

Effects of water scarcity and salinity on the anatomy of the Tunisian table olive cultivar ‘Meski’

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Abstract

The table olive cultivar ‘Meski’ was subjected to two stresses related to water, scarcity, and salinity. Anatomical adaptations of leaves, stems and roots were studied and compared, to value the water use efficiency of the tree. Two stress levels were adopted corresponding to moderate and severe levels. Thus, the trees behaviour was influenced by the stress type and intensity. The aerial part of the trees showed more adaptation modes than the underground part. Under both stresses, plants have fortified the protection of the leaf tissues by developing upper envelope and multiplying the trichomes. Plants reinforced the support tissues by multiplying the collenchyma and sclereids, and have amplified the transport tissues by enhancing vascularity through multiplying the number of conductive vessels. However, different behaviours seemed to be specific to each stress such an enlargement of liber and reduction of wood in the drought stress and a restriction of liber and wood tissues in salt stress. Additionally, a retraction of the palisade parenchyma and an extension of the spongy parenchyma in drought stress inversely to salt stress were noted. In the treated stems and roots, development of stomata, suber, pericyclic fiber and liber, and a restriction of wood especially in severe stress were observed. The plants developed important changes in moderate stresses; however, in the severe, the plants seemed to be stressed, by presenting no significant changes relatively to the control.

Keywords: anatomical adaptations; drought; olive; moderate stress; salinity; severe stress

Introduction

The Mediterranean is one of the most vulnerable regions in the world to the impacts of global warming (IPPC, 2013). North Africa is the most exposed to climate change impacts. Scenarios predict an average rise in annual temperatures, higher than the average expected for the planet (Radhouane, 2013). North Africa would be particularly affected by droughts that would be more frequent, more intense and longer-lasting. Rising temperatures associated with climate change are expected to decrease the land areas suitable for agriculture, shorten the length of growing seasons and reduce crop yields.

Tunisia is among the top 10 impacted countries showing increasing dramatic effects of climate change (Dasgupta *et al.*, 2007). The number and intensity of droughts are increasing; water resources are declining causing loss of groundwater reserves, loss in arable cropland, and loss of rain-fed forested areas in southern

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Tunisia. Many of Tunisian ecosystems are not managed sustainable and therefore have little resilience and are extremely vulnerable. The main risks are erosion, increased salinity and submersion, the whole Tunisian coast is exposed to these risks. The rocky coast and cliffs are the least vulnerable (Dasgupta *et al.*, 2007). The development of salinity becomes a serious problem. High sodium bicarbonate concentrations can cause dissolution of organic fertilizers, weaken the physical properties of soil, and render the soil unsuitable for growing plants (Foster *et al.*, 2008; Hassen *et al.*, 2016; Pan *et al.*, 2017; Pan *et al.*, 2019).

Olive has been widely grown around the Mediterranean Basin for around 5000 years. In Tunisia, the olive tree is one of the most widely diffused and economically important crops. In the first ten months of the last crop year 2019/20, Tunisia is the first world supplier of olive oil (IOC, 2020). The olive tree is well-known for its tolerance to prolonged drought periods, although very long and severe droughts may have significant effects on olive production (Galán *et al.*, 2008). Some differences among olive cultivars have been observed concerning their ability for adaptation and production under harsh conditions (Chartzoulakis *et al.*, 1999). Bacelar *et al.* (2004, 2006, 2007 and 2009) studied Portuguese and Spanish cultivars subjected to hard conditions. They identified several mechanisms to cope with drought at the morpho-structural leaf level. Therefore, thick cuticle and trichome layers, high density and smaller stomata, high-density foliar tissue, developed sclerophyll, thick palisade parenchyma, developed upper and/or lower epidermis were observed. These structures increased water use efficiency, by increasing the resistance of the leaf boundary layer, and allowing the leaves to benefit from light rain or water condensation (Savé *et al.*, 2000). Mesophyll compactness leads to low cellular conductance thereby providing an efficient system to limit cellular water loss during drought (Bongi *et al.*, 1987). Besides, olive trees showed a high resistance to drought-induced embolism, essentially due to the small diameter of the xylem vessels and high density, leading to low xylem hydraulic conductivity that limit transpiration (Bacelar *et al.*, 2007; Torres-Ruiz *et al.*, 2013; Dichio *et al.*, 2013). According to Fernández *et al.*, (1994), rain-fed olive trees needed to explore larger soil volumes than irrigated trees to collect similar amounts of water, the total root system and central cylinder radii were larger in drought than in irrigation conditions. Additionally, no difference was found between treatments in metaxylem vessel diameter.

'Meski' is a Tunisian table olive, known for the very good taste of its fruits (Khabou *et al.*, 2009). This main cultivar is concentrated mainly in the north region. Planting densities are between 100 and 110 trees/ha and generally rise from 200 to 450 trees/ha in irrigated conditions (Ben Abdallah *et al.*, 2014). It seemed interesting to undertake the evaluation of the impact of deficiency in water quantity and quality on the anatomy and ultrastructure of this cultivar to improve the water-use efficiency of the tree. The hereby study focused on the adaptation strategy provided by anatomical changes in the different organs of the cultivar 'Meski', suspected to water scarcity and water salinity.

Materials and Methods

Plant material

Drought stress

'Meski' fresh leaves, stems and roots were gathered from plants growing in costal zones located in the north (Mornag 36°40'51"N, 10°17'25"E), the center (Chott Mariem (35°56'08"N, 10°33'26"E) and the south of Tunisia (Zarzis 33°30'N; 11°07'E).

The climatic parameters specific to the previous sites were detailed in Table 1. The data was determined from a standard meteorological station located in the experimental field.

Table 1. Climatology of different studied coastal areas, Mornag, Chott Mariem and Zarzis, located respectively in the superior semiarid, inferior semiarid and inferior arid of Tunisia, in two years (2012 and 2013)

Area	T min (°C)	T Max (°C)	H min (%)	H max (%)	Precipitation (mm)
Mornag	15.03±0.1 ^c	24.70±0.1 ^b	38.65±0.6 ^b	86.25±0.9 ^a	31.10±1.8 ^a
Chott Mariem	16.98±0.1 ^b	24.21±0.5 ^b	45.46±0.5 ^a	83.91±1.9 ^b	25.33±3.2 ^b
Zarzis	17.27±0.0 ^a	25.67±0.0 ^a	26.61±0.8 ^c	35.77±1.0 ^c	9.63±0.0 ^c

Tmax: maximal temperature; Tmin: minimal temperature; Hmax: maximal relative humidity; Hmin: minimal relative humidity. Values followed by the same letter are not significantly different at $P < 0.05$ according to Tukey test.

Each zone had specific climatic parameters. Especially, a great difference was recorded between precipitation and maximal relative humidity of Zarzis (9.63 mm and 35.77% respectively) located in the south of Tunisia and corresponding to the inferior arid stage and those of Mornag located in the north of Tunisia and corresponding to the superior semiarid stage (31.10 mm and 86.25% respectively).

Rainfall and temperatures were irregular, varying considerably from the relatively humid coastal area, strongly influenced by the Mediterranean Sea, to the desert like and Sahara conditions in the south. The cultivar 'Meski' was grown on rain-fed orchards, with densities up to 100 trees/ha. The north, 'Meski's' cradle, was considered as area where 'Meski' cultivar grew in no drought stress (D0); while the center was considered as area where 'Meski' was subjected to moderate drought stress (D1) and the south to severe drought stress (D2).

Young trees of about 30 years old were studied. Leaves, stems and roots were gathered, in the year 2012 at flowering stage, in accordance with four tree orientations corresponding to the north, the east, the south and the west. Mature leaves were chosen. Stems and roots were selected about three mm diameters. Roots were collected around 0.5 m far from the tree trunk. Three replicates were realized.

Salt stress

'Meski' plants were transplanted into 1.5 L pots filled with perlite, then well irrigated regularly to field capacity for a period of one month with the complete nutrient solution of Hoagland. Hydroponic system was adopted in a greenhouse at the Olive Tree Institute (Tunisia, 35° 49'N, 10° 38'E) under normal day-light conditions. The recycled nutrient solutions were continuously checked and corrected. Next, the plants were randomly divided into three groups of six plants. Three levels of salinity were applied for 35 days. The first corresponded to the control receiving a complete nutrient solution, the second corresponded to a moderate salt stress treatment of 4 g/L and the third corresponded to a severe salt stress treatment of 6 g/L.

Light microscopy and histology

Cross-sections through the fresh vegetative organs using a sharp blade were executed. Locquin and Langeron (1996) method was adopted for the histological and anatomical studies. After emptying the cell contents with sodium hypochlorite, the sections were washed with distilled water then with water mixed with few drops of concentrated acetic acid to fix the future color. Consequently, samples were doubly colored with green iodine and alum carmine.

The changes in sample anatomy under stress conditions were analysed and photographed by trinocular microscope Leica (model DM-1000), combined with a camera photo Leica (DFC-280).

The thicknesses of all tissues of leaf, stem and root of 'Meski' were measured using Mesurim Pro software. Tissues of both leaf midrib and blade were examined. Several magnifications of the scanning microscope were used such x40, x100 and x200. Three measures were realized for each tissue.

Statistical analysis

The percentage of each tissue was calculated based on the thickness of the entire cross-sectional area of the leaf blade, stem, or root. Data variance was analysed using ANOVA procedure in the SPSS, version 20. Differences between tissue thicknesses for each type of stress were compared using Tukey test. The values marked with the same letter do not differ significantly for $p < 0.05$. Principal component analysis (PCA) was carried out in order to study the patterns of variation of the analysed parameters, as well as to highlight the anatomical variables which contribute most strongly to the adaptation to imposed stress.

Results

Effects of drought and salt stresses on leaf anatomy

Effects of drought stress on leaf anatomy

Anatomical study of the leaves of the cultivar 'Meski' indicated more collenchyma development under severe drought stress (D2) compared to the moderate drought (D1) and the no drought (D0) conditions. This tissue is a support tissue, thus ensuring better protection (Figure 1 A1, A2 and A3).

An increase in vascularization in 'Meski's' leaves of D1 was also noted compared to D0. An augmentation in the number of secondary conducting vessels was revealed in moderate stress (D1) to facilitate the circulation of the sap. However, this vascularization decreased in 'Meski' leaves of D2. This variety appeared to be stressed during increased aridity and severe drought characterizing the south of Tunisia (Figure 1 B1, B2 and B3).

The palisade parenchyma in D2 leaves appeared very limited, while the spongy parenchyma appeared very developed, occupying almost $\frac{3}{4}$ of the leaf (Figure 1 B1, B2, B3, C1, C2 and C3). Additionally, in spongy parenchyma, many lacunas appeared, facilitating gas exchange (Figure 1 C3).

The stomata were embedded in invaginations located in the epidermal layer, which was also protected by a thick cuticle. The trichomes covered the stomata to ensure their best protection (Figure 1 B2 and C3). Trichomes were present especially on the lower epidermidis of D1 and D2 leaves (Figure 1 B2 and C3).

Elongated sclereids were multiplied, crossing the cells of the lacunar parenchyma of 'Meski' leaves of D1 and D2, thus consolidating the leaf protection (Figure 1 B2 and C2).

The modifications in tissues sizes, in midrib, were more pronounced in moderate water stress. Indeed, a significant development of the upper cuticle (3.43 against 1.74% for the control) and the liber (11.47 against 8.97%) and the decrease of the wood (26.10 against 36.36%) were observed. Liber/wood ratio was thus improved. These changes were accompanied with significant fiber reduce (Table 2).

In response to moderate drought stress (D1), the upper epidermis and thus the whole upper envelope were well developed (Table 2). Drought stress reduced the upper palisade parenchyma, reaching 32.33% and 25.48% respectively for D1 and D2, comparatively to 46.66% of D0; and developed the spongy parenchyma, as 45.23 and 52.01% respectively for D1 and D2, comparatively to 35.96% of D0. Hence, the ratio of the palisade tissues /spongy tissue was significantly restricted.

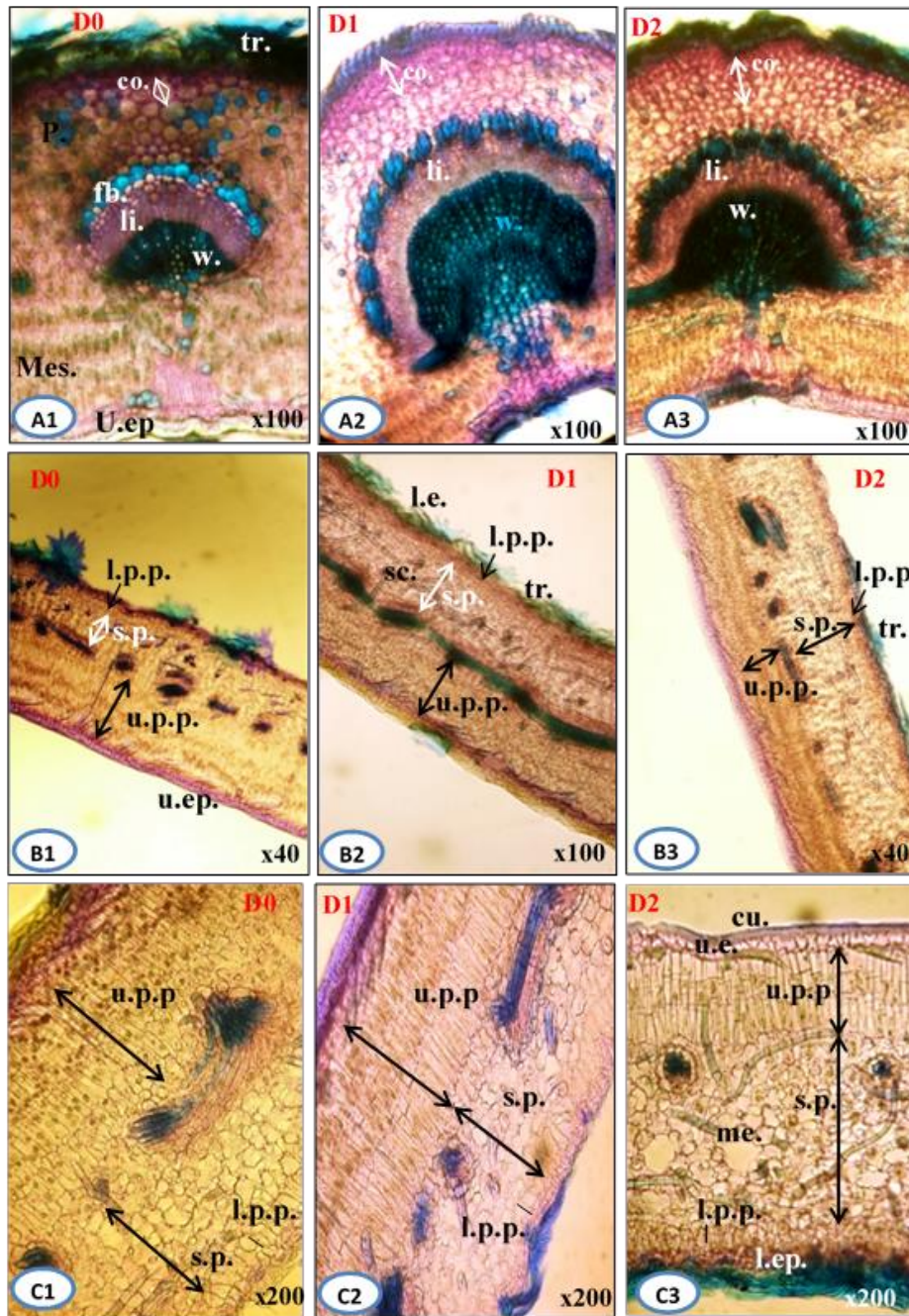


Figure 1. Cross sections of the leaf of the olive cultivar ‘Meski’ under drought stress
 cu: cuticle; fb: fiber; lep.: lower epidermis; Lp.p: lower palisade parenchyma; me: meatus; mes: mesophyll; p: parenchyma; sc: sclereids; tr: trichomes; s.p: spongy parenchyma; u.e: upper epidermis u.p.p: upper palisade parenchyma; w: wood. D0: no drought; D1: moderate drought; D2: severe drought.

Effects of salt stress on leaf anatomy

Microscopic leaf anatomy subjected to different salt concentrations showed an increase in vascularization of both treated leaves compared to the controlled ones. The number of secondary conducting vessels became more and more numerous depending on the severity of the applied salt stress (Figure 2 A1, A2 and A3).

Trichomes became richer in the upper face of the leaves of S1 and S2 treatments, differently to the control S0 where they seemed rare (Figure 2 A1, A2 and A3).

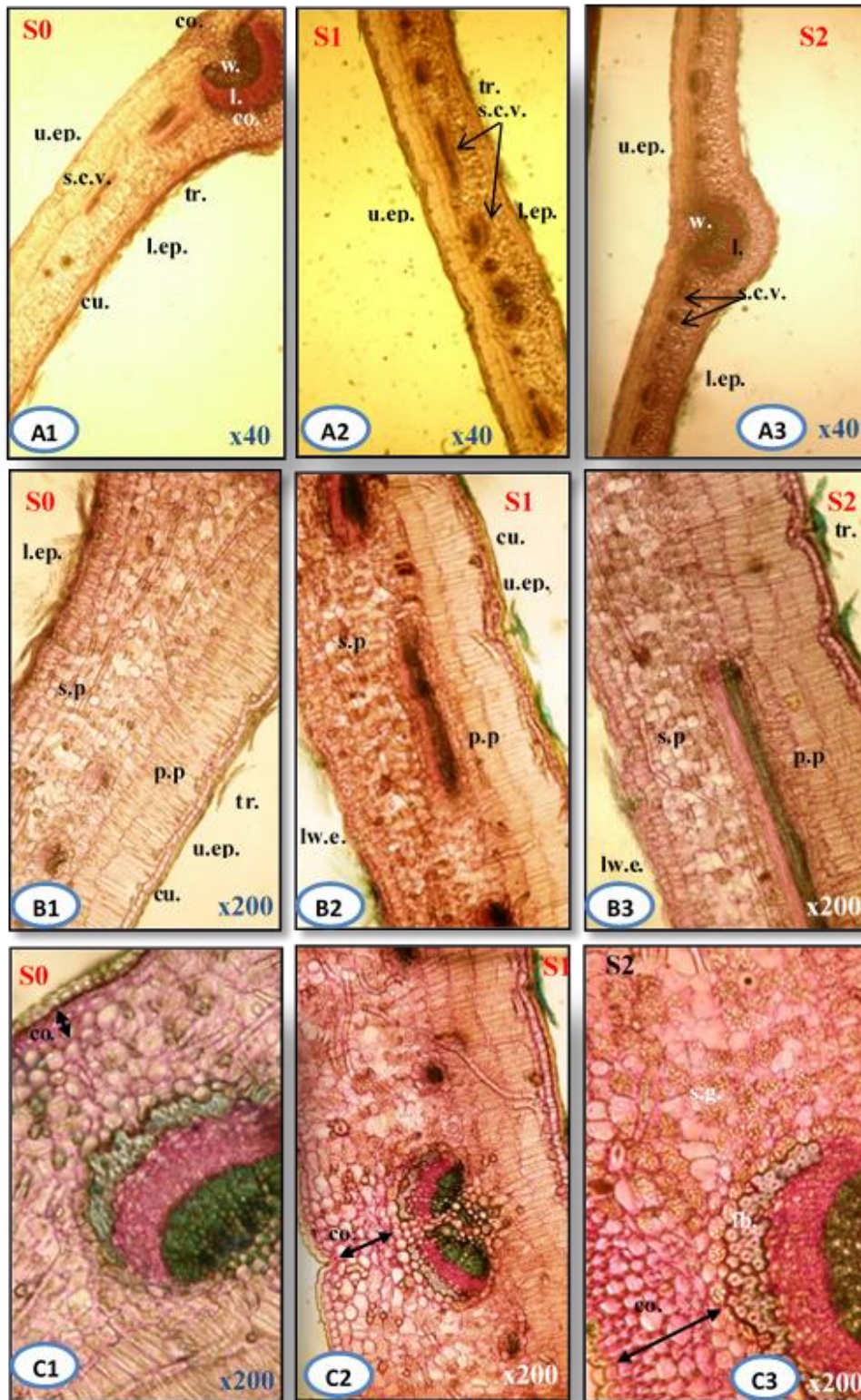


Figure 2. Cross sections of the leaf of the olive cultivar ‘Meski’ under salt stress
 co: collenchyma; cu: cuticle; fb.: fiber; lep.: lower epidermis; l: liber; p.p. palisade parenchyma; s.c.v.: secondary conductive vessel; s.g.: starch grain inclusion; s.p.: spongy parenchyma; tr.: trichomes; u.ep.: upper epidermis; w: wood.

S0: no salt stress; S1: salt stress level 1 (4g/l); S2: salt stress level 2 (6g/l).

Furthermore, trichomes were placed in invaginations in both sides of the treated leaves. These invaginations were more pronounced on the upper face to minimize contact with the sun's rays (Figure 2 B3). The trichomes cover the stomata, which were located at the bottom of the invagination to ensure their best protection.

The spongy parenchyma was narrowed while the palisade parenchyma was developed, forming an additional third layer for the leaves of S2 treatment (Figure 2 B3).

The collenchyma was more developed in the S1 and S2 treated leaves compared to the control ensuring them better protection (Figure 2 C1, C2 and C3).

Under moderate salt condition (4 g/L), 'Meski' cultivar developed its upper envelope including the cuticle (3.19%) and the epidermis (3.32%) to enhance its protection and minimized the evaporation phenomenon (Table 2). Thus, a significant increase was noted (6.51%) compared to the control.

Table 2. Leaf anatomical characteristics of 'Meski' cultivar under drought and salt conditions

	D0	D1	D2	S0	S1	S2
	No drought	Moderate drought	Severe drought	Salt Control	4 g/L	6 g/L
Midrib						
Tissue thickness (%)						
Upper cuticle	1.74±0.33 ^b	3.43±0.45 ^a	1.63±0.07 ^b	1.91±0.15 ^b	3.19±0.29 ^a	2.09±0.41 ^b
Upper epidermis	3.95±0.31 ^a	3.52±0.32 ^a	4.89±0.06 ^a	1.90±0.14 ^b	3.32±0.29 ^a	1.86±0.10 ^b
Upper envelope	5.46±0.64 ^a	6.95±0.77 ^a	6.52±0.13 ^a	3.81±0.29 ^b	6.51±0.59 ^a	3.95±0.51 ^b
Mesophyll	14.61±2.00 ^a	15.24±2.76 ^a	14.91±1.91 ^a	21.82±1.58 ^b	28.44±0.44 ^a	24.49±2.33 ^b
Wood	36.36±2.80 ^a	26.10±5.22 ^b	33.06±2.99 ^a	30.96±1.50 ^a	20.94±0.44 ^b	28.89±1.97 ^a
Liber	8.97±0.30 ^b	11.47±0.83 ^a	11.55±2.22 ^a	16.42±0.46 ^a	7.65±0.80 ^c	13.24±0.06 ^b
Liber/Wood	0.27±0.04 ^b	0.43±0.13 ^a	0.31±0.09 ^b	0.53±0.04 ^a	0.37±0.03 ^c	0.46±0.03 ^b
Fibers	5.35±0.80 ^a	3.28±0.09 ^b	6.19±0.67 ^a	4.66±0.65 ^{ab}	3.64±0.05 ^b	5.37±0.78 ^a
Rib parenchyma	15.20±1.36 ^a	15.43±2.22 ^a	12.77±1.09 ^a	7.87±1.13 ^a	2.80±0.01 ^c	4.97±1.14 ^b
Collenchyma	8.79±0.89 ^a	6.38±0.24 ^a	6.38±0.09 ^a	13.06±0.21 ^c	23.85±1.68 ^a	21.07±1.08 ^b
Lower envelope	5.70±0.44 ^a	5.75±0.29 ^a	5.72±0.54 ^a	3.47±0.70 ^b	5.85±0.99 ^a	1.71±0.09 ^c
Leaf blade						
Upper cuticle	3.43±0.83 ^a	4.18±1.91 ^a	3.94±0.59 ^a	2.15±1.17 ^b	3.05±1.68 ^a	3.50±0.62 ^a
Upper epidermis	5.79±1.02 ^b	8.77±1.39 ^a	5.85±1.48 ^{ab}	1.68±0.00 ^b	5.67±1.61 ^a	2.00±0.50 ^b
Upper envelope	9.23±1.03 ^a	12.96±3.31 ^a	9.79±1.07 ^a	3.83±1.17 ^c	8.72±3.29 ^a	5.50±1.12 ^b
Upper palisade parenchyma	46.66±2.91 ^a	32.33±4.18 ^b	25.48±2.44 ^c	29.02±0.56 ^c	33.02±0.77 ^b	38.86±1.85 ^a
Spongy parenchyma	35.96±1.70 ^a	45.23±0.11 ^a	52.01±2.88 ^a	54.71±0.85 ^a	50.3±0.84 ^b	39.67±0.98 ^c
Lower palisade parenchyma	5.36±1.54 ^a	5.63±1.06 ^a	5.75±0.92 ^a	6.38±0.63 ^a	5.85±1.34 ^a	5.34±1.25 ^a
Sum of palisaded	52.02±2.13 ^a	37.97±4.81 ^b	31.23±1.61 ^b	35.40±1.02 ^c	38.87±1.57 ^b	44.19±1.30 ^a
Palisade tissues /spongy tissue ratio	1.44±0.02 ^a	0.83±0.04 ^b	0.60±0.01 ^c	0.65±0.02 ^c	0.77±0.04 ^b	1.14±0.06 ^a
Upper palisade tissue /spongy tissue ratio	1.29±0.02 ^a	0.71±0.03 ^b	0.48±0.02 ^c	0.53±0.01 ^c	0.65±0.01 ^b	0.98±0.08 ^a
Lower envelope	4.43±1.28 ^a	5.61±0.65 ^a	4.77±0.41 ^a	4.43±1.28 ^a	3.74±0.80 ^a	4.74±0.29 ^a

Values followed by the same letter are not significantly different at $P < 0.05$ according to Tukey test.

Additionally, a significant modification was noted in vascular tissue especially in moderate salt stress, summarized by an important decrease in liber (7.65%) and wood tissues (20.94%) comparatively to the control (16.42 and 30.96% respectively). However, under a stress of 6 g/L, the decrease in the dimension of vascular tissue was attenuated additionally to a significant development of fibers as a support tissue.

Similarly, to the midrib of the leaf, the blade presented a developed upper envelope in stressed leaf, especially in the moderate stress comparatively to the control. Indeed, the thickness of the upper envelope reached 8.72% at a concentration of 4 g/L comparatively to 3.83% of the control (Table 2).

Effects of drought and salt stresses on stem anatomy

Effects of drought stress on stem anatomy

According to the cross sections of the 'Meski' stems growing in several drought levels (D0, D1 and D2), a multiplication of tissue layers around 'Meski' stem, was noted increasingly from D0 to D2 (Figure 3 A1, A2 and A3). Some trichomes appeared and lined the outer suber layer, in 'Meski' stems growing in D1 and D2 conditions. These trichomes covered the stomata that protected them (Figure 3 A3 and B2).

The collenchyma seemed to be more developed in the cortical zone of 'Meski' stem under D1 and D2 compared to D0, which reinforced the stem protection (Figure 3 B1, B2 and B3).

Besides, lignified tissue was abundant in the medullary zone of 'Meski' stems of D0, formed by wood vessels, fibers, lignified parenchyma and medullary rays (Figure 3 A1 and C1).

This lignified tissue was shrunken from D0 to D2, in opposite to the medullary parenchyma which became more and more important (Figure 3 A1, A2, A3, C1 and C3). In D1 conditions, starch grain inclusions seemed to be abundant in the medullary zone of the stem (Figure 3: C2).

The response of 'Meski' to a moderate water stress was much expressed in its stem. Several modifications in different tissues were noted (Table 3). Indeed, a significant enlargement of suber (6.46 against 2.88% for control), cortical parenchyma (7.40 against 4.32% for control), pericyclic fiber (4.08 against 2.11% for control) and liber (7.21 against 4.89% for control) were remarked.

These changes were accompanied with a wood restriction, which was enhanced with stress severity (6.37 and 3.61 for moderate and severe stress respectively against 13.69% for control).

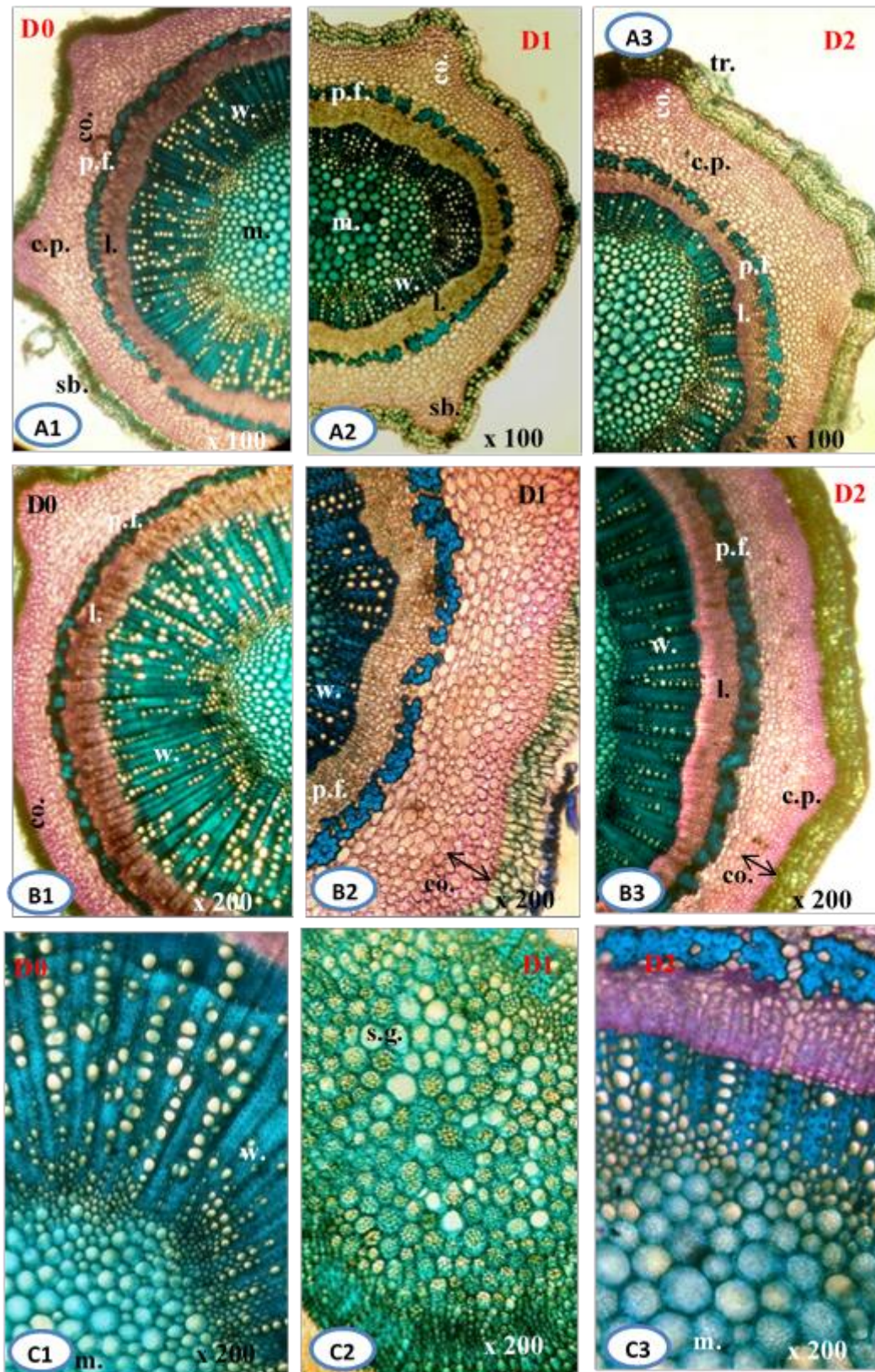


Figure 3. Cross sections of the stem of the olive cultivar 'Meski' under drought stress
 c.p.: cortical parenchyma; co: collenchyma; l: liber; m: medulla; p.f.: pericyclic fibers; s.g: starch grain inclusion; sb: suber; tr: trichomes; w: wood.
 D0: no drought; D1: moderate drought; D2: severe drought.

Table 3. Stem anatomical characteristics of 'Meski' cultivar under drought and salt conditions

	D0	D1	D2	S0	S1	S2
	No drought	Moderate drought	Severe drought	Salt control	4 g/L	6 g/L
Suber	2.88±0.37 ^b	6.46±0.50 ^a	5.54±0.81 ^a	4.64±0.61 ^{ab}	5.24±0.36 ^a	4.15±0.52 ^b
Collenchyma	3.27±0.25 ^a	4.76±0.90 ^a	3.06±0.62 ^a	2.98±0.31 ^b	4.18±0.58 ^a	4.03±0.57 ^a
Cortical parenchyma	4.32±0.92 ^b	7.40±0.81 ^a	4.79±0.89 ^b	5.91±0.92 ^a	5.43±1.06 ^a	6.24±0.97 ^a
Pericyclic fiber	2.11±0.22 ^b	4.08±0.99 ^a	1.69±0.28 ^b	2.21±0.15 ^a	1.51±0.11 ^b	1.98±0.27 ^a
Liber	4.89±0.66 ^b	7.21±1.21 ^a	3.19±0.95 ^b	7.47±1.12 ^a	8.75±1.25 ^a	7.86±0.20 ^a
Wood	13.69±0.25 ^a	6.37±1.95 ^b	3.61±0.8 ^b	12.87±0.82 ^a	12.20±1.19 ^a	9.17±0.57 ^b
Medulla	33.72±3.54 ^{ab}	30.60±4.50 ^b	41.09±3.75 ^a	29.20±1.64 ^a	25.71±1.22 ^a	25.64±1.07 ^a

Values followed by the same letter are not significantly different at $P < 0.05$ according to Tukey test.

Effects of salt stress on stem anatomy

Microscopic examination of olive stems subjected to different levels of salt stress did not show any significant anatomical change. It can be summed up in inclusions of starch grains which increased with the degree of salinity in the cortical zone (Figure 4 A1, A2 and A3) and the medullary zone (Figure 4 B1, B2 and B3). A discontinuity in the pericyclic fibers of S2 was revealed (Figure 4 B3 and C3), accompanied with an accentuated development of the collenchyma in the cortical zone of S1 and S2 compared to the control S0, thus strengthening the stem support (Figure 4 B2 and B3). Indeed, under salt stress, collenchyma, the supporting tissue, was significantly developed. It was 4.18 and 4.03% for 4 and 6 g/L, and 2.98% for the control (Table 3). Conversely, pericyclic fibers were more reduced in salt stress conditions, significantly in the moderate stress (1.51% for 4 g/L). A feeble development of liber and feeble restriction of wood were noted in moderate salt stress. Wood restriction became more pronounced in severe salt stress (9.17% for 6 g/L).

Effects of drought and salt stresses on root anatomy

Effects of drought stress on root anatomy

Anatomical microscopic examination of the roots of 'Meski' growing under D0, D1 and D2 conditions, indicated a gradual development of the suber in the roots of 'Meski' from D0 to D2 (Figure 5 A1, A2, A3, B1, B2 and B3), thus strengthening their protection.

Pericyclic fibers were remarkably abundant at the level of D1, invading almost the entire cortical area (Figure 5 A2, B2 and C2). The liberian tissue of D2 was developed, consisted by the conductive vessels of the phloem, the parenchyma and the fibers.

This tissue contained several inclusions (Figure 5 A3, B3 and C3). 'Meski' roots of D2 were characterized by an abundance of starch grain inclusions in the cortical zone (Figure 5 A3, B3 and C3).

A variation in the frequency of wooden vessels in the woody tissue was also noted. In fact, wooden vessels were abundant in the roots of D0, they decreased in number in D1, and multiplied enormously in D2 (Figure 6 A1, A2, A3).

Under moderate drought stress a development of suber, pericyclic fiber and medulla were noted (11.42, 12.51 and 7.51% respectively against 4.73, 4.83, 2.90% for the control) (Table 4). While, vascular tissues were restricted in their dimension. 5.92 and 20.29% were registered for dimensions of the liber and the wood comparatively to 10.26 and 27.58 for the control. An enlargement of the whole dimension of the root was supposed.

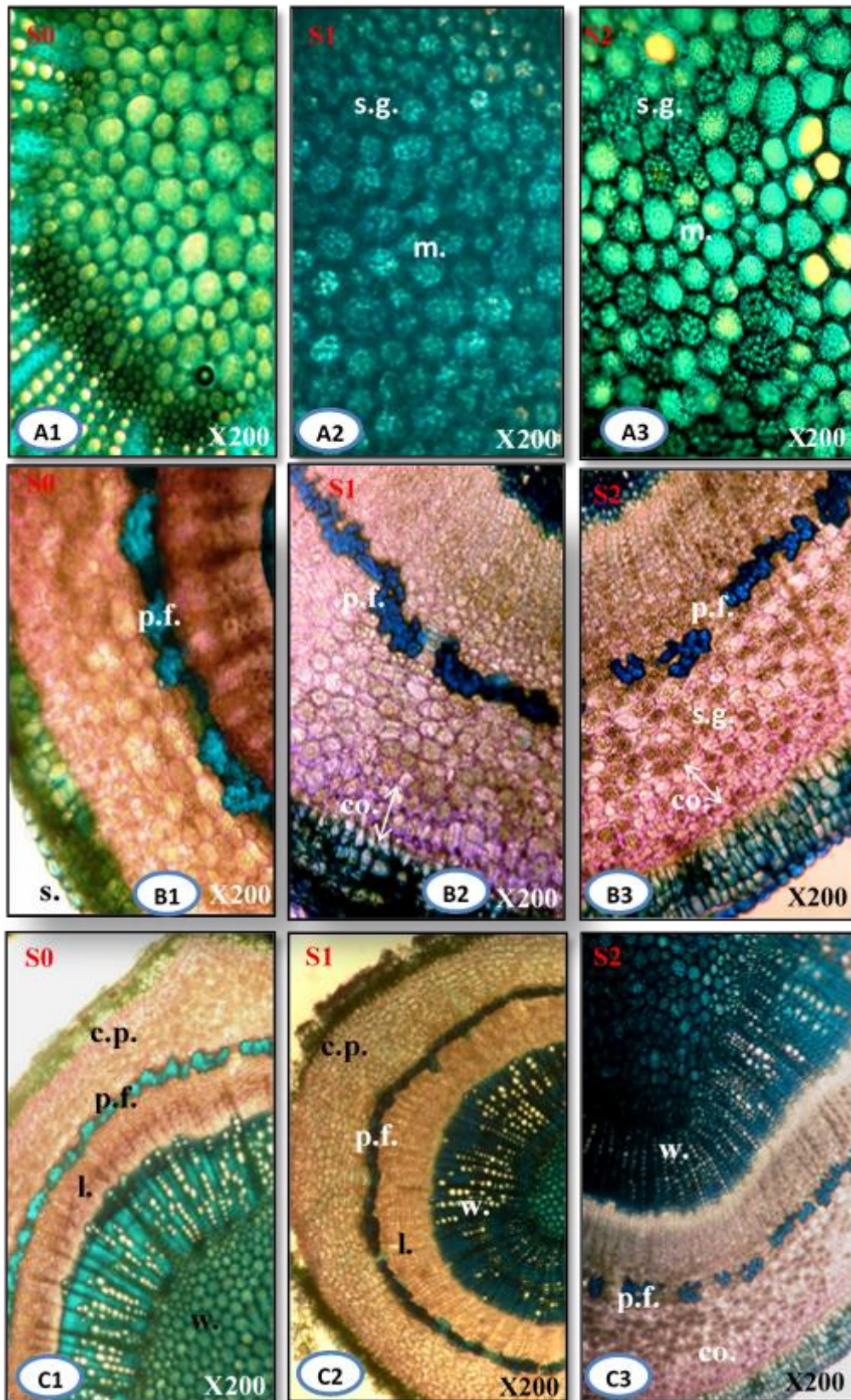


Figure 4. Cross sections of the stem of the olive cultivar 'Meski' under salt stress
 c.p.: cortical parenchyma; co.: collenchyma; l.: liber; m.: medulla; pe.f.: pericyclic fibers; s: suber; s.g.: starch grain inclusions; w.: wood.

S0: no salt stress; S1: salt stress level 1 (4g/l); S2: salt stress level 2 (6g/l).

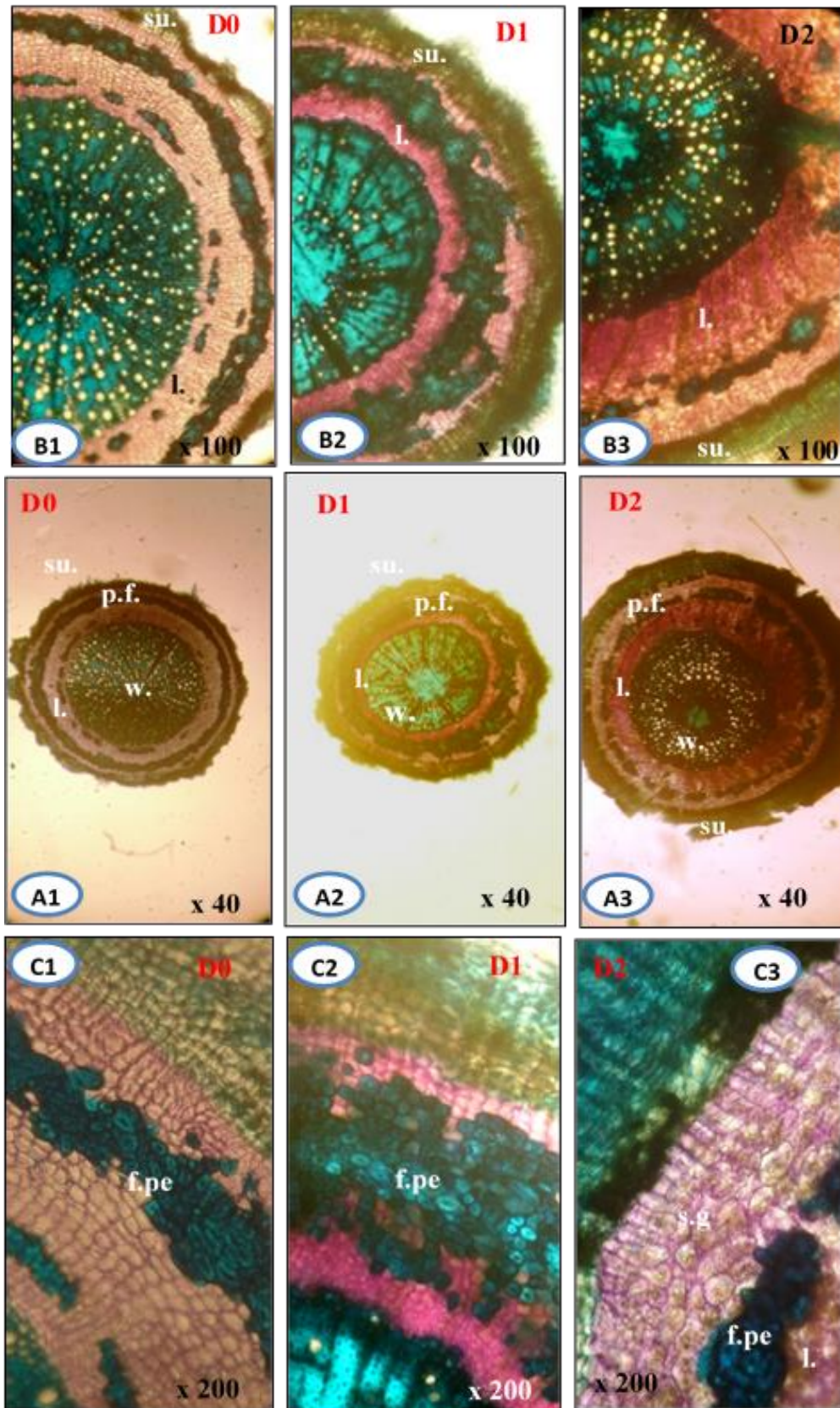


Figure 5. Cross sections of the root of the olive cultivar 'Meski' under drought stress
 l.: liber; p.f.: pericyclic fibers; s.g.: starch grain inclusion; sb: suber; w: wood.
 D0: no drought; D1: moderate drought; D2: severe drought.

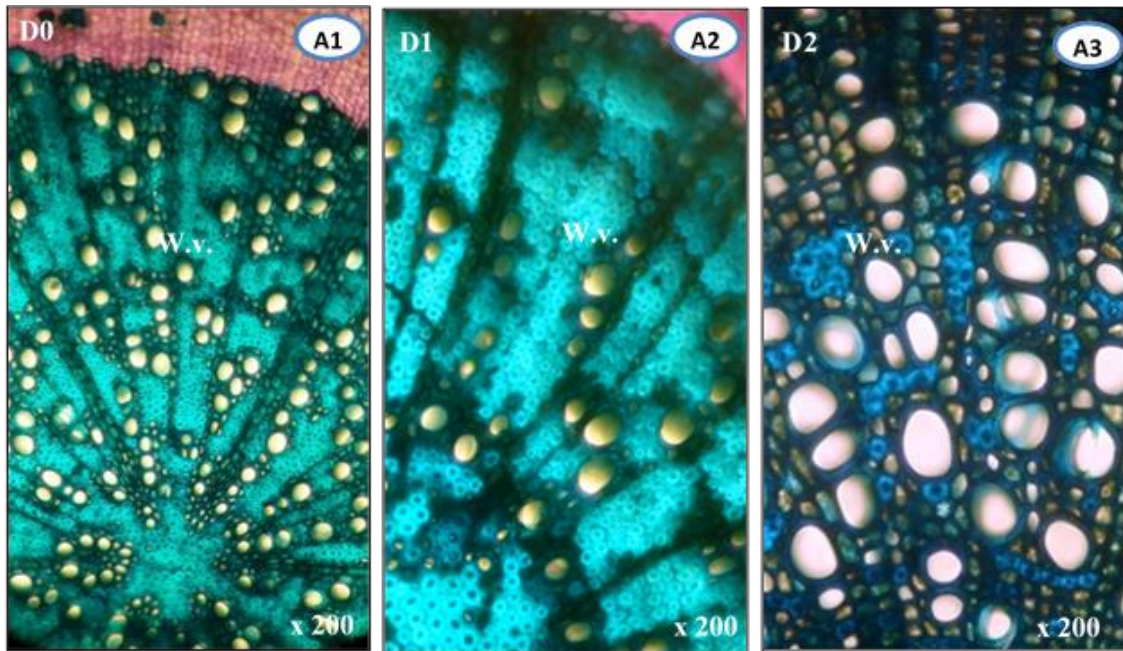


Figure 6. Cross sections of the root of the olive cultivar 'Meski' under drought stress
W v.: wood vessels. D0: no drought; D1: moderate drought; D2: severe drought.

Table 4. Root anatomical characteristics of Meski cultivar under drought and salt conditions

	D0	D1	D2	S0	S1	S2
	No drought	Moderate drought	Severe drought	Salt Control	4 g/L	6 g/L
Suber	4.73±0.48 ^c	11.42±2.89 ^a	7.95±0.76 ^b	26.97±0.80 ^a	14.17±1.95 ^b	16.14±2.10 ^b
Cortical parenchyma	2.32±0.95 ^b	2.25±1.12 ^b	4.24±0.47 ^a	13.05±2.05 ^a	4.52±0.72 ^b	7.16±1.42 ^b
Pericyclic fiber	4.83±0.92 ^b	12.51±2.22 ^a	3.48±0.80 ^b	5.53±2.28 ^a	3.77±0.09 ^a	4.51±0.82 ^a
Liber	10.26±0.65 ^a	5.92±1.07 ^b	8.89±2.28 ^a	13.15±1.25 ^a	7.20±1.63 ^b	9.23±0.35 ^b
Wood	27.58±1.53 ^a	20.29±1.01 ^b	20.61±0.24 ^b	32.11±4.31 ^a	20.62±0.31 ^b	15.36±0.45 ^c
Medulla	2.90±0.83 ^b	7.51±1.95 ^a	3.59±0.25 ^b	17.07±2.23 ^a	3.80±0.59 ^b	5.15±0.28 ^b

Values followed by the same letter are not significantly different at $P < 0.05$ according to Tukey test.

Effects of salt stress on root anatomy

Microscopic observation of the roots of the olive tree subjected to salt stress allowed noting many modifications. The suber was made up of several superimposed layers. It appeared to be continuous in the root of the control treatment S0, while it became discontinuous during the treatments S1 and S2 where it was interspersed with lenticels thus allowing the respiration of the root (Figure 7 A1, A2 and A3).

Following treatments S1 and S2, the pericyclic fibers formed a continuous ring around the liber, differently from those of the control (Figure 7 A1, A2, A3, B1, B2 and B3). Inclusions of starch grains were present at the root and increased with the degree of salinity (Figure 7 B2, B3, C2 and C3).

Diameter of the wood vessels has decreased, increasingly with the level of stress (Figure 7 C1, C2 and C3). This can induce a slowing down of the circulation of raw sap relative to an increase in resistance and the resulting pressure.

The liber was well developed (Figure 7 B3 and C3), which counterbalanced the pressure exerted by the circulation of the raw sap. The return of the elaborated sap was thus facilitated by the development of the liberian zone. The salt stress effect on root anatomy was very pronounced, affecting all aspects of tissues. A general and significant narrowing was noted for all tissues signifying the shrinkage of the root (Table 4).

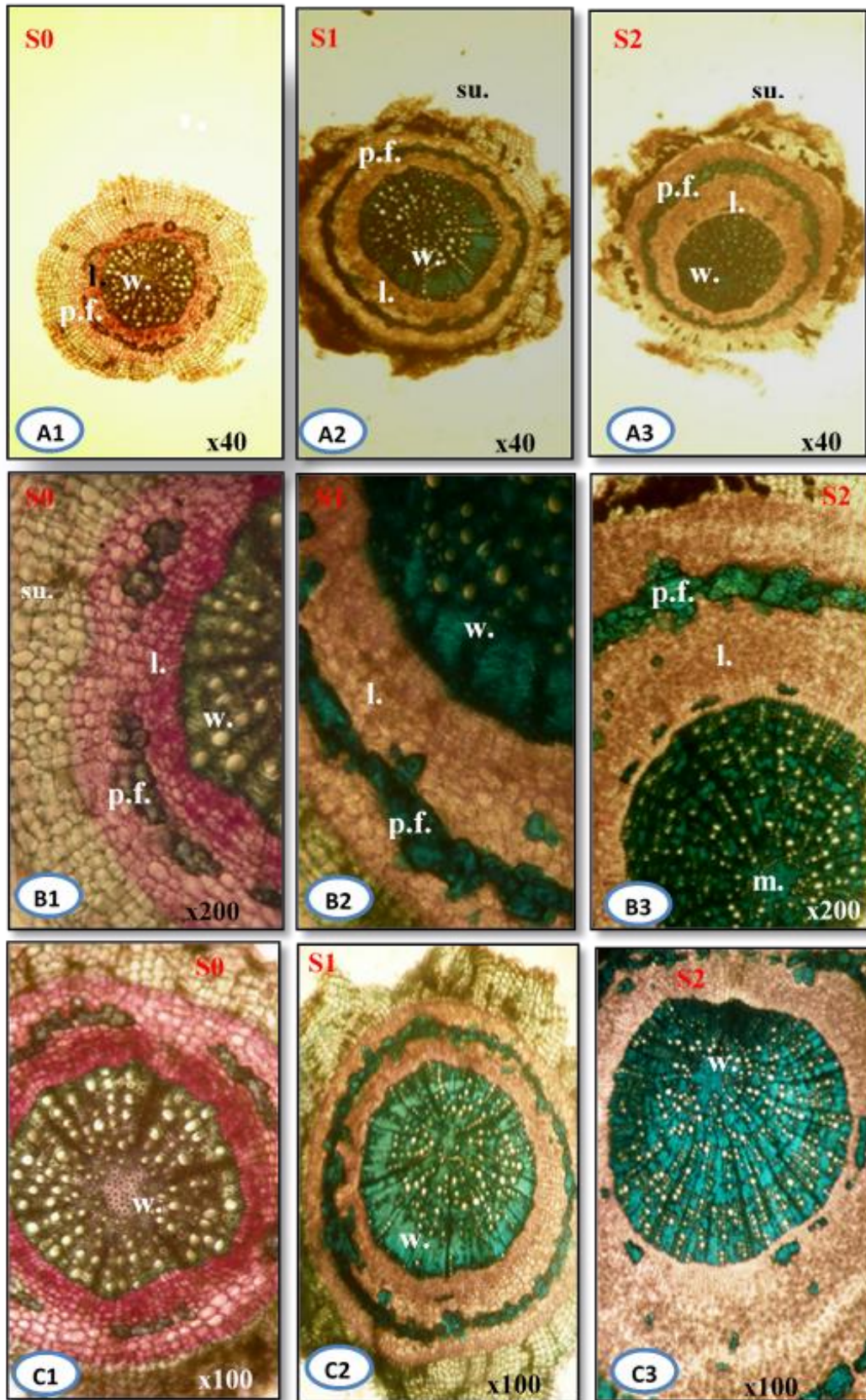


Figure 7. Cross sections of the root of the olive cultivar 'Meski' under salt stress

l.: liber; m: medulla; p.f.: pericyclic fibers; su.: suber; w.: wood.

S0: no salt stress; S1: salt stress level 1 (4g/l); S2: salt stress level 2 (6g/l).

Principal Component Analysis (PCA)

In order to a better understanding of the anatomy tissue variations according the different organs of the olive tree under two types of stress (water and salt one), a principal component analysis (PCA) was performed. The two first components showed 47.77% of total variance (Figure 8).

As shown in Figure 8, the first factor explained by 28.62% of the variability, showed the two opposite groups of variables. The first one included the upper and lower envelope characterizing either the leaf at the midrib (MUEN) or at the blade (LBUEN), the well-developed lower (MUDEP) and upper (MUEP, LBUEP) epidermis of the protective leaf tissue; this group showed a strong positive contribution to axis 1.

On the opposite site, another group of highly correlated variables was characterized like the internal tissues of the midrib of the leaf such as collenchyma (MCO), mesophyll (MMES) and liber (ML), also root tissues such as suber (RSU), cortical parenchyma (RCRP) and medulla (RM), and finally for stems wood tissue (SW). This axis seemed to oppose on the one hand the development of the external protective leaf tissues to opposite its internal tissues of the midrib indicating leaf shrinkage, and on another hand, the roots tissue shrinkage.

The second axis was correlated positively in one hand with the suber (SSU), collenchyma (SCO), cortical parenchyma (SCRCP) and pericyclic fibers (SPFB), which constituted the cortex of the stem and in other hand with the cuticle of the leaf (MUCU). However, this axe was correlated negatively with leaf lignified tissues such wood (MW) and pericyclic fibers (MFP).

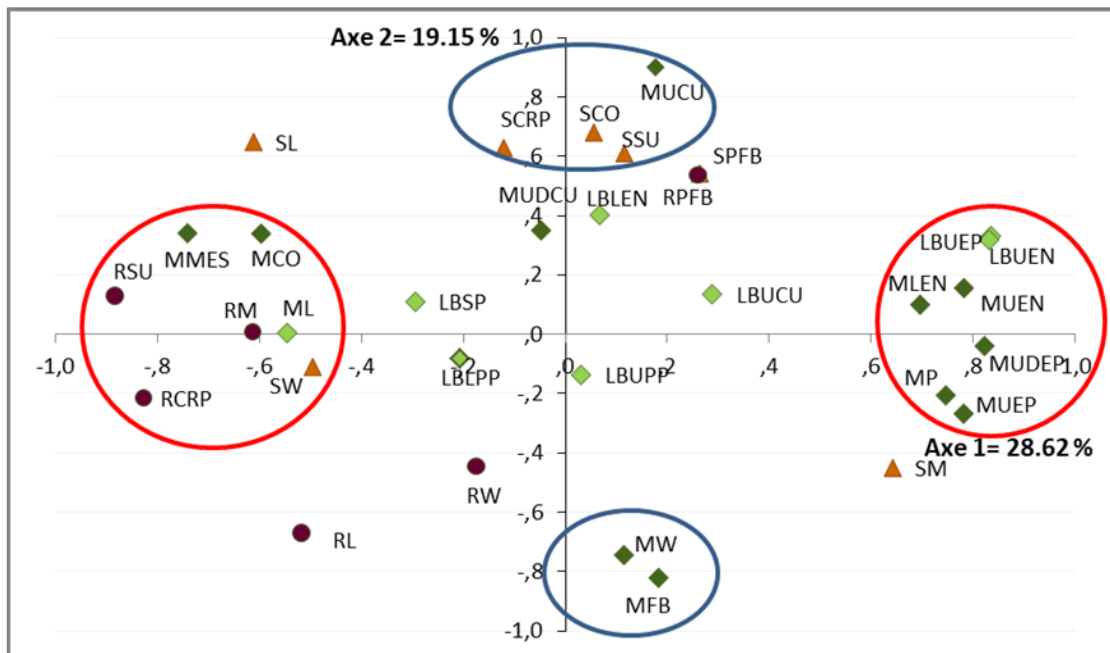


Figure 8. Principal component analysis of leaf, stem and root tissue thicknesses of 'Meski' olive cultivar subjected to drought stress and salt stress

LBLEN: Leaf blade Lower Envelope; **LBLPP:** Leaf blade Lower Palisade Parenchyma; **LBSP:** Leaf blade Spongy Parenchyma; **LBUCU:** Leaf blade Upper Cuticle; **LBUEN:** Leaf blade Upper Envelope; **LBUEP:** Leaf blade Upper Epidermis; **LBUPP:** Leaf blade Upper Palisade Parenchyma; **MCO:** Midrib Collenchyma; **MFB:** Midrib Fibers; **ML:** Midrib Liber; **MLEN:** Midrib Lower Envelope; **MP:** Midrib Parenchyma; **MUCU:** Midrib Upper Cuticle; **MUDCU:** Midrib Under Cuticle; **MUDEP:** Midrib Under epidermis; **MUEN:** Midrib Upper Envelope; **MMES:** Midrib Mesophyll; **MUEP:** Midrib Upper Epidermis; **MW:** Midrib Wood; **RCRP:** Root Cortical Parenchyma; **RL:** Root Liber; **RM:** Root Medulla; **RPFB:** Root Pericyclic Fiber; **RSU:** Root Suber; **RW:** Root Wood; **SCO:** Stem Collenchyma; **SCRCP:** Stem Cortical Parenchyma; **SL:** Stem Liber; **SM:** Stem Medulla; **SPFB:** Stem Pericyclic Fiber; **SSU:** Stem Suber; **SW:** Stem Wood.

The biplot plan showed three distinct groups (Figure 9). The first group identified the first level of the water stress (D1) which was characterized by high anatomy adaptation of the leaf envelope and the stem bark. The second group enclosed the non-water stress (D0) and the second level of the stress (D2) which noted also high adaptation of the leaf anatomy but in opposite no development of the stem cortex.

In the third group were included the results at S0 (no salt stress) and S2 (second level of salt stress) which noted a poorly developed envelope for the leaf, small leaf and root tissues.

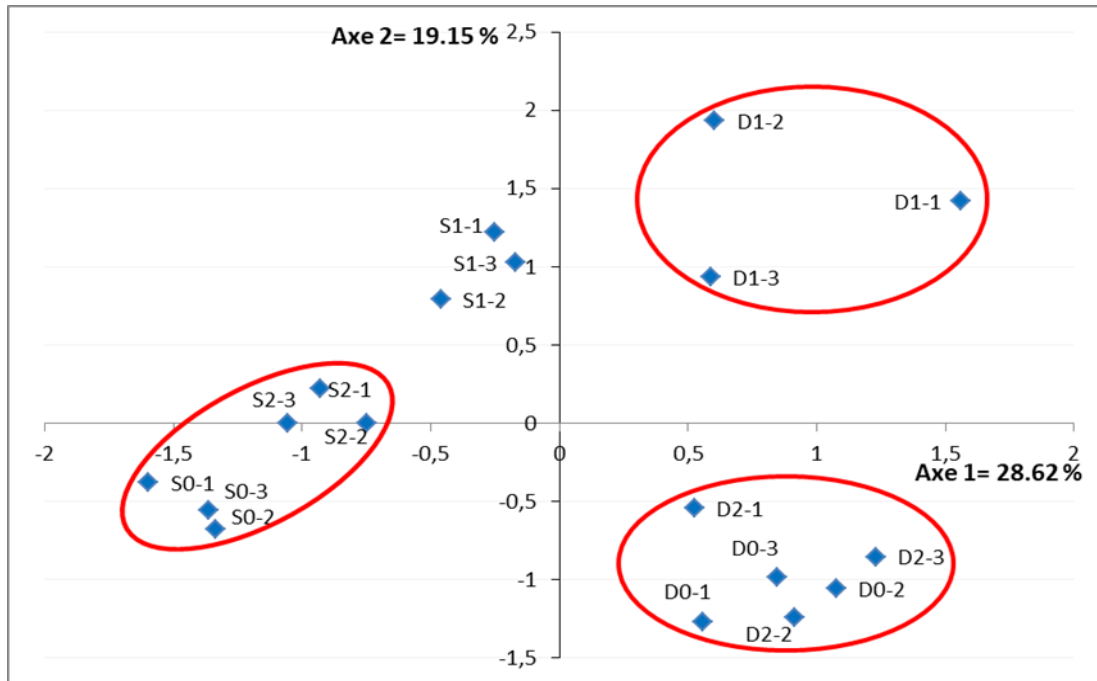


Figure 9. Groups of individuals contributing to axes 1 and 2

D0 1-3: no drought; D1 1-3: moderate drought; D2 1-3: severe drought;

S0 1-3: no salt stress; S1 1-3: salt stress level 1 (4g/l); S2 1-3: salt stress level 2 (6g/l).

Discussion

The cultivar 'Meski' was tested for its behavior under drought and salt stress. Anatomical changes were described in according its leaves, stems and roots.

Regarding leaves, olive leaves was characterized by long filiform sclereids and large scutiform trichomes, that created a thin cover limiting water loss by transpiration (Ennajeh *et al.*, 2010), providing thus more protection against desiccation for the inner leaf tissues (Bacelar *et al.*, 2004). According to Karabourniotis *et al.* (1994), sclereids act as synthetic optical fibers and, in addition to other functions, they can help improve the light microenvironment in the mesophyll of the thick and compact sclerophyllous leaves of *Olea*. The thickness of the trichome layer was increased, in 'Meski' cultivar, by water and salt stress, additionally to development of several sclereids. However, according to Ennajeh *et al.* (2010) and under drought conditions, trichome density remained unchanged in 'Meski' cultivar comparatively to the 'Chemlali' and the control.

'Meski' developed significantly its leaf upper envelope, either in the midrib, or in the blade especially when subjected to the moderate salt stress (4 g/L) comparatively to the severe. The upper cuticle (3.05%) and epidermis (5.67%) became significantly thicker comparatively to the control (2.15 and 1.68% respectively).

The development of the upper envelope was less detected in the case of water stress. Indeed, 'Meski' presented only a thicker upper epidermis in its leaf blade and a thicker cuticle in its midrib when subjected to

a moderate water stress. Response under salt stress in this level was more significant. Therefore, no significant modification was noted in the lower envelope under both stresses. Just a development of the lower envelope was noted in the midrib (5.58 against 3.47% for the control) when subjected to a moderate salt stress. According to Fayek *et al.* (2018), in the Egyptian olive cultivars 'Picual', 'Hamed', 'Kalamata' and 'Toffah', the upper and lower epidermis thickness of the olive leaf clearly increased in plants received the high saline water (9 g/L) compare to the control plants irrigated with tap water. However, according to Ennajeh *et al.* (2010) and under drought stress, the thickness of the upper and lower epidermis increased in the leaves of 'Chemlali' cultivar but not in 'Meski', which is in concordance with our result. The lasted authors found cultivar-dependent differences in leaf anatomical adaptations to drought stress, and accorded that the cuticle and epidermis characteristics are promising criteria which can help the selection of drought tolerant cultivars (Luković *et al.*, 2009).

Water stress induced the reduction of the upper palisade parenchyma and the development of the spongy parenchyma in correlation with stress severity. On the contrary, under salt stress, the upper palisade parenchyma was developed significantly and increasingly with severity of stress. Inversely, spongy parenchyma was shrunk progressively and significantly with stress severity. According to Ennajeh *et al.* (2010), the thickness of the upper palisade and spongy parenchyma layers in 'Meski' subjected to drought stress increased by 9% and 13% respectively, comparatively to the control. Additionally, 'Meski's' spongy parenchyma was 20% thicker than that of 'Chemlali' cultivar. The thickness of palisade tissue decreased as salinity treatment (9 g/L), according to the research results of Fayek *et al.* (2018), such decrease was much low in salt tolerant genotypes. The palisade cell length was decreased besides the appearance of spaces in mesophyll of salinity treatment. Such result was generally in harmony with those reported by Mohsen *et al.* (1987), Nomir (1994) and Sherin (2002). On the opposite, spongy tissue thickness, was increased with saline water treatment, compare to control. Karimi *et al.* (2009) found on olive a marked increase in spongy mesophyll thickness due to salt stress along with a slight reduction in palisade mesophyll length and thickness. Parida *et al.* (2004) also found that mesophyll thickness of mangrove decreased with salinity due to a decrease in length of palisade cells and number of spongy cell layers. Spongy cell diameter also decreased with salinity, but they were denser. Consequently, the amount of intercellular space in spongy tissue was lower in salt treated leaves. A thicker palisade parenchyma, such observed in 'Meski' cultivar, could form an obstacle which reinforce the protection of the plant. This tissue could contain, according to Fayek *et al.* (2018), larger numbers of CO₂-fixation sites per unit leaf area. While a thicker spongy parenchyma, which is a tissue for gas exchange with the atmosphere during photosynthetic assimilation, could result in easier diffusion of CO₂ through the inter-cellular spaces from the sub-stomatal cavity to the outer surface of the mesophyll cells. Therefore, the upper palisade parenchyma and the spongy parenchyma are considered key structural features of leaves that give the ability of a tree to withstand water stress. The degree of differentiation of the mesophyll and the proportion of palisade and spongy parenchyma vary according to plant species and habitat (Esau, 1965).

Under moderate drought stress, an increase in the number of vascular vessels was revealed in the leaf blade. However, this vascularization decreased in 'Meski' leaves when grown in severe stress. In the midrib of 'Meski' leaves subjected to a moderate water stress, a significant development of liber and a decrease of wood were observed.

These changes were accompanied with significant fiber reduce. According to Luković *et al.* (2009), in drought stress, the genotypes with a larger number of vascular bundles per mm² of the midrib area have a larger number of smaller vessels as well as a lower proportion of sclerenchyma. Sclerenchymatic tissues along the vascular bundle contributed to avoiding collapse of vascular bundle elements under turgor loss conditions (Grill *et al.*, 2004). Such criteria could be used according to Luković *et al.* (2009) in the selection of drought tolerant genotypes. Whereas, Jacobsen *et al.* (2007) thought that xylem density may be a useful tool in estimating the xylem characteristics and drought tolerance of large number of species. Chen *et al.* (2006) concluded that the size of the vascular bundle is strongly negatively correlated with the soil water content, whereas net photosynthesis, night respiration, and stomatal conductance are highly positively correlated with

soil water content. Besides, under salt stress, an increase in vascularization was observed in the leaf blade, accompanied with a decrease in the size of liber and wood in the midrib comparatively to the control. Nevertheless, under the severe salt stress (6 g/L), a decrease in the dimension of vascular tissue was attenuated in the midrib additionally to a significant development of fibers. Accordingly, Fayek *et al.* (2018) declared that, in response to the salinity, the thickness of xylem, phloem and fibers clearly reduced as salinity changes in the tolerate olive cultivar Hamed and its hybrid H61. Additionally, leaf midrib thickness as well as number and area of xylem vessels were found to decrease due to water salinity treatment. In the same way, Hassani *et al.* (2014) affirmed that the increase of salinity level reduced the xylematic vessel diameter in the proportion of 10 – 18% according to the genotype. In severe salt stress, ‘Meski’ cultivar appeared stressed, by maintaining similar vascular tissue to the control and the development of fibers tissue. The fiber tissue contains sclerenchyma which decreases the cellular flexibility under turgor loss conditions (Grill *et al.*, 2004).

As regards leaves and stems, collenchyma was more developed especially in moderate and severe salt stress providing better protection. According to Ambronn (1881), the collenchyma underwent plastic deformations at relatively low stresses compared to the fibers, which regained their original length after tensile stresses which were up to 18 times higher. Flexibility of collenchyma tissues is an advantage for any part of the plant that is subjected to mechanical stress which would damage its tissues (Leroux, 2012). Similarly, to leaves and under salt and water stresses, an increase in the number of stomata headed with trichomes was observed at the level of stems. This behavior could increase the capacity of this cultivar, to exchange gas with the external environment.

Concerning stems and roots, the cultivar ‘Meski’ concentrated starch grain in its both stems and roots, when subjected to salt stress or drought stress. The accumulation of starch grains has been frequently reported (Keiper *et al.*, 1998, Locy *et al.*, 1996, Salama *et al.*, 1994). This accumulation of starch observed in the chloroplasts of clover accompanied by an increase in the contents of soluble sugars can be attributed at least in part to a first denaturation reaction of sucrose phosphate synthase in the cytosol, and also to an intrachloroplastic inhibition of enzymes involved in starch degradation following a change in the ionic composition of chloroplasts (Salama *et al.*, 1994). Thalmann and Santelia (2017) have considered the starch as a key molecule in mediating plant responses to abiotic stresses, such as water scarcity, high salinity or extreme temperatures. Under these challenging environmental conditions, plants generally remobilize starch to provide energy and carbon at times when photosynthesis may be potentially limited. The released sugars and other derived metabolites maintain plant growth under stress, and function as osmoprotectants and compatible solutes to mitigate the negative effect of the stress (Krasensky and Jonak, 2012). Sugars can also act as signaling molecules, which interact with the ABA-dependent signaling pathway to activate downstream components in the stress response cascade (Rook *et al.*, 2006). Generally, degradation of starch in response to stress often has been considered as an improved tolerance. Concentration of soluble carbohydrates and starch content in the leaves were affected by the drought-stress treatment. In another way and according to Karimi *et al.* (2018), drought stress significantly increased the concentration of soluble carbohydrates in the olive cultivars Fishomi, Amigdalolia, and Conservolia. However, starch content significantly decreased in their leaves under drought stress.

Subjected to water and salt stress, ‘Meski’ cultivar presented modifications in fiber tissues. The area of fiber was restricted in the stems under both stresses. However, in roots fibers seems to be well developed forming a continuous ring around the liber. According to Lopez and Barclay, (2017), fibers are composed with sclerenchyma cells, characterized with thickened lignified walls, which make them strong and waterproof. Support sclerenchyma is comprised of sclereids and fibers. Makbul *et al.* (2011) explained that in stressed plants, the fibers spread as a continuous layer between cortex and phloem, however in unstressed plants, they occur as grouped layers. So, their numbers increase under drought stress. Yentür (2003) specified that sclerenchyma tissue provides an advantage against the loss of water.

The principal component analysis (PCA) summarized an important development of protective tissues of leaves at both midrib and blade levels, a significant development of the stem cortex but a general root narrowing in salt stress and a decrease in vascular tissue, mainly wood tissue in all organs of the tree.

Most of the anatomical changes were revealed during the first stress level corresponding to moderate stress. These modifications were expressed above all in leaves, whether in midrib or blade and afterwards in stems, especially during drought stress. In both severe stresses, 'Meski' cultivar presented similar tissues dimensions than those of the non-stress which suggested that this cultivar did not undergo adaptive anatomical changes to overcome the severe state of stress. No adaptation or strategy to deal with severe salt and water stresses, which proposed that 'Meski' was stressed at this level of stress. Accordingly, no significant modifications were noted in the most cases of severe stresses, which showed the vulnerability of this cultivar to this level. Drought and salinity stresses inhibit plant growth and development by imposing an osmotic stress (the lowering of the external water potential) that compromises a plant's ability to take up water (Zhao *et al.*, 2020). According to Wu and Cosgrove (2000), when plants are subjected to low water potentials, the growth of leaves and stems is rapidly inhibited. In contrast, roots may continue to elongate which completely inhibit shoot growth. This differential response of roots and shoots to low water potential is considered to be an adaptation of plants to dry conditions since continued root elongation facilitates water uptake from the soil.

Conclusions

'Meski' cultivar showed several changes in its anatomy which testify many forms of stress and adaptation to the both stresses. Many similitudes and differences were revealed in response to water stress and salt stress.

Leaves were the most touched, testifying great changes under applied stresses. 'Meski' cultivar reinforced its tissues of protection by developing its upper envelope and multiplying trichomes, strengthened its tissues of support by enlarging collenchyma and sclereids; and amplified its tissues of transport by increasing its vascularization and multiplying the number of conductor vessels. These behaviors were similar for water stress or salt stress and were clear especially when they were in moderate level. However, there was a big difference in the tissue of filling in the leaf blade, where palisade parenchyma was retracted while spongy parenchyma was extended in drought condition inversely to which was observed in salt conditions. Although the vascularization seemed to be enhanced by multiplying the number of conductor vessels, there was narrowing of liber and wood tissues in salt stress and enlargement of liber and narrowing of wood in drought stress. Pericyclic fiber changed differently according to the type of stress and its intensity.

In the treated stems and roots, development of stomata, suber, pericyclic fiber and liber, was accompanied with narrowing of wood especially in severe stress.

'Meski' developed important changes in moderate stress. However, this cultivar seemed to be stressed in severe stresses. This suggestion should be proved by future eco-physiological and biochemical studies.

Authors' Contributions

Conceptualization; Methodology; Data curation; Writing: DSN – Methodology; ACP analysis and interpretation: SBG - Supervision: MB. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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