Using brassinolide and girdling combined application as an alternative to ethephon for improving color and quality of ‘Crimson Seedless’ grapevines

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Abstract
The color of ‘Crimson Seedless’ berries is a major constraint in expanding marketing and export potential. The target of this investigation is to enhance the color and quality of ‘Crimson Seedless’ by using brassinolide (BL) and girdling treatments, individually and in combination, as an alternative to ethephon over two consecutive seasons. The obtained results revealed that the combined treatment BL at 2.0 mg/L and girdling recorded the highest significant yield values (17.78 ± 0.29 and 18.26 ± 0.23 kg/vine) in both seasons, respectively, in contrast to ethephon and girdling treatments which had no significant influence. Besides, BL 2.0 mg/L + girdling presented high levels of physicochemical characteristics of clusters and berries compared to the control. Concerning berry color assessment, ethephon followed by BL at 2.0 mg/L + girdling recorded the highest significant improvement in the red color, as indicated by Color Index of Red Grapes and visual assessment, and had similar and elevated levels of anthocyanin content. On the other hand, flavonoids (3.46 ± 0.03 and 3.66 ± 0.02 mg/100 g FW), and antioxidant capacity (2.37 ± 0.02 and 2.43 ± 0.06 IC₅₀) were obviously increased with BL 2.0 mg/L + girdling, with a slight decrease than ethephon application. As for phenylalanine ammonia-lyase activity, the data show that it increased by ethephon (7.91 ± 0.02 and 8.01 ± 0.02 U mg⁻¹ protein) and BL 2.0 mg/L + girdling (7.77 ± 0.04 and 7.79 ± 0.07 U mg⁻¹ protein), compared to the control (3.69 ± 0.02 and 3.80 ± 0.01 U mg⁻¹ protein) in both seasons, respectively. In terms of polyphenol oxidase activity, the increase in PPO was closely associated with PAL. Moreover, BL 2.0 mg/L + girdling was the most effective treatment for increasing dormant season parameters in both seasons, while ethephon spraying had no influence. All these confirmed that the combined treatment BL 2.0 mg/L + girdling exhibited a synergistic effect in improving the color and quality of ‘Crimson Seedless’.

Keywords Anthocyanins · Brassinolide · Crimson Seedless · Ethephon · Girdling · Phenylalanine ammonia-lyase · Polyphenol oxidase

1 Introduction

Red grape varieties occupy a high marketing position in the Egyptian market, as they are widely accepted by the consumer (Mohamed and Sayed 2021). The main red cultivars produced in Egypt are ‘Red Globe’, ‘Flame Seedless’, ‘Red Romy’, ‘King Ruby’, and ‘Crimson Seedless’. The latter is the most profitable and fills a commercial gap in the market, as it is a seedless substitution for red seeded grape and late-maturing cultivar, the texture of the berries is firm, attractive, crisp, has a high sugar content and its flavor is excellent (Ramming et al. 1995; El-Boray et al. 2019). However, this cultivar faces a major production problem as its berries lack adequate color intensity, and heterogeneous berry pigmentation in the clusters as red color in some berries while
others remain green and immature (Cameron 2005). This is attributed to the non-conductive conditions for anthocyanin biosynthesis, which is often affected by many factors, such as genetic regulation, phytohormones, and global climate change (Global Warm), which negatively affects fruit maturation indexes (Ferrara et al. 2014). Anthocyanins are considered the most essential flavonoid pigments and synthesized through a biosynthetic pathway organized by enzymes such as; phenylalanine ammonia-lyase (PAL) (Xi et al. 2013). Besides, the native physiological functions of polyphenol oxidase (PPO) have been examined and it was found that PPO was implicated in the biosynthesis of some specific pigments (Nakayama et al. 2001), and performs a novel and essential role in secondary metabolism in walnut (Araji et al. 2014).

Farmers resorted to carrying out some agricultural practices as well as the use of some plant hormones that would improve the coloring and quality of the red grape varieties. Girdling at the véraison stage is the most common agricultural practice adopted by farmers to increase the accumulation of sugar level and the coloring of red grape varieties by enhancing the soluble solids content (SSC) and total phenolics content (Pereira et al. 2020). Also, ethephon has been used successfully to advance fruit maturation in many cultivars (Roberto et al. 2013). Application of ethephon at véraison stage enhances color development in fruits, increases SSC, and reduces acidity (Yahuaca et al. 2006). However, ethephon in special is extremely poisonous and is categorized according to the Environmental Protection Agency of the United States in the third category of toxicity (Category III) (Bhadoria et al. 2018). Also, the application of this compound has been found to have a negative effect on the firmness and causes softening of grape-berries, leading to berry abscission and disturbing its sugar/acid ratio. Besides, it leads to deterioration of quality and the shorter shelf-life of grapes (Ferrara et al. 2016). Moreover, Roberto et al. (2013) showed that ethephon is drifting and gets absorbed by the leaves and becomes responsible for yellowing and defoliation of the leaves, which negatively affects the productivity of vines both in the current year and in the following year. The European Union has expressed concern about ethephon residues on grapes and other fruits (Grealyling 2007).

Currently, it can be said that it is safe to use brassinosteroids (BRs), in agriculture (Babalık et al. 2020), as they are characterized as part of the sixth category of plant hormones which are safe for the environment and non-toxic (Kang and Guo 2011). BRs include many compounds; the most important are brassinolide (BL), castasterone (CS), and 24-epibrassinolide because of their potent biological activity and wide distribution (Bartwal et al. 2013). BL exhibited a broad range of physiological and molecular responses in plants, such as cell elongation, cell division, photomorphogenesis, regulation of anthocyanin biosynthesis, vascular differentiation, gene expression, and protein synthesis (Sasse 2003). BRs have been reported to enhance PAL and PPO activities in jujube (Zhu et al. 2010), grape (Asghari and Rezaei-Rad 2018; Xi et al. 2013), and radish (Sharma et al. 2014). To the best of our knowledge, this is the first report in which BR improved anthocyanin by enhancing PAL and PPO activities in 'Crimson Seedless' grapevines.

Encouraged by the above facts and in the quest to improve the nutritional quality of berries using safe and environmentally ways, in this study we used brassinolide and girdling treatments individually and in combination to enhance the quality and color development of 'Crimson Seedless' grapevines, as an alternative to ethephon.

2 Materials and methods

2.1 Plant and experimental procedure, treatments protocol, and weather conditions

During two successive seasons of 2018 and 2019, sixty-three 4-year-old uniform vines of 'Crimson Seedless' grapevines grafted on Freedom rootstock were selected from the experimental station vineyard of the Horticultural Research Institute in Mansoura region, Dakhilia Governorate, Egypt. The vines were cultivated in soil characterized by being; texture (clay), organic matter (1.89%), pH (7.8), electrical conductivity (0.66 mmhos cm−1), CaCO3 (1.76%), N content (30.6 mg/L), P content (13.28 mg/L), and K content (312.7 mg/L). All vines irrigated by a surface irrigation system, planted at cultivation distances of 2 × 3 m with quadrilateral cordon trellis system and cane pruning, leaving 7 canes 12 eyes each and 7 renewal spurs 2 eyes each, the total bud load was about 98 buds and supported by Spanish baron system. After berry set stage, 22 clusters/vine were the adjusted crop load of all treatments. For crop management, all the recommendations of the Ministry of Agriculture have been applied to all the vines. Seven treatments were applied in this study, they were organized in a completely randomized blocks design, and each treatment was applied in three replicates, three vines/ replicate, and applied to the same vines during the two seasons of the study. Regarding the climate status through the experiment time was as follows: average air temperature (17–29 °C), relative humidity (52–60%), and daily sunshine hours (10.8–11.9 h). The vines were either treated by brassinolide (BL) (Blank ®, Spanish) at a rate of 1.0 and 2.0 mg/L or ethephon (Eth) (48% w/v) at a rate of 250 mg/L as foliar applications. BL was used at three times: after complete set of berries, 15 days after set of berries, and at the beginning of berries coloring (at the véraison stage) (Champa et al. 2015), while, ethephon was sprayed at véraison stage when the berries reached about 10–20% coloration. Girdling treatment...
was carried out at véraison by removing a narrow ring of the bark (4 mm) entirely around the trunk.

The treatments used in this study were applied as follows:

T1. Spraying with tap water (Control).
T2. Spraying with BL at 1.0 mg/L.
T3. Spraying with BL at 2.0 mg/L.
T4. Girdling.
T5. Spraying with BL at 1.0 mg/L + Girdling.
T6. Spraying with BL at 2.0 mg/L + Girdling.
T7. Spraying with Ethephon at 250 mg/L.

2.2 Yield, cluster and berry physicochemical characteristics

The grape samples were collected at the time of harvest when the soluble solids content (SSC) in the control treatment reached about 16–17%. To measure the average of yield/vine (kg), representative sample of 6 clusters/vine was weighted and then multiplied the average weight of the cluster by the number of clusters/vine. Also, these clusters used for the following measurements: cluster length and width (cm), weight (g) and volume (cm³) of 100 berries, berry diameter (cm), and finely berries firmness was measured by using Push/Pull (Dynamometer Model DT 101) and the results were expressed as (g/cm²). For chemical maturity parameters, SSC was determined by using a hand refractometer and titratable acidity (TA) by titration against 0.1 N NaOH results were expressed as (%) then SSC/acid ratio was calculated as defined maturity index also, total sugars (%) were measured according to the method described by (AOAC 2005).

2.3 Berry color assessment

2.3.1 Visual assessment parameters

Visual assessment parameters (I) were measured using the CR-10 colorimeter (Konica Minolta, Tokyo, Japan), and the following variables of its equatorial portion were obtained: L* (lightness), C* (chroma), and h° (hue). Lightness values range from 0 (black) to 100 (white). Chroma indicates the saturation of the color, that is, the distance from gray (achromatic) to a pure color, and is calculated from the a* and b* values of the CIELab scale system. Hue refers to the color wheel and is measured in angles; green, yellow, and red correspond to 180, 90, and 0, respectively (Lancaster et al. 1992). From these data, the Color Index of Red Grapes (CIRG) was calculated as CIRG = (180-h°)/(C* + L*). Based on this index, the berries were classified into five categories: green-yellow (CIRG < 2); pink (2 < CIRG < 4); red (4 < CIRG < 5); dark red (5 < CIRG < 6); and blue-black (CIRG > 6).

Visual assessment parameters (II) were measured according to (El-Kenawy 2018) as follow:
- Green berries%: The number of green berries was divided by the total berries number.
- Pink berries%: The number of pink berries was divided by the total berries number.
- Red berries%: The number of red berries was divided by the total berries number.

2.3.2 Chemical assessment parameter (anthocyanin measurement)

To measure the anthocyanin content in berries, samples of 0.5 g of berry skin were softly detached from the flesh using a sharp blade and added to 30 mL of acidified methanol (HCl 1% + methanol 99%), and left in the dark for 48 h then evaluated by spectrophotometer at 520 nm, results were expressed as (mg/100 g FW) according to (Husia et al. 1965).

2.4 Total phenols, flavonoids and antioxidant activity determinations

2.4.1 Preparation of the methanol extract

Two grams of berries skin tissue (randomly collected from 30 berries/replicate) were extracted by shaking at 150 rpm for 12 h with 20 mL methanol (80%) and filtered through filter paper No. 1. The methanol extract will be used for estimating total phenols and flavonoids and antioxidant activity.

2.4.2 Estimation of total phenols, total flavonoids and antioxidant capacity

Total phenols, flavonoids contents, and free radical scavenging activity were determined using a modified colorimetric method using a spectrophotometer. Regarding the measurement of the total phenol content, 50 μL of the previous methanol extract, 100 μL Folin-Ciocalteu reagent plus 850 μL of methanol were mixed and kept at ambient temperature for 5 min., then sodium carbonate was added (500 μL of 20%) to react for 30 min. A calibration curve of known concentrations of gallic acid was measured to quantify the total phenols, measured at a wavelength of 750 nm, and the results were expressed as mg/100 g FW gallic acid equivalent (Hoff and Singleton 1977). Regarding the measurement of flavonoids, 250 μL of the previous methanol extract, 1.25 mL of distilled water and 75 μL of 5% NaNO₂ were mixed and kept at ambient temperature for 6 min. Then 150 μL of 10% AlCl₃ solution, 0.5 mL of 0.1 N NaOH, and 275 μL of distilled water were added to the above mixture 5 min later. A
calibration curve of known concentrations of catechin was measured to quantify the total flavonoids, measured at a wavelength of 510 nm and the results expressed as mg/100 g FW catechin equivalent (Zhishen et al. 1999). The activity of free radical scavenging of methanol extract was evaluated according to Ao et al. (2008) method using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). Freshly prepared DPPH (0.9 mL of 0.1 mM) was mixed with the previous methanol extract (0.1 mL) and kept at room temperature in the dark for 30 min, then measured at a wavelength of 517 nm. The activity of scavenging percentage (IC50) was defined as µg phenolics of the test sample that decreases 50% of initial radical and was calculated using the shown below formula: DPPH radical scavenging% = [(Abs control – Abs sample)/Abs control100 ×].

2.5 Enzymes measurements

2.5.1 Phenylalanine ammonia-lyase (PAL)

One gram of skin was mixed with borate buffer (mL of 50 mM), 2-mercaptoethanol (5 mM) and polyvinylpyrroolidone (400 mg) at 8.5 PH. Then the above mixture was centrifuged for 15 min at 16,000 rpm under cooling (4 °C) to obtain a pure mixture. Then, L-phenylalanine (700 µL of 100 mM) and borate buffer (3 mL of 50 mM) was added to the pure mixture. After that, the resultant supernatant fraction (300 µL) was incubated for 60 min at 40 °C. To block more of the enzyme response, HCL (100 µL of 5 mM) have been added. The activity of PAL was measured at a wavelength of 290 nm at room temperature, regarding the formation of cinnamic acid (Ke and Salveit 1986).

2.5.2 Polyphenoloxidase assay (PPO)

PPO was assayed by using 1.0 g of skin sample that homogenized at 7.0 pH with buffer; Tris- Hydrochloride (20 mM), then centrifuged at 16,000 rpm for 6 min under cooling (4 °C), and the pure extraction was kept below -20 °C. The catechol substrate was used to measure PPO activity. The extract (200 µL) was directly added to 3 mL of 20 mM catechol dissolved in 100 mM Na PO4 − buffer, PH 7.0 (Jiang et al. 2002). The increased activity was reported at 400 nm on a spectrophotometer for 3 min. The activity was presented with a 0.1 min−1 difference in absorbance. The mg−1 units of protein were used to express the specific activity of enzymes, hence the method according to Bradford (1976) to determine the total content of soluble proteins in the enzyme extract.

2.6 Dormant season parameters

Wood ripening coefficient (%) measured by tagging twelve shoots for each replicate of the current season’s growth, then dividing length of the mature part by the total length of the shoot. Total carbohydrates in canes (g/100 g DW) were determined according to Hodge and Hofreiter (1962). Pruning wood weight was taken in both seasons of study during the period of winter pruning and the obtained data were recorded as kg/vine.

2.7 Statistical analysis

Data were analyzed using The Co-Stat software package, Ver. 6.303 (789 lighthouse Ave PMB 320, Monterey, CA, 93,940, USA). The significance of differences between each treatment was determined by one-way analysis of variance (ANOVA) and Tukey’s HSD test at p < 0.05. Data were expressed as the mean values of triplicate experiments and different letters indicate a significant difference between treatment and the control. Each value represents the mean of three replicates ± SE (n = 3). Principal component analysis (PCA) and Pearson’s correlation matrix were employed to analyze the relationships among the color-related parameters already analyzed.

3 Results

3.1 Yield, cluster, and berry physicochemical characteristics

The response of various applications on yield and physical properties of clusters and berries are presented in Tables 1 and 2. The results show that the BL treatments at three times i.e., after complete set of berries, 15 days after set of berries, and at the beginning of berries coloring (at the véraison stage), individually or combined with girdling, gave the greatest significant increase (p < 0.05) compared to the control in both seasons. Concerning girdling application, no significant differences were noticed in yield or cluster weight as compared to the control in the first season. Ethephon had no influence on all parameters except cluster length and berry firmness, and it produced no significant difference with the control. It was observed that the cluster length increased slightly in the first season in response to the ethephon application, while decreased significantly during the second season. As for berry firmness, which is considered an important factor contributing to postharvest quality of grapes, ethephon proved to be less effective than the control in both seasons, and grape berries taken from the BL 2.0 mg/L + girdling treated clusters were significantly firmer (186 ± 6.11 and 194 ± 3.05 g/cm²) than those of the ethephon treated clusters (112 ± 5.29 and 130 ± 4.16 g/cm²), respectively. Further observing the results showed a prominent increase in yield/vine, cluster weight, volume of 100 berries, and berry firmness in both seasons by the combined treatments compared
to the individual treatments. The effect of concentration is also crucial, where increasing the concentration (from 1.0 to 2.0 mg/L) leads to increasing the effect. It was also observed that the yield and physical characteristics of clusters and berries were increased by all treatments in the second season than the first season. In general, the BL 2.0 mg/L + girdling treatment was the most effective treatment, where it recorded the highest yield (17.78 ± 0.29 and 18.26 ± 0.23 kg/vine), compared to the control (14.74 ± 0.24 and 15.14 ± 0.26 kg/vine) in both seasons, respectively. Meanwhile, the highest physical characteristics of clusters and berries were obtained from the same treatment.

The berry chemical characteristics such as soluble solids content (SSC), titratable acidity (TA), SSC/acid ratio, and total sugars% were also examined as quality criteria (Table 3). The data of both seasons presented a significant effect by all applications as compared to the untreated vines. Ethephon and BL 2.0 mg/L + girdling had similar and elevated levels of SSC%, SSC/acid ratio, and total sugars% compared to control. Concomitant with enhance in SSC%, a decrease in TA was noticed, where ethephon (0.48 ± 0.01 and 0.46 ± 0.01%) and BL 2.0 mg/L + girdling (0.50 ± 0.02d and 0.47 ± 0.02d %) treatments significantly reduced the TA, compared to the control (0.63 ± 0.01 and 0.61 ± 0.01%) in both seasons, respectively. It was also observed that the SSC, SSC/acid ratio and total sugars were increased by all treatments in the second season than the first season, and no such effect was observed in TA.

### 3.2 Berry color assessment

Figure 1 expresses the visual berry color assessment (I) in numerical terms along Lightness (L* value), Chroma (C*), intensity of color), and hue angle (h°) within the CIELAB color sphere which are usually mathematically combined to calculate the color indexes. Concerning the berry color analyzed at the harvest time, it can be noticed that the applications used in this study led to lower average for L*

| Treatments | Yield/vine (kg/vine) | Cluster weight (g) | Cluster length (cm) | Cluster width (cm) |
|------------|----------------------|--------------------|--------------------|-------------------|
|            | 2018                 | 2019               | 2018               | 2019              | 2018               | 2019               |
| Control    | 14.74 ± 0.24 e       | 15.14 ± 0.26 f     | 670 ± 11.0 e       | 688 ± 12.1 f      | 19.0 ± 0.57 e      | 21.3 ± 0.66 d      |
| BL 1.0 mg/L| 16.42 ± 0.31 d       | 16.85 ± 0.22 d     | 746 ± 14.5 e       | 766 ± 10.0 d      | 22.3 ± 0.88 c      | 22.7 ± 0.94 c      |
| BL 2.0 mg/L| 16.76 ± 0.43 c       | 17.16 ± 0.34 c     | 762 ± 19.6 c       | 780 ± 15.8 c      | 23.0 ± 0.57 bc     | 25.3 ± 0.33 b      |
| Girdling   | 15.05 ± 0.19 e       | 15.52 ± 0.16 e     | 682 ± 11.0 e       | 705 ± 12.6 e      | 22.3 ± 0.33 c      | 24.3 ± 0.33 b      |
| BL 1.0 mg/L + Gird | 17.26 ± 0.34 b   | 17.78 ± 0.26 b     | 784 ± 15.8 b       | 808 ± 12.0 b      | 24.0 ± 0.91 b      | 27.0 ± 0.57 a      |
| BL 2.0 mg/L + Gird | 17.78 ± 0.29 a  | 18.26 ± 0.23 a     | 808 ± 13.3 a       | 830 ± 10.7 a      | 25.3 ± 0.33 a      | 27.0 ± 0.57 a      |
| Ethephon   | 14.96 ± 0.24 c       | 15.31 ± 0.26 ef    | 680 ± 11.0 e       | 696 ± 12.2 ef     | 20.3 ± 0.66 d      | 20.0 ± 0.57 e      |

Table 1 Effect of brassinolide (BL), girdling and ethephon applications on yield, cluster weight, cluster length and width of 'Crimson Seedless' grapes in 2018 and 2019 seasons

| Treatments | 100 Berry weight (g) | 100 Berry volume (cm³) | Berry diameter (cm) | Berry firmness (g/cm²) |
|------------|----------------------|------------------------|---------------------|-----------------------|
|            | 2018                 | 2019                   | 2018               | 2019                  | 2018                   | 2019                   |
| Control    | 384 ± 9.22 e         | 392 ± 6.42 f           | 363 ± 6.11 f        | 371 ± 4.37 f          | 1.78 ± 0.03 e          | 1.84 ± 0.03 e          |
| BL 1.0 mg/L| 448 ± 9.01 c         | 462 ± 8.32 d           | 426 ± 7.41 d        | 438 ± 6.42 d          | 2.14 ± 0.08 c          | 2.28 ± 0.03 c          |
| BL 2.0 mg/L| 464 ± 8.32 b         | 482 ± 6.11 c           | 438 ± 6.42 c        | 455 ± 6.35 c          | 2.26 ± 0.04 b          | 2.36 ± 0.03 b          |
| Girdling   | 418 ± 7.21 d         | 428 ± 5.29 e           | 393 ± 6.35 e        | 422 ± 6.08 e          | 1.98 ± 0.06 d          | 2.10 ± 0.02 d          |
| BL 1.0 mg/L + Gird | 469 ± 8.41 b   | 490 ± 6.42 b           | 454 ± 7.42 b        | 464 ± 5.29 b          | 2.38 ± 0.03 b          | 2.42 ± 0.04 b          |
| BL 2.0 mg/L + Gird | 482 ± 8.71 a  | 504 ± 6.66 a           | 460 ± 6.42 a        | 472 ± 7.08 a          | 2.48 ± 0.07 a          | 2.54 ± 0.03 a          |
| Ethephon   | 392 ± 6.00 e         | 398 ± 3.46 f           | 368 ± 7.21 f        | 372 ± 6.92 f          | 1.78 ± 0.02 e          | 1.85 ± 0.17 e          |

Table 2 Effect of brassinolide (BL), girdling and ethephon applications on physical properties of berries of 'Crimson Seedless' grapes in 2018 and 2019 seasons

Different letters within the same column are indicated significant differences at p < 0.05 (Tukey’s HSD test). Values are expressed as means ± standard error.
readings, indicating that the berries of 'Crimson Seedless' had darker coloration. In contrast, the highest average for L* reading was noticed in the control, showing that the color of the berries presented a lower intensity. Meanwhile, the h° significantly decreased along with L* after applying all treatments, implying a diversion from the green to red color of the berries. Besides, we noticed that combined BL and girdling treatments resulted in higher effects than individual BL treatments. Interestingly, the changes in the anthocyanin content in berry skins followed a pattern similar to that noticed in the visual assessment.

Table 3 Effect of brassinolide (BL), girdling and ethephon applications on SSC, TA, SSC/acid ratio and total sugars (%) of 'Crimson Seedless' grapes in 2018 and 2019 seasons

| Treatments          | SSC (%) 2018 | SSC (%) 2019 | TA (%) 2018 | TA (%) 2019 | SSC/acid ratio 2018 | SSC/acid ratio 2019 | Total sugars (%) 2018 | Total sugars (%) 2019 |
|---------------------|--------------|--------------|-------------|-------------|---------------------|---------------------|----------------------|----------------------|
| Control             | 16.7 ± 0.17f | 17.0 ± 0.30e | 0.63 ± 0.01a | 0.61 ± 0.01a | 26.2 ± 0.53e        | 27.5 ± 0.82e        | 14.3 ± 0.31e         | 14.6 ± 0.45e         |
| BL 1.0 mg/L         | 17.8 ± 0.11e | 17.9 ± 0.46d | 0.58 ± 0.01b | 0.57 ± 0.03b | 30.3 ± 1.02d        | 31.4 ± 2.66d        | 15.7 ± 0.30d         | 15.9 ± 0.55d         |
| BL 2.0 mg/L         | 18.1 ± 0.17de| 18.2 ± 0.11 cd| 0.55 ± 0.02bc| 0.53 ± 0.01bc| 32.8 ± 1.61 cd      | 34.2 ± 0.99 cd      | 16.3 ± 0.26c         | 16.5 ± 0.13c         |
| Girdling            | 18.5 ± 0.35 cd| 18.4 ± 0.35bcd| 0.53 ± 0.01 cd| 0.52 ± 0.02c | 33.8 ± 1.38c        | 35.4 ± 1.97c        | 16.4 ± 0.46c         | 16.6 ± 0.46c         |
| BL 1.0 mg/L + Gird  | 18.7 ± 0.06b c| 18.7 ± 0.35bc| 0.52 ± 0.02 cd| 0.51 ± 0.02 cd| 35.4 ± 1.12c        | 36.8 ± 2.62c        | 16.7 ± 0.14bc        | 16.9 ± 0.40bc        |
| BL 2.0 mg/L + Gird  | 19.0 ± 0.20ab| 18.9 ± 0.13ab| 0.50 ± 0.02de| 0.47 ± 0.02de | 37.1 ± 1.22ab       | 39.9 ± 1.67ab       | 16.9 ± 0.23ab        | 17.4 ± 0.20ab        |
| Ethephon            | 19.0 ± 0.01a | 19.5 ± 0.20a | 0.48 ± 0.01e | 0.46 ± 0.01e | 39.3 ± 1.51a        | 41.8 ± 1.015a       | 17.5 ± 0.01a         | 17.8 ± 0.20a         |

Different letters within the same column are indicated significant differences at p < 0.05 (Tukey’s HSD test). Values are expressed as means ± standard error.

The increased availability of color-measuring instruments now makes possible a more objective notation of specimen colors. The influence of the different applications on the visual (II) and chemical assessment parameters color is presented in Fig. 2. These scales were measured to illustrate the influence of the tested applications on the homogeneity of the berry color of 'Crimson Seedless' grape, which suffers from the heterogeneity of its color at the harvest stage. Shifting to Fig. 2, the visual assessment (II) (green, pink, and red berries percentage) was greatly affected by the different treatments used in this study compared to the control, where a significant enhance in the percentage of red and pink berries was obtained, in both seasons, by all the treatments in parallel with a decrease in the percentage of green berries. Besides, the effect of BL treatments, alone or combined, on 'Crimson Seedless' grape coloring was different depending on the applied rate, where increasing the concentration (from 1.0 to 2.0 mg/L) leads to increasing the coloring. Moreover, we noticed that combined BL and girdling treatments resulted in higher effects than individual BL treatments. Interestingly, the highest significant improvement in the red and pink color of the berries was observed by ethephon and BL 2.0 mg/L + girdling treatments, meanwhile, homogeneity of the color of the clusters increased. As was observed in visual assessment, data in Fig. 2 show also that the anthocyanin content was significantly affected by the different treatments as compared to the control in two seasons, where it was significantly higher in the ethephon and BL 2.0 mg/L + girdling treated berries, at par among themselves, than in the control. Also, the combined BL and girdling treatments exceptionally improved the anthocyanin content in berry skins compared to the individual BL treatments. Interestingly, the changes in the anthocyanin content of berry skins followed a pattern similar to that noticed in the visual assessment.

3.3 Total phenols, flavonoids, and antioxidant activity determinations

The data in Fig. 3 show that total phenols and flavonoids were significantly (p < 0.05) changed depending on the treatments as compared to the control in both seasons, with the highest values were recorded by ethephon and BL 2.0 mg/L + girdling. Also, total phenols and flavonoids were increased with nearly 1.5-fold by the BL + girdling combined treatments compared to the individual. Regarding total phenols, BL 2.0 mg/L + girdling was equally effective as ethephon especially in the first season, however in
the second season there was little difference, and the relative increase between these treatments was nearly threefold with the control. Concomitantly with the increase in total phenols an increase in flavonoids was also observed.

In connection with this, the data show that the effect was much apparent in vines treated with ethephon, where it gave in both seasons the highest values of total flavonoids (3.68 ± 0.07 and 3.92 ± 0.06 mg/100 g FW) followed by

Fig. 1 Effect of brassinolide (BL), girdling, and ethephon applications on visual berry color assessment (I), L* (lightness), C* (chroma), and h° (hue). T1: control T2: BL 1.0 mg/L, T3: BL 2.0 mg/L, T4: Girdling, T5: BL 1.0 mg/L + Girdling, T6: BL 2.0 mg/L + Girdling, T7: Ethphon. Each value represents the mean of three replicates ± SE (n = 3) replicates. The different letters at p < 0.05 represent the significance between treatment means using the Tukey-HSD test. Vertical bars (error bars) represent mean ± SE (n)
Fig. 2 Effect of brassinolide (BL), girdling, and ethephon applications on visual berry color assessment (II), red berries%, pink berries%, and green berries% and chemical berry color assessment, anthocyanin contents. T1: control T2: BL 1.0 mg/L, T3: BL 2.0 mg/L, T4: Girdling, T5: BL 1.0 mg/L + Girdling, T6: BL 2.0 mg/L + Girdling, T7: Ethphon. Each value represents the mean of three replicates ± SE (n = 3) replicates. The different letters at p < 0.05 represent the significance between treatment means using the Tukey-HSD test. Vertical bars (error bars) represent mean ± SE (n = 3).
BL 2.0 mg/L + girdling combined treatment (3.46 ± 0.03 and 3.66 ± 0.02 mg/100 g FW) with slight differences were observed between them.

The antioxidant capacity (IC$_{50}$ values) of berries determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method was influenced significantly by the applied treatments (Fig. 3), where the highest antioxidant capacity gives lest IC$_{50}$ values. In this respect, ethephon treatment gave the highest antioxidant capacity (2.23 ± 0.02 and 2.26 ± 0.02 IC$_{50}$), while the control recorded the lowest capacity.

**Fig. 3** Effect of brassinolide (BL), girdling, and ethephon applications on total phenols, flavonoids, and antioxidant activity. T1: control T2: BL 1.0 mg/L, T3: BL 2.0 mg/L, T4: Girdling, T5: BL 1.0 mg/L + Girdling, T6: BL 2.0 mg/L + Girdling, T7: Ethphon. Each value represents the mean of three replicates ± SE (n = 3) replicates. The different letters at p < 0.05 represent the significance between treatment means using the Tukey-HSD test. Vertical bars (error bars) represent mean ± SE (n = 3).
Application of BL and girdling improved the DPPH through increasing accumulation of flavonoids accompanied by the improvement of phenolic compounds. Combined application of BL 2.0 mg/L and girdling imparted a prominent impact (2.37 ± 0.02 and 2.43 ± 0.06 IC50) (two-fold of control) with a slight decrease compared to ethephon application. Besides, the antioxidant capacity of combined treatments was higher in both seasons than the individual treatments. It was also observed that the effect of concentration is also crucial, where increasing the concentration (from 1.0 to 2.0 mg/L) leads to increasing the antioxidant capacity.

### 3.4 The activities of PAL and PPO enzymes in grape skins

Promoting the red pigmentation process occurs by stimulating the anthocyanin-associated enzymes such as PAL that plays essential and vital role in the biosynthetic pathway of anthocyanin. As mentioned previously, PPO was contributory in the biosynthesis of some specialized pigments and plays a novel and essential role in secondary metabolism in walnut. The influence of all application on PAL and PPO enzyme activities is described in Fig. 4. Data show that the two enzymes activities were increased gradually in both seasons by all applications compared to the control. Also, the combined treatments remarkably enhanced the PAL and PPO activities in berries in both seasons than the individual treatments. The enzymes activities measured in this study increased with an increase in the BL treatments concentration and the maximum was at the concentration of 2.0 mg/L as compared with control. As for PAL activity, the greatest increase in activity was detected from the berries applied with ethephon (7.91 ± 0.02 and 8.01 ± 0.02 U mg⁻¹ protein) and BL 2.0 mg/L + girdling (7.77 ± 0.04 and 7.79 ± 0.07 U mg⁻¹ protein), with slightly different rates between them, and the relative increase was three-fold the control (3.69 ± 0.02 and 3.80 ± 0.01 U mg⁻¹ protein) in both seasons, respectively. In terms of PPO activity, increasing in PPO was closely associated with PAL. It was observed that

![Fig. 4 Effect of brassinolide (BL), girdling, and ethephon applications on the activities of PAL and PPO enzymes in grape skins. T1: control, T2: BL 1.0 mg/L, T3: BL 2.0 mg/L, T4: Girdling, T5: BL 1.0 mg/L + Girdling, T6: BL 2.0 mg/L + Girdling, T7: Ethphon. Each value represents the mean of three replicates ± SE (n = 3) replicates. The different letters at p < 0.05 represent the significance between treatment means using the Tukey-HSD test. Vertical bars (error bars) represent mean ± SE (n = 3)
ethephon and BL 2.0 mg/L + girdling were the most effective treatments in increasing the PPO activity, and they had the same activity in the first season, while in the second season showed slightly different activity.

### 3.5 Dormant season parameters

Coefficient of wood ripening, total carbohydrates in canes, and pruning wood weight are considered important parameters that determine the vigor of vines and fruit quality in the next season. When wood ripening coefficient and pruning wood weight increase, the vines will be mature well and reach full production in the following season. This sets the vine into a continuous vegetative growth cycle, and better production of wood, leaf, and fruit. Also, early spring growth requires carbohydrates in the form of starch to be remobilized and sent to the shoot apex to increase the percentage of fruit set, berries per cluster, cluster weight, and yield per vine (Vasconcelos & Castagnoli 2000). It is obvious from Table 4 that the coefficient of wood ripening, total carbohydrates in canes (g/100 g DW), and pruning wood weight (kg) were significantly increased with all treatments used, except ethephon, compared to control. The arrangement was as follow: combined treatments BL + girdling > girdling > individual treatments BL > control in both seasons. Additionally, ethephon spraying had no influence, and it produced no significant difference with the control. In general, BL 2.0 mg/L + girdling was the most effective treatment, where it recorded increasing in coefficient of wood ripening (85.2 ± 0.22 and 87.4 ± 0.27%), total carbohydrates in canes (24.3 ± 0.19 and 24.2 ± 0.21 g/100 g D.W.), and pruning wood weight (2.59 ± 0.11 and 2.72 ± 0.14 kg/vine) in both seasons, respectively, and consequently, this treatment recorded higher yield and physical characteristics of cluster and berries in the second season than the first season (Tables 1 and 2). Moreover, no significant difference was found between BL 2.0 mg/L + girdling and BL 1.0 mg/L + girdling treatments coefficient of wood ripening and pruning wood weight in both seasons, while there was a slight difference in the total carbohydrates in canes between them.

### 3.6 Multivariate analysis of the color-related parameters

Relationships between the color-related parameters were investigated by Pearson’s correlation matrix (Table 5) and principal component analysis (PCA) (Fig. 5) during two growth seasons (2018 and 2019) on ‘Crimson Seedless’ grapes. The PC1 explained 90.5% of the variability in the data, while PC2 explained 6.42% variability. The present results in Fig. 5A showed the strong positive correlations between increasing in phenolic compounds content, the activities of PAL and PPO enzymes and increasing in berry coloring of ‘Crimson Seedless’ grape. Mean values were used to create a correlation matrix from which standardized principal components (PCs) scores were extracted and those PCs with Eigen values greater than 1.00 were selected. Correlations between the original traits and the respective PCs were calculated. Scatter plot was prepared according to the PC1 and PC2 that reflected relationship among accessions in terms of characteristics.

### 4 Discussion

#### 4.1 Yield, cluster, and berry physicochemical characteristics

The results showed that the BL 2.0 mg/L + girdling recorded a significant increase in yield through increasing the weight, size, and diameter of the berries, consequently causing an

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**Table 4** Effect of brassinolide (BL), girdling and ethephon applications on the coefficient of wood ripening, total carbohydrates in canes, and pruning weight of ‘Crimson Seedless’ grapes in 2018 and 2019 seasons

| Treatments       | Coefficient of wood ripening (%) | Total Carbohydrates (g /100 g DW) | Pruning wood weight (kg/vine) |
|------------------|----------------------------------|-----------------------------------|-------------------------------|
|                  | 2018                             | 2019                             | 2018                          | 2019                          | 2018                          | 2019                          |
| Control          | 74.1 ± 1.18e                      | 75.7 ± 0.72e                     | 19.5 ± 0.20f                   | 20.2 ± 0.20f                   | 1.82 ± 0.14d                   | 1.88 ± 0.14d                   |
| BL 1.0 mg/L      | 77.2 ± 0.43d                      | 79.9 ± 0.80d                     | 20.8 ± 0.11e                   | 21.5 ± 0.11e                   | 2.19 ± 0.15c                   | 2.28 ± 0.02c                   |
| BL 2.0 mg/L      | 79.1 ± 0.45c                      | 81.6 ± 0.36c                     | 21.1 ± 0.14d                   | 21.8 ± 0.15d                   | 2.28 ± 0.21c                   | 2.37 ± 0.06bc                  |
| Gird             | 82.5 ± 0.33b                      | 84.5 ± 0.61b                     | 21.9 ± 0.19c                   | 22.8 ± 0.19c                   | 2.33 ± 0.21bc                  | 2.44 ± 0.09b                   |
| BL 1.0 mg/L + Gird | 84.1 ± 0.17a                  | 86.6 ± 0.40a                     | 22.8 ± 0.21b                   | 23.7 ± 0.12b                   | 2.48 ± 0.16ab                  | 2.62 ± 0.09a                   |
| BL 2.0 mg/L + Gird | 85.2 ± 0.22a                 | 87.4 ± 0.27a                     | 24.3 ± 0.19a                   | 24.2 ± 0.21a                   | 2.59 ± 0.11a                   | 2.72 ± 0.14a                   |
| Ethephon         | 75.1 ± 0.15e                      | 76.4 ± 1.14e                     | 19.7 ± 0.19f                   | 20.2 ± 0.14f                   | 1.84 ± 0.09d                   | 1.91 ± 0.02d                   |

Different letters within the same column are indicated significant differences at $p < 0.05$ (Tukey’s HSD test). Values are expressed as means ± standard error.
excess in the cluster weight. Also, the same treatment led to an increase in the cluster dimensions, and this increase could be attributed to the positive dual impact of BL and girdling. The BR plays an essential role in stimulating division and elongation of the cell (Catterou et al. 2001), regulation of enzymes accountable for the modification of cell wall activity and enlargements, cellulose synthase, and sucrose synthase (Ashraf et al. 2010), carbohydrate assimilation by improving the activities of different enzymes, increasing RNA and DNA content, polymerase activity, protein synthesis and ATP activity (Sasse 2003), thus improves vegetative growth and physiological status. This reflects on enhancing the physical characteristics of cluster and berries and increasing yield. The beneficial effect of BR application on increasing firmness of berries may be due to increasing Ca$^{2+}$, proteopectin, and pectin of cell walls (Peng et al. 2004). Maintenance of higher firmness with BR application has been reported by Peng et al. (2004) in litchi and Zhu et al. (2010) in jujube. As for the girdling, increment in grape berry size is attributed to the higher accumulation of assimilates especially carbohydrates throughout the canopy above the girdle because the transport of sugars from leaves to the root system is blocked by girdling application, which acts as a rich source of energy for all the stages of reproductive development including fruit enlargement and ripening (Goren et al 2004). Also, metabolic profiling by NMR following girdling of ‘Italia’ grapes pointed to potential modulation of amino acid levels (Ferrara et al 2014). Böttcher et al. (2018) investigated the impact of peduncle girdling of Shiraz grapevine and concluded that it had severe effects on the aggregation of some amino acids and cytokinins in fruits. Our results are in agreement with Champa et al. (2015) on

Table 5  Pearson’s correlation matrix among the color-related parameters on ‘Crimson Seedless’ grape

| Variables          | Red berries | CIRG | Anthocyanin | Total sugar | TPC | Flavonoids | DPPH | PAL | PPO |
|--------------------|-------------|------|-------------|-------------|-----|------------|------|-----|-----|
| Red berries        | 1           |      |             |             |     |            |      |     |     |
| CIRG               | 0.9841      | 1    |             |             |     |            |      |     |     |
| Anthocyanin        | 0.9012      | 0.8659 | 1           |             |     |            |      |     |     |
| Total sugar        | 0.693       | 0.623 | 0.8996      | 1           |     |            |      |     |     |
| Total phenols      | 0.9552      | 0.9815 | 0.8565      | 0.6476      | 1   |            |      |     |     |
| Flavonoids         | 0.9871      | 0.9572 | 0.9192      | 0.7556      | 0.9365 | 1        |      |     |     |
| DPPH               | −0.8886     | −0.9034 | −0.8354    | −0.6523     | −0.9218 | −0.9023 | 1    |     |     |
| PAL                | 0.9763      | 0.9741 | 0.9214      | 0.745       | 0.9738 | 0.9775 | −0.9442 | 1   |
| PPO                | 0.9835      | 0.9691 | 0.9087      | 0.7355      | 0.9617 | 0.9765 | −0.8868 | 0.9841 | 1   |

Values express average values of the treatments. The correlations are estimated by the Row-wise method.

Fig. 5 The principal component analysis (PCA) representing relationship between parameters, plotted with the contribution of treatments with the contribution of each parameter on the two PCA axes (A) and the color-related parameters measured (B)
'Flame Seedless', and Babalık et al. (2020) on 'Alphonse Lavallée' grape. Oguzhan et al. (2015) reported that girdled Red Globe grapevines were accomplished for improving desirable increases in berry diameter and length. On the other hand, ethephon did not influence yield and physical properties in our study. These results are in accordance with those obtained by Sabry et al. (2013), who proved that yield/ wake, cluster weight, length, and width of 'Flame Seedless' were not affected by applying ethephon. The negative effect of ethephon application at the véraison stage on berry firmness attributed to being a senescence promoter, leading to fruit softening at maturity in harvest stage and cold store (Yahuaca et al. 2006). High rates of ethephon maybe also increase the enzymes associated with cell wall metabolism of grapefruit and lead to the raise of grape berries softening (Dong et al. 2020). It was found that ethephon application resulted in a significant decrease in berry firmness as stated by Sabry et al. (2013) on 'Flame Seedless' grape and Ferrara et al. (2016) on 'Thompson Seedless' and 'Crimson Seedless' grapes. In our study, BL 2.0 mg/L+ girdling had elevated levels of SSC, SSC/acid ratio, and total sugars, concomitant with a reduction in TA like ethephon. Xu et al. (2015) has shown that sugar content increasing may be interpreted by the overexpression of hexose transporter genes as a consequence of BR application. Our results are in line with Xi et al. (2013), Champa et al., (2015), and Asghari and Rezaei-Rad (2018) who found that there was a significant improvement in the chemical properties of berries such as SSC, SSC/ acid ratio and total sugars, concurrent with a reduce in TA when the BR was applied with appropriate rates. Girdling leads to an increment in the sugar contents in grape berry, this is attributed to the higher aggregation of carbohydrates in the canopy above the girdle (sources), which supply a substrate for the growth and maintenance of non-photosynthetic tissues (sinks) (Ainsworth and Bush 2011). When carbohydrate demand reduces or sugar transport is prevented, sugar aggregation in leaves raises the expression of genes shared in carbohydrate storage, utilization and inhibits the expression of genes involved in photosynthesis (Lu et al. 2020). It was reported that girdling is an efficient horticultural application to improve sugar content in table grape cultivars (Basile et al. 2018; Xi et al. 2020). Our results are in agreement with Belal et al. (2016) and El-Kenawy (2018) showed that girdling the trunk significantly increased SSC, SSC/acid ratio, and total sugar, while decreasing TA in berry juice.

4.2 Berry color assessment

The present study demonstrates the effect due to combined between BL and girdling, especially the BL 2.0 mg/L + girdling in improving the coloring of 'Crimson Seedless' grapes through induction of sugar accumulation and related enzymes. Previous studies showed that preharvest BR applications lead to an increase in the total amount of anthocyanin in berries through stimulation of the expressions of the structural and regulatory genes involved in the synthesis of anthocyanin, which indicates that BR increment the interrelationships among the developmental and environmental signaling pathways, which improved berry coloration (Champa et al. 2015; Xi et al. 2013). Moreover, a statistically significant relationship was noticed between CIRG and total anthocyanin content and concentration (Vergara et al. 2018). These results are agreement with Roghabadi and Pakkish, (2014) who noticed that foliar applications of BR raised skin color of sweet cherry by enhancing total anthocyanin content. Girdling of 'Crimson Seedless' grapevine increased the anthocyanin aggregation in the berry skin by stimulating the supply of photosynthates to the grape berries, and consequently promoted the berry coloration (Brar et al. 2008). These results are accordance with Ferrara et al. (2014) and Soltekin et al. (2016) who stated that the color parameters (L, h° and CIRG index) values were raised by girdling at beginning of the color stage. Also, El-Kenawy (2018) revealed that application of girdling solely or combination with jasmonic acid significantly increased the percent of the red and pink color of berries. In addition, Pereira et al. (2020) found that girdling improved the anthocyanin accumulation in berry skin of berries.

4.3 Total phenols, flavonoids, and antioxidant activity determinations

Application of BL + girdling improved the DPPH through increasing accumulation of flavonoids accompanied by the improvement of phenolic compounds. Burin et al. (2010) explained that the most effective compounds in terms of adding better properties, such as flavor and color in grapes, are phenolic compounds, including flavonoids. The researchers propose that the exogenous BR affects the enzymes and genes involved in phenolic compounds biosynthesis since it works as a signaling molecule and stimulates secondary metabolite accumulation. Reports on exogenous application of BR strongly suggest that these steroidal hormones can increase the activities of antioxidative enzymes (Hayat et al. 2010) and modify them (Sharma et al. 2014). Besides, the effect of BR on improving berry quality parameters and phytochemical contents such as phenolics, flavonoids, and antioxidants is principally due to the impacts on photosynthesis reaction (Asghari and Rezaei-Rad 2018). Overall, BR treatment improves the plant’s potential to combat the toxic effects imposed by the stress by tightly regulating the accumulation of ROS, which was reflected in the improved redox state of antioxidants (Kaur et al 2018). In corroborate to our results, Xu et al. (2015), and Babalık et al. (2020) demonstrated that BR treated vines as recorded high phenols, flavonoid contents, and antioxidant capacity. Girdling
application increased total phenolic content in peach fruits (Kubota et al. 1993). Pereira et al. (2020) indicated that girdling at veraison increased flavonol concentrations in skin/pulp tissues of 'Cabernet Sauvignon' berries grapes.

4.4 The activities of PAL and PPO enzymes in grape skins

In our study, combined application of BL + girdling imparts significant effects on the enzymes activities (PAL and PPO) involved in anthocyanin synthesis. PAL is considered one of the regulating factors for anthocyanin accumulation in mature and ripe fruit (Xi et al. 2013) through increasing phenylpropanoid derivatives (Winkel-Shirley 2002). On the other hand, PPO is implicated in the biosynthesis of some particular pigments (Nakayama et al. 2001). Also, the native physiological roles of PPO in intact plant cells have been explained in a study on walnut which gives a rich array of phenolic compounds and possesses a single PPO enzyme, strikingly, the silencing of PPO caused major alterations in the metabolism of phenolic compounds and their derivatives and the expression of phenylpropanoid pathway genes. So, these results suggest that PPO plays a novel and essential role in secondary metabolism in walnut (Araji et al. 2014). It was noticed that BR injection in the strawberry at pink stage increases the ripening and mainly in a phenylpropanoid pathway (Ayub et al. 2018). Exogenous BR causes an increase in the enzymes activities by increasing the rate of photosynthesis which increases the production of carbohydrates that are used for the synthesis of various bioactive compounds (Koch 1996). Also, BR treatment increases the activity of PAL and PPO and this is attributed to the enhanced activity of secondary metabolism-related enzymes, production of secondary metabolites, and subsequent ROS scavenging (Asghari and Rezaei-Rad 2018; Xi et al. 2013). In corroborations to our results, Zhu et al. (2010) suggested that BR enhances the activities of PAL and PPO of jujube fruit. Also, Ahanger et al. (2020) found that tomatoes treated with BR displayed high PAL activity. Moreover, PPO was notably improved in response to BR application in grapes (Sharma et al. 2014 and Asghari and Rezaei-Rad 2018). Interestingly, girdling resulted in an increase in genes associated with starch and carbohydrate metabolism, photosynthesis, and hormone signals, including α-aminoase (α-AL) (Lu et al. 2020). Tyagi et al. (2020) showed that girdling increases the expression of phenylpropanoid pathway genes in both 'Sable' and 'Superior' grapes including PAL gene.

4.5 Dormant season parameters

Another significant encouraging effect of BL combined with girdling treatment was observed on dormant season parameters. The positive effect of BR on improving total carbohydrates in canes %, ripening wood %, and pruning wood weight could be due to its main role in stimulating cell division and elongation, carbohydrate assimilation, photosynthesis, nucleic acid, and protein synthesis (Catterou et al. 2001; Sasse 2003) and this reflected in improving vegetative growth, physiological status and subsequently, reflected in improving the previous properties. These results are inconsistent with those found by Işçi and Gokbayrak (2015), who reported that spraying 'Alphonse Lavallée' grape cultivar with BR had improved pruning wood weight. As for girdling application, Belal et al. (2016) and El-Kenawy (2018) reported that girdling trunk at the véraison stage increased the coefficient of ripening wood and total carbohydrates in canes. Regarding the effect of ethephon, González et al., (2018) revealed that the vigor of the vineyard (pruning weight) was not affected by spraying with ethephon.

5 Conclusion

Conclusively, the results of this investigation demonstrated that the application of BL at 2.0 mg/L + girdling treatment to 'Crimson Seedless' grapevines could significantly promote color and quality as an alternative to ethephon, which has negative effects. This treatment had a prominent impact in improving the yield and physical characteristics of cluster (cluster length, width, and weight) and berries (volume of 100 berry, diameter, and berry firmness), compared to the control. Besides, it recorded elevated levels of SSC, SSC/acid ratio, and total sugars, notably reducing the TA. The same treatment notably improved the red color homogeneity of the berry color as indicated by CIRG and visual assessment and recorded elevated levels of the anthocyanin content. The combined effect of BL at 2.0 mg/L + girdling increased the contents of total phenols and flavonoids and significantly improved the antioxidant capacity as well as the activities of PAL and PPO enzymes. Moreover, it is the most effective treatment in increasing the coefficient of wood ripening, total carbohydrates in canes, and pruning wood weight, compared to the control. This work opens a new perspective for studying the effect of BL at 2.0 mg/L + girdling in increasing the efficiency of the genes responsible for coloring, making it one of the most promising compounds that may help solve the problem of coloration heterogeneity in many fruits due to climate changes.

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Declarations

Conflict of interest All authors confirm that they have no conflict of interest.

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