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Research Article

Effect of Water Stress on Photosynthetic Assimilation and Biomass Accumulation in Olive Tree

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Abstract

The climate change that we are attending in recent years requires a revision of the water resources management in order to ensure a sustainable supply. For this reason, in order to test the plants ability to face water stress, three water stress intensities (100% (T0), 50 (T1) and 25% (T2) field capacity (fc)), for 42 days in a greenhouse under semi-controlled conditions, were applied on 18 olive plants (*Olea europaea* L. cv 'Meski') of one year old. An evaluation of the response of the olive tree at different water supplies was conducted by monitoring the photosynthetic assimilation, chlorophyll index measured by SPAD, plant growth and dry matter accumulation. The results showed that photosynthesis has increased after 42 days for T0 plants while it increased during the first two weeks before dropping to the 42th day of the application of water restriction (AWR) for T1 plants; a decrease of 26% compared to control treatment. The photosynthetic assimilation of T2 plants has increased at a rate slower until the 28th day AWR then it has dropped after 42 days a decrease of 46% from the witness thus increasing the irrigation dose results in improved photosynthesis. The chlorophyll index fell 2, and 7% compared to the control treatment for T1 and T2, respectively, after 42 days AWR showing that fluid restriction affects the integrity of chlorophylls. Plant growth was shown by the development of a longer root for T2 plants explained by the search for minimum quantities of water available. The results showed that the control plants have accumulated more dry matter than stressed plants in the roots and the stem while the underground part/aerial part report seems indifferent to water stress. Water restriction has affected thereby biomass of roots and stems in favor of the leaves, the first operator of the photosynthetic activity.

Keywords: Olea europaea L.; Water Stress; Photosynthetic Assimilation; Dry Matter Accumulation; Chlorophyll Index

Introduction

The Mediterranean climate is characterized by hot and dry summers, leading to a seasonally recurring drought stress [1] that strongly limits plant photosynthetic productivity [2,3].

Water is the most limiting resource in the Mediterranean region, where the climate is typically characterized by high potential evaporation and low and highly variable rainfall during the growing season. The agricultural sector is the largest water consumer accounting for about 70% of all extracted water [4].

Olive has become a major crop in wide arid and semi-arid areas due to both its capacity to grow and produce acceptable yields under harsh environmental conditions and the demand for olive products, especially olive oil, which is considered by an increasing number of consumers as a key ingredient for a healthy diet. In addition, olive has shown a marked response to improved crop management practices. Both circumstances explain the substantial increase, since the 1980s, in the number of research groups focused on understanding the biology of this species and its response to the environment, as well as on using the acquired knowledge to improve crop management practices and to design new cropping systems for more sustainable olive orchards. As a consequence, a substantial amount of information on olive biology and olive growing has been published in the last decades. Main findings have been summarized in comprehensive reviews on biology and physiology [5], response to environmental stimuli [6] and water use and irrigation [7-9].

Materials and Methods

Plant material and experimental design

18 olive plants (*Olea europaea* cv. 'Meski') of one year old were grown in 1.6L plastic pots in a greenhouse at the Tunisian Olive Tree Institute (Tunisia, 35 49′N, 10 38′E) under normal daylight conditions. Prior to the start of the experiment, trees 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 perlite and peat (1/3 and 1/3 volume ratio respectively). Trees were watered daily to field capacity for a period of 4weeks with a full-strength Hoagland solution. Plants were subjected to drought stress from 18 March 2013 to 29 April 2013. Three drought stress levels were considered that are: T0 control treatment: irrigation at 100% of field capacity (FC); T1: 50% FC and T2: 25% FC. During the drought stress experiment the mean day and night temperature was 25°C and 18°C and the mean air humidity was 40%. Control and drought-stressed trees were arranged in a complete randomized design with six replications.

Gas exchange measurements

Fully expanded leaves were used to measure simultaneously maximum net photosynthetic assimilation rate Amax (µmol CO_2 m^{-2} s^{-1}), stomatal conductance for water vapour gs (mol H_2O m^{-2} s^{-1}) and transpiration E (mmol H_2O. m^{-2} s^{-1}) using a portable gas exchange system (LI-6400, LI-COR, Lincoln, NE, USA). Measurements were performed weekly in 3 replicates for each treatment. Measurements of Amax and gs were performed at light saturation (1500 µmol PAR m^{-2} s^{-1}) at midday and at a fixed CO_2 concentration (Ca of 400 µmol mol^{-1}), leaf temperature (25°C) and relative humidity (50%).

The A_{max}/gs ratio was calculated and used as an estimate of the intrinsic water use efficiency (WUE) according to Mediavilla., *et al.* (2002). Also, The A_{max} /E ratio was calculated as the extrinsic water use efficiency.

Relative water content (RWC)

Relative leaf 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 24h. For TW determination, leaves were rehydrated by immersing the petiole in distilled water in a beaker sealed with parafilm. Full rehydration was achieved after 24h in complete darkness at 4°C.

Chlorophyll index measurements by non-destructive SPAD-502 method

The SPAD-502 chlorophyll meter (Minolta, Spectrum Technologies, IL, USA) is an example of non-destructive measurement based on the amount of chlorophyll present in a leaf. This instrument measures the leaf transmittance in the red (wavelength of maximum amplitude: approximately 650 nm) and the near-infrared (wavelength of maximum amplitude: approximately 940 nm). It calculates the ratio of transmittance and converts it into a SPAD value, corresponding to the chlorophyll content of the leaf. The measurement time is very fast, about three seconds per measurement.

Biomass measurements

At the end of the application of water stress (April, 29^{th} 2013), destruction of plants to obtain fresh and dry biomass and to determine the dry weight body (leaves, root and stem) were done.

Results and Discussion

Gas exchange

Photosynthesis

Figure 1 shows photosynthetic assimilation rates (A; µmol CO_2 m^{-2} s^{-1}) of olive tree leaves (*Olea europaea* L. cv Meski) under three water regimes. For control plants (irrigation at 100% of FC), photosynthesis rates have increased, compared to the experiment starting, to reach 14,5 µmol CO_2 m^{-2} s^{-1} 42 days after applying water stress. T1 plants have shown a rapid increase 14 days after applying the water stress (13,25 µmol CO_2 m^{-2} s^{-1}) then rates tumbled progressively to attain 10,71 µmol CO_2 m^{-2} s^{-1} at the end of the experiment, that means a depletion of 26.13% compared to control plants. For stressed plants, photosynthesis rate has increased in an un-accelerated way to attain 8,98 µmol CO_2 m^{-2} s^{-1} after 28 days of applying the water stress and then it declined to 7.73 µmol CO_2 m^{-2} s^{-1}, 42 days after applying the water stress. For stressed plants, this results show a decline of 46.68% compared to control plants. A significant difference was shown between the three water treatments throughout the experiment period demonstrating that the increase in the irrigation dose results in an improvement in photosynthesis. The water supply is not the only survival factor for plants. Indeed, the maximum photosynthetic activity is dependent on other environmental factors such as temperature, intensity of solar radiation and humidity [10].

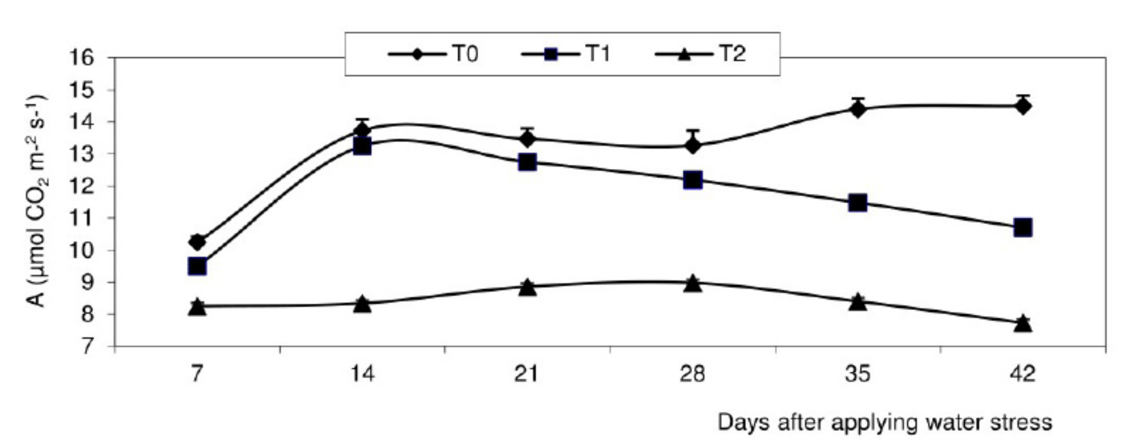


Figure 1: Evolution of photosynthesis (A; µmol CO_2 m^{-2} s^{-1}) from the leaves of *Olea europaea* L. cv Meski subjected to three water regimes. Each value represents the mean ± standard deviation of three measurements.

Stomatal conductance

Figure 2 shows stomatal conductance (gs; mol H_2O m^{-2} s^{-1}) of olive tree leaves (*Olea europaea* L. cv Meski) under three water regimes. An increase in stomatal conductance values has been indicated for the three water treatments depending on the water stress period progression. 42 days after applying the water stress, there was a decrease by 6 and 51.85% for T1 and T2 plants respectively, compared to control treatments (T0). Statistical analysis showed that the treatments T0 and T1 have no significant difference between them, but they differ significantly in the processing T2 except for the 35th day after the application of stress. These results do not coincide with those mentioned by Boussadia [11], Boujnah [12] and Braham [13] that mention an important and early elevation of stomatal resistance.

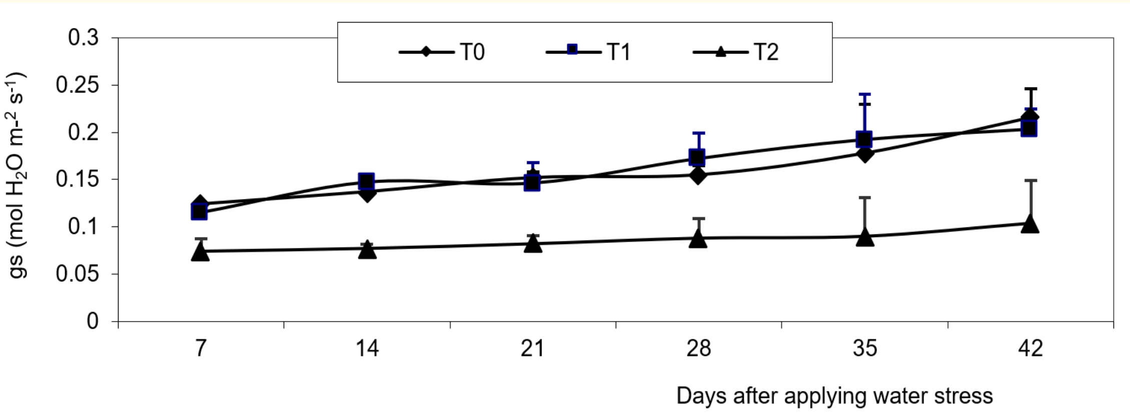


Figure 2: Evolution of stomatal conductance (gs; H20 mol m-2s-1) from the leaves of Olea europaea L. cv Meski subjected to three water regimes. Each value represents the mean ± standard deviation of three measurements.

Transpiration

Figure 3 shows transpiration (E; mol H_20 m^{-2} s^{-1}) of olive tree leaves (*Olea europaea* L. cv Meski) under three water regimes. The T0 treatment plants have the highest transpiration while those in the treatment T2 have the lowest one. T1 treatment plants have shown a significant decrease of 42% at the end of the experiment. 28 days after applying the water stress, control plants have shown a significant difference compared to stressed plants (T2) resulting in a decrease of 47.21% that was maintained until the end of the experiment. Statistical analysis showed that the treatments T0 and T1 does not significantly differ from each other but differ with the T2 treatment for the first measurement date. These results confirm those found by Choné., *et al*. [14] showing that the transpiration decreases depending on the degree of stress and increases with fluid intake [12].

Relative Water Content (RWC)

Figure 4 shows the Relative Water Content (RWC, %) evolution of olive tree leaves (*Olea europaea* L. cv Meski) under three water regimes. The control plants leaves (T0) showed a stability depending on the progression of the stress period relative to those of treatments T1 and T2 where the RWC had a decreased from 86 to 81% and 75 to 82% respectively for T1 and T2 at the end of the experiment. After 21 days from the application of stress, the RWC were 84 and 78% for T1 and T2 respectively so a significant reduction of 6.66 and 13.33% compared to control plants. After 28 days from the application of stress, a significant decrease was also recorded in the order of 8.79 and 17.58% for T1 and T2 respectively. These results were confirmed by Boussadia [11].

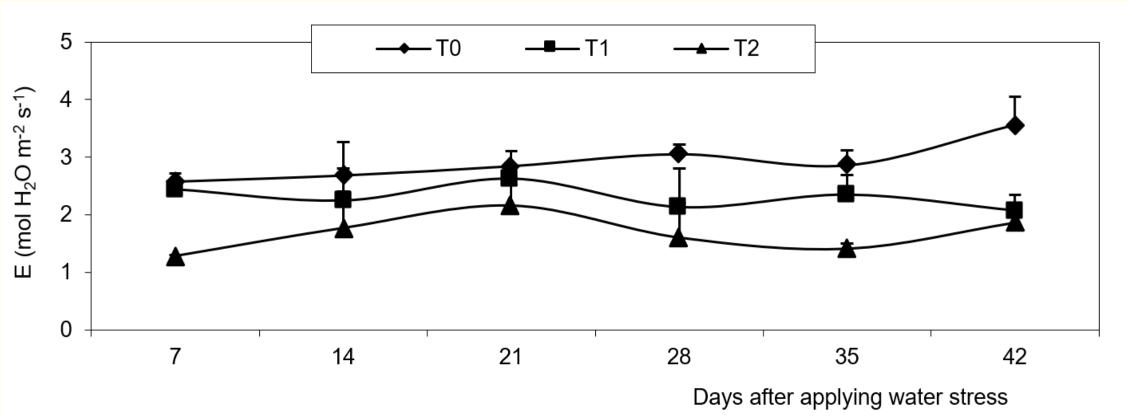
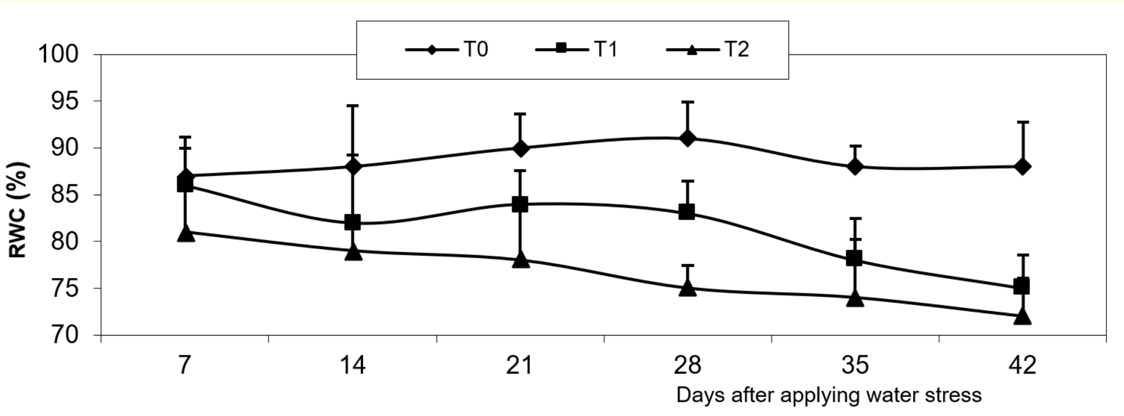


Figure 3: Evolution of transpiration rate (E; H_20 mol m^{-2} s^{-1}) of olive tree leaves (*Olea europaea* L. cv Meski) subjected to three water regimes. Each value represents the mean ± standard deviation of three measurements.



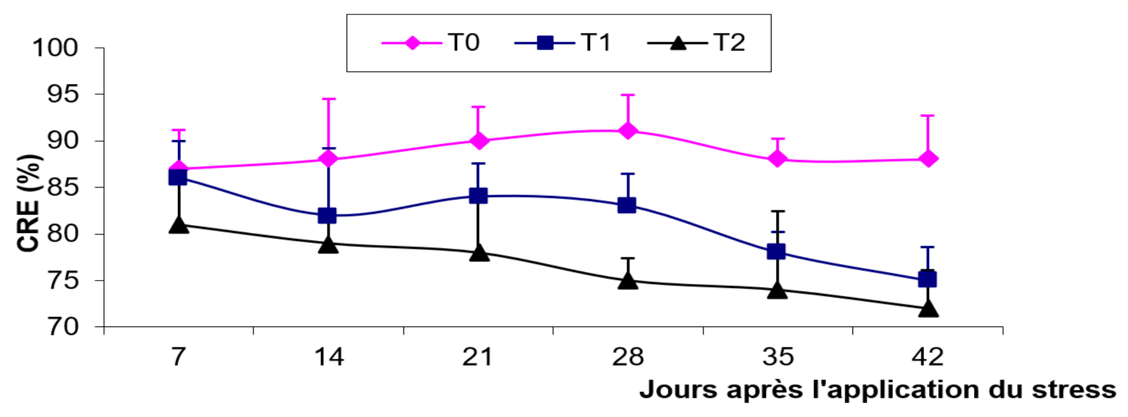


Figure 4: RWC (%) evolution of olive tree leaves (*Olea europaea* L. cv Meski) subjected to three water regimes. Each value represents the mean ± standard deviation of three measurements.

Chlorophyll index measured by non-destructive SPAD-502 method

Figure 5 shows the chlorophyll index evolution of olive tree leaves (*Olea europaea* L. cv Meski) under three water regimes. In the first two weeks, there were not any significant difference between the three water treatments but starting from the 14th after applying water stress, a significant difference was found between the control treatment and the other two treatments T1 and T2 where they showed a decrease of 7.83 and 9.47% respectively compared to the control. This significant difference was maintained until the end of the experiment on which T1 and T2 treatments decreased 2.11 and 7% compared to the control (T0) on the 42nd day of stress.

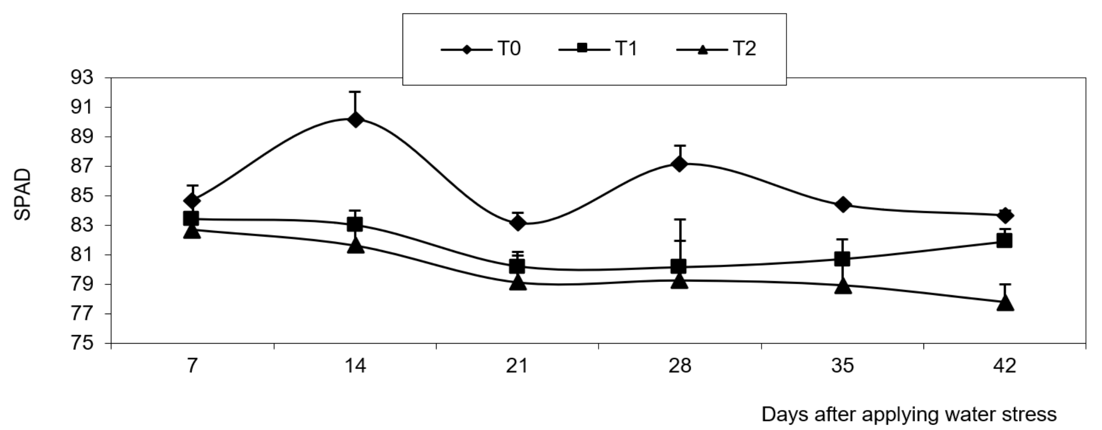


Figure 5: Chlorophyll index evolution of olive tree leaves (Olea europaea L. cv Meski) under three water regimes. Each value represents the mean ± standard deviation of six measurements.

Water restriction seems to affect the integrity of chlorophylls measured by the index of SPAD. Guerfel., *et al*. [15] confirms that water stress reduces the chlorophyll concentration predicted in our case by the SPAD index.

Plant growth and dry matter accumulation

Plant growth Plant growth

Table 1 shows the effects of water treatment on the length of roots and stem measured 42 days after applying water stress. The more stressed plants (T2) show a significant difference in root length compared to the other plants. This significant difference could be explained by the development of a longer root in search of minimal amounts of water available. For the stem, there is no significant difference between treatments. Water restriction has positively influenced root growth despite the vegetative growth.

	Stem length (cm)	Root length (cm)
T0	55,83 ± 8,89	21,17 ± 1,04
T1	45,33 ± 14,44	31,4 ± 2,42
T2	44,1 ± 4,38	36,25 ± 3,18

Table 1: Effects of water treatment on the length of roots and stem measured 42 days after applying water stress. Each value represents the mean ± standard deviation of five measurements.

Dry matter accumulation

Table 2 show the effect of water treatment on dry weight of roots, stem and leaves and the ratio root part/aerial part 42 days after applying stress. For the leaves dry weight, no significant difference has been observed between the three water treatments. However, for the roots and stem dry weight, a significant difference is proven where control plants show a significant difference compared to T1 and T2 plants. In general, there are no significant differences between treatments for the root part/aerial part ratio.

	Roots dry weight (g/plant)	Stem dry weight (g/plant)	Leaves dry weight (g/plant)	Root part/aerial part ratio
T0	8,36 ± 1,82[a]	12,62 ± 2,1[a]	12,27 ± 1,64[a]	0,28 ± 0,09[a]
T1	4,05 ± 1,13[b]	7,17 ± 2,59[b]	8,87 ± 2,49[a]	0,26 ± 0,04[a]
T2	3,91 ± 0,81[b]	6,63 ± 0,77[b]	9,08 ± 2,77[a]	0,25 ± 0,02[a]

Table 2: Effect of water treatment on dry weight of roots, stem and leaves and the ratio root part/aerial part 42 days after applying stress. Each value represents the mean ± standard deviation of five measurements.

Water restriction affected the biomass of roots and stems in favor of leaves, the first operator of the photosynthetic activity.

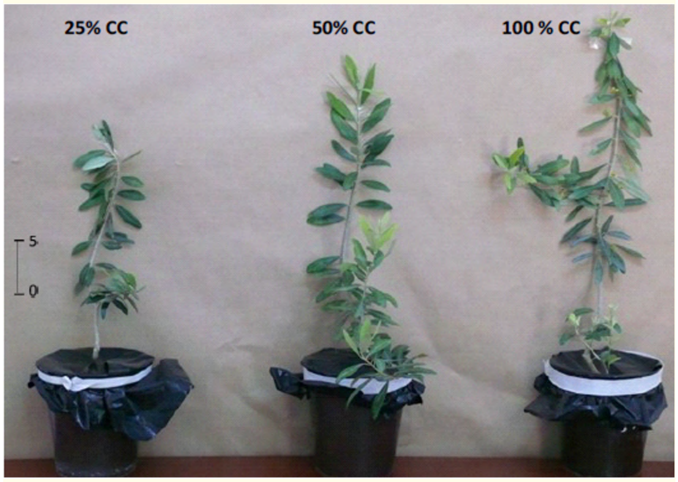


Figure 6: Effects of water treatments on stem length of olive plants (Olea europaea L. cv Meski) 42 days after application of the water stress.

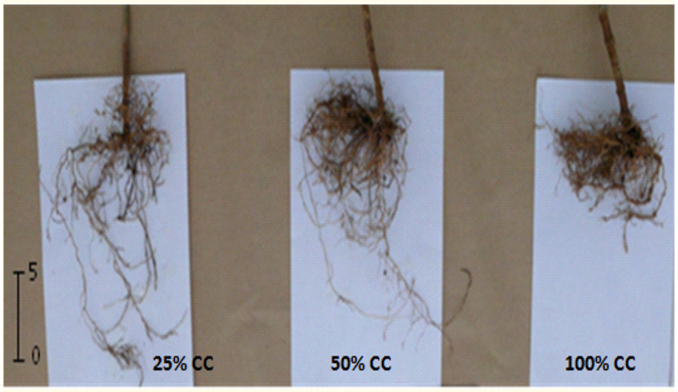


Figure 7: Effects of water treatments on root length of olive plants (Olea europaea L. cv Meski) 42 days after application of the water stress.

Water Use Efficiency (WUE)

Intrinsic water use efficiency

Figure 8 shows the intrinsic water use efficiency (WUE; µmol CO_2 (mol H_2O)$^{-1}$) calculated by the ratio Photosynthesis (A)/stomatal conductance (gs) of olive plants under three water treatments.

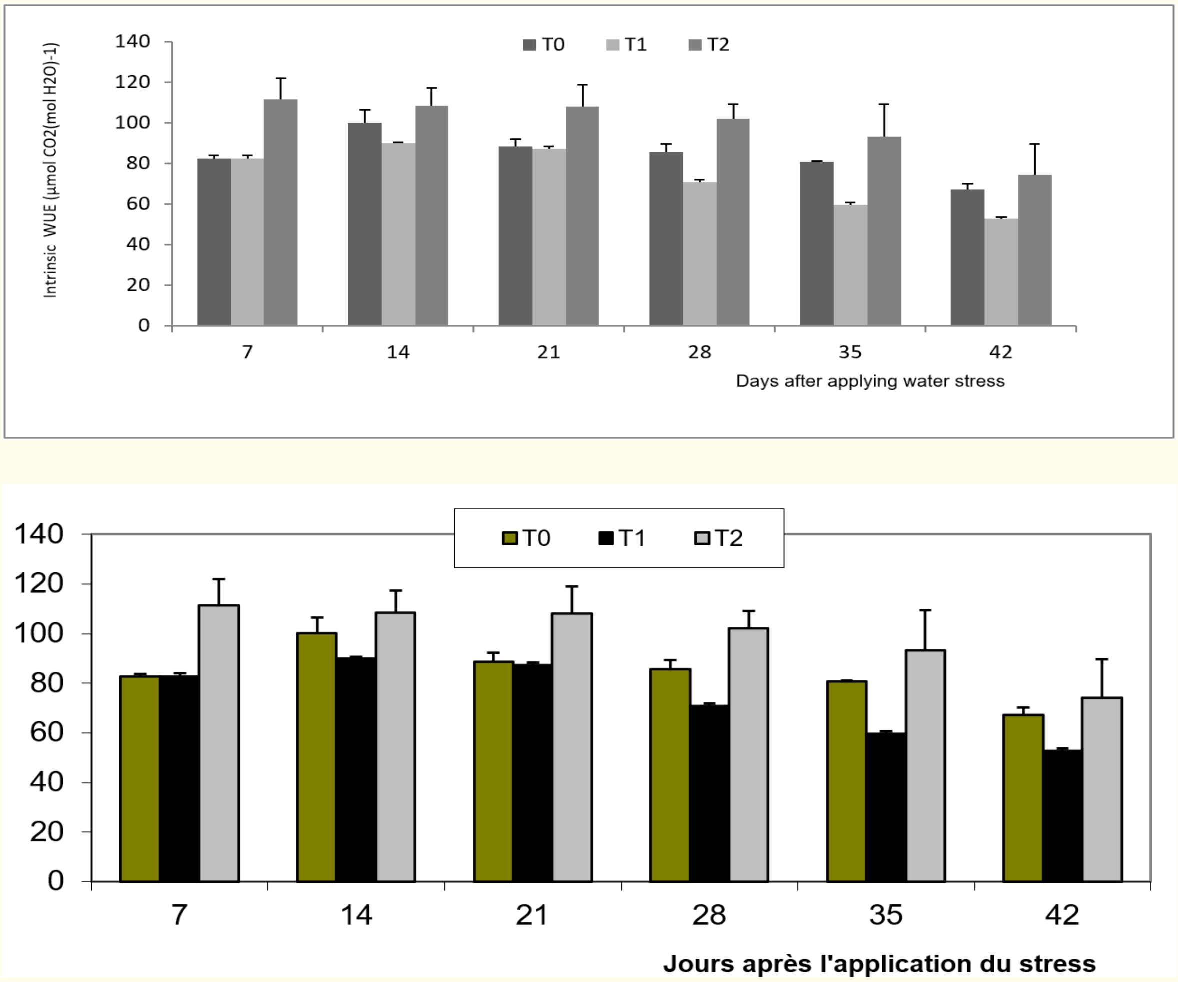


Figure 8: Effects of water water stress on the intrinsic water use efficiency (WUE; µmol CO2 (mol H2O)-1) calculated by the ratio Photosynthesis (A)/stomatal conductance (gs) of olive plants under three water treatments. Each value represents the mean ± standard deviation of three measurements.

During all the experiment period, stressed plants (T2) are the most efficient even if there is no significant difference proven between the three water treatments starting from 21 days after applying the water stress. These results are inconsistent with those mentioned by Boussadia [11] which show an increase of the water use efficiency 20 days after applying the water stress.

Extrinsic Water Use Efficiency (WUE)

Figure 9 shows the extrinsic water use efficiency (WUE; µmol CO_2 (mol H_2O)-1) calculated by the ratio Photosynthesis (A)/ transpiration (E) of olive plants under three water treatments. During all the experiment period (except for the 14^{th} day after applying water stress), the more stressed plants (25% FC) are the most efficient. This results confirm those mentioned by Arquero., *et al*. [16] how shows that well-watered plants have less of water use efficiency than plants with a water deficit.

Water Use Efficiency calculated by the ratio dry matter accumulation/water consumed

Table 3 show the olive plants nutritive solution (ml) consumption for the three water treatments. A significant difference was maintained throughout the experiment between the control treatment and treatments that have suffered water restriction 50 and 75%.

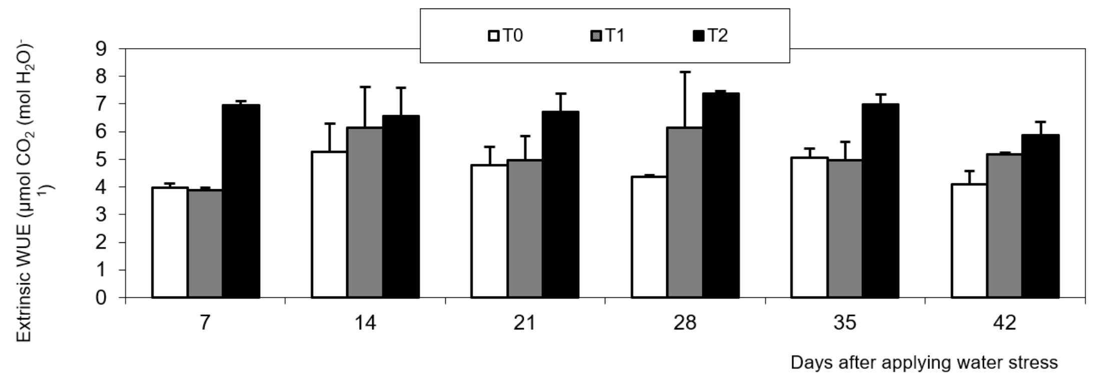


Figure 9: Effect of water treatments on the extrinsic water use efficiency (WUE; µmol CO2 (mol H20)-1) calculated by the ratio Photosynthesis (A)/transpiration (E) of olive plants. Each value represents the mean ± standard deviation of three measurements.

	7	14	21	28	35	42
T0	290 ± 6.12	583 ± 24.65	877 ± 54.04	1228 ± 80.67	1584 ± 91.41	1890 ± 113.14
T1	121 ± 37.15	286 ± 85.54	482 ± 134.38	718 ± 201.08	958 ± 264.49	1169 ± 330.01
T2	87 ± 20.8	243 ± 57.84	426 ± 106.44	648 ± 166.76	880 ± 224.81	1087 ± 274.22

Table 3: Weekly olive plants nutritive solution (ml) consumption for the three water treatments. Each value represents the mean ± standard deviation of five measurements.

Figure 10 show the effects of water treatments on the olive plants water use efficiency calculated by the ratio dry matter accumulation/water consumed where a significant difference is proven between the stressed plants (T2) and, control and T1 plants. T2 plants can produce 23.34g of dry matter for each one liter of nutritive solution consumed then these plants are the most efficient. There is no significant difference between control plants (irrigation at 100% of field capacity) and T1 plants (irrigation at 50% of field capacity) so we can advise irrigation at 50% of field capacity instead of irrigation at 100% of field capacity and then we can save 50% of water applied.

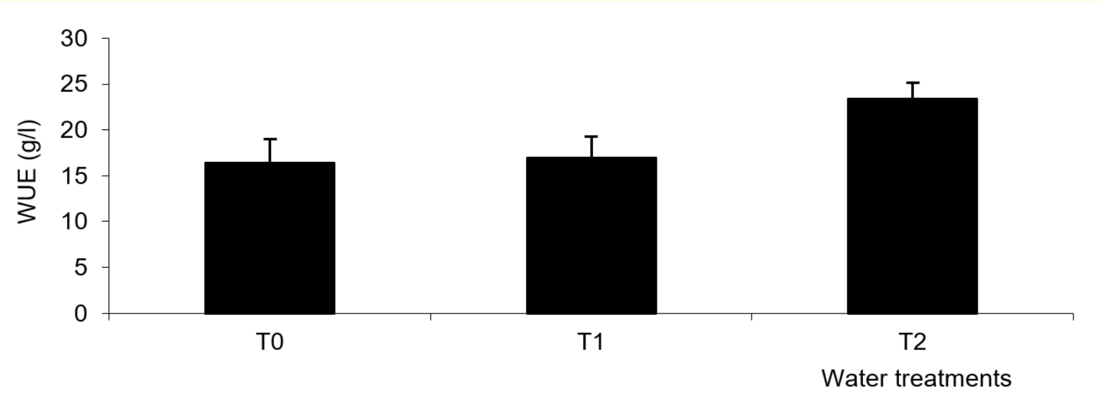


Figure 10: Effect of water treatments on the olive plants water use efficiency calculated by the ratio dry matter accumulation/water consumed. Each value represents the mean ± standard deviation of six measurements.

Conclusion

The competition on water resources in Tunisia imposes an adaptation of good water management plan to ensure the sustainability of agricultural practice. Indeed, our test which is oriented to the development of eco-physiological responses of plants Olea europaea L. cv Meski subjected to three water regimes showed good adaptation.

All results relating to gas exchange shows that the olive variety 'Meski' shows a rather stomatal adaptation to adjust his behavior with the stress intensity. Consequently the efficiency of water use calculated by the different possible relationships has shown that a restriction of 50% field capacity does not affect the yield of photosynthesis and biomass. This further indicates a margin of water management.

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