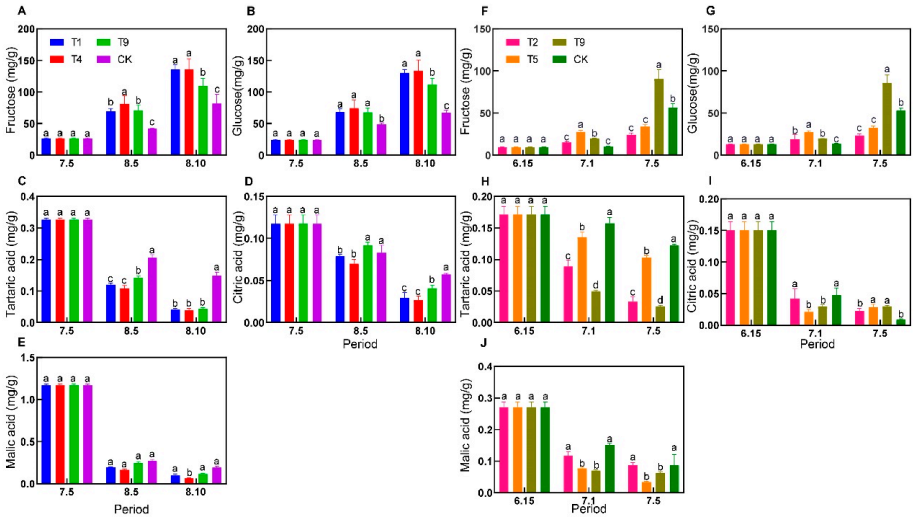


Figure 5. Effects of different PGRs on soluble sugars and organic acid contents of 'Kyoho' (**A**–**E**) and 'Summer Black' (**F**–**J**) fruits. 'Period' refers to the fruit developmental stages represented by the sampling dates shown in the figures. Values are mean ± SE (n = 3). Different letters indicate significant differences among treatments according to Duncan's multiple range ($p < 0.05$). CK: control group; T1: 900 mg L^{-1} ABA; T2: 600 mg L^{-1} ABA; T4: 300 mg L^{-1} 6-BA; T5: 200 mg L^{-1} 6-BA; T9: 50 mg L^{-1} ALA.

In 'Kyoho', all PGR treatments significantly enhanced fructose and glucose accumulation after véraison,, and these effects were maintained through full ripening. Among the treatments, T1 and T4 resulted in the greatest increases in sugar contents, followed by T9 (Figure 5A,B). In 'Summer Black', significant increases in fructose and glucose contents after véraison, were observed mainly under T5 and T9 treatments. Notably, at the full ripeness stage, berries treated with T9 exhibited the highest levels of both fructose and glucose, which were approximately 65% higher than those of CK (Figure 5F,G).

Three major organic acids—malic acid, tartaric acid, and citric acid—were detected in both cultivars, with malic acid being the most abundant. In 'Kyoho', tartaric acid content was significantly reduced by T1, T4, and T9 after the véraison, while citric acid content was significantly decreased under T1 and T4. No significant changes in malic acid content were observed at this stage. At the full ripeness stage, tartaric and citric acid concentrations were further reduced by all PGR treatments, whereas a significant decrease in malic acid was detected only under T4 (Figure 5C–E). In 'Summer Black', organic acid composition showed a stronger response to PGR application. At véraison,, tartaric acid content was significantly reduced under T2, T5, and T9, while T5 and T9 simultaneously decreased both citric and malic acid contents. At the full ripeness stage, tartaric and malic acid concentrations further declined under all PGR treatments. Among them, T9 exhibited the strongest reduction in tartaric acid, whereas T5 showed the greatest decrease in malic acid content. In contrast, citric acid content was higher in all PGR-treated berries than in CK at the full ripeness stage (Figure 5H–J).

These results indicate that the tested PGR treatment regimes affected sugar accumulation and organic acid dynamics in treatment-, cultivar-, and developmental stage-dependent manner, with particularly pronounced responses to 6-BA in 'Kyoho' and to ALA in 'Summer Black'.

3.6. Gene Expression and Correlation Analysis of Soluble Sugar and Acid-Related Genes

PGR treatments altered the expression level of several genes involved in sugar and organic acid metabolism in both cultivars. In 'Kyoho', T1 showed higher mean expression of

VvSUT1, *VvSUT2*, *VvAI* and *VvNI* than CK at full ripening, while T9 showed higher means for these genes at both the véraison and fully ripen. T4 had higher *VvSUT1* and VvNI means at the véraison stage, but lower means for all six sugar-related genes at the full ripeness stage. Acid-related genes showed contrasting responses: T4 and T9 had lower *VvPEPC* and *VvIDH* means but higher *VvMDH* means at both stages. T1 had lower *VvMDH* and higher *VvPEPC* means at both stages, whereas *VvIDH* was slightly lower at the véraison stage and higher at the full ripeness stage (Figure 6A). Across the 21 matched treatment-stage means, *VvAI* was positively correlated with fructose and glucose (r = 0.416 for both; Figure 6B). *VvMDH*, *VvPEPC* and *VvIDH* were positively correlated with malic acid content (r = 0.633, 0.592 and 0.526, respectively; Figure 6C).

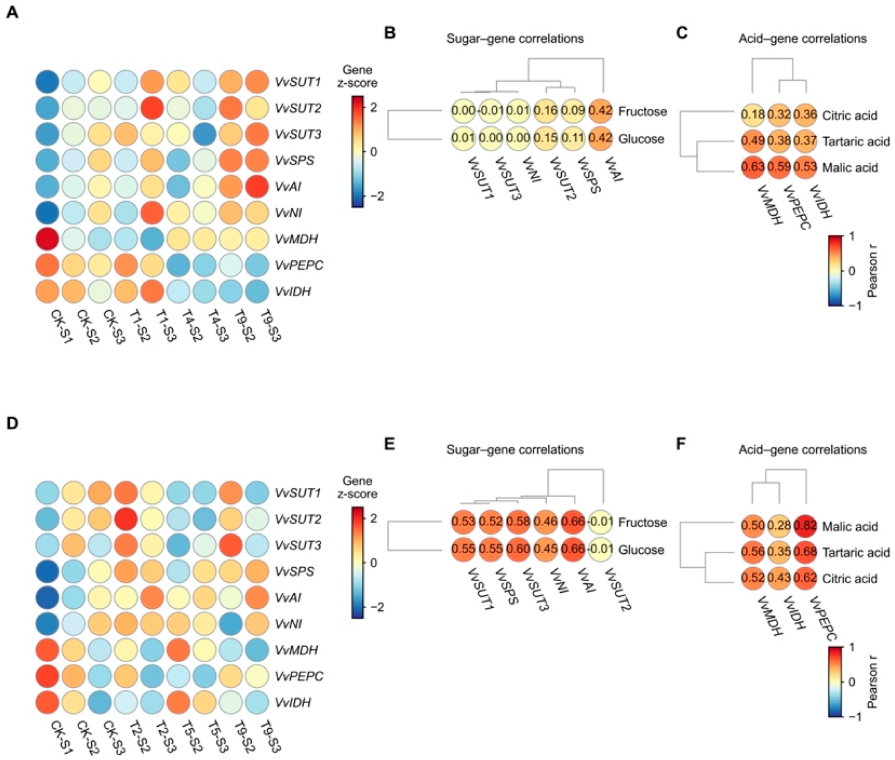


Figure 6. Effects of different PGRs on expression of sugar and organic acid metabolism-related genes and correlation analysis in 'Kyoho' (**A**–**C**) and 'Summer Black' (**D**–**F**) berries. Expression profiles and correlation analyses of sugar- and acid-related genes in Kyoho (**A**–**C**) and Summer Black (**D**–**F**) grape berries. (**A**,**D**) Gene expression profiles across nine treatment-stage conditions; colours indicate within-gene z-scores of $\log_2$-transformed expression means. (**B**,**E**) Correlations between sugar contents and gene expression. (**C**,**F**) Correlations between organic acid contents and gene expression. Correlations were calculated using 21 matched condition means per cultivar, with the shared CK-S1 baseline counted once. Colours and numbers indicate Pearson correlation coefficients (r), presented descriptively. Sampling stages: pre-véraison, (S1), véraison (S2), and fully ripen (S3). CK: control group; T1: 900 mg L^{-1} ABA; T2: 600 mg L^{-1} ABA; T4: 300 mg L^{-1} 6-BA; T5: 200 mg L^{-1} 6-BA; T9: 50 mg L^{-1} ALA.

In 'Summer Black', T2 showed higher *VvSUT1* and *VvSUT2* expression means than CK at the véraison stage but lower means at the full ripeness stage, while VvAI was higher at both stages. T5 and T9 also had higher *VvAI* and *VvSPS* means at both stages, although their VvSUT1 and *VvSUT2* means were lower than CK at the full ripeness stage. Acid-related genes did not show uniform decreases at full ripening: T5 had higher *VvMDH* and *VvIDH* means, T2 had a higher *VvIDH* mean, and T9 had higher *VvPEPC* and *VvIDH* means than CK (Figure 6D). Across the 21 matched conditions, fructose and glucose were positively

correlated with *VvAI* (r = 0.660 and 0.656) and *VvSUT3* (r = 0.579 and 0.598; Figure 6E). *VvPEPC* was positively correlated with malic, tartaric and citric acid contents (r = 0.817, 0.678 and 0.616, respectively; Figure 6F).

These findings indicate that the changes in sugar and organic acid contents associated with PGR treatments were accompanied by gene-specific expression responses, further supporting cultivar-, treatment-, and developmental stage-dependent response patterns.

4. Discussion

4.1. Selected ABA Treatments Were Strongly Associated with Enhanced Berry Coloration

Exogenous application of ABA, 6-BA and ALA significantly promoted berry coloration in both 'Kyoho' and 'Summer Black', confirming that exogenous PGRs effectively accelerate the onset of berry coloration through enhanced anthocyanin accumulation [19,27,29]. However, rather than exhibiting similar physiological effects, the three selected PGR treatment regimes produced distinct response patterns during berry ripening. Among them, the selected ABA treatment regime for each cultivar showed the most pronounced coloration response under the tested conditions, as indicated by earlier increases in CIRG, greater anthocyanin accumulation, and stronger induction of several anthocyanin-related genes. (Figures 1–3).

ABA is widely recognized as a central regulator of ripening in non-climacteric fruits. Previous studies have demonstrated that ABA activates anthocyanin biosynthesis by inducing R2R3-MYB transcription factors, including VvMYBA1/2, which subsequently activate the expression of structural genes such as *UFGT*, *F3′5′H* and *GST*, thereby promoting anthocyanin biosynthesis and vacuolar sequestration [30,31]. Consistent with this regulatory model, ABA markedly increased the expression levels of *VvUFGT* and *VvGST* in both cultivars, while selectively inducing *F3′5′H* in 'Kyoho' and *PAL* and *4CL* in 'Summer Black' indicating cultivar-dependent regulation within the anthocyanin biosynthetic pathway (Figure 3). These transcriptional responses were accompanied by increased accumulation of malvidin- and peonidin-derived anthocyanins. Notably, ABA preferentially promoted methylated anthocyanins such as malvidin-3-O-glucoside, which possess greater chemical stability and contribute to the characteristic dark purple coloration of ripe berries [32,33].

Although the selected ABA concentration differed between cultivars, the ABA treatment selected for each cultivar consistently produced more pronounced berry pigmentation than the selected 6-BA and ALA treatment regimes under the tested conditions. Ethylene signaling may also contribute to grape berry coloration, as ethephon has been reported to promote berry coloration and anthocyanin accumulation [34,35]. Nevertheless, ethephon was not included in the present study, which focused on ABA, 6-BA and ALA. Therefore, the observed changes in anthocyanin-related gene expression should be interpreted as responses associated with PGR treatment rather than direct evidence of transcriptional regulation by these compounds. Interactions between ABA, cytokinin, ALA-related metabolic signaling and ethylene may also contribute to the regulation of berry ripening.

Although ABA effectively promoted berry coloration, its potential effects on leaf senescence and defoliation should also be considered in practical production. These effects were not evaluated in the present study and warrant further investigation, particularly regarding the longer-term effects of ABA application on grapevine growth. Overall, these findings suggest that the selected ABA treatment regime was more strongly associated with the visual component of berry ripening through enhanced anthocyanin biosynthesis, while cultivar-specific responses may be related to differences in genetic background, berry skin characteristics and endogenous hormonal levels.

4.2. Cultivar-Dependent Effects of Selected 6-BA and ALA Treatments on Internal Fruit Quality

In contrast to its dominant effect on berry coloration, ABA showed comparatively limited effects on several internal chemical parameters under the selected treatment regimes, whereas the selected 6-BA and ALA treatment regimes were associated with more pronounced changes in certain internal quality parameters. These contrasting response patterns suggest that different PGR treatment regimes may affect different components of berry quality to varying degrees.

In 'Kyoho', 6-BA was associated with relatively pronounced changes in several internal quality parameters, as indicated by higher TSS, increased TSS/TA ratios and a broader reduction in tartaric, citric and malic acids (Figures 4A–C and 5C–E). These changes corresponded to upregulation of sugar transporters and metabolic genes (*VvSUTs*, *VvAI*, *VvNI*) and downregulation of organic acid-related genes (*VvMDH*, *VvPEPC*, *VvIDH*) (Figure 6A). These observations suggest that the response to the selected 6-BA treatment may have been associated with changes in carbohydrate transport and metabolism and organic acid-related processes. This interpretation is consistent with previous reports describing the roles of cytokinins in assimilate allocation and fruit quality [36–39].

By comparison, the selected ALA treatment was associated with more pronounced changes in several internal quality parameters in 'Summer Black' than those observed under the corresponding ALA treatment in 'Kyoho'. Glucose and fructose contents increased markedly during ripening, accompanied by reduced titratable acidity and suppression of *VvPEPC* expression (Figures 5F,G and 6D–F). As a key precursor of tetrapyrrole biosynthesis, ALA enhances chlorophyll formation and photosynthetic performance, thereby increasing carbon assimilation and assimilate availability [40,41]. Previous studies have also demonstrated that ALA promotes primary metabolism and accelerates ripening, leading to enhanced sugar accumulation and reduced acidity [42]. However, because photosynthetic performance and carbon assimilation were not directly measured in the present study, these processes remain possible explanations rather than demonstrated mechanisms underlying the observed quality responses.

Taken together, the results suggest that the selected ABA treatment produced a more pronounced response in berry coloration, whereas selected 6-BA and ALA showed relatively stronger responses in several parameters associated with internal fruit quality. This observed functional differentiation among PGR treatment regimes provides a physiological basis for considering external coloration and internal quality as related but partially separable quality dimensions in PGR-mediated quality management.

4.3. Differential Responses of Visual and Internal Quality Parameters to PGR Treatments

Our results indicate that visual coloration and internal quality parameters did not respond uniformly to exogenous PGR treatments during grape berry ripening. Although all three PGR treatments promoted aspects of berry ripening, their effects on different components of the ripening process varied in magnitude and timing. Under the selected treatment conditions, ABA treatment was associated with a more pronounced coloration response, whereas changes in soluble sugars and TSS/TA ratios were comparatively moderate at corresponding developmental stages. Thus, the selected ABA treatment produced a more pronounced response in visual coloration than in several measured parameters of internal quality at corresponding developmental stages. This phenomenon is consistent with previous reports showing that anthocyanin biosynthesis, sugar accumulation, and organic acid degradation are temporally coordinated but can be partially uncoupled during grape berry ripening. Such temporal differences in developmental responses may contribute to differences between external coloration and internal quality development [43–45]. Sugar accumulation in grape berries depends on coordinated source–sink relationships,

phloem unloading, sugar transport, and enzymatic conversion processes, involving multiple transport and metabolic steps [46–48]. Therefore, changes in berry coloration and internal quality may exhibit different temporal response patterns because they involve interconnected but distinct metabolic and regulatory processes. In the present study, the selected ABA treatment was more strongly associated with berry coloration, whereas the selected 6-BA and ALA treatments showed relatively stronger effects on selected parameters associated with internal fruit quality. These differential responses indicate that external coloration and internal quality development do not always progress synchronously under exogenous PGR treatment.

From a practical perspective, these findings highlight the importance of selecting PGRs according to specific production objectives rather than seeking a universally superior regulator. Under the tested treatment regimes, the selected ABA treatment was more strongly associated with berry coloration and market appearance, whereas selected 6-BA and ALA treatments produced greater responses in several parameters related to sweetness and sugar–acid balance. Whether combined or sequential applications could improve both external coloration and internal quality remains to be investigated in future studies. However, the practical use of these PGRs should comply with local regulatory requirements, and only products registered and labeled for the intended use should be applied in commercial grape production.

Finally, the different responses observed between 'Kyoho' and 'Summer Black' suggest that cultivar characteristics may influence responses to PGR treatments. However, because the selected ABA and 6-BA concentrations differed between cultivars, the present results cannot fully separate cultivar effects from dose-related effects. Furthermore, the treatment regimes were selected on the basis of coloration responses, and no additional independent validation experiment using a separate set of biological materials was conducted. These factors limit the extent to which the observed response patterns can be generalized beyond the specific conditions examined here. Future studies should therefore use independently designed concentration series, include additional biological materials and growing seasons, and evaluate multiple grape cultivars to distinguish the effects of PGR identity, concentration and cultivar. Although the present study was conducted using three-year-old vines in a single growing season, and endogenous hormone and ethylene levels were not measured, future studies using mature vines, multiple seasons, and integrated transcriptomic, metabolomic and hormone analyses will help validate the reproducibility of these responses and investigate potential crosstalk among ABA-, cytokinin-, ethylene-, and ALA-related signaling pathways during grape berry ripening.

5. Conclusions

In conclusion, this study showed that ABA, 6-BA and ALA produced distinct response patterns in berry coloration and internal quality under the selected treatment regimes during table grape ripening. The selected ABA treatments produced more pronounced responses in berry coloration and anthocyanin accumulation, accompanied by stronger induction of key anthocyanin-related genes, particularly *VvUFGT* and *VvGST*. In contrast, selected 6-BA and ALA treatments produced stronger responses in several internal quality parameters, including soluble sugar accumulation, TSS/TA ratio, and organic acid metabolism, with more pronounced responses to 6-BA in 'Kyoho' and to ALA in 'Summer Black'. These findings characterize the responses of the particular treatment regimes examined rather than establishing universal, regulator-specific functions in controlling external and internal fruit quality.

These findings indicate that the measured components of berry coloration and internal quality responded to the selected PGR treatment regimes to different degrees. The observed

response patterns varied with the treatment regime and cultivar, although the effects of treatment concentration could not be fully separated from cultivar effects. Rather than identifying a universally superior regulator, this study highlights the importance of considering PGR type, concentration and cultivar when targeting specific quality parameters.

From an applied perspective, the complementary response patterns observed for different PGR treatment regimes provide opportunities for targeted ripening management in table grape production. Combined or sequential application of different PGRs may represent a potential strategy to better coordinate external coloration and internal quality development, thereby improving fruit commercial value and consumer acceptance. However, whether such combinations produce additive, synergistic, or antagonistic effects remains to be determined experimentally. Future studies should further evaluate different PGR concentrations across multiple grape cultivars and growing seasons. Integrated molecular, metabolic, and hormone analyses may also help clarify the interactions between exogenous PGR treatments and endogenous hormonal pathways, and further elucidate the mechanisms underlying the differential and coordinated responses of berry coloration and internal quality development during grape ripening.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae12111433/s1. Figure S1. Effects of different PGR treatments on coloration and firmness of 'Kyoho' (A, C) and 'Summer Black' (B, D) berries. Table S1: Plant growth regulator treatments and concentrations used in the experiment. Table S2: Primers used for quantitative real-time PCR analysis. Table S3: Effects of different PGR treatments on anthocyanin composition in grape skins of 'Kyoho'. Table S4: Effects of different PGR treatments on anthocyanin composition in grape skins of 'Summer Black'. Table S5: The relative expression value of anthocyanin biosynthesis genes in 'Kyoho'. Table S6: The relative expression value of anthocyanin biosynthesis genes in 'Summer black'.

Author Contributions: Conceptualization: X.F., L.W. and L.S.; Methodology: X.F. and L.W.; Investigation: X.F., L.W. and M.T.; Data curation: X.F. and L.W.; Formal analysis: X.F., L.W., W.C. and M.T.; Validation: X.F. and L.W.; Writing—original draft: X.F.; Writing—review and editing: X.F., M.T., J.F. and L.S. All authors have read and agreed to the published version of the manuscript.

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