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Brassinosteroid-induced quality enhancement in 'Flame Seedless' grapevine: impact on harvest and post-harvest attributes under open and covered cultivation systems

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ABSTRACT

Early grape maturation is an important trait for Egypt's grape export program to meet international market demands on time. A plastic-covering technique along with exogenous brassinosteroids (BR) treatments was used on 'Flame Seedless' grapes, in a two-year experiment to assess fruit quality at harvest and during storage. The results indicated that vines protected by plastic cover benefit from early maturation irrespective of BR treatment, with a mean harvest time two weeks earlier than that of grapes grown without the cover. However, this also resulted in a decrease in grape anthocyanin content. The application of BRs could partly compensate for this insufficiency, concurrently enhancing the grapes' qualitative attributes and storage potential. The upregulation of selected anthocyanin biosynthesis-related genes such as chalcone synthase *(CHS)*, flavanone 3-hydroxylase *(F3H)*, and UDP-glucose-flavonoid 3-*O*-glucosyltransferase *(UFGT)* was confirmed to be in a linear and concentration-dependent relationship with BR treatments and associated with significantly increased anthocyanin content. Based on our research, it appears that the combined use of a plastic cover with elevated concentrations of brassinosteroids (0.8 ppm) is a potentially effective strategy for expediting the harvest season and preserving shelf life and the quality of grapes during cold storage.

KEYWORDS: gene expression, plastic cover, antioxidant, grape, cold storage

INTRODUCTION

Worldwide grape (*Vitis vinifera* L.) production was 73,524,196 metric tons in 2021, reflecting a decrease of 4.5 % compared to the 76,997,321 metric tons produced in 2020 (Food and Agriculture Organization of the United Nations, 2023). China held the top position as the primary producer of grapes, accounting for 15.2 % of total global production (Food and Agriculture Organization of the United Nations, 2023). Italy was the second highest contributor with 11.1 %, while Spain followed closely with 8.3 % (Food and Agriculture Organization of the United Nations, 2023; Mishra *et al.*, 2024). Egypt, on the other hand, ranked fifteenth, contributing 1.4 %. Grapes are a widely cultivated fruit crop in Egypt, ranking second in popularity only to citrus fruits. In the marketing year 2023-2024, the output of table grapes in Egypt is predicted to reach 1.57 million metric tons, with an estimated export volume of 170,000 metric tons (Food and Agriculture Organization of the United Nations, 2023).

The 'Flame Seedless' grape is praised as a top choice among consumers and is a widely acknowledged grape cultivar in the Egyptian market due to its early ripening and excellent cluster quality. The appearance, quality, cluster size, and form of 'Flame Seedless' grapes determine their commercial value (Abdel-Sattar *et al.*, 2022). Hence, it holds considerable importance for local and international markets, particularly for exports to European countries (Doaa, 2018). Given the fact that most global markets do not have access to a year-round supply of grapes in Egypt, early maturation is a key component of Egypt's grape export program.

Grape growers worldwide face significant challenges associated with climate change and its detrimental effects on grape quality (Müller *et al.*, 2023). Grapevines are perennial plants with a distinct geographical distribution pattern occupying certain regions. They are situated in climate sectors which pose relatively higher risks of both short and long-term climatic variations. Grapevines are sensitive to extreme temperature conditions (Venios *et al.*, 2020), and the effects of high temperatures on *V. vinifera* have been thoroughly studied in earlier research (Greer & Weedon, 2013). Like many plant species, grapevines respond to temperature stress via built-in physiological adaptations. However, processes such as plant respiration, transpiration, and photosynthesis are sensitive to transient temperature changes. Photosynthesis, the plant's most important physiological process, is both directly and indirectly affected by temperature (Venios *et al.*, 2020). Delayed berry development, late ripening, and substantially lower berry quality are among several consequences of high temperatures, signifying a subsequent economic impact for growers (Greer & Weedon, 2013). To lessen these impacts, it is essential to develop methods that decrease the impact of high temperatures.

Given the progression of climate change, research programs have focused their objectives on identifying and analysing high-quality production strategies within certain climatic zones, more effectively addressing challenges posed by these environmental changes. The shading approach using materials like plastic films to protect vineyards by altering the microclimate can be a feasible strategy to regulate grape ripening according to market demand (Vox *et al.*, 2014). As reported earlier, covering grapevines with polyethylene film significantly enhanced the regulation of phenological phases, in particular the ripening time of 'Flame Seedless' grape in Egypt (Salem *et al.*, 2021). Cultivating grapevines beneath plastic covers presents a promising opportunity for achieving early ripening and improving the possibility of exporting to European markets, leading to increased profitability. Managing contemporary greenhouse microclimates to provide the minimal cooling requirement of 'Flame Seedless' table grapes allows producers to strategically plan the harvest for early June (in the northern hemisphere), when prices are significantly higher. The controlled environment of shaded vineyards can provide enhanced growing conditions by reducing direct sun exposure and buffering day and night temperature fluctuations, resulting in earlier harvests and better quality of grapes for export (Alonso *et al.*, 2021). Anthocyanin content and grapevine fruit ripening periods are significantly influenced by temperature (Ryu *et al.*, 2020). Therefore, optimising the grape maturation period should simultaneously produce berries of high nutrient content and post-harvest stability without compromising quality.

As the main indicator of grape berry colour and quality, anthocyanins were the subject of several studies on content and composition enhancement using exogenous stimulants such as abscisic acid (Shahab *et al.*, 2020; Salama *et al.*, 2023), allantoin (Moriyama *et al.*, 2020), brassinosteroids (Vergara *et al.*, 2018), (+)-cis,trans-abscisic acid and ethephon (İşçi *et al.*, 2020), methyl jasmonate, ethephon, and melatonin (Salama *et al.*, 2023), and gibberellin (Xue *et al.*, 2022). Brassinosteroids, a recently discovered glass of plant hormones, can be safely incorporated into table grape production to regulate several physiological processes and enhance the overall quality of the fruit (Asghari & Rezaei-Rad, 2018).

This study presents a practical method for identifying the applicability of a covered system to regulate required temperatures for the 'Flame Seedless' grape variety, from budbreak through maturity. We also investigated the effect of brassinosteroid applications on fruit quality parameters such as anthocyanin content. Anthocyanin biosynthesis pathway gene expression was also explored to link observed fruit quality traits to their corresponding molecular mechanisms.

MATERIALS AND METHODS

1. Field and experimental design conditions

This research was conducted in Egypt in a commercial vineyard during the 2022 and 2023 seasons. Seven-year-old uniform and disease-free vines of the 'Flame Seedless' table grape cultivar on 'Freedom' rootstock were chosen for their high-quality berries. The vines are located at the Cairo-Alexandria desert road, Wadi El Natrun, Beheira governorate, Egypt (30° 19' 11" N 30° 32' 01" E).

The vines were planted with a designated spacing of 2 meters between individual plants and 3 meters between adjacent rows, similar to the system reported by Ghoneem *et al.* (2024). A drip irrigation system was installed to provide water for the experimental plants. The system adhered to the standard agricultural methods for vineyards in the area, which include soil fertilisation, pruning, and pest control. 'Flame Seedless' vines were trained using the *pergola* system. Vine pruning occurred throughout the first week of January, leaving a load of 82 buds/vine (12 fruiting canes × 6 buds + 5 spurs × 2 eyes). The experimental vines were shielded with plastic coverings from mid-January to the end of April during both study seasons. The plastic covering used was a 100-µm-thick, 90 % light-transmitting semi-transparent polyethylene film (AL-Kuds for Plastic Products Company, Egypt).

The plastic covering was set at a height of 3.30 m above the sandy soil surface to regulate the internal climate of the vineyard rows, as shown in Figures 1 and 2. The greenhouse was closed until the interior temperature reached 35 °C, then opened to prevent overheating. Passive ventilation was maintained by lifting and lowering plastic sheet levels on the sides as needed until the vines reached the veraison stage. The plastic cover was then gradually detached until it was completely removed. Meteorological data from the Sadat City Meteorology Station were obtained to monitor the external climatic factors. Concurrently, a sophisticated data logger (TM-305U, Tenmars Electronics Co., Ltd, Taipei, Taiwan) was deployed beneath the plastic covering to measure real-time temperature and humidity, ensuring the precision and reliability of the environmental data recorded during the experiment (Table S1).



FIGURE 1. BR-treated `Flame Seedless' table grapevines grown under plastic covering.



FIGURE 2. BR-treated `Flame Seedless' table grapevines grown in the open field.

The trials were conducted as a factorial experiment using completely randomized blocks designed, in triplicate, with two factors: first, the effect of the plastic covering, and second, the effect of foliar treatments of differing brassinosteroid concentrations (0, 0.2, 0.4, 0.6, and 00.8 ppm BRs; 10 treatments × 3 replicates × 3 vines) following the recommendations of Babalık *et al.* (2020). The brassinosteroid (22(S),23(S)-Homobrassinolide) was obtained from Sigma Aldrich Co., USA, and solutions were prepared by sequentially dissolving the BRs first in 500 µl ethanol and then to their designated final concentrations using tap water. To improve the absorption and distribution of the BR solution, Tween 20® (Sigma Aldrich Co., USA) was used as a surfactant (0.1 % concentration). Control plants received the same preparation without adding the BRs. On the experimental plants, five liters of the BR solutions were sprayed onto the clusters at the pea stage (berry size of approximately 4-5 mm in diameter) and then during veraison when approximately 10 % of the berries in 50 % of the clusters turned soft and start changing color (Champa *et al.*, 2015). Spraying was carried out in the morning using a sprayer machine (Power Sprayer Machine, Honda, Tanta, Egypt) until solution runoff occurred.

2. Measurements and analysis

2.1. Harvest and post-harvest weight stability assessment

Clusters were meticulously collected at commercial maturity from each block replication for the control treatment (4-5 clusters per box) based on their soluble solids content that was measured using an RFM 340-T refractometer (SSC) > 16-17 %) according to El-kenawy (2017). There were four carton boxes, representing the experimental treatments, each containing 2 kg of clusters (4-5 clusters per box). The clusters from each treatment were packaged into plastic crates and transferred in an air-conditioned vehicle to the laboratory of the Sakha Research Station in Kafr El-Sheikh.

Following the harvest, clusters that were free of discernible defects were selected and separated into two groups. One group was subjected to an initial assessment of quality (day 0), while the other group were stored in 40 × 25 × 15 cm carton boxes containing encased dual-release SO_2-producing pads (Grapage® 40 %-Sodium Metabisulphite, JK Enterprises, Pune, India). Each treatment was represented by three replicate carton boxes, each containing 2 kg of grape clusters, equivalent to approximately 4-5 clusters per box. This represents a total of 12-15 clusters per treatment used for analysis. To assess post-harvest quality each box's weight was recorded four times on the day of harvest and then every 10 days after to evaluate the decrease in cluster weight during cold storage (1 ± 1 °C, relative humidity 90-95 %). The weight loss percentage was then calculated after four days of storage at 23 ± 3 °C to simulate real life conditions for each of the five indicated groups (0, 10, 20, 30, and 40 days).

2.2. Physical and chemical parameters

Cluster weights and the combined weight of 100 individual berries were measured on a digital scale. Fruit dimensions, including length and breadth, were determined using a Vernier calliper. The firmness of randomly selected berries (n = 30) was assessed using a penetrometer pressure tester (handheld Shimpo digital force gauge, FGV-50XY, Wilmington, NC, USA), which was equipped with a 1 mm-diameter probe. Results were expressed in Newton per square centimetre (N/cm^2). Berry removal force was measured using a penetration pressure gauge and expressed in Newtons (El-Abbasy *et al.*, 2015). Grape berry juice is made by hand-pressing a total of 16 berries chosen and pooled from each cluster's upper, middle, and lower portions. The juice was analysed for SSC determination by an RFM 340-T refractometer (KEM Kyoto E.M. Co. Ltd., Japan) and expressed in degrees Brix (°Brix), corresponding to the percentage of SSC. Titratable acidity (TA) was determined by measuring tartaric acid concentration (%) with an automated titrator (TitroLine, TL 5000, SI Analytics, Weiheim, Germany). Total anthocyanins were quantified using a spectrophotometer (1800 UV-VIS Shimadzu Inc., Kyoto, Japan) at 520 nm using extracts obtained from a half-gram of berry skins, using 95 % ethanol and 1.5 M HCl (85:15 *v/v*) (Iland *et al.*, 2004). The mixture was incubated under dark conditions at room temperature for 24 hours to ensure complete pigment extraction.

Physiological weight loss was estimated by subtracting the final weight of grape clusters from the initial, presented as a percentage of the original weight. For each cluster, the extent of berry shatter was determined by dividing the weight of loose berries by the total weight of their corresponding cluster. Berry decay rate was quantified by dividing the weight of the rotten berries by the total weight of their corresponding cluster, expressed in percentage (%).

2.3. Relative gene expression analysis

Total RNA isolation, cDNA synthesis, and qRT-PCR were conducted on berry peels sampled at harvest from the vines treated with brassinosteroids under both open fields and plastic cover conditions. The RNeasy Mini Kit was used to extract RNA from 500 mg of berry peels that were instantly frozen using liquid N_2 (Elmoslemany *et al.*, 2021). DNase treatment was conducted using DNase I enzyme according to the manufacturer's protocol (Thermo Scientific). To produce cDNA, 4 µg of normalized intact total RNA (assessed by NanoDrop 1000 spectrophotometer/260 nm) was reverse-transcribed by Quantiscript reverse transcriptase enzyme (Thermo Scientific, Waltham, MA, USA) and used as a template for relative expression analysis of anthocyanin biosynthesis pathway genes in a 'Step One Plus' real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). The primer sequences of *F3H*, *CHS*, *UFGT*, and β-actin are listed in Table S2 (Salama *et al.*, 2023). The PCR reaction consisted of 2 × QuantiTect SYBR Green qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), cDNA template, forward and reverse primers, and nuclease-free water. The target gene's threshold cycles was normalised using β-actin and fold changes were determined by the $2^{-\Delta\Delta Ct}$ method (Rao *et al.*, 2013).

3. Statistical analysis

The data were first assessed for normality and variance homogeneity using Shapiro-Wilk's and Levene's tests, respectively. A randomised block design was used with two growth conditions (open fields (uncovered) and plastic covering (covered)) × five brassinosteroid treatments × three replicates, totalling 50 plots. A combined analysis was conducted on the data from two consecutive years (Moore & Dixon, 2015). Statistical analysis was performed using SPSS Statistics version 26 (SPSS, Inc., Chicago, IL, USA), and value differences were considered significant at a 95 % probability level ($p < 0.05$). Data homogeneity was confirmed, as the error mean squares (EMS) of the two years differed by less than a factor of 10. The data were therefore considered homogeneous before merging the variables. Duncan's multiple range test was used to compare the means, with a significance threshold of 0.05 (Duncan, 1955).

RESULTS

This research examined the impact of five different concentrations of brassinosteroids (0, 0.2, 0.4, 0.6, and 0.8 ppm) on the quality of 'Flame Seedless' grapes at harvest and during cold storage. The grapes were grown in an open field with and without plastic covering. By carefully observing how 'Flame Seedless' grapes grow and ripen during the growing seasons of 2022 and 2023, it was found in both years that plastic covering accelerated all phenological stages, including bud burst, blooming, veraison, and ripening. Covered vines were ready to be picked 10 and 12 days earlier in the first and second seasons, respectively, compared to vines without any cover (data not shown).

1. Cluster and berry weights

The effects of varying concentrations of BRs on grape cluster weight and the weight of 100 individual berries under uncovered and plastic-covered conditions are illustrated in Table 1. This study revealed high variations in cluster weight between the different treatments. Under uncovered conditions, the control group (no BRs) had the lowest cluster weight (429.0 g), while the presence of a plastic cover resulted in a slightly greater average weight (439.6 g). Brassinosteroid treatments resulted in concentration-dependent yields of 'Flame Seedless' grape. Treatments with 0.6 and 0.8 ppm BRs resulted in the highest average (covered and uncovered) cluster weights of 619.5 g and 626.9 g, respectively. The highest BR concentration (0.8 ppm) increased the cluster weight from both uncovered and covered vines by 45.5 % and 43.0 %, respectively, when compared with their control (no BRs) counterparts. Similarly, the plastic cover slightly increased the weight of 100 berries from 329.0 g (open field) to 335.2 g (plastic cover) in control groups. Brassinosteroid treatments significantly improved the average weight of 100 berries under both covered and uncovered conditions Under open field conditions, only the highest BR concentration (0.8 ppm) resulted in a significant increase in 100 berries weight, but interestingly under the plastic cover system, all of the BR treatments, including the 0.2 ppm, significantly improved this trait (Table 1). This confirmed the significantly positive effect of BRs at 0.6 and 0.8 ppm on grape yield under both open and protected conditions.

2. Berry length and diameter at harvest

The impact of BR treatments on berry length and diameter from covered and uncovered vines is illustrated in Table 2. Without BR treatment, the covered group had slightly, but not significantly, higher berry length (18.44 mm) than the control group (17.61 mm); berry diameter increased significantly ($p < 0.05$). An apparent relationship between brassinosteroid concentrations and their impact was noted.

Under both uncovered and covered conditions, only the 0.6 and 0.8 ppm BR treatments resulted in significantly increasing berry length (Table 2). While all of the BR treatments significantly increased the berry diameter under uncovered conditions, the 0.2 ppm treatment did not significantly improve berry diameter. Comparing the mean values from both covered and uncovered conditions demonstrated the significant positive effects of all BR concentrations in improving berry length and diameter (Table 2).

TABLE 1. Impact of foliar brassinosteroid (BR) treatments on cluster weight and 100 berry weight of `Flame Seedless' grapevines grown under uncovered and covered conditions at harvest.

	Cluster weight (g)			100 berries weight (g)		
	Uncovered	Covered	Mean	Uncovered	Covered	Mean
Control	429.0 ^{b}	439.7 ^{b}	434.3 ^{b}	329.0 ^{b}	335.3 ^{b}	332.2 ^{b}
0.2 ppm BRs	471.8 ^{ab}	469.3 ^{b}	470.6 ^{b}	331.2 ^{b}	456.7 ^{a}	393.9 ^{a}
0.4 ppm BRs	461.8 ^{ab}	555.2 ^{ab}	508.5 ^{b}	338.5 ^{b}	460.0 ^{a}	399.2 ^{a}
0.6 ppm BRs	623.0 ^{a}	616.0 ^{a}	619.5 ^{a}	349.5 ^{ab}	466.7 ^{a}	408.1 ^{a}
0.8 ppm BRs	625.0 ^{a}	628.8 ^{a}	626.9 ^{a}	396.7 ^{a}	455.0 ^{a}	425.8 ^{a}

Different letters in the same column denote significant differences among treatments within one growing condition according to Duncan's test ($p < 0.05$, n = 12-15).

TABLE 2. Impact of foliar brassinosteroid (BR) treatments on the berry length (mm) and berry diameter (mm) of uncovered and covered `Flame Seedless' grapevines at harvest.

	Berry length (mm)			Berry diameter (mm)		
	Uncovered	Covered	Mean	Uncovered	Covered	Mean
Control	17.61 ^{c}	18.44 ^{c}	18.02 ^{c}	16.25 ^{d}	16.05 ^{b}	16.15 ^{c}
0.2	18.28 ^{bc}	19.03 ^{c}	18.65 ^{c}	17.03 ^{c}	17.85 ^{ab}	17.45 ^{b}
0.4	18.80 ^{abc}	19.73 ^{bc}	19.26 ^{b}	17.88 ^{bc}	18.11 ^{a}	17.99 ^{b}
0.6	19.84 ^{ab}	21.03 ^{b}	20.43 ^{b}	18.17 ^{b}	18.67 ^{a}	18.42 ^{a}
0.8	20.20 ^{a}	23.77 ^{a}	21.99 ^{a}	19.20 ^{a}	18.31 ^{a}	18.75 ^{a}

Different letters in the same column denote significant differences among treatments within one growing condition according to Duncan's test ($p < 0.05$, $n = 12$-15).

3. Anthocyanin content and expression of *UFGT, F3H,* and *CHS* genes

The control 'Flame Seedless' grapes and those treated with different BR concentrations from covered and uncovered systems are shown in Figure 3. The grapes harvested from the covered system had lower anthocyanin content than their uncovered counterparts. Across all treatments, the average anthocyanin concentration in the grapes from uncovered vines exceeded that of grapes from vines under plastic cover by 26 % ($p < 0.05$) (Figure 4A). Furthermore, anthocyanin content increased in a consistent linear relationship with applied BR concentrations for both covered and uncovered grapevine berries (Figure 4B). The highest average anthocyanin content (1411.0 mg/l), measured from the berries of vines treated with 0.8 ppm BRs, was approximately 37 % higher than the no-BR controls. Under uncovered conditions, anthocyanin content was significantly higher ($p < 0.05$) and concentration-dependent, even at the lowest BR treatment (0.2 ppm) (Figure 4C). The vines under plastic cover also produced more anthocyanins, but the increase was significant only from 0.6 ppm BRs (Figure 4D). These results highlight the significant impact of brassinosteroid application on promoting anthocyanin formation.

To further explore the core effects of BRs on anthocyanin biosynthesis, selected genes were targeted for transcriptional analysis. The expression of three genes implicated in anthocyanin biosynthesis was investigated in 'Flame Seedless' berry skins after BR treatments under both uncovered and covered systems (Figure 5).

The obtained data demonstrate that plastic covering and applied BR treatments have a significant effect on the expression of UDP-glucose-flavonoid 3-*O*-glucosyltransferase *(UFGT)*, flavanone 3-hydroxylase *(F3H)*, and chalcone synthase *(CHS)* genes in grape berry skins. On average, the covered group exhibited greater expression of the selected genes than the uncovered group (Figures 5A, 5B and 5C). Under both covered and uncovered systems, compared to the non-BR-treated control plants, BR treatment at all concentrations significantly increased *UFGT*, *F3H,* and *CHS* gene expression in berry skins (Figures 4D, 4E and 4F).

This pattern indicates that these biosynthesis genes respond to BR treatment independently of the covering system. The observed similarity in trends between the open-field and plastic cover groups implies a basic relation between BRs signalling and gene expression. In case of the uncovered group, applying 0.2 ppm BRs caused a significant ($p < 0.05$) 1.95-fold increase in *UFGT* expression. Subsequently, the fold change values showed a linear relationship with BR concentrations, reaching 4.14-, 5.98-, and 8.63-fold for 0.4 ppm, 0.6 ppm, and 0.8 ppm BRs, respectively (Figure 5G). The same pattern was detected with *F3H* and *CHS* expression (Figures 4H and 4I). Similarly, in the covered group, *UFGT* biosynthesis gene expression displayed an observable response to BR treatment, with the application of 0.2 ppm BRs resulting in a 2.48-fold increase in *UFGT* expression (Figure 5J). *UFGT* expression fold changes were 5.90, 7.89, and 11.24 for 0.4 ppm, 0.6 ppm, and 0.8 ppm BRs, respectively (Figure 5J). The same pattern of gene expression induction was also observed with *CHS* and *F3H* (Figures 5K and 5L).

4. Berry firmness and removal force

As shown in Figure 6, grape berries showed notable differences in firmness and removal force both at harvest and during cold storage, depending on the covering system and BR application. The impact of BR treatment on fruit firmness and removal force varied between covered and uncovered systems, as well as with BR concentrations. The plastic covering significantly improved fruit firmness and removal force of berries simultaneously treated with 0.8 ppm BRs. Grapes treated with the highest concentration of BR (0.8 ppm) showed a significant increase in firmness and removal force compared to those of the control groups (uncovered and 0 ppm BRs). The slope of decrease in fruit firmness was less linear in berries from the covered system paired with higher BR treatments. After 40 days of storage, 0.8 ppm BR-treated plants produced berries with considerably higher firmness (Figure 6A) and removal force (Figure 6B) compared to the controls of both covered and uncovered berries.

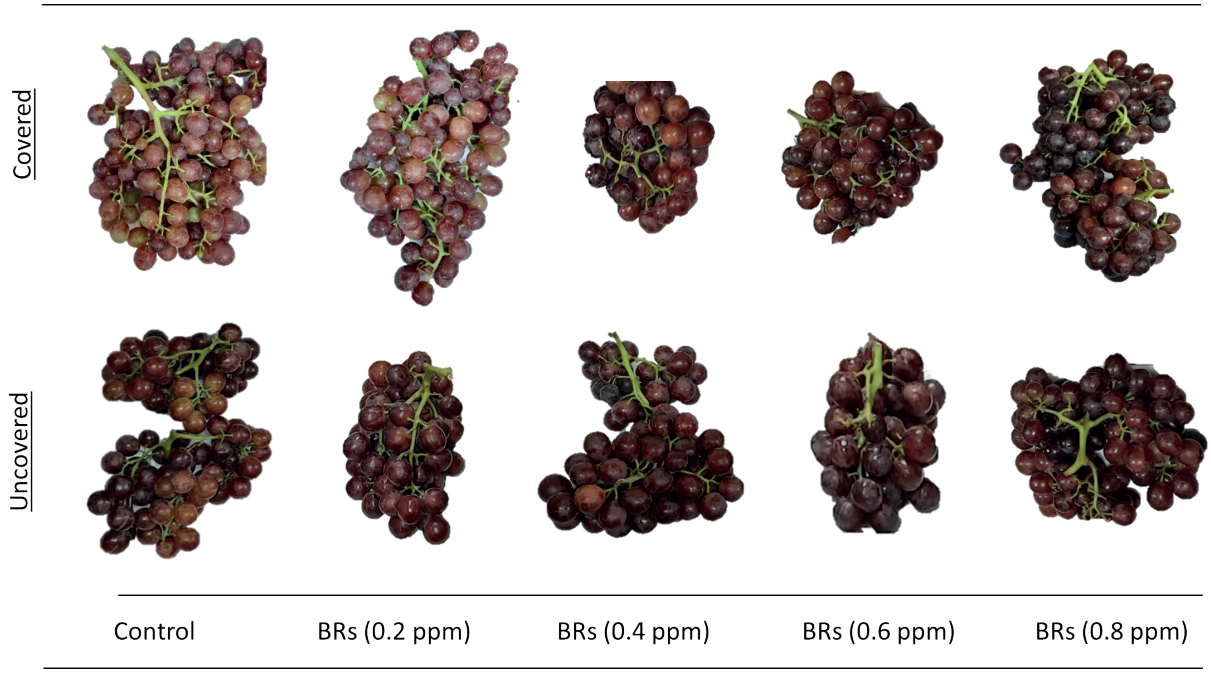


FIGURE 3. `Flame Seedless' grape clusters from covered and uncovered systems with and without different concentrations of foliar brassinosteroid (BR) treatments at harvest time.

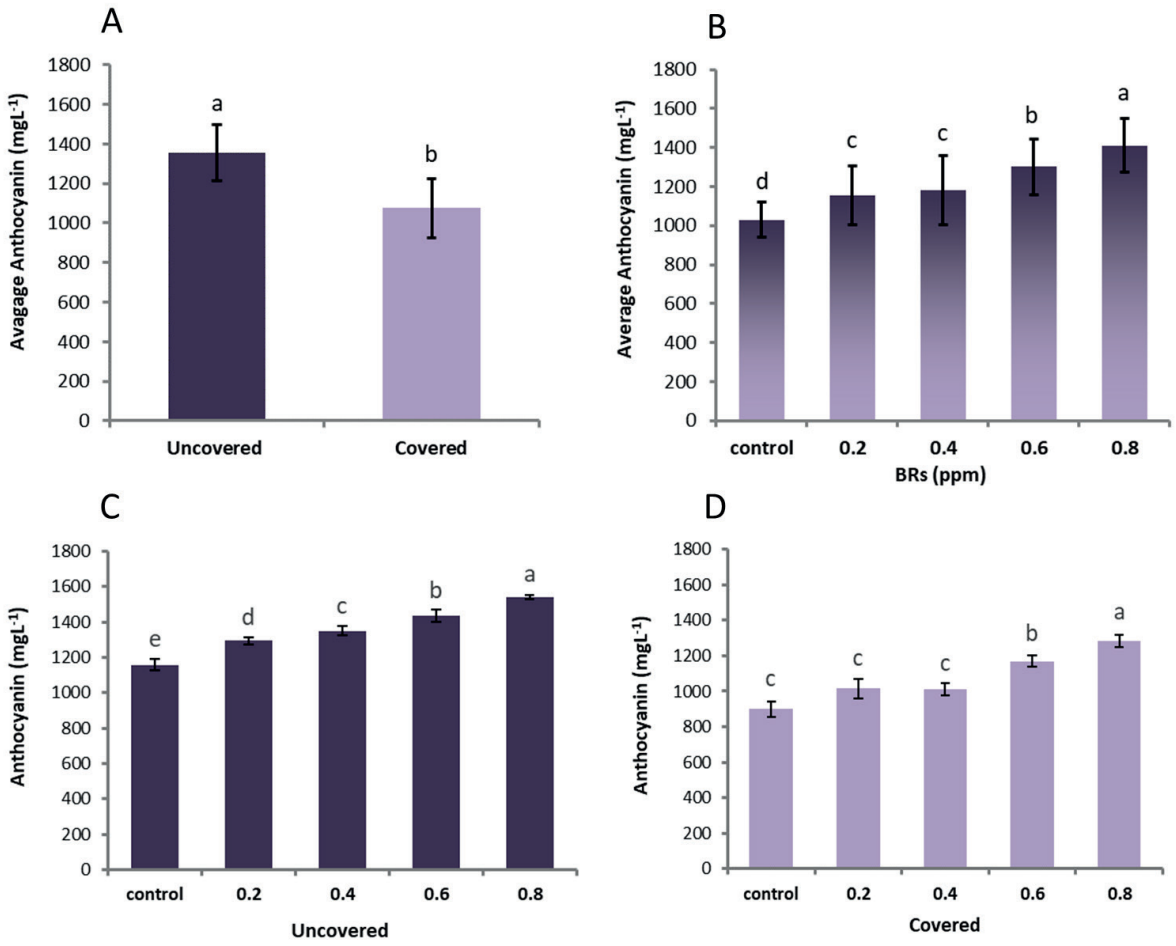


FIGURE 4. Impact of uncovered and covered (A), brassinosteroid (BR) treatments (B), and their combined effect (C & D) on the anthocyanin content of `Flame Seedless' grape berries at harvest.

Different letters indicate significant differences according to Duncan's test at $p < 0.05$ (n = 12-15).

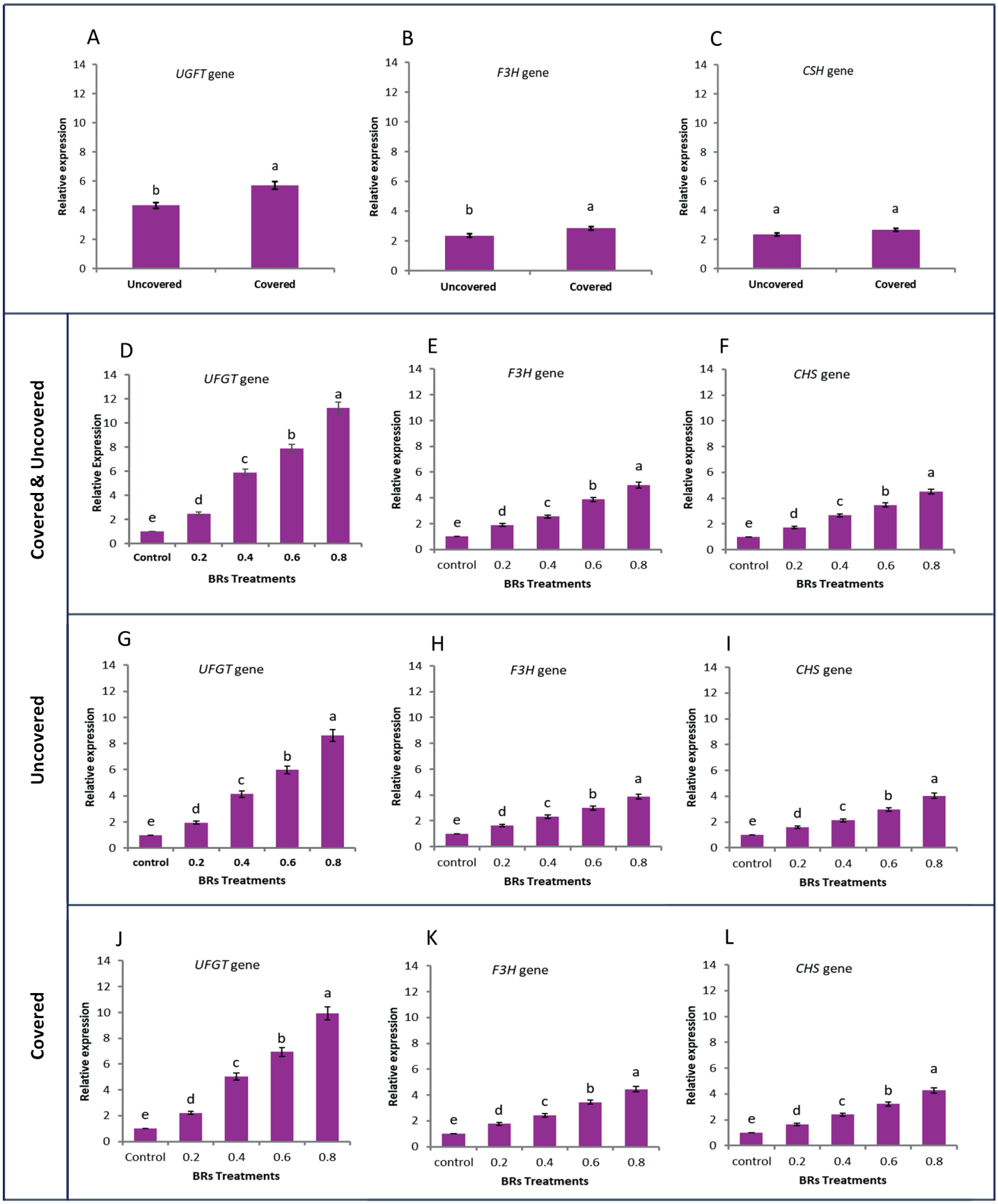


FIGURE 5. Quantitative Real-Time qRT-PCR analysis of the expression of *UFGT*, *F3H*, and *CHS* genes in `Flame Seedless' grape berries under uncovered and covered conditions (A-C), BR treatments (D-F), under BR treatments of uncovered (G-I) and covered (J-L) `Flame Seedless' grapes berries.

The different letters indicate significant differences between the values according to Duncan's test at $p < 0.05$ ($n = 3$).

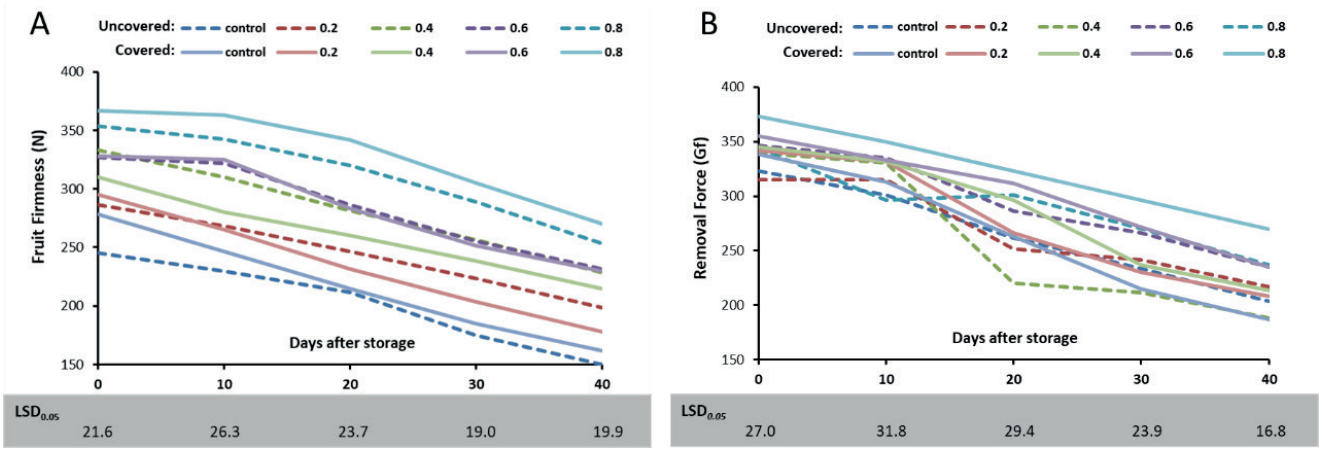


FIGURE 6. Impact of brassinosteroid treatments (0.2, 0.4, 0.6, and 0.8 ppm) on fruit firmness (A) and removal force (B) of covered and uncovered `Flame Seedless' grapevines at harvest and during cold storage at 1 ± 1 °C (n = 12-15).

5. Soluble solid content and titratable acidity

Brassinosteroids treatment significantly affected the soluble solids concentration (SSC %) and titratable acidity levels (TA %) in grapes (Figures 7A and 7B). At harvest and during cold storage, the uncovered group consistently exhibited higher SSC % values than the covered group (Figure 7A). After 40 days of storage, the uncovered group treated with the highest concentration of BRs (0.8 ppm) exhibited the highest SSC (21.05 %), slightly higher than that of the covered group (18.6 %), suggesting BRs have a regulatory effect on grape soluble solid content.

The data revealed distinct variations in TA % between the two growing conditions and in response to BR treatments (Figure 7B). The administration of BRs caused a decrease in the acidity of grapes from the covered system more consistently than in the uncovered groups. Berry acidity decreased during the storage period and reached lower levels in both covered and uncovered berries.

6. Berry decay and shatter percentage at harvest and during storage

BRs' influence on berry decay and shatter was observed between the uncovered and covered groups. Berry decay rate comparisons between the uncovered and covered groups revealed that BRs exhibit a regulatory effect on this important trait (Figure 7C). Under uncovered conditions, without BRs treatment, the berry decay rate increased to 3.24 % after 40 days of storage, higher than that of grapes from the covered system (2.68 %). Treatment with 0.8 ppm BRs had the most pronounced effect on reducing berry decay after 40 days of cold storage in both covered and uncovered berries. Brassinosteroid treatments at 0.4, 0.6, and 0.8 ppm had the greatest impact on reducing fruit decay rate during storage, especially in berries from the covered system.

The covered group showed a significantly lower (approximately 50 %) berry shatter rate after 40 days of cold storage (Figure 7D). BR treatments resulted in significantly decreasing berry shatter in a concentration-dependent manner under both uncovered and covered conditions (Figure 7D). Across the BR concentrations applied, 0.8 ppm BRs produced the most significant reduction in berry shatter rate.

7. Weight loss percentage during storage

'Flame Seedless' grapes treated with 0.6 and 0.8 ppm of BRs showed the lowest rate of weight loss after 40 days of cold storage at 1 ± 1 °C and 90 % relative humidity (RH) in both uncovered and covered conditions (Figure 7E). At 10, 20, and 30 days of cold storage, grapes progressively increased weight loss percentage, as typically expected.

When grapes were stored for four days at 22 ± 2 °C and 40-45 % RH, berries from the 0.8 ppm treatment paired with the covered system demonstrated the lowest rate of weight loss. (Figure 7F). Similar to the cold storage group, increasing weight loss was also observed after four days of shelf life.

DISCUSSION

1. `Flame Seedless' grape maturation and quality

Climate change is likely to cause more extreme weather events, which most of Egypt is preparing for. These actual and anticipated events should be the primary considerations of the grape industry as it seeks to adapt to climate change. Consequently, it is of the utmost importance to develop efficient methods to mitigate the negative effects of climate change-induced temperature fluctuations.

One short-term adaptation method is to implement coverings; this strategy aims to reduce risks and maximise benefits in response to actual and expected consequences of regional climate change (Hewer & Brunette, 2020). Although covering systems for grapevine cultivation have been widely studied and implemented in several viticultural regions, it remains relatively underutilised in Egypt, where it could represent a potentially lucrative vineyard management method.

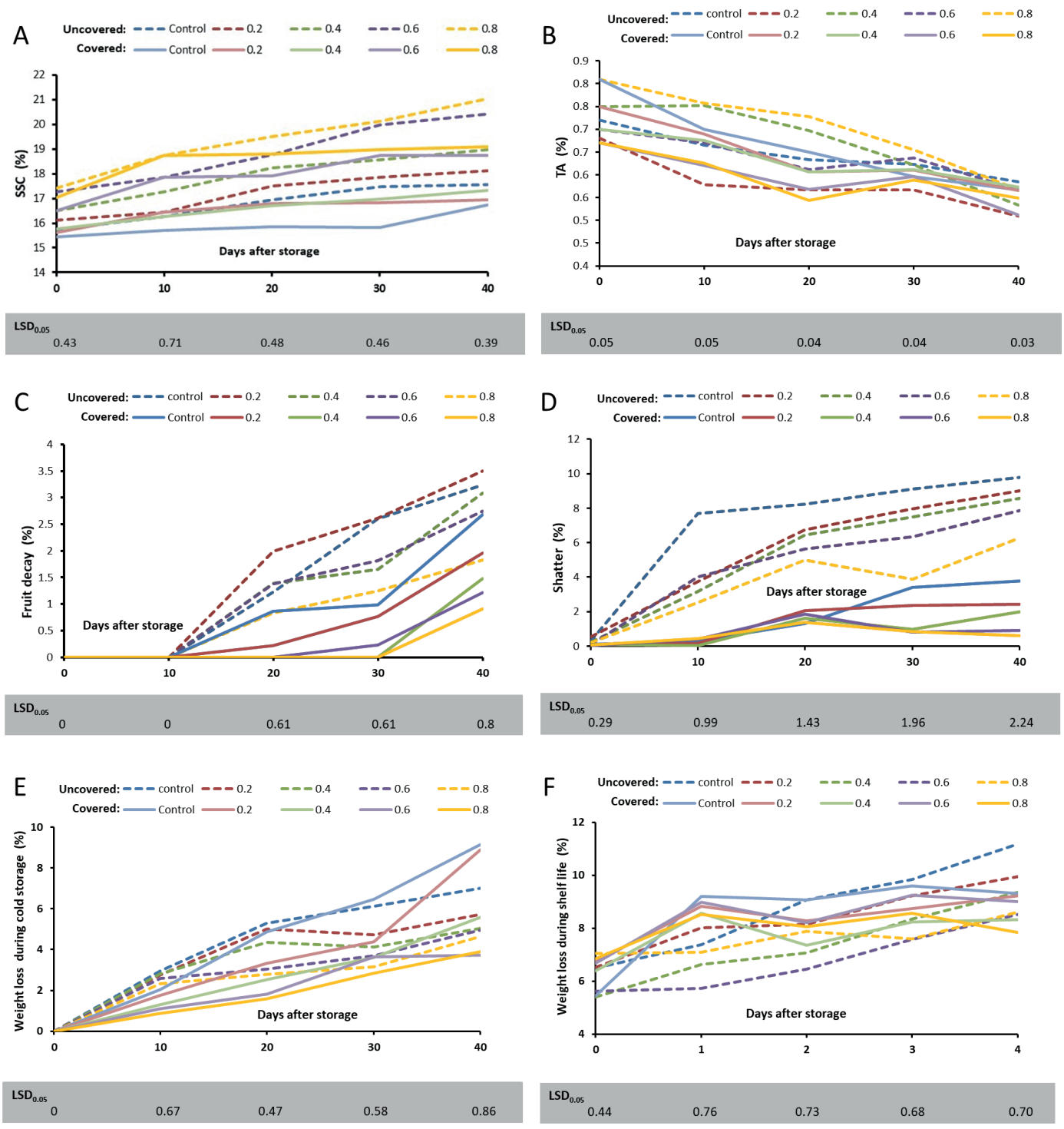


FIGURE 7. Impact of brassinosteroid (BR) treatments on uncovered and covered `Flame Seedless' grapevines on A: soluble solid content (SSC %), B: titratable acidity (TA %), C: fruit decay (%), D: berry shatter rate (%), E: weight loss during 40 days of cold storage (%), and F: weight loss during four days of shelf life. *n* = 12-15.

This technique promotes grape maturation and quality (Alonso *et al.*, 2021), thereby improving the likelihood of exporting grapes to European markets in a more timely manner (Mohamed El-Saeed *et al.*, 2015; Salem *et al.*, 2021). Our findings show a consistent pattern over the experimental timeframe, which aligns with previous results on improved harvest times and berry quality (Alonso *et al.*, 2021; Mohamed El-Saeed *et al.*, 2015; Salem *et al.*, 2021). Our research indicates that using plastic as a protective covering for vines leads to accelerated ripening and harvest times by approximately 10 and 12 days in both seasons, respectively, compared to leaving them uncovered. Using this idea, Mohamed El-Saeed *et al.* (2015) demonstrated that employing plastic coverings in grape cultivation establishes a microenvironment characterised by diminished solar radiation, elevated maximum temperatures,

and heightened saturation deficit levels, leading to earlier grape maturation and enhanced prospects for exporting to the European market. Salem *et al.* (2021) showed that plastic coverings improve grapevine growth and development at every stage. Coverings elevate January temperatures under the plastic sheets, leading to more precocious budbreak.

Regarding brassinosteroids, Zheng *et al.* (2020) reported that natural brassinosteroid levels increase in table grapes from the onset of ripening, indicating these phytohormones play a positive role in berry growth and ripening. Furthermore, Champa *et al.* (2015) discovered that external BRs may enhance skin colour and sugar accumulation, facilitating the ripening process. This study's results demonstrated that BRs can enhance fruit ripening, accelerate harvest dates while simultaneously maintaining fruit quality, and delay fruit decay during storage. In both climacteric fruits, such as pears (Ji *et al.*, 2021) and non-climacteric fruits such as grapes (Xu *et al.*, 2015) and litchi (Ma *et al.*, 2021). BRs were shown to regulate fruit ripening in similar ways (Xu *et al.*, 2015; Champa *et al.*, 2015; Ma *et al.*, 2021; Li *et al.*, 2023a). Another possible ripening benefit of BRs is on monosaccharide synthesis, which hastens the breakdown of cell wall components, the primary cause of fruit softening (Pei *et al.*, 2023).

Our experiments on 'Flame Seedless' berries showed that plastic covering and BR treatment have positive effects on berry traits, cluster weights, and 100 berry weight (Tables 1 and 2). This indicates a positive regulatory effect of BRs on cluster development, which was particularly pronounced under the protective environment of the plastic cover, which creates a conducive microenvironment (Alonso *et al.*, 2021). The observed increase in cluster weights and 100 berry weight also suggests BRs play a regulatory role in grapevine reproductive development (Babalık *et al.*, 2020; Li *et al.*, 2023a). Sweetman *et al.* (2014), however, reported that the decrease in berry weight in high-temperature conditions may be attributed to an increased deficit in vapour pressure in environments with elevated temperatures and lower humidity levels. After performing a comparative analysis, it was shown that the use of plastic coverings resulted in increased berry lengths and diameters compared to those in open field conditions. Application of varying BR concentrations both to the open field and plastic cover setups resulted in longer berries. However, this effect became more noticeable with increased concentrations of BRs. This suggests a synergistic association of plastic coverings and BRs, augmenting berry size, which agrees with the findings of both Bedrech *et al.* (2022) and Salem *et al.* (2021). The parallel trends observed between the open-field and plastic cover groups highlight the regulatory influence of BRs on the morphology of grapevine berries, in particular on berry length and diameter. Plastic cover, which acts to create a protected and modulated environment (Mohamed El-Saeed *et al.*, 2015), appears to amplify the positive effects of BRs on berry development. These observations may be attributed to the amplifying effect of BR-induced berry size enlargement combined with a plastic cover (Li *et al.*, 2023b).

As mentioned previously, our findings are also in line with those of Tadayon and Moafpourian (2019), Champa *et al.* (2015), and Belal (2019). All of which demonstrated that compared to the control group, berries sprayed with a high concentration of BRs had greater cluster weight, berry weight, and berry length. Additionally, BRs also promote plant growth and development, as described by Nolan *et al.* (2020), who reported that these hormones increase cell elongation by controlling cell growth, and Oh *et al.* (2020) who similarly found that BRs are essential for cell growth and elongation. Another study by Li *et al.* (2023c) revealed that BRs affect cell wall composition and characteristics by cleaving and restitching xyloglucan endotransglucosylase/hydrolase, essential for cell expansion and elongation.

2. Anthocyanin content and *CHS*, *F3H*, and *UFGT* gene expression in grapes

Understanding how BRs may affect anthocyanin biosynthetic pathway gene expression is of high interest for viticulture. A comparative analysis of the open field and plastic cover systems indicated that the open field environment often increased anthocyanin levels (Figure 3A). The average anthocyanin content of grapes from the open field system was approximately 19.22 % greater than those under the plastic cover. This disparity indicates that environmental elements linked to the open field, such as light exposure and temperature variations, may promote anthocyanin synthesis in grapes. These results agree with those of Chorti *et al.* (2010), who reported that reduced sunlight over the period from veraison to harvest led to a decrease in anthocyanin production in Nebbiolo grapes and that shading had an observable effect on anthocyanin composition and acylation levels. This led to a decrease in anthocyanins with a 3'-hydroxyl group and an increase in anthocyanins with a 3',5'-hydroxyl group and 3-coumaroyl-glucosides (Chorti *et al.*, 2010).

Multiple research studies have shown that there is a negative relationship between shading and the development of phenolic compounds in berries of different grape cultivars, even when exposed to different environmental conditions (Pallotti *et al.*, 2023). Shading had a negative effect on phenolic and anthocyanin concentrations in Pinot noir grapes (Ranjitha *et al.*, 2015), as well as on total anthocyanin levels in Touriga Nacional grapes (Oliveira *et al.*, 2014) and total extractable flavonols in Chardonnay grapevines (Ghiglieno *et al.*, 2020). Furthermore, environmental temperature is critical in regulating the formation of anthocyanins in grape berries (Mori *et al.*, 2007). Light manipulation and hormone therapies were used to increase key gene activity in the anthocyanin synthesis pathway, resulting in increased red pigment production in table grapes (Afifi *et al.*, 2023).

Using plastic covers over grapevines to maintain a higher temperature throughout the night was already a common practice. Research conducted by Ryu *et al.* (2021) investigated the influence of night temperatures during veraison on berry skin colour in 'Kyoho' grapevines.

Night temperatures greater than 24 °C were shown to inhibit colour development of berry skins and alter anthocyanins levels and types, in addition to reducing the ABA/GA ratio and soluble solids content in the fruit. Another study by Yan *et al.* (2020) explored the effects of various temperature conditions on anthocyanin and flavonol concentrations in Merlot grapes. They found that higher day temperatures had a more pronounced effect on anthocyanin and flavonol levels than cooler night temperatures. In addition, higher diurnal temperatures led to an increase in the fraction of methoxylated and acylated anthocyanin species. A 25 °C differential between diurnal and noctural temperatures was necessary to influence anthocyanin levels, and genes associated to anthocyanin and flavonol biosynthesis, including flavonoid 3'5'-hydroxylase *(VviF3'5'Hf)*, flavonol synthase *(VviFLS5)*, *VviUFGT*, anthocyanin MATE transporter *(VviAM3)*, and *VviMybA*, showed increased expression levels at night compared to daytime. This investigation assessed the impact of two different temperature conditions on berry maturation from veraison to harvest. High temperatures at the start of verasion increase sugar accumulation but significantly decrease anthocyanin and flavonol levels (Pastore *et al.*, 2017).

Another study by Afifi *et al.* (2023) found that using white ground cover to regulate light quality increased the expression of *UFGT*, a key gene implicated in anthocyanin biosynthesis in grapes, as determined by real-time PCR data. Specifically, *UFGT* gene expression increased by approximately 27-fold when ethylene treatment was combined with white ground cover and silicon. Alternatively, we observed relatively higher anthocyanin biosynthesis gene expression but lower anthocyanin content in covered grapes. The same observation was previously reported by Mori *et al.* (2007) and is believed to be influenced by elevated temperatures, which may degrade or chemically modify these compounds.

Our results show that brassinosteroids have a significant influence on increasing *CHS*, *F3H*, and *UFGT* gene expression in grape berries. Higher concentrations of BRs resulted in greater increases in gene expression under plastic cover conditions than under open field conditions.

Red table grape varieties face challenges in warmer climates due to a lack of skin pigmentation. (Pinillos *et al.*, 2020). Heat stress decreases the expression of genes involved in metabolic activity and cell wall formation. According to Goda *et al.* (2002), BRs can upregulate genes that encode proteins that modify cell walls, and genes that encode enzymes involved in cell elongation. BRs can also mitigate heat stress *via* various pathways, potentially enhancing protective protein expression and restoring developmental protein synthesis inhibited by heat stress.

Vergara *et al.* (2018) reported that BRs can enhance colour, soluble solids concentration, and anthocyanin levels in "Red Globe" grape berries. BR analogues and their commercial formulations promote dihydroxylated anthocyanin synthesis, which is believed to be responsible for red and pink berry colouration, leading to hue intensification. Differential anthocyanin accumulation regulates toward 2-OH anthocyanins, which contribute to pink and red colours, and are predominant in "Red Globe" grape berries. 2-OH:3-OH anthocyanin ratios in treated berries were significantly different from those in control berries, indicating a selective increase in 2-OH anthocyanins. In red grapes, a significant correlation was found between the colour index and total anthocyanin concentration. (Vergara *et al.*, 2018).

Further evidence supporting the role of BRs in enhancing fruit colour development was shown in a study by Zhou *et al.* (2018), which demonstrated that BRs enhance grape pericarp pigmentation at the molecular level. In general, applying external BRs increased total anthocyanin accumulation in "Cabernet-Sauvignon" grape berries in bright light conditions compared to dark. Additionally, in bright light conditions, BRs enhanced berry outer layer color through increased expression of genes involved in anthocyanin production, such as chalcone isomerase1 *(VvCHI1)*, chalcone synthase3 *(VvCHS3)*, flavonoid-3', 5'-hydroxylase *(VvF3',5'-H)*, dihydroflavonol reductase *(VvDFR)*, and *VvUFGT*, by approximately 1.2-3 times. These findings support the present study on open-field assessments of anthocyanin levels in plants treated with BRs. The pattern parallelism observed between the open-field and plastic cover groups suggests a fundamental correlation between BR signalling and the activation of genes encoding *CHS*, *F3H*, and *UFGT* transcripts.

Similarly, Salama *et al.* (2023) showed that genes responsible for anthocyanin biosynthesis, such as *CHS*, *F3H*, and *UFGT*, play a crucial role in regulating anthocyanin formation in plants. The expression levels of these genes are strongly associated with anthocyanin accumulation in plant tissue.

3. Assessment of grape berry quality at harvest and during the storage period

At harvest, there is an inverse relationship between total soluble solids concentration and total acids in ripe fruits. A study conducted by Bedrech *et al.* (2022) revealed that vines grown under plastic cover exhibit higher values for reproductive growth parameters, including bud break, sprouting, and all chemical parameters. Furthermore, the findings corroborate those of Salem *et al.* (2021), who demonstrated that grapes harvested from covered vines have higher overall quality.

BR treatments considerably improved berry quality indicators (SSC % and TA), which is in line with the results of Champa *et al.* (2015) and Ghorbani *et al.* (2017), who also reported improved grape berry quality after concentrated BR treatments. This observation aligns with the findings of Pakkish *et al.* (2019) and Vergara *et al.* (2018), who similarly noted a rise in sugar levels and soluble solids in grape berries after BR application. Research by Li *et al.* (2023c) revealed that BRs might promote CH_4 production for

enhancing cell wall flexibility. Furthermore, BRs may also regulate sugar and carbohydrate metabolism in grape berries during veraison. A study by Xu *et al.* (2015) revealed that external administration of BRs increased soluble sugar concentration in grape berries, stimulating invertase and sucrose synthase gene expression, thus influencing sucrose metabolism and transport.

The experimental findings corroborated another study that examined 'Thompson Seedless' grapes (Asghari & Rezaei-Rad, 2018). The results indicated that applying BRs resulted in significantly increasing soluble solids concentration, phenolics, and total organic and ascorbic acids, as well as antioxidants.

In this study, SSC % fluctuated during the storage period, but trended upwards. Simultaneously, fruit acidity also changed significantly across preharvest treatments, then decreased during the storage period due to the gradual increase of free sugars and the respiratory oxidation of organic acids. (Khaliq *et al.*, 2015; Parven *et al.*, 2020). During ripening, organic acids are consumed as an energy source to support increased metabolic activity of the tricarboxylic acid cycle, resulting in a decrease in acidity (Batista-Silva *et al.*, 2018).

These findings also indicate that higher BR concentrations (0.6 and 0.8 ppm) under both open and covered conditions increase SSC % during the storage period. However, the covering system on SSC % had a more pronounced effect than the BRs. These results were inconsistent with those of Sen *et al.* (2016), who reported that during storage, the soluble solids concentration of uncovered grapes was greater than that of covered grapes. Nevertheless, in applying 0.8 ppm BRs, TA levels were significantly reduced under covered conditions compared to those under uncovered. Furthermore, it has been shown that BRs influence fruit ripening and ethylene production. According to Zhu *et al.* (2010), BR-treated climacteric fruits, such as jujube *(Ziziphus jujuba)*, produced far less ethylene during storage.

In addition, Ji *et al.* (2021) examined how BRs delay ripening in apple and pear fruits by inhibiting ethylene production. According to Ma *et al.* (2021), BRs protect litchi fruitlets from falling off by decreasing ethylene production and blocking expression of *LcACS1/4* and *LcACO2/3*, two genes involved in ethylene biosynthesis in the fruitlet abscission zone.

Furthermore, Zhu *et al.* (2015) also indicated that BR treatments may reduce postharvest disease occurrence in citrus fruit by activating stress-related genes, thus stimulating the accumulation of H_2O_2 and stress-related protective compounds such as osmolites.

BR application increased berry firmness and removal force of our 'Flame Seedless' grapes. According to Peng *et al.* (2004), BRs increase cell wall Ca^{2+}, protopectin and pectin, improving fruit structural integrity.Overall, elevating BR concentrations under a plastic cover increased firmness and removal force, and decreased shatter percentage, berry decay, and weight reduction. This finding agrees with that of Sen *et al.* (2016), who reported that the use of a covering during storage increases grape quality during storage. Our study highlights the potential of brassinosteroids, particularly at elevated concentrations, in reducing fruit weight loss during grape storage. The results agreed with those obtained by Pakkish *et al.* (2019), who indicated that BR treatments preserve the grape quality and shelf life of the grape. Possible causes for this include antioxidant enzyme activation, which protects cell membranes from oxidative damage. Additionally, lipid peroxidation and H_2O_2 levels decreased in the grape berries. The significant reduction in fruit weight can be attributed to elevated malondialdehyde (MDA) concentrations, which indicates cell membrane damage from lipid peroxidation (Elmenofy *et al.*, 2023; Zhao *et al.*, 2021). Grape quality assessment and adaptation are complex issues that are constantly affected by environmental and genetic factors, which are comprehensively addressed by Poni *et al.* (2018).

CONCLUSIONS

Ensuring the marketability of 'Flame Seedless' grapes and prolonging shelf life while minimising detrimental impacts on berry quality is a critical concern for Egypt's grape production industry. Our research showed that the application of plastic covering combined with brassinosteroids (BRs) improves grape quality as evidenced by increased cluster weight, 100 berry weight, berry length, fruit firmness and removal force. Results showed that when covered in plastic film, the 'Flame Seedless' grape variety could be harvested earlier while still maintaining high quality. Additionally, the use of exogenous BRs appears to be a feasible and valuable method for enhancing table grape quality, offering potential advantages for grape growers and the grape industry. In addition, upregulation of ripening and anthocyanins-related *F3H*, *CHS*, and *UFGT* genes was strongly correlated with elevated BR concentrations. Hence, this research recommends BR application and installing plastic covering to ensure higher quality and shelf life of 'Flame Seedless' grapes cultivated in Egypt.

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