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Improvement in Physiochemical Characteristics of 'Prime Seedless' Grapes by Basal Defoliation with Foliar-Sprayed Low-Biuret Urea and Cyanocobalamin under Mediterranean Climate

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Abstract: In viticulture, the main target is to achieve high yield and good fruit quality without compromising vine growth. Methods to achieve this balance will vary with regard to climate and cultivar. A two-year study was conducted on five-year-old 'Prime Seedless' grapevines to evaluate the effect of leaf defoliation and the foliar application of low-biuret urea (LBU) and cyanocobalamin (CCA) on berry set percentage, the compactness coefficient of the clusters and the overall quality of clusters and berries. The removal of the first four basal leaves was conducted at the full-bloom (FB) stage, while LBU (5 $g \cdot L^{-1}$) and CCA (40 $mg \cdot L^{-1}$) were sprayed at three phenological stages: (1) when the cluster length reached ~10 cm long, (2) at FB and (3) one week after the fruit set. The results demonstrated that the sole application of basal leaf removal (BLR) or in combination with LBU and/or CCA improved the vegetative growth, total yield and physiochemical characteristics of clusters and berries, whereas the same treatments decreased berry set and shot berry percentages and the compactness coefficient of the clusters, which in turn led to looser clusters compared to the control. The most pronounced effect was recorded for the combined application of BLR, LBU and CCA, which revealed the highest values of shoot length, leaf area and the contents of chlorophyll, proline, N, P, K, Ca, Mg, Fe and Zn. The same treatment recorded the lowest berry set and shot berry percentages, compactness coefficient of clusters and decay percentage. Overall, this treatment was the best in terms of total yield, cluster weight, berry firmness, soluble solid content (SSC), the SSC/acid ratio, total sugars, total carotenoids, total phenols, phenylalanine ammonialyase and polyphenol oxidase.

Keywords: basal leaf removal; bunch quality; cluster compactness; urea; vitamin B12

1. Introduction

Grapes, *Vitis vinifera* L., are considered one of the most important fruit crops around the world in terms of total production and economic importance [1]. The total cultivated area worldwide is about 6,730,179 ha with a total annual production of 74,943 million ton·ha^{-1}. In Egypt, total cultivated areas occupy about 79,092 ha with a total annual production of approximately 1.572 million tons with an average yield of 19.87 ton·ha^{-1} [2]. The Egyptian climate and soils are well suited for grape growth and production. Cultivated areas exist across the country since grapes are considered among the most suitable fruit crops for sandy soils and reclaimed lands with the production of many early and late cultivars during the May–November period. In terms of total production, grapes are considered second to citrus. The Behira and El-Sadat regions are the main production areas of grape exports to the European Union, the major importer of Egyptian grapes. The most popular cultivars are 'Thompson Seedless', 'Flame Seedless', 'Early Superior', 'Superior', 'Crimson' and 'Roomy' [3], plus some newly introduced ones such as 'Prime Seedless', 'Early Sweet' and 'Star Light' [4].

'Prime Seedless' grapes, an early-season cultivar, are some of the recent commercial seedless white cultivars that have spread to many areas worldwide. They are well known for their high fertility, good eating quality, round medium-sized berries, creamy-green color, good sugar level and mild Muscat flavor, which have increased their marketability in local and foreign markets. One of the main problems of this cultivar is cluster compactness, which leads to the development of bunch rot due to gray mold that is caused by *Botrytis cinerea* during the fruit maturity stage. Other problems include uneven berry size in the cluster due to the presence of an excessive number of small berries (shot berries) along with full-sized berries [5]. Consequently, this cultivar requires additional studies to improve the cluster characteristics under Egyptian conditions. Improving the quality of early grapes is very important for either local markets or exportation [6].

Basal leaf defoliation (BLD) is one of the most widely used viticulture techniques for grapevine canopy management that is used to improve yield in high-crop cultivars. When carried out at the pre-bloom stage, it reduces the carbohydrate/nitrogen ratio (C:N ratio) in the flowers and hence reduces the fruit set [7–9]. Leaf removal also alters the canopy microclimate and increases fruit exposure to sunlight, as well as air penetration into the cluster zone of the canopy [10], thereby reducing the percentage of yield loss due to cluster rot diseases, such as gray mold (botrytis rot), which particularly happens in compacted cluster cultivars, and represents about 30–40% of total decay [11]. Early leaf removal enhanced cluster and berry quality [12,13]. The severity of leaf removal affected the fruit set, as well as berry growth and development [14]. The removal of six and eight leaves led to a reduced fruit set and improved fruit quality, while the removal of two leaves only had a very minor effect in this regard. In contrast, the removal of ten leaves induced severe carbon stress and decreased total yield. Recent worldwide research revealed that basal leaf removal at the bloom stage in vigorous vines reduced fruit set percentage and bunch compactness and improved physiochemical characteristics [14–21]. Similar results were reported when defoliation was performed at the full-bloom (FB) stage but without a reduction in yield [22]. Late BD applied at the pea-size stage reduced fruit soluble solid and sugar contents and decreased acidity as a consequence of the faster rate of malic acid catabolism [23]. Late defoliation applied by the beginning of berry coloring (veraison stage) showed an incidence of sunburn with a negative effect on the biosynthesis of anthocyanins and thus berry color [24].

Nitrogen (N) represents about 1–4% of the grapevine dry mass and plays a key role in growth and development as a main component of proteins, DNA and chlorophyll [25]. Urea is the most commonly used N source for producing synthetic fertilizers worldwide [26]. It is a non-electrically charged N molecule suitable for foliar application, since it easily penetrates the epicuticular waxes and the cutin layer of leaves. The effectiveness of foliar urea application is related to crop load and the timing of application, which could also play a vital role in basal bud fertility and stored N reserves. It elevates arginine biosynthesis and induces flowering and may also affect volatile components and phenolic substances without influencing the yield and its components [27,28]. Spraying urea compounds at the pre-bloom or FB stages has been widely used to reduce berry set percentage, induce berry thinning and improve overall cluster characteristics [5,29–31]. The foliar application of urea was also suggested to increase amino acid contents in grapevines [32]. The urea manufacturing process sometimes resulted in fertilizers with high biuret concentrations (≈1.5–2%) due to the combining of two urea molecules into one (bi-uret; bi means two), which is considered toxic, interferes with N metabolism and inhibits protein synthesis. Typically, biuret concentrations 1–1.3% do not cause problems to plants; however, some plants are sensitive and require lower concentrations (<1%), so low-biuret urea [LBU] ($CO(NH_2)_2$) should be used for foliar application in these cases [33]. Cluster thinning is a cultural practice accomplished by spraying chemicals at the pre-bloom, FB or fruit set (FS) stages to reduce cluster compactness and improve berry quality [29]. Using LBU significantly reduced the fruit set due to a negative effect on ovary fertilization. It also improved fruit thinning, which might be attributed to phytotoxicity in the peduncle region of the berries [34].

Cyanocobalamin (CCA) is the manufactured version of cobalamin, also known as vitamin B12 ($C_{63}H_{88}CoN_{14}O_{14}P$, molecular weight = 1355.38 g·mol^{-1}), a water-soluble vitamin that is naturally found in animal cells. Plant cells do not synthesize it, but it was found to accumulate within plant cell organs like cytosol, plastids and mitochondria, providing definitive evidence that some plants can absorb and transport cobalamin [35–37] that was synthesized by some microorganisms (e.g., archaea, bacteria, fungi) that live in the plant root zone through microbial interaction [38]. Cobalamin is a cobalt-containing tetrapyrrole cofactor involved in intramolecular rearrangement and necessary for the regulation of DNA synthesis during plant cell division [39,40]. It plays a vital role in the conversion of homocysteine to methionine [41], 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 [42,43]. Cobalamin has been suggested to be an intercellular antioxidant that reduces the levels of intercellular reactive oxygen species (ROS) and molecule damage (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 [40,44,45]. Cyanocobalamin has been used to improve fruit color and other quality attributes of kaki [46], 'Thompson seedless' grapes [47], mango [48], guava [49], banana [50], 'Le-Conte' pear [51] and 'Crimson Seedless' grapes [52].

Previous reports on commonly used cultural practices such as trunk girdling and the foliar spray of ethephon [53], mechanical cluster thinning [54], the foliar spray of ABA [55], cover cropping and deficit irrigation [56], leaf removal in the cluster area [57] and moderate deficit irrigation [58] did not show that much improvement in vine growth or fruit quality. Therefore, this research aimed to use some efficient practices and determine whether the combined application of basal leaf removal (BLR), LBU and CCA could be used as a new sustainable solution to improve the productivity and fruit quality of 'Prime Seedless' grapes under the Mediterranean climate of Egypt. Most of the previous reports have focused on the sole use of BLR [18–21], LBU [59] or CCA [46,51,52] on the growth and productivity of different fruit trees, including grapes.

2. Materials and Methods

2.1. Experiment

This research was conducted on six-year-old 'Prime Seedless' grapevines, *Vitis vinifera* L., grafted on 'Freedom' grape rootstocks, in a sandy soil under a drip irrigation system and groundwater table of 1.5 m, at a private grape orchard in El-Sadat City, Menoufia governorate, Egypt (30°22′30′′ N and 30°30′1′′ E), for two consecutive seasons (2022 and 2023). The climatic conditions of the experimental site are shown in Table 1 [60]. Soil samples were randomly collected from the root zone (0–90 cm) for analysis [61]. The Nile River was the source of irrigation, and water samples were also collected for analysis [62–65]. Soil and water analysis values are displayed in Table 2. A drip irrigation system was utilized per the recommended program for 'Prime Seedless' grapes, released by the grape growers in the area. The quantity of water per vine and hectare is displayed in Table 3.

Table 1. Average monthly weather data for El-Sadat City, Menoufia governorate, Egypt, from September 2021 to August 2023.

Month	Year	Temperature (°C)	Humidity (%)	Rainfall (mm·Month^{-1})	Wind Speed (km·h^{-1})	Cloud (%)	Sun (h·Month^{-1})	UV Index
September	2021	34.2	53	0.0	12.1	5	379	7
	2022	31.5	51	0.0	13.2	5	378	7
October	2021	31.5	55	0.8	11.0	10	389	6
	2022	33.0	59	0.0	11.8	11	389	5
November	2021	27.8	62	2.6	11.1	8	378	6
	2022	29.1	57	1.8	10.3	27	359	6
December	2021	19.7	66	4.2	13.1	22	380	5
	2022	21.5	65	5.3	10.2	19	370	4
January	2022	17.0	54	4.1	13.9	31	369	4
	2023	21.5	59	3.0	13.0	19	385	5
February	2022	18.2	57	10.0	12.2	31	334	4
	2023	21.7	60	4.6	12.5	27	324	4
March	2022	20.5	58	2.3	14.6	23	371	7
	2023	23.3	62	6.7	14.2	14	394	5
April	2022	23.9	51	0.8	13.3	15	382	7
	2023	29.2	51	3.8	15.3	9	386	8
May	2022	29.0	38	0.0	14.2	10	393	7
	2023	39.9	37	0.0	13.3	3	398	8
June	2022	32.6	44	0.0	13.5	7	386	8
	2023	39.3	41	0.0	12.9	1	386	9
July	2022	36.7	46	0.0	12.5	8	398	8
	2023	39.7	41	0.0	13.2	2	398	9
August	2022	36.1	47	0.0	12.8	4	398	8
	2023	36.0	43	0.0	11.8	2	398	8

The experiment was carried out on sixty-three uniform grapevines planted at a distance of 2 m × 3 m. Grapevines were free from any symptoms of physiological disorders or nutrient deficiencies. They were trellised on a Spanish baron trellis with a quadrilateral cordon training system of short spurs. Winter pruning was performed during the first week of January in both seasons. Spur pruning was performed for all grapevines, keeping 68 buds per vine (four cordons, each cordon with three spurs of five buds [60 buds total], in addition to one renewal spur of two buds beside each cordon). Under the Egyptian conditions, total buds usually grow to 45–48 shoots that eventually produce about 30–35 clusters [66]. To achieve good cluster weight and quality for marketing and exportation, the growers usually perform cluster thinning right after FS stage (≈late April − early May) and maintain an average number of 18–24 clusters per vine [personal communication]. In the present research, the total number of clusters was maintained at 22–23 in both seasons.

The selected vines were subjected to the same cultural practices as the entire orchard in both seasons. The annual soil fertilization program per hectare was set to include 300 kg calcium superphosphate ($CaH_6P_2O_9$) + 119 kg sulfate ($SO_4{}^{2-}$) applied once at the beginning of vegetative growth (VG) during the first week of March, 119 kg of calcium nitrate ($Ca(NO_3)_2$) applied 10 days before the beginning of bloom (first week of April) and at full FS stage during the fourth week of April, 553.5 kg potassium sulfate (K_2SO_4) + 215 kg ammonium nitrate ($NH_4NO_3{}^{-}$) applied three times (beginning of VG, at FS and after harvest [mid-June]), 119 kg magnesium sulfate ($MgSO_4$) applied monthly from April to

August (23.8 kg·month^{-1}) and 24 kg zinc sulfate ($ZnSO_4$) applied after harvest. Vines also received foliar application of 1785 mg·L^{-1} micronutrients (595 mg·L^{-1} chelated-Fe, 595 mg·L^{-1} chelated-Zn and 595 mg·L^{-1} Mn) at the beginning of the VG, at 30–45 cm-shoot length stage (third week of March) and again after two weeks by the beginning of bloom (first week of April), while 400 mg L^{-1} borax (source of B) was foliarly sprayed twice, one week before bloom (fourth week of March) and after harvest.

Table 2. Average soil and water analysis for the experimental farm, El-Sadat City, Menoufia governorate, Egypt, where 'Prime Seedless' grapevines were grown.

Parameter	**Soil Depth (cm)**			**Water**	
	0–30	**30–60**	**60–90**		
Clay (%)	4.29	4.29	4.29	Transparency (cm)	132.5
Silt (%)	3.36	3.36	3.36	Permeability index (%)	55.64
Sand (%)	92.31	92.31	92.31	Water quality index	21.54
Texture	Sandy	Sandy	Sandy	pH	7.33
Field capacity (%)	13.77	13.71	13.71	Total dissolved salts (mg·L^{-1})	204.9
Permanent wilting point (%)	6.65	6.62	6.62	E.C. (μmhos·cm^{-1})	558.8
pH (1:2.5 extract)	8.08	8.05	8.01	O_2 (%)	95.80
Organic material (%)	2.10	0.55	0.35	$CaCO_3$ (mg·L^{-1})	100.6
E.C. (dS·m^{-1}) [1:5 extract]	2.03	2.01	2.01	HCO_3^- (mg·L^{-1})	159.5
$CaCO_3$ (%)	1.83	1.41	1.88	CO_3^{2-} (mg·L^{-1})	7.00
HCO_3^- (meq·100 g^{-1})	0.30	0.37	0.40	SO_4^{2-} (mg·L^{-1})	10.13
CO_3^{2-} (meq·100 g^{-1})	0.00	0.00	0.00	SiO_2 (mg·L^{-1})	1.21
SO_4^{2-} (meq·100 g^{-1})	3.17	4.04	4.13	Cl^- (mg·L^{-1})	0.4.0
Cl^- (meq·100 g^{-1})	0.96	0.98	1.08	Na^+ (mg·L^{-1})	29.20
Na^+ (meq·100 g^{-1})	0.48	0.66	1.42	Ca^{2+} (mg·L^{-1})	6.00
Ca^{2+} (meq·100 g^{-1})	0.80	0.20	1.25	Mg^{2+} (mg·L^{-1})	0.70
Mg^{2+} (meq·100 g^{-1})	0.33	0.97	1.16	N (mg·L^{-1})	1.56
N (mg·kg^{-1})	3.00	2.00	1.00	P (mg·L^{-1})	0.094
P (mg·kg^{-1})	1.00	2.00	1.00	K (mg·L^{-1})	8.81
K (mg·kg^{-1})	27.0	24.0	23.0	Fe (mg·L^{-1})	0.23
Fe (mg·kg^{-1})	1.48	1.21	111	Mn (mg·L^{-1})	0.005
Mn (mg·kg^{-1})	1.10	150	1.21	Zn (mg·L^{-1})	0.60
Zn (mg·kg^{-1})	0.18	0.11	0.11	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

Seven different treatments were applied to the selected vines, as follows: the control, distilled water-sprayed vines (T1), four BLR at FB stage [second week of April] (T2), foliar spray of LBU at 5 g·L^{-1} (T3), foliar spray of CCA at 40 mg·L^{-1} (T4), T2 + T3 (T5), T2 + T4 (T6) and T2 + T3 + T4 (T7). The experimental design was a randomized complete block design (RCBD) with three replicates, three vines each. Each group of nine vines received the same treatment in both seasons. Basal leaf removal was conducted by detaching the first four leaves at the bottom section of the shoot during FB stage (second week of April [≈30–35 days after the beginning of VG]) in both seasons. At FS stage (≈10–14 days after FB), cluster numbers were reduced to 22 and 23 per vine for all treatments during the 2021/2022 and 2022/2023 seasons, respectively. Distilled water, low-biuret urea or cyanocobalamin was supplemented with Tween 20 as a surfactant (0.1% *v:v*) and applied to the selected vines using a 25 L Knapsack power sprayer (model HT-767, TaizhouTianyi

Agricultural and Forestry Machinery Co., Taizhou, China). The entire vine was sprayed until dripping at three different times: when the cluster length reached approximately 10 cm in length (≈first week of March), FB stage and one week after FS (pea-size berry stage {6–8 mm} ≈ first week of May [≈21–25 days from FB]). Veraison stage was recorded on the third week of May during both seasons (≈30–35 days from FB). Low-biuret urea fertilizer (99–100% urea [46% N], <0.4% biuret, water-soluble, white granules, slight ammonia smell) was imported from Planta Düngemittel GmbH (Munich, Germany). Cyanocobalamin and all other chemicals used in this research were imported from Sigma Aldrich (St. Louis, MO, USA).

Table 3. Average monthly irrigation water applied to 'Prime Seedless' grapevines in El-Sadat City, Menoufia governorate, Egypt, during the 2021/2022 and 2022/2023 seasons.

Month	Dripper Discharge Amount ($L \cdot h^{-1}$)	Number of Drippers per Vine	Irrigation Period ($h \cdot Day^{-1}$)	Daily Water Quantity ($L \cdot Vine^{-1}$)	Monthly Water Quantity ($L \cdot Vine^{-1}$)
September	4	4	1 h, 45 m	28.00	840.00
October	4	4	0 h, 45 m	12.00	372.00
November	4	4	0 h, 30 m	7.98	239.40
December	4	4	0 h, 08 m	2.13	66.03
January	4	4	0 h, 09 m	2.40	74.40
February	4	4	0 h, 10 m	2.68	75.04
March	4	4	1 h, 00 m	16.00	496.00
April	4	4	1 h, 29 m	23.73	711.90
May	4	4	2 h, 00 m	32.00	992.00
June	4	4	2 h, 11 m	34.93	1047.90
July	4	4	2 h, 13 m	35.47	1099.57
August	4	4	2 h, 15 m	36.00	1116.00
Annual water quantity ($m^3 \cdot vine^{-1}$) = 7.13 m^3					
Annual water quantity ($m^3 \cdot hectare^{-1}$) = 11,883.73 m^3					

2.2. Vegetative Growth

At pea-size berry stage, two non-fruiting shoots off the renewal spurs, at each side of the vine, were randomly chosen and marked to measure shoot length, using a 1000 cm tape (Fisher Scientific, Waltham, MA, USA) to calculate the average shoot length (cm). Two mature leaves (i.e., the 6th and 7th from the shoot tip) on each marked shoot were collected to measure leaf area (cm^2) using LI-3100 leaf area meter (LI-COR, Inc., Lincoln, NE, USA), and average leaf area was calculated.

2.3. Leaf Analysis

Same eight leaves were also used to determine leaf chlorophyll and proline contents.

2.3.1. Chlorophyll ($C_{55}H_{72}MgN_4O_5$) Content

The chlorophyll analysis was conducted [67], and the absorbance of the extract was measured using a UV/Vis spectrophotometer, model UV-9100-B (LabTech Inc., Hopkinton, MA, USA), at 663 and 646 nm for chlorophyll 'a' and chlorophyll 'b', respectively. Chlorophyll contents ($\mu g \cdot mL^{-1}$) were calculated using the following equations:

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

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

where E is the optical density at the indicated wavelength.

Accordingly, total chlorophyll content ($mg \cdot g^{-1}$ fw) was calculated using the following equation:

$$\text{Total chlorophyll} = (([\text{chlorophyll a} + \text{chlorophyll b}] \times \text{extract volume}) \div [1000 \times \text{fw}]) \tag{3}$$

2.3.2. Proline ($C_5H_9NO_2$) Content

A fresh leaf sample (0.5 g) was homogenized with 3 mL sulfosalicylic acid ($C_7H_6O_6S$), [3% *w*:*v*] using a porcelain mortar and pestle set (Fisher Scientific, Waltham, MA, USA). The mixture was centrifuged at 18,000× *g* for 15 min using LRF-C20 benchtop centrifuge (Labtron Equipment Ltd., Camberley, UK) at 22 °C. The supernatant (1 mL) was then mixed with 2 mL glacial acetic acid (CH_3COOH) and 2 mL freshly made acid ninhydrin reagent (1.25 g ninhydrin ($C_9H_6O_4$) dissolved in 30 mL glacial acetic acid and 20 mL orthophosphate (PO_4^{3-}) [6 M] in a test tube [Thermo Fisher Scientific, Waltham, MA, USA]). The tubes were incubated in 'PrecisionTM General Purpose' water bath (Thermo Fisher Scientific, Waltham, MA, USA) at 100 °C for 1 h and then left to cool at room temperature (≈20–22 °C) for 24 h. Afterward, the solution was mixed with toluene ($C_6H_5CH_3$) [4 mL] using a Vortex-Genie 1 mixer (Scientific Industries, Inc., Bohemia, NY, USA) for 20 s, and tubes were left in upright position for at least 10 min until the separation of the toluene and the aqueous phase. The toluene phase was then carefully pipetted out into a cuvette, and a spectrophotometer, model UV-120-20 (Shimadzu Corp., Kyoto, Japan), was used to measure the absorbance at 520 nm. Later, a proline standard curve was used to calculate the proline concentration as $mg{\cdot}g^{-1}$ fw [68].

2.3.3. Nutrient Contents

A sample of 20 leaf petioles per replicate from leaves opposite the cluster was used to determine the content of macro- and micronutrients. Leaf samples were collected two weeks after FS (second week of May) and dried at 65 °C for 72 h until constant weight using a bench-top Heratherm GP oven (Thermo Fisher Scientific, Waltham, MA, USA). Consequently, dried leaves were pulverized using the mortar and pestle set, and the powder was digested with concentrated sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) [69]. This mixture was then used to colorimetrically determine N and P using the spectrophotometer [70,71]. Potassium (K) concentration was determined using a flame photometer, model FP910 (PG instruments, Leicestershire, UK) [72]. Other nutrients like Ca, Mg [73], Fe and Zn [74] were determined using the atomic absorption spectrophotometer, model AA990 (PG Scientific, Inc., Auburn, CA, USA). All values of macro- and micronutrients were expressed as percentage (%) and $mg{\cdot}kg^{-1}$ dry weight (dw) of leaves, respectively.

2.4. Berry Set and Total Yield

At bloom, two uniform clusters at each side of the vine were randomly selected and marked. The total number of blossoms in each cluster was counted to calculate the average blossom number per cluster. At the pea-size berry stage, the total number of berries per cluster was counted to calculate the average number of berries per cluster. Accordingly, the percentage of berry set was calculated, as follows [75]:

$$\text{Berry set (\%)} = (\text{number of berries per cluster} \div \text{number of blossoms per cluster}) \times 100 \tag{4}$$

All clusters were harvested when soluble solid content (SSC) reached 16% [76] by mid-June of both seasons (≈60–70 days from FB). A hand-held refractometer [reading 0–32%], model N-1E (Atago Co., Ltd., Tokyo, Japan), was used to determine SSC in the field. Clusters on each vine per replicate were weighed using a field digital scale [200 kg capacity] (VEVOR Equipment and Tools, Rancho Cucamonga, CA, USA), and then average fruit yield ($kg{\cdot}vine^{-1}$) was calculated for each treatment [77].

2.5. Fruit Physiochemical Characteristics

2.5.1. Cluster Parameters

Ten clusters per vine were randomly selected during harvest to assess cluster and berry parameters. Each cluster was weighed, using a PC-500 bench-top digital scale (Doran scales, Batavia, IL, USA), to calculate average cluster weight (g). The length (cm) of each cluster was also measured from the uppermost berry to the most bottom berry, using a 30 cm stainless steel ruler (Apuxon, Shenzhen, China), and average cluster length was calculated. In addition, the number of berries per cluster (including shot berries) was counted to calculate the average number of berries per cluster, and hence the compactness coefficient of the cluster and the percentage of shot berries (small, green and round shriveled berries equal to the size of black pepper or smaller [78]) per cluster were calculated using the following equations [79]:

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

$$\text{Shot berries per cluster (\%)} = (\text{number of shot berries} \div \text{total number of berries}) \times 100 \tag{6}$$

Each cluster was then shaken well by the same person [80], and the abscised berries were collected and counted to calculate shattering (excessive shedding of berries) percentage [81], as follows:

$$\text{Berry shattering (\%)} = (\text{number of abscised berries} \div \text{total number of berries}) \times 100 \tag{7}$$

2.5.2. Decay

Same 10 clusters were used to determine the decay index (%) of the cluster (due to natural infection by *Botrytis cinerea* and other species), according to the number of infected berries [82]. Decay severity scores of the clusters were rated using a scale of 0–5, where 0 is healthy cluster with zero decay symptoms, 1 is decay on ≤5% of the cluster, 2 is decay on 6–25% of the cluster, 3 is decay on 26–50% of the cluster, 4 is decay on 51–75% of the cluster, and 5 is decay on 76 to 100% of the cluster [83]. Afterward, the following formula was used to calculate decay index [82]:

$$\text{Decay index (\%)} = (\Sigma\ [n \times v] \div [N \times D] \times 100) \tag{8}$$

where n = number of decayed clusters in each scale, v = decay severity score, N = total number of examined clusters, and D = the highest decay severity score.

The incidence of naturally occurring gray mold (Botrytis rot, *Botrytis cinerea*) on berries was calculated using the following formula [84]:

$$\text{Botrytis incidence (\%)} = (\text{number of decayed berries} \div \text{total number of berries}) \times 100 \tag{9}$$

2.5.3. Berry Parameters

The bench-top digital scale was used to weight 100 randomly selected berries from each cluster and then calculate the average weight of 100 berries per vine (g). Fifty out of the hundred berries were used to measure berry diameter (mm) using a digital caliper [0.01 accuracy] (Grizzly Industrial, Chicago, IL, USA), and then the average berry diameter per vine was calculated. Twenty berries were randomly selected from the same set of 50 berries to determine berry firmness twice at the equatorial area of the berry [85] using a hand-held digital penetrometer, model FT-02, fitted with a 2 mm plunger tip (QA Supplies LLC, Norfolk, VA, USA), and firmness was expressed in newton (N).

The remaining 50 berries were used to determine SSC in Brix using the hand-held refractometer at room temperature (≈20–22 °C). The actual values were re-calculated and recorded in percentages after making a temperature correction to 20 °C [86]. Total 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 indicator [86], and the SSC:TA ratio was calculated. Total sugars were colorimetrically determined [87] at 490 nm using the spectrophotometer, and the concentration was calculated as grams glucose·100 g^{-1} fw (expressed as a percentage). The total carotenoid contents in berry peel were also determined [88] and calculated as mg·g^{-1} fw.

Total phenols were estimated in berry peel [89] by homogenizing a peel sample (2 g) with 3 mL methanol [CH_3OH] (80%) and stirring the mixture on a hot plate stirrer

[1000 rpm], model RT2 (ThermoFisher Scientific, Waltham, MA, USA), at 70 °C for 15 min. A small portion (0.1 mL) of the extract was then mixed with 2 mL sodium carbonate [Na_2CO_3] (2%), incubated at room temperature (≈20–22 °C) for 5 min, consequently mixed with 0.1 mL of the Folin–Ciocalteu reagent [$C_{10}H_5NaO_5S$] and then incubated at room temperature for another 10 min. Total phenols were colorimetrically determined at 765 nm using the spectrophotometer and expressed as milligrams of gallic acid [$C_7H_6O_5$] (mg·100 g^{-1} fw) using a gallic acid standard curve.

Phenylalanine ammonialyase (PAL) was determined using a peel sample (1 g) mixed with 3 mL borate buffer [$B_4Na_2O_7$] (50 mM), 0.05 mL 2-mercaptoethanol [C_2H_6OS] (5 mM) and 400 mg polyvinylpyrrolidone $(C_6H_9NO)_n$ at pH level of 8.8. The mixture was then centrifuged at 16,000 rpm and 4 °C for 15 min. Subsequently, the mixture was mixed with 700 µL L-phenylalanine [$C_9H_{11}NO_2$] (100 mM) and 3 mL borate buffer (50 mM). Afterward, the supernatant (300 µL) was incubated at 40 °C for 60 min and then mixed with 100 µL hydrochloric acid [HCl] (5 mM) to block any additional enzyme response. Eventually, PAL activity (as a formation of cinnamic acid [$C_9H_8O_2$]) was measured colorimetrically at 290 nm using the spectrophotometer at room temperature (≈20–22 °C) [90], and results were expressed as U·mg^{-1} protein.

Polyphenoloxidase (PPO) was assayed using another peel sample (1 g) homogenized with sodium phosphate buffer [$NaPO_4^-$] (0.1 M) and Tris-Hydrochloride [$C_4H_{11}NO_3HCl$] (20 mM) at pH = 6.8. The mixture was then centrifuged at 16,000 rpm and 4 °C for 6 min, and the pure extract was directly kept at −25 °C in a low-temperature freezer, model LF45–408 (Infitek Inc., Spokane, WA, USA). To measure PPO activity, 200 µL of the pure extract was added to 3 mL of 4-methylcatechol substrate [$C_7H_8O_2$] (20 mM) dissolved in 1.49 mL of sodium phosphate buffer (0.1 M) at pH = 6.8 [91]. Later, the increased activity of PPO was recorded at 400 nm for 3 min using the spectrophotometer. The activity was presented with a 0.1 min^{-1} difference in absorbance, and the protein units (mg) were used to express the specific activity of PPO against the total content of soluble proteins in the enzyme extract. Results were expressed as U·mg^{-1} protein [92].

2.6. Statistical Analysis

Data were tested for numerical normality and homogeneity of variance using the Shapiro–Wilk and Levene tests, respectively. Data calculated as percentages were transformed to the Arcsine square root values before running the analysis of variance (ANOVA), and results were presented as back-transformed means. The ANOVA test was carried out using the CoStat software package, version 6.311 (CoHort software, Monterey, CA, USA). Means were compared using Tukey's honestly significant difference (HSD) test at probability (p) $\leq$ 0.05 [93].

3. Results

3.1. Vegetative Growth

The results in Figure 1 indicate that all used treatments, except T2 (removing four basal leaves), had a significant effect on vegetative growth indices (i.e., shoot growth and leaf area) compared to the control during both seasons. No significant differences were recorded among treatments T3–T7 on shoot length in both seasons, except that between T4 and T7 during the second season only. However, the combined application of BLR, LBU and CCA (T7) significantly improved leaf area compared to T4 during both seasons.

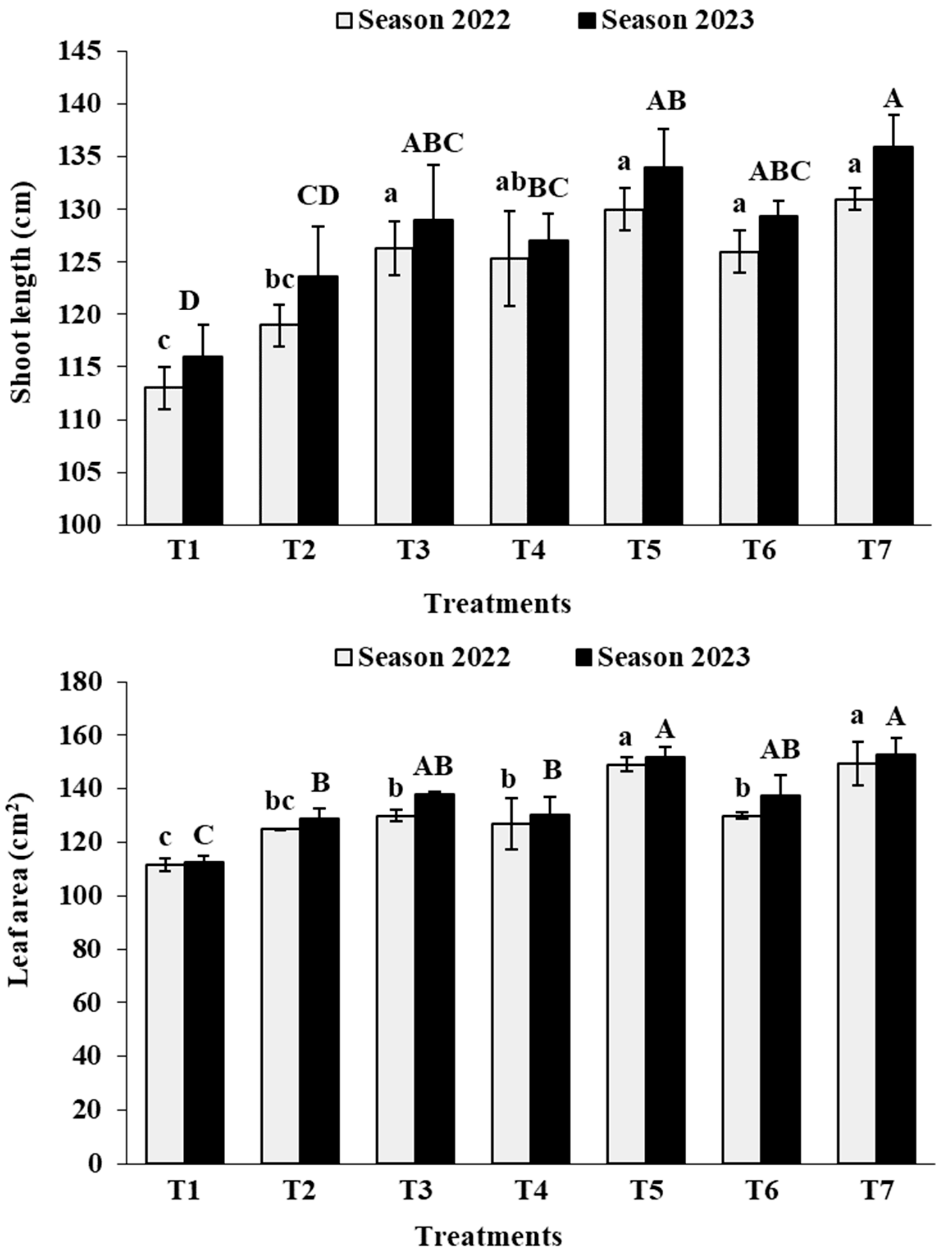


Figure 1. Effect of basal leaf removal (BLR) alone or combined with foliar spray of low-biuret urea (LBU) or cyanocobalamin (CCA) on shoot length and leaf area of 'Prime Seedless' grapevines during 2022 and 2023 seasons. T1 = distilled water (control), T2 = four BLR at full bloom (FB), T3 = LBU at 5 $g \cdot L^{-1}$, T4 = CCA at 40 $mg \cdot L^{-1}$, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4. Values are means of three replicates (n = 3) $\pm$ standard deviation (SD). Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's honestly significant difference (HSD) test.

3.2. Leaf Analysis

Leaf analysis indicated a positive effect on both chlorophyll and proline contents, in response to all treatments, compared to the control during both seasons (Figure 2). The most pronounced effect compared to the control and all treatments was found in the combined application of BLR, LBU and CCA (T7), although the difference was insignificant in chlorophyll content between T5 and T7 during the first season only.

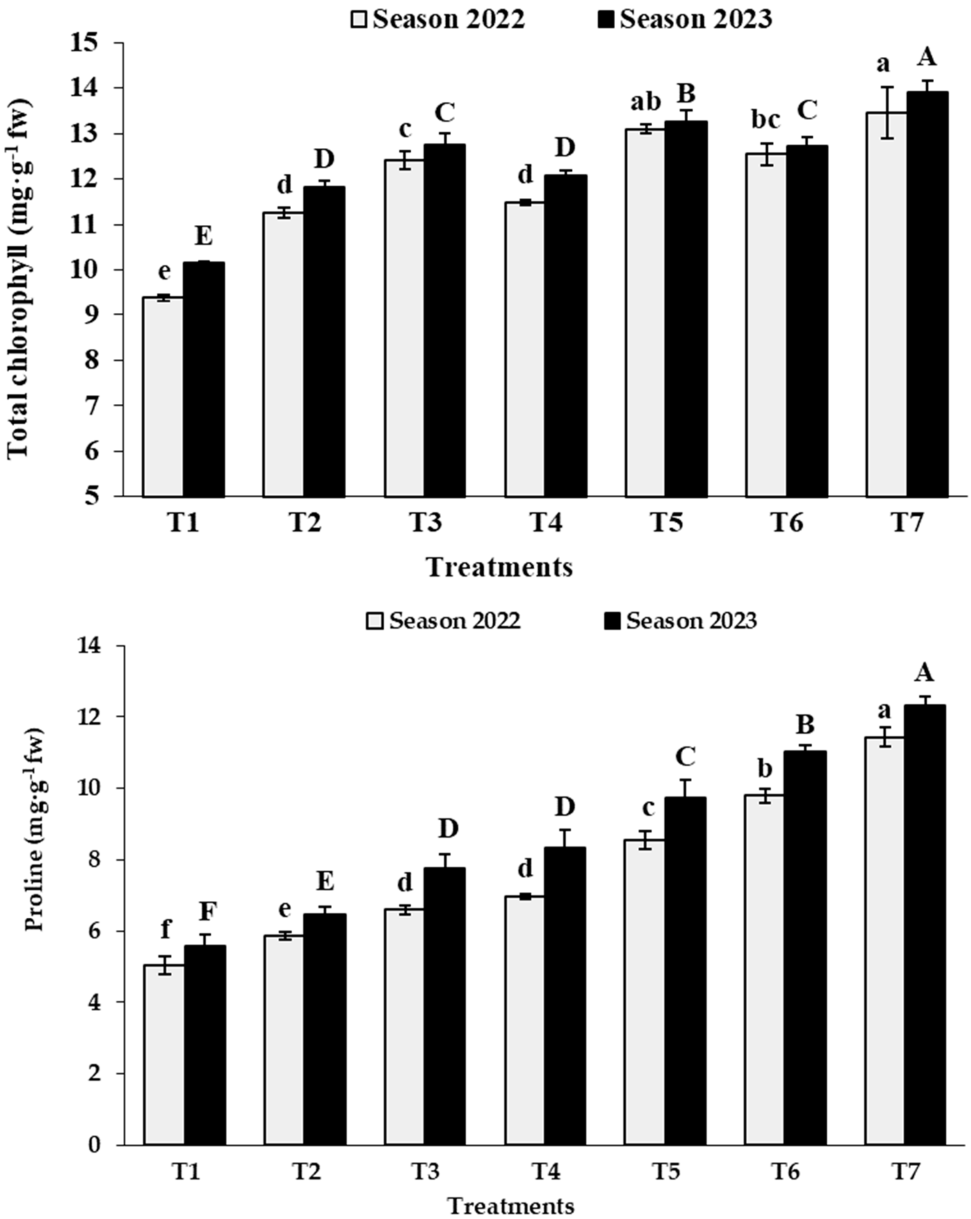


Figure 2. Effect of BLR alone or combined with foliar spray of LBU or CCA on leaf chlorophyll and proline contents of 'Prime Seedless' grapevines during 2022 and 2023 seasons. T1 = control, T2 = four BLR at FB, T3 = LBU at 5 g·L^{-1}, T4 = CCA at 40 mg·L^{-1}, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4. Values are means of three replicates (n = 3) ± SD. Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's HSD test.

Likewise, all treatments significantly improved the levels of macronutrients (Figure 3) and micronutrients (Figure 4) in leaves, although there was no significant difference between T2 and the control in regards to K, during both seasons. In all cases, the most pronounced effect was recorded for T7 during both seasons; however, the difference was insignificant between T5 and T7 in regards to N, K, Ca, Mg and Zn.

3.3. Fruit Set and Total Yield

The lower the vegetative growth and the contents of chlorophyll, proline and nutrients, the higher the percentage of the fruit set was, as indicated in Figure 5. Control grapevines recorded the highest fruit set percentage but the lowest total yield compared to all treatments during both seasons. In regards to the total yield, the difference was insignificant between the control and T2 in the second season only. In this regard, T7 recorded the lowest fruit set but the highest yield compared to all treatments, but insignificant differences were noticed compared to T5 during both seasons.

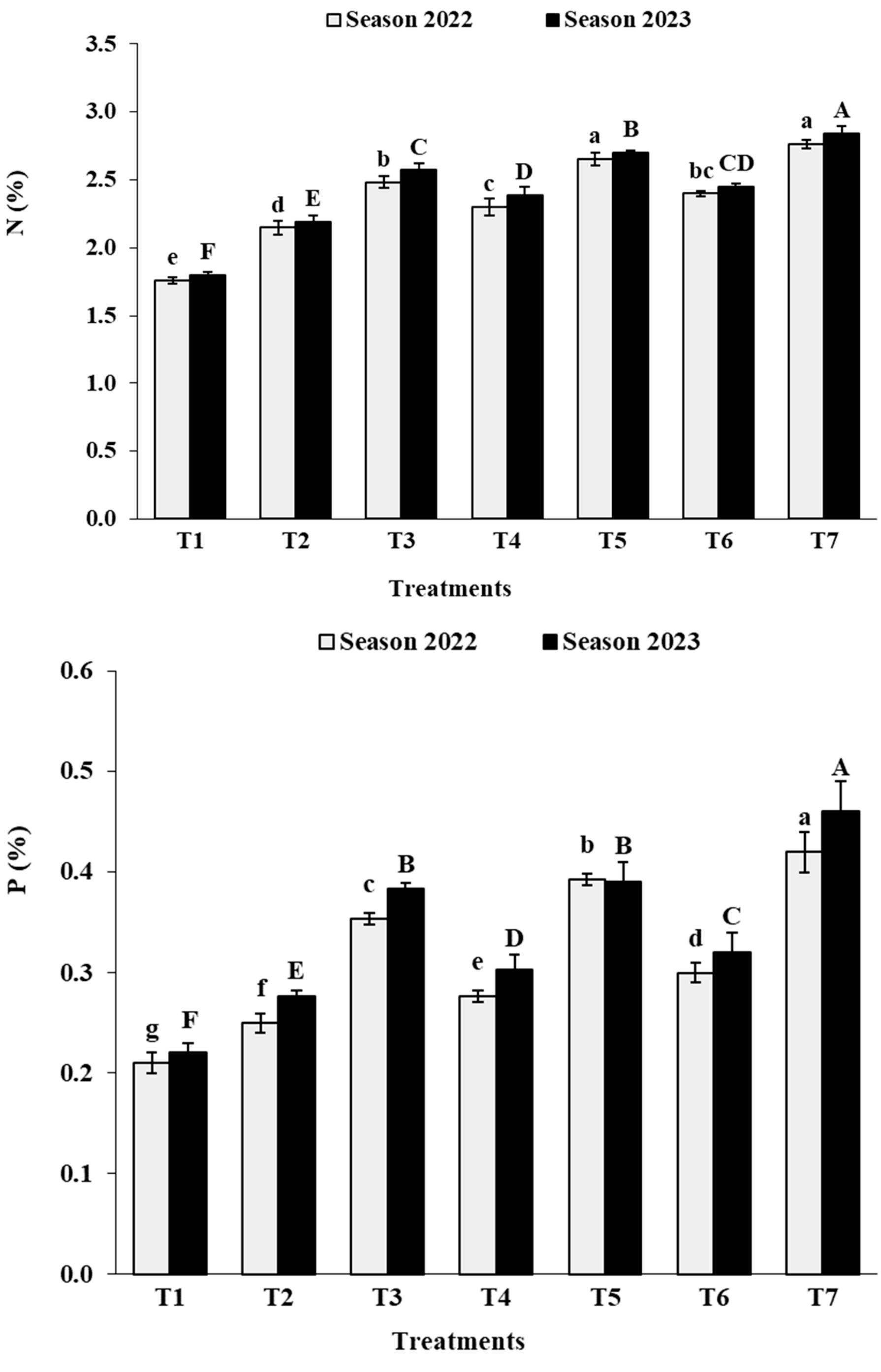


Figure 3. *Cont.*

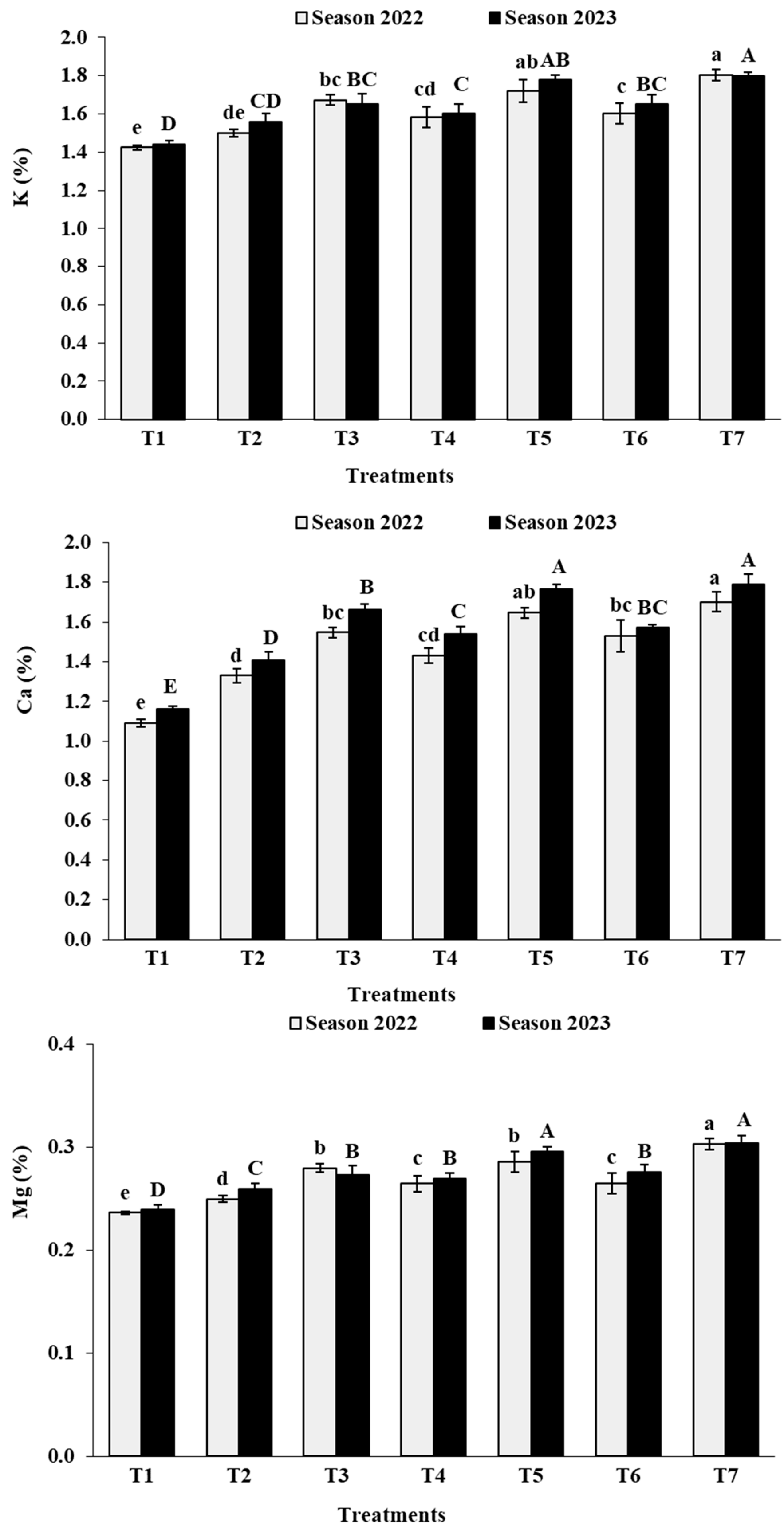
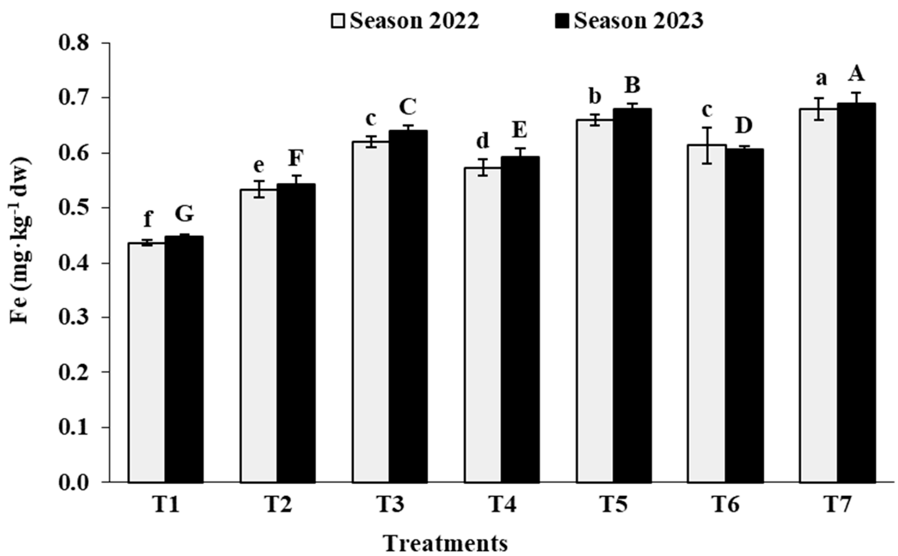


Figure 3. Effect of BLR alone or combined with foliar spray of LBU or CCA on leaf macronutrient contents of 'Prime Seedless' grapevines during 2022 and 2023 seasons. T1 = control, T2 = four BLR at FB, T3 = LBU at 5 g·L^{-1}, T4 = CCA at 40 mg·L^{-1}, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4.

Values are means of three replicates (n = 3) $\pm$ SD. Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's HSD test.



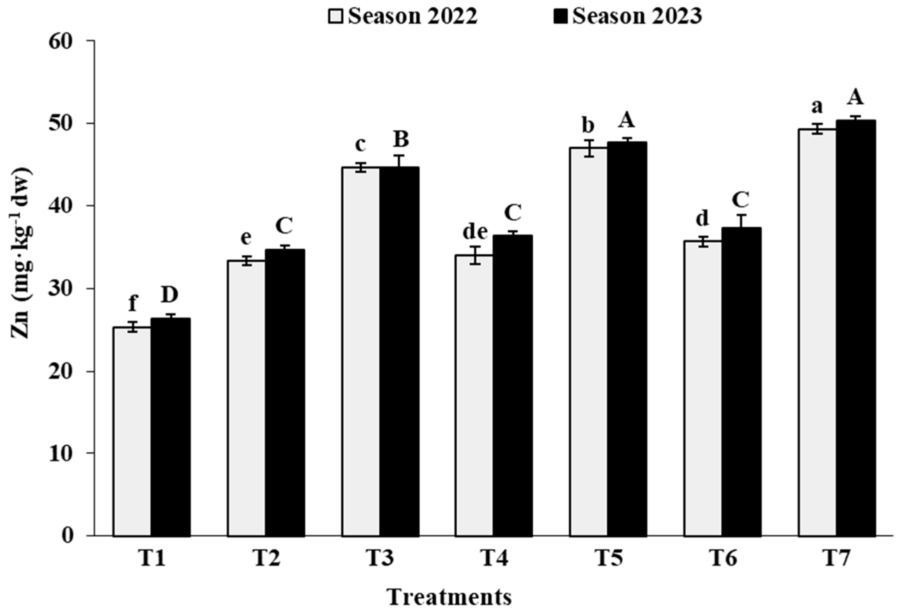


Figure 4. Effect of BLR alone or combined with foliar spray of LBU or CCA on leaf micronutrient contents of 'Prime Seedless' grapevines during 2022 and 2023 seasons. T1 = control, T2 = four BLR at FB, T3 = LBU at 5 g·L^{-1}, T4 = CCA at 40 mg·L^{-1}, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4. Values are means of three replicates (n = 3) $\pm$ SD. Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's HSD test.

3.4. Cluster Physical Characteristics

The positive effect of the applied treatments on total yield could be due to the improvement in overall cluster weight and length, compared to the control (Figure 6). Likewise, the most pronounced effect was recorded for T7, although the difference was insignificant compared to T5 only in both seasons. Likewise, clusters collected from the T7-treated vines were the least compacted ones; however, the difference was insignificant compared to T5 in both seasons. Those clusters from T7 also had the lowest shot berry and shattering percentages, while control clusters had the highest percentages in this regard (Figure 6). The effect of T7 on shot berries was insignificant compared to T6 in both seasons, as well as T2, T4 and

T5 in the second season only. No significant differences were noticed in berry shattering percentages among T3, T4, T5, T6 and T7 in both seasons.

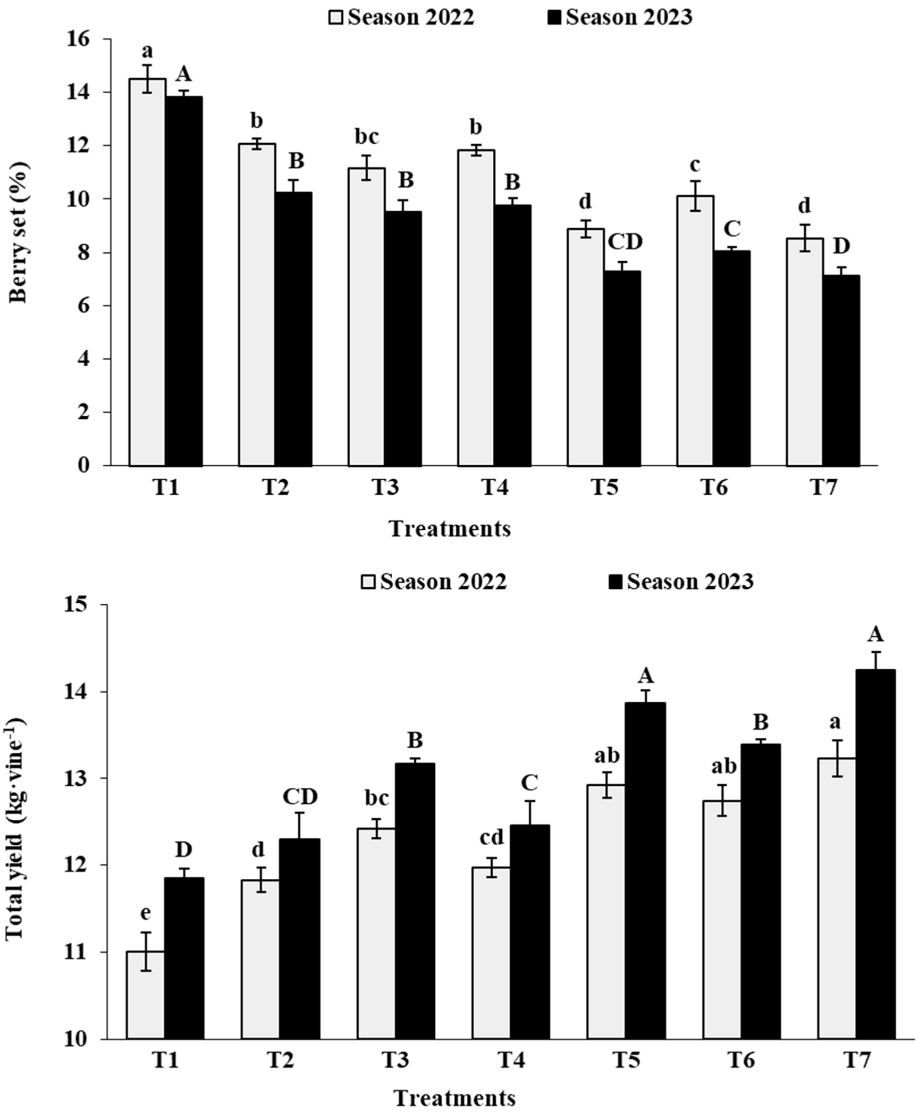


Figure 5. Effect of BLR alone or combined with foliar spray of LBU or CCA on berry set and total yield of 'Prime Seedless' grapevines during 2022 and 2023 seasons. T1 = control, T2 = four BLR at FB, T3 = LBU at 5 g·L^{-1}, T4 = CCA at 40 mg·L^{-1}, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4. Values are means of three replicates (n = 3) ± SD. Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's HSD test.

3.5. Decay Percentage

The percentages of fruit decay were the highest in control vines during both seasons, as indicated by the decay index of the clusters and the incidence of botrytis rot on berries (Figure 7). However, the difference in decay index was insignificant among the control, T2 and T3. Clusters collected from T7-treated vines had the lowest decay index and botrytis incidence compared to the control and all other treatments in both seasons, even though the difference in botrytis incidence between T6 and T7 was insignificant during the first season only.

3.6. Berry Physical Characteristics

The improvement in cluster weight (Figure 6) and hence total yield (Figure 5) in T7-treated vines could also be attributed to the highest values of berry weight and diameter, compared to other treatments and the control in both seasons (Figure 8). However, the difference in berry weight was insignificant between T5 and T7, as well as between the control and T2 in both seasons. Insignificant values were also recorded between the control and T2 in regards to berry diameter during both seasons. In terms of berry firmness, the control and T7 recorded the lowest and the highest values, compared to all other treatments during both seasons (Figure 8).

3.7. Berry Chemical Characteristics

The results indicated that all applied treatments positively affected berry taste, in terms of improved SSC and reduced TA compared to the control in both seasons (Figure 9). Accordingly, the SSC: TA ratio was also improved. The same effect was also noticed for total sugars, which was eventually reflected in SSC. However, no significant differences were noticed in SSC and total sugars among T4, T5, T6 and T7 in both seasons. The same treatments showed insignificant differences in TA, except for the difference between T5 and T7 during the first season which was significant.

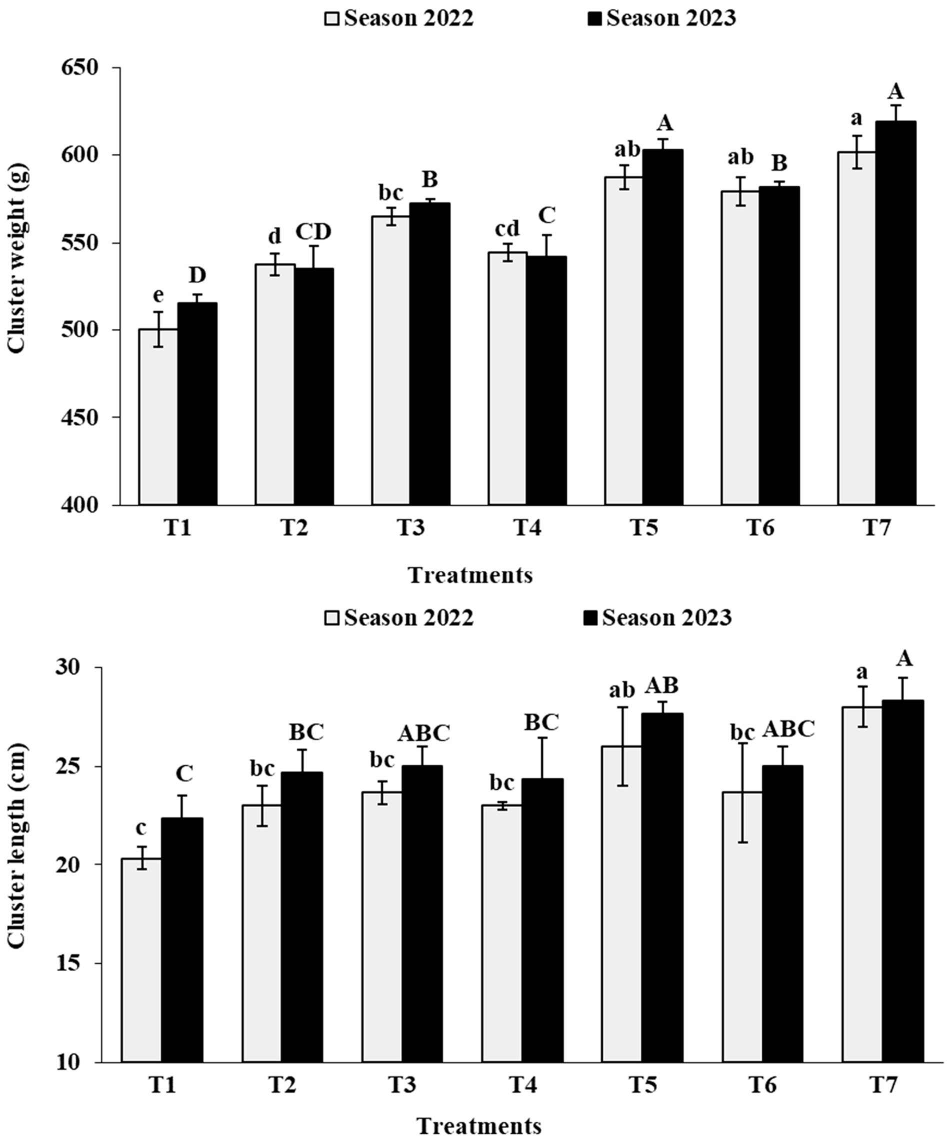


Figure 6. *Cont.*

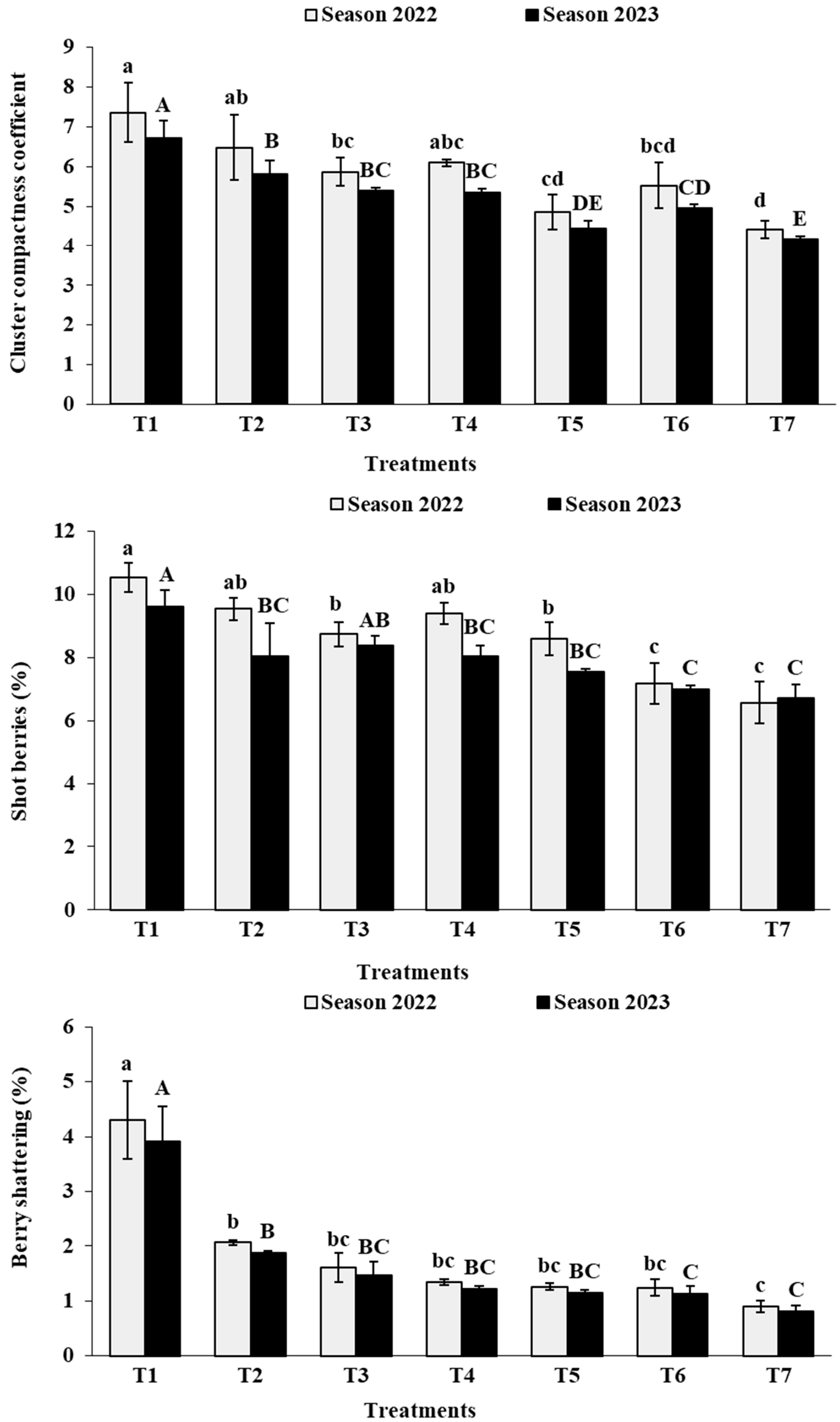


Figure 6. Effect of BLR alone or combined with foliar spray of LBU or CCA on cluster physical characteristics of 'Prime Seedless' grapes during 2022 and 2023 seasons. T1 = control, T2 = four BLR at FB, T3 = LBU at 5 g·L^{-1}, T4 = CCA at 40 mg·L^{-1}, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4. Values are means of three replicates (n = 3) ± SD. Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's HSD test.

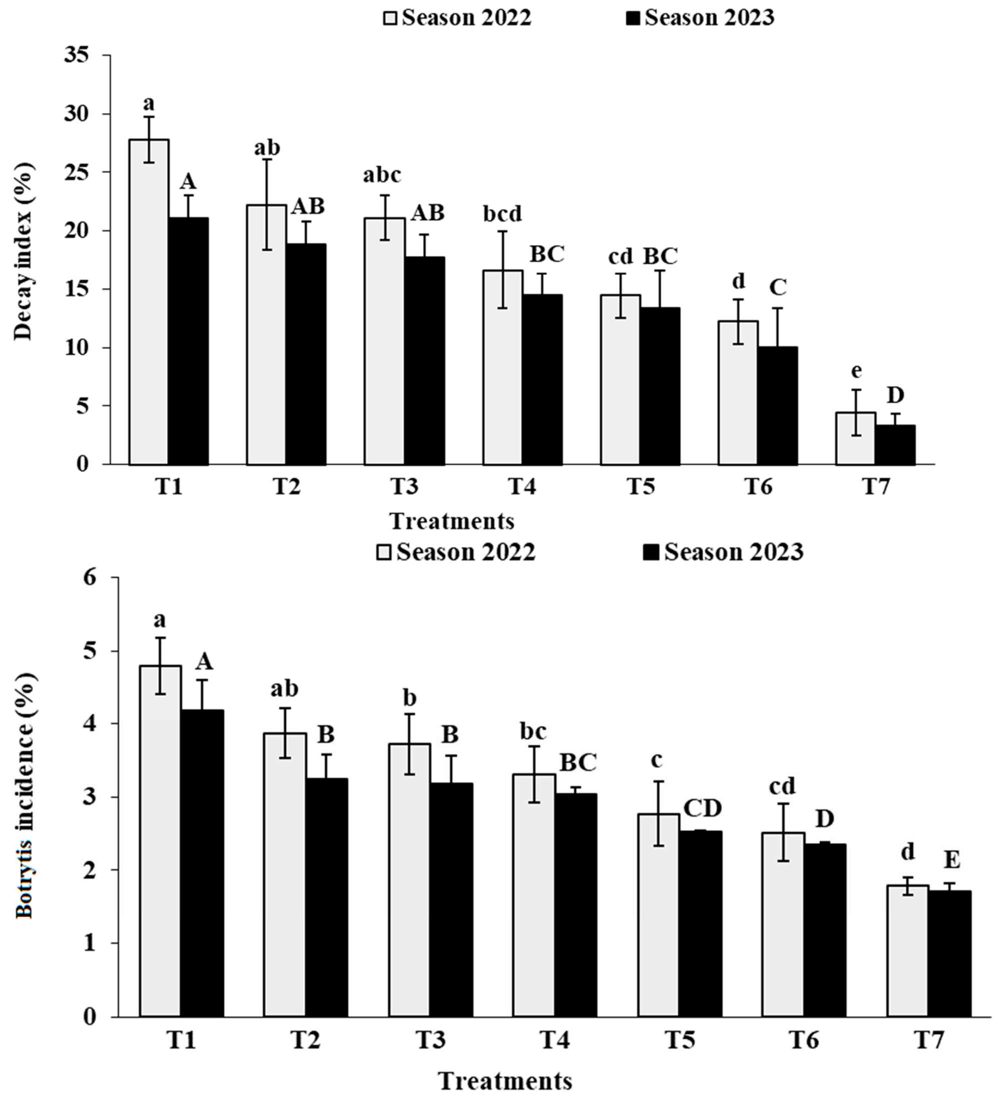


Figure 7. Effect of BLR alone or combined with foliar spray of LBU or CCA on decay index of clusters and botrytis incidence on berries of 'Prime Seedless' grapes during 2022 and 2023 seasons. T1 = control, T2 = four BLR at FB, T3 = LBU at 5 g·L^{-1}, T4 = CCA at 40 mg·L^{-1}, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4. Values are means of three replicates (n = 3) ± SD. Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's HSD test.

The analysis of berry peel indicated a significant and positive effect for all treatments over the control, in terms of the total carotenoid and phenol contents, as well as on the activity of phenylalanine ammonialyase and polyphenol oxidase during both seasons (Figure 10). In all cases, the most remarkable effects were noticed with the application of T7, although insignificant differences in total carotenoids and phenylalanine ammonialyase were recorded between T6 and T7 during the second season only.

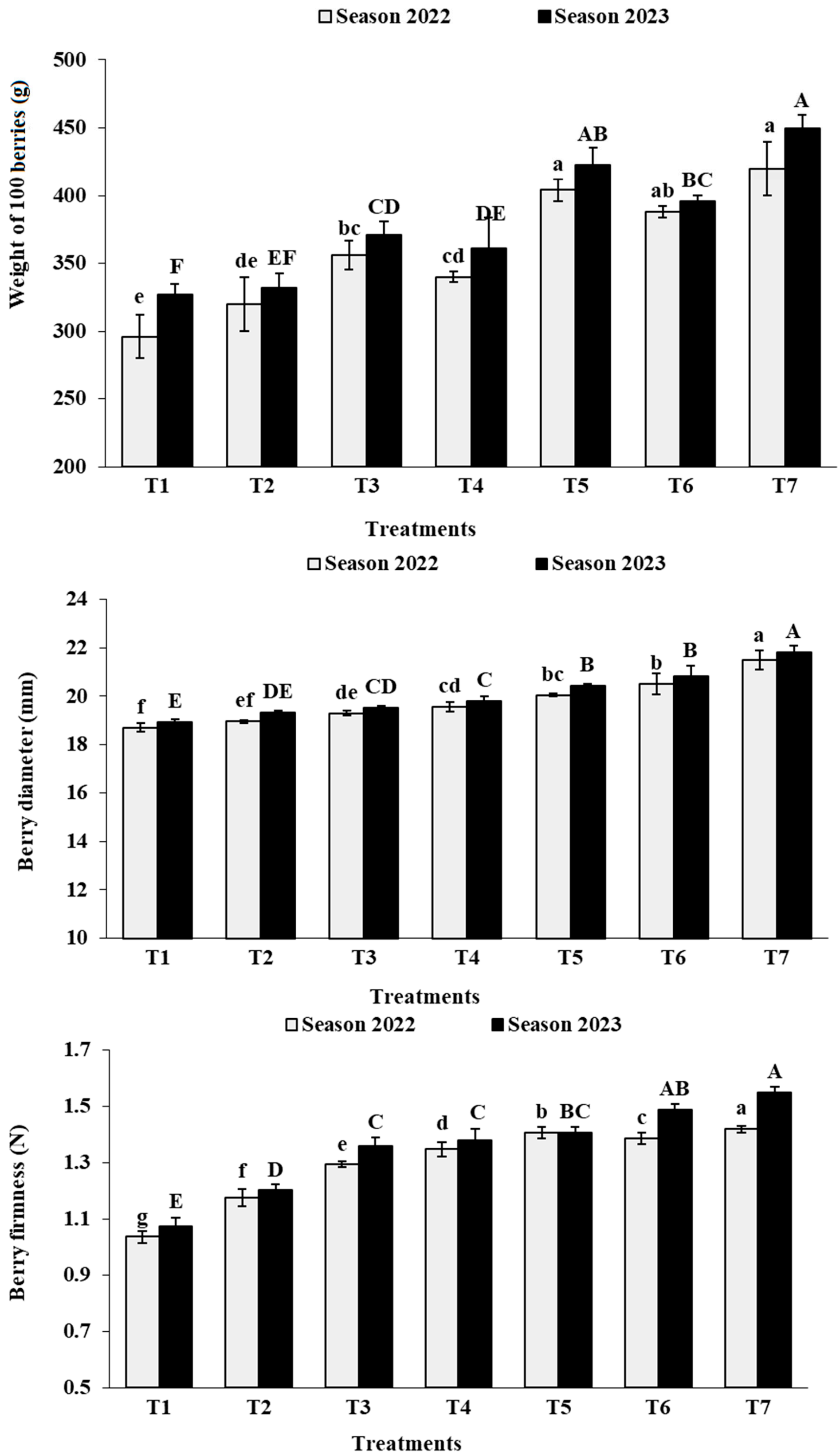


Figure 8. Effect of BLR alone or combined with foliar spray of LBU or CCA on berry physical characteristics of 'Prime Seedless' grapes during 2022 and 2023 seasons. T1 = control, T2 = four BLR at FB, T3 = LBU at 5 $g \cdot L^{-1}$, T4 = CCA at 40 $mg \cdot L^{-1}$, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4. Values are means of three replicates (n = 3) ± SD. Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's HSD test.

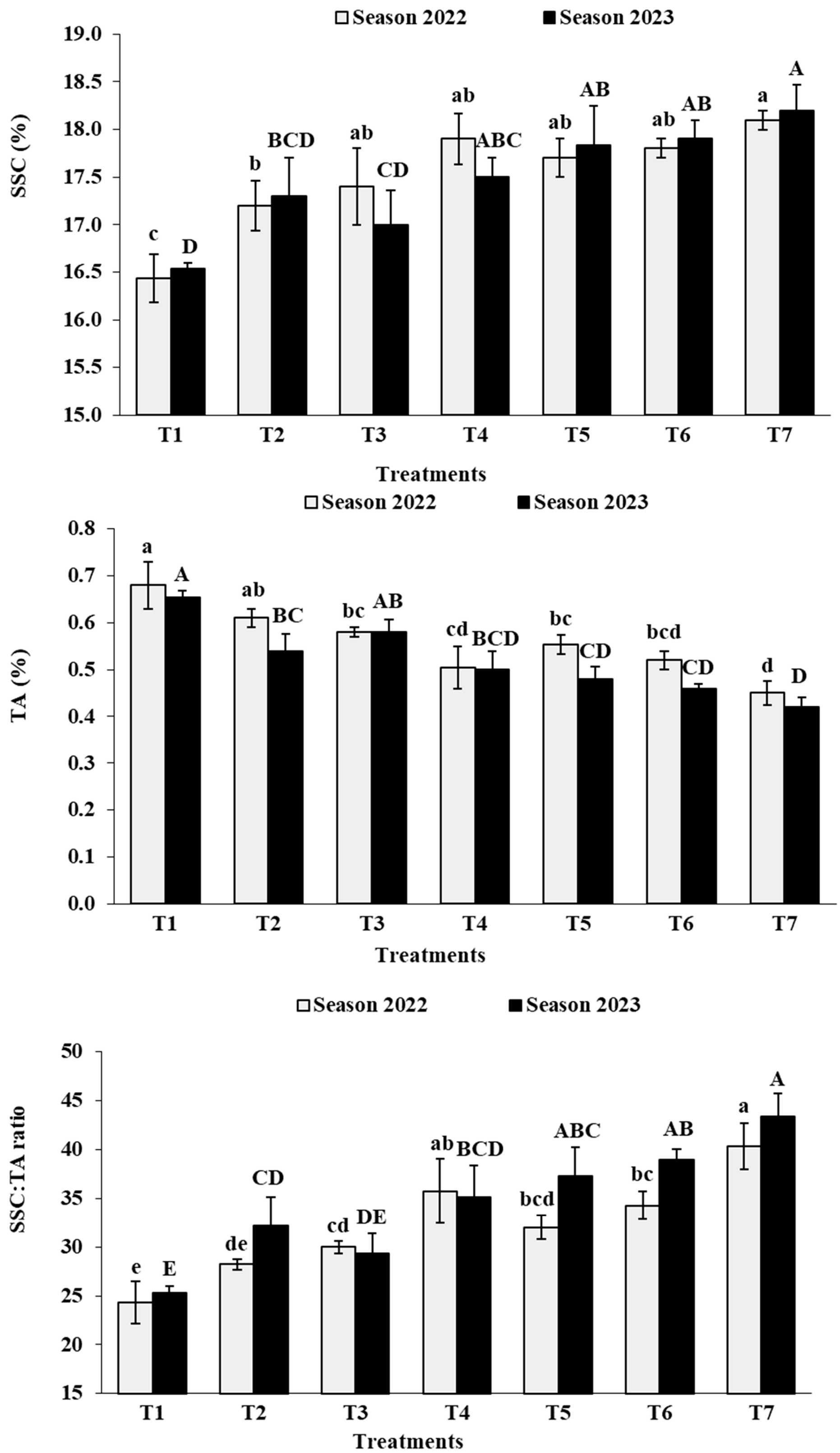


Figure 9. *Cont.*

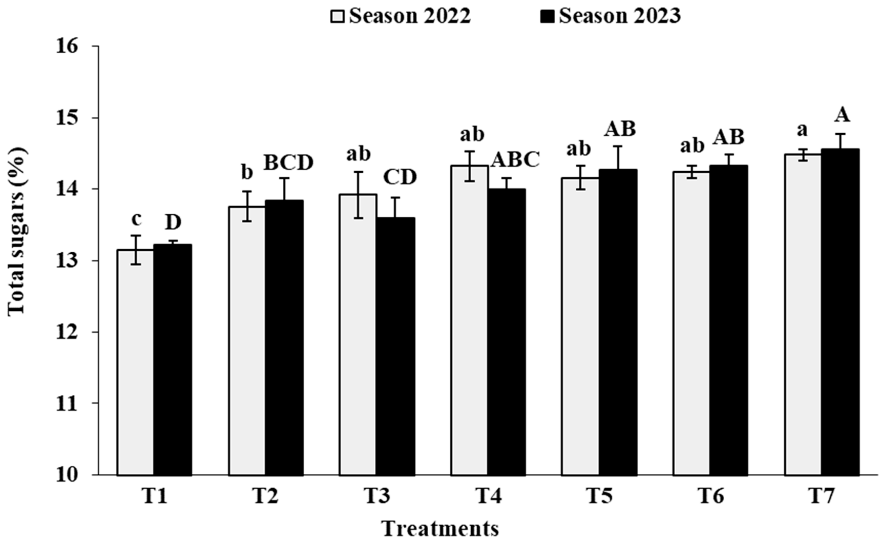


Figure 9. Effect of BLR alone or combined with foliar spray of LBU or CCA on berry chemical characteristics of 'Prime Seedless' grapes during 2022 and 2023 seasons. T1 = control, T2 = four BLR at FB, T3 = LBU at 5 g·L^{-1}, T4 = CCA at 40 mg·L^{-1}, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4. Values are means of three replicates (n = 3) ± SD. Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's HSD test.

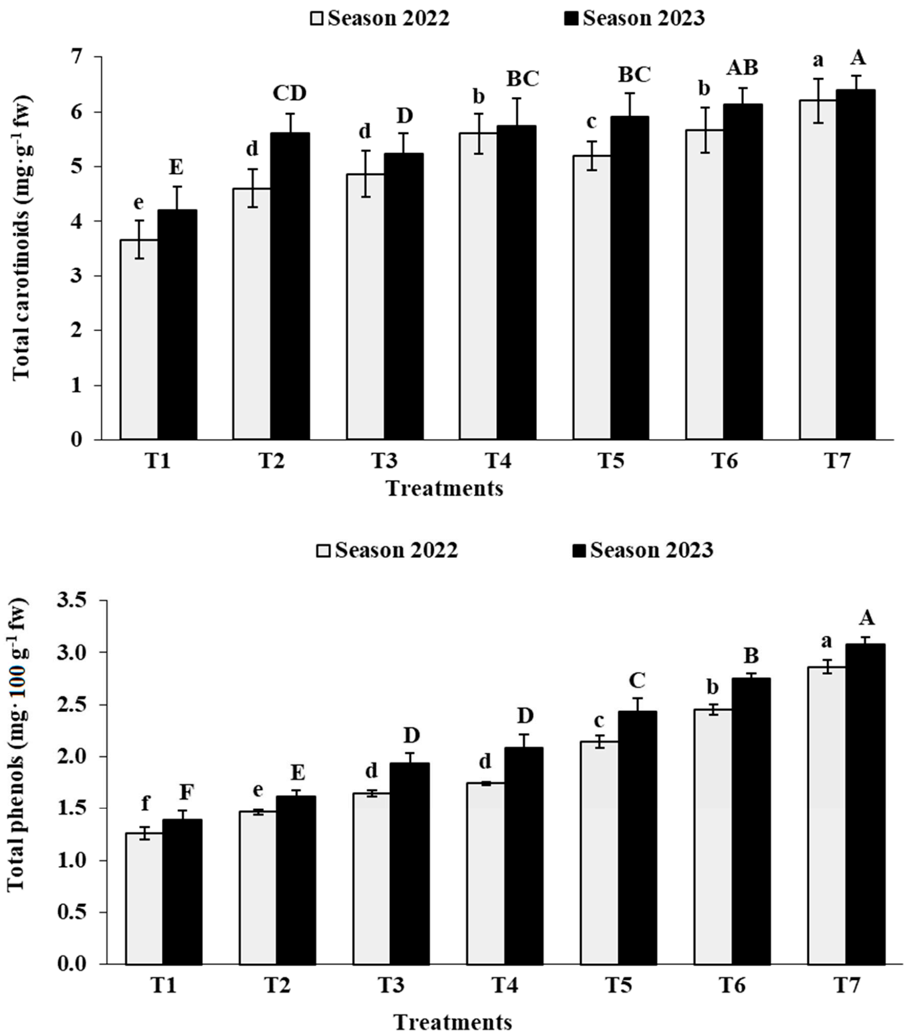


Figure 10. *Cont.*

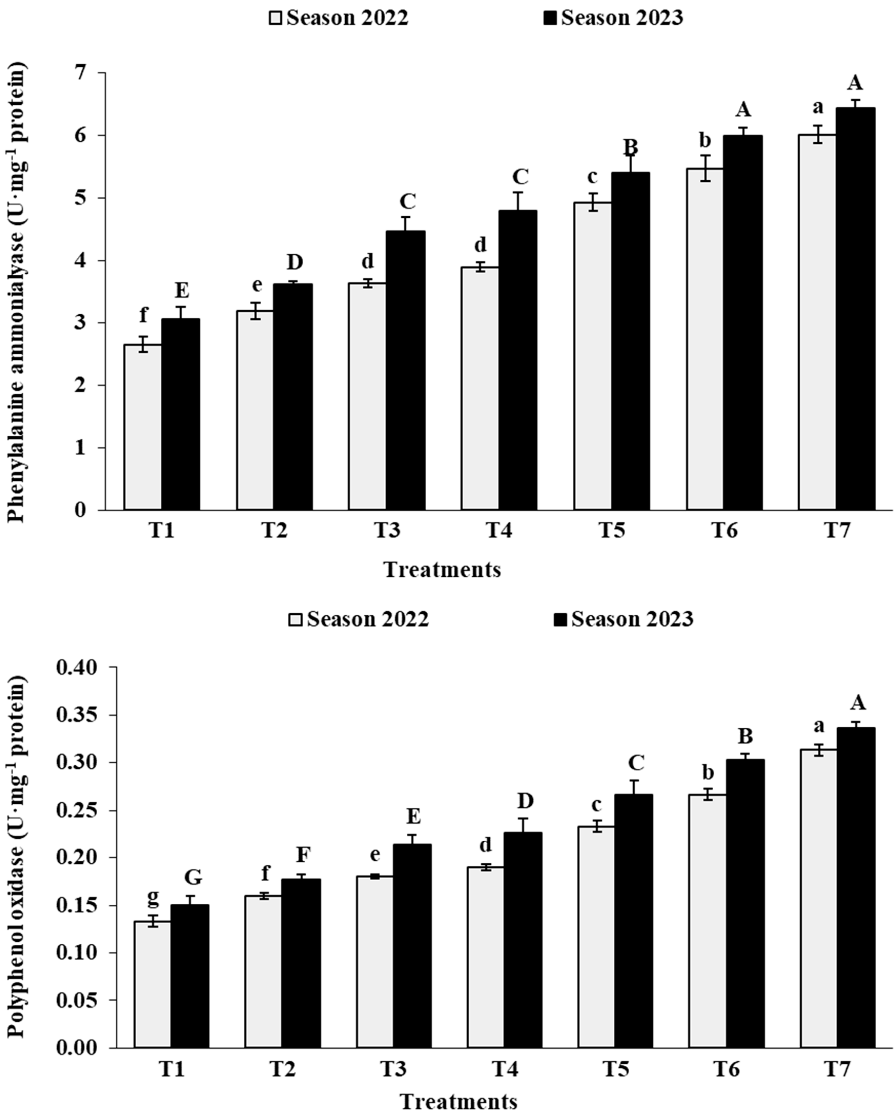


Figure 10. Effect of BLR alone or combined with foliar spray of LBU or CCA on berry peel analysis of 'Prime Seedless' grapes during 2022 and 2023 seasons. T1 = control, T2 = four BLR at FB, T3 = LBU at 5 g·L^{-1}, T4 = CCA at 40 mg·L^{-1}, T5 = T2 + T3, T6 = T2 + T4 and T7 = T2 + T3 + T4. Values are means of three replicates (n = 3) ± SD. Means with same lowercase or uppercase letters in 2022 or 2023 seasons, respectively, are not significantly different at $p \leq 0.05$ using Tukey's HSD test.

4. Discussion

The present findings indicated an effective role of the combined application of BLR, LBU and CCA (T7), over other treatments and the control, in the vegetative growth, physiological status, productivity and fruit quality of 'Prime Seedless' grapevines grown under the Mediterranean climate (Figures 1–10). The positive effect of BLR on the vegetative growth parameters (i.e., shoot length, leaf area) may be attributed to the stimulation of growth and initiation of summer lateral shoots [94,95]. These newly developed summer lateral shoots with younger leaves characterized by higher photosynthetic activity, plus leaf defoliation, improved root absorption rate and supplied the remaining and developing leaves with more water, nutrients and phytohormones, which were in turn reflected in the overall plant vegetative growth [20,94,96]. The results are consistent with previously reported findings that revealed that early mechanical leaf removal improved the vegetative growth and development in 'Pinot noir' grapes, compared to late/no defoliation [97]. Removing the first four basal leaves during bloom significantly increased the total leaf area per shoot of 'Riesling' grapes [8,17]. Leaf defoliation by the onset of bloom induced new growth and enhanced leaf area [17–20]. Early defoliation at the pre-bloom stage resulted in the largest leaf area per vine in 'Crimson seedless' grapes [20]. The beneficial effect of BLR on total yield and fruit characteristics could be due to the increased levels of photosynthesis and respiration in the remaining leaves to mitigate the impact of leaf removal [23] and adjust the source/sink ratio by increasing the water and nutrient supplies from roots toward the developing shoots and leaves, instead of the mature ones [95,96]. In addition, BLR improves the vine's microclimate by enhancing the light intensity and air circulation around the canopy, which in turn enhance the photosynthetic capacity and increase the carbohydrate accumulation in the remaining shoots and leaves. This actually results in an increase in the translocation of carbohydrates to clusters and is reflected in total yield and berry quality [98]. When compared to shaded clusters, sunlight-exposed clusters had greater sugars and phenolic compounds, as well as reduced acidity [94]. The amount of solar radiation around the canopy had a significant influence on carotenoid concentrations in clusters [99]. Leaf defoliation in the cluster zone changed the microenvironment (i.e., solar radiation, temperature, air circulation) around the clusters and influenced berry physiochemical characteristics [19]. Carotenoid concentrations in berries were mainly affected by exposure to sunlight and the ripening stage [100]. Defoliation before the onset of bloom improved cluster and berry weight and reduced decay percentage [12,101]. It also reduced berry TA but increased SSC and total sugar levels in 'Lambrusco' and 'Barbera' grapes [102] due to an enhanced canopy microenvironment [12]. Sugars constitute the major component of SSC [12,103]. Leaf defoliation at the flowering stage was more effective compared to that at the berry set stage, in terms of improving the SSC: TA ratio [95]. Defoliation at pre-bloom [104] or right by bloom onset [18] effectively increased SSC, compared to that conducted at FB or FS stages, due to improved shoot growth and carbohydrate accumulation [18]. Removing four basal leaves by bloom onset resulted in the highest SSC in 'Crimson seedless' grapes [20].

The positive action of LBU to enhance vegetative growth parameters and leaf chlorophyll content may be due to the increase in leaf N levels [105]. Nitrogen has many functions such as encouraging cell division and protein synthesis, and it is a main component of protoplasm structure, enzymes, organic compounds, nucleic acids (e.g., DNA and RNA), nucleoproteins (e.g., chromosomes, ribosomes), amino acids (e.g., proline) and chlorophyll [32,106]. Therefore, it eventually improved overall plant growth and development [59]. Similar results were reported on 'Superior' [30] and 'Red Globe' grapevines [107]. The foliar spray of LBU at FB efficiently reduced the berry set (Figure 5) and cluster compactness coefficient (Figure 6), which could be attributed to improved shoot growth that altered the source/sink ratio, enhancing the uptake of water and nutrients toward newly grown shoots, instead of the reproductive structures [6]. The reduced fruit set in response to LBU could also be attributed to the inhibition effect on ovary fertilization due to phytotoxicity in the peduncle region of the berries [34]. In addition, improved chlorophyll content and photosynthesis capacity increased carbohydrate assimilates and led to improved cluster physical characteristics and berry physiochemical characteristics [3,18,31,32,106–108]. Spraying urea at 5000 mg·L^{-1} reduced cluster compactness and berry TA while improving the yield, cluster physical characteristics and berry physiochemical characteristics of 'Red Globe' grapes [107]. Spraying urea at 1.5% when cluster length reached about 10–12 cm positively affected the total yield and improved the fruit quality of 'Superior Seedless' grapes [31].

Cyanocobalamin also effectively improved the vegetative growth of the vines (Figure 1). Similar results were also reported on 'Le-Conte Pear' [51], which could be related to its physiological role in improving the enzyme activities responsible for carbohydrate synthesis [101], increasing carbohydrate accumulation in the shoots and subsequently enhancing shoot length, leaf area [102], total yield and fruit quality [109]. It was reported that CCA increases carbohydrate accumulation via the induction of the Krebs cycle and the pentose phosphate pathway [46]. In addition, CCA was found to play a vital role in the regulation of DNA synthesis during plant cell division [39,40] and enhance the photosynthesis capacity [42,43]. Cobalamin has been suggested to be an intercellular antioxidant that improves plant resistance to environmental stresses [40,44,45], and hence this could be the reason for improved proline content in leaves (Figure 2). Proline is an amino acid that plays a vital role in plants exposed to various biotic and abiotic stress conditions [110], and it was found to improve plant growth and yield characteristics [111] and maintain the plant water and nutrient status by promoting the uptake of water, N, P, K and Ca [112]. These findings could justify the effect of CCA on plant nutrient status (Figures 3 and 4), yield characteristics (Figures 5, 6 and 8) and decay percentage (Figure 7). When compared to the sole use of BLR (T2), LBU (T3), CCA (T4) and the combinations (T5–T7) effectively improved the nutritional status of the vines (Figures 3 and 4), which was helpful in reducing the percentage of shot berries (a physiological disorder) via enhancing nutrient accumulation (particularly K) in the cluster's stalk [113].

The present results could also infer that vines might be slightly stressed in response to BLR, which found to impart a kind of physiological stress by suddenly restricting the availability of photosynthates needed for growth and production [114], and therefore vines might exhibit some morphological mechanisms (e.g., root development) and physiological mechanisms (e.g., chlorophyll synthesis, redistribution of carbohydrates and nutrients) [115]. Such physiological mechanisms were enhanced and reflected in improved vine growth and productivity, in response to the combined application of BLR with LBU or/and CCA, as indicated by treatments T5, T6 and T7, with most effects related to T7 in all studied parameters. The results showed that BLR at FB either alone or combined with the foliar spray of LBU or/and CCA significantly reduced the berry set (Figure 5), cluster compactness coefficient (Figure 6) and decay percentage (Figure 7). The reduced berry set led to less compacted clusters, and hence the percentage of infected clusters was also reduced. A lack of carbohydrate assimilates, due to the induced physiological stress in response to BLR [114], resulted in a low C:N ratio and hence reduced berry set that subsequently led to loose clusters [7,12]. The carbohydrate supply at the bloom stage is thought to be a major factor in the fruit set [116]. Basal defoliation removes the most photosynthetic-active and enlarged leaf area, which certainly affects the total carbohydrate supply [117]. The more severe the post-flowering defoliation was, the more loose the clusters were [118]. Basal defoliation during flowering reduces cluster compactness without affecting the next-season bud fertility [94] and may alter the cluster shape [14,20,119]. The lower the percentage of the berry set, the higher the weight of the berry was (Figure 8) and hence the cluster weight (Figure 6) which was eventually reflected in the total yield (Figure 5) and overall berry chemical characteristics (Figures 9 and 10).

Reduced carbohydrate supply during flowering and the fruit set enhanced the percentage of flower abscission [18,120]. Carbohydrate stress can induce longan fruit abscission, which may be mediated by the production of ROS such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl (OH) and singlet oxygen ($^1O_2$) [121,122]. Previous findings showed that H_2O_2 plays a role in the cell-wall degradation process via improving the activity of cell-wall-degrading enzymes (e.g., cellulase, pecticlyase, polygalactosidase) and ethylene production [123]. This might be the reason for the high shattering percentage in BLR-treated vines (T2) that was significantly lowered with the combined application of BLR, LBU and CCA (T7) in both seasons (Figure 6). Figure 10 revealed the positive role of all treatments on PAL and PPO activities. Phenylalanine ammonialyase plays a key role in the metabolism of secondary phenylpropanoids that have a vital role in plant development. Phenylpropanoids are precursors to a wide range of phenolic compounds such as phenols, flavonoids, isoflavonoids, phytohormones, phytoalexins and lignins, which enable plant defense mechanisms against various abiotic and biotic stresses [124]. This could explain the presence of a low decay percentage in all treated vines compared to the control, with the most conspicuous effect related to T7 (Figure 7). The increase in PAL has been shown to be responsible for the biosynthesis of phenols [125], which are one of the most important groups of secondary metabolites that act as antioxidants to protect cell structure and improve plant tolerance to stressful conditions [126]. As a complex phenolic polymer, lignin acts as an essential component of the cell wall that enhances cell-wall rigidity and hydrophobic properties, promotes nutrient transport through the vascular bundle and protects the cell against pathogen attacks [127]. This could also be a reason for the reduced decay percentage (Figure 7) in response to improved berry firmness (Figure 8). Polyphenol oxidase is a Cu-containing phenolase that also has an important role in plant stress resistance and physiological metabolism. It catalyzes the oxidation of phenolic compounds into highly reactive quinones that enhance plant stress resistance [128,129]. It is mostly found in the chloroplasts of photosynthetic cells and is relatively more abundant in young tissues [128]. So, this might be the reason for improved PPO levels after BLR due to improved vegetative growth and the formation of young tissues (Figure 1). Phenolic compounds have also been reported to be the major influence on fruit sensory attributes (e.g., color, flavor, taste) [130].

5. Conclusions

To meet the growers' concerns with the current quality issues (i.e., compacted clusters, shot berries and decay) that reduce the marketability of 'Prime Seedless' grapes grown under the Mediterranean climate of Egypt, the present research suggested a combined application of BLR, LBU and CCA to overcome the high percentage of the fruit set, and results in loose and less infected clusters with very few shot berries. Such improvements had an added value on this cultivar that increased its marketability at local and international levels. Future research may incorporate molecular studies to determine how leaf defoliation may result in a carbohydrate-stressed grapevine and means to ameliorate such physiological stress at the molecular level.

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