



## Research article

## Pomegranate plasticity to water stress: attempt to understand interactions between cultivar, year and stress level



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## ABSTRACT

This work investigated the effects of sustained deficit irrigation (SDI) on yield components and fruit physico-biochemical proprieties of two pomegranate cultivars, 'Sefri' and 'Wonderful' 21 trees each, in Sais plain (northern Morocco) over two consecutive seasons (2018–2019). Irrigation treatments consisted of a control, irrigation applied to fully satisfy crop evapotranspiration (100% ET<sub>C</sub>), and two SDI treatments: 70% ET<sub>C</sub> (SDI<sub>70</sub>) and 50% ET<sub>C</sub> (SDI<sub>50</sub>). The effects of the SDI treatments differed between cultivars and years. During the first year, yield and fruit weight were significantly reduced in 'Sefri' under SDI<sub>50</sub>. The same effect was also observed in the second year under SDI<sub>70</sub>. In 'Wonderful', a significant decrease in yield occurred in the second year under both SDI regimes. Fruit juice content was reduced in both cultivars, particularly under SDI<sub>50</sub>, with no significant effect on fruit aril content and aril weight. As for juice chemical properties, it was observed a decrease in total soluble solids, especially under SDI<sub>50</sub>. Furthermore, a significant decrease in juice soluble sugars content was observed in the first year for both cultivars. Total polyphenols content has significantly diminished in the second year in both cultivars. Likewise, total anthocyanins level displayed the same pattern, particularly in 'Wonderful'. The findings suggest that SDI decreases yield and fruit quality even under moderate regime of 70% ET<sub>C</sub>. These negative effects may be dramatic depending on pomegranate genotypes, as observed in 'Sefri', compared to Wonderful variety. The great impact on species behavior toward water stress was particularly driven by the irrigation treatment, of which the interaction with the cultivar factor had the higher impact on the model. As many areas of how sustained deficit irrigation impacts pomegranate tree resilience are still needed to be investigated further, this study is a first attempt to explore water management in pomegranate in the Mediterranean semiarid lands.

## 1. Introduction

Pomegranate (*Punica granatum* L.) has been cultivated since ancient times, valued as a food source and for dietetic purposes in ancient Egypt and was later spread by the Greeks and other civilizations around many parts of the world (Legua et al., 2012; Lansky and Newman, 2007). Nowadays, pomegranate is one of the most important emerging fruit crops in Morocco, cultivated from North to Southwest with an overall production ranged between 85 000 and 140 000 tons for an area of 12.700 ha (MAPMDREF, 2018). However, the actual climate change, including more frequent periods of drought, is challenging the promotion of this species, particularly the yield regularity alongside the fruit quality components. This frequent yield irregularity, which is witnessed

nowadays in several fruit trees, including pomegranate is most often due to the occurrence of stressful climatic factors, particularly drought (Schilling et al., 2012; Martinez et al., 2012).

The dramatic decrease in water availability, combined with a high-water demand, forces the farmers to adopt deficit irrigation and to overexploit groundwater, which is expected to be increased in the future years according to climate change predictions (Stour and Agoumi, 2008). Indeed, agricultural stakeholders must acquire practical knowledge on orchard management with a focus on yield irregularity and fruit quality under deficit irrigation. Besides, they need to develop reflections for sustainable strategies for water saving, particularly for the most water-demanding crops, including a large part of fruit trees (Henry, 2010). One of the promising approaches is to identify optimal regimes for

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sustained deficit irrigation (SDI) to save water without yield penalties or quality loss (Pena et al., 2013). The SDI is an irrigation strategy based on the application of only a fraction of the plant water requirements continuously over one or two seasons of the year or during the entire irrigation period. In Morocco, the active growth of pomegranate tree extends from April to October (Benassi, 2008), overlapping with a rainfall deficit period during which the SDI can be envisioned. It can be limited to the summer season, from June to August, where rainfall deficit becomes more pronounced or to the period of fruit growth after ensuring a full irrigation in flowering period.

Pomegranate is known to be fairly drought tolerant, although it requires normal irrigation for high yields and fruit quality (Intrigliolo et al., 2013; Holland et al., 2009). However, this tolerance is being questionable in the context of the actual climate change. Therefore, more studies are needed to further investigate the plasticity of this species toward the water stress (Galindo et al., 2017). In Morocco, pomegranate is cultivated in both the less and well-watered areas where it constitutes an important source of income for the local population. However, very little is known about pomegranate orchard water management and tree performance under deficit irrigation. The few studies carried out on pomegranate response to water restrictions showed mixed results, due to differences in genotypes investigated and environmental conditions. According to Adiba et al. (2021) significant differences were reported regarding the response to water stress among eleven pomegranate cultivars growing in the same area. On the other hand, Martinez-Nicolas et al. (2019) have reported similar yield levels in the Spanish variety 'Mollar de Elche' growing on sandy clay loam soil under sustained deficit irrigation of 50% ET<sub>C</sub> and control trees watered at 100% ET<sub>C</sub>. However, a statically significant decrease in fruit yield of this variety was observed by Intrigliolo et al. (2012) which was attributed to the decrease in fruit weight.

Ambiguous results were also found regarding pomegranate juice quality under deficit irrigation. Some studies concluded negative impacts of deficit irrigation on some biochemical traits such as a decrease in total anthocyanin and polyphenol content (Mena et al., 2012). Contrary, Mellisho et al. (2012) observed that the pomegranate juice color changed to a more perceptible red as a consequence of the increasing total anthocyanin content in response to deficit irrigation. On the other hand, Centofanti et al. (2017) concluded that deficit irrigation strategies, as low as 35% ET<sub>C</sub>, did not significantly affect the juice color, pH, anthocyanin and non-anthocyanin compounds, total phenolic compounds, concentration of soluble solids and mineral elements.

The studies on pomegranate response to deficit irrigation might therefore be specific to each particular agro-ecosystem. In Morocco, very little information is available regarding pomegranate orchard water management, and to the best of our knowledge, there has been no scientific assessment of pomegranate juice quality under deficit irrigation. This first report was carried out on the local cultivar 'Sefri' and the exotic variety 'Wonderful' as being widely cultivated in Morocco. The objective was to evaluate the effects of two SDI regimes applied during the whole fruit growth period on yield level, fruit physical traits and pomegranate juice quality, compared to a full irrigation regime.

## 2. Materials and methods

### 2.1. Plant material and experimental design

The plant material consisted of 42 pomegranate trees: 21 trees of Sefri cultivar, a local clone widely cultivated in Morocco and 21 trees of Wonderful variety introduced from Northern America and mainly cultivated for industrial processing purpose. The trees were planted at 5 × 3 m spacing and pruned to a goblet canopy shape. They received the same fertilization, namely 150, 60 and 150 kg ha<sup>-1</sup> year<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O respectively. Pest control was those usually used by the growers, and no weeds were allowed to develop within the orchard.

This experiment was conducted over two consecutive years (2018–2019) in Ain Taoujdate station of the National Institute for

Agricultural Research (INRA) in Northern Morocco (33°56'E, 5°13'N; 499 m). The soil is sandy clay with an average of 3% CaCO<sub>3</sub>, low available potassium and phosphorus levels and moderately rich in organic matter along with a useable water reserve of 1.7 mm cm<sup>-1</sup> (Table 1).

Local climate is semiarid Mediterranean with hot and dry summers. Weather data was recorded from an automated weather station near the experimental orchard. The annual averages of rainfall and reference evapotranspiration (ET<sub>0</sub>) during the growing seasons between 2018 and 2019 were 432 and 1015 mm, respectively. However, the monthly distribution of rainfall and ET<sub>0</sub> shows that the rainfall deficit was more pronounced between March and August in 2018 and from February to October in 2019 (Figure 1). The average value of ET<sub>0</sub> was 775 mm during the active growth season of pomegranate (April–October).

Crop evapotranspiration (ET<sub>C</sub>) was scheduled according to daily ET<sub>0</sub> and the crop coefficient values determined by Bhantana and Lazarovitch (2010), adjusted to tree canopy cover (Sc) using the reduction coefficient (Kr) recommended for almond trees by Fereres et al. (1981), expressed as Eq. (1), where D is the average of canopy cover diameters and N is the planting density.

$$Kr = \frac{2 \cdot Sc}{100} \quad \text{with} \quad Sc = \frac{\pi D^2 N}{100} \quad (1)$$

During the rainy days, it was considered the effective rainfall, equivalent to 80% of the recorded rainfall. Each tree was drip-irrigated daily with two emitters. The water debit was changed since fruit set of each pomegranate cultivar to give two SDI treatments, 50% ET<sub>C</sub> (SDI<sub>50</sub>) and 70% ET<sub>C</sub> (SDI<sub>70</sub>), and a control of 100% ET<sub>C</sub>. The experiment was conducted in completely randomized blocks, each with seven replicates (trees). The five central trees from each water treatment were used for measurement and the other trees acted as buffer plants.

### 2.2. Yield and fruit physical traits

In late October, for each tree, 30 fully ripened fruits were randomly collected from arbitrarily selected fruiting branches to determine mean fruit weight (150 fruits per treatment). This method of fruit sampling was adopted as it considers the fruit size variability within individual trees. Sub-samples of 10 fruits each (50 fruits per treatment) were then hand-peeled to determine aril content, aril weight and aril dry matter. Fruit juice content was determined using a commercial pomegranate juicer on sub-samples of 6 fruits (30 fruits per treatment), previously weighted. Fruit yield was calculated as product of fruit weight and fruit number counted per tree.

### 2.3. Juice chemical and biochemical proprieties

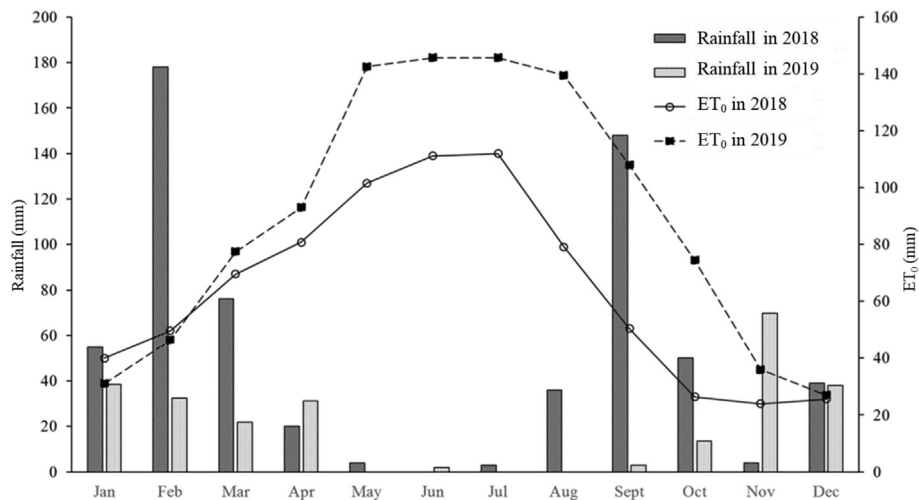
Total soluble solids (TSS), titratable acidity (TA) and pH were systematically measured on juice samples according to AOAC methods; each measurement was performed in triplicate (AOAC, 2006). The TSS content was determined with a digital refractometer (PR-101 ATAGO, Norfolk, VA, USA). The TA was expressed as % citric acid and determined by titrating 1 mL of juice with 0.1 M NaOH. The whole juice samples were then frozen at -20 °C for later extraction and determination of total soluble sugars, amino acids, phenolic compounds and anthocyanins contents.

Extraction was based on a method described by Xie and Bolling (2014). First, 1 mL aliquots of juice sample were transferred to polypropylene tubes and homogenized in 20 mL of ethanol and ultra-pure water (80:20, v/v) at 4 °C for 15 min using an IKA T-18 Basic Ultra-Turrax homogenizer (IKA Werke GmbH & Co., Staufen, Germany). The homogenate was then centrifuged for 10 min at 4 °C at 3000 g, and the supernatant was removed from the residue. The supernatants were then combined and filtered using Whatman No.1 filter paper.

Total soluble sugars content was estimated using the phenol sulfuric acid method of Dubois et al. (1956) with a glucose solution as a standard.

**Table 1.** Physical and chemical proprieties of the soil in the experimental orchard.

Soil depth	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	CaCO <sub>3</sub> (%)	P <sub>2</sub> O <sub>5</sub> (ppm)	K <sub>2</sub> O (ppm)	pH	EC (mS cm <sup>-1</sup> )
0–35 cm	43.0	10.2	46.8	2.51	3.0	73.36	458.87	7.30	0.10
35–70 cm	37.6	16.1	46.3	1.58	3.1	15.12	222.48	8.06	0.07

**Figure 1.** Monthly rainfall and reference crop evapotranspiration in the experimental orchard over the two years of the study.

Indeed, 100  $\mu$ L of pomegranate juice diluted in the ratio of 1:100 with distilled water was mixed with 500  $\mu$ L of phenol, 2.5 mL of sulfuric acid 96% and the mixtures were shaken and left to stand for 10 min then placed in a water bath for 20 min at 30 °C. Absorbance of the resulting solution was measured at 485 nm (UV-1700 Shimadzu, Japan). The results were expressed as mg of glucose equivalent per liter of juice (g GE L<sup>-1</sup>).

Total amino acids content was measured calorimetrically according to the method of [Yemm and Cooking \(1955\)](#) using glycine solution as a standard. 50  $\mu$ L of juice was mixed with 0.5 mL of 80% ethanol and 0.5 mL of citrate buffer (0.2 M, pH 5), 1 mL of solution of ninhydrin-acetone solution (1 g ninhydrin in 125 mL acetone). The mixture was incubated for 15 min at 95 °C. After incubation and cooling, 8 mL of distilled water were added to the solution and the absorbance was measured at 570 nm. The results were expressed as mg of glycine equivalent per liter of juice (g GlyE L<sup>-1</sup>).

Total phenolic content was determined using Folin–Ciocalteu method described by [Singleton et al. \(1999\)](#) using gallic acid solution as a standard. 300  $\mu$ L of diluted juice in the ratio of 1:100 with methanol-water solution (6:4) was mixed with 1.5 mL of 10-fold-diluted Folin–Ciocalteu reagent and 1.2 mL of 7.5% sodium carbonate. After 90 min, the absorbance was measured at 760 nm. The results were expressed as g of gallic acid equivalent per liter of juice (g GAE L<sup>-1</sup>).

Total anthocyanin content was quantified according to the pH differential method ([Giusti et al., 1999](#)). Absorbance was measured at 520 nm and 700 nm in buffers at pH 1.0 and pH 4.5 and the results were expressed as mg L<sup>-1</sup>.

#### 2.4. Statistical analysis

Data analysis was performed using SPSS v22. Prior to data treatment, all variables were normalized so they can have a comparable scale. Then, they were checked for their normality, which was confirmed using Shapiro-Wilk normality test. Afterward, for each pomegranate cultivar, analysis of variance was computed to test significant differences among the irrigation treatments. Student-Newman and Keuls test (SNK) was applied to compare between the irrigation treatments at  $p \leq 0.05$ . Data

were then subjected to three-way analysis of variance to assess the interactions between the factors (cultivar, year experiment and irrigation treatment) and the magnitude of their impacts over the herein investigated variables.

### 3. Results

#### 3.1. Yield components

Yield and fruit weight of the two studied pomegranate cultivars in response to SDI treatments are given in [Table 2](#). Compared to the control, Wonderful variety did not display any change in the abovementioned traits during 2018. However, in 2019 a consistent decrease of about 27% in yield was observed under SDI<sub>70</sub> treatment, compared to the control trees. Under SDI<sub>50</sub>, the yield decreases were more pronounced (42% in average), combined with a reduction of 13% in fruit weight. Contrary, yield and fruit weight decreased dramatically in Sefri cultivar in 2018 under the SDI<sub>50</sub> treatment by an average of 67% and 41% respectively, while they remain unchanged under SDI<sub>70</sub>. In 2019, the impact of SDI treatments remained spectacular under SDI<sub>50</sub> for ‘Sefri’ with a decrease of 61% in yield and of 44% in fruit weight. Likewise, under SDI<sub>70</sub>, yield was diminished by 55%, whereas the fruit weight was decreased by 22%.

Among the aril physical traits, the weight of a single aril was not affected by the SDI treatments in both pomegranate cultivars ([Table 3](#)). Compared to the control treatment, aril content per fruit was reduced only in 2019 in Sefri cultivar under the both SDI treatments by about 20%, while it was not significantly affected in ‘Wonderful’ variety. As for fruit juice content, it decreased in ‘Wonderful’ under the both SDI treatments over the two consecutive years of the experiment by average rates of 9% under SDI<sub>70</sub> and 13% under SDI<sub>50</sub>. In ‘Sefri’, the decrease in juice content was more accurate although it was not significantly affected under SDI<sub>70</sub> in 2018. Considering the results of the two years, the average rates of juice content decrease in Sefri cultivar were about 11% under SDI<sub>70</sub> and 19% under SDI<sub>50</sub>. The decrease in juice content was combined to an increase of aril dry matter, with similar rates under the both SDI treatments, of 0.66% in ‘Wonderful’ and 1.98% in Sefri as an additional rate compared to the control treatment.

**Table 2.** Effect of different irrigation treatments (Control, SDI<sub>70</sub> and SDI<sub>50</sub>) on fruit and juice yields.

Cultivar	Season	Treatment	Fruit yield (kg tree <sup>-1</sup> )	Juice yield (kg tree <sup>-1</sup> )	Fruit weight (g)
'Wonderful'	2018	Control	16.31	1.80	209.43
		SDI <sub>70</sub>	17.03	1.85	208.01
		SDI <sub>50</sub>	17.61	1.83	198.26
	2019	Control	25.52 <sup>c</sup>	15.71 <sup>c</sup>	313.14 <sup>b</sup>
		SDI <sub>70</sub>	18.52 <sup>b</sup>	9.75 <sup>b</sup>	301.24 <sup>b</sup>
		SDI <sub>50</sub>	14.82 <sup>a</sup>	7.51 <sup>a</sup>	271.65 <sup>a</sup>
'Sefri'	2018	Control	14.73 <sup>b</sup>	2.45 <sup>b</sup>	281.49 <sup>b</sup>
		SDI <sub>70</sub>	14.49 <sup>b</sup>	2.27 <sup>b</sup>	246.91 <sup>b</sup>
		SDI <sub>50</sub>	4.89 <sup>a</sup>	0.21 <sup>a</sup>	164.76 <sup>a</sup>
	2019	Control	16.11 <sup>c</sup>	8.85 <sup>c</sup>	440.60 <sup>c</sup>
		SDI <sub>70</sub>	7.32 <sup>b</sup>	2.57 <sup>b</sup>	342.84 <sup>b</sup>
		SDI <sub>50</sub>	6.32 <sup>a</sup>	1.40 <sup>a</sup>	245.16 <sup>a</sup>

Values with different letters indicate significant difference ( $p < 0.05$ , SNK test) with respect to irrigation treatment for each cultivar and season independently; -: non-significant difference; \*: significant difference at  $p < 0.05$ ; \*\*: significant difference at  $p < 0.01$ .

**Table 3.** Effect of different irrigation treatments (Control, SDI<sub>70</sub> and SDI<sub>50</sub>) on fruit weight, fruit aril content, aril weight, aril dry matter and fruit juice content.

Cultivar	Season	Treatment	Fruit aril content (%)	Aril weight (mg)	Aril dry matter (%)	Fruit juice content (%)
'Wonderful'	2018	Control	51.86	308.16	7.75 <sup>a</sup>	54.42 <sup>c</sup>
		SDI <sub>70</sub>	49.09	310.83	8.38 <sup>b</sup>	46.13 <sup>b</sup>
		SDI <sub>50</sub>	46.45	362.25	8.43 <sup>b</sup>	39.71 <sup>a</sup>
	2019	Control	62.15	318.85	7.90 <sup>a</sup>	61.59 <sup>b</sup>
		SDI <sub>70</sub>	59.04	289.85	8.54 <sup>b</sup>	52.68 <sup>a</sup>
		SDI <sub>50</sub>	56.59	300.80	8.59 <sup>b</sup>	50.74 <sup>a</sup>
'Sefri'	2018	Control	47.27	339.50	7.02 <sup>a</sup>	50.02 <sup>b</sup>
		SDI <sub>70</sub>	46.06	340.83	8.79 <sup>b</sup>	47.30 <sup>b</sup>
		SDI <sub>50</sub>	45.02	357.50	9.12 <sup>b</sup>	34.90 <sup>a</sup>
	2019	Control	55.64 <sup>b</sup>	345.95	7.37 <sup>a</sup>	54.96 <sup>c</sup>
		SDI <sub>70</sub>	36.02 <sup>a</sup>	320.73	9.22 <sup>b</sup>	35.17 <sup>b</sup>
		SDI <sub>50</sub>	34.44 <sup>a</sup>	308.55	9.57 <sup>b</sup>	32.62 <sup>a</sup>

Values with different letters indicate significant difference ( $p < 0.05$ , SNK test) with respect to irrigation treatment for each cultivar and season independently; -: non-significant difference; \*: significant difference at  $p < 0.05$ ; \*\*: significant difference at  $p < 0.01$ .

### 3.2. Juice biochemical proprieties

The SDI effects on juice total soluble solids (TSS), pH and titratable acidity (TA) varied depending on the cultivar and the year of the experiment (Table 4). In 'Wonderful', no significant change was observed in 2018 on all the above-mentioned traits. However, in 2019, TSS content decreased by 0.86 °Brix under SDI<sub>70</sub> and 1.46 °Brix under SDI<sub>50</sub> compared to control trees. A similar effect was recorded in 'Sefri' only in the first year of SDI application (2018), contrary to Wonderful variety. The TSS content decrease rates observed in 'Sefri' were relatively higher than those recorded in Wonderful variety, with respective averages of 1.12 °Brix and 1.90 °Brix under SDI<sub>70</sub> and SDI<sub>50</sub> respectively, compared to the control treatment. Data of 2018 showed some upward trend in juice pH in response to SDI, which was only significant in Sefri cultivar under SDI<sub>50</sub>. On the other hand, titratable acidity remained unaffected in the both cultivars.

Significant changes were observed in pomegranate juice composition with regard to soluble sugars content, amino acids, polyphenols and

anthocyanins in response to SDI (Table 5). Data showed a reducing trend of these compounds in response to SDI, although some differences were not significant. Indeed, in Sefri cultivar, changes were significant in soluble sugars and polyphenols only, while in 'Wonderful' there were significant differences on all the measured traits.

A linear relationship between soluble sugars content (SSC) decrease and SDI intensity increase was observed in both cultivars. The impact of both SDI treatments was important in 'Sefri', which displayed a remarkable decrease in SSC up to 17% as an average of the two consecutive years. However, in 'Wonderful' variety, SSC decreased significantly under SDI<sub>70</sub> and SDI<sub>50</sub> by average rates of about 4% and 9%, respectively. Likewise, amino acids content (AAC) decreased significantly in 'Wonderful', particularly under SDI<sub>50</sub> by about 30% compared to the control trees in the two years experiments; Whereas AAC remained unchanged in Sefri cultivar in response to the same SDI regime. However, under SDI<sub>70</sub>, changes in AAC were ambiguous since they displayed a significant increase in 2018 of about 22% versus a remarkable decrease of 23% in 2019 compared to the control treatment. This means that

**Table 4.** Effect of different irrigation treatments (Control, SDI<sub>70</sub> and SDI<sub>50</sub>) on total soluble solids content (TSS, °Brix), pH and titratable acidity (TA, % citric acid) of pomegranate juice.

Cultivar	Season	Treatment	TSS	pH	TA
'Wonderful'	2018	Control	17.30	3.64	1.89
		SDI <sub>70</sub>	16.80	3.66	1.74
		SDI <sub>50</sub>	16.35	3.69	1.74
	2019	Control	17.13 <sup>c</sup>	3.50	1.31
		SDI <sub>70</sub>	16.27 <sup>b</sup>	3.63	1.60
		SDI <sub>50</sub>	15.67 <sup>a</sup>	3.67	1.74
'Sefri'	2018	Control	17.70 <sup>b</sup>	4.01 <sup>b</sup>	0.23
		SDI <sub>70</sub>	16.40 <sup>ab</sup>	3.94 <sup>b</sup>	0.20
		SDI <sub>50</sub>	15.50 <sup>a</sup>	4.70 <sup>a</sup>	0.20
	2019	Control	16.50 <sup>ab</sup>	3.63	0.28
		SDI <sub>70</sub>	15.57 <sup>ab</sup>	3.63	0.28
		SDI <sub>50</sub>	14.90 <sup>a</sup>	3.70	0.25

Values with different letters indicate significant difference ( $p < 0.05$ , SNK test) with respect to irrigation treatment for each cultivar and season independently; -: non-significant difference; \*: significant difference at  $p < 0.05$ ; \*\*: significant difference at  $p < 0.01$ .

**Table 5.** Effect of different irrigation treatments (Control, SDI<sub>70</sub> and SDI<sub>50</sub>) on total content of soluble sugars (SSC, g GE L<sup>-1</sup>), amino acids (AAC, g GlyE L<sup>-1</sup>), polyphenols (TPC, g GAE L<sup>-1</sup>) and anthocyanins (TAC, mg L<sup>-1</sup>) in pomegranate juice.

Cultivar	Season	Treatment	SSC	AAC	TPC	TAC
'Wonderful'	2018	Control	113.76 <sup>b</sup>	2.11 <sup>ab</sup>	2.11	106.98
		SDI <sub>70</sub>	109.96 <sup>ab</sup>	2.57 <sup>b</sup>	3.26	104.99
		SDI <sub>50</sub>	105.40 <sup>a</sup>	1.72 <sup>a</sup>	1.20	99.69
	2019	Control	102.74 <sup>c</sup>	3.26 <sup>b</sup>	2.95 <sup>b</sup>	117.00 <sup>b</sup>
		SDI <sub>70</sub>	97.80 <sup>b</sup>	2.52 <sup>ab</sup>	2.36 <sup>b</sup>	87.39 <sup>a</sup>
		SDI <sub>50</sub>	91.40 <sup>a</sup>	1.92 <sup>a</sup>	2.07 <sup>a</sup>	81.27 <sup>a</sup>
'Sefri'	2018	Control	75.24 <sup>b</sup>	2.31	2.78	32.14
		SDI <sub>70</sub>	68.09 <sup>a</sup>	2.24	3.47	23.67
		SDI <sub>50</sub>	63.50 <sup>a</sup>	2.11	1.76	21.84
	2019	Control	70.84 <sup>b</sup>	2.23	3.15 <sup>c</sup>	41.02
		SDI <sub>70</sub>	61.44 <sup>a</sup>	2.04	2.48 <sup>b</sup>	49.71
		SDI <sub>50</sub>	50.45 <sup>a</sup>	1.59	2.09 <sup>a</sup>	41.91

GE: glucose equivalent; GlyE: glycine equivalent; GAE: gallic acid equivalent.

Values with different letters indicate significant difference ( $p < 0.05$ , SNK test) with respect to irrigation treatment for each cultivar and season independently; -: non-significant difference; \*: significant difference at  $p < 0.05$ ; \*\*: significant difference at  $p < 0.01$ .

probably the year factor had the strongest weight among interactions between the experimental design factors (Table 5). It is noteworthy that in case of Sefri cultivar, the differences were not significant, but data show a decreasing trend of AAC in juice in response to SDI. Statistical analysis highlighted the absence of significant effect of SDI on total polyphenols content (TPC) in the first year of the experiment. However, in the second year, TPC recorded a significant decrease in the both cultivars. The SDI<sub>70</sub> treatment decreased TPC in 'Sefri' juice by about 21%, in comparison with the control trees, without significantly affecting their level in 'Wonderful' variety. Under SDI<sub>50</sub>, the TPC decreases were more pronounced, with respective averages of 30% and 34% in 'Wonderful' and 'Sefri', respectively. As for total anthocyanins content (TAC), it was not affected by SDI in 'Sefri'. However, in 'Wonderful', TAC was significantly decreased under both SDI treatments in the second year by an average rate of 28%, compared to the control treatment. However, both

SDI treatments did not have any significant impact on the TAC during the first year of the experiment.

### 3.3. Interactions between the experimental design factors

In order to further understand the findings above-stressed, the interactions between the experimental design factors, irrigation treatment (I), cultivar (C) and year experiment (Y) and their impacts on the herein investigated traits were assessed using the three-way analysis of variance (Table 6). As being disposed of having the most general applicability, the Wilks's lambda ( $\lambda$ ) was used to evaluate the significance of each interaction level between the model factors (Todorov and Filzmoser, 2010; Hssaini et al., 2020). All factors and their interactions showed statistically significant impact on the whole model ( $p < 0.001$ ). Since lambda value is inversely proportional to the impact score within the built model,



**Table 6.** Multiple analysis of variance (MANOVA) showing the interactions between the experimental design factors.

Multivariate tests <sup>a</sup>					
Effect	Wilks' Lambda Value	F	Hypothesis df	Error df	Sig.
Intercept	0.000	360315.017 <sup>b</sup>	14.000	11.000	0.000
Irrigation (I)	0.000	149.757 <sup>b</sup>	28.000	22.000	0.000
Cultivar (C)	0.004	205.811 <sup>b</sup>	14.000	11.000	0.000
Year (Y)	0.007	106.959 <sup>b</sup>	14.000	11.000	0.000
I x C	0.000	65.970 <sup>b</sup>	28.000	22.000	0.000
I x Y	0.003	12.895 <sup>b</sup>	28.000	22.000	0.000
C x Y	0.012	63.135 <sup>b</sup>	14.000	11.000	0.000
I x C x Y	0.003	13.159 <sup>b</sup>	28.000	22.000	0.000

<sup>a</sup> Design: Intercept + I + C + Y + I x C + I x Y + C x Y + I x C x Y; b. Exact statistic.

it is noteworthy that the interaction 'C x Y' have displayed the lowest impact on the whole model, as it has exhibited the highest Wilks's lambda (0.012). This interaction was significantly important for juice content, aril content, aril weight and aril dry matter along with the following biochemical traits: pH, TA, AAC and TAC (Table 7). However, the interaction 'I x C' has the highest effect on the model ( $\lambda = 0.000$ ), and has been shown significant over fruit weight, aril weight, aril dry matter, juice content and AAC. Among these variables, aril weight, juice content and AAC were particularly significantly affected by the three-level interaction 'I x C x Y'. These findings are interesting as they describe the magnitude of each factor effect on the herein investigated traits and how this magnitude was affected following the interactions levels. This was remarkable when comparing the Wilks's lambda values of different interactions levels.

#### 4. Discussion

Fruit yield is considered the most interesting trait for the fruit industry, so it was selected as the target parameter to evaluate tree productivity in response to SDI. To the best of our knowledge, this is the first study to investigate deficit irrigation effects on pomegranate under the Moroccan conditions with focus on fruit yield and quality. The results clearly showed that the marketable yields in Sefri and Wonderful cultivars were negatively affected by SDI, even under moderate level of 70% ET<sub>C</sub>. Similar result was observed in 'Mollar de Elche' in Spain and Rabab cultivar in Iran (Galindo et al., 2014a; Parvizi et al., 2016). The recorded

declines in pomegranate yield under SDI were not linked only to the decrease in fruit weight, since their reduction rates were not equal, but also linked to fruit drop emphasis in response to water stress. According to Galindo et al. (2014b), the decrease in yield and fruit weight under deficit irrigation is mainly related to a deceleration in fruit growth and fruit cracking incidence, which can be attributed to a turgor loss in fruit, since a direct relationship between turgor and growth of fruit has been proved in various species (Serpe and Matthews, 2000; Matthews and Shackel, 2005). However, data herein reported showed that the total yield decreased in response to deficit irrigation, while the mean fruit weight was maintained, as observed in 'Wonderful' under SDI<sub>70</sub>. Therefore, the fruit yield reduction cannot be attributed to the decrease in fruit weight, but to the fruit drop from stressed trees. Similar outcomes were reported by Mills et al. (1996) who reported that some fruits act as strong sinks of photosynthates and end up falling under drought conditions.

On the other hand, in a review of similar studies on different pomegranate cultivars, ambiguous results were reported with regard to changes in yield and its components under water stress. Indeed, Centofanti et al. (2017) did not observed any statistically significant effect on yield and fruit weight in Wonderful variety under a severe water deficit of 35% ET<sub>C</sub> applied continuously during two years in Central California. Comparable results have been also reported by Intrigliolo et al. (2013) under the Spanish climate in Mollar de Elche cultivar with a SDI of 50% ET<sub>C</sub> over three consecutive years. Likewise, Martinez-Nicolas et al. (2019) have reported similar tendencies when the pomegranate trees were subjected to water withholding during flowering-fruit set period

**Table 7.** Analysis of variance showing the significance of the factors and their interactions on the studied variables.

Source of variation	Irrigation (I)	Cultivar (C)	Year (Y)	I x C	I x Y	C x Y	I x C x Y
Fruit yield	<b>0.001</b>	<b>0.000</b>	0.666	0.308	0.061	0.154	0.124
Juice yield	<b>0.000</b>	<b>0.000</b>	0.146	0.750	<b>0.026</b>	<b>0.018</b>	0.249
Fruit weight	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.063	0.244	0.432
Fruit aril content	<b>0.004</b>	<b>0.000</b>	0.134	0.263	0.093	<b>0.001</b>	0.104
Aril weight	<b>0.013</b>	0.229	0.086	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>
Aril dry matter	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.277	<b>0.000</b>	0.377
Juice content	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.001</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>
pH	0.217	<b>0.016</b>	<b>0.013</b>	0.437	0.508	<b>0.046</b>	0.396
TSS	<b>0.039</b>	0.279	0.162	0.835	0.999	0.634	0.881
TA	0.722	<b>0.000</b>	0.104	0.454	0.079	<b>0.009</b>	0.092
TPC	0.149	0.547	0.853	0.974	0.339	0.710	0.980
SSC	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.401	0.667	0.339	0.871
AAC	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>
TAC	0.242	<b>0.000</b>	0.446	0.474	0.851	<b>0.040</b>	0.291

TSS: total soluble solids content, TA: titrable acidity, TPC: total polyphenols content, SSC: soluble sugars content, AAC: amino acids content, TAC: total antioxidant capacity.

Significant p-values (<0.05) are marked in bold.

over two consecutive years. The mechanisms involved in maintaining fruit weight and yield of pomegranate under drought stress condition remain until now understood, although some studies reported certain tolerance and avoidance mechanisms. Indeed, [Aseri et al. \(2008\)](#) reported that under water deficit conditions, pomegranate leaf conductance decreases first in order to control water loss via transpiration and to avoid cells leaf turgor loss. However, under severe levels of water stress, pomegranate leaves trigger active osmotic adjustment in order to maintain leaf turgor, which can ensure normal photosynthetic activity, thereby maintaining yield and fruit weight. In addition, [Rodríguez et al. \(2012\)](#) reported that water stress tolerance mechanisms commonly seen in xeromorphic plants such as high relative apoplastic water content (42–58%) and the retention of water at low leaf water potentials can be also observed in pomegranate trees. However, a significant genotypic difference was proved regarding the expression of these physiological mechanisms in pomegranate ([Pourghayoumi et al., 2017](#)), thereby explaining the contradictory results. These mechanisms are also influenced by the environmental conditions, since contrasting results were recorded for the same cultivar in different areas.

Data show that the decrease in fruit weight under SDI may result from reduction in peel weight, aril content or both. In ‘Wonderful’, the fruit weight decrease was originating from a proportional reduction in peel and total aril weight, because there was no significant effect of the SDI treatment on fruit aril content. However, in ‘Sefri’ in 2019, it resulted from a significant reduction in weight of total aril rather than that of fruit peel. In previous study, this effect was also related to the pollination factor ([Gharaghani et al., 2017](#)). However, in this research, this statement may not be valid as the experiment was conducted under homogeneous environmental and growing conditions. Furthermore, the decrease in aril content under SDI was linked to a regression in number of arils per fruit since no significant change was observed in aril weight. In fact, data showed that under SDI, pomegranate produced arils with similar unit weight than in well-watered trees, but were less juicy. This means that SDI induces an increase in aril dry matter without causing a significant loss in aril weight, thereby decreasing the aril juice content and consequently the fruit juice yield. Particularly in Sefri cultivar, this effect on aril juiciness, combined with a reduction of aril content, decreased significantly fruit juice content that, coupled with a lower fruit yield, caused a substantial reduction in juice yield. [Selahvarzi et al. \(2017\)](#) observed similar results on Shahvar cultivar under a severe water deficit of 50% ET<sub>C</sub>.

In the two studied pomegranate cultivars, juice was less sweet under SDI than in control treatment, which is most often due to the significant decrease in TSS values. Similar result was found in the varieties ‘Grenade Rouge’ and ‘Mollar de Elche’ growing in Morocco and Spain, respectively ([Adiba et al., 2021](#); [Ghosh et al., 2015](#)). The SDI impact on juice traits seems to be strongly dependent to the genotypic factor and experimental conditions, since in similar work on Mollar de Elche cultivar, TSS values have been observed rather increased in response to deficit irrigation ([Galindo et al., 2014a](#)). In addition, other studies found that deficit irrigation had no significant effect on sugars content of pomegranate juice ([Pena et al., 2013](#); [Mellisho et al., 2012](#); [Centofanti et al., 2017](#)). A similar result was observed in ‘Sefri’ in the first year of the SDI application, but which became significant in the second year. On the other hand, it seems that there was a slight decrease of the sour taste in pomegranate juice under severe SDI regime, as indicated by a significant rise of juice pH in Sefri cultivar under SDI<sub>50</sub>. However, the data on ‘Wonderful’ showed insignificant effects on both pH and titratable acidity, thus involving the genotype as a determinant factor for pomegranate juice traits under water stress. In this sense, similar studies on Mollar de Elche cultivar indicated a stability of these traits under deficit irrigation. The fact that amino acids content varied according to the irrigation levels in Wonderful variety although titratable acidity remained stable, suggests that there were significant decreases in certain organic acids other than citric acid. Furthermore, the results showed an imperative decrease of AAC in ‘Wonderful’ as a response to SDI<sub>50</sub>, while

AAC variation under SDI<sub>70</sub> did not allow drawing clear conclusions regarding their tendency under moderate deficit irrigation. The stability of AAC in ‘Sefri’ under water stress indicates the fact that the species plasticity to drought is remarkably dependent to the genotypic factor. This result, particularly observed in ‘Sefri’, was in agreement with those of [Centofanti et al. \(2017\)](#) reported on Wonderful variety.

The results regarding total polyphenols showed that moderate water deficit, applied during fruit growth, leads to a significant decrease in their concentration in ‘Sefri’ fruit juice. Whereas, they remained unchangeable in Wonderful variety. However, the decrease in TPC under severe water stress, as in SDI<sub>50</sub>, was substantial for both cultivars. The decrease in TPC has been related to an accentuation of the enzymatic oxidation of polyphenols under water stress ([Kulkarni and Aradhya, 2005](#); [Fawole and Opara, 2013](#)). On the other hand, the fact that the reduction in TPC occurred in the second season suggests a cumulative effect of water stress on these compounds over the years. This could explain the results of some similar studies conducted over a single season where water stress did not significantly affect juice TPC, as reported by [Centofanti et al. \(2017\)](#) and [Mellisho et al. \(2012\)](#).

The decrease in total anthocyanins content in response to water deficit was often observed in various plants, including pomegranate, particularly under severe regime. [Schwartz et al. \(2009\)](#) showed that pomegranate fruits from desert areas exhibited lower levels of TAC compared to fruits grown under Mediterranean conditions. Furthermore, [Gil et al. \(1995\)](#) linked the decrease of anthocyanin biosynthesis under desert areas to high levels of temperature and sunlight. Actually, under water stress conditions, pomegranate fruits could be more exposed to these environmental factors due to a reduction in shoot and leaf growth ([Martinez-Nicolas et al., 2019](#)), thereby explaining the observed reduction of TAC in ‘Wonderful’ fruits. [Laribi et al. \(2013\)](#) reported similar result on Mollar de Elche cultivar, which displayed a weak red color, and consequently a lower visual attraction of the juice. In contrast, our results showed that TAC in pomegranate juice from Sefri cultivar was not affected by water deficit, suggesting that there is a genetic variation regarding anthocyanin biosynthesis under water stress in pomegranate. In this sense, the TAC low level in ‘Sefri’ compared to ‘Wonderful’ under all water treatments may indeed be a probable source of this genotypic variation, which deserves further exploration.

In fact, it is well known that water stress improves fruit quality in various plants such as almond, peach, pear and plum through an increase in SSC, AAC and TPC ([Razouk et al., 2020](#); [Guizani et al., 2019](#); [Marsal et al., 2012](#)). Contrary, in pomegranate, there was globally a significant decrease in these parameters under SDI with significant phenotypic effect that varies depending on the SDI regime and the targeted traits, and this can probably lead rather to an opposite effect of water stress on pomegranate juice quality.

The present study was also aiming to address potential interactions between all studied factors on the targeted variables as means to understand the species behavior as various water stress levels were applied. The effect magnitude of the study design factors and their interactions were examined using MANOVA test and the results were summarized in Tables 6 and 7. The fact that ‘I x C’ interaction was among the ones having the highest impact on the model ( $\lambda = 0.000$ ), may make sense as the phenomenon was particularly driven by the irrigation treatment factor, which the lambda value was extremely low compared to those of other factors. This assumption may be due mainly to the lowest impact recorded by the cultivar ( $\lambda = 0.004$ ) and the year ( $\lambda = 0.007$ ) factors, of which the impact magnitude was not as large as that induced by the irrigation treatment. In fact, based on meteorological data previously reported, the two experiment years seemed similar with only very few contrasts ([Figure 1](#)). This seems not being sufficient enough to induce larger impact among cultivars under the same irrigation treatment. Furthermore, the two cultivars herein examined, ‘Sefri’ and ‘Wonderful’, are in fact somewhat distant genetically, as previously raised by [Ajal et al. \(2015\)](#) using AFLP markers. Through this study conducted on the same field under the same growing conditions, the phenotypic similarity was

greater between the aforementioned cultivars, which may explain the magnitude of the cultivar factor on the model as being less important compared to the irrigation treatment factor.

Even though, all previously mentioned interactions levels were revealed having statistically significant impact on the yield components and biochemical traits of pomegranate and its juice following various magnitude levels, it is actually difficult to untangle the complexity of the species behavior toward different irrigation strategies, cultivars and years.

## 5. Conclusion

The current work is the first report describing the pomegranate plasticity to moderate (SDI<sub>70</sub>) and severe (SDI<sub>50</sub>) deficit irrigation and its impact on yield components and fruit quality under the Moroccan growing conditions. Under both deficit regimes, pomegranate trees showed a significant decrease in fruit growth, leading to lower final fruit weight along with the total yield, especially in the local cultivar 'Sefri'. Arils did exhibit any change during the two years field experiment in term of unit weight, but were less juicy, leading to lower juice yield, which was accentuated by fruit yield decrease. The both SDI treatments induced a significant decrease in soluble sugars and polyphenols, particularly in Wonderful variety which displayed also a remarkable decrease in total anthocyanins content. From a nutritional point of view, this means that juice yielded by pomegranate trees under SDI was of lower quality and less nutritional compared to that from well-watered trees. Therefore, the application of SDI during the whole period of fruit growth is not appropriate for pomegranate. However, it is important to emphasize that the herein observed decrease in yield and fruit quality were less important under SDI of 70% ET<sub>C</sub>, which could make this deficit irrigation regime an efficient technical option for growing pomegranate in semiarid lands. The whole experimental design showed that the tree plasticity to drought was particularly driven by the applied stress levels. The latter, when interacted with cultivar factor displayed the highest magnitude effect on trees behavior.

## Declarations

### Author contribution statement

Atman Adiba: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lahcen Hssaini: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abdelmajid Haddioui: Conceived and designed the experiments.

Anas Hamdani; Jamal Charafi; Salma El Iraqui: Contributed reagents, materials, analysis tools or data.

Rachid Razouk: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### Data availability statement

Data included in article/supplementary material/referenced in article.

### Declaration of interests statement

The authors declare no conflict of interest.

## Additional information

No additional information is available for this paper.

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