

# Effect of irrigation practices upon yield and fruit quality of four grapefruit (*Citrus paradisi* Mac.) cultivars

G. Morianou, V. Ziogas, N. N. Kourgialas and G. P. Karatzas

## ABSTRACT

Two strategies of irrigation were applied during the phase of fruit growth in 30-year-old grapefruit trees (*Citrus paradisi* Mac.) of four varieties (cv. Marsh SRA 8, cv. Shambar SRA 22, cv. Frost Marsh, cv. Ruby). For the first strategy (T1), the trees were irrigated every week at 100% crop evapotranspiration (ETc), while for the second strategy (T2) at 60% ETc. At harvest time, tree yield was estimated along with fruit quality and water productivity. T1 strategy significantly increased the tree yield, the fruit weight and the juice content. Although an increase in water productivity (crop yield/total water use) was achieved during the water stress experiment for three out of the four varieties. The main effect of the T2 strategy was a significant increase of qualitative (commercial and nutritional) attributes like total soluble solids (TSS), citric acid, ascorbic acid (Vitamin C) and phenolic content. In addition, strategy T2 increased the maturation index and the fruits had sweeter flavor for two varieties. These results lead to the conclusion that in mature grapefruit trees, optimal irrigation practice provided better fruit yield, while water stress practices favor the accumulation of specific nutritional elements and improve specific fruit quality parameters, especially for cv. Ruby.

**Key words** | agronomic practices, citrus, deficit irrigation, phenols, water management

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## HIGHLIGHTS

- Assess the effects on grapefruit yield, fruit quality, and water productivity under different irrigation treatments.
- Investigate differences under full irrigation and deficit irrigation among four grapefruit cultivars.

## INTRODUCTION

Citrus is one of the most-economically-important crops in the world and they have great value in terms of nutrition. Grapefruits (*Citrus paradisi* L.) are an especially rich source of vitamin C, phenolic and flavonoids, carotenoids (provitamin A) and other nutritional elements. They are also famous for their antioxidant properties, which strengthen the human body against various diseases (Navarro *et al.* 2015). In Greece, citrus occupies 45,400 ha

of agricultural land and the production during the year 2018 reached 1,179,555 tonnes. Grapefruit cultivation is limited in Greece, occupying an area of only 160 ha, and the production during 2018 reached 3,160 tonnes (FAO 2019). Grapefruits, like all citrus, have in general high demands for annual irrigation. The annual water requirement of citrus varies between 900 and 1,200 mm depending on the soil, climate and the age of the tree (Doornbos & Kassam 1979; Kourgialas & Karatzas 2015).

Scarcity of water resources, due to rapidly growing cultivation demands and climate change, is one of the major factors limiting irrigated agriculture (Navarro *et al.* 2010).

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doi: 10.2166/ws.2021.115

Globally, agriculture is the largest user of water, with nearly 70% of withdrawals worldwide (FAO 2019). The limited water supply worldwide and increased irrigation cost, especially in arid and semi-arid areas, demands a more efficient and optimized use of irrigation water. The aim is the maximization of water savings and the improvement of fruit yield and quality. One of the most promising irrigation strategies for accomplishing this objective might be Deficit Irrigation (DI). Deficit irrigation is based on the application of amounts of irrigation water lower than the crop evapotranspiration needs (ETc) (Romero *et al.* 2006; Fereres & Soriano 2007; García-Tejero *et al.* 2010a). DI has been examined thoroughly as a valuable and feasible way to implement sustainable agriculture in semi-arid regions (Ruiz-Sanchez *et al.* 2010). Following this trend, several authors have stated the advantages of DI strategies with regard to improving water use efficiency and fruit quality in many emblematic citrus species (Romero *et al.* 2006; García-Tejero *et al.* 2010b).

Several studies have shown that under deficit irrigation (DI), fruit quality is improved in citrus trees (Ballester *et al.* 2013; Pérez-Pérez *et al.* 2014; Navarro *et al.* 2015). The majority of the studies show that the response of citrus trees to DI treatments depends mainly on the phenological stage (cell division, cell elongation, ripening-harvest) that is applied (Ginestar & Castel 1996; García-Tejero *et al.* 2010a; Navarro *et al.* 2010, 2015). Previous studies have showed that the application of water stress during flowering, as well as during the initial stage of fruit growth, reduced fruit yield (Romero *et al.* 2006; Pérez-Pérez *et al.* 2008, 2014; García-Tejero *et al.* 2010b). Also, when water stress was applied during the stage of fruit growth, it negatively affected fruit size, yield and delayed maturation, while improving some quality parameters (Navarro *et al.* 2010, 2015; Ballester *et al.* 2013; Pérez-Pérez *et al.* 2014). Finally, applying water stress during fruit maturation mainly had a reflection on the fruit quality parameters. Citrus fruit quality characteristics such as total soluble solids and titratable acidity were positively affected, while no significant delay in maturation time or fruit yield were observed (Pérez-Pérez *et al.* 2009; García-Tejero *et al.* 2010b; Navarro *et al.* 2010, 2015). However, the citrus response to DI strategies depends not only on the phenological stage that the DI is applied, but also upon the severity of the water deficit

(García-Tejero *et al.* 2010b; Aguado *et al.* 2012; Ballester *et al.* 2013; Gasque *et al.* 2016). One of the main effects of severe water stress is in the change of the maturity index. Many authors observed that DI strategies can improve water productivity (García-Tejero *et al.* 2010a; Aguado *et al.* 2012). Moderate water stress strategies are suitable for saving water and obtaining similar yield and citrus fruit quality similar to no DI strategies (García-Tejero *et al.* 2010a).

Most of the DI studies in citrus have been carried out mainly for sweet oranges and mandarin, but little work has been done upon the response of grapefruits to water stress. In order to implement an orthological DI strategy that saves water but at the same time sustains fruit quality, a comparative study is needed in order to study the effect of DI in different varieties. Pérez-Pérez *et al.* (2014) and Navarro *et al.* (2015) evaluated the sensitivity to DI of different fruit growth stages in adult ‘Star Ruby’ grapefruit trees. The aim of this work was to apply a Deficit Irrigation (DI) treatment versus a Full Irrigation (FI) – control treatment in order to assess the effects on yield, fruit quality, and water productivity (WP) in an experimental 30-year-old orchard with grapefruit trees (*Citrus paradisi* Mac.) of four different varieties: (i) cv. Marsh SRA 8, (ii) cv. Shambar SRA 22, (iii) cv. Frost Marsh and (iv) cv. Ruby.

## MATERIAL AND METHODS

### Orchard, soil and climatic characteristics

The study was carried out during 2019 at the experimental field of the Institute of Olive Tree, Subtropical plants and Viticulture (IOSV) of the Hellenic Agricultural Organization ‘DIMITRA’ (ELGO-DIMITRA), in Chania, Crete (south Greece). The experiment was performed on 30-year-old grapefruit trees (*Citrus paradisi* Mac.) of four varieties (cv. Marsh SRA 8, cv. Shambar SRA 22, cv. Frost Marsh, cv. Ruby) grafted onto sour orange (*C. aurantium* L.) rootstock. The soil texture was loam, with 45.28% sand, 19.34% clay and 35.38% silt, an organic matter content of 1.35%, an electrical conductivity of 0.19 dS m<sup>-1</sup>, 0.32% total CaCO<sub>3</sub> and a pH of 7.3. The N-NO<sub>3</sub> was 8.02 mg kg<sup>-1</sup> and the phosphorus was 18.28 mg P kg<sup>-1</sup> Olsen. Soil water content at field capacity was 300 mm m<sup>-1</sup> and the wilting

point was  $110 \text{ mm m}^{-1}$ , respectively, with an available soil-water content of 190 mm in the root zone (0.6 m from the soil surface). Finally, the bulk density was  $1.64 \text{ g/cm}^3$ .

Climatic data were provided by the IOSV's meteorological station in Chania (Greece), which is located at a distance of less than 200 m from the experimental plots. The climate of the area is sub-humid Mediterranean with humid and relatively cold winters, and dry – warm summers. The annual rainfall ranges from 300 to 700 mm/year, distributed mainly during late autumn to early spring. The mean annual air temperature ranges around  $18^\circ\text{C}$ .

### Experimental design and description of irrigation treatments

The experimental design was two randomized complete blocks: each block had four rows with six trees per row (26 trees per block). Two irrigation treatments were applied during this experiment, a control – FI treatment (T1) and a DI treatment (T2). The DI treatments are based on the theory of non-irrigation or irrigation with less water during different phenological stages of fruit growth (Phase I: bloom and cell division; Phase II: cell elongation, rapid fruit growth period; Phase III: ripening and harvest). Phase I for grapefruits lasts from late April to early June, Phase II from early June to early October and finally Phase III lasts from October until harvest. In Crete, due the local climate conditions, the crop needs at phases I and III are mainly covered by precipitation amounts. Thus, both T1 and T2 treatments were applied from May to early October, at the fruit growth stage (during the second half of Phase I and during Phase II). T1 was applied in the first block of trees and involved irrigation every week at 100% of the crop needs ( $ET_c$ ). T2, applied to the second block of trees, also involved irrigation every week at 60% of the crop needs. Irrigation was applied using 12 emitters for each tree, with a discharge rate of  $4 \text{ L h}^{-1}$ . Emitters were located in a circle 25 cm away from the tree trunk at an equal distance from each other.

Water meters were used to measure the volume of irrigation applied to each treatment. The seasonal values of  $ET_o$  were determined by the FAO56 version of the Penman equation (Allen et al. 1998) using the climatic data recorded

by the automated weather station near the orchard. In this approach, evapotranspiration of the crop ( $ET_c$ ) was estimated using the following equation:

$$ET_c = k_c \cdot ET_o$$

where the  $k_c$  coefficient incorporates crop characteristics and averaged effects of evaporation from the soil. In our case, the  $k_c$  ranged between 0.5 from March to May; 0.55 from June to October and 0.5 in November and December, respectively, in accordance with BEWARE project (2005) and Kourgialas et al. (2019).

The fertilization applied, for both treatments, was according to soil and leaf analysis. The fertilizer applied in the experimental orchards was ammonium nitrate fertilizer – 0.5 kg per tree. All optimum agricultural practices, such as pruning, weed control and fertilizer application, were also applied for both irrigation strategies.

### Fruit measurements

When fruits had reached commercial maturation, the total number of citrus fruits was recorded in order to estimate the tree yield. Then, ten fruits per variety were selected randomly from a subsample of four trees per treatment. Specifically, five fruits per side (east and west sides) were picked, at the height of the human chest. The net weight of the fruits, the equatorial diameter (mm), the fruit shape, the external fruit color, the rind thickness (mm) and the flesh firmness (Kg) and juice content (%) were measured in the laboratory. Flesh firmness was quantified by using a 10 mm cylinder Fruit Pressure Tester digital penetrometer and the fruit shape was calculated according to:

$$\text{Fruit shape} = \frac{\text{polar diameter (mm)}}{\text{equatorial diameter (mm)}}$$

The external fruit Color Index (CI) was measured in ten fruits per treatment, using a tristimulus color difference meter (Minolta CR300 colorimeter), at four locations around the fruit. The Hunter parameters  $a^*$ ,  $b^*$  and  $L^*$  were reported by the colorimeter, obtaining the CI using

the following equation:

$$Cl = \frac{1000 \cdot a^*}{L^* \cdot b^*}$$

$L^*$  parameter gives a value of the luminance or brightness of the sample;  $a^*$  parameter indicates the area of variation between red and green spectrum and  $b^*$  parameter refers to the area of variation between the yellow and blue spectrum.

Subsequently, fruits were cut in the equatorial area and peel thickness was measured at four points.

### Juice measurements

The fruits were squeezed with an electric citrus fruit-juicer and the juice was strained through a 1-mm-mesh sieve. The juice from three fruits were combined and considered as a single sample for further chemical analysis. Thus, there were three (3) replications per variety, for each DI strategy. The juices were analyzed for Titratable Acidity (citric acid content), pH, Total Soluble Solids, Ascorbic Acid (Vitamin C) and Total Phenolics. The Maturity Index, which is connected to the citrus fruit flavor, was also estimated.

The content of total soluble solids (TSS) in the juice was measured at 25 °C with a digital refractometer (Atago) and titratable acidity (TA) was determined by titration with NaOH and phenolphthalein indicator according to conventional methods (Kimball 2012). The maturity index (MI) was expressed as  $TSS \times 10/TA$ .

Ascorbic acid analysis in the fruit juice was determined according to the Official Methods of Analysis for Ascorbic Acid in Vitamin Preparations and Juices (method number 967.21, chapter 45, AOAC). Fruit juice samples were centrifuged at 1,650 g for 5 min at 4 °C. The supernatant was used for determination of the ascorbic acid content by titration with 2,6-dichlorophenolindophenol sodium salt hydrate (DIF). The results were expressed as mg ascorbic acid 100 mL<sup>-1</sup> juice.

The total phenolics were determined by the Singleton *et al.* (1999) method. According to this method, 1 mL of fruit juice was extracted with 9 mL of 80% methanol for 30 min at room temperature. After centrifugation at 5,000 rpm for 10 min, the supernatant was taken for the

determination of total phenolics by the Folin–Ciocalteu method. A reagent blank using H<sub>2</sub>O was prepared. Folin–Ciocalteu phenol reagent (0.5 mL) was added to the mixture and it was shaken vigorously. After 5 min, 5 mL of 5% Na<sub>2</sub>CO<sub>3</sub> solution were added, mixing at the same time. The solution was immediately diluted to 25 mL with distilled water, mixed thoroughly and then allowed to stand for 60 min before measurement: the absorbance was measured at 750 nm versus the prepared blank. The total phenolic content of the sample (three replicates per treatment) was expressed as mg mL<sup>-1</sup> of Gallic Acid Equivalent (GAE).

### Water productivity

Water productivity in agriculture and landscape irrigation may be generally defined as the ratio between the actual crop yield achieved ( $Y_a$ ) and the total water use ( $TWU = \text{irrigation} + \text{rainfall}$ ) expressed in kg/m<sup>3</sup> (Pereira *et al.* 2012):

$$WP = \frac{Y_a}{TWU}$$

WP indicator express the benefit derived from the consumption of water and can be used for assessing the impact of on-farm strategies under water-scarce conditions. It provides a vision of the water that could be saved with simultaneous increase in crop yield (Singh *et al.* 2006).

### Statistical analysis

Statistical analyses were performed using the SPSSv17 package (SPSS Inc., Chicago IL) with a one-way analysis of variance (ANOVA), with four treatments for each DI strategy and six replicate trees per treatment. When there was a significant difference ( $P$ -value <0.05), means were separated using Duncan's test.

A principal-component analysis (Ihaka & Gentleman 1996) was performed using SPSS v17 package (SPSS Inc., Chicago IL). Principal Component Analysis (PCA) is a factor analysis protocol that is used in order to identify variables or underline factors that better explain the correlation or covariance matrix of several attributes (Davis & Sampson 1986).

## RESULTS AND DISCUSSION

### Yield and water productivity

The application of water stress treatment during Phase II (T2) decreased tree fruit yield compared with the control treatment (T1), in all grapefruit varieties except for cv. Frost Marsh (Table 1). Under water stress, the overall tree yield was reduced significantly by 14, 23 and 56% in cv. Marsh SRA 8, Shambar SRA 22 and Ruby, respectively, while cv. Frost Marsh remained unaffected. This is mainly due to the reduction of fruit weight in all the grapefruit varieties, since fruit load was not significantly affected by the water stress (Table 1). Although a decrease in tree yield was observed, a significant increase in water productivity was achieved during the experiment for the three out of four varieties, namely Marsh SRA 8, Shambar SRA 22 and cv. Frost Marsh (Table 1). In the case of the Ruby cultivar, the lower water productivity during DI T2 treatment is attributed to the significant decrease of both fruit yield and fruit weight. Increasing water productivity may be a means of achieving efficient and effective water use. In agriculture, the interest is to have higher yield with less water, due to water scarcity and the necessity to use water in the most optimum way during agricultural practices (Navarro et al. 2015).

**Table 1** | Fruit yield parameters during the experiment period for the control (T1) and the deficit irrigation (T2) treatments

Cultivar	Treatments	Yield (Kg tree <sup>-1</sup> )	Fruit load	Fruit weight (g)	WP (Kg m <sup>-3</sup> )
cv. Marsh SRA 8	T1	163 d	427 bc	381.6 cd	24.03
	T2	141 bc	409 abc	343.7 c	32.63
	ANOVA	sd	nsd	nsd	
cv. Shambar SRA 22	T1	160 cd	468 c	341.9 cd	23.62
	T2	124 b	440 c	280.7 b	28.74
	ANOVA	sd	nsd	sd	
cv. Frost Marsh	T1	160 cd	371 ab	432.2 e	23.64
	T2	143 bcd	418 abc	344.4 c	32.41
	ANOVA	nsd	nsd	sd	
cv. Ruby	T1	187 e	470 c	398.9 de	27.52
	T2	82 a	357 a	229.0 a	18.78
	ANOVA	sd	sd	sd	

Values are the mean of 3 replicates per variety. Values followed by different letter within a column are significant different (sd) and values followed by the same letter are not significantly different (nsd) at the 0.05 level of probability, according to the Duncan's test.

Field studies related to the effect of deficit irrigation upon the tree yield are contradictory, since in the work of Pérez-Pérez et al. (2012), the tree yield of lemon trees was not affected when they received partial root deficit irrigation treatment compared to fully irrigated ones. Furthermore, in the work of Faber & Lovatt (2012) when navel oranges received limited water in the root zone, the fruit yield and fruit size were significantly reduced. In the work of Navarro et al. (2010), the application of DI upon grapefruit trees, during Phase I and via the use of drip irrigation did not significantly reduce the overall tree yield. But in mature oranges, when DI was applied every 3 days during Phase II and every 17 days, during Phase III, the citrus trees maintained their yield and a limited reduction in fruit size was observed (Hutton et al. 2007). Furthermore, in the work of Pérez-Pérez et al. (2014), the application of DI water stress in grapefruit trees, during Phase II, caused a significant decrease of 25% in fruit yield, which exceeded the saving of irrigation water, compared to optimum irrigated trees, indicating that the saving of irrigation water during this stage severely affected tree yield. The different effect of DI when it is applied during Phase II upon the fruit yield of grapefruit trees, compared to other citrus species, could be due to the different pattern of vegetative-reproductive resource distribution in response to drought stress (Pérez-Pérez et al. 2014). The witnessed differences among these studies and our results could be due to the volumes and time of applied water stress treatment and the examined citrus species, or even cultivars put under test.

### Fruit physiological and quality affect by deficit irrigation

Many authors have stated that the application of DI treatments in citrus improves fruit quality via the increase or decrease of the most important quality parameters related with taste, flavor, nutritional value and osmotic adjustment (García-Tejero et al. 2010b; Navarro et al. 2010).

In this work, fruit quality was affected by the water stress treatment during this experiment. DI significantly reduced the equatorial diameter of all the tested grapefruit cultivars (Table 2); but at the same time, the market-desired commercial equatorial diameter of near 10 cm was retained for both strategies (T1 and T2). The reduction of equatorial diameter by water stress is in line with the lower fruit



**Table 2** | Fruit physiological parameters during the experiment period for the control (T1) and the water stress (T2) treatments

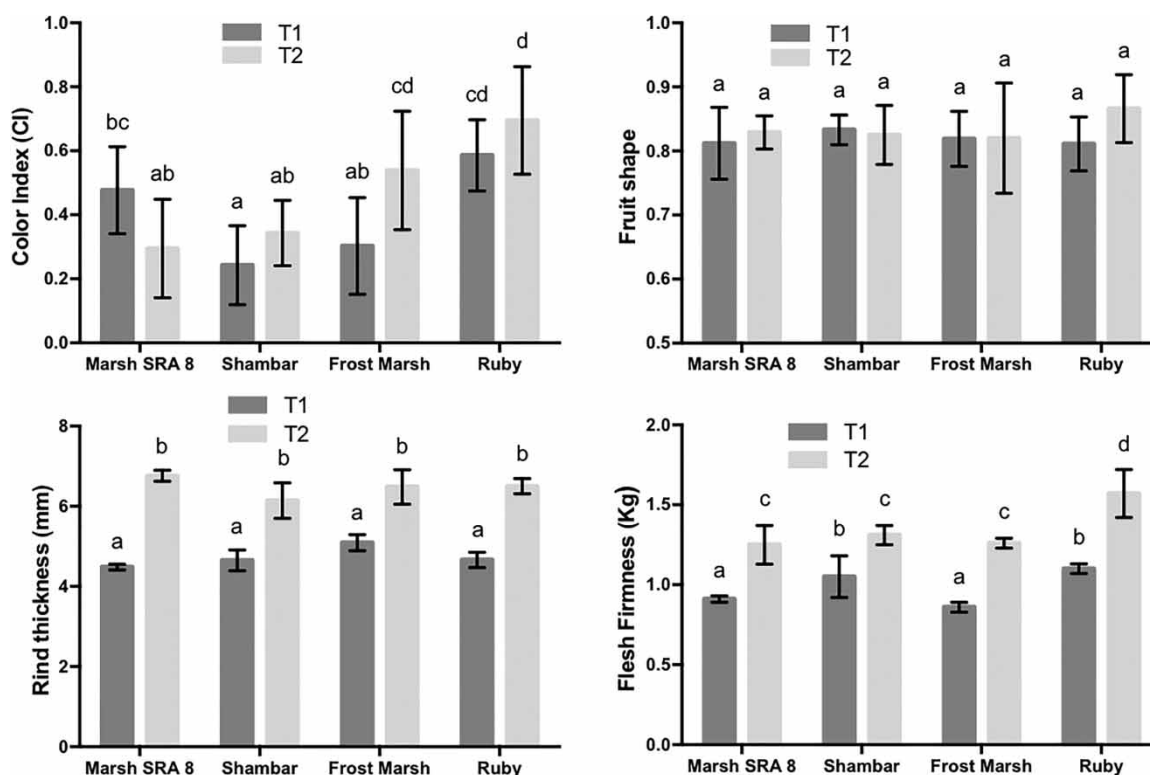
Cultivar	Treatments	Eq. D (mm)	Fr. Sh.	CI	R. th. (mm)	Fl. Fir (Kg)	MI
cv. Marsh SRA 8	T1	100.5 d	0.812 a	0.477 bc	4.48 a	0.91 a	5.31 a
	T2	94.9 c	0.829 a	0.295 ab	6.76 b	1.25 c	6.11 d
	ANOVA	sd	nsd	nsd	sd	sd	sd
cv. Shambar SRA 22	T1	95.9 c	0.833 a	0.243 a	4.65 a	1.05 b	5.76 c
	T2	88.0 b	0.825 a	0.343 ab	6.14 b	1.31 c	5.41 ab
	ANOVA	sd	nsd	nsd	sd	sd	sd
cv. Frost Marsh	T1	105.7 e	0.819 a	0.303 ab	5.09 a	0.86 a	5.47 abc
	T2	96.0 c	0.820 a	0.539 cd	6.48 b	1.26 c	5.76 c
	ANOVA	sd	nsd	sd	sd	sd	nsd
cv. Ruby	T1	103.9 de	0.811 a	0.586 cd	4.66 a	1.10 b	5.68 bc
	T2	81.1 a	0.866 a	0.695 d	6.50 b	1.57 d	6.11 d
	ANOVA	sd	nsd	nsd	sd	sd	sd

External parameters analyzed are: equatorial diameter (Eq. D.), fruit shape (Fr. Sh.), color index (CI), rind thickness (R. Th.) flesh firmness (Fl. Fir.), maturation index (MI).

Values are the mean of three replicates per variety. Values followed by different letters within a column are significantly different (sd) and values followed by the same letter are not significantly different (nsd) at the 0.05 level of probability, according to Duncan's test.

weight. The fruit shape, which is the ratio between the equatorial and polar diameter, was not clearly affected by the DI treatment (T2), as all grapefruit varieties maintained the same aspect ratio (Figure 1). Regarding citrus

plants, it is common to observe the phenomenon of smaller mean fruit diameter under low level of water irrigation regimes (Hutton *et al.* 2007; Melgar *et al.* 2010; Hutton & Loveys 2011). In grapefruit, severe water stress during



**Figure 1** | External fruit Color Index (CI), Fruit shape, Rind thickness and Flesh firmness of all the studied grapefruit cultivars. Comparison between control (T1) and DI (T2) treatments. Values are the mean of three replicates, bars with the same letter are not significantly different at the 0.05 level of probability, according to Duncan's test.

Phase II significantly decreases the fruit size (Navarro *et al.* 2015). It is well documented that the reduction of fruit size in drought-stressed grapefruit trees is due to the fact that water-stressed fruits accumulate less dry matter than non-stressed citrus fruits due to active competition among the fruit tissue and other sink organs of the tree structure (Cohen & Goell 1988).

The external citrus fruit color index (CI) was also measured during this study. The obtained results did not show significant differences between irrigation treatments (T1 and T2), except for cv. Frost Marsh, which exhibited a slightly more yellowish color under DI treatment (Figure 1). This occurred due to the fact that citrus peel color changes fast during ripening period (Phase III), while the trees of both treatments received the main amount of water from precipitation. Compared to all tested grapefruit varieties, cv. Ruby exhibited, under DI and control regimes, increased citrus peel Color index. This is due to the fact that this variety is enlisted among the pink varieties. It is accepted that peel color of citrus fruit is under the tight control of environmental and nutritional factors, which control the alternation of peel chloroplasts into chromoplasts, a process that is orchestrated via the activity of various hormonal signaling molecules such as ethylene, gibberellic acid and abscisic acid (Rodrigo *et al.* 2013).

The application of DI strategy T2 significantly increased the rind thickness of all the examined cultivars (Table 2). Namely, under DI during Phase II, it increased the peel thickness of cv. Marsh SRA 8 by 51%, of cv. Shambar SRA 22 by 32%, of cv. Frost Marsh by 27% and of cv. Ruby by 39%, with respect to control. Thick peel is not a desirable feature for marketable products; some traders are interested in it for transportation and maintenance reasons. In the work of Pérez-Pérez *et al.* (2009), the implementation of DI during Phase III of orange cv. Lane Late fruit growth significantly reduced the peel thickness. Also, in the work of García-Tejero *et al.* (2010b), the peel thickness of orange fruit cv. Navellina remained unaffected when trees were under the effect of DI. While in the work of Mostert & Van Zyl (1998), peel thickness from oranges of cv. Valencia and grapefruit cv. Nelruby were significantly increased when the trees were under poor irrigation regimes.

Also, in this work, application of DI strategy during Phase II significantly increased the citrus flesh firmness of all the tested cultivars. In detail, the fruit firmness of

cv. Marsh SRA 8 was increased by 37.8%, of cv. Shambar SRA 22 by 24.1%, of cv. Frost Marsh by 45.7% and of cv. Ruby by 42.7%, with respect to control. Among the tested varieties under DI regime, the flesh firmness of cv. Ruby was the one that exhibited the highest significant value. In the work of Kusakabe *et al.* (2016), the application of partial root drying in grapefruit trees during Phase II resulted in a decrease in citrus fruit firmness, but this parameter was not correlated to any other fruit or juice quality attribute.

The maturation index (MI) is considered the fine balance between the Total Soluble Solids (TSS) and the Titratable Acidity (TA) and is the most useful indicator of internal citrus fruit maturation stage (except lemons), since during ripening the TA decreases due to catabolism of citric acid and dilution phenomena and TSS (sugars) increase. In this work, under the effect of DI strategy, the MI of cv. Marsh SRA 8 and cv. Ruby exhibited an increase, while the MI of cv. Shambar SRA 22 was decreased and MI of cv. Frost Marsh was not significantly altered with respect to control. The fine balance of TSS and TA is a widely accepted method to determine citrus fruit maturation stage, and is also the most relative attribute that correlates fruit quality with consumer acceptance (Harding & Fisher 1945). In the work of Navarro *et al.* (2010), DI strategy during Phase III of citrus development increased the values of TSS and TA of fruit juices, with respect to the control fruits, and exhibited improved flavor and thus could contribute to the improvement of the commercial quality and consumer acceptance of the citrus fruit (Fellers *et al.* 1988). An increase of MI is also considered as a positive point for the commercial value of juice, and the benefit of an early harvest. Most of the previous studies on citrus showed an increase of TSS and TA values under DI strategies in citrus, although maturity index was not affected or decreased (Romero *et al.* 2006; Pérez-Pérez *et al.* 2009; Navarro *et al.* 2015). Exceptional scientific results highlight the fact that severe DI regimes implemented during citrus fruit maturity improve juice quality attributes without decreasing the maturity index (García-Tejero *et al.* 2010b). It is generally accepted that during citrus fruit ripening, external and internal ripening coincide and the peel and pulp behave in many cases as distinct organelles. Under DI strategies the delay or advance of citrus fruit maturation stage is tightly depended upon the stage of the fruit growth

when the stress was applied, since it can alter the balance of TSS and TA (Pérez-Pérez *et al.* 2009; Aguado *et al.* 2012).

The T1 (full irrigation) treatment gave better results in terms of juice content. For the varieties Frost Marsh and Ruby, there is no significant difference between the two strategies, but there is a positive trend of higher juice content under the full irrigation strategy (Table 3; Figure 2). Under the effect of DI strategy, the fruit juice content of cv. Marsh SRA 8 and cv. Shambar SRA 22 were significantly reduced by 16.4% and 9.7%, respectively, with respect to control. Similar results were also reported by Navarro *et al.* (2015), where the application of DI during Phase I in grapefruit trees caused a significant reduction in fruit juice content. Also, in mandarin fruits, water stress during the Phase II of the citrus fruit developmental stage significantly decreased the juice percentage by 27% with respect to control fruits (Navarro *et al.* 2010). In the work of Pérez-Pérez *et al.* (2014), DI applied during Phase II upon grapefruit trees significantly decreased the fruit juice percentage, but considering the fact that no symptoms of water stress were observed upon the tree canopy during fruit harvest leads the authors to the fact that low juice content could be attributed to internal changes of fruit structure rather than dehydration processes.

The application of DI increased Total Soluble Solids (TSS) and Titratable Acidity (TA) – citric acid content, (Table 3; Figure 2). In detail, under DI strategy, TSS and

TA of all studied varieties were increased. The highest % increase of TSS was witnessed in cv. Marsh SRA 8 and cv. Ruby, where the increase was 24.3% and 28.2%, respectively, while the highest percentage increase of TA was witnessed in cv. Shambar SRA 22 and cv. Ruby, by 22.3% and 19.2%, respectively (Table 3). It is well established that under water deficiency citrus fruit quality attributes are affected (Yakushiji *et al.* 1998) and the main effects are reflected in organoleptic parameters in juice, such as increase of Total Soluble Solids content and acidity (Chartzoulakis *et al.* 1999).

Former data indicate that the increase of TSS in juice from citrus fruits subjected to mild drought stress is due to the attempt to achieve osmotic adjustment (Yakushiji *et al.* 1998). Similar results were also reported in the work of Navarro *et al.* (2010) in clementine mandarins. The application of DI water stress, during Phase II, resulted in increased levels of TSS and TA, which were maintained for more than 3 months, resulting in passive dehydration of the juice sacs and providing significantly lower juice percentage, thus increasing the values of TSS and TA with respect to the control. In grapefruit, severe water stress during Phase II significantly increases the citrus fruit acidity (Navarro *et al.* 2015). It has been proposed that the increased levels of TA during DI regimes may be due to *de novo* biosynthesis of organic acids in an overall attempt to achieve osmotic adjustment in the fruit matrix (Navarro *et al.*

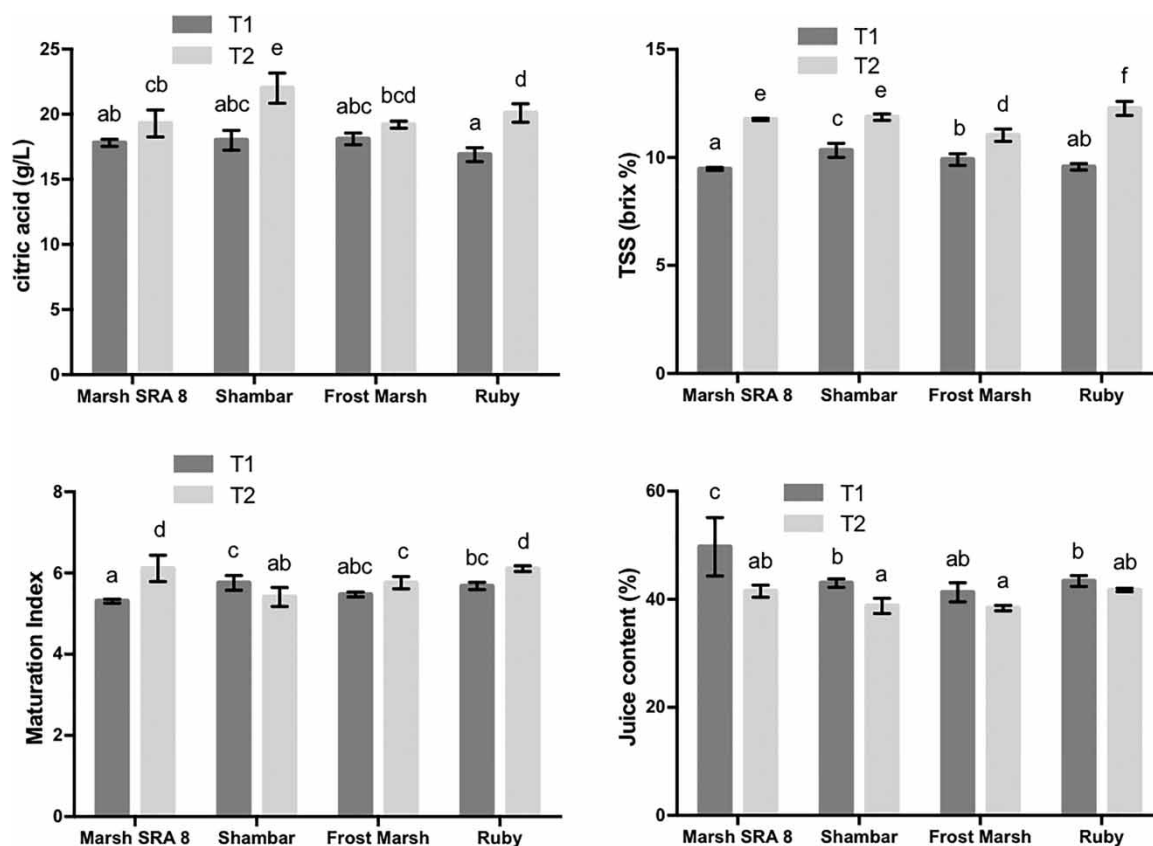
**Table 3** | Fruit quality parameters during the experiment period for the control (T1) and the water stress (T2) treatments

Cultivar	Treatments	Juice (%)	TSS (°Brix)	TA (g L <sup>-1</sup> )	Vitamin C (mg/100 mL)	T. Phenols. (mg GAE/mL)
cv. Marsh SRA 8	T1	49.7 c	9.5 a	17.8 ab	39.0 a	1.06 a
	T2	41.5 ab	11.8 b	19.3 cd	42.6 bcd	1.24 b
	ANOVA	sd	sd	sd	sd	sd
cv. Shambar SRA 22	T1	43.0 b	10.3 a	18.0 abc	40.0 ab	1.10 a
	T2	38.8 a	11.9 b	22.0 e	45.5 d	1.35 c
	ANOVA	sd	sd	sd	sd	sd
cv. Frost Marsh	T1	41.3 ab	9.9 a	18.1 abc	39.7 ab	1.18 b
	T2	38.4 a	11.0 b	19.2 bcd	43.3 cd	1.24 b
	ANOVA	nsd	sd	nsd	sd	nsd
cv. Ruby	T1	43.4 b	9.6 a	16.9 a	41.6 abc	1.09 a
	T2	41.7 ab	12.3 b	20.1 d	49.1 e	1.40 c
	ANOVA	nsd	sd	sd	sd	sd

Parameters analyzed are: total soluble solids (TSS); titratable acidity (TA), ascorbic acid (vitamin C), phenols content (T. phenols).

Values are the mean of three replicates per variety. Values followed by different letter within a column are significantly different (sd) and values followed by the same letter are not significantly different (nsd) at the 0.05 level of probability, according to Duncan's test.



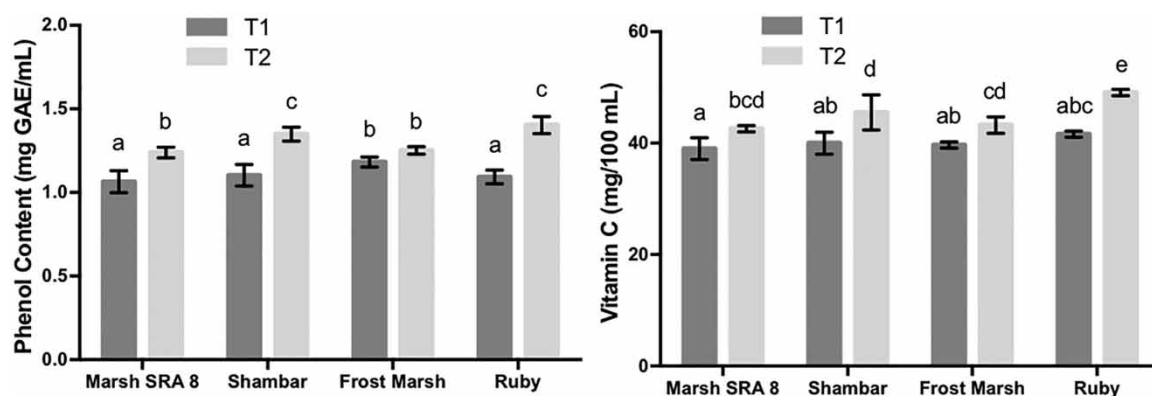


**Figure 2** | Citric acid (Titratable acidity), Total Soluble Solids (TSS), Maturity Index and Juice content (%) of all the studied grapefruit cultivars. Comparison between control (T1) and DI (T2) treatment. Values are the mean of three replicates, bars with the same letter are not significantly different at the 0.05 level of probability, according to Duncan's test.

2010). Higher values of TSS and TA in juice from fruits of the DI treatment could have improved the flavor of the juice.

Vitamin C has a beneficial role in human nutrition and the juice of citrus fruits provides an important source of ascorbic acid, thus is a key parameter for the chemical

analysis of juice. In the present work, the ascorbic acid content of the fruit juice of all the grapefruits varieties was also affected by the water stress treatment (Table 3; Figure 3). In detail, under the applied DI strategy, the vitamin C content (ascorbic acid) was increased by 9.1%, 13.8%, 9% and 17.8%



**Figure 3** | Phenol Content and Vitamin C (ascorbic acid) of all the studied grapefruit cultivars. Comparison between control (T1) and DI (T2) treatment. Values are the mean of three replicates, bars with the same letter are not significantly different at the 0.05 level of probability, according to Duncan's test.

in the fruit juice of cv. Marsh SRA 8, cv. Shambar SRA 22, cv. Frost Marsh and cv. Ruby, respectively. This is in accordance with a study by Navarro *et al.* (2010), which stated that the application of DI water stress during Phase II of citrus fruit developmental stage increased the ascorbic acid content in mandarins by 15% with respect to the control juice. The witnessed effect could be related to overall attempt of the tree to combat the occurring water stress via the *de novo* synthesis of ascorbic acid (Navarro *et al.* 2010).

Several studies have highlighted the significance and importance of citrus fruit juices in dietary health (Roussos 2011), via their composition in bioactive compounds that exert beneficial effect to human health via disease prevention (Tripoli *et al.* 2007). Citrus fruits have been characterized as having a relatively high amount of phenolic content compared to other fruits and vegetables (Hunlun *et al.* 2017; Zhang *et al.* 2018). In our current work, the fruits under T2 water strategy had significantly increased levels of total phenolics compared with the control (Table 3; Figure 3). In detail, under DI the total phenolic content in the juice of cv. Marsh SRA 8 was increased by 16.3%, from cv. Shambar SRA 22 by 22.3%, from cv. Frost Marsh by 5.9% and from cv. Ruby by 28.4%. Similar results were reported by Navarro *et al.* (2010) in mandarin fruits, where the juice of clementine mandarin exhibited increased levels of total phenolics under the effect of DI treatments. The documented increase of total phenolics in fruit under the negative effect of drought stress could be attributed to the activation of cascades leading to new synthesis (Roby *et al.* 2004; Stefanelli *et al.* 2010) and the lower juice content (Navarro *et al.* 2010).

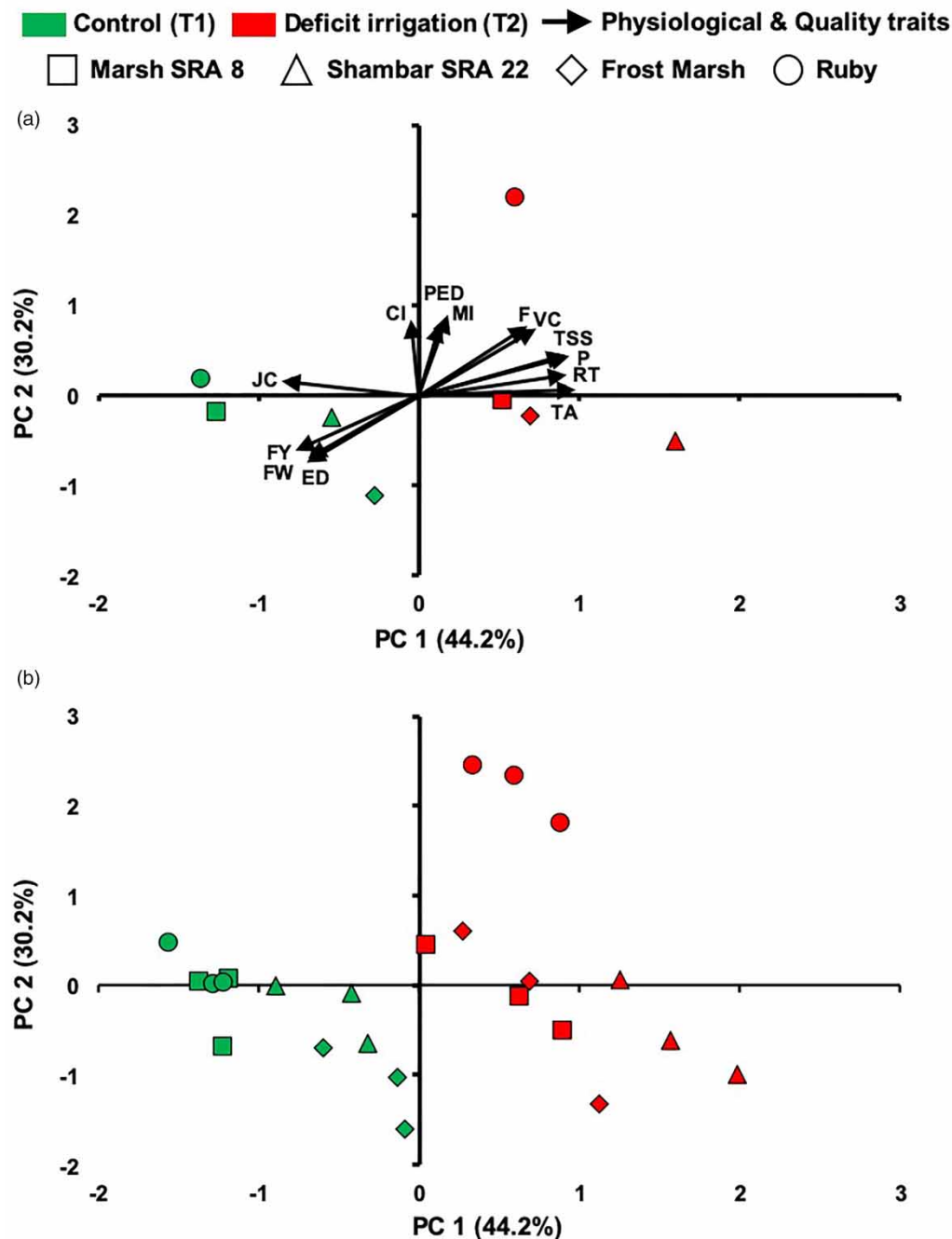
### Principal component analysis of the overall data

Principal components analysis (PCA) was used to detect any patterns, groupings, and differences in full irrigated (T1) and deficit irrigation (T2) regimes, among the four (4) grapefruit cultivars (Marsh SRA 8, Shambar SRA 22, Frost Marsh and Ruby). PCA analysis was performed using 13 variables related to fruit physiology and quality (Figure 4, PCA A). The variance of data that was explained by the PCA model was 74.4%; in particular, PC1 explained 44.2% and PC2 30.2% of the variance. The Kaiser Meyer Olkin measure of sampling adequacy (KMO) on the traits score data was

0.768. The observed changes in PC1 scores coincided with an increasing irrigation regime, suggesting that PC1 may be associated with levels of irrigation, green color for full irrigation (T1) and red color for deficit irrigation (T2). This hypothesis was demonstrated by the fact that the juice content (JC) is closely related to PC1 and reduced sufficiently in fruits with deficit irrigation schedule (T2); on the other hand, titratable acidity (TA) was increased in the fruits under deficit irrigation schedule (red color – T2) indicating a possible way of inducing acids accumulation during fruit internal maturation (Pérez-Pérez *et al.* 2014). Moreover, other fruit quality traits such as total soluble solids (TSS), total phenols (P), and rind thickness (RT) have also participated in the structure of PC1, demonstrating a strong increase under deficit irrigation regimes. On the contrary, PC2 is more closely linked to cultivars separation mainly based on three (3) certain variables with high loadings in the PC model: these variables were Color Index (CI), maturation index (MI), and fruit shape (PED: polar to equatorial diameter ratio) (Figure 4, PCA A). In general, an obvious separation between treatments via PCA analysis on the score plot has been observed, where PC1 is mostly responsible for the observed effect (Figure 4, PCA B). A clear pattern separation between the deficit irrigation schedule (T2) upon fruits of cv. 'Ruby' over the other examined fruit cultivars was observed, while this pattern is also maintained under full irrigation, highlighting the qualitative superiority of cv. Ruby among the examined cultivars, independent of the irrigation regime (Figure 4, PCA B).

### CONCLUSIONS

Lately grapefruit (*Citrus paradisi* Macf.) have been proposed as a very profitable alternative citrus cultivation compared to traditional ones that are widely cultivated. Grapefruit are characterized by increased irrigation demands for the production of quality fresh fruits. This fact drives most farmers to seek methods and implement agricultural practices that limit the applied irrigation water. Thus, there is a specific need to investigate and evaluate the impact of DI farming practices upon the most important fruit nutritional quality attributes that provide added value to the consumed fruits.



**Figure 4** | Principal Component Analysis (PCA) of physiological and quality traits of four grapefruit cultivars and irrigation stress (T2) using a biplot (A) and a score plot (B) data presentation. Control – T1: green, deficit irrigation treatment T2: red, cv. 'Marsh SRA 8' square, cv. 'Shambar SRA 22' triangle, and cv. 'Frost Marsh' rhombus, cv. 'Ruby' circle. Physiological and quality traits are represented with a black arrow: Fruit yield (FY); fruit weight (FW); equatorial diameter (ED); fruit shape (PED); color index (CI); rind thickness (RT); flesh firmness (F), maturation index (MI); juice content (JC); total soluble solids (TSS); titratable acidity (TA); ascorbic acid content (VC); total phenols content (P).

The implementation of DI during Phase II of grapefruit development strongly affects qualitative parameters in several ways. The fact that under DI cv. Ruby increased

significant quality criteria like TSS, TA, ascorbic acid content and total phenolics, with respect to cv. Frost Marsh and cv. Marsh SRA 8, clearly highlights the fact that the

response is tightly dependent on the cultivar genotype. Further work should be done, via the use of -omic technologies (genomic, metabolic and transcriptomic analysis) so as to investigate and pinpoint the genes and cascades that participate in the enhancement of quality parameters in grapefruit during drought stress.

## ACKNOWLEDGEMENTS

The authors thank Dr Michalis Michailidis for his assistance on statistical data management and Mrs Georgia Leontopoulou for her assistance with field and laboratory measurements.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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First received 31 January 2021; accepted in revised form 4 April 2021. Available online 15 April 2021