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Communication

Eco-Physiological Behavior of Five Tunisian Olive Tree Cultivars under Drought Stress

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Abstract: Tunisia is known to be a country with poor water resources, and water scarcity is evident in certain regions. In the long term, this situation could worsen, given the increased risk of drought. The olive tree (*Olea europaea* L.) is one of the plants best adapted to this climate, and numerous studies have been carried out to assess the effects of water stress. The aim of this work was to study and compare the ecophysiological behavior of a main Tunisian olive cultivar (Chetoui) and four rare Tunisian olive cultivars (Chemchali, Besbessi, Sayali and Jarboui) under drought stress and to identify the main parameters while comparing the tolerance of the cultivars studied to this abiotic stress. One-year-old olive trees grown in pots in a greenhouse were subjected to four drought treatments (i.e., 15, 30, 45 and 60 days of drought stress) in comparison with control trees. The evaluation of the response of the olives to this induced stress was based on five parameters: relative water content (RWC), stomatal resistance (SR), photosystem PSII, maximal photochemical efficiency (F_V/F_M), performance index on absorption basis (*PI*), measured by the handy PEA, and chlorophyll index, measured by SPAD. The relative water content (RWC) of the five cultivars decreased with increasing drought stress. Jarboui showed lower RWC values than Chemchali, especially under severe drought stress. This result was confirmed by changes in fluorescence characteristics. F_V/F_M, *PI* and SPAD index decreased during the development of drought stress. These observations are discussed in relation to the strategies developed by the cultivars to grow under drought stress. The Principal Component Analysis allowed the parameter with the strongest loading factor, which is F_V/F_M, to be highlighted and the cultivars most tolerant to drought stress to be distinguished. These cultivars, Besbessi and Sayali in the north of Tunisia and Chemchali in the south, can present a possible alternative to replace the local or foreign cultivars most cultivated in the country, which are characterized by high water needs.

Keywords: chlorophyll index; drought stress; *Olea europaea* L.; performance index; relative water content

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1. Introduction

Climate change is one of the major challenges facing humanity nowadays. The Mediterranean region is described as a climate change "hot spot" view of the increasing warming [1]. In fact, a 40% reduction in water availability is predicted by the end of this century along the coastal areas of the Mediterranean countries [2,3].

Tunisia as a southern Mediterranean country is characterized by low rainfall and limited renewable water resources, which is accompanied by overexploited groundwater resources [4,5]. In fact, the climate change impact in Tunisia is predicted to induce an increase of 1.1 °C in annual average temperature and a considerable decrease in annual precipitation, which is estimated to reach 15% by 2050 in the southern regions of the country [6–8].

The investigation of a set of scenarios combining future water availability in Tunisia and water use efficiency show that the agricultural sector would be negatively affected and that the mostly affected regions would be the north east, central west and southern areas [9]. Olive cultivation is the main agricultural activity in the country, and olive farms are extended over one-third of the total crop area, where the climate varies from arid to semi-arid, representing 1.85 million hectares [10,11]. A study of the social and private profitability of tree-based adaptation options to climate change in Tunisia showed that irrigated olive tree production is profitable for farmers, while rainfed plantation is not profitable at all. It is therefore necessary to adopt strategies that ensure that farmers increase their income without increasing the use of agricultural water [12]. In this context, the investigation of minor varieties which have proven the high potential for quality improvement of oils [13,14], in addition to the major varieties in the country Chemlali and Chetoui, is of great importance, and especially in terms of their adaptability to water scarcity conditions [15].

Olive trees are one of the most suitable and adapted species to the Mediterranean-type climate. Nowadays, olive trees face new challenges and threats related to climate change, especially the increase in the occurrence of extreme weather events linked with an increased warming and drought [16,17]. These severe conditions predicted in future climate scenarios may induce physiological changes as well as alterations in the phenological responses of olive trees [18–20].

One of the main detrimental effects of drought is a decrease in soil water potential, which impairs the plant's ability to absorb water, thereby reducing the ability of roots to absorb nutrients from the soil and transport water to shoots [21].

Among all the resistances that water have to overcome through the plant, leaves are considered as the major ones since they play an important role in the regulation of stomata [22,23]. Olive leaves can withstand very low water potentials, maintaining full rehydration capacity even when losing nearly 40% of their water content [24]. The stomatal aperture regulation is one of the primary drought responses allowing water losses reduction and the maintenance of an appropriate plant water status [25,26]. The most related trait to this stomatal conductance regulation is the leaf hydraulic conductance, which decreases exponentially with leaf water potential [27–29]. Olive trees are characterized by a tight control mechanism that can reduce excessive water loss by closing their stomata progressively when soil water availability decreases, which significantly decreases transpiration rate and contributes to preserving positive turgor pressure of the cells [20,26,30–32]. The plant's response to water deficit differs depending on the stage at which it is imposed. In fact, if it started in the beginning of the plant development, the inhibition of cell expansion results in a reduced leaf area, while if it started after a significant leaf area has developed, leaves will senesce and can fall off. These responses restrict the photosynthetic zone and contribute to the decline in the whole-canopy photosynthesis [25,33]. The reduction in photosynthesis, linked to the decrease in leaf water potential, depends on the stomata closure, with the consequence of a reduction in the conductance of the CO_2 diffusion [34–36]. The extent of damage to the photosynthetic mechanism, particularly to photosystem II (PSII), can be evaluated by the Chlorophyll *a* fluorescence technique, which is a non-destructive and rapid testing method [37,38]. Further advances in chlorophyll fluorescence techniques allowed the introduction of new parameter that takes into account all of the main photochemical processes, such as absorption and trapping of excitation energy, electron transport further than primary plastoquinone (QA) and dissipation of excess excitation energy [39]. This parameter is performance index (*PI*). It is a suitable and sensitive parameter that reflects the functionality of both photosystems I and II and that gives quantitative information on the current state of plant performance under different abiotic and biotic stress conditions [40–42].

The objectives of the present study were: to understand the ecophysiological behavior of 5 Tunisian cultivars in response to drought stress; to decide which parameter(s) to use for early screening of cultivars for real-time monitoring of their tolerance; and to jeopardize the future supply of olive production by selecting more tolerant cultivars.

2. Materials and Methods

2.1. Plant Material and Experimental Design

This study was conducted in 2021 at the Olive Institute at the specialized unit in Sousse. The research station latitude and longitude were 10° E and 35° N, respectively. One year-old plants of five cultivars 'Chemchali', 'Sayali', 'Jarboui', 'Besbessi' and 'Chetoui' were used in this experiment. All plants of the studied cultivars were transferred to 4 L pots containing peat and perlite in a 1:1 ratio, maintained for three months in full irrigation and nutritional conditions in a greenhouse.

The plants were homogeneous and of the same size, about 1.2 m high. Mean average day- and night-time temperatures in the greenhouse were 32 °C and 18.8 °C, respectively, while day- and night-time humidities were 65 and 85%, respectively.

Plants were subjected to drought stress from 2 May 2021 until 30 June 2021. The drought stress treatments were gradually imposed by withholding water. Four drought stress levels were considered and compared to a control treatment (T0), in which the soil water potential was kept constant because plants were well-watered daily. Drought-stressed plants showed mild water deficit after 15 days without watering (T15), moderate stress after 30 days without watering (T30), severe stress after 45 days without watering (T45) and more severe stress after 60 days without watering (T60). Control and drought-stressed trees were arranged in a complete randomized design with four replications. Drought stress (five levels, including the control treatment) and cultivars (five) were considered as treatments. In total, 100 olives trees were used.

2.2. Relative Water Content

The water status of the plants was identified by measuring the leaf relative water content (RWC). This RWC was calculated as: %RWC = 100 × (FW − DW)/(TW − DW). Where FW = fresh weight, DW = dry weight after 48 h drying at 80 °C, TW = turgid weight obtained after 48 h in distilled water at 4 °C in the dark [33,34]. RWC was performed with eight replicates for each treatment and for each cultivar.

2.3. Stomatal Resistance

Stomatal resistance (SR, s·cm^{-1}) was measured using a porometer (AP4, Delta-T Device, Burwell, Cambridge, UK). The measurements were carried out on leaves from the middle part of branches of each olive tree plant. Stomatal resistance was performed between 10:30 and 12:30 a.m. with 8 repetitions for each treatment and each cultivar.

2.4. Chlorophyll Fluorescence

Chlorophyll *a* fluorescence was measured by a Plant Efficiency Analyser Handy-PEA Plant Efficiency Analyser, (Hansatech Instruments Ltd., Norfolk, UK). After leaves' adaptation to darkness (30 min), the fluorescence of the Chlorophyll *a* was induced by applying a pulse of saturating red light (peak at 650 nm, 3000 mmol m^{-2}·s^{-1}). The data obtained were used to identify two biophysical parameters that describe the PSII maximal photochemical efficiency (F_V/F_M) and the performance index on absorption basis (*PI*).

PI parameter was calculated according to Strasser et al. [43] as follows:

$$PI = \frac{1-(F_0/F_M)}{(M_0/V_J)} \times \frac{F_M - F_0}{F_0} \times \frac{1-V_J}{V_J}$$

where F_0 is the fluorescence intensity at 50 µs, F_J is fluorescence intensity at the J step (at 2 ms), F_M represents maximal fluorescence intensity, V_J is relative variable fluorescence at 2 ms calculated as $V_J = (F_J - F_0)/(F_M - F_0)$ and M_0 represents initial slope of fluorescence kinetics, which can be derived from the equation: $M_0 = 4 \times (F_{300\mu s} - F_0)/(F_M - F_0)$. Moreover, F_V/F_M parameter is calculated according to the equation: $F_V/F_M = (F_M - F_0)/F_M$.

F_V/F_M and PI were performed with eight replicates for each treatment and for each cultivar.

2.5. SPAD Index

The leaf chlorophyll index or SPAD index was measured using the SPAD-502 m (Spectrum Technologies, Inc., Aurora, IL, USA). This non-destructive method was performed in the greenhouse with 10 replicates for each treatment and for each cultivar as described by Moula et al. [44].

2.6. Dry Matter Accumulation

At the end of the experiment, four plants were randomly harvested per cultivar. The plants were divided into root and shoot fractions. Dry mater was determined after drying the root and shoot fractions at 70 °C for 72 h.

2.7. Statistical Analysis

All the results were reported as the mean values of separate replications and a test of equal variances was conducted using Levene's test. The various parameters measured were the subject of a one-way analysis of variance (ANOVA) and a multiple comparison according to the Tukey's post hoc test with statistical significance at a 95% confidence level ($p < 0.05$). Two-way ANOVA was performed to see the interaction between the effect of water stress and the effect of cultivar. Multivariate analysis was performed by principal component analysis (PCA). All statistical analysis were processed with the program MINITAB (Minitab Inc. Version 18, Coventry, UK).

3. Results

3.1. Effect of Drought Stress on Relative Water Content

Table 1 shows that drought stress decreased the relative water content (RWC) of the leaves of the five cultivars studied. At the beginning of the experiment, the leaves of all plants were characterised by almost the same relative water content between 90.14% and 92.67%.

Table 1. Effect of drought stress treatments on five Tunisian olive cultivars.

Cultivar	T	RWC (%)	SR (s·cm^{-1})	F_V/F_M	PI	SPAD Index
Besbessi	T0	92.67 ± 1.31 ^{A,a}	1.39 ± 0.21 ^{B,d}	0.828 ± 0.003 ^{CD,a}	12.27 ± 0.41 ^{A,a}	90.41 ± 1.58 ^{A,a}
	T15	92.84 ± 1.27 ^{A,a}	2.03 ± 0.34 ^{B,d}	0.828 ± 0.004 ^{A,a}	11.68 ± 0.65 ^{A,a}	88.63 ± 2.80 ^{A,ab}
	T30	88.00 ± 0.99 ^{A,b}	3.76 ± 0.46 ^{B,c}	0.827 ± 0.006 ^{AB,a}	8.39 ± 0.68 ^{B,b}	84.03 ± 1.89 ^{B,bc}
	T45	78.99 ± 1.66 ^{A,c}	6.86 ± 0.52 ^{B,b}	0.801 ± 0.011 ^{A,b}	5.20 ± 0.65 ^{BC,c}	84.96 ± 1.22 ^{A,abc}
	T60	55.00 ± 1.20 ^{A,d}	13.73 ± 0.71 ^{A,a}	0.790 ± 0.01 ^{B,b}	3.72 ± 0.04 ^{D,d}	82.39 ± 1.30 ^{AB,c}
Chemchali	T0	92.17 ± 0.33 ^{AB,a}	1.37 ± 0.15 ^{B,d}	0.834 ± 0.004 ^{BC,a}	12.54 ± 0.67 ^{A,a}	89.34 ± 2.30 ^{A,ab}
	T15	90.15 ± 0.56 ^{AB,a}	2.03 ± 0.59 ^{B,d}	0.832 ± 0.007 ^{A,a}	11.33 ± 0.24 ^{A,ab}	90.39 ± 3.09 ^{A,a}
	T30	79.95 ± 2.81 ^{B,b}	44.9 ± 0.47 ^{AB,c}	0.830 ± 0.0006 ^{AB,a}	10.58 ± 0.31 ^{A,b}	89.13 ± 2.20 ^{A,ab}
	T45	73.33 ± 3.21 ^{A,b}	7.75 ± 0.65 ^{AB,b}	0.817 ± 0.005 ^{A,b}	8.056 ± 0.92 ^{A,c}	86.41 ± 2.09 ^{A,ab}
	T60	54.99 ± 6.61 ^{A,c}	11.69 ± 0.46 ^{B,a}	0.830 ± 0.0006 ^{A,a}	6.60 ± 0.44 ^{A,c}	82.97 ± 2.12 ^{AB,b}
Chetoui	T0	90.14 ± 0.22 ^{B,a}	1.25 ± 0.14 ^{B,d}	0.821 ± 0.002 ^{D,a}	12.52 ± 0.23 ^{A,a}	89.38 ± 1.74 ^{A,a}
	T15	87.91 ± 1.06 ^{B,a}	3.25 ± 0.41 ^{AB,c}	0.825 ± 0.002 ^{A,a}	10.89 ± 0.44 ^{AB,b}	89.47 ± 2.54 ^{A,a}
	T30	72.77 ± 2.25 ^{C,b}	6,00 ± 0.87 ^{A,b}	0.817 ± 0.010 ^{B,ab}	8.56 ± 0.58 ^{B,c}	84.18 ± 1.62 ^{B,ab}
	T45	60.70 ± 2.46 ^{C,c}	8.42 ± 0.32 ^{A,a}	0.799 ± 0.002 ^{A,b}	5.40 ± 0.16 ^{B,d}	79.45 ± 1.22 ^{B,b}
	T60	49.71 ± 1.33 ^{A,d}	9.66 ± 0.42 ^{C,a}	0.722 ± 0.012 ^{D,c}	1.57 ± 0.03 ^{C,e}	79.24 ± 2.58 ^{B,b}
Jarboui	T0	91.49 ± 0.77 ^{AB,a}	2.00 ± 0.14 ^{A,c}	0.841 ± 0.004 ^{B,a}	11.66 ± 0.45 ^{a,a}	89.11 ± 1.88 ^{A,a}
	T15	92.11 ± 2.00 ^{A,a}	4.48 ± 0.66 ^{A,b}	0.831 ± 0.004 ^{A,a}	10.21 ± 0.18 ^{b,b}	87.18 ± 1.22 ^{A,a}
	T30	77.27 ± 0.61 ^{BC,b}	5.94 ± 0.83 ^{A,a}	0.836 ± 0.014 ^{AB,a}	9.13 ± 0.23 ^{b,c}	86.87 ± 0.23 ^{AB,a}
	T45	55.00 ± 1.70 ^{C,c}	6.81 ± 0.20 ^{B,a}	0.800 ± 0.007 ^{A,b}	3.68 ± 0.06 ^{c,d}	85.43 ± 2.43 ^{A,ab}
	T60	37.00 ± 1.00 ^{B,d}	7.10 ± 0.40 ^{D,a}	0.745 ± 0.005 ^{C,c}	1.51 ± 0.33 ^{c,e}	81.07 ± 1.89 ^{AB,b}
Sayali	T0	92.50 ± 0.68 ^{A,a}	1.56 ± 0.13 ^{B,d}	0.851 ± 0.001 ^{A,a}	12.30 ± 0.128 ^{A,a}	91.14 ± 1.57 ^{A,a}
	T15	92.02 ± 2.11 ^{A,a}	2.46 ± 0.30 ^{B,d}	0.839 ± 0.010 ^{A,ab}	10.99 ± 0.19 ^{AB,b}	88.52 ± 0.92 ^{A,a}
	T30	86.49 ± 2.80 ^{A,b}	3.53 ± 0.26 ^{B,c}	0.842 ± 0.005 ^{A,a}	10.46 ± 0.15 ^{A,b}	90.10 ± 1.10 ^{A,a}
	T45	67.30 ± 0.64 ^{B,c}	5.00 ± 0.55 ^{C,b}	0.795 ± 0.021 ^{A,c}	4.92 ± 0.67 ^{BC,c}	88.60 ± 0.436 ^{A,a}
	T60	53.00 ± 1.090 ^{A,d}	8.03 ± 0.39 ^{D,a}	0.811 ± 0.002 ^{A,bc}	3.14 ± 0.52 ^{B,d}	85.03 ± 0.97 ^{A,b}

Table 1. *Cont.*

Cultivar	T	RWC (%)	SR (s·cm^{-1})	F_V/F_M	*PI*	SPAD Index
p-value	Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Treatment	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Cultivar × Treatment	<0.0001	<0.0001	<0.0001	<0.0001	0.009

Values represent the means ± standard deviations. Different capital letters in the same column indicate significantly different values per $p < 0.05$ between different cultivars for the same treatment and different lower-case letters in the same column indicate significantly different values per $p < 0.05$ between different treatments for the same cultivar. All cultivars have the same value for this treatment and there is no significant difference.

(T15) showed no significant effect on RWC for any of the cultivars. RWC decreased by 5%, 15% and 40%, respectively, for Besbessi olive trees under moderate, severe and severe water stress compared to well-watered plants. For Chemchali cultivar, RWC decreased by 13%, 20% and 40% for T30, T45 and T60 treatments, respectively, compared to T0. For Sayali cultivar, the decrease was quantified at 6.5%, 28% and 43% for T30, T45 and T60 treatments, respectively. For Jarboui, the most significant decrease was observed in T60, followed by Chetoui with 64% and 45%, respectively.

3.2. Effect of Drought Stress on Stomatal Resistance

The monitoring of stomatal resistance showed an increase in this parameter according to the intensity of the drought stress. This increase was progressive in all cultivars.

However, after 2 months of stress (T60), statistical analysis showed significant differences in the increase of SR between the five cultivars (Table 1). The highest values were around 13.7 s·cm^{-1} and 11.7 s·cm^{-1} for Besbessi and Chemchali, respectively.

3.3. Effect of Drought Stress on PSII Maximal Photochemical Efficiency (F_V/F_M) and Performance Index (PI)

Table 1 shows the evolution of (F_V/F_M) during the experiment. The decrease of F_V/F_M started at T45. However, there was no significant difference between the five cultivars. However, after 60 days of progressive drought stress, Besbessi, Chetoui and Jarboui showed a significant decrease in F_V/F_M compared to the other two cultivars.

The performance index (*PI*) is a sensitive indicator of drought stress in plants [40]. Table 1 shows the evolution of the *PI* during the stress treatments. At the beginning of the treatment, the *PI* showed stability for all cultivars. A decrease was then observed during the course of the drought stress. The T60 treatment showed a reduction in *PI* of 87.5%, 87%, 74%, 70% and 47% for Chetoui, Jarboui, Sayali, Bessbassi and Chemchali, respectively.

3.4. Effect of Drought Stress on SPAD Index

At the beginning of the drought stress treatment, a slight decrease in the SPAD index values was observed, but no significant difference was found between the cultivars studied (Table 1). During the T30 treatment, Chetoui starts to be the most affected cultivar in terms of SPAD index until it reaches the lowest value towards the end of the experiment with 79.24.

3.5. Effect of Drought Stress on Dry Matter Accumulation

At the end of the experimental period, dry matter (DM) accumulation was signif cantly affected by the drought stress treatments (Table 2). DM showed a drastic decrease for all cultivars. A pronounced decrease was observed for Jarboui (85%) compared to the control plants. Significant differences were observed among cultivars.

3.6. Principal Component Analysis

Principal component analysis (PCA) was applied to the five measured physiological parameters for all the cultivars studied. The first two principal components (PCs) explained 93.9% of the total variance (PC1: 85.3%; PC2: 8.6%). The loading plot in Figure 1A shows that the majority of the studied traits are positively correlated with the first component PC1, and only the stomatal resistance parameter is negatively correlated with PC1. Regarding the second component PC2, there is a negative correlation with four traits, with the highest eigenvalue registered for F_V/F_M, which is considered to be the strongest loading factor, followed by SR and a positive correlation with two traits, biomass and relative water content. Figure 1B shows a clear separation between the physiological behavior of the olive plants in the initial state before the drought stress treatment T0 and the olive plants after 60 days of progressive drought stress.

Table 2. Effect of drought stress treatments on dry matter accumulation of five Tunisian olive cultivars.

Cultivar	Treatment	
	T0	T60
Besbessi	139.69 ± 19.21 ^{D,a}	27,91 ± 0.38 ^{C,b}
Chemchali	177.01 ± 8.54 ^{B,a}	31.075 ± 0.79 ^{B,b}
Chetoui	149.13 ± 0.85 ^{CD,a}	27.03 ± 1.98 ^{C,b}
Jarboui	251.78 ± 2.47 ^{A,a}	37.130 ± 0.36 ^{A,b}
Sayali	174.80 ± 15.81 ^{BC,a}	32.11 ± 0.43 ^{B,b}

Values represent the means ± standard deviations. Different capital letters in the same column indicate significantly different values per $p < 0.05$ between different cultivars for the same treatment, and different lower-case letters in the same row indicate significantly different values per $p < 0.05$ between different treatments for the same cultivar.

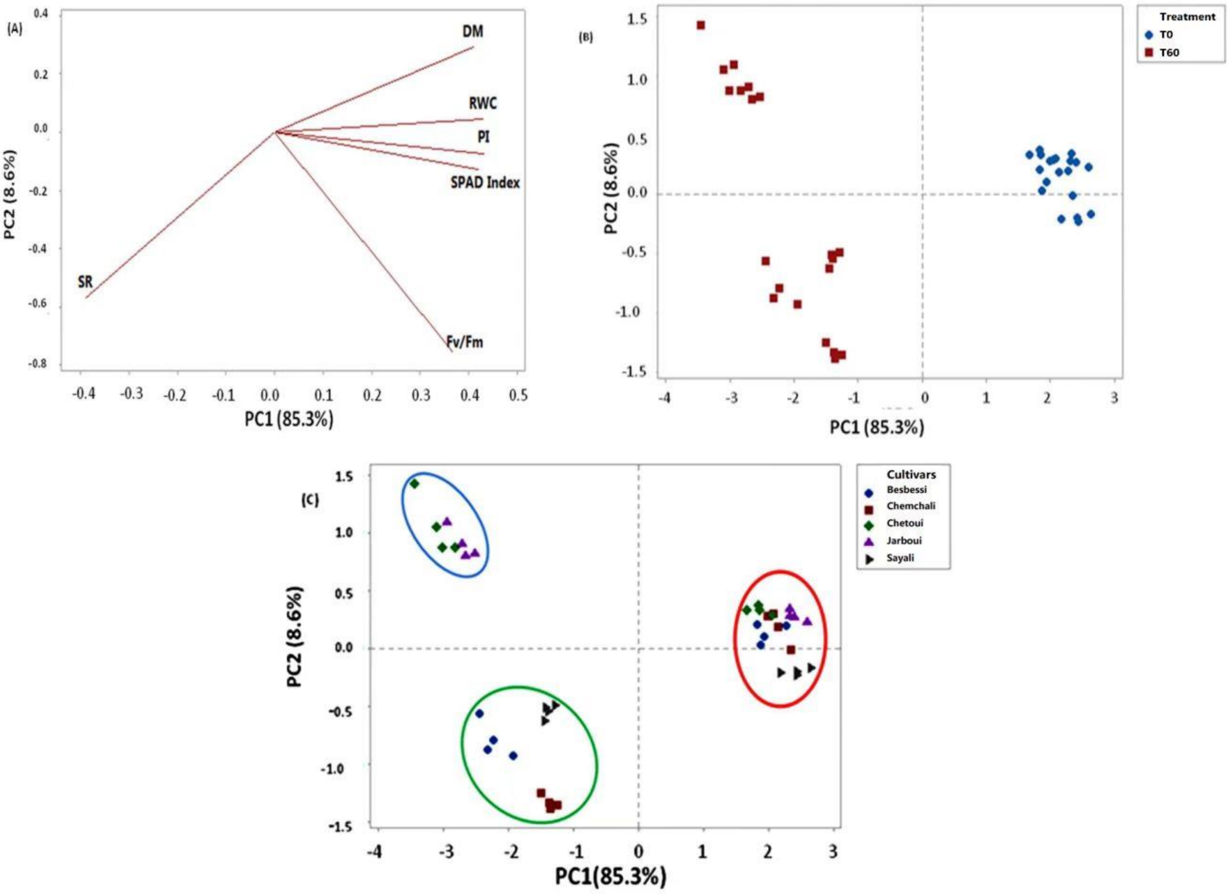

Figure 1. Principal component analysis of olive samples from five cultivars according to six agronomical and physiological parameters: (**A**) loading plot of samples and studied parameters; (**B**) samples distribution according to the measurement dates T0 and T60; (**C**) samples distribution according to cultivars.

The distribution of these samples on the axes of the first and second principal components allowed three groups to be distinguished (Figure 1C): The group of well-watered olive trees (in the red circle), the group of varieties Chemchali, Sayali and Besbessi (in the green circle) and the group of varieties Chetoui and Jarboui (in the blue circle). The cultivars in the third group were most affected by drought stress and were characterized by the highest decrease in (F_V/F_M).

4. Discussion

Leaf relative water content (RWC) is a commonly used indicator to evaluate plant water status and drought resistance [45]. In this sense, RWC provides measurement of the 'water deficit' of plant leaves and may indicate a degree of stress expressed under unfavorable conditions such as drought [46]. Previous studies used RWC measured for both control and stressed olive cultivars as a first indication about the response to drought stress [19,20,47,48]. In the present study, a significant decrease of RWC was found after 30 days for all the cultivars. After 60 days of drought stress, Besbessi, Chemlali, Sayali and Chetoui cultivars show a similar RWC. Only Jarboui reached very low level of RWC (<50%).

The reduction of the water status observed for all the studied cultivars has been followed by a stomatal resistance (SR) increase. This can be explained by the closing of the stomata due to drought stress [19,49]. In fact, stomatal closure is an efficient way to preserve water under this situation, and to adjust CO_2 input sufficiently for optimal photosynthesis [50].

A significant correlation was found between RWC and SR under increasing levels of drought stress (Figure 1A). The distribution of the values showed a linear relationship. Increasing levels of drought stress led to decreasing values of RWC and increasing SR simultaneously, compared to control plants.

The distribution of the values showed a linear relationship. Maximum amounts of RWC and minimum values of SR were achieved in control plants T0. In our study, non-stressed plants presented the values of SR < 4 $S{\cdot}m^{-1}$ and the values of RWC > 80%.

Based on the regression illustrated in Figure 2A, two drought stress ranges could be identified for all cultivars amongst increasing drought levels. The first range is "moderate stress", which corresponded to values of RWC in the interval of 80–60% and of values SR in the interval of 4–8 S m^{-1}. The second range is "severe stress", which presented the values of RWC < 60% and the values of SR < 8. Practically, plants grown under "moderate stress" were drought stressed of 30 and 45 days and those grown under "severe stress" were stressed with 60 days of drought.

As reported for several Mediterranean species, including olive [19,20,51,52], chlorophyll was negatively affected by stress imposition in all cultivars. In this sense, leaf Chlorophyll meter (SPAD-502) has been used with various crops as an indirect indicator of plant Chlorophyll content [53].

Jarboui and Chetoui present a low SPAD index (Table 1) after 60 days of drought stress. Chlorophyll loss, due to pigment degradation, may be a consequence of oxidative stress increases [19,48,54]. Moreover, it may represent an adaptative feature to cope with stress, particularly in species usually exposed to excess of excitation energy as olive trees, resulting in lower leaf light absorbance that contribute to photoprotection [52]. The fact that F_V/F_M and *PI* were negatively affected, simultaneously with chlorophyll, also suggests that this stress induced damages in the photosynthetic apparatus of all cultivars, and specifically for sensitive ones, which supports its higher susceptibility.

Exposure of leaves to increasing drought stress resulted in subsequent decreases in F_V/F_M (Table 1). F_V/F_M and *PI* decreased only slightly for Besbessi, Chemchali and Sayali, and more substantially for Jarboui and Chetoui, after 60 days of drought stress. The decreases in F_V/F_M and *PI* can be described as a down-regulation of PSII that reflects the protective or regulatory mechanism to avoid photodamage of the photosynthetic apparatus [19].

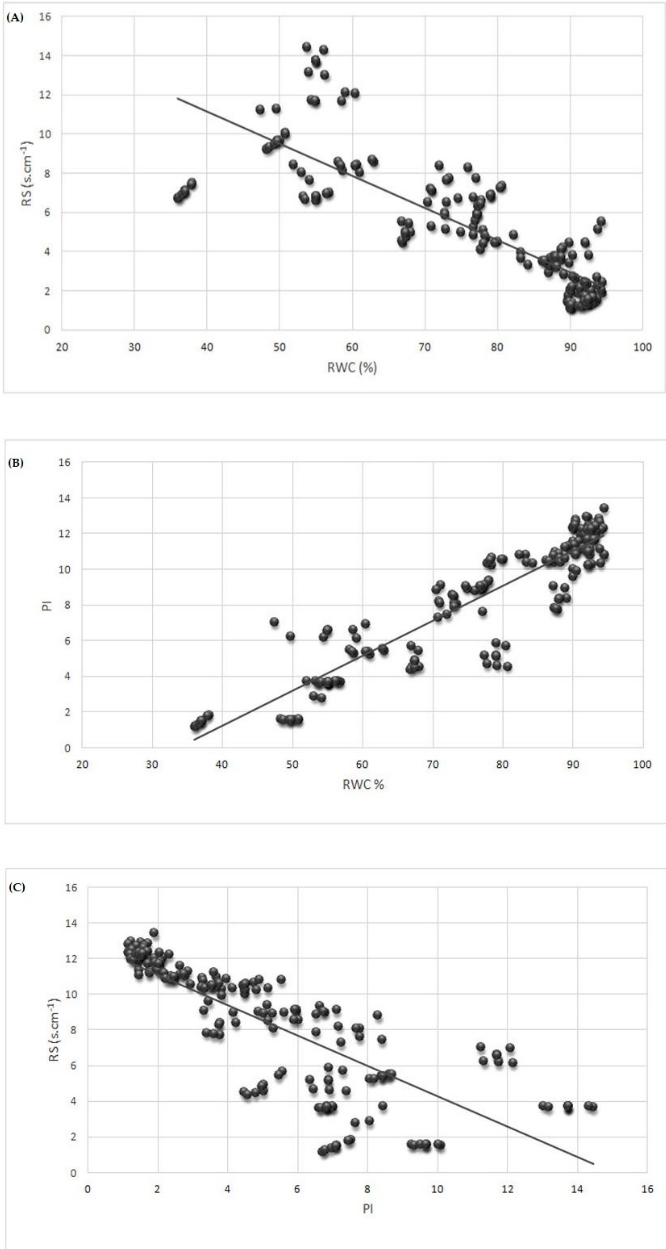

Figure 2. The relationship between SR and RWC (**A**), between *PI* and RWC (**B**) and between SR and *PI* (**C**). Regression lines are fitted to the data. The equation of these relationships is SR = −0.153 RWC + 16.79 (r^2 = 0.69; n = 200; p = 0.001); *PI* = 0.195 RWC − 6.55 (r^2 = 0.86; n = 200; p < 0.0001); SR = −0.917 *PI* + 13.03 (r^2 = 0.64; n = 200; p < 0.0001).

A significant relationship was determined between SR, *PI* and RWC (Figure 2B,C). The *PI* is therefore a sensitive parameter to water statute for olive trees. In contrast to this, a continuous decrease in *PI* parameter was observed from the very beginning of dehydration following the decrease of RWC [40,55]. Statistically, significant differences were also found in the *PI* parameter among all tested olive cultivars. The results show that *PI* may serve as an index of plant/cultivar vitality and/or sensitivity to drought stress reflecting their different drought tolerance.

To classify cultivars according to their degrees of tolerance to drought stress, Lepeduš et al. [39] confirmed that the performance index is one of the main reliable parameters of chlorophyll fluorescence.

To demonstrate the sensitivity of these non-destructive parameters to drought stress, the dry matter accumulation (DM) was assessed. DM showed that the most affected cultivar is Jarboui and the most tolerant is Besbessi. The integration of DM in the PCA confirmed the classification of cultivars according to their tolerance to drought stress.

According to the results of the present study, Chetoui and Jarboui are the most sensitive cultivars to drought stress, while Chemchali, Besbessi and Sayali were the most tolerant ones. These conclusions were confirmed by the PCA.

Finally, the above-mentioned parameters were ranked according to their sensitivity to detect stress, allowing decision-making on which parameter(s) should be used for early screening of tolerant cultivars. F_V/F_M and SR were considered as the strongest loading factor of screening drought.

These cultivars, Besbessi and Sayali in the north of Tunisia and Chemchali in the south, can present a possible alternative to replace the local or foreign cultivars most cultivated in the country which are characterized by high water needs.

In conclusion, the introduction of new cultivars far from their traditional growing areas without any previous testing in the new locations cannot be considered as good agricultural practice. The tolerance and adaptation of these cultivars to even dryer conditions need to be investigated. The handy PEA and the SPAD-502 can be used as a rapid tool to support site-specific water management in olive orchards. Furthermore, this study indicates that the nondestructive parameters will be able to help in the investigation of tolerant cultivars.

5. Conclusions

After analyzing the data, we can conclude that the five studied cultivars showed different drought-tolerance levels when subjected to water stress. In fact, two groups were distinguished, the most drought-sensitive cultivars Chetoui and Jarboui, and the drought-tolerant ones Besbessi, Sayali and Chemchali. These tolerant cultivars could be better known and appreciated by Tunisian olive growers for large-scale plantations in future as a strategy to replace water-intensive cultivars and address drought issues.

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