



Article

Morphological and Physio-Biochemical Responses of Watermelon Grafted onto Rootstocks of Wild Watermelon [*Citrullus colocynthis* (L.) Schrad] and Commercial Interspecific Cucurbita Hybrid to Drought Stress

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Citation: Bikdeloo, M.; Colla, G.; Rouphael, Y.; Hassandokht, M.R.; Soltani, F.; Salehi, R.; Kumar, P.; Cardarelli, M. Morphological and Physio-Biochemical Responses of Watermelon Grafted onto Rootstocks of Wild Watermelon [*Citrullus colocynthis* (L.) Schrad] and Commercial Interspecific Cucurbita Hybrid to Drought Stress. *Horticulturae* **2021**, *7*, 359. <https://doi.org/10.3390/horticulturae7100359>

Academic Editor: Francisco M. del Amor Saavedra

Received: 20 August 2021
Accepted: 22 September 2021
Published: 3 October 2021

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Abstract: This study aimed to assess the morphological and physio-biochemical responses of a commercial watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) cv. 'Crimson Sweet' grafted onto a drought-tolerant rootstock of wild watermelon (bitter apple, *Citrullus colocynthis* (L.) Schrad, 'Esfahan') in comparison with an ungrafted 'Crimson Sweet' watermelon or one grafted onto a commercial interspecific *Cucurbita* hybrid (*Cucurbita maxima* Duch. × *Cucurbita moschata* Duch.) rootstock ('Shintoza') under water stress. The experiment was conducted in pots under a controlled environment in a greenhouse, and water stress was imposed by maintaining moisture level in pots at 100% (well water (WW)) or 50% (water deficit (WD)) of container capacity (CC). WD significantly decreased most of the morphological traits in ungrafted and grafted plants, while the decrease in growth traits was lower in grafted plants than ungrafted plants. The response of grafted plants onto wild watermelon rootstock ('Esfahan') for most of the affected parameters (shoot fresh and dry weight, vine length and internodal length) was, however, comparable to those grafted onto commercial *Cucurbita* hybrid rootstock ('Shintoza'). Plants grafted onto bitter apple (wild watermelon) exhibited a relatively lower decrease in growth and biomass, besides showing higher antioxidant activity (e.g., guaiacol peroxidase) concomitant with the lower accumulation of malondialdehyde and electrolyte leakage in the leaf tissues in comparison with ungrafted plants. The overall growth performance, as well as those under water stress conditions in commercial rootstock-grafted watermelon, was related to its better plant water status (e.g., high relative water content) which was likely ascertained by its greater root efficiency. This suggests that watermelons grafted onto bitter apple rootstock and *Cucurbita* hybrid rootstock were constitutively more resistant to drought, with higher efficiency in mitigating oxidative stress than ungrafted treatment. The above findings demonstrated that bitter apple, a well-adapted desert species, can be used as an alternative rootstock to commercial rootstocks (e.g., 'Shintoza') for watermelon grafting under water stress conditions. In addition, bitter apple rootstock can be involved in rootstock breeding programs to improve drought tolerance in watermelon.

Keywords: *Citrullus colocynthis*; rootstocks; grafting; drought tolerance; MDA; antioxidant enzymes

1. Introduction

Grafting vegetables, including cucurbits (e.g., cucumber, melon, and watermelon), has become a common practice in many countries in recent years. Among the cucurbits, watermelon holds a prime position in the adoption of grafting; some countries are currently using from 95 to 100% grafted seeding in watermelon cultivation [1]. The primary motive of watermelon grafting has been to overcome the soilborne disease problem, especially Fusarium wilt; with this being the reason for the first instance of grafting application in vegetables (i.e., watermelon), which emerged about a century ago in Japan and Korea [2]. Grafting application in other fruiting vegetables, however, succeeded later, with the realization of its potential to successfully tackle numerous other production issues. The expansion of grafting application across the globe in recent decades is probably due to advancements in grafting research to deal with various aspects including grafting techniques, rootstock development, graft union formation and scion–rootstock interaction, and develop an understanding of issues ranging from morphological to physio-biochemical to molecular mechanisms in the leading grafted plants' tolerance to various stresses. The literature indicates the increasing instances of grafting applications successfully controlling certain biotic [3–5] and abiotic stresses [6–12], as well as increasing the nutrient- and water-use efficiency of vegetables [13–15].

Nevertheless, limited research has been carried out on the effects of grafting on increasing resistance to water stress. In fact, drought or water stress is the most widespread problem across the globe, with implications for sustainable food production, including vegetable crops [15,16]. Its prevalence and intensity are expected to increase, as predicted by the climate change models; this will lead to a reduction in agricultural production in some areas [17,18]. The severity of drought is likely to increase due to increased evapotranspiration rather than precipitation, as a consequence of increasing instances of warm weather [19]. It is widely documented that drought depresses crop growth and yield by causing several alterations in the subjected plants, which include disturbed plant water balance, and a disruption of photosynthesis, and water and nutrient uptake [20–22]. At the cellular level, water deficit causes oxidative stress as a result of the generated reactive oxygen species, which affect membrane stability and lipid peroxidation. Additionally, the inactivation of metabolic enzymes and the severity may lead to damage to the nucleic acid and, finally, cell death [21,23]. Some coping mechanisms for drought stress have been found to be dependent on the plants' (variety or species) ability to withstand drought, namely, better root architecture, maintained plant water status, photosynthetic efficiency, and a higher antioxidant system in tolerant genotypes [15,21].

Retaining the high yield potential of vegetable cultivars under water stress is the main challenge in water-scarce areas. Watermelon is a widely cultivated all over the world, mainly in a semi-arid to arid climate and, like many other vegetables, it is sensitive to drought since it has relatively high water requirements [24,25]. The literature indicates that, for optimum production of watermelon, seasonal water requirements ranged between 520 mm and 660 mm, depending on the climate of the area and length of the growing period [25–27]. Grafting, being an easily applicable cultural technique in fruit vegetables and efficient in quickly combining the resistant traits of rootstock genotypes with susceptible scion cultivars, is the preferred tool over classical and modern breeding techniques [15,28]. This became possible with the search for suitable rootstock genotypes, with targeted resistance and a good compatibility reaction with scion. Rootstock breeders evaluate diversity among the germplasm for the release of new rootstock cultivars with the highest water stress tolerance [29].

Drought-tolerant accessions might exist in the gene pools of various plant species. *Citrullus colocynthis* (L.) Schrader is a highly drought-tolerant species with a very extensive and deep root system, which is widely distributed in the Sahara-Arabian region in Africa and the Mediterranean region [30]. It grows in the sandy lands of the south-west, south-east and central parts of Iran [31]. Its occurrence is also widespread in the Indian Thar desert, where it naturally grows in the barren lands on arid dunes and plains [32]. This wild

species is closely related to domesticated watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) and commonly known as bitter apple. The genotypes of wild species of watermelon may be used as a source of resistance to desired characters. There are numerous studies which reveal the drought-tolerance characteristics of wild watermelon, the bitter apple. For instance, dynamic gene expression analysis performed in *C. colocynthis* root tissues by using a cDNA-amplified fragment length polymorphism (cDNA AFLP) Si et al. [33] revealed that this species has a great potential to improve the drought tolerance of other cucurbit species.

The most common rootstocks used for grafting watermelon are cultivars of interspecific hybrid squash (*Cucurbita maxima* Duch. × *Cucurbita moschata* Duch.) and cultivars of bottle gourd (*Lagenaria siceraria* (Molina) Standl.) [3]. One of the most famous interspecific hybrid squash rootstocks is ‘Shintoza’, which has a very vigorous plant habit and root system. However, there is also need to harness the potential of existing germplasms with copious drought tolerance as an alternative rootstock to increase tolerance to drought in commercial watermelon. The potential of a drought-tolerant bitter apple accession ‘Esfahan’, identified in our previous research [34] as a rootstock, was compared with a commercial rootstock, ‘Shintoza’, regarding grafting watermelon for morphological and physio-biochemical traits under water-stress conditions in a greenhouse.

2. Materials and Methods

2.1. Plant Material and Growing Environment

The experiment was carried out in a greenhouse situated at the Experimental Farm of Tuscia University, Central Italy (42°25' N; 12°08' E) from 10 April to 30 May 2015. The greenhouse was maintained at daily temperatures between 18 and 33 °C, and relative humidity was maintained in the range from 55 to 85%. Watermelon cultivar ‘Crimson Sweet’ (Asgrow Vegetable Seeds, Creve Coeur, MO, USA) was used as the scion. This scion cultivar was grafted onto rootstocks, ‘Shintoza’ (*Cucurbita maxima* Duch. × *Cucurbita moschata* Duch., Syngenta, Basel, Switzerland), a commercial and widely used hybrid pumpkin rootstock and ‘Esfahan’ (*Citrullus colocynthis* (L.) Schrad), an Iranian wild accession of bitter apple. Seeds of ‘Crimson Sweet’ (scion) and ‘Esfahan’ were sowed five days earlier than seeds of the ‘Shintoza’ to ensure uniformity in the hypocotyls diameter of both the scion and rootstocks. The ‘splice grafting’ method was used to graft the scion ‘Crimson Sweet’ onto the ‘Esfahan’ and ‘Shintoza’ rootstocks. For the graft-healing process, immediately after grafting, seedlings were placed in sealed transparent boxes and shifted to a growth chamber maintained at 25 °C/20 °C (day/night) temperature. The relative humidity inside boxes was maintained close to 100% for 4 days and then gradually reduced to about 85% by regulating the lid placement. After 7 days of grafting, seedlings were removed from boxes and kept in the open environment of growth chamber for 2 days, then shifted to the greenhouse environment for hardening.

2.2. Experimental Layout and Stress Treatment

Grafted and ungrafted seedlings were transferred into plastic pots (d 30 cm, h 30 cm) at the two–three true leaf stage. The potting mixture was composed of quartziferous sand, field soil and peat in 1:1:1 ratio. The experiment was designed as a factorial combination comprising of three grafting combinations: ungrafted watermelon, ‘Crimson Sweet’ grafted onto commercial rootstock ‘Shintoza’ and onto wild watermelon accession ‘Esfahan’, and two irrigation rates based on container capacity (CC), normal irrigation (100 % CC) and water stress conditions (50% CC). All treatments were given uniform optimal irrigation (100% CC) for 2 weeks after transplantation to promote root system establishment without water stress; during the first 2 weeks after planting a nutrient solution, drainage of 30% was assured to avoid buildup of salinity in the substrate. Afterwards, water stress treatment was expressed as the weight loss of the last weighing of the individual pots every day for 4 weeks. When the weight of pot was less than the pot weight in 50% CC, we irrigated the pot. Control plants were grown under normal conditions for the same period of time

and were irrigated to keep the container capacity at 100%. The pots were fertilized using modified Hoagland and Arnon formulation as supplementary nutrition during the first two weeks before starting the experiment [35].

2.3. Record of Morphological Parameters

The experiment was terminated 40 days after transplanting (30 May). At that day, the measurement of plant height and internode lengths was made on three randomly selected plants from each treatment in each replication. The leaf area was determined using an electronic area meter (Delta-T Devices Ltd., Cambridge, UK). Shoot fresh mass was collected for each treatment in replicates separately and placed in an oven to dry until constant weight was achieved.

2.4. SPAD Index and Chlorophyll Fluorescence

The SPAD and chlorophyll fluorescence (F_v/F_m) measurements were carried out 39 d after transplanting. A portable chlorophyll meter (SPAD-502, Minolta Corporation, Ltd., Osaka, Japan) was used to measure the relative leaf chlorophyll concentration as a rational unit. The photosystem II efficiency, as the variable to maximum chlorophyll fluorescence ratio (F_v/F_m) of leaves, was estimated by chlorophyll fluorometer Handy PEA (Hansatech Instruments Ltd., Norfolk, UK). The measurement was made on the adaxial surface of six random leaves (third–fourth leaflets from the top) per plot after at least 20 min dark adaptation. The maximum quantum yield of open PSII (F_v/F_m) was calculated as $(F_m - F_0)/F_m$, as described by Maxwell and Johnson [36]. Six measurements per experimental unit were performed in the fully expanded leaves from the top for both SPAD and chlorophyll fluorescence.

2.5. Leaf Relative Water Content

Relative water content (RWC) of leaves was determined by the method described by Smart and Bingham [37], which was calculated as $RWC = 100 \times [FW - DM]/[TW - DM]$. FW and DM denote fresh weight (g) and dry weight (g). Turgid weight (TW) was calculated after fully hydrating fresh leaves in darkness at 4 °C for 24 h. Results were expressed as percentages.

2.6. Biochemical Analyses

On the last day of experiment, the plant samples were collected in liquid nitrogen and stored in −80 °C for further laboratory analysis of leaf pigments, antioxidant enzymes and lipid peroxidation (MDA). The leaf pigments were extracted by homogenization of 0.5 g fresh leaf tissues in 80% acetone, adding a small amount of $MgCO_3$. Then, this was centrifuged for two cycle each of 10 min at $4800 \times g$. The absorbance of the supernatant was taken at 470, 647, and 664 nm by an ultraviolet-visible spectrophotometer (Perkin Elmer Inc., Norwalk, CT, USA). The chlorophyll and carotenoid contents were calculated as per the Lichtenhaler and Wellburn [38], and the content was expressed on a fresh weight basis ($mg\ g^{-1}$).

Antioxidant enzymes, catalase (CAT) and guaiacol peroxidase (GPX) activities were determined by using frozen samples, according to Kumar et al. [10]. In brief, enzyme extractions were performed with two volumes of an ice-cold extraction buffer (0.05 M potassium phosphate buffer, K-PB, pH 7.0) containing 0.1% (*w/v*) ascorbic acid, 1% (*w/v*) polyvinylpyrrolidone, 1 mM Na_2-EDTA , and 0.1% (*v/v*) Triton X-100. The sample mixture was centrifuged ($15,000 \times g$ at 4 °C for 30 min); then, the supernatant was determined for the enzyme activity and protein content using a spectrophotometer (Perkin Elmer, Norwalk, CT, USA). For catalase (CAT) activity, the assay mixture (1 mL) contained 0.1 mL of 125 mM H_2O_2 and 20 μL of the crude extract in 0.05 M K-PB (pH 7.0). Activity of CAT was evaluated following the decomposition of H_2O_2 (240 nm for 1 min) and calculated using the extinction coefficient ($0.036\ mM^{-1}\ cm^{-1}$). For guaiacol peroxidase activity (GPX), the assay mixture (1 mL) contained 0.1 mL of 90 mM guaiacol, 0.1 mL of 125 mM H_2O_2 ,

and 50 μL of the crude extract in 0.05 M K-PB (pH 7.0). GPX activity was evaluated following the guaiacol oxidation (470 nm for 40 s) and was calculated using the extinction coefficient ($26.6 \text{ mM}^{-1} \text{ cm}^{-1}$). The specific enzyme activity for both the enzymes was expressed as $\text{mmol mg}^{-1} \text{ protein min}^{-1}$.

The drought-induced oxidative damage (membrane lipid peroxidation) was estimated by measuring the malondialdehyde (MDA) concentrations in frozen leaf samples, as described by Kumar et al. [10]. In brief, leaf tissues were homogenized in 0.1% (*w/v*) trichloroacetic acid (TCA) solution in 1:3 ratio. After centrifugation ($12,000 \times g$ at 4°C for 15 min), an aliquot of the supernatant was added to 0.5% thiobarbituric acid (TBA) made in 20% TCA and heated (95°C for 30 min). After rapid cooling on ice, the mixture was centrifuged ($10,000 \times g$ for 10 min). The MDA concentration was calculated from the difference between the measured absorbance values at 532 and 600 nm. Leaf electrolyte ion leakage (EL) was determined on ten randomly chosen mature leaves per treatment, taken and cut into 1 cm segments as described by Kumar et al. [39]. Leaf samples were incubated (in vial, at 25°C) for 24 h). The electrical conductivity of bathing solution (EC_1) was read after incubation. Then, the same samples were autoclaved (120°C for 20 min) and a second reading of the EC (EC_2) was made after cooling the solution to room temperature. The EL was calculated as EC_1/EC_2 and expressed as a percentage.

2.7. Statistical Analysis

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). Duncan's multiple range test was performed at $p = 0.05$ on each of the significant variables measured.

3. Results

3.1. Morphological Parameters

Drought stress (50% CC) significantly decreased all morphological parameters (i.e., shoot fresh and dry weight, leaf area, vine length and internode length), except the number of branches, of grafted and ungrafted watermelon plants (Table 1). The drought-induced decrease in growth parameters was more conspicuous in ungrafted plants ('Crimson Sweet') as compared to grafted plants on both the rootstocks ('Esfahan' and 'Shintoza').

Grafting positively influenced watermelon plant growth, especially shoot fresh and dry weight, vine- and internode-length, which were significantly affected by grafting (Table 1). Compared to well-watered conditions (WW, 100% CC), the decrease in these growth parameters under drought stress (50% CC) was lower in grafted than ungrafted watermelon plants. The lowest decrease in shoot fresh or dry weight, and vine and internode length was recorded in graft combinations involving bitter apple rootstock 'Esfahan' (27.6%, 26.9%, 28.8%, 24.2%, respectively), followed by those involving commercial *Cucurbita* rootstock 'Shintoza' (38.6%, 39.0%, 18.4%, 34.2%, respectively), while the highest decrease was noted in ungrafted plants (41.5%, 39.0%, 39.6%, 45.3%, respectively) under drought stress in comparison to their respective well-watered conditions (Table 1).

Drought stress and graft combination significantly affected the plant growth parameters independently of each other, but no interaction effect of drought and grafting treatments was observed for any growth parameter (Table 1).

3.2. Physiological Parameters

Similar to the response of drought exerted on morphological parameters of watermelon, drought stress resulted in significant decreases in plants physiological characteristics, such as leaf carotenoids, chlorophyll a/b ratio, SPAD index and maximum quantum use efficiency of PSII (F_v/F_m) in both ungrafted and grafted plants (Table 2). However, the effect of grafting was not clearly evident on most of these physiological parameters, except the SPAD index, which was found to be higher in ungrafted watermelon plants or graft combinations involving bitter apple rootstock ('Crimson Sweet'/'Esfahan') than those involved commercial *Cucurbita* rootstock ('Crimson Sweet'/'Shintoza'). There was no inter-

action effect of drought treatment and grafting treatment in the measured physiological parameters (Table 2).

Table 1. Effects of graft combination and drought treatment on shoot fresh and dry weight, leaf area, vine length, internode length and number of branches of watermelon plants.

Treatment	Shoot		Leaf Area (cm ²)	Vine Length (cm)	Internode Length (cm)	Number of Branches (n. plant ⁻¹)
	Fresh Weight (g plant ⁻¹)	Dry Weight (g plant ⁻¹)				
Drought treatment (D)						
100% CC	58.68 a	8.17 a	25.62 a	109.71 a	9.45 a	3.49
50% CC	37.60 b	5.33 b	17.27 b	78.44 b	6.18 b	3.36
Graft combination (G)						
Ungrafted 'Crimson Sweet'	42.94 b	5.81 b	20.38	83.75 b	7.35 b	3.14
'Crimson Sweet'/'Shintoza'	52.40 a	7.33 a	21.72	105.76 a	7.70 ab	3.47
'Crimson Sweet'/'Esfahan'	49.09 a	7.10 a	22.24	92.72 ab	8.29 a	3.67
D × G						
100% CC × Ungrafted 'Crimson Sweet'	54.17	7.21	24.29	104.40	9.51	3.17
100% CC × 'Crimson Sweet'/'Shintoza'	64.93	9.08	25.80	116.45	9.40	3.61
100% CC × 'Crimson Sweet'/'Esfahan'	56.95	8.21	26.78	108.28	9.44	3.69
50% CC × Ungrafted 'Crimson Sweet'	31.71	4.40	16.46	63.09	5.20	3.11
50% CC × 'Crimson Sweet'/'Shintoza'	39.87	5.58	17.64	95.07	6.19	3.33
50% CC × 'Crimson Sweet'/'Esfahan'	41.23	6.00	17.70	77.15	7.16	3.64
Significance ^a						
Drought treatment	**	**	**	**	**	NS
Graft combination	*	*	NS	*	*	NS
D × G	NS	NS	NS	NS	NS	NS

^a, NS, *, ** Nonsignificant or significant at $p \leq 0.05$ or 0.01 , respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

Table 2. Effects of graft combination and drought treatment on total chlorophyll (Chl.), carotenoids (Car.), chlorophyll a/b ratio, SPAD index and maximum quantum use efficiency of PSII (F_v/F_m) of watermelon plants.

Treatment	Leaf Pigments (mg g ⁻¹ FW)		Chl a/b	SPAD Index	F_v/F_m
	Chl.	Car.			
Drought treatment (D)					
100% CC	1.54	0.33 a	3.26 a	44.09 a	0.82 a
50% CC	1.42	0.29 b	2.81 b	42.38 b	0.80 b
Graft combination (G)					
Ungrafted 'Crimson Sweet'	1.48	0.32	3.14	44.18 a	0.807
'Crimson Sweet'/'Shintoza'	1.57	0.31	2.83	42.37 b	0.808
'Crimson Sweet'/'Esfahan'	1.39	0.30	3.13	43.15 ab	0.818
D × G					
100% CC × Ungrafted 'Crimson Sweet'	1.55	0.34	3.22	45.24	0.81
100% CC × 'Crimson Sweet'/'Shintoza'	1.59	0.33	3.22	43.22	0.82
100% CC × 'Crimson Sweet'/'Esfahan'	1.48	0.33	3.32	43.83	0.83
50% CC × Ungrafted 'Crimson Sweet'	1.40	0.30	3.05	43.12	0.80
50% CC × 'Crimson Sweet'/'Shintoza'	1.56	0.29	2.43	41.53	0.80
50% CC × 'Crimson Sweet'/'Esfahan'	1.29	0.27	2.94	42.48	0.81
Significance ^a					
Drought treatment	NS	**	**	**	**
Graft combination	NS	NS	NS	*	NS
D × G	NS	NS	NS	NS	NS

^a, NS, *, ** Nonsignificant or significant at $p \leq 0.05$ or 0.01 , respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$).

Drought treatment significantly decreased relative water content (RWC), but increased the level of lipid peroxidation (MDA) and electrolyte leakage (EL) in watermelon leaves of both grafted and ungrafted plants (Table 3). Grafting also significantly influenced RWC, MDA and EL. The RWC was significantly higher in grafted plants (onto both rootstocks) than ungrafted plants, whereas the levels of MDA and EL were the lowest in ‘Crimson Sweet’/‘Esfahan’ graft combination, regardless of drought treatment (Table 3).

Table 3. Effects of grafting combination and drought treatment on catalase (CAT), and guaiacol peroxidase (GPX) activities, malondialdehyde content (MDA), electrolyte leakage (EL) and leaf relative water content (RWC) of watermelon plants.

Treatment	Antioxidant Enzymes (mmol mg protein ^{−1} min ^{−1})		MDA (nmol g ^{−1} FW)	EL (%)	RWC (%)
	CAT	GPX			
Drought treatment (D)					
100% CC	82.67 b	1.56 b	5.91 b	70.96 b	82.67 b
50% CC	137.36 a	3.06 a	8.25 a	81.34 a	137.36 a
Graft combination (G)					
Ungrafted ‘Crimson Sweet’	102.43	2.08 b	7.21 a	78.35 a	102.43
‘Crimson Sweet’/‘Shintoza’	119.96	2.20 ab	7.23 a	77.72 a	119.96
‘Crimson Sweet’/‘Esfahan’	107.66	2.63 a	6.81 b	72.38 b	107.66
D × G					
100% CC × Ungrafted ‘Crimson Sweet’	66.98	1.38	6.18 c	73.53 c	66.98
100% CC × ‘Crimson Sweet’/‘Shintoza’	88.84	1.47	5.61 c	71.14 d	88.84
100% CC × ‘Crimson Sweet’/‘Esfahan’	92.19	1.81	5.95 dc	68.19 e	92.19
50% CC × Ungrafted ‘Crimson Sweet’	137.88	2.79	8.24 ab	83.16 a	137.88
50% CC × ‘Crimson Sweet’/‘Shintoza’	151.08	2.94	8.84 a	84.30 a	151.08
50% CC × ‘Crimson Sweet’/‘Esfahan’	123.14	3.44	7.68 b	76.56 b	123.14
Significance ^a					
Drought treatment	**	**	**	**	**
Graft combination	NS	*	**	**	*
D × G	NS	NS	*	*	*

^a, NS, *, ** Nonsignificant or significant at $p \leq 0.05$ or 0.01 , respectively. Different letters within each column indicate significant differences according to Duncan’s multiple-range test ($p = 0.05$).

The interaction effects of drought × grafting for RWC, MDA and EL (Table 3) indicate that the level of these parameters varied between WW and drought stress for different graft combinations. RWC was statistically similar in grafted and ungrafted plants under WW conditions, whereas, under drought stress, the highest RWC was recorded in the ‘Crimson Sweet’/‘Shintoza’ graft combination, followed by the ‘Crimson Sweet’/‘Esfahan’ graft combination, and the lowest RWC was recorded in ungrafted plants. Similarly, MDA content was similar between grafted and ungrafted plants under WW conditions, while its level increased considerably under drought stress, with the highest levels recorded in the ‘Crimson Sweet’/‘Shintoza’ graft combination. This was statistically similar with ungrafted plants. The lowest MDA content was recorded in the ‘Crimson Sweet’/‘Esfahan’ graft combination (Table 3). The level of EL significantly varied among graft combinations under both WW and drought stress conditions. Under WW conditions, the highest level of EL was recorded in ungrafted plants, followed by the ‘Crimson Sweet’/‘Shintoza’ graft combination, while the lowest level was recorded in the ‘Crimson Sweet’/‘Esfahan’ graft combination (Table 3). Under stress conditions, EL was significantly lower in the graft combination involving bitter apple rootstock (‘Crimson Sweet’/‘Esfahan’) than the other graft treatments, which all had similar ELs (Table 3).

The changes in the antioxidant enzymes activities were mainly due to the drought stress treatment and, to a lesser degree, to the effect of the grafting combination. The activities of antioxidant enzymes, catalase (CAT) and guaiacol peroxidase (GPX), were significantly increased in water stressed plants. Among the grafting combinations, the GPX activity was significantly higher in grafted plants involving wild watermelon rootstock 'Esfahan' compared to those grafted onto 'Shintoza' rootstock or ungrafted plants, which showed similar GPX activities. The CAT activities were not affected by grafting (Table 3).

4. Discussion

Watermelon is a popular fruiting vegetable, which is often grown in semi-arid and arid areas, and it is prone to water stress. Water-limiting conditions can have significant impact on its production [6]. It is widely recognized that yields are the results of the cumulative effect of growth and physiology of plants under a given set of conditions; hence, depression in the physiological and growth parameters of vegetable plants under water stress conditions may lead to yield losses. Among the various suggestions to alleviate water stress in vegetables, grafting commercial cultivars onto resistant rootstocks is an efficient and sustainable approach [15]. Fortunately, the highest proportion of grafted seedlings (up to 95%) to the total seedling use in several countries is found in watermelon, and the main reason for grafted seedlings' use has been to manage soil-borne pathogens [1]. In most cases, the watermelon is grafted onto interspecific *Cucurbita* (pumpkin) hybrid rootstocks, including 'Shintoza', which is also included in this study.

In the present investigation, drought stress (50% CC) caused a significant depression in the growth and biomass production of watermelon plants in both grafted and ungrafted plants, in comparison to well-watered (WW) plants (100% CC). However, the effect of drought was less pronounced in grafted plants than ungrafted plants, suggesting that, unlike the ungrafted 'Crimson Sweet', which was susceptible to drought, plants grafted onto both the rootstocks were more able to tolerate drought stress. The response of grafting to drought stress, however, depends upon the rootstock genotypes and scion–rootstock combinations that determine growth and physiology [6,15].

Shoot fresh and dry weights are considered among the most important morphological traits to screen and identify tolerant genotypes; these decrease under water stress, depending on the responses of genotypes [40]. The rates (%) of decrease in shoots' fresh and dry weight were similar under drought stress in all graft combinations, whereas relative decreases in these parameters, along with some other parameters (e.g., vine length, internodal length and number of branches), were lowest in grafted plants involving wild watermelon rootstock 'Esfahan' (Table 1). This indicates that the responses of the rootstock-based grafts to drought stress was seemingly, but not certainly, different, as there was no clear evidence of their interactive effects on different growth parameters. However, the research findings of a previous experiment carried out by Bigdelo et al. [34], using the same graft combinations under field conditions, indicates that commercial rootstock 'Shintoza' was more advantageous in increasing the fruit size and yield of grafted watermelon; the above findings were related to the intrinsic ability of *Cucurbita* hybrid rootstock 'Shintoza' to impart increases in plant vigor rather than tolerance to stresses. Furthermore, this rootstock has also shown to increase water and nutrient uptake in grafted watermelon that was likely associated with greater rootstock's vigorous root system [6,34]. Grafting 'Crimson Sweet' onto commercial *Cucurbita* hybrid rootstock ('Shintoza') and wild watermelon accession 'Esfahan' rootstock presented a constitutive response as described by Kumar et al. [15], since these graft combinations, besides presenting good tolerance to drought, were also able to produce higher amount of growth and biomass under well-watered conditions as compared to ungrafted controls. It has been reported that constitutive response is effective under low and intermediate degrees of drought, while responsive traits affect yield only under severe water-stressed conditions [15].

Water stress reduces plant growth by affecting various physiological processes, such as plant water status, photosynthesis, the accumulation of reactive oxygen species (ROS) and

lipid peroxidation [21]. A ramified root system has been implicated in the drought tolerance and high biomass production, primarily due to its ability to extract more water from soil and transport it to aboveground parts, contributing to better physiological functioning [16]. Therefore, some reports showed that different rootstocks can change the physiological parameters' responses to drought stress [41]. Leaf RWC is an important physiological indicator defining water stress tolerance in plants based on tissue and cell dehydration [40]. The higher level of RWC in the leaves of grafted watermelon plants (onto both rootstocks) than non-grafted plants is indicative of the better ability of grafted plants to maintain plant water status, particularly under water stress condition. Relative to ungrafted watermelon 'Crimson Sweet' plants, the higher fresh biomass production in grafted plants is an indication of better accumulation of water in plant tissues under drought stress. Further, the higher generation of dry mass by grafted plants can be explained by the better carbon assimilation, which is likely due to the improved photosynthetic efficiency and stomatal regulation in grafted plants than ungrafted plants under water stress conditions [6,15]. Furthermore, the slightly lower values of the Chl a/b ratio recorded in both watermelon rootstocks indicates less damage to chlorophyll b and, hence, better protection of PSII under drought stress conditions [10].

The production and accumulation of ROS in plant cells is another common (oxidative) form of damage under drought stress conditions [7]. The common resultant products of oxidative stress are the accumulation of malondialdehyde (MDA) and electrolyte leakage (EL). To assess cell membrane stability, MDA and EL have been widely adopted as a benchmark to differentiate stress-susceptible and -tolerant species/genotypes; higher membrane stability was associated with tolerance to certain abiotic stress in vegetable crops [8,10]. Drought stress induced oxidative stress, as indicated by its resultant effects on the increased levels of MDA and EL in all graft combinations of watermelon under drought stress. However, the stress-related damage varied in different graft combinations; the distinctly low levels of MDA and EL in 'Crimson Sweet'/'Esfahan' are clearly evident of the least oxidative damage being caused by drought in this graft combination (Table 3). As a coping mechanism for oxidative stressors, plants employ an antioxidant defense system, depending on their ability to respond to abiotic stresses [15,21,39]. Together with the distinctly lower levels of MDA and EL in 'Crimson Sweet'/'Esfahan', the higher induction of antioxidant enzymes (e.g., GPX) in this graft combination is indicative of its better ability to cope up the oxidative stress under stressful conditions (Table 3).

Another mechanism of drought tolerance in these grafted plants could be the increased ability of rootstocks to take up water and nutrients more efficiently, due to their specialized root system of the rootstocks. Since this study was conducted in pots in which the restriction of roots limits the configuration of the responses of drought related to root traits and their impact on mineral uptake, but the available literature suggests that root architecture changes may occur and, accordingly, influence grafted plants' efficiency under drought stress. As explained earlier, rootstock's root architecture may influence the performance of grafted plants under stressful conditions [15]. For instance, watermelon grafting onto interspecific *Cucurbita* rootstock (e.g., 'Shintoza') has resulted in a higher concentration of nutrients in aerial parts [34], which was thought to be associated with grafted plants' more developed root system (higher root surface area, root volume, root forks and number of root tips), in comparison with ungrafted plants [42].

Overall, the lower relative decrease in morphological growth parameters under both drought stress and WW conditions, concomitant with the better antioxidant defense mechanisms in wild watermelon 'Esfahan' rootstock-grafted plants, explain the better ability of 'Esfahan' rootstock to tolerate drought compared to ungrafted plants. 'Crimson Sweet', grafted onto 'Esfahan', may represent a valuable alternative to the commercial rootstock 'Shintoza'.

5. Conclusions

Drought stress invariably decreased most of the plant growth parameters, including the biomass production of ungrafted and grafted watermelon plants. However, the effect of water stress was less damaging in grafted plants, as grafting displayed more maintained physiological plant processes in the scion of grafted watermelon under water stress. Grafting watermelon onto interspecific *Cucurbita* hybrid rootstock ('Shintoza') produced greater morphological growth attributes both under well-watered and stressful conditions. However, wild watermelon/bitter apple ('Esfahan') rootstock-grafting displayed greater resistance to drought stress, as indicated by the lower decrease in growth and biomass accumulation. The resistance response of bitter apple ('Esfahan') rootstock-grafted watermelon was ascribed to its better antioxidative defense mechanism at the cellular level, as reflected by the higher level of antioxidant enzyme (i.e., GPX), concomitant with the lower oxidative stressors (MDA, EL), particularly under water stress conditions. However, commercial *Cucurbita* rootstock-based grafted plants displayed a better ability to maintain plant water status (higher RWC), likely due to the vigorous roots of rootstock helping to extract water and nutrients more efficiently. Finally, it was inferred that the drought-adapted accession of bitter apple ('Esfahan') can be used as a potential rootstock to improve drought tolerance in watermelon. It can also be used in rootstock breeding programs to improve drought tolerance in watermelon.

Author Contributions: Conceptualization, G.C., M.C. and Y.R.; methodology, G.C., M.B., M.R.H., F.S., R.S., P.K., M.C. and Y.R.; software, M.B., M.R.H., R.S., F.S. and P.K.; validation, M.B., M.R.H., R.S., F.S., M.C. and P.K.; formal analysis, M.B., M.R.H., R.S., F.S., M.C. and P.K.; investigation, M.B., P.K. and M.C.; resources, G.C.; data curation, M.B., P.K. and M.C.; writing—original draft preparation, M.B., P.K. and M.C.; writing—review and editing, G.C., M.B., M.R.H., R.S., F.S., P.K., M.C. and Y.R.; visualization, G.C., Y.R. and M.C.; supervision, P.K., M.C. and G.C.; project administration, G.C.; funding acquisition, G.C., M.B., R.S., M.R.H. and F.S. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully acknowledge MIUR (Ministry for Education, University and Research) for financial support of DAFNE, University of Tuscia (Law 232/216, Department of Excellence).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated for this study are available on request to the principal or corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

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