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Volatiles Composition, Anatomical and Mineral Changes of the Olive Tree Cultivar Chemlali Under Different Climatic Conditions

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Abstract: **To identify new indicators of stress imposed by climate changes, Chemlali, the main Tunisian olive tree, was studied for its leaf and root volatiles in the north and the south of the country. Great changes in volatiles composition were noted in both aerial and underground organs summarized by enrichment in phenolic and carbonylic compounds and reduction in hydrocarbons and fatty acids in south samples. Anatomic study of leaves, woods and roots of Chemlali grown in the north and the south was realized and compared. Several anatomical changes were identified namely the important development of collenchyme, the invagination of stomata, the multiplication of trichomes, the development of pericyclic fibers, the variations in the proportions of palisade and spongy parenchyma. Additionally, mineral analysis was performed in the different olive tree organs in both regions which demonstrate an important variation of mineral content and distribution. The Chemlali cultivar tries to maintain its mineral balance, and satisfies its nitrogen and potassium needs.**

Keywords: **Chemlali, Climate Changes, Leaf and Root Volatiles, Anatomical Changes, Mineral Analysis.**

I. INTRODUCTION

The olive tree is native and well adapted to the Mediterranean basin where it is most often grown in nutrient poor, shallow, rocky hillside soils and where extended periods of drought during the summer are common [1], [2]. Additionally this species is often grown under rain-fed conditions and is well known for its resistance to water stress [3], [4]. In fact, the olive tree has developed adaptive anatomical, physiological and biochemical mechanisms to withstand drought stress [5], [6], [7], [8].

Anatomical characteristics can be efficiently used as an indicator of drought tolerance [9]. In the majority of cases, there is a correlation between some of xeromorphic features and dry conditions of the habitat [10], [11].

Several studies have shown that modifications of cell wall polymers such as polyphenolics (lignin), long chain aliphatic polymers (suberin), pectins, and proteins help to create barriers to water, solutes, gases, and pathogens in plants exposed to unfavorable biotic and abiotic stress conditions [12], [13], [14]. Casparian bands and suberin lamellae, the major components of apoplastic barriers in plant roots, are laid down in radial transverse and tangential walls in response to different habitat conditions such as drought, anoxia, salinity, heavy metal and nutrient stress [15].

Furthermore, nutritional response of the olive tree was strongly dependent on environmental conditions [16].

Macro and microelements change quantitatively, and qualitatively. The nutrients available in the nutritional environment are also capable of changing volatiles yield and composition [17], [18].

The aim of this study is to indicate anatomical and nutritional changes of olive tree under different climatic conditions and to search new biochemical indicators to stress based on qualitative and quantitative changes in its volatiles.

II. MATERIAL AND METHODS

A. *Plant material:*

The olive tree, cultivar Chemlali is harvested from coastal regions situated in the North, the Center and the South of Tunisia (Mornag, Chott Mariem and Jarzis respectively). The experimental sites belong to different bioclimatic stages (Table1). Leaves, stems and roots of Chemlali are collected.

Table 1. Climatology of different sampling sites

Region	Coordinates	Bioclimatic stage	Localisation
Mornag	36° 40′ 51″ Nord 10° 17′ 25″ Est	Subhumid to Higher semiarid	Coastal North East zone
Chott Meriem	35° 56′ 08″ N 10° 33′ 26″ E	Higher semiarid	Coastal zone in Sahel
Zarzis	33° 30′ N 11° 07′ E	Lower arid	Coastal South zone

B. *Anatomical analysis:*

Transverse sections of fresh leaf, stem and root of Chemlali cultivar, grown in the North, Center and South, are hand-sectioned by razor blade, as described by

Locquin and Langeron, [19]. 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 are examined by trinocular microscope Leica, model DM-1000, coupled with a camera photo Leica (DFC-280), resulting in images of 1024 × 1280 pixels of resolution.

C. Mineral analysis:

Nitrogen (N) was determined in leaf, stem and root tissues by the Kjeldahl method [20].

Potassium (K), calcium (Ca) and sodium (Na) contents (% DW) were determined according to Martin-Prével et al. [20]. Leaf, stem and root were dried at 70°C for 48 h and then ground. One gram of those materials has undergone calcination. The mineral content was determined using a flame photometer (Jenway, England).

D. Volatiles extraction:

The fresh leaves and roots, gathered from Chemlali of the North and the South of Tunisia, were cut in little pieces, weighted and submitted to steam distillation for 6 h. The recovered solution was extracted with the hexane. After drying the extract over anhydrous $MgSO_4$, the solvent was removed and the oil samples were stored prior to analysis. Yield based on fresh weight of the sample was calculated.

E. GC/MS:

The analysis of the volatile constituent were run on a Hewlett-Packard GC-MS system (GC: 5890 series II; MSD 5972). The fused-silica HP-5 MS capillary column (30m0.25mm ID, film thickness of 0.25 mm) was directly coupled to the MS. Oven temperature was programmed (50 °C for 1 min, then 50–280 °C at 5 °C/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).

F. Mass spectrometer:

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.

G. 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) [21], [22].

III. RESULTS

A. Climatic change effects on anatomical feature:

The leaf cross sections of Chemlali, grown in the North, the Centre and the South, indicate an increase in leaf vascularity from the North to the South (Fig. 1, N_1, C_1 and S_1).

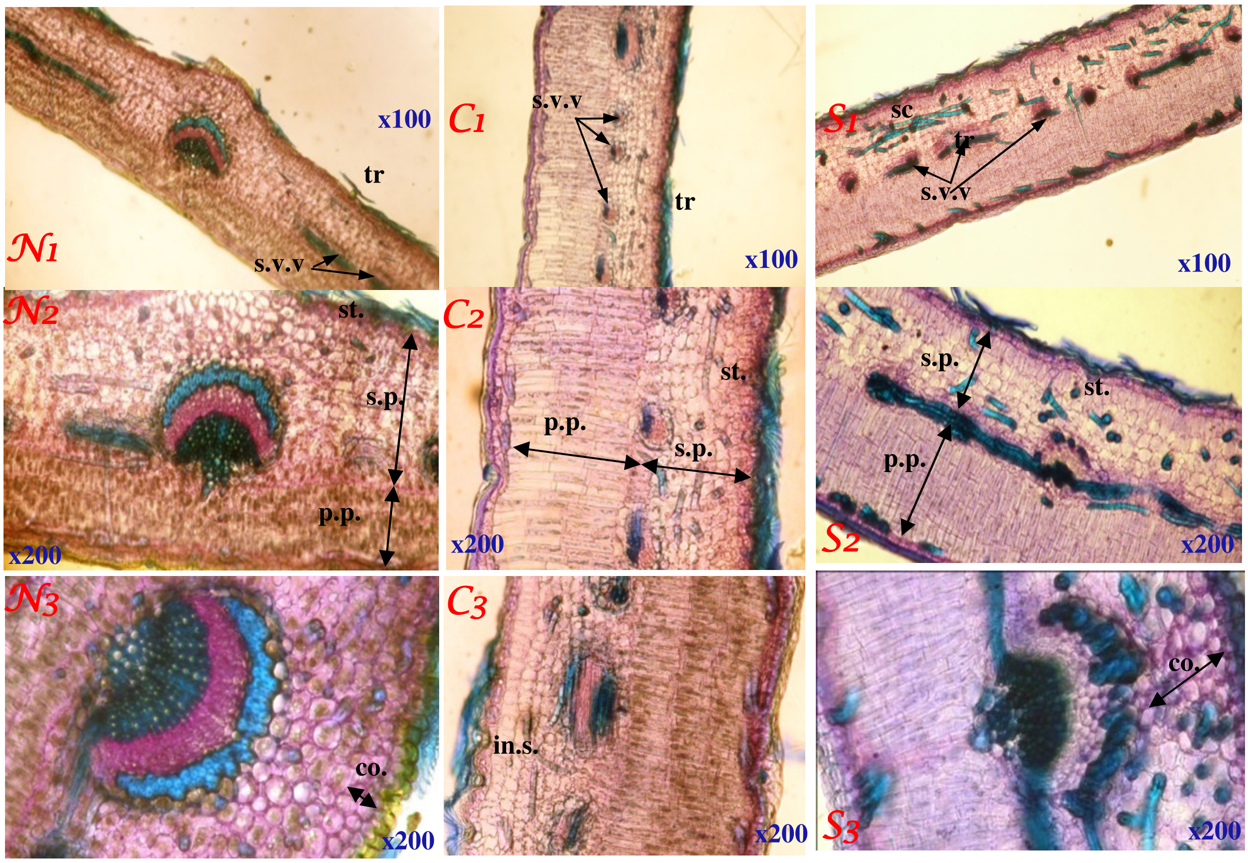

Fig. 1. Cross-sections of the Chemlali leaf in the different regions.

$N_{1,2,3}$: Northern Chemlali; $C_{1,2,3}$: Center Chemlali; $S_{1,2,3}$: Southern Chemlali. Sc: sclerites; tr: trichomes; s.v.v: Secondary vascular vessels; s.p.: spongy parenchyma; p.p: palisade parenchyma; st: stomata, co: collenchyme, in.s: invaginated stomata.

The secondary conductor vessels seem to be multiplied. To enhance the protection, an enrichment of trichomes is noted in abaxial surfaces of Chemlali leaves, increasingly from the north to the south areas.

Trichomes overcome stomata, to avoid their direct contact with sunlight. However, in leaves adaxial surfaces, stomata are invaginated in all tested area (Fig. 1, N_2, C_3 and S_2). Sclerites are more abundant in the spongy parenchyma of the South leaves, consolidating additionally their support (Fig. 1, S_1).

In the other way, there is an increasing development of palisade parenchyma at the expense of the spongy's one, increasingly from the North to the South. The palisade parenchyma forms a barrier that minimizes receiving sunlight and strengthens leaf protection (Fig. 1, N_2, C_3 and S_2).

The microscopic examination of the Chemlali wood, present in three regions belonging to the North, Center and South of Tunisia, show a greater development of the cuticle layer surrounding Chemlali stem of the Centre and the South compared to the North's one. Some trichomes are present at the cuticle layer of Chemlali belonging to the Centre and the South (Fig. 2, C_2 and S_2).

On the other hand, at the stem of Chemlali growing in South, the liberian tissue is smaller compared to the other regions (Fig. 2, $N_{1,2}$, $C_{1,2}$ and $S_{1,2}$). Below the cuticle layer, the collenchyme shows an important development especially in the Chemlali stem of the Centre and the South (Fig. 2, N_3, C_3 and S_3). Several inclusions of starch grain are detected in southern sample, comparatively to the others (Fig. 2, N_4, C_4 and S_4). According to the root cross-sections of Chemlali growing in the North, Center and South, there is a development of the pericyle, especially in roots of the Center and the South comparatively to the North's one, inducing thus the sclerotization of the cortex and the increase of its rigidity (Fig. 3, $N_{1,2}$, $C_{1,2}$ and S_1).

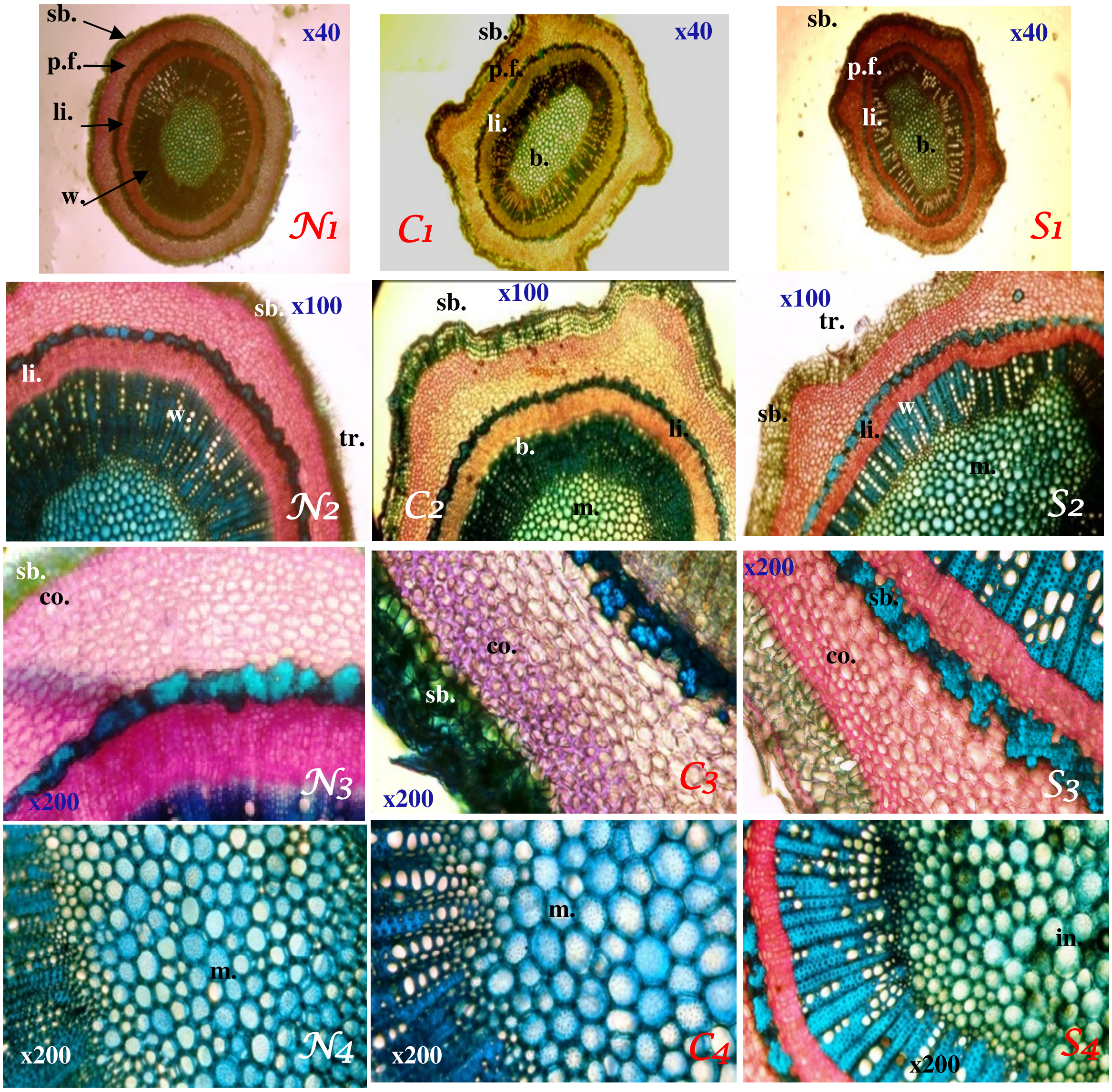

Fig. 2. Cross-sections of the Chemlali wood in the different regions.
$N_{1,2,3}$: Northern Chemlali; $C_{1,2,3}$: Center Chemlali; $S_{1,2,3}$: Southern Chemlali; Co: collenchyme; w: wood; p.f.: pericyclic fibers; li: liber; sb: suber; m medulla; tr: trichomes, in: inclusion.

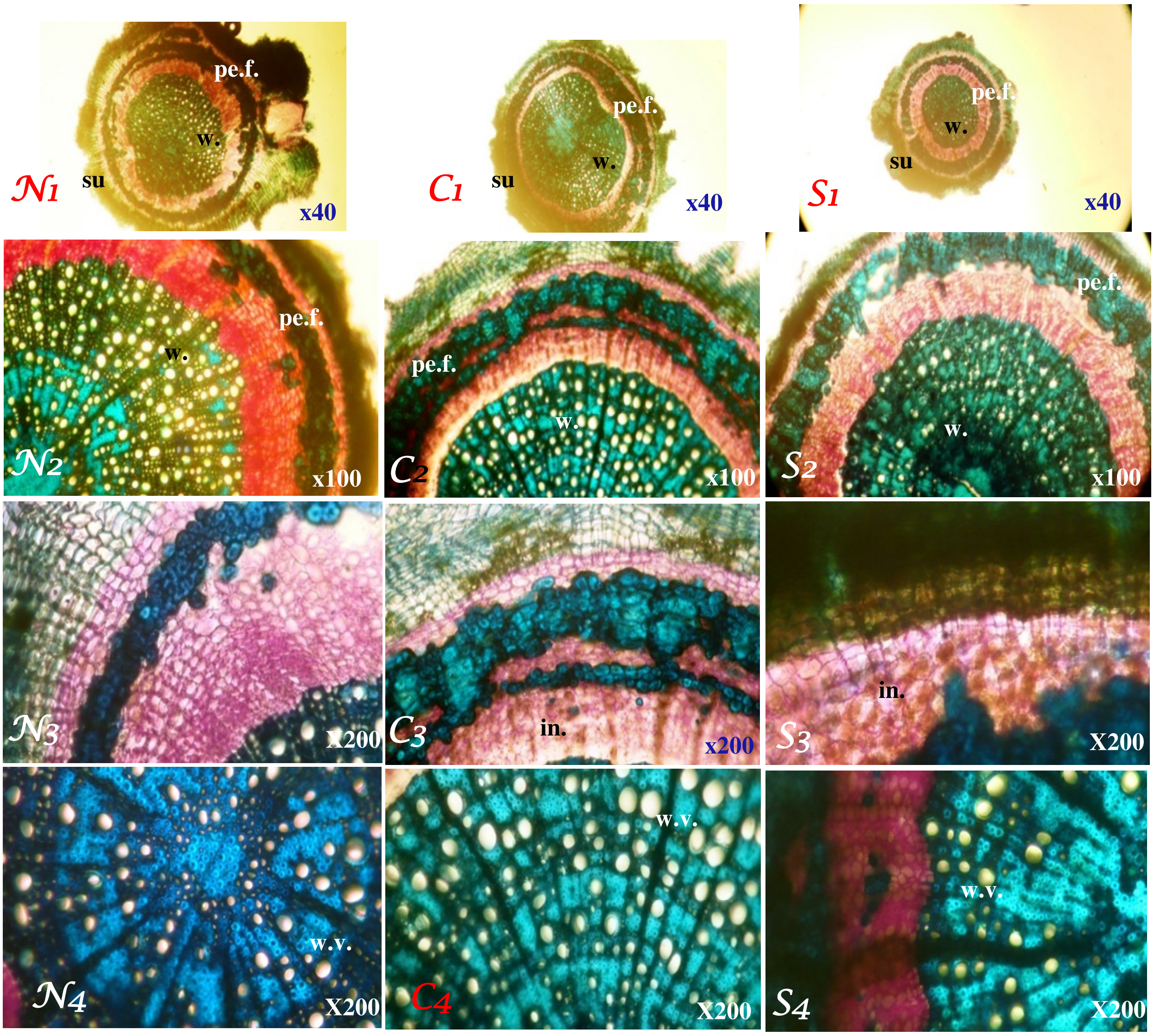

Fig. 3. Cross-sections of the Chemlali Root in the different regions

$N_{1,2,3}$: Northern Chemlali; $C_{1,2,3}$: Center Chemlali; $S_{1,2,3}$: Southern Chemlali; w: wood; pe.f: pericyclic fibers; sb: suber; s.g. starch grains; W.V. wood vessels.

Inclusions of starch grains are present in both liberian (Fig. 3, C3) and cortical zones (Fig. 3, S3).

In the medulla zone of Chemlali roots, an increase of wooden vessels is noted in the Center (Fig. 3, C_4), differently from the South, where vessels are less abundant in the medulla zone which is covered with a well developed and lignified tissue (Fig. 3, S_4).

B. Climatic change effects on mineral statute:

1. Evolution of calcium content:

There are a decrease of 17.55 and 5.98 % in the calcium rate in both leaves and woods of Chemlali, growing in the Centre area. However, significant increases of 15; 28.65 and 25.25% are noted in the calcium levels in all Southern Chemlali parts (leaves, woods and roots) comparatively to the Northern one. Chemlali limits the variation rate of the calcium, in its leaves, comparatively to the other organs, especially in the Center, which restricts the interaction of the calcium on the absorption of the other vital minerals (Fig. 4).

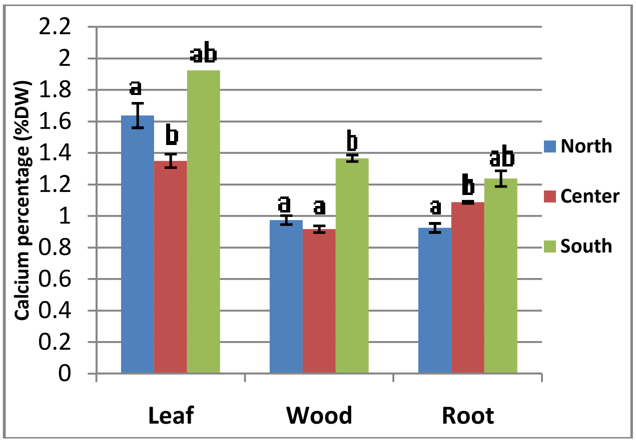

Fig. 4. Calcium content change of Chemlali growing in different geographic areas.

Letters change: the difference is significant. Same letter, the difference is not significant at 5%.

2. Evolution of sodium content:

Regional comparison shows no significant differences in the concentrations of sodium, for Chemlali cultivar in the wood and the root of Chemlali. Exceptionally, only one peak (1 %) was detected in the leaves of the cultivar of the South, where a significant increase of 40% is detected comparatively to the North's (Fig. 5).

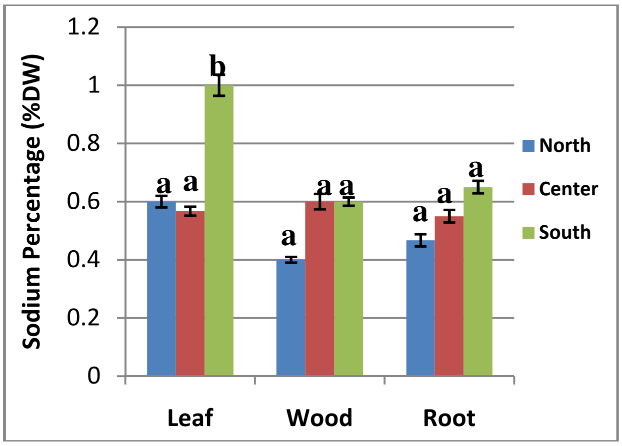

Fig. 5. Sodium content change of Chemlali growing in different geographic areas.

3. Evolution of potassium content:

The potassium is concentrated similarly in the different organs of Northern Chemlali as 1.05, 1.02 and 0.87% respectively for leaves, wood and roots (Fig. 6). Conversely, the potassium content of Central and Southern Chemlali appears higher than that of the North, in all tested organs. Indeed, increases of 19.74; 14.68 and 22.79% are respectively recorded for leaves, woods and roots of Central Chemlali and 14.28; 28.65 and 16.66% for Southern Chemlali, comparatively to the Northern one (Fig. 6).

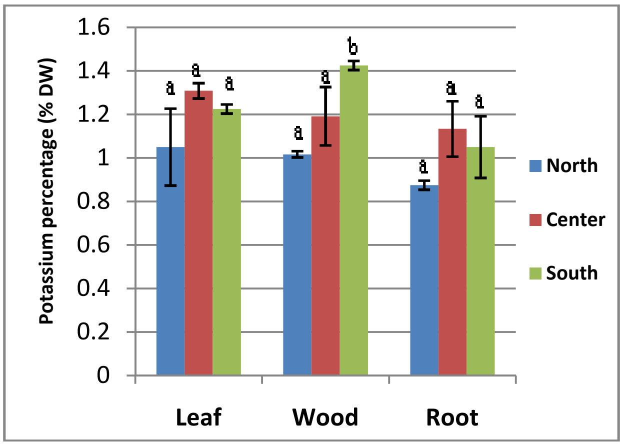

Fig. 6. Potassium content change of Chemlali growing in different geographic areas.

4. Evolution of phosphorus content:

Phosphorus is accumulated especially in leaves, reaching 0.07; 0.06 and 0.03% in Northern Chemlali leaves. Contrary to the other mineral, the phosphorus stored in the South sample is in the lowest levels; a reduction of over 50% is unregistered for leaves of Southern Chemlali compared to the Northern cultivar. For Chemlali woods and roots, phosphorus is dominant in the Centre, reaching 0.04%; reductions of 44.80 and 53.81% are respectively noted in the samples of the South comparatively to the Centre. Thus phosphorus is a very sensitive element to high temperatures and dry climate (Fig. 7).

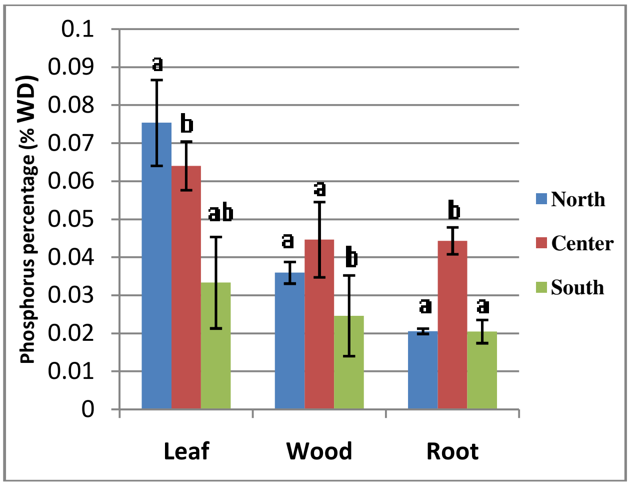

Fig. 7. Phosphorus content change of Chemlali growing in different geographic areas.

5. Evolution of Nitrogen content:

Chemlali cultivar is rich in nitrogen. The highest values are recorded in Northern and Southern Chemlali leaves (1.56%) (Fig. 8). Nitrogen rate seems to be more important in Center and Southern Chemlali wood, reaching 0.8%. However, it is concentrated essentially in the root of Northern Chemlali (1.2%).

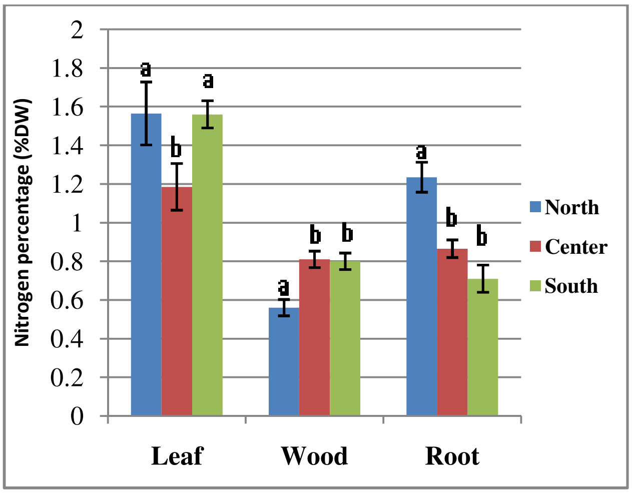

Fig. 8. Nitrogen content change of Chemlali growing in different geographic areas.

C. Climatic change effects on Chemlali volatiles:

1. Volatiles yield:

The volatile yields are generally very low. The obtained yields are summarized in Fig. 9. The highest volatile yield is stored in Chemlali leaves of Center (0.035%), followed by the amount extracted from Chemlali wood of North (0.032%) (Fig. 9a). Furthermore, it is notable that the volatile productivity is more important in the roots of the Southern Chemlali compared to other regions. Indeed, increases of 55.44 and 66.8% are recorded for the Southern cultivar compared respectively to the Northern and the Center one (Fig. 9a). Chemlali growing in the Center seems to be the richest in volatiles (0.058 %), contrarily to the Southern one, which presents the lowest volatile yield (0.029%) (Fig. 9b).

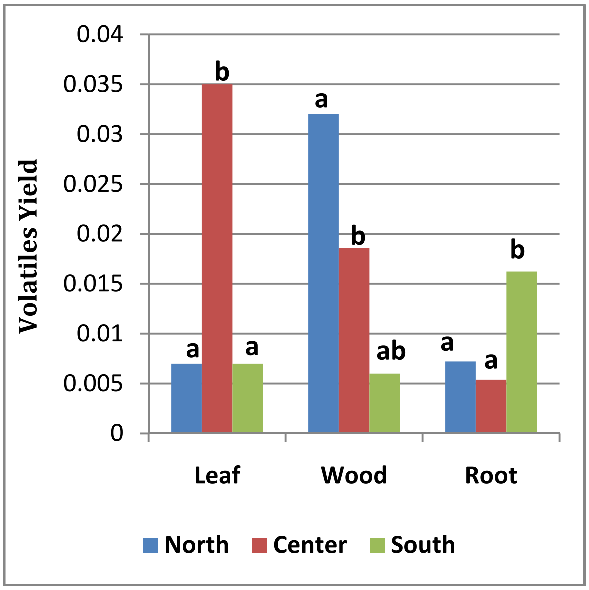

Fig. 9.(a)

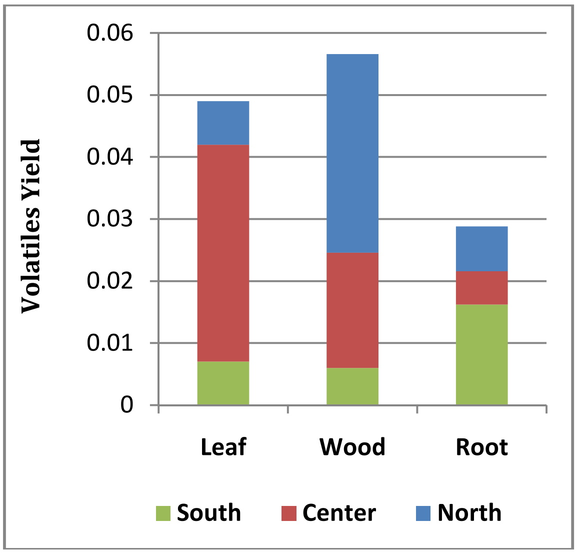

Fig. 9.(b)

Fig. 9. Volatiles yield of Chemlali growing in different geographic areas.

2. Volatiles chemical composition:

The chemical composition of the cultivar Chemlali volatiles, grown in the North and the South of Tunisia, is analyzed by CPG- FID and CG-SM (Table 2).

60.83 and 69.95% of compounds are identified in leaves and roots of Chemlali of North, while 80.4 and 72.84 are identified in Southern Chemlali. Several compounds are common in all studied samples, such some hydrocarbons N Tetradecane, 1- Tetradecene , pentadecane; several aromatic compounds, as Benzeneethanol, Phenol, 2,4-bis(1,1-dimethylethyl); fatty acids as Nonanoic acid, Hexadecanoic acid, Octadecanoic acid and 9-Octadecenoic acid. However, these compounds are present in different proportions.

Although it is the same cultivar grown, in pluvial conditions, in the North and the South of Tunisia, there is an important difference in the composition of the leaves and the roots of this cultivar, in these areas. 1-Pentadecene, Hexadecane, 1-Docosene and 9,12,15-Octadecatrienoic acid, methyl ester are only identified in the leaves and the roots of Northern Chemlali; while 1H-Indene, octahydro-, cis-; Benzene, 1,2,4-trimethyl-; Benzene, 1,2-dimethyl-; C3-Benzene are identified only in the leaves and the roots of Southern Chemlali. These compounds are specific to the studied area and depend directly on the weather and the soil conditions residing in this area. Certainly they play an important role to overcome the hard conditions.

Numerous aromatic compounds are only specific to Southern Chemlali leaves, especially Benzene, 1-ethyl-3-methyl- (1.01%); *p*-cymène (0.23%), Benzoic acid ; 2-hydroxy-, methyl ester; 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-; 2(3H)-Benzothiazolethione (1.95%) and 3-(4-Methoxyphenyl)-2-Methyl-1-Propene (0.96%).

However the proportion of aromatic compounds in Northern Chemlali leaves (71.93%) is much higher than that of the South (26.58%). Additionally, many Ketones characterize Southern Chemlali leaves such as 2,3,4,5-Tetramethyl-2-Cyclopent1-one, 5,8-dimethoxythiochroman-4-one;1,3,6,7,8-Pentamethyl-5,6,7,8-Tetrahydropteri-2,4,-dione in the proportion respectively of 1.64; 3.64 and 1.73%.

Other products characterize only the leaves of Chemlali, namely 1-Hexadecene; beta.-Ionone, Dodecanoic acid and 2-bis (methylthio) methylene-1-phren yl-4-methyl-4-penten-1-one. While, Cyclohexasiloxane, dodecamethyl; Heptadecane; Benzene, 1-ethyl-2-methyl and l-.alpha.-Terpineol are identified only in the roots of this cultivar.

IV. DISCUSSION

In Tunisia, Chemlali is the main cultivar of olive oil. This olive tree accounts for 80 percent of the national production of olive oil and is grown in the centre and the south of the country, in areas with low rainfall (lower than 250 mm per year). To adapt to these areas and to oppose to the climatic fluctuation specific to it, Chemlali has developed many arrangement including anatomical, mineral, physiological and biochemical changes.

Table 2. Volatile composition of leaves and roots of Chemlali growing in the North and the South of Tunisia

		North		South	
	BPX-5				
Compounds	**DB wax**	**Leaves**	**Roots**	**Leaves**	**Roots**
Phenolic compounds					
1H-Indene, octahydro-, cis-	1035.1			0.16	0.97
o-Xylene	1179.3			0.71	0.66
p-cymène	1217.6			0.23	
o-Ethyltoluene	1341		0.16		4.82
1,2,4-Trimethylbenzene	1348			1.47	5.89
m- Xylene, 4-ethyl-	1395.07				0.14
3-Pyridinecarboxylic acid, 5-ethenyl, methyl ester	1509		4.07		4.8
Benzoic acid, 2-hydroxy-, methyl ester	1771.7			2.48	
Benzeneethanol	1890.9	2.3	0.49	13.87	
Phenol	1981.9			2.17	
Cuminic alcohol	2079		2.79		2.78
CIS-3-HEXENYL BENZOATE	2106			3.16	
Methyl alpha Deutriocinnamate	2114.3		5.14		
5-Acetyl-6-Methyl-Benzimidazolone		4.5			
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	2313.3			0.45	
Phenol, 2,4-bis(1,1-dimethylethyl)	2423.6		0.92	3.25	1.42
3-(4-Methoxyphenyl)-2-Methyl-1-Propene	2692.4			0.96	
4-methyl –Methylphenanthrene		2			
Phenanthrene, 2,5-dimethyl			1.07		
Total		**8.8**	**14,64**	**28.91**	**21.48**
Terpenoid					
Oxygenated monoterpen					
CIS-3-HEXENYL BUTYRATE	1465.8			1.14	
Octyl Trifluroacetate			0.7		
Benzene, 1-ethenyl-4-methoxy-	1670				1.02
l-.alpha.-Terpineol	1693.4		2.27		0.54
Sesquiterpenoid hydrocarbons					
Dodecane, 2,6,11-trimethyl- 53	1689.3				0.69
Oxygenated Sesquiterpen					
Cubebene			1.92		
Oxygenated Diterpen					
Decyl ether		0.13			
Cis-Linalool oxide	1720.7		0.74		
Linalool	1548.6		2.14		
Triterpenoid hydrocarbons					
Squalene	3101.5				0.37
Total		**0.13**	**7.77**	**1.14**	**2.62**
Alcohols					
CIS-3-HEXENOL	1382.1			0.43	
1-Hexanol, 2-ethyl-	1483.8		1.25		
Ethanol, 2-(hexadecyloxy)-	3720.7				0.13
Total			**1.25**	**0.43**	**0.13**
Carbonylic compounds					
Nonyl aldehyde	1391.7	-	1.02	1.94	1.44
2,4 Heptadienal	1491.5			1.43	
Decanal	1510				0.74
Camphenilone	1539.8			8.03	
2,3,4,5-Tetramethyl-2- cyclopenten 1-one A					1.64
E-2-decenal	1700.2			1.65	
beta.-Damascenone	1832	2.09		10.63	0.94
Geranyl acetone	1852.8	0.13			
Dihydro-.alpha.-ionone	1830		3.57		
beta.-Ionone	1921.7	3.57		2.49	
(Z)-non-6-en-11-one		0.45			

p-Carbomethoxybenzaldehyde	2249.2			6.35	
5,8-dimethoxythiochroman-4-one	2352.2				3.64
1-Oxaspiro(4.5)decan-2-one				2.65	
1,3,6,7,8-Pentamethyl-5,6,7,8-	2423.6				1.73
2(3H)-Benzothiazolethione	2442.1			1.95	
BHT-aldehyde	1774		1.58		
2-bis (methylthio)methylene-1-phren yl-4-methyl-4-penten-1-one	3362.3	0.18		0.25	
Total		**6.42**	**6.17**	**37.37**	**10.13**

		North		South	
	BPX-5				
Compounds	**DB wax**	**Leaves**	**Roots**	**Leaves**	**Roots**
Hydrocarbons					
Dodecane	1200	0.26			
N-Tetradecane	1400	2.36		0.81	1.32
1-Tetradecene	1440	0.97			1.5
Cyclopropane, nonyl-					0.43
Pentadecane	1500	1.5			0.49
1-Pentadecene	1545	2.66	2.04		
Hexadecane	1600	0.82	1.5		
1-Hexadecene	1644	0.28		2.78	
Heptadecane	1700		3.38		1.88
1-Heptadecene	1725				1.9
Octadecane	1800		0.76		2.54
Nonadecane	1900				1.45
1-Nonadecene	1956	0.99			
Heneicosane	2100		1		
pentadecane	1500		3.24		
1-Docosene	2194	1.47	1.11		
Tricosane	2300		1.55		
Tetracosane	2400		1.64		
1-Octadecene	2670				2.31
Hexacosane	2600		1.16		
1-Hexadecene	1644				1.36
1-methyl-2-(3-methyl-2-buten-1-yl) -1-(4-methyl-3-penten-1-yl)oxetane	2871.5				1.69
N -Docosane	2200		0.65		
Triacontane	3000				0.1
Heptadecane	1700		0.42		
1,4-Dimethyl-Anthracene		0.23			
Phenanthrene, 2,5-dimethyl		0.84			
Total		**12.32**	**18.45**	**3.59**	**16.97**
Fatty acids					
Octanoic Acid	2048.4	3.23			
Nonanoic acid	2143.6	9.05	3.09	2.29	8.44
Decanoic acid	2271.9	3.31			
Geranic Acid	2327.7	5.05			
Benzoic acid, 4-nitro-		1.46			
Dodecanoic acid	2488.4	1.68		0.82	
Pentadecanoic acid	2812				1.01
Hexadecanoic acid	2899	4.7		3.01	4.5
Tetradecanoic acid	2724		6.39		
Palmitic acid	2899		1.89		
Heptadecanoic acid	3022.2				0.55
Octadecanoic acid	3181	1.07	2.97	0.21	1.19
Oleic acid	3184	1.9	4.18	0.35	2.91
Linoleic acid	3154.9			0.26	0.83
Total		**31.45**	**18.52**	**6.94**	**19.43**
Others					

Triallylsilane		0.17			
Cyclopentasiloxane, decamethyl-: D5	1183		0.42		
Cyclohexasiloxane, dodecamethyl: D6	1365		0.45		0.53
α-Ionene	1567	0.32			
Theaspirane A	1528,5	0.43			
Methyl linolenate	2565.1			0.58	
1-Heneicosyl formate	2691		0.73		
Beta.-H-Pregna	2702.9				1.55
Farnesyl acetate 78	2998			0.12	
Murrayafoline A	3065,06			0.18	
Methy l linolenate	2565.18	0.67	1.55		
Ethyl Linoleolate	3275.25			1.14	
Cholesta-3,5-diene		0.12			
Total		**1.71**	**3.15**	**2.02**	**2.08**
Identified compounds		**60.83**	**69.95**	**80.4**	**72.84**

The leaf of Chemlali seems to contain more abundant vessels in the South area to facilitate sap circulation. The secondary conductor vessels are multiplied. According to Luković et al [23], the genotypes of sugar beet, with a larger number of vascular bundles per mm^2 of the cross-section area of the main vein have a larger number of smaller vessels as well as a lower proportion of sclerenchyma.

Jacobsen et al. [24] think that xylem density may be a useful tool in estimating the xylem characteristics and drought tolerance of large number of species.

Palisade parenchyma appears more developed in Chemlali leaves growing in the southern area. Generally, the degree of differentiation of the mesophyll and the proportion of palisade and spongy parenchyma vary in relation to plant species and habitat [25]. According to Martinez et al. [26], the size of the spongy cell parenchyma of the leaves of *Phaseolus vulgaris* L., under water stress treatment, represented only 40% of the size of the palisade cell.

Since significant correlations were recorded between the size of the vascular bundle of the main vein, number and length of stomata on abaxial side, palisade cells height and physiological traits, cited structural characteristics may point to the adaptation ranges to drought [27].

The cuticle layer is more developed in the Center and the Southern Chemlali stem. The waxy layer varies in continuity and in thickness with the age of the leaf and with growing conditions [28]. Cuticular waxes play an important role in drought tolerance [29]. The increase in wax content might be used to improve drought resistance and water use efficiency.

The Liberian tissue becomes narrow in stems of Chemlali growing in south, comparatively to the other regions. This data is in accordance with Joyce et al., [30] finding, as the stress significantly causes a reduction of the number of cells in all tissues.

Generally speaking, it is known that shoots are not the main resistance to water flow, about half of the total plant resistance lies in roots, and most of the other half may be in leaves [31]. Reinhardt and Rost [32] showed that the absolute amounts of suberin and lignin in the exodermal cell walls of cotton seedling roots are markedly increased by external stresses such as salt stress, osmotic stress, and heavy metal stress. In the case of the olive tree Chemlali, a lignified tissue is well developed in medulla zone, which contains vascular vessels with small size.

The relationships between olive mineral nutrition, flowering, and productivity are complex and dependent on environmental factors such as water availability and winter chilling [16]. The different elements are in direct correlation; increased calcium causes a decrease in the other elements, which is confirmed by several authors [33]. According to Therios and Sakedalliaris [34], the existence of a competition between minerals at transporter may be the cause of positive and negative interactions. Thus, in the different organ of the cultivar, the calcium, sodium, potassium, phosphorus and nitrogen vary according to the weather conditions, but remain in equilibrium. Braham [35] reported that within the same variety grown in two different regions, there are not the same balances but in each zone, the olive tree tends to achieve a specific balance.

An increase of sodium concentration is detected in the leaves of Southern Chemlali, which confirms the results of Ezzili [36] who reports that in Tunisia, in a dry year, there is an accumulation of sodium in the leaves of the olive tree taking into account the very dry climate of southern Tunisia (Fig. 5). Sodium is the most common ion causing salinity, but other ions can also be found in toxic level. The antagonism between Na^+ and the three cations, which are essential nutrients (K^+, Ca^{2+}, and Mg^{2+}) is well documented in the literature [37], [38], [39], [40].

According to Bouat [41] and Braham [42], the maximum level of potassium in olive tree leaves is 1.99%. Additionally, a sufficiency threshold of 0.8% is presented by Fernández-Escobar [43]. In our study, potassium is acceptable; it is concentrated in the rate of 1.3% in Chemlali leaves (Fig. 6).

Potassium (K) is considered as one of the most important minerals in olive nutrition [44], [45], probably as a result of the high concentration of K found in the fruit flesh [43]. Reports on K deficiencies are common in the Mediterranean basin, even on relatively deep fertile soils

[2], [43]. Potassium is considered as the element causing the most severe nutritional disorders in olives when environmental levels are low [46].

Several increases of K rate are noted in all Chemlali parts when it is growing in the Center and the South. According to Boussadia et al. [47], K accumulated substantially in olive tree, apparently to help the tolerance mechanism. K is indeed known to be an activator for enzymes related to photosynthesis and respiration and promotes osmoregulation and stomatal regulation.

According to Braham [42] and Fernández-Escobar [43], the minimum value of foliar phosphorus is 0.11%; however, the stored content in our study is smaller and hasn't even reached the minimum value (0.07%). Similarly, for the wood, the majority of recorded values do not exceed the value quoted by these authors. Phosphorus (P) is a scarce and nonrenewable resource; its acquisition by plants decreases when soil moisture declines, as anticipated under climate-change scenarios. Frequent soil drying is likely to induce a decrease in nutrients particularly P due to reduced diffusion and poor uptake, in addition to restrictions in available water, with strong interactive effects on plant growth and functioning.

The nitrogen level of Chemlali leaves remain within the limits given by Bouat [41] (1.01 to 2.55%), while the wood level is high compared with the levels cited by Braham [42] (0.48 -0.47%), but lower than that of leaves. This is due probably to the high demand for nitrogen during flowering stage [35], [48] (Braham, 1984; Ben Khelil, 2010).

The yield is varied according to the studied region; it's influenced by soil and climatic conditions [49], [50]. To oppose the harsh weather conditions, synthesis of volatiles is stimulated, demonstrating their important role in adaptation to the severe conditions.

The volatile composition of Chemlali leaves, stems and roots changes qualitatively and quantitatively according to the studied region. Aldehyds and ketones seem to be accumulated especially in the Southern Chemlali leaves, while aromatic compounds and fatty acids seem to be in the Northern Chemlali leaves.

The methyl alpha deutriocinnamate is accumulated only in Chemlali leaves in important proportion (5.14%), when this cultivar is grown in the South. This compound is known being involved in lignifications, which increase in drought conditions [51], [52].

The beta-Damascenone is a Ketone concentrated especially in Southern Chemlali leaves (10.63%). This compound is derived from the degradation of carotenoids. The degradation prevents the oxidative stress by inhibiting oxidation of several biological substrates, including polyunsaturated fatty acids [53].

Terpene concentrations have been generally found to increase in drought conditions [54], [17]. This result is in concordance with our founding. Terpenes are more accumulated in leaves of Chemlali when it is grown in South (1.14%) than in North (0.13%). However, the contra result is found in roots (7.77% in North opposing to 2.62% in South). Terpenes in this fact are more concentrated in roots than in leaves.

V. CONCLUSION

This research is, in our Knowledge, the first report on the effect of the climatic changes on olive tree volatiles. Many compounds are newly secreted in the leaves and the roots of the olive tree, especially cultivar Chemlali to adapt to these conditions. Such compounds could be considered as new indicators of adaptation to stress. Anatomical proprieties of Chemlali under hard conditions are summarized to the development of tissues of protection, support and conduction. Additionally, Chemlali tries to maintain its mineral state in equilibrium with the preservation of nitrogen and potassium rates.

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REFERENCES

[1] S. Lavee, "Biology and physiology of the olive", in *International Olive Oil Council* (Eds.), World Olive Encyclopedia, International olive oil council (IOOC), Madrid, Spain, 1996, pp. 59–106.

[2] Z. Wiesman, "Desert olive oil cultivation", in *Advanced Biotechnologies,* Elsevier Inc., New York, 2009.

[3] C. Ben Ahmed, B. Ben Rouina, M. Boukhris,. Effects of water deficit on olivetrees cv. Chemlali under field conditions in arid region in Tunisia. Sci. Hortic.113, 2007, pp. 267–277.

[4] D. Barranco, R. Fernandez-Escobar, L. Rallo, *Olive Growing*. Mundi-Prensa/Australian Olive Association Ltd., Junta de Andalucía, 2010, 756 pp.

[5] A. Bosabalidis, G. Kofidis,. Comparative effects of drought on leaf anatomy of two olive cultivars. Plant Sci. 163, 2002, pp. 375–379.

[6] B. Dichio, C. Xiloyannis, K. Angelopoulos, V. Nuzzo, S. A. Bufo, G. Celano, Drought-induced variations of water relation in Olea europaea. Plant Soil 257, 2003, 381–389.

[7] E. A. Bacelar, D. L. Santos, J. M. Moutinho-Pereira, B. C. Gonçalves, H. F. Ferreira, C. M. Correia, Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. Plant Sci. 170, 2006, pp. 596–605.

[8] M. Ennajeh, T. Tounekti, A.M. Vadel, H. Khemira, H. Cochard, Water relation and drought-induced embolism in olive (*Olea europaea* L.) varieties "Meski" and "Chemlali" during severe drought. Tree Physiol. 28, 2008, pp. 971–976.

[9] M.B.G. Martins, R. Zieri, Leaf anatomy of rubber-tree clones. Sci. Agric. 60, 2003, pp. 709–713.

[10] Z. Ristic, D. D. Cass, Leaf anatomy of *Zea mays* L. in response to water shortage and high temperature: a comparasion of drought-resistant and drought sensitive lines. Bot. Gaz. 152, 1991, pp. 173–185.

[11] S. Belhadj, A. Derridj, T. Aigouy, C. Gers, T. Gauquelin, J. P. Mevy,. Comparative morphology of leaf epidermis in eight populations of atlas Pistachio (*Pistacia atlantica* Desf., *Anacardiaceae*). Microsc. Res. Technol. 70, 2007, pp. 834–846.

[12] D. E. Enstone, C. A. Peterson, F. Ma, Root endodermis and exodermis: structure, function, and responses to the environment. J. Plant Growth Regul. 21, 2003, pp. 335–351.

[13] C. A. Moore, H. C. Bowen, S. Scrase-Field, M. R. Knight, P. J. White, The deposition of suberin lamellae determines the magnitude of cytosolic Ca^{2}+ elevations in root endodermal cells subjected to cooling. Plant J. 30, 2002, 457–465.

[14] R. P. Sabba, E. C. Lulai, Histological analysis of the maturation of native and wound periderm in potato tuber. Ann. Bot. 90, 2002, pp. 1–10.

[15] C. Kun-Ming, Y-H. Feng Wang, C. Wang Tong, H. Yu-Xi, L. Jin-

Xing. Anatomical and chemical characteristics of foliar vascular bundles in four reed ecotypes adapted to different habitats. Flora 201, 2006, pp. 555–569.

[16] R. Erel, U. Yermiyahu, J. Van Opstal, A. Ben-Gal, A. Schwartz, A. Dag, The importance of olive (*Olea europaea* L.) tree nutritional status on its productivity. Scientia Horticulturae 159, 2013, pp. 8–18.

[17] J. Llusià, J. Peňuelas,. Changes in terpene content and emission in potted Mediterranean woody plants under severe drought. Can. J. Bot. 76, 1998, pp. 1366-1373.

[18] R. Nurzyńska-Wierdak, Does mineral fertilization modify essential oil content and chemical composition in medicinal plants?. *Acta Sci. Pol., Hortorum Cultus 12(5), 2013, 3-16.*

[19] Locquin M., Langeron M., 1996. Manuel de Microscopie. Masson, 2ème édition, Paris. 350 p.

[20] P. Martin-Prével, J. Gonard, P. Gautier, "Méthodes analytique de référence, in *L'analyse végétale dans le contrôle de l'alimentation des plantes tempérées et tropicales*. Edition Lavoisier TEC & DOC, 1984.

[21] T. Shibamoto, "Retention indices in essential oil analysis", in *Capillary Gas Chromatography in Essential Oil,* Sandra P, Bicchi C (Eds.), , 1987, pp. 259–275. Dr. Alfred Heuthig.

[22] R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. Allured, Carol Stream, IL, 1995.

[23] J. Luković, I. Maksimović, L. Zorić, N. Nagl, M. Perĉić, D. Polić, M. Putnik-Delić, Histological characteristics of sugar beet leaves potentially linked to drought tolerance. Industrial Crops and Products 30, 2009, pp. 281–286.

[24] L. A. Jacobsen, L. Agenbag, J. K. Esler, R. B. Pratt, W. F. Ewers, D. S. Davis, Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa. J. Ecol. 95, 2007, 171–183.

[25] K. Esau, (Ed.), *Plant Anatomy*. John Wiley & Sons, Inc., New York/London/Sydney, 1965.

[26] J.P. Martinez, H. Silva, J.F. Ledent, M. Pinto, Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L). Eur. J. Agron. 26, 2007, pp. 30–38.

[27] Lj. Merkulov, B. Krstić, L. Kovaĉev, J. Ivezić, S. Pajević, Source-sink relationship in sugar beet lines. In: Proceeding for Natural Sciences, Matica Srpska Novi Sad, 91, 1996, pp. 73–81.

[28] B. G. Bystrom, R. B. Glater, F. M. Scott, S. C. Bowler, Leaf surface of *Beta vulgaris* electron microscope study. Bot. Gaz. 129, 1968, pp. 133–138.

[29] K. D. Cameron, M. A. Teece, E. Bevilacqua, L.B. Smart, Diversity of cuticularwax among *Salix* species and *Populus* species hybrids. Phytochemistry 60, 2002, pp. 715–725.

[30] D. C. Joyce, D. Aspinall, G. R. Edwards, Water deficit and the growth and anatomy of the radish flesehy axis. New Phytol. 93, 1983, pp. 439–446.

[31] F.C. Meinzer, Coordination of vapour and liquid-phase water transport properties in plants. Plant Cell Environ. 25, 2002, pp. 265–274.

[32] D.H. Reinhardt, T.L. Rost, Salinity accelerates endodermal development and induces an exodermis in cotton seedling roots. Environ. Exp. Bot. 35, 1995, pp. 563–574.

[33] F.A.O., *Les engrais et leurs applications, Précis à l'usage des agents de vulgarisation agricole*, Quatrième édition, Editions F.A.O., I.F.A. (Paris, France) et IMPHOS (Casablanca, Maroc), 2003**,** 84 P.

[34] I. N. Therios, S. D. Sakellariadis, Some Effects of varied magnesium nutrition on the growth and composition of olive plants (cultivar '*Chondrolia Chalkirdikis*'). Scientia Horticulturae, 17, 1982, pp. 33-41.

[35] M. Braham, *Evolution des réserves minérales et carbonées chez les variétés d'Olivier à huile « Chétoui » et « Chemlali » (Olea europaea. L).* Memoire de 3$^{\text{éme}}$ Cycle de spécialisation Oléiculture-Oléotechnie de l'INAT. 1984, 142 p. A.

[36] B. Ezzili, Effect of gibberellins on fertility and mineral content in leaves of black Grenache grapes (*Vitisvinifera* L.), 8 (23), 1996, 34-39.

[37] P. M. Kopittke, F. P. Blamey, T. B. Kinraide, P. Wang, S. M. Reichman, N. W. Menzies,. Separating multiple, short-term, deleterious effects of saline solutions on the growth of cowpea seedlings. New Phytol. 189, 2011, pp. 1110–1121.

[38] P. M. Kopittke, Interactions between Ca, Mg, Na and K: alleviation of toxicityin saline solutions. Plant Soil 352, 2012, pp. 353–362.

[39] R. Munns, M. Tester, Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 2008, 651–681.

[40] M. Tester, R. Davenport, Na+ tolerance and Na+ transport in higher plants. Ann. Bot. 91, 2003, 503–527.

[41] Bouat. *Le diagnostic foliaire et son utilisation dans les problèmes de conduite, de régénération et fumure de l'Olivier.* Inf. Oléi. Int, n°8, 1959, pp. 25 – 34.

[42] M. Braham, Evaluation des exportations en azote, en phosphate et en Potassium d'un hectare d'olivier 'Chemlali' *(Olea europaea L.).* Revue Ezzaitounia 5, 1999, pp. 1-2.

[43] R. Fernández-Escobar, "Fertilization", in *Olive Growing*, D. Barranco, R. Fernández-Escobar, , L. Rallo, (Eds.),. RIRDC, Australia, 2010, pp. 267–297.

[44] M. Freeman, K. Uriu, H.T. Hartmann, "Diagnosing and correcting nutrient problems", in *Olive Production Manual,* Sibbet, G.S., Ferguson, L. (Eds.), University of California, Agriculture and Natural Resources, Oakland, 2005, pp. 83–92.

[45] I. Klein, S. Lavee, "The effect of nitrogen and potassium fertilizers on olive production", in: Proceeding of the 13th Colloquium of the International Potash Institute, York, England, 1977, pp. 295–304.

[46] R. Fernández-Escobar, A. Ortiz-Urquiza, M. Prado, H.F. Rapport, Nitrogen status influence on olive tree flower quality and ovule longevity. Environ. Exp. Bot. 64, 2008, pp. 113–119.

[47] O. Boussadia, A. Bchir, K. Steppe, M.-C. Van Labeke, R. Lemeur, M. Braham,. Active and passive osmotic adjustment in olive tree leaves during drought stress. European Scientific Journal, 9 (24), 2013, pp. 1857 – 7881.

[48] M. Ben Khelil, 2010. *Evaluation du statut nutritionnel de l'Olivier (Olea europaea) par la méthode du diagnostique floral*, Thèse de Doctorat : Institut National d'Agronomie de Tunis.

[49] A. Belouad. *Plantes médicinales d'Algérie*. Office des Publications Universitaires, Alger, 2001, pp : 5-10.

[50] G. Gilly, Les plantes à parfum et huiles essentielles à Grasse ». L'HARMATTAN, Paris, 1997, pp: 11-19.

[51] H. Shimazono, W.J. Schubert, F. F. Nord, Investigations on Lignins and Lignification. XX. 1a The Biosynthesis of Methyl p-Methoxycinnamate from Specifically Labeled D-Glucose by *Lentinus lepideus. J. Am. Chem. Soc.*, 80 (8), 1958, 1992–1994.

[52] W. J. Schubert, F. F. Nord, Investigations on lignin and lignification. XV. Heterogeneity of Native and enzymatically liberated lignins as established by electrophoresis and paper chromatography. PNAS, 41 (3), 1955, pp. 122-127.

[53] C .I. Cazzonelli, Carotenoids in nature: insights from plants and beyond**.** *Functional Plant Biology*, 38, 2011, pp. 833–847.

[54] P. Kainulainen, J. Oksanen, V. Palomäki, J.K. Holopainen, T. Holopainen, Effect of drought and waterlogging stress on needle monoterpene of *Picea abies*. Can. J. Bot. 70: 1991, 1613-1616.

[55] L. Bertschinger, G. Christian, J. P. Ryser, A. Häseli, R. Neuweiler, W. Pfammatter, A. Schmid, F. Weibel, *Données de base pour la fumure en arboriculture fruitière, Fruits à pépins, fruits noyau, kiwis, baies d'arbustes*, Edition: Eidgenössische Forschungsanstalt, Postfach 185, CH-8820 Wädenswil, 2003, 48 P.