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Effect of Different Climate Conditions on the Physiological and Biological Responses of Young Olive Trees (*Olea Europaea L.*)

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Abstract

The effects of variable climatic conditions on physiological and biological traits were investigated in 2-year-old olive plants *(*Olea europaea L cvs. Chemlali and Chetoui) grown in pots. The studies were carried out during 2013 and 2014 growing seasons in three Tunisian different olive growing areas (North, Central and South). Vegetative growth and leaf measurements included chlorophyll fluorescence parameters, stomatal conductance; stomatal density, trichome density and leaf density were investigated. Based on our results, we found considerable genotypic differences between the two cultivars.

Chemlali exhibited more tolerance to different climatic conditions, with a higher stomatal and trichome densities in the south of Tunisia. Also, Chemlali leaves revealed lower leaf area and had higher density of foliar tissue. The morphological and structural characteristics of the leaves are in accordance with physiological observations (better stomatal regulation and photosynthetic activity as expressed by the measurement of the maximum quantum yield of PSII (Fv/Fm)) and contribute to the interpretation of why the olive cv. Chemlali is well-adapted to different climate conditions than Chetoui cultivar. Furthermore, from the behavior of Chemlali plants we consider this cultivar very promising for cultivation in arid areas.

Keywords: Olea Europaea L; Climatic Conditions; Leaf Anatomy; Cultivar; Stomatal Conductance; Adaptation

Abbreviations: FW: Fresh Weight; TW: Turgid Weight; DW: Dry Weight; VPD: Vapour Pressure Deficit; RWC: Relative Water Content.

Introduction

Agriculture sector is highly exposed to climate change, as farming activities directly depend on climatic conditions. Climate change affects agriculture in a number of ways, including through changes in average temperatures, rainfall, and climate extremes such as heat waves. The Mediterranean has been considered as one of the regions of the world to be more affected by climate change during the course of the 21st century [1]. Indeed, reported a significant increase in minimum temperatures and moderate increase in maximum ones. Precipitation of North Africa is characterized by a wet season in winter and dry conditions in summer. The rainy season, from October to April, has its maximum in December, January and February [2-4] is a schlerophyllous evergreen Mediterranean tree species capable to acclimate to long dry periods, with little or no precipitation, to high temperatures and high irradiance [5].

Among these environmental factors, soil and atmospheric water deficit are the most important factors that limit photosynthesis, growth and survival of plants growing under these climate conditions [6]. Olive culture is one of Tunisia's main agricultural activities. It plays a fundamental social and economic role, given that 60% of the country's farmers work in this area and draw all or part of their revenues from it. In Tunisia, more than 95% of the olive-growing area is under rainfed conditions and 70% of this area is located in semi-arid, arid or desert areas. There are two main Tunisian olive cultivars, Chemlali and Chetoui. 'Chemlali' cultivar is cultivated in the south and the centre of the country, areas characterized by a low rainfall (<250 mm per year), and 'Chetoui' in the north. These two cultivars account for 95% of the total olive tree orchards and contribute more than 90% of the national production of olive oil.

Although the olive tree represents a typical drought-stress tolerant plant that has developed adaptive anatomical, physiological and biochemical mechanisms to withstand environmental stress [6-10] the responses to abiotic stress have proved to be quite variable among cultivars, due to the high genetic diversity of the species [11].The current situation and the prediction trend indicate a general decline in water availability for agriculture as a result of both the increase in domestic use and global environmental change [12].For these reasons, advancement in the current understanding of the responses of olive trees to environmental stress has become a major target for research in order to improve management practices and breeding efforts in agriculture and to predict the fate of natural vegetation under climate change. The aim of this work is to evaluate the effects of different climatic conditions on several physiological and biological parameters of the two main Tunisian olive cultivars, Chemlali and Chetoui. Specifically, we compared these two olive cultivars under different climatic conditions (North, central and South of Tunisia) to identify morphological and structural adaptations.

Materials and Methods

Plant material and experimental design

The experiment was carried out from September 2013 to August 2014. It was conducted in three different geographical locations; in the north of Tunisia at the Higher School of Agriculture of Kef (36°10'56''N; 8°42'53"E), in central Tunisia at the Olive Tree Institute of Sfax (34°44'N; 10°46'E) and in the Tunisian south at the Institute of Arid Regions in Medenine (33°21'17"N ; 10°30'19"E). Two-years-old own-rooted olive trees (Olea europaea L. cvs. Chemlali and Chetoui) were produced in the National Office of Olive Oil, then, transplanted in 20-L plastic pots containing freely drained light soil with a pH of 7.6, a field capacity of 25% (-0.02 MPa) and permanent wilting point of 11 % (-1.5 MPa). After 3 months of acclimatization in a greenhouse in Sousse, olive plants were placed in each location. Plants were homogeneous (of 25 cm in height) grown under natural conditions and irrigated twice a week with tap water. For each location, 18 plants per cultivar were used. After three months of plant acclimatization at each site, the experiment began in September 2013 and measurements were made in November (2013), April (2014) and July (2014).

Climatic conditions

Thirty year records from weather stations located at the three stations (Kef, Sfax and Medenine) were used to characterize their climate. Averages of rainfall and air temperature of thirty years were recorded (Figure 1). According to Emberger's classification [4] the climate at the experimental sites was semi-arid in the north and arid in the Central and the south of Tunisia. The Emberger's pluviometric quotient (Q), which expresses the aridity of the Mediterranean climate (the smaller the quotient, the drier the climate), was 46.4, 19.1 and 16.2 for Kef, Sfax and Medenine, respectively. The minimum temperature of the coldest month was 3 °C for Kef and 7 °C for both Sfax and Medenine. The mean of annual rainfall was 319 mm in Kef, 143 mm in Sfax and 135 in Medenine (mean of 30 years), mainly distributed from November to January as evident from (Figure 1) (Walter and Lieth 1967). The climatic conditions (Mean temperature and precipitation) over the study period were monitored using a meteorological station installed in each location. Annual precipitations of 2013 and 2014 were 574 and 441 mm in Kef, 284 and 251 mm in Sfax and 217 and 221 mm in Medenine.

Growth measurements

During the experiment, eighteen plants per cultivar for every location were selected. The length of the main shoot (in cm) was measured with a tape. These measurements were performed during November (2013), April (2014) and July (2014).

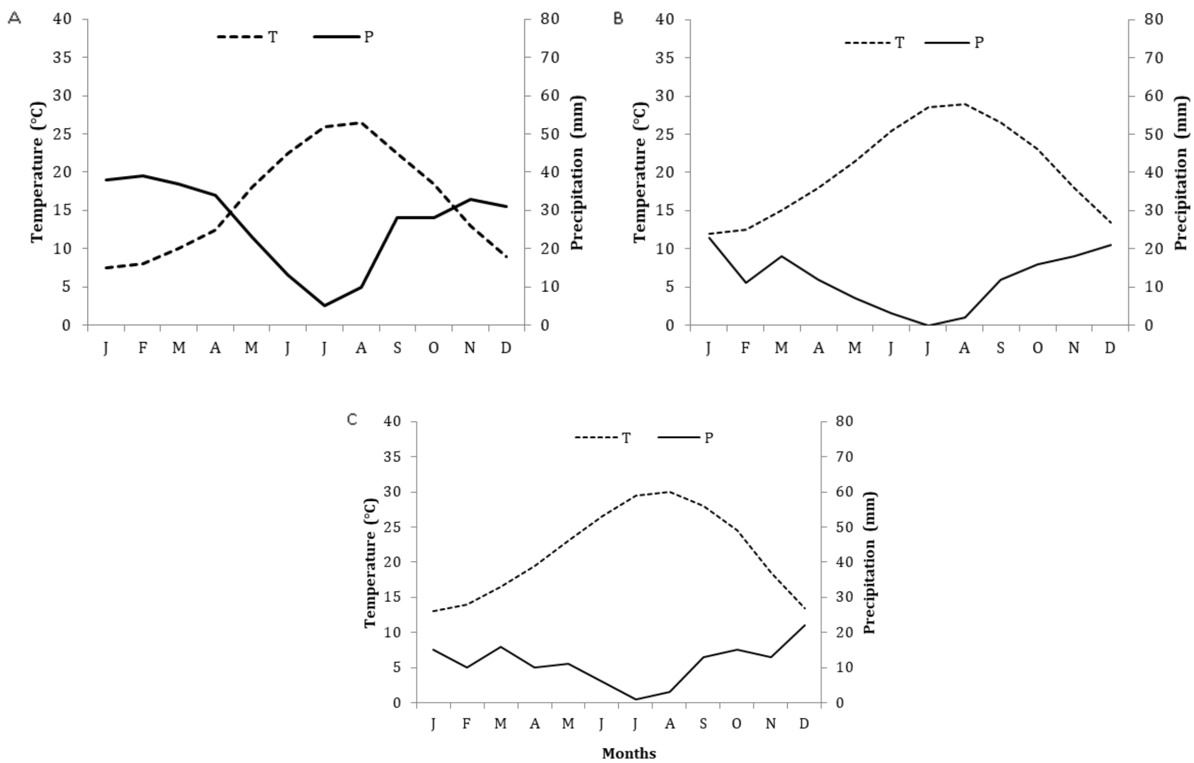


Figure 1: Walter and Lieth's climate diagram determined over the 1984–2014 period at Kef (A), Sfax (B) and Medenine (C). The broken line represents the monthly mean temperature, the solid line monthly precipitations. The area between the lines indicates the dry season.

Leaf characteristics

At the end of the experiment, leaf area per plant was measured with a WinDIAS Colour Image Analysis System (Delta-T Devices Ltd, Cambridge, UK) and leaf density (D) was calculated as:

$$D = (DM/FM) * 1000$$

Where
DM: Dry matter
FM: Fresh matter (Dijkstra, 1989).

Stomatal density was assessed by using an optical Leitz microscope (Leitz DIA LUX 22EB), equipped with a digital camera (Hitachi KP-D 40 Color Digital). Stomata were counted with the analysis software program for Image Analysis (Delta-T Devices Ltd., Cambridge, UK). Leaf imprints were taken from abaxial leaf surface, using nail polish, on 6 leaves per cultivar per location (3 separate regions per leaf). These imprints were later examined under a high magnifications (250×) microscope, and stomatal density (number of cells per surface area) was counted. This parameter was measured throughout the experiment (autumn, spring and summer).

Leaf relative water content

During the experiment, five leaves per plant in a similar position were detached to determine their relative water content (RWC) with six replicate plants for each cultivar. After cutting, the petiole was immediately immersed in distilled water inside of a glass tube, which was immediately sealed. The tubes were then taken to t he laboratory where the increased weight of the tubes was used to determine leaf fresh weight (FW). After 48 h in dim light, the leaves were weighed to obtain the turgid weight (TW). The dry weight (DW) was then measured after oven drying at 80 °C for 48 h, and RWC was calculated as:

$$RWC = 100\times((FW-DW)/(TW-DW))$$

Chlorophyll fluorescence measurements

Chlorophyll a fluorescence was measured with a portable fluorometer (OS1-FL Modulated Fluorometer) at midday on eight attached intact leaves from each cultivar in each location. Prior to the measurements, a small part of the leaves was kept in the dark for 30 min using clips for dark adaptation. A 5-s light pulse at 400 μmol m^{-2} s^{-1} was used. We evaluate the minimal fluorescence (F0), which occurs when all PSII reaction centres are open, and maximal fluorescence (Fm), which occurs when all PSII reaction centres are closed. The difference between F0 and Fm is variable fluorescence, Fv. Maximum quantum yield of PSII was estimated by the Fv/Fm ratio (Maxwell and Johnson). Chlorophyll fluorescence measurements were made during November 2013, April 2014 and July 2014.

Photosynthetic pigments

During the experiment, leaf discs were taken from five fully expanded leaves of comparable physiological age. Leaf sections were ground in 80% acetone for chlorophyll and carotenoid determination. Total chlorophyll Chl (a+b) concentration was determined according to Arnon (1949) and total carotenoids (Car) concentration as described in [14]. Measurements were made during November 2013, April 2014 and July 2014.

Stomatal conductance

Stomatal conductance (gs, mol m^{-2} s^{-1}) was measured with an 'AP4 leaf porometer' (Delta-T Devices, UK). Measurements, made during November (2013), April and July (2014), were carried out between 11:00 h and 12:00 h on five well-exposed leaves per cultivar in each location.

Statistics

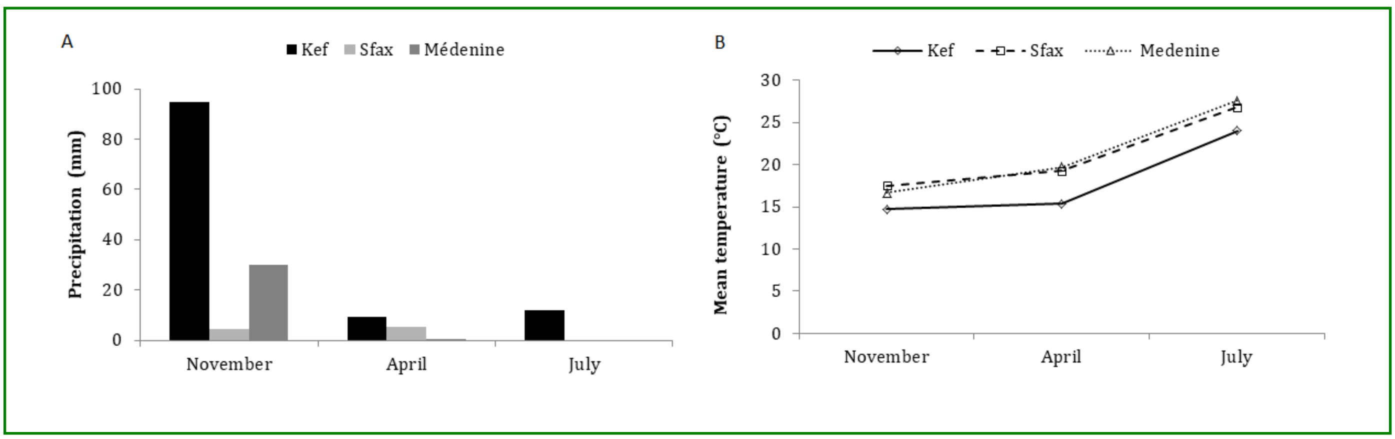
A two-way analysis of variance (ANOVA) was used to examine cultivar and geographical station effects on physiological and biological traits of olive plants using the SPSS for windows 16.0software. Significant different means were separated using the Tukey's test calculated at 5% level. At least, 3 replicates were used for each laboratory tests.

Result

Environmental parameters of the experimental sites

Weather data are summarized in (Figure 2) as mean temperatures (°C), rainfall (mm) and vapour pressure deficit (VPD). July and August were the hottest months and December and January the coldest. Rainfall occurred mainly from September to December while June, July and August were typically dry (Data not shown). No rainfall was recorded in July or August 2014 in both Sfax and Medenine. The rainfall pattern in the trial period was characterized by a little rain in autumn (from September to November) and in spring.

The most important quantity (152 mm) was recorded in Sfax at the end of autumn (November). The experimental period was characterized by a moderate temperature during spring time (16, 20 and 22.3 °C, respectively in Kef, Sfax and Medenine) and a high temperature average from June to August (23, 27.6 and 30.4 °C, respectively in Kef, Sfax and Medenine). Measurements of VPD during November, April and July showed that Kef had the lowest values comparing to Sfax and Medenine. The highest VPD (2.1 kPa) was recorded in July in Medenine.



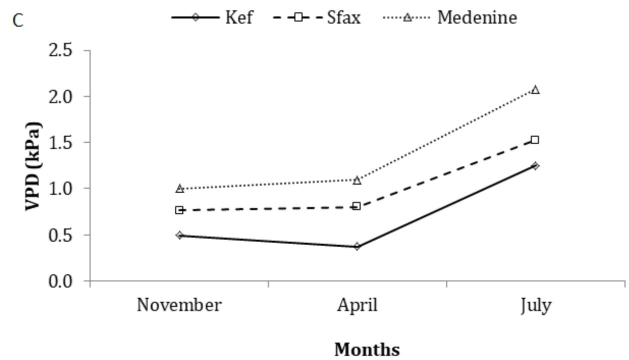


Figure 2: Monthly precipitations (A), mean air temperature (B) and vapour pressure deficit (VPD) (C) values in the three locations (Kef, Sfax, Medenine) during November, April and July.

Stomatal conductance (gs)

Significant differences in stomatal conductance (gs) were observed among cultivars (figure 3). Chetoui had the highest values of gs during the trial period except in Kef where Chemlali showed higher values of conductance (0.65, 0.61 and 0.56 mol m^{-2} s^{-1} in November, April and July, respectively) than Chetoui. For both periods of measurements, the interactions between cultivar and station were highly significant ($P < 0.001$) (Data not shown).

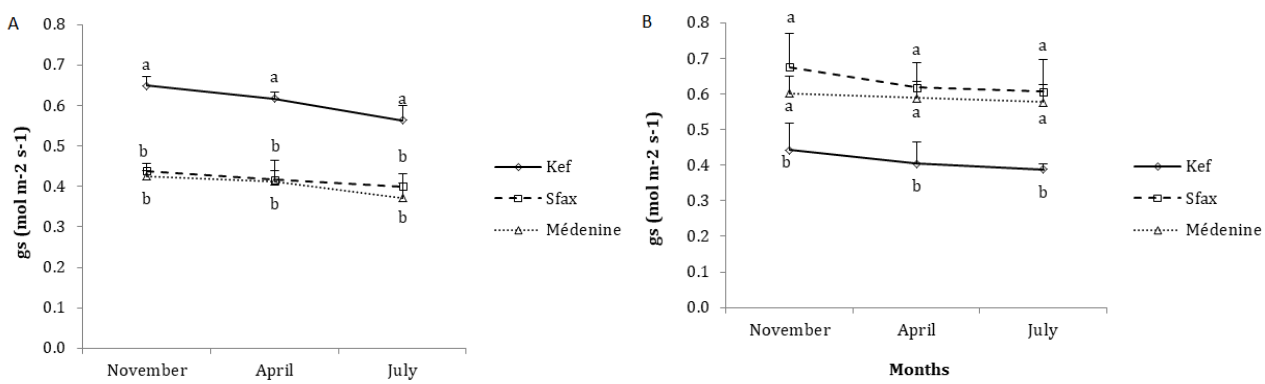


Figure 3: Measurements of the stomatal conductance (gs) in two olive cultivars; Chemlali (A) and Chetoui (B) in the three locations (Kef, Sfax, Medenine) during November, April and July. Means followed by different letter are not significantly different at $P \leq 0.05$.

Leaf relative water content

During the experimental period, leaf relative water content showed highly significant differences between stations. During November, significant difference was recorded between cultivars only in Medenine (Figure 4). While, in April and July, when the interaction between genotype and station were significant, cultivars showed significant changes in leaf relative water content (RWC) in both Kef and Medenine. Chemlali presented the most important decrease of RWC in Kef, from 99% in autumn to 77% in summer. Also, Chetoui exhibited a high decline of RWC in Medenine of about 9% from autumn to summer.

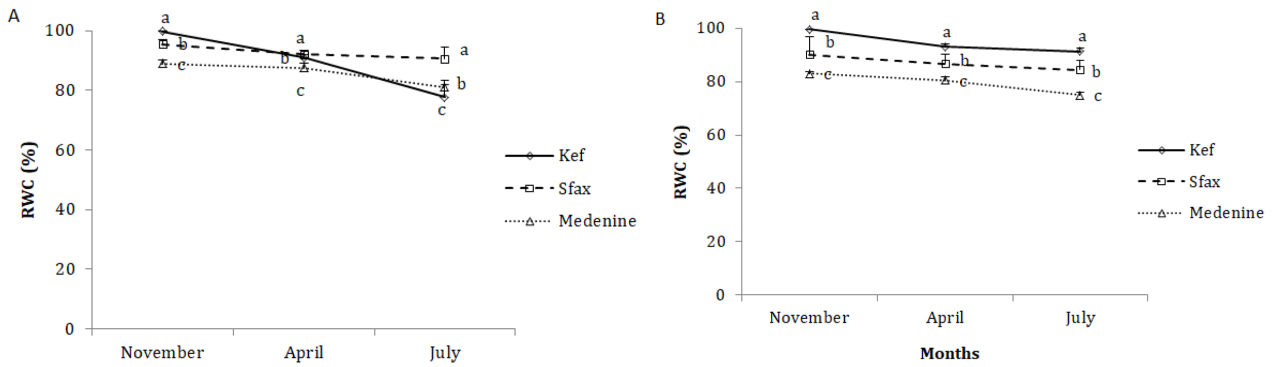


Figure 4: Measurements of leaf relative water content (%) in two olive cultivars; Chemlali (A) and Chetoui (B) in the three locations (Kef, Sfax, Medenine) during November, April and July. Means followed by the same letter are not significantly different at P ≤0.05.

Chlorophyll fluorescence measurements

As indicated in (Figure 5) the maximum quantum yield of PSII (Fv/Fm) showed few differences between cultivars. During November, no significant change was observed between cultivars, while a high significant difference was recorded between stations (P < 0.001). In April, we note that Fv/Fm exhibited great significant changes between cultivars in both Kef and Medenine. In summer (July), differences between station, cultivars and the interaction between these two variables were statistically significant.

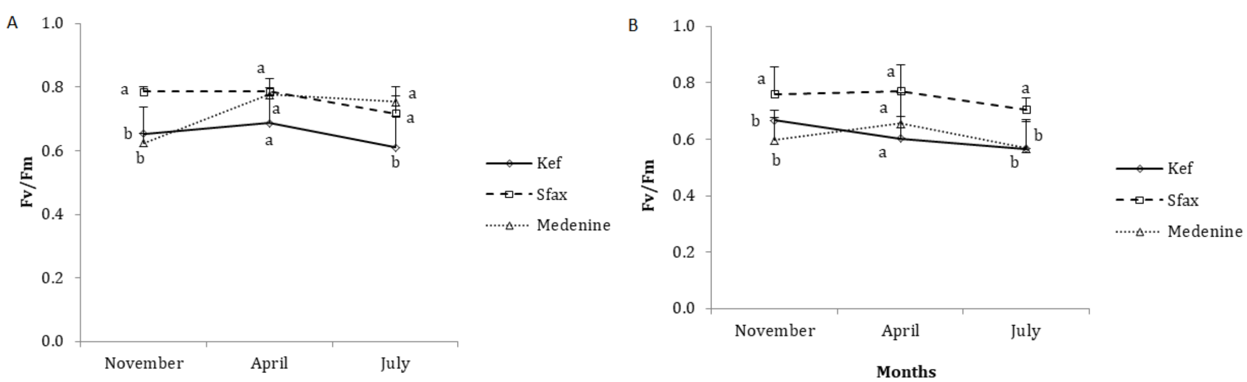


Figure 5: Measurements of the shoot elongation (cm) in two olive cultivars; Chemlali (A) and Chetoui (B) in the three locations (Kef, Sfax, Medenine) during November, April and July. Means followed by the same letter are not significantly different at P ≤0.05.

Discussion

Climatic conditions (Monthly mean temperature and precipitation) during the experiment were typically of a Mediterranean environment and representative of the study areas. During summer, high air temperatures induced a decrease of atmospheric humidity and an increase of transpiration. Atmospheric vapour pressure deficit (VPD) is amongst the most important environmental variables affecting leaf gas exchange, and their effects on reducing gs have been observed in many species[22,37]. The effect of VPD on plant physiology has been shown to be especially important in woody plants, where it is the main variable affecting their diurnal evolution of transpiration and stomatal conductance. In the present study, Kef area presented the lowest VPD values comparing to Sfax and Medenine. For the Chetoui cultivar, gs was higher in Kef when VPD was the lowest. Whereas, for the Chemlai cultivar, gs was the highest when VPD was higher. These findings show a differentiation between cultivars against VPD. In fact, changes in stomatal conductance (gs) in response to environmental and physiological signals represent the primary way that plants regulate gas exchange and water flow through the soil-plant-atmosphere continuum over the short-term [2]. As vapor pressure deficit between leaf and air increases, stomata generally respond through partial closure. In most cases, stomatal conductance decreases exponentially with increasing VPD [18].There are contradictory observations on the response of olive trees to VPD under field conditions. Indeed the stomatal closure response to increasing VPD generally results in a non-linear rise in transpiration rate (per unit leaf area, E) to a plateau and in some cases a decrease at high VPD [19-21].

By escaping high transpiration that would be caused by increasing atmospheric vapour pressure deficit, stomatal closure avoids the corresponding decline in plant water potential [22]. It is a reasonable premise that the closure response evolved to prevent excessive dehydration and physiological damage. While [24] found no correlation between gs and VPD, Bongi and [23] found low responsiveness of gs to VPD and [1] reported a negative upper-bound relationship when VPD was lower than 3.5 kPa. The reciprocal offset with the response of gs to other environmental factors correlated to VPD, such as radiation [24] may explain why VPD seemed not to affect gs. For the 'Chemlali' cultivar, our results were supported by the study of [10] who found a gradual decrease in gs and the response was earlier and more marked in 'Chemlali' than in 'Meski', suggesting a more efficient stomatal response to stressful conditions [3]. Confirmed that leaf water status interacts with gs and transpiration [25]. Reported that leaf stomatal conductance, in olive trees, was high during autumn and low during summer when vapour pressure deficit was high.

The stomatal regulations influenced RWC that decreased significantly when VPD and air temperature increased as well for plants in Kef (North of Tunisia) as ones in Medenine (South of Tunisia). In July, the RWC of Chetoui placed in Sfax and Medenine reached, respectively, 84% and 75%. For Chemlali placed in Kef, it was about 77% which is considered the turgor loss point for olive (Hinckley et al. 1980) [26] found that a reduction in RWC from 96 to 65% induced an 85% reduction in photosynthesis of potted olive trees. Thus, the low RWC of both cultivars likely inhibits photosynthesis during the summer. Since leaves are the main organs of internal water removal, stressed olive plants undertake leaf anatomical alterations in order to save water as described by [11]. According to our results, leaf area was higher for Chemlali plants placed in Sfax and Medenine, whereas it was lower for ones in the north (Kef). Because small leaf areas transpire less water than large leaf areas, the production of small leaves may help reduce water loss in Chetoui cultivar. The reduction in total leaf area can be considered as a dehydration avoidance mechanism, which minimises water loss by transpiration, enabling plants to resist long dry periods while keeping the leaves photosynthetically active [9]. In olive plants, chlorophyll fluorescence measurements have been shown to be a useful technique for studying changes in the performance of the photosynthetic apparatus under the effect of adverse environmental factors [23,27]. These changes may be an important step for enhancing olive cultivation, particularly in arid regions. Suffering from high temperatures and high vapour pressure deficit. Reductions in photosynthetic performance under high VPD have also been observed by [6,28,29]. In this study, we found that the significant changes in the maximal efficiency of PSII photochemistry (Fv/Fm) were observed between cultivars and locations especially in November and July. Chemlali cultivar showed a lower reduction in Fv/Fm during July and maintained better values of this ratio during the experiment as compared to Chetoui cv. Which is in agreement with the results of Angelopoulos et al. showing that during the development of stressful conditions a decline of the ratio Fv/Fm occurred. In addition, [30] reported that during dry hot seasons, harsh environmental conditions limit photosynthetic activity.

The decrease was a consequence of many environmental factors. Under high VPD, a decrease of atmospheric humidity and an increase of transpiration occured. Declining Fv/Fm values implies that photochemical conversion efficiency is altered and could indicate the possibility of photoinhibition [31,32].On the other hand, this decrease in Fv/Fm can be ascribed as a down-regulation of PSII that reflect the protective or regulatory mechanism to avoid photodamage of photosynthetic apparatus[33]. During the experiment, highly significant changes of Chl (a+b) were recorded between cultivars and stations in both periods of measurements. The best concentrations of chlorophylls were observed in Chemlali cultivar during November. However, there was not a large reduction in leaf chlorophylls and carotenoid concentrations. Both the chlorophyll a and b are prone to soil dehydration [34] Decreased or unchanged chlorophyll level during abiotic stress has been reported in many species, depending on the duration and severity of the stress [35,36].

An important role associated with the survival of the plants grown under harsh climatic conditions is played by the leaf stomata. In olive leaves, the stomata are located on the abaxial side (hypostomatous), below the trichome layer, which prevents dehydration [13]. Leaves developed under hard conditions usually have smaller and more numerous stomata than leaves that develop under favorable conditions [37,38]. In the present study, the stomatal density changed significantly between stations over the experiment. Chemlali cultivar indicates the highest percentage of promotion except in Kef [8]. Showed that the rise of the density of stomata contributes to a better control of transpiration. This feature is thought to increase water use efficiency by increasing leaf boundary layer resistance [6] and decreasing transpirational water loss [13,39]. Observed that trichomes are a barrier to the diffusion of CO_2 and H_2O, and that they lower the boundary layer conductance in the air surrounding the stomata. From this conclusion we can [30]conclude that Chemlali is more protected against water loss than Chetoui in both Sfax and Medenine as indicated by the high trichome densities. Trichomes may also function as an effective filter, protecting the underlying tissues against damage from ultraviolet-B radiation [40].

Trichomes may allow olive leaves to take advantage of light rain or condensation of water [41] thereby increasing the probability of water uptake by leaves [3]. During the experiment, Chemlali cultivar showed the best shoot elongation in both Sfax and Medenine compared to those in Kef. For Chetoui plants, vegetative growth, as expressed by shoot length, was higher in Kef than those placed in Sfax or Medenine, reflecting, thereby, the origin of each cultivar. Indeed, growth of olive tree is a complex phenomenon, governed by exogenous and endogenous factors. The cyclic growth pattern occurs over two growing seasons [42]. The two main periods of active growth are occurring in the spring (before flowering) then in the autumn when wet conditions prevail. However, time and rates may vary according to location and year [29]. The seasonal temperature may accelerate or decelerate the growth rate, but it does not modify the cyclic growth pattern, which is highly influenced by the daily absolute temperatures and the seasonal radiant energy accumulation [39,43].In fact, it has been shown that shoot growth is not only highly correlated to the maximum and minimum daily temperatures but also to root development and their ability to extract water and nutrients from the surrounding area [44,45].

Conclusion

In summary, we have investigated the changes in physiological and biological parameters in two olive cultivars grown under different environmental conditions (North, Central and South of Tunisia). Our results show that Chemlali cultivar is well adapted to different climate conditions comparing to Chetoui. Indeed, the Chemlali olive cultivar is considered as an arid active species which can cope with severe environmental conditions (high temperatures and high VPD) and its activity can be established when clement climatic conditions prevail. This work provides some further evidence, as known, that the olive tree has to rest in summer and winter in order to preserve it survival mechanisms. Thus, its most intensive activity is occurring in spring and to a lesser extend in autumn in conjunction with favorable climatic conditions which is in accordance with our findings supporting the hypothesis that Chemlali olive cultivar native to dry regions, such as Sfax and Medenine, has more capability to acclimate to different climate conditions than Chetoui cultivar originated in regions with a more temperate climate, like Kef. In order to clarify the mechanisms by which olive cultivars cope with different climatic conditions, it will be necessary to relate the results obtained in this study to data from future field trials.

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