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Influence of Water Stress on Hydraulic Properties and Biochemical Changes of Oueslati and Jarboui Olive Cultivars (*Olea Europaea L.*)

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Abstract

Fluorescence parameters, xylem hydraulic properties, polyphenolic contents and antioxidant activity were studied on Two Tunisian olive cultivars (Ouslati, Jarboui) grown under water deficit conditions. Our results showed that water stress caused a decline in chlorophyll content, maximum quantum yield of photosystem II (*Fv/Fm*), linear electron transport rate (ETR) and quantum efficiency of PSII electron transport (ΦPSII). Oueslati variety was less affected by water stress but all these parameters decreases considerably in Jarboui variety. In addition, water stress induced an increase in xylem vessel frequency. The phenolic and flavonoid contents as well as antioxidant activities increase in the two cultivars under water stress conditions. Oueslati cultivar may be considered as the most tolerant cultivar showing the highest phenolic (43.65 mg GAE/g) and flavonoid (18.87 mg CE/g) contents and had the best antioxidant activity by both DPPH and ABTS methods with antioxidant activity of respectively 84.56% and 82.1%.

Keywords: Olea Europea L; Water Stress; Jarboui; Xylem Hydraulic Conductivity; Activities; Ouslati; Olive Cultivar; Water Stress; Photosynthetic; Xylem; Polyphenolic

Abbreviations: ETR: Electron Transport Rate; OD: Optical Density; VF: Vessel Frequency; VD: Vessel Diameter; RC: Relative Hydraulic Conductivity.

Introduction

Olea europaea belonging to the family Oleaceae is a small evergreen tree, from 12 to 20 feet high, with rigid branches, and a grayish bark. It is best adapted to the semi-arid Mediterranean environment. They are tolerant to drought and salinity and have low nutritional requirements. Drought adaptations of olive trees depends on several anatomic characteristics such as leaf cuticular waxes, stomata present only in the abaxial position and covered by trichomes and

physiological mechanisms such as stomatal closure [1] resulting in a reduction in photosynthetic rate [2].

Many mechanisms were investigated by which olive tree resists to more or less extended drought periods [3,4], but there are some differences among olive cultivars have been observed concerning their capability for adaptation under water stress conditions [3]. The leaf is the most adaptable organ in its response to environmental conditions [5]. Leaf structures reflect the effects of water stress more clearly than those of stems or roots. Two olive cultivars planted widely in Tunisia (Ouslati and Jarboui) were used in the present study. But despite their economic importance in Tunisia there isn't any information documenting their drought tolerance. So, the objective of this study is to evaluate the effects of drought on several physiological and biochemical parameters of these two olive cultivars. In order to compare them by variety and resistance to water deficit.

Material and Methods

Site description and plant material

This research was carried out with two Tunisian olive cultivars (Olea europaea L. cvs. Jarboui (centre Tunisia) and Ouslati (Kairouan) (Figure 1). Two year old plants were grown in a greenhouse situated in the olive institute in Sousse (Tunisia; 35°N,10° E) in 10 dm^3 pots (one plant per pot)) filled with a mixture of sandy soil and manure (2:1, v/v), with a pH of 7.6, a field capacity(FC) of 35% and permanent wilting point of 15%(WP). During the experiment the temperature and humidity were 25/32°C and 65/55% under the greenhouse. Four plants from each variety were used as controls (Watered) and irrigated once a week to field capacity. An additional four plants from each cultivar were stressed by withholding water during two months (May and June) until the soil water content almost reached less than the wilting point (6.5%). The experiment comprised four treatments of two cultivars and two watering regimes in a factorial design.

Figure 1: The Two olive Tunisian cultivars.

Determination of pigment content

The procedure was carried out at 4°C and in the dark. Leaf sample (0.25g) were mashed in a pestle and mortar with 80% acetone (v/v).The extract was filtered through two layers of nylon and centrifuged in sealed tubes at 15,000 x g for 5min .The supernatant was collected and the absorbance was read at 663 and 645 nm for chlorophyll a and chlorophyll b, respectively. The total chlorophyll Chl(a + b) concentration was given in µg ml^{-1} of extract solution according to the equations of Lichtenthaler and Buschmann (2001):

$$\text{Total Chlorophyll} = 20.2(A645) + 8.02(A663)$$

Measurement chlorophyll fluorescence parameters

Six flag leaves for each cultivar were selected to measure chlorophyll fluorescence parameters. Dark adaptation period for all the measurements was about 30 min, and chlorophyll fluorescence was measured using a portable fluorescence spectrometer Handy PEA (Hansatech instruments, Norfolk, UK) following the manufacturer's instruction. Recorded fluorescence values included: quantum yield of electron transport at PSII (ΦPSII), and electron transport rate (ETR), and Fv/ Fm, which represents the maximum quantum efficiency of PSII photochemistry and is highly correlated with the quantum yield of net photosynthesis. They were all determined according to Genty et al [6].

Xylem anatomical analyses

Stem samples of similar diameter were collected in four plants of each cultivar for microscopic investigation of xylem anatomy. Shoot transverse sections, approximately 3 mm thick, were cut at the same distance from the apex with a hand microtome, stained in a combination of alum carmine and iodine green [7]. This double staining brought out the lignified elements in green and the cellulose in pink. Measurements of xylem vessel frequency and xylem vessel diameter were made on each cross section.

Vessel frequency (vessels mm^{-2}) represents the mean of 16 fields per cultivar and vessel diameter (µm) was calculated from the average of two orthogonal measurements of vessel lumen. Efficiency to damage during water conduction was evaluated by determination of the hydraulic conductivity [8]. The relative hydraulic conductivity was estimated using a modified Hagen–Poiseuille equation [9]: $RC = r^4 VF$, where RC is the relative hydraulic conductivity, r the vessel radius and VF the vessel frequency.

Preparation of methanolic extracts and determination of total phenolic content

Fully developed leaves from the mid-section of each cultivar were immediately transferred to the laboratory delete and lyophilized. An aliquot of 250 mg from each variety was extracted in 10 mL of 80% methanol on a shaker at 200 rpm for30 min. The mixture was filtered and all extracts were stored at -20°C prior to experimentation. The total phenolic content was determined by the Folin-Ciocalteau colorimetric method with minor modifications [10]. To 100 µL of extract, 7.9 mL of deionized water and 0.5 mL of Folin-Ciocalteau reagent (F9252, Sigma Aldrich, St Louis, MO were added, mixed on a vortex mixer, and 1.5 mL of 1.85 M Na_2CO_3 was added after 15 min. Absorbance of samples was measured at 765 nm after 2 h. Gallic acid (GA) was used as a standard and results were expressed as mg of GAE per g of extract.

Determination of total flavonoids

The total flavonoid content (TFC) of the leaf extracts were determined according to the colorimetric assay developed by Zhishen et al [11]. One ml of leaf extract was mixed with 5 ml of distilled water. After that 300 µl of (5%, w/v) $NaNO_2$ was added. After 5min, 300µl of (10%, w/v) $AlCl_3$was added. At 6 min, 2ml of 1M solution of NaOH were added. Thereafter the volume of the mixture was adjusted to 10 ml with distilled water. Finally the absorbance was read at 510 nm. The results were also expressed on a dry weight basis as mg Quercetin equivalents (mg QE)/g of sample.

Antioxydant Activity by DPPH Method

The free radical scavenging activity was determined by measuring the bleaching of purple-coloured methanol solution of DPPH•. The radical scavenging activity was determined according to the method of Kontogiorgis and Hadjipavlou-Litina [12].

Antioxidant activity by ABTS assay

For the determination of the antiradical activity, a protocol based on the ABTS free radical decolourisation assay was used, as described previously. Five milliliter ml of a 7.0 mM ABTS solution was treated overnight in the dark with 88.0 µl of a 140 mM potassium persulfate solution to yield the ABTS radical cation. After that, the ABTS radical cation was diluted with ethanol to an initial absorbance of about 0.700 at 734 nm. Free radical-scavenging activity was assessed by mixing 1.0 ml of diluted ABTS radical cation with 10 µl of methanol extracts. The reaction mixture was kept at room temperature. Trolox was used as positive control. The optical density (OD) of the solution was measured at 734 nm, after 30 min. All tests were carried out in triplicate.

Statistics

A two-way analysis of variance (ANOVA) was used to examine cultivar and water availability treatment effects on fluorescence parameters, xylem hydraulic properties, polyphenol and flavonoid contents and antiradical activity of olive plants using Stat Plus 2007 software. Significant different means were separated using the Fisher's L.S.D. test ($P < 0.05$).

Results

Chlorophyll Content

According to Table 1 significant reductions in total chlorophyll for stressed olives were observed in comparison to the watered plants. These reductions were 70.3% and 78.5% for, Ouslati and Jarboui, respectively. Statistical analysis of this parameter showed significant differences between water treatment effects.

Varieties	Treatment	Ch(a+b) (µg ml-1)
Ouslati	Watered	31.6 ± 1.5a
	Stressed	9.5± 0.5b
Jarboui	Watered	34.8± 1.2a
	Stressed	7.5± 1.6b

Table 1: Effects of water stress on total Chlorophyll Ch(a + b) (µg /ml) in the two olive varieties under irrigated and stressed water regimes. Means± S.E. (n = 6). Means with different letters are significantly different at $P < 0.05$.

Measurement Chlorophyll Fluorescence Parameters

The result from Figure 2, 3 and 4 showed that there was no significant difference between the two varieties under watered condition but overall there was a significant decreased in fluorescence parameters under stressed condition. This shows that the PSII in these varieties can be damaged in different degrees under drought stress and that the primary reaction of photosynthesis may be inhibited. As summarized in figures 2-4 there was a significant decline in the Fv/Fm, ETR and in **Φ**PSII for Ouslati and jarboui cultivar. But a significant decrease was observed for Jarboui than Ouslati cultivar with 0.31 for Fv/Fm, 1.16 for ETR and 0.32 for ΦP_{SII}. Adaptability to drought stress is thus higher at Ouslati compared to Jarboui.

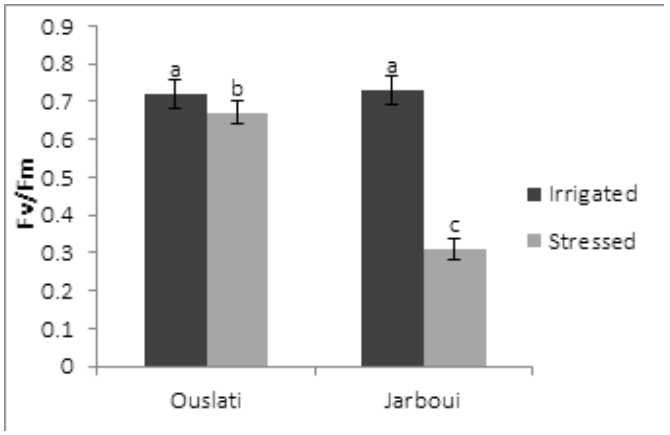

Figure 2: Maximal photochemical ef*fi*ciency of PSII (Fv/Fm) of olive cultivars (Ouslati, and Jarboui), Values represent averages ± standard deviations for triplicate experiments.

1.

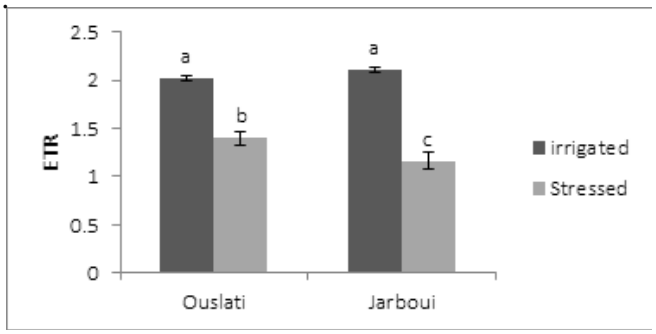

Figure 3: Electron transport rate (ETR) of olive cultivars (Ouslati and Jarboui), Values represent averages ± standard deviations for triplicate experiments.

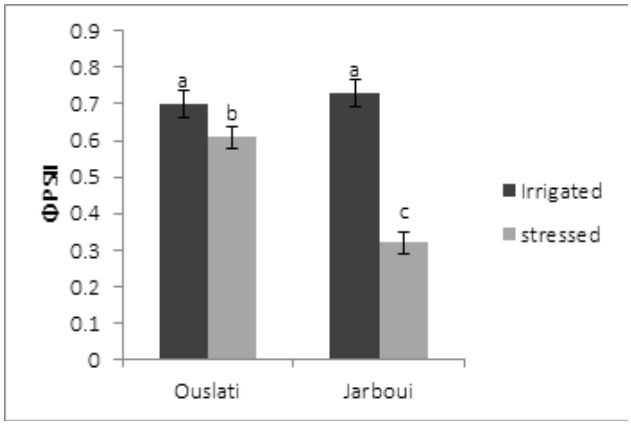

Figure 4: Quantum yield of photosystem II electron transport (***Φ***PSII) of olive cultivars (Ouslati, Jarbooui), Values represent averages ± standard deviations for triplicate experiments. Columns ***fl***anked by the same letter are not significantly different at P < 0.05.

Xylem Hydraulic Conductivity

According to Table 2 water stress generated an increase in VF. Moreover VD showed significant decrease between the four cultivars. As shown in Table 2 and under stressed condition, Jarboui variety showed a significant reduction in VD but Ouslati had the highest relative hydraulic conductivity (RC). This last decreased in the two varieties of olive because of the drop of VD. We can observe a signi**fi**cant differences were recorded among cultivars and water regimes.

		VF (vessels/ mm2) mm−2)	VD (µm)	RC (µm4 106)
Ouslati	Irrigated	343.75d	368a	0.39
Ouslati	Stressed	625a	267.1c	0.19
Meski	Irrigated	437.5c	314.4b	0.26
Jarbouii	Stressed	531b	223.4d	0.08

Table 2: Stem xylem vessel frequency (VF), vessel diameter (VD) and relative hydraulic conductivity (RC) of olive cultivars under contrasting water availability regimes (*n* = 6). Represent averages ± standard deviations. Means with different letters are signi**fi**cantly different at P < 0.05.

Determination of Total Phenolic and Flavonoid Contents

The total phenolic and flavonoid contents of methanolic leaves extracts were different among olive cultivars (Figure 5 and 6) under watered conditions. Ouslati had the highest total phenolic (43.65 mg GAE/g extract) and flavonoid (10.64 mg QE/g extract) contents and Ouslati had the lowest ones. But under water deficit, total phenolic and flavonoid contents increased significantly in the two cultivars. Signi**fi**cant differences were recorded among cultivars and water regimes.

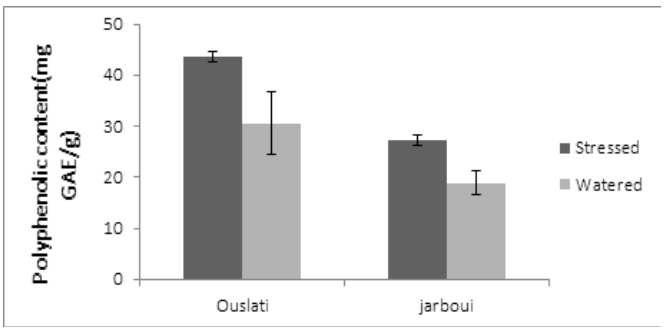

Figure 5: Total phenolic content of methanolic leaves extracts of olive cultivars.Vertical bars represent means of 3 replications ±S.E. Columns flanked by the same letter are not significantly different at P < 0.05.

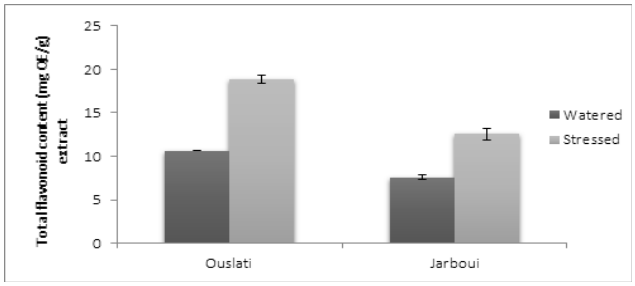

Figure 6: otal Flavonoid content of methanolic leaves extracts of the two olive cultivars. Vertical bars represent means of 3 replications ±S.E. Columns **fl**anked by the same letter are not significantly different at $P < 0.05$.

Antiradical Activity

DPPH and ABTS methods: Figure 7 and 8 showed the antioxidant activity of methanol leaves extracts of the olive cultivars.

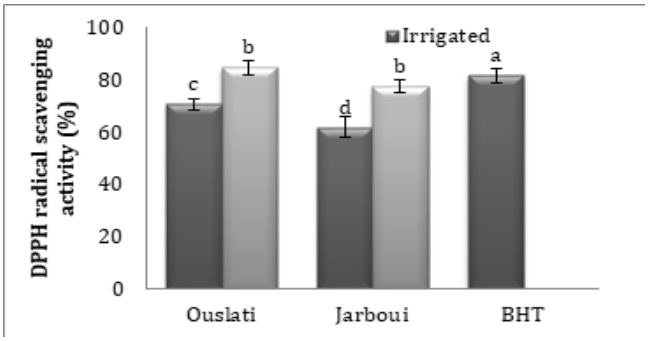

Figure 7: Free radical-scavenging capacities of methanol extracts of olive cultivars measured in DPPH assay. Vertical bars represent means of 3 replications ±S.E. Columns **fl**anked by the same letter are not significantly different at $P < 0.05$.

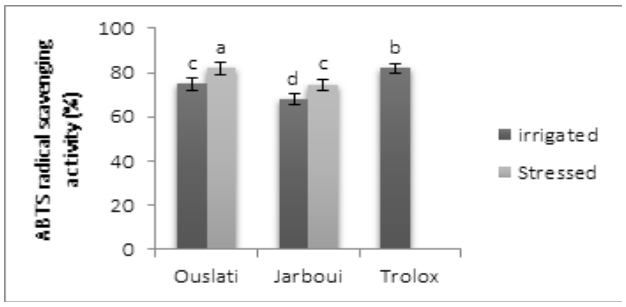

Figure 8: Free radical-scavenging capacities of methanol extracts of olive cultivars measured in ABTS assay. Results are means of three different experiments. Vertical bars represent means of 3 replications ±S.E. Columns **fl**anked by the same letter are not significantly different at $P < 0.05$.

In watered condition, Ouslati variety had the best antioxydant activity by both DPPH and ABTS methods with antioxydant activity of respectively 70.47% and 74.7%. Under water stress condition the two cultivars showed an important increase in antioxydant activity. With ATBS method ouslati possess the highest antioxydant activity compared to controls Trolox, followed by Ouslati (Figure 7 and 8).

Discussion

Water deficit, temperature, nutrient deficiency and attack by pathogens influence the development of the plants and reduce photosynthesis. For that reason analyses of chlorophyll content and chlorophyll fluorescence parameters (ETR, Fv/Fm, **Φ**PSII) are considered important approaches for evaluating the internal apparatus during photosynthetic process within a leaf [13] they provide a rapid way to quantify plants tolerance to drought stress [14].

In this study we evaluated the chlorophyll content, ETR, Fv/Fm, **Φ**PSII, xylem hydraulic properties, total phenolic content and antiradical activity in four Tunisian olive varieties under water stress conditions. The significant decrease in total chlorophyll content can be attributed to the sensitivity of this pigment to increasing environmental stresses, especially salinity and drought [15].

The chl(a+b) content in Ouslati variety showed a larger reduction of this parameter under water deficit compared to other varieties. All chlorophyll fluorescence parameters, Fv/Fm, ETR and **Φ**PSII declined in all four varieties under water deficit condition. The decrease in Fv/Fm ratio indicates a reduction in the photochemical eff**i**ciency of the PSII complex, which could be due to ineff**i**cient energy transfer from the light-harvesting Chl a/b complex to the reaction center [16,17]. In addition, the present study shows a decrease of quantum yield of photosystem II electron transport (**ΦPSII**) under water stress conditions, which is correlated with the quantum yield of non-cyclic electron transport observed in plants.

The decrease of ETR can explain that drought limits the photosynthetic electron transport and consequently results in a decrease in NADPH and ATP synthesis [18]. It may be suggested that differences exist in the reaction of the photosynthetic apparatus to drought as we observed that in Ouslati variety the photosynthetic process has a higher tolerance to drought stress. However, the Jarboui variety is apparently sensitive to water stress. This is in good agreement with Araus, et al. [19] who showed that chlorophyll fluorescence can be used as a good indicator of adaptation to drought stress in wheat.

The results of xylem hydraulic conductivity showed great

differences among the four cultivars. Xylem hydraulic properties play an essential role in supporting growth and photosynthesis and influence sensitivity to environmental conditions such as drought and freezing. Furthermore, stem hydraulic conductance may be used as a comparative measure of overall hydraulic adaptation across species and to assess the impact of environmental variations, especially drought, on water transport [20]. In addition, we observed that in all cultivars, water stress induced an increase in VF, which is known to provide a greater security of xylem sap conduction under drought conditions [21].

The cultivar from Ouslati showed rather the highest vessel frequency. As it is the most adapted variety to water stress the abundant vessels permit the functioning of the conduction system when some vessels are disabled by cavitation [8]. Plants from Jarboui had the lowest VF indicating that there is probably a dysfunction in its water flow system. Under water stress condition, all varieties showed a significant reduction in VD. Vessels with thin diameters are less susceptible to embolism [22]. In addition, RC decreased in all varieties but mostly in Jarboui. The low hydraulic conductivity of xylem seems to play an important role in the olive-water relations as it allows the tree to avoid water loss on days of high atmospheric demand [23].

In our study, total phenolic and flavonoid contents increased under water stress in all leaves cultivars. But Gregorova et al. [24] reported that responses of phenolics to drought were different in Shoots and Roots. They observed an important increase in total phenols in shoots compared to roots in Triticum aestivum plants exposed to drought over 20 days. In rice grains, prolonged drought even depleted the amount of total phenols, indicating tissue or species-specific differences [25]. This phenomenon can be explained by the fact that the plant protection is generally secured by phenolics which accumulate during drought due to an increase in the levels of ROS in plant cells [26].

It is well known that an important function of flavonoids and phenolic acids are of great importance in plant defense mechanisms [27]. Phenolics are involved in protection against oxidative stress under adverse environmental conditions. In addition, we can observed that'Oslati' had higher antioxidant activity than Jarboui cultivar, suggesting that the ability of olive plants to scavenge ROS is cultivar dependent. Furthermore, ROS are involved in the photodamage to PSII [28-33]. It seems that Ouslati variety was the best cultivar with respect to its behavior against water stress and its contribution in the antioxidant scavenging mechanism.

Conclusions

Our study can be considered as the first report on the effect of water stress on fluorescence parameters, xylem hydraulic properties, and antiradical activity of Ouslati and Jarboui cultivars. Our results demonstrated that water stress affects the physiological and biochemical parameters of Tunisian olive cultivars. Ouslati variety appears the most adapted variety to drought and occupies the first position, followed by Jarboui, which is the most sensitive cultivar to drought. This selection will be continued in a future work by other anatomical and biochemical criteria, in order to obtain a more complete picture of the drought resistance strategies of this species.

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