

Article

Spatiotemporal Differences of 24-Epibrassinolide Regulating Anthocyanin and Proanthocyanidin Biosynthesis in *Vitis vinifera* ‘Cabernet Sauvignon’

Dandan Li ¹, Hao Chen ¹, Kenan Zhang ¹, Chan Li ¹, Hanmei Su ¹, Mengyao Han ¹  and Zhumei Xi ^{1,2,*}

¹ College of Enology, Northwest A&F University, Yangling 712100, China; lidandan2018@nwafu.edu.cn (D.L.); haochen9805@nwafu.edu.cn (H.C.); kenanzhang@nwafu.edu.cn (K.Z.); chanli987@163.com (C.L.); suuu@nwafu.edu.cn (H.S.); hanmengyao970905@163.com (M.H.)

² Shaanxi Engineering Research Center for Viti-Viniculture, Yangling 712100, China

* Correspondence: xizhumei@nwafu.edu.cn; Tel.: +86-029-8709-2107

Abstract

Brassinosteroids are recognized regulators of anthocyanin and proanthocyanidin biosynthesis in grapevine; however, their spatiotemporal effects remain insufficiently characterized. This study examined the stage-specific impacts of exogenous 24-epibrassinolide and brassinazole on these phenolic compounds in Cabernet Sauvignon. Treatments were applied at fruit set and veraison, with skin and seed tissues collected across six developmental stages. Berry ripening and quality parameters were evaluated, and phenolic profiles were quantified via HPLC. The results revealed that both 24-epibrassinolide and brassinazole significantly influenced grape maturation and phenolic biosynthesis in a timing-dependent manner. Specifically, 24-epibrassinolide application at fruit set increased the content of proanthocyanidins and trihydroxylated subunits, as well as the galloylation percentage, in both skins and seeds, while also altering their composition and subunit architecture. In contrast, veraison-stage treatment reduced these parameters in seeds but promoted them in skins, highlighting a tissue-specific response within the same developmental window. Moreover, compared with fruit-set treatment, 24-epibrassinolide application at veraison more significantly enhanced total anthocyanin content in skins, predominantly through the accumulation of specific monomeric forms. Together, based on two consecutive growing seasons (2022–2023) in *Vitis vinifera* L. cv. Cabernet Sauvignon, these findings demonstrate that brassinosteroid regulation of anthocyanin and proanthocyanidin biosynthesis in grape berries depends not only on the tissue but also on the phenological stage of application. In conclusion, this study reveals distinct spatiotemporal patterns in the regulation of phenolic biosynthesis by 24-epibrassinolide in grapevine, providing new insights into the hormone-mediated modulation of secondary metabolism and suggesting a potential agronomic strategy for precisely shaping the phenolic profile of wine grapes through stage-targeted brassinosteroid application.



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Keywords: phytohormone; brassinosteroid; phenolic composition; flavonoid accumulation; stage-specificity; tissue-specificity; grape; fruit quality

1. Introduction

Grapevine (*Vitis vinifera* L.) is a cornerstone of global viticulture, with its berries constituting the essential raw material for wine production. Among red wine cultivars, Cabernet Sauvignon is one of the most widely cultivated and economically important worldwide,

renowned for yielding wines with robust structure and aging potential. The quality of red wine is fundamentally determined by the composition of flavonoid compounds in the berry skin, primarily anthocyanins and proanthocyanidins (PAs, also known as condensed tannins) [1]. Anthocyanins are the pigments responsible for red wine color, whose final intensity and stability are governed by their concentration and specific profile, such as the proportion of methoxylated derivatives [2,3]. Proanthocyanidins, which are polymers of flavan-3-ol subunits, critically influence wine mouthfeel by imparting bitterness and astringency [4–6]. They also contribute to long-term wine structure through interactions with anthocyanins and proteins [7]. The biosynthesis of these phenolic compounds is subject to strict spatiotemporal regulation during berry development. PAs accumulate predominantly in seeds and skins during the early green phase, peaking around veraison [8,9]. In contrast, anthocyanin synthesis is specifically activated in the skin layers after veraison, a process synchronized with berry softening and soluble solids accumulation [8,10,11]. In addition to their enological significance, anthocyanins and proanthocyanidins are recognized for their potential health benefits, including antioxidant, anti-inflammatory, and cardioprotective activities, which have attracted increasing interest from both consumers and the scientific community [12–14].

Brassinosteroids (BRs) are a class of natural steroid hormones that are essential for multiple aspects of plant growth and development [15–17]. First identified in rape pollen (*Brassica napus* L.), they are now known to be ubiquitous in higher plants, occurring in pollen, seeds, stems, leaves, fruits, and roots [18–20]. Recognized for their potent regulatory activity at very low concentrations, BRs were formally classified as the sixth group of plant hormones in 1998, following auxins, gibberellins, cytokinins, ethylene, and abscisic acid [17]. BRs participate in a wide range of critical physiological processes, including photomorphogenesis, skotomorphogenesis, and thermomorphogenesis [15,21]. They also enhance plant resilience to various biotic and abiotic stresses, such as chilling, heat, drought, and pathogen attack [15,22,23]. Furthermore, BRs promote cell elongation and division, thereby driving the development of plant organs—from seed germination and pollen tube growth to fruit ripening [21,24]. These attributes have led to their widespread use as plant growth regulators aimed at improving stress tolerance and accelerating fruit maturation. Exogenous application of BRs has been shown to promote ripening and coloration in several fruit crops, including tomato (*Solanum lycopersicum* L.) [25,26], mango (*Mangifera indica*) [27], banana (*Musa acuminata* L.) [28,29], persimmon (*Diospyros kaki* L.) [30], apple (*Malus × domestica* Borkh.) [31] and strawberry (*Fragaria × ananassa*) [32]. In grapevine (*Vitis vinifera* L.), endogenous BR levels rise markedly at the onset of ripening (veraison) [33,34]. Consistent with this, treatment with 24-epibrassinolide (EBR) at veraison accelerates berry ripening and elevates the skin content of anthocyanins and other phenolic compounds [35,36]. Conversely, application of brassinazole (BRZ), a specific BR biosynthesis inhibitor, delays grape maturation and reduces coloring rates [37,38]. These findings collectively indicate that BR signaling plays a key role in regulating the accumulation of anthocyanins and proanthocyanidins during grape berry development.

Several critical knowledge gaps remain regarding the spatiotemporal regulation of phenolic biosynthesis by BRs in grape berries. Previous studies have demonstrated that applying EBR at veraison enhances anthocyanin accumulation and modulates its monomeric profile in berry skins [39], while single application at fruit set or double application (at fruit set and 14 days before veraison) promotes proanthocyanidin biosynthesis and influences its structural characteristics in both skins and seeds [37]. However, these investigations were confined to either single treatment timings or the cumulative effect of two applications, without systematically comparing the effects of EBR applied at distinct single phenological stages (fruit set vs. veraison) within a unified experimental framework. Accordingly, this study aimed to address these gaps by comprehensively investigating the stage- and

tissue-specific effects of exogenous EBR and its inhibitor BRZ on the accumulation, composition, and structural features of anthocyanins and proanthocyanidins in *Vitis vinifera* L. cv. Cabernet Sauvignon. This work also provides a foundation for future research aimed at elucidating the molecular mechanisms underlying BR-mediated regulation of phenolic biosynthesis in grape berries.

Specifically, this study aims to address the following three key questions: (1) Stage-specific effects: How does the application timing of EBR (fruit set vs. veraison) differentially affect the biosynthesis of anthocyanins (in only the fruit peel) and proanthocyanidins (in both the fruit peel and seeds)? (2) Tissue-specific regulation: Are there any differences in the regulation of proanthocyanins in the fruit peel and seeds? (3) Optimal application window: Based on the above temporal and spatial differences, what is the most effective timing for EBR application to achieve targeted modulation of the phenolic profile under practical vineyard conditions?

Elucidating how BRs regulate anthocyanin and proanthocyanidin biosynthesis in grape berries is crucial for advancing the control of ripening, coloration, and ultimately wine quality. In large-scale production, uniform application precisely at veraison is often logistically challenging due to operational constraints and variable environmental factors. Moreover, accurately identifying the onset of veraison is inherently difficult, as this critical transition occurs non-synchronously across individual berries and varies with cultivar. Determining the most effective phenological window for EBR application is therefore essential to maximize anthocyanin and proanthocyanidin accumulation in Cabernet Sauvignon and to develop robust, vineyard-ready strategies for improving grape phenolic quality.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

The experiment was conducted in the Rixin Agricultural Vineyard, Jingyang County, Shaanxi Province, China (34°53' N, 108°84' E, altitude 600 m), from 2022 to 2023. The vines used were own-rooted Cabernet Sauvignon (*Vitis vinifera* L.), planted in 2010 and trained to a single-trunk, two-arm cordon system with north–south row orientation and spacing of 1.0 m × 3.0 m. Based on key phenological stages of grape development, two time points were selected for treatment application: the fruit-setting stage (E-L 27) and veraison (E-L 35). The following treatments were applied: (1) 0.40 mg/L 24-epibrassinolide (EBR); (2) 1.31 mg/L brassinazole (BRZ), a BR biosynthesis inhibitor; and (3) distilled water as control (CK). Berry samples were collected at six developmental stages: E-L 27 (fruit set), E-L 31 (pea size), E-L 35 (onset of veraison), E-L 36 (mid-veraison), E-L 37 (late veraison), and E-L 38 (harvest maturity). The first sampling was conducted 24 h after treatments, with subsequent samplings performed at key phenological stages thereafter. Harvest (E-L 38) occurred on 7 September 2022 and 21 September 2023. Skins and seeds were manually separated from fresh berries, immediately frozen in liquid nitrogen, and stored at −80 °C until analysis. Three biological replicates were prepared for each sample.

2.2. Reagents and Standards

Analytical-grade ethanol, sodium acetate, NaOH, KCl, HCl and Tween-80 were obtained from a local commercial supplier. HPLC-grade acetonitrile, acetone, methanol, and formic acid were purchased from Thermo Fisher Scientific Inc. (Fairlawn, NJ, USA). Ascorbic acid and phloroglucinol were supplied by Sigma-Aldrich (Steinheim, Germany). Ultrapure water was prepared using a Milli-Q purification system (Millipore, Billerica, MA, USA). 24-Epibrassinolide was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China), and brassinazole was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). For anthocyanin and proanthocyanidin quantification, malvidin-3-

O-glucoside and epicatechin standards were sourced from Sigma-Aldrich LLC (St. Louis, MO, USA).

2.3. Measurement of Fruit Traits and Color Parameters

At each sampling point, a random set of 100 berries was selected for the measurement of basic fruit traits. The longitudinal and equatorial diameters were determined using a vernier caliper, and the 100-berry weight was recorded with an analytical balance. The grape shape index was calculated as the ratio of longitudinal diameter to equatorial diameter. Total soluble solids (TSS) content was measured with a handheld refractometer, while titratable acidity (TA) was quantified by titration with NaOH. Berry color characteristics were assessed using a CM-5 spectrophotometer (Konica Minolta, Inc., Tokyo, Japan). Thirty berries were randomly selected, and each berry was measured at three different positions on its surface. The CIELAB color parameters were recorded, including L^* (lightness), a^* (red–green), b^* (yellow–blue), hue angle (h°) and chroma (C^*). Finally, the Color Index of Red Grape (CIRG) was calculated using the following formula [40]:

$$\text{CIRG} = (180 - h^\circ) / (L^* + C^*) \quad (1)$$

2.4. Extraction and HPLC Analysis of Anthocyanins

Anthocyanin monomers in the grape skin extracts were analyzed by high-performance liquid chromatography (HPLC) [41,42]. The extract was filtered through a 0.45 μm PTFE syringe filter (Thermo Scientific, #44513-NP, Waltham, MA, USA), and 1.0 mL of the filtrate was transferred into a 1.5 mL amber HPLC vial for analysis. Anthocyanin monomers were separated and quantified using a Shimadzu LC-20AT HPLC system equipped with a photodiode array detector (PAD; Shimadzu, Kyoto, Japan) and an Agilent TC-C18(2) column ($250 \times 4.6 \text{ mm}$, 5 μm ; Agilent, Santa Clara, CA, USA). The mobile phase consisted of (A) formic acid/acetonitrile/water (1:1:8, $v/v/v$) and (B) formic acid/acetonitrile/water (5:10:8, $v/v/v$). Separation was achieved with the following gradient program at a flow rate of 1.0 mL/min: 0–15 min, 0–15% B; 15–30 min, 15–25% B; 30–45 min, 25–40% B; 45–46 min, 40–100% B; 46–50 min, 100% B; 50–51 min, 100–0% B, followed by 9 min re-equilibration at 0% B. The column temperature was maintained at 30 $^\circ\text{C}$, detection was performed at 520 nm, and the injection volume was 20 μL . The calibration curve for malvidin-3-O-glucoside demonstrated excellent linearity over the concentration range of 1–500 mg/L, with a regression equation of $y = 0.0613x + 0.7084$ and a correlation coefficient (R^2) of 0.9993.

As a typical *Vitis vinifera* cultivar, Cabernet Sauvignon predominantly accumulates anthocyanins as 3-O-monoglucosides and their acylated derivatives, in contrast to certain hybrid varieties where 3,5-O-diglucosides may also be present [43,44]. Accordingly, the present study focused on the nine major 3-O-monoglucoside anthocyanins. Anthocyanin monomers were quantified relative to an external standard of malvidin-3-O-glucoside, with results expressed as malvidin-3-O-glucoside equivalents [41,42]. Each sample was analyzed in triplicate.

2.5. Extraction and HPLC Analysis of Proanthocyanidins

Proanthocyanidins were extracted from 1.0 g of dry powder in a 150 mL Erlenmeyer flask using 10 mL of an acetone/water solution (2:1, v/v). The flask was purged with nitrogen, sealed, and placed in a thermostatic shaker for 12 h at low speed. The mixture was then transferred to 50 mL centrifuge tubes and centrifuged at $5000 \times g$ for 10 min at room temperature. The supernatant was collected, and the pellet was re-extracted twice with 10 mL of the same acetone/water solution. All steps were performed under light-protected conditions. The combined supernatants were concentrated using a rotary evaporator at 34 $^\circ\text{C}$ and then dissolved in 3 mL of methanol for subsequent phloroglucinol

derivatization. For derivatization, 200 μ L of the skin or seed extract was mixed with 200 μ L of phloroglucinol reagent (containing 12 M HCl, 100 g/L phloroglucinol, and 20 g/L ascorbic acid in methanol) and incubated at 50 °C for 20 min. The reaction was terminated by adding 1000 μ L of 40 mM sodium acetate solution. The mixture was filtered through a 0.45 μ m PTFE syringe filter, and 0.5 mL of the filtrate was transferred into a 1.5 mL amber HPLC vial for analysis. Proanthocyanidin derivatives were separated using a Shimadzu LC-20AT HPLC system equipped with a photodiode array detector (PAD) and two serially connected Chromolith RP-18e columns (100 $\times$ 4.6 mm, 2 μ m; Millipore, Burlington, MA, USA). The mobile phases consisted of (A) glacial acetic acid/water (1:99, *v/v*) and (B) glacial acetic acid/acetonitrile (1:99, *v/v*). The following gradient was applied at a flow rate of 1.0 mL/min: 0–4 min, 3% B; 4–14 min, 3–18% B; 14–16 min, 18–80% B; 16–18 min, 80–3% B, followed by 5 min re-equilibration at 3% B. The column temperature was maintained at 30 °C, and detection was performed at 280 nm with an injection volume of 20 μ L. Quantification was based on an external calibration curve of epicatechin, and results were expressed as (-)-epicatechin equivalents [9,45]. All samples were analyzed in triplicate.

2.6. Statistical Analysis

All statistical analyses were conducted using Microsoft Excel (Microsoft, Redmond, WA, USA) and IBM SPSS Statistics 26 (IBM, Armonk, NY, USA). Graphs were plotted with OriginPro2026 (OriginLab, Northampton, MA, USA). Statistical analyses were performed using one-way analysis of variance (ANOVA) to compare differences among different treatments (EBR, BRZ, and control) within the same phenological stage (fruit set or veraison), followed by Duncan's multiple range test for post hoc comparisons when ANOVA indicated significant differences ($p < 0.05$). Independent-samples *t*-tests were employed to evaluate differences for the same treatment between the two application stages (fruit set vs. veraison). Significance was defined at $p < 0.05$. Data are presented as mean $\pm$ standard error (SE) of three biological replicates.

3. Results

3.1. Fruit Traits

This study examined the effects of EBR and BRZ application timing on the growth and development of grape berries. In *Vitis vinifera* cv. Cabernet Sauvignon, changes in berry weight and size were evaluated following EBR and BRZ treatments administered at fruit set and veraison. As shown in Figures 1–4, 100-berry weight and vertical and horizontal diameters increased progressively from fruit set through veraison until full maturity. EBR-F (EBR application at fruit set) significantly increased 100-berry weight at E-L 31 by 34.07% and BRZ-F (BRZ application at fruit set) decreased it by 26.42% in 2022 (Figure 1A). Similarly in 2023 (Figure 1B), EBR-F observably increased 100-berry weight at E-L 31 by 15.77%. Both EBR-F and EBR-V (EBR application at veraison) remarkably enhanced 100-berry weight at maturity (E-L 38), with increases of 5.98% and 6.20% in 2022 (Figure 1A), as well 5.89% and 6.78% in 2023 (Figure 1B); no significant difference was observed between the two application timings.

Vertical and horizontal diameters of grapes at E-L 31 were signally enlarged by 13.63% under EBR-F and reduced by 11.89% under BRZ-F (Figures 2 and 3). On the contrary, EBR-V and BRZ-V (BRZ application at veraison) had less observable effects on berry size during grape ripening. Obviously, berry size of grapes was affected by the timing of the EBR application, especially at fruit set. Moreover, grape shape index was significantly influenced by EBR-F treatment in both years, whereas EBR-V treatment showed no notable

effect (Figure 4). This further indicated that the timing of BR application is critical for grape fruit growth and development.

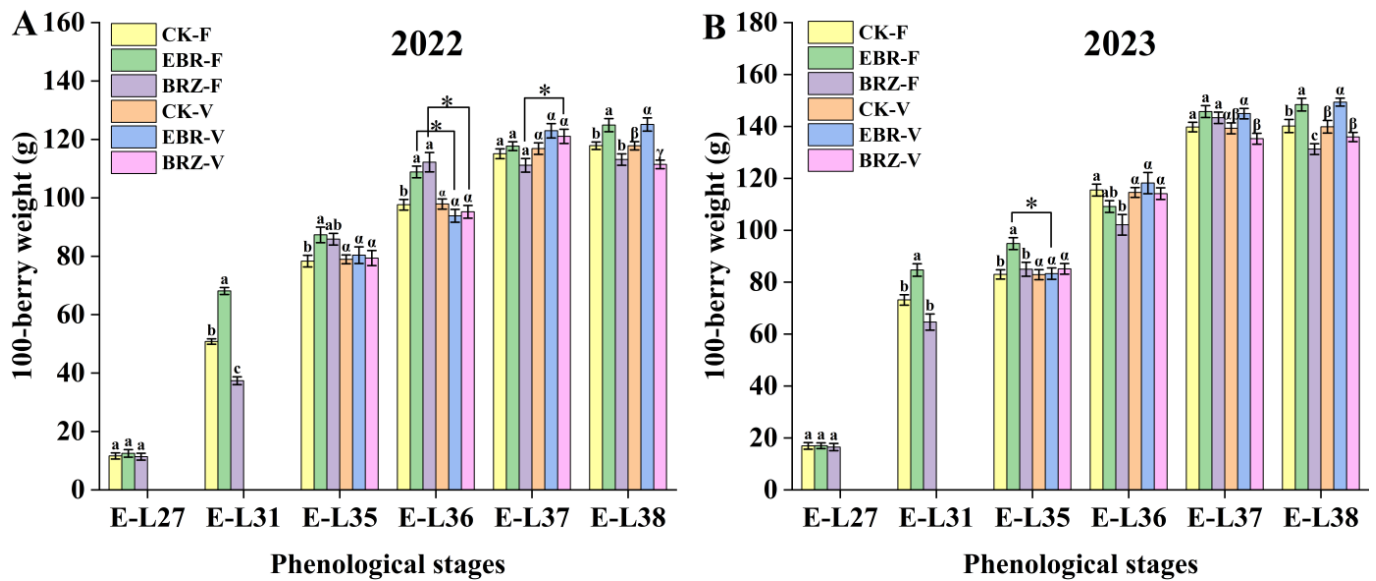


Figure 1. Changes in 100-berry weight of grapes under different treatments in 2022 (A) and 2023 (B). Lowercase letters indicate significant differences among treatments at fruit set, and Greek letters at veraison ($p < 0.05$). Asterisks (*) mark significant differences for the same treatment between stages ($p < 0.05$). Significance was determined by ANOVA and independent-samples t -tests.

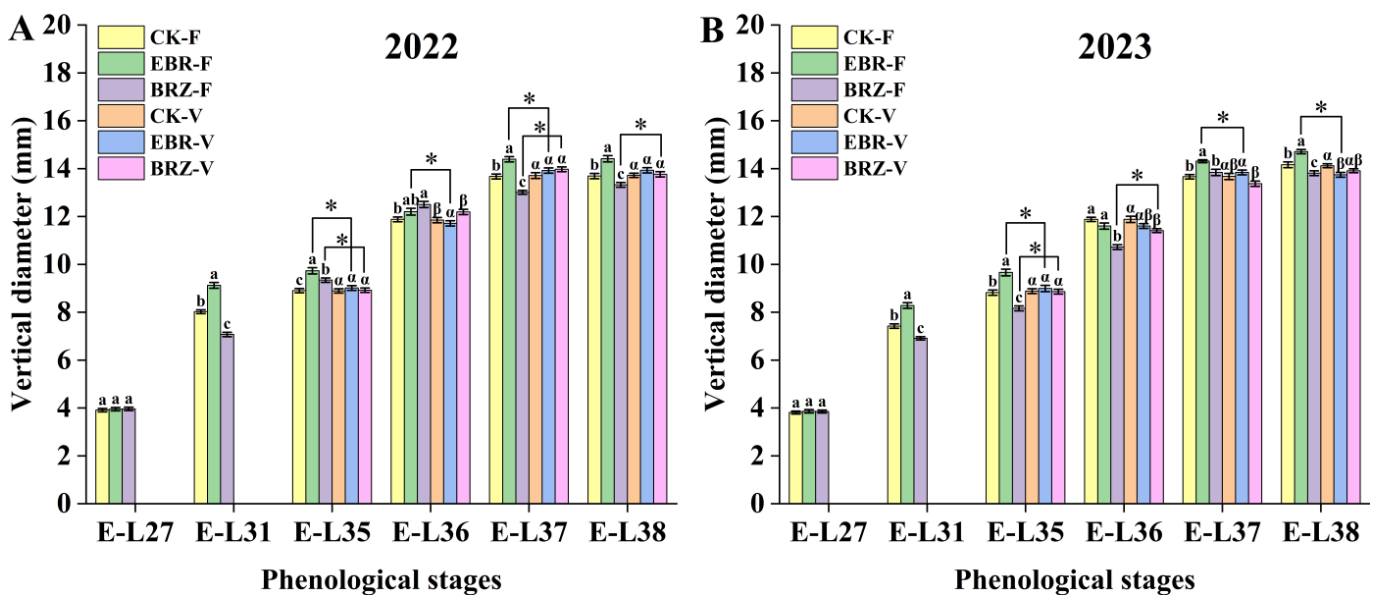


Figure 2. Changes in vertical diameter of grapes under different treatments in 2022 (A) and 2023 (B). Lowercase letters indicate significant differences among treatments at fruit set, and Greek letters at veraison ($p < 0.05$). Asterisks (*) mark significant differences for the same treatment between stages ($p < 0.05$). Significance was determined by ANOVA and independent-samples t -tests.

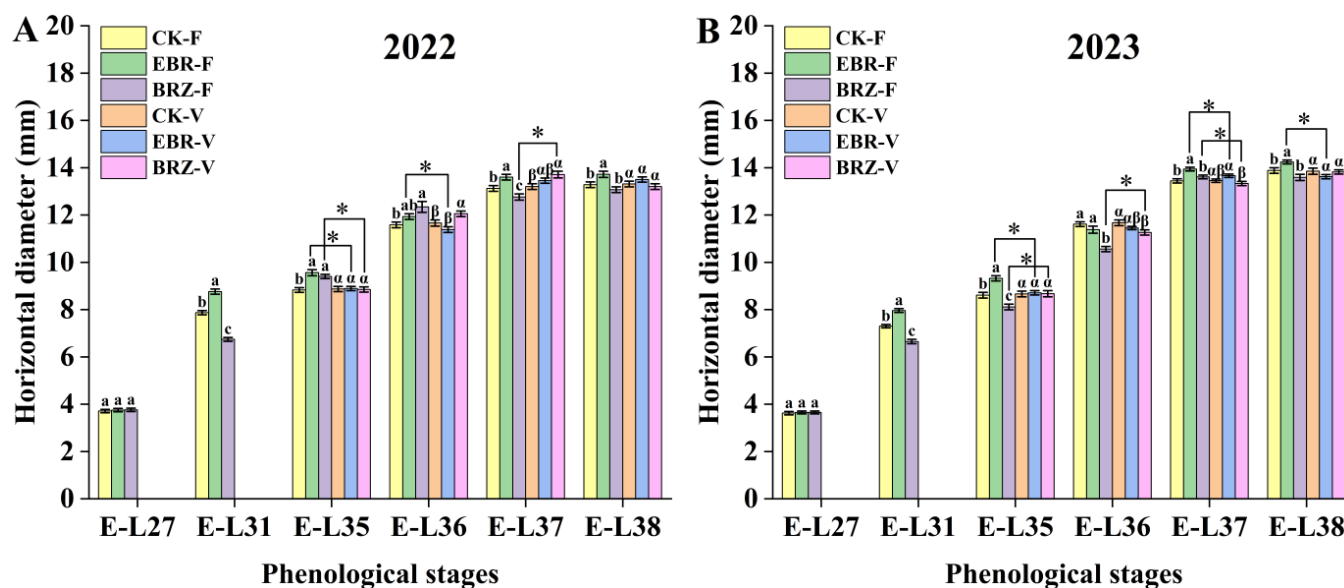


Figure 3. Changes in horizontal diameter of grapes under different treatments in 2022 (A) and 2023 (B). Lowercase letters indicate significant differences among treatments at fruit set, and Greek letters at veraison ($p < 0.05$). Asterisks (*) mark significant differences for the same treatment between stages ($p < 0.05$). Significance was determined by ANOVA and independent-samples *t*-tests.

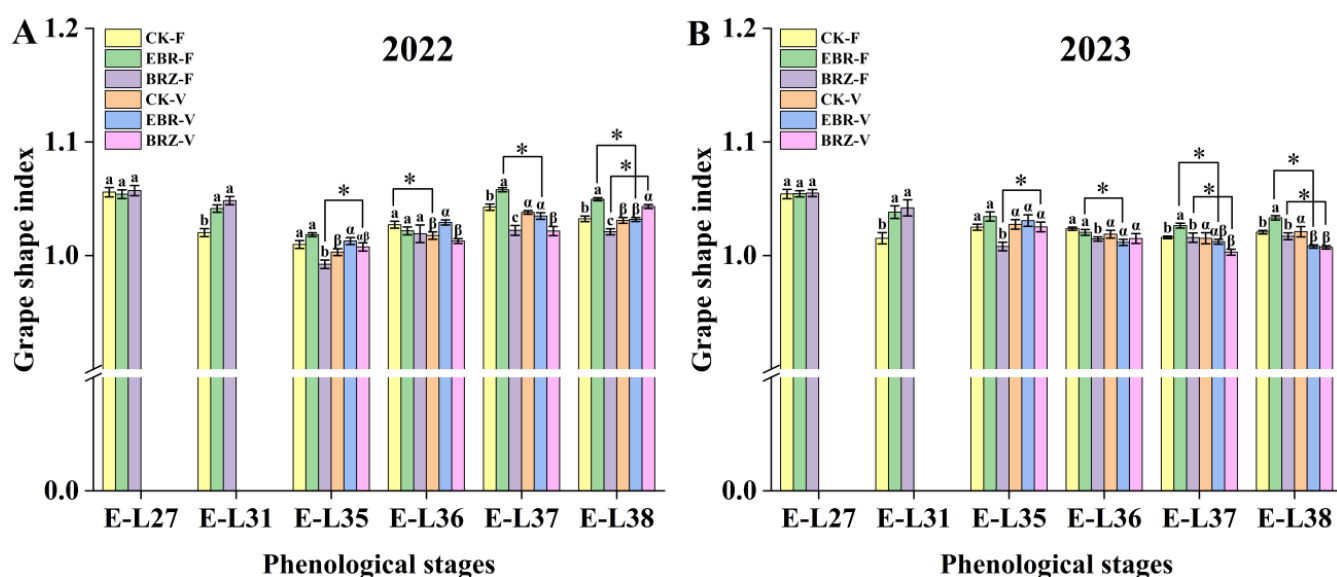


Figure 4. Changes in shape index of grapes under different treatments in 2022 (A) and 2023 (B). Lowercase letters indicate significant differences among treatments at fruit set, and Greek letters at veraison ($p < 0.05$). Asterisks (*) mark significant differences for the same treatment between stages ($p < 0.05$). Significance was determined by ANOVA and independent-samples *t*-tests.

3.2. Color Parameters

Color is also one of the important evaluation indicators for the quality of grapes [3]. As shown in Tables 1 and 2, compared with the control at fruit set (CK-F), EBR-F treatment significantly reduced the luminance of grape berries at fruit set, but the differences gradually disappeared as fruit developed in 2023. However, EBR-V significantly decreased L^* value of grapes at harvest, and the opposite results of BRZ-V further confirmed this point. Except for E-L 36 in 2023, EBR-F and BRZ-F treatments did not have significant impacts on the a^* value at any time. However, EBR-V significantly increased the a^* value of grapes at E-L 37 in 2023. At the last maturity, the a^* value in EBR-V was higher than that in CK-V, but

no significant difference was observed. At E-L 31, grapes treated by EBR-F exhibited lower b^* and C^* values compared to CK-F in both years. EBR-F significantly increased the h° value of grapes at E-L 36 compared to CK-F. From the beginning of veraison to the middle of veraison (E-L 35 and E-L 36), the stage of grape color change dramatically; the h° value of grape under BRZ-F was obviously higher than that under BRZ-V. CIRG is calculated based on the above parameters. Ultimately, EBR-F treatment significantly decreased the CIRG of grape at E-L 36 in both years, yet the significant differences gradually disappear as grape mature. However, EBR-V obviously enhanced the CIRG of grape at harvest.

Table 1. Chromatic characteristic parameters of grape under different treatments in 2022.

Index	Treatments	E-L27	E-L31	E-L35	E-L36	E-L37	E-L38
L^*	CK-F	51.41 ± 0.30 a	47.32 ± 0.44 a	43.49 ± 0.42 a	32.90 ± 1.53 a	26.06 ± 0.64 ab	24.74 ± 0.38 a
	EBR-F	48.42 ± 0.38 b	46.97 ± 0.47 a	41.82 ± 1.18 ab	29.01 ± 0.81 b	24.82 ± 0.85 b	23.56 ± 0.40 b
	BRZ-F	51.25 ± 0.49 a	46.54 ± 0.44 a	38.85 ± 1.49 b	35.33 ± 1.58 a	28.14 ± 1.50 a	25.35 ± 0.34 a
	CK-V			43.00 ± 1.120 α	32.84 ± 1.51 β	26.56 ± 1.12 α	24.95 ± 0.36 β
	EBR-V			43.23 ± 0.78 α	29.70 ± 1.38 β	23.74 ± 0.39 β	21.97 ± 0.31 γ^*
	BRZ-V			43.32 ± 0.83 α^*	37.61 ± 1.67 α	27.59 ± 1.13 α	26.70 ± 0.42 α^*
a^*	CK-F	−1.46 ± 0.19 a	−1.31 ± 0.12 a	−0.58 ± 0.23 a	2.69 ± 0.70 a	4.90 ± 0.49 a	4.91 ± 0.31 a
	EBR-F	−1.49 ± 0.13 a	−1.36 ± 0.12 a	−0.89 ± 0.36 a	1.98 ± 0.46 a	5.41 ± 0.46 a	5.23 ± 0.36 a
	BRZ-F	−1.47 ± 0.11 a	−1.25 ± 0.11 a	−0.57 ± 0.67 a	2.87 ± 0.66 a	5.02 ± 0.47 a	5.44 ± 0.34 a
	CK-V			−0.50 ± 0.53 α	2.68 ± 0.63 $\alpha\beta$	5.05 ± 0.58 α	4.98 ± 0.52 $\alpha\beta$
	EBR-V			−0.59 ± 0.48 α	3.89 ± 0.50 α^*	5.77 ± 0.43 α	6.02 ± 0.41 α
	BRZ-V			−0.63 ± 0.28 α^*	2.01 ± 0.36 β	4.40 ± 0.44 α	4.37 ± 0.20 β^*
b^*	CK-F	29.02 ± 0.35 a	24.06 ± 0.35 a	21.07 ± 0.46 a	8.67 ± 1.61 a	3.02 ± 0.69 a	1.04 ± 0.30 a
	EBR-F	27.89 ± 0.39 a	22.37 ± 0.37 b	17.03 ± 1.06 b	4.02 ± 0.86 b	3.06 ± 0.90 a	0.75 ± 0.15 a
	BRZ-F	28.73 ± 0.45 a	23.87 ± 0.40 a	12.73 ± 1.50 c	11.46 ± 1.62 a	5.03 ± 1.44 a	1.39 ± 0.29 a
	CK-V			21.04 ± 0.56 α	8.68 ± 1.63 β	3.03 ± 0.58 α	1.09 ± 0.25 α
	EBR-V			21.47 ± 0.83 α^*	7.09 ± 1.43 β	0.72 ± 0.19 β^*	0.85 ± 0.22 α
	BRZ-V			21.20 ± 0.89 α^*	13.42 ± 1.76 α	4.40 ± 1.09 α	1.48 ± 0.29 α
h°	CK-F	−1.31 ± 0.14 a	−1.52 ± 0 a	−0.60 ± 0.26 a	−0.09 ± 0.18 b	0.49 ± 0.04 a	0.20 ± 0.05 a
	EBR-F	−1.52 ± 0 a	−1.51 ± 0.01 a	−0.86 ± 0.21 a	0.48 ± 0.14 a	0.26 ± 0.08 b	0.12 ± 0.02 a
	BRZ-F	−1.52 ± 0 a	−1.52 ± 0 a	0.09 ± 0.24 b	−0.05 ± 0.21 b	0.41 ± 0.10 ab	0.23 ± 0.05 a
	CK-V			−0.93 ± 0.21 α	0.25 ± 0.19 β	0.35 ± 0.08 α	0.15 ± 0.02 β
	EBR-V			−0.53 ± 0.25 α	0.26 ± 0.16 β	0.09 ± 0.03 β^*	0.11 ± 0.03 β
	BRZ-V			−0.40 ± 0.27 α^*	0.85 ± 0.18 α^*	0.51 ± 0.10 α	0.31 ± 0.04 α
C^*	CK-F	29.08 ± 0.34 a	24.10 ± 0.35 a	21.12 ± 0.47 a	10.75 ± 1.39 a	6.09 ± 0.76 a	5.25 ± 0.32 a
	EBR-F	27.94 ± 0.39 a	22.42 ± 0.38 b	17.29 ± 0.98 b	5.36 ± 0.81 b	6.96 ± 0.83 a	5.31 ± 0.37 a
	BRZ-F	28.77 ± 0.45 a	23.91 ± 0.40 a	13.32 ± 1.48 c	13.46 ± 1.28 a	8.59 ± 1.22 a	5.83 ± 0.34 a
	CK-V			21.26 ± 0.53 α	10.22 ± 1.52 β	6.45 ± 0.66 α	5.15 ± 0.56 $\alpha\beta$
	EBR-V			21.72 ± 0.75 α^*	9.57 ± 1.18 β^*	5.86 ± 0.45 α	6.16 ± 0.43 α
	BRZ-V			21.29 ± 0.86 α^*	14.68 ± 1.46 α	7.23 ± 0.95 α	4.81 ± 0.25 β^*
CIRG	CK-F	2.26 ± 0.02 a	2.55 ± 0.03 a	2.81 ± 0.04 b	4.69 ± 0.30 b	5.81 ± 0.19 a	6.06 ± 0.11 ab
	EBR-F	2.38 ± 0.02 a	2.63 ± 0.03 a	3.26 ± 0.19 b	5.48 ± 0.19 a	5.97 ± 0.22 a	6.31 ± 0.13 a
	BRZ-F	2.28 ± 0.02 a	2.58 ± 0.03 a	3.81 ± 0.23 a	4.10 ± 0.25 b	5.47 ± 0.28 a	5.82 ± 0.10 b
	CK-V			2.85 ± 0.06 α	4.71 ± 0.27 $\alpha\beta$	5.74 ± 0.22 $\alpha\beta$	6.08 ± 0.14 β
	EBR-V			2.84 ± 0.10 α	5.10 ± 0.28 α	6.18 ± 0.14 α	6.48 ± 0.14 α
	BRZ-V			2.87 ± 0.11 α^*	3.93 ± 0.3 β	5.49 ± 0.21 β	5.75 ± 0.09 β
HEX Code	CK-F	#8C8568	#7E785B	#736E52	#564F40	#4B3F33	#483E33
	EBR-F	#827B60	#7B7558	#6A654B	#494437	#493D31	#463C31
	BRZ-F	#8B8467	#7C7659	#5F5B44	#5D5543	#504436	#4C4034
	CK-V			#726D51	#564F40	#4C4034	#483E33
	EBR-V			#736D51	#504938	#473D31	#443A2F

Data are presented as mean ± standard error. Lowercase letters indicate significant differences among treatments at fruit set, and Greek letters at veraison ($p < 0.05$). Asterisks (*) mark significant differences for the same treatment between stages ($p < 0.05$). Significance was determined by ANOVA and independent-samples t -tests. Table background color is the direct visual representation of the HEX code.

Table 2. Chromatic characteristic parameters of grapes under different treatments in 2023.

Index	Treatments	E-L27	E-L31	E-L35	E-L36	E-L37	E-L38
L*	CK-F	50.71 ± 0.38 a	47.44 ± 0.44 b	39.54 ± 0.50 a	32.41 ± 0.51 a	26.65 ± 0.38 a	22.24 ± 0.3 ab
	EBR-F	48.28 ± 0.57 b	47.81 ± 0.44 b	39.61 ± 0.66 a	33.72 ± 0.49 a	26.21 ± 0.40 a	21.39 ± 0.30 b
	BRZ-F	50.99 ± 0.59 a	49.09 ± 0.41 a	41.25 ± 0.63 a	33.00 ± 0.41 a	27.26 ± 0.41 a	22.41 ± 0.32 a
	CK-V			39.24 ± 0.70 α	32.65 ± 0.89 αβ	26.85 ± 0.43 β	22.10 ± 0.36 β
	EBR-V			39.13 ± 0.74 α	29.87 ± 0.90 β*	23.66 ± 0.36 γ*	19.59 ± 0.33 γ*
	BRZ-V			39.61 ± 0.64 α	34.05 ± 1.26 α	28.36 ± 0.52 α	24.17 ± 0.28 α*
a*	CK-F	−1.25 ± 0.09 a	−0.87 ± 0.14 a	0.41 ± 0.12 a	3.22 ± 0.19 a	5.18 ± 0.49 a	4.61 ± 0.46 a
	EBR-F	−1.29 ± 0.10 a	−0.88 ± 0.15 a	0.58 ± 0.13 a	2.53 ± 0.17 b	6.29 ± 0.40 a	4.85 ± 0.30 a
	BRZ-F	−1.22 ± 0.15 a	−0.91 ± 0.12 a	0.64 ± 0.10 a	2.04 ± 0.17 b	5.66 ± 0.42 a	3.98 ± 0.34 a
	CK-V			0.45 ± 0.24 α	3.31 ± 0.48 α	5.27 ± 0.49 β	4.69 ± 0.46 αβ
	EBR-V			0.55 ± 0.27 α	2.77 ± 0.31 α	6.66 ± 0.20 α	5.60 ± 0.26 α
	BRZ-V			0.39 ± 0.24 α	2.52 ± 0.46 α	5.65 ± 0.33 αβ	4.01 ± 0.30 β
b*	CK-F	26.57 ± 0.38 a	26.14 ± 0.43 a	22.19 ± 0.33 b	12.13 ± 0.44 b	3.32 ± 0.71 a	0.59 ± 0.28 a
	EBR-F	26.17 ± 0.37 a	23.58 ± 0.32 b	22.55 ± 0.49 b	14.48 ± 0.36 a	2.82 ± 0.37 a	0.77 ± 0.30 a
	BRZ-F	25.90 ± 0.51 a	23.43 ± 0.43 b	24.82 ± 0.52 a	10.68 ± 0.32 c	3.36 ± 0.60 a	0.75 ± 0.34 a
	CK-V			22.23 ± 0.45 α	12.16 ± 0.74 β	3.30 ± 0.51 α	0.61 ± 0.28 α
	EBR-V			22.08 ± 0.45 α	9.67 ± 0.66 γ*	2.56 ± 0.22 α	0.79 ± 0.25 α
	BRZ-V			22.48 ± 0.43 α*	15.3 ± 0.56 α*	3.34 ± 0.60 α	0.66 ± 0.35 α
h°	CK-F	−1.42 ± 0.10 a	−1.12 ± 0.19 a	0.71 ± 0.25 b	1.3 ± 0.02 b	0.47 ± 0.11 a	0.01 ± 0.09 a
	EBR-F	−1.42 ± 0.10 a	−1.01 ± 0.21 a	1.12 ± 0.19 ab	1.39 ± 0.01 a	0.44 ± 0.06 a	0.16 ± 0.07 a
	BRZ-F	−1.42 ± 0.10 a	−1.11 ± 0.19 a	1.33 ± 0.14 a	1.37 ± 0.02 a	0.47 ± 0.08 a	0.18 ± 0.09 a
	CK-V			0.29 ± 0.28 α	1.09 ± 0.13 α	0.54 ± 0.08 α	0.15 ± 0.09 α
	EBR-V			0.08 ± 0.28 α*	1.26 ± 0.04 α*	0.37 ± 0.03 α	0.13 ± 0.05 α
	BRZ-V			0.19 ± 0.28 α*	0.88 ± 0.20 α*	0.44 ± 0.08 α	0.12 ± 0.09 α
C*	CK-F	26.60 ± 0.38 a	26.16 ± 0.43 a	22.21 ± 0.33 b	12.63 ± 0.39 b	7.14 ± 0.54 a	5.01 ± 0.41 a
	EBR-F	26.20 ± 0.37 a	23.61 ± 0.32 b	22.57 ± 0.49 b	14.74 ± 0.34 a	7.20 ± 0.38 a	5.19 ± 0.29 a
	BRZ-F	25.94 ± 0.51 a	23.46 ± 0.43 b	24.83 ± 0.52 a	10.92 ± 0.31 c	7.25 ± 0.47 a	4.52 ± 0.30 a
	CK-V			22.27 ± 0.45 α	12.85 ± 0.75 β	6.77 ± 0.51 α	5.12 ± 0.40 αβ
	EBR-V			22.14 ± 0.45 α	10.26 ± 0.62 γ*	7.23 ± 0.20 α	5.82 ± 0.25 α
	BRZ-V			22.53 ± 0.43 α*	15.70 ± 0.55 α*	7.18 ± 0.43 α	4.49 ± 0.30 β
CIRG	CK-F	2.35 ± 0.02 b	2.47 ± 0.03 a	2.92 ± 0.04 a	4.01 ± 0.08 a	5.38 ± 0.11 a	6.69 ± 0.15 a
	EBR-F	2.44 ± 0.03 a	2.54 ± 0.02 a	2.90 ± 0.05 a	3.71 ± 0.06 b	5.42 ± 0.10 a	6.83 ± 0.12 a
	BRZ-F	2.37 ± 0.03 ab	2.50 ± 0.03 a	2.73 ± 0.05 b	4.10 ± 0.06 a	5.26 ± 0.10 a	6.71 ± 0.09 a
	CK-V			2.94 ± 0.04 α	4.01 ± 0.11 β	5.39 ± 0.10 β	6.69 ± 0.14 β
	EBR-V			2.95 ± 0.04 α	4.53 ± 0.11 α*	5.84 ± 0.08 α*	7.15 ± 0.13 α
	BRZ-V			2.91 ± 0.04 α*	3.81 ± 0.28 β	5.1 ± 0.09 γ	6.32 ± 0.09 γ*
HEX Code	CK-F	#898266	#807A5D	#6C654C	#595141	#4D4135	#443A30
	EBR-F	#827C60	#7D775B	#6D664D	#5C5443	#4E4234	#43392F
	BRZ-F	#888165	#80795E	#716A50	#564F3F	#504436	#443A30
	CK-V			#6B644C	#5A5241	#4E4236	#443A30
	EBR-V			#6B644C	#514A3B	#493F33	#3F362C

Data are presented as mean ± standard error. Lowercase letters indicate significant differences among treatments at fruit set, and Greek letters at veraison ($p < 0.05$). Asterisks (*) mark significant differences for the same treatment between stages ($p < 0.05$). Significance was determined by ANOVA and independent-samples *t*-tests. Table background color is the direct visual representation of the HEX code.

3.3. The Contents of Total Soluble Solids and Titratable Acidity

The ripening process in grapes involves a marked transition in sugar–acid metabolism, where increasing sugars and decreasing acids collectively form a key maturation index [37]. During berry development, TSS content increased continuously, reaching a maximum of 20.50 °Brix at maturity. Concurrently, TA content declined steadily, attaining a minimum value of 6.04 g/L at full maturation. From the results shown in Figures 5 and 6, EBR-V treatment persistently increased TSS and decreased TA in both experimental years, whereas BRZ-V treatment produced the opposite effect. And TSS content in grapes at harvest reached its average peak of 20.17 °Brix for two years, 9.21% more than CK-V. Simultaneously, TA content in grapes at harvest reached its lowest point of 6.13 g/L, 13.51% less than CK-V. EBR-F had little effect on TSS but significantly affected TA, especially before veraison.

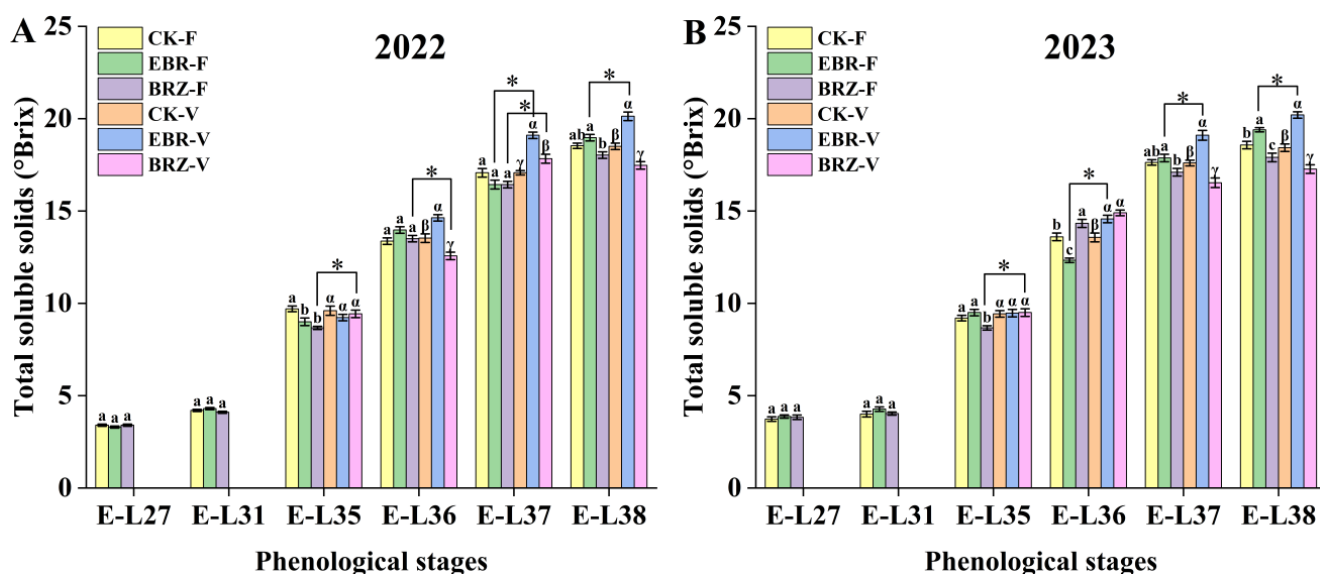


Figure 5. Changes in total soluble solids of grapes under different treatments in 2022 (A) and 2023 (B). Lowercase letters indicate significant differences among treatments at fruit set, and Greek letters at veraison ($p < 0.05$). Asterisks (*) mark significant differences for the same treatment between stages ($p < 0.05$). Significance was determined by ANOVA and independent-samples *t*-tests.

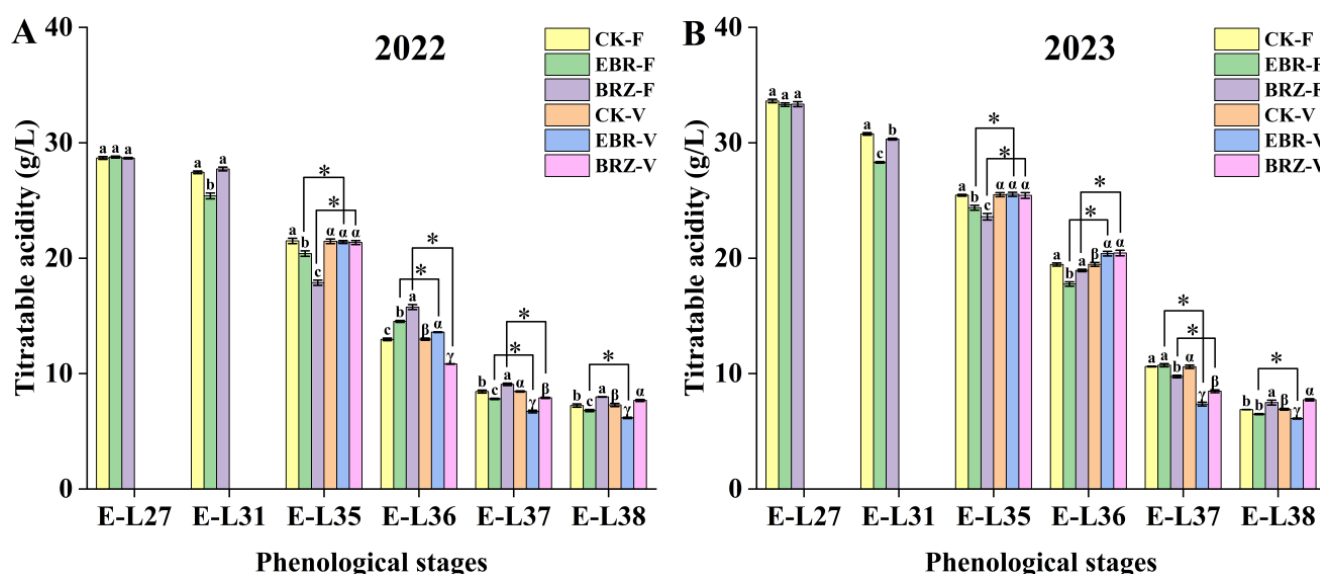


Figure 6. Changes in titratable acidity of grapes under different treatments in 2022 (A) and 2023 (B). Lowercase letters indicate significant differences among treatments at fruit set, and Greek letters at veraison ($p < 0.05$). Asterisks (*) mark significant differences for the same treatment between stages ($p < 0.05$). Significance was determined by ANOVA and independent-samples *t*-tests.

As shown in Figure 7, the ratio of TSS/TA gradually rose after veraison and underwent significant changes during late veraison (E-L 37) and harvest (E-L 38). The TSS/TA ratio in mature grapes exceeded 22 in both years. EBR-F and EBR-V treatments further elevated this ratio, particularly EBR-V which dramatically increased TSS/TA by 48.66% and 26.24% at the last two stages. These results indicate that both EBR treatments, particularly EBR-V, significantly promote grape berry maturity.

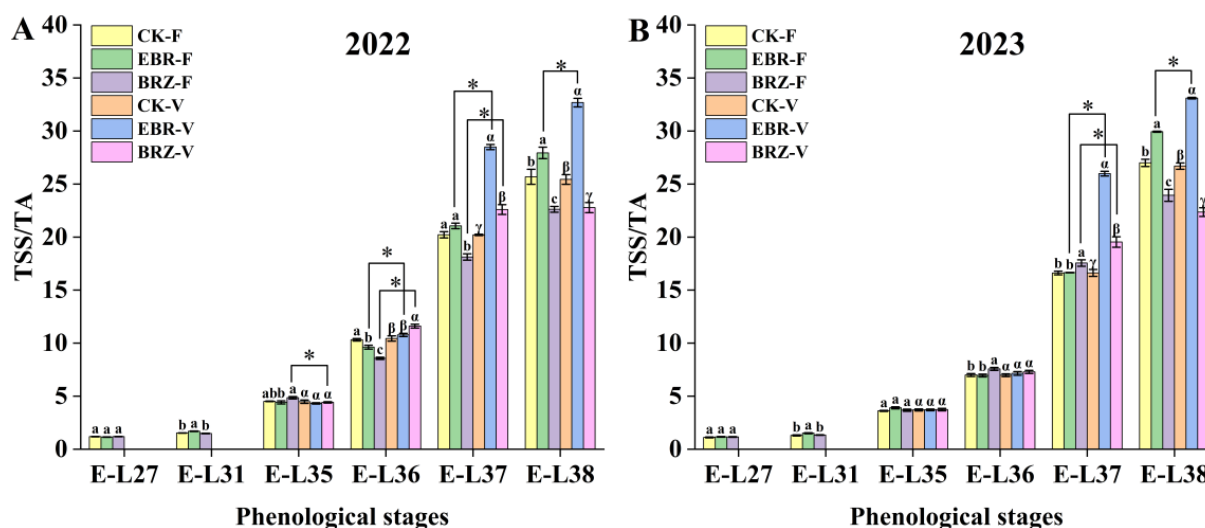


Figure 7. Changes in the ratio of TSS/TA from grapes under different treatments in 2022 (A) and 2023 (B). Lowercase letters indicate significant differences among treatments at fruit set, and Greek letters at veraison ($p < 0.05$). Asterisks (*) mark significant differences for the same treatment between stages ($p < 0.05$). Significance was determined by ANOVA and independent-samples *t*-tests.

3.4. Anthocyanins Content and Profiles

In grapes, the synthesis and accumulation of anthocyanins in the skin commence at veraison [10]. Subsequently, this diverse array of anthocyanin compounds is further modified through glycosylation, methylation, and acylation [7]. In this study, nine individual anthocyanins and total anthocyanins were detected via HPLC in Cabernet Sauvignon grapes at three developmental stages: early veraison (E-L 35), mid-veraison (E-L 36), and maturity (E-L 38). The individual compounds quantified were as follows: delphinidin-3-O-glucoside (Dp), cyanidin-3-O-glucoside (Cy), petunidin-3-O-glucoside (Pt), peonidin-3-O-glucoside (Pn), malvidin-3-O-glucoside (Mv), peonidin-3-O-(6-O-acetyl)-glucoside (Pn-acet), malvidin-3-O-(6-O-acetyl)-glucoside (Mv-acet), peonidin-3-O-(6-O-trans-p-coumaroyl)-glucoside (tPn-coum), and malvidin-3-O-(6-O-trans-p-coumaroyl)-glucoside (tMv-coum). As shown in Tables 3 and 4, at the maturity, the total anthocyanins content was maximum 22.42 mg/g Dry Weight (mg/g DW) in 2022 and 25.85 mg/g DW in 2023, both in grape skin under EBR-V treatment. Nevertheless, the total anthocyanins content in grape skin by EBR-F was 75.72% more than that in CK at E-L 35, which revealed EBR application at fruit set accelerated the coloring process of grapes. As grapes developed into late veraison, the effect of EBR-F on regulating the anthocyanin synthesis was gradually weakening; instead, EBR-V started to exert a strong enhancement on anthocyanin synthesis in grape skin.

Mv and Mv-acet accounted for the highest concentrations of anthocyanins in both vintages, collectively forming the predominant profile in Cabernet Sauvignon grapes. Consistent results across both experimental years demonstrated that EBR-F treatment significantly increased the contents of Mv-acet by 114.45% and tMv-coum by 68.67% at the onset of veraison (E-L 35), a trend that was consistent with the accumulation of total anthocyanins under EBR-F treatment. Subsequently, at mid-veraison (E-L 36) and maturity (E-L 38), Mv-acet was still mainly induced by EBR-F, with a higher concentration than the CK and other treatments. Notably, the highest Mv-acet content in grape skin at harvest was observed under EBR-F treatment, indicating that the accumulation of this particular anthocyanin was primarily enhanced by EBR-F application. The tMv-coum content paralleled the total anthocyanin accumulation and remained the highest among all samples under EBR-V treatment. Moreover, EBR-V treatment exerted a similar pronounced effect on the synthesis of Dp, Cy, Pt, Pn, Mv and tPn-coum at the last two stages, resulting in the final highest concentration of total anthocyanins.

Table 3. Anthocyanin content and profiles from grape skin in 2022.

		Dp/ (mg/g DW)	Cy/ (mg/g DW)	Pt/ (mg/g DW)	Pn/ (mg/g DW)	Mv/ (mg/g DW)	Pn-acet/ (mg/g DW)	Mv-acet/ (mg/g DW)	tPn-coum/ (mg/g DW)	tMv-coum/ (mg/g DW)	Total/ (mg/g DW)
E-L 35	CK	0.1924 ± 0.002 d	0.1021 ± 0.0004 b	0.1546 ± 0.0009 c	0.2077 ± 0.0011 d	0.6063 ± 0.0017 d	0.1031 ± 0.0005 d	0.4349 ± 0.0006 b	0.0794 ± 0.0002 d	0.1636 ± 0.0005 d	2.0440 ± 0.0014 c
	EBR-F	0.2760 ± 0.0018 a	0.1272 ± 0.0014 a	0.2236 ± 0.0037 a	0.2998 ± 0.0034 a	0.9887 ± 0.0052 a	0.1834 ± 0.0028 a	0.8019 ± 0.0012 a	0.1250 ± 0.0013 a	0.2887 ± 0.0051 a	3.3144 ± 0.0136 a
	BRZ-F	0.1092 ± 0.0004 e	0.0665 ± 0.0008 c	0.1091 ± 0.0005 d	0.1484 ± 0.0003 e	0.4450 ± 0.0003 e	0.0966 ± 0.0011 e	0.3324 ± 0.0003 c	0.1040 ± 0.0002 b	0.1962 ± 0.0005 c	1.6073 ± 0.0011 e
	EBR-V	0.2215 ± 0.0006 c	0.1227 ± 0.0036 a	0.1840 ± 0.0012 b	0.2776 ± 0.0009 b	0.7380 ± 0.0046 b	0.1092 ± 0.0014 c	0.1495 ± 0.0025 d	0.1139 ± 0.0011 c	0.2083 ± 0.001 b	2.1246 ± 0.0098 b
	BRZ-V	0.2304 ± 0.0016 b	0.1068 ± 0.0016 b	0.1793 ± 0.0014 b	0.2191 ± 0.002 c	0.7249 ± 0.0029 c	0.1151 ± 0.0012 b	0.1154 ± 0.0022 e	0.1028 ± 0.0007 c	0.2045 ± 0.0011 b	1.9983 ± 0.006 d
E-L 36	CK	0.5681 ± 0.0019 c	0.1571 ± 0.0005 d	0.4913 ± 0.0016 c	0.5824 ± 0.0012 b	3.7439 ± 0.0061 c	0.2301 ± 0.0017 a	2.5259 ± 0.0045 b	0.1915 ± 0.0016 d	0.8562 ± 0.0027 c	9.3465 ± 0.0107 c
	EBR-F	0.8494 ± 0.0012 b	0.1480 ± 0.0005 e	0.5278 ± 0.0018 b	0.5433 ± 0.0002 d	5.0166 ± 0.002 b	0.2006 ± 0.0004 c	2.5390 ± 0.0015 a	0.3156 ± 0.0017 b	1.4097 ± 0.0002 b	11.5500 ± 0.0011 b
	BRZ-F	0.5505 ± 0.0014 d	0.1767 ± 0.0005 c	0.4378 ± 0.0031 e	0.5475 ± 0.0003 c	2.8197 ± 0.0028 d	0.2207 ± 0.0013 b	1.9535 ± 0.0016 c	0.1498 ± 0.0005 e	0.5980 ± 0.0009 e	7.4542 ± 0.0021 d
	EBR-V	1.6725 ± 0.0042 a	0.4936 ± 0.0005 a	1.3262 ± 0.0017 a	1.4899 ± 0.0004 a	7.7562 ± 0.0055 a	0.1293 ± 0.001 d	0.5649 ± 0.0029 d	0.4731 ± 0.0023 a	1.6349 ± 0.0015 a	15.5406 ± 0.0015 a
	BRZ-V	0.5393 ± 0.0015 e	0.2058 ± 0.0003 b	0.4531 ± 0.0007 d	0.5802 ± 0.0012 b	2.6911 ± 0.003 e	0.0726 ± 0.0008 e	0.2355 ± 0.0004 e	0.2208 ± 0.0003 c	0.6635 ± 0.0019 d	5.6620 ± 0.0014 e
E-L 38	CK	0.8583 ± 0.0043 c	0.3216 ± 0.002 b	0.7442 ± 0.0019 c	1.3187 ± 0.0014 b	6.8906 ± 0.0066 c	0.52 ± 0.001 a	4.7447 ± 0.0048 b	0.5282 ± 0.0007 b	1.8012 ± 0.0041 c	17.7275 ± 0.0102 c
	EBR-F	0.9047 ± 0.0017 b	0.2448 ± 0.0015 d	0.7613 ± 0.0053 b	1.2026 ± 0.0009 c	7.8773 ± 0.0021 b	0.4839 ± 0.0005 b	5.6455 ± 0.0025 a	0.4662 ± 0.0019 c	1.7955 ± 0.0028 c	19.3818 ± 0.0009 b
	BRZ-F	0.8400 ± 0.0044 d	0.2844 ± 0.0008 c	0.7584 ± 0.0024 b	1.0270 ± 0.0014 e	6.3240 ± 0.007 e	0.3433 ± 0.0009 c	3.8633 ± 0.005 c	0.4490 ± 0.0016 d	2.4154 ± 0.0016 b	16.3050 ± 0.0213 d
	EBR-V	1.5003 ± 0.0068 a	0.5427 ± 0.0015 a	1.3113 ± 0.0017 a	2.2970 ± 0.0016 a	11.9111 ± 0.0266 a	0.1145 ± 0.0011 d	0.9647 ± 0.0011 d	0.8509 ± 0.0012 a	2.9324 ± 0.0025 a	22.4249 ± 0.0236 a
	BRZ-V	0.8216 ± 0.0047 e	0.2846 ± 0 c	0.7561 ± 0.0028 b	1.1617 ± 0.0011 d	6.7405 ± 0.0218 d	0.0873 ± 0.0018 e	0.5191 ± 0.0037 e	0.4530 ± 0.0011 d	1.7382 ± 0.0016 d	12.5624 ± 0.0177 e

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

Table 4. Anthocyanin content and profiles from grape skin in 2023.

		Dp/ (mg/g DW)	Cy/ (mg/g DW)	Pt/ (mg/g DW)	Pn/ (mg/g DW)	Mv/ (mg/g DW)	Pn-acet/ (mg/g DW)	Mv-acet/ (mg/g DW)	tPn-coum/ (mg/g DW)	tMv-coum/ (mg/g DW)	Total/ (mg/g DW)
E-L 35	CK	0.0935 ± 0.0005 d	0.0209 ± 0.0001 d	0.0799 ± 0.0003 d	0.1019 ± 0.0007 c	0.6767 ± 0.0015 d	0.0581 ± 0.0001 d	0.5695 ± 0.002 b	0.0420 ± 0.0001 c	0.2406 ± 0.0006 d	1.8830 ± 0.0043 d
	EBR-F	0.1106 ± 0.0027 c	0.0286 ± 0.0007 c	0.1297 ± 0.0010 b	0.1545 ± 0.0004 b	1.1899 ± 0.0011 b	0.1057 ± 0.0006 b	1.3924 ± 0.0048 a	0.0661 ± 0.0003 b	0.3866 ± 0.0011 a	3.5641 ± 0.0051 a
	BRZ-F	0.0352 ± 0.0004 e	0.0155 ± 0.0002 e	0.0390 ± 0.0003 e	0.0400 ± 0.0002 d	0.2671 ± 0.0018 e	0.0286 ± 0.0004 e	0.2489 ± 0.0015 d	0.0215 ± 0.0001 d	0.0818 ± 0.0004 e	0.7775 ± 0.0051 e
	EBR-V	0.1563 ± 0.0012 a	0.0863 ± 0.0014 a	0.1354 ± 0.0011 a	0.2310 ± 0.0036 a	1.2231 ± 0.0018 a	0.1164 ± 0.0012 a	0.4281 ± 0.0076 c	0.1281 ± 0.0076 a	0.3489 ± 0.0061 b	2.8535 ± 0.0414 b
	BRZ-V	0.1351 ± 0.0001 b	0.0754 ± 0.0004 b	0.1117 ± 0.0001 c	0.1522 ± 0.0005 b	0.8313 ± 0.0023 c	0.0722 ± 0.0003 c	0.2515 ± 0.0014 d	0.0715 ± 0.0014 b	0.2736 ± 0.0012 c	1.9745 ± 0.0069 c
E-L 36	CK	0.2829 ± 0.0024 d	0.0535 ± 0.0004 b	0.3094 ± 0.0025 d	0.4078 ± 0.0047 d	4.3014 ± 0.0288 c	0.2182 ± 0.0009 c	3.5506 ± 0.0227 b	0.1654 ± 0.0013 c	1.1940 ± 0.0087 c	10.4831 ± 0.0715 c
	EBR-F	0.3135 ± 0.0057 c	0.0604 ± 0.0007 b	0.3848 ± 0.0019 c	0.5456 ± 0.0020 c	4.9330 ± 0.0358 b	0.3262 ± 0.0039 b	4.1610 ± 0.0359 a	0.2556 ± 0.0026 b	1.6106 ± 0.0119 b	12.5907 ± 0.0995 b
	BRZ-F	0.2594 ± 0.001 e	0.0555 ± 0.0007 b	0.2739 ± 0.0045 e	0.3666 ± 0.0048 e	3.3670 ± 0.0448 e	0.1954 ± 0.0031 d	2.7688 ± 0.0323 c	0.1345 ± 0.0017 d	0.8861 ± 0.0042 d	8.3070 ± 0.0887 d
	EBR-V	0.5879 ± 0.0059 a	0.2453 ± 0.0046 a	0.6857 ± 0.0202 a	1.5059 ± 0.0230 a	8.4564 ± 0.0076 a	0.4102 ± 0.0041 a	2.0535 ± 0.0444 d	0.4535 ± 0.0107 a	2.2788 ± 0.0383 a	16.6771 ± 0.1278 a
	BRZ-V	0.3635 ± 0.0076 b	0.2454 ± 0.0061 a	0.4156 ± 0.0029 b	0.5770 ± 0.0111 b	3.5788 ± 0.0355 d	0.1082 ± 0.0019 e	0.9562 ± 0.0119 e	0.1682 ± 0.0064 c	0.9223 ± 0.0085 d	7.3352 ± 0.0883 e
E-L 38	CK	0.5469 ± 0.0081 c	0.1197 ± 0.0019 c	0.608 ± 0.0056 c	1.1553 ± 0.0095 c	8.8406 ± 0.0791 c	0.6397 ± 0.0053 b	6.6841 ± 0.0576 b	0.4894 ± 0.0077 c	2.4317 ± 0.0184 c	21.5153 ± 0.1782 c
	EBR-F	0.4646 ± 0.0155 d	0.1268 ± 0.0035 c	0.5628 ± 0.0066 d	1.2930 ± 0.0062 b	9.5409 ± 0.0571 b	0.7222 ± 0.0072 a	7.1811 ± 0.0582 a	0.6302 ± 0.0039 b	2.8803 ± 0.0204 b	23.4020 ± 0.1615 b
	BRZ-F	0.4191 ± 0.0049 e	0.1173 ± 0.0007 c	0.4548 ± 0.0027 e	1.0075 ± 0.0057 d	7.3964 ± 0.0476 e	0.5869 ± 0.0061 c	5.9878 ± 0.0396 c	0.5022 ± 0.0045 c	2.3707 ± 0.0163 c	18.8428 ± 0.1271 d
	EBR-V	0.7998 ± 0.0028 a	0.6219 ± 0.0064 a	0.7794 ± 0.0035 a	2.0325 ± 0.0209 a	13.1538 ± 0.1076 a	0.4117 ± 0.0065 d	3.5291 ± 0.033 d	1.1713 ± 0.0098 a	3.3464 ± 0.0380 a	25.8460 ± 0.1226 a
	BRZ-V	0.6430 ± 0.0084 b	0.3674 ± 0.0052 b	0.6546 ± 0.0159 b	1.1470 ± 0.0131 c	7.6464 ± 0.0856 d	0.1648 ± 0.0051 e	2.5499 ± 0.0457 e	0.5082 ± 0.0041 c	2.1176 ± 0.0173 d	15.7988 ± 0.1885 e

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

3.5. Proanthocyanidin Characteristics in Grape Skin and Seeds

The content and structural characteristics of proanthocyanidins in the skin of Cabernet Sauvignon grapes across different phenological stages under various treatments in 2022 and 2023 are summarized in Tables 5 and 6. The contents of proanthocyanidin by phloroglucinol method (PA-Phl) and trihydroxy subunit (Tri-OH) in grape skin were gradually reduced during grape maturation with similar dynamic trends. The juvenile fruiting stage is an important period for the synthesis and accumulation of proanthocyanidins. Compared to the control, the PA-Phl content in grape skin under EBR-F was increased by 23.90% and 22.62% at the pea-size stage of grapes (E-L 31) in both years, respectively. Simultaneously, BRZ-F made the PA-Phl content in grape skin decreased by 24.03% and 19.64%. The content of Tri-OH in grape skin was also increased by EBR-F to 2712.67 mg/L and 4431.67 mg/L at E-L 31 in the two consecutive years, as well as decreased to 1663.33 mg/L and 2904.33 mg/L by BRZ-F. From mid-veraison (E-L 36) to maturity (E-L 38), the contents of PA-Phl and Tri-OH in grape skin were highest under EBR-F and secondly were under EBR-V. These results indicated that exogenous BR application on grape at fruit set could regulate the synthesis of proanthocyanidins during grape development. In contrast, the mean degree of polymerization (mDP), molecular mass estimated by gel permeation chromatography (MM-GPC), and the percentage of galloylation exhibited complex, demonstrate year-dependent variations across treatments. At maturity (E-L 38), the degree of galloylation in grape skin was increased significantly by EBR-F.

Table 5. Proanthocyanidin content and characteristics of grape skin in 2022.

Phenological Stage	Treatment	PA-Phl/ (mg/g Skin DW)	Tri-OH/ (mg/L)	mDP	MM-GPC/ (mg/mol)	Gallic Acylation%
E-L 27	CK	59.41 ± 1.91 a	1980.00 ± 63.55 a	24.81 ± 0.13 b	7597.00 ± 39.5 b	8.58 ± 0.17 a
	EBR-F	57.89 ± 0.83 a	1929.67 ± 28.05 a	25.03 ± 0.10 b	7664.33 ± 28.75 b	7.44 ± 0.03 b
	BRZ-F	58.44 ± 1.09 a	1948.33 ± 36.45 a	26.48 ± 0.37 a	8110.00 ± 111.86 a	8.36 ± 0.08 a
E-L 31	CK	65.68 ± 1.81 b	2189.00 ± 60.14 b	27.42 ± 0.24 a	8396.33 ± 71.50 a	6.83 ± 0.19 b
	EBR-F	81.38 ± 3.23 a	2712.67 ± 107.6 a	22.76 ± 0.41 b	6969.00 ± 125.70 b	10.32 ± 0.48 a
	BRZ-F	49.90 ± 1.04 c	1663.33 ± 34.46 c	20.59 ± 0.03 c	6305.00 ± 9.61 c	6.34 ± 0.18 b
E-L 35	CK	50.73 ± 1.97 ab	1690.67 ± 65.82 ab	32.84 ± 8.74 a	10,056.33 ± 2675.84 a	5.37 ± 0.20 b
	EBR-F	46.51 ± 1.84 b	1550.33 ± 61.2 b	17.83 ± 0.19 b	5460.67 ± 56.96 b	5.87 ± 0.23 ab
	BRZ-F	53.99 ± 2.08 a	1799.33 ± 69.29 a	23.43 ± 0.46 ab	7175.33 ± 141.47 ab	6.18 ± 0.21 a
	EBR-V	53.86 ± 1.29 a	1795.33 ± 43.13 a	27.50 ± 0.35 ab	8424.33 ± 107.29 ab	5.26 ± 0.24 b
	BRZ-V	52.39 ± 0.29 a	1746.67 ± 9.84 a	27.89 ± 0.21 ab	8539.67 ± 63.52 ab	5.36 ± 0.05 b
E-L 36	CK	33.62 ± 0.67 c	1120.67 ± 22.1 c	16.80 ± 0.72 c	5144.67 ± 221.18 c	5.20 ± 0.04 a
	EBR-F	44.69 ± 2.76 a	1489.67 ± 92.03 a	19.98 ± 0.83 b	6118.33 ± 253.67 b	5.25 ± 0.15 a
	BRZ-F	39.43 ± 1.35 b	1314.33 ± 45.24 b	11.91 ± 0.29 d	3648.67 ± 87.56 d	4.81 ± 0.13 a
	EBR-V	32.93 ± 1.36 c	1097.67 ± 45.50 c	23.63 ± 0.85 a	7238.33 ± 260.94 a	3.34 ± 0.24 b
	BRZ-V	23.23 ± 0.03 d	774.00 ± 1.15 d	23.05 ± 0.32 a	7059.00 ± 97.21 a	2.19 ± 0.03 c
E-L 37	CK	11.81 ± 0.16 d	393.67 ± 5.49 d	20.44 ± 0.38 ab	6259.33 ± 117.63 ab	1.09 ± 0.03 c
	EBR-F	23.67 ± 0.41 a	789.33 ± 13.74 a	21.12 ± 0.53 a	6467.33 ± 164.00 a	2.36 ± 0.05 a
	BRZ-F	12.98 ± 0.49 c	432.33 ± 16.25 c	14.64 ± 0.34 c	4485.67 ± 104.72 c	1.73 ± 0.05 b
	EBR-V	17.12 ± 0.06 b	570.67 ± 2.19 b	20.78 ± 0.54 a	6363.33 ± 163.96 a	1.71 ± 0.02 b
	BRZ-V	8.10 ± 0.03 e	270.00 ± 1.15 e	19.30 ± 0.33 b	5911.33 ± 102.12 b	0.70 ± 0.01 d
E-L 38	CK	8.31 ± 0.39 c	277.00 ± 13.08 c	17.06 ± 0.37 a	5224.67 ± 112.17 a	0.85 ± 0.03 c
	EBR-F	20.24 ± 0.80 a	674.67 ± 26.59 a	8.22 ± 0.26 d	2516.67 ± 80.15 d	3.53 ± 0.27 a
	BRZ-F	7.88 ± 0.35 c	262.67 ± 11.35 c	5.95 ± 0.28 e	1823.33 ± 86.33 e	1.76 ± 0.11 b
	EBR-V	14.08 ± 0.08 b	469.33 ± 2.40 b	12.68 ± 0.09 c	3883.33 ± 26.62 c	1.88 ± 0.01 b
	BRZ-V	5.53 ± 0.04 d	184.67 ± 1.20 d	13.51 ± 0.15 b	4138.67 ± 46.28 b	0.67 ± 0.01 c

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

Table 6. Proanthocyanidin content and characteristics of grape skin in 2023.

Phenological Stage	Treatment	PA-Phl/ (mg/g Skin DW)	Tri-OH/ (mg/L)	mDP	MM-GPC/ (mg/mol)	Gallic Acylation%
E-L 27	CK	70.54 ± 0.64 b	2351.33 ± 21.37 b	7.66 ± 0.14 a	2291.33 ± 43.48 a	3.64 ± 0.12 a
	EBR-F	75.54 ± 1.36 a	2518.33 ± 45.37 a	7.06 ± 0.25 b	2110.67 ± 74.66 b	3.71 ± 0.11 a
	BRZ-F	67.87 ± 0.89 c	2262.00 ± 29.48 c	7.90 ± 0.08 a	2346.33 ± 22.84 a	2.38 ± 0.21 b
E-L 31	CK	108.43 ± 1.86 b	3614.33 ± 61.9 b	20.04 ± 2.44 a	5942.00 ± 725.13 a	2.97 ± 0.22 a
	EBR-F	132.96 ± 1.66 a	4431.67 ± 55.34 a	21.07 ± 0.27 a	6233.00 ± 78.62 a	2.54 ± 0.25 a
	BRZ-F	87.13 ± 0.98 c	2904.33 ± 32.95 c	13.95 ± 0.13 b	4140.33 ± 40.37 b	2.78 ± 0.17 a
E-L 35	CK	95.32 ± 2.7 a	3177.33 ± 89.84 a	7.48 ± 0.16 bc	2249.33 ± 49.76 bc	6.90 ± 0.16 a
	EBR-F	110.41 ± 0.92 b	3680.33 ± 30.89 b	8.53 ± 0.16 a	2552.67 ± 47.4 a	6.61 ± 0.21 ab
	BRZ-F	83.57 ± 4.92 c	2785.67 ± 163.92 c	7.79 ± 0.19 b	2333.00 ± 54.69 b	5.52 ± 0.22 c
	EBR-V	89.60 ± 1.65 bc	2986.33 ± 55.25 bc	7.05 ± 0.02 c	2119.67 ± 6.23 c	6.64 ± 0.03 ab
	BRZ-V	90.76 ± 1.29 bc	3025.33 ± 42.94 bc	7.70 ± 0.1 b	2310.67 ± 29.04 b	5.96 ± 0.34 bc

Table 6. Cont.

Phenological Stage	Treatment	PA-Phl/ (mg/g Skin DW)	Tri-OH/ (mg/L)	mDP	MM-GPC/ (mg/mol)	Gallic Acylation%
E-L 36	CK	53.35 ± 0.73 b	1778.33 ± 24.55 b	9.68 ± 0.26 b	2935.67 ± 80 b	4.78 ± 0.26 a
	EBR-F	68.27 ± 3.91 a	2276.00 ± 130.41 a	10.33 ± 0.23 b	3116.33 ± 65.58 ab	5.21 ± 0.2 a
	BRZ-F	42.11 ± 0.34 c	1403.33 ± 11.39 c	10.81 ± 0.45 a	3268.67 ± 129.77 a	3.57 ± 0.29 b
	EBR-V	41.19 ± 0.7 c	1372.67 ± 23.13 c	9.86 ± 0.14 b	2986.00 ± 36.77 b	3.61 ± 0.16 b
	BRZ-V	39.07 ± 0.47 c	1302.33 ± 15.68 c	8.65 ± 0.2 c	2632.67 ± 59.89 c	4.06 ± 0.1 b
E-L 37	CK	42.38 ± 1.06 c	1412.67 ± 35.38 c	8.33 ± 0.39 bc	2558.33 ± 118.18 bc	5.58 ± 0.57 ab
	EBR-F	57.27 ± 0.83 a	1908.67 ± 27.72 a	9.72 ± 0.3 a	2953.33 ± 86.36 a	5.53 ± 0.36 ab
	BRZ-F	42.84 ± 0.52 c	1427.67 ± 17.32 c	9.08 ± 0.14 ab	2770.33 ± 44.69 ab	4.77 ± 0.08 b
	EBR-V	46.74 ± 0.6 b	1558.00 ± 19.86 b	8.40 ± 0.25 bc	2590.33 ± 76.59 bc	6.00 ± 0.04 a
	BRZ-V	30.57 ± 0.35 d	1019.00 ± 11.59 d	7.81 ± 0.27 c	2413.67 ± 79 c	4.58 ± 0.20 b
E-L 38	CK	29.03 ± 0.4 c	967.33 ± 13.38 c	6.05 ± 0.17 bc	1868.00 ± 53.52 bc	4.31 ± 0.29 b
	EBR-F	47.03 ± 0.33 a	1567.67 ± 10.73 a	6.76 ± 0.15 ab	2067.33 ± 39.28 ab	5.66 ± 0.52 a
	BRZ-F	17.93 ± 0.16 e	597.67 ± 5.46 e	6.26 ± 0.29 abc	1937.67 ± 86.9 abc	2.71 ± 0.14 c
	EBR-V	34.42 ± 0.44 b	1147.33 ± 14.43 b	5.87 ± 0.04 c	1813.67 ± 12.12 c	5.09 ± 0.13 ab
	BRZ-V	23.97 ± 0.4 d	799 ± 13.01 d	6.85 ± 0.32 a	2111.67 ± 97.42 a	3.42 ± 0.11 c

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

The proanthocyanidin content and structure in the seeds of Cabernet Sauvignon grapes across different phenological stages and treatments in 2022 and 2023 are summarized in Tables 7 and 8. In grape seeds, the contents of PA-Phl and Tri-OH were obviously more than those in grape skin, as well as the percentage of galloylation. At fruit set (E-L 27), the PA-Phl, Tri-OH, mDP and MM-GPC were all increased by EBR-F, although PA-Phl and Tri-OH did not reach a significant level in 2023. This result indicated that proanthocyanidins in seeds respond to exogenous EBR faster than those in grape skin. In grape seeds, EBR-F treatment significantly improved the contents of PA-Phl and Tri-OH at E-L 31, E-L 37, and E-L 38, whereas the EBR-V treatment markedly decreased them at E-L 35, E-L 37, and E-L 38. The dynamic trends for mDP and MM-GPC in grape seeds were complex and showed no clear pattern across phenological stages following different treatments. EBR-F enhanced the degree of galloylation in seeds at E-L 31 and E-L 38, while EBR-V reduced it at E-L 38. From a general trend perspective, these results indicated that the timing of EBR application distinctly influenced both the contents and structural properties of proanthocyanidins in grape seeds, even playing the opposite roles.

Table 7. Proanthocyanidin content and characteristics of grape seeds in 2022.

Phenological Stage	Treatment	PA-Phl/ (mg/g Seeds DW)	Tri-OH/ (mg/L)	mDP	MM-GPC/ (mg/mol)	Gallic Acylation%
E-L 27	CK	355.03 ± 52.45 b	1980.00 ± 63.55 a	6.69 ± 0.12 b	2050.33 ± 35.67 b	223.10 ± 32.28 a
	EBR-F	375.60 ± 16.9 a	1929.67 ± 28.05 a	7.48 ± 0.14 a	2289.67 ± 41.18 a	238.34 ± 15.17 a
	BRZ-F	354.26 ± 1.39 b	1948.33 ± 36.45 a	6.56 ± 0.06 b	2009.00 ± 17.09 b	218.14 ± 0.95 a
E-L 31	CK	211.12 ± 1.88 b	2189.00 ± 60.14 b	8.64 ± 0.10 a	2646.33 ± 29.19 a	109.23 ± 0.95 b
	EBR-F	274.30 ± 7.59 a	2712.67 ± 107.60 a	8.11 ± 0.02 b	2485.67 ± 6.44 b	143.40 ± 4.10 a
	BRZ-F	194.07 ± 10.14 b	1663.33 ± 34.46 c	8.62 ± 0.12 a	2639.67 ± 37.64 a	99.76 ± 5.52 b
E-L 35	CK	132.54 ± 3.87 b	1690.67 ± 65.82 ab	4.42 ± 0.01 b	1352.67 ± 3.28 b	66.81 ± 2.09 b
	EBR-F	166.19 ± 3.75 a	1550.33 ± 61.20 b	4.59 ± 0.06 a	1404.67 ± 17.70 a	90.91 ± 2.12 a
	BRZ-F	108.63 ± 3.76 c	1799.33 ± 69.29 a	4.42 ± 0.05 b	1353.00 ± 14.57 b	56.79 ± 1.91 c
	EBR-V	95.92 ± 3.53 d	1795.33 ± 43.13 a	3.59 ± 0.03 c	1099.67 ± 8.09 c	42.54 ± 1.40 d
	BRZ-V	99.26 ± 1.13 cd	1746.67 ± 9.84 a	3.23 ± 0.01 d	989.00 ± 3 d	43.23 ± 0.87 d
E-L 36	CK	101.21 ± 1.43 a	1120.67 ± 22.10 c	3.42 ± 0.01 c	1048 ± 2.65 c	49.63 ± 0.48 ab
	EBR-F	111.18 ± 5.10 a	1489.67 ± 92.03 a	2.97 ± 0.02 d	909.33 ± 5.36 d	50.97 ± 2.35 a
	BRZ-F	101.12 ± 2.39 a	1314.33 ± 45.24 b	3.29 ± 0.03 c	1008.67 ± 9.96 c	52.28 ± 1.04 a
	EBR-V	95.61 ± 3.88 a	1097.67 ± 45.50 c	4.66 ± 0.03 a	1428.33 ± 9.26 a	43.48 ± 1.47 b
	BRZ-V	77.42 ± 8.32 b	774.00 ± 1.15 d	4.43 ± 0.12 b	1358.33 ± 36.8 b	34.32 ± 3.75 c
E-L 37	CK	82.62 ± 0.82 b	393.67 ± 5.49 d	4.08 ± 0.02 b	1249.67 ± 6.36 b	36.22 ± 0.04 b
	EBR-F	99.12 ± 4.80 a	789.33 ± 13.74 a	4.58 ± 0.4 b	1403.33 ± 121.40 b	42.75 ± 3.59 a
	BRZ-F	79.79 ± 1.76 b	432.33 ± 16.25 c	4.20 ± 0.03 b	1286.33 ± 9.53 b	35.91 ± 0.82 b
	EBR-V	64.84 ± 0.54 c	570.67 ± 2.19 b	5.40 ± 0.17 a	1653.67 ± 51.14 a	26.51 ± 0.59 c
	BRZ-V	75.93 ± 2.26 b	270.00 ± 1.15 e	4.59 ± 0.19 b	1404.67 ± 57.48 b	32.87 ± 1.13 b
E-L 38	CK	88.32 ± 2.36 b	277.00 ± 13.08 c	5.05 ± 0.05 b	1547.33 ± 15.62 b	40.59 ± 0.99 b
	EBR-F	119.01 ± 3.95 a	674.67 ± 26.59 a	4.24 ± 0.00 d	1298.00 ± 0.58 d	51.35 ± 2.07 a
	BRZ-F	72.75 ± 2.93 c	262.67 ± 11.35 c	4.68 ± 0.14 c	1432.00 ± 43.29 c	35.20 ± 1.35 c
	EBR-V	67.68 ± 1.05 c	469.33 ± 2.40 b	5.32 ± 0.05 a	1628.67 ± 14.50 a	31.63 ± 0.37 c
	BRZ-V	72.51 ± 1.77 c	184.67 ± 1.20 d	5.06 ± 0.03 b	1548.67 ± 10.35 b	32.27 ± 0.79 c

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

Table 8. Proanthocyanidin content and characteristics of grape seeds in 2023.

Phenological Stage	Treatment	PA-PhI/ (mg/g Seeds DW)	Tri-OH/ (mg/L)	mDP	MM-GPC/ (mg/mol)	Gallic Acylation%
E-L 27	CK	422.73 ± 8.79 a	14,090.67 ± 292.82 a	8.31 ± 0.25 b	2499.33 ± 73.44 b	42.23 ± 1.88 a
	EBR-F	434.41 ± 28.79 a	14,480.33 ± 959.49 a	9.31 ± 0.34 a	2791.00 ± 96.25 a	39.26 ± 0.48 a
	BRZ-F	397.71 ± 6.81 a	13,257.00 ± 227.05 a	8.32 ± 0.19 b	2502.67 ± 55.47 b	39.99 ± 1.83 a
E-L 31	CK	301.59 ± 3.47 b	10,053.00 ± 115.81 b	2.76 ± 0.00 b	871.00 ± 1.53 b	70.01 ± 0.91 b
	EBR-F	355.51 ± 2.92 a	11,850.00 ± 97.25 a	3.25 ± 0.04 a	1019.67 ± 9.49 a	76.94 ± 1.42 a
	BRZ-F	252.20 ± 3.42 c	8406.67 ± 113.83 c	2.20 ± 0.00 c	701.00 ± 0.00 c	65.11 ± 0.89 c
E-L 35	CK	166.39 ± 1.79 a	5546.33 ± 60.03 a	3.46 ± 0.08 a	1077.33 ± 19.46 a	33.00 ± 1.90 a
	EBR-F	150.48 ± 3.36 b	5016.00 ± 112.26 b	2.54 ± 0.05 c	805.67 ± 12.99 c	36.79 ± 1.48 a
	BRZ-F	126.65 ± 4.95 c	4221.67 ± 165.02 c	3.00 ± 0.12 b	956.67 ± 31.35 b	32.40 ± 3.21 a
	EBR-V	153.85 ± 1.71 b	5128.33 ± 56.83 b	3.17 ± 0.01 b	996.00 ± 1.53 b	33.82 ± 0.39 a
	BRZ-V	152.5 ± 2.73 b	5083.00 ± 90.75 b	3.46 ± 0.03 a	1083.33 ± 9.28 a	32.23 ± 1.28 a
E-L 36	CK	185.79 ± 1.72 b	6193.00 ± 57.30 b	4.97 ± 0.02 b	1504.00 ± 6.25 b	20.39 ± 0.10 a
	EBR-F	223.63 ± 2.25 a	7454.33 ± 74.70 a	6.27 ± 0.12 a	1879.33 ± 32.04 a	19.12 ± 1.06 ab
	BRZ-F	143.96 ± 1.97 d	4799.00 ± 65.52 d	4.53 ± 0.01 b	1370.33 ± 5.24 b	16.61 ± 0.66 c
	EBR-V	150.53 ± 2.00 c	5017.67 ± 66.68 c	4.74 ± 0.02 b	1436.33 ± 4.67 b	17.47 ± 0.41 bc
	BRZ-V	182.07 ± 1.96 b	6069.33 ± 65.24 b	4.73 ± 0.60 b	1428.00 ± 181.00 b	19.02 ± 0.17 ab
E-L 37	CK	202.12 ± 3.16 b	6737.33 ± 105.41 b	5.75 ± 0.05 b	1727.00 ± 13.43 b	18.85 ± 1.05 a
	EBR-F	223.05 ± 2.46 a	7435.33 ± 81.78 a	7.11 ± 0.03 a	2121.00 ± 9.50 a	15.72 ± 0.42 b
	BRZ-F	147.40 ± 1.55 d	4913.67 ± 51.80 d	5.02 ± 0.02 d	1513.67 ± 6.94 d	14.98 ± 0.36 b
	EBR-V	189.33 ± 1.32 c	6311.00 ± 43.92 c	4.47 ± 0.01 e	1344.33 ± 3.93 e	18.48 ± 0.12 a
	BRZ-V	193.12 ± 2.21 c	6437.33 ± 73.90 c	5.50 ± 0.02 c	1660.67 ± 5.36 c	20.25 ± 0.51 a
E-L 38	CK	192.84 ± 2.62 b	6428.00 ± 87.49 b	6.17 ± 0.12 b	1854.00 ± 36.56 b	17.40 ± 0.42 b
	EBR-F	251.56 ± 2.38 a	8385.33 ± 79.30 a	8.31 ± 0.06 a	2479.67 ± 18.12 a	18.52 ± 0.18 a
	BRZ-F	147.99 ± 2.18 c	4932.67 ± 72.78 c	5.32 ± 0.13 c	1602.33 ± 39.94 c	13.99 ± 0.07 c
	EBR-V	147.84 ± 2.13 c	4927.67 ± 70.92 c	6.08 ± 0.10 b	1824.67 ± 30.48 b	13.20 ± 0.02 c
	BRZ-V	195.82 ± 3.56 b	6527.00 ± 118.53 b	6.14 ± 0.07 b	1844.00 ± 20.53 b	18.07 ± 0.33 ab

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

3.6. Subunit Composition of Proanthocyanidins

The subunit composition of proanthocyanidins was also affected by EBR and BRZ treatments (Tables 9–12). Proanthocyanidin subunits are classified into extension subunits and terminal subunits [45]. The extension subunits primarily comprised epicatechin (EC-ext), epigallocatechin (EGC-ext), epigallocatechin gallate (ECG-ext), and catechin (C-ext). The terminal subunits consisted mainly of catechin (C-ter), epigallocatechin gallate (ECG-ter), and epicatechin (EC-ter). The composition of proanthocyanidin subunits varies among different grape tissues [45]. For example, EC-ext and EGC-ext dominated proanthocyanidin subunits in skin of Cabernet Sauvignon while in grape seeds, EC-ext still stayed the highest percentage, but EGC-ext became a smaller portion of proanthocyanidin subunits [45]. Since proanthocyanidins are complex macromolecular polymers, their structural composition is influenced by various external and internal factors, like interannual effects and so on [9].

Table 9. Subunit composition of proanthocyanidins from grape skin in 2022.

Phenological Stage	Treatment	Extension Subunits/ (Molar %)				Terminal Subunits/ (Molar %)		
		EGC-ext	C-ext	EC-ext	ECG-ext	C-ter	EC-ter	ECG-ter
E-L 27	CK	16.78 ± 0.11 c	1.69 ± 0.22 a	68.44 ± 0.11 a	9.05 ± 0.13 a	2.63 ± 0.04 a	0.39 ± 0.03 b	1.01 ± 0.01 a
	EBR-F	19.80 ± 0.28 a	1.90 ± 0.04 a	66.38 ± 0.37 b	7.91 ± 0.08 b	2.54 ± 0.03 a	0.45 ± 0.02 a	1.00 ± 0.03 a
	BRZ-F	18.43 ± 0.04 b	1.86 ± 0.09 a	66.94 ± 0.19 b	8.99 ± 0.2 a	2.36 ± 0.05 b	0.43 ± 0.01 ab	0.99 ± 0.01 a
E-L 31	CK	23.85 ± 0.13 b	2.52 ± 0.03 a	63.55 ± 0.06 a	6.43 ± 0.13 b	2.45 ± 0.04 c	0.45 ± 0.02 a	0.74 ± 0.01 c
	EBR-F	24.09 ± 0.01 a	1.70 ± 0.04 b	61.99 ± 0.16 b	7.83 ± 0.08 a	2.94 ± 0.05 b	0.48 ± 0.04 a	0.98 ± 0.02 b
	BRZ-F	22.14 ± 0.06 c	1.85 ± 0.1 b	63.59 ± 0.23 a	7.57 ± 0.1 a	3.11 ± 0.01 a	0.50 ± 0.02 a	1.25 ± 0.01 a
E-L 35	CK	22.73 ± 0.24 b	2.13 ± 0.35 a	65.13 ± 0.88 a	6.58 ± 0.55 b	2.22 ± 0.7 b	0.45 ± 0.04 bc	0.77 ± 0.01 c
	EBR-F	22.22 ± 0.12 c	1.64 ± 0.04 ab	62.65 ± 0.1 b	7.87 ± 0.18 a	4.04 ± 0.04 a	0.66 ± 0.01 a	0.91 ± 0.01 b
	BRZ-F	23.50 ± 0.13 a	1.93 ± 0.17 ab	63.42 ± 0.27 b	6.88 ± 0.05 b	2.76 ± 0.1 b	0.46 ± 0.01 bc	1.05 ± 0.01 a
	EBR-V	23.39 ± 0.10 a	1.47 ± 0.06 b	65.27 ± 0.13 a	6.24 ± 0.18 b	2.65 ± 0.03 b	0.51 ± 0.02 b	0.47 ± 0.00 d
	BRZ-V	22.35 ± 0.04 bc	1.52 ± 0.01 b	66.00 ± 0.05 a	6.55 ± 0.02 b	2.67 ± 0.03 b	0.43 ± 0 c	0.49 ± 0.01 d
E-L 36	CK	19.49 ± 0.06 e	1.87 ± 0.09 ab	62.89 ± 0.52 b	9.78 ± 0.23 a	3.83 ± 0.14 b	1.07 ± 0.03 b	1.07 ± 0.1 b
	EBR-F	22.07 ± 0.25 c	1.98 ± 0.14 ab	63.75 ± 0.31 b	7.18 ± 0.38 b	3.22 ± 0.14 c	0.80 ± 0.03 c	1.01 ± 0.05 b
	BRZ-F	21.20 ± 0.14 d	2.28 ± 0.37 a	61.60 ± 0.40 c	6.52 ± 0.09 bc	5.01 ± 0.06 a	1.46 ± 0.15 a	1.93 ± 0.10 a
	EBR-V	25.71 ± 0.14 a	1.45 ± 0.1 b	62.59 ± 0.31 bc	6.02 ± 0.23 c	2.87 ± 0.12 c	0.42 ± 0.02 d	0.95 ± 0.03 b
	BRZ-V	22.94 ± 0.11 b	1.45 ± 0.04 b	65.04 ± 0.15 a	6.22 ± 0.08 c	3.11 ± 0.11 c	0.98 ± 0.06 bc	0.25 ± 0.02 c
E-L 37	CK	25.54 ± 0.49 a	1.71 ± 0.05 a	62.90 ± 0.50 b	4.95 ± 0.08 c	2.79 ± 0.05 b	0.74 ± 0.06 b	1.37 ± 0.01 b
	EBR-F	26.26 ± 0.20 a	1.81 ± 0.18 a	61.52 ± 0.26 b	6.67 ± 0.02 a	3.59 ± 0.16 a	0.94 ± 0.05 ab	0.21 ± 0.01 b
	BRZ-F	21.62 ± 0.62 c	1.80 ± 0.08 a	62.72 ± 1.03 b	7.01 ± 0.21 a	3.58 ± 0.09 a	1.01 ± 0.13 a	2.24 ± 0.06 a
	EBR-V	25.59 ± 0.09 a	0.79 ± 0.05 b	62.94 ± 0.15 b	5.87 ± 0.06 b	3.07 ± 0.13 b	0.74 ± 0.01 b	1.01 ± 0.01 c
	BRZ-V	23.25 ± 0.13 b	1.07 ± 0.07 b	65.61 ± 0.17 a	4.89 ± 0.09 c	3.07 ± 0.04 b	1.06 ± 0.03 a	1.05 ± 0.05 c
E-L 38	CK	24.15 ± 0.25 b	1.20 ± 0.06 b	64.25 ± 0.27 a	4.53 ± 0.26 c	2.84 ± 0.13 d	0.53 ± 0.05 c	2.50 ± 0.03 d
	EBR-F	26.26 ± 0.02 a	1.71 ± 0.37 ab	55.30 ± 0.27 c	4.54 ± 0.07 c	3.33 ± 0.13 c	1.08 ± 0.11 b	7.79 ± 0.55 b
	BRZ-F	20.13 ± 0.72 d	2.91 ± 0.8 a	54.51 ± 0.41 c	5.58 ± 0.04 b	4.34 ± 0.05 a	2.04 ± 0.16 a	10.49 ± 0.78 a
	EBR-V	22.73 ± 0.26 c	0.89 ± 0.03 b	64.07 ± 0.4 a	4.42 ± 0.08 c	2.18 ± 0.01 e	0.84 ± 0.05 b	4.87 ± 0.02 c
	BRZ-V	24.26 ± 0.31 b	2.11 ± 0.04 ab	59.76 ± 0.22 b	6.47 ± 0.06 a	3.72 ± 0.04 b	1.75 ± 0.09 a	1.93 ± 0.03 d

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

Table 10. Subunit composition of proanthocyanidins from grape skin in 2023.

Phenological Stage	Treatment	Extension Subunits/ (Molar %)			Terminal Subunits/ (Molar %)			
		EGC-ext	C-ext	EC-ext	ECG-ext	C-ter	EC-ter	ECG-ter
E-L 27	CK	19.26 ± 0.25 c	1.90 ± 0.27 a	64.47 ± 0.44 b	2.30 ± 0.70 a	3.25 ± 0.07 b	2.00 ± 0.19 a	6.81 ± 0.70 ab
	EBR-F	21.92 ± 0.04 b	2.25 ± 0.10 a	63.56 ± 0.09 c	1.96 ± 0.24 a	3.68 ± 0.12 a	1.96 ± 0.04 a	4.66 ± 0.47 c
	BRZ-F	19.70 ± 0.14 c	1.93 ± 0.21 a	65.35 ± 0.24 a	1.99 ± 0.24 a	3.38 ± 0.11 b	1.95 ± 0.06 a	5.69 ± 0.04 bc
E-L 31	CK	27.20 ± 0.11 a	1.65 ± 0.19 a	57.09 ± 0.16 d	2.14 ± 0.24 a	3.43 ± 0.02 ab	2.03 ± 0.19 a	6.81 ± 0.19 ab
	EBR-F	19.43 ± 0.08 c	1.66 ± 0.01 a	63.27 ± 0.25 c	2.80 ± 0.13 a	3.19 ± 0.01 b	1.99 ± 0.14 a	7.67 ± 0.48 a
	BRZ-F	17.87 ± 0.12 c	2.18 ± 0.08 a	60.49 ± 0.32 b	2.90 ± 0.22 a	6.68 ± 0.38 a	2.42 ± 0.27 b	7.46 ± 0.54 a
E-L 35	CK	19.18 ± 0.15 a	2.49 ± 0.17 a	60.82 ± 0.47 b	2.70 ± 0.13 ab	7.33 ± 0.21 a	1.86 ± 0.23 b	5.62 ± 0.67 b
	EBR-F	18.54 ± 0.29 b	2.24 ± 0.49 a	61.14 ± 0.94 b	2.05 ± 0.23 b	4.05 ± 0.30 b	3.48 ± 0.64 a	8.50 ± 0.29 a
	BRZ-F	18.10 ± 0.03 ab	2.30 ± 0.03 a	60.01 ± 0.10 b	2.55 ± 0.23 ab	6.87 ± 0.01 a	2.39 ± 0.01 b	7.77 ± 0.09 a
	EBR-V	17.64 ± 0.18 c	1.63 ± 0.32 a	63.46 ± 0.68 a	2.60 ± 0.17 ab	5.13 ± 0.72 b	2.21 ± 0.02 b	7.32 ± 0.08 a
	BRZ-V	19.26 ± 0.25 c	1.90 ± 0.27 a	64.47 ± 0.44 b	2.30 ± 0.70 a	3.25 ± 0.07 b	2.00 ± 0.19 a	6.81 ± 0.70 ab
E-L 36	CK	21.92 ± 0.04 b	2.25 ± 0.10 a	63.56 ± 0.09 c	1.96 ± 0.24 a	3.68 ± 0.12 a	1.96 ± 0.04 a	4.66 ± 0.47 c
	EBR-F	19.70 ± 0.14 c	1.93 ± 0.21 a	65.35 ± 0.24 a	1.99 ± 0.24 a	3.38 ± 0.11 b	1.95 ± 0.06 a	5.69 ± 0.04 bc
	BRZ-F	27.20 ± 0.11 a	1.65 ± 0.19 a	57.09 ± 0.16 d	2.14 ± 0.24 a	3.43 ± 0.02 ab	2.03 ± 0.19 a	6.81 ± 0.19 ab
	EBR-V	19.43 ± 0.08 c	1.66 ± 0.01 a	63.27 ± 0.25 c	2.80 ± 0.13 a	3.19 ± 0.01 b	1.99 ± 0.14 a	7.67 ± 0.48 a
	BRZ-V	17.87 ± 0.12 c	2.18 ± 0.08 a	60.49 ± 0.32 b	2.90 ± 0.22 a	6.68 ± 0.38 a	2.42 ± 0.27 b	7.46 ± 0.54 a
E-L 37	CK	19.18 ± 0.15 a	2.49 ± 0.17 a	60.82 ± 0.47 b	2.70 ± 0.13 ab	7.33 ± 0.21 a	1.86 ± 0.23 b	5.62 ± 0.67 b
	EBR-F	18.54 ± 0.29 b	2.24 ± 0.49 a	61.14 ± 0.94 b	2.05 ± 0.23 b	4.05 ± 0.30 b	3.48 ± 0.64 a	8.50 ± 0.29 a
	BRZ-F	18.10 ± 0.03 ab	2.30 ± 0.03 a	60.01 ± 0.10 b	2.55 ± 0.23 ab	6.87 ± 0.01 a	2.39 ± 0.01 b	7.77 ± 0.09 a
	EBR-V	17.64 ± 0.18 c	1.63 ± 0.32 a	63.46 ± 0.68 a	2.60 ± 0.17 ab	5.13 ± 0.72 b	2.21 ± 0.02 b	7.32 ± 0.08 a
	BRZ-V	19.26 ± 0.25 c	1.90 ± 0.27 a	64.47 ± 0.44 b	2.30 ± 0.70 a	3.25 ± 0.07 b	2.00 ± 0.19 a	6.81 ± 0.70 ab
E-L 38	CK	21.92 ± 0.04 b	2.25 ± 0.10 a	63.56 ± 0.09 c	1.96 ± 0.24 a	3.68 ± 0.12 a	1.96 ± 0.04 a	4.66 ± 0.47 c
	EBR-F	19.70 ± 0.14 c	1.93 ± 0.21 a	65.35 ± 0.24 a	1.99 ± 0.24 a	3.38 ± 0.11 b	1.95 ± 0.06 a	5.69 ± 0.04 bc
	BRZ-F	27.20 ± 0.11 a	1.65 ± 0.19 a	57.09 ± 0.16 d	2.14 ± 0.24 a	3.43 ± 0.02 ab	2.03 ± 0.19 a	6.81 ± 0.19 ab
	EBR-V	19.43 ± 0.08 c	1.66 ± 0.01 a	63.27 ± 0.25 c	2.80 ± 0.13 a	3.19 ± 0.01 b	1.99 ± 0.14 a	7.67 ± 0.48 a
	BRZ-V	17.87 ± 0.12 c	2.18 ± 0.08 a	60.49 ± 0.32 b	2.90 ± 0.22 a	6.68 ± 0.38 a	2.42 ± 0.27 b	7.46 ± 0.54 a

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

Table 11. Subunit composition of proanthocyanidins from grape seeds in 2022.

Phenological Stage	Treatment	Extension Subunits/ (Molar %)			Terminal Subunits/ (Molar %)			
		EGC-ext	C-ext	EC-ext	ECG-ext	C-ter	EC-ter	ECG-ter
E-L 27	CK	0.25 ± 0.01 a	2.39 ± 0.04 b	39.4 ± 0.10 a	43.01 ± 0.34 ab	4.54 ± 0.13 b	0.75 ± 0.05 a	9.66 ± 0.10 a
	EBR-F	0.19 ± 0.00 c	2.41 ± 0.03 b	39.21 ± 1.11 a	44.81 ± 1.37 a	4.26 ± 0.12 c	0.78 ± 0.01 a	8.35 ± 0.14 b
	BRZ-F	0.21 ± 0.00 b	2.59 ± 0.02 a	40.43 ± 0.08 a	41.52 ± 0.07 b	4.84 ± 0.02 a	0.67 ± 0.02 b	9.74 ± 0.13 a
E-L 31	CK	0.24 ± 0.00 b	3.60 ± 0.05 a	50.54 ± 0.12 a	34.04 ± 0.10 a	3.41 ± 0.01 b	0.92 ± 0.00 b	7.25 ± 0.12 b
	EBR-F	0.22 ± 0.01 b	3.68 ± 0.04 a	49.76 ± 0.06 b	34.01 ± 0.07 a	3.58 ± 0.05 a	0.95 ± 0.01 a	7.80 ± 0.05 a
	BRZ-F	0.26 ± 0.01 a	3.61 ± 0.01 a	50.75 ± 0.22 a	33.77 ± 0.07 b	3.47 ± 0.02 b	0.95 ± 0.01 a	7.18 ± 0.14 b
E-L 35	CK	0.22 ± 0.01 c	3.38 ± 0.04 b	46.47 ± 0.10 a	27.29 ± 0.16 c	6.29 ± 0.02 c	3.64 ± 0.04 c	12.71 ± 0.12 b
	EBR-F	0.17 ± 0.00d	3.56 ± 0.04 a	44.35 ± 0.10 c	30.11 ± 0.17 a	5.36 ± 0.06 d	2.36 ± 0.06 e	14.10 ± 0.20 a
	BRZ-F	0.27 ± 0.01 b	3.4 ± 0.01 b	45.46 ± 0.02 b	28.23 ± 0.24 b	6.11 ± 0.04 c	2.95 ± 0.03 d	13.58 ± 0.20 a
	EBR-V	0.31 ± 0.00 a	3.62 ± 0.03 a	43.75 ± 0.12 d	24.48 ± 0.12 d	11.38 ± 0.18 b	6.60 ± 0.07 b	9.87 ± 0.19 c
	BRZ-V	0.27 ± 0.00 b	3.60 ± 0.06 a	41.62 ± 0.21 e	23.55 ± 0.28 e	13.33 ± 0.14 a	7.58 ± 0.06 a	10.05 ± 0.11 c
E-L 36	CK	0.20 ± 0.00 cd	3.49 ± 0.1 b	40.23 ± 0.28 b	26.84 ± 0.41 b	11.08 ± 0.12 b	6.28 ± 0.04 c	11.87 ± 0.13 b
	EBR-F	0.22 ± 0.01 c	3.64 ± 0.06 b	37.89 ± 0.11 c	24.57 ± 0.07 c	14.23 ± 0.13 a	8.31 ± 0.07 a	11.13 ± 0.06 c
	BRZ-F	0.18 ± 0.00d	3.94 ± 0.05 a	37.10 ± 0.12 d	28.43 ± 0.36 a	11.31 ± 0.06 b	6.21 ± 0.03 c	12.84 ± 0.25 a
	EBR-V	0.30 ± 0.01 b	3.96 ± 0.05 a	46.11 ± 0.16 a	28.20 ± 0.18 a	8.07 ± 0.08 d	6.18 ± 0.05 c	7.19 ± 0.18 d
	BRZ-V	0.35 ± 0.01 a	3.76 ± 0.12 ab	46.27 ± 0.43 a	27.04 ± 0.36 b	8.53 ± 0.22 c	6.78 ± 0.16 b	7.27 ± 0.21 d
E-L 37	CK	0.29 ± 0.01 ab	4.39 ± 0.01 a	44.88 ± 0.13 c	25.92 ± 0.23 c	9.66 ± 0.14 a	6.90 ± 0.10 ab	7.95 ± 0.12 a
	EBR-F	0.28 ± 0.01 b	4.15 ± 0.11 a	45.16 ± 1.01 c	28.29 ± 0.64 a	9.81 ± 0.17 a	7.51 ± 0.16 a	4.81 ± 2.09 ab
	BRZ-F	0.27 ± 0.00 b	4.37 ± 0.04 a	43.72 ± 0.08 a	27.83 ± 0.12 ab	9.79 ± 0.03 a	6.92 ± 0.02 ab	7.10 ± 0.17 ab
	EBR-V	0.27 ± 0.00 b	4.02 ± 0.03 a	50.28 ± 0.35 b	26.87 ± 0.69 abc	7.89 ± 0.06 c	6.34 ± 0.51 b	4.33 ± 0.13 b
	BRZ-V	0.32 ± 0.02 a	4.36 ± 0.29 a	47.10 ± 0.82 c	26.34 ± 0.26 bc	8.97 ± 0.32 b	5.88 ± 0.48 b	7.02 ± 0.26 ab
E-L 38	CK	0.28 ± 0.00 a	4.96 ± 0.13 a	46.36 ± 0.23 ab	28.61 ± 0.14 b	7.02 ± 0.08 c	5.56 ± 0.06 d	7.21 ± 0.30 a
	EBR-F	0.20 ± 0.00 b	4.55 ± 0.10 b	44.57 ± 0.15 bc	27.08 ± 0.24 c	10.2 ± 0.04 a	7.25 ± 0.04 a	6.15 ± 0.08 b
	BRZ-F	0.28 ± 0.01 a	4.12 ± 0.08 c	43.35 ± 1.51 c	30.83 ± 0.85 a	7.61 ± 0.14 b	6.51 ± 0.12 b	7.31 ± 0.43 a
	EBR-V	0.27 ± 0.00 a	4.30 ± 0.12 ab	46.48 ± 0.25 ab	30.14 ± 0.04 a	6.40 ± 0.14 d	6.00 ± 0.07 c	6.41 ± 0.34 ab
	BRZ-V	0.29 ± 0.00 a	4.35 ± 0.16 ab	47.41 ± 0.14 a	28.17 ± 0.01 bc	7.33 ± 0.11 bc	6.15 ± 0.10 c	6.31 ± 0.23 ab

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

Table 12. Subunit composition of proanthocyanidins from grape seeds in 2023.

Phenological Stage	Treatment	Extension Subunits/ (Molar %)				Terminal Subunits/ (Molar %)		
		EGC-ext	C-ext	EC-ext	ECG-ext	C-ter	EC-ter	ECG-ter
E-L 27	CK	1.78 ± 0.02 a	1.57 ± 0.09 a	83.76 ± 0.36 b	0.84 ± 0.02 a	4.42 ± 0.13 a	1.70 ± 0.10 a	5.94 ± 0.17 a
	EBR-F	1.76 ± 0.03 a	1.37 ± 0.12 a	85.30 ± 0.50 a	0.80 ± 0.04 a	3.98 ± 0.07 b	1.43 ± 0.04 a	5.37 ± 0.30 a
	BRZ-F	1.80 ± 0.02 a	1.62 ± 0.09 a	83.67 ± 0.16 b	0.88 ± 0.04 a	4.33 ± 0.11 a	1.75 ± 0.22 a	5.95 ± 0.26 a
E-L 31	CK	1.70 ± 0.11 b	0.88 ± 0.05 b	60.67 ± 0.12 b	0.48 ± 0.06 a	19.71 ± 0.14 b	0.46 ± 0.01 a	16.09 ± 0.09 b
	EBR-F	1.32 ± 0.01 c	0.67 ± 0.00 c	66.76 ± 0.32 a	0.46 ± 0.03 a	15.47 ± 0.05 c	0.42 ± 0.01 a	14.9 ± 0.39 c
	BRZ-F	2.27 ± 0.02 a	1.11 ± 0.06 a	50.75 ± 0.07 c	0.37 ± 0.03 a	26.85 ± 0.01 a	0.41 ± 0.05 a	18.23 ± 0.04 a
E-L 35	CK	2.22 ± 0.03 a	1.10 ± 0.02 a	66.96 ± 0.65 a	0.75 ± 0.02 a	15.15 ± 0.24 c	0.59 ± 0.04 a	13.23 ± 0.77 c
	EBR-F	1.83 ± 0.03 b	0.77 ± 0.01 b	57.47 ± 0.76 c	0.54 ± 0.01 b	21.93 ± 0.27 a	0.47 ± 0.01 a	16.99 ± 0.63 ab
	BRZ-F	2.25 ± 0.09 a	1.02 ± 0.09 a	62.54 ± 1.26 b	0.79 ± 0.04 a	15.16 ± 0.33 c	0.65 ± 0.03 a	17.57 ± 1.34 a
	EBR-V	2.34 ± 0.02 a	1.08 ± 0.00 a	64.26 ± 0.07 b	0.76 ± 0.00 a	16.06 ± 0.01 b	0.63 ± 0.00 a	14.86 ± 0.07 bc
	BRZ-V	2.27 ± 0.03 a	1.15 ± 0.01 a	66.93 ± 0.27 a	0.72 ± 0.01 a	14.11 ± 0.07 d	0.58 ± 0.00 a	14.24 ± 0.37 c
E-L 36	CK	3.16 ± 0.03 d	0.93 ± 0.00 c	74.83 ± 0.10 b	0.99 ± 0.12 a	12.84 ± 0.02 a	0.75 ± 0.04 ab	6.50 ± 0.04 ab
	EBR-F	3.16 ± 0.04 d	0.78 ± 0.02 d	79.39 ± 0.34 a	0.72 ± 0.02 a	10.38 ± 0.14 a	0.51 ± 0.03 c	5.07 ± 0.32 c
	BRZ-F	3.46 ± 0.04 b	0.98 ± 0.01 b	72.31 ± 0.15 b	1.15 ± 0.25 a	14.53 ± 0.02 a	0.83 ± 0.04 a	6.74 ± 0.02 a
	EBR-V	4.36 ± 0.03 a	1.03 ± 0.00 a	72.51 ± 0.05 b	1.00 ± 0.07 a	13.32 ± 0.02 a	0.84 ± 0.00 a	6.95 ± 0.07 a
	BRZ-V	3.35 ± 0.02 c	0.90 ± 0.00 c	72.78 ± 3.16 b	1.04 ± 0.05 a	15.13 ± 3.18 a	0.71 ± 0.00 b	6.09 ± 0.01 b
E-L 37	CK	4.30 ± 0.04 b	0.96 ± 0.00 c	76.19 ± 0.40 b	1.13 ± 0.25 a	11.23 ± 0.04 c	0.98 ± 0.18 ab	5.20 ± 0.04 b
	EBR-F	4.48 ± 0.04 a	0.82 ± 0.00 d	79.70 ± 0.13 a	0.93 ± 0.13 a	9.42 ± 0.03 d	0.82 ± 0.05 b	3.82 ± 0.01 c
	BRZ-F	4.48 ± 0.04 a	0.99 ± 0.01 b	73.62 ± 0.11 d	0.98 ± 0.12 a	12.78 ± 0.03 b	1.20 ± 0.09 a	5.94 ± 0.04 a
	EBR-V	4.40 ± 0.02 ab	0.99 ± 0.01 b	70.89 ± 0.06 e	1.33 ± 0.02 a	16.03 ± 0.07 a	1.06 ± 0.00 ab	5.31 ± 0.02 b
	BRZ-V	4.39 ± 0.02 ab	1.05 ± 0.00 a	75.10 ± 0.14 c	1.29 ± 0.05 a	11.25 ± 0.02 c	1.06 ± 0.00 ab	5.87 ± 0.10 a
E-L 38	CK	4.73 ± 0.07 ab	0.99 ± 0.02 b	76.69 ± 0.38 b	1.38 ± 0.12 a	10.15 ± 0.28 b	1.32 ± 0.13 ab	4.74 ± 0.10 ab
	EBR-F	4.35 ± 0.04 c	0.74 ± 0.01 c	81.81 ± 0.11 a	1.06 ± 0.09 b	7.08 ± 0.01 b	1.06 ± 0.09 b	3.91 ± 0.02 c
	BRZ-F	4.97 ± 0.07 a	1.08 ± 0.01 a	73.62 ± 0.33 c	1.55 ± 0.12 a	12.49 ± 0.42 ab	1.42 ± 0.11 a	4.89 ± 0.02 a
	EBR-V	4.53 ± 0.13 bc	1.03 ± 0.03 ab	76.52 ± 0.19 b	1.47 ± 0.04 a	18.04 ± 4.08 a	1.38 ± 0.01 a	4.59 ± 0.05 b
	BRZ-V	4.77 ± 0.04 ab	1.03 ± 0.02 ab	76.49 ± 0.21 b	1.41 ± 0.04 a	10.15 ± 0.22 b	1.29 ± 0.05 ab	4.86 ± 0.02 a

Data are presented as mean ± standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among treatments. Statistical significance was determined using ANOVA.

Over the two experimental years, treatment-induced differences in skin subunit composition were more pronounced in 2022 (Table 9) than in 2023 (Table 10). In grape skin, the molar percentage of EGC-ext was significantly higher under EBR-F than CK or other treatments at E-L 27, E-L 31, and E-L 38. Conversely, at E-L 27, the molar percentage of EC-ext was markedly lower under EBR-F than in CK and BRZ-F. At E-L 31, BRZ-F treatment substantially increased the molar percentages of C-ter and ECG-ter while reducing that of EGC-ext in the skin.

In seeds, EBR-F treatment decreased the molar percentage of EGC-ext at E-L 35 and E-L 38 and lowered that of C-ext at final maturity (Tables 11 and 12). At veraison (E-L 35), the molar percentage of EC-ext was lower under EBR-F, BRZ-F, and EBR-V treatments compared to CK. Furthermore, at E-L 38, the molar percentages of EC-ext under BRZ-F and of ECG-ext under EBR-F were also reduced relative to CK. Regarding terminal subunits in seeds, the molar percentage of C-ter was decreased by EBR-F at fruit set (E-L 27) but increased by BRZ-F at maturity (E-L 38). The molar percentages of ECG-ter were elevated by both EBR-F and BRZ-F treatments at veraison (E-L 35) but were subsequently reduced by EBR-F at later stages.

4. Discussion

4.1. The Effects of Applying BR at Different Phenological Stages on the Maturity and Quality of Grapes

From fruit set to maturity, grape berries undergo significant physiological and morphological changes. During the green-fruit period, rapid cell expansion and volume increase occur, accompanied by substantial accumulation of organic acids and proanthocyanidins, while sugar and anthocyanin synthesis remain minimal [46]. Berry size and weight are largely determined by cell division and expansion, processes regulated by both endogenous and exogenous hormones [47,48]. Brassinosteroids are known to promote cell expansion and division [49]. Consistent with this, the present study showed that EBR application at

fruit set significantly increased 100-berry weight and berry diameters at the pea-size stage (E-L 31), indicating that EBR treatment during the green phase can effectively enhance early berry development by targeting the period of active cell division. These results align with previous findings on grapes, where exogenous BR application improved yield and fruit quality parameters, including berry weight and dimensions [50–52]. However, this promotive effect was timing-dependent, as EBR application at veraison did not significantly enhance berry size, suggesting that the responsiveness of berry tissues to BR signaling varies across developmental stages.

Veraison initiates berry coloring and ripening, a critical phase for nutrient accumulation in grape berries [53]. Although a secondary expansion occurs post-veraison, developmental emphasis shifts from physical growth to metabolic activity [54]. During this period, metabolite levels increase substantially, driven by active biosynthesis and accumulation of soluble solids and phenolic compounds [53]. Consequently, exogenous EBR application at veraison is unlikely to enhance berry enlargement further. Instead, brassinosteroid signaling is redirected toward the metabolic and biosynthetic pathways that are dramatically activated during ripening, thereby promoting anthocyanin accumulation and fruit maturation [33,35].

As key determinants of flavor and color, soluble sugars represent the second most abundant component in grape berries after water, serving as fundamental substrates for fruit development and wine fermentation [55]. Their content and composition significantly influence both berry quality and the final wine profile, particularly in determining alcohol concentration [55]. Similarly, organic acids—primarily tartaric acid, constituting approximately 90% of total acidity—are crucial for stabilizing wine pH, thereby affecting sensory attributes and aging potential [56]. The sugar-to-acid ratio is a principal maturity index, reflecting the dynamic physiological shift from acid accumulation to sugar import that characterizes berry ripening [57].

During early development, acid content is high, declining slightly through the green fruit stage before dropping sharply at veraison [53]. Conversely, sugar levels remain low initially but increase rapidly just before veraison, supplying energy for subsequent coloration and ripening [53]. In our experiments, exogenous EBR treatment consistently elevated total TSS and reduced TA in berries, whereas BRZ application exerted the opposite effect. Notably, EBR applied at veraison exerted a stronger and more stable impact on sugar–acid metabolism than application at fruit set, resulting in a significantly higher sugar–acid ratio. This indicates that exogenous BR application at veraison effectively promotes sugar accumulation and acid degradation, thereby accelerating ripening—a finding consistent with prior reports [34,37]. The underlying mechanism involves EBR-enhanced sugar unloading and modulation of enzyme activities in sugar metabolism pathways, likely through crosstalk with endogenous hormone signaling [37]. The veraison period represents a metabolic transition during which exogenous BR signaling integrates with active biosynthetic and degradative pathways, thereby intensifying the ripening process [34,58].

Fruit quality encompasses visual, gustatory, and nutritional attributes, with color being a primary evaluation criterion for red grape cultivars. In our two-year trial, EBR application at veraison, but not at fruit set, significantly improved berry coloration, as reflected by lower brightness, higher redness values, and an increased CIRG. These results align with studies demonstrating BR-mediated promotion of ripening and anthocyanin accumulation in various fruits [34,58,59]. The color development in red grapes—from green to pink, red, and ultimately dark purple—is predominantly governed by the composition and concentration of skin anthocyanins, which also determine wine color [41,60].

4.2. The Temporal and Spatial Differences in the Regulation of Anthocyanin and Proanthocyanidin Synthesis in Grapes by BR During Different Periods

Anthocyanins and proanthocyanidins represent the most significant phenolic compounds in wine grapes and wines [1]. Anthocyanins, with diverse modification reactions in grape skins, impart color to red wines [2,44,61], whereas proanthocyanidins primarily derived from seeds contribute astringency and structure, enhancing both sensory complexity and aging potential [9,62,63]. Proanthocyanidins interact with sugars and acids to shape flavor profiles and can also form stable complexes with anthocyanins [9,45]. These interactions are crucial for stabilizing wine color and preserving sensory attributes during aging. Anthocyanins and proanthocyanidins can interact to form polymeric pigments through direct condensation or acetaldehyde-mediated cross-linking, resulting in enhanced color expression and greater stability [64,65]. In addition to the acetaldehyde-mediated pathway, anthocyanins and proanthocyanidins can also form direct C–C bonds [66]. Importantly, accumulating evidence suggests that the formation of such polymeric pigments may begin earlier than previously thought—potentially as early as during berry maturation [67]. Kennedy et al. [67] demonstrated that pigment incorporation into proanthocyanidins increases with fruit maturity, accompanied by an apparent rise in the average degree of polymerization, indicating that non-flavan-3-ol terminal subunits accumulate during ripening. This suggests that some degree of polymerization already occurs within the berry prior to harvest, which may directly influence wine taste and aging potential [68]. In the present study, the mDP of proanthocyanidins in both skins and seeds varied significantly across treatments and developmental stages. The observed increases in mDP, particularly following EBR-F treatment at specific stages, may reflect an enhanced potential for subsequent polymeric pigment formation during winemaking and aging. This could ultimately contribute to improved color stability in the resulting wines.

An additional factor influencing the stability and extractability of anthocyanins and proanthocyanidins is the pH of the grape berry and subsequent wine matrix [69]. Anthocyanins exist in equilibrium between several structural forms—flavylium cation (red), quinoidal base (purple/blue), carbinol pseudobase (colorless), and chalcone (colorless)—with the relative proportions of each form being highly pH-dependent [69,70]. At the typical pH of grape berries (around 3.0–4.0), the flavylium cation predominates, favoring stable red coloration [71,72]. However, as pH increases toward wine pH (typically 3.2–3.8), structural transformations can occur, affecting color intensity and stability [73]. Proanthocyanidins are also influenced by pH, with higher pH conditions promoting autooxidation and facilitating their interactions with anthocyanins to form polymeric pigments [74]. In the present study, TA, which is closely related to pH, was influenced by EBR treatment. Specifically, EBR-V treatment significantly decreased TA and thereby increased the sugar/acid ratio, which may indirectly affect anthocyanin stability by modulating the pH microenvironment within the berry.

Building on prior findings that exogenous brassinosteroids (BRs) regulate flavonoid biosynthesis in grape berries—including the accumulation of anthocyanins and proanthocyanidins [34,58]—the present study demonstrates that EBR application at either fruit set or veraison enhances the synthesis of both compound classes in *Vitis vinifera* L. cv. Cabernet Sauvignon, consistent with earlier reports. This study demonstrates that EBR application at either fruit set or veraison enhances the synthesis of both compound classes in *Vitis vinifera* L. cv. Cabernet Sauvignon, consistent with earlier reports [35,36,75]. However, a distinct temporal specificity was observed: EBR application at veraison exerted a stronger promotive effect on anthocyanin accumulation, whereas application at fruit set was more effective in stimulating proanthocyanidin synthesis. This aligns with the natural ontogenetic patterns of these metabolites: proanthocyanidins are predominantly

synthesized in green berries prior to veraison, while anthocyanin accumulation is initiated post-veraison, often coinciding with a decline in proanthocyanidin content. Thus, exogenous BR appears to preferentially enhance the biosynthetic pathway that is naturally active at the time of application.

Notably, seeds—which accumulate proanthocyanidins but not anthocyanins—also exhibited a stage-specific response to EBR treatment. Application at fruit set significantly promoted proanthocyanidin accumulation in seeds, whereas treatment at veraison failed to increase—and even slightly reduced—seed proanthocyanidin content. This novel finding underscores the temporally and spatially compartmentalized regulation of phenolic biosynthesis by exogenous BR within the berry.

Furthermore, the timing of EBR application significantly influenced not only anthocyanin concentration but also its compositional profile. Application at veraison was more effective in increasing both total anthocyanin content and the proportion of key derivatives. Notably, the composition of individual anthocyanin monomers was differentially modulated: for instance, the acetylated derivative of malvidin (Mv-acet) was highest following fruit-set application, whereas the acetylated derivative of peonidin (Pn-acet) was significantly suppressed by veraison treatment. This indicates a timing-dependent regulation of specific modification steps within the anthocyanin biosynthetic pathway.

As the primary source of condensed tannins in wine, seed proanthocyanidins are critical for wine structure and aging potential [62]. Proanthocyanidins in grapes are polymeric compounds derived from flavanol monomers such as catechin, epicatechin, and their galloylated derivatives [45]. In this study, the composition of skin and seed proanthocyanidins was analyzed following EBR and BRZ treatments across two vintages. However, the effects on proanthocyanidin composition exhibited considerable variability between years and developmental stages. This variability may be attributed to the inherent structural complexity of proanthocyanidin polymers, whose full characterization remains challenging, as well as to interactions with environmental factors, cultivation practices, and inter-plant variability, all of which are known to influence flavonoid biosynthesis.

Unlike previous studies that examined cumulative effects of sequential EBR applications at fruit set and veraison [75], the present study provides a novel contribution by systematically comparing single EBR applications at each stage (fruit set vs. veraison) within a unified framework, thereby revealing previously unrecognized stage-specific regulatory patterns in anthocyanin and proanthocyanidin biosynthesis. This approach enabled us to delineate the stage-specific regulatory roles of BRs, rather than merely evaluating the cumulative effects of combined treatments. Our findings reveal a critical “temporal window” for BR application: its promotion of anthocyanin accumulation is most effective during ripening, whereas its influence on seed proanthocyanidin composition is largely determined prior to veraison. In contrast to the traditional single-time-point experimental paradigm, this work underscores the dynamic and temporally precise nature of BR regulation in grape phenolic metabolism. These insights provide a physiological foundation for optimizing BR application strategies in precision viticulture. Furthermore, they lay the groundwork for future studies aimed at elucidating the molecular mechanisms by which BRs regulate anthocyanin and proanthocyanidin biosynthesis in grape berries.

4.3. Limitations and Future Perspectives

This study provides comprehensive insights into the spatiotemporal regulation of phenolic biosynthesis by BRs in grape berries, demonstrating that the effects of EBR application are dependent on both the phenological stage (fruit set vs. veraison) and tissue type (skins vs. seeds). However, several limitations of the present study should be acknowledged.

First, while this study provided a systematic and comprehensive analysis of anthocyanin and proanthocyanidin in grape berries using HPLC, it did not determine the content of other phenolic compounds or evaluate antioxidant activity—parameters that could also reflect the impact of exogenous BRs on grape berry quality. Therefore, some spectrophotometric analyses (i.e., total polyphenol content and total flavonoid content) and antioxidant capacity assays should be involved and increase the scientific soundness of future studies, which are also preferable to support the evidence of BR regulation of phenolics in grapes.

Second, although we systematically analyzed phenolic profiles at the metabolic level—including anthocyanin monomers and proanthocyanidin structural characteristics—the underlying molecular mechanisms remain unexplored. Specifically, this study did not investigate the expression of genes involved in either the flavonoid biosynthetic pathway or BR signaling transduction. Consequently, while we can describe the phenotypic outcomes of EBR treatment, we cannot explain the regulatory events that lead to these tissue- and stage-specific responses. Transcriptomic analyses (e.g., RNA-seq) and targeted gene expression studies are needed to elucidate the molecular basis for the observed spatiotemporal differences. Such integrated approaches, combining molecular data with physiological and biochemical analyses, would comprehensively reveal the mechanisms by which BRs regulate phenolic biosynthesis in grapes.

Third, this study focused exclusively on phenolic composition in grape berries. While these metabolites are key determinants of wine quality, we did not evaluate the sensory attributes of wines produced from EBR-treated grapes. Future research should explore how the modulated phenolic profiles—particularly changes in anthocyanin monomer composition and proanthocyanidin structural features—translate into wine sensory properties, including color stability, mouthfeel, astringency, and bitterness.

Addressing these limitations in future studies will build upon the physiological findings of the present work. The spatiotemporal differences in phenolic accumulation observed here, particularly the stage- and tissue-specific responses to EBR treatment, provide a foundation for investigating the underlying molecular mechanisms. By integrating transcriptomic and targeted gene expression analyses, the regulatory network through which BR signaling modulates anthocyanin and proanthocyanidin biosynthesis can be progressively elucidated. Ultimately, a comprehensive understanding of this process—from physiological phenomena to molecular regulation—will offer a scientific basis for optimizing BR application strategies in viticulture, with the potential to enhance grape and wine quality in a targeted and sustainable manner.

5. Conclusions

Based on two consecutive growing seasons (2022–2023) in *Vitis vinifera* L. cv. Cabernet Sauvignon, exogenous BR application at fruit set exerts a stronger influence on berry growth and development, whereas its effect on fruit coloration is less pronounced compared to treatment at veraison. The latter timing most effectively promotes anthocyanin accumulation in berry skins during ripening. The timing of BR application proves even more critical for proanthocyanidin metabolism. BR treatment at fruit set increased proanthocyanidin content in both skins and seeds, along with tri-hydroxylated subunit levels and the galloylation percentage. In contrast, veraison application produced opposite effects in seeds. Together, these findings reveal a sophisticated spatiotemporal regulatory pattern in BR-mediated phenolic biosynthesis, providing a foundation for future molecular studies. They also establish a physiological rationale for employing BRs as a precision viticultural tool—applied at specific stages to selectively enhance color intensity or modulate wine astringency.

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Abbreviations

The following abbreviations are used in this manuscript:

BRs	Brassinosteroids
PAs	Proanthocyanidins
CK	Distilled water as control
EBR	24-Epibrassinolide
BRZ	Brassinazole-
HPLC	High-Performance Liquid Chromatography
CK-F	The control at fruit set
EBR-F	24-Epibrassinolide application at fruit set
BRZ-F	Brassinazole application at fruit set
CK-V	The control at veraison
EBR-V	24-Epibrassinolide application at veraison
BRZ-V	Brassinazole application at veraison
TSS	Total soluble solids
TA	Titrateable acidity
CIRG	Color Index of Red Grape
Dp	Delphinidin-3-O-glucoside
Cy	Cyanidin-3-O-glucoside
Pt	Petunidin-3-O-glucoside
Pn	Peonidin-3-O-glucoside
Mv	Malvidin-3-O-glucoside
Pn-acet	Peonidin-3-O-(6-O-acetyl)-glucoside
Mv-acet	Malvidin-3-O-(6-O-acetyl)-glucoside
tPn-coum	Peonidin-3-O-(6-O-trans-p-coumaroyl)-glucoside
tMv-coum	Malvidin-3-O-(6-O-trans-p-coumaroyl)-glucoside
PA-Phl	Proanthocyanidin by phloroglucinol method
Tri-OH	Trihydroxy subunit
mDP	Mean degree of polymerization
MM-GPC	Molecular mass estimated by gel permeation chromatography
EC-ext	Epicatechin extension subunit
EGC-ext	Epigallocatechin extension subunit
ECG-ext	Epigallocatechin gallate extension subunit
C-ext	Catechin extension subunit
C-ter	Catechin terminal subunit
ECG-ter	Epigallocatechin gallate terminal subunit
EC-ter	Epicatechin terminal subunit

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