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Abdelaziz BOUIZGAREN1*, Lhassane SIKAOUI1, Hakim BOULAL2, Abderraouf EL ANTARI1, Mohammed KARROU2, Vinay NANGIA3 and Theib OWEIS3
1Unit of Plant Breeding, National Institute for Agronomic Research (INRA), PO. Box 533, Gueliz 40000, Marrakesh, Morocco.
2International Center of Agricultural Research in Dry Area (ICARDA)
3International Center of Agricultural Research in Dry Area (ICARDA), Amman, Jordan
*Corresponding author, Email: bouizgaren_abdelaziz@yahoo.fr

Abstract
The scarcity of irrigation is the major constraint facing the olive production in Morocco. Under such conditions, deficit irrigation is one of the techniques leading to maximizing olive yields per unit of irrigation water. This work was conducted to investigate physiological responses, to deficit irrigation, of young olive trees (Olea europaea L.) cultivated under semi-arid conditions of Morocco. The experiment was carried out at the Experiment Station of INRA Marrakech. Three watering regimes were studied: FDI= Full drip irrigation (100% of ETc), DDI= Deficit drip irrigation (70% of ETc) and FU= Flood irrigation (farmer’s conventional technique). The physiological parameters, leaf relative water content (LRWC), Leaf chlorophyll content (LCC), total leaf mineral content (TLMC), leaf protein content (LProtC), leaf soluble sugar content (LSSC), leaf proline content (LProIC) and stomatal conductance were measured at three phenological stages: Flowering, fruiting and fruit set. The results showed that at the three phenological stages, the low values of LRWC, LCC, TLMC and stomatal conductance were recorded under FU regime. The High values of LProIC, LSSC, LProtC were obtained in recorded by Fun regime, suggesting that the young trees are more exposed to water stress under this irrigation technique despite the high amount of applied water. For most of those physiological parameters no significant differences were noted between FDI and DDI regimes. These latter regimes allowed, respectively, 30% and 80% of water saving compared to FU regime.

Key words: young olive trees, deficit irrigation, physiological parameters

Introduction
The scarcity of water resources in Morocco is of growing concern for the agriculture sector, particularly in semi-arid areas, because of the increasing population, high drought frequency and severity due to the effects of climate change. Moreover, these areas are characterized by high evaporative demand (about 1500 mm/ year), and low and irregular rainfall (200-300 mm/year). In this semi arid area, the new olive orchards are planted under irrigation to ensure of the supply of water required for growth and development of the crop. However, a large newly planted area is irrigated by a traditional inefficient surface irrigation system which leads to high water losses.

Under conditions of water scarcity and drought, deficit irrigation at selected phenological phases can lead to saving water and maximizing yields per unit of water for a given crop (Tognetti et al. 2006) without significant yield losses. Therefore, to understand how young olive trees respond to water restrictions and drought, on a study
was conducted on the physiological responses of this crop to deficit irrigation under semi arid conditions of Morocco.

Material and methods
The experiment was conducted in a three years old olive orchard (Olea europaea L. cv Menara) installed in 2010 at Sâada Experiment station of INRA, Morocco, located 9 km from Marrakech city (32° N, 8°08 W, 411 m altitude). The climate is a typical Mediterranean. Spring and summer seasons at the experimental site are normally characterized by severe drought stress associated with high temperatures (Table 1). The soil has a loamy clay texture and it is over 2 m deep.

Table 1: Mean climatic variables in the experimental year.

<table>
<thead>
<tr>
<th>Climatic variable</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall September–March (mm)</td>
<td>164.2</td>
</tr>
<tr>
<td>Rainfall April– August June (mm)</td>
<td>26.8</td>
</tr>
<tr>
<td>Mean daily min temp January–February (°C)</td>
<td>11.5</td>
</tr>
<tr>
<td>Mean daily max temp July–August (°C)</td>
<td>45.5</td>
</tr>
<tr>
<td>Total rainfall 12 months (mm)</td>
<td>191.0</td>
</tr>
<tr>
<td>Total 12 months ET₀ (mm)</td>
<td>1508</td>
</tr>
</tbody>
</table>

Three watering regimes were studied: FDI= Full drip irrigation (100% x ETc), DDI= Deficit drip irrigation (70% x ETc), and FU=Flood irrigation (farmer’s practice).

For drip irrigation treatments (FDI and DDI), the frequency of irrigation was two days; while it was around one month in the case of flood irrigation during summer.

The full drip irrigation regime received the crop water requirement (ETc). The ETc was calculated using the equation: ETc = ETo x Kc x Kr /Ne
Where ETo is the reference evapotranspiration, Kc is the crop coefficient for olive tree, Kr is the coefficient to correct for incomplete soil cover and Ne is the efficiency of irrigation network.

ET₀ was estimated using Penman-Monteith equation and daily meteorological data collected from an automatic weather station located 50 m away from the experiment plot. The Kc values used are the ones reported by Orgaz and Fereres (1997); while the value of Kr was estimated at 0.8 (Fereres and Golhamer, 1990).

The amounts of irrigation applied for the three watering regimes FDI, DDI and FU were 116 mm, 81.2 mm and 538 mm, respectively.

A randomized complete-block design was used with three replications of 21 trees each. The spacing between trees was 8 m x 8 m.

The physiological parameters were measured at flowering, fruit set and fruiting stages:

Leaf relative water content (LRWC): LRWC was defined as follows (Makela et al., 1998): LRWC(%)=((FW-DW)/(TW-DW)) x100. Where FW (fresh weight), TW (turgid weight) and DW (dry weight).

Leaf proline content (LProlC): is determined using the method described by Bates et al. (1973).

Leaf total Chlorophyll contents (LTCC): Chlorophyll concentration was measured by the method described by Arnon (1949).
Leaf protein content (LprotC): LProtC was determined using the method described by Bradford (1976).

Leaf soluble sugar content (LSSC): The content of soluble sugars was determined according to the method of Dubois et al. (1956).

Stomatal conductance: Stomatal conductance to H$_2$O (g mm s$^{-1}$) were measured in leaves, using leaf porometer (model sc-1).

**Statistical analysis**
Data were statistically analyzed using the analysis of variance and mean comparisons were carried out using the Student-Newman-Keuls test when significant treatment effects occurred.

**Results**
Leaf relative water content (LRWC): A significant variation was shown among the three studied irrigation regimes at flowering, fruiting, and fruit set stages (Figure 1). The high values of this parameter were obtained under FDI; but the difference with DDI was not statistically significant. The lowest values of LRWC were obtained under FU regime.

![Figure 1: LRWC of young olive trees under three irrigation regimes for the three phenological stages (Values with the same letter do not differ significantly (P > 0.05))](image)

Leaf proline content (LProlC): At the three phenological stages, the accumulation of proline in leaves varied significantly between the three irrigation regimes (Table 2). The FU regime induced high values of LPC which reached 21.1, 23.6 and 31.1 mg. g FW$^{-1}$ at flowering, fruit set and fruiting stages respectively. The FDI and DDI regimes did not differ significantly.

Leaf chlorophyll content (LTCC): The variation of the irrigation regimes induced a significant effect on LTCC of young olive trees (Table 2). The high values of this parameter were obtained under FDI regime with 22.7, 15.1 and 23.3 mg. ml$^{-1}$ at flowering, fruit set and fruiting stages, respectively that did not differ significantly with those obtained under DDI regime.

Leaf Proteins content (LProtC): Water regime affected significantly the LProtC of young olive trees at fruit set and fruiting stages (Table 2). The high accumulation of proteins in young olive leaves was noted under FU regime with 14.9, 23.3, and 54.3 mg. mg FW$^{-1}$ at flowering, fruit set and fruiting stages, respectively.
Soluble sugar (LSSC): A significant variation was shown among the irrigation regimes for the LSSC at the three phenological stages (table 2). Within the same phenological stage, the low and high values were achieved under FDI and FU regimes, respectively. At fruiting stage, the LSSC varied between 289.2 and 350.1 mg. g FW\(^{-1}\) for FDI and FU regimes, respectively, and we did not observe any significant difference between DDI and FDI regimes.

Table 2: Physiological parameters of young olive trees measured at three phenological stages and under three different water regimes.

<table>
<thead>
<tr>
<th>Phenological stages</th>
<th>Irrigation regimes</th>
<th>LProC (mg. g FW(^{-1}))</th>
<th>LTCC (mg. ml(^{-1}))</th>
<th>LProtC (mg. mg FW(^{-1}))</th>
<th>LSSC (mg. g FW(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering</td>
<td>FDI</td>
<td>15.4 a</td>
<td>22.7 a</td>
<td>14.2 a</td>
<td>160.6 a</td>
</tr>
<tr>
<td></td>
<td>DDI</td>
<td>17.2 a</td>
<td>22.4 a</td>
<td>14.8 a</td>
<td>174.4 ab</td>
</tr>
<tr>
<td></td>
<td>FU</td>
<td>21.1 b</td>
<td>16.4 b</td>
<td>14.9 a</td>
<td>185.3 b</td>
</tr>
<tr>
<td></td>
<td>(P (\alpha=5%))</td>
<td>0.018</td>
<td>(&lt;0.001)</td>
<td>0.402</td>
<td>0.023</td>
</tr>
<tr>
<td>Fruit set</td>
<td>FDI</td>
<td>20.1 a</td>
<td>15.1 a</td>
<td>18.6 a</td>
<td>162.5 a</td>
</tr>
<tr>
<td></td>
<td>DDI</td>
<td>20.3 a</td>
<td>14.6 a</td>
<td>18.7 a</td>
<td>195.5 b</td>
</tr>
<tr>
<td></td>
<td>FU</td>
<td>23.6 b</td>
<td>13.1 b</td>
<td>23.3 b</td>
<td>242.5 c</td>
</tr>
<tr>
<td></td>
<td>(P (\alpha=5%))</td>
<td>0.03</td>
<td>0.045</td>
<td>0.013</td>
<td>0.004</td>
</tr>
<tr>
<td>Fruiting</td>
<td>FDI</td>
<td>12.3 a</td>
<td>23.3 a</td>
<td>37.2 a</td>
<td>289.2 a</td>
</tr>
<tr>
<td></td>
<td>DDI</td>
<td>16.3 a</td>
<td>21.1 a</td>
<td>41.4 a</td>
<td>290.9 a</td>
</tr>
<tr>
<td></td>
<td>FU</td>
<td>31.1 b</td>
<td>18.2 b</td>
<td>54.3 b</td>
<td>350.1 b</td>
</tr>
<tr>
<td></td>
<td>(P (\alpha=5%))</td>
<td>0.015</td>
<td>0.015</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values with the same letter do not differ significantly between the irrigation regimes for a given phenological stage (\(P>0.05\)).

Stomatal conductance: The average values of stomatal conductance measured at flowering, fruit set, and fruiting stages are shown in figure 2. Significant variations were recorded among the three irrigation regimes during the three phenological stages. The high and low values were recorded under FDI and FU regimes, respectively.

Figure 2: Stomatal conductance of young olive trees in relation with irrigation regimes

Total mineral leaves content (TMLC): The total mineral content in leaves of the young olive trees measured at fruiting stage varied significantly with the variation of the irrigation regimes (Figure 3). The highest mean values of TMLC were observed under full drip irrigation, while the lowest were obtained under flood irrigation.
Discussion

In the semi arid area where the experiment was carried out proper management of irrigation water, such as the application of deficit irrigation, increases water use efficiency and decreases water consumption. However, water stress causes changes in a number of physiological and biochemical processes governing plant growth and productivity (Alexieva et al., 2001).

Our results showed that at the three phenological stages, the LRWC depended on the irrigation regime. The lowest values of this parameter were recorded under FU regime, because the low frequency of irrigation with high amounts of water in summer under high temperatures certainly increased the loss of water by percolation and evaporation and created the conditions of water deficit in the root zone.

The stomatal conductance was negatively affected by FU regime. Fernández (1997) showed that the olive tree is able to restrict water loss by closing its stomata. DDI induced a stomatal close in comparison with FDI. This is in relation with the soil water potential which controlled significantly stomatal conductance (Lu and Zhang 1999).

We noted that FU regime induced a significant increase in accumulation of proline and sugar in leaves. The proline accumulation is frequently observed during drought stress and is thought to play a multifunctional role in defence mechanisms (Boussadia et al., 2013). Sugars are also among the important osmolytes contributing to the osmotic adjustment of plants under water or saline stress (Ashraf and Harris, 2004).

A significant decrease in LCC was shown under FU regime in comparison with drip irrigation regimes. In general, this decrease is related to water deficit which occurred under flood irrigation and caused a decrease in the concentration of chlorophyll pigments in the leaves of young olive trees. This phenomenon can be explained either by the degradation of the pigments by the hydrolytic enzymes, or by the inactivation of the biosynthesis of these pigments (Farooq et al., 2009).

We noted an increase in proteins accumulation under FU regime at fruit set and fruiting stages. Galau and closed (1992) reported that water deficit induced an increase in proteins content in olive trees by an increase of nitrogen (Garg, 2003). This induction of proteins by water deficit depends on the stage and the genotype of the tree (Riccardi et al., 1998).

The total mineral content in leaves of the young olive trees varied significantly with irrigation regimes and the highest values were noted under FDI, while the lowest were obtained under FU regime which induce a limitation in nutrient uptake and decreasing their concentrations in leaves tissue. Similar results were reported by Mehanna et al., (2012) when the applied water stress on olive orchard.

We conclude that deficit drip irrigation did not induce a significant effect on all physiological parameters measured in comparison with full drip irrigation. It allowed...
30% of water saving compared to full drip irrigation and 80% of water saving compared to flood irrigation techniques.

References