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Maximize Irrigation Amount Before Spraying with Hydrogen Cyanamide on Bud Behavior and Hormonal Content of Crimson Seedless Grape Cultivar

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Abstract: This experiment was carried out during two successive seasons 2020and 2021 with a preliminary season 2019,in a private vineyard located at Berkash, Giza governorate on seven year-old Crimson Seedless grapevines grown in a sandy soil and trellised by Gable shaped-system were used in this investigation to study the effect of different levels of irrigation before spraying with Hydrogen Cyanamide of Crimson Seedless grape cultivar in terms of bud behavior and bud content of hormones, which include indole acetic acid (IAA), gibberellic acid (GA_3), cytokinin (CK) and abscisic acid (ABA). The vines were irrigated through drip irrigation system and spaced 2 x 3 m. Vines were cane pruned during the second week of February with bud load of 72 buds/vine(6 canes x 12 bud/cane) Five different irrigation levels were applied based on the reference evapotranspiration (ETo) as follow: 0, 60, 100, 140 and 180% of ETo. All irrigation treatments were performed one day before spraying with Hydrogen Cyanamide. The study demonstrated that irrigation with 60% ETo before spraying with Hydrogen Cyanamide gave the best results in terms of obtaining the highest percentages of bud burst, fruitful buds and bud magnitude of IAA, GA_3 and CK and the least bud magnitude of ABA compared to the other irrigation levels, whereas water stress represented by the non-irrigated vines (0% ETo) resulted in the least percentages of bud burst, fruitful buds and bud magnitude of IAA, GA_3 and CK and the highest bud magnitude of ABA in both seasons. On the other hand, excessive irrigation water represented by vineyards irrigated with 100, 140 and 180% of ETo is useless due to the high cost, although its results are similar or less than the vineyards irrigated with 60% Eto.

Key words: Irrigation・Hydrogen Cyanamide・Crimson Seedless・Grape・Bud burst・Hormones

INTRODUCTION

Crimson Seedless (*Vitis vinifera* L.), is a late ripening red table grape cultivar with an important economic fruit variety widely cultivated around the world [1].

Bud dormancy is an essential adaptation, which allows temperate woody perennials to survive adverse environmental conditions during winter. During dormancy, plants experience arrested growth and reduced metabolic activities [2].

In most fruit trees prolonged dormancy is considered to be the major obstacle to economic production in warm winter regions. In these regions, the need for artificial means to compensate for lack of natural chilling becomes a dominant factor for maintaining economic production. Hydrogen Cyanamide has been found to be the most extremely effective for breaking dormancy of vegetative buds in many deciduous fruit crops [3].

Good irrigation scheduling means applying the proper amount of water at the right time. By minimizing over-irrigation, scheduling improves irrigation efficiency, which frequently results in less costly expenses, ideal water use and higher crop yields. Irrigation during winter did not fully offset the decline in production. Refilling the soil profile at the end of winter increased vigor and reduced yield. Reduced soil moisture prior to spring delayed the time of bud burst [4].

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Plant hormones or phytohormones, are naturally occurring small signaling molecules that influence plant physiological metabolism at low concentrations [5]. Plant hormones regulate growth and growths processes throughout the plant life cycle and also trigger adaptive responses triggered by external stimuli such as environmental changes and biotic or abiotic stresses. Traditional plant hormones include five main groups: abscisic acid (ABA), gibberellins (GA), ethylene (ET), Auxin (indole-3-acetic acid, IAA) and cytokinin (CK) [6].

IAA is one of the first plant hormones isolated from living plant tissues, it plays extremely significant role in the elongation of cells and plants as well as the prevention of flowers and berries dropping [7]. GA_3 could significantly promote elongation of stems and germination of seeds as well as acceleration of blossom [8]. ABA is another widely studied plant hormone which has been demonstrated to play important role in accelerating abscission of leaves and berries, dormancy of buds and formation of stress resistance [9, 10]. Cytokinins (CK) are a class of plant hormones that promote cell division, or cytokinesis, in plant roots and shoots. They are involved primarily in cell growth and differentiation, but also affect apical dominance, axillary bud growth and leaf senescence [11].

Many changes in some chemical components in buds, particular the contents of endogenous hormones (IAA, GA_3, CK and ABA) found to occur for playing a vital role in regulating dormancy and bud burst. Endogenous hormones help plants to respond to the environmental signals [12]. Several studies focused on the relationship between the endogenous hormones and dormancy in buds [13].

The positive action of Hydrogen Cyanamide on breaking dormancy is mainly attributed to its effect in removing buds scales and reducing ABA, catalase and oxidized glutathione as well as enhancing free water, IAA, GA_3, cytokinin, amino acids, total indoles, oxidative stress, H_2O_2, total free polyamines and respiratory key enzymes activities [14].

Understanding the physiological and molecular bases of grapevine responses to mild to moderate water deficits is fundamental to optimize deficit irrigation management and identify the most suitable varieties to those conditions [15].

Spraying grapevines with Hydrogen Cyanamide started in Egypt in 1987 to stimulate bud break after dormancy resulting in uniform flowering and maturity. This method was used till 1994, when the Chilean expertise recommended to irrigate the vine extremely range between 6-20 hours which equivalent to 200-600m3/ Feddan one day before spraying with Hydrogen Cyanamide and this method is applied in the sandy soil only not for the clay soil.

The haphazard application of irrigation before spraying with Hydrogen Cyanamide has actually been the main reason for the undesired and unreliable obtained results. Using the optimum irrigation will undoubtedly affect the bud behavior and hormones accumulation of the vines.

Thus, the purpose of this study is to maximize the effect of irrigation amount before spraying with Hydrogen Cyanamide on bud behavior and bud hormonal content of Crimson Seedless grape cultivar.

MATERIALS AND METHODS

This study was carried out during the two successive seasons, 2020 and 2021 with a preliminary season 2019 in a private vineyard located at Berkash, Giza governorate on seven year-old Crimson seedless grapevines to evaluate the effect of different irrigation levels before spraying with Hydrogen Cyanamide on bud behavior and endogenous hormonal content. All vines were grown in a sandy soil and irrigated through drip irrigation system. The vines were trellised by Gable shaped-system spaced at 2 x 3 m and pruned during the second week of February with a bud load of 72 buds/vine (6 canes x 12 bud/cane), in addition to 6 spurs x 2 buds/spur. Sixty vines were chosen for this study (5 treatments x 3 replicates x 4 vines / replicate). The vines were uniform in vigor and received common horticultural practices.

Five different irrigation levels were applied based on the reference evapotranspiration (ETo) as follow:

- 0.0% ETo (without irrigation)
- 60% ETo (6 hours irrigation)
- 100% ETo (10 hours irrigation)
- 140% ETo (14 hours irrigation)
- 180% ETo(control) (18 hours irrigation)

All irrigation treatments were performed one day before spraying with Hydrogen Cyanamide.

Monthly average of agro-meteorological data at the experimental site and ETo values for the two growing seasons are presented in Table 1.

The soil physical, chemical properties and soil-moisture constants at the experimental site, determined according to Page, *et al*. [16] and Klute [17] are listed in Tables 2 and 3.

Table 1: Monthly average meteorological data of Giza Research weather station during 2020 and 2021 seasons.

Months	Temperature (°C) Max.	Min.	Relative humidity (%)	Wind speed (m/sec)	Sunshine (h)	ETo (mm/day)
	2020 season					
January	19.3	8.1	46.0	1.9	10.3	2.87
February	21.2	9.4	45.3	1.9	11.0	3.44
March	24.9	12.0	41.3	2.5	11.8	4.99
April	29.1	14.3	34.0	2.0	12.8	6.00
May	34.6	19.1	29.3	2.4	13.5	7.85
June	36.7	21.3	32.3	2.1	13.9	8.03
July	37.3	23.5	40.7	2.2	13.7	8.06
August	37.4	24.1	39.7	2.3	13.1	7.85
September	36.8	24.7	40.0	2.1	12.2	6.88
October	33.0	21.8	44.0	2.3	12.4	5.78
November	25.9	15.2	45.7	2.0	10.4	3.67
December	31.9	11.1	46.7	1.9	11.0	3.5
	2021 season					
January	21.4	13.5	59.1	2.6	10.3	2.84
February	22.3	9.2	61.5	2.4	11	3.16
March	14.4	10.7	62.4	2.7	11.8	3.75
April	30.6	13.7	50.2	3.2	12.8	5.66
May	36.5	20.1	36.6	3.1	13.5	6.30
June	36.5	20.1	41.4	3.5	13.9	7.42
July	37.2	25.4	41.1	3.0	13.7	7.86
August	37.8	24.9	42.8	2.8	13.1	7.64
September	33.7	22.7	51.0	3.2	12.2	6.38
October	20.1	16.7	55.2	3.0	12.4	4.47
November	27.6	16.9	61.7	2.3	10.4	3.54
December	20.1	11.2	68.4	2.6	11	2.35

Table 2: Soil physical and chemical properties at the experimental site in 2019/2020 and 2020/2021 seasons

Soil properties		Soil depth (cm)		
Particle size distribution	Unite	0-20	20-40	40-60
Sandy	%	91.00	90.40	90.20
Silt	%	3.70	3.85	4.00
Clay	%	5.30	5.75	5.80
Texture		Sandy	Sandy	Sandy
pH (1:2.5)		7.70	7.50	7.68
EC, soil past extract	dS m^{-1}	0.36	0.35	0.34
Soluble cations				
Ca^{++}	meq l^{-1}	1.22	1.24	1.47
Mg++	meq l^{-1}	0.62	0.50	0.43
Na+	meq l^{-1}	1.60	1.66	1.45
K+	meq l^{-1}	0.22	0.16	0.10
Soluble anions				
CO_3^-	meq l^{-1}	--	--	--
HCO_3^-	meq l^{-1}	1.20	1.03	1.08
Cl^-	meq l^{-1}	1.72	1.74	1.75
SO_4^{--}	meq l^{-1}	0.76	0.68	0.62

* According to Klute [17] **According to Page, *et al*. [16]

Table 3: Soil water constants and bulk density at the experimental site

Depths (cm)	Field capacity (%)	Wilting point (%)	Available moisture (%)	Bulk density (g cm^{-3})
0-15	13.5	5.6	7.9	1.50
15-30	12.7	5.1	7.7	1.70
30-45	12.3	5.0	7.3	1.75
45-60	10.2	4.4	5.8	1.80

Soil Water Relations:

Reference Crop Evapotranspiration (Et_o): ET_o values were calculated based on local meteorological data of the experimental site (Table 2) and according to the penman-monteith equation [18]. Calculations were performed using the CROPWAT model [19].

$$ET_o \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T+273} u_2 (e_s - e_a)}{\Delta + \gamma(1+0.3u_2)}$$

where:

ET_o: reference evapotranspiration (mm day^{-1}), R_n: net radiation at the crop surface (MJ m^{-2} day^{-1}), G: soil heat flux density (MJ m^{-2} day^{-1}), T: mean daily air temperature at 2 m height (°C), u_2:wind speed at 2 m height (ms^{-1}), e_s: saturation vapor pressure (kPa), e_a: actual vapor pressure (kP) e_s-e_a: vapor pressure deficit (kPa), Δ: slope of the vapor pressure-temperature curve (kPa °C^{-1}), γ: psychrometric constant (kPa °C^{-1}).

Amount of Applied Irrigation Water (AIW): The amount of applied water was measured by a flow meter and was calculated according to the following equation [20]:

$$AIW = \frac{S_p \ XS_l \ X \ ET_o \ X \ Kr \ X \ I\text{interval}}{Ea} + LR$$

where:

AIW = Applied irrigation water depth (liters/day).

Sp = Distance between plants in the same line (m).

S_l = Distance between lines (m).

Et_o = Reference evapotranspiration (mm day^{-1})

K_r = Reduction factor that depends on the crop cover

E_a = Irrigation efficiency of the drip system (assumed as 0.9)

$I_{interval}$ = Irrigation intervals (days) = 1 day for the experimental site.

$$\text{LR = Leaching requirements} = \frac{EC_w}{2MaxEC_e}$$

where:

Ec_w = Electrical conductivity of the irrigation water (1.2 dS/m).

Max Ec_e = Maximum tolerable electrical conductivity of the soil saturation extract for grape crop (5 dS/m).

Bud Behavior: At the onset of bud burst of each season, the number of buds burst /vine and fruitful buds were counted, then the percentages of bud burst and fruitful buds were calculated according to Bessis [21].

$$\text{Bud burst (\%)} = \frac{\text{(No. of burst buds} \times 100)}{\text{Total number of bud/vine}}$$

$$\text{Fruitful buds (\%)} = \frac{\text{(No. of fruitful buds} \times 100)}{\text{Total number of bud burst/vine}}$$

Bud Hormones Content: Samples of buds were collected at the onset of bud burst for each season. Plant hormones including indole acetic acid (IAA), gibberellic acid (GA_3), cytokinin (CK) and abscisic acid (ABA) were determined (mg/100g D.W.) in plant samples as methods of Durley *et al.* [22] and Wurst *et al*. [23] using HPLC Agilent infinity better 1250 Model.

Experimental Design and Statistical Analysis: The experiment arranged in a randomized complete block design. According to Snedecor and Cochran [24], the statistical analysis of the present data was performed. Usage the new LSD values at 5% level, averages were compared [25].

RESULTS AND DISCUSSIONS

Amount of Applied Irrigation Water (AIW): The effect of tested irrigation treatments on applied irrigation water expressed as m^3/fed/month for the 2019/2020 and 2020/2021 growing seasons is presented in Table 4. The grapevines were irrigated with 25 % ETo as follows: October and November: three times/month; December, January: two times/month. During winter, vines are dormant, or their metabolism is slowed significantly; therefore, irrigation can be less frequent.

Irrigation treatments started after vines received the winter irrigation on February i.e., starting from bud swelling stage. Additional amount of water were applied in this trial in the period before spraying with Hydrogen Cyanamid. Results show that amounts of applied irrigation water were 0.0, 100, 167, 233 and 300 m3/fed./yr in first season and 0.0, 97, 162, 226 and 291 m3/fed./yr in second season for the I_1 (0.0 % Eto), I_2 (60 % ETo), I_3 (100 % ETo), I_4 (140 % ETo) and I_5 (180 % ETo) irrigation treatments, respectively. The obtained amounts equal 0.0, (143 -139), (238 -231), (333 -323) and (428 - 416)

Table 4: Effect of irrigation treatments on the amounts of applied irrigation water for the 2020 and 2021 growing seasons.

		Applied irrigation water									
		2019/2020					2020/2021				
		I_1	I_2	I_3	I_4	I_5	I_1	I_2	I_3	I_4	I_5
Month	AIW	Dormancy period					Dormancy period				
		25 % Eto					25 % ETo				
Oct.	L/ vine	110	110	110	110	110	86.6	86.6	86.6	86.6	86.6
	m^3/fed	77.3	77.3	77.3	77.3	77.3	60.6	60.6	60.6	60.6	60.6
Nov.	L/ vine	69.4	69.4	69.4	69.4	69.4	66.4	66.4	66.4	66.4	66.4
	m^3/fed	48.6	48.6	48.6	48.6	48.6	46.5	46.5	46.5	46.5	46.5
Dec.	L/ vine	67.8	67.8	67.8	67.8	67.8	45.5	45.5	45.5	45.5	45.5
	m^3/fed	47.5	47.5	47.5	47.5	47.5	31.9	31.9	31.9	31.9	31.9
Jun.	L/ vine	54.3	54.3	54.3	54.3	54.3	55.0	55.0	55.0	55.0	55.0
	m^3/fed	38.0	38.0	38.0	38.0	38.0	38.5	38.5	38.5	38.5	38.5
	Before spray with Hydrogen Cyanamide						Before spray with Hydrogen Cyanamide				
		0.0 % Eto	60 % ETo	100 % ETo	140 % ETo	180 % ETo	0.0 % ETo	60 % ETo	100 % ETo	140 % ETo	180 % ETo
Feb.	L/ vine	0.0	143	238	333	428	0.0	139	231	323	416
	m^3/fed	0.0	100	167	233	300	0.0	97	162	226	291

Table 5: Effect of irrigation amount before spraying with Hydrogen Cyanamide on bud behavior of Crimson Seedless grapevines during 2020 and 2021 seasons

	Bud burst (%)		Fruitful buds (%)	
Treatments	2020	2021	2020	2021
0.0% ETo (without irrigation)	65.4	68.2	58.1	59.3
60% ETo	84.2	87.5	82.3	84.6
100% ETo	79.9	82.4	77.1	79.1
140% ETo	75.1	79.1	75.6	77.2
180% ETo(control)	74.6	68.9	73.4	75.9
New LSD at 0.05 =	4.7	5.2	5.6	6.1

L/vine /month in both seasons for the same respective treatments, respectively. The values showed that water applied by grape are higher in the first than in the second season. Such results are mainly due to differences in climatic factors. The obtained results were in harmony with those reported by Musick, *et al*. [26] who concluded that benefits of preseason irrigation are likely to be greatest when the soil profile is dry and growing season irrigation is reduced. In general, the conclusions were that in-season irrigation was more beneficial than preseason irrigation and that often preseason irrigation was not warranted [27].

Bud Behavior: As shown in Table (5), it is noticed that irrigation amount before spraying with Hydrogen Cyanamide markedly affected on bud behavior represented by the percentages of bud burst and fruitful buds different levels of irrigation in both seasons.

Bud Burst %: The highest significant bud burst percentage was obtained from vines irrigated with 60% ETo followed by100% ETo with insignificant differences between them was observed followed by vines irrigated with 140% Eto then 180% ETo. On the other hand, the non-irrigated vines (0 % ETo) had the lowest percentage of this one in both seasons.

These results are in accordance with those stated by Abd El-Maksoud [28] on Thompson Seedless grape, Ali, *et al*. [29] and El-Gendy [30] on Superior Seedless grape and Gaser, *et al*. [31] on Flame Seedless grape, they found that bud burst percentage decreased by increasing irrigation water levels.

Fruitful Buds %: Vines irrigated with 60% ETo significantly improved the percentage of fruitful buds followed by 100% ETo, however no significant differences was noticed between them, followed by vines irrigated with 140% ETo then 180% ETo, while the lowest percentage of this one was acquired from the non-irrigated vines (0% ETo) in both seasons. The increment of fruitful buds percentage could be ascribed mainly to the increase in bud burst percentage.

The obtained results are in agreement with those reported by Ali, *et al*. [29] on Superior Seedless grape and Gaser, *et al*. [31] on Flame Seedless grape; they found that fruitful buds percentage decreased by increasing irrigation water levels.

Table 6: Effect of irrigation amount before spraying with Hydrogen Cyanamide on bud hormones content of Crimson Seedless grapevines during 2020 and 2021 seasons

	IAA (mg/100g D.W.)		GA_3 (mg/100g D.W.)		CK (mg/100g D.W.)		ABA (mg/100g D.W.)	
Treatments	2020	2021	2020	2021	2020	2021	2020	2021
0.0% Eto (without irrigation)	123.2	117.3	277.5	234.3	15.6	13.1	0.81	0.86
60% ETo	171.7	162.9	393.9	322.3	32.9	34.8	0.52	0.63
100% ETo	157.3	143.4	323.4	289.8	30.7	25.4	0.58	0.71
140% ETo	145.2	127.1	309.1	271.7	25.6	21.3	0.73	0.76
180% ETo(control)	132.9	125.8	284.9	239.8	19.3	17.5	0.74	0.79
New LSD at 0.05 =	16.7	19.8	71.6	49.2	5.7	8.3	0.04	0.03

Bud Hormones Content: The data in Table (6) showed that the measurements of bud content of hormones *i.e.* indole acetic acid (IAA), gibberellic acid (GA_3), cytokinin (CK) and abscisic acid (ABA) were significantly affected by different levels of irrigation before spraying with Hydrogen Cyanamide in both seasons.

Indole Acetic Acid (IAA): The highest significant bud IAA content was obtained from vines irrigated with 60% ETo followed by 100% ETo with insignificant differences between them was observed followed by vines irrigated with 140% ETo then 180% ETo. On the other hand, the non-irrigated vines (0% ETo) had the lowest content of this one in both seasons.

Gibberellic Acid (GA_3): Vines irrigated with 60% ETo significantly increased the buds content of GA_3followed by 100% ETo significantly, however no significant differences was noticed between them, followed by vines irrigated with 140% ETo then 180% ETo, while the lowest content of this one was acquired from the non-irrigated vines (0% ETo) in both seasons.

Cytokinin (CK): The highest significant bud CK content was obtained from vines irrigated with 60% ETo followed by 100% ETo with insignificant differences between them was observed followed by vines irrigated with 140% ETo then 180% ETo. On the other hand, the non-irrigated vines (0% ETo)resulted in the lowest content of this one in both seasons.

Abscisic Acid (ABA): Vines irrigated with 60% ETo significantly reduced the bud content of ABA followed by 100% ETo, however no significant differences was noticed between them, followed by vines irrigated with 140% ETo then 180% ETo, while the highest content of this one was acquired from the non-irrigated vines (0% ETo) in both seasons.

Plants respond to water deficit to drought stress through various physiological and biochemical changes, including changes of the endogenous phytohormone levels especially that of IAA, GA_3 CK and ABA [32].

The results obtained are consistent with those stated by Ndung, *et al*. [33], Shawky *et al*. [34] and El-Gendy [30], in which they indicated that increased water stress significantly reduced the concentrations of IAA, GA_3 and IAA, while significant increases occurred in the concentration of ABA.

The Relationship Between Bud Burst Percentage and the Changes of Hormonal Balance: Data illustrated in Figures (1, 2, 3 & 4) indicate the existence of a highly positive correlation between bud burst percentage and IAA content (r =0.9537 &0.9007), between bud burst percentage and GA_3content (r = 0.8645& 0.9867) and between bud burst percentage and CK content (r = 0.9392& 0.9469), while it was noticed that the presence of a highly negative correlation between bud burst percentage and ABA content (r = - 0.9450&- 0.9399) in the both seasons, respectively.

In general, plant hormones play critical roles in the responses of grapevines to water stress and its reflection on bud behavior, especially the percentage of bud burst. The changes of hormonal balances were associated with reduced IAA, GA_3 and CK levels and elevated ABA levels in the vines subjected to water stress.

Economic Justification of the Recommended Treatment (Irrigation with 60 % Eto) Compared with Control (Irrigation with 180 % ETo): It is clear from the data in Table (7) that irrigation with 60% ETo achieved the lowest cost up to one-third compared to the control (irrigation with 180% ETo) in both seasons. Excessive irrigation water represented by vineyards irrigated with 100, 140 and 180% of ETo is useless due to the high cost, although its results are similar or less than the vineyards irrigated with 60% Eto.

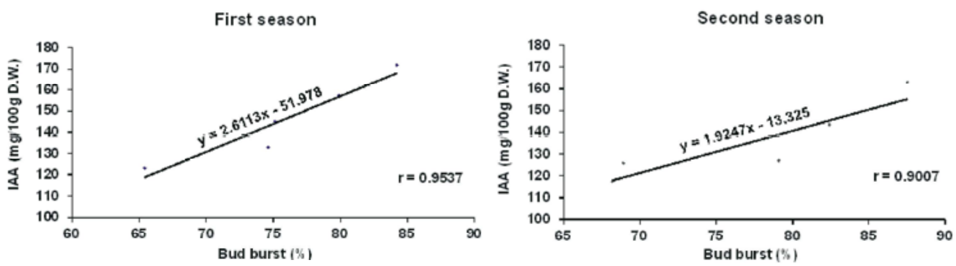

Fig. 1: The relationship between the bud burst (%) and IAA (mg/100g D.W.)

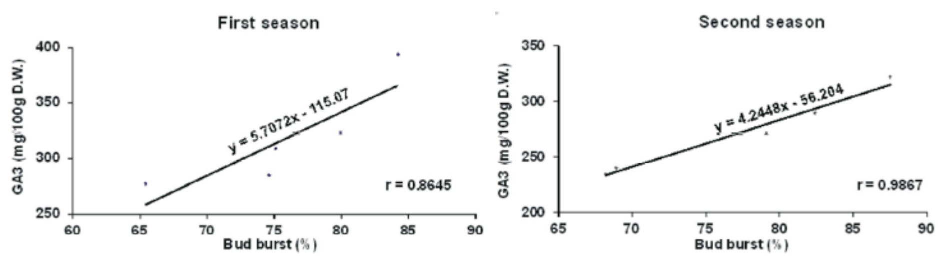

Fig. 2: The relationship between the bud burst (%) and GA3 (mg/100g D.W.)

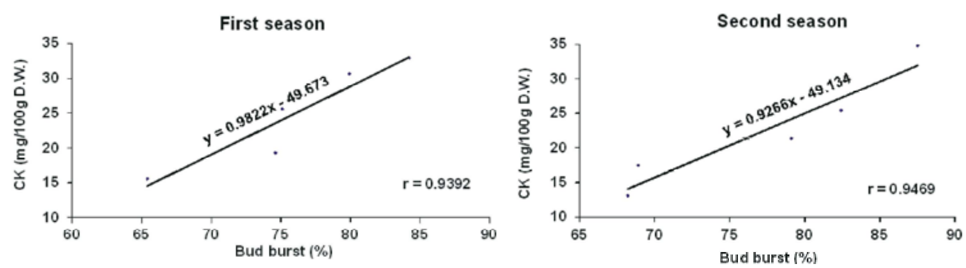

Fig. 3: The relationship between the bud burst (%) and CK (mg/100g D.W.)

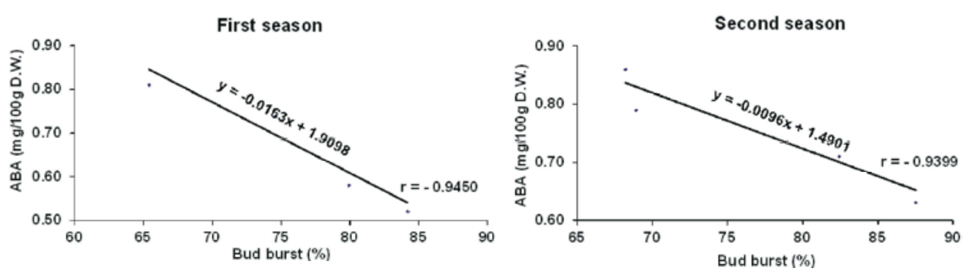

Fig 4: The relationship between the bud burst (%) and ABA (mg/100g D.W.) in b

Table 7: Economic justification of the recommended treatment (irrigation with 60 % ETo) compared with control (irrigation with 180 % ETo) for the 2020 and 2021 growing seasons.

	First season					Second season				
	0.0 % Eto	60 % ETo	100 % ETo	140 % ETo	180 % ETo	0.0 % ETo	60 % ETo	100 % ETo	140 % ETo	180 % ETo
The amount of irrigation water (m^3/Fed)	0.0	100	167	233	300	0.0	97	162	226	291
The Price of irrigation water (L.E./m^3)	0.6					0.6				
The total cost of irrigation water (L.E./Fed)	0.0	60.0	100.2	139.8	180.0	0.0	58.2	97.2	135.6	174.6

CONCLUSION

The research deals with one of the important and necessary points that focus on one of the problems that the Arab Republic of Egypt suffers from, but rather the whole world, which is water shortage or scarcity. This research dealt with this problem in a good scientific manner and an integrated scientific study and the research showed good results that could result in building useful recommendations in this field, as this research is considered important in dealing with water shortage or scarcity.

From the previous results, it can be recommended that irrigation with 60% ETo before spraying with Hydrogen Cyanamide of Crimson Seedless grapevines to give it the best results in terms of obtaining the highest percentages of bud burst, fruitful buds and bud magnitude of IAA, GA_3 and CK and the least bud magnitude of ABA compared to the other irrigation levels.

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