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Basal defoliation, salicylic acid and cyanocobalamin to ameliorate the physiological and biochemical characteristics of flood-irrigated 'Crimson Seedless' grapevines in a semi-arid Mediterranean climate

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ABSTRACT

There is a high retail demand for 'Crimson Seedless' grape. Cluster shape, berry size, colour, and sugar contents influence the overall fruit quality and marketability. In many commercial vineyards of flood-irrigated clay soils under warm and humid semi-arid climates, adequate irrigation may lead to an enhanced fruit set that could potentially be associated with restricted berry growth, compact clusters, and poor berry colour and taste. To assess the role of some agronomic practices that may influence the canopy microclimate, and affect primary and secondary metabolites, seven treatments arranged in a randomised complete block design (RCBD) system with three replicates each (three vines per replicate) were applied as follow; the control (T1), 5-leaf basal defoliation at pre-bloom (BDPB) (T2), BDPB + foliar spray (FS) of 200 mg · L^{-1} salicylic acid (SA) (T3), BDPB + FS of 20 mg · L^{-1} cyanocobalamin (CCA) (T4), 5-leaf basal defoliation at full bloom (BDFB) (T5), BDFP + SA (T6) and BDFB + CCA (T7). Foliar applications were applied at 1) 2 weeks after the beginning of vegetative growth, 2) full bloom and 3) veraison stages. The analysis of variance (ANOVA), the principle component analysis (PCA) and the two-way hierarchical cluster analysis (HCA) indicated that BDPB (T2) generally has a better effect than that of BDFB (T5). The most pronounced effect on vegetative growth (shoot length and leaf area), photosynthesis activity (leaf chlorophyll and carbohydrate contents), fruit weight and dimensions, and total yield was observed in the case of T3, followed by T6, whereas concerning berry firmness, colour (anthocyanins) and sensory characteristics (soluble solids: acids ratio, total sugars and phenols), the most pronounced effect was observed in the case of T4, followed by T7.

Keywords: anthocyanins, colour, firmness, irrigation, phenols, photosynthesis, *Vitis vinifera*

Abbreviations: BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FB, full bloom; FS, foliar spray; PB, pre-bloom; SA, salicylic acid; SSC, soluble solid contents; TA, titratable acidity.

INTRODUCTION

The common grape, *Vitis vinifera* L., family Vitaceae, native to Southwestern Asia, Central Europe and the Mediterranean region, is one of the world's most important fruit crops in terms of total production and economic

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value (Vivier and Pretorius, 2002). The total cultivated area of this crop in 2020 was about 6,950,930 ha with a total production of 78,034,332 t. The top 15 grapevines producers are China, Italy, Spain, France, the USA, Turkey, India, Chile, Argentina, South Africa, Iran, Uzbekistan, Egypt, Australia and Brazil. Egypt ranked the 13th with a total cultivated area of 71,889 ha and a total production of 1,586,342 t, yielding an average of 22.07 $t \cdot ha^{-1}$ (FAO, 2020).

In Egypt, table grapes are ranked second to citrus in terms of production. The semi-arid Mediterranean climate of Egypt is well suited for grape production. Grape cultivation is spread throughout the country, mostly in heavy soils next to the Nile River, as well as sandy soils and newly reclaimed lands in the deserts, which ensure the prolonged availability of fresh fruit in the market from May to November. Climate conditions, soil types and production technology are the main factors that give table grapes the ability to grow all over the country. 'Thompson Seedless' and 'Flame Seedless' dominate the production as they remain the most popular varieties in the European Union markets, the major importer of the Egyptian grapes; however, producers are expanding towards other varieties including 'Early Superior', 'Superior', 'Roomy' and 'Crimson Seedless' to supply other markets such as Argentina and New Zealand (Omar, 2020).

'Crimson Seedless' is a vigorous cultivar with five to six non-fruiting basal buds, classified as a mid- to late-season maturing grape based on weather conditions (mid-September to mid-November in the northern hemisphere). Similar to most grape cultivars, 'Crimson Seedless' is classified as moderately resistant to drought and salinity conditions (Dokoozlian et al., 2016). The berries are bright red, large, have a pit characterised by a cylindrical-oval shape and are seedless. They have a high nutritional value, and are not highly susceptible to early or late season cracking. A primary factor driving the growth of this variety is the high retail demand (Dokoozlian et al., 2016); however, cluster weight and shape, berry size, soluble solid contents:titratable acidity ratio (SSC/TA ratio), aroma and colour are all factors that influence overall fruit quality (García-Pastor et al., 2020).

Failure to develop adequate red colour at maturity is a common issue associated with 'Crimson Seedless' grape, especially in warm and humid conditions of the semi-arid climates (Spayed et al., 2002; Abd El-Rahman et al., 2018). In Egypt, most growers of 'Crimson Seedless' grape irrigate their vineyards well to ensure adequate water supply (≈4,500–5,000 $m^3 \cdot ha^{-1}$) that satisfies the maximum field capacity (≈0.328 $m^3 \cdot m^{-3}$) during the ripening stage due to hot summer (mid-July to late-August); however, they experience poor berry colour and low sugar contents (El-Ansary, 2017; Temnani et al., 2021). Most clusters usually show variability in berry colour, which leads to depreciation of their market value (Cantín et al., 2007; Lurie et al., 2010; El-Sayed, 2013). In many commercial 'Crimson Seedless' vineyards of clay soils and traditional surface irrigation, adequate water in root zone may lead to an enhanced fruit set that could potentially restrict berry growth and results in tightly filled and compact clusters, which become more susceptible to bunch rots, in addition to poor colour and taste quality. These problems could be overcome with proper cultural practices such as trunk girdling and ethephon foliar application (Dokoozlian et al., 1995), gibberellic acid foliar application (Dokoozlian, 2001), ABA foliar application (Ferrara et al., 2013), combined ABA and sucrose foliar application (Ferrara et al., 2015), shoot thinning (Dokoozlian et al., 2016) and deficit irrigation (Pinillos et al., 2016; El-Ansary, 2017; Temnani et al., 2021). In this regard, this research is pointing out to vineyards in clay soils that are still subjected to surface (also known as flood or furrow) irrigation. It is not the most efficient irrigation method, since it uses more amount of water and water loss is higher compared to new irrigation methods, but it is cheap (USGS, 2018).

It is well known that clay soils are less permeable than other soil types. This indicates the absence of natural drainage, and consequently with continuous irrigation, the soil will suffer from waterlogging conditions. Waterlogging is more prolonged and more severe in heavy textured soils, and it is associated with temporary aeration stress, particularly with higher levels of groundwater table, which indirectly affects plant growth and fruit quality (Yadav et al., 2013). Waterlogging and salinisation of soils are common problems associated with flood irrigation, especially in the arid and semi-arid conditions. The greater the aridity of the region, the larger the quantity of irrigation water used, and the result of such water-intensive irrigation practice is an eventual increase in waterlogging and concentrate salts, drawn up from lower in the soil profile, in the plants' root zone. In addition, the rivers in arid and semi-arid regions tend to become progressively saline from their headwaters to their mouths, and excess salinity within the root zone reduces plant growth, particularly with the small rainfall percentage that is not enough to leach away the accumulated salts. Therefore, adequate drainage system could solve soil-related problems. Waterlogging can also be minimised using the micro-irrigation techniques that apply water more precisely and easily limit quantities to no more than the plant needs (FAO, 1997). In Egypt, for example, the Nile River is the main water resource, and the installation of good drainage systems or the use of new irrigation techniques in some small-scale farming activities would be somewhat expensive (Agricultural Statistics of Egypt, 2014). This was a reason to look for other agricultural practices that improve grapevine growth, productivity and fruit quality.

All agronomic practices that influence the canopy microclimate and the grape yield to leaf area ratio may influence the berry primary and secondary metabolites (Tardaguila et al., 2008; Gambacorta et al., 2011;

Baiano et al., 2015; Suriano et al., 2016; Tarricone et al., 2017). Basal defoliation (BD), the practice of leaf removal in the cluster zone, is one of the most widely used canopy management techniques applied to modify fruit-zone microclimates, and the impact of the timing and intensity differs across varieties and climates. This practice regulates yield components in high-yielding cultivars via reduced fruit set percentage when performed immediately before anthesis (Poni et al., 2006; Intrieri et al., 2008; Sabbatini and Howell, 2010). This procedure eventually affects vine carbohydrate supply by reducing carbohydrates in the flowers at anthesis (altering source: sink ratio) (Petrie et al., 2003; Vasconcelos et al., 2009) or by altering the canopy microclimate to increase fruit exposure to sunlight and improve air penetration into the clusters (Austin and Wilcox, 2011). Early defoliation in vigorous grapevines noticeably affected vine performance, and thus reduced cluster compactness, weight and yield, lowered the incidence of *Botrytis* bunch rot and improved fruit composition (Tardaguila et al., 2010; Yorgos et al., 2012; Beslic et al., 2013; Diego et al., 2014; Sabbatini, 2015; Acimovic et al., 2016; Intrieri et al., 2016; Frioni et al., 2017; Shalan and Hamza, 2020). Similar results were reported when defoliation was performed at full-bloom stage, but without a reduction in yield (Sivilotti et al., 2016). Late BD applied at the pea-size stage reduced fruit soluble solids and sugar contents, and decreased acidity as a consequence of faster rate of malic acid catabolism (Petrie et al., 2003). Late defoliation applied by the beginning of berry colouring (veraison stage) showed an incidence of sunburn with a negative effect on the biosynthesis of anthocyanins, and thus berry colour (Mosetti et al., 2016). Basal defoliation is widely used in cool climates, characterised by low solar heat accumulation and high air humidity and rainfall (Frioni et al., 2017). Such conditions can increase polyphenols and anthocyanins up to 18% due to better UV radiation in the cluster zone that may positively affect phenol synthesis by stressing the berry skin (Šuklje et al., 2014).

Anthocyanins are water-soluble vacuolar pigments that belong to the flavonoids group and are responsible for grape colour (García-Beneytez et al., 2002). In 'Crimson Seedless' grape, these pigments accumulate during the 'veraison' stage, which represents the beginning of the ripening process (Cantín et al., 2007). In general, most table grapes grown in warm climates have less red colour than those grown in cold climates (Spayed et al., 2002). The increase in interior canopy leaves results in lack of light penetration, and thus leaf removal is considered an important canopy practice (Tarricone et al., 2017). Defoliation is considered one of the main factors affecting the accumulation of anthocyanins (Downey et al., 2006) through changes in microclimate temperature, humidity, wind speed and leaf wetness around the clusters (English et al., 1990). Clusters exposed to sunlight had higher sugars, anthocyanins and phenols, while acidity was lower when compared to the shaded clusters (Diago et al., 2012; USGS, 2018). Possible negative effects of leaf removal are reduction in leaf area per vine and berry weight, decrease in total acidity due to reduction in malic acid content, and decrease in bud differentiation in the following season (Lemut et al., 2013; Mosetti et al., 2016). Substantial BD of more than six leaves has not been thoroughly evaluated (Verdenal et al., 2018; Tarricone et al., 2020).

Salicylic acid (SA), 2–hydroxybenzoic acid ($C_7H_6O_3$, molecular weight = 138.121 g · mol^{-1}), a phenolic compound, natural growth regulator and antioxidant in vascular plants, stimulates many physiological processes that regulate plant growth and development such as nutrient uptakes, membrane permeability, enzyme activities, photosynthesis capacity, stomatal conductance and transpiration under various biotic and abiotic stresses (Hayat et al., 2010; Rady et al., 2015; Rady et al., 2017). It is an important secondary metabolite in grapes that activates the expression of several defence-related genes (Loake and Grant, 2007), and effectively improves berry colour, flavour, astringency, bitterness, size and weight, and delays softening through inhibiting the activity of cell wall degrading enzymes such as cellulase, polygalacturonase and xylanase (Srivastava and Dwivedi, 2000; Zhang et al., 2003), suggesting that SA has an anti-senescent effect (Harindra Champa et al., 2015). Application of SA to 'Sahebi' grapes at pre-veraison stage increased total phenol, flavonoid and anthocyanin contents (Oraei et al., 2019).

Cyanocobalamin (CCA) is the manufactured version of cobalamin, also known as vitamin B_{12} ($C_{63}H_{88}CoN_{14}O_{14}P$, molecular weight = 1 355.38 g · mol^{-1}), a water-soluble vitamin that is naturally found in animal cells. It is not synthesised by plant cells, but it was found to accumulate within plant cell organs such as cytosol, plastids and mitochondria, providing definitive evidence that some plants can absorb and transport cobalamin (Roje, 2007; Asensi-Fabado and Munne-Bosch, 2010; Lawrence et al., 2018) that was synthesised by some microorganisms (e.g. archaea, bacteria, fungi) that live in the plant root zone through microbial interaction (Antony, 2018). Cobalamin is a cobalt-containing tetrapyrrole cofactor involved in intramolecular rearrangement, and necessary for the regulation of DNA synthesis during plant cell division (Smith et al., 2007; Ferrer et al., 2016). It plays a vital role in the conversion of homocysteine to methionine (Allen, 2012), which is a precursor for a variety of metabolic processes in plants, including polyamine, protein and ethylene synthesis. Methionine synthase is required for methionine biosynthesis, and it serves as a link between the methionine biosynthesis pathway and the one-carbon cycle pathway related to photosynthesis capacity (Zeh et al., 2002; Fontecave et al., 2004). Cobalamin has been suggested to be an intercellular antioxidant that reduces the levels of intercellular reactive oxygen species (ROS) and molecule damages (e.g. DNA, RNA, proteins, lipids, enzyme cofactors), and improves overall cell physiology stimulating the growth and survival of cells

exposed to extremely oxidative environmental stresses (Jones et al., 2013; Ferrer et al., 2016; Vasquez et al., 2022). Cyanocobalamin has been used to improve fruit colour and other quality attributes of kaki (Lo'ay, 2010), 'Thompson Seedless' grapes (Lo'ay, 2011), mango (Samaan et al., 2011), guava (Samaan et al., 2012), banana (El-Baz et al., 2016) and 'Le Conte' pear (Abd El-Bary et al., 2017).

Due to limited research on clay-soil grown 'Crimson Seedless' grapevines that demonstrate quality problems under flood irrigation in semi-arid conditions, and in an attempt to use some efficient cultural practices, instead of commonly used methods such as trunk girdling and foliar spray of ethephon (Dokoozlian et al., 1995), mechanical cluster thinning (Tardaguila et al., 2008), foliar spray of ABA (Lurie et al., 2010), cover cropping and deficit irrigation (Gambacorta et al., 2011), leaf removal in the cluster area along with the East and West geographical directions (Baiano et al., 2015) and moderate deficit irrigation (Tarricone et al., 2017) that did not show that much improvement in vine growth or fruit quality under such conditions, this research aimed to determine whether the combined application of BD with either SA or CCA could be used as a new sustainable solution that would meet the growers' concerns on this cultivar. Most of the previous reports have focussed on the role of BD by itself, as well as the solo role of foliarly sprayed SA or CCA on the growth and productivity of different fruit trees, including grapes. In this regard, the present research is considered the first to study the combined role of BD with either SA or CCA on the growth, productivity and fruit quality of 'Crimson Seedless' grapevines grown under the semi-arid Mediterranean climate of Egypt. It is also noteworthy that this study is comparing the effective roles of two stress-reducing substances (SA and CCA) in terms of price and nature. The cheap price of SA [≈\$52 · 500 g^{-1}] (Sigma Aldrich, St. Louis, MO, USA) in Egypt, in conjunction with its natural origin, makes it a favourable cultural practice to the growers, compared to CCA that has a synthetic origin and a higher price [≈\$121 · 1 g^{-1}] (Sigma Aldrich, St. Louis, MO, USA), although it was reported safe for human use.

MATERIALS AND METHODS

Experiment

This research was conducted on 5-year-old 'Crimson Seedless' grapevines (*Vitis vinifera* L.) grafted on

Table 1. Weather data of Al-Baramon, Mansoura, Dakahlia, Egypt from November 2019 to October 2021.

Season		Temperature (°C)	Humidity (%)	Rainfall (mm · month^{-1})	Wind speed (km · h^{-1})	Cloud (%)	Sun (h · month^{-1})	UV index
November	2019	24	57	0.0	11.2	9	358	6
	2020	22	63	9.7	10.4	28	339	6
December	2019	18	62	12.2	13.2	23	360	5
	2020	19	62	4.7	10.3	20	350	4
January	2020	14	70	9.6	14.0	32	349	4
	2021	17	64	3.7	13.1	20	365	5
February	2020	16	69	9.6	12.3	32	308	4
	2021	18	66	8.9	12.6	28	298	4
March	2020	19	63	20.6	14.7	24	345	7
	2021	20	60	0.6	14.3	15	368	5
April	2020	23	60	0.9	13.4	16	356	7
	2021	24	52	0.0	15.4	10	360	8
May	2020	28	51	0.6	14.3	11	367	7
	2021	30	46	0.0	13.4	4	372	8
June	2020	30	56	0.1	13.6	8	360	8
	2021	31	52	0.0	13.0	2	360	9
July	2020	33	63	0.0	12.6	9	372	8
	2021	34	54	0.0	13.3	3	372	9
August	2020	33	64	0.0	12.9	5	372	8
	2021	35	55	0.0	11.9	2	372	8
September	2020	32	65	0.0	12.2	6	359	7
	2021	30	60	0.0	13.3	6	358	7
October	2020	28	61	14.1	11.1	11	369	6
	2021	26	61	1.3	11.9	12	369	5

'Freedom' grape rootstocks, in a clay soil with no artificial drainage system and a groundwater table of 1.5 m, at the experimental farm of the Horticulture Research Institute, Agricultural Research Center, Al-Baramon, Mansoura, Dakahlia, Egypt (31°11′98″ N, 31°45′13″ E, 15 m elevation above sea level) for two consecutive seasons (2020 and 2021). The climatic conditions of the experimental site (World Weather Online, 2021) are shown in Table 1. Soil samples were randomly collected from the root zone (0–90 cm) for analysis, according to the methodology adopted in the study of Wilde et al. (1985), and are displayed along with water analysis values (Ali et al., 2014; Abuzaid, 2018; El-Sayed et al., 2020) in Table 2.

Sixty-three uniform grapevines planted at a 2 m × 3 m spacing, free from any sign of physiological disorders or nutrient deficiencies, were chosen for this experiment. Grapevines were grown in a Spanish baron trellis with a quadrilateral cordon training system of long canes. Winter pruning was carried out by mid-February in both seasons by reducing the number of canes on each vine to six canes of 12 buds each, and six renewal spurs of 2 buds each, with a total number of 84 buds per vine (Figure 1). Irrigation started during the first week of March, and the vegetative growth started by the first week of April (≈1–3 April) in both seasons. All selected grapevines received the same common agricultural practices, as did the entire

Table 2. Soil and water analysis of the experimental site at Al-Baramon, Mansoura, Dakahlia, Egypt.

Soil depth (cm)	0–30	30–60	60–90	Water	
Clay (%)	49.25	50.55	51.15	Transparency (cm)	132.5
Silt (%)	27.69	26.72	26.11	Permeability index (%)	55.64
Sand (%)	23.06	22.66	21.55	Water quality index	21.54
Texture	Clay	Clay	Clay	pH	8.27
Field capacity (%)	15.3	15.7	15.8	Total dissolved salts (mg · L^{-1})	204.9
Permanent wilting point (%)	7.4	7.6	7.7	EC (µmhos · cm^{-1})	558.8
pH (1:2.5 extract)	7.7	7.11	7.11	O_2 (%)	95.8
Organic material (%)	2.3	0.55	0.35	$CaCO_3$ (mg · L^{-1})	100.6
EC (dS · m^{-1}) [1:5 extract]	0.61	0.61	0.61	HCO_3^- (mg · L^{-1})	159.5
$CaCO_3$ (%)	1.83	1.41	1.88	CO_3^{2-} (mg · L^{-1})	7.0
HCO_3^- (meq · 100 g^{-1})	0.30	0.37	0.40	SO_4^{2-} (mg · L^{-1})	15.13
CO_3^{2-} (meq · 100 g^{-1})	0.0	0.0	0.0	SiO_2 (mg · L^{-1})	1.21
SO_4^{2-} (meq · 100 g^{-1})	3.17	4.04	4.13	Cl^- (mg · L^{-1})	32.4
Cl^- (meq · 100 g^{-1})	0.96	0.98	1.08	Na^+ (mg · L^{-1})	29.2
Na^+ (meq · 100 g^{-1})	0.48	0.66	1.42	Ca^{2+} (mg · L^{-1})	27.8
Ca^{2+} (meq · 100 g^{-1})	0.80	0.20	1.25	Mg^{2+} (mg · L^{-1})	14.7
Mg^{2+} (meq · 100 g^{-1})	0.33	0.97	1.16	N (mg · L^{-1})	1.56
N (mg · kg^{-1})	32	24	18	P (mg · L^{-1})	0.094
P (mg · kg^{-1})	13	22	13	K (mg · L^{-1})	8.81
K (mg · kg^{-1})	271	240	230	Fe (mg · L^{-1})	0.23
Fe (mg · kg^{-1})	2.48	2.21	2.11	Mn (mg · L^{-1})	0.005
Mn (mg · kg^{-1})	4.10	3.50	3.21	Zn (mg · L^{-1})	0.60
Zn (mg · kg^{-1})	1.18	0.61	0.51	Cu (mg · L^{-1})	0.018
Cu (mg · kg^{-1})	4.24	2.10	0.75	Co (mg · L^{-1})	1.56
				Pb (mg · L^{-1})	0.77
				B (mg · L^{-1})	0.03
				Mo (mg · L^{-1})	0.009
				Al (mg · L^{-1})	0.03
				Ni (mg · L^{-1})	0.014
				Se (mg · L^{-1})	0.021
				As (mg · L^{-1})	0.044
				V (mg · L^{-1})	0.014

orchard, during both seasons. The annual fertilisation program per hectare has included the following: 150 kg calcium superphosphate ($CaH_6P_2O_9$) + 119 kg sulphate (SO_4^{2-}) applied once by the beginning of the vegetative growth; 553.5 kg potassium sulphate (K_2SO_4) + 215 kg ammonium nitrate (NH_4NO_3) applied by the beginning of vegetative growth, at fruit set and after harvest; 119 kg magnesium sulphate ($MgSO_4$) applied monthly from April to August (23.8 kg · month^{-1}); 119 kg of calcium nitrate ($Ca(NO_3)_2$) applied before bloom and after fruit set; 24 kg zinc sulphate ($ZnSO_4$) applied after harvest; 1785 mg · L^{-1} micronutrients (595 mg · L^{-1} chelated-Fe, 595 mg · L^{-1} chelated-Zn and 595 mg · L^{-1} Mn) applied foliarly by the beginning of the vegetative growth, at 30–45 cm-shoot length stage and 2 weeks after; and 400 mg · L^{-1} borax (source of B) applied foliarly before bloom and after harvest. The flood irrigation system was applied as per the recommended program used in the area, for both 'Crimson Seedless' and 'Flame Seedless' grape cultivars, with a total rate of ≈12,000 m^3 · ha^{-1} during the growing season (March–October). The total calculated amount was based on the rate of water flow (2 L · h^{-1}) for 55 min per each irrigation time. The total amount of water was 1090.9 m^3 · ha^{-1} per each irrigation time out of 11 times during the entire season. For both seasons, irrigation frequency was once a month, except for May, June and July where it was twice a month (Gaser et al., 2018).

The selected grapevines were subjected to seven treatments: the control vines sprayed with distilled water (T1), 5-leaf basal defoliation at pre-bloom (BDPB) stage (T2), BDPB + foliar spray (FS) of 200 mg · L^{-1} SA (T3), BDPB + FS 20 mg · L^{-1} CCA (T4), 5-leaf basal defoliation at full bloom (BDFB) stage (T5), BDFP + FS 200 mg · L^{-1} SA (T6) and BDFB + FS 20 mg · L^{-1} CCA (T7). Treatments were arranged in a randomised complete block design (RCBD) system with three replicates each, and three vines represented each replicate. The same nine vines were subjected to the same treatment in both seasons.

Basal defoliation was performed by removing the first five leaves at the shoot base, at either pre-bloom (PB) stage on the first week of May (≈30–33 days after the beginning of vegetative growth), or full bloom (FB) stage on the third week of May (≈47–50 days after the beginning of vegetative growth) of both seasons. At full fruit set stage, by the first week of June of both seasons (≈10–13 days after FB), crop load for all treatments was adjusted to 18 clusters per vine. Foliar application of distilled water, SA or CCA, supplemented with polysorbate 20 (0.1%) (Sigma Aldrich, St. Louis, MO, USA) as a surfactant, was carried out using a 25 L knapsack power sprayer (Model HT-767; Taizhou Tianyi

Figure 1. The quadrilateral cordon training system of 'Crimson Seedless' grapevines.

Agricultural and Forestry Machinery Co., Zhejiang, China). The whole vine was sprayed until dripping at three different times; 2 weeks after the beginning of vegetative growth when shoot length reached about 20–30 cm (≈mid-April), FB stage (≈third week of May) and beginning of berry colouring (veraison stage) during the second week of July (≈50–55 days after FB). All chemicals used in this research were imported from Sigma Aldrich (St. Louis, MO, USA).

Vegetative growth

At the stage of pea-size berry during the third week of June (≈25–30 days after FB), four non-fruiting shoots off the renewal spurs were randomly marked; two shoots were chosen at each side of the vine to measure shoot length using a wind-up measuring tape (1,000 cm) (Fisher Scientific, Waltham, MA, USA), and average shoot length (cm) was calculated. The mature leaves (i.e. the 6th and 7th from the shoot tip) on each selected shoot were collected to measure leaf area (cm^2) using a leaf area meter (Model LI-3100; LI-COR, Inc., Lincoln, NE, USA).

Vine chlorophyll and carbohydrate contents

The same eight leaves used to determine leaf area were used for chlorophyll analysis, according to the protocol of Wellburn (1994). The absorbance of the extract was measured at 663 nm for chlorophyll *a* and 646 nm for chlorophyll *b* using a UV/VIS spectrophotometer (Model UV-9100-B; LabTech Inc., Hopkinton, MA, USA), and chlorophyll contents (μg · mL^{-1}) were calculated using the following equations:

$$\text{Chlorophyll } a = (12.21\ E663 - 2.81\ E646) \quad (1)$$

$$\text{Chlorophyll } b = (20.13\ E646 - 5.03\ E663) \quad (2)$$

where E is the optical density at the indicated wave length.

Accordingly, total chlorophyll content (mg · g^{-1} fw) was calculated, as follows:

$$\text{Total chlorophyll} = ((\text{chlorophyll } a + \text{chlorophyll } b) \times \text{extract volume})/(1{,}000 \times \text{fw}) \quad (3)$$

Four non-fruiting shoots off the renewal spurs, two shoots at each side of the vine, were randomly collected by the end of the growing season in late October (≈150–155 days after FB) to determine total carbohydrates, according to Hodge and Hofreiter (1962). The middle portion of the cane was chopped into small pieces using a knife, and heat-dried at 70°C for 72 hr, until a constant weight was achieved, using a bench-top Herathermal GP oven (ThermoFisher Scientific, Waltham, MA, USA). The dried cane pieces were ground using a porcelain mortal and pistil set (Fisher Scientific, Waltham, MA, USA). A powder sample (0.2 g) was then hydrolysed with 15 mL HCL (1 M) for 6 hr. The solution was colourimetrically measured at 490 nm using the spectrophotometer, and total carbohydrates were expressed as a percentage of dry weight.

Berry set and total yield

At FB, four uniform clusters per vine (two from each side of the vine) were randomly selected and marked. The total number of blossoms per cluster was counted, and the average blossom number was calculated. At the stage of pea-size berry during the third week of June, the total number of berries per cluster was counted and averaged. The percentage of berry set was then calculated according to Mohamed and El-Sese (2004), as follows:

$$\text{Berry set} = (\text{number of berries per cluster/number of blossoms per cluster}) \times 100 \quad (4)$$

All clusters were harvested when SSC reached 16–17 °Brix (Guelfat-Reich and Safran, 1971) by the first week of September in both seasons (≈100–105 days from FB). Soluble solid contents were determined using a hand-held refractometer, 0%–32% (Model N-1E; Atago Co., Ltd., Tokyo, Japan). The 18 clusters of each vine in a replicate were weighted using a regular field digital scale (200 kg capacity; VEVOR Equipment and Tools, Rancho Cucamonga, CA, USA), and then average fruit yield/vine per each treatment was calculated (kilograms per vine) (Mohamed et al., 2017).

Fruit physiochemical characteristics

At harvest, 10 clusters per vine were randomly selected, and individually weighted using a bench-top digital scale (Model PC-500; Doran scales, Batavia, IL, USA), and average cluster weight (g) was calculated and recorded. Cluster length (cm) was also measured using a regular 30-cm stainless steel ruler (Apuxon, Shenzhen, Guangdong, China) from the uppermost berry to the most bottom berry, and then average length was calculated. The number of berries per each cluster was counted and averaged, and hence the compactness coefficient of the cluster was calculated using the following equation (Fawzi et al., 2019):

$$\text{Cluster compactness coefficient} = \text{number of berries/cluster length} \quad (5)$$

Overall cluster colour was visually evaluated and classified as per the majority of red, pink or green berries, using the following equation (El-Kenawy, 2018):

$$\text{Overall cluster colour} = (\text{number of berries in a specific colour/total number of berries}) \times 100 \quad (6)$$

As indication of average berry weight, the weight (g) of randomly selected 50 berries per each cluster of the 10 selected clusters was measured using the bench-top digital scale, and averaged per vine. Twenty berries out of the 50 berries were used to measure berry length and diameter (mm) using a digital calliper with a 0.01 accuracy (Grizzly Industrial, Chicago, IL, USA), and average length and diameter were calculated. The same 20 berries were used to determine berry firmness twice at the equatorial area of the berry (Watkins and Harman, 1981) using a hand-held digital penetrometer fitted with a 2 mm plunger tip (Model FT-02; QA Supplies LLC,

Norfolk, VA, USA), and firmness was expressed in Newton.

Berry colour was also estimated for another 20 berries, out of the 50 berries, on two opposite sides at the equatorial area of the berry, as *hue* angles (°h = $\tan^{-1}$ (b*/a*)) referring to the colour wheel measured in angles, giving rise to values of 180, 90 and 0 that correspond to green, yellow and red, respectively; and as colour lightness (L*) coordinates (ranging from 0 [black] to 100 [white]) (Lancaster, 1992) using a colourimeter (Model CR-400; Konica Minolta, Tokyo, Japan). The two coordinates of *hue* angle are the red/green coordinate (a*) with +a* for red colour and –a* for green colour, and the yellow/blue coordinate (b*) with +b* for yellow colour and –b* for blue colour (McGuire, 1992).

The same 20 berries were used to evaluate SSC (°Brix) using the hand-held refractometer at room temperature, ≈20–22°C (Lancaster, 1992). Titratable acidity (TA) was determined as a percentage of tartaric acid ($C_4H_6O_6$) in 10 mL juice using NaOH (0.1 N), and phenolphthalein as an indicator (AOAC, 2005), and SSC/TA ratio was thereby calculated. Total sugars were colourimetrically determined using the phenol and sulphuric acid protocol described by Dubois et al. (1956). The absorbance was measured at 490 nm using the spectrophotometer and the concentration of total sugars was calculated as grams of glucose per 100 grams fresh weight, and expressed as a percentage.

As indication of berry colour, total anthocyanins in berry skin were determined using methanolic HCL extraction solvent. The peel of the remaining 10 berries, out of the 50 berries, was separated from the pulp and mixed together. A peel sample (1 g) was randomly collected, mixed with acidic methanol (30 mL) and left in dark conditions at room temperature (≈20–22°C) for 48 hr. The absorbance was measured at 520 nm using the spectrophotometer and results were expressed as mg · 100 g^{-1} fw (Lee and Francis, 1972).

Total phenols were estimated using the Folin–Ciocâlteu method (Slinkard and Singleton, 1977) by homogenising a sample of the peel (2 g) with 3 mL methanol (80%). The mixture was then stirred using a hot plate stirrer at 1,000 rpm (Model RT2; ThermoFisher Scientific, Waltham, MA, USA) and 70°C for 15 min. An aliquot of the extract (0.1 mL) was added to 2 mL sodium carbonate 2% (Na_2CO_3), and the mixture was incubated at room temperature (≈20–22°C) for 5 min. The Folin–Ciocâlteu reagent (0.1 mL) was added to the mixture, and incubated again at room temperature for 10 min. The absorbance of the blue colour against a blank was measured at 765 nm using the spectrophotometer, and results were expressed as microgram gallic acid ($C_7H_6O_5$) equivalents per gram fresh weight using a gallic acid standard curve.

Statistical analysis

Data were preliminary tested for numerical normality and homogeneity of variance using Shapiro–Wilk's and Levene's tests, respectively. Data calculated as percentages were first transformed to the Arcsine square root values before performing the analysis of variance (ANOVA), and results were presented as back-transformed means. The ANOVA was performed using the CoStat software package, version 6.311 (CoHort software, Monterey, CA, USA). Mean comparisons were conducted using Tukey's honestly significant difference (HSD) test at probability (p) ≤ 0.05 (Snedecor and Cochran, 1990). The score and loading plots for plant and fruit characteristics were generated using a principal component analysis (PCA) (Jolliffe, 2011). The two-way hierarchical cluster analysis (HCA) and heat map were generated using the means of the data matrices (Michie, 1982). Both PCA and HCA were performed using JMP Pro 16 (SAS Institute, Cary, NC, USA).

RESULTS

Vegetative growth

As indicators of plant growth and vigorous state, control plants recorded the least values of shoot length (Figure 2A) and leaf area (Figure 2B) in both 2020 and 2021 seasons. All treatments positively improved both parameters compared to the control; however, the difference in shoot length was insignificant between the control and T5 during the second season, as well as the difference in leaf area between the control and both T2 and T5 during the first season. The application of BDPB or BDFB in combination with foliar application of SA (T3 and T6, respectively) slightly improved shoot length (Figure 2A) with insignificant differences compared to T2, T4 and T7 in both seasons. The most conspicuous effect on leaf area (Figure 2B) was recorded for vines subjected to T3 or T6, although the difference was insignificant when compared to T4 in both seasons. Results indicated that SA or CCA was more effective on shoot length when combined with BDFB, compared to BDPB. Regarding leaf area, SA was more effective than CCA when combined with BDFB.

Vine chlorophyll and carbohydrate contents

Similarly, control recorded the lowest values of leaf chlorophyll by mid-season (Figure 3A), and cane carbohydrate contents by the end of the growing seasons (Figure 3B) of both 2020 and 2021. All treatments significantly improved both parameters, with the most pronounced effect recorded for T3 followed by T6 in both seasons. In this regard, the difference between both treatments was only significant on chlorophyll content during the 2021 season (Figure 3A). No significant differences on carbohydrate contents were noticed between both treatments in both seasons (Figure 3B). Results indicated that SA was more effective than CCA when combined with BDPB.

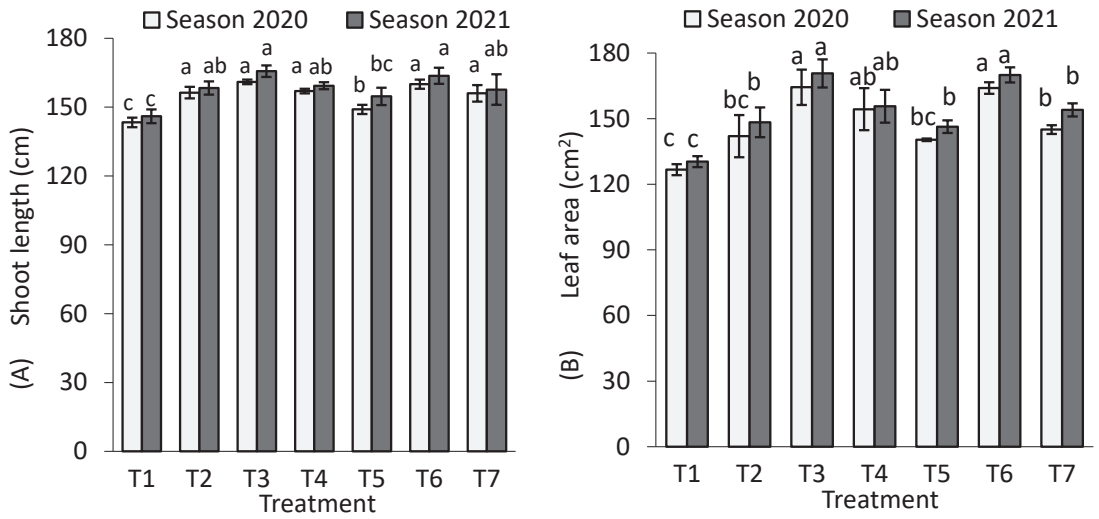

Figure 2. Effect of BD alone or combined with FS of either SA or CCA on shoot length (A) and leaf area (B) of 'Crimson Seedless' grapevines. T1 = distilled water (control), T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA. Values are the means of three replicates (n = 9) ± SD. Means with the same letters in each season are insignificantly different at probability (p) ≤ 0.05 using Tukey's HSD test. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HSD, honestly significant difference; SA, salicylic acid; SD, standard deviation.

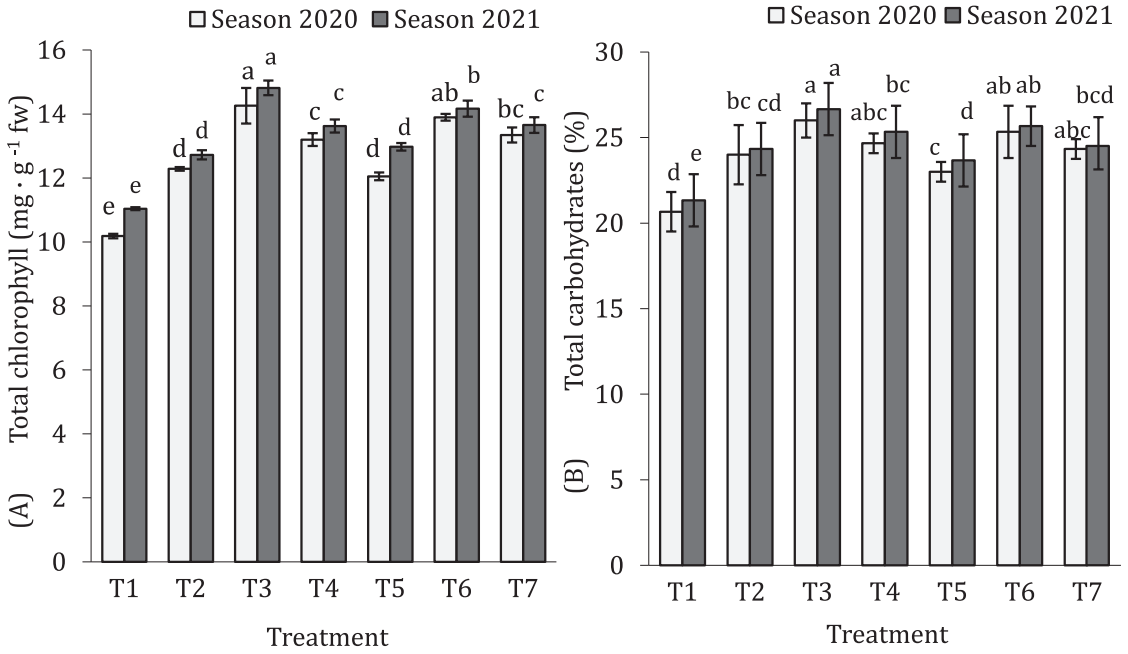

Figure 3. Effect of BD alone or combined with FS of either SA or CCA on total chlorophyll (A) and carbohydrate (B) contents of 'Crimson Seedless' grapevines. T1 = control, T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA. Values are the means of three replicates (n = 9) ± SD. Means with the same letters in each season are insignificantly different at $p \leq 0.05$ using Tukey's HSD test. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HSD, honestly significant difference; SA, salicylic acid; SD, standard deviation.

Berry set and total yield

It is interesting to note that the control (T1) recorded the highest percentage of fruit set (Figure 4A), but the lowest total yield (Figure 4B) compared to all other treatments during both seasons. Unlike vegetative growth (Figure 2), chlorophyll and carbohydrates (Figure 3), treatments T3 and T6 significantly recorded the lowest fruit set (Figure 4A) percentage compared to all other treatments. However, both treatments recorded the highest yield (Figure 4B) in both seasons. The difference between T3 and T6 was only significant during the second season, in terms of total yield. All other treatments were mediocrity effective in these regards. Results indicated that SA was more effective than CCA when combined with BDPB or BDFB.

Cluster weight, length and compactness

Accordingly, cluster weight (Figure 5A) was the lowest in control vines (T1), and the highest in T3-, followed by T6-treated vines with a significant difference between both treatments during the second season only. Results indicated that SA was more effective than CCA on cluster weight, when combined with either BDPB or BDFB during both seasons. In terms of cluster length (Figure 5B), control recorded the shortest clusters, which was significantly different from T3-treated vines that recorded the maximum cluster length with insignificant differences compared to all other treatments, except for T2 during the second season only.

The higher the fruit set percentage was (Figure 4), the more compacted the clusters were during both seasons, as expected (Figure 5C). Similarly, T3- and T6-treated vines recorded the lowest compacted clusters compared to the control during both seasons, and all other treatments during the first season only.

Berry weight, dimensions and firmness

As indicators of total yield, the average weight of 50 berries (Figure 6A) as well as berry dimensions (Figure 6B and Figure 6C) were the lowest in the control vines (T1) that eventually affected the cluster weight (Figure 5A), and hence total yield (Figure 4B). Similarly, BDPB associated with foliarly sprayed SA (200 mg · L^{-1}) (T3) was the most effective treatment improving berry weight and dimensions in both seasons, although the difference in berry weight was insignificant compared to T4 and T6 during the first season only. Moreover, no significant differences were noticed in berry length between T3, T5, T6 and T7, or in berry diameter between T3, T4, T6 and T7 in both seasons.

Interestingly, the more compacted clusters (Figure 5C) showed the lowest berry firmness (Figure 6D) as in the control vines (T1). Results indicated that all treatments positively improved berry firmness compared to the control in both seasons, particularly those applied at the PB stage, compared to the FB stage. Application of BDPB associated with foliarly sprayed CCA (20 mg · L^{-1}) (T4) was the most effective treatment with insignificant difference compared to T3 in both seasons.

Berry sensory characteristics

Colour

Results indicated that improved berry firmness (Figure 6D) was associated with more colourful berries (Figure 7), which reflected on the overall cluster colour.

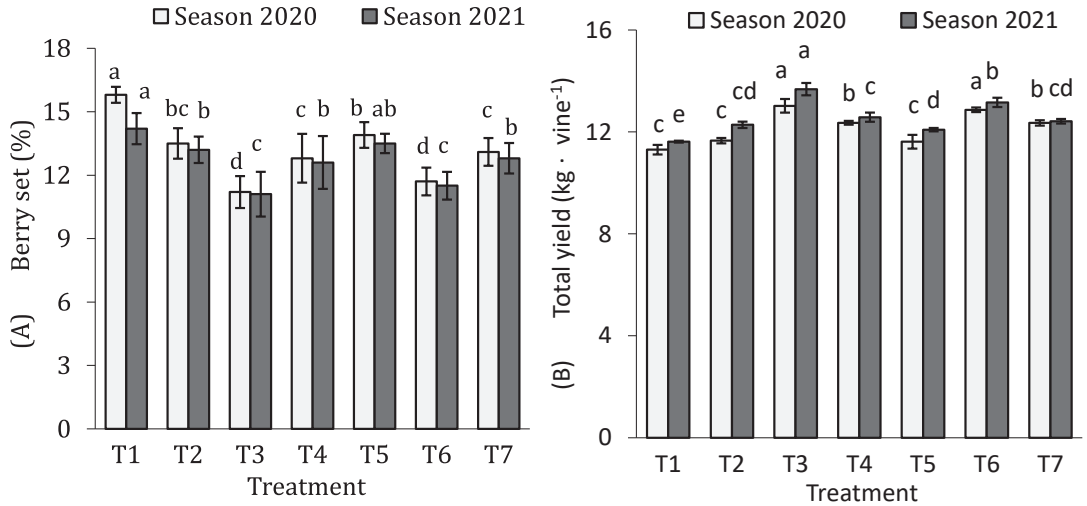

Figure 4. Effect of BD alone or combined with FS of either SA or CCA on berry set (A) and total yield (B) of 'Crimson Seedless' grapevines. T1 = control, T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA. Values are the means of three replicates ($n = 9$) ± SD. Means with the same letters in each season are insignificantly different at $p \leq 0.05$ using Tukey's HSD test. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HSD, honestly significant difference; SA, salicylic acid; SD, standard deviation.

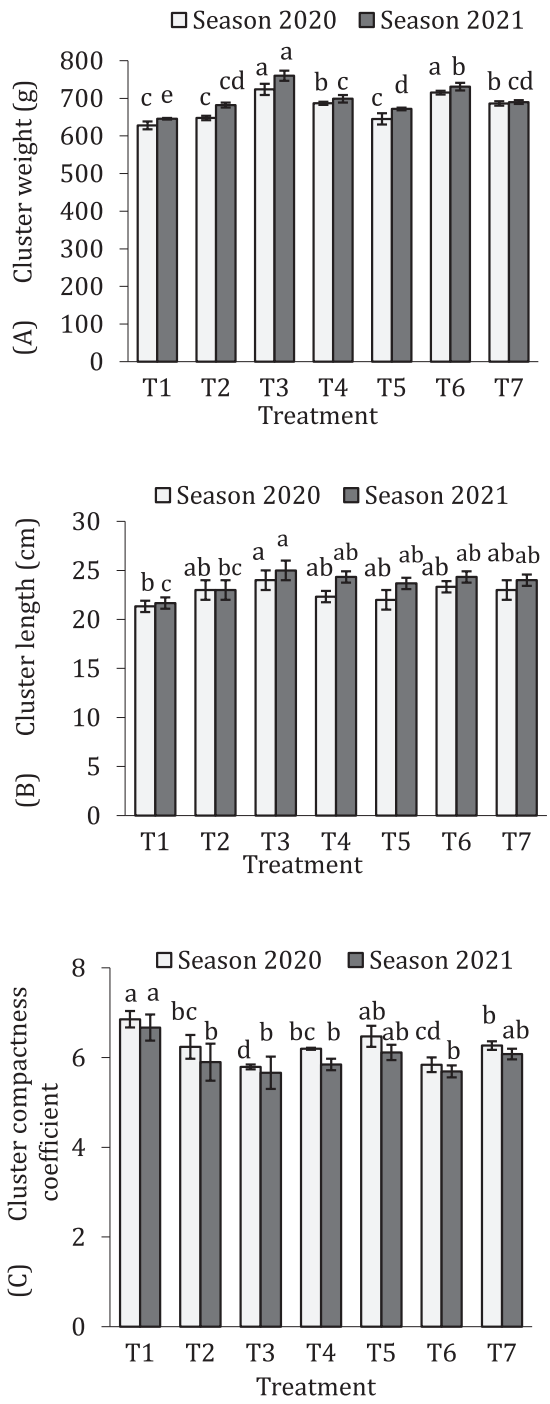

Figure 5. Effect of BD alone or combined with FS of either SA or CCA on cluster weight (A), length (B) and compactness (C) of 'Crimson Seedless' grapes. T1 = control, T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA. Values are the means of three replicates (n = 9) ± SD. Means with the same letters in each season are insignificantly different at $p \leq 0.05$ using Tukey's HSD test. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HSD, honestly significant difference; SA, salicylic acid; SD, standard deviation.

The most pronounced effect on berry colour was noticed with the combined application of either BDPB or BDFP, and CCA (20 mg · L^{-1}), corresponding to T4 or T7, respectively. Both treatments increased the percentage of red (Figure 7A) and pink (Figure 7B) berries, but decreased the percentage of green berries (Figure 7C), in comparison to the control and other treatments during both seasons.

Colour improvement was also determined by the decreased and increased values of *hue* angle (Figure 7D) and colour lightness (Figure 7E), respectively, in both T4- and T7-treated vines, due to the improved levels of anthocyanin synthesis (Figure 7F), compared to the control during both seasons. No significant difference was noticed between T4 and T7 during both seasons, except for anthocyanin levels (Figure 7F). No significant differences were noticed in the percentage of red berries (Figure 7A) and colour lightness (Figure 7E) between T3, T4 and T7 in both seasons.

Taste

Berry taste markers indicated that the best values were associated with the combined application of BDPB and CCA (T4), and control vines (T1) represented the least taste quality in both seasons (Figure 8). This was represented by improved levels of SSC (Figure 8A) and reduced levels of TA (Figure 8B) with enhanced SSC/TA ratio (Figure 8C) and sugar contents (Figure 8D) in T4-treated plants during both seasons. However, the effect of T4 on SSC (Figure 8A) was insignificant compared to all other treatments, except T5. In addition, the differences between T4 and both T3 and T7 were insignificant in regards to TA (Figure 8B) and SSC/TA ratio (Figure 8C) during both seasons. Results also indicated that the sole application of BDPB (T2) or BDFP (T5) was not highly effective on SSC, TA and SSC/TA ratio, in comparison to the control during both seasons. Both T4 and T3 showed a similar effect on total sugars during both seasons (Figure 8D).

Phenols

As an indicator of berry sensory quality (e.g. colour, flavour, taste), phenols were positively improved with all applied treatments, compared to the control in both 2020 and 2021 seasons (Figure 9). The most pronounced effects were noticed with the application of BDPB + CCA (T4), followed by a similar effect of BDPB + SA (T3) and BDFB + CCA (T7) during both seasons.

PCA and HCA

The goal of using PCA and HCA was to obtain a broader picture about the effect of BD, SA and CCA on 'Crimson Seedless' grapevines' growth, development and fruit quality under such environmental conditions. With regard to the PCA (Figure 10), the score plot showed that all treatments were different from the control, affecting plant and fruit characteristics during both seasons (Figure 10A and Figure 10B); however, the most pronounced effects on vines' vegetative growth,

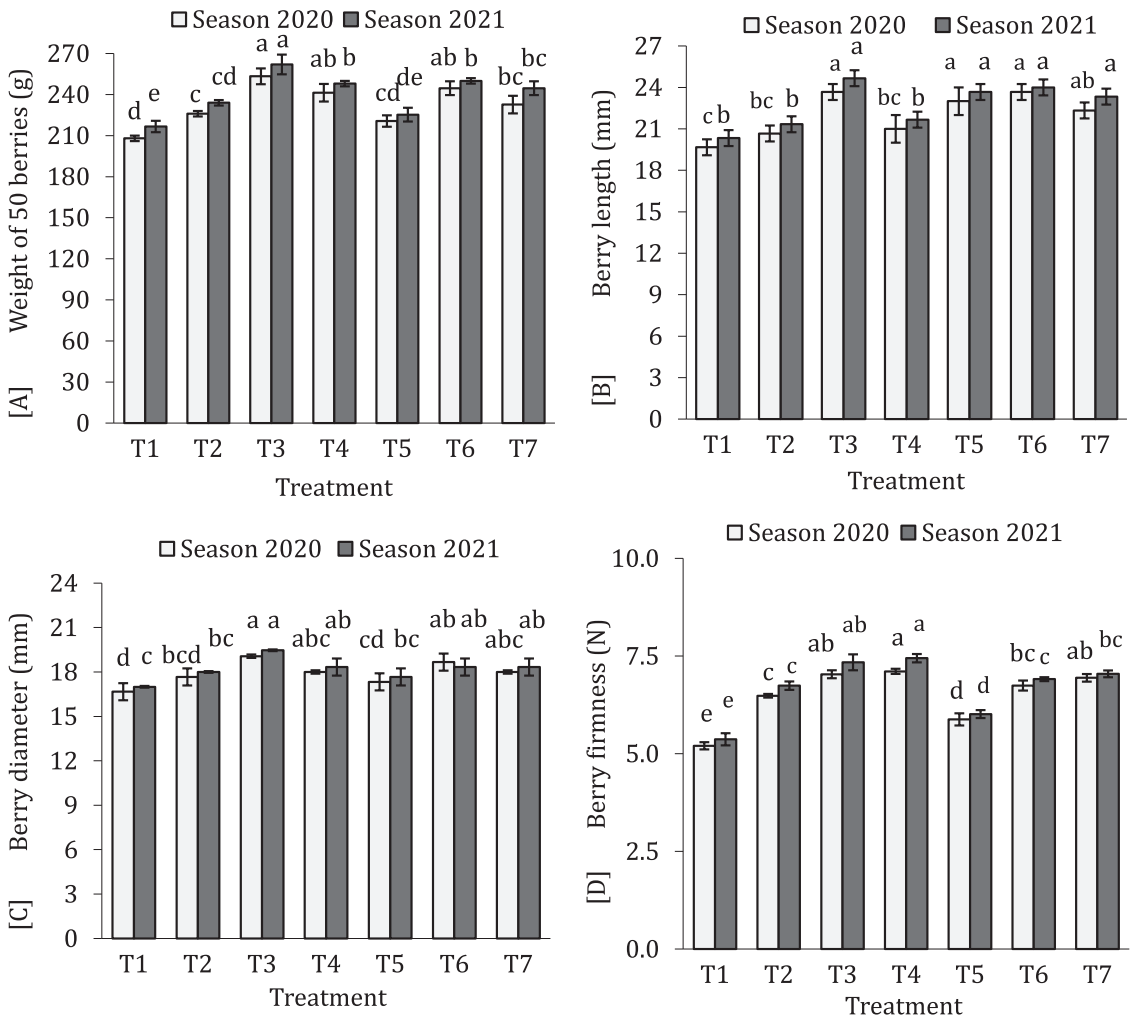

Figure 6. Effect of BD alone or combined with FS of either SA or CCA on berry weight (A), length (B), diameter (C) and firmness (D) of 'Crimson Seedless' grapes. T1 = control, T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA. Values are the means of three replicates ($n = 9$) ± SD. Means with the same letters in each season are insignificantly different at $p \leq 0.05$ using Tukey's HSD test. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HSD, honestly significant difference; SA, salicylic acid; SD, standard deviation.

physical characteristics of cluster and berry, and total yield were recorded for T3 followed by T6, whereas berry firmness, colour and taste were mostly related to T4 during both seasons (Figure 10C and Figure 10D). There is also indication that T7 had some slight effect on vegetative growth, physical characteristics of cluster and berry, and total yield during the 2020 season (Figure 10C), as well as on berry firmness, colour and taste during the 2021 season (Figure 10D). Principal components 1 and 2 accounted for 96.04% and 96.51% of the total variance in the 2020 and 2021 seasons, respectively (Figures 10A–Figure 10D).

Similarly, the cluster analysis (HCA) (Figure 11) indicated four different clusters with the control (T1) represented by a separate sub-cluster (sub-cluster 1). Other treatments are grouped in three sub-clusters, including T2 and T5 (sub-cluster 2), T3 and T6 (sub-cluster 3), and T4 and T7 (sub-cluster 4), which confirm the PCA results. The heat map of HCA showed that sub-cluster 1 recorded the minimum values in all studied parameters, except fruit set, cluster compactness, green berries, *hue* angle and TA during both seasons (Figure 11A and Figure 11B) that are normal results for a control treatment, which confirm previous results in Figures 2–9. Sub-cluster 2 of the heat map also revealed a better effect than the control, but this effect was still less effective compared to other treatments, indicating that the sole application of BDPB (T2) or BDFB (T5) was not highly effective in both seasons. As additional confirmation of PCA results, sub-cluster 3 (T3 in particular) was mainly related to vines' vegetative growth, physical characteristics of cluster and berry, and total yield, and

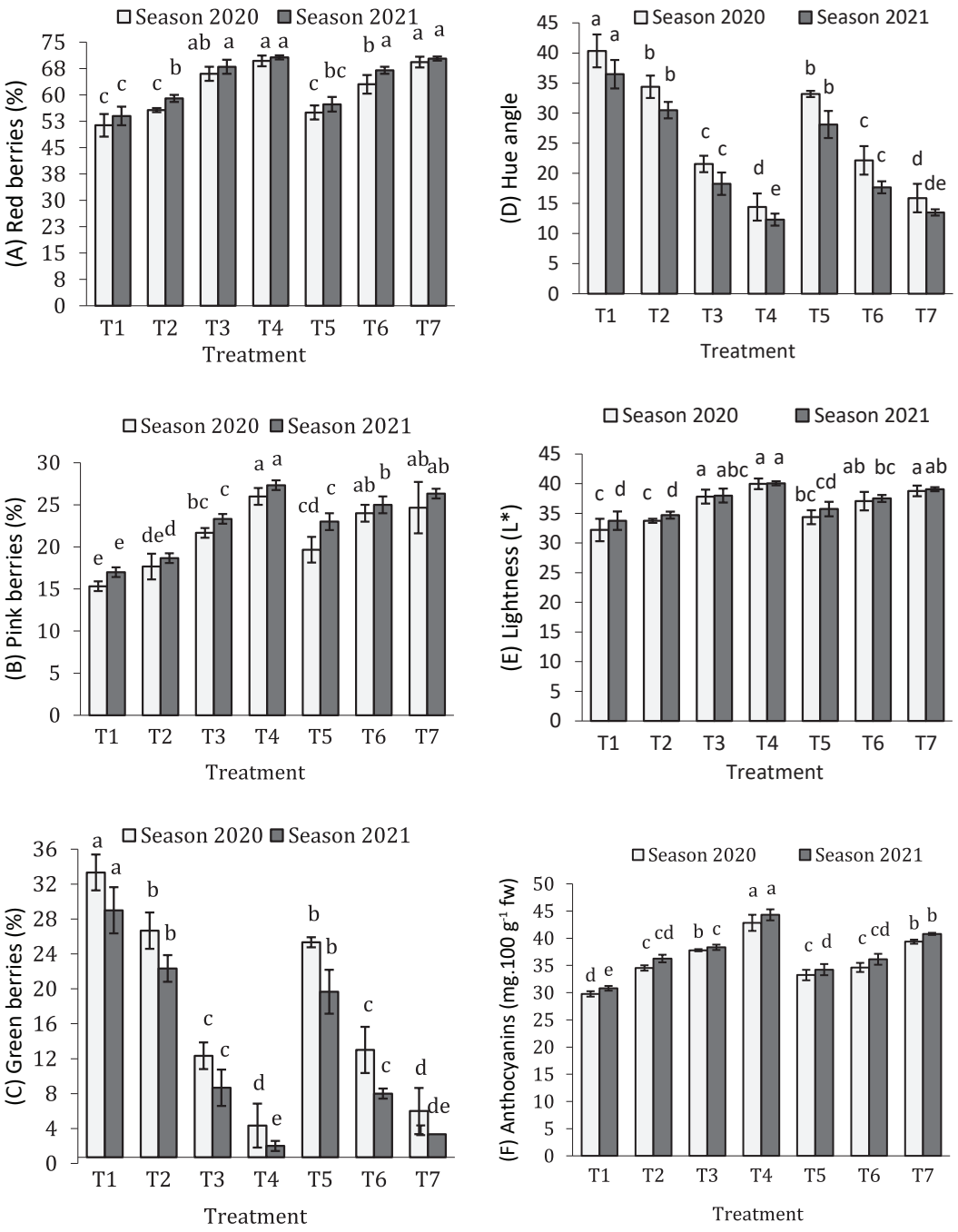

Figure 7. Effect of BD alone or combined with FS of either SA or CCA on berry colour (red [A], pink [B], green [C]), hue angle (D), lightness (E), and anthocyanin contents (F) of 'Crimson Seedless' grapes. T1 = control, T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA. Values are the means of three replicates (n = 9) ± SD. Means with the same letters in each season are insignificantly different at $p \leq 0.05$ using Tukey's HSD test. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HSD, honestly significant difference; SA, salicylic acid; SD, standard deviation.

sub-cluster 4 (T4 in particular) was mainly effective on berry firmness, colour and taste during both seasons.

DISCUSSION

Under the Egyptian semi-arid Mediterranean climate and flood irrigation conditions, clay-soil grown 'Crimson Seedless' grapevines manifested poor fruit quality (i.e. compact clusters, pale colour, low sugars, high acidity), which means low-grade fruit during marketing (Spayed et al., 2002; Cantín et al., 2007; El-Sayed, 2013; El-Ansary, 2017; Abd El-Rahman et al., 2018). This present study aimed to understand whether the combined application of BD with either SA or CCA

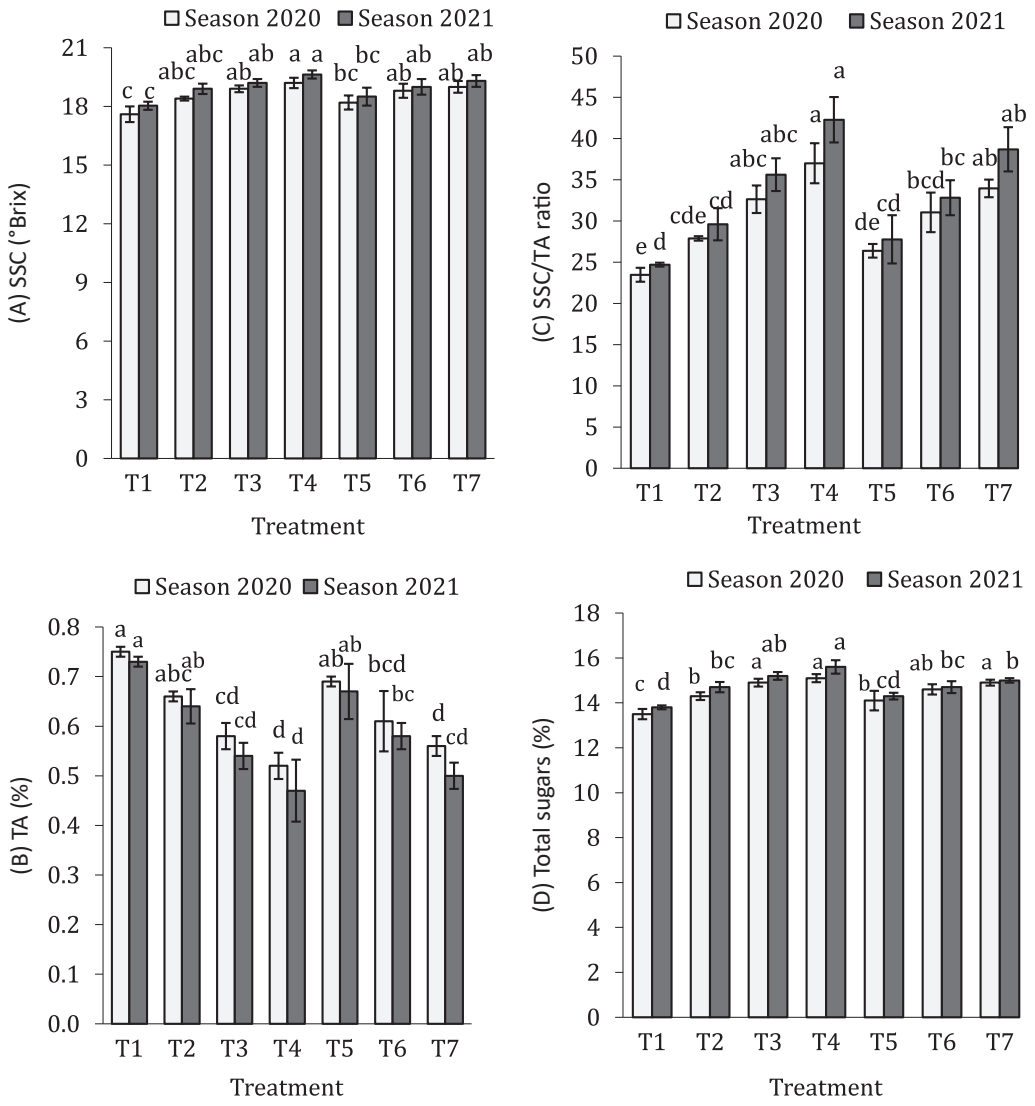

Figure 8. Effect of BD alone or combined with FS of either SA or CCA on berry SSC (A), TA (B), SSC/TA ratio (C) and total sugars (D) of 'Crimson Seedless' grapes. T1 = control, T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA. Values are the means of three replicates ($n = 9$) ± SD. Means with the same letters in each season are insignificantly different at $p \leq 0.05$ using Tukey's HSD test. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HSD, honestly significant difference; SA, salicylic acid; SD, standard deviation; SSC, soluble solid contents; TA, titratable acidity.

would solve the growers' concerns on this cultivar. Results indicated that 'Crimson Seedless' grapevines treated with either BDPB or BDFB, along with foliarly sprayed SA (T3 and T6, respectively), showed a positive enhancement in plant growth parameters (i.e. shoot length, leaf area) (Figure 2), whereas the photosynthesis markers (i.e. chlorophyll and carbohydrate contents) were more affected by T3 in both seasons (Figure 3). It was reported that leaf removal resulted in enhanced chlorophyll and carbohydrate contents by the end of the growing season to compensate the increased levels of photosynthesis and respiration in the remaining leaves, so that plants can mitigate the impacts of leaf removal (Petrie et al., 2003; Palliotti et al., 2011). Leaf removal has also been reported to increase the supply of water, nutrients and hormones from the roots to the developing leaves, leading to more new flushes with highly efficient tissues (Tardaguila et al., 2010; Palliotti et al., 2011), which eventually affect overall plant growth (Figure 2), productivity (Figure 4) and fruit physiochemical characteristics (Figures 5–9). Previous findings reported a positive role of SA or CCA on total chlorophyll content of 'Bez El-Naka' grapes (Abdel-Salam, 2016) and 'Le Conte' pear (Abd El-Bary, 2017), respectively. It was reported that SA improved plant height, chlorophyll content (Ghasemi et al., 2016), net photosynthesis and carbohydrate accumulation of heat-stressed grapevines (Wang et al., 2010). Also, CCA induced the antioxidant capacity, inhibited lipid peroxidation and enhanced

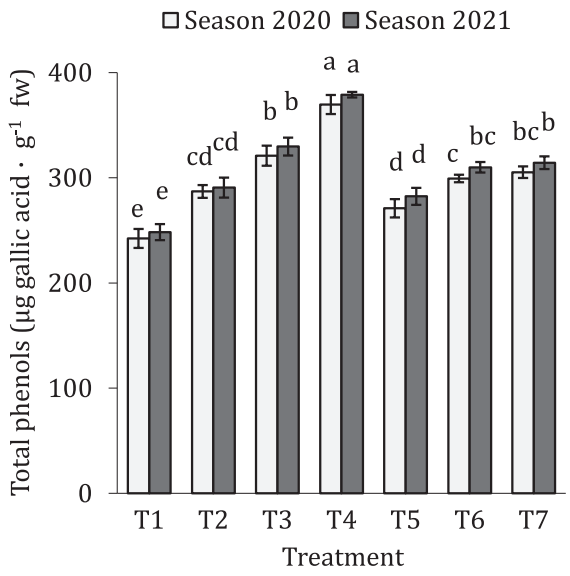

Figure 9. Effect of BD alone or combined with FS of either SA or CCA on berry total phenol contents of 'Crimson Seedless' grapevines. T1 = control, T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA. Values are the means of three replicates ($n = 9$) ± SD. Means with the same letters in each season are insignificantly different at $p \leq 0.05$ using Tukey's HSD test. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HSD, honestly significant difference; SA, salicylic acid; SD, standard deviation.

the biosynthesis of chlorophyll and carotenoids in salt-stressed common bean plants (Keshavarz and Moghadam, 2017).

The reduction in berry set percentage with BD has shown to be overcome and led to the maximum yield per vine with the application of either SA or CCA, with best results achieved with SA at either BDPB (T3) or BDFB (T6) (Figure 4). This was also represented with enhanced cluster weight, along with less compactness (Figure 5), as well as average berry weight, particularly with T3 (Figure 6). Berry firmness has shown similar responses to SA and CCA, with best results at BDPB (T3 and T4, respectively) in both seasons (Figure 6). In this context and regardless of the stage, BD has led to lack of carbohydrates, which in turn reduced the percentage of fruit set, and hence resulted in loose clusters (Poni et al., 2006; Intrieri et al., 2008; Tardaguila et al., 2010). It was reported that the carbohydrate supply at bloom stage is the major factor affecting fruit set (Caspari and Lang, 1996). Reduced carbohydrate supply during the phenological stages of flowering and fruit set has resulted in early abortion, and hence reduced percentage of berry set (Poni et al., 2005; Intrieri et al., 2008). Basal defoliation at the PB stage has been shown to reduce the photosynthates supply to flowers, resulting in reduced berry set (Caspari et al., 1998). This could explain the slight reduction in fruit set percentage and cluster compactness with improved yield when applying BDPB, compared to BDFB (Figure 4 and Figure 5), as also previously reported on 'Crimson', 'Tempranillo', 'Grenache' and 'Carignan' grapes (Diago et al., 2010; Tardaguila et al., 2010; Shalan and Hamza, 2020). It was reported that severe defoliation resulted in fewer number of berries, and hence less compacted clusters in 'Merlot' grapes (Yorgos et al., 2012). In addition, BD during the flowering stage has reduced the number of berries per cluster, and hence clusters' compactness, without affecting the next season's bud fertility in 'Cabernet Sauvignon' and 'Prokupac' grapes (Beslic et al., 2013). Defoliation before or during the flowering stage reduced cluster compactness, compared to that performed during fruit set stage (Diego et al., 2014). Defoliation during bloom or fruit set stages resulted in considerably fewer number of berries per cluster, which altered the cluster form and reduced compactness (Sabbatini, 2015; Sternad et al., 2015; Acimovic et al., 2016). The findings of the present study are inconsistent with the previous reports on 'Crimson Seedless' grapes that showed a positive improvement in cluster weight and size, as well as berry weight and size when defoliation was performed before the flowering stage (BDPB); however, the present results confirmed the role of BDPB on berry firmness (T2 vs T5) (Figure 6) as being in conformity with that elucidated in previous reports (Abd El-Razek et al., 2010; Tardaguila et al., 2010). Removing 30% of the leaf area with some shoots and inflorescences led to fewer berries per cluster, and thus less compacted clusters and firmer berries with less vulnerability to decays (Intrieri et al., 2016).

As a phytohormone, SA regulates the physiological and biochemical processes, improves chlorophyll content, photosynthesis, total carbohydrates and hence plant growth, flowering, setting, productivity and fruit quality of various crops, including grapes (Hayat et al., 2010; Abdel-Salam, 2016; Farhood et al., 2016; Ali et al., 2017; Roustakhiz and Saboki, 2017; Abou-zaid and Badawy, 2018; Ghattas, 2020). Preharvest application of SA has been reported to improve antioxidant levels, colour and yield in low-pigmented 'Magenta' and 'Crimson Seedless' grapes, and preserve quality traits during storage (García-Pastor et al., 2020). Berry firmness is one of the most critical physical quality criteria in determining customer acceptance (Poni et al., 2018). The nature of cell wall, water content and turgor pressure play a vital role in berry firmness (Payasi et al., 2009). Results indicated that BDPB positively improved berry firmness compared to BDFB (Figure 6). The combined application of BDPB with either SA (T3) or CCA (T4) similarly improved berry firmness, compared to the solo application of BDPB (T2). The effect of SA or CCA could be due to their antioxidant roles, which preserve insoluble pectin contents, inhibit the activity of primary cell wall degrading enzymes such as xylanase, cellulose and polygalacturonase, and stimulate cell division and enlargement (Eichel et al.,

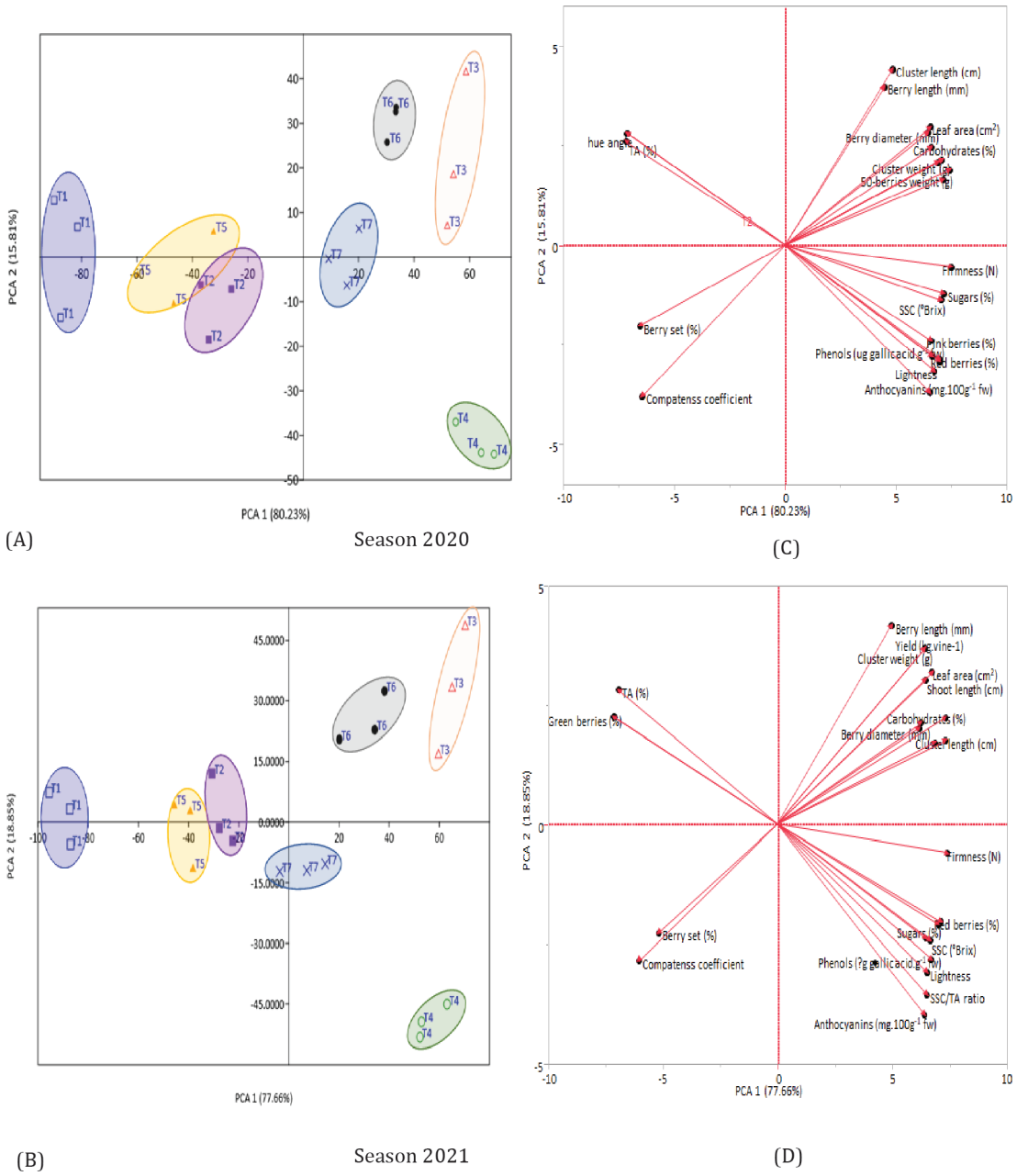

Figure 10. PCA showing the score plots (A, B) of the treatments: T1 = control, T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA (n = 9), and their associated loading plots (C, D) of various 'Crimson Seedless' plant and fruit characteristics during the 2020 and 2021 seasons, respectively. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HCA, hierarchical cluster analysis; HSD, honestly significant difference; PCA, principal component analysis; SA, salicylic acid; SD, standard deviation.

1995; Hayat et al., 2010), thus preserving berry firmness. It was reported that SA effectively maintained fruit firmness (Ennab et al., 2020) due to its anti-senescent role that resulted in reduced endogenous ethylene production (Nissen, 1994), hence protecting cell wall structure (Kazemi et al., 2011) and maintaining cell turgor pressure through the accumulation of defence-related metabolites (Zhu et al., 2016), including a salicylate-dependent pathway, resveratrol synthesis and cell-wall strengthening components (Serrano et al., 2017). Salicylic acid was also reported to improve plant antioxidant capacity and prevent cell-wall degradation through its positive effect on phenols and antioxidant enzymes (Cui et al., 2020). It was also suggested that CCA effectively increased cell wall polysaccharides during berry development (Lo'ay, 2010). Cyanocobalamin is an intercellular antioxidant

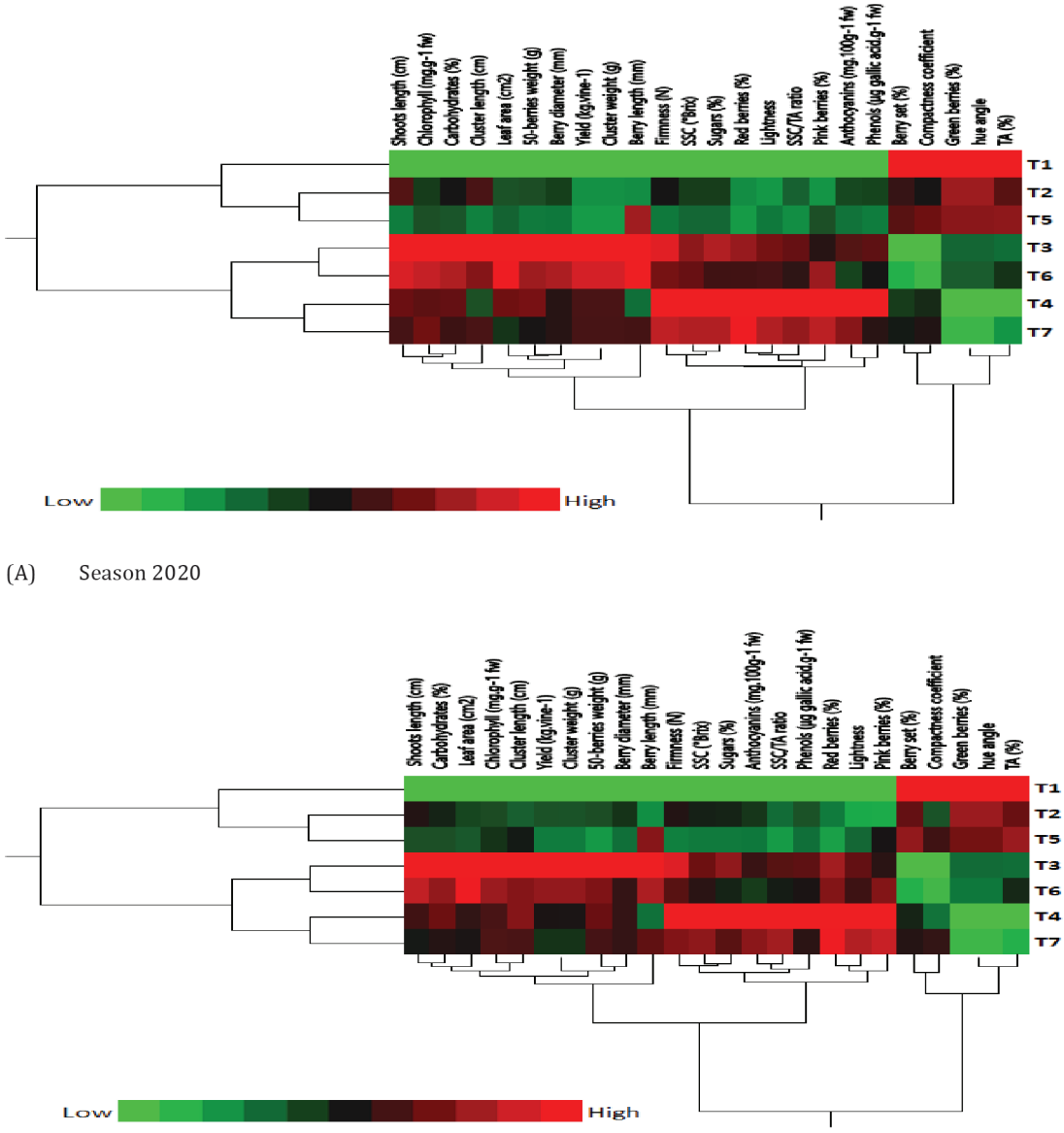

Figure 11. Two-way HCA and heat map showing the effect of BD alone or combined with FS of either SA or CCA on various 'Crimson Seedless' plant and fruit characteristics during the 2020 (A) and 2021 (B) seasons. Rows represent the treatments: T1 = control, T2 = BDPB, T3 = BDPB + 200 mg · L^{-1} SA, T4 = BDPB + 20 mg · L^{-1} CCA, T5 = BDFB, T6 = BDFB + 200 mg · L^{-1} SA and T7 = BDFB + 20 mg · L^{-1} CCA. Columns represent the plant and fruit characteristics. Higher peak areas are coloured red, and lower peak areas are coloured green. BD, basal defoliation; BDFB, basal defoliation at full bloom; BDPB, basal defoliation at pre-bloom; CCA, cyanocobalamin; FS, foliar spray; HCA, hierarchical cluster analysis; HSD, honestly significant difference; SA, salicylic acid; SD, standard deviation.

that reduces the levels of intercellular ROS and molecule damages (e.g. cell wall lipids, enzyme cofactors) (Jones et al., 2013; Ferrer et al., 2016; Ghasemi et al., 2016; Vasquez et al., 2022), which could also be the reason for maintaining cell wall integrity, and hence overall berry firmness, as previously reported in 'Thompson Seedless' grapes (Lo'ay, 2011) and 'Le Conte' pear (Abd El-Bary, 2017). Cyanocobalamin also plays a role in methionine biosynthesis (Ferrer et al., 2016), which is a precursor of polyamines synthesis (Zeh et al., 2002) that was reported to maintain cell turgor pressure and improve berry firmness (Mirdehghan and Rahimi, 2016).

Berry colour characteristics indicated a positive role of CCA over SA, in combination with either BDPB (T4) or BDFB (T7) (Figure 7). However, the difference between T4 and T7 was insignificant for all colour characteristics, except for anthocyanins in both seasons (Figure 7). Improved colour of 'Crimson Seedless'

grapes was represented by the improved anthocyanins and colour lightness (L*), but decreased values of *hue* angles (Figure 7), which negatively correlate with colour development, as previously confirmed (Ruiz et al., 2005; Elmenofy et al., 2021). Anthocyanins are the main pigment responsible for berry colour in 'Crimson Seedless' grapes (García-Beneytez et al., 2002). The higher the anthocyanin contents, the redder the colour of the berries were (Figure 7), and this could be attributed to the role of CCA in activating the cDNA and cobalamin-independent methionine synthase during berry development (Ali et al., 2017) that plays a vital role in methionine biosynthesis (Allen, 2012), which is the precursor of polyamine, protein and ethylene synthesis (Boselli et al., 2019). Methionine synthase has also a role in the one-carbon cycle pathway related to photosynthesis capacity (Zeh et al., 2002; Fontecave et al., 2004) and the biosynthesis of polysaccharides (Krook et al., 2000), and other secondary metabolites such as phenolic compounds, particularly flavonoids (i.e. flavonols, flavones and anthocyanins) (Ni et al., 2020). Anthocyanins are synthesised via the flavonoid pathway (He et al., 2010), and there are different types of anthocyanins that differ in their colours based on the cellular pH, such as malvidin (purple-red), delphinidin (pink), peonidin (purple-blue), cyanidin (red) and petunidin (purple) (García-Beneytez et al., 2002). Anthocyanins accumulation is also affected by environmental conditions such as light (i.e. photoperiod, intensity, composition in wavelengths of the light spectrum) that play an important role in the expression of genes along the flavonoid pathway, and related transcription factors that regulate the accumulation of anthocyanins in grape berries (Koyama et al., 2012; Kondo et al., 2014; Zoratti et al., 2014; Cheng et al., 2015). High temperature also inhibits anthocyanins accumulation, whereas lower temperature enhances the expression of flavonoid pathway genes (Ubi et al., 2006; Lin-Wang et al., 2011; Kuhn et al., 2014). It was noticed that CCA improved fruit colour via increased accumulation of peonidin and acylated derivatives of anthocyanins in grape peel (González-Neves et al., 2005). Salicylic acid was not as highly effective as CCA on fruit colour (Figure 7), and this could be related to the role of SA in delaying fruit maturity via the inhibition of ethylene biosynthesis, as well as its relationship with other phytohormones that affect overall plant metabolism (Arif et al., 2020). There is a positive role of CCA on methionine biosynthesis (Allen, 2012), which is the precursor of ethylene biosynthesis (Zeh et al., 2002; Fontecave et al., 2004). Both ethylene and abscisic acid (ABA) are commonly recognised to act antagonistically in the control of plant growth and development (Wright, 1980; Li et al., 2011). However, several studies on ethylene and ABA suggested that they can operate in parallel or even interact positively (Luo et al., 2014; Li et al., 2019), and this could justify the colour improvement in CCA-treated plants, compared to SA-treated plants (data not shown), since ABA is primarily responsible of the accumulation of anthocyanins and colour development in grapes (Ban et al., 2003).

The combined application of BDPB and CCA (T4) was also the most effective on berry SSC, TA, SSC/TA ratio and total sugars; however, this effect was followed by similar effects for both T3 and T7 (Figure 8). Phenols are one of the most important groups of secondary metabolites in plants that act as antioxidants, which protect cell structure and improve plant tolerance against stress (Boud, 2007). They also have been shown to be the major influence on the sensory quality of the fruit (i.e. colour, flavour, taste) (Balasundram et al., 2006). Anthocyanins are the prominent phenolic compounds responsible for berry colour, and contribute to the plant antioxidant capacity more than any other phenolic compounds (Mazza, 1995; Winkel-Shirley, 2001; Koes et al., 2005; Kristl et al., 2011). In addition, anthocyanins can offer slight astringency to the taste of the grapes, and can interact with some aroma substances (Dufour and Sauvaitre, 2000; Vidal et al., 2004a; Vidal et al., 2004b). The most pronounced effect on phenols was recorded for T4, followed by a similar effect for both T3 and T7 (Figure 9), which confirm the positive role of BDPB over BDFB. In general, this could be attributed to enhanced light penetration within the vine due to leaf removal, associated with stimulated root and shoot growth, along with improved water uptake, which in turn led to an enhanced level of photosynthesis and carbohydrate accumulation (Hunter and Le Roux, 1992; Tardaguila et al., 2010; Intrieri et al., 2016; Shaker, 2016). Previous reports have shown that leaf removal resulted in increased levels of solar radiation on both canopy and clusters, which effectively improved the following characteristics of berry contents: SSC/TA ratio, sugars, anthocyanins and phenols (Bergqvist et al., 2001; Poni et al., 2006; Poni et al., 2009; Tardaguila et al., 2010; Diago et al., 2012; Sun et al., 2012). Previous findings have also confirmed that BDPB plays a positive role in accelerating the ripening process, and that in the case of 'Crimson Seedless' grapes, suitable application of this technique further contributes to an improved SSC/TA ratio, anthocyanins and phenols (Poni et al., 2005; Tardaguila et al., 2010; Yorgos et al., 2012; Beslic et al., 2013), particularly with four-leaf BD (Shalan and Hamza, 2020). The solo application of CCA was reported to improve 'Crimson Seedless' grapes' berry contents of SSC, sugars, anthocyanins and phenols (Lo'ay, 2017). Similarly, CCA improved fruit set, SSC, TA and SSC/TA ratio in 'Le Conte' pear (Abd El-Bary, 2017). The role of SA in the enhancement of berry chemical characteristics could be ascribed to its positive effect on leaf area, chlorophyll content, photosynthesis capacity and carbohydrate contents (Abd El-Razek et al, 2010; Diago et al., 2010; Hayat et al., 2010). Preharvest spray of SA induced berry ripening, which was evidenced by improved SSC/TA ratio, total phenols (Champa et al., 2015; Abdel-Salam, 2016) and anthocyanins (García-Pastor et al., 2020), whereas the application at pre-

veraison stage increased total phenols, flavonoids and anthocyanins, particularly malvidin-3-glucoside at harvest (Oraei et al., 2019).

CONCLUSIONS

The inflicted fruit quality of 'Crimson Seedless' grapevines grown under the conditions of heavy clay soils, flood irrigation and semi-arid climate could be related to improper physiological and biochemical processes that were expressed by reduced vegetative growth, photosynthesis rate, productivity and hence fruit quality. Under such growing conditions, the sole use of BD or its use in combination with FS of either SA (200 mg · L^{-1}) or CCA (20 mg · L^{-1}) was found beneficial for improving overall plant growth and productivity. The improved shoot length and leaf area that were obtained pursuant to the use of BD treatments enhanced light penetration within the vine, and hence photosynthesis activity and carbohydrate accumulation. This effect was reflected on total yield and fruit quality, and definitely increased when SA or CCA was combined with the BDPB or BDFB stages. The most pronounced effect on vegetative growth, photosynthesis activity, cluster and berry weight and dimensions, and total productivity was mainly observed in the case of BDPB+SA (T3). Berry firmness, colour and sensory characteristics were more related to BDPB+CCA (T4). Both treatments could be suggested as proper applications to improve the quality of 'Crimson Seedless' grape crop subjected to flood irrigation under semi-arid conditions. Future research could incorporate the use of BDPB, SA and CCA as one application to compensate for the reduction in some quality characteristics attributable to the sole use of each treatment. More research is also needed: (i) to determine whether the low berry quality (and resultant poor marketability) observed in 'Crimson Seedless' grapes cultivated under the aforementioned growing conditions could be attributed to partially stressed vines; and (ii) to enhance vine growth and berry characteristics, particularly given the fact of reduced amounts of irrigation water, since water scarcity is, of late, becoming a problem in Egypt, and may become a limiting factor for the overall fruit industry in the future.

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AUTHOR CONTRIBUTIONS

All authors have significantly contributed to finalising the research. A.F.A.E.-K., M.A.E.-K. and B.E.B. designed the experimental set-up. M.A.E.-K. and B.E.B. performed the field experiments and relevant laboratory analyses. A.F.A.E.-K., I.F.H. and S.M.A.-E. performed software and statistical analysis. I.F.H., H.M.H.-V. and S.M.A.-E. performed data curation and visuality. A.F.A.E.-K., I.F.H. and H.M.H.-V. wrote the first draft. S.M.A.-E. wrote, reviewed and edited the final draft, which was read and approved by all the authors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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