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Influence of deficit irrigation and crop load on the yield and fruit quality in *Wonderful* and *Mollar de Elche* pomegranates

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Abstract

BACKGROUND: The working hypothesis of the present study was that, by proper simultaneous control of irrigation (hydroSOStainable products) and crop load (thinning), it is possible to promote the accumulation of bioactive compounds and improve fruit appearance (size and weight). The effects of (i) irrigation status [T0, 120% ETc (estimated crop evapotranspiration); T1, 60% ETc during fruit growth and ripening] and (ii) crop load (A0, no thinning; A1, thinning) on yield and fruit quality were evaluated in two pomegranate cultivars (*Wonderful*, *Wond* and *Mollar de Elche*, *ME*).

RESULTS: Thinning was effective in increasing the size and weight of fruits. Unfortunately, neither punicalagin, nor total polyphenolic content were positively affected by irrigation and thinning. T1A1 *Wond* fruits were characterized by high sugar content (glucose and fructose), together with high fruit size and weight. Furthermore, T1A1 *ME* fruits were characterized by high contents of alcohols and monoterpenoids (providing vegetal and citric flavor notes) and key sensory attributes (color, fruity and fresh pomegranate).

CONCLUSION: The final recommendation was to use the treatment T1A1 [simultaneous combination of deficit irrigation during fruit growth and ripening (T1) and thinning (A1)], although the positive results were cultivar-dependent.

Keywords: fruit thinning; hydroSOStainable fruits; *Punica granatum* L.; water deficit

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a very interesting crop because its fruits are a source of valuable compounds, such as hydrolysable tannins (punicalagins), anthocyanins (ACNs) and phenolic acids (ellagic acid).[1] These compounds have a major impact on (i) fruit quality and (ii) antioxidant activity, and have been linked to its health promoting properties basically related to the prevention of oxidative stress.[2] Moreover, pomegranate tree exhibits high adaptability to water deficit in arid and semiarid areas because it possesses drought tolerance characteristics.[3] Nonetheless, to reach optimal vegetative growth, yield and fruit size, the crop requires regular irrigation throughout the dry season.

Spain is the largest European pomegranate producer, yielding 56 185 tons in 2015[4]; the main cultivars being farmed are *Mollar de Elche* (*ME*) and *Wonderful* (*Wond*). The major criteria for the commercial quality of pomegranate fruits are fruit size, external color and shape. Fruit size is mainly affected by crop load and plant water status, which must be controlled to obtain large fruits. It is important to consider that the amount of fresh water available for agricultural use worldwide is decreasing; thus, pomegranate farming must adopt the use of deficit irrigation (DI) strategies, leading to an improved water-use efficiency. Only a few studies have evaluated the response of pomegranate fruit to DI and their conclusions are

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not unanimous, even though they agree that water deficit effects depend on the stage of fruit growth at which DI is applied, as well as the water deficit level achieved. In this sense, sustained deficit irrigation (SDI) applied throughout the pomegranate season reduces total yield per tree, the number of fruits per tree and the size of the fruits.[5] Furthermore, DI can advance the optimal harvest time by approximately 7–8 days, which can be of interest for the pomegranate industry in early ripening cultivars together with the fact that they have high contents of bioactive compounds. In a similar way, SDI under moderate water stress showed changes in color and chemical characteristics, related to earlier ripening.[6] However, pomegranate juice obtained from SDI trees, under severe water stress, was of lower quality and less healthy than that that from fully irrigated trees.[7] Recently, however, other studies[8] concluded that pomegranates from SDI trees had good sensory quality, a higher content of most of the bioactive compounds, and suffered less chilling injury during cold storage, and also had a longer shelf-life than fully irrigated fruits. Moreover, it was shown that pomegranates from SDI trees, submitted to mild water stress during flowering and fruit set and more severe water stress during the linear phase of fruit growth and ripening, had a redder peel and juice with a higher level of total soluble solids.[9]

On the other hand, previous studies with fruits such as pistachios[10,11] and table olives,[12,13] grown under deficit irrigation strategies were shown to have a proper and specific identity and were termed 'hydroSOStainable' products; they have a special sensory profile and chemical composition. In this way, it is expected that pomegranate fruits could also be improved on some key chemical parameters and sensory attributes by DI.

Considering all of the previously reported information, the present study aimed to develop knowledge on the simultaneous effects of deficit irrigation (during fruit growth and ripening) and crop load on yield and fruit quality in the two most popular pomegranate cultivars in Spain: *ME* and *Wond*. The quality was studied by evaluating (i) physical characteristics, (ii) chemical characteristics and (iii) descriptive sensory attributes of fruit.

MATERIALS AND METHODS

Plant material, experimental conditions and treatments

The experiment was carried out in 2016 in a pomegranate (*Punica granatum* L.) orchard at the CEBAS-CSIC experimental station in Santomera (Murcia, Spain) (38°11′, 1°03′). The trees were own-rooted 6-year-old *Wond* and *ME* with only one trunk and spaced at 3 × 5 m. Yearly, trees were lightly pruned to encourage fruit production. Sprouts and suckers were removed as they appeared and dead and damaged wood was removed in late winter. The soil is a paralithic mollic-calciorthid very stony (33%, w/w) and shallow with a clay-loam texture. Micrometeorological data (air temperature, solar radiation, air relative humidity, rainfall and wind speed 2 m above the soil surface) was collected by an automatic weather station located at the experimental farm; the station has been operating for more than 20 years and it is located on a soil with grass cover.

The design of the field experiment was completely randomized with four replications, with each replication consisting of three adjacent tree rows, each with seven trees. Samples for the morphological, physical, chemical and sensory analyses were taken on the inner tree of the central row of each replicate, which were very similar in appearance (leaf area, trunk cross-sectional area, height, ground shaded area, etc.), whereas the other trees served as border trees. Each plot had a separate irrigation system and a meter to measure the volume of water applied; the plots were separated by the rows of border trees.

Two *irrigation treatments* (T0 and T1) were used to study the effects on the plant water status. From 19 April 2016 [day of the year (DOY) 109] to 6 October (DOY 279), control pomegranate trees (T0) of both cultivars were irrigated daily above the estimated crop evapotranspiration (120% ETc) to obtain non-limiting soil water conditions. Deficit irrigated plants of both cultivars (T1) were irrigated at 120% ETc from the beginning of the experiment to fruit setting (DOY 168) and at 60% ETc from then to harvest (fruit growth and ripening). Crop irrigation requirements were determined using the daily crop reference evapotranspiration (ETo), as calculated using the Penman–Monteith equation (FAO method),[14] and a crop factor based on the time of the year[15] and also the percentage of ground area shaded by the tree canopy.[16] Irrigation was carried out during the night using a drip irrigation system, with one lateral pipe per tree row and four emitters (spaced 75 cm and each delivering 4 L h^{-1}) per plant, and adjusting the irrigation hours. The total irrigation water amounts, measured with in-line water meters, applied to each treatment were 516 mm (T0) and 317 mm (T1) in *Wond* trees and 537 mm (T0) and 327 mm (T1) in *ME* trees.

There were two *thinning* treatments (A0 and A1) used to study the effects on the crop load. Pomegranate A0 trees were not thinned, and A1 trees were manually thinned, leaving 20–25 cm between fruits and avoiding the presence of 'double fruits' (two fruits fused together).

Pomegranate fruits from each treatment and cultivar ($n = 20$ fruits per tree) were manually harvested on DOY 280, when commercial maturity was reached (*Wond* 15 °Brix and *ME* 12 °Brix), with exact values being: (i) *Wond*: 16.8 °Brix, 0.781 g 100 mL^{-1}, maturity index (MI) [TSS (°Brix)/TA (g 100 mL^{-1})] 21.5 ± 6.3 and (ii) *ME*: 12.7 °Brix, 0.140 g 100 mL^{-1}, MI 90.8 ± 11.4. Fruits were immediately transported under ventilated conditions to the laboratory and stored under controlled conditions (5 °C and 90%, relative humidity) for less than 1 week, until the analysis.

The marketable yield was calculated by weighting all fruits from the three central trees of the central row (three rows per field plot) of each one of the four replications of this experiment (3 trees × 4 replications, giving a total of 12 trees per treatment), and after removing fruits not reaching commercial size, those affected by pest attack and having physiopathies. Then, 20 fruits per tree were used for the morphological, physical, chemical and sensory analyses.

Plant water status

The water relationships of the leaves were measured at midday (12 h solar time). Fully expanded leaves from the south-facing side and middle height of four trees per treatment were selected for measurements. Midday leaf conductance (g_{leaf}) was measured with a porometer (Delta T AP4; Delta-T Devices, Cambridge, UK) on the abaxial surface of two leaves per tree. Midday stem water potential (Ψ_{stem}) was measured in two leaves similar to those used for g_{leaf}, which were enclosed in a small black plastic bag covered with aluminum foil for at least 2 h before the measurements were made using a pressure chamber (PMS 600-EXP; PMS Instruments Company, Albany, OR, USA).[5]

Morphological, physical and chemical parameters

The moisture (*M*) percentage of arils was determined in accordance with Alcaraz-Mármol *et al.*[17] Fruit diameter was measured

with an electronic digital slide gauge (Mitutoyo, Kawasaki, Japan). Ten fruits were carefully cut at the equatorial zone with a sharp knife, and then arils were manually extracted to obtain the fresh pomegranate juice. The color density (CD) and the percentage of polymeric color (PC) were using a previous method proposed by Giusti and Wrolstad.[18] All analyses were run in four replications.

From the 20 fruits taken, three batches of six fruits were randomly prepared and all arils from each batch were manually extracted and mixed to prepare the juices; thus, three juices were prepared for each of the four field replications per treatment. Then, a total of 12 measurements per treatment were made for all the analyses described below, although the mean values represent four replications per treatment.

Total polyphenol content (TPC) and punicalagin content (Pn)

Methanol extract was prepared as follows: pomegranate juices (1 mL) were mixed with 10 mL of MeOH/water (80:20, v/v) + 1% HCl, sonicated at 20 °C for 15 min, and left for 24 h at 4 °C. Then, the extract was again sonicated for 15 min, and centrifuged at 15 000 × *g* for 10 min. TPC was quantified using Folin–Ciocalteu reagent.[19] Absorption was measured using a UV–Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). The extraction and quantification of the Pn content (sum of α and β isomers) in the pomegranate juices were analyzed using the method proposed by Cano-Lamadrid *et al.*[20]

Sugar and organic acid content

The extraction and quantification of sugar and organic acids in pomegranate juices were conducted as described for a recent study.[17] Standards of sugars (glucose and fructose) and organic acids (citric and ascorbic) were obtained from Sigma (St Louis, MO, USA) and calibration curves were prepared and showed good linearity ($r^2 \geq 0.999$).

Extraction and chromatographic analysis of volatile compounds

Headspace solid phase micro-extraction comprised the isolation technique used to study the volatile composition of the pomegranate juice. Pomegranate juice (5 mL), ultrapure water (10 mL), 1-octanol (10 μL of 1000 mg L^{-1}, internal standard) and NaCl (15% w/v, weight/volume) were placed into a 50-mL vial with polypropylene caps and polytetrafluoroethylene/silicone septa. The vial was placed in a water bath at controlled temperature (40 °C) and automatic stirring. After allowing time for equilibration, a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fiber was exposed to the sample headspace for 50 min at 40 °C.

The chromatographic set up and conditions were identical to those reported recently,[21] with the only exception that the column used was a Restek Rxi-1301 Sil MS (Restek Corporation, Palo Alto, CA, USA) with an internal diameter of 30 m × 0.25 mm and a film thickness of 0.25 μm.

The identification and semi-quantification of volatiles was conducted using gas chromatography–mass spectroscopy and gas chromatography-flame ionization detection, respectively. The volatile composition analysis was run in four replications for each treatment and the results are expressed as a percentage of the total area represented by each one of the volatile compounds.

Sensory analysis by trained panel

Eight trained panelists (aged 30–55 years; four females and four males) with more than 500 h of training in sensory testing from the department of Agro-Food Technology (UMH) participated in the study. The sample serving and analysis procedures (using the suitable lexicon and reference products) were conducted.[22] A scale from 0 to 10, with increments of 0.5, was used, where 0 represented no intensity and 10 represented extremely strong intensity. Sensory analysis was run in four replications per treatment (four sessions).

Statistical design and analysis

Data were analyzed using StatGraphics Plus, version 5.0 (Manugistics, Inc., Rockville, MD, USA). A two-way analysis of variance (irrigation treatment and crop load as factors) was performed and means values were compared by Tukey's multiple range test. Ψ_{stem} and g_{leaf} values for each replicate were averaged before the mean ± SE of each treatment were calculated. Percentage values were arcsin-transformed before statistical analysis. Instrumental parameters correlated with sensory descriptors were used for establishing a principal component analysis (PCA regression map) using XLSTAT Premium 2016 (Microsoft Corp., Redmond, WA, USA); only those parameters showing significant differences in any of the two factors under study were included in the PCA.

RESULTS AND DISCUSSION

Climate and plant water status

During the experiment, the average daily maximum and minimum air temperatures were 29.4 and 17.8 °C, respectively, and the average mean relative humidity was 60.9%. The total rainfall was extremely low, and reached a total added value of 15 mm, after 4 days of rain, and the total ETo reached 773 mm (DOY 109–279).

Ψ_{stem} and g_{leaf} values for T0 plants in both cultivars were high and almost constant during the experimental period (Fig. 1), implying that the irrigation applied to these treatments in both cultivars was sufficient to avoid any water deficit during the measurement period. The differences in Ψ_{stem} and g_{leaf} values between T0 and T1 plants were characterized by the gradual decrease of their values in T1 plants from the beginning of the experiment (DOY 109), reaching minimum values near the end of the measurement period, DOY 230–279 (Fig. 1), clearly indicated a significant water deficit situation in T1 plants.

Regarding crop load, it has been indicated that high crop load may increase transpiration rates,[23] stomatal conductance,[24] leaf photosynthesis[25] and tree water use.[26] However, studies also reported a reduction in water uptake as a result of a high crop load.[27] Finally, further studies concluded that the effect of crop load on tree water status is not obvious[28] or is apparent only under severe deficit irrigation conditions.[28] In this sense, the results from the current experiment indicate that Ψ_{stem} and g_{leaf} values in A0 plants were very similar to those of A1 plants not only in full irrigated plants (T0), but also in deficit irrigated plants (T1). Thus, it can be concluded that pomegranate plant water status was not influenced by crop load under these particular experimental conditions.

Marketable yield and fruit morphology

It is important to note that the marketable yield only includes fruits with a commercial size and with no pest-attack or physiopathies. Both cultivars showed similar response of the marketable yield (production) and fruit morphology to deficit irrigation and thinning (Table 1). Fruit thinning decreased the marketable yield in both cultivars, although the fruits remaining on the tree showed

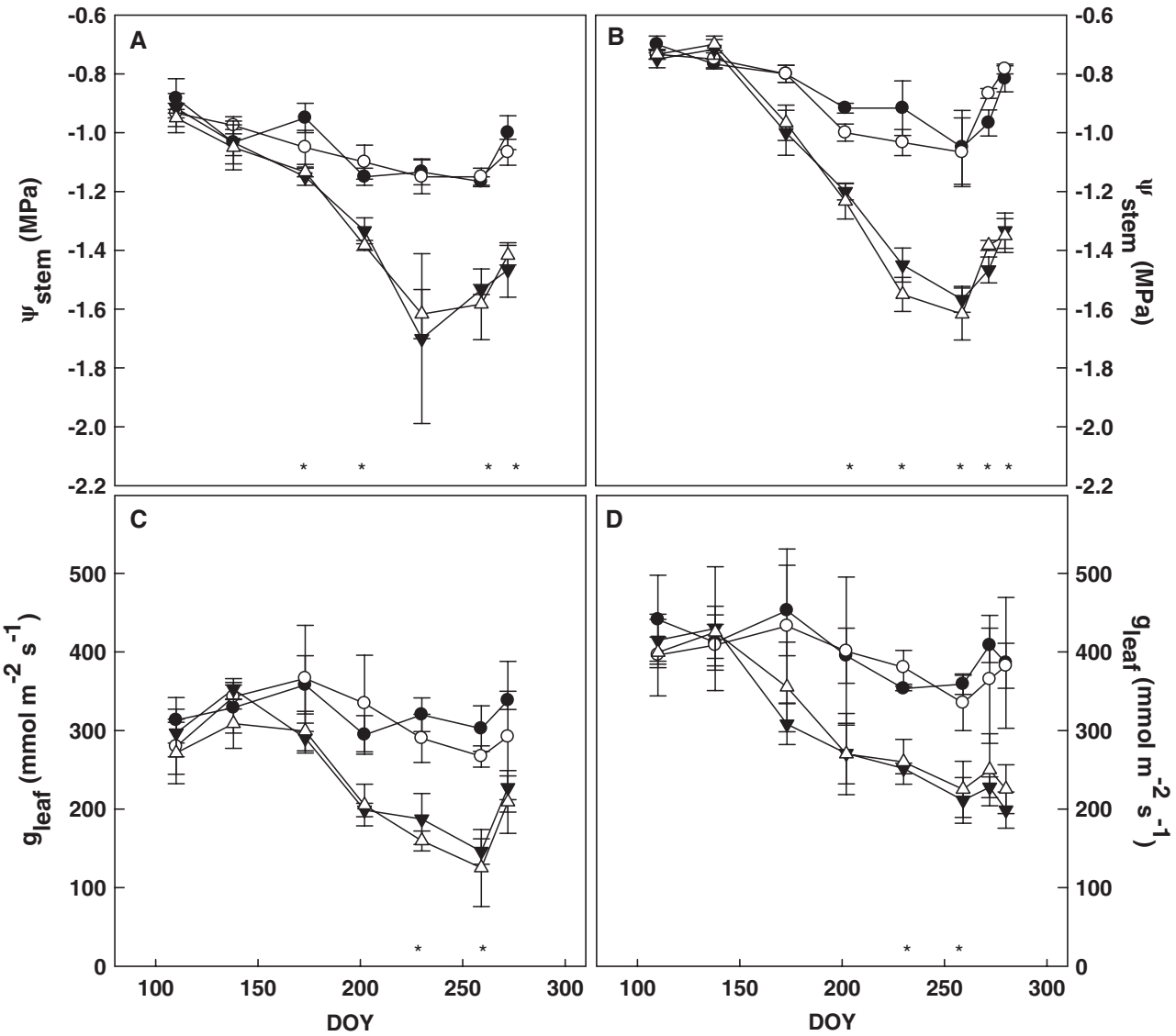

Figure 1. Midday stem water potential (Ψ_{stem}) and leaf conductance (g_{leaf}) values in control (T0, circles) and deficit irrigated (T1, triangles) *Wond* (A, C) and *ME* (B, D) pomegranate trees, which were hand-thinned (A1, open symbols) or non-thinned (A0, closed symbols), during the experimental period (DOY: day of the year). Each value is the mean of four measurements. Asterisks indicate significant differences among treatments according to Tukey's test ($P < 0.05$).

higher weight and size. Thus, it is expected that a higher price can be obtained for these final commercial fruits. However, DI reduced significantly the marketable yield but not the weight and size.

As expected, marketable yield of pomegranates decreased significantly by the plant water deficit effect (Fig. 1), as has been shown previously.[5,6] However, water deficit did not affect fruit weight and size. In this respect, and taking into consideration the results shown previously,[5] probably the maximum water deficit achieved in the current experiment was lower than that necessary to produce fruit turgor loss and, consequently, to decrease fruit growth.

Color density, polymeric color and phytochemical compounds

The importance of the copigmentation in pomegranate juice is a result of the importance of the color with respect to determining consumer acceptance of pomegranates and pomegranate-based products (Table 1). Copigmentation is the reaction among ACNs (responsible of color to pomegranate juices) with copigments (e.g. phenolic acids), producing a hyperchromic effect in the absorption spectrum.

CD, which has a high correlation with catechin-phlorogucinol and monomeric ACNs,[29] was affected by irrigation and thinning in the two pomegranate cultivars. The value of CD in *Wond* fruits (2.69–5.89) was higher than that in *ME* fruits (2.18–4.08) because of the higher content of monomeric ACNs, especially cyanidin-3-glucoside.[30] Color density was reduced (increasing polymerization) when water deficit was applied (T1) in *Wond*; however, the trend was the opposite in *ME* fruits. Crop load affected CD only in *Wond* fruits, by reducing polymerization in remaining fruits; no effect was observed in the *ME* cultivar.

In general, the percentage of polymeric color, PC (high values indicate a high degree of ACN polymerization, leading to a less intense red color) of fresh pomegranate juices should be less than 10%.[18] In the present study, the values were higher (16.4–85.9%) as a result of different factors, including the use

Table 1. Production (yield), morphology, total polyphenolic content (TPC, mg GAE kg^{-1} FW), and, punicalagin (Pn, mg mL^{-1} FW) of *Wonderful* and *Mollar de Elche* pomegranates as affected by deficit irrigation and thinning treatments

Treatment	Production (kg)	Fruit weight (g)	Diameter (mm)	Color density, CD	Polymeric color, PC (%)	TPC (mmol L^{-1} Trolox FW)	Pn (mg mL^{-1} FW)
				WONDERFUL			
				ANOVA test			
Irrigation	*	NS	NS	*	**	NS	NS
Thinning	*	**	***	*	NS	**	NS
Irrigation x Thinning	*	**	NS	**	***	**	NS
Tukey's multiple range test							
Irrigation							
T0	49.7 a	387	91.4	4.45 a	22.4 b	704	3.14
T1	29.3 b	372	90.1	3.11 b	34.8 a	714	2.96
Thinning							
A0	42.3 a	360 b	88.5 b	3.10 b	31.4	728 a	2.98
A1	36.7 b	400 a	92.9 a	4.46 a	25.9	689 b	3.11
Irrigation × Thinning							
T0A0	53.9 a	387 ab	90.3	3.51 ab	25.4 c	754 a	3.15
T0A1	45.5 b	387 ab	92.4	5.89 a	16.4 d	654 c	3.03
T1A0	30.8 c	333 b	86.7	2.69 b	37.6 a	704 b	2.99
T1A1	27.9 c	412 a	93.5	3.53 ab	32.3 b	724 ab	2.80
				MOLLAR DE ELCHE			
				ANOVA Test			
Irrigation	*	NS	NS	**	**	NS	NS
Thinning	*	*	*	NS	NS	***	NS
Irrigation × Thinning	*	*	NS	***	***	***	NS
Tukey's multiple range test							
Irrigation							
T0	39.0 a	424	93.8	2.21 b	50.2 b	676	3.04
T1	29.6 b	398	92.2	3.47 a	74.1 a	661	3.23
Thinning							
A0	37.1 a	392 b	91.7 b	3.17	67.9	692 a	2.98
A1	31.5 b	431 a	94.3 a	2.52	56.4	644 b	3.29
Irrigation × Thinning							
T0A0	39.9 a	405 ab	92.6	2.26 b	50.0 c	724 a	3.04
T0A1	38.1 a	443 a	95.0	2.18 b	50.4 c	628 c	2.77
T1A0	34.4 a	378 b	90.8	4.08 a	85.9 a	662 b	2.92
T1A1	24.9 b	418 ab	93.6	2.86 ab	62.4 b	659 b	3.80

NS, not significant at $P < 0.05$; significant at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, respectively.
Values followed by the same lowercase letter, within the same column and factor, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.
Values for punicalagin (Pn) content are the sum of α and β punicalagin isomers.
FW, fresh weight.

of different cultivars, and probably also because pomegranate juices were stored at −18 °C during 2 weeks and then defrosted at 4 °C. Previous studies indicated that a prolonged storage at 4 °C, or even at −18 °C, produced the polymerization of ACNs with other compounds, mainly condensed tannins.[29,31] The values of PC of *Wond* (16.4–37.6%) were significantly lower than those of *ME* (50.0–85.9%), being attributed to the difference in phenolic compounds and the stability between cultivars (ACNs from *Wond* juices were more stable than those from *ME*).[17] Water stress increased the value of PC in *Wond* and *ME* cultivars, allowing polymerization and thus deterioration of the red color.

Regarding TPC, the experimental results demonstrated that thinning had no positive effect in the remaining fruits, and even led to slight but significant reductions (6% and 8% in *Wond* and *ME*, respectively) (Table 1). Moreover, juices from *Wond* cultivar presented higher values of TPC than *ME* because of differences in the polyphenol profile.[32] On the other hand, water deficit did not affect either TPC or Pn content, in contrast to that reported previously in pomegranate fruits under moderate and severe SDI[30]; probably, the level of water stress reached in the previous experiment was higher than that reached in the present study.

No correlation was found between TPC and Pn, in contrast to previously reported data,[20] implying that Pn (isomers α and β) was not the only compound implied in the total polyphenolic content; other compounds behind this experimental trend could be anthocyanins and other ellagitannins. Only trace levels of ellagic acid were found in the present study, and cannot account for this lack of correlation.

Table 2. Sugars and organic acid profiles of *Wonderful* and *Mollar de Elche* pomegranates as affected by deficit irrigation and thinning treatments

	Sugars		Organic acids	
	Glucose	Fructose	Citric acid	Ascorbic acid
Treatment	(g L^{-1} FW)			
	WONDERFUL			
	ANOVA test			
Irrigation	*	*	NS	NS
Thinning	*	*	NS	NS
Irrigation x Thinning	***	***	NS	NS
Tukey's multiple range test				
Irrigation				
T0	121 b	142 b	1.47	0.32
T1	143 a	171 a	1.17	0.20
Thinning				
A0	141a	143 b	1.23	0.20
A1	123 b	169 a	1.40	0.32
Irrigation × Thinning				
T0A0	119 b	138 c	1.45	0.21
T0A1	123 b	146 b	1.47	0.23
T1A0	126 b	149 b	1.04	0.23
T1A1	159 a	192 a	1.33	0.44
	MOLLAR DE ELCHE			
	ANOVA test			
Irrigation	NS	NS	NS	NS
Thinning	NS	NS	NS	NS
Irrigation x Thinning	NS	NS	***	NS
Tukey's multiple range test				
Irrigation				
T0	119	151	0.93	0.43
T1	120	142	0.32	0.47
Thinning				
A0	120	148	0.93	0.44
A1	118	144	0.32	0.47
Irrigation × Thinning				
T0A0	118	151	1.54 a	0.40
T0A1	120	151	0.32 b	0.46
T1A0	122	145	0.32 b	0.47
T1A1	117	138	0.32 b	0.47

NS, not significant at $P < 0.05$; significant at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, respectively.
Values (mean of three replications) followed by the same lowercase letter, within the same column and factor, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.
FW, fresh weight.

Sugars and organic acids

In accordance with previous data,[17,32] two sugars (glucose and fructose) and two organic acids (citric and ascorbic) were identified in the *Wond* and *ME* pomegranate juices (Table 2).

In terms of sugar profile, the glucose/fructose ratios were 0.84 and 0.82 in *Wond* and *ME*, respectively; similar results were reported previously.[17,32] An increase of glucose and fructose in *Wond* cultivar under water stress (T1) was found. The level of water stress played a role in fruit maturity; an accelerated early fruit maturity can be hypothesized based on the stimulation of the conversion of sucrose (disaccharide combining glucose and fructose) to the noted monosaccharides.[33] The interaction on SDI and thinning (T1A1) gave rise to the highest increase in glucose and fructose in the *Wond* cultivar. By contrast, sugars from *ME* cultivar were not affected, probably because *ME* trees are better adapted to the cultivation area and to water stress than the *Wond* ones.[9]

The content of citric acid was higher in the *Wond* fruits than in the *ME* fruits, which agreed with a previous study stating that citric acid predominated in sour varieties, such as *Wond*.[17] On the other hand, the level of ascorbic acid was low in all treatments and in both cultivars as a result of the loss of this compound from metabolic activity during ripening, generating polymeric compounds.[34] Both identified organic acids were not affected by either the irrigation treatment (water stress) or the thinning treatment (crop load) in any of the two cultivars, probably because of the low level of water stress reached.

Volatile compounds

A total of 12 and 14 different compounds were identified in the *Wond* and *ME* juices under study, respectively. Although the number of the volatile compounds found in the present study was lower than those previously found in other studies (18 compounds), it is a normal value for this fruit with a low odor/aroma intensity and complexity.[35] Table 3 shows the retention times and indices used for the identification of the compounds (together with the simultaneous use of standards) found in the pomegranate juices, as well as the main sensory descriptors of each one of the volatile compounds.[36,37]

For clarity, the pomegranate volatile compounds have been grouped into five chemical families:

(i) *Aldehydes* (ALDs, total aldehydes): hexanal (V1), octanal (V2) and nonanal (V3);

(ii) *Esters* (ESTs): benzyl acetate (V4), ethyl hexanoate (V5) and ethyl octanoate (V6);

(iii) *Aliphatic alcohols* (ALCs): 2-ethyl-1-hexanol (V7), 3-hexen-1-ol (V8) and 1-hexanol (V9); and.

(iv) *Monoterpenes* (MTEs): β-pinene (V10), *p*-cymene (V11) and α-terpineol (V12); and (v) *Monoterpenoids* (MTOs): linalool (V13) and limonene (V14).

The relative abundance of each chemical family (sum of the percentages of all the members of the family) was significantly different between cultivars, and followed the order (Table 4):

- *Wond*: ALCs (mean of all treatments 67.0%) >> MTOs (11.5%) > MTEs (10.1%) ≈ ALDs (9.9%) > ESTs (1.4%)
- *ME*: ALDs (mean of all treatments 29.6%) > MTOs (26.7%) > ALCs (23.6%) > MTEs (13.9%) > ESTs (6.2%).

In general, aliphatic alcohols (ALCs) were the predominant family [mainly, 1-hexanol (V9) and 3-hexen-1-ol (V8)] in *Wond* fruits (Table 4); these results agreed with those reported previously.[38] On the other hand, aldehydes, ALDs [mainly hexanal (V1) and nonanal (V3)] played an important role and were the most abundant chemical family in the *ME* juices, also in agreement with previous data.[39]

Changes on volatile composition as affected by irrigation or thinning treatments have been found in different fruits, such as jujube,[40] table olive[13] and pistachio,[10] amongst others. However, the present study is the first to evaluate the combined effects of these two factors on the volatile profile of pomegranate juices; different trends were found for each cultivar under study.

In the *Wond* cultivar, when water stress was applied, a reduction in ALD (only as a result of hexanal) and EST (as a result of benzyl

Table 3. Retention time (min) and indices and sensory descriptors (SAFC, 2012) of the volatile compounds of pomegranate juices, cultivars *Wonderful* and *Mollar de Elche*

Code	Compounds	Retention time (min)	Retention indices		Descriptors
			Experimental	UMH database	
Aldehydes					
V1	Hexanal	6.64	826	835	Green
V2	Octanal	14.30	1036	1029	Herbaceous, citrus
V3	Nonanal	19.16	1140	1154	Citrus, vegetable
Esters					
V4	Benzyl acetate	22.37	1206	1210	Apple, floral, fruity, sweet
V5	Ethyl hexanoate	13.38	1016	1018	Apple, banana, pineapple
V6	Ethyl octanoate	22.61	1211	1212	Floral, pear, pineapple
Aliphatic alcohols					
V7	2-Ethyl-1-hexanol	15.72	1067	1070	Rose, sweet
V8	3-Hexen-1-ol	8.70	898	902	Banana, green, vegetable
V9	1- Hexanol	8.98	906	912	Green, herbaceous
Monoterpenes					
V10	β-Pinene	12.33	992	998	Woody
V11	*p*-Cymene	14.63	1043	1051	Citrus
V12	α-Terpineol	24.03	1240	1250	Lilac
Monoterpenoids					
V13	Linalool	18.94	1135	1142	Lemon, orange, sweet
V14	Limonene	14.46	1039	1046	Lemon, orange, sweet

The UMH research group (Universidad Miguel Hernández) has created their own library of standards to provide proper retention indices for the identification of the volatile compounds found in different food matrixes. All 14 compounds found in the pomegranate juices have been identified by using Sigma-Aldrich (Merk KGaA, Darmstadt, Germany) standards.

acetate) contents, as well as an increase of MTEs (β-pinene), was observed; however, in the *ME* cultivar, ALDs also decreased (hexanal and nonanal), although MTEs decreased as well (β-pinene and α-terpineol) and MTOs increased (limonene). Probably, the reduction of ALDs was a result of the effect of water stress on the synthesis of the compounds from this chemical family. The synthesis starts with the C18 fatty acids, linolenic and linoleic acids.[41] Furthermore, the effects seen in the MTEs family could be explained by the effects of water stress on the activity of the enzyme linalool synthase or in the contents of its substrates, mainly carotenoids.[42] The reduction of hexanal was observed in other fruits such as grapes under water deficit during grape development and this was associated with fruit maturity.[43]

On the other hand, the only common effect of thinning on the volatile composition of both pomegranate cultivars was the decrease of the EST content (ethyl hexanoate), whereas the behaviors of ALCs and MTEs were different. The effects on thinning (crop load) on the volatile composition of *Wond* fruits was limited to the noted effects on esters; however, in ME fruits, a smaller crop load implied reductions of ESTs and ALCs but an increase of MTEs.

PCA

For a better understanding of the relationships among all the studied variables for the different treatments (interaction between irrigation and thinning treatments), a PCA was run for the *Wond* and *ME* cultivars, respectively (Fig. 2). All sensory data have been included in the PCA and are discussed here to avoid duplication (Table 5).

The first principal component (F1) accounted for 55.19% and 40.77% of the total data variance in *Wond* (Fig. 2A) and *ME* (Fig. 2B), respectively, whereas the second principal component (F2) accounted for 27.72% and 32.36% of the total variance, respectively.

It is important to note that the higher the distance between two parameters, the lower their correlation. Considering F1 as the dimension that explained the main differences among treatments, in *Wond* fruits (Fig. 2A), T0A0 was positively linked with TPC, ∑MTOs (MTOs normally have citric flavor notes), ∑ALDs (which have vegetable flavor notes), ∑ESTs (with fruity flavor notes), production (yield) and key descriptive sensory parameters (sweetness, color, fruity, floral and pomegranate ID). In *Wond* fruits, all other three treatments (T0A1, T1A0 and T1A1) were positively correlated with the sugar content, ∑ALCs (vegetable notes), fruit weight, diameter, polymeric color, and apple and pear flavor notes.

On the other hand, in *ME* fruits (Fig. 2B), treatment T1A1 (trees under water stress and subjected to thinning) was positively correlated with ∑ALCs (vegetable notes), ∑MTOs (citric notes) and key descriptive sensory parameters (color, sourness, fruity, floral and pomegranate ID). The other treatments (T0A0, T0A1 and T1A0) were positively correlated with ∑ALDs (vegetable notes), ∑MTEs (citric notes), ∑ESTs (fruity notes), production and citric acid content.

CONCLUSIONS

Sustained deficit irrigation (T1) caused water stress in pomegranate trees. Although a decrease of marketable yield was observed after deficit irrigation (T1) and thinning (A1), the fruits remaining on the tree presented a higher weight and size, which is a positive fact and could be linked to the higher price of the commercial fruits. *Wond* fruits were more sensitive to changes in the sugar profile, with the values of glucose and fructose being increased by deficit irrigation strategies (T1),

Table 4. Volatile compounds (% of total area) of *Wonderful* and *Mollar de Elche* pomegranates as affected by deficit irrigation and thinning treatments

	Aldehydes				Esters				Aliphatic alcohols				Monoterpenes				Monoterpenoids		
Compounds	V1±	V2	V3	∑ALD	V4	V5	V6	∑EST	V7	V8	V9	∑ALC	V10	V11	V12	∑MTE	V13	V14	∑MTO
	Relative abundance (%)																		
WONDERFUL																			
ANOVA test																			
Irrigation	*	NS	NS	*	***	NS	NS	**	NS	*	*	NS	*	NS	NS	*	NS	NS	NS
Thinning	NS	NS	NS	NS	NS	*	NS	*	*	NS	*	NS	NS	**	*	NS	NS	NS	NS
Irrigation x Thinning	*	NS	*	*	*	*	NS	*	*	NS	*	**	*	NS	*	*	*	*	*
Tukey's multiple range test																			
Irrigation																			
T0	6.15 a	0.00	4.66	10.8 a	1.27 a	0.92	0.00	2.18 a	1.15	26.3 b	39.1 a	66.6	4.44 b	0.25	3.39	8.08 b	1.61	10.8	12.4
T1	3.08 b	0.00	5.19	8.26 b	0.00	0.70	0.00	0.70 b	1.08	34.5 a	31.6 b	67.2	7.65 a	0.31	4.14	12.1 a	1.14	9.77	10.9
Thinning																			
A0	4.72	0.00	5.49	10.2	0.76	1.21 a	0.00	1.97 a	0.79 b	29.5	38.2 a	68.5	5.86	0.56 a	4.28 a	10.7	1.87	11.3	13.2
A1	4.49	0.00	4.35	8.84	0.51	0.40 b	0.00	0.91 b	1.44 a	31.3	32.4 b	65.1	6.23	0.00 b	3.26 b	9.49	0.88	9.22	10.1
Irrigation × Thinning																			
T0A0	6.25 a	0.00	5.66 a	11.9 a	1.51 a	1.03 ab	0.00	2.54 a	1.12 b	23.2 c	34.9 b	59.2 c	6.98 b	0.50	3.80 b	11.3 b	2.34 a	12.7 a	15.1 a
T0A1	6.04 a	0.00	3.66 b	9.70 ab	1.02 a	0.80 b	0.00	1.82 b	1.18 b	29.4 b	43.3 a	73.9 a	1.90 d	0.00	2.99 b	4.88 d	0.87 c	8.84 b	9.71 b
T1A0	3.16 b	0.00	5.28 a	8.44 b	0.00 b	1.31 a	0.00	1.31 b	0.40 c	36.6 a	29.8 c	66.8 b	5.47 c	0.58	4.84 a	10.9 b	1.43 b	9.37 b	10.8 b
T1A1	2.93 b	0.00	5.05 ab	7.98 b	0.00 b	0.00 c	0.00	0.00 c	1.71 a	33.3 ab	33.2 bc	68.2 ab	9.83 a	0.00	3.53 b	13.4 a	0.90 c	9.60 b	10.5 b
MOLLAR DE ELCHE																			
ANOVA test																			
Irrigation	*	NS	**	***	NS	*	**	NS	*	*	NS	NS	*	NS	**	*	**	**	**
Thinning	NS	NS	NS	NS	NS	*	***	*	NS	NS	*	*	**	**	**	*	*	NS	NS
Irrigation x Thinning	*	**	*	*	*	**	**	**	**	***	**	***	**	*	*	**	*	*	*
Tukey's multiple range test																			
Irrigation																			
T0	16.2 a	1.43	17.2 a	34.8 a	3.17	2.06 b	0.68 b	5.91	3.89 a	9.96 b	9.73	23.6	7.15 a	2.15	5.57 a	14.9 a	3.63 a	17.9 b	21.5 b
T1	13.5 b	1.41	9.51 b	24.4 b	2.67	3.02 a	0.86 a	6.55	2.97 b	11.8 a	9.49	24.3	6.57 b	2.10	4.06 b	12.7 b	1.76 b	30.3 a	32.1 a
Thinning																			
A0	14.4	1.54	12.5	28.4	3.16	3.13 a	0.49 b	6.78 a	3.44	11.6	10.7 a	25.7 a	4.97 b	1.88 b	5.63 a	12.5 b	2.94 a	23.7	26.6
A1	15.2	1.30	14.3	30.8	2.68	1.95 b	1.05 a	5.68 b	3.42	10.2	8.52 b	22.1 b	8.76 a	2.37 a	4.00 b	15.1 a	1.76 b	24.5	26.3
Irrigation × Thinning																			
T0A0	17.4 a	0.86 b	13.8 b	32.0 b	3.09 a	1.98 b	0.47 c	5.54 bc	2.07 c	14.5 a	13.3 a	29.8 a	3.62 c	1.76 ab	6.22 a	11.6 c	4.64 a	16.4 c	21.0 c
T0A1	15.0 ab	1.95 a	20.7 a	37.6 a	3.26 a	2.15 ab	1.24 a	6.64 b	3.87 b	5.44 c	6.20 c	15.5 c	10.7 a	2.55 a	4.91 b	18.2 a	2.62 b	19.4 c	22.0 c
T1A0	11.3 b	1.86 a	11.6 b	24.8 c	3.17 a	4.43 a	0.44 c	8.04 a	4.49 a	8.16 b	7.66 c	20.3 b	7.13 b	1.57 b	4.81 b	13.5 b	1.05 c	32.3 a	33.3 a
T1A1	15.4 ab	0.66 b	7.90 c	24.0 c	2.11 b	1.76 b	0.85 b	4.72 c	2.96 bc	14.9 a	10.8 b	28.7 a	6.83 c	2.19 ab	3.09 c	12.1 bc	0.90 c	29.6 b	30.5 b

NS, not significant.
V1, hexanal; V2, octanal; V3, nonanal; ∑ALD, total aldehydes; V4, benzyl acetate; V5, ethyl hexanoate; V6, ethyl octanoate; ∑EST, total esters; V7, 2-ethyl-1-hexanol; V8, 3- hexen-1-ol; V9, 1-hexanol; ∑ALC, total aliphatic alcohols; V10, β-pinene; V11, *p*-cymene; V12, α-terpineol; ∑MTE, total monoterpenes; V13, linalool; V14, limonene; ∑MTO, total monoterpenoids.

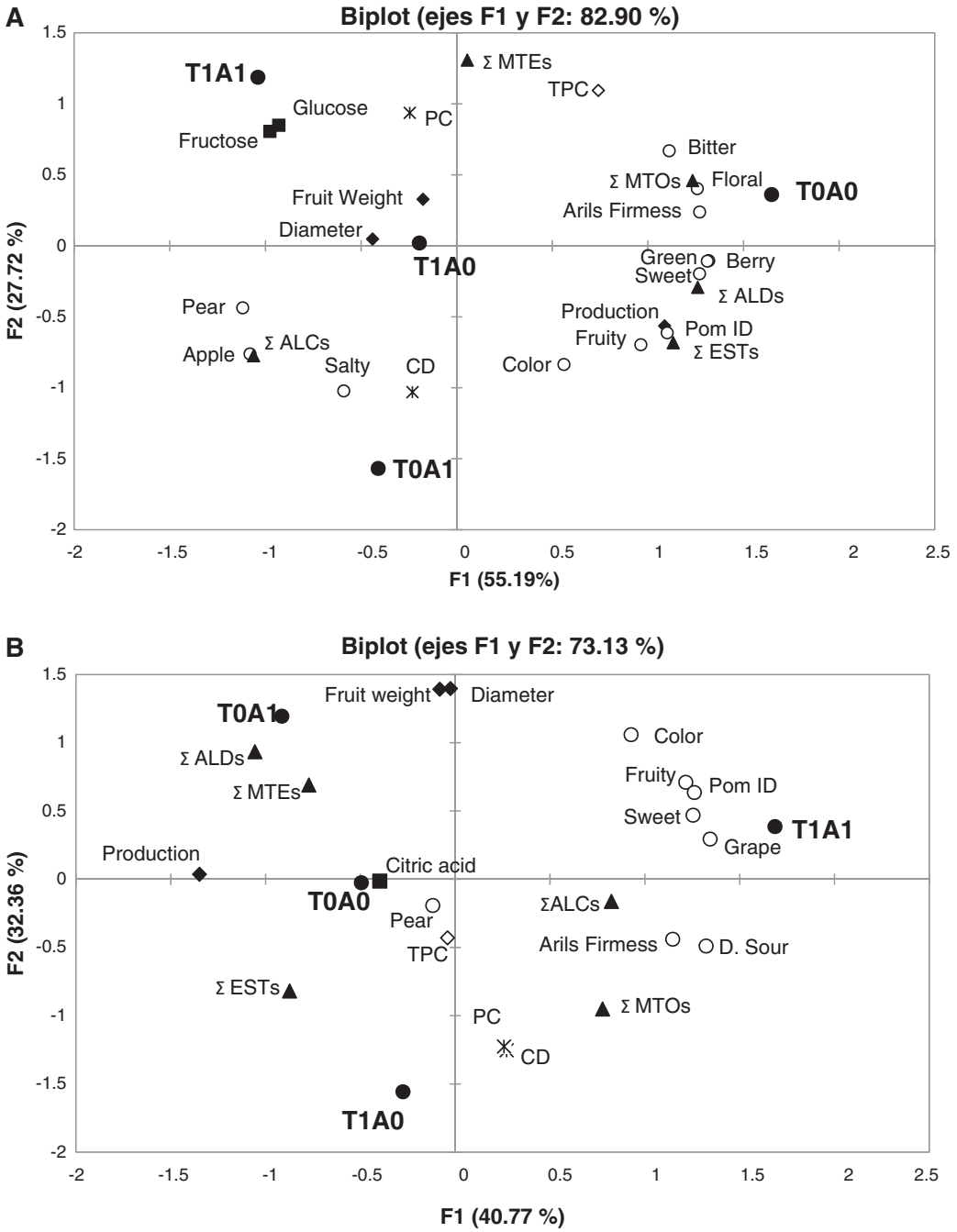

Figure 2. PCA scores plot showing the relationship among production, morphological parameters, total polyphenolic content, polymeric color, organic acids and sugars contents, volatile composition, and descriptive sensory analysis and treatments (T0A0, T0A1, T1A0 and T1A1) in cultivars *Wond* (A) and *ME* (B). ◇, Production and morphologic parameters: fruit weight and diameter; ■, total polyphenolic content (TPC); ▲, organic acids and sugars content; ⁎, color density (CD) and% polymeric color (PC); ○, Volatile composition: total aldehydes (∑ALDs), total esters (∑ESTs), total aliphatic alcohols (∑ALCs), total monoterpenes (∑MTEs) and total monoterpenoids (∑MTOs); ○, descriptive sensory attributes.

which is important because of the strong sourness of these fruits. Furthermore, T1 caused a reduction of total aldehydes (mainly hexanal) and terpenoids in both cultivars, losing vegetables notes. In the *Wond* cultivar, fruits of the treatment T1A1 were positively linked with the highest fruit weight and fruit diameter and high contents of glucose and fructose, which is essential for a sour cultivar, whereas control *Wond* fruits, T0A0, were linked with total phenolic content, monoterpenes (citric notes), aldehydes (vegetable notes), esters (fruity notes), production and key descriptive sensory parameters (sweetness, color, fruity, floral and pomegranate ID). In the *ME* cultivar, the interaction between water stress and commercial thinning (T1A1) was positively correlated with aliphatic alcohols (vegetable notes), monoterpenoids (citric notes) and key descriptive sensory parameters (color, sourness, fruity, floral and pomegranate ID), whereas control *ME* fruits, T0A0, were only linked to parameters such as total phenolic content, content of citric acid and the pear flavor. Thus, the final recommendation is that the best treatment for both

Table 5. Descriptive sensory analysis of *Wonderful* and *Mollar de Elche* pomegranates as affected by deficit irrigation and thinning treatments

	Color	Fruity	Pom ID	Apple	Pear	Grape	Berry	Green	Earthy	Salty	Sweet	Sour	Bitter	Arils Firmesss
							WONDERFUL							
							ANOVA test							
Irrigation	***	NS	NS	NS	NS	NS	*	NS	NS	NS	*	NS	NS	NS
Thinning	NS	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	*	NS
Irrigation x Thinning	***	**	**	**	**	NS	**	**	NS	***	**	*	**	**
Tukey's multiple range test														
Irrigation														
T0	9.3 a	4.7	5.6	1.8	0.7	1.9	2.4 a	1.8	0.2	3.8	4.2 a	1.3	1.7	7.1
T1	7.3 b	4.1	5.0	2.1	1.2	1.5	1.5 b	1.2	0.4	3.9	3.3 b	1.4	1.4	6.7
Thinning														
A0	8.0	4.8	5.6	1.4 b	0.8	1.8	2.4 a	1.9	0.2	3.7	4.0	1.3	1.8 a	7.1
A1	8.5	4.0	5.0	2.6 a	1.2	1.6	1.5 b	1.1	0.4	4.0	3.5	1.4	1.3 b	6.7
Irrigation × Thinning														
T0A0	9.0 a	4.9 a	5.7 a	0.7 c	0.2 b	1.9	3.1 a	2.4 a	0.1	2.9 b	4.6 a	1.6 a	2.1 a	7.5 a
T0A1	9.5 a	4.6 a	5.4 a	3.0 a	1.3 a	1.8	1.8 b	1.3 b	0.3	4.8 a	3.8 ab	1.1 b	1.2 b	6.8 b
T1A0	7.0 b	4.8 a	5.4 a	2.1 b	1.4 a	1.8	1.8 b	1.5 b	0.2	4.6 a	3.4 b	1.0 b	1.4 b	6.8 b
T1A1	7.5 b	3.5 b	4.6 b	2.1 b	1.1 a	1.3	1.3 b	0.8 c	0.6	3.2 b	3.2 b	1.7 a	1.5 b	6.7 b
							MOLLAR DE ELCHE							
							ANOVA test							
Irrigation	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
Thinning	***	NS	**	NS	**	NS	NS	NS	NS	NS	*	NS	NS	NS
Irrigation x Thinning	***	***	**	NS	**	*	NS	NS	NS	NS	**	NS	NS	**
Tukey's multiple range test														
Irrigation														
T0	6.4	3.3	3.1	1.6	2.7	0.9	0.3	0.4	1.6	1.4	4.9 b	0.9	0.8	6.8
T1	6.4	3.7	3.6	1.7	2.2	1.3	0.5	0.6	1.5	1.5	5.5 a	1.3	1.0	7.3
Thinning														
A0	4.9 b	3.0	2.8 b	1.4	2.9 a	0.9	0.3	0.4	1.7	1.5	4.8 b	1.1	0.9	7.1
A1	7.8 a	4.0	3.8 a	1.8	2.0 b	1.3	0.5	0.6	1.5	1.5	5.6 a	1.1	0.8	7.0
Irrigation × Thinning														
T0A0	6.0 b	3.1 b	3.0 b	1.3	3.6 a	0.9 b	0.4	0.4	1.9	1.2	4.6 b	1.0	0.8	7.3 ab
T0A1	6.8 b	3.4 b	3.1 b	1.9	1.8 b	1.0 b	0.2	0.5	1.4	1.5	5.1 b	0.8	0.8	6.3 b
T1A0	3.9 c	2.9 b	2.6 b	1.6	2.1 b	1.0 b	0.3	0.4	1.5	1.6	4.9 b	1.1	1.1	7.0 ab
T1A1	8.9 a	4.5 a	4.5 a	1.8	2.3 b	1.6 a	0.8	0.8	1.5	1.3	6.1 a	1.4	0.9	7.6 a

NS, not significant at $P < 0.05$; significant at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, respectively.
Values (mean of three replications) followed by the same lowercase letter, within the same column and factor, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

pomegranate cultivars under study (*Wond* and *ME*) was T1A1, which comprises the simultaneous application of soft deficit irrigation during fruit growth and ripening (T1) and the application of thinning (A1).

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