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ORIGINAL PAPER

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BIOCHEMICAL, MINERAL AND ANATOMICAL CHARACTERISTICS OF THE OLIVE TREE CV. CHETOUI GROWING IN SEVERAL TUNISIAN AREAS

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ABSTRACT

To tolerate harsh climatic conditions, olive tree Chetoui has developed some anatomic, physiologic and biochemical mechanisms. The aim of this study was to determine the indicators of stress in leaves, stems and roots growing under various climatic conditions. To protect against stress conditions this cultivar increased cuticle thickness, protective structures and building parenchyma tissues of leaves, woods and roots from the North to the South. The volatile compounds, extracted from northern and southern Chetoui leaves and roots, were analyzed by GC-FID and GC-MS. Great changes in volatiles were illustrated in the studied organs, by enrichment in phenolics and fatty acids for leaves and in hydrocarbons for roots of southern Chetoui. Also, a reduction in terpenes, alcohols and carbonylic compounds was noted in both southern samples. Moreover, minerals of all organs of Chetoui, varied in content and allocation, but their levels are the highest in leaves. The changes in volatiles might be affected by changes in the mineral elements uptake or accumulation under environment stress. A significant correlation was noted between phenolic compounds and sodium, nitrogen, and calcium contents. However, terpenoids was highly correlated with phosphorus content for all organs and studied areas. The detection of new volatiles, anatomical and mineral changes seem to be efficient indicators of adaptation of Chetoui to environment stress conditions.

Key words: *Olea europaea* L., anatomical changes, minerals, volatile compounds, climate changes

INTRODUCTION

The olive tree (*Olea europaea* L.) is an important crop grown throughout the Mediterranean basin [Boulal et al. 2013]. More than 95% of the world's olive production comes from this area [COI 2009]. In Tunisia, olive trees cover over 33% of the agricultural area with 1,800,000 ha, making it second in the world in terms of olive cultivated area next to Spain [Boulal et al. 2013, DGPA 2015]. Their cultivation is of great socio-economic importance, is spread from the north to the south in varying bioclimatic conditions. Olive trees are well adapted to abiotic stress. This crop tolerate severe environmental conditions especially drought and extreme temperatures imposed by the typical Mediterranean climate, such us prolonged summer and water shortage [Zhang et al. 2011].

Plant responses to water shortage are complex, involving adaptive changes or deleterious effects

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[Chaves et al. 2002, Vieira Areal Bacelar 2006], or both. In fact, the perception of stresses and the consequent adaptation by plants include physiological, biochemical and morphological characterizes which largely depend on factors such as severity of stress, plant developmental stage and their genotype [Agarwal and Zhu 2005]. Also, various anatomical changes, in leaves, stems and roots, can be efficiently used as an indicator of drought tolerance [Martins and Zieri 2003, Rossi et al. 2013].

In the majority of suitcases, there is a correlation between some of xeromorphic skin and dry conditions of the territory [Ristic and Cass 1991, Belhadjet al. 2007]. To resist against unfavorable biotic and abiotic stress conditions, Olive tree revealed many structural modifications of cell wall polymers such as lignin (polyphenolics), pectins and suberin [Enstone et al. 2003, Vieira Areal Bacelar 2006]. Therefore, Kun-Ming et al. [2006] suggested that the variations in the deposition of components such as lignin and suberin, the major components of apoplastic barriers in plant root, in different vascular bundle cell walls may be involved in the adaptation of plants to various drought and salinity stress.

Nutrition plays a key role in the growth and development of all crop plants. Moreover, the nutritious of the olive tree was strongly influenced by environmental conditions [Erel et al. 2013]. The macro and micro-nutrients available in the nutritional environment of plants are also capable of changing volatile compounds yield and composition and some of them contribute to increased tolerance of plants to salt stress [Nurzyńska-Wierdak 2013].

In addition to the mineral contents, olive leaves are wealthy in secondary metabolite such us, polyphenols, flavonoids, oleuropein and volatile compounds. Volatiles especially phenolic compounds are present in all parts of the plant, but their nature and concentration vary greatly between the various tissues. It has been considered that leaves are the primary site of plant metabolism at the level of both primary and secondary plant products [Ryan et al. 2002]. Also, volatiles content are dependent on different factors, such as the olive cultivar, climatic conditions, plant mineral contents and geographic area [Brahmi et al. 2012]. These compounds, such us polyphenols, played an important role in the plant response to environmental stress, being an important defense compounds against oxidative stress [Abaza et al. 2017] and showed a significant antibacterial and antifungal effects [Brahmi et al. 2011].

The aim of the present study was to comparatively investigate the anatomical, biochemical and nutritional changes taking place in the leaves, stems and roots of olive tree under various climatic conditions and to search new biochemical indicators to stress basing on qualitative and quantitative changes in its volatiles.

MATERIAL AND METHODS

Plant material

Samples of Chetoui cultivar were collected from twelve olive trees, about thirty years old, localized in three coastal zones of Tunisia in Morneg (36°40'51"N; 10°17'25"E), Chott Mariem (35°56'08"N; 10°33'26"E) and Jarzis (33°30'N; 11°07'E) (fig. 1). This cultivar has been grown on extensive, non irrigated farming, in orchards characterized with densities up to 100 trees/ha. This study was carried out on leaves, stems and roots of trees on flowering stage. Theses samples were selected according to four orientations of the trees (North, East, South and West). The diameters of stems were about three mm, while roots were collected around 0.5 m far from the tree trunk. The temperature, the relative humidity and the precipitation of the studied areas were estimated from a standard meteorological station adjacent to the experimental field (fig. 2).

Anatomical analysis

Transverse sections of fresh leaf, stem and root of Chetoui cultivar, grown in the North, Center and South, were hand-sectioned by razor blade, as described by Locquin and Langeron [1996]. After destruction of the cell contents with sodium hypochlorite, the sections were washed and double-colored with green iodine and alum carmine. The changes in sample anatomy under the different bioclimatic conditions were examined by trinocular microscope Leica, model DM-1000, coupled with a camera photo Leica (DFC-280), resulting in images of 1024 × 1280

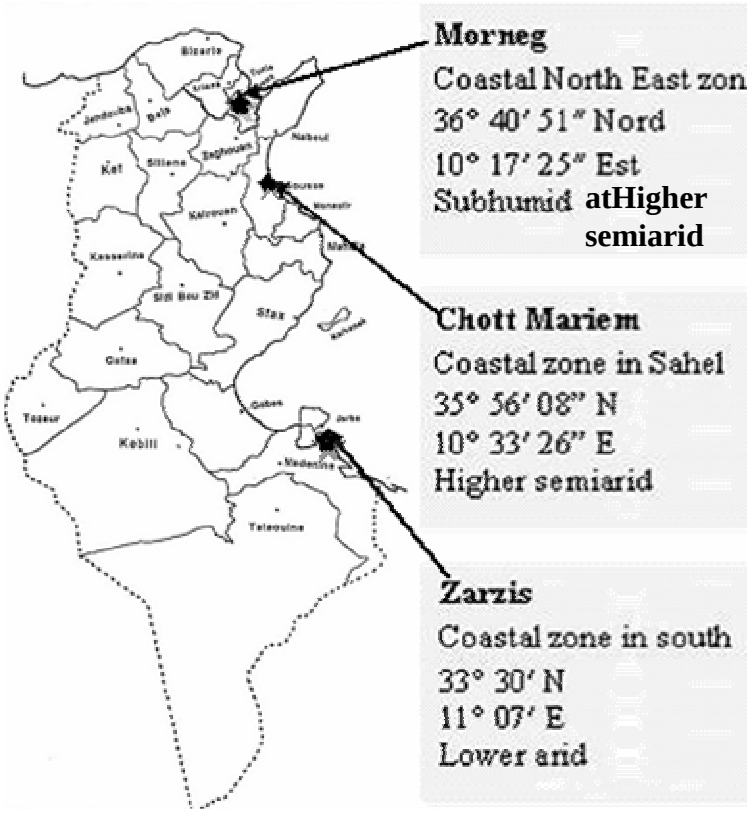


Fig. 1. Description of three Tunisian growing areas from Chetoui variety. Plant collected zones are shown in asterisk

pixels of resolution. Measurements of various cells and tissues thickness were taken with an ocular micrometer, three replications were adopted.

Mineral analysis

The samples of leaf, stem and root, collected in different location, were dried at 70°C for 48 h and then grounded. One gram of those materials has undergone calcinations. The mineral content was determined using flame photometer (Jenway, England). The nitrogen level (N) was determined in leaf, stem and root tissues according to the Kjeldahl method [Martin-Prével et al. 1984]. In addition, potassium (K), calcium (Ca) and sodium (Na) contents (percentage dry weight (%DW) was analyzed using a flame photometer according to Martin-Prével et al. [1984]. Three replications were applied for all samples.

Volatile compounds extraction

The fresh leaves and roots were collected from Chetoui of the North and the South of Tunisia. Each sample was sectioned, in little pieces, weighted and submitted to steam distillation for 6 hours. The recovered solution was extracted with the hexane. After drying the extract over anhydrous MgSO4, the solvent was removed and the oil samples were stored prior to analysis. Yield based on fresh weight of the sample was calculated. Three replications were adopted.

Gas chromatography (GC)/ mass spectrometry (MS) analysis

The analysis of the volatile component were run on a Hewlett-Packard GC-MS system (GC: 5890 series II; MSD 5972). The fused-silica HP-5 MS capillary column (30 m × 0.25 mm ID, film thickness

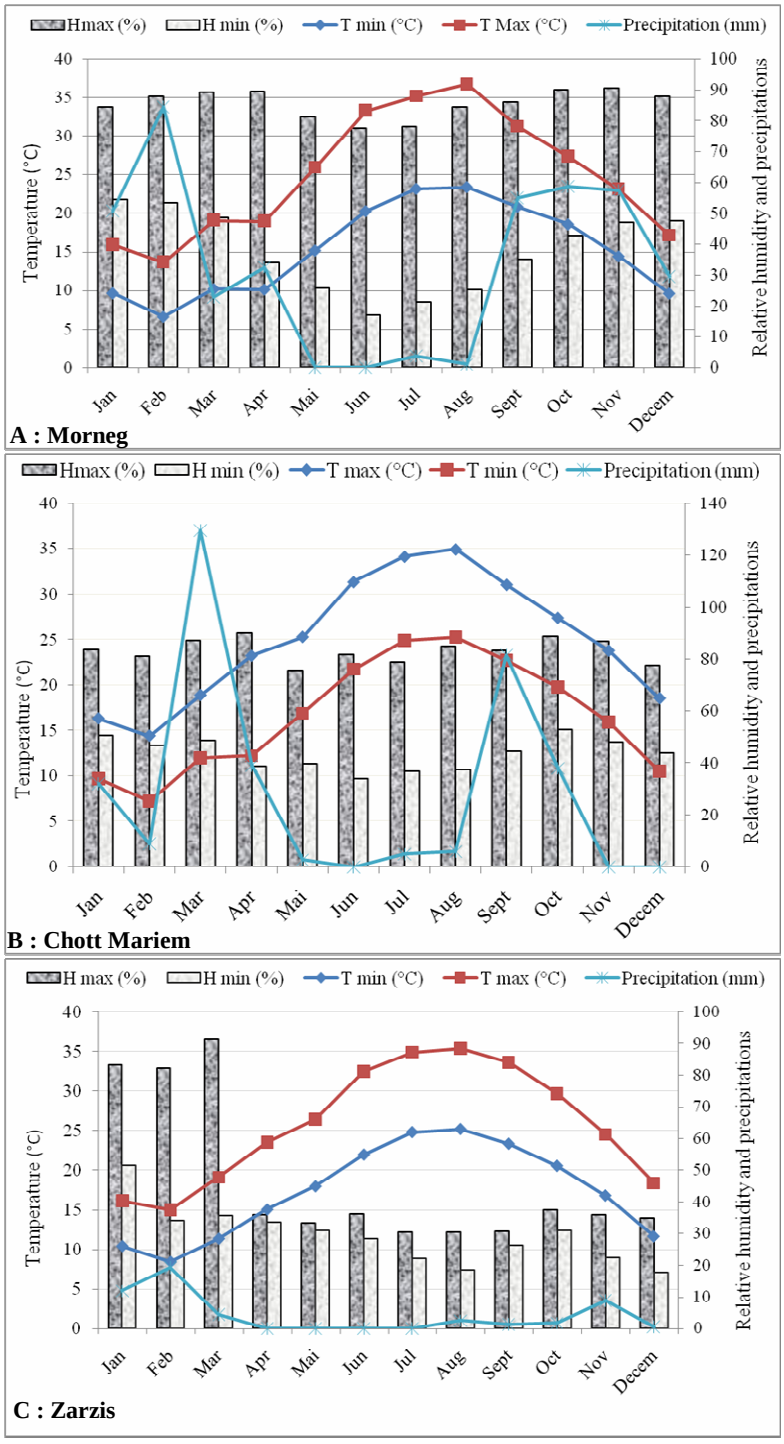


Fig. 2. Monthly average temperature, relative humidity and precipitations of three Tunisian areas during the culture 2012 of olive tree Chetoui (T max: maximal temperature; T min: minimal temperature and HR: relative humidity)

of 0.25 µm) was directly coupled to the MS and equipped with flame ionization detectors (FID). The oven temperature was programmed (50°C for 1 min, then 50–280°C at 5°/min) and subsequently, held isothermal for 20 min. Injector port: 250°C, detector: 280°C, split ratio 1 : 50. Volume injected: 0.1 ml of 1% solution (diluted in hexane). The HP5972 recording at 70 eV; scan time 1.5 s; mass range 50–550 amu. Software adopted to handle mass spectra and chromatograms was a HP Chem-Station.

Identification of the compounds

The components of the volatile fractions were identified by comparing their mass spectra with those of a computer library (Wiley 275 library). Further confirmation was done by referring to retention indices data generated from a series of alkanes (C9–C28) [Shibamoto 1987, Adams 1995]. Percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction.

Statistical Analyses

Data were subjected to statistical analysis using "SPSS 20" statistical program package. The percentages of volatile compounds are means of three experiments; the one-way analysis of variance (ANOVA) followed by Duncan multiple range test was employed and the differences between individual means were seem to be significant at $p < 0.05$. Principal Component Analysis (PCA) was used to evaluate the associations among the volatile compounds and the minerals stress from the progeny.

RESULTS

Effect of climatic changes on anatomical characteristics

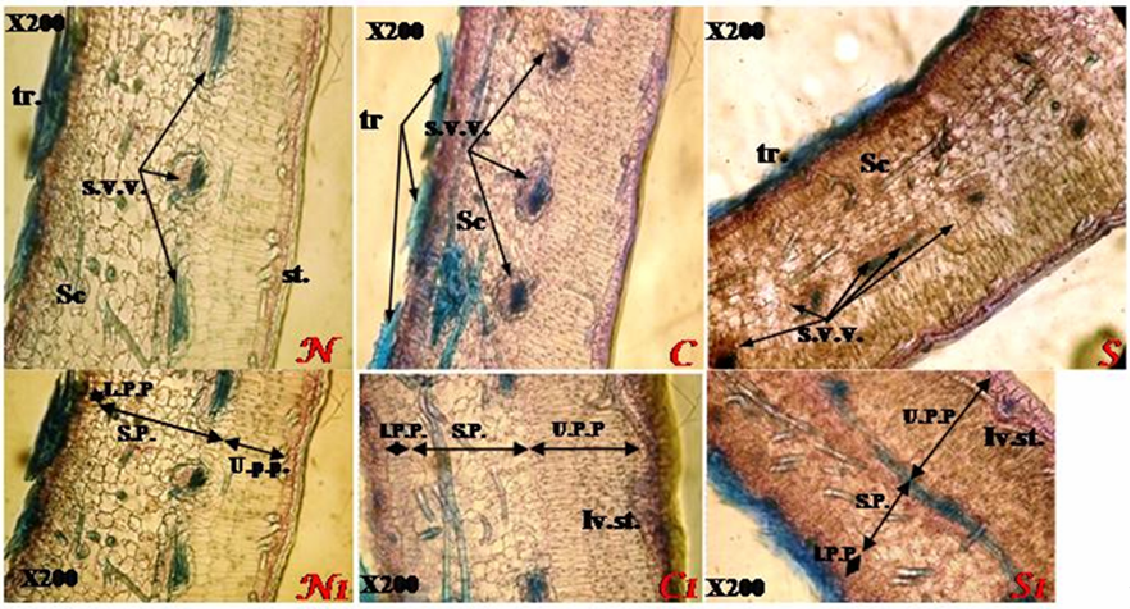
Leaf tissue structure. A change in anatomical characteristics of Chetoui leaves of northern, center and southern areas are presented in Table 1. Under water deficit availability, the cross section of olive leaves showed an increase of all tissue thickness from the North (Morneg) to the South (Zarzis), except total lamina thickness which is thinner in Zarzis (941.33 µm) than in Morneg (998.17 µm).

The southern Chetoui leaves developed more structural adaptations to protect against water shortage. To enhance their sclerophilly, southern leaves increased protective structure like the upper cuticule, and both the upper and lower epidermis. Thus, for southern Chetoui, the upper epidermis and lower epidermis thickness increased significantly by 37.03 and 43.62 µm compared to the northern one such as 15.12 and 27.12 µm, respectively (tab. 1). To develop the protection, an enrichment of trichomes was noted in abaxial surfaces of Chetoui leaves, increasingly from the North to the South areas (fig. 3 N, C, S).

Table 1. Mean values of different tissue thickness (µm) in transverse sections of leaves of Chetoui grown in three coastal zones of Tunisia

	Component thickness (µm)						
Zone	upper epidermis	UPP	UPP/SP	SP	LPP	lower epidermis	total leaf thickness
North	15.12 ±1.7 b	261.2 ± 11 c	0,95 b	318.7 ± 13.29 a	42.2 ±2.17 c	27.117 ±5.8 b	998.17 ±9.75 a
Center	35.37 ±3.23 a	293.8 ±4.5 b	0,93 b	370.3 ±29.3 a	52.8 ±3.9 b	32.067 ±5.6 b	931.17 ±17.04 b
South	37.03 ±0.36 a	432.2 ±12.6 a	1,76 a	293.2 ±5.11 b	86.2 ±5.25 a	43.617 ±3.8 a	941.33 ±13.43 b

The values represent the mean of 5 replications. Means followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test). UPP: upper palissade parenchyma; SP: spongy parenchyma; LPP: lower palisade parenchyma



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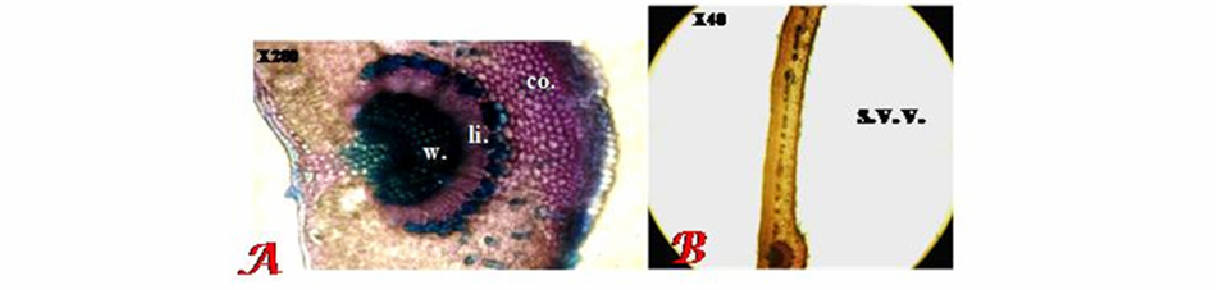
N, N_1: northern Chetoui; C, C_1: center Chetoui; S, S_1: southern Chetoui. Sc: sclerites; tr: trichomes; s.v.v: secondary vascular vessels; S.P.: spongy parenchyma; U.P.P: upper palisade parenchyma; L.P.P: lower palisade parenchyma; st: stomata, In.st.: invaginated stomata

Fig. 3. Cross-sections of the Chetoui leaf in different regions



co: collenchyme; w: wood; li: liber; s.v.v: secondary vascular vessels

Fig. 4. Cross-sections of the southern Chetoui leaf

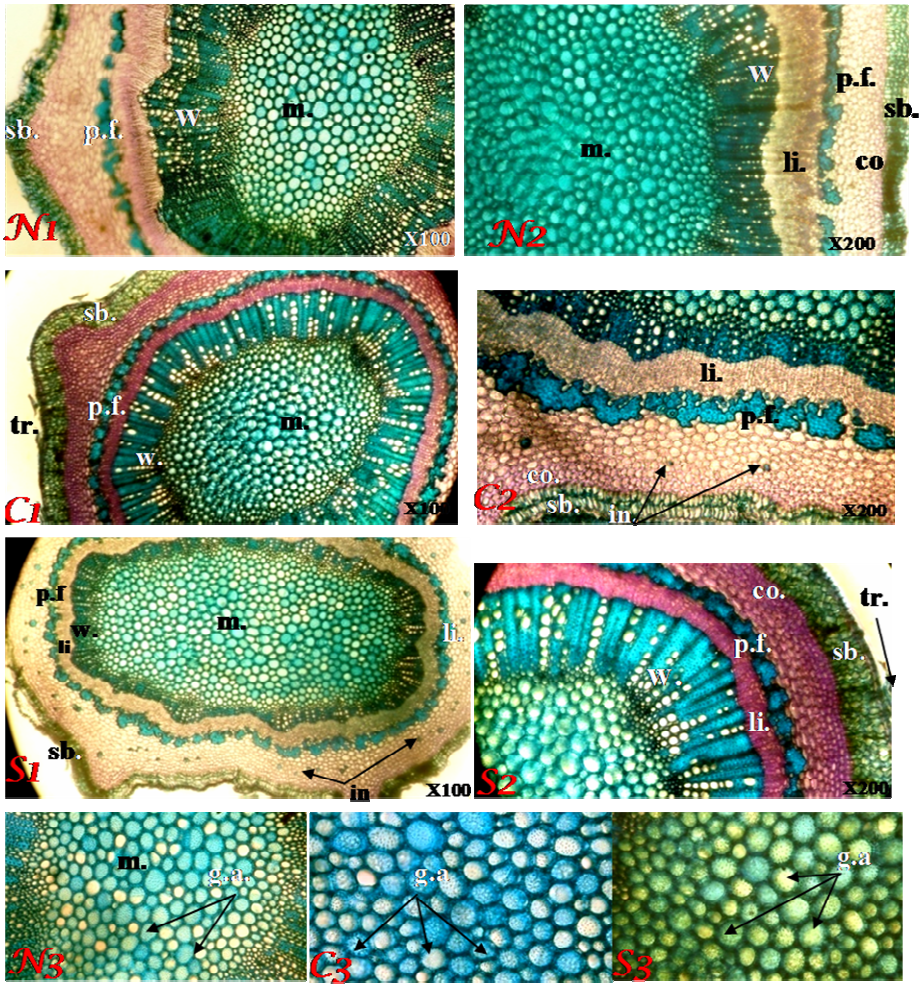
The leaf anatomical section (fig. 3 N_1, C_1 and S_1; tab. 1) showed an improvement of palisade parenchyma at the expense of the spongy's one, increasingly from the North to the South. Also the southern leaves had the highest palisade/spongy parenchyma ratio (1.76). Thus, the palisade parenchyma formed a barrier that minimized receiving sunlight and strengthened leaf protection. In fact, under water stress lower and upper palisade parenchyma became considerably thicker in southern Chetoui leaves.

Moreover, the cross section showed a more abundance of the sclerites in spongy parenchyma and the collenchymas in the central cylinder of southern leaves for enhancing the adaptation to water stress (fig. 4 A, B).

Table 2. Mean values of stem tissue thicknesses (µm) of Chetoui cultivars grown in three coastal zones of Tunisia: North, Center and South, on extensive dry farming

Zone	Component thickness (µm)						
	wood cross section	suber	cortex	liber + xylem + medulla	liber	xylem	medulla
North	1423.8 ±23.8a	67.18 ±2.5 c	415.2 ±25.9 a	981.4 ±20.3 a	148.2 ±7.9 a	401.0 ±10.56 a	458.0 ±13.35 b
Center	981.8 ±6.5 b	84.76 ±3.74 b	273.2 ±4.32 b	668 ±4.95 b	65.1 ±2.37 b	219.4 ±7.83b	463.4 ±12.6 b
South	982.2 ±8.7 b	117.8 ±8.5a	287.6 ±5.32 b	675.6 ±14.6 b	70.88 ±2.11b	132.6 ±13.28b	540.0 ±17.3a

The values represent the mean of 5 replications. Means followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test)



$N_{1,2,3}$: northern Chetoui; $C_{1,2,3}$: center Chetoui; $S_{1,2,3}$: southern Chetoui; Co: collenchyme; w: wood; p.f.: pericyclic fibers; li: liber; sb: suber; m: medulla; tr: trichomes; in: inclusion; g.a: starch grains

Fig. 5. Cross-sections of the Chetoui wood in the different regions. N: northern Chetoui; C: center Chetoui; S: southern Chetoui

Stem tissue structure. The measurements related to the stem anatomical parameters are given in Table 2. The result showed a decrease of the wood thickness from northern (1423.8 µm) to southern area (982.2 µm) of Chetoui cultivar. Also, we noted a greater development of suber layer surrounding stems of the Center and the South compared to the North's one (fig. 4). On the other hand, xylem and liberian tissue thickness in southern area showed significant reduction in the stem compared to the North's one (fig. 5, $N_{1,2}$, $C_{1,2}$ and $S_{1,2}$; tab. 2).

Below the cuticle layer, the collenchyme showed an important development especially in the Centre and the South areas (fig. $5N_2$, C_2, and S_2). The anatomical sections of the stem showed several inclusions of starch grains which were detected in all samples (fig. 5 N_3, C_3, S_3).

Root tissue structure. The root cross-section characteristics of Chetoui, illustrated in Table 3, showed a development of mainly tissue thickness from the North to the South. In fact, cortex and pericyclic fibers of Center and southern roots were more abundant than the northern ones, inducing thus the sclerotization of the cortex and the increase of its rigidity (fig. 6 $N_{1,2}$, $C_{1,2}$, $S_{1,2}$). The diameter wood vessel increased according to the increase in the severity of the climate. In the medulla zone of Chetoui roots, an increase of wooden vessels was noted in the South (fig. 6 C_4), differently from the North. Wherever, vessels were less abundant in the medulla zone which was covered with a well developed and lignified tissue (fig. 6 S_4). In addition, the liberian tissue in the Chetoui roots of the center was more abundant compared to the other regions (fig. 6 C2, C3). Inclusions of starch grains were present in both liberian (fig. 6 C_3) and cortical zones (fig. 6 S_3).

Climatic change effects on mineral status

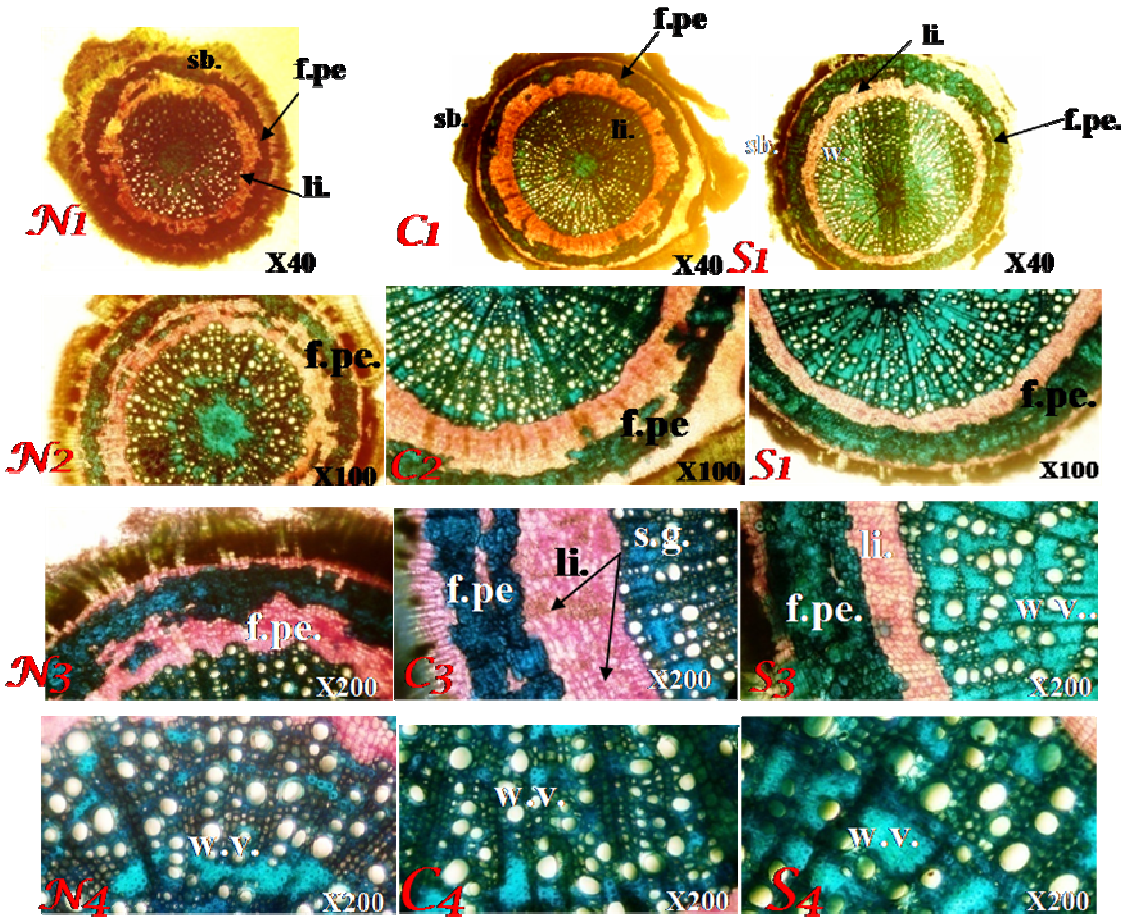
Evolution of sodium content. The evaluation of sodium percentage (%) originated from diverse regions; North, Center and South, showed no significant differences for Chetoui cultivar in the leaves and the woods. While, in southern Chetoui roots, the % Na noted a significant increase of 45% comparatively to the North's one (fig. 7). This result suggested an increase of Na level with draught stress and the severity of the climate.

Evolution of nitrogen content. Chetoui cultivar was reach in nitrogen. Compared to that of stems and roots, leaves displayed higher accumulation of N ions. The nitrogen values ranged from 1.55 % in leaves to 0.30 % in roots for all regions. The highest values were recorded in and center Chetoui leaves (1.55 % and 1.34% respectively) (fig. 7). Nitrogen seemed to be more important in center and northern Chetoui wood, reaching 0.95 and 0.79% respectively. However, it was concentrated essentially in the Southern roots of Chetoui (0.85%).

Table 3. Mean values of root tissue thickness (µm) of Chetoui olive cultivars grown, on extensive dry farming, in Morneg, Chott Mariem and Zarzis

	Component thickness (µm)							
Zone	root cross section	cortex	suber	sclerenchyme	pericyclic fibre	liber	stele thickness	xylem vessel diameter
North	1126.0 ±16.5 b	346.3 ±13.6 a	198.67 ±10.7 b	36.8± 6.8 b	141.3 ±10.5 c	84.2 ±4.4 a	478.3 ±6.5c	25.3 ±1.0 a
Center	1778.3 ±70.3 a	484.7 ±16.4 b	407.7 ±13.20 a	76.1 ±9.5 a	314.0 ±20.0 b	82.6 ±9.6 a	909.3 ±15.3 b	46.6 ±0.6 b
South	1772.0 ±47.4 a	595.7 ±17.9 c	172.0 ±15.10 b	73.6 ±16.5a	377.7 ±3.5 a	92.9 ±7.5 a	1269.7 ±59.0 a	51.0 ±1.7 c

The values represent the mean of 5 replications. Means followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test)



$N_{1,2,3,4}$: northern Chetoui; $C_{1,2,3,4}$: center Chetoui; $S_{1,2,3,4}$: southern Chetoui; w: wood; f.pe.: pericyclic fibers; sb: suber ; s.g.: starch grains; w.v.: wood vessels; li: liber

Fig. 6. Cross-sections of the Chetoui Root in the different regions

Evolution of phosphorus content. The mean phosphorus percentage for the Chetoui trees in each part varied greatly among experimental site (fig. 7). Phosphorus was accumulated especially in leaves and stems reaching 0.085% in northern Chetoui leaves and 0.094% in Center Chetoui stems. Contrary to the other studied minerals, the phosphorus stored in the South sample was in the lowest levels; a reduction of over 50% is unregistered for leaves of southern Chetoui compared to the northern cultivar. For Chetoui woods, phosphorus was dominant in the Center, reaching 0.094%; reductions of 77.53% and 53.84% were noted in the sample of the South comparatively to the Center and the North respectively. For Chetoui roots, phosphorus was dominant in the north, reaching 0.045%; a reduction of 58.33% was noted in the sample of the South comparatively to the North. Thus, phosphorus was a very sensitive element to high temperatures and dry climate (fig. 7).

Evolution of potassium content. Chetoui cultivar was rich in Potassium. For all tissues, the rate of this element seemed to be more important in center and southern areas and was more concentrated in leaves and woods than in roots. However, southern Chetoui

woods (1.43%) and leaves (1.23%) accumulated the highest values of potassium. This richness was attributed to the mobility of this element in tissues of greater metabolic intensity.

Evolution of calcium content. The calcium percentage in different part of Chetoui trees was illustrated in (fig. 7). These results showed a significant difference between geographic areas for leaves, woods and roots. For all tissues, calcium accumulated the highest level in Center Chetoui areas; conversely the southern one showed a deficiency in this element. There was a significant decrease of 81.02, 89.82 and 92.64% of calcium content in southern leaves, woods and roots of Chetoui respectively. This result may promote more environmentally effect of calcium deficiency in Chetoui trees. Chetoui limited the variation rate of the calcium, in its leaves, comparatively to the other organs, especially in the center, which restricted the interaction of the calcium on the absorption of the other vital minerals (fig. 7).

Effect of climatic change on volatile compounds

Chetoui volatile yields. The volatiles yield, presented in the Figure 8, revealed a significant difference between organs and studies areas. The highest volatiles yield was stored in southern Chetoui woods (0.019%), followed by the amount of southern leaves (0.013%) (fig. 10). In fact, for all tissues an increase of 67.71% was recorded for the southern cultivar compared to the northern ones.

Chemical composition of volatiles. The CPG-FID and GC-MS analysis of Chetoui volatiles, grown in various areas of Tunisia, was illustrated in Table 4. The results showed that 93.95 and 69.26% of volatile compounds were identified in northern leaves and roots of Chetoui cultivar; while 73.89 and 69.82% were indentified in Southern ones.

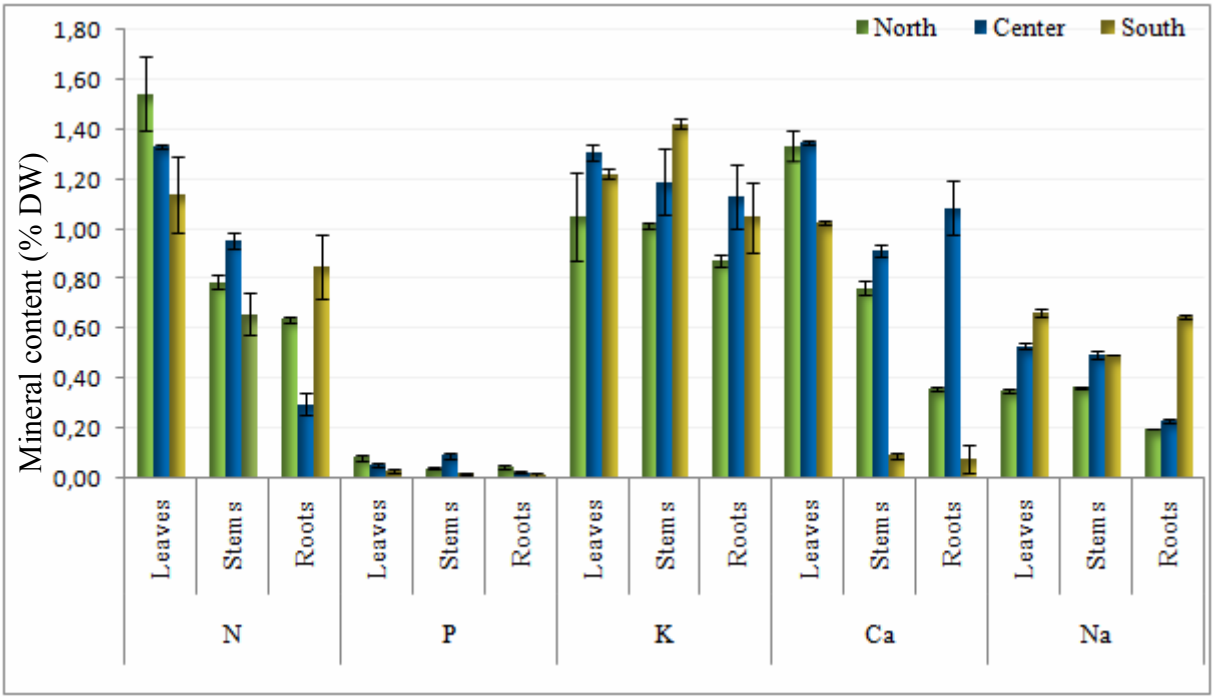
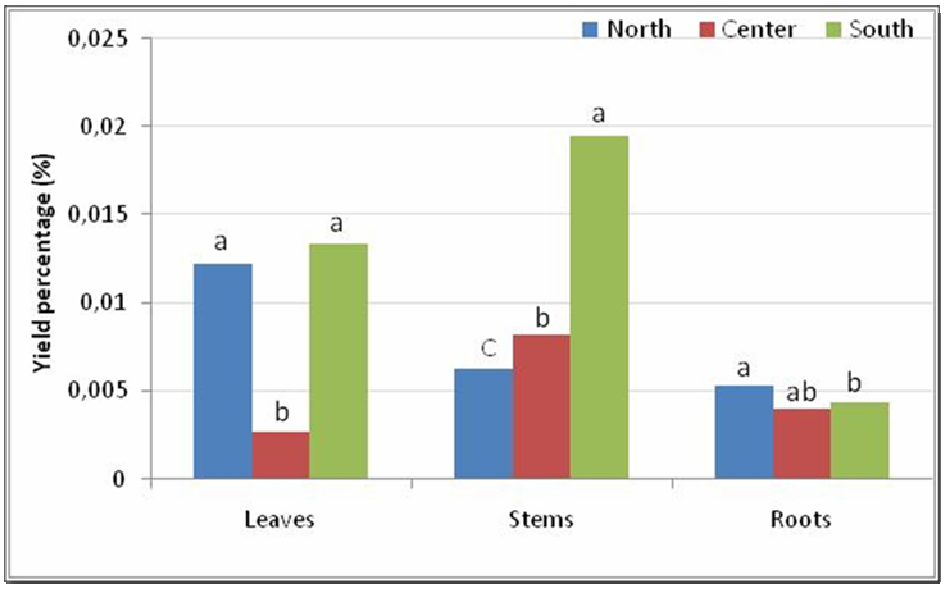


Fig. 7. Macro-element contents (% of dry weight) in leaves, stems and roots of Chetoui cultivar grown in different geographic areas. Statistical differences are calculated by ANOVA followed by Duncan's post-hoc test ($P < 0.05$). Data are means of 3 replicates ± standard deviation



Letters change: the difference is significant; same letter: the difference is not significant at 5%

Fig. 8. Volatile yields of Chetoui growing in different geographic areas

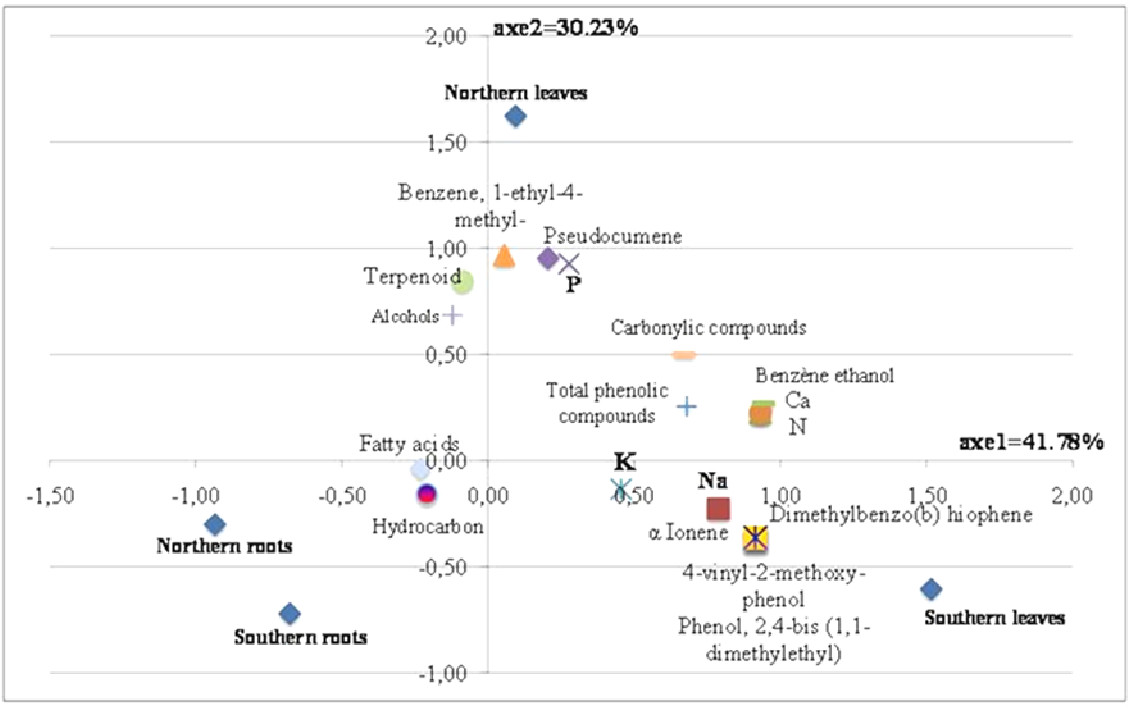


Fig. 9. Principal component analysis (score plot) of minerals and volatile compounds of leaves and roots of olive tree Chetoui growing in northern and southern Tunisian area

Several compounds were common in all studied samples, such hydrocarbons, as N tetradecane, 1-nonadecene, heptadecane, 1-hexadecene; some aromatic compounds, as benzene 1-ethyl-3-methyl, benzene ethanol; fatty acids as hexadecanoic acid, 9-octadecanoic acid (z) and linoleic acid. However, these compounds were present in different proportions.

Although, growing in pluvial conditions, northern and southern Chetoui cultivar showed an important difference in the composition of their leaves and roots, in these areas. Hexadecane, Tricosane were only identified in the leaves and the roots of southern Chetoui; whereas 1-Hexanol 2-ethyl, methyl alpha-deuteriocinnamate; Geranic acid were identified only in the leaves and the roots of northern Chetoui. These compounds are specific to the studied area and depend directly to the weather and the soil conditions residing in this region. Indeed, they played an important function to opposite the hard conditions.

For all areas, various products characterized only the leaves of Chetoui, namely 1,2,4-trimethylbenzene; 1H-indene, octahydro-cis; dodecane, 2,6,11-trimethyl-62; trans-beta damascenone; beta-ionone. Whereas, 1-tetradecene; octadecane and 1-octadecene were identified only in the roots of this cultivar.

However, the amount of aromatic compounds in southern Chetoui leaves (34.35%) was very higher than of the northern ones (24.02%). Many aromatic compounds were specific only to southern Chetoui leaves, especially benzene, 1,2-dimethyl-(CAS) (0.1%); isopropylbenzene (0.29%); α-ionene (2.55); benzene, 1-methyl-4-(1-methylethenyl) (0.71); 4-vinyl-2-methoxy-phenol (5.82%); phenol, 2,4-bis(1,1-dimethylethyl) (11.12%); 5-acetyl-4-methyl-benzimidazolone (2.89%) and dimethyltbenzo[b]hiophene (3.85%).

Also, many molecules characterized northern Chetoui leaves such as benzene, 1-ethyl-4-methyl (5.38 %); benzene, 1-methyl-3-propyl; benzene, 1,2,3-trimethyl; benzene, 1-ethyl-2,3-dimethyl; benzene, 4-ethyl-1,2-dimethyl (1.43%); benzamide, N-(1,1-dimethylethyl) (2.09 %) and 2-pentanone, 1-(2,4,6-trihydroxyphenyl).

Correlation between mineral elements and volatile compounds contents. Pearson correlation was used to determine the relation between volatile compounds content and mineral concentration. A positive and significant correlation between total phenolic compound and Na, N and Ca was illustrated (tab. 4). Such as dimethyltbenzo[b]hiophene, 4-vinyl-2-methoxy-phenol, benzene ethanol, α-ionene, 4-vi- nyl-2--methoxy-phenol, phenol 2.4-bis (1.1-dimethylethyl) showed a high significant correlation (between 0.65 and 0.92) with Na, N and Ca. The accumulation of these elements could be contributed to increase the level of phenolic compounds. Also, for all studied areas, carbonylic compounds and alcohols were positively and significantly correlated with N and Ca. Although, phosphorus was positively and significantly correlated with terpenoid, pseudocumene and benzene 1-ethyl-4-methyl of 0.99; 0.96 and 0.74 respectively, for northern and southern leaves and roots. The level of production and synthesis of these compounds might be dependant in the balance of phosphorus.

Principal Component Analysis (PCA). Principal component analysis (PCA) was used to better understand the changes of volatilesand minerals content of leaves and roots collected from different studied areas. The means values of volatile compounds and macro-element were used to build the PCA. For Chetoui, organs exposed to different area, the multivariate data processing for mineral elements and volatile compounds agreeing to for a large group of diverse data samples was explained in 41.78% and 30.23% as the first and second principal components. Two components, axe 1 and axe 2, were extracted describing approximately 72.01% of the common variance. This illustrates that there are significant differences between northern, southern leaves and roots on the level of minerals and volatile compounds composition.

Compounds such as total phenolics and hydrocarbons, benzene ethanol, α-ionene, 4-vinyl-2-methoxy-phenol, phenol 2.4-bis (1.1-dimethylethyl), Ca, N, Na and K permitted the classification of the samples (North leaves) according to axis 2. While, terpenoid, pseudocumene, alcohols and benzene 1-ethyl-4-methyl and phosphorus allowed the classification of the samples (South leaves) according to axis 1.

According to PCA, the response of Chetoui cultivar to climatic variability and organs was different.

In southern area, the production of total polyphenol and the accumulation of N, K, Ca and Na increased in leaves. Although in northern ones, leaves produced more terpenoid, alcohols, pseudocumene and increased the level of phosphorus element. Also, the study noted that macro-element content varied differently with organs and climatic conditions. As well, the severity of climate positively affected the concentration of total phenolics and hydrocarbons which varied differently according to the organs.

Table 4. Volatiles composition (%) of leaves and roots of Chetoui growing in the North and the South of Tunisia

Regions		North		South	
Compounds	RI*	leaves	roots	leaves	roots
1	2	3	4	5	6
Phenolic compounds					
2-pentanone,1-(2,4,6-trihydroxyphenyl)	850	0.70			
Phenol	976.0	2.46			
1H-indene, octahydro-, cis-	989.5	0.81		0.18	0.81
Hemellitol	996	0.91			
p-cymene	1027			0.71	
Toluene	1029		0.09	1.02	
Benzene, 1-methyl-3-propyl-	1040.6	0.40			
Benzeneethanol	1096.1	3.12	0.58	4.84	
2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-	1150				0.18
o-xylene	1179.3			0.1	
Isopropylbenzene = cumene	1260			0.29	
5-acetyl-4-methyl-benzimidazolone	1300			2.89	
Cuminic alcohol = cuminol	1306		2.94		
Pseudocumene	1348	6.29		0.98	
Benzene, 1,2,3,4-tetramethoxy-5-(2) -propenyl)-	1394		4.53		
o-xylene, 3-ethyl-	1395	0.43			
Benzene, 4-ethyl-1,2-dimethyl-	1412	1.43			
α-ionene	1465			2.55	
Benzamide,N-(1,1-dimethylethyl)-	1510	2.09			
Phenol, 2,4-bis(1,1-dimethylethyl)	1512			11.12	
Benzene, 1-ethyl-4-methyl-	1527.1	5.38			
Dimethyltbenzo[b]hiophene	1540			3.85	
2-ethyldibenzothiophene thiophene, 2-ethyl-	1550		0.93		
Phenanthrene	1776.3		0.66		
Phenanthrene, 2,5-dimethyl-	1810		0.72		
Methyl 6,6-dimethyl-2,4-heptadyinoate	2056		5.09		
4-vinyl-2-methoxy-phenol	2156			5.82	
Total		24.02	15.54	34.35	0.99
Terpenoids					
Non-oxygenated monoterpen					
Naphthalene, decahydro-	1099		1.53		
Oxygenated monoterpenoids					
Linalool	1095	0.43			
1,8-menthadien-4-ol	1187	0.81			
l-alpha-terpineol	1189		1.84		

Table 4 cont.

1	2	3	4	5	6
Beta-Ionone	1487	2.11		1.22	
Ethanone,1-(1,4-dimethyl-3-cycloh exen-1-yl)-	2550	0.81			
p-menth-8(10)-en-9-ol, cis-	2670		0.06		
Oxygenated sesquiterpenoids					
Oxirane [(dodecyloxy)methyl]-	1380			0.16	
Sesquiterpène acyclique					
Farnesol	1722				0.80
Non oxygenated sesquiterpen					
Methyl-phenanthrene	1896	0.45			
Total		4.61	3.59	1.22	0.80
Alcohols					
1-octanol, 2-butyl-	1063	4.11			0.27
1-hexanol, 2-ethyl	1483.8	2.58	1.93		
1-(1,3-butadiene-2-YL)-cyclopentanol	1490			0.56	
1-dotriacontanol	1936				1.60
Total		6.69	1.93	0.56	1.87
Hydrocarbons					
Cyclohexane, 1,1'-(1,2-dimethyl-1, 2-ethanediyl) bis-	840		0.41		
Isoterpinolene	1090		2.67		
2-cyclohexyl-5,5-dimethyl-1-hexen 3-yne	1150	1.45			
Dodecane	1200		0.24		
Dodecane, 2,6,11-trimethyl-	1250	0.32		0.66	
Tridecane	1300			0.18	
Tetracyclo[4.4.0.0(2, 4).0(3,7)] decane	1395			3.46	
N-tetradecane	1400		1.61	0.43	
Anthracene, 9-methyl-	1436		2.11		
1-tetradecene	1440		1.78		1.32
Pentadecane	1500			2.10	
Pentadecane 1-bromo-tadecylbromide	1500				1.03
1-pentadecene	1545				1.23
Cyclotridecane	1559				0.63
1-hexadecene	1593.5	0.56		4.45	3.42
Hexadecane	1600		2.38	0.53	5.03
Hexadecane, 2,6,10,14-tetramethyl-	1635				1.30
Cyclotetradecane	1679				1.78
Heptadecane	1700	1.47	1.77	2.91	11.89
1-heptadecene	1725	0.69			
1-octadecene	1789		2.70		14.14
Octadecane	1800	0.85	1.39		3.66
Nonadecane	1900		2.80		2.36
1-nonadecene	1956		1.95	0.34	
Eicosane	2000				1.02
1-docosene	2194				3.56
Docosane	2200				1.80
Tricosane	2300			2.36	3.46

1	2	3	4	5	6
Squalane	2400				1.94
Tetracosane	2400			2.45	
Pentacosane	2500				0.52
Hexacosane	2600			3.18	
Octacosane	2800			0.62	0.59
1-methyl-2-(3-methyl-2-buten-1-yl)-1-(4-methyl-3-penten-1-yl) oxetane	2871.5				
Nonacosane	2900				0.29
Triacontane	3000				0.28
18,18'-Bi-1,4,7,10,13,16-hexaoxacyclononadecane	3200				0.26
18,19-dihydroxy-1,4,7,10,13,16-hexaoxocycloencosane	3400				0.11
17-pentatriacontene	3508	34.03			1.57
Hexatriacontane	3600				0.76
Total		42.96	21.81	23.67	63.69
Carbonylic compounds					
Nonanal	1100			0.38	
Nonyl aldehyde	1105.6	1.10			
2-methyl-6-oxo-2,4-heptadienal	1125			1.29	
p-carbomethoxybenzaldehyde	1362	3.70			
Trans-beta-damascenone	1386	3.34		3.31	
BHT-aldehyde	1856			0.37	
Total		8.50	0	5.35	0
Fatty acids					
Decanoic acid	1364		3.07		
Geranic acid	1347.5	0.91	1.84		
Dodecanoic acid	1565	0.62			
Tridecanoic acid	1664	0.59			
Hexadecanoic acid	1959		3.26	1.34	
Linoleic acid	2070		1.48	0.28	
Oleic acid	2141	0.30	4.09	1.18	
Total		2.42	13.74	2.8	0
Others					
Phosphonic acid, dioctadecyl ester	805	0.38			
Acetamide, N-methyl-N-[4-[4-methox y-1- -hexahydropyridyl]-2-butynyl]	1110				2.47
endo-2(R)-methylbicyclo[3.2.1]octan-3-one	1265		0.96		
CIS-3-hexenyl benzoate	1587	0.73			
3-pyridinecarboxylic acid, 5-ethenyl-, methyl ester	1605			5.94	
Methyl-alpha-deuteriocinnamate	1614	4.00	4.51		
Hexadecanoic acid, methyl ester	1963		2.09		
Ethyl linoleolate	2098		1.07		
10-octadecenoic acid, methyl ester	2120		3.88		
2-bis(methylthio)methylene-1-phrenyl-4-methyl-4-penten-1-one	2205		0.14	0.72	
Total		5.11	12.65	6.66	2.47
Identified compounds (%)		**93.95**	**69.26**	**73.89**	**69.82**

Components are listed according to their elution on apolar column (HP-5). * RI: retention indices relative to C8–C22 n-alkanes on the apolar HP-5; GC/MS: identification based on comparison of mass spectra; Column-GC: identiication based on retention time comparison to authentic compounds.

Table 5. Correlation between volatile compounds (%) content and mineral concentrations (%) of different parts of Chetoui cultivar growing in various Tunisian areas

	Na	N	P	K	Ca	T.ph	α-ionene	Benz ethan.	P.C.	Phe-2,4 bis	Benz	Benzo iophen	V. Ph.	Terp	Alcoh ols	H C	Carb Comp	Fatty acids	Organ	Zone
Na	1																			
N	0.64*	1																		
P	–0.08	0.52	1																	
K	0.3	0.47	0.13	1																
Ca	0.64*	1**	0.52	0.47	1															
Total phenolic compounds	0.63*	0.62*	0.31	0.09	0.62*	1														
α-ionene	0.75**	0.73**	–0.08	0.4	0.73**	0.44	1													
Benzeneethanol	0.65*	0.92**	0.51	0.36	0.92**	0.63*	0.8**	1												
Pseudocumene	–0.1	0.46	0.99**	0.11	0.46	0.32	–0.18	0.44	1											
Phenol.2.4-bis(1.1-dimethylethyl)	0.77**	0.74**	–0.1	0.41	0.74**	0.53	0.99**	0.79**	–0.18	1										
Benzene. 1-ethyl-4-methyl-	–0.21	0.33	0.96**	0.04	0.33	0.22	–0.33	0.30	0.99**	–0.33	1									
dimethyltbenzo[b]hiophene	0.77**	0.74**	–0.1	0.4	0.74**	0.52	0.99**	0.79**	–0.18	.999**	–0.33	1								
4-vinyl-2-methoxy-phenol	0.77**	0.74	–0.09	0.4	0.74**	0.51	0.99**	0.79**	–0.18	.997**	–0.33	0.99**	1							
Terpenoid	–0.22	0.07	0.74**	–0.08	0.07	0.09	–0.34	0.20	0.7**	–0.34	0.78**	–0.34	–0.34	1						
Alcohols	0.04	–0.03	0.49	–0.54	–0.03	0.33	–0.37	0.05	0.54	–0.38	0.57	–0.38	–0.38	0.51	1					
Hydrocarbons	0.21	–0.16	–0.23	–0.23	–0.16	–0.01	–0.23	–0.32	–0.18	–0.24	–0.14	–0.23	–0.23	–0.25	0.44	1				
Carbonylic compounds	0.55	0.66*	0.57	–0.10	0.66*	0.71**	0.45	0.77**	0.54	0.43	0.44	0.44	0.44	0.26	0.62*	0.04	1			
Fatty acids	–0.04	–0.41	–0.23	–0.29	–0.41	–0.11	–0.07	–0.12	–0.24	–0.07	–0.22	–0.07	–0.07	0.41	0.06	–0.25	–0.13	1		
Organ	–.50	–.92**	–0.74**	–0.35	–0.92**	–.68*	–.57	–.94**	–0.7*	–0.58*	–0.57	–0.58*	–0.57	–0.37	–0.22	0.29	–0.80**	0.25	1	
Zone	0.60*	0.36	–0.48	0.35	0.36	0.13	0.57	0.17	–0.51	.58*	–0.57	0.58*	0.57	–0.72**	–0.37	0.50	–0.02	–0.42	–0.26	1

Na: sodium, N: nitrogen, P: phosphorus, K: potassium, Ca: calcium, T.ph: total phenolic compounds, Benz ethan.: benzeneethanol, P.C.: pseudocumene, Phe-2,4 bis: phenol.2.4-bis(1.1-dimethylethyl), Benz: benzene. 1-ethyl-4-methyl-, Benzoiophen: dimethyltbenzo[b]hiophene, 4-vinyl-2-methoxy-phenol, V.Ph.: 4-vinyl-2-methoxy-phenol, Terp: terpenoid, HC: hydrocarbons, Carb Comp.: carbonylic compounds. The compounds are expressed in %.

* significant correlation (0.05)

** significant correlation (0.01)

DISCUSSION

In Tunisia, the Chetoui cultivar is widespread in the North of the country, occurring in plains as well as in mountainous regions [Guerfel et al. 2002]. However, in the Center and the South of the country, areas with low rainfall, the growing of this cultivar is limited. To adapt to these areas and to oppose to the climatic fluctuation specific to it, Chetoui has developed many arrangement including anatomical, physiological and biochemical changes. The capacity of olive to take up water and nutrients not only depends on root distribution, but also on root growth dynamics and activity.

To acclimatize to dryness and high temperature, Chetoui reduced total leaf thickness from the North to the South on the way to reduce water loss at the whole-plant level. Also this cultivar increased upper cuticle and both the upper and lower epidermis thickness. According to Bacelar et al. [2006] Cobrançosa, Manzanilla and Negrinha varieties exhibited a good protection against water loss by increasing cuticle thickness, protective structures and building parenchyma tissues. This response to environmental stimuli contributes to high adaptation to atmospheric demand [Fernandez 2014].

Southern Chetoui leaves showed an increase in the thickness of the upper and lower palisade parenchyma, mesophyll compactness leads to low cellular conductance there by providing an efficient system to limit cellular water loss in order to reduce transpiration during drought [Bosabalidis and Kofidis 2002]. Spongy mesophyll anatomy greatly depends on leaf water status [Ehrenberger et al. 2012]. This result was in accordance with those of Ennajeh et al. [2010] who has suggested that drought caused an increase in the thickness of the upper palisade parenchyma, which was more important in 'Chemlali'. This should increase the number of CO_2 assimilation sites per unit leaf area, helping to maintain high absorbance values despite the low stomatal conductance values caused by drought. Also, the same authors showed a strong positive correlation between stomatal density and the thickness of spongy parenchyma in 'Chemlali' under drought conditions [Ennajeh et al. 2010].

Southern leaves developed more conductor vessels than northern ones. Generally, leaves with high tissue density are better able to survive in a severe drought because of a higher resistance to physical damage by desiccation [Mediavilla et al. 2001]. Xylem density may be a useful tool to estimate drought tolerance of large number of species [Jacobsen et al. 2007].

The cross section of the wood showed that cuticle layer was more developed in the center and the southern Chetoui stem. Also, suber was more developed in southern area; which played an important role in drought tolerance [Cameron 2002]. Cortex, xylem tissue and phloem tissue thickness of stems were reduced significantly at moderate to severe drought. Moreover, the liberian tissue became narrow in southern Chetoui stems, comparatively to the other regions. Besides, in southern condition xylem tissue became thinner than the northern ones. These results are in agreement with those of Bougalleb et al. [2014], indicating a significantly reduction in the cortex, xylem tissue and vessels, phloem tissue and sclerenchyma thickness of *Astragalus gombiformis* stems with water deficit. Also, Rossi et al. [2013] noted that in rainfed, olive trees produce more vessels with lower diameters. Since, when the stress increases, greater flows are recorded deeper into the xylem [Fernández et al. 2010].

It is known that shoots are not the most resistant to water flow, about half of the total plant resistance lies in roots, and most of the other half may be in leaves [Meinzer 2002]. In this study, a lignified tissue was well developed in medulla zone of the southern Chetoui root, which contained vascular vessels with bigger size, than the northern ones. Others showed that greater root cavitations resistance was correlated with greater vessel implosion resistance [Pratt et al. 2007].

The correlation between olive mineral nutrition flowering and productivity are complex and depend on environmental factors [Erelet al. 2013]. Such as, water limitations caused reduction in uptake of potassium, phosphorus, magnesium and calcium due to lower mobility in soils and decrease in absorption and transport ability to the organs under stress conditions [Cetinkayaa et al. 2016]. According to Therios and Sakedalliaris [1982], the existence of a competition between minerals

at transporter maybe the cause of positive and negative interactions. In the same conditions the level of minerals differed with variety and organs.

The effect of mineral nutrition, as reflected in leaf content, on flowering, on fruit set and oil production of olive trees. According to Al-Absi et al. [2009] the concentrations of nutriments were found to be statistically different in the leaves and roots depending on the cultivar. As well, Gonzalezet al. [1968], Braham [1984] and Braham and Mhiri [1997] noted an existence of a regional nutrient balance, established between the mineral elements in the leaves of the olive tree.

The sodium concentration increased with regions; southern Chetoui leaves and roots showed the highest level. This result was in agreement with that of Ezzili [1996] who reported that in Tunisia, in drought conditions, there was an accumulation of sodium in the leaves of the olive tree taking into account the very dry climate of southern Tunisia. Other research reports indicated that water stress resulted to increased level of sodium in plant parts [Martinez et al. 2003, Paranychianakis and Angilakis 2008]. Therefore, Na^+ appears to interfere with essential nutrients such as K^+ metabolism and Ca^{2+} reduces this obstruction [Kopittke 2012]. Although, Kchaou et al. [2010] showed, in salinity conditions, the Na^+ concentrations were higher in roots than in shoots and leaves in most of five olive tree cultivars. This accumulation was attributed to minimize sodium accumulation in leaves and concentrate it in its underground parts [Saidana et al., 2014].

In all geographic areas, the N concentration in Chetoui leaves was higher than the roots and stems. Nitrogen level of Chetoui leaves remained in the lower limits given by Connell and Vossen [2007] (1.4 to 2%), while the wood level seemed to be higher compared with the levels cited by Braham [1999] (0.48–0.47%), but lower than that of leaves. This is due to the high demand for nitrogen during flowering period [Braham 1984, Ben Khelil 2010]. Since, the critical period for N availability is floral induction, and more specifically, before flower bud differentiation.

Potassium is one of the most important nutrients for olive trees [Freeman et al. 2005]. In environmental stress conditions, K was considered the element causing the most severe nutritional disorders [Fernandez-Escobar et al. 2008]. High accumulation of K by different organs in center and southern areas, during optimal growing conditions, may be considered as an "insurance strategy" to enable the plant to better survive in a sudden stress [Kafkafi1990] and to help the tolerance mechanism [Boussadia et al. 2013]. The K was indeed known to be an activator for enzymes related to photosynthesis and respiration and promotes osmo-regulation and stomatal regulation.

The Phosphorus is an essential macro-element; it is not commonly applied in olive cultivation [Freeman et al. 2005, Fernandez-Escobar et al. 2010]. According to Braham [1999] and [Fernández-Escobar et al. 2010], the lowest value of foliar phosphorus is 0.11%; however, in our study their content is less important than the minimum value (0.07%). Phosphorus of Chetoui wood center showed the highest level; but the values do not exceed the level quoted by these authors. Although, the P amount in leaves was above the critical threshold established by Buchman et al. [1958] in irrigated Tunisian olive groves. This element is necessary for many life processes such as photosynthesis and metabolism of carbohydrates. It helps plants, speeds-up the maturity process and drought-stress resistance. Phosphorus (P) is an insufficient and nonrenewable resource; its acquisition by plants decreases when soil moisture declines, as anticipated under climate-change scenarios [Saidana et al. 2015].

The sodium concentration increased with regions; Southern Chetoui leaves and roots showed the highest level. While, in all studied area, leaves presented the highest Ca concentration. This result was in agreement with that of Ezzili [1996] who reported that in Tunisia, in drought conditions, there was an accumulation of sodium in the leaves of the olive tree taking into account the very dry climate of southern Tunisia. Therefore, Na^+ appears to interfere with essential nutrients such as K^+ metabolism and Ca^{2+} reduces this obstruction [Kopittke 2012]. Although, Kchaou et al. [2010] showed, in salinity conditions, the Na^+ concentrations were higher in roots than in shoots and leaves in most of five olive tree cultivars. This accumulation was attributed to minimize sodium accumulation in leaves and concentrate it in its underground parts [Saidana et al. 2014]. Also, in arid environment, adequate concentrations of Ca are required in olive to protect against Na toxicity [Bustan et al. 2013].

Environmental stresses increased the level of Reactive Oxygen Species in plant cells, including drought and salinity [Petridis et al. 2012]. To protect against drought stress, plants produced anti-oxidant compounds, such as phenolics. In our study, southern leaves and roots of olive tree Chetoui produced more volatile compounds than northern ones; this can be explained by the very dry climate to response to oxidative stress. The most of volatile compounds, polyphenols, flavonoides, terpenes, and a part from their role as antioxidants, phenolic compounds have been considered to act as screening agents against especially water deficit [Petridis et al. 2012].

Terpenes were more accumulated in leaves of Chetoui when it was grown in the North than in the South. Although, this result is in disagreement with other studies that showed, terpene concentrations have been generally found to increase in drought conditions [Llusià and Peñuelas 1998]. Their accumulation in water-stressed leaves may have ecological functions such as defense or storage [Penuelas and Estiarte1998].

The accumulation of hydrocarbons was three times more important in Southern Chetoui roots than in the northern ones. In fact, the areas influenced significantly the amounts of heptadecane and octadecene; in particular, the southern roots permitted to obtain Hydrocarbons containing the highest values. This expansion appeared to be an indicator of drought stress.

Under hard climatic conditions, characterized by water shortage and high temperature, the changes in polyphenol content might be affected by changes in the accumulation of macro-elements. Results showed that the response of Chetoui cultivar was different with the area, the organs and mineral concentration. Total phenolic content was positively correlated with contents of Ca, N and Na. According to Cetinkaya et al. [2016] an imbalance of minerals would change the secondary metabolites like polyphenols content; such as phosphorus deficiency might lead to an increase of flavonoid concentration [Lillo et al. 2008]. The production of metabolites might be due to the role of element in activating enzymes that are responsible for enhancing or decreasing biosynthesis of metabolites Cetinkaya et al. [2016]. The management of these macronutrients may therefore be used to control the levels of desirable compounds and improve plant quality [Lillo et al. 2008].

CONCLUSION

Olive tree have evolved an important adaptation mechanism to climatic conditions, heat, dryness and high irradiation, at anatomical, physiological and biochemical levels. To survive under hard conditions, Chetoui developed several tissues of protection, support and conduction in leaves stems and roots. The findings of this study showed that the climatic changes have a notable effect on volatile compounds and minerals in different parts of Chetoui. This cultivar maintained its mineral statute in equilibrium, by way of the preservation of nitrogen and potassium rates. Many volatiles are newly detected in the leaves and the roots of Chetoui cultivar to adapt to these conditions. Such compounds could be considered as new indicators of adaptation to stress. Phenolic compounds were positively correlated with sodium, nitrogen, and calcium contents. However, terpenoids was highly correlated with phosphorus content for all organs and studied areas. Management of these macronutrients may therefore be used to control the volatile levels and improve plant adaptation and quality product. The treatment by volatiles could be a solution to improve the adaptation capacity of olive tree to mitigate abiotic stress effect.

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