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# Agro-physiological responses of the pistachio (*Pistacia vera* L., cv. Mateur) to partial root drying (PRD) irrigation

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#### ABSTRACT

Purpose: Water irrigation regimes strongly influence the agrophysiological parameters in pistachio. This study aims to investigate the impact of the partial root drying on the yield, vegetative growth, physiological parameters, water status and biochemical traits of the pistachio cv. Mateur budded on P. atlantica rootstocks during the growing season (2021). Research Method: The agro-physiological responses of the pistachio trees located in the experimental orchard of the Regional Center of Agriculture Research (CRRA, Sidi Bouzid, Tunisia), were studied. Three water treatments were applied; T0: 100% Partial root drying (PRD) during all the season, T1; 75% PRD during all the season and T2; 50% PRD during all the growing season. The leaf gas exchange parameters were determined using a portable photosynthesis system (CI-340 handheld photosynthesis system, USA). Findings: Results showed the stomatal conductance (gS) of pistachio leaves ranged from 320 to 760 mmol H2O m-2s-1 in the 100% PRD treatment whereas the water regimes 75% PRD and 50% PRD presented a clear decrease in this parameter. The proline and the soluble sugar content reached its maximum value (2.10 µmol g-1 FW and 275.60 µg g-1 FW, respectively) under the 50 % PRD treatment during the month of August. Research limitations: No limitations were found. Originality/Value: The 75% PRD treatment was the most efficient as it did not show significant differences with the 100% PRD treatment while 25% of the irrigation water was saved. The partial root drying strategy can be used in pistachio orchards under semi-arid conditions.



#### **INTRODUCTION**

The pistachio (*Pistacia vera* L.) is the most economically important species in the genus *Pistacia*. Pistachio world production reached 915.7 thousand tons in a harvested area of around 817 thousand ha in 2021 (FAOSTAT, 2023). The main pistachio producing countries are Iran, USA, Turkey, China and Syria contributing over 90% of the world production (FAOSTAT, 2023). In Tunisia, pistachio production reached 3123 tons with harvested area of around 27810 ha in 2021 (FAOSTAT, 2023). The most productive zones are Kasserine, Sidi Bouzid, Gafsa and Sfax presenting more than 80% of the national production. Among the most cultivated varieties, the cv. Mateur is the most important showing a high production, a high pomological nut quality traits and adaptability to the soil and climatic conditions.

Stressful environmental factors such as water deficiency represents a major constraint limiting crop growth and yield worldwide (Abboud et al., 2021). Pistachio tree has the reputation of being drought tolerant and saline-resistant species cultivated under rainfed conditions in its region of origin (Rieger, 1995). In pistachio, the nut development is characterized by three different periods (Goldhamer, 1995): stage I starts at the beginning of the nut growth and ends when its maximum size is reached; during stage II the shell hardening takes place and finally, the stage III is the period of kernel growth. The impact of drought stress on tree agrophysiological parameters is a complex process (Fereres et al., 2012). Hence, plants have evolved morphological, anatomical, physiological, biochemical and molecular adaptive responses that enable them to adapt to drought under water shortage conditions (Abboud et al., 2021). The primary effects of drought are the reduction of plant stomatal conductance, water potential, leaf area and leaf gas exchange (Abboud et al., 2021; Jiménez et al., 2013). Goldhamer and Beede (2004) reported that water deficiency in pistachio decreases tree growth, nut yield, affects nut quality by decreasing the proportion of split nuts and increases the alternate bearing intensity. Others responses of pistachio tree to the water deficiency is relative water content decrease (Sajjadinia et al., 2010), proline accumulation (Anjum et al., 2011), soluble sugars accumulation in leaves (Kempa et al., 2008). Galindo et al. (2017) reported that pistachio tree exposed to water stress also developed stress avoidance and stress tolerance mechanisms. Hence, during pistachio fruit stages I and II, when the soil water content is quite high and the evaporative demand of the atmosphere is low, the tree showed higher net photosynthesis and leaf conductance values. In contrast, during fruit stage III, at which the evaporative demand of the atmosphere is higher, the pistachio plants showed lower net photosynthesis and leaf conductance values (Memmi et al., 2016b).

In pistachio trees, irrigation increases the yield and improves the nut quality (Kanber et al., 1993). However due to the prolonged drought periods, there are scarce water resources for crop irrigation (Giorgi & Lionello, 2008). The implementation of irrigation strategies that improve the water use efficiency (WUE), without yield and quality reduction is of a great interest (Fereres & Soriano, 2007). Partial root drying (PRD) consists in alternate the irrigation periodically in the two parts of the root zone (Dry et al., 1996). The PRD has been successfully applied to a large number of crops taking into consideration variety-rootstock interaction, type and characteristics of soil, agricultural practice, and specific agro-climatic conditions, demonstrating that the main benefit of PRD irrigation is the reduced use of water for irrigation (Jovanovic et al., 2017). The fully hydrated roots maintain a favorable plant water status, while the dried ones send chemical signals (abscisic acid) to the shoots to induce partial stomatal closure and thus reduce the relevant water demand (Abboud et al., 2019). Several studies reported that the PRD practice induced a decrease in leaf water potential in olive (Centritto et al., 2005), a slight decrease in the average shoot length and yield as



compared to the full-irrigated treatment (Wahbi et al., 2005), a decrease in stomatal conductance and subsequently leaf water potential (Abboud et al., 2019).

With recurring decrease of precipitation and prolonged drought periods in major pistachio orchard under semi-arid areas, water deficit become a critical factor for irrigated high-density pistachio plantations. Therefore, the use of water-saving irrigation strategies and the selection of the best adapted cultivars are crucial for a sustainable production and an efficient water use under water scare conditions. The aim of this study was to evaluate the agro-physiological and biochemical responses of the pistachio cultivar Mateur conducted with fewer than three partial root drying water regimes (100% PRD, 75% PRD, and 50% PRD).

# MATERIALS AND METHODS

#### Plant material and Experimental design

The trial was carried out in the experimental orchard of the Regional Center of Agriculture Research (CRRA, Sidi Bouzid) in west central Tunisia (9°43'E, 35°01'N; altitude 354 m). Fifteen-years-old pistachio trees cultivar Mateur grafted on *P. atlantica* rootstock were studied. Trees were planted at a spacing of  $6\times 6$  m and grown under standard conditions of fertilization, pruning, pollination and pest and disease control. The surveyed trees were selected for uniform trunk and canopy size. The experiment was designed as a complete randomized block design with 9 trees (8 females and 1 male) per experimental plot. The production area is the semi-arid Mediterranean climate with a low annual rainfall of 200 mm irregularly distributed over the growing season and a reference evapotranspiration (ETo) of more than 1300 mm (Fig. 1).

#### Treatments for partial root drying

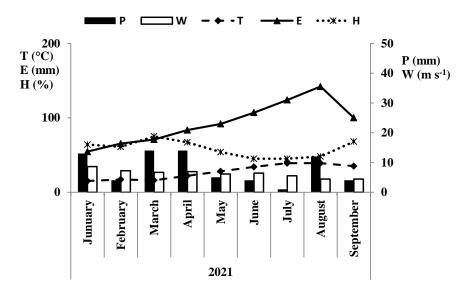
The trees were conducted under the partial root drying (PRD) irrigation strategy. During the experiment period, trees had only one side of their root zones irrigated and irrigation was alternated every two weeks. The pistachio trees were grown under three water regimes (100% PRD, 75% PRD, and 50% PRD). In the control treatment (100% PRD), trees received water equivalent of 100% of crop evapotranspiration (ETc). For the treatment 75% PRD, trees received 75% of crop evapotranspiration. In the treatment 50% PRD, trees received 50% of crop evapotranspiration. Two drip lines were employed for each tree row one on each side with drippers located at 60 cm from the trunk and discharged an average flow rate of 2.0 L/h. The irrigation scheduling was applied from March to September with a frequency of 3 times per week. The amount of water provided (Table 1) was calculated on the basis of the crop evapotranspiration and the crop coefficient according to the FAO method demand using the following formula (Allen et al., 1998).

$$ETc = ETo \times Kc \times Kr$$

(1)

With ETc: crop evapotranspiration, Kc: crop coefficient values (0.4; 1.06 and 1.14 during the stage I, II and III respectively (Fereres & Goldhamer, 1990).





**Fig 1.** Climatic conditions during pistachio nut development. Abbreviations: T: temperature; P: precipitation; E: evapotranspiration; W: wind; H: humidity; m: meter; mm: millimeter; s: second.

**Table 1.** Crop evapotranspiration rate (ETc) and water applied to the three water regimes (T0=100 % PRD, T1=75 % PRD and T2=50 % PRD).

Year	ET0 (mm)	P (mm)	Water regime (mm)							
			100% PRD	75% PRD	50% PRD					
2021	1350	186	650	487.5	325					
Abbreviations: ET0= reference evapotranspitation; T0= 100% PRD; T1= 75% PRD;										

T3= 50% PRD; P= precipitation.

# Agronomical measurements

#### Trees vigor

The trees vigor parameters were assessed in the three water regimes during the growing season 2021 as reported in Abidi et al. (2023). The tree height, canopy and the trunk cross-sectional area (TCSA) were measured during the dormant season at 30 cm above the graft union. The yield (Kg/tree), cumulative yield per tree, and yield efficiency (cumulative yield in kilograms per final TCSA) were determined.

#### **Phenological traits**

The initial blooming (5% of flowers are opened), full blooming, final blooming, harvesting dates (when hull separates easily from the shell) and nut development period were recorded during the growing season (2021) according to International Plant Genetic Resources Institute (IPGRI, 1997) descriptors for pistachio.

#### Vegetative growth

The vegetative growth parameters under the applied treatments were assessed as described in Abboud et al. (2019). In each tree, four shoots were selected in four orientations around the canopy. Then the shoot length, shoot diameter, internode number and leaf area were recorded each month during the growing season, from April to August on the tagged shoots.

#### Leaf gas exchange parameters

The leaf gas exchange parameters were assessed in the trees of the cv. Mateur under the three water regimes as described in Abboud et al. (2019). The photosynthesis rate ( $P_n$ ), stomatal conductance (gs), transpiration rate (E) were measured using a portable photosynthesis system



(CI-340 handheld photosynthesis system, USA) with a flow rate of 0.2 1 min<sup>-1</sup> and leaf temperature within 2–3 °C of ambient air temperature (25–35 °C). Water use efficiency (WUEi) was calculated was calculated as the ratio of net photosynthesis to stomatal conductance and expressed in  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>/mol m<sup>-2</sup> s<sup>-1</sup>. All Measurements were made from the tip of the youngest fully expanded leaf (usually the third or fourth leaf from the apex) between 11:00 and 12:00 h every month from April to August. Three leaves per tree were monitored in three experimental trees for each treatment.

# Water status

# Leaf relative water content

Fully mature and healthy leaves were used to determine the leaf relative water content (RWC). Leaves of similar age were collected from three trees for each per experimental plot and the fresh weight (FW) was immediately determined. Then leaves were placed in similar volumes of distilled water for 24 hours to re-hydrate (Cameron et al., 1999). After that leaf turgid weight (TW) was measured and then leaves were dried at 80°C for 48 hours to obtain the dry weight (DW). The leaf relative water content (RWC) was calculated using the formula: LRWC = 100x (FW-DW) / (TW-DW) as described in Yamazaki and Dillenburg (1999).

# Midday leaf water potential

The midday leaf water potential ( $\Psi$ md) was measured following the methodology described in Abboud et al. (2019). The  $\Psi_{md}$  was determined at 10 to 12 am under clear sky every 4 weeks from April to August. The measurements were performed on current-year shoot from the mid canopy of the trees that had been enclosed in plastic bags covered with aluminum foil at least 2 hours before measurements in order to reduce leaf transpiration (Shackel et al., 1997) and to equilibrate foliar and stem water potential. Shoots were then detached, and  $\Psi$ stem measurements were performed using a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA).

# **Biochemical measurement**

# **Proline content**

Proline contents were determined using the ninhydrin method described by Troll and Lindsley (1955). Three 200 mg samples were extracted for 30 min in 5 ml 40% (v/v) methanol heated to 80°C in hermetically sealed tubes. A 1 ml aliquot of extract was mixed with 2 ml glacial acetic acid, 1 ml 25 mg ml<sup>-1</sup>ninhydrin solution and 2 ml of a mixture consisting of 24% (v/v) distilled water, 60% (v/v) glacial acetic acid and 16% (v/v) orthophosphoric acid. The mixture was boiled for 30 min then cooled on ice and 3 ml toluene added before shaking vigorously. Two phases were obtained. The upper phase was saved and dehydrated with anhydrous Na<sub>2</sub> SO<sub>4</sub>. The extracts were kept in the dark for a minimum of 2 h before their absorbance was measured at 528 nm and free proline concentration was calculated from a calibration curve using proline as a standard (Sigma-Aldrich). Free proline content was reported as mg g<sup>-1</sup>FW.

# Total soluble sugar

Three samples (200 mg) of leaves were extracted in 5 ml 80% (v/v) methanol and heated to 70 °C for 30 min. The extract was then centrifuged at 5,000 rpm for 15 min and the supernatant was assayed for soluble sugars using the phenol-sulphuric acid method (Robyt & White, 1987). One ml of extract was shaken with1 ml 5% phenol and 5 ml concentrated sulphuric acid. Once the extract had cooled, its absorbance was determined at 640 nm with spectrophotometer (Jenway 6300).



#### **Statistical analysis**

Analysis of variance (ANOVA) was performed to test the effect of PRD irrigation regimes on phonological traits, agro-physiological parameters, water status, and biochemical traits, using SPSS Statistics 20.0 for Windows. Posthoc analysis was performed using the Scheffe test.

# **RESULTS AND DISCUSSION**

#### **Phenological traits**

The dates of initial blooming, full blooming, end blooming, harvesting dates and the fruit development period were shown in Table 2. The cv. Mateur presented an initial blooming in March, 24 whereas the full blooming date was in April, 06 and the end of blooming was in April, 13. The harvesting date was inAugust16 and the fruit development period was of 145 days. The male tree presented a blooming date from 15 to 20 March. The full blooming dates of the male and female trees were not in total synchronization. Results showed that the cv. Mateur showed similar blooming and harvesting dates under the three applied water regimes. In this line, Vargas et al. (1995) reported that 'Mateur' cultivar was among the earliest female cultivars evaluated, with the mean blooming period from March, 30 to April, 13. In another study, Monastra et al. (1998) reported that the flowering process development in irrigated trees was two years earlier than in non-irrigated. The authors reported that the important branch growth with presence or absence of inflorescences reduced the alternate bearing that is especially higher in non-irrigated trees.

#### **Agronomical traits**

The tree height, tree canopy and the trunk cross sectional area were shown in Table 3. The tree height varied from 2.2 to 2.9 m. The tree canopy was in the range of 3.0 to 3.2 m. The trunk cross sectional area varied from  $60.6 \text{ cm}^2$  in the treatment T2 (50% PRD) to  $66.5 \text{ cm}^2$  in the treatment T1 (75% PRD). The water regime has no effect on the tree vigor parameters. In this line, Monastra et al. (1998) indicated that irrigation with 50% of evaporative demand could support trunk growth equal to that in the fully irrigated trees. Our findings are not in accordance with the study of Egea et al. (2010) showing a negative impact of regulated deficit irrigation on trunk growth.

The analysis of the impact of the three irrigation regimes on yield and fruit weight (FW) showed significant difference between T2 (50% PRD) and the two treatments T0 (100% PRD) and T1 (75% PRD) as shown in Table 3. The mean yield was slightly higher in the control treatment as compared to T1 (75% PRD) and the stressed treatment (T2). The yield ranged from 4.5kg per tree under the treatment T2 to 6.5kg/tree under the control treatment. These results are similar to the study of Memmi et al. (2016) showing that the mean yield of pistachio trees under regulated deficit irrigation was not reduced in T1 and T2 compared to the control treatment with water savings of 40 % in T1 and 45 % in T2. The nut fresh weight was affected by the water regime with the treatment T2 showing statistically significant (P<0.05) difference with the control and T1. Regarding the nut fresh weight (FW) the highest value (0.75g) was observed under the control treatment whereas the lowest value (0.50g) was shown under the T2 treatment. In almond trees, Egea et al. (2010) reported that with exception of the partial root drying (PRD<sub>70</sub>) treatment, the nut weight was significantly reduced in remaining deficit irrigated treatments.



Table 2. Blooming and harvesting dates of the cultivar 'Mateur'.

Traits	Mateur T0	Mateur T1	Mateur T2			
Initial blooming	24 March	24 March	24 March			
Full blooming	06 April	06 April	06 April			
End blooming	13 April	13 April	13 April			
Harvest day	16 August	16 August	16 August			
Development period (day)	145	145	145			
T0 – 100% PRD: T1 – 75% PRD: T3 – 50% PRD						

T0 = 100% PRD; T1 = 75% PRD; T3 = 50% PRD

Table 3. Tree vigor and agronomical measurements of the cv. Mateur under three water regimes.

Treatment	Height	Canopy	TCSA	Yield	FW	Y.E
T0 (100% PRD)	$2.9\pm0.1^{a}$	$3.2\pm0.5^{\rm a}$	$65.9\pm5.1^{a}$	$6.5\pm1.2^{a}$	0.75±0.2ª	$0.09 \pm 0.01^{a}$
T1 (75% PRD)	$2.5\pm0.1^{a}$	$3.1\pm0.6^{\rm a}$	$66.5\pm1.4^{\rm a}$	$5.8\pm1.0^{a}$	$0.65 \ \pm 0.5^a$	$0.08\pm0.02^{\rm a}$
T2 (50% PRD)	$2.2\pm0.1^{a}$	$3.0\pm0.2^{\rm a}$	$60.6\pm0.9^{\text{a}}$	$4.5\pm0.7^{b}$	$0.50\pm0.3^{b}$	$0.07\pm0.02^{\rm a}$

Values are mean  $\pm$  Standard error. Abbreviations: Tree height (m); Canopy (m); TCSA= Trunk cross sectional area (cm<sup>2</sup>); Average yield (kg/tree); Yield efficiency (kg/cm<sup>2</sup>); FW=fresh weight (g).

Mean separation within columns by Scheffe test at ( $p \le 0.05$ ).

wheat separation within columns by Scheric test at  $(p \ge 0.05)$ .

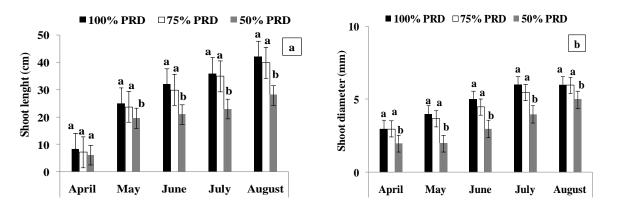
Different letters indicate statistically significant (P<0.05) differences between treatments.

#### Vegetative growth

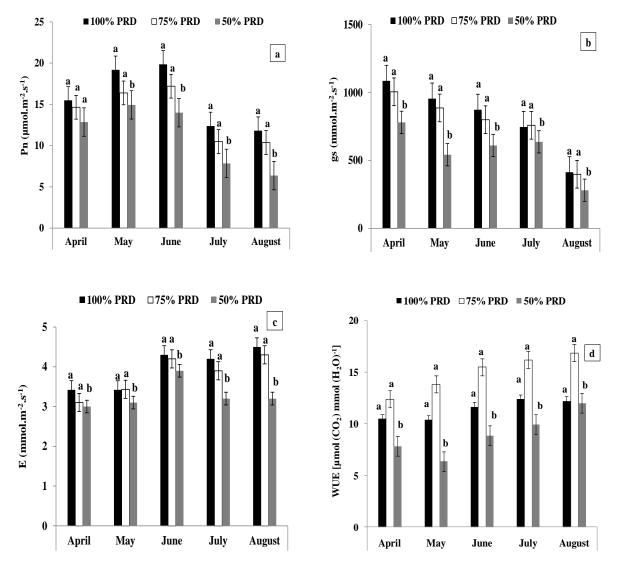
The apical shoot lengths of trees were affected by the water restriction generated under the treatment T2 (50% PRD) as observed in Figure 2a. The measurements made in May, presented a shoots length of 26 cm in the treatment T0, 21 cm in the treatment T1, and 19 cm in the treatment T2. The control treatment (100% PRD) and the treatment T1 (75% PRD) showed similar pattern of shoot length and diameter whereas the treatment T2 showed statistically significant differences (P<0.05) in all measurements from May to August.

The shoot diameter (Fig. 2b) presented the same behavior as shoot length being similar between T0 and T1 treatments whereas the T2 presented statistically significant (P<0.05) lower values. Hence, this parameter varied from 2 mm in April to 5 mm in August for the 50% PRD treatment.

Our results are in accordance with the findings of Robyt and White (1987), reporting that shoot growth ranged from 11 to 25 cm being higher in the control treatment. Spann et al. (2007) reported that the length of shoot growth produced from terminal buds on mature trees was quite variable, with the shortest shoots being less than 10 cm long and the longest shoots approaching a meter in some years. Baccari et al. (2020) reported that the effects of water stress on growth may be considered the first line of defense, due to the inhibition of cell elongation by the interruption of water flow from the xylem to the surrounding cells and serves to reduce the amount to total water transpired by the plant under drought conditions. Abboud et al. (2019) reported that the lowest shoot length was observed under the treatment 50% PRD. Similar results were found by Grattan et al. (2006), showing a clear reduction in shoot growth of olive trees under the lowest irrigation treatments. The PRD irrigation regime (50% PRD) decreased the vegetative growth and was consistent with several reports on partial root zone drying experiments on olive tree (Abboud et al., 2019; Dbara et al., 2016). Hence, Dbara et al. (2016) reported that the PRD50 induced a slight reduction of shoot elongation compared to control, while those of the PRD100 treatment did not statistically differ from the control.



**Fig 2.** Shoot length (a) and diameter (b) of the pistachio cultivar Mateur. Different letters indicate significant differences among treatment.



**Fig. 3.** Evolution of the photosynthetic assimilation (a), stomatal conductance (b), transpiration rate (c), and photosynthetic efficiency (d) of the pistachio cultivar Mateur. Different letters indicate significant differences among treatments.



# Leaf gas exchange parameters

# Photosynthetic assimilation

The photosynthetic assimilation ( $P_n$ ) of the cultivar 'Mateur' under the PRD treatments was presented in Figure 3a. The Pn increased gradually during the rapid growth and the stone hardening stages of the pistachio nut (April, May and June). During the kernel growth stage (July and August), the Pn decreased gradually showing the high-water demand during this period of nut development. The treatment (T2) showed significantly (P<0.05) lower values of  $P_n$ .

# Stomatal conductance

The stomatal conductance (gs) of pistachio leaves (Fig. 3b) ranged from 320 to 760 mmol  $H_2O \text{ m}^{-2}\text{s}^{-1}$  in the 100% PRD treatment, showing statistically significant (P<0.05) differences among the three water regimes except for the values registered in April (Fig. 2b). Results showed that the water stressed treatment (T2) affected gs in all the nut development period whereas the treatment T1 (75% PRD) was less affected.

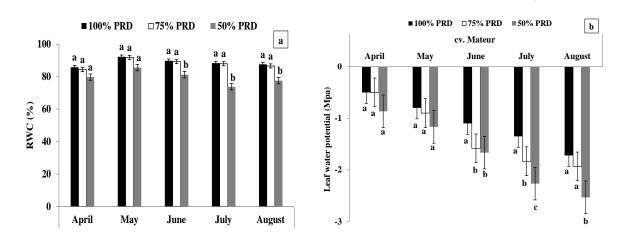
# Transpiration (E)

The evolution of transpiration in the cv. Mateur is presented in Figure 3c. The evapotranspiration showed high values in August, being 4.5, 4.3 and 4.2 mmol  $m^{-2} s^{-1}$  for 100% PRD, 75 and 50% PRD treatments, respectively.

# Water use efficiency (WUE)

The intrinsic water use efficiency (WUE) was significantly (P<0.05) affected by the irrigation regimes (Fig. 3d). The high values of WUE were detected under the 75% PRD. The lowest value of WUE (6.36  $\mu$ mol (CO<sub>2</sub>) mol (H<sub>2</sub>O)<sup>-1</sup> was observed under the 50% PRD treatment during the month of May.

It has also been reported that the decrease in leaf photosynthesis in summer could be due to the temperature damage of the photosystems and the increase in the rate of photorespiration (Angelopoulos et al., 1996). Reddy et al. (2004) reported that the reduction of  $P_n$  in drought stressed plants could be attributed to the stomatal limitation and may also be explained by inhibited leaf photochemistry as well as metabolic impairment. According to Gijón et al. (2011) water stress applied during stages II (shell hardening) and III (kernel growth) were the most adversely circumstances for the plant physiological response. Leaf gas exchange is among the first processes that are affected by water deficit, through reducing the photosynthetic productivity, osmotic adjustment, and the capacity of plants to cope with drought (Ranjbar et al., 2021). Guerrero et al. (2006) reported that the RDI affected g<sub>S</sub> later than  $\Psi$ stem, and the greatest reduction in gs (60% of control) was at the end of the regulated deficit irrigation period. Interestingly, the close relationship between Pn and gs reflects the role of gs in regulating the supply of CO<sub>2</sub> to the site of carboxylation and suggests that the decline in net photosynthesis over the season is largely a consequence of stomatal limitation (Abboud et al., 2019). Ranjbar et al. (2021) reported that the water use efficiency defined as the ratio of dry accumulation matter to water consumption during a season or the ratio of Pn to gs, is a significant indicator in assessing drought resistance.



**Fig. 4.** Relative water content (RWC) (a), and leaf water potential (b) in leaves of the pistachio cultivar Mateur. Different letters indicate significant differences among treatments.

#### Water status

#### *Relative water content*

The Relative water content (RWC) of pistachio leaves under the three PRD treatments is presented in Figure 4a. The control treatment (T0) in our experiment maintained high RWC during all the growing season. In the current study, the PRD irrigation significantly (P<0.05) affected the tree water status. Under the treatment (T2), the RWC was significantly (P<0.05) reduced, especially during the months of June, July and August.

#### Leaf water potential

The leaf water potential of the cv. Mateur was evaluated during the growing season (Fig. 4b). At the beginning of the experiment, the leaf water potential values (about -1 MPa) no varied among the three treatments. During the stone hardening (June), the leaf water potential declined in the stressed treatments (75% PRD and 50% PRD). The greatest treatment differences in leaf water potential values occurred during July. At the end of the kernel growth stage, the control treatment (100% PRD) and the treatment (75% PRD) presented similar values.

Water is one of the main limiting factors of pistachio production in Tunisia. Under water deficiency, drought affects different physiological, biochemical, and molecular traits of the pistachio trees, whereas irrigation enhances the yield and improves the tree's nut quality (Haghighi et al., 2021). Several studies carried out in Prunus species reported a decrease in leaf water status and photosynthetic parameters (Jiménez et al., 2013; Escobar-Gutiérrez et al., 1998). Baccari et al. (2020) reported that the plant growth decrease is mainly due to the loss of turgor pressure through the decrease of trees water status attaining leaf water potential values between -6 and -4 MPa, thus indicating severe water deficit. These results are in accordance with the study of Abboud et al. (2021) in three olive cultivars conducted under partial root drying. Behboudian et al. (1986) reported that pistachio trees are able to pursue their photosynthetic activity even when leaf registers extraordinary low water potential (Yleaf) values due to the unusual capability for leaf thermoregulation. Indeed, these authors showed that although photosynthesis declined with decreasing leaf water potential, plants continued to photosynthesize until a leaf water potential of as low as -5 MPa was reached which is a typical response of xerophytic plants. In the same line, Germana (1997) highlighted the great ability of pistachio to swiftly compensate water losses without displaying visible water stress symptoms. Additionally, Memmi et al. (2016b) considered that the rootstock P.

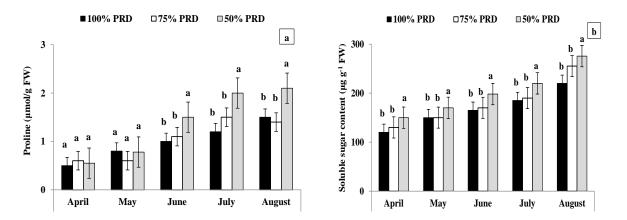


*atlantica* is adequate for deficit irrigated plantations due to its reputation as permissive to water stress under rain fed system.

#### **Biochemical analysis**

Results showed that the proline content (Fig. 5a) in pistachio leaves was significantly higher (P < 0.05) under the 50% PRD treatment as compared to 100 % PRD and 75% PRD treatments. Proline content reached its maximum value in the month of August under 50 % PRD (2.10  $\mu$ mol g<sup>-1</sup>FW). The proline accumulation was generally higher from June to the end of the trial. These findings are in accordance with the study of Abboud et al. (2021) showing that the proline accumulation was significantly (P<0.05) higher under the stressed water regime (50% PRD). This result matches previous works on olives (Abboud et al., 2021; Ben Ahmed et al., 2009) and citrus (Zandalinas et al., 2016), in which tolerant cultivars accumulate higher amounts of proline.

The total soluble sugars content was influenced by the irrigation treatments (Fig. 5b). The applied water regimes caused a significant increase (P<0.05) in sugar content for the treatment 50% PRD during the growing season. Furthermore, the maximum value of soluble sugar was 275.60  $\mu$ g g<sup>-1</sup> FW, belonged to the 50 % PRD treatment during the month of August. These results are in line with the study of Abboud et al. (2021) showing a total sugar accumulation in leaves of the three olive cultivars. Proline has been considered to act as an osmolyte, and ROS scavenger, its accumulation has been described as a tolerance mechanism used by plants to face drought stress (Jiménez et al., 2013). Dutra et al. (2017) reported that in addition to its conventional role as an osmolyte, proline protects membrane integrity and prevents enzyme/ protein denaturation by functioning as a potent ROS scavenger. Regarding the soluble sugar evolution, Escobar-Gutiérrez et al. (1998) showed that sorbitol rather than sucrose is preferentially photosynthesized at the low photosynthetic rates of drought-stressed peach leaves. Owing the putative role of soluble sugar and proline as antioxidants (Jimenez et al., 2013), they could improve deleterious effects of drought-induced oxidative stress by protecting membranes and enzymes.



**Fig. 5.** Proline (a) and soluble sugar content (b) in the leaves of the pistachio cultivar Mateur. Different letters indicate significant differences among treatments.



# CONCLUSION

The cv. Mateur presented quite similar response concerning the phenological traits and the tree vigor under the three PRD (100% PRD, 75% PRD, and 50% PRD) water regimes. A differential response to PRD irrigation was observed in the vegetative growth, water status, and the physiological parameters. However, the 75% PRD and 100% PRD showed similar behavior whereas the 50% PRD treatment led to a reduction in vegetative growth and leaf gas exchange. The reduction of irrigation volumes by 25% over the control (100% PRD) could be an efficient irrigation strategy to be implemented in high density pistachio orchards in arid and semi-arid conditions.

# **Conflict of interest**

Authors declare no conflict of interest.

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