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Partial Root-Zone Drying and Deficit Irrigation Effect on Growth, Yield, Water Use and Quality of Greenhouse Grown Grafted Tomato

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Abstract: The tomato is an important horticultural crop, the cultivation of which is often under influence of abiotic and biotic stressors. Grafting is a technique used to alleviate these problems. Shortage of water has stimulated the introduction of new irrigation methods: deficit irrigation (DI) and partial root-zone drying (PRD). This study was conducted in two spring–summer season experiments to evaluate the effects of three irrigation regimes: full irrigation (FI), PRD and DI on vegetative growth, leaf gas-exchange parameters, yield, water-use efficiency (WUE), nutrients profile and fruit quality of grafted tomatoes. In both years, the commercial rootstocks Emperador and Maxifort were used. In the first year, the scion cultivar Clarabella was grown on one stem and in the second year the cultivar Attiya was grown on two stems. Self-grafted cultivars were grown as a control. In both experiments, higher vegetative traits (leaf area and number, height, shoot biomass) were recorded in tthe plants grafted on commercial rootstocks. The stomatal conductance and transpiration rate were higher under FI. Under DI, transpiration was lowest and photosynthetic WUE was highest. Photosynthetic rate changed between irrigation treatments depending on plant type. In both years, the total yield was highest in grafted plants as result of more and bigger fruits per plant. In the 2nd year, grafted plants under FI had higher yield compared to PRD, but not to DI, while self-grafted plants did not differ between irrigation treatments. WUE was highest in DI and PRD treatments and in grafted plants. Leaf N, P, K and Ca was highest in tthe plants grafted on Emperador and Maxifort, while more Mg was measured in self-grafted plants. More Ca and Mg were recorded in tthe plants under DI and PRD. Fruit mineral concentrations were higher in tthe plants grafted on commercial rootstocks. Total soluble solids differed between irrigation regarding plant types, while fruit total acidity was higher in Emperador and Maxifort. In conclusion, our study showed that grafted plants could be grown under DI with minor yield reduction with 30–40% less water used for irrigation. Moderate DI could be used before PRD for cultivation of grafted tomato and double stemmed plants did not show negative effect on tomato yield so it can be used as standard under reduced irrigation.

Keywords: reduced irrigation; rootstocks; yield traits; leaf gas exchange

1. Introduction

The tomato (*Solanum lycopersicum* L.) is a leading vegetable and one of the most important horticultural crops. The world production of tomatoes is second to only potatoes, with an estimated production of 160 million tons [1]. Tomato production problems with abiotic and biotic stressors as results of intensive monoculture often create problems in tomato production. Tomato production losses caused by unfavorable growing conditions can be reduced by grafting onto specific rootstocks. Commercial vegetable grafting started at the beginning of the 20th century, with the primary intention to achieve tolerance to soil pathogens [2]. The substantial proportion of total tomato production in Europe and Asia currently include usage of grafted tomatoes [3]. In addition, the widespread use of grafting was expected to improve crop response to water, salt, nutrient deficiency and temperatures stresses and to improve fruit quality [4,5].

Water availability is decreasing worldwide—especially where agriculture uses between 50% and 90% of all water, such as semi-arid Mediterranean areas. In the context of climate change, improvement of irrigation practices is needed, by use of new sources (e.g., wastewater) or by application of new irrigation techniques [6]. Two main methods regarding the use of reduced irrigation are introduced, namely deficit irrigation (DI) and partial rootzone-drying (PRD) to improve water-use efficiency (WUE). These practices expose the plant to moderate drought stress, could increase abscisic acid (ABA) levels that leads to greater increase in WUE [7]. DI supplies less water to the entire rootzone than the amount lost by evapotranspiration, while PRD involves alternate wetting and drying of the root zones. PRD has been shown to improve DI and has resulted in substantial water savings, improved water-use efficiency and it is superior to DI in terms of yield maintenance in greenhouse or processing tomato [8,9].

Grafted plants showed better uptake of minerals and water than un-grafted plants due to vigorous root growth by the chosen rootstock [10]. Grafted tomato under deficit irrigation showed increased yield and WUE [11,12]. The tomato cultivar Boludo grafted on 144 tomato rootstocks showed higher shoot fresh weight under water deficit in 38% of combinations [13]. Tomato cultivars Belle and Clarabelle grafted on the rootstock He-man had similar vegetative growth and yield under PRD conditions in commercial greenhouse [14]. The breeding of commercial rootstocks like 'Maxifort' on the other hand was more directed to increase growth capacity and to alleviate soilborne diseases, instead of water economy [2], although it showed similar performance compared to drought tolerant cultivars [15]. Sink limitation is more pronounced in grafted tomato plants compared to non-grafted ones. One of the ways to avoid sink limitations is growing double-stemmed grafted plants. Grafting with two stems became the standard growing method for sustaining tomato production, as it decreased the costs per unit area by reducing the number of plants grown in a greenhouse by one-half [16,17].

The effects of grafting on tomato fruit quality are showing inconsistencies in of the results, mostly affected by the rootstock–scion combination [5]. Similarly, scarce reports showed that grafted plants under water stress differ in total soluble solids (TSS) and total acidity (TA) [11,14,18].

In the first study, we evaluated the effect of PRD on the growth, yield and quality of grafted tomato grown in a commercial greenhouse [14]. As proper evaluation of reduced irrigation methods apart PRD include DI, the two-year studies included the deficit irrigation treatments with similar amount of water applied as in PRD, but evenly to the whole root system. Since two stems are the standard practice for cultivation of grafted tomato, we also included stem number as a factor in our experiments. Finally, the purpose of our studies was to compare the responses of grafted tomato plants subjected to PRD or DI by evaluating vegetative growth, leaf gas-exchange parameters, yield traits, WUE and leaf and fruit mineral profile and fruit quality in a greenhouse located in a Mediterranean climate.

2. Material and Methods

Two experiments were conducted on tomatoes in spring–summer season in commercial greenhouses in the Split area (Mediterranean area of Croatia). The first experiment used plants grown on one stem, and in the second experiment two-stem plants were used.

2.1. Experiment I: Single Stem Plants (2016)

The first experiment was established in an unheated greenhouse in the Trogir (43°31′ N, 16°15′ E) used for intensive vegetable production for many years. The soil type was an alkaline clay with 8.09 pH

(H_2O), 7.51 pH (KCl), 4.4% soil organic matter and 120 mg of available P_2O_5, and 48 mg K_2O/100 g of soil. The greenhouse had side ventilation and the roof was 3 m high.

Tomato (*Solanum lycopersicum* L., cv. Clarabella F1, Rijk Zwaan, The Netherlands) plants were either self-grafted or grafted onto the rootstocks "Emperador" (*S. lycopersicum* × *S. habrochaites*, Rijk Zwaan) or "Maxifort" (*S. lycopersicum* × *S. habrochaites*, De Ruiter Seeds, Amsterdam North, The Netherlands). Both rootstocks, as noted by the seed companies, have high to medium vigor and are resistant to *Fusarium*, *Verticillium* and ToMV.

The scion seeds were sown on 17 January 2016 and rootstock seeds on 20 January 2016 in polystyrene plug trays with cell volume of 40 mL in an organic substrate (Brill Type 4, Brill Substrate, Georgsdorf, Germany). As scion and rootstocks have variable growth vigor and to ensure optimum stem diameter between scion and rootstock seedlings at grafting time the scion seeds were sown 3 days earlier than the rootstock. The trays with sown seeds were put in a heated greenhouse (day/night 25 °C).

The cv. 'Clarabella' seedlings were self-grafted and grafted onto both rootstocks at 25 days after sowing using the "splice grafting" method. Grafted seedlings were maintained under reduced light conditions (10% of the daily light intensity) at a relative humidity above 95% and temperature from 22 °C to 25 °C until callus formation. After callus formation, the seedlings were maintained as standard tomato transplant. Seedlings were grown in research greenhouse at the Institute for Adriatic Crops (Split, Croatia).

Tomato seedlings with four to five true leaves were transplanted 65 days after sowing (24 March 2016) in a two-row system (90 cm apart) with rows 60 cm apart with plants were spaced 50 cm in each row, for a total of 2.7 plants/m^2. The plants were drip irrigated with drippers (pressure-compensating emitters) set in opposite lines of row plants (15 cm from plants). After transplanting, plants were irrigated per the standard practice in the area. During the trial, plants were fertilized twice with Mg, as other nutrients had high available soil concentrations, as confirmed by N and K petiole sap analysis during growth phases.

Three irrigation treatments were started 30 days after transplanting: full irrigation (FI), deficit irrigation (DI) and partial-root zone drying (PRD). The soil moisture content was measured by tensiometers (Blumat digital, Leingarten, Germany) which were laboratory calibrated for conducted soil. The tensiometers were placed at 25–30 cm depth. In FI treatment, plants were watered to a soil moisture content of 65–75% of field capacity. DI treatments received 50% water used in FI by using drippers with half capacity than in FI. With PRD, half of the root system was irrigated at 65–75% of field water capacity (FWC), while other half of the roots were dried until soil moisture reached 35–40% of field capacity and then irrigation was shifted between two sides of the root system. PRD also received 50% water from FI. Different irrigation treatments were divided by placing PE folia to the depth of 45–50 cm to stop horizontal water movement. Taking into account water supplied before start of irrigation treatment, in total DI and PRD got 60% water supplied to FI that received 214 L per plant.

The plant height and the number of leaves (longer than 5 cm) were determined for 7 weeks after transplanting. Harvest started 70 days after transplanting (DAT) and lasted for 55 days—including 12 harvests of fruits as they matured (light red color) The average fruit weight and fruit number were recorded. The first four harvests were calculated as early yield. On the day of the last harvest, the aboveground parts of the subsample plants (3 plants per treatment) were removed, divided into leaves, stems and green fruits and weighed for fresh biomass (FM). After measuring the leaf area with leaf area meter LI-3000 (LI-COR, Bad Homburg, Germany), samples were put into an oven and dried at 70 °C to constant weight to obtain the DM. The yield divided by supplied water was used to find the yield WUE (WUEy).

2.2. Experiment II: Double Stem Plants (2017)

The greenhouse in the split was used for the second experiment (43°30′ N, 16°30′ E). The soil type was a clay loam with 8.71 pH (H_2O), 7.46 pH (KCl), 2.9% soil organic matter, 16.5% high active lime and 27 mg of available P_2O_5 and 31 mg K_2O/100 g of soil. Same rootstocks were used as in

Experiment I, and scion cultivar was Attiya (Rijk Zwaan, De Lier, the Netherlands) due to resistance to TSWV (Tomato spotted wilt virus) that influenced growth and yield in previous years on different tomato cultivars in used greenhouse. The scion and rootstocks seeds were sown on 19 January 2016 and 23 January 2016, respectively. Grafting was done on 17 February 2016; all other procedures for seedling production were done as in previous year. In this experiment, two types of seedlings were produced: self-grafted Attiya grown on one stem (ATT) and plants grown on two stems: self-grafted Attiya (AT), Attiya grafted on Emperador (EM) and Attiya grafted on Maxifort (MX), which in total gave four plant types. Two-stemmed seedlings were formed from side-shoots of cotyledons.

The tomato seedlings were transplanted 55 days after sowing (16 March 2016) in two systems: one stem seedlings as in previous years in a two-row system and two-stem seedlings in a one-row system with rows 120 cm apart. In each row, the plants were spaced 50 cm, which in both systems gave 2.7 stems/m^2.

Irrigation treatments started 50 DAT and included FI, DI and PRD. In this year, the soil moisture content was controlled by Maxi Rain soil moisture sensors (Elektronik Jeske, Windorf, Germany), which were set up to open electromagnetic valve when soil moisture was lower than 65% field water capacity and was irrigated until reached 80% FWC. DI treatment received 60% water supplied to FI using drippers with lower capacity. To better measure water needs in the PRD, this treatment had its own sensors for controlling irrigation and switching sides. The PRD used 50% of the water used in FI. In total—including water applied before irrigation treatment start—the DI used 70% and PRD 65% water of the FI. The FI in total received 233 L/plant, DI 170 L/plant and PRD 153 L/plant. House-made lysimeters (60 cm × 100 cm × 5 cm) were put below every irrigation treatment at depth of 60 cm to control possible leaching if plants were over-irrigated.

Plants were fertilized by irrigation system with N, K and Mg, depending on growth phases and plant needs. Plant height and leaf number were measured each week. Harvest started at 80 DAT (7 June) and lasted 45 days, consisting of 11 harvests. At the end, plants were divided to determine biomass partitioning, as in previous year.

Leaf nutrient concentrations were determined in the youngest fully developed leaves after the leaves were dried at 70 °C and then ground. The micro-Kjeldahl digestion method (Kjeltec System 1026, Tecator, Höganas, Sweden) was used to measure total leaf N concentration. Dry ash of grounded samples from a muffle furnace were dissolved in 2 mL HCl to extract the P, K, Ca and Mg. The K concentration was measured using a flame photometer (Model 410, Sherwood Scientific, Cambridge, UK). The vanadate-molybdate yellow color method using a UV-visible spectrophotometer was used to determine the P concentration (Cary 50 Scan, Varian, Palo Alto, CA, USA) at 420 nm. The Ca and Mg in solution were determined by atomic absorption spectrometry (SpectrAA 220, Varian, Palo Alto, CA, USA).

The quality parameters of fruits from each treatment were analyzed in the second experiment. For the tomato juice, the total soluble solids (TSS) content was determined by a DR 201–95 refractometer (Kruss optronic, Hamburg, Germany) and expressed in Brix at 20 °C. Acidity was determined by juice titration with 0.1-M NaOH was used for determination of acidity and results were expressed as citric acid. Gas-exchange parameters were measured using LI-6400 infrared gas analyzer (LI-COR, Inc., Lincoln, NE, USA) in youngest fully expanded leaves. Measurements were performed on six leaves per treatment 20 days after different irrigation techniques were applied in whole experiment. Measurements were conducted under constant light (PAR 750 μmol m^{-2} s^{-1}) and CO$_2$ concentration (400 μmol mol^{-1}). The environmental conditions in the greenhouse ranged from 22 °C to 33 °C for air temperature and from 33% to 42% for relative humidity (RH). The greenhouse light conditions (PAR) ranged from 300 to 1100 μmol m^{-2} s^{-1}. The transpiration rate (E) and photosynthetic rate (A) were determined from gas exchange measurements and were used to determine the photosynthetic/instantaneous water-use efficiency (PWUE) as the ratio between A and E [19].

The experiments were set up in a randomized block design, consisting of three replications. Each treatment (irrigation × plant type) was comprised of 12 plants. The data were evaluated by

ANOVA and when F-tests were significant, the means of the main factors (rootstock/plant type and irrigation technique) and their interactions were compared using the least significant difference test at $p \leq 0.05$. The data were statistically analyzed using StatView ver. 5.0 (SAS Institute, Cary, CA, USA).

3. Results

3.1. Plant Growth and Biomass Production

In the first year of the experiment, plants grown on one stem showed significant differences in vegetative parameters regarding rootstock type used (Table 1). Plants grafted on Emperador rootstock had highest leaf area, number of leaves, plant height and leaf and shoot dry biomass (DM) compared to self-grafted plants. The irrigation method and interaction between irrigation and graft did not show significant differences.

Table 1. Effect of rootstock type and irrigation treatment on tomato vegetative characteristics in the first year of the experiment with plants grown on one stem.

Treatments	Leaf			Shoot	Plant Height (70 DAT) (cm)
	Number (70 DAT)	Area (cm^2 plant^{-1})	DM (g plant^{-1})	DM (g plant^{-1}) *	
Rootstock (R)					
Self-grafted	24.4 b [†]	10,406 b	108.1 b	183.7 b	172.5 b
Emperador	26.7 a	19,117 a	188.6 a	296.9 a	184.1 a
Maxifort	26.2 ab	16,521 a	158.1 ab	248.7 ab	177.7 ab
Irrigation (I)					
Full	25.3	15,621	149.9	245.9	175.2
Deficit	26.7	14,990	144.4	234.1	180.8
PRD	26.2	15,433	160.5	249.2	178.3
I × R	ns	ns	ns	ns	ns

* DM—dry mass; [†] Significant differences between treatments (LSD test at $p \leq 0.05$) are indicated with different letters within columns. ns—non-significant.

In the second year, statistical analyses for vegetative growth are presented in Table 2. Results are presented per stem for two-stemmed grafted plants with plants of one stem. Similar to the first experiment year, most measured traits were significantly affected by rootstock type. Additionally, the leaf number showed differences in irrigation technique applied. Leaf and shoot DM per stem was significantly highest in Emperador and lowest in Attiya. No difference in these traits were found between one-stem Attiya and Maxifort. It can be concluded that Maxifort produces two times more DM per whole plant.

Table 2. Effect of rootstock type and irrigation treatment on tomato vegetative characteristics in the second year of the experiment with plants grown on one and two stem.

Treatments	Leaf			Shoot	Plant Height (60 DAT) (cm)
	Number (60 DAT)	Area (cm^2 plant^{-1})	DM (g stem^{-1})	DM (g stem^{-1})	
Rootstock (R)					
One-stem SG *	25.8 b [†]	14,039 a	118.5 b	177.8 b	166.2 b
Two-stem SG	22.2 c	7758 b	77.3 c	124.4 c	154.8 b
Emperador	27.3 a	11,669 a	147.8 a	252.4 a	199.8 a
Maxifort	26.7 ab	13,916 a	117.5 b	195.5 b	192.8 a
Irrigation (I)					
Full	25.5 ab	12,308	117.3	189.8	178.1
Deficit	26.1 a	12,064	111.2	178.3	181.6
PRD	24.8 b	11,164	117.3	194.6	177.9
I × R	ns	ns	ns	ns	ns

* SG—self-grafted plants; DM—dry mass; [†] significant differences between treatments (LSD test at $p \leq 0.05$) are indicated with different letters within columns. ns—non-significant.

3.2. Leaf Gas Exchange

The leaf gas-exchange parameters measured 20 days after initiation of reduced irrigation treatments (DI + PRD) are in Table 3. Stomatal conductance (gs), intercellular CO_2 (Ci), transpiration rate (E) and photosynthetic WUE (PWUE) were significantly affected by irrigation rate. The values of gs, Ci and E were highest under FI and lowest under DI treatment, while PWUE had highest value under DI that differed from FI and PRD. In addition, only stomatal conductance differed ($p \leq 0.05$) between rootstocks, with plants grafted on Emperador having the highest values. The effect of the interaction of the rootstock/plant type × irrigation was recorded for photosynthetic rate (A), photosynthetic WUE (Figure 1A,B) and intercellular CO_2 (data not shown). The highest A was measured for one stem self-grafted Attiya under DI, while lowest was found for same plants under FI. On average, PWUE was highest at DI and as shown by interaction did not differed between plant types under DI, while differences in two other irrigation treatments was influenced by rootstock.

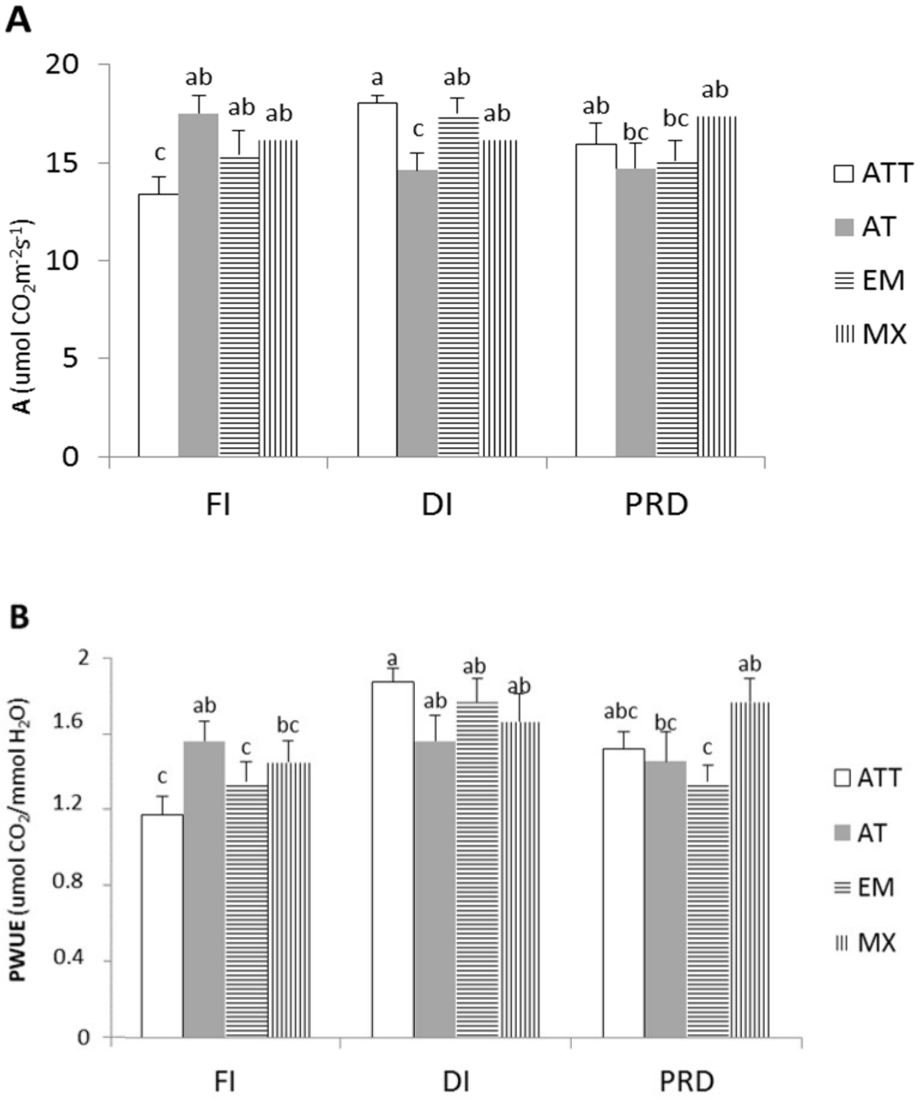

Figure 1. Photosynthetic rate (**A**,**B**) photosynthetic water-use efficiency—PWUE of grafted tomato plants grown with one or two stems under three irrigation techniques. Vertical bars represent SE values ($n = 6$). a—significant difference by the LSD test at $p \leq 0.05$ are indicated by different letters above column; ATT—one-stem Attiya; AT—two-stem Attiya; EM—Emperador, MX—Maxifort.

Table 3. Leaf gas-exchange parameters and photosynthetic water-use efficiency of grafted tomatoes grown with one and two stems under three irrigation techniques in the second year of the experiment.

Treatments	Photosynthetic Rate (A) (µmol CO_2 m^{-2} s^{-1})	Stomatal Conductance (gs) (mol H_2O m^{-2} s^{-1})	Intercellular CO_2 (Ci) (µmol CO_2 mol^{-1})	Transpiration Rate (E) (mmol H_2O m^{-2} s^{-1})	Photosynthetic WUE (PWUE) (µmol CO_2/mmol H_2O)
Rootstock (R)					
One-stem SG	15.8*	0.81 b	336.8	10.9	1.45
Two-stem SG	15.6	0.85 b	339.5	10.8	1.44
Emperador	16.0	0.92 a	341.5	11.4	1.40
Maxifort	16.6	0.84 b	335.9	10.7	1.55
Irrigation (I)					
Full	15.8	0.93 a	342.0 a	11.7 a	1.35 b
Deficit	16.5	0.79 b	334.7 b	10.1 c	1.63 a
PRD	15.8	0.85 b	338.7 ab	10.9 b	1.45 b
I × R	*	ns	*	ns	*

* Significant differences between treatments (LSD test at $p \leq 0.05$) are indicated with different letters within columns. ns—non-significant.

3.3. Yield and Water-Use Efficiency

The effects of rootstock type and irrigation technique on tomato yield traits and WUE in the first experiment are presented in Table 4, Average fruit weight, fruit number per plant and total yield were affected by rootstock and were higher in grafted plants than in self-grafted ones. Early yield was significantly affected by irrigation technique and highest values were noted for DI plants differing only with plants cultivated under PRD regime. WUE was affected by rootstock and irrigation. As expected, plants under DI and PRD had almost double values of WUE. Emperador and Maxifort plants significantly differed from self-grafted ones and on average had 40% higher WUE values.

Table 4. Yield parameters and water-use efficiency of grafted tomato plants grown with one stem under three irrigation techniques in the first year of the experiment.

Treatments	Fruit Mean Weight (g)	Number $Plant^{-1}$	Early Yield $Plant^{-1}$ (g)	Yield $Plant^{-1}$ (g)	WUEy (g L^{-1})
Rootstock (R)					
Self-grafted	243 b *	19.3 b	761	4749 b	34.1 b
Emperador	293 a	24.1 a	821	7089 a	50.0 a
Maxifort	304 a	23.1 a	847	7064 a	40.9 a
Irrigation (I)					
Full	278	21.0	805 ab	5953	27.8 b
Deficit	279	43853.0	938 a	6583	53.5 a
PRD	283	43912.0	687 b	6366	51.8 a
I × R	ns	ns	ns	ns	ns

* Significant differences between treatments (LSD test at $p \leq 0.05$) are indicated with different letters within columns. ns—non-significant.

Yield parameters and WUE results for second year are shown in Table 5. As in the first year, plants grafted on commercial rootstocks had significantly ($p \leq 0.001$) higher fruit mean weight and also number of fruits per stem. Early yield was affected ($p \leq 0.001$) by plant type and highest was noted for self-grafted Attiya grow with one stem. Yield WUE was significantly ($p \leq 0.001$) higher for Emperador and Maxifort grafted plants and also for plants grown under both types of reduced irrigation (DI + PRD).

Total yield results for second year are shown in Figure 2. Although, the interaction between factors was not significant, it was important to compare yield of same plant type under different irrigation. This analysis showed that grafted plants with commercial rootstocks grown under FI had highest total yield that differed from same plants type grown under PRD, but not significantly different from DI. In addition, one stem self-grafted plants from all irrigation regimes did not differed to each other and two stem ones had lowest yield had under PRD.

Table 5. Yield parameters and water-use efficiency of grafted tomato plants grown with one and two stems under three irrigation techniques in the second year of the experiment.

Treatments	Fruit Mean Weight (g)	Number stem^{-1}	Early Yield Stem^{-1} (g)	Yield Stem^{-1} (g)	WUEy (g L^{-1})
Rootstock (R)					
One-stem SG	210 b *	17.6 b	1415 a	3696 b	41.1 b
Two-stem SG	226 b	14.8 c	786 b	3347 b	36.8 b
Emperador	261 a	20.4 a	604 b	5279 a	58.1 a
Maxifort	271 a	19.7 a	699 b	5342 a	59.5 a
Irrigation (I)					
Full	256	18.4	772	4769 a	40.8 b
Deficit	239	18.7	799	4436 ab	52.2 a
PRD	239	17.5	949	4187 b	54.9 a
I × R	ns	ns	ns	ns	ns

* Significant differences between treatments (LSD test at $p \leq 0.05$) are indicated with different letters within columns. ns—non-significant.

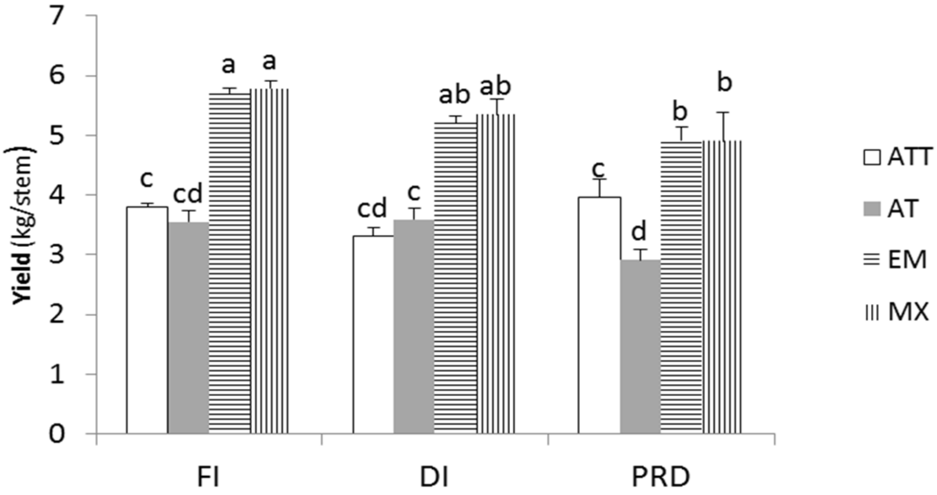

Figure 2. Total yield (kg stem^{-1}) of grafted tomato plants grown with one or two stems under three irrigation techniques. Vertical bars represent SE values ($n = 4$). Different letters above column indicate a significant difference by the LSD test at $p < 0.05$. ATT—one-stem Attiya; AT—two-stem Attiya; EM—Emperador; MX—Maxifort.

3.4. Mineral Content and Fruit Quality

In the second experiment, all leaf mineral concentrations significantly differed by rootstock type, while Ca and Mg were affected also with irrigation method (Table 6). Plants grafted on commercial rootstocks had highest values for N, P and K ($p \leq 0.001$). Ca was lowest in self-grafted plants grown with one-stem, while Mg was highest in same plants. Plants grown under both reduced irrigation techniques had significantly ($p \leq 0.01$) highest Ca and Mg leaf concentrations. Fruit mineral concentration in same year was significantly affected by rootstock. Plants grafted on commercial rootstocks had higher values than both types of self-grafted plants (Table 7).

The effects of rootstock and irrigation on fruit quality traits are presented in Table 6. Both TSS and TA differed by rootstock type ($p < 0.05$). There were no differences among irrigation techniques in these traits, but the effect of the interaction of the rootstock × irrigation was found for TSS ($p < 0.05$) (Figure 3). Highest TSS was recorded in one-stem Attiya grown under DI and differ from values same plants under FI and PRD. Plants grafted on Emperador had higher TSS under PRD than other irrigation treatments. TA was higher in fruits grown on commercial rootstocks.

Table 6. Leaf mineral concentrations of self-grafted and grafted tomatoes grown under three irrigation techniques in the second year.

Treatments	N	P	K	Ca	Mg
	g kg^{-1} DW				
Rootstock (R)					
One-stem SG	28.9 b	2.01 b	15.0 b	23.1 b	5.34 a
Two-stem SG	31.5 b	2.32 ab	14.2 b	29.3 a	4.98 ab
Emperador	36.5 a	3.23 a	21.0 a	28.3 a	4.5 bc
Maxifort	37.7 ab	3.31 a	21.8 a	28.5 a	4.45 c
Irrigation (I)					
Full	33.9	2.82	17.5	24.7 b	4.41 b
Deficit	32.8	2.73	17.6	28.3 a	5.17 a
PRD	34.2	2.7	18.9	29.0 a	4.87 a
I × R	ns	ns	ns	ns	ns

Significant differences between treatments (LSD test at $p \leq 0.05$) are indicated with different letters within columns. ns—non-significant.

Table 7. Effect of rootstock type and irrigation treatment on tomato fruits mineral concentrations and quality parameters in the second year of the experiment with plants grown on one and two stems.

Treatments	N	P	K	Ca	Mg	TSS (Brix^{o})	TA (g L^{-1})
	g kg^{-1} DW						
Rootstock (R)							
One-stem SG	18.2 b	2.08 b	29.9 b	1.22 b	1.37 c	4.7 a	4.5 b
Two-stem SG	18.1 b	2.51 b	30.5 b	1.39 ab	1.41 bc	4.4 b	4.6 b
Emperador	22.1 a	3.26 a	37.7 a	1.59 a	1.61 a	4.3 b	5.6 ab
Maxifort	19.9 a	3.19 a	35.4 a	1.51 a	1.57 ab	4.4 b	5.9 a
Irrigation (I)							
Full	20.1	2.81	33.9	1.46	1.55	4.3	5
Deficit	19.5	3.04	33.1	1.42	1.43	4.5	5.8
PRD	19.4	2.56	33.5	1.47	1.50	4.5	4.8
I × R	ns	ns	ns	ns	ns	*	ns

* Significant differences between treatments (LSD test at $p \leq 0.05$) are indicated with different letters within columns. ns—non-significant.

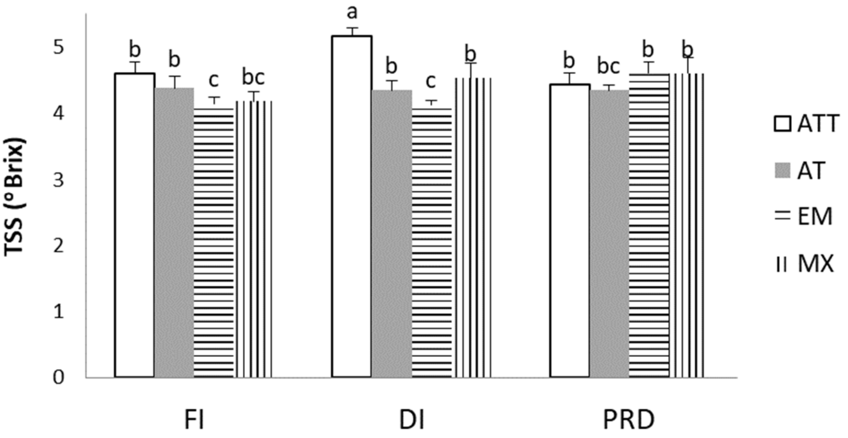

Figure 3. Total soluble solids (TSS) of tomato fruits sampled from different grafted plants grown with three irrigation techniques. Vertical bars represent SE values ($n = 4$). Different letters above column indicate a significant difference by the LSD test at $p \leq 0.05$. ATT—one-stem Attiya; AT—two-stem Attiya; EM—Emperador; MX—Maxifort.

4. Discussion

In general, the use of commercial rootstocks resulted in highly improved plant vigor in terms of excessive vegetative growth. In our studies, the plant height and leaf number measured at 60 or 70 DAT were highest with plants grafted on Emperador and Maxifort rootstocks; the difference was even more pronounced in the second trial. Comparing height with leaf number, it can be assumed that interleaf nodes interval was not influenced by grafting or irrigation. These findings partially differed from other studies [14,20]. Similarly, vegetative biomass production (as leaf area, leaf and shoot DM) was bigger in grafted plants. For example, shoot DM in year one was 40% and 60% higher in Maxifort and Emperador grafted plants compared to self-grafted ones. Similar values were found in the second year on stem basis, but when we include all plant production (both stems) vegetative biomass is at least two or three times higher. Vigorous rootstocks had enough capacity to provide satisfactory vegetative growth by roots that can supply needed water and nutrients for assimilates production. On average, leaf area was not influenced by irrigation, as others found that tomato under DI and PRD had smaller leaf area than control and explained as soil drying affected roots reaction and production of chemical signals, i.e., changed ABA concentration or xylem sap pH that leads to stomatal closure and decreases leaf expansion growth [21].

Interactive effect between rootstock type and irrigation treatment showed that plants grafted on commercial rootstocks did not differ in photosynthetic rate (A), while both types of self-grafted ones differed depending on the applied irrigation technique. It can be concluded that grafted plant had better assimilative processes. The optimization of A under water stress could be modified by the rootstock through action on biochemical and biophysical processes [22]. Stomatal conductance and intercellular CO_2 measured 20 days after starting irrigation treatments was lower under DI and PRD. These effects of reduced irrigation in some cases were noted later after initiation of irrigation [23], while in another study differences between treatments disappeared with time [24]. Valerio et al. [23] showed that lower stomatal conductance was related with leaf ABA accumulation, more ABA reduced stomatal conductance. Reducing stomatal conductance is a typical response to soil drying as stomatal closure is primary response to water deficit so plants could better contol water loss due to transpiration [7]. Stomatal closure reduced transpiration rate which was more pronounced in DI. Although, stomatal conductance was similar in both DI and PRD, it was expected that transpiration will be similar in both of them suggesting the response is mostly to the overall amount of water supplied to roots [21]. In contrast, in our study DI received 5% more water than PRD, so it seems that hydraulic signal is an important factor because plants under PRD on the wet side of the root can absorb enough water to keep higher level of transpiration [23]. Photosynthetic WUE had highest values under DI as result of lowest transpiration rate and similar photosynthetic rate to other irrigation. This can lead to lower biomass production as was noted in our study as reduced shoot biomass (although not significant) under DI and others found similar [21].

Rootstocks may affect tomato productivity positively or negatively, although in most cases yield increased both under non and stress conditions and depended on rootstock/scion combination [24]. In our experiment, plants grafted on commercial rootstocks had highest yield as a result of more and bigger fruits per plant, as was found in other studies [11,17,25]. Enhanced fruit production could be clearly related with higher plant biomass [15]. Early yield was different between years, in the first year highest early yield was noted under DI which can be related to a more pronounced water stress that hastened fruit ripening in this treatment. Topcu et al. [26] found higher tomato early yield in PRD than DI plants in experiment with more water reduction (50%) comparing our 40%. In experiment with two stems (second year), early yield was highest in one-stem-grafted Attiya what is result of longer period of growth for two stems plants because they were trained as side-shoots from cotyledons. In both experiments, cultivars grafted on commercial rootstocks had highest total yield under all irrigation treatments. In the second year comparing yield of these plants, it was found that under FI yield did not differ from DI but differ from PRD (Figure 1). It seems that rootstocks due to its vigor have enough capacity for water uptake to sustain yield under DI. It is important to notice that growing on two stems (2nd experiment) did not reduce yield markedly when comparing with one stem plants (1st experiment), although different cultivars were used what should be taken into account. Rahmatian et al. [16] found dry matter allocation was not influenced by grafting or stem numbers and that good balance between vegetative and generative growth can depend on rootstocks. Other studies done with ungrafted greenhouse and processing tomato mostly obtained higher yield using PRD than DI [8] or similar to DI and FI [24].

WUE is the main indicator of plant water relations and is regulated by physiological mechanisms. In both years WUEy calculated as ratio between yield and water applied per treatment was higher in rootstock-grafted plants and as expected under PRD and DI. In these treatments higher fruit yield and lower water use resulted in improved WUE. It was not shown that PRD improved WUE better than DI, which means that irrigation volume is more important than used irrigation technique in determining yield or all crop growth as was suggested before [21,23,24], although other found irrigation technique can be more important [9]. Comparing two experiments it can be seen that in double stemmed plants (2nd experiment) WUE was higher leading to conclusion water use was optimized. In addition, in 1st Experiment WUEy was much lower in FI than in the second year what can be related to use of different soil moisture meters: tensiometers and soil sensors. The tensiometers was used for hand-operated irrigation, which could have led to overirrigation in the first year. It was shown that automatic operated tensiometers was more effective, which can be compared with sensors with automatic valves in our study [27].

The leaf mineral concentrations of P and K were under range of sufficiency while others were in range (N and Mg) or above (Ca) as proposed for greenhouse tomato. Grafting is considered as an effective tool for improving nutrient uptake and use efficiency in vegetables, although those were observed under optimal nutrient status in the root zone. N, P and K had higher concentrations in the plants grafted on commercial rootstocks what was expected and already confirmed in other studies that showed that nutrient uptake depends on rootstock–scion combinations [28]. Higher leaf P in grafted plants were reported for grafted eggplant and watermelon [29,30]. Grafted plants had more vigorous root system, which could be reason for increase in active uptake of P that has low mobility in soils. Self-grafted and grafted plants had low leaf K (under sufficiency range) because fertilization was not intensive as in commercial production. Potassium is nutrient normally required in the largest amount in tomato production. Grafting promote better growth and K uptake even under low K supply as was shown by Schwarz et al. [31]. These nutrients (N, P, K) concentrations were not affected by water supply rate, although opposite was shown for N in other studies for PRD or DI in non-grafted tomatoes [32]. Increase in K concentrations under water stress was found in some non-grafted and grafted tomatoes explaining that K accumulation improves stomatal resistance which improve drought tolerance [33]. In other case, decrease in grafted tomato leaf K was noted with increase in water stress level [12].

Regarding Ca^{2+} and Mg^{2+}, a significant increase in tomato Ca leaf concentration was found due to grafting what is in line with other reports [29,31]. In addition, both DI and PRD resulted in more leaf Ca than plants under FI. It was found that tomatoes under PRD had increased Ca uptake due to higher plant water status and lower stomatal conductance [34]. Higher Ca uptake induced by grafting are important for the tomato fruits due to the possibility of blossom-end rot incidence. Different than Ca, in grafted tomato was found lower leaf Mg what is in line with previous studies and could be also influenced by rootstock and cultivars used [5,14]. It seems that grafting somehow decrease Mg uptake in grafted vegetables, but reason it is not yet clear. Possible higher Ca uptake reacts antagonistically to Mg uptake, which could be related to specific transport systems [35]. Under reduced irrigation treatments higher leaf Mg was measured and same was found for mini watermelons [30]. Mg^{2+} ion has largest hydrated radius among cations and this property makes Mg^{2+} bind weakly to negatively charged soil colloids and root cell walls [36], which could lead to decreased Mg uptake under FI conditions due to leching in sub-root zones. The fruit mineral concentrations was influenced by rootstock type showing that highest values in the plants grafted on Emperador and Maxifort. Other found effect of rootstock, but also influence of water stress on fruit minerals [18].

Higher TSS was affected by plant type with highest values in one stem plants. Interactive analysis showed it is mostly result of highest values of same plant type under DI. The enhanced TSS in that treatment could be result of water stress, although it is not clear why similar was not found in self-grafted two stem plants. Self-grafted double stemmed plants possible use more assimilates for additional vegetative growth [16]. Grafted plants had lower TSS what is often found even when used different cultivars and rootstocks [25]. For grafted plants vigorous roots can be additional sinks for assimilates and also better water uptake can result in dilution effect of fruits sugars [10]. Under PRD all plant types had similar TSS so it can be concluded that self and grafted plants with this irrigation type changed mechanisms responsible for results recorded under DI. Grafting on commercial rootstocks decreased Mg leaf content, which can possible lead to latent Mg deficiency influencing carbohydrate partitioning requiring for obtaining maximum yield and ensuring sugar accumulation in fruits [37]. In our study, both rootstocks increased the TA. Their increase by grafting was also found in many other experiments under different conditions [5]. Grafting under regular and low K resulted in higher TA, independent of K in fruits [31], while in our study K in fruits grafted on both rootstocks was higher compared to self-grafted plants. It is known that K concentration in fruits can be positively related with acid content, although further investigations are needed.

5. Conclusions

In the present experiment, we evaluated growth, gas-exchange parameters, yield, WUE and leaf mineral concentrations and fruit quality of self-grafted and tomato grafted on two commercial rootstocks cultivated in greenhouses in Mediterranean climate under three irrigation techniques: FI, DI and PRD. First year plants were grown with one stem and in the second with two stems.

In conclusion, these studies for the first time demonstrates the effects of parallel usage of different reduced irrigation techniques on grafted tomato vegetative and generative traits. Grafting onto commercial rootstocks improved plants growth and yield both in cultivation with one or two stems. Grafted plants under DI had minimal yield reduction compared to FI in double stemmed plants. WUE was highly improved with grafting and application of PRD and DI. That was more pronounced in experiment with two stem plants and could be result of different biomass partitioning and irrigation scheduling based on soil moisture sensors. Leaf mineral concentrations were higher in grafted plants as possible better uptake of vigorous rootstocks while Mg was reduced what imply contrasting rootstock–scion interactions. These findings indicate that grafted plants can be grown under moderate DI before PRD and that two stem plants could be used under that irrigation regime.

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