

Effect of three water treatments on water status and biomass of olive tree (*Olea europaea* L cv Meski)



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Abstract - The objective was to evaluate the effect of three water treatments on biomass by monitoring water status as an indicator of stress. The study consists of applying three water treatments (100, 50 and 25% field capacity (FC)) on one-year-old olive plants (*Olea europaea* L. cv Meski) during 42 days in a greenhouse under semi-controlled conditions. The evaluation of olive tree response was carried out by monitoring water and osmotic potentials, osmotic adjustment, relative water content and accumulation of dry matter.

The water potential decreased by 16 and 28% compared to control plants (T100%) at the end of the experiment, respectively for T50% and T25% plants. The osmotic potential decreased by 50 and 64% compared to control plants respectively for T50% and T25% plants to reach -4.54 and -4.98 MPa due to regression of water stock. As a result, the osmotic adjustment increased to 1.51 and 1.95 MPa respectively for T50% and T25% plants at the end of the experiment. Olive tree decreases its water and osmotic potentials and the osmotic adjustment would be more important according to the progression of the degree of stress. T50% and T25% plants had a passive osmotic adjustment of 79 and 89% respectively. Control plants accumulated more dry matter in the roots and stem, while the root/ shoot ratio seemed indifferent to water stress. The water restriction has affected root and stem dry matter in favor of leaves, the first operator of photosynthetic activity.

These results indicate that a water restriction for Meski cultivar implies a specific adaptation characterized by a reduction in osmotic potential involving an increase in osmotic adjustment and a shift in the distribution of dry matter maintaining the leaves dry matter of stressed plants similar to those of control plants at the expense of roots and stem to ensure photosynthetic activity.

Keywords: *Olea europaea* L., Water stress, Leaf water potential, Leaf osmotic potential, Relative water content, Root/shoot ratio.

1. Introduction

Tunisia is a southern Mediterranean country; it is characterized by a spatio-temporal variability of rainfall with a marked contrast of hydro-climatic conditions: a humid climate in the North West to a structural deficit in the Southeast. Aware of these issues, Tunisia began an extensive program of mobilization of water resources, with a partial interconnection of hydraulic structures, in order to satisfy a growing water demand for the demographic development.

Tunisia also has the reputation of being prone to very high erosion risk. Indeed, water erosion is a complex phenomenon threatening water and soil resources of the country. The physical and climatic characteristics of Tunisia are favourable for triggering the water erosion phenomenon. On the other hand, water erosion in Tunisia has been accelerated by socio-economic conditions by crops subsidies agriculture manifested by a great change in the land cover.

Land cover change seriously affects water resources. As the most direct expression of the interaction between human activities and the natural environment, land cover affects the condition of water resources and agricultural economic growth, thereby affecting the process of watershed hydrology and water resource cycles.



Recently, SWAT model has been applied to study climate change scenarios, for vulnerable situation of water availability, specifically, in the regions where more prolonged droughts and more frequent and more intensive floods will occur. In this study the impacts of climate change on stream flow and water resources management using data from the Rmel watershed which is located in the north-eastern of Tunisia to test the effectiveness of the proposed methodology above using downscaled GCM climate predictors in the SWAT hydrological model for water resources predictions, taking into account the evolution of land cover scenarios. Thus, the objective of this study is to improve the efficiency of future water assessment in a climate change context. The output of this study can then be used as an input for further decision support for water resources planning in the watershed

2. Material and methods

2.1. Plant material

One-year-old olive trees (*Olea europaea* L. cv Meski) were grown in 1.6 L plastic pots in a greenhouse at the Tunisian Olive tree Institute (Tunisia, 35 49'N, 10 38'E) under normal day-light conditions with an average temperature of 25° C and relative humidity of 40%. Prior to the start of the experiment, trees with a height of about 1 m were selected and lifted from a soil mix of organic material, sand and clay. Roots were washed and plants were transplanted into a substrate mixture of peat and perlite (2/3 volume ratio). A full-strength before starting, the plants were watered daily to 100% of Field Capacity (FC) for a period of 4 weeks with a Hoagland solution. Then, plants were divided into two parts the first one (control plants) was subjected to irrigation to 100% of FC and the second one to drying until reaching 50% of FC.

2.2. Experimental design

The experiment was conducted from March, 18th to April, 29th 2013. Three water treatments were applied:

- T100%: control treatment: Daily irrigation at 100% of Field Capacity (FC),
- T50%: Daily irrigation at 50% of FC,
- T25%: Daily irrigation at 25% of FC.

Control and drought-stressed plants were arranged in a complete randomized design with six replications for each water treatment. In total, 18 olive plants were used.

2.3. Parameters of control

2.3.1. Leaf water status

Plant water status was determined by measuring the total leaf water potential (Ψ w) and the osmotic potential (Ψ \pi) on fully expanded sunlit leaves (taken from the mid-section of the shoots). Leaf water potential measured at midday is the most widely accepted indicator and it has been proposed as a suitable approach to determine plant water status for irrigation scheduling of the majority of the fruit trees (Fereres and Goldhamer 2003; Moriana et al. 2012). Measurements require little training and are easy to perform. The technique, however, is destructive, slow, labour intensive, discontinuous and unsuitable for automation.

Three plants per treatment were measured at predawn (6 am) (only for the last measurement date) and at midday (12 am) with a thermocouple psychrometer (sample chambers type C52; Wescor, Logan, Utah, USA) following the method described by Chazen et al. (1995). For each plant and at each measurement event, two leaf disc samples were taken. One sample (surface area of the leaf disc = 0.25 cm²) was used to measure Ψ w, whereas the second one, taken from the same leaf, was protected in aluminum foil and kept in a freezer at -20°C for 24 h in order to disrupt the cell membranes. After thawing, the second leaf disc was used to measure $\Psi\pi$. Osmotic adjustment (OA) was calculated by taking the difference between midday $\Psi\pi$ measured at control (T100%) and stressed (T50% and T0%) plants.

Active osmotic adjustment (AOA) was defined as the difference between $\Psi\pi$ at full turgor measured at predawn for the 100% FC treatment ($\Psi\pi$ 100) and the two other treatments T50% and T25% ($\Psi\pi$ 100(T50% and T25%)) plants.

The contribution of passive osmotic adjustment (POA) to OA via the loss of symplastic water was determined as:

$$POA = OA - AOA$$



The osmotic adjustment and its compounds were taken in the end of the experiment (April, 29th 2013), in three replicates for each water treatment.

2.3.2. Relative Water Content (RWC)

Leaf Relative Water Content (RWC) was determined at midday on fully expanded leaves of similar age. Three replicates per treatment were applied and values of RWC were calculated by the following equation:

RWC = [(FW - DW)/(TW - DW)]*100

where FW, DW, and TW are fresh weight, dry weight and turgid weight (g), respectively. DW was determined after drying the leaf sample at 80°C for 24 h. For TW determination, leaves were rehydrated by immersing the petiole in distilled water in a beaker sealed with parafilm. Full rehydration was achieved after 24 h in complete darkness at 4°C.

2.3.3. Dry matter accumulation measurements

At the end of the water stress application (April , 29th 2013), Plants destruction was done in order to obtain fresh and dry biomass and to determine the dry weight body (leaves, root and stem).

3. Results and discussion

3.1. Effect of water treatments on water status

3.1.1. Effect on leaf water potential

Figure 1 shows the effect of three water treatments on leaf water potential of olive tree (*Olea europaea* L. cv Meski) during 42 days after applying water treatments. The results showed that T100% plants had the highest leaf water potential while T25% plants the lowest one throughout the experiment period. The T100% plants had a relatively constant leaf water potential during the experiment period, compared to the two water treatments T50% and T25%. The leaf water potential of these two water treatments (T50% and T25%) decreased progressively throughout the experiment period to be less than the control treatment (T100%) by 16 and 28%, 42 days after applying water treatments. This decrease can be explained by a regression of the water stock. Our results are similar with those obtained by Giron et al. (2015) despite that the experiment was conducted in field conditions and showed lower values mainly in the middle of the water stress period (from DOY 207 until 220), though values on the stress treatment tended to be lower throughout the water stress period. According to Marino et al. (2014), under severe drought, the leaf water potential ranged between \sim -4.5 MPa (predawn) and \sim -6.4 MPa (midday). Braham (1997) mentioned that other factors could modify the leaf water potential especially climatic conditions such as temperature, air humidity and solar radiation.



Figure 1. Leaf water potential (Ψ w; - MPa) of olive tree (*Olea europaea* L. cv Meski) under three water treatments during 42 days after applying water treatments. Values with different letters are significantly different at P < 0.05 (means of three replicates) according to SNK test; error bars = SD.



3.1.2. Effect on leaf osmotic potential

Figure 2 shows the effect of three water treatments on leaf osmotic potential of olive tree (*Olea europaea* L. cv Meski) during 42 days after applying water treatments. The leaf osmotic potential of T100% plants ranged between - 2.4 and -3.03 MPa from the 7th to the 42nd day after applying water treatments while it decreases from -2.84 and -2.91MPa, 7 days after applying water treatments, to -4.54 and - 4.98 MPa, 42 days after applying water treatments, respectively for T50% and T25% plants. These results indicated that, the cultivar Meski decreased its leaf osmotic potential throughout the experiment for plants receiving less water (50% and 25% FC) than control plants (100% FC). At the end of the experiment, the leaf osmotic potential of T50% and T25% plants decreased by 50 and 64% respectively, compared to control plants (T100%). The ability of olive tissues to lose water in transpiration stream causes an increment of cell solute concentration and, hence, osmotic potential decline with increasing drought stress (Chartzoulakis et al., 1999). This accumulation of compatible solutes in olive trees allows maintenance of the cell turgor and, thus, the opening of the stomata during periods of drought (Chartzoulakis et al., 1999; Fernandez, 2014).



Figure 2. Leaf osmotic potential ($\Psi\pi$; - MPa) of olive tree (*Olea europaea* L. cv Meski) under three water treatments during 42 days after applying water treatments. Values with different letters are significantly different at P < 0.05 (means of three replicates) according to SNK test; error bars = SD.

3.1.3. Effect on osmotic adjustment and components

Figure 3 shows the effect of water treatments on leaf osmotic potential of olive tree (*Olea europaea* L. cv Meski) during 42 days after applying water treatments. T25% plants showed a higher osmotic adjustment compared to T25% plants during the whole period of the experiment. In addition, plants of both water treatments T50% and T25% showed a progressive increase of their leaf osmotic adjustment throughout the experiment period. In fact, the leaf osmotic potential increased from 0.44 and 0.51 MPa, 7 days after applying water treatments to 1.51 and 1.95 MPa, 42 days after applying water treatments, for T50% and T25% plants respectively. Our results confirm those of Chaves et al. (2003) which indicated that the ability of olive trees to reach extremely negative osmotic potential values is partially due to osmotic adjustment that allows plants to tolerate temporary or prolonged periods of water shortage and is one of the crucial processes involved in plant adaptation to drought. According to Dichio et al. (2003), osmotic adjustment is one of the early mechanisms of trees in respond to water stress (i.e. in olive trees). Giron et al. (2015) showed that the physiological response of the trees (osmotic adjustment and trunk dehydration) compensated the decrease in water potential.





Figure 3. Leaf osmotic adjustment (OA; MPa) of olive tree (*Olea europaea* L. cv Meski) during 42 days after applying water treatments. Values with different letters are significantly different at P < 0.05 (means of three replicates) according to SNK test; error bars = SD.

Figure 4 illustrates the effect of water treatments on leaf osmotic adjustment components of olive tree (*Olea europaea* L. cv Meski), 42 days after applying water treatments. The results showed that plants of both water treatments (T50% and T25%) had a passive osmotic adjustment at 79 and 89%, respectively. This passive osmotic adjustment is not significant however the active osmotic adjustment is significant between the two water treatments. These results do not confirm those of Boussadia (2009) showing an active adaptation strategy. This difference could be due to the water restriction application method.



Figure 4. Leaf osmotic adjustment components (OA, AOA, POA; MPa) of olive tree (*Olea europaea* L. cv Meski) 42 days after applying water treatments. Values with different letters are significantly different at P < 0.05(means of three replicates) according to SNK test; error bars = SD.



3.1.4. Effect on Relative Water Content (RWC)

Figure 5 shows the effect of water treatments on leaf relative water content of olive tree (*Olea europaea* L. cv Meski) during 42 days after applying water treatments. Control plants showed relatively stable relative water content compared to T50% and T25% plants throughout the experiment period due to the daily irrigation at 100% of field capacity. The RWC decreased from 86 and 81%, at the beginning of the experiment to 75 and 82%, at the end of the experiment for T50% and T25% plants, respectively. After 21 days of applying water treatments, the RWC was 84 and 78% for T50% and T25% plants, respectively, which indicated a significant regression by 6.7 and 13.3% compared to control plants. One week after (28 days after applying water treatments), the RWC of T50% and T25% plants showed a significant decrease by 8.8 and 17.6% respectively. Our results confirm those of Boussadia (2009).



Figure 5. Leaf relative water content (RWC; %) of olive tree (*Olea europaea* L. cv Meski) during 42 days after applying water treatments. Values with different letters are significantly different at P < 0.05 (means of three replicates) according to SNK test; error bars = SD.

3.2. Effect of water treatments on plant dry matter accumulation

Table 1 shows the effect of three water treatments on the root, stem and leaves dry weight and root/shoot ratio of olive tree (Olea europaea L. cv Meski), 42 days after applying water treatments. No significant difference was mentioned between the three water treatments on leaves dry weight however, T100% plants showed the highest roots and stem dry weights compared to the two other water treatments. The water restriction has affected root and stem dry matter in favor of leaves, the first operator of photosynthetic activity. This result was statistically significant. In general, for the root/shoot ratio, no significant difference was determined between the three water treatments. Di Vaio et al. (2013) mentioned that, under different water regimes applied on young pot-grown olive trees, the dry matter was affected by the water regime and cultivar. Indeed, the cv Leccino, full irrigated, displayed a greater accumulation of total dry matter and fruit dry matter, while these two parameters were greatly reduced under the other water regimes (T50 and T25). By contrast, the cv Racioppella always showed a lower accumulation of dry matter and a more balanced canopy/root ratio. Nevertheless, water stress is not the only parameter that affects dry matter distribution in the plant; in particular, many agronomic and genetic factors, such as rootstock, cultivar, training system, pruning and planting density have been shown to influence growth and to modify the distribution of biomass between roots and canopy (canopy/roots ratio) and between the different plant organs (Caruso et al., 1997, 2001, 2008; Zucconi, 1992; Xiloyannis et al., 2007; Di Vaio et al., 2012; Weibel and Reighard, 2012; Yano et al., 2002).



Table 1. Root, stem and leaves dry weight (g/plant) and root/shoot ratio of olive tree (*Olea europaea* L. cv Meski) under three water treatments, 42 days after applying water treatments.

	Roots Dry Weight (g/plant)	Stem Dry Weight (g/plant)	Leaves Dry Weight (g/plant)	Root/shoot ratio
T100%	8.36*	12.62*	12.27	0.28
Т50%	4.05	7.17	8.87	0.26
T25%	3.91	6.63	9.08	0.25
* significant	at $P < 0.05$			

4. Conclusion

Cultivar Meski showed a good adaptation to water restriction by decreasing its leaf water and osmotic potentials to ensure a progressive increase of osmotic adjustment which is one of the early mechanisms of trees in respond to water stress. In addition, at the 42^{nd} day after applying water treatments, the osmotic adjustment of the two water treatments T50% and T25% was passive rather than active. The leaf relative water content of stressed plants decreased throughout the experiment due to water shortage. Also, a water restriction affected the root and stem dry matter in favor of leaves, the first operator of photosynthetic activity and consequently the root/shoot ratio was not affected.

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