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Effect of canopy regulation and new generation bio-regulators on berry colouration and bio-active compounds in crimson seedless grape

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Abstract
A filed experiment on Studies on the influence of cane regulation and bio-regulators on growth, yield and quality parameters of grape *cv.* Crimson Seedless was conducted at Fruit Orchard, Division of Fruit Science, MHREC, University of Horticultural Sciences, Bagalkote, during 2021-22 and 2022-23. The combination of cane regulation at 25-33 canes per vine with 300-400 ppm of ethrel treatment at veraison and 30 days after veraison recorded the highest colour (a*) value (7.08 and 6.91), maximum anthocyanins in skin and berry (67.77 and 65.71 mg/100 g and 26.97 and 23.79 mg/100 g), highest total phenolic content (59.60 and 57.73 mg GAE/100 g) and flavonoid (65.58 and 63.48 mg GAE/100 g) content.

Keywords: Cane regulation, growth, bio-regulators, bio-active, veraison

1. Introduction
Crimson Seedless grape (*Vitis vinifera* L.) is a late season, attractive, red seedless grape with naturally small berries. Cultivar is the result of five generations of hybridization at the USDA Fresno location, starting in 1926. The source of seedlessness is from Thompson Seedless which was used as parent in the first generation of crossing. C33-199, a late-ripening white seedless grape (With all white grapes in its parentage) was used in final hybridization with emperor to produce `Crimson seedless`. But inadequate colouration and a small size might detract from their quality, because these small berries do not meet the size requirements of the export market. 'Crimson Seedless' vines are vigorous and moderately productive when cane-pruned. Seven to eight canes may be necessary on mature vines to provide commercial production. Spur-pruned vines are unproductive (Ramming and Tarailo, 1995) [16].

Cane regulation is an essential form of canopy management practice in vineyard operation. This is mainly done to regulate the current season growth, yield and quality of grapes. Crop load adjustment should be considered as one of the technical cultural practices suitable to modify grapevine physiology and plant production towards a defined goal (Matti and Ferrini, 2005) [9]. Higher number of shoots per vine *i.e.,* increased shoot density impairs the productivity of shoots. Therefore, April pruning is done to develop shoots at this rate and their vigour may be curtailed by either pinching or thinning of shoots. While in October pruning, depending upon variety more number of canes are retained on vigorous vines, less is retained on less vigorous ones. Hence, cane thinning is considered as a technique which could lead to improvement in grape (Bravdo *et al.*, 1985) [1].

Poor skin colour in red table grape cultivars is one of the most laborious difficulties faced by grape growers. Table grapes are usually cultivated in warm-climate regions of the world where the high temperatures hinder anthocyanin accumulation, leading to preventing sufficient colour development in some coloured grapes (Peppi *et al.* 2006) [15]. Foliar sprayings of grapevines with different new generation bio-regulators were evaluated for determining grape skin colour, flavonoids and phenolic compounds content and enhancing table grape quality characteristics of *cv.* Crimson Seedless. Grape skin colour is one of the most important quality factors for export table grapes. Sometimes, it can be viewed difficulties in skin colouration of some red table grape cultivars grown in various grape growing regions of the world and poor skin colouration of red table grapes is a frequent trouble that reduces production efficiency. Despite the fact that, it is utilized from some canopy management practices, use of new generation bio-regulators, recently used for improving anthocyanin accumulation in growing of some table grape cultivars.

Plant bio-regulators can be extremely useful in improving the red grape colour at low concentration can greatly modify the expression of genes involved in the anthocyanin biosynthesis pathways. Abscisic acid (ABA) is plant hormone involved in stress response in plants especially water stress. ABA treatment enhances the expression of UFGT F_3 5H and CHI in anthocyanin biosynthesis pathway leading to improving the colour of grapes. Additionally, the ABA treatment alters the anthocyanin composition by increasing the formation of petunidin and malvidin type anthocyanins which results in darker colour of grape skins. Another popular plant bio-regulator used to enhance the grape colouration is ethylene, treatments of grape berries with 2-chloroethyl phosphonic acid (Ethrel) can induces the expression of CHS F3H ANS and UFGT in the anthocyanin biosynthesis pathway so hastens anthocyanin accumulation.

2. Materials and Method

The present investigation "Studies on the influence of cane regulation & bi-regulators on growth, yield and quality parameters of Grape *cv.* 'Crimson Seedless' was carried out during 2021-22 and 2022-23 at grape orchard, MHREC, Sector no-70, UHS, Bagalkote. The experimental design adopted for the present investigation was split plot design with four main treatments, seven sub treatments and replicated thrice. In each vine five canes were selected randomly and tagged for detailed observation.

2.1 Total anthocyanins skin & berry (mg/100 g)

Total anthocyanin content in grape was determined by macerating the pulp in aqueous methanolic HCL solvent prepared by mixing methanol (95%) 85 parts and 15 parts 1.5N HCL. The extract were centrifuged and OD value recorded at 530 nm and 675 nm in UV visible spectrophotometer. Anthocyanin content was calculated based on standard formula as follows.

$$\text{Anthocyanin (Mg/100 g)} = \frac{\text{(OD 530)-0.33(OD 657) x 100}}{\text{W}}$$

W= Weight of sample in gram

2.2 Total phenols content (mg/100 g berries as gallic acid equivalent)

It was based on Folin-Ciocalteau reagent. An aliquot of fruit material was extracted with 80% methanol and agitated for fifteen min at 70 °C. The extract was added to 2% sodium bi-carbonate (Na_2co_3).after incubation for five min. Folin-Ciocalteau reagent was added the solution was again incubated for 10 min. The absorbance of the blue colour was measured by spectrophotometer at 760 nm and was expressed as ug Gallic acid equivalent /g FW.

2.3 Total flavonoid content (mg QUE/100 g)

The total flavonoid content was measured through spectrophotometer. In this procedure, 1 ml sample of extract was drawn from each treatment. The each sample was added to a 10 ml volumetric flask containing 4 ml of distilled water and also added 0.3 ml of 5% $NaNO_2$ and allowed to stand for 5 min at ambient conditions. Another 0.3 ml portion of 10% $AlCl_3$ was also added to the mixture and allowed to stand for 6 min at ambient conditions. Finally 2 ml of 1N NaOH was added and the solution was diluted to the desired volume (10 ml) with distilled water. The absorbance of the solution was measured at 510 nm immediately by Spectrophotometer (UV 5704SS, ECIL, India). The quantity was calculated and expressed as quercetin equivalent (QE) Using a standard curve (absorbance versus concentration).

2.4 Colour values (L*a*b* values)

The popular colour spaces used for measuring the external colour of red grapes are the CIE L*a*b*, it is based on a colour opponent theory that an object cannot be green or red and blue or yellow at the same time in this system L*a* and b* describe three rectangular dimension of colour space in which L* is lightness and ranges from 0 to 100, a* is the green–red coordinate and b* is blue-yellow coordinate. The value of a* and b* can vary from -60 to +60 in which positive a* indicates the red colour negative a* indicates green colour positive b* indicates yellow colour and negative b* indicates the blue colour. The colour space can be used effectively to evaluate the fruits whose colour varies between red and yellow.

3. Results and Discussion

3.1 Colour values (L* a* b*)

The pooled data registered significant results with respect to colour (a*) value presented in Table 1. Among the cane regulation treatments, the highest colour (a*) value was recorded in C_4 (6.80) which was at par with C_3 (6.35), cane regulation treatment results in better penetration of sun light and proper aeration results in better photosynthesis, results in better colour development in grape.

The pooled data showed significant results with respect to colour value recorded after harvesting. Among the bio-regulator treatments application of ABA and Ethrel at veraison stage results in highest colour (a*) value (6.91) in G_7 which was at par with G_4 (6.52). Increase in colour (a*) value mainly because of ethrel induces endogenous ethylene production which leads to transcription of genes encoding important enzymes which enhance the petunidin and malvidin anthocyanins by cholcone synthase(CHS) and Flavonone 3-hydroxylase (F3H) and Urindine diphosphate glucose flavonoid glucosyltransferase enzymes (Cantin *et al.* (2007) [19]. Similar results were obtained by Isci *et al.* (2020) [5] in Crimson Seedless grape, Mohamed *et al.* (2021) [12] in Red Globe grape and Nanthakumar *et al.* 2021 [13] in Red Globe grape.

Interaction effect between canopy and bio-regulator treatments with respect to colour (a*) value showed significant results in pooled data of both the years. The highest colour (a*) value (7.08) was recorded in C_4G_7 which was on par with C_3G_7 (6.50), C_3G_6 (6.43), C_3G_7 (6.41)combination treatments helps in increasing colour (a*) value mainly due the application of new generation bio-regulators along with cane regulation increases the phenylalanine ammonia lyase (PAL) activity in grapes results in more accumulation of anthocyanins. Similar findings are noticed by Nanthakumar *et al.* 2021 [13] in Red Globe grape.

3.2 Total anthocyanins in skin (mg/100 g)

Assessment of anthocyanins in skin as one of the most important quality parameters of Crimson Seedless grapes was also done during two seasons is presented in Table 2. The data obtained in respect of total anthocyanins in skin was influenced by various cane regulation and bio-regulator treatments. The data indicated significant results with respect to total anthocyanins in skin. Among the cane regulation

treatments, the highest total anthocyanins in skin was recorded in C_4 (47.91 mg/100 g) which was at par with C_3 (44.06 mg/100 g).Cane regulation helps in better penetration of sunlight and proper aeration helps in increasing the anthocyanins levels. Similar results were obtained by Han *et al.* (2019) [4] and Singh *et al.* (2017) [17] in Flame Seedless grape.

The application of abscisic acid and ethrel showed significant differences with respect to total anthocyanins in skin. Among the treatments, the highest total anthocyanins in skin (63.03 mg/100) was recorded in G_7 which was at par with G_4 (58.65 mg/100 g).Increase in anthocyanins content in skin mainly because of ethrel induces endogenous ethylene production which leads to transcription of genes encoding important enzymes which enhance the petunidin and malvidin anthocyanins by cholcone synthase (CHS) and Flavonone 3-hydroxylase (F3H) and Urindine diphosphate glucose flavonoid glucosyltransferase enzymes. Similar results were obtained by Omran *et al.* (2010) [14] in Red Globe grape and Mohamed *et al.* (2021) [12] in Red Globe grape.

The interaction effect between cane regulations and bio-regulator treatments was found significant with respect to total anthocyanins in skin. The highest total anthocyanins in skin (67.77 mg/100 g) was recorded in C_4G_7 followed by C_3G_7 (65.71 mg/100 g).The combined effect of cane regulation and bio-regulators enhances the anthocyanins levels in berry skin. Combined effect was supported by Kaur *et al*. (2013) [6] in Flame Seedless grape and Singh *et al*. (2017) [17] in Flame Seedless grape.

3.3 Total anthocyanins in berry (mg/100 g)

The data obtained in respect of total anthocyanins in berry was influenced by various cane regulation and bio-regulator treatments are presented in the Table (2). The data showed significant results with respect to total anthocyanins in berry. Among the cane regulation treatments, the highest total anthocyanins in berry were recorded in C_4 (18.51 mg/100 g) which was at par with C_3 (15.60 mg/100 g). Cane regulation helps in better penetration of sunlight and proper aeration helps in increasing the anthocyanins levels. Similar results were obtained by Kaur *et al.* (2013) [6] in Flame Seedless grape.

The bio-regulator treatments abscisic acid and ethrel showed significant differences with respect to total anthocyanins in berry. Among the bio-regulator treatments, application of ABA and ethrel at veraison stage results in highest total anthocyanins in berry (22.14 mg/100 g) in G_7 which was at par with G_4 (17.52 mg/100 g). Increase in anthocyanins content in berry mainly because of ethrel induces endogenous ethylene production which leads to transcription of genes encoding important enzymes which enhance the cyanidin and peonidin anthocyanins by cholcone synthase (CHS) and Flavonone 3-hydroxylase (F3H) and Urindine diphosphate glucose flavonoid glucosyltransferase enzymes. Similar results were obtained by Metre *et al.* (2021) [10] in Beauty seedless grape and Jia *et al.* (2023) [20] in grape *cv.* Baoguang and Cuiguang.

The interaction effect between cane regulations and bio-regulator treatments was found significant with respect to total anthocyanins in berry. The highest total anthocyanins in berry (26.97 mg/100 g) were recorded in C_4G_7, which was on par with C_4G_6 (23.93 mg/100). The combined effect of cane regulation with 25 canes per vine and ethrel 400 ppm enhances the anthocyanins levels in berry. Similar findings were obtained by kaur *et al.* (2013) [6] in Flame Seedless grape and Singh *et al.* (2017) [17] in Flame Seedless grape.

3.4 Total phenolic content (mg GAE/100 g)

The data regarding total phenolic contentin berryas influenced by various cane regulation and bio-regulator treatments are presented in Table (3). The data showed significant results with respect to total phenolic contentin berry. Among the cane regulation treatments, the highest total phenolic contentwas recorded in C_4 (56.09 mg GAE/100 g**)** which was at par with C_3 (54.64 mg GAE/100 g**)**. Similar results were noticed with Han *et al.* (2019) [4] in Cabernet Sauvignon.

The bio-regulator treatments showed significant differences with respect to total phenolic content in berry. Among the bio-regulator treatments, the highest total phenolic content (56.35 mg GAE/100 g**)** was recorded in G_7 which was followed by G_4 (55.83 mg GAE/100 g).Exogenous application of abscisic acid and ethrel promotes anthocyanin biosynthesis and increased expression of flavonoid synthesis genes resulted in a higher accumulation of total anthocyanins in the skin of berries and increased the gene expression of CHI F3H DFR, and UFGT and of the VvMYBA1 and VvMYBA2 transcription factors (Koyama *et al.* (2018) [7]. Similar results were obtained by Lopez *et al.* (2021) [8] in Tempranillo grape and Mhetre *et al.* (2022) [11] in Flame Seedless.

The interaction effect between cane regulations and bio-regulator treatments was found significant with respect to total phenolic in berry. The highest total phenolic content (59.60 mg GAE/100 g**)** was recorded in C_4G_7which was on par with C_4G_6 (57.85 mg GAE/100 g). Similar results were obtained by Ferrara *et al.* (2015) in Crimson Seedless.

3.5 Total flavonoid content (mg QUE/100 g)

The data obtained in respect of total flavonoid contentin berry was influenced by various cane regulation and bio-regulator treatments is shown in Table (3). The data showed significant results with respect to total flavonoid contentin berry. Among the cane regulation treatments, the highest total flavonoid content were recorded in C_4 (61.89 mg QUE/100 g) which was at par with C_3 (60.09 mg QUE/100 g). Similar results were obtained by Yamamoto *et al.* (2015) [18] and Guidoni *et al.* (2022) [3] in grape *cv.* Nebbiolo**.**

The bio-regulator treatments showed significant differences with respect to total flavonoid content in berry. Among the bio-regulator treatments application of ABA and ethrel at veraison stage results in highest total flavonoid content (62.76 mg QUE/100 g) in treatment G_7 which was followed by G_6 (61.03 mg QUE/100 g). Exogenous abscisic acid and ethrel promotes anthocyanin biosynthesis and increased expression of flavonoid synthesis genes resulted in a higher accumulation of total anthocyanins in the skin of berries and increased the gene expression of CHI, F3H, DFR, and UFGT and of the VvMYBA1 and VvMYBA2 transcription factors (Koyama *et al.* (2018) [7]. The results are agreement with Mhetre *et al.* (2022) [11] in Flame Seedless.

The interaction effect between cane regulations and bio-regulator treatments was found significant with respect to total flavonoid contentin berry. The highest total flavonoid content (65.58 mg QUE/100 g) was recorded in C_4G_7 which was followed by C_4G_6 (64.65 mg QUE/100 g).

Table 1: Colour value (L*, a*, b*) in grapes cv. Crimson Seedless as influenced by cane regulation and bio-regulators

Treatment	*L**			*a**			*b**		
	2021	**2022**	**Pooled**	**2021**	**2022**	**Pooled**	**2021**	**2022**	**Pooled**
Main plot (Cane regulation)									
C_1 (Control)	30.31	30.25	30.28	6.16	6.16	6.16	2.34	2.35	2.35
C_2 (50 canes vine^{-1})	29.87	29.84	29.85	6.20	6.19	6.19	2.38	2.38	2.38
C_3 (33 canes vine^{-1})	28.27	28.18	28.23	6.35	6.35	6.35	2.57	2.55	2.56
C_4 (25 canes vine^{-1})	26.73	26.73	26.73	6.81	6.80	6.80	2.67	2.66	2.66
S. Em ±	0.22	0.41	0.30	0.08	0.05	0.02	0.04	0.04	0.03
CD at 5%	0.76	1.41	1.03	0.28	0.17	0.07	NS	NS	NS
Sub plot (Bio- regulators)									
G_1 (Control)	30.04	30.02	30.03	5.46	5.46	5.46	2.16	2.16	2.16
G_2 (ABA 200 ppm)	29.64	29.56	29.60	6.41	6.40	6.40	2.56	2.55	2.55
G_3 (ABA 300 ppm)	29.41	29.43	29.42	6.43	6.42	6.43	2.52	2.51	2.52
G_4 (ABA 400 ppm)	28.57	28.48	28.52	6.52	6.51	6.52	2.49	2.48	2.49
G_5 (Ethrel 300 ppm)	29.11	29.08	29.09	6.44	6.44	6.44	2.66	2.65	2.65
G_6 (Ethrel 350 ppm)	27.91	27.81	27.86	6.47	6.48	6.47	2.53	2.52	2.53
G_7 (Ethrel 400 ppm)	26.89	26.88	26.89	6.92	6.91	6.91	2.52	2.51	2.51
S. Em ±	0.52	0.46	0.37	0.10	0.11	0.07	0.05	0.04	0.03
CD at 5%	1.48	1.31	1.05	0.27	0.32	0.19	NS	NS	NS
Interactions (Main plot × Sub plot)									
C_1G_1	31.53	31.50	31.52	5.29	5.31	5.30	2.01	2.03	2.02
C_1G_2	31.33	31.31	31.32	6.18	6.17	6.17	2.41	2.44	2.42
C_1G_3	31.00	31.05	31.03	6.20	6.19	6.20	2.39	2.38	2.39
C_1G_4	28.83	28.49	28.66	6.23	6.22	6.23	2.35	2.34	2.35
C_1G_5	31.15	31.15	31.15	6.19	6.19	6.19	2.51	2.50	2.51
C_1G_6	30.34	30.25	30.30	6.21	6.21	6.21	2.38	2.37	2.37
C_1G_7	27.98	27.96	27.97	6.83	6.83	6.83	2.37	2.35	2.36
C_2G_1	30.44	30.43	30.44	5.35	5.33	5.34	2.11	2.11	2.11
C_2G_2	30.43	30.42	30.43	6.19	6.18	6.18	2.44	2.42	2.43
C_2G_3	30.44	30.40	30.42	6.21	6.20	6.21	2.40	2.39	2.40
C_2G_4	30.06	30.08	30.07	6.31	6.31	6.31	2.37	2.36	2.36
C_2G_5	29.83	29.72	29.77	6.23	6.21	6.22	2.54	2.53	2.54
C_2G_6	29.43	29.41	29.42	6.25	6.25	6.25	2.42	2.41	2.42
C_2G_7	28.44	28.41	28.42	6.84	6.83	6.84	2.41	2.40	2.41
C_3G_1	29.18	29.18	29.18	5.41	5.40	5.41	2.15	2.16	2.16
C_3G_2	28.76	28.51	28.64	6.39	6.38	6.39	2.66	2.63	2.64
C_3G_3	28.75	28.72	28.73	6.41	6.40	6.41	2.61	2.60	2.60
C_3G_4	28.48	28.46	28.47	6.50	6.49	6.50	2.58	2.57	2.58
C_3G_5	28.01	28.00	28.01	6.41	6.41	6.41	2.72	2.71	2.71
C_3G_6	27.51	27.14	27.33	6.43	6.43	6.43	2.63	2.61	2.62
C_3G_7	27.20	27.25	27.23	6.92	6.90	6.91	2.61	2.60	2.60
C_4G_1	29.01	28.95	28.98	5.79	5.79	5.79	2.36	2.35	2.35
C_4G_2	28.04	28.02	28.03	6.88	6.87	6.88	2.73	2.72	2.72
C_4G_3	27.46	27.53	27.49	6.90	6.88	6.89	2.68	2.67	2.68
C_4G_4	26.89	26.88	26.89	7.03	7.02	7.03	2.66	2.65	2.66
C_4G_5	27.44	27.43	27.44	6.95	6.94	6.95	2.86	2.85	2.85
C_4G_6	24.34	24.42	24.38	7.00	7.02	7.01	2.70	2.69	2.70
C_4G_7	23.93	23.91	23.92	7.09	7.07	7.08	2.69	2.68	2.68
S. Em ±	1.04	0.92	0.74	0.19	0.23	0.13	0.09	0.08	0.06
CD at 5%	2.97	2.63	2.10	0.55	0.64	0.38	NS	NS	NS

Table 2: Total anthocyanins in skin (mg/100 g) and total anthocyanins in berry (mg/100 g) in grapes *cv.* Crimson Seedless as influenced by cane regulation and bio- regulators

Treatment	Total anthocyanins in skin(mg/100 g)			Total anthocyanins in berry(mg/100 g)		
	2021	2022	Pooled	2021	2022	Pooled
Main plot (Cane regulation)						
C_1 (Control)	36.62	36.38	36.50	10.44	10.43	10.43
C_2 (50 canes vine^{-1})	40.76	40.73	40.75	13.21	13.34	13.28
C_3 (33 canes vine^{-1})	44.15	43.96	44.06	15.52	15.67	15.60
C_4 (25 canes vine^{-1})	46.95	48.88	47.91	18.47	18.55	18.51
S. Em ±	1.33	1.69	1.04	0.88	0.79	0.39
CD at 5%	4.59	5.83	3.59	3.04	2.74	1.35
Sub plot (Bio- regulators)						
G_1 (Control)	20.10	20.26	20.18	4.30	4.44	4.37
G_2 (ABA 200 ppm)	34.03	34.02	34.02	10.82	10.93	10.87
G_3 (ABA 300 ppm)	37.74	39.86	38.80	14.92	15.06	14.99
G_4 (ABA 400 ppm)	58.81	58.49	58.65	17.51	17.53	17.52
G_5 (Ethrel 300 ppm)	35.79	35.45	35.62	12.11	12.14	12.13
G_6 (Ethrel 350 ppm)	45.12	46.53	45.83	19.07	19.11	19.09
G_7 (Ethrel 400 ppm)	63.26	62.80	63.03	22.14	22.29	22.21
S. Em ±	1.81	1.85	1.35	0.96	0.75	0.62
CD at 5%	5.14	5.26	3.83	2.72	2.12	1.77
Interactions (Main plot × Sub plot)						
C_1G_1	18.21	18.38	18.30	3.36	3.52	3.44
C_1G_2	27.36	27.33	27.35	6.94	6.96	6.95
C_1G_3	32.10	32.08	32.09	10.33	10.38	10.36
C_1G_4	52.25	52.05	52.15	12.95	12.83	12.89
C_1G_5	29.65	29.43	29.54	7.90	7.95	7.92
C_1G_6	39.85	39.03	39.44	14.18	13.93	14.06
C_1G_7	56.92	56.37	56.64	17.44	17.43	17.44
C_2G_1	19.46	19.50	19.48	4.00	4.15	4.08
C_2G_2	33.05	33.35	33.20	9.63	9.65	9.64
C_2G_3	36.35	36.81	36.58	13.26	13.31	13.28
C_2G_4	56.95	56.32	56.63	15.43	15.45	15.44
C_2G_5	33.86	33.80	33.83	11.73	11.78	11.76
C_2G_6	43.46	43.57	43.51	17.94	18.20	18.07
C_2G_7	62.22	61.76	61.99	20.45	20.86	20.66
C_3G_1	20.62	21.01	20.81	4.60	4.72	4.66
C_3G_2	36.18	35.83	36.00	11.83	11.86	11.85
C_3G_3	39.37	39.50	39.44	16.40	16.78	16.59
C_3G_4	61.22	61.26	61.24	18.69	18.80	18.75
C_3G_5	38.47	37.93	38.20	13.26	13.25	13.25
C_3G_6	47.18	46.83	47.01	20.33	20.30	20.32
C_3G_7	66.05	65.36	65.71	23.55	24.02	23.79
C_4G_1	22.12	22.15	22.14	5.24	5.37	5.31
C_4G_2	39.51	39.58	39.55	14.89	15.24	15.06
C_4G_3	43.15	51.06	47.10	19.69	19.77	19.73
C_4G_4	64.82	64.34	64.58	22.96	23.04	23.00
C_4G_5	41.18	40.62	40.90	15.55	15.59	15.57
C_4G_6	50.00	56.71	53.36	23.84	24.02	23.93
C_4G_7	67.83	67.70	67.77	27.11	26.83	26.97
S. Em ±	3.62	3.70	2.69	1.91	1.49	1.25
CD at 5%	10.28	10.53	7.66	5.44	4.24	3.55

Table 3: Total phenolic content (mg GAE/100 g) and total flavonoid content(mg QUE/100 g) ingrapes *cv.* Crimson Seedless as influenced by cane regulation and bio-regulators

Treatment	Total phenolic content(mgGAE/100 g)			Total flavonoid content(mgQUE/100 g)		
	2021	2022	Pooled	2021	2022	Pooled
Main plot (Cane regulation)						
C_1 (Control)	51.21	51.40	51.31	57.00	57.17	57.09
C_2 (50 canes vine^{-1})	53.11	53.45	53.28	57.61	57.66	57.64
C_3 (33 canes vine^{-1})	54.61	54.67	54.64	60.04	60.13	60.09
C_4 (25 canes vine^{-1})	55.93	56.25	56.09	61.85	61.92	61.89
S. Em ±	0.61	0.70	0.54	0.70	0.48	0.48
CD at 5%	2.10	2.42	1.86	2.42	1.64	1.66
Sub plot (Bio- regulators)						
G_1 (Control)	48.96	48.85	48.91	53.40	53.30	53.35
G_2 (ABA 200 ppm)	52.75	52.88	52.81	57.01	57.12	57.06
G_3 (ABA 300 ppm)	54.57	54.68	54.63	59.50	59.64	59.57
G_4 (ABA 400 ppm)	55.73	55.92	55.83	60.84	60.94	60.89
G_5 (Ethrel 300 ppm)	52.85	53.56	53.21	59.48	59.63	59.55
G_6 (Ethrel 350 ppm)	54.97	55.18	55.07	60.95	61.12	61.03
G_7 (Ethrel 400 ppm)	56.16	56.53	56.35	62.68	62.83	62.76
S. Em ±	0.83	0.86	0.60	0.97	1.08	0.86
CD at 5%	2.37	2.46	1.71	2.76	3.07	2.45
Interactions (Main plot × Sub plot)						
C_1G_1	49.75	49.40	49.58	53.11	52.96	53.04
C_1G_2	50.00	50.04	50.02	55.30	55.30	55.30
C_1G_3	51.77	52.13	51.95	57.54	57.56	57.55
C_1G_4	52.54	52.73	52.64	59.01	59.18	59.09
C_1G_5	50.04	50.38	50.21	55.61	56.03	55.82
C_1G_6	51.79	52.09	51.94	58.01	58.55	58.28
C_1G_7	52.56	53.04	52.80	60.42	60.62	60.52
C_2G_1	48.73	48.65	48.69	53.11	52.90	53.00
C_2G_2	52.34	52.73	52.54	55.31	55.31	55.31
C_2G_3	54.26	54.47	54.37	57.93	58.02	57.98
C_2G_4	54.50	55.03	54.76	59.72	59.90	59.81
C_2G_5	52.35	53.25	52.80	57.08	57.08	57.08
C_2G_6	54.55	54.55	54.55	58.77	58.87	58.82
C_2G_7	55.03	55.48	55.26	61.35	61.58	61.47
C_3G_1	48.80	48.70	48.75	53.28	53.21	53.24
C_3G_2	53.67	53.45	53.56	57.70	57.88	57.79
C_3G_3	55.26	55.12	55.19	60.07	60.42	60.25
C_3G_4	56.85	56.92	56.89	61.87	61.85	61.86
C_3G_5	54.10	54.69	54.40	61.51	61.68	61.60
C_3G_6	55.84	56.06	55.95	62.38	62.39	62.39
C_3G_7	57.72	57.74	57.73	63.47	63.48	63.48
C_4G_1	48.56	48.65	48.61	54.11	54.12	54.11
C_4G_2	54.98	55.30	55.14	59.74	59.97	59.86
C_4G_3	57.00	57.00	57.00	62.46	62.54	62.50
C_4G_4	59.02	59.02	59.02	62.78	62.81	62.80
C_4G_5	54.90	55.93	55.42	63.69	63.72	63.71
C_4G_6	57.69	58.01	57.85	64.65	64.65	64.65
C_4G_7	59.32	59.87	59.60	65.50	65.65	65.58
S. Em ±	1.67	1.73	1.20	1.94	2.16	1.72
CD at 5%	4.74	4.91	3.41	5.51	6.14	4.90

4. Conclusion

From this study, it can be clearly concluded that, the cane regulation is essential form of thinning in vineyard operation and considered as a technique which could lead to tremendous improvement in quality parameters of Crimson Seedless as it is very vigourus cultivar. Application bio-regulator is one of important operations to get export quality standards and has more demand in market. In case of bio- regulator treatments, application ethrel 300-400 ppm at veraison stage has positively influenced berry colouration and bioactive compounds in grape, also early harvesting was possible. Among the interaction effects, the vines regulated with 25-33 canes per vine with the application of ethrel 300-400 ppm at veraison stage followed by vines regulated with 25-33 canes per vine with the application of abscisic acid 200-400 ppm at veraison stage was found superior over the control and other interactions.

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