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Grafting onto an Appropriate Rootstock Reduces the Impact on Yield and Quality of Controlled Deficit Irrigated Pepper Crops

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Abstract: In this study, hybrid pepper rootstock NIBER® is tested for its ability to overcome water stress situations under soil conditions. The impact of deficit irrigation (DI) on yield and fruit quality, irrigation water use efficiency is evaluated, and consequently, the agronomic impact of employing water-stress tolerant rootstock is compared to ungrafted pepper plants. For this purpose, plants of the California-type sweet pepper 'Maestral F1' grafted onto NIBER® underwent a sustained DI regime during seasons 2018 and 2019 and were compared to their respective controls. Plants were drip-fertirrigated, and volumetric soil water content was continuously monitored by capacitance sensors. Gas exchange and leaf water potential measurements were taken early in the morning and midday 58, 79, and 114 days after transplanting. Plant and fruit dry biomass, marketable quality, blossom-end rot incidence and harvest index were also determined. For consecutive years, our results confirmed that grafting a pepper cultivar onto an appropriate rootstock (NIBER® in this case) as part of a DI strategy can overcome the negative effects of sustained water stress conditions. The plant biomass production and fruit yields of grafted plants were less affected by DI due to less sensitivity to water stress. This can be attributed to a less marked reduction in shoot dry weight in the grafted plants, which allowed greater whole photosynthesis by maintaining sink activity compared to ungrafted plants.

Keywords: grafting; *Capsicum annum* L.; sustained deficit irrigation; drought stress; abiotic stress; volumetric soil water content; gas exchange; leaf water potential; biomass

1. Introduction

Sweet pepper (*Capsicum annuum* L.) is an important vegetable crop around the world, with production at approximately 36 Mt obtained from 2 million ha. Currently, the leading sweet pepper-producing countries are China, Mexico, and Turkey, while Spain, Italy, and Romania are the main producers in Europe [1].

Drought stress is an important limiting factor for vegetable crop production. Water is becoming increasingly scarce worldwide, which seriously affects agricultural production, especially in arid and semiarid areas [2,3], due to increased global temperature and evapotranspiration (ET) and less precipitation, which would consequently increase water demands [4–6]. Already existing drought conditions are expected to worsen, particularly in regions where water scarcity is a concern, such as the Mediterranean Region [7]. The Mediterranean climate presents mild winter temperatures and long hot, dry summers, with interannual and seasonal-depending variable precipitation, which make irrigation essential for crop production [8–10].

Globally speaking, agriculture is the largest freshwater consumer and represents approximately 68% of water use [11]. By 2050, the world population is expected to be 9 billion people, which will involve a 60% increase in agricultural production and a 15% increase in water withdrawal [12], which would increase competition for water resources by urban and industrial users. To mitigate these water shortage effects, researchers are attempting to increase water productivity by different approaches [10,13–15]. Nowadays, it is even more important to maximize crop water productivity rather than crop yield per unit area [16].

Drought severity and duration affect yields in many ways by reducing plant growth, disturbing plant water relations, and affecting water-use efficiency. Due to different physiological processes, some parameters like leaf water potential, stomatal resistance, and transpiration rate, strongly influence plant-water relations. These, in turn, affect mineral nutrition, photosynthesis, and oxidative damage [17]. As these parameters depend on the cultivars and environment in which they are grown to a large extent, irrigation management should be adapted to each variety and environmental condition.

One important tool to reduce irrigation water use in agriculture is deficit irrigation (DI), which consists of applying water below full crop-water requirements. Fereres and Soriano [18] reviewed several cases about successful DI use for different crops, and stated that DI can not only increase water productivity, but also farmers' profits. However, DI may also mean major yield reductions [18], and therefore, crop responses to water deficit must be evaluated to achieve efficient water use, while obtaining adequate yields [3].

Another tool to cope with yield reductions related to water stress in some vegetables is to employ grafting technology. Grafting enables plants to overcome biotic and abiotic stresses, including water stress [19–21]. This fact has been particularly studied in tomato [22] and melon [23]. In recent years, several studies have also been conducted on pepper. Some rootstocks can confer pepper cultivars tolerance to water restrictions [24] as they can maintain stomatal conductance, and consequently, the photosynthetic rate under these conditions [25,26]. This water-stress tolerance leads to comparable yields to those of fully irrigated ungrafted plants [27], due to osmotic adjustment via, i.e., proline accumulation and/or the protective role of this substance [28]. More recently, López-Serrano et al. [29] stated that water stress severity in pepper plants was alleviated by using tolerant accessions as rootstocks, capable of partially opening stomata, preserving the relative water content, and consequently, both lowering oxidative stress and diminishing lipid peroxidation. These accessions were inserted in a classic breeding program to obtain more uniform hybrids in terms of germination, growth, and highest vigor to be used as rootstocks under water stress conditions, one of them is NIBER®.

Nevertheless, and as far as we know, no studies have been conducted on water restrictions using these rootstocks in pepper crops under greenhouse conditions, monitoring water availability for plants.

Therefore, it would be advisable to study the response of sweet pepper cultivars grafted onto drought-tolerant rootstocks to DI. Hence, the objective of this study was to evaluate the impact of DI on the yield and fruit quality of a green pepper cultivar using NIBER®, an F1 hybrid, as a rootstock, on irrigation water use efficiency, and consequently, on the agronomic impact that can lead to better profits with sweet pepper crops.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted during two consecutive growing seasons (GS; 2018 and 2019) at the Instituto Valenciano de Investigaciones Agrarias (IVIA) Research Institute in Moncada (Valencia, Spain; latitude: 39.589517, longitude: –0.395550, elevation: 37 m). The climate is the Mediterranean, distinguished by warm wet winters and hot, dry summers. Temperature and relative humidity values along the experimental seasons are presented in Figure S1. Trials were conducted in an unheated plastic greenhouse (30 m long × 7.5 m wide) that was E-W oriented, and passively ventilated by opening side panels and roof vents. The soil composition within a 20-centimeter depth was sandy clay loam (68% sand, 11% clay, 21% silt) and contained 0.61% organic matter, 0.051% total N, 8 mg kg^{-1} of P, 301 mg kg^{-1} of K, and 2.87 meq 100 g^{-1} of assimilable Mg. Soil electrical conductivity (1:5) was 0.29 dS m^{-1}, and pH was 8.1. Irrigation was applied with a drip system with one single line per plant row and one emitter every 0.2 m with a 2.5 L h^{-1} discharge rate. Irrigation water had an electrical conductivity of 0.3 dS m^{-1}, 16.5 mg L^{-1} of NO_3^- content, very low concentrations of other macro- and micronutrients, and a pH of 8.1. Nutrients were applied during both crop seasons through the irrigation system according to the following nutritional plan and in line with Maroto [30]: 170 kg ha^{-1} of N; 40 kg ha^{-1} of P_2O_5; 200 kg ha^{-1} of K_2O.

2.2. Plant Material

Sweet pepper 'Maestral F1' (California-type Rijk Zwaan, The Netherlands) grafted onto rootstock F1 NIBER® (V/N) (obtained from the Universitat Politècnica de València (UPV)-IVIA, Spain) and ungrafted (V) plants were used in the experiments.

Sowing took place on 22 January 2018 and 25 January 2019, in 104-cell polystyrene trays on peat moss-based substrate (70% blonde, 30% dark), which is recommended for vegetable seedbeds (Pindstrup Mosebrug S.A.E., Sotopalacios, Spain). Trays were maintained in a Venlo-type greenhouse. On 1 March 2018 and 5 March 2019, plants were grafted by the tube-grafting method [27].

Seedlings were transplanted on 27 March 2018 and 2 April 2019 inside the plastic greenhouse in raised beds spaced 1.10 m apart with one plant row per ridge and 0.5 m spacing between plants within rows (1.8 plants m^{-2}). The raised part of the raised bed was 0.2 m wide, 6 m long, and 0.10 m high. Plants were horizontally supported by two nylon guide cords laid parallel to both sides of the plant line, as described by Maroto [30], and no pruning was performed.

The experiments were laid out to be complete randomized with three replicates. Each replicate consisted of 13 plants.

2.3. Irrigation Strategies

Two irrigation strategies (IS) were adopted: Irrigation with 100% and 50% of the estimated crop water requirements (control (C) and DI, respectively). Irrigation during the first four weeks after plantation was applied to assure the correct plant establishment. Irrigation management consisted of fixed 30-minute irrigation events, applied as often as necessary, depending on the VSWC and irrigation strategy. Crop irrigation water requirements were obtained by estimating crop evapotranspiration (ET_c) by the K_c–ET_o method [31]. The reference evapotranspiration (ET_o; in mm day^{-1}) was calculated by the calibrated radiation method, which is often used in the greenhouse industry in SE Spain [32]. This method requires daily solar radiation outside greenhouses and cladding greenhouse transmissivity (σ, in %). Solar radiation was obtained from an agroclimatic weather station located near the greenhouse, while transmissivity was calculated by using the radiation data collected before the crop period with a CMP3 pyranometer (Kipp and Zonen, Delft, The Netherlands), whose value was 43% during both seasons. The crop coefficient (K_c) values were those determined by Orgaz et al. [33] for sweet pepper. Initially, K_c took a value of 0.2 and rose to 1.4 at harvest.

The applied irrigation water, and its distribution according to growth stages, are presented in Table 1. For the environmental conditions during the experimental period, the ET_o calculated from outdoor solar radiation was 261.4 mm in 2018 and 265.1 mm in 2019. When applying the proposed K_c, the estimated water requirements were 230.5 mm in 2018 and 228.4 mm in 2019. The total applied water amount in the control strategy was 216.3 mm and 206.8 mm, respectively (Table 1), which means that the irrigation enforced in the control strategy was around 94% and 91% of the ET_c value in 2018 and 2019, respectively. The DI (V-DI and V/N-DI) received 58% and 56% of the control doses (V-C and V/N-C) in 2018 and 2019, respectively.

Table 1. Crop evapotranspiration (ET_c) and irrigation water applied in the different growth stages for the plants under the control and deficit irrigation conditions during 2018 and 2019.

Growth Stages		Duration (days)	ET_c (mm)	Irrigation Water Applied (mm)	
				Control	Deficit Irrigation
2018					
	Vegetative growth	34	22.1	25.7	21.9
	Fruit development	29	45.0	54.0	27.0
	Harvesting period	49	163.4	136.6	76.5
	Total	112	230.5	216.3	125.4
2019					
	Vegetative growth	34	19.7	20.2	22.6
	Fruit development	43	82.9	76.2	33.3
	Harvesting period	38	125.8	110.4	60.5
	Total	115	228.4	206.8	116.4

2.4. Soil Moisture

The volumetric soil water content (VSWC; m^3 m^{-3}) was continuously monitored by ECH_2O EC-5 capacitance sensors connected to an Em50 data logger using the ECH_2O Utility software (Decagon Devices, Inc., Pullman, WA, USA). VSWC values were used directly because factory calibration provides ± 3% accuracy for mineral soils. One sensor per replicate was placed below the dripline at a 20-centimeter depth, where the maximum root density was located and was equidistant between two adjacent emitters. The VSWC was measured and stored every 30 min, and its variation was used to determine in situ field capacity (FC) [34,35]. The permanent wilting point (PWP) was determined by using Richards Plates (Set for pF-determination; Eijkelkamp, Giesbeek, The Netherlands) [36]. To compare VSWC between irrigation strategies and growing seasons, their values are presented as available water content (AWC; %) before each irrigation event, and were determined as reported by Fernández et al. [37]:

$$AWC = (1 - [(VSWC_{FC} - VSWC_a) / (VSWC_{FC} - VSWC_{PWP})]) \times 100, \quad (1)$$

considering the subscripts a, FC and PWP correspond to the actual (before irrigation), field capacity, and permanent wilting point soil water content, respectively. When the $VSWC_a$ was greater than the $VSWC_{FC}$, the last one was considered, given that AWC cannot be greater than 100%. The values for the VSWC at FC and PWP were determined for the different GS and IS and are shown in Table 2.

Table 2. Volumetric soil water content (m^3 m^{-3}) at field capacity (FC) and permanent wilting point (PWP) for each growing season (2018 and 2019), irrigation strategy (control (C) and deficit irrigation (DI)) and plant type (ungrafted (V) and grafted (V/N)).

	Plant Type	2018		2019	
		FC	PWP	FC	PWP
Control	V-C	0.19	0.08	0.24	0.13
	V/N-C	0.17	0.08	0.23	0.13
Deficit irrigation	V-DI	0.23	0.08	0.19	0.13
	V/N-DI	0.22	0.08	0.19	0.13

2.5. Physiological Measurements

Physiological measurements were taken during GS 2019. The gas exchange measurements were taken early in the morning (9.30 am to 10.30 am GMT) and at midday (1 pm to 2 pm) with three plants per replicate (9 per treatment) after 58, 79, and 114 days after transplanting (DAT). The net CO_2 assimilation rate (A_N, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), and transpiration rate (E_{leaf}, mmol H_2O m^{-2} s^{-1}) were determined on fully expanded leaves (3rd–4th leaf from the apex) in the steady-state under saturating light conditions (1000 µmol m^{-2} s^{-1}) and with 400 ppm CO_2 by an LI-6400 infrared gas analyzer (LI-COR, Nebraska, USA) at 24 ± 2 °C and 65 ± 10% relative humidity. The average values of leaf vapor pressure deficit (considering 58, 79, and 114 DAT) were 1.68 ± 0.22 kPa and 2.50 ± 0.25 kPa at early morning and midday, respectively. Parameters A_N/g_s and A_N/E_{leaf} were calculated as intrinsic water efficiency and instantaneous water use efficiency, respectively.

The predawn (5.00 am to 6.00 am) and midday leaf water potential (1 pm to 2 pm) ($\Psi_{predawn}$ and Ψ_{leaf}, respectively) were determined by a Schölander-type pressure chamber (model 3000; Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Determinations were made on fully expanded leaves in an identical physiological state as that used in the gas exchange measurements of three plants per replicate (9 per treatment) after 58, 79, and 114 DAT.

Plant conductance of the hydraulic water flow from root systems to leaves (whole-plant hydraulic conductivity, Kh_{plant}) was calculated by analogy to Ohm's law [38]:

$$Kh_{plant} = E_{leaf\text{-}md} / (\Psi_{soil} - \Psi_{leaf}) \quad (2)$$

where $E_{leaf\text{-}md}$ is the maximum transpiration rate in the leaf measure in LICOR-6400 from 1 pm to 2 pm by assuming that $\Psi_{soil} = \Psi_{predawn}$. Kh_{plant} was measured at 58, 79, and 114 DAT.

2.6. Biomass and Fruit Yield

Harvests consisted of four passes between mid-June and the end of July in both years. The commercial production quality was evaluated following the criteria described by the European Regulations [39], insofar as the yield was partitioned into three categories: «Extra» Class, Class I (together hereafter referred to as marketable yield; MY) and Nonmarketable, the fruits which, given their defects, mainly due to blossom-end rot (BER), did not match the former categories.

Biomass was analyzed at the end of crop cycles. The aboveground plant part was partitioned into two parts and separately analyzed: Vegetative, including shoots with all their leaves (hereafter referred to as shoot dry weight (SDW)), and fruit (including all the fruit of all the passes of the harvests). The sum of both terms was the total dry weight (TDW). Each part was dried at 65 °C in a forced-air oven (Oven 100-800, Memmert, Germany) until constant weight and weighed on an analytical scale, which measures dry weights. The harvest index (HI) was determined as the ratio of the total yield to the total aboveground biomass (TDW) on a dry mass basis [37].

2.7. Statistical Analysis

The biomass and fruit yield data were subjected to an ANOVA analysis, including the three factors: IS, plant type (PT), and GS. The physiological parameters were separately analyzed for each measurement time (58, 79, and 114 DAT) as a multiway ANOVA (Statgraphics Centurion for Windows, Statistical Graphics Corp.). With these parameters, after verifying the significance of the interaction for each variable (data are not shown), a one-way ANOVA was performed by joining the plant combination and treatment for each studied time. Means were compared by the Fisher's least significance difference (LSD test) at $p < 0.05$. No significant differences were found among the replicates for each measured parameter.

3. Results

3.1. Soil Moisture

Figure 1 presents the weekly average values for the available water content (AWC) before each irrigation event. For both growing seasons (GS) and irrigation strategies (IS), AWC increased before the differential irrigation strategies started (25 and 24 April in 2018 and 2019, respectively), reaching values of 100% of the AWC in 2018, as a consequence of plants being over irrigated to assure their establishment. After this day, during both GS the soil moisture in the deficit irrigation (DI) treatment decreased to constant values at the end of the cycle [40% in 2018 and 26.5% (on average for both plants types (PT)) in 2019], which was lower than those reached in the control treatment. In 2018, this drop in AWC was more marked for V/N-DI than for V-DI. From early June, soil moisture also decreased in the control treatment in the two PT and during both GS. It is noteworthy that in 2019, some problems were encountered with the irrigation system, which involved an unplanned lack of irrigation for two days. Consequently, the AWC in the control treatments dropped below 50%, and rose to previous levels when irrigation was reestablished. It is also noteworthy that the standard error was generally lower for DI than for the control plants as soil moisture remained at constant values.

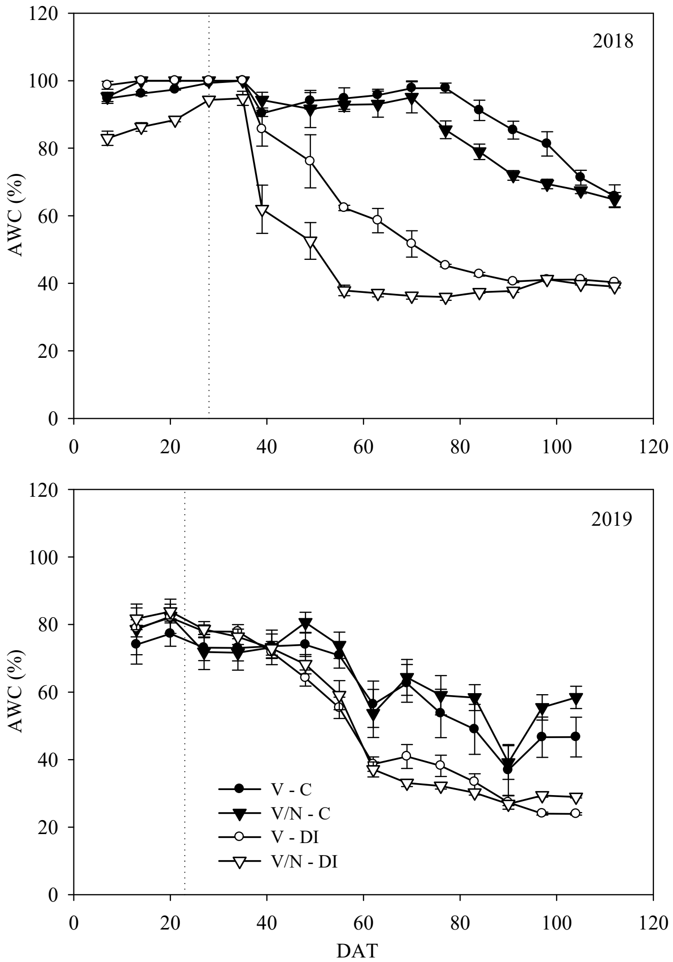

Figure 1. Weekly average soil moisture (expressed as a percentage of the available water content, AWC) during each growing season (2018 and 2019) for plant type, grafted (V/N) and ungrafted (V), and for both irrigation strategies: Control (C) and deficit irrigation (DI). DAT are days after transplanting. The vertical line represents the start of irrigation treatments.

3.2. Plant Water Relations

The predawn leaf water potential ($\Psi_{predawn}$) values (Figure 2A) after 58 DAT were not significantly different between the control and DI treatments. At 79 DAT and at the end of the experiment (114 DAT), the V-C and V/N-C values were higher (with no significant differences between them) compared to V/N-DI and V-DI, of which the latter had the lowest $\Psi_{predawn}$ values.

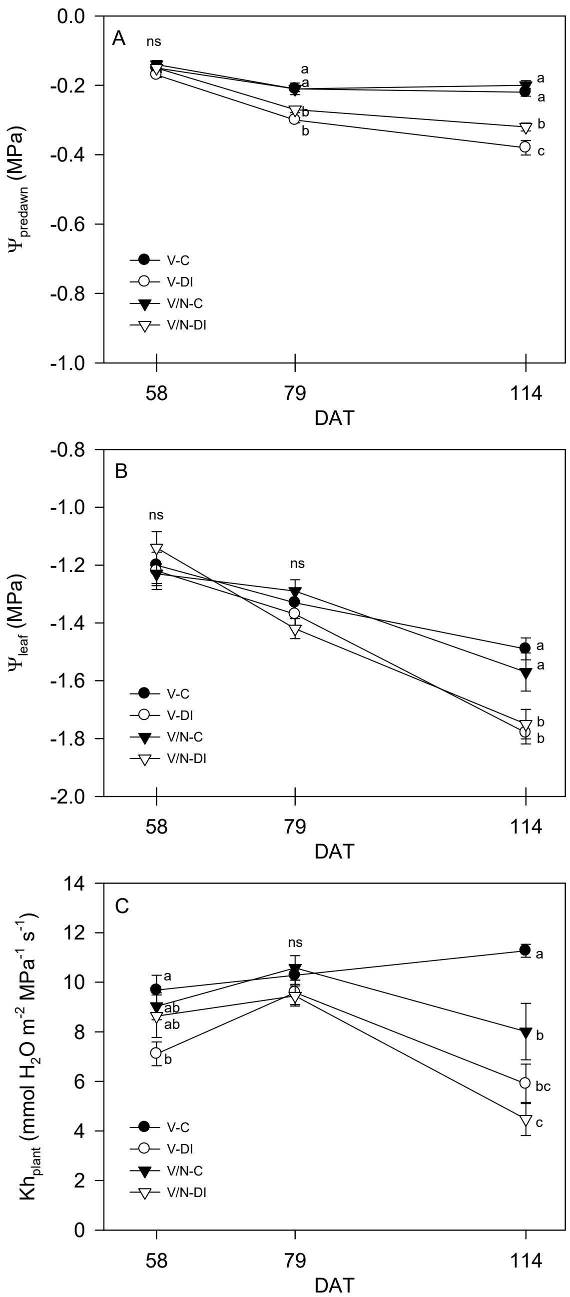

Figure 2. Predawn leaf water potential ($\Psi_{predawn}$) (**A**), midday leaf water potential (Ψ_{leaf}) (**B**), and whole plant hydraulic conductivity (Kh_{plant}) (**C**) in the ungrafted pepper plants (cultivar 'Maestral', V) and the plants grafted onto NIBER® (V/N) grown under well-irrigated (C) and deficit-irrigated (DI) conditions. Measurements were taken on 58, 79, and 114 days after transplanting (DAT). Data are the mean values for n = 9 ± SE. For each studied time, different letters indicate differences at $p \leq 0.05$ (LSD test). n.s. indicate no significant difference.

The midday leaf water potential Ψ_{leaf} values progressively lowered during the experiment as plant size enlarged in all the IS (Figure 2B). The application of the DI strategy significantly decreased Ψ_{leaf} in the DI plants, but only at the end of the experiment (114 DAT), with no differences appearing between the grafted and ungrafted plants.

Whole plant hydraulic conductivity (Kh_{plant}) (Figure 2C) was not constant during the experiment. At 58 DAT, values ranged between 7 and 10 mmol H_2O m^{-2} MPa^{-1} s^{-1}, and the lowest values were for the V-DI plants, and the highest ones were measured for the V-C plants, whereas the grafted plants had intermediate values. At 79 DAT, no significant differences between treatments were observed for all the Kh_{plant} values. However, at the end of the experiment (114 DAT), differences between treatments were found. When comparing only the control treatment plants, the V plants had higher Kh_{plant} than the V/N plants. The DI treatment decreased Kh_{plant} (46% lower) in the V and V/N groups of the plants compared to the control, with the lowest values for the V/N-DI plants.

3.3. Photosynthetic Parameters

The net CO_2 assimilation rate (A_N) measured early in the morning (Figure 3A) showed no significant differences between plants and treatments during the crop cycle. Nevertheless, the A_N values obtained at midday (Figure 3B) were grouped for the significant differences in the control and DI plants at 58 DAT and 79 DAT. The lowest values were for the DI plants at both these times. At 114 DAT, the maximal A_N was observed in the V-C plants followed by V/N-C, V-DI, and V/N-DI with the lowest values.

The transpiration rate (E_{leaf}) early in the morning (Figure 3C) increased from 58 DAT to 79 DAT, but with no significant differences between treatments at both DATs. At the end of the experiment, the DI strategy lowered the E_{leaf} values in the V and V/N plants compared to the control strategy. At midday, the E_{leaf} values of the control plants were higher than the values obtained early in the morning (Figure 3C,D). The measurements taken at 58 DAT and 79 DAT did not show any changes in the E_{leaf} of the V-C and V/N-C plants, but the E_{leaf} values were higher in the V-C plants than in the V/N-C plants at the end of the experiment. The application of the DI strategy led to equal E_{leaf} values for both the V and V/N plants at 58 DAT (Figure 3D). At 79 DAT, the DI strategy only reduced E_{leaf} in the V-DI plants. At the end of the experiment, the differences in E_{leaf} between IS were more evident, with the highest values for V-C and the lowest ones for V/N-DI.

Stomatal conductance (g_s) was measured early in the morning (Figure 3E) and only showed significant differences between IS at the end of the experiment, with similar reductions in the V and V/N plants compared to the control. The influence of IS on g_s was more evident at midday (Figure 3F). Although the measurements taken at 58 DAT and 79 DAT did not indicate any changes in g_s of the V-C and V/N-C plants, g_s was higher in the V-C than the V/N-C plants at the end of the experiment. At 58 DAT, the reduction in g_s accomplished by the DI strategy was equal for the V and V/N plants (Figure 3F). At 79 DAT, the DI strategy only reduced g_s in the V-DI plants. At the end of the experiment, the differences in g_s between IS were more evident, with the highest values for V-C and lowest ones for V/N-DI.

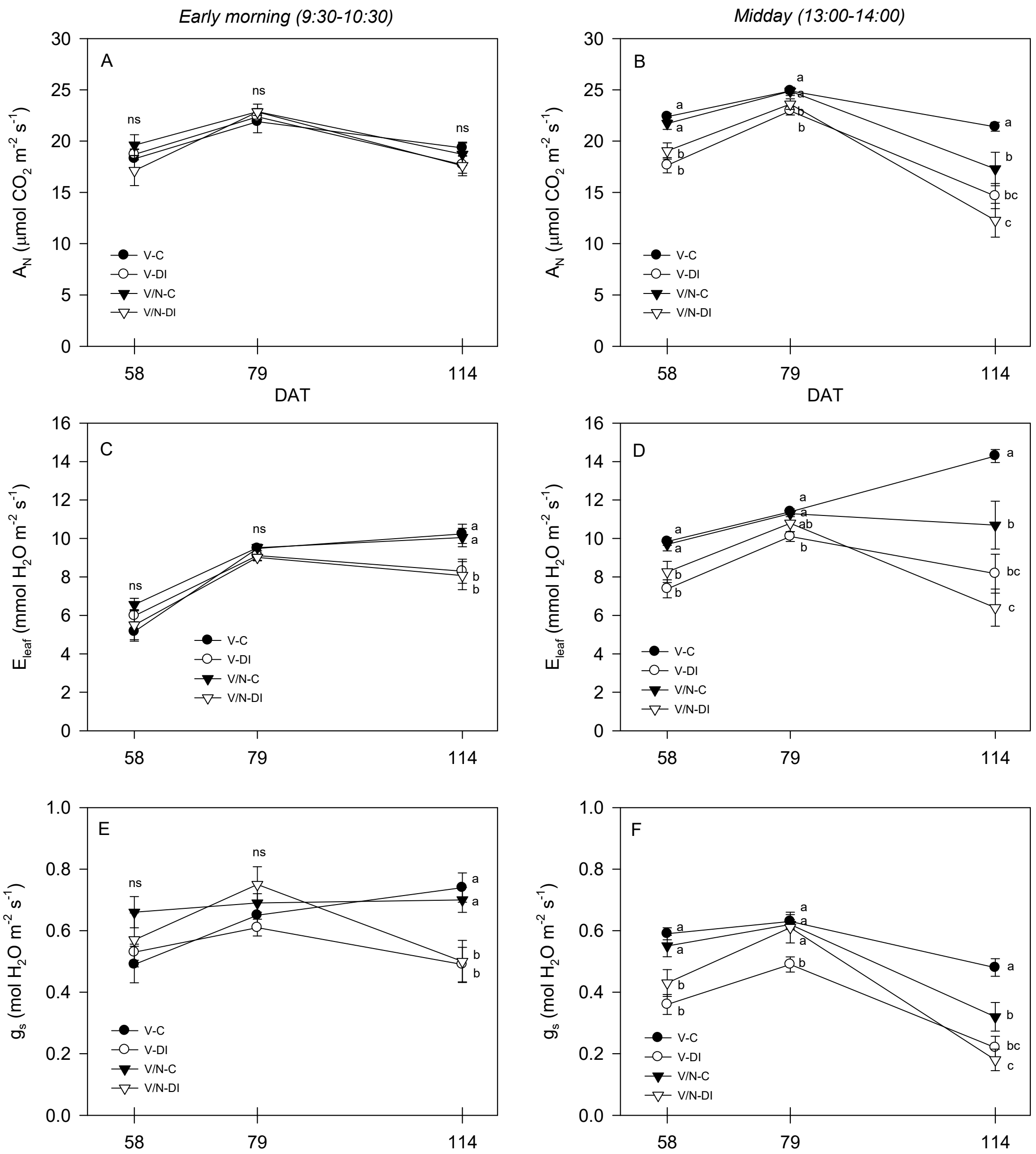

Figure 3. Net CO_2 assimilation rate (A_N) (**A**,**B**), leaf transpiration rate (E_{leaf}) (**C**,**D**), and stomatal conductance (g_s) (**E**,**F**) measured at two representative day times (early morning, midday) in the ungrafted pepper plants (cultivar 'Maestral', V) and the plants grafted onto NIBER® (V/N) grown under well-irrigated (C) and deficit-irrigated (DI) conditions. Measurements were taken on 58, 79, and 114 days after transplanting (DAT). Data are the mean values for n = 9 ± SE. For each studied time, different letters indicate differences at $p \leq 0.05$ (LSD test). n.s. indicate no significant difference.

Instantaneous water use efficiency (A_N/E_{leaf}) progressively decreased early in the morning for all the plant combinations during the experiment, with minimum values at 114 DAT (Figure 4A). There were no significant differences between IS at 58 DAT and 79 DAT, whereas A_N/E_{leaf} increased in the DI plants (V and V/N) at 114 DAT compared to the control. A similar response occurred at midday (Figure 4B). Regarding intrinsic water use efficiency (A_N/g_s), a different shape behavior was observed during the experiment to the values measured early in the morning (Figure 4C) and at midday (Figure 4D). Early in the morning (Figure 4C), it was only at the end of the experiment when the A_N/g_s values exhibited significant differences, with the highest values for the DI plants (V-DI and V/N-DI).

From 79 DAT to 114 DAT, the A_N/g_s values obtained at midday significantly increased (Figure 4D), where the DI plants obtained the maximum values compared to the control plants (V and V/N).

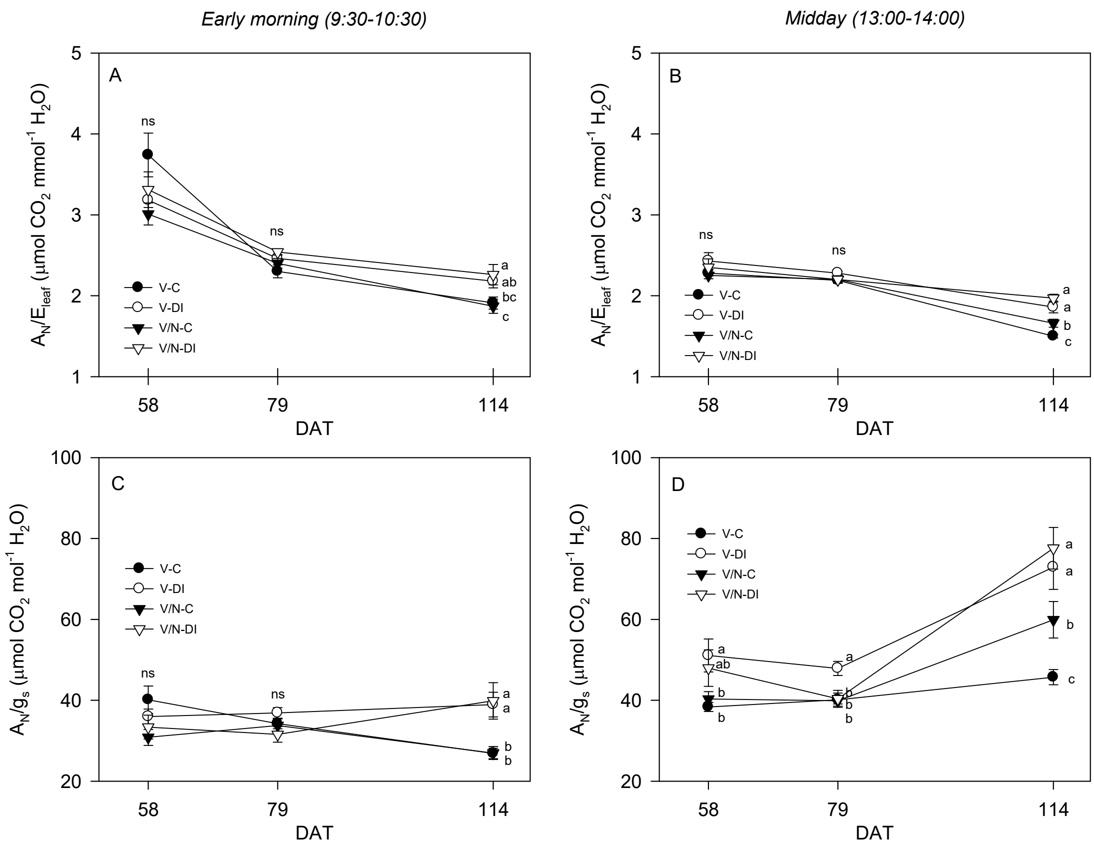

Figure 4. Instantaneous water use efficiency (A_N/E_{leaf}) (**A**,**B**) and intrinsic water use efficiency (A_N/g_s) (**C**,**D**) measured at two representative day times (early morning, midday) in the ungrafted pepper plants (cultivar 'Maestral', V) and the plants grafted onto NIBER® (V/N) grown under well-irrigated (C) and deficit-irrigated (DI) conditions. Measurements were taken on 58, 79, and 114 days after transplanting (DAT). Data are the mean values for n = 9 ± SE. For each studied time, different letters indicate differences at $p \leq 0.05$ (LSD test). n.s. indicate no significant difference.

3.4. Plant Biomass and Fruit Yield

Shoot dry weight (SDW) was significantly affected by IS ($p \leq 0.01$; Table 3), with a 20% decrease for the stressed plants versus the control ones, but the IS × PT interaction for this parameter was statistically significant ($p \leq 0.05$; Table 3) as the differences in SDW between DI and C were small (down to 50%) in the V/N plants in relation to V (Figure 5). Total dry weight (TDW) was also significantly affected by IS ($p \leq 0.01$; Table 3), with an average 22% decrease for the DI plants in relation to the control for both seasons. No significant IS × PT interaction was detected. The harvest index (HI) was affected by neither any analyzed factor nor their interaction for any season.

Table 3. Effect of irrigation strategy, plant type, and growing season on the shoot dry weight (SDW), total dry weight (TDW), and harvest index (HI).

	SDW (g plant^{-1})		TDW (g plant^{-1})		HI (-)	
Irrigation strategy (IS)						
C	176.3	a	311.7	a	0.437	
DI	140.5	b	240.8	b	0.416	
Plant type (PT)						
V	158.0		270.8		0.418	
V/N	158.8		281.7		0.434	
Growing season (GS)						
2018	157.8		272.5		0.421	
2019	158.9		280.0		0.432	
ANOVA (*df*)	**% Sum of the Squares**					
IS (1)	69.85	**	81.01	**	13.18	n.s.
PT (1)	0.03	n.s.	1.89	n.s.	7.61	n.s.
GS (1)	0.06	n.s.	0.91	n.s.	3.56	n.s.
IS*PT (1)	6.51	*	1.89	n.s.	0.00	n.s.
IS*GS (1)	3.57	n.s.	0.55	n.s.	9.30	n.s.
PT*GS (1)	2.74	n.s.	2.52	n.s.	9.30	n.s.
IS*PT*GS (1)	3.01	n.s.	1.89	n.s.	0.19	n.s.
Residuals (16)	14.22		9.33		0.53	
Std. Dev.$^{(+)}$	9.9		14.7		0.026	

The mean values followed by the different lowercase letters in each column indicate significant differences at $p \leq 0.05$ using the LSD test. ** and * indicate significance at $p \leq 0.01$ and $p \leq 0.05$, respectively, n.s. no significant difference. $^{(+)}$ Standard deviation, calculated as the square root of the residual sum of squares. *df* degrees of freedom.

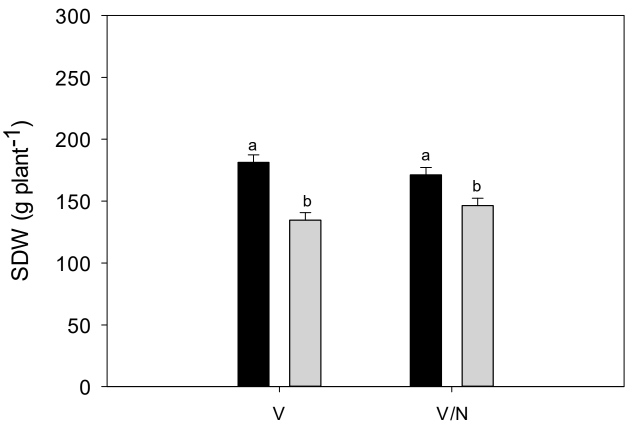

Figure 5. Shoot dry weight (SDW, g plant^{-1}) for the deficit irrigation plants (gray bars) and the control plants (black bars) in the ungrafted pepper plants (V) and the cultivar grafted onto NIBER® (V/N). Data are the mean values for n = 6. In each plant combination, different letters indicate significant differences at $p \leq 0.05$. Error bars represent the LSD value of the interaction.

The marketable yield (MY) and «Extra» class yield were also significantly affected by IS ($p \leq 0.01$; Table 4) and by the IS × PT interaction ($p \leq 0.05$; Table 4). Deficit irrigation reduced yields in relation to the control, down to 24% and 31% for the MY and «Extra» class yield, respectively. In both cases for the DI plants, higher yields were maintained in the V/N plants compared to V (Figure 6). Class I yield was affected by neither any analyzed factor nor their interaction.

Table 4. Effect of irrigation strategy, plant type, and growing season on marketable yield (MY), «Extra» Class yield («Extra» Class), and Class I yield (Class I).

	MY (kg plant^{-1})		**«Extra» Class (kg plant^{-1})**		**Class I (kg plant^{-1})**	
Irrigation strategy (IS)						
C	2.189	a	1.770	a	0.421	
DI	1.652	b	1.219	b	0.432	
Plant type (PT)						
V	1.889		1.466		0.425	
V/N	1.952		1.523		0.428	
Growing season (GS)						
2018	1.906		1.484		0.423	
2019	1.935		1.505		0.430	
ANOVA (*df*)	**% Sum of the Squares**					
IS (1)	66.03	**	66.19	**	0.04	n.s.
PT (1)	0.89	n.s.	0.69	n.s.	0.74	n.s.
GS (1)	0.19	n.s.	0.09	n.s.	0.36	n.s.
IS*PT (1)	8.32	*	8.52	*	0.22	n.s.
IS*GS (1)	3.62	n.s.	4.10	n.s.	20.93	n.s.
PT*GS (1)	0.00	n.s.	0.67	n.s.	0.99	n.s.
IS*PT*GS (1)	2.48	n.s.	2.75	n.s.	0.53	n.s.
Residuals (16)	18.45		16.98		76.19	n.s.
Std. Dev. $^{(+)}$	0.174		0.171		0.067	n.s.

The mean values followed by the different lowercase letters in each column indicate significant differences at $p \leq 0.05$ using the LSD test. ** and * indicate significance at $p \leq 0.01$ and $p \leq 0.05$, respectively, n.s. no significant difference. $^{(+)}$ Standard deviation, calculated as the square root of the residual sum of squares. *df* degrees of freedom.

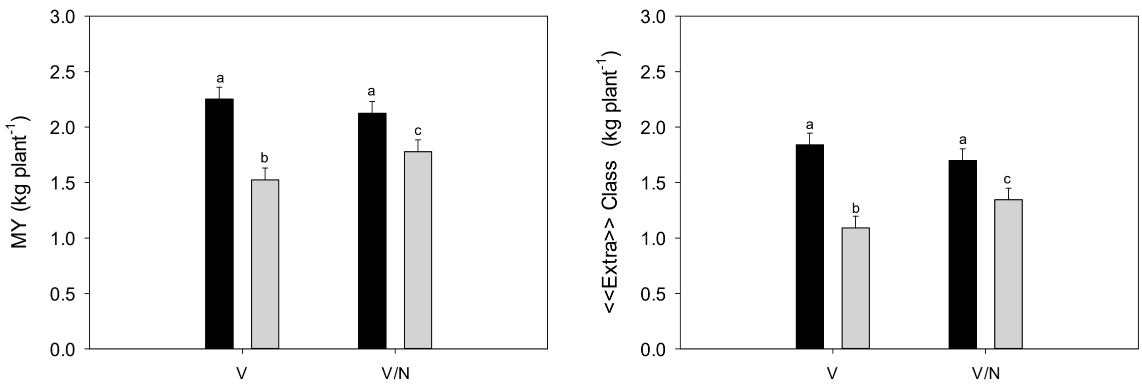

Figure 6. Marketable yield (MY, kg plant^{-1}) and «Extra» Class yield («Extra» Class, kg plant^{-1}) for the deficit irrigation plants (gray bars) and the control plants (black bars) in the ungrafted pepper plants (V) and the cultivar grafted onto NIBER® (V/N). Data are the mean values for n = 6. In each plant combination, different letters indicate significant differences at $p \leq 0.05$. Error bars represent the LSD value of the interaction.

The «Extra» class number of fruits per plant and average fruit weight were only affected by IS (Table 5) as the DI values lowered by 25% and 8%, respectively, in relation to the control. As previously mentioned, in 2018, no fruits were affected by BER, and were not affect by any analyzed factor in 2019 (Table S1).

Table 5. Effect of irrigation strategy, plant type, and growing season on fruit number of «Extra» Class (No. «Extra» Class) and average fruit weight of «Extra» Class (AFW «Extra» Class).

	No. «Extra» Class (fruits plant^{-1})		AFW «Extra» Class (g fruit^{-1})	
Irrigation strategy (IS)				
C	8.16	a	218.5	a
DI	6.06	b	201.0	b
Plant type (PT)				
V	7.11		204.6	
V/N	7.11		214.9	
Growing season (GS)				
2018	6.91		213.7	
2019	7.31		205.7	
ANOVA (*df*)	**% Sum of Squares**			
IS (1)	50.43	**	26.15	*
PT (1)	0.00	n.s.	9.07	n.s.
GS (1)	1.80	n.s.	5.41	n.s.
IS*PT (1)	8.06	n.s.	0.96	n.s.
IS*GS (1)	5.45	n.s.	0.04	n.s.
PT*GS (1)	0.08	n.s.	2.73	n.s.
IS*PT*GS (1)	4.26	n.s.	0.14	n.s.
Residuals (16)	29.93		55.49	
Std. Dev. (+)	0.99		15.6	

The mean values followed by the different lowercase letters in each column indicate significant differences at $p \leq 0.05$ using the LSD test. ** and * indicate significance at $p \leq 0.01$ and $p \leq 0.05$, respectively, n.s. no significant difference. (+) Standard deviation, calculated as the square root of the residual sum of squares. *df* degrees of freedom.

4. Discussion

For two consecutive years, our results demonstrated that grafting a pepper cultivar onto the new hybrid rootstock, NIBER®, can overcome the negative effects of sustained water stress in biomass and yield terms.

The water applied during both GS was similar because the meteorological conditions were comparable that, in turn, led to equivalent water requirements. Harvesting is, as usual, the longest growth stage, and it presents the maximum daily water requirements. This means having to apply the largest amount of water, which represents 58% of the total amount.

For both GS and IS, AWC maintained close to 100% from planting to the establishment, which indicates that the VSWC came close to FC. The reduction of AWC after the establishment was more marked for V/N-DI than for V-DI, in 2018, and was probably related to the fact that water uptake was greater in the grafted plants. Afterward, the AWC in the DI strategies dropped to 40% and 50% in 2018 and 2019, respectively. These values were lower than 55%, the value established by Fernández et al. [37] as the threshold AWC, below which pepper ET_c lowers in response to soil water deficit. Therefore, it can be stated that DI plants were water-stressed, and as the AWC for both plant types was similar during each GS, the stress level was similar for both PT. So, it can be hypothesized that the higher yield obtained in V/N in relation to V could be due to the grafted plants' better response to water stress conditions.

In fact, DI reduced both shoot and total dry weight by an average 20–22% for the DI plants (V/N-DI and V-DI) in relation to the control for both seasons, which is a similar decrease to that observed by López-Marín et al. [24], and comes close to the reduced plant yields reported by several studies for pepper crops [40,41] and tomato [42]. What is more, the NIBER® rootstock had a beneficial effect on MY and «Extra» Class by maintaining a bigger yield than the ungrafted plants under stress conditions. These results, together with those previously obtained with water stress tolerant rootstocks [29], confirm our hypothesis on NIBER® water stress tolerance, now under field conditions, and monitoring soil water content.

Fereres and Soriano [18] pointed out that when water stress increases in severity terms at a sustained DI, with biomass production losses over 40%, the HI can be affected in many crops. However, in the present study, the HI was not affected by either IS or PT, which indicates that yield was lowered by DI to a similar extent as vegetative biomass. Similar results have been obtained by Abdelkhalik et al. [41], who observed differences for the HI between treatments, but only when sever DI was applied, and these authors did not observe any differences for a moderate deficit.

The reduction in SDW between the deficit and control irrigation regimes in the grafted plants was much less marked than in the ungrafted plants, around 56% less. This indicates the ability of some rootstocks to overcome the effects of water stress, as previously found by López-Marín et al. [24]. According to Lee et al. [43], the root system of selected rootstocks is usually much larger and more vigorous than that of cultivars, which allows them to more efficiently absorb water and nutrients compared to ungrafted plants. The NIBER® rootstock has shown better root development in several experiments (Reference [44] and unpublished data). As stated before, water content monitoring in the soil in our experiments affirms the better response of the NIBER® rootstock to water stress in biomass formation and yield terms.

We also tested the physiological processes associated with grafting and DI by focusing on water and photosynthetic relations to explain the obtained agronomic results. Under well-irrigated conditions, plant water status ($\Psi_{predawn}$ and Ψ_{leaf}) was apparently similar between the V/N and V plants. Similar results have been reported in other studies with mini-watermelon, where the leaf water potential in both ungrafted and grafted plants showed comparable values upon optimal irrigation [23]. However, the use of pepper plants grafted onto a drought-tolerant rootstock had a significant influence on plant water transport capacity compared to the ungrafted plants. The hydraulic conductance of the entire plant in the control treatment under high atmospheric demand conditions (at noon) was lower in V/N-C, and was accompanied by lower g_s values. Changes in hydraulic properties and stomatal behavior enable grafted plants to better regulate the water used by the plant by reducing the water lost by transpiration, which improves leaf water use efficiency (A_N/E_{leaf} and A_N/g_s). This can be attributed to the differences in the root system's water absorption capacity, as found in grafted melon plants [45].

We observed that DI strongly influenced the plant water status both in the grafted and ungrafted plants, especially at the end of the experiment (114 DAT), which coincides with the fruit ripening stage, and a similar plant water stress level was obtained (based on $\Psi_{leaf} \approx -1.8$ MPa). The DI effects were also reflected in lower Kh_{plant}, which means that plant water transport capacity diminished compared to well-irrigated plants, and was similarly affected in the V and V/N plants. Changes in plant water relations affected g_s in both plant groups, with maximum stomatal regulation at midday. Stomatal limitation partially reduced the plant's water use, but also lowered the CO_2 assimilation rate similarly in both the V and V/N plants. In both plant groups, water stress had less impact on photosynthesis than in g_s and E_{leaf}, which indicates that stomatal closure occurred earlier than CO_2 fixation [26,46], and consequently and similarly enhanced water use efficiency (A_N/E_{leaf} and A_N/g_s).

The physiological alterations generated by DI significantly reduced yield and biomass production in both plant groups (V and V/N). This reduction has been associated mainly with changes in all the photosynthetic parameters and water relations, as observed in different crops like mini-watermelon [23], tomato [47] or pepper [24,27,29] when comparing grafted vs. ungrafted plants. Nevertheless, the highest fruit yields and SDW under DI were recorded in the grafted plants (V/N-DI) compared to the V-DI plants, despite no significant differences being observed between them from the physiological point of view. The greater SDW reduction in the V-DI plants (25.7%) versus V/N-DI (14.5%), could lead to a more marked decrease in the intercepted radiation in the V-DI plants and could, thus, reduce the whole canopy photosynthesis in relation to V/N-DI. Although root biomass was not monitored in this experiment, SDW decreased in V-DI plants, and this could be explained by differences in biomass partitioning between the aerial part and roots. On the other hand, the capacity to maintain sink activity in the vegetative V/N-DI parts could be an adaptive advantage that leads to more shoot growth and bigger yields through regulating source-sink relations [24] with DI. Other mechanisms not associated to maintenance photosynthetic activity have been described to explain the better biomass and yield performance in grafted plants under DI as osmotic adjustment [48], modulation of antioxidant defenses [49] or hormonal signaling [50].

5. Conclusions

These results confirm that using appropriate rootstocks in pepper crops is a good strategy to better tolerate sustained DI, which promotes water stress in pepper, compared to ungrafted plants. This is a consequence of an adaptation morphology and physiology being capable of maintaining photosynthesis levels, and consequently, biomass production. They also confirm that hybrid NIBER® is an interesting rootstock for this purpose.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/10/1529/s1, Figure S1: Temperature (°C) and relative humidity (%) values for 2018 (black line) and 2019 (red line). Table S1: Effect of irrigation strategy and plant type on BER yield (BER) and the fruit number of BER (No. BER).

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References

1. Food and Agriculture Organization. Faostat, Food and Agriculture Data. 2018. Available online: http://www.fao.org/faostat/es/#home (accessed on 28 July 2020).
2. Mancosu, N.; Snyder, R.; Kyriakakis, G.; Spano, D. Water Scarcity and Future Challenges for Food Production. *Water* **2015**, *7*, 975–992. [CrossRef]
3. Chai, Q.; Gan, Y.; Zhao, C.; Xu, H.L.; Waskom, R.M.; Niu, Y.; Siddique, K.H.M. Regulated deficit irrigation for crop production under drought stress. A review. *Agron. Sustain. Dev.* **2016**, *36*, 1–21. [CrossRef]
4. Turral, H.; Burke, J.; Faures, J.M.; Faures, J.M. *Climate Change, Water and Food Security*; Food and Agriculture Organization: Rome, Italy, 2011; ISBN 9251067953.
5. IPPC Intergovernmental Panel on Climate Change (IPCC). Available online: https://www.ipcc.ch/ (accessed on 28 July 2020).
6. Kahil, M.T.; Dinar, A.; Albiac, J. Modeling water scarcity and droughts for policy adaptation to climate change in arid and semiarid regions. *J. Hydrol.* **2015**, *522*, 95–109. [CrossRef]
7. Iglesias, A.; Garrote, L. Adaptation strategies for agricultural water management under climate change in Europe. *Agric. Water Manag.* **2015**, *155*, 113–124. [CrossRef]
8. Turner, N.C. Agronomic options for improving rainfall-use efficiency of crops in dryland farming systems. *J. Exp. Bot.* **2004**, *55*, 2413–2425. [CrossRef]
9. Daccache, A.; Ciurana, J.S.; Rodriguez Diaz, J.A.; Knox, J.W. Water and energy footprint of irrigated agriculture in the Mediterranean region. *Environ. Res. Lett.* **2014**, *9*, 124014. [CrossRef]
10. Galindo, A.; Collado-González, J.; Griñán, I.; Corell, M.; Centeno, A.; Martín-Palomo, M.J.; Girón, I.F.; Rodríguez, P.; Cruz, Z.N.; Memmi, H.; et al. Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems. *Agric. Water Manag.* **2018**, *202*, 311–324. [CrossRef]

11. Food and Agriculture Organization Aquastat. *AQUASTAT-FAO's Global Information System on Water and Agriculture*; Food and Agriculture Organization: Rome, Italy, 2018; Available online: http://www.fao.org/nr/water/aquastat/data/query/index.html?lang=en (accessed on 18 August 2020).
12. WWAP (United Nations World Water Assessment Programme). *The United Nations World Water Development Report 2015: Water for a Sustainable World*; United Nations Educational, Scientific and Cultural Organization: Paris, France, 2015.
13. Molden, D.; Oweis, T.; Steduto, P.; Bindraban, P.; Hanjra, M.A.; Kijne, J. Improving agricultural water productivity: Between optimism and caution. *Agric. Water Manag.* **2010**, *97*, 528–535. [CrossRef]
14. Levidow, L.; Zaccaria, D.; Maia, R.; Vivas, E.; Todorovic, M.; Scardigno, A. Improving water-efficient irrigation: Prospects and difficulties of innovative practices. *Agric. Water Manag.* **2014**, *146*, 84–94. [CrossRef]
15. Kang, S.; Hao, X.; Du, T.; Tong, L.; Su, X.; Lu, H.; Li, X.; Huo, Z.; Li, S.; Ding, R. Improving agricultural water productivity to ensure food security in China under changing environment: From research to practice. *Agric. Water Manag.* **2017**, *179*, 5–17. [CrossRef]
16. Ruiz-Sanchez, M.C.; Domingo, R.; Castel, J.R. Deficit irrigation in fruit trees and vines in spain. *Spanish J. Agric. Res.* **2010**, *8*, 5–20. [CrossRef]
17. Farooq, M.; Wahid, A.; Kobayashi, N.; Fujita, D.; Basra, S.M.A. Plant drought stress: Effects, mechanisms and management. *Agron. Sustain. Dev.* **2009**, *29*, 185–212. [CrossRef]
18. Fereres, E.; Soriano, M.A. Deficit irrigation for reducing agricultural water use. *J. Exp. Bot.* **2007**, *58*, 147–159. [CrossRef] [PubMed]
19. Colla, G.; Rouphael, Y.; Leonardi, C.; Bie, Z. Role of grafting in vegetable crops grown under saline conditions. *Sci. Hortic. (Amsterdam)* **2010**, *127*, 147–155. [CrossRef]
20. Huang, Y.; Bie, Z.; He, S.; Hua, B.; Zhen, A.; Liu, Z. Improving cucumber tolerance to major nutrients induced salinity by grafting onto *Cucurbita ficifolia*. *Environ. Exp. Bot.* **2010**, *69*, 32–38. [CrossRef]
21. Martínez-Ballesta, M.C.; Alcaraz-López, C.; Muries, B.; Mota-Cadenas, C.; Carvajal, M. Physiological aspects of rootstock-scion interactions. *Sci. Hortic. (Amsterdam)* **2010**, *127*, 112–118. [CrossRef]
22. Sánchez-Rodríguez, E.; Romero, L.; Ruiz, J.M. Accumulation on free polyamines enhanced antioxidant response in fruit of grafting tomato plants under water stress. *J. Plant Physiol.* **2016**, *190*, 72–78. [CrossRef]
23. Rouphael, Y.; Cardarelli, M.; Colla, G.; Rea, E. Yield, Mineral Composition, Water Relations, and Water Use Efficiency of Grafted Mini-watermelon Plants Under Deficit Irrigation. *HortScience* **2008**, *43*, 730–736. [CrossRef]
24. López-Marín, J.; Gálvez, A.; del Amor, F.M.; Albacete, A.; Fernández, J.A.; Egea-Gilabert, C.; Pérez-Alfocea, F. Selecting vegetative/generative/dwarfing rootstocks for improving fruit yield and quality in water stressed sweet peppers. *Sci. Hortic. (Amsterdam)* **2017**, *214*, 9–17. [CrossRef]
25. Penella, C.; Nebauer, S.G.; López-Galarza, S.; San Bautista, A.; Rodríguez-Burruezo, A.; Calatayud, A. Evaluation of some pepper genotypes as rootstocks in water stress conditions. *Hortic. Sci.* **2014**, *41*, 192–200. [CrossRef]
26. López-Serrano, L.; Penella, C.; Bautista, A.S.; López-Galarza, S.; Calatayud, A. Physiological changes of pepper accessions in response to salinity and water stress. *Spanish J. Agric. Res.* **2017**, *15*. [CrossRef]
27. Penella, C.; Nebauer, S.G.; Bautista, A.S.; López-Galarza, S.; Calatayud, Á. Rootstock alleviates PEG-induced water stress in grafted pepper seedlings: Physiological responses. *J. Plant Physiol.* **2014**, *171*, 842–851. [CrossRef] [PubMed]
28. Penella, C.; Nebauer, S.G.; López-Galarza, S.; Quiñones, A.; San Bautista, A.; Calatayud, Á. Grafting pepper onto tolerant rootstocks: An environmental-friendly technique overcome water and salt stress. *Sci. Hortic. (Amsterdam)* **2017**, *226*, 33–41. [CrossRef]
29. López-Serrano, L.; Canet-Sanchis, G.; Selak, G.V.; Penella, C.; Bautista, A.S.; López-Galarza, S.; Calatayud, Á. Pepper rootstock and scion physiological responses under drought stress. *Front. Plant Sci.* **2019**, *10*, 1–13. [CrossRef] [PubMed]
30. Maroto, J.V. *Horticultura Herbácea Especial*; Mundi-Prensa: Madrid, Spain, 2002.
31. Allen, R.G.; Pereira, L.S.; Raes, D. *Crop Evapotranspiration-Guidelines for Computing Crop Water Requirements-FAO Irrigation and Drainage Paper 56 Table of Contents*; Food and Agriculture Organization: Rome, Italy, 1998.
32. Bonachela, S.; González, A.M.; Fernández, M.D. Irrigation scheduling of plastic greenhouse vegetable crops based on historical weather data. *Irrig. Sci.* **2006**, *25*, 53–62. [CrossRef]

33. Orgaz, F.; Fernández, M.D.; Bonachela, S.; Gallardo, M.; Fereres, E. Evapotranspiration of horticultural crops in an unheated plastic greenhouse. *Agric. Water Manag.* **2005**, *72*, 81–96. [CrossRef]
34. Veihmeyer, F.J.; Hendrickson, A.H. The moisture equivalent as a measure of the field capacity of soils. *Soil Sci.* **1931**, *32*, 181–193. [CrossRef]
35. Thompson, R.B.; Gallardo, M.; Agüera, T.; Valdez, L.C.; Fernández, M.D. Evaluation of the Watermark sensor for use with drip irrigated vegetable crops. *Irrig. Sci.* **2006**, *24*, 185–202. [CrossRef]
36. Richards, L.A. Physical conditions of water in soils. In *Methods of Soil Analysis: Part 1 Physical and Mineralogical Properties*; Including Statistics of Measurement and Sampling; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 1965; pp. 128–152.
37. Fernández, M.D.; Gallardo, M.; Bonachela, S.; Orgaz, F.; Thompson, R.B.; Fereres, E. Water use and production of a greenhouse pepper crop under optimum and limited water supply. *J. Hortic. Sci. Biotechnol.* **2005**, *80*, 87–96. [CrossRef]
38. Van Den Honert, T.H. Water transport in plants as a catenary process. *Discuss. Faraday Soc.* **1948**, *3*, 146–153. [CrossRef]
39. Ofcial Journal of the European Union (2011) Commission Implementing Regulation (EU) No 543/2011 of 7 June 2011 Laying Down Detailed Rules for the Application of Council Regulation (EC) No 1234/2007 in Respect of the Fruit and Vegetables and Processed Fruit and Vegetables Sectors. Part 8: Marketing Standard for Sweet Peppers. Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:157:0001:0163:EN:PDF (accessed on 28 July 2020).
40. Sezen, S.M.; Yazar, A.; Tekin, S. Physiological response of red pepper to different irrigation regimes under drip irrigation in the Mediterranean region of Turkey. *Sci. Hortic. (Amsterdam)* **2019**, *245*, 280–288. [CrossRef]
41. Abdelkhalik, A.; Pascual, B.; Nájera, I.; Domene, M.A.; Baixauli, C.; Pascual-Seva, N. Effects of deficit irrigation on the yield and irrigation water use efficiency of drip-irrigated sweet pepper (*Capsicum annuum* L.) under Mediterranean conditions. *Irrig. Sci.* **2020**, *38*. [CrossRef]
42. Pogonyi, Á.; Pék, Z.; Helyes, L.; Lugasi, A. Effect of grafting on the tomato's yield, quality and main fruit components in spring forcing. *Acta Aliment.* **2005**, *34*, 453–462. [CrossRef]
43. Lee, J.M.; Kubota, C.; Tsao, S.J.; Bie, Z.; Echevarria, P.H.; Morra, L.; Oda, M. Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Sci. Hortic. (Amsterdam)* **2010**, *127*, 93–105. [CrossRef]
44. López-Serrano, L.; Canet-Sanchis, G.; Selak, G.V.; Penella, C.; San Bautista, A.; López-Galarza, S.; Calatayud, Á. Physiological characterization of a pepper hybrid rootstock designed to cope with salinity stress. *Plant Physiol. Biochem.* **2020**, *148*, 207–219. [CrossRef]
45. Agele, S.; Cohen, S. Effect of genotype and graft type on the hydraulic characteristics and water relations of grafted melon. *J. Plant Interact.* **2009**, *4*, 59–66. [CrossRef]
46. Delfine, S.; Tognetti, R.; Loreto, F.; Alvino, A. Physiological and growth responses to water stress in field-grown bell pepper (*Capsicum annuum* L.). *J. Hortic. Sci. Biotechnol.* **2002**, *77*, 697–704. [CrossRef]
47. Nilsen, E.T.; Freeman, J.; Grene, R.; Tokuhisa, J. A Rootstock Provides Water Conservation for a Grafted Commercial Tomato (*Solanum lycopersicum* L.) Line in Response to Mild-Drought Conditions: A Focus on Vegetative Growth and Photosynthetic Parameters. *PLoS ONE* **2014**, *9*, e115380. [CrossRef]
48. Munns, R. Why measure osmotic adjustment? *Aust. J. Plant Physiol.* **1988**, *15*, 717–726. [CrossRef]
49. Sánchez-Rodríguez, E.; Rubio-Wilhelmi, M.D.M.; Blasco, B.; Leyva, R.; Romero, L.; Ruiz, J.M. Antioxidant response resides in the shoot in reciprocal grafts of drought-tolerant and drought-sensitive cultivars in tomato under water stress. *Plant Sci.* **2012**, *188–189*, 89–96. [CrossRef]
50. Gaion, L.A.; Monteiro, C.C.; Cruz, F.J.R.; Rossatto, D.R.; López-Díaz, I.; Carrera, E.; Lima, J.E.; Peres, L.E.P.; Carvalho, R.F. Constitutive gibberellin response in grafted tomato modulates root-to-shoot signaling under drought stress. *J. Plant Physiol.* **2018**, *221*, 11–21. [CrossRef] [PubMed]