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Irrigation water saving during pomegranate flowering and fruit set period do not affect *Wonderful* and *Mollar de Elche* cultivars yield and fruit composition

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A B S T R A C T

Adult pomegranate trees [*Punica granatum* (L.) cultivars *Wonderful* and *Mollar de Elche*] were submitted to different irrigation treatments during the 2015 and 2016 seasons. Control (T0) plants were drip irrigated to guarantee non-limiting soil water conditions; T1 plants were subjected to water withholding during flowering-fruit set period for 64 (2015) and 53 (2016) days, resuming irrigation as in T0 plants subsequently. In both cultivars, the results demonstrated that during the flowering-fruit set period the sensitivity to water stress is very small, being possible to suppress or reduce irrigation (at least, while plant water status maintains similar levels to those reported in this study) without affecting marketable yield and fruit size and composition. Thus, this period can be considered as a clearly non-critical period. In this sense, water saving was around 19–30% and water productivity (WP) increased around 4–14% in *Wonderful* and 10–16% *in Mollar de Elche*. Water stress increased flowering, but not the number of viable hermaphrodite flowers, and decreased shoot growth (TSG), which could favour a compensatory young fruit growth when irrigation was resumed due to a shift in the carbon allocation pattern. This WP increase, the reduction in pruning cost (TSG decrease), and the redder arils in T1 plants were key aspects to increase consumers' acceptance and farmers' crop revenues.

1. Introduction

The availability of water for agricultural uses is the main challenge for optimal fruit trees farming under Mediterranean conditions, because rainfall has to be supplemented by irrigation to avoid harmful plant water deficit reducing regular fruit yield and enhancing alternate bearing pattern. Moreover, climate change will inevitably lead to very frequent and severe droughts in a near future (Collins et al., 2009).

In agreement with Galindo et al. (2018), the main strategies to cope with water scarcity are (i) the use of improved, innovative and precise deficit irrigation management practices able to minimize the impact on fruit yield and quality, and (ii) the use of new plant materials less water-demanding or able to withstand deficit irrigation with minimum impact on yield and fruit quality. In relation to these plant materials, it is important also to underline that agriculture has been focused on the cultivation of a very limited number of tree crop species and many others crops have been relegated to the status of neglected or under-utilized crop species, damaging the necessary agricultural biodiversity (Padulosi et al., 2001; Chivenge et al., 2015).

In relation with this last aspect, pomegranate (*Punica granatum* L.),

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one of the oldest known edible fruits and one of the seven kinds of fruit mentioned in the Bible, is mainly grown in semi-arid mild-temperate to subtropical climates (Blumenfeld et al., 2000). It is originally from Central Asia and actually the total area dedicated to its cultivation in the world is more than 302,000 ha with more than 76% found in five countries (India, Iran, China, Turkey and USA) (Quiroz, 2009; Melgarejo et al., 2012). Spain is the main producer and exporting country of pomegranates in the European Union, and its cultivation has increased greatly in the past few years, with a total surface in 2015 of 4,753 ha, from which 3,197 ha are in production and yield 56,185 tons (MAGRAMA, 2016). This cultivation increase is due to a great consumers' demand for pomegranate mainly because of the fruit beneficial effects on health (e.g. Lansky and Newman, 2007) and to the perfect acclimation of the crop to Mediterranean edaphoclimatic conditions, supporting heat and thriving even under desert conditions (Aseri et al., 2008; Intrigliolo et al., 2013; Rodríguez et al., 2012). Nevertheless, to reduce the incidence of some fruit physiopathies (e.g. fruit cracking and splitting) (Galindo et al., 2014; Rodríguez et al., 2018) and to reach optimal growth, yield and fruit quality (Levin, 2006; Holland et al., 2009), pomegranate for commercial production requires regular irrigation throughout the season.

Regulated deficit irrigation (RDI) is probably the most useful deficit irrigation strategy to improve water saving and even harvest quality, inducing minimum impacts in marketable yield. RDI is based in reducing irrigation or non-irrigating during the water stress-tolerant phenological periods (non-critical periods) and supplying full irrigation during the water stress-sensitive phenological periods (critical periods) (Chalmers et al., 1981; Geerts and Raes, 2009; Galindo et al., 2018).

Intrigliolo et al. (2013) studied the pomegranate response to RDI involving irrigation water restrictions during different fruit stages and concluded that water deficit during flowering-fruit set period is the best RDI strategy for pomegranate trees, and that the linear fruit growth phase and the last part of fruit growth and ripening are critical periods. Nevertheless, these last authors concluded that the water stress achieved was very mild and the water saving very scarce (9–14%). Then, research focussed on the response of pomegranate trees to severe water deficit during flowering-fruit set it is necessary to optimize RDI in pomegranate trees.

The research reported in this paper was conducted to test the hypotheses that (i) irrigation withholding during flowering-fruit set period can improve water saving and even fruit quality, without affecting marketable yield, and that (ii) RDI could constitute a tool to increase pomegranate consumers' acceptance and farmers' crop revenues.

2. Materials and methods

2.1. Plant material, experimental conditions and treatments

Two different but complementary experiments were conducted in 2015 and 2016 in the same experimental farm located near the city of Murcia (38° 6′ N, 1° 2′ W). The plant material was own rooted seven-year old pomegranate trees (*P. granatum* L.) of cultivars *Mollar de Elche* and *Wonderful*, each cultivar in a different orchard at a planting spacing of 3 m x 5 m.

The sandy clay loam soil of the experimental site (1.20% organic matter, 4.80 mmol kg^{-1} available potassium, 1.85 mmol kg^{-1} available phosphorus, 49% lime content and pH of 7.9) is characterized by a high stone content (39% by weight) and 8.0 cm h^{-1} saturated hydraulic conductivity, which provides excellent internal drainage. Available soil water and bulk density were 200 mm m^{-1} and 1.58 g cm^{-3}, respectively. The volumetric soil water content at saturation, field capacity and permanent wilting point were 0.44, 0.24 and 0.14 $m^3 m^{-3}$, respectively. During the experimental period, the irrigation water used had electric conductivity and Cl- in the ranges 0.8–1.0 dS m^{-1} and 59–73 mg L^{-1}, respectively. Fertilizers were supplied with the irrigation water and the doses were 110, 60 and 80 kg ha^{-1} $year^{-1}$ of N, P_2O_5 and K_2O, respectively. Pest control practices were those regularly used by local growers, and no weeds were allowed to develop within the orchard.

The irrigation was conducted daily during the night, using a drip irrigation system, with a lateral irrigation line per tree row and four drippers per tree, set at a rate of 4 l h^{-1}. During both growing seasons, control plants (treatment T0) were irrigated to achieve crop irrigation requirements (crop evapotranspiration, ETc), 115% ETc, which were calculated as ETc = ETo × Kc (Appendix A, Annexes 2 and 3), being ETo the crop reference evapotranspiration calculated using the Penman-Monteith equation (Allen et al., 1998) and Kc the crop coefficient for each phenological period and cultivar (Bhantana and Lazarovitch, 2010). T1 treatment plants were irrigated as T0 except during the flowering-fruit set periods, which took place mainly from DOY (day of the year) 120 to 184 in 2015 season and from DOY 115 to 168 in 2016 season, when irrigation was withheld. The total irrigation water amounts, measured with in-line water-meters, applied to each treatment in the 2015 season were 446 mm (T0) and 313 mm (T1) in *Wonderful* trees and 458 mm (T0) and 325 mm (T1) in *Mollar de Elche* trees, whereas in the 2016 season were 430 mm (T0) and 343 mm (T1) in *Wonderful* trees and 447 mm (T0) and 360 mm (T1) in *Mollar de Elche* trees (Appendix A, Annexes 2 and 3).

The experimental design was a randomized complete block, with four replicates per treatment. Each experimental plot consisting of three adjacent tree rows, each with at least six trees very similar in appearance (ground shaded area, height, leaf area, trunk cross sectional area, etc.). The inner plants of the central row of each replicate were used for measurements (Appendix A, Annex 1).

2.2. Climate, plant water status, vegetative growth and flowering

Micrometeorological data, namely air relative humidity, air temperature, solar radiation, rainfall and wind speed 2 m above the soil surface, were collected by an automatic weather station (Adcon Telemetry Gmb. Vienna, Austria) located near the experimental site. Mean daily air vapour pressure deficit (VPDm, kPa) and ETo (mm) were calculated according to Allen et al. (1998). ETo, Rainfall (P), effective rainfall (Pe) (Smith, 1992) are reported in Appendix A (Annexes 2 and 3).

The water relations of the leaves were measured at midday (12 h solar time). Fully expanded leaves from the south-facing side and middle third of the inner tree of the central row of each replicate (4 trees per treatment) were used for measurements. Midday stomatal conductance (g_s, mmol $m^{-2} s^{-1}$) was measured with a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface of 2 leaves per tree. Midday stem water potential (Ψ_{stem}, MPa) were measured in two leaves similar to those used for g_s. Leaves for Ψ_{stem} measurements were enclosed in a small black plastic bag covered with aluminium foil for at least 2 h before the measurements were made in a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA).

Total shoot growth (TSG, cm) was measured in 1 tree per replicate, using 4 shoots per tree (1 per each point of the compass). The increase of each shoot was calculated as the difference between the last (at the end of the experimental period) and the first (at the beginning of the experimental period) measurement of the shoot.

The total number of flowers (TNF) was estimated counting the number of viable hermaphrodite flowers (VHF) and non-viable or staminate hermaphrodite flowers (NVHF) in every monitored tree (4 trees per replicate). To control the flower and young fruit fall, a blanket was placed under these trees and weekly all flowers and fruit dropped were collected. These flowers were also identified as VHF or NVHF. Moreover, when pomegranate trees were hand-thinned to reach the regular commercial crop load, removed fruitlets were controlled.

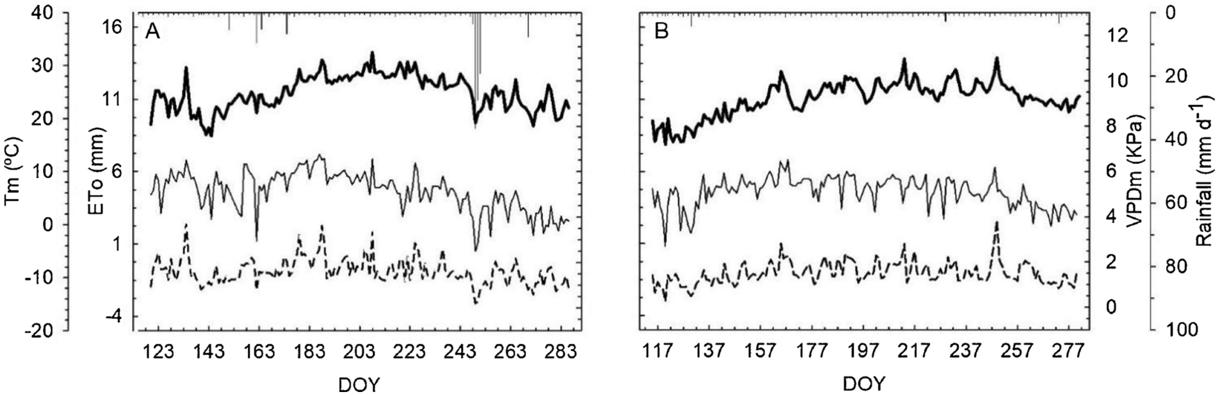

Fig. 1. Daily mean air temperature (Tm, solid line), daily crop reference evapotranspiration (ETo, thin line), mean daily air vapour pressure deficit (VPDm, medium-medium line) and daily rainfall (vertical bars) during the 2015 (A) and 2016 (B) experimental periods.

2.3. Yield and fruit quality

When fruit commercial maturity was reached (commercial fruit size and total soluble solids > 14°Brix), pomegranate fruits were manually harvested DOY 245 and 273 for *Wonderful* and *Mollar de Elche* cultivars, respectively, in the 2015 season and DOY 253 and 280, respectively, in the 2016 season), and fruits showing some physiopathies (peel cracking and/or splitting and sunburn) were discarded to estimate the marketable yield (MY). Twenty fruits from each replicate were immediately transported in ventilated plastic pallet boxes to the laboratory (a 15 min trip) and stored under controlled conditions (5 °C and 90% relative humidity, RH) for less than a week, until analysis. The mean fruit weight (FW) of MY was determined according to the weight and number of fruits per box in 2 randomly selected boxes per replicate.

Pomegranate peel colour was assessed at 4 equidistant points of the equatorial region of individual fruit using a Minolta CR 2000 colorimeter (Osaka, Japan). The arils obtained to calculate aril weight in each fruit were extended on a white plate and their colour was assessed in 10 different places on the plate, expressing the results using the CIE$L^*a^*b^*$ system. The mean values for lightness (L^*), green-red (a^*) and blue-yellow (b^*) coordinates for each fruit were calculated. The objective colour was calculated as chromaticity or chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($H^{o} = \arctan(b^*/a^*)$).

Juices from each treatment were obtained by pressure from arils inside a nylon mesh. pH, total soluble solids (TSS, ºBrix) and titratable acidity (TA, g of anhydrous citric acid L^{-1}) contents were measured as previously reported by Legua et al. (2012). The maturity index (MI) was calculated as the ratio between TSS/TA. All the analyses were performed in triplicate to ensure accuracy, and results were expressed as the mean.

The extraction and quantification of α- and β-punicalagin isomers, and ellagic acid from pomegranate juices were conducted using the method proposed by Cano-Lamadrid et al. (2018). Punicalagin (α and β) and ellagic acid contents were identified and quantified using a Hewlett-Packard-series 1200 high performance liquid chromatograph, HPLC (Woldbronn, Germany), equipped with a LiChroCART 100 RP-18 reversed-phased column (250 × 4 mm, particle size, 5 μm; Merck, Darmstadt, Germany), and a pre-column C18 (LiChrospher 100 RP-18, 5 μm; Merck, Darmstadt, Germany). Eluents were analysed using a UV–vis Diode Array detector. The compounds were quantified through calibration curves of standard compounds as the mean of 3 replicates.

The total phenol content (TPC, mg GAE 100 g^{-1} dw) of pomegranate aril juice was estimated using the Foling–Ciocalteu reagent and following the recommendations of Singleton et al. (1999). Absorption was measured at 760 nm using a UV–vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). Calibration curves, with a concentration range between 0 and 0.25 g GAE L^{-1}, were used for the quantification of TPC, and showed good linearity ($R^2 \geq 0.996$).

The antioxidant activity was evaluated with three different methodologies ($ABTS^+$, FRAP and DPPH˙, mmol Trolox kg^{-1} dw). The $ABTS^+$ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation and ferric-reducing antioxidant power (FRAP) methods were applied according to Re et al. (1999), and Benzie and Strain (1996), respectively. The radical scavenging activity was evaluated using the DPPH˙ radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams et al. (1995) with a modification in the reaction time (Nuncio-Jáuregui et al., 2015).

2.4. Statistical analysis

Statistical analysis was performed by an analysis of variance (ANOVA) using the general linear model (GLM) of SPSS v. 12.0 (SPSS Inc., 2002), for which two independent variables or factors [(i) irrigation and (ii) season], each one having two different levels (T0 and T1 for irrigation factor and 2015 and 2016 for season factor), were considered. To check statistical hypothesis (linearity, homoscedasticity, normality and independency) Kolmogorov–Smirnov with the Liliefors correction was used. Shapiro–Wilk and Levene tests were used to evaluate normality and homoscedasticity on the typified residuals, respectively. Independency was assumed by the experimental desing. Ψ_{stem} and g_s values for each replicate were averaged before the mean and the standard error of each treatment was calculated.

3. Results

During both experimental periods, the meteorological characteristics were similar to those of the Mediterranean climate. VPDm ranged from 0.21 to 3.69 kPa and 0.28 to 3.84 kPa in 2015 and 2016, respectively, and ETo was 775 (2015) and 761 (2016). Also, in 2015 experimental period, average daily maximum and minimum air temperatures were 33 and 17 °C, respectively, and in 2016 experimental period, these values were 32 and 15 °C, respectively. Total rainfall amounted to 124 mm in 2015 experimental period, which fell mainly on DOY 249 (36 mm), 250 (28 mm) and 251 (19 mm), whereas in 2016 experimental period total rainfall was extremely low (15 mm), which fell mainly on DOY 130 (4 mm), 229 (3 mm) and 273 (3 mm) (Fig. 1).

Considering the total irrigation water amounts applied to T0 and T1 plants, water savings obtained by irrigation withholding effect (T1) in *Mollar de Elche* plants were of 29% in 2015 and 19% in 2016, whereas in *Wonderful* plants water saving reached a 30% and 20% in 2015 and 2016, respectively (Annexes 2 and 3). Thus, throughout the 2015 and 2016 experimental periods, there were differences in Ψ_{stem} values between T0 and T1 plants from both cultivars. Ψ_{stem} values in T0 plants were high and near constant, having mean values of −0.93 MPa (2015) and -1.06 MPa (2016) in *Wonderful* plants while these values for the

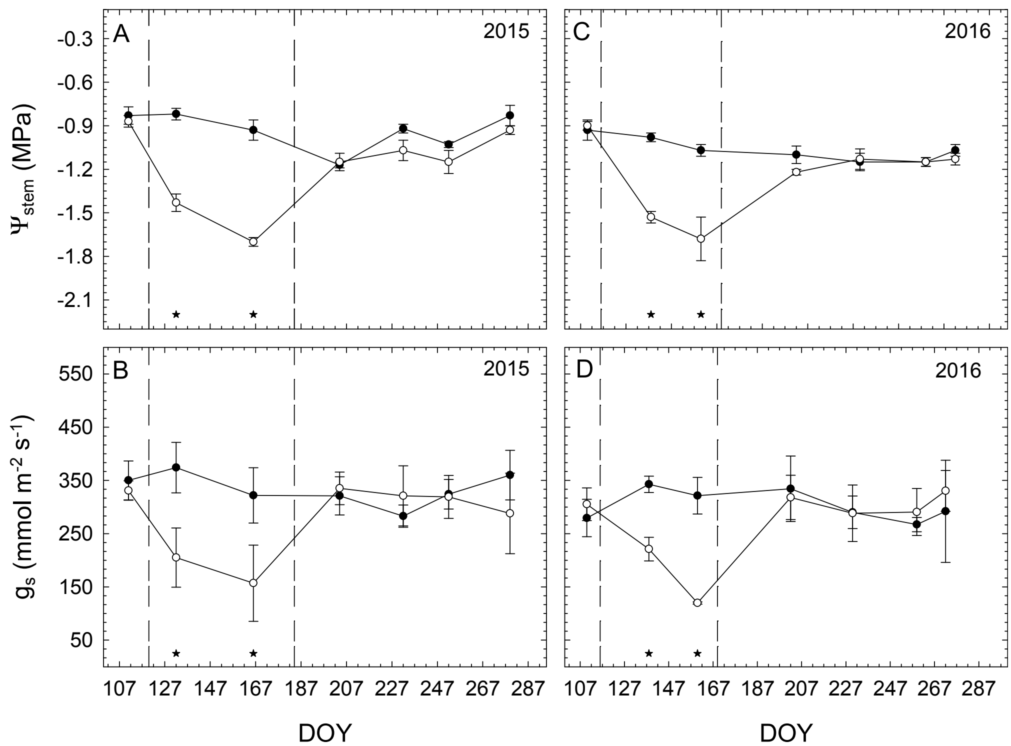

Fig. 2. Midday stem water potential (Ψ_{stem}) and midday stomatal conductance (g_s) in T0 (closed circles) and T1 (open circles) *Wonderful* pomegranate plants during 2015 and 2016 seasons. Vertical lines, from left to right, represent the flowering-fruit set period. Vertical bars are twice the overall mean standard error (S.E.). Asterisks indicate statistically significant differences between treatments.

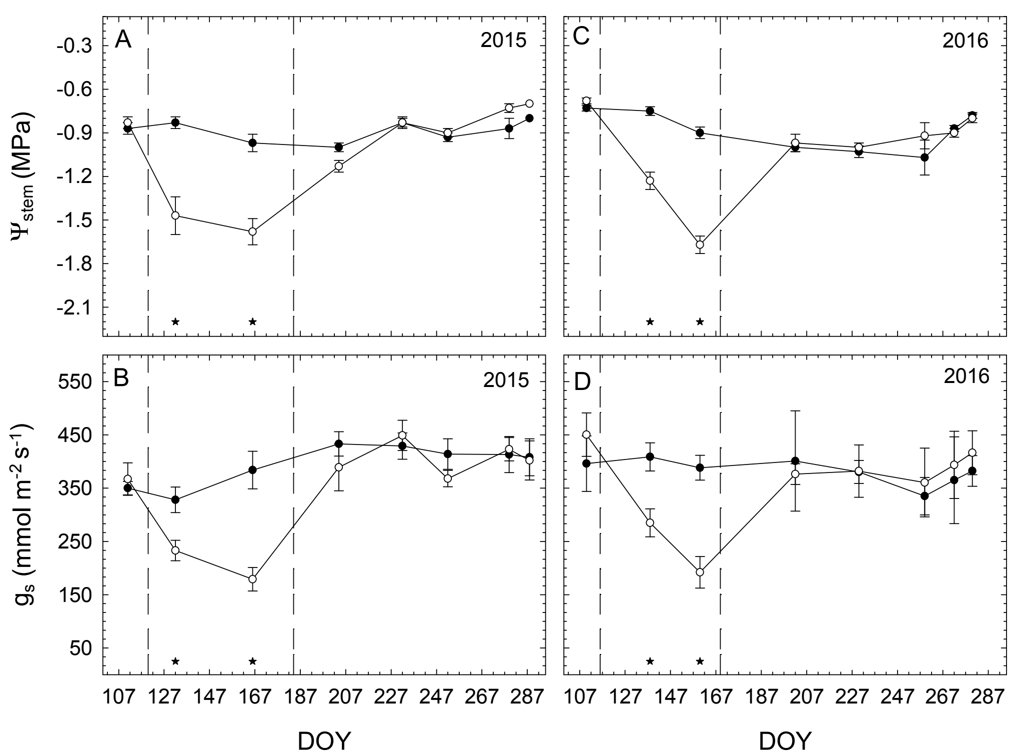

Fig. 3. Midday stem water potential (Ψ_{stem}) and midday stomatal conductance (g_s) in T0 (closed circles) and T1 (open circles) *Mollar de Elche* pomegranate plants during 2015 and 2016 experimental periods. Vertical lines, from left to right, represent the flowering-fruit set period. Vertical bars are twice the overall mean standard error (S.E.). Asterisks indicate statistically significant differences between treatments.

Mollar de Elche trees were −0.89 MPa (2015) and -0.89 MPa (2016) (Figs. 2 and 3). On the contrary, Ψ_{stem} values in T1 plants progressively decreased during the water withholding periods, reaching in *Wonderful* plants minimum values of −1.70 MPa (2015) and −1.68 MPa (2016) and in *Mollar de Elche* plants minimum Ψ_{stem} values were of −1.58 MPa (2015) and −1.67 MPa (2016). When irrigation was resumed, Ψ_{stem} values in T1 plants increased very rapidly reaching values similar to those observed in T0 plants (Figs. 2 and 3).

Differences in g_s values between T0 and T1 plants gradually increased due to the response of T1 plants to the water withholding. Thus, in *Wonderful* plants minimum g_s values of 156.7 and 119.7 mmol m^{-2} s^{-1} for 2015 and 2016, respectively, while in *Mollar de Elche* minimum g_s values were 178.7 and 192.0 mmol m^{-2} s^{-1}, respectively (Figs. 2 and 3), before water restriction was finished (Figs. 2 and 3).

TSG showed similar values in both cultivars and was significantly higher in the 2015 than in 2016 season. Moreover, TSG was characterized by an important reduction as a consequence of the water deficit (T1), which was more important in the Mollar de Elche cultivar

Table 1
Effect of different irrigation treatments (T0 and T1) on pomegranate total shoots growth (TSG, cm), total number of flowers per tree (TNF), number of viable hermaphrodite flowers per tree (VHF), total fruit yield (TY, kg $tree^{-1}$), marketable fruit yield (MY, kg $tree^{-1}$), number of fruits per tree (NF), average fruit weight (FW, g) and water productivity (WP, kg m^{-3}). Mean values within a column for each cultivar and season that do not have a common letter are significantly different at P ≤ 0.05. Asterisks indicate a statistically significant effect of season within a cultivar.

Cultivar	Season	Treatment	TSG	TNF	VHF	TY	MY	NF	FW	WP
Mollar de Elche	2015	T0	156.4a*	941b*	332	45.9	40.6	98.7	413.4	5.9b
		T1	85.0b	2474a	485	39.6	31.6	76.0	421.6	6.5a
	2016	T0	91.5a	2856b	373	42.6	35.7	90.0	397.1	5.3b
		T1	46.8b	3964a	353	41.4	34.2	88.7	387.2	6.3a
Wonderful	2015	T0	176.4a*	605b	178	42.7	38.5	124.3	308.3	5.8b*
		T1	165.4b	800a	218	37.4	31.4	105.7	297.1	6.7a
	2016	T0	97.5a	590b	209	61.2	54.5	161.7	326.7	8.4b
		T1	61.1b	974a	212	60.1	45.2	150.7	300.0	8.8a

as compared to that of the Wonderful cultivar (Table 1).

VHF values were similar in both cultivars and were not affected by water deficit (Table 1). However, TNF values were higher in Mollar de Elche than in Wonderful cultivar and increased by water stress effect (Table 1).

Both cultivars showed similar TY, MY, NF and FW values in 2015 and 2016, and a clear absence of the water deficit effect. On the other hand, in both cultivars WP values were significantly higher in T1 than in T0 plants. In addition, in Wonderful plants a seasonal effect was observed, being WP values in 2016 higher than in 2015 (Table 1).

Peel colour was not significantly affected by water stress (Table 2), even though T1 fruits increased arils a∗ values in both cultivars and C∗ values in Wonderful fruits (Table 3). Wonderful peel fruit showed seasonal differences only in C∗ values, which were higher in 2015 than in 2016 season, whereas the season effect was significant in arils L∗, a∗, b∗ and C∗ values, which were also higher in 2015 than in 2016 season (Table 3). Mollar de Elche peel fruit showed seasonal differences increasing L^* and H^o values in 2016 and a^* values in 2015, whereas the seasonal effect was significant in arils L^* and b^* values, which decreased in 2016 respect to 2015 (Tables 2 and 3).

The water deficit in T1 plants did not affect the fruit arils chemical composition (Tables 4 and 5), even though the seasonal effect was significant when some compounds were considered. In particular, *Mollar de Elche* arils showed higher MI and EA values but lower $ABTS^+$ and FRAP values in 2016 than in 2015 season, and *Wonderful* arils showed higher α-PUN, β-PUN and EA values and lower MI and FRAP values in 2016 than in 2015 season (Tables 4 and 5). Moreover, fruit arils chemical composition showed some important differences between both pomegranate cultivars. In fact, *Wonderful* arils showed substantially higher TA, CA and SUC values than *Mollar de Elche* arils (Table 4).

Table 2
Effect of different irrigation treatments (T0 and T1) on pomegranate peel lightness (CIE L^*), red/greenness (CIE a^*), yellow/blueness (CIE b^*), chroma (C^*) and hue angle (H^o) values. Mean values within a column for each cultivar and season that do not have a common letter are significantly different at P ≤ 0.05. Asterisks indicate a statistically significant effect of season within a cultivar.

Cultivar	Season	Treatment	L^*	a^*	b^*	C^*	H^o
Mollar de Elche	2015	T0	64.3*	28.4*	32.8	44.1	50.0*
		T1	63.3	28.5	35.1	45.8	51.3
	2016	T0	72.3	16.4	35.5	39.6	65.3
		T1	67.8	21.0	36.2	42.3	60.2
Wonderful	2015	T0	55.4	37.9	31.4	50.0*	41.1
		T1	50.8	42.8	30.2	53.1	36.7
	2016	T0	53.8	37.3	28.9	47.5	38.3
		T1	53.9	38.0	26.4	46.6	35.5

Table 3
Effect of different irrigation treatments (T0 and T1) on pomegranate arils lightness (CIE L^*), red/greenness (CIE a^*), yellow/blueness (CIE b^*), chroma (C^*) and hue angle (H^o) values. Mean values within a column for each cultivar and season that do not have a common letter are significantly different at P ≤ 0.05. Asterisks indicate a statistically significant effect of season within a cultivar.

Cultivar	Season	Treatment	L^*	a^*	b^*	C^*	H^o
Mollar de Elche	2015	T0	35.0*	18.1b	14.4*	23.1	38.5
		T1	44.4	20.2a	16.2	25.9	38.7
	2016	T0	29.6	12.3b	10.0	15.9	39.1
		T1	32.0	20.2a	10.9	23.0	28.6
Wonderful	2015	T0	33.1*	29.0b*	13.3*	31.9b*	24.7
		T1	34.0	31.3a	13.6	34.2a	23.4
	2016	T0	31.1	19.9b	9.1	21.9b	24.7
		T1	28.5	24.7a	10.2	26.7a	22.7

4. Discussion

The irrigation scheduling of the *Mollar de Elche* and *Wonderful* T0 plants allowed high values of Ψ_{stem} and g_s during both experimental periods (Figs. 2 and 3), suggesting the absence of limiting factors for the ETc to be fulfilled (Rodríguez et al., 2012; Galindo et al., 2013, 2014). However, T1 plants on both cultivars reached an important water deficit level during the irrigation withholding periods as indicated by Ψ_{stem} and g_s values; whereas, similar Ψ_{stem} and g_s values to those observed in T0 plants were reached during the periods of full irrigation (Figs. 2 and 3). Additionally, considering that seasonal rainfall was very scarce, mainly in the 2016 season (Fig. 1), it is evident that irrigation was the main factor affecting the pomegranate water relations during the season (Figs. 2 and 3). In agreement with the results obtained by Rodríguez et al. (2012), the g_s decrease in response to irrigation withholding and the fast g_s recovery when irrigation was restored indicated that stomatal aperture was controlled directly only by a hydroactive mechanism. Nevertheless, under a more severe water stress or a longer stress period situation hormonal changes at leaf levels could have taken place delaying stomatal aperture after rehydration (Mansfield, 1987; Davies and Zhang, 1991; Ruiz-Sánchez et al., 1997).

Intrigliolo et al. (2013) suggested that mild water deficit during flowering-fruit set period, saving a 9–14% irrigation water, was the best RDI strategy for Mollar de Elche pomegranate trees because minimal negative effects on fruit yield take place. Current results confirmed that flowering-fruit set period is a non-critical period for pomegranate culture. However, irrigation water restrictions and plant water stress levels were far more important than those reported previously by Intrigliolo et al. (2013), because water saving were between 19% and 30% in 2015 and 2016, respectively, and the plant water stress was clearly important (Figs. 2 and 3). This water saving increased 4–14% and 10–16%, in Wonderful and in Mollar de Elche, respectively, WP values because marketable yield and fruit size were not affected (Table 1) (Parvizi

Table 4
Effect of different irrigation treatments (T0 and T1) on pomegranate arils total soluble solids (TSS, ºBrix). titratable acidity (TA, g citric acid L^{-1}). maturity index (MI, TSS/TA), citric acid (CA, g 100 m L^{-1}), ascorbic acid (AA, g L^{-1}), malic acid (MA, g L^{-1}), sucrose (SUC, g L^{-1}), glucose (GLU, g L^{-1}) and fructose (FRU, g L^{-1}). Mean values within a column for each cultivar and season that do not have a common letter are significantly different at P ≤ 0.05. Asterisks indicate a statistically significant effect of season within a cultivar.

Cultivar	Season	Treatment	TSS	TA	MI	CA	AA	MA	SUC	GLU	FRU
Mollar de Elche	2015	T0	14.3	2.8	5.11*	0.3	0.4	4.4	3.2	108.5	130.0
		T1	14.9	2.9	5.14	0.3	0.4	4.5	3.1	108.7	131.2
	2016	T0	13.8	2.6	5.31	0.3	0.5	4.6	2.7	115.0	141.0
		T1	14.6	2.7	5.41	0.3	0.4	4.4	2.8	112.0	135.0
Wonderful	2015	T0	16.8	17.9	0.94*	1.5	0.4	4.1	35.1	143.8	158.4
		T1	17.0	16.8	1.01	1.3	0.3	3.7	29.9	137.4	152.1
	2016	T0	16.3	18.9	0.86	1.5	0.3	4.0	32.0	133.0	158.0
		T1	16.7	17.8	0.94	1.5	0.3	3.8	31.8	146.0	176.0

et al., 2014). Thus, this WP increase and the reduction in the pruning cost (TSG reduction) (Table 1) (Khattab et al., 2012) constitutes a very important finding to increase the farmers' crop revenues.

Considering that in pomegranate trees spring vegetative growth and flowering-fruit happen simultaneously, the reduction in TSG values by water stress (Table 1) could decrease the competition between both processes favouring flowering. TSG decrease can be considered as an advantageous result because a compensatory young fruit growth could be favoured when irrigation was resumed due to a shift in the carbon allocation pattern, whereof similar FW values in both irrigation treatments were attained (Table 1) (Abrisqueta et al., 2008).

In both cultivars, there was a similar incidence of peel cracking or splitting and sunburn fruit physiopathies in T0 and T1 plants (data not shown), even though fruit sunburn was much more important than the other physiopathies, which were very low. This similar incidence of fruit physiopathies in T0 and T1 plants explained the absence of irrigation effect also in MY values from both cultivars (Table 1). Moreover, in agreement with Galindo et al. (2014, 2018), the low peel cracking or splitting incidence could be due to the fact that all treatments were adequately irrigated from the end of fruit set to harvest.

It is known that irrigation can modify processes related to fruit trees floral biology such as the duration of phenological stages, flowering intensity and fruit set (e.g. Borroto and Rodríguez, 1977; Maranto and Hake, 1985; Ruiz-Sánchez et al., 1988). At first sight, the increase in pomegranate TNF values in T1 plants could be interpreted as an evolutive process to ensure the survival of the species under severe water stress conditions. However, under the current experimental conditions, this assumption was unsubstantiated because the increase in TNF values in T1 plants was due to an increase in NVHF values because no differences between treatments were observed in VHF values and, consequently, TY was similar in T0 and T1 treatments (Table 1).

Despite the expected significant effect of cultivar and season on some fruit characteristics, the water withholding effect was very scarce inducing significant changes only in some fruit colour parameters (Tables 2–5). Specifically, T1 fruits showed reddish arils, which could be due to an anthocyanins content increase, which can be considered an advantageous characteristic leading to a better consumers' acceptance of pomegranate fruits. This is always important but mainly in Mollar de Elche cultivar fruits, in which red colour has important implications for their market price.

The reduced vegetative growth in water withheld trees (Table 1) could have induced a higher sunlight fruits exposure and, as a consequence, a higher red appearance. Similar results have been showed by Li et al. (1989) and Gelly et al. (2003) in peach fruits and Laribi et al. (2013) in pomegranate fruits. Moreover, Castellarin et al. (2007a, b) suggested a direct effect of water stress on the anthocyanin biosynthesis pathway in grapevines,

5. Conclusions

The above mentioned results showed that in *Wonderfu*l and *Mollar de Elche* pomegranate trees the flowering-fruit set phenological period can be considered as a non-critical period because the sensitivity to water stress is small, which makes possible to suppress or reduce irrigation (at least, while plant water status maintains similar levels to those reported in this study). Under our experimental conditions, irrigation withholding during this period led to clearly important water deficit levels, water saving of around 19–30% and a WP increase of 4–16%, without affecting marketable yield and fruit size. The water stress achieved led to an increase in TNF values, due only to higher NVHF values, and to a TSG reduction, which could favour a compensatory young fruit growth when irrigation was resumed due to a shift in the carbon allocation pattern. The WP increase and the pruning cost reduction (TSG decrease) in T1 plants constitute very important aspects to increase the farmer' crop revenues. Despite irrigation withholding effect on fruit characteristics was very scarce; the fact that T1 fruits showed reddish arils constitute other complementary advantage to improve consumer's acceptance and commercial price of pomegranate fruits, mainly that of *Mollar de Elche* fruits.

Table 5
Effect of different irrigation treatments (T0 and T1) on pomegranate arils total polyphenols content (TPC, mg GAE 100 g^{-1} dw), α-punicalagin (α-PUN, mg L^{-1}), β-punicalagin (β-PUN, mg L^{-1}), ellagic acid (EA, mg L^{-1}) and antioxidant activity by ABTS method ($ABTS^{+}$, mmol Trolox kg^{-1} dw), by $DPPH^{\cdot}$ method ($DPPH^{\cdot}$, mmol Trolox kg^{-1} dw) and by FRAP method (FRAP, mmol Trolox kg^{-1} dw). Mean values within a column for each cultivar and season that do not have a common letter are significantly different at P ≤ 0.05. Asterisks indicate a statistically significant effect of season within a cultivar.

Cultivar	Season	Treatment	TPC	α-PUN	β-PUN	EA	$ABTS^{+}$	$DPPH^{\cdot}$	FRAP
Mollar de Elche	2015	T0	808.7	1.2	1.1	0.04*	21.8*	21.8	39.6*
		T1	791.1	1.1	0.8	0.04	19.5	19.5	44.2
	2016	T0	632.2	1.5	1.4	0.07	16.2	22.2	29.9
		T1	649.1	1.8	1.5	0.09	16.4	22.1	31.4
Wonderful	2015	T0	779.2	0.8*	0.8*	0.03*	21.0	21.0	47.4*
		T1	783.4	0.9	0.8	0.03	16.4	19.4	47.0
	2016	T0	748.2	1.7	1.7	0.06	18.2	21.3	19.2
		T1	792.2	1.6	1.6	0.06	22.5	22.5	19.8

Declaration of Competing Interest

The authors declare no conflict of interest

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Appendix A. Supplementary data

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