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Application of Exogenous Polyols, Amino Acids and Girdling to Improve (*Vitis vinifera* L.) Crimson Seedless cv. Berries Coloration, and Postharvest Quality

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Abstract: The present study was conducted for two successive seasons 2017 and 2018 in a private vineyard located at Cairo-Alexandria desert road. Ten-year-old 'Crimson Seedless' grapevines were used to examine the effect of polyols (Sorbitol and Mannitol) at 10 and 20 mM, Amino acids (phenylalanine and methionine) at 250 and 500 ppm along with Ethrel (Ethephon) at 480 ppm, with or without girdling on berry characteristics and storability. At harvest the clusters were stored for 21 days, and postharvest measurements were recorded. Results showed significant effects of girdling on the physical and chemical characteristics of berries, besides improving the berry anthocyanin, coloration, and storage characteristics enhancement. Generally, Phenylalanine 250 and 500 ppm showed the best results were obtained from treating the vines at the harvest and after the storage period.

Key word: Grapes · *Vitis vinifera* · Polyols · Amino acids · Girdling Crimson seedless · Coloration · Postharvest quality

INTRODUCTION

The berries quality is the most important criterion in grape local marketing and exporting. Grape berries' color is the major limiting factor of the fruit quality for consumer satisfaction [1-3]. Crimson seedless is one of the important cultivars around the world, it is famous for its late maturity, crimson color, crispy firmness, and long life of storability [4, 5]. However, the coloration of its' berries face undesirable mal-coloration that affects the marketing of that variety. This mal-coloration includes berries in the same cluster or even between different clusters on the same vine. Vines that are grown in relatively higher temperatures climates showed nonmarketable berries' red color and they required exogenous application of products that could improve the coloration [6, 7]. Additionally, grape berries' characteristics and ripening are conducted by many other factors such as nutrients, hormones, environmental factors, and horticulture technical practices [9].

Moreover , anthocyanin is the major pigment in the grape berries' skin (exocarp) which is one of flavonoids that gives the berries their color [8, 9]. It is considered an antioxidant substance and the most important component of table grapes and wine grapes. Light, UV, nutrient conditions, hormones, elicitors, osmotic stress, and carbohydrates can affect the production of anthocyanins, the high temperature decreases the accumulation of anthocyanin in grape berries' skin [10]. Some sugars showed increasing in the anthocyanin production of grapes as a result of inducing the phenylalanine ammonia-lyase (PAL) enzyme [6]. Sugars could be gone through another pathway in increasing anthocyanin while decreasing phenylalanine as a precursor of anthocyanin production [11]. Subsequently, there is a crosstalk between sugars and hormones in the process of anthocyanin production [8, 9].

Polyols are monosaccharide and disaccharide molecules in which the aldehyde group is replaced by hydroxyl. Polyols, such as Sorbitol and Mannitol, are used to provide sweet taste in food. A major advantage of polyols is the functional ability to provide sweetness with fewer calories per gram [12].

They participate in many different physiological processes such as a source of carbon and energy, and they make the plants tolerant to water deficiency stress and adapted to osmotic stress (Osmo-protective substances) in some plants and microorganisms [13-16].

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Sugar alcohol can be used as a foliar application in improving plant growth, and as a chelation substance of nutrients to increase the absorption and metabolism of nutrients such as Calcium, thus improving the fruit quantity and quality characteristics [17]. Sorbitol and Mannitol are sugar alcohols that are polyols with low molecular weight which are naturally located in many fruits and vegetables [18]. They can be used exogenously in developing the growth characteristics of plants [19]. They have a role in the activity of the phenylalanine ammonia-lyase (PAL) enzyme [20]. That consequently impacts anthocyanin production. However, they generally may increase the anthocyanin content in plant cells under various conditions; they may have a negative effect under different conditions [21-23].

Studying the role of amino acids will lead to understanding their impact on not only Crimson Seedless cv. but also all the other varieties. Phenylalanine is a vital amino acid in viticulture and enology as it has various roles in many physiological processes. It works as a precursor of phenolic compounds biosynthesis and anthocyanin production [11, 24-26]. Studies found that the foliar application by phenylalanine enhanced the anthocyanin, furthermore, application at veraison stage is significantly efficient when anthocyanin is started to be synthesized and accumulated [4, 25, 26]. Besides, phenylalanine affects the acid contents of the grape berries, which reflects the quality of the fruits [27]. Methionine is considered one of the important amino acids besides phenylalanine with its different roles in plant cells. The application of methionine may increase soluble solid content (SSC), Total soluble solids (TSS), and some sugar contents, in addition, to increasing the anthocyanin content in grape berries or conserving the fruit anthocyanin during storage [28, 29].

Additionally, girdling is one of the common horticultural practices on fruit trees. It is shown an effective method to enhance the quality of fruits and lead to the accumulation of various substances inside the fruit tissues such as acids, antioxidants, sugars, phenolic compounds such as anthocyanin, and others. Thus, it can improve the quality of the fruits and enhance the coloration of berries [30, 31].

The aim of this study was to investigate new alternative substances that can be used instead of ethephon to improve the quality of grape berries.

MATERIAL AND METHODS

The experiment was implemented at a vineyard located at the Cairo-Alexandria desert road for two successive seasons 2017 and 2018 on 10-year-old Crimson seedless grape cultivar (*Vitis vinifera* L.) grown in sandy soil. They were normally irrigated under drip irrigation, fertilized, and trellised by Spanish Barron training system. Vines were spur pruned and spaced at 3×4 m between vines and rows respectively.

Experimental design and treatments: One hundred uniform vines were used (20 treatments x 5 replicates x one vine/replicate). The 20 treatments were divided into 2 main groups; girdled vines and non-girdled vines, each 10 treatments comprised foliar application of polyols (Sorbitol and Mannitol) at 10 and 20 mM, Amino acids (phenylalanine and methionine) at 250 and 500 ppm along with Ethrel at 480 ppm, as follow:

1. Control (water spray)
2. Ethrel (Ethephon) at 480 ppm as the same as used in the orchard application.
3. Polyol (Sorbitol) at 10 mM
4. Polyol (Sorbitol) at 20 mM
5. Polyol (Mannitol) at 10 mM
6. Polyol (Mannitol) at 20 mM
7. Amino acid (Phenylalanine) at 250 ppm
8. Amino acid (Phenylalanine) at 500 ppm
9. Amino acid (Methionine) at 250 ppm
10. Amino acid (Methionine) at 500 ppm

The foliar application of Ethrel, Polyols and amino acids was applied two times at the veraison stage by using a back-carrying sprayer. All vines were sprayed until the saturation of leaves and the beginning of leaching dropped.

The girdling was done before the veraison stage at the truck (main stem) 30 cm away from the initiation of arms. The clusters were collected at the full maturity stage with the common grape maturity indices [4].

Physical Measurements of Berries:

a. Brush length (cm)
b. Pedicel length (mm)
c. Tours width (mm) had been measured by using caliper tool.
d. Detachment force (g) and Firmness of berries Kp/cm^2

Chemical measurements of berries:

a. The total soluble solids (TSS as Brix°) were measured by a manual refractometer for the juice of the berries [32].

b. Titratable acidity (TA) % was recorded by titrating according to Tyl and Sadler *et al.* [33].
c. TSS/ acid ratio was calculated subsequently to Sabir, *et al*. [32].
d. Skin color was evaluated by the color charts, while the anthocyanin (mg/100g) was measured by spectrophotometer according to Hsia *et al.* [34] and Aleixandre-Tudo, *et al.* [35].

Postharvest Characteristics Berries after the Storage Period: Number of clusters stored at 0°C±2 and RH 85-90 % for 21 days as a storage period and then the following criteria were measured:

a. Excluded clusters %
b. Dropped berries %
c. Shrunken berries %
d. Rotten berries %
e. Changing cluster weight %
f. Changing in TSS %

Statistical Analysis: Statistical analysis of Split plot design (SPD), analysis of variance (ANOVA) and Duncan's multiple range test was used to determine the significance of the differences among samples ($p < 0.05$). Stat software had been used for the statistical analysis.

RESULTS AND DISCUSSION

Physical Measurements of Berries:
Berries Brush Length, Pedicel Length and Tours Width: The results in Tables 1, 2 and 3 revealed that brush length, pedicel length and tours width were significantly decreased in the girdled vines compared to the non-girdled ones in both seasons.

Regarding to the foliar application treatments in table 1, Phenylalanine at 500 ppm significantly increased the brush length in first season, however Phenylalanine at 250 ppm had the highest values in the second season compared to the ethrel treatment and the control. Similarly, the other treatments gave higher values compared to the control for both seasons.

In an opposite trend as mentioned in Table 2, Phenylalanine significantly decreased the pedicel length in the first season, while there was not a significant difference in the second season compared to both the control and ethrel treatments. Generally, it is obvious that the pedicel length has significant differences between the control and all other treatments except for the ethrel treatment in the first season, whereas in the second

Table 1: Effect of Polyols, amino acids, and girdling on brush length /cm of Crimson seedless cv. berries.

Treatment	Non-Girdling	Girdling	Means
Season 1			
Control	1.20b-e	0.83f	1.02E
Ethrel	1.43b-d	1.10d-f	1.27CD
Mannitol 10 mM	1.37b-e	1.07e-f	1.22DE
Mannitol 20 mM	1.40b-e	1.47bc	1.43BC
Sorbitol 10 mM	1.53b	1.50bc	1.52B
Sorbitol 20 mM	1.50bc	1.30b-e	1.40B-D
Methionine 250 ppm	1.50bc	1.17c-f	1.33B-D
Methionine 500 ppm	1.53b	1.47bc	1.50B
Phenylalanine 250 ppm	1.43b-d	1.27b-e	1.35B-D
Phenylalanine 500 ppm	2.27a	1.23b-e	1.75A
Means	1.52A	1.24B	
Season 2			
Control	1.54b-f	1.30 gh	1.42DE
Ethrel	1.27h	1.38 f-g	1.32E
Mannitol 10 mM	1.65a-d	1.59 a-e	1.62AB
Mannitol 20 mM	1.65a-d	1.51c-f	1.58BC
Sorbitol 10 mM	1.75a	1.56b-f	1.66AB
Sorbitol 20 mM	1.71ab	1.59a-e	1.65AB
Methionine 250 ppm	1.76a	1.57b-e	1.66AB
Methionine 500 ppm	1.64a-d	1.63a-e	1.63AB
Phenylalanine 250 ppm	1.72ab	1.69a-c	1.71A
Phenylalanine 500 ppm	1.45e-g	1.49d-f	1.47CD
Means	1.61A	1.53B	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

Table 2: Effect of Polyols, amino acids, and girdling on pedicle length/cm of Crimson seedless cv. berries.

Treatment	Non-Girdling	Girdling	Means
Season 1			
Control	0.667a	0.533ab	0.600A
Ethrel	0.533ab	0.467ab	0.500AB
Mannitol 10 mM	0.467ab	0.433b	0.450BC
Mannitol 20 mM	0.433b	0.433b	0.433BC
Sorbitol 10 mM	0.400b	0.467ab	0.433BC
Sorbitol 20 mM	0.400b	0.433b	0.417BC
Methionine 250 ppm	0.400b	0.367b	0.383BC
Methionine 500 ppm	0.400b	0.500ab	0.450BC
Phenylalanine 250 ppm	0.433b	0.433b	0.433BC
Phenylalanine 500 ppm	0.367b	0.367b	0.367C
Means	0.450A	0.443A	
Season 2			
Control	0.543bc	0.490c	0.517B
Ethrel	0.547bc	0.567bc	0.557AB
Mannitol 10 mM	0.533bc	0.553bc	0.543AB
Mannitol 20 mM	0.747a	0.513bc	0.630A
Sorbitol 10 mM	0.610a-c	0.523bc	0.567AB
Sorbitol 20 mM	0.743a	0.480c	0.612A
Methionine 250 ppm	0.577bc	0.567bc	0.572AB
Methionine 500 ppm	0.577bc	0.657ab	0.617A
Phenylalanine 250 ppm	0.567bc	0.643ab	0.605AB
Phenylalanine 500ppm	0.577bc	0.620a-c	0.598AB
Means	0.602A	0.561B	

*Different letters indicate significant differences by Dunkn's ($P \leq 0.05$)

Table 3: Effect of Polyols, amino acids, and girdling on tours width /cm of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
Season 1			
Control	0.127c	0.103c	0.115BC
Ethrel	0.283a	0.117c	0.200A
Mannitol 10 mM	0.167bc	0.130c	0.148A-C
Mannitol 20 mM	0.200a-c	0.113c	0.157A-C
Sorbitol 10 mM	0.267ab	0.107c	0.187A-B
Sorbitol 20 mM	0.107c	0.100c	0.103C
Methionine 250 ppm	0.100c	0.100c	0.100C
Methionine 500 ppm	0.100c	0.100c	0.100C
Phenylalanine 250 ppm	0.267ab	0.100c	0.183A
Phenylalanine 500ppm	0.267ab	0.100c	0.183A
Means	0.188A	0.107B	
Season 2			
Control	0.333a	0.210d-h	0.272AB
Ethrel	0.270a-e	0.270a-e	0.270AB
Mannitol 10 mM	0.290a-c	0.210d-h	0.250A-C
Mannitol 20 mM	0.180f-h	0.220c-g	0.200D
Sorbitol 10 mM	0.210d-h	0.233b-f	0.222CD
Sorbitol 20 mM	0.283a-c	0.143h	0.213CD
Methionine 250 ppm	0.300ab	0.157gh	0.228B-D
Methionine 500 ppm	0.243b-f	0.200e-h	0.222CD
Phenylalanine 250 ppm	0.270a-e	0.280a-d	0.275A
Phenylalanine 500ppm	0.200e-h	0.247b-f	0.223CD
Means	0.258A	0.217B	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

Table 4: Effect of Polyols, amino acids, and girdling on detachment force (g) of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
Season 1			
Control	320.67F	213.67g	267.17E
Ethrel	214.00g	272.00fg	243.00E
Mannitol 10 mM	620.00de	581.00de	600.50CD
+Mannitol 20 mM	530.00e	581.33de	555.67D
Sorbitol 10 mM	671.00b-d	653.33cd	662.17B
Sorbitol 20 mM	548.67e	736.00a-c	642.33BC
Methionine 250 ppm	762.00ab	772.33a	767.17A
Methionine 500 ppm	772.67a	803.33a	788.00A
Phenylalanine 250 ppm	825.33a	820.00a	822.67A
Phenylalanine 500ppm	591.00ed	595.67de	593.33CD
Means	585.53B	602.87A	
Season 2			
Control	357.33F	521.33e	439.33D
Ethrel	279.00F	335.33f	307.17E
Mannitol 10 mM	581.00e	624.33c-e	602.67C
Mannitol 20 mM	574.33e	598.33e	586.33C
Sorbitol 10 mM	751.00ab	720.00b-d	735.50B
Sorbitol 20 mM	783.00ab	791.00ab	787.00AB
Methionine 250 ppm	731.00a-c	732.00a-c	731.50B
Methionine 500 ppm	790.67ab	795.67ab	793.17AB
Phenylalanine 250 ppm	822.00ab	836.33a	829.17A
Phenylalanine 500ppm	601.00e	607.67d-e	604.33C
Means	627.03B	656.20A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

Table 5: Effect of Polyols, amino acids, and girdling on the firmness (Kp/cm^2) of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
Season 1			
Control	0.193Bc	0.217a-c	0.205B
Ethrel	0.180C	0.200a-c	0.190B
Mannitol 10 mM	0.193Bc	0.220a-c	0.207B
Mannitol 20 mM	0.227a-c	0.200a-c	0.213AB
Sorbitol 10 mM	0.190bc	0.220a-c	0.205B
Sorbitol 20 mM	0.207a-c	0.193bc	0.200B
Methionine 250 ppm	0.210a-c	0.223a-c	0.217AB
Methionine 500 ppm	0.250a	0.233ab	0.242AB
Phenylalanine 250 ppm	0.200a-c	0.233ab	0.217AB
Phenylalanine 500ppm	0.217a-c	0.217a-c	0.217AB
Means	0.207B	0.216A	
Season 2			
Control	0.197b	0.227ab	0.212AB
Ethrel	0.223ab	0.213ab	0.218AB
Mannitol 10 mM	0.200ab	0.210ab	0.205AB
Mannitol 20 mM	0.217ab	0.193b	0.205AB
Sorbitol 10 mM	0.193b	0.217ab	0.205AB
Sorbitol 20 mM	0.207ab	0.183b	0.195B
Methionine 250 ppm	0.210ab	0.223ab	0.217AB
Methionine 500 ppm	0.250a	0.213ab	0.232A
Phenylalanine 250 ppm	0.213ab	0.223ab	0.218AB
Phenylalanine 500ppm	0.207ab	0.200ab	0.203AB
Means	0.213A	0.212A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

season, there were no significant differences between the ethrel treatment and all the other treatments, while there were significant differences between both types of Polyols; mannitol and sorbitol at 20 mM and the control.

Concerning the effect of phenylalanine on tours width in Table 3, there was a significant increase in both seasons, although there was no significance with the control in the second season.

Overall, there was a great variation in treatments interactions between girdling effect and the sprayed treatments.

There are many factors and elements affecting the coloration, sweetness, and quality characteristics of berries such as brush length, pedicel width and tours width. Consequently, that may affect other factors related to the quality and storability of clusters during a definite period [36].

Detachment Force (g) and Firmness of Berries Kp/cm^2: The results in Tables 4 and 5 demonstrated that girdling significantly increased the detachment force in both seasons whereas it significantly increased the firmness in the first season, while there was no significant difference for the berry firmness in the second season.

Table 6: Effect of Polyols, amino acids, and girdling on TSS (Brix°) of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
	Season 1		
Control	15.80b-d	15.50b-e	15.67CD
Ethrel	15.30c-d	14.70e	15.00E
Mannitol 10 mM	16.00bc	14.80de	15.42DE
Mannitol 20 mM	16.50b	17.70a	17.08A
Sorbitol 10 mM	16.30cb	16.20bc	16.25BC
Sorbitol 20 mM	16.50b	16.00bc	16.25BC
Methionine 250 ppm	16.50b	16.20bc	16.33B
Methionine 500 ppm	16.00cb	16.00bc	16.00BD
Phenylalanine 250 ppm	16.00cb	16.00bc	16.00BD
Phenylalanine 500ppm	16.30cb	16.00bc	16.17BC
Means	16.13A	15.90B	
	Season 2		
Control	20.33b-f	23.00ab	21.67A
Ethrel	19.67d-f	21.00a-f	20.33A
Mannitol 10 mM	20.00c-f	22.67a-c	21.33A
Mannitol 20 mM	19.00f	23.67a	21.33A
Sorbitol 10 mM	19.33e-f	21.67a-f	20.50A
Sorbitol 20 mM	19.33e-f	22.33a-d	20.83A
Methionine 250 ppm	19.00f	22.33a-d	20.67A
Methionine 500 ppm	20.00c-f	22.00a-e	21.00A
Phenylalanine 250 ppm	19.00f	22.33a-d	20.67A
Phenylalanine 500ppm	19.33e-f	20.67b-f	20.00A
Means	19.50B	22.17A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

Table 7: Effect of Polyols, amino acids, and girdling on acidity g/100mL of Crimson seedless cv. berries.

Treatment	Non-Girdling	Girdling	Means
	Season 1		
Control	0.393a-d	0.350a	0.372BC
Ethrel	0.217b-d	0.383a	0.300CD
Mannitol 10 mM	0.417b-d	0.300ab	0.358BC
Mannitol 20 mM	0.600c-d	0.250ab	0.425BC
Sorbitol 10 mM	0.233c-f	0.207a-c	0.220D
Sorbitol 20 mM	0.230c-f	0.233a-c	0.232D
Methionine 250 ppm	0.450c-g	0.433a-d	0.442BC
Methionine 500 ppm	0.500c-g	0.350a-d	0.425BC
Phenylalanine 250 ppm	0.233c-h	0.473a-d	0.353BC
Phenylalanine 500ppm	0.617d-h	0.533a-d	0.575A
Means	0.389A	0.351B	
	Season 2		
Control	0.303b	0.477a	0.390A
Ethrel	0.320b	0.343ab	0.332A
Mannitol 10 mM	0.333ab	0.323ab	0.328A
Mannitol 20 mM	0.323ab	0.307b	0.315A
Sorbitol 10 mM	0.333ab	0.360ab	0.347A
Sorbitol 20 mM	0.300b	0.367ab	0.333A
Methionine 250 ppm	0.320b	0.333ab	0.327A
Methionine 500 ppm	0.293b	0.373ab	0.333A
Phenylalanine 250 ppm	0.340ab	0.330ab	0.335A
Phenylalanine 500ppm	0.293b	0.400ab	0.347A
Means	0.316B	0.361A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

According to the results in Tables 4 and 5, the Ethrel treatment showed the lowest mean of detachment force in both seasons. the treatment of Ethrel had the lowest values of berries firmness in the first season, however this was not clear in the second season. Phenylalanine at 250 ppm recorded the highest detachment force means in both seasons with significant difference for either the control or ethrel treatment. It was noticed that Methionine whether at 250 or 500 ppm, showed significant differences among the other treatments in the first season but did not follow the same trend in the second season. Phenylalanine and Methionine at 250 ppm and 500 ppm showed higher values of firmness in both seasons.

In the interactions between treatments in Tables 4 and 5, all treatments with or without girdling showed an increment in detachment force compared to both of control and Ethrel treatments. The ethrel treatment had the lowest means in the first season whereas Methionine and Phenylalanine at 250, 500 ppm had the highest values, however in the second season the means were relatively close to Ethrel treatment.

Chemical Measurements of Berries:

Total Soluble Solids (TSS), Acidity, and TSS Acid Ratio: the results in Table 6 revealed that in the first season Mannitol 20 mM has the highest mean of TSS compared to the control and the other treatments in both seasons. There were significant differences between Mannitol 20 mM and the other treatments in the first season however there was not significance in the second season [13, 15, 17, 37].

Whereas in Table 7 expressed that the lowest acidity values were recorded from treating the vines with sorbitol 10 and 20 mM with approximately significant differences with all the other treatments.

in the first season sorbitol 10, 20 mM was the highest TSS/acid ratio % ratio with significant difference with all the other treatment and the control as well, while in the second season there was not any significant differences between all the treatments of polyols or amino acids as mentioned in Table 8. According to the interaction between treatments, Sorbiltol 10 mM with girdling treatment showed the highest mean with significant difference compared to the control. It is noticed that sorbitol 10, 20 mM with and without girdling interactions showed higher means compared to control. In the second season, sorbitol 20mM and its interaction with girdling showed highest mean with significant difference with the interaction of control with girdling.

Table 8: Effect of Polyols, amino acids, and girdling on TSS/Acid ratio % of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
	Season 1		
Control	40.56de	44.90c-e	42.73CE
Ethrel	71.67ab	39.84de	55.76BC
Mannitol 10 mM	40.30de	53.33a-d	46.82CD
Mannitol 20 mM	27.84de	70.67a-c	49.26CD
Sorbitol 10 mM	70.83a-c	78.41a	74.62A
Sorbitol 20 mM	72.25ab	71.11a-c	71.68AB
Methionine 250 ppm	36.93de	37.41de	37.17DE
Methionine 500 ppm	32.00de	46.35b-e	39.17DE
Phenylalanine 250 ppm	71.11a-c	34.26de	52.68CD
Phenylalanine 500ppm	26.52e	30.22de	28.37E
Means	49.00A	50.65A	
	Season 2		
Control	67.86ab	50.03b	58.95A
Ethrel	63.14ab	63.56ab	63.35A
Mannitol 10 mM	61.11ab	70.17ab	65.64A
Mannitol 20 mM	59.00ab	77.22a	68.11A
Sorbitol 10 mM	58.26ab	60.31ab	59.28A
Sorbitol 20 mM	65.38ab	62.22ab	63.80A
Methionine 250 ppm	59.60ab	67.78ab	63.69A
Methionine 500 ppm	68.26ab	59.30ab	63.78A
Phenylalanine 250 ppm	57.49ab	68.24ab	62.87A
Phenylalanine 500ppm	65.95ab	51.67ab	58.81A
Means	62.61A	63.05A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

Table 9: Effect of Polyols, amino acids, and girdling on anthocyanin (g/L) of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
	Season 1		
Control	1.34d	1.59cd	1.46B
Ethrel	2.76ab	2.47ab	2.62A
Mannitol 10 mM	2.38ab	2.84ab	2.61A
Mannitol 20 mM	2.12a-d	2.74ab	2.43A
Sorbitol 10 mM	2.06b-d	2.88a	2.47A
Sorbitol 20 mM	2.10a-d	2.87a	2.48A
Methionine 250 ppm	2.35a-c	2.59ab	2.47A
Methionine 500 ppm	2.51ab	2.71ab	2.61A
Phenylalanine 250 ppm	2.15a-c	2.79ab	2.47A
Phenylalanine 500ppm	2.61ab	2.72ab	2.67A
Means	2.24B	2.62A	
	Season 2		
Control	1.36f	1.41f	1.39D
Ethrel	2.55b-e	2.94a-c	2.74A-C
Mannitol 10 mM	2.48c-e	2.74a-d	2.61BC
Mannitol 20 mM	2.69a-e	2.24de	2.47C
Sorbitol 10 mM	2.13e	2.64a-e	2.39C
Sorbitol 20 mM	2.70a-e	2.64a-e	2.67BC
Methionine 250 ppm	2.59b-e	2.45c-e	2.52BC
Methionine 500 ppm	2.38c-e	2.71a-d	2.54BC
Phenylalanine 250 ppm	2.56b-e	3.11ab	2.84AB
Phenylalanine 500ppm	2.92a-c	3.19a	3.06A
Means	2.44B	2.61A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

Table 10: Effect of Polyols, amino acids, and girdling on color % of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
	Season 1		
Control	2.17f-g	3.00d-f	2.58D
Ethrel	4.33a-c	3.17c-f	3.75AB
Mannitol 10 mM	1.67g	4.33a-c	3.00B-D
Mannitol 20 mM	1.67g	5.00a	3.33B-D
Sorbitol 10 mM	2.17fg	4.00a-d	3.08B-D
Sorbitol 20 mM	2.83d-g	4.67a-b	3.75AB
Methionine 250 ppm	2.33fg	4.83a-b	3.58A-C
Methionine 500 ppm	3.67b-e	4.83a-b	4.25A
Phenylalanine 250 ppm	2.83d-g	2.83d-g	2.83DC
Phenylalanine 500ppm	3.83a-e	2.67e-g	3.25B-D
Means	2.75B	3.93A	
	Season 2		
Control	2.33e	2.83de	2.58C
Ethrel	3.67a-e	2.83de	3.25A-C
Mannitol 10 mM	2.83de	4.33a-d	3.58A-C
Mannitol 20 mM	2.83de	5.00a	3.92AB
Sorbitol 10 mM	2.33e	3.67a-e	3.00BC
Sorbitol 20 mM	3.33b-e	4.83ab	4.08A
Methionine 250 ppm	3.00c-e	4.83ab	3.92AB
Methionine 500 ppm	3.33b-e	4.67ab	4.00AB
Phenylalanine 250 ppm	3.33b-e	4.00a-d	3.67AB
Phenylalanine 500ppm	4.50b-c	3.83a-e	4.17A
Means	3.15B	4.08A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$).

Sorbitol and mannitol had different effects on some berries physical and chemical characteristics [13]. In our experiments, effects on physical characteristics of berries, anthocyanin content, TSS and acidity have been noticed. They showed differences with ethrel application in the characteristic of berries during storage as well.

Anthocyanin and Color %: Girdling clearly improved the coloration of cluster berries [30, 31]. but the interaction between Girdling and the application of polyols and amino acids showed an effect on the anthocyanin in berries besides the ethrel treatment.

Girdling significantly increased the anthocyanin and consequently Crimson color of berries in both seasons (showed in Tables 9 and 10) [38, 39].

All the treatments values showed an increment in the anthocyanin content compared to the control, besides there were no significant differences between ethrel treatment and all the other treatments in both seasons [29, 38].

In the first season the highest values of anthocyanin for were recorded from interaction between girdling and mannitol 10, 20 mM but there was not any significant differences with the other interaction treatments. There was a significant difference between the control with and

Table 11: Effect of Polyols, amino acids, and girdling on dropped berries % of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
	Season 1		
Control	4.52b-e	2.85c-f	3.69BC
Ethrel	11.83a	12.78a	12.30A
Mannitol 10 mM	6.98b	2.88c-f	4.93B
Mannitol 20 mM	2.69c-f	5.03b-d	3.86BC
Sorbitol 10 mM	6.07bc	3.47b-f	4.77B
Sorbitol 20 mM	4.10b-f	1.37d-f	2.74B-D
Methionine 250 ppm	2.64c-f	1.64d-f	2.14CD
Methionine 500 ppm	3.02c-f	1.35d-f	2.18CD
Phenylalanine 250 ppm	1.61d-f	2.70c-f	2.16CD
Phenylalanine 500ppm	0.64f	1.01ef	0.82D
Means	4.41A	3.51A	
	Season 2		
Control	1.28de	1.71c-e	1.50B
Ethrel	11.19a	8.44b	9.82A
Mannitol 10 mM	1.65c-e	2.58cd	2.11B
Mannitol 20 mM	1.17de	0.81de	0.99B
Sorbitol 10 mM	0.90de	3.00cd	1.95B
Sorbitol 20 mM	0.70de	1.05de	0.87B
Methionine 250 ppm	0.64de	2.52c-e	1.58B
Methionine 500 ppm	1.11de	0.74de	0.92B
Phenylalanine 250 ppm	0.00e	2.86cd	1.43B
Phenylalanine 500ppm	0.00e	3.85c	1.92B
Means	1.86B	2.76A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

Table 12: Effect of Polyols, amino acids, and girdling on shrunken berries % of Crimson seedless cv. berries

Treatment	Non Girdling	Girdling	Means
	Season 1		
Control	4.51b	3.29b	3.90B
Ethrel	14.17a	12.58a	13.38A
Mannitol 10 mM	3.38b	2.11b	2.74B
Mannitol 20 mM	1.67b	4.00b	2.84B
Sorbitol 10 mM	1.82b	1.50b	1.66B
Sorbitol 20 mM	4.12b	3.37b	3.74B
Methionine 250 ppm	2.45b	4.56b	3.51B
Methionine 500 ppm	2.28b	2.16b	2.22B
Phenylalanine 250 ppm	1.83b	2.60b	2.22B
Phenylalanine 500ppm	1.71b	1.20b	1.46B
Means	3.79A	3.74A	
	Season 2		
Control	3.82cd	4.01cd	3.91B
Ethrel	13.88a	8.75b	11.32A
Mannitol 10 mM	3.86cd	2.36c-e	3.11BC
Mannitol 20 mM	2.40c-e	4.39c	3.40BC
Sorbitol 10 mM	4.55c	4.21cd	4.38B
Sorbitol 20 mM	2.77c-e	4.35c	3.56B
Methionine 250 ppm	4.15cd	4.60c	4.38B
Methionine 500 ppm	2.54c-e	2.94c-e	2.74B-D
Phenylalanine 250 ppm	0.95e	1.67de	1.31D
Phenylalanine 500ppm	0.67e	3.03c-e	1.85CD
Means	3.96A	4.03A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

without girdling and all the other treatments with and without treatments. This go through the principle of the crosstalk between sugars and plant hormones in anthocyanin producing [8, 9, 23].

There were many researches explained the role of phenylalanine in anthocyanin biosynthesis [40, 41].

Phenylalanine 500 ppm had the highest values of the anthocyanin in both seasons. There were significant differences between phenylalanine 500 ppm and the control in both seasons. The control means were the lowest with and without girdling, besides there were significant differences between control and all other treatments with and without girdling process.

According to the percentage of color % which was depended on the perception test of visual judgement, it was go in the same trend, but there was a little bit different variation in the significance differences between treatment. The variation differences in the treatments of girdling, other substances application, and the interaction between them.

Postharvest Characteristics Berries after the Storage Period: Recently the storage periods can be adjusted and determined because of the progress in logistics and marketing technology. One of the most important traits of grapes is the color of berries and storability. The common material for improving color in grapes has been ethrel (ethephon) but its residual in berries is harmful in grapes. Ethephon can as a growth regulator can deteriorate berries characteristics during the storage period. Our experiment highlighted that other substances and girdling can be alternatives to ethrel in improving coloration without decreasing the berries characteristics during storage of grapes.

According to Singh *et al*. [30] girdling significantly improves the grape berries characteristics before the storage period, while its effect was unclear after the storage period. It increased the brush length and tour width and consequently increased the detachment force, and subsequently decreased the overall dropped berries.

In the same trend, phenylalanine increased the brush length and decreased the pedicle length which may affect increase the detachment force and firmness of the clusters. Methionine has effects on the firmness and detachment force as well, which decreased the dropping of berries during the storage period.

The results recorded after storage in Table 11 explained that the interaction between phenylalanine 500 ppm and non-girdling had the lowest means of dropped berries in both seasons. It significantly decreased the

Table 13: Effect of Polyols, amino acids, and girdling on rotten berries % of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
Season 1			
Control	0.29b	0.00b	0.15B
Ethrel	2.06a	2.24a	2.15A
Mannitol 10 mM	0.30b	0.22b	0.26B
Mannitol 20 mM	0.00b	0.00b	0.00B
Sorbitol 10 mM	0.33b	0.25b	0.29B
Sorbitol 20 mM	0.00b	0.00b	0.00B
Methionine 250 ppm	0.00b	0.00b	0.00B
Methionine 500 ppm	0.30b	0.19b	0.25B
Phenylalanine 250 ppm	0.00b	0.00b	0.00B
Phenylalanine 500ppm	0.00b	0.18b	0.09B
Means	0.33A	0.31A	
Season 2			
Control	4.01c	3.60c-e	3.81B
Ethrel	12.62a	6.41b	9.52A
Mannitol 10 mM	1.80de	1.49e	1.65D
Mannitol 20 mM	1.50e	3.07c-e	2.28CD
Sorbitol 10 mM	2.98c-e	3.72cd	3.35BC
Sorbitol 20 mM	2.45c-e	2.66c-e	2.55B-D
Methionine 250 ppm	2.96c-e	2.30c-e	2.63B-D
Methionine 500 ppm	2.10c-e	2.94c-e	2.52B-D
Phenylalanine 250 ppm	1.58de	4.04c	2.81B-D
Phenylalanine 500ppm	1.66de	1.99c-e	1.83D
Means	3.37A	3.22A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

Table 14: Effect of Polyols, amino acids, and girdling on excluded clusters % of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
Season 1			
Control	6.67b	13.33b	10.00B
Ethrel	13.33b	33.33a	23.33A
Mannitol 10 mM	0.00b	0.00b	0.00C
Mannitol 20 mM	0.00b	0.00b	0.00C
Sorbitol 10 mM	0.00b	0.00b	0.00C
Sorbitol 20 mM	0.00b	0.00b	0.00C
Methionine 250 ppm	0.00b	0.00b	0.00C
Methionine 500 ppm	0.00b	0.00b	0.00C
Phenylalanine 250 ppm	0.00b	0.00b	0.00C
Phenylalanine 500ppm	0.00b	0.00b	
Means	2.0A	4.67A	
Season 2			
Control	13.33ab	13.33ab	13.33B
Ethrel	33.33a	33.33a	33.33A
Mannitol 10 mM	0.00b	0.00b	0.00B
Mannitol 20 mM	0.00b	0.00b	0.00B
Sorbitol 10 mM	0.00b	0.00b	0.00B
Sorbitol 20 mM	0.00b	0.00b	0.00B
Methionine 250 ppm	0.00b	0.00b	0.00B
Methionine 500 ppm	0.00b	0.00b	0.00B
Phenylalanine 250 ppm	0.00b	0.00b	0.00B
Phenylalanine 500ppm	0.00b	0.00b	0.00B
Means	4.67A	4.67A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

Table 15: Effect of Polyols, amino acids, and girdling on changing cluster weight % of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
Season 1			
Control	37.15b	29.51c	33.33B
Ethrel	43.38a	45.42a	44.40A
Mannitol 10 mM	28.30cd	24.09de	26.19C
Mannitol 20 mM	29.62c	22.13e	25.88C
Sorbitol 10 mM	30.00c	22.29e	26.15C
Sorbitol 20 mM	30.00c	25.30c-e	27.65C
Methionine 250 ppm	30.00c	24.79c-e	27.39C
Methionine 500 ppm	29.70c	25.00c-e	27.35C
Phenylalanine 250 ppm	20.52ef	17.05f	18.79D
Phenylalanine 500ppm	6.96g	7.37g	7.17E
Means	28.56A	24.30B	
Season 2			
Control	26.05d-f	32.36c	29.20B
Ethrel	41.50b	48.15a	44.83A
Mannitol 10 mM	21.84fg	27.49c-e	24.67CD
Mannitol 20 mM	26.34d-f	25.14d-f	25.74BC
Sorbitol 10 mM	25.23d-f	23.12e-g	24.18CD
Sorbitol 20 mM	29.82cd	24.69d-f	27.26BC
Methionine 250 ppm	26.91d-f	24.03e-g	25.47C
Methionine 500 ppm	23.67e-g	19.34gh	21.50D
Phenylalanine 250 ppm	16.72hi	14.65hi	15.68E
Phenylalanine 500ppm	8.31j	12.16ij	10.23F
Means	24.64A	25.11A	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

total dropped berries % during the storage period compared to the ethrel treatment in both seasons, and with the control treatment in the first season. The other treatments have significant effects also in the decreasing dropping of berries compared to the ethrel which was the highest treatment for dropping berries. In both seasons the interaction of phenylalanine and non-girdling was the lowest mean with significant difference.

Shrunken berries percentage (%) in Table 12 were significantly increased during the storage period by the ethrel treatments with or without girdling in both seasons. the girdling did not have any solo effect in shrinkage trait during the storage period. Phenylalanine 500 ppm were the lowest means in first season with significant differences with ethrel treatments, however both 250 and 500ppm concentrations of phenylalanine showed the lowest values in the second season.

In the same trend, the most susceptible berries to rot were the treated with Ethrel with significant differences with all treatments (as showed in Table 13). On the other hand, all the treatments showed less numbers of rotten berries with significance to each other in both seasons. The highest numbers excluded clusters % in both seasons was ethrel treatment (as showed in Table 14). There were significant differences between ethrel treatment and all the

Table 16: Effect of Polyols, amino acids, and girdling on changing in TSS % of Crimson seedless cv. berries

Treatment	Non-Girdling	Girdling	Means
Season 1			
Control	11.91bc	11.00bc	11.45B
Ethrel	8.89bc	6.75c	7.82C
Mannitol 10 mM	13.54b	13.52b	13.53B
Mannitol 20 mM	14.06b	10.62bc	12.34B
Sorbitol 10 mM	9.19bc	11.46bc	10.32BC
Sorbitol 20 mM	9.28bc	10.63bc	9.95BC
Methionine 250 ppm	10.08bc	13.45b	11.76B
Methionine 500 ppm	12.50b	12.50b	12.50B
Phenylalanine 250 ppm	12.50b	12.34b	12.42B
Phenylalanine 500ppm	22.47a	22.92a	22.70A
Means	12.44A	12.52A	
Season 2			
Control	10.67a-c	2.24f	6.46BC
Ethrel	5.09d-f	3.25f	4.17C
Mannitol 10 mM	8.33a-e	6.62b-f	7.48AB
Mannitol 20 mM	8.77a-e	5.65c-f	7.21A-C
Sorbitol 10 mM	9.52a-d	9.24a-d	9.38AB
Sorbitol 20 mM	9.52a-d	8.18a-e	8.85AB
Methionine 250 ppm	11.52ab	8.98a-e	10.25A
Methionine 500 ppm	9.17a-d	6.14c-f	7.65AB
Phenylalanine 250 ppm	11.40ab	4.00ef	7.70AB
Phenylalanine 500ppm	12.11a	8.16a-e	10.13A
Means	9.61A	6.25B	

*Different letters indicate significant differences by Duncan's ($P \leq 0.05$)

other treatments even control. That may be not clear in the first days of storage but appeared clear after the storage period. Girdling had not significant effects on the number of excluded clusters% in both seasons. in the interactions of the two factors, Ethrel with girdling treatment was the highest mean of excluded clusters % in the first season with significant difference compared to the control and the other interaction treatments.in the second season ethrel with or without were the lowest means without significant difference with control but with significant difference with all the other treatments.

Phenylalanine 500 ppm showed the highest mean in TSS changing % with significant differences compared to the control and the ethrel treatments.

CONCLUSION

The Girdling improve the berries coloration and quality . Phenylalanine 250 and 500 ppm enhance the berries quality characteristics, coloration and the berries quality after the storage period.

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