Journal of Plant Biochemistry & Physiology
ISSN: 2329-9029
Edziri et al., J Plant Biochem Physiol 2017, 5:3
DOI: 10.4172/2329-9029.1000193
Research Article
OMICS International

The Effect of Water Stress on Leaf Phenolic Composition, Fluorescence Parameters, Xylem Hydraulic Properties and Antiradical Activity of Four Tunisian Olive (*Olea europaea* L.) Cultivars

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Abstract

Chlorophyll content, fluorescence parameters, xylem hydraulic properties, total phenolic content and antiradical activity were studied on young plants of four Tunisian olive cultivars (Chetoui, Ouslati, Jarboui and Meski) grown under water deficit conditions for two months. Water stress caused a decline in chlorophyll content, in maximum quantum yield of photosystem II (Fv/Fm), in linear electron transport rate (ETR) and in quantum efficiency of PSII electron transport (Φ PSI). Chetoui variety was the less affected by water stress but all these parameters decreases considerably in Ouslati, Jarboui and Meski. In all cultivars, water stress induced an increase in xylem vessel frequency. Water stress also increased the phenolic and flavonoid contents and antiradical activity in all cultivars. Chetoui cultivar may be considered as the most tolerant cultivars among the tested cultivars showing higher phenolic and flavonoid contents and an important antiradical activity under water deficit. Meski cultivar is the most sensitive one.

Keywords: *Olea europea* L.; Water stress; Fluorescence; Xylem hydraulic conductivity; Antiradical activity; Phenols

Introduction

The olive tree (*Olea europaea* L.) is one of the hypostomatous species, which is best adapted to the semi-arid Mediterranean environment. Olive trees can attain a height of 15 to 20 m, are extremely long-lived (up to 1000 years). 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 resulting in a reduction in photosynthetic rate [1]. Many mechanisms were investigated by which olive tree resists to more or less extended drought periods [2,3], but there are some differences among olive cultivars have been observed concerning their capability for adaptation under water stress conditions [4]. 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.

Four olive cultivars planted widely in Tunisia (Chetoui, Meski, Ouslati and Jarboui) were used in the present study. Our choice was based more on their physiological properties than on their economic importance. Chetoui and Meski cultivars differ greatly in their ability to withstand water stress. The cultivar 'Chetoui' is known for its drought tolerance, whereas 'Meski' is drought-sensitive [6,7]. But Ouslati and Jarboui cultivars despite their economic importance in Tunisia there isn't any information documenting their drought tolerance, our study can be considered as the first report on the physiological and biochemical parameters of these two cultivars under water stress. So, the objective of this study is to evaluate the effects of drought on several physiological and biochemical parameters of four olive cultivars (Ouslati, Jarboui, Chetoui and Meski). In order to classify them by variety and resistance to water deficit.

Materials and Methods

Site description and plant material

This research was carried out with three Tunisian olive cultivars (*Olea europeae* L. cv. Meski (North), cv. Jarboui (Teboursok) and cv. Ouslati (Kairouan). Two years old plants were grown in a greenhouse situated in the olive institute in Sousse (sahel Tunisia; 35°N,10°E) in 10 dm pots (one plant per pot) with a pH of 7.6, a field capacity of 35% and permanent wilting point of 15%. The greenhouse temperature was 25-32 °C. 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 for two months (May and June) until the soil water content almost reached less than the wilting point.

Determination of pigment content

The procedure was carried out at 4°C and in the dark. A leaf sample (0.25 g) were mashed in a mortar and pestle 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 5 min. The supernatant was collected and the absorbance was read at 663 and 647 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 [8].

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Received July 02, 2017; **Accepted** July 14, 2017; **Published** July 18, 2017

Citation: Edziri H, Mastouri M, Cheheb H, Laameri S, Boujnah D (2017) The Effect of Water Stress on Leaf Phenolic Composition, Fluorescence Parameters, Xylem Hydraulic Properties and Antiradical Activity of Four Tunisian Olive (*Olea europaea* L.) Cultivars. J Plant Biochem Physiol 5: 193. doi: 10.4172/2329-9029.1000193

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 (Φ PSII), 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. [9].

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 [10]. 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 [11]. The relative hydraulic conductivity was estimated using a modified Hagen–Poiseuille equation [12]: $RC=r^4$ VF, where RC is the relative hydraulic conductivity, r the vessel radius and VF the vessel frequency. The vulnerability index (VI) was calculated as proposed by Carlquist [11].

Preparation of methanolic extracts and determination of total phenolic content

Fully leaves from the mid-section of each cultivar were immediately transferred to the laboratory and lyophilized. An aliquot of 250 mg from each variety was extracted in 10 mL of 80% methanol on a shaker at 200 rpm for 30 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 colourimetric method with minor modifications [13]. 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. [14]. 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 5 min, 300 µl of (10%, w/v) $AlCl_3$ was added. At 6 min, 2 ml of 1 M 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.

Dpph radical scavenging 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 [15].

ABTS radical scavenging activity

For the determination of the antiradical activity, a protocol based on the ABTS free radical decolourization assay was used, as described previously [15]. 5.0 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. Prior to use in the assay, the ABTS radical cation was diluted with ethanol to an initial absorbance of about 0.700 (ratio of 1:88) at 734 nm, with 30°C. 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

Soil moisture

As shown in Figure 1, soil moisture was about 35% at the beginning of the experiment, at the field capacity in watered condition, and then decreased progressively to reach 6.5%, less than the wilting point in stressed condition.

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 66.13%, 70.3%, 78.5%, and 87.2% for Chetoui, Ouslati, Jarboui and Meski, respectively. Statistical analysis of this parameter showed significant differences between water treatment effects.

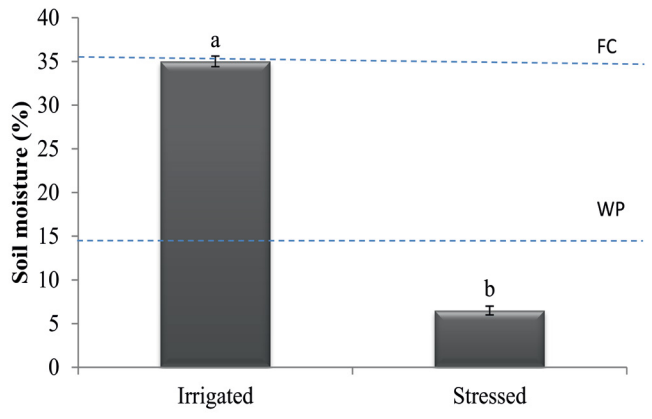

Figure 1: The soil moisture in irrigated and stressed conditions, mean with different letter are significantly different at P<0.05 (EDZIRI H).

Measurement chlorophyll fluorescence parameters

The result from Figures 2-4 showed that there was no significant difference between the different varieties under watered condition but overall there is a significant decreased in fluorescence parameters under stressed condition. This shows that the PSII in all varieties can be damaged in different degrees under drought stress and that the primary reaction of photosynthesis may be inhibited. There is no significant decrement for Chetoui in Fv/Fm (2.7%), ETR (10%) and in Φ PS (4.1%) and for Ouslati in Fv/Fm (6.1%) and in Φ (9.1%). However a significant decrease was observed for Jarboui and even more for Meski reaching respectively (57.5% for Fv/Fm, 54.02% for ETR and 56.1% for Φ P and 67.14% for Fv/Fm, 45.16% for ETR and 69.7% for Φ P. The order of the values for Fv/Fm, ETR and Φ under water stress are Chetoui, Ouslati, Jarboui and Meski. Adaptability to drought stress is thus higher at Chetoui and Ouslati compared to Jarboui and Meski.

Varieties	Treatment	Ch(a+b) (mg g^{-1} DW)
Chetoui	Watered	37.5 ± 1.7a
	Stressed	12.7 ± 1.1b
Ouslati	Watered	31.6 ± 1.5a
	Stressed	9.5 ± 0.5b
Jarboui	Watered	34.8 ± 1.2a
	Stressed	7.5 ± 1.6b
Meski	Watered	37.5 ± 0.2a
	Stressed	4.8 ± 1.7b

Means ± SE (n=4); Means with different letters are significantly different at P<0.05

Table 1: Effects of water stress on Chl(a+b) (µg/ml) in the four olive varieties under irrigated and stressed water regimes.

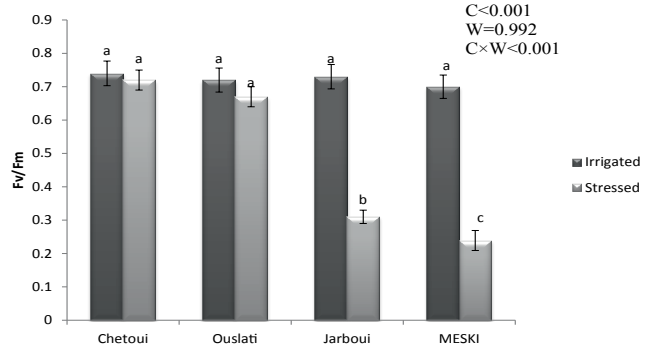

Figure 2: Maximal photochemical efficiency of PSII (Fv/Fm) of four olive cultivars (Ouslati, Jarbouii, Meski, Chetouii). Values represent averages ± standard deviations for triplicate experiments (EDZIRI H).

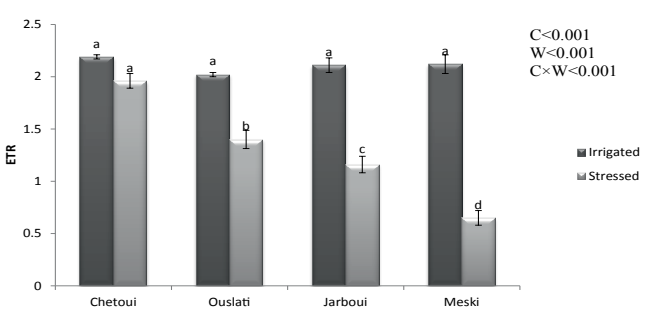

Figure 3: Electron transport rate (ETR) of four olive cultivars (Ouslati, Jarbouii, Meski, Chetouii). Values represent averages ± standard deviations for triplicate experiments (EDZIRI H).

Xylem hydraulic conductivity

According to Table 2 water stress generated an increase in VF. Moreover, VD showed significant decrease between the four cultivars. Meski variety showed a significant reduction (50.7%) in VD under stressed condition. Meski also had the highest relative hydraulic conductivity(RC), which is decreased in the four varieties of olive because of the drop of VD. We can observe a significant differences were recorded among cultivars and water regimes. Chetoui showed a decrease of 75% but Meski showed a greater decrease (93.45%) in RC.

Determination of total phenolic and flavonoid contents

The total phenolic and flavonoid contentsof methanolic leaves extracts were different among olive cultivars (Figures 5 and 6). Under watered condition Chetoui had the highest total phenolic (37.4 mg GAE/g extract) and flavonoid (13.25 mg QE/g extract) contents and Meski had the lowest ones. But under water deficit, total phenolic and flavonoid contents increased significantly in all cultivars. Significant differences were recorded among cultivars and water regimes. In general Chetoui showed the highest total phenol (73.5 mg GAE/g extract) and flavonoid contents (24.87 mg QE/g extract) followed by Ouslati, Jarbouii and Meski.

Antiradical activity

DPPH and ABTS methods: Figures 7 and 8 showed the antioxidant activity of methanol leaves extracts of the four olive cultivars. In watered condition, Chetoui variety had the best antiradical activity by

		VF ((Vessels/mm²) mm^{-2})	VD (µm)	RC (µm^4 10^6)
Chetoui	irrigated	637a	424.9a	1.29
	stressed	787b	282.1c	0.41
Ouslati Ouslati Meski	irrigated	458c	368b	0.52
	stressed	565d	267.1c	0.17
Jarbouii	irrigated	437c	321.3c	0.25
	stressed	559d	215.8d	0.18
Meski	irrigated	458c	290.2c	0.20
	stressed	539d	144.5e	0.014

Values represent averages ± standard deviations for triplicate experiments. Means with different letters are significantly different at P<0.05

Table 2: Stem xylem vessel frequency (VF), vessel diameter (VD), relative hydraulic conductivity (RC) and vulnerability index (VI) of four olive cultivars under contrasting water availability regimes (*n*=5).

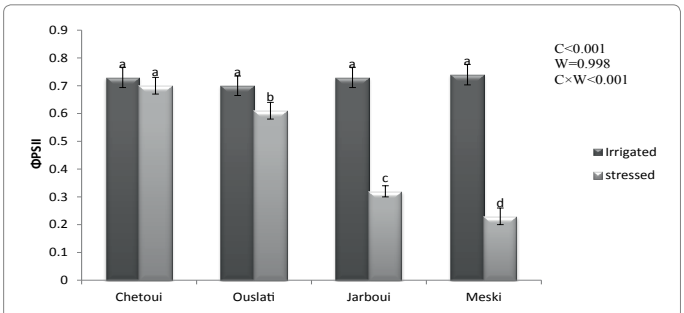

Figure 4: Quantum yield of photosystem II electron transport (Φ PSII) of four olive cultivars (Ouslati, Jarbouii, Meski, Chetouii), Values represent averages ± standard deviations for triplicate experiments. Columns flanked by the same letter are not significantly different at *P*<0.05. The *P*-values for cultivar (*C*), watering regime (*W*), and cultivar × watering regime (*C*×*W*) are shown in the upper right of the panel (EDZIRI H).

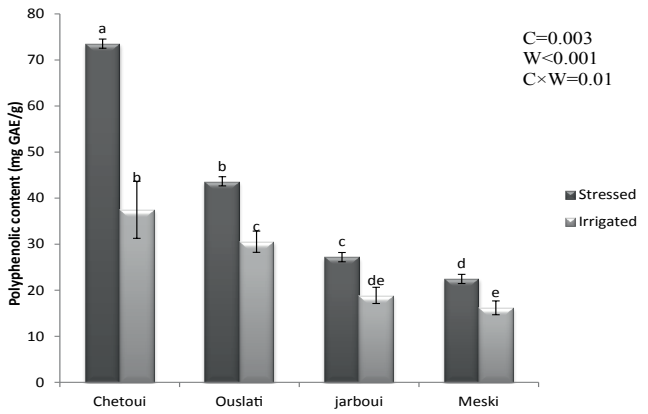

Figure 5: Total phenolic content of methanolic leaves extracts of four olive cultivars (EDZIRI H). Values represent averages ± standard deviations for triplicate experiments. Vertical bars represent means of 3 replications ± S.E. Vertical bars represent means of 3 replications ± S.E. Columns flanked by the same letter are not significantly different at *P*<0.05. The *P*-values for cultivar (*C*), watering regime (*W*), and cultivar × watering regime (*C* × *W*) are shown in the upper right of the panel (EDZIRI).

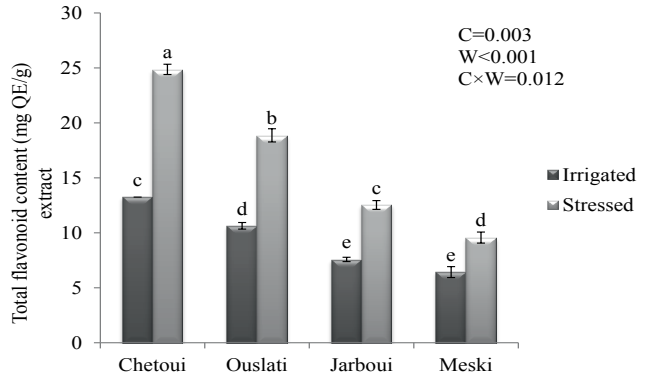

Figure 6: Total Flavonoid content of methanolic leaves extracts of four olive cultivars (EDZIRI). Values represent averages ± standard deviations for triplicate experiments. Vertical bars represent means of 3 replications ± S.E. Vertical bars represent means of 3 replications ± S.E. Columns flanked by the same letter are not significantly different at *P*<0.05. The *P*-values for cultivar (*C*), watering regime (*W*), and cultivar × watering regime (*C* × *W*) are shown in the upper right of the panel (EDZIRI).

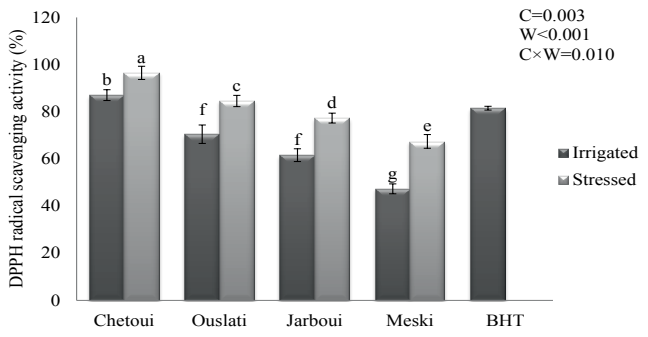

Figure 7: Free radical-scavenging capacities of methanol extracts of four Tunisian olive cultivars measured in DPPH assay. Results are means of three different experiments. Vertical bars represent means of 3 replications ± S.E. Vertical bars represent means of 3 replications ± S.E. Columns flanked by the same letter are not significantly different at *P*<0.05. The *P*-values for cultivar (*C*), watering regime (*W*), and cultivar × watering regime (*C* × *W*) are shown in the upper right of the panel (EDZIRI H).

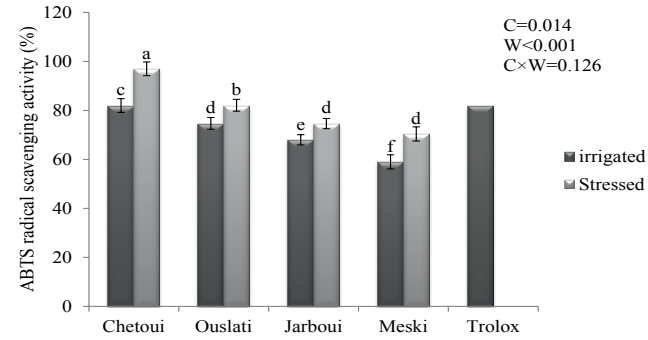

Figure 8: Free radical-scavenging capacities of methanol extracts of four Tunisian olive cultivars measured in ABTS assay. Results are means of three different experiments. Vertical bars represent means of 3 replications ± S.E (p<0.05). Vertical bars represent means of 3 replications ± S.E. Columns flanked by the same letter are not significantly different at *P*<0.05. The *P*-values for cultivar (*C*), watering regime (*W*), and cultivar × watering regime (*C* × *W*) are shown in the upper right of the panel (EDZIRI H).

both DPPH and ABTS methods with antiradical activity of respectively 80.05% and 81.99%, Under water stress condition all cultivars showed an important increase in antiradical activity. With both methods Chetoui possess the highest antiradical activity compared to controls BHT and Trolox, followed by Ouslati, Jarbouii and Meski (Figures 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 [16]. They provide a rapid way to quantify plants tolerance to drought stress [17].

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 [18]. The chl(a+b) content in Meski 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 efficiency of the PSII complex, which could be due to inefficient energy transfer from the light-harvesting Chl a/b complex to the reaction center [19,20]. In addition the present study shows a decrease of quantum yield of photosystem II electron transport (Φ PSII) 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 [21].

It may be suggested that differences exist in the reaction of the photosynthetic apparatus to drought as we observed that in Chetoui variety the photosynthetic process has a higher tolerance to draught stress. However, the Meski variety is apparently the most sensitive to water stress. This is in good agreement with Araus et al. [22] who showed that chlorophyll fluorescence can be used as a good indicator

of adaptation to draught 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 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 [23]. 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 [24]. The cultivar from Chetoui revealed 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 [25]. Plants from Meski possessed 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 [26]. In addition, RC decreased in all varieties but mostly in Ouslati, jarboui and Meski. 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 [27]. Concerning the total phenolic and flavonoid contents which increased under water stress. The plant protection is generally secured by phenolics which are accumulated during drought. It is well known that an important function of flavonoids and phenolic acids is their action in plant defense mechanisms [28]. Phenolics are implicated in protection against oxidative stress under adverse environmental conditions. Water stress induced an increase in the levels of ROS in plant cells [29]. In addition we can observed that'Chetoui' had higher antioxidant activity than the other tested cultivars (Ouslati, Jarbouii and Meski), suggesting that the ability of olive plants to scavenge ROS is cultivar dependent. Furthermore, ROS are involved in the photodamage to PSII [30].

It seems that Chetoui variety was the best cultivar with respect to its behavior against water stress and its participation in the antioxidant scavenging mechanism.

Conclusion

In conclusion, our study can be considered as the first report on the effect of water stress on leaf phenolic composition, 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 studied in this work. So, we could classify the four varieties according to their tolerance to water stress. Chetoui variety appears the most adapted variety to drought and occupies the first position, followed by Ouslati and Jarboui. But Meski variety occupies the last position because it 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.

Acknowledgements

This study was supported by the Ministry of Higher Education, Ministry of Scientific Research of Technology and Skills Development and Institute of the Olivier de Sousse.

References

1. Giorio P, Sorrentino G, Andria R (1999) Stomatal behaviour leaf water status and photosynthetic response in field-grown olive trees under water deficit. Environ Exp Bot 42: 95-104.
2. Chartzoulakis K, Patakas A, Bosabalidis A (1999) Changes in water relations, photosynthesis and leaf anatomy induced by intermitten drought in two olive cultivars. Environ Exp Bot 42: 113-120.
3. Bacelar EA, Correia CM, Moutinho-Pereira JM, Goncalves BC, Lopes JM, et al. (2004) Sclerophylly and leaf anatomical traits of five field-grown olive cultivars growing under drought conditions. Tree Physiol 24: 233-239.
4. Bacelar EA, Dario L, Santos JM, Moutinho P, Berta CG, et al. (2006) Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: Changes on structure and chemical composition of foliage and oxidative damage. Plant Sci 170: 596-605.
5. Marchi S, Tognetti R, Minnocci A, Borghi M, Sebasiani L (2008) Variation in mesophyll anatomy and photosynthetic capacity during leaf development in a deciduous mesophyte fruit tree (Prunus persica) and an ever- green sclerophyllous Mediterranean shrub (Olea europaea L.). Trees 22: 559-571.
6. Ennajeh M, Vadel AM, Khemira H, Mimoun M, Hellali R (2006) Defense mechanisms against water deficit in two olive (Oleaeuropaea L.) cultivars 'Meski' and 'Chemlali'. J Hortic Sci Biotechnol 81: 99-104.
7. Ennajeh M, Tounekti T, Vadel AM, Khemira H, Cochard H (2008) Water relations and drought-induced embolism in olive (Oleaeuropaea) varieties 'Meski' and 'Chemlali' during severe drought. Tree Physiol 28: 971-976
8. Lichtenthaler HK, Buschmann C (2001) Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current Protocols in Food Analytical Chemistry.
9. Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990: 87-92.
10. Deysson G (1965) Eléments D'anatomie Des Plants Vasculaires. Société d'édition d'enseignement supérieur, Paris.
11. Carlquist S (1977) Ecological factors in wood evolution: a floristic approach. Am J Bot 64: 887-896.
12. Fahn A (1986) Structural and functional properties of trichomes of xeromorphic leaves. Ann Bot 57: 631-637.
13. Singleton VL, Rossi JAJ (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Viticult 16: 144-158
14. Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64: 555-559.
15. Kontogiorgis C, Hadjipavlou-Litina D (2005) Synthesis and antiinflammatory activity of coumarin derivatives. J Med Chem 48: 640.
16. Barber J (2009) Photosynthetic energy conversion: natural and artificial. Chem Soc Rev 38: 185-196.
17. Perciva GC, Sheriffs CN (2002) Identification of droughttolerant woody perennials using chlorophyll fluorescence. J Arb 28: 215-223.
18. Younis ME, El-Shahaby OA, Abo-Hamed SA, Ibrahim AH (2000) Effects of water stress on growth, pigments and 14CO2 assimilation in three sorghum cultivars. Agron Crop Sci 185: 73-82.
19. Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ 25: 275-294.
20. Whatley FR, Tagawa K, Arnon DI (1963) Separation of the light and dark reactions in electron transfer during photosynthesis. Proc Natl Acad Sci 49: 266.
21. Epron D, Dreyer E, Bréda N (1992) Photosynthesis of oak trees Quercus petraea) (Matt.) Liebl.) during drought under field conditions: diurnal course of net CO2 assimilation and photochemical efficiency of photosystem II. Plant Cell Environ 15: 809-820.
22. Araus JL, Amaro T, Casadesus J, Asbati A, Nachit MM (1998) Relationships between ash content, carbon isotope discrimination and yield in durum wheat. Aust J Plant Physiol 25: 835-842.
23. Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. New Phytol 119: 345-360.
24. Terral JF, Mengual X (1999) Reconstruction of Holocene climate in southern France and eastern Spain using quantitative anatomy of olive wood and archaeological charcoal. Palaeogeogr Palaeoclimatol Palaeoecol 153: 71-92.

25. Tognetti R, Andria R, Morelli G, Calandrelli D, Fragnito F (2004) Irrigation effects on daily and seasonal variations of trunk sap flow and leaf water relations in olive trees. Plant Soil 263: 249-264.

26. Lovisolo C, Schubert A (1998) Effects of water stress on vessel size and xylem hydraulic conductivity in Vitis vinifera L. J Exp Bot 49: 693-700.

27. Fernandez JE, Moreno F, Giron IF, Blasquez OM (1997) Stomatal control of water use in olive tree leaves. Plant Soil 190: 179-192.

28. Grace SC (2005) Phenolics as antioxidants. Antioxidants and reactive oxygen species in plants 141168.

29. Smirnoff N (1993) The role of active oxygen in the response to water deficit and desiccation. New Phytol 125: 27-58.

30. Nishiyama Y, Yamamoto H, Allakhverdiev SI, Inaba M, Yokota A, et al. (2001) Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery. EMBO J 20: 5587-5594.