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The Effect of Exogenous Application of Gibberellic Acid and Girdling on Characters of Grapes (*Vitis vinifera*) cv. Yaghoot

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ABSTRACT

'Yaghoot' grape cultivar is the earliest cultivar known in the history of Iran. The main problem of this cultivar is the compactness and small size of its berries, under the influenced genes that are controlled mainly by Gibberellic Acid (GA_3). Those undesirable traits reduce customer interest and profitability. To alleviate these problems, we used GA_3 and carried out Girdling (G) to enhance the quantitative and qualitative characteristics. The GA_3 treatment was used at four concentrations, *i.e.:* 0, 60, 90, and 120 mg/l, and was sprayed on plants three times (*i.e.:* 10 days before flowering, in the midst, and after flowering). The G treatment has achieved the proximity of the branch bases during the formation of berries. In this experiment, various morphological and biochemical traits were measured. The results exhibited that the GA_3 and G treatments significantly positively affected the fruits' quantitative and qualitative characteristics. Both treatments were observed to increase the berry length, berry weight, berry diameter, berry width, cluster width, cluster length, cluster weight, titratable acidity, total soluble solids, proline, malondialdehyde, H_2O_2 content, total phenolic content, anthocyanin content, ascorbate content, flavonoids content, antioxidant capacity, catalase activity, peroxidase activity, polyphenol oxidase activity, superoxide dismutase activity, and ascorbate peroxidase activity. The interaction between the GA_3 and G showed that they significantly increased the berry weight, proline content, antioxidant capacity, ascorbate content, peroxidase activity, polyphenol oxidase activity, ascorbate peroxidase activity, catalase activity, and superoxide dismutase activity. Those treatments are promising treatments that can help improve the quantitative and qualitative characteristics of 'Yaghoot' grapes.

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Introduction

Grapes (*Vitis*) have traditionally been prominent horticultural crops in the world producing about 75 million tons of crops annually (Jadhav *et al.*, 2020). In particular, grapes are important commercial fruit crops in temperate and subtropical regions. Initially, their fruits were grown because of their juicy and delicious berries. However, over time, more attention has been given to their high nutritional value, good taste, and various other benefits (Sangeetha *et al.*, 2015). In addition to its properties such as size, soluble solids, acidity, phenols, flavonoids, anthocyanins and vitamins that make grapes popular, its fruit quality is endowed with a rich content of antioxidant that contribute to human health (Xia *et al.*, 2010). Most grape cultivars produce fruits with berries that are naturally arranged in a highly compact manner (Tello and Ibanez, 2014) and, as a result, cannot compete well in the market because of this compact arrangement of berries and small berry size

(Jadhav *et al.*, 2020). When a fruit bunch is crowded with berries, the ones that lie inward do not receive enough sunlight in the compact cluster. This makes the berries ripen at different times of the season and, ultimately, has a negative effect on the total quality of the fruit cluster, while affecting the primary and secondary metabolites (Figueiredo-González *et al.*, 2013). Several strategies such as cluster thinning, leaf removal (Silvestre *et al.*, 2017), canopy shading, Plant Growth Regulators (PGR) and girdling have been mentioned in previous research that aimed at reducing the cluster compactness, often through a decrease in the number of berries and fruit set (Gao *et al.*, 2020). The GA_3 has long been applied as a plant growth regulator to increase the size of the grapes and to produce commercially acceptable grapes of the world-class quality. In addition, gibberellins are known to reduce the compression of grapes, as they naturally contain compounds that increase cell division and elongation (Gao *et al.*, 2020). Morphological and chemical properties of the grapes and their berry clusters can be improved using the GA_3 (Jadhav *et al.*, 2020). In a study, Guerios *et al.* (2016) stated that the GA_3 can substantially increase the berry diameter, Titratable Acidity (TA), soluble solids content, and eventually, lead to larger grapes with better quality. In the contrary, oscillation in auxin and gibberellin signaling and expression of analogous genes to their transport showed the importance of such hormones on the size, number and compactness of the clusters and berries of grapes (Shiri *et al.*, 2020). In another study, it was shown that the GA_3 treatment had a significant and positive effect on the number of various metabolites (Jadhav *et al.*, 2020), such as proline and anthocyanin in grapes, compared to untreated samples (Ferrara *et al.*, 2014; Reynolds *et al.*, 2016). The effect of the GA3 on the cluster compactness and size of berries in grapes can also be studied from a genetic point of view. One of the important genes in the grape cluster compactness is AGAMOUS, affecting the expression of the GA gene to increase the berries size and cluster length (Shiri *et al.*, 2018). The down-regulation of the GA gene is a key factor in the compactness and small berry size of the Yaghooti grape, with a significant effect on the gibberellin action at the time of cluster development of grape (Chai *et al.*, 2014). *POM1* gene is essential for the natural growth, development, and regulation of the cell size and differentiation of the plants. At the time of the second developmental stage of the clusters, this gene has been up-regulated under the gibberellin (Shiri *et al.*, 2020).

Girdling (G) involves the removal of a ring phloem tissue of the grape plant, which limits the translocation of the plant sap from the aerial part of the vine to the base (*i.e.*: from the shoots to the roots) and, therefore, the phloem sap remains largely active in the upper part of the plant *i.e.:* buds, flowers and fruits above the G region. Nonetheless, the effect of G is usually temporary on the flow of the phloem sap and, after a few days, the vine can revitalize the flow of phloem by means of callus formation that heals the G region (Ferrara *et al.*, 2014). In one study, the G was performed after fruit set to increase the size and weight of the berries (which increased the amount of carbohydrates, anthocyanins and soluble solids in the berries (Abu-Zinada, 2015), ultimately improving the fruit quality (Basile *et al.*, 2018). The application of the GA_3 on the grapes of the 'Shirazi' cultivars showed that the phenol compounds increased in the fruits (Kok and Bal, 2017), whereas another study on similar matters addressed how G and GA_3 treatments could positively influence the berry development during ripening, as well as accumulating Total Soluble Solids (TSS) and organic acids in the developed table grapes (El-kenawy, 2018). In high amounts, the GA_3 and G have reportedly increased the amount of reactive oxygen species and eventually caused cell death (Cheng *et al.*, 2013). Therefore, the current study was performed to evaluate the effects of the GA_3 and G on the berry quality, enzymatic antioxidant compounds such as Catalase (CAT) and Per-Oxidase (POD), Super-Oxide Dismutase (SOD), polyphenol oxidase (PO), Ascorbate Peroxidase (APX), non-enzymatic enzymes and phenolic compounds in the 'Yaghoot' grapes.

Materials and Methods

Plant materials and experimental procedure

This investigation was carried out as a completely randomized design with three replications on the Yaghoot grape in the vineyards of Shahid Beheshti Company in

Dezful. The average temperature and relative humidity in an 18-year period was 26ºC and 54-60%. The elevation was 134 meters and the average annual rainfall was 300 mm. The soil of the vineyard was loamy. The GA_3 was used at four levels of 0, 60, 90 and 120 ppm three times. The first treatment was done 10 days before flowering, the second treatment in the middle of flowering almost when 50% of the flowers were open, and the third treatment was done in the final flowering. This experiment included three treatments and each treatment included three time steps. The first treatment included 15 ppm in the first stage, 20 ppm in the second stage and 25 ppm in the third stage, the second treatment included 22.5 ppm in the first stage, 30 ppm in the second stage and 37.5 ppm in the third stage, and then the third treatment included 30 ppm in the first stage, 40 ppm in the second stage and 50 ppm in the third stage. The spraying was continued until runoff by handheld sprayers on the plants leaves. The G treatment was done with a diameter of 2.5 mm near the base of the branches in the stage of berry formation (diameter of berries 5 mm) with a sharp knife. At the harvest stage, treated and control samples were randomly selected from the experimental rows.

Measurement of physical parameters

To determine the physical and chemical factors of the recent experiment, 50 berries were randomly selected. Physical factors, including the berry weight (g), berry length (mm), berry diameter (mm), berry width (mm), cluster weight (g) and cluster size (mm) were measured.

Measurement of TSS, TA and Vitamin C

To measure the TSS of the grapes, berry juice was used and measured by digital Refractometer (HRT -32, Germany) and expressed as a percentage. For Titratable Acidity (TA), acidity was determined by titration method. Initially, 10 ml of fruit juice was added to phenolphthalein indicator solution and the TA of the grape juice was determined by titration to an end point of pH 8.1 with 0.1 N NaOH. Finally, it was read as a percentage of the TA.

The amount of vitamin C was determined by the oxidation of ascorbic acid and the 2,6-dichlorophenol endophenol dye was used and finally the results of the analysis were read in mg in 100 ml of fruit juice (Khazaei *et al.*, 2020).

Measurement of anthocyanin

Anthocyanins were measured using the pH change method (Reynolds *et al.*, 2016). The measurements of the absorption changes were performed at 520 nm between the grape extracts at pH 1.0 and 4.5. Anthocyanin was obtained from the following equation (mg/L) = A520 (pH 1) - A520 (pH4.5) / 0.0042

Preparation of methanolic extract

Two grams of berry tissue with 20 ml of 80% methanol were shaken at 150 rpm for 12 hours until the extraction was performed, then the extract was filtered with filter paper No. 1 and this extract was used to measure the proline, phenols, flavonoids and antioxidant activity (Alrashdi *et al.*, 2017).

Measurement of total phenol and flavonoids

The total phenol content was measured by Hoff and Singleton's method (1977). Then, 100 µl of methanol extract was added to 200 µl of Folin-Ciocalteu reagent, 100 µl of methanol and placed at room temperature for 5 min, then 550 µl of 20% sodium carbonate was added and placed at 25ºC for 30 min. the absorption changes were read at 750 nm. The total phenolic content was determined by the mg ml^{-1} of fresh material.

The total amount of the flavonoids was determined by Zhishen *et al.*'s (1999) method with slight changes. First, 250 µl of methanolic extract was mixed with 1.2 ml of distilled water and 80 µl of 5% $NaNO_2$ solution and placed at room temperature for 6 min, then the resulting mixture was mixed with 160 µl of 10% $AlCl_3$ solution, 0.5 ml of 1 mM NaOH and 280 µl of distilled water and placed at room temperature for 5 min, then the absorption was read at 510 nm. The total amount of flavonoids was determined by quercetin mg g^{-1} of fresh material.

Measurement of proline content

Bates *et al.*'s (1973) method was used to measure the proline content. In a test tube containing a solution of ninhydrin acid (1.25 g of ninhydrin solution in 20 ml of 6 mM orthophosphoric acid and 30 ml of glacial acetic acid) and 2 ml of glacial acetic acid, add 2 ml of

methanolic extract. The above test tubes are then kept in the boiling water for 60 min and then placed at room temperature to cool. Absorption changes were recorded at 520 nm. The amount of proline is presented in µg g^{-1} of µg g^{-1} FW.

Measurement of antioxidant capacity

The method of Khazaei *et al.* (2020) was used to evaluate the antioxidant activity. Then, 0.1 ml of methanol extract was added to 0.9 ml of 0.1 mM DPPH solution in methanol. Then kept in the darkness for 30 minutes at room temperature, the absorbance was read at 517 nm. The antioxidant activity was determined using the following equation:

Antioxidant capacity = [(control absorption - sample absorption) ÷ control absorption] × 100.

Measurement of MDA content

The amount of the MDA was assessed by the method of Zhao *et al.* (1993) with slight changes. To measure the MDA content, the grape samples were mixed with 5 ml of 5% (w / v) trichloroacetic acid solution until getting homogeneous, then, they were centrifuged at 10,000 rpm for 15 minutes at 4°C. Next, 2 ml of the above solution was added to 2 ml of 5% trichloroacetic acid containing 0.67% (w / w) thiobarbituric acid solution. Then it was heated for 10 min at 100°C, and cooled over time and the absorption changes were read at 450, 523 and 600 nm. The MDA was recorded in µmol g^{-1} FW.

Measurement of H_2O_2 content

H_2O_2 was assessed by Khan *et al.* (2014) with slight variations. One gram of grape sample was added into a mortar and mixed with 5 ml of 1% (w/w) TCA and then homogenized by centrifugation at 4°C for 15 min at 10,000 rpm. Then, 1 ml of the above solution was combined with 1 ml of 0.1 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 mM potassium iodide (KI). Then, it was located in the darkness at 25°C for 1 hour. The absorption changes of the reaction were evaluated at 390 nm and the amount of H_2O_2 was recorded in µmol g^{-1} FW.

Measurement of enzymes activity

First, the enzymes extracting from one gram of grape seed was added to 5 ml of 20 mM Tris-HCl buffer with a pH of 7.5, and mixed until homogeneous. Then, it was centrifuged at 10,000 rpm for 4 minutes at 4°C. Finally, the supernatant was isolated and used to measure the POD, PO, SOD, and APX enzymes activity (Alrashdi *et al.*, 2017).

The POD activity was evaluated by Zhou and Leul's (1998) method. Then, 2 ml of the reaction mixture containing 0.023 ml of 0.97 M H_2O_2, 0.23 ml of 0.5 M guaiacol, 0.75 ml of 0.2 M sodium acetate buffer (pH 5.5) and 1 ml of enzymatic extract was added. The activity of the enzyme was expressed as min^{-1} g^{-1}fresh at 25 ± 2°C. The change in the solution absorption was read at 470 nm. The amount of absorption was expressed in U g^{-1}FW.

We used the method of Khazaei *et al.* (2020) to evaluate the activity of the PO enzyme activity. Then, 200 µl of the enzymatic extract was added to the mixture containing 2.8 ml of 20 mM catechol solution in 0.01 M sodium phosphate buffer (pH 6.8), then the absorption changes were explained at 400 nm. The amount of absorption was read in U g^{-1}FW in absorbance per min.

For the measurement of CAT activity, the method of Dhindsa *et al.* (1981) was used. About 150 µl of enzyme extract was mixed with 1000 µl of 100 mM potassium phosphate buffer (pH 7.8) and 1500 µl of 15 mM H_2O_2. The absorption changes were recorded at 240 nm. The amount of absorption was expressed in U g^{-1}FW. The method of Xu *et al.* (2013) was used for the measurement of the activity of the SOD enzyme. For this purpose, 200 µl of the enzyme extract with 1000µl of 100 mM phosphate buffer (pH 7.8), 700 µl of 55 mM methionine, 200 µl of Nitro-Blobrazo-Trazolium (NBT) 0.75 mM and 600µl were added into the test tube.

The test tubes containing the reaction mixture were then exposed to 40 µmol m^{-2} s^{-1} fluorescent light for 10 minutes. Absorption changes were measured at 560 nm. One unit of the SOD enzyme activity was expressed as the amount of enzyme that inhibits 50% of the NBT light transmission. The amount of absorption was expressed in U g^{-1}FW.

Nakano and Asada's (1981) method was used to evaluate the APX activity with some modifications. 200 µl of the enzyme extract was poured into a mixture containing 1,500 µl of 50

mM phosphate buffer (pH 7.0), 300 µl of 0.2 mM ascorbic acid, 200 µl of 20 mM H_2O_2, and 0.1 mL of EDTA. Absorption changes were recorded at 290 nm. The amount of absorption was read in U g^{-1}FW.

Statistical analysis

In this study, data analysis was performed in SPSS 18.1 Software using Duncan multi-range tests. The analyses were carried out to determine the significant differences between the means at a significance level of $P \leq 0.05$.

Results and Discussion

In this experiment, both treatments had considerable effects on the studied factors. The combined effects of both treatments caused enhancements in the studied factors, compared to the simple effect of each treatment alone. The GA_3 plays an important role in regulating various biological activities in the plants, fruit growth, cellular division and cellular growth. The weight and size of the fruits were the most important factors of quality among the clusters and berries that affect the marketing of grapes (Fortes *et al.*, 2015). Gibberellins revealed the most effect at cluster development and subsequently the increase of the cellular activity in the cluster tissue (Shiri *et al.*, 2020). Photosynthesis enhanced largely in the cluster cells under gibberellin. Consequently, the growth speed of the clusters increased and led to cumulate the photosynthetic materials in the cluster tissues, enhancing the cell divisions and ultimately producing larger clusters compared by the control samples (Wientjes *et al.*, 2011). The GA3 improved the growth and development of chloroplasts, increased the total chlorophyll content and photosynthetic productivity, leading to increased yield by acerbating the weight and size of fruit (Pal *et al.*, 2016). The application of the GA3 was important for better results and berry development, cell enlargement phase and enhanced berry weight (Anjum *et al.*, 2020). The prior researches have shown that treatment with the GA_3 increases the expression of genes which are corresponded with DNA replication (histones *h1* and *h2b*) and cell elongation (expansin and α-tubulin) (Van den Heuvel *et al.*, 2001). The genes ERD10, RED14, COR47 were up-regulated during the first stage of cluster formation under the gibberellin (Kovacs *et al.*, 2008). The cell wall plasticity was increased to 150% by treatment with the GA; the increase of the plasticity of the cell wall was with hydrolysis of starch to sugars, which decreased the water potential of cell and the entry of more water into the cell, causing the cell to lengthen (Nowsheen *et al.*, 2017). Here, the GA_3 and G treatments caused positive significant effects on the characteristics of the clusters and berries; however, the GA_3 treatment had more effects. Both treatments caused a significant increase in the berry weight, length, diameter and width, compared to the control (Fig 1, Table 1).

a study by El-kenawy (2018) showed that the GA_3 had positive effects on the grape cultivars. The GA_3 was also found to play a key role in enhancing the N, P and K content, and foliar spray with the GA3 improved the ratio of carbon to nitrogen (Soad and Ibrahim, 2005).

The cluster size and weight increased in response to the GA_3 and G treatments, compared to the control (Table 1), confirming the previous results by Eleonora *et al.* (2018) on *Vitis vinifera* L. An evaluation of both treatments on the grapes (*Vitis vinifera* L.) of 'Victorian' and 'Italian' cultivars revealed that a combined treatment of the GA_3 and G yielded the best results in terms of grape cluster weight (Eleonora *et al.,* 2018), thereby confirming the results of this research. The G altered hormone levels and had positive effects on the grape growth, creating larger berries, with greater diameters and lengths (El-kenawy, 2018). In another study, Ferrara *et al.,* (2014) showed that the gibberellins and G enhanced the vegetative qualities of the grape berries. This finding was in line with the current results on 'Yaghoot' grapes.

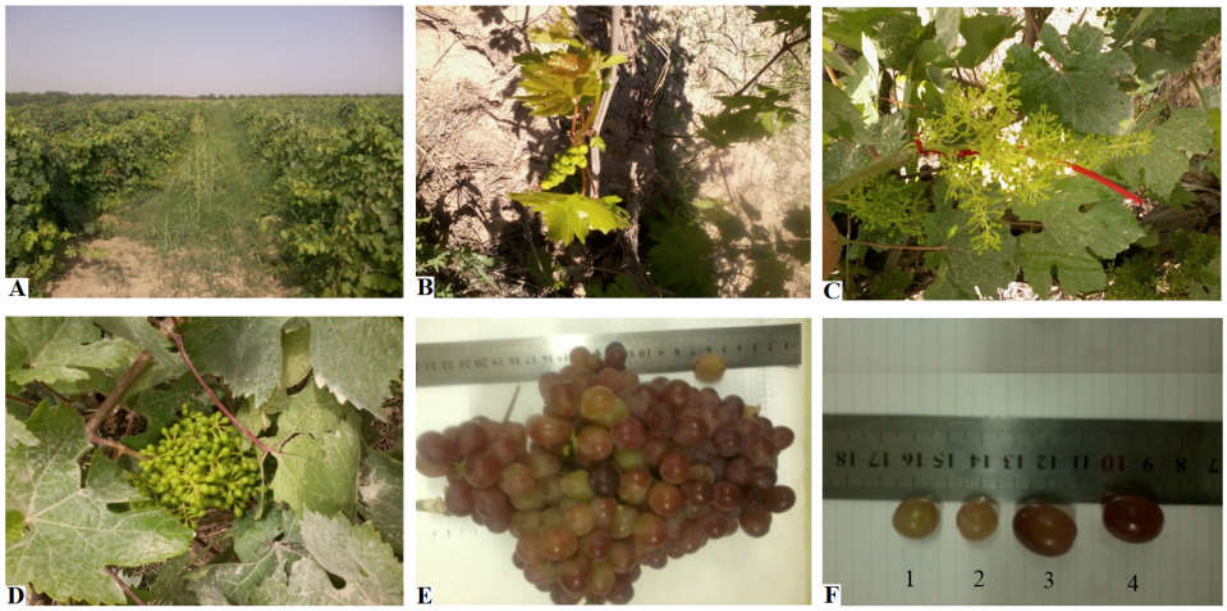

Fig. 1. The effect of GA_3 on Berry diameter: A) Research location, B) Exogenous application in the early flowering stage, C) flowering stage, D) at the stage of berry formation. E) The size of berries after exogenous application with GA_3: 3, 4 and 1, 2 controls. F) The size of clusters after being treated by GA.

Table 1. A comparison of the mean GA_3 and G effects on physicochemical parameters of Yaghoot grape

Treatments	Weight of 20 berry (g)	Berry Length (mm)	Berry width (g)	Berry diameter (mm)	Cluster weight (mm)	Cluster Length (mm)	Cluster width (mm)	TSS (%)	TA (%)	TSS/TA
0 (control)	18.35± 0.8h	14.08± 0.2e	8.69± 0.5f	18.6± 0.6f	284.3± 3.1f	13.6± 0.2f	130± 2.2f	18.2± 0.5e	1.5± 0.02g	12.08± 0.5g
GA60 mM	22.97± 0.5.f	17.48± 0.36c	11.27± 0.41e	21.8± 0.9de	334± 5.5d	21.1± 0.5d	210± 5.5d	27.6± 0.6d	1.7± 0.002ef	15.19± 0.6e
GA90 mM	23.04± 0.4e	18.08± 0.41c	13.07± 0.4d	22.5± 0.3cde	337± 4.9d	21.5± 0.8d	210± 2.8d	28.2± 0.5d	1.7± 0.02e	16.63± 0.8c
GA120 mM	24.9± 0.45d	18.71± 0.35c	14.28± 0.61c	23.1± 0.7cd	346± 4.8cd	23.2± 0.7c	230± 3.7c	35.54± 0.92b	1.8± 0.03d	19.74± 0.6b
G	20.05± 0.9g	16.1± 0.5d	9.66± 0.4f	21.1± 0.8e	320± 4.1e	19.4± 0.2e	194± 2.2e	35.49± 0.7b	1.6± 0.02fg	21.55± 0.61a
GA60+ G	27.28± 0.9c	20.45± 0.4b	16.05± 0.4b	23.5± 0.7bc	353± 5.6bc	24.6± 0.3b	246± 5.3b	32.58± 0.95c	1.9± 0.03c	16.19± 0.7c
GA90+ G	28.68± 0.6b	20.51± 0.4b	16.9± 0.5ab	24.7± 0.6b	360± 6 b	24.7± 0.4b	247± 5.4b	35.96± 0.86b	2.1± 0.04b	16.09± 0.87c
GA120+ G	32.82± 0.8a	23.44± 0.35a	17.61± 0.41a	27.2± 0.6a	375.3± 5 a	26.4± 0.5a	264± 6.5a	38.62± 0.42a	2.2± 0.03a	17.55± 0.86c

In each column, the same letters do not show a significant difference at the 5% probability level in the Duncan test.

Both treatments affected the growth factors due to the effect of the GA_3 on the stimulating cell division and expansion, thereby leading to an increase in the berry size and weight (length and width). The GA_3 can also increase the flexibility of the cell wall, accelerate the transfer rate of nutrients inside the grape berries, and re-distribute the carbohydrate, thereby increasing the weight and size of the berries (Cheng *et al.,* 2013; Fortes *et al.,* 2015).

In this study, treatment G caused an increase in the concentration of soluble solids in the grape berries. The G treatment increased the production rate of the Plant Growth Regulators (PGR) in the fruit. TSS is an important measure of fruit quality. It includes soluble solids such as sucrose, glucose, fructose and acids (Tyagi *et al.,* 2020). Soluble sugars play a key role in the synthesis of antioxidant compounds such as AsA (Rivas *et al.,* 2008).

In this regard, Muñoz-Robredo *et al.* (2013) suggested that this change is due to fluctuations in the concentration of sugars and organic matter. Therefore, it can be a good indicator of grape ripening for the information of consumers. The statistical results of this study showed that

the combination of the GA_3 and G caused increases in the TSS, TA and TSS/TA (Table 1). Kumar *et al.,* (2017) showed that applying the GA_3 helped to increase the fruit size, fruit weight, total soluble solids and ascorbic acid level.

The G treatment caused an increase in the carbohydrates and TSS in the fruits after preventing the transfer of carbohydrates to the roots of grapes or by the effect of high endogenous levels of phytohormones that occured after the two treatments (Teszlak *et al.,* 2013). Kaplan *et al.* (2019) showed how treating grapes with the GA_3 can cause a significantly higher ratio of TSS/acid and, also, a higher level of total acidity, compared to the control.

In many fruits, anthocyanin pigments are liable for the colors of red, purple and blue; they are beneficial to human health because of their antioxidant (Boyce, 2011). In fact, the antioxidant content capacity of the fruits plays a key role in determining the marketability of the grapes and in increasing the likelihood of the consumer acceptance (Kok and Bal, 2017; Kaplan *et al.,* 2019).

In our study, after the GA_3 and G treatments, there was an increase in the anthocyanins content in fruits, compared to the control. These results are consistent with those of a recent study on the grapes treated by the GA_3 (Alrashdi *et al.,* 2017; Sangeetha *et al.,* 2015) and G (Tyagi *et al.,* 2020).

The statistical analysis showed that the GA_3 and G caused significant increases in the carbohydrate content of the grapes (Table 2) and more accumulation of carbohydrates caused more synthesis of anthocyanins (Teszlak *et al.,* 2013). In previous studies, the GA_3 reportedly reduced the ability of the adsorbents at flowering, while increasing the intake of carbohydrates in the fruits (Pereira *et al.,* 2011). The G treatment temporarily interrupted the transmission through the phloem, thereby increasing the carbohydrates and other metabolites above the girdling area (Basile *et al.,* 2018), which partly explain the observations of this study.

Table 2. A comparison of the mean GA_3 and G effects on biochemistry and physiological parameters of Yaghoot grape

Treatments	AC (%)	TPC (mg g^{-1}FW)	ASA (µmolg^{-1}FW)	Anthocyanin (mol g^{-1} FW)	TSC (mgg^{-1} FW)	Flavonoids (mg g^{-1} FW)	Proline (µg g^{-1} FW)	H_2O_2 (µmol g^{-1} FW)	MDA (µmol g^{-1} FW)
0 (control)	10.3± 0.5f	1.57± 0.1e	9.93± 0.2g	0.13± 0.02d	1.13± 0.2d	1.31± 0.02e	11.6± 0.1g	0.15± 0.05e	1.61± 0.08g
GA60 Mm	15.2± 0.6ef	2.2± 0.2e	12.74± 0.3f	0.15± 0.03d	1.33± 0.1d	2.02± 0.08e	15.3± 0.3f	0.2± 0.03e	1.94± 0.06fg
GA90 mM	17.3± 0.3e	3.39± 0.1d	16.7± 0.3e	0.20± 0.01cd	1.42± 0.1d	2.2± 0.05e	18.7± 0.2e	0.30± 0.01d	2.65± 0.08ef
GA120	26.12± 0.6d	4.29± 0.1c	22.11± 0.5d	0.21± 0.05cd	1.9± 0.05cd	3.54± 0.11d	23.4± 0.2d	0.31± 0.04d	3.41± 0.1e
G	35.88± 0.5c	4.95b± 0.1c	22.89± 0.7d	0.27± 0.08bc	2.65± 0.1bc	4.17± 0.09d	26.1± 0.3c	0.36± 0.05bc	4.9± 0.1d
GA60+G	41.7± 0.5bc	5.3± 0.3b	26.56± 0.3c	0.28± 0.03bc	2.8± 0.08bc	6.15± 0.3c	27.9± 0.3c	0.37± 0.06bc	6.24± 0.2c
GA90+G	43.0± 0.5b	5.34± 0.1b	29.59± 0.6b	0.32± 0.02ab	3.45± 0.3b	7.24± 0.3b	30.8± 0.4b	0.45± 0.02ab	7.75± 0.3b
GA120+G	50.43± 0.7a	6.95± 0.2a	34.64± 0.6a	0.37± 0.07a	5.1± 0.3a	10.31± 0.4a	35± 0.55a	0.48± 0.06a	11.41± 0.5a

The repeated letters in each column indicate no significant variation at the 5% probability level in the Duncan test; TSC= Total soluble carbohydrates.

The results of our study showed that the GA_3 and G increased the ASA content in the treated samples compared to the control (Table 2). Ascorbic acid is one of the organic acids that increase in response to the GA_3 treatment and G in fruits (Rivas *et al.,* 2008; Jadhav *et al.,* 2020). It is one of the non-enzymatic antioxidants that can play an important role in the activity of several enzymes (Jadhav *et al.,* 2020). In addition, it is effective in various plant functions, including the regulation of cell division and growth, as well as its involvement in signals transduction (Kapłan *et al.,* 2019; Alrashdi *et al.,* 2017). Table 2 shows that the GA_3 and G increased the antioxidant capacity of the treated

grapes, compared to the control. An increment in the amount of the reactive oxygen species could trigger a rise in the antioxidant enzymes (Khazaei *et al.,* 2020). Therefore, enzymes such as CAT, SOD and POD tend to increase to mitigate the effects of the oxidative stress from the reactive oxygen species (Rivas *et al.,* 2008). The use of the GA_3 improved the antioxidant enzymes activities and antioxidant enzyme genes expression such as SOD, POD, and CAT (Loreti *et al.,* 2008). The presence of the secondary metabolites, such as phenols, anthocyanins and flavonoids can help determine the antioxidant capacity of the grapes (Kaplan *et al.,* 2019).

The GA_3 abolished the positive effects on the expression of several anthocyanin biosynthetic genes in the anthocyanin pathway (Loreti *et al*., 2008). In agreement with our results, Alrashdi *et al.* (2017) stated that the GA_3 increased the antioxidant capacity in table grapes. In other studies, Boyce (2011) showed that the G increased the antioxidant capacity in *Syzygium samarangense* and *Citrus reticulata*, respectively. Proline is known to increase in plants when stress is imposed. It helps maintain the osmotic balance, thereby reducing or eliminating the amount of the reactive oxygen species (Cai *et al.,* 2019). Our results showed that the GA_3 and G caused an increase in the proline amounts. Previous results have shown that the GA_3 (Jadaw *et al.,* 2020) and G (Rivas *et al.,* 2008) increased the proline content, thereby confirming the results of this experiment. In general, stresses increase reactive oxygen species, including hydrogen peroxide which oxidizes lipids and damages cells (Xu *et al.,* 2013). Stresses also trigger the production of H_2O_2 as a signaling molecule involved in regulating the plant growth (Foyer and Noctor, 2005). In our study, the H_2O_2 and MDA contents in the 'Yaghoot' grapes were affected and increased by the GA_3 and G treatments, compared to the control (Table 2). In a study by Cheng *et al.* (2013), the application of the GA_3 on *Vitis vinifera* L. increased the amounts of H_2O_2 and MDA. Similar results were observed by Tang *et al.* (2016) after carrying out the G treatment on *Alhagi sparsifolia*, being in agreement with our results. Antioxidants include enzymatic and non-enzymatic essential constituents for scavenging free radicals in the plants (Khazaei *et al.,* 2020). The enzyme superoxide dismutase transforms the superoxide radical to a hydrogen peroxide radical, which is then reduced to water and oxygen molecules by CAT and peroxidase enzymes (Kadkhodaie *et al.,* 2013). Thus, by increasing the free radicals, the SOD enzyme is stimulated in the plant, and then other antioxidant enzymes are activated to continue deactivating the reactive oxygen species (Alscher *et al.,* 2002). The CAT enzyme plays an important role in the glutathione ascorbate cycle, which removes the H_2O_2 produced by the SOD enzyme in different parts of the cell. APX is another antioxidant enzyme that plays an important role in regulating the intracellular ROS levels (Khazaei *et al.,* 2020). In addition to catalase and peroxidase, the enzyme PPO reduces the amount of H_2O_2 and maintains the membrane stability (Khazaei and Estaji, 2020). As shown in Table 3, samples treated with the GA_3 and G enhanced the activity of SOD, CAT, POD, APX, and PPO enzymes, compared to the control samples.

An increase in antioxidant enzymes were observed by Wang *et al.* (2019) after the GA_3 treatment on cherry tomatoes. Similarly, such observations were made after carrying out the G treatments on citrus, olives (Rivas *et al.,* 2008) and grapes (El-kenawy, 2018) in confirmation of our results.

Table 3. A comparison of the mean GA_3 and G effects on biochemistry and physiological parameters of Yaghoot grape.

Treatments	SOD ($Ug^{-1}FW$)	CAT ($Ug^{-1}FW$)	POD ($Ug^{-1}FW$)	PPO ($Ug^{-1}FW$)	APX ($Ug^{-1}FW$)
0 (control)	0.71±0.08f	1.48±0.06f	0.74±0.068e	30.18±0.9e	11.43±0.35g
GA60 Mm	1.61±0.05e	2.33±0.09f	1.78±0.098e	39.85±0.85d	19.38±0.5f
GA90 mM	1.72±0.09e	3.86±0.2e	3.02±0.11d	42.75±0.52d	33.21±0.5e
GA120	2.32±0.09d	4.95±0.22d	3.03±0.29d	46.09±0.35d	39.68±0.4d
G	3.54±0.1c	5.78±0.29d	3.76±0.31cd	57.47±0.6c	42.47±0.5d
GA60+G	3.61±0.3c	6.83±0.3c	4.28±0.41c	61.27±0.8bc	58.05±0.8c
GA90+G	4.12±0.3b	8.73±0.45b	6.02±0.52b	67.86±0.8ab	72.43±0.9b
GA120+G	6.59±0.6a	13.76±0.43a	10.14±0.81a	73.83±0.86a	79.24±0.9a

The repeated letters in each column indicate no significant difference at the 5% probability level in the Duncan test.

Conclusion

Our data show that the GA_3 and G treatments increase the level of lipoxidation and H_2O_2 content in the Yaghoot grapes. However, since both treatments also increase the activity of the antioxidant enzymes such as CAT, POD, SOD, APX and PPO and phenolic compounds such as anthocyanin and flavonoids, they reduce the oxidative damage in the grapes.

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Conflicts of interest

The authors declared no conflict of interest for this work.

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