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Anatomical adaptations of *Astragalus gombiformis Pomel.* under drought stress

Research Article

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Abstract: The present study was designed to study the effect of drought on root, stem and leaf anatomy of *Astragalus gombiformis* Pomel. Several root, stem and leaf anatomical parameters (cross section diameter, cortex, root cortical cells, pith, leaf lamina and mesophyll thickness) were reduced under moderate to severe water deficit (20-30 days of withheld irrigation). The stele/cross section root ratio increased under moderate water deficit. The root's and stems vascular systems showed reduced xylem vessel diameter and increased wall thickness under water deficit. In addition, the root xylem vessel density was increased in these drought conditions while it was unchanged in the stems. The stomata density was increased under prolonged drought conditions whereas the stomata size was untouched. The leaf vascular system showed reduced xylem and phloem tissue thickness in the main vein under moderate to severe water deficit. However, in the lamina the vascular tissue and the distance between vascular bundle were unaffected. Our findings suggest a complex network of anatomical adaptations such as a reduced vessel size with increased wall thickness, lesser cortical and mesophyll parenchyma formation and increased stomata density. These proprieties are required for the maintenance of water potential and energy storage under water stress which can improve the resistance of *A. gombiformis* to survive in arid areas.

Keywords: *Anatomical Changes • Water Deficit • Vascular System • Astragalus gombiformis*

1. Introduction

Plants respond to drought stress through deleterious and/or adaptive changes of morphological, anatomical and physiological nature [1-2]. Drought stress cause substantial negative effects in plant growth, reducing the productivity [3-4]. Moreover, in several species, plant development is proportional to the intensity of water deficit such as wheat, *Triticum durum* [5] and lemon, *Citrus limon* [6]. Plant water status was affected under drought condition. Thus, plants grown under water deficit have low leaf Ψ_w and turgor potential (Ψ_p) [7]. Therefore, the maintenance of appropriate plant water status during water deficit is essential for continued growth and this process can be achieved by stomatal regulation [8]. Thus, stomatal closure significantly decreases transpiration rate and so, contributes to maintaining positive turgor pressure of the cells [9].

Various anatomical changes induced by water deficit depend on adaptive characteristics expressed through diverse mechanisms. Thus, plant tissues responses to drought depend on the anatomic characteristics that regulate the transmission of the water stress effect to the cells [10]. Roots can be compared to sensors that detect water status changes in the soil through tissue dehydration and play an important role in plant resistance to water deficit [11]. However, fewer works on the anatomical modifications induced by drought

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stress were interested to changes in the anatomy of root and stem tissue. For example, in common bean (*Phaseolus vulgaris* L.), the thickness of epidermis and the parenchymatic cell area diminished in wild and domesticated beans growing under drought stress. At the same time, the number of cells in the cortex and the thickness of the xylem wall increased in both wild and domesticated beans. The diameter of xylem vessels and transverse root area diminished in the cultivar, but in the wild common bean were not affected [12]. Stem and cortex diameter, vascular tissue thickness and xylem vessel diameter were reduced under drought stress [13-14]. Some of the above root and stem anatomical responses seem to be adaptations that enhance plant survival in hostile environments [15]. Leaf tissues exposed to drought shows various responses. Thus, in many species such as, *Ctenanthe setosa* and *Triticum aestivum*, the entire lamina and mesophyll thickness were reduced under drought stress [2-14], while it was unaffected by water stress in *salvia spendens* and *Glycine max* [13-16]. Bussotti *et al*. [17] found that limited soil moisture caused a major thickening of the mesophyll, especially in the leaf cuticle and spongy parenchyma of the beech plant, *Fagus sylvatica*. Multiple characteristics of vascular structure have been investigated, such as modifications of the vascular bundle number and area and alteration of xylem/phloem ratio, which are thought to be involved in the resistance of the plant to environmental stresses [18]. Several studies have shown that xylem and/or phloem vessel diameter was reduced in *Ctenanthe setosa* and *Triticum aestivum* plants subjected to drought stress [2-14]. Thus, xylem with narrow vessels is physiologically better protected against cavitation [19].

Astragalus gombiformis Pomel. a wild species of the genus *Astragalus* belongs to the Fabaceae and known in Tunisia as “Helbet Elbel” [20]. It is traditionally used against the bites of snakes and scorpions [21]. In addition, Teyeb *et al*. [22] showed recently that the leaves of *A. gombiformis* are biologically active and the leaf extracts have high cytotoxic activity with high antimicrobial activity against several bacterial strains. However, little information is available about the anatomical responses of *A. gombiformis* to water deficit. In this study, we examined the effects of drought on root, stem and leaf anatomy, water status, and growth in this wild psammophytic species under different periods of withheld irrigation. Therefore, the main objective is to evaluate the responses of *A. gombiformis* to different water deficit levels and to identify the degree of tolerance developed to confront drought stress. On the other hand we test the hypothesis of the importance of eventual anatomical adaptations used by this species to cope with drought stress.

2. Experimental Procedures

2.1 Plant growth conditions

A. gombiformis seeds were collected in 2010 from Douz in southern Tunisia. They were naturally air-dried, purified then stocked at 25°C in the seeds bank of the laboratory. Seeds were surface sterilized for 5 min in 3g/L calcium hypochlorite solution and then thoroughly washed with deionised water. They were sown in 5L plastic pots in a 2:1 mixture of sandy soil and peat in a growth chamber at 25/18°C day/night temperatures, at 65–85% relative humidity, with a photosynthetic photon flux density of 1200 $\mu mol m^{-2} s^{-1}$ and a 16/8 h photoperiod at the Arid Region Institute of Medenine (Tunisia). Initially 8–10 seeds were planted in each pot; 2 weeks after germination the seedlings were thinned to three per pot. Plants were watered using tap water twice weekly to maintain soil moisture close to field capacity before initiation of water treatment. Potted 90-day-old plants were subjected to drought stress (irrigation withheld: Treated) or continuously grown under field capacity conditions (Control) for 30 days. The pots were arranged in a randomized complete block design with four replicates per treatment and three plants per pot for every date of harvest corresponding to 10, 20 and 30 days of drought. Waterlogging was avoided by drainage holes in the bottom of the pot, which permitted soil aeration and drainage of excess water. Day 0 of the experiment was considered as the beginning of the drought period. Every 10 days, plants (control and treated) were harvested and divided into aerial part and roots prior to use for analyses. During this experiment, growth and water relations and anatomical parameters were measured.

2.2 Growth activity and plant water status

The dry mass (DM) was measured after the fresh material was dried at 70°C for 48 h. Midday leaf water potential (Ψ_w) was measured using 3rd to 4th fully expanded leaf counting from the terminal shoot apex, using a Sholander pressure chamber (Skye Instruments, Powys, UK). Each replicate was the average of three measures corresponding to three plants per pot.

2.3 Anatomical study

This study was carried out on mature leaves and roots of plants subjected or not to 10, 20, and 30 days of withholding irrigation. Small pieces of leaf tissue (approx. 5×5 mm), from the midportion of laminate leaves, and roots tissue pieces (approx. 5 mm) were excised. Cut tissues were fixed in freshly prepared FAA (formaldehyde: glacial acetic acid: 70% ethanol 5:5:90 by volume) overnight at room temperature. After washing with a 0.1 M phosphate buffer (pH 7.4), they were dehydrated by passage through a tertiary butyl alcohol series (15–100%), and embedded with warm (56–58 °C) paraffin. The resulting blocks were then cut in 10 µm sections with rotary microtome and stained with 2% safranine O and fastgreen 0.2%. Observations were performed under a light microscope (Leitz, Germany), and photographed with a digital camera (Cannon, USA). Measurements of various cells and tissues were taken with an ocular micrometer and exact values were calculated with a factor derived by comparing ocular with stage micrometers.

2.4 Statistical analysis

The data were subjected to analysis of variance (ANOVA), and comparisons between the mean values of treatments were made by the least significant difference (Duncan post hoc) test ($P < 0.05$). Statistical analyses were performed using the SPSS statistical package (SPSS 13).

3. Results

3.1 Growth and water potential

The changes in the biomass accumulation and water plant status with increase in water-deficit period are presented in Figure 1. In the present study, the shoot biomass accumulation was reduced significantly only after 20 to 30 days of withheld irrigation. The depressive effect of water deficit was more pronounced when prolonging water deficit periods. Thus, in the plants receiving these former water regimes, the dry matter production was 79.4 and 62.5% of the controls, respectively. The Ψw was significantly lower in plants subjected to water-deficit stress than in controls. After 30 days of water deficit, Ψw reached the most negative values (-1.8 MPa).

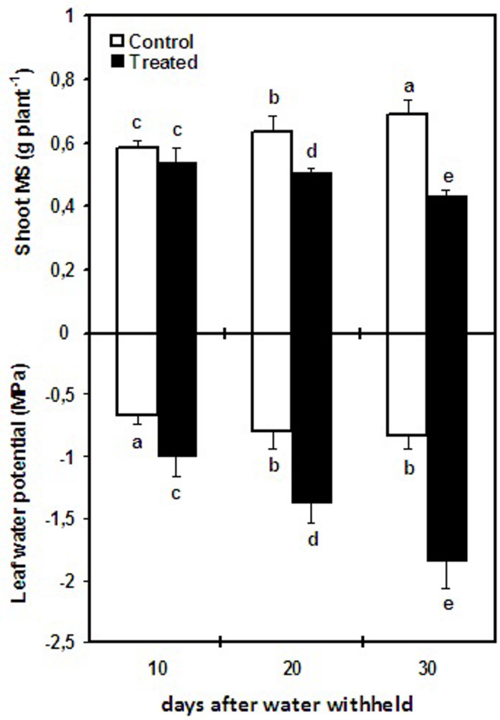

Figure 1. (A) Shoot biomass production (MS, g plant-1), and (B) leaf water potential (ΨW, 3 MPa) of A. gombiformis grown over time under control (field capacity) and treated (withheld irrigation) conditions. Bars followed by the same letter do not differ statistically at $p < 0.05$ (Duncan post hoc test). Bars represent the standard error of the mean (n=4).

3.2 Root Anatomy

Changes in root anatomical parameters under water deficit are shown in Figure 2. Prolonged water deficit (20-30 days) caused decreases in the root cross-sectional and cortex thickness. As compared with the control, root total thickness decreased by 9.1 and 37.9% in plants subjected to irrigation withheld of 20 and 30 days, respectively (Table 1). After 30 days of water deficit, cortex thickness reached 77.2% of the control plants. Drought also had a profound effect on cortical cell area, which was significantly less under moderate (171.2 µm^2) and severe water deficit (170.6 µm^2) than in the control (273.4 and 269.5 µm^2, respectively). However, the epidermis thickness decreased slightly when plants are subjected to prolonged water deficit (20-30 days). On the other hand, stele thickness showed significant increase (11.4%) in the roots subjected to irrigation withheld of 20 days nevertheless, after 30 days the stele thickness decreased significantly by 37.5% as compared with the control (Table 1). In addition, the stele/root ratio increased significantly only at water deficit of 20 days. Drought caused decreases in the xylem vessel diameter

Table 1. Effects of 10, 20 and 30 days of water withheld on root anatomical parameters of *A. gombiformis*.

Characters	10 days		20 days		30 days	
	Control	Treated	Control	Treated	Control	Treated
Root cross-section (µm)	1045.1 ±20.2[d]	1020.2 ±30.2[d]	1481.2 ±23.9[b]	1346.2 ±24.3[c]	1518.7 ±21.9[a]	942.5 ±22.5[e]
Epidermis thickness (µm)	41.1 ±1.2[d]	40.2 ±0.9[d]	59.2 ±1.3[ab]	58.3 ±1.6[b]	60.9 ±1.4[a]	59.4 ±1.1[ab]
Cortex thickness (µm)	208.5 ±5.8[c]	207.6 ±5.2[c]	377.5 ±15.3[a]	282.4 ±12.4[b]	362.6 ±10.4[a]	280.2 ±8.5[b]
Cortical cell area (μm^2)	180.1 ±9.3[b]	176.0 ±8.7[bc]	273.4 ±11.2[a]	171.2 ±8.1[c]	269.5 ±12.5[a]	170.6 ±8.5[c]
Stele thickness (µm)	418.7 ±13.4[d]	416.2 ±12,5[d]	611.3 ±16.4[b]	681.4 ±15.9[a]	576.1 ±9.7[c]	356.3 ±8.3[e]
Stele/Root ratio (%)	40.1 ±1.7[bc]	40.8 ±2.3[b]	41.3 ±2.4[b]	50.6 ±2.5[a]	37.9 ±1.9[d]	37.8 ±2.1[d]
Xylem vessel diameter (µm)	29.7 ±0.6[ab]	23.8 ±0.4[c]	30.5 ±1.5[a]	18.4 ±0.5[d]	29.1 ±0.9[b]	18.6 ±0.7[d]
Xylem vessel density (nu mm^{-2})	803.1 ±15.2[d]	796.3 ±14.5[d]	826.6 ±11.2[d]	1329.7 ±18.6[a]	920.2 ±13.9[c]	1249.8 ±16.3[b]
Vessel wall thickness (µm)	1.84 ±0.05[c]	1.86 ±0.05[c]	1.83 ±0.07[c]	2.09 ±0.05[b]	1.88 ±0.04[c]	2.20 ±0.08[a]

Data are means values ± SE of four measurements. Values in each line with the same letter are not significantly different (P = 0.05) as described by Duncan's test.

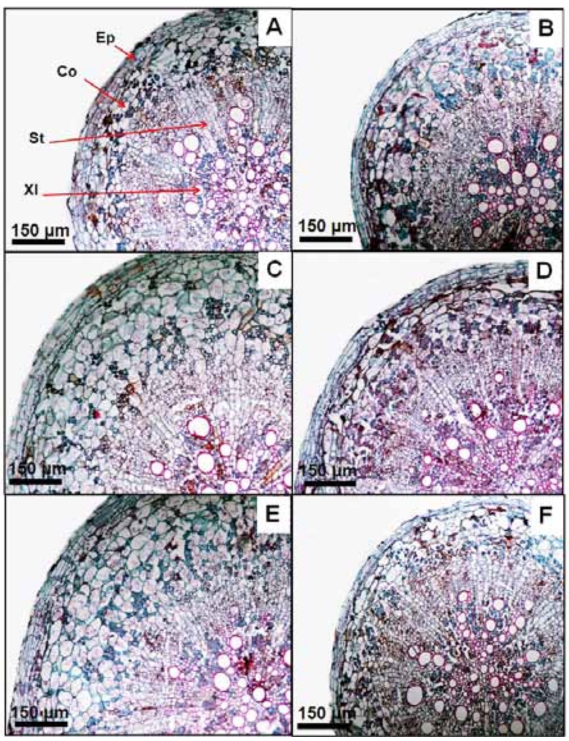

Figure 2. Root cross-sections showing root thickness changes in *A. gombiformis* plants grown under control (A, C and E) and drought conditions (B, D and F) at the periods 10 (A and B), 20 (C and D) and 30 (E and F) days of treatment. Bars = 150µm. Co: cortex, Ep: epidermis, St: stele, Xl: xylem

and this reduction increased with improving drought stress severity. As compared with the control, xylem vessel diameter decreased by 19.8%, 39.6% and 36% in plants subjected to irrigation withheld of 10, 20 and 30 days, respectively. The vessel wall thickness was 2.09 µm under moderate water deficit and 2.20 µm under the severe ones, while it was only 1.83 to 1.88 µm in the controls. However, xylem vessel density was markedly improved under irrigation withheld of 20 and 30 days when the *increase reached* 1.5 and 1.36-fold relative to controls, respectively (Table 1; Figure 2)

3.3 Stem Anatomy

The detailed measurements related to the stem anatomical parameters are given in Table 2. The stem cross-sectional and pith diameter decreased with the increasing intensity of drought. As compared with the control, these previous parameters decreased by 51.5 and 45% in plants subjected to irrigation withheld of 30 days, respectively. In addition the cortex thickness was reduced significantly (P< 0.05) at prolonged water deficit (20-30 days). After these periods of irrigation withheld, cortex thickness reached 70% of the control plants (Table 2). However, under water deficit, *A. gombiformis* stem did not show a difference in the thickness of epidermis. On the other hand, xylem tissue thickness and xylem vessel diameter showed significant reduction in the stems of the plants subjected to prolonged water deficit (20-30 days). After 30 days of water deficit, xylem

Table 2. Effects of 10, 20 and 30 days of water withheld on the stem anatomical parameters of *A. gombiformis.*

Characters	10 days		20 days		30 days	
	Control	Treated	Control	Treated	Control	Treated
Stem cross-section (μm)	1791.6 ±24.9[c]	1721.3 ±29.1[d]	2353.5 ±23.9[b]	1490.4 ±34.5[e]	2513.6 ±47.7[a]	1245.1 ±27.1[f]
Epidermis thickness (μm)	21.1 ±0.9[a]	21.5 ±0.8[a]	21.3 ±0.7[a]	21.8 ±0.6[a]	20.9 ±0.8[a]	22.0 ±0.5[a]
Cortex thickness (μm)	108.1 ±5.7[b]	106.6 ±5.9[b]	149.2 ±9.4[a]	105.4 ±4.5[b]	146.5 ±9.1[a]	103.4 ±7.2[b]
Pith diameter (μm)	953.3 ±23.7[c]	910.1 ±20.7[d]	1065.2 ±18.3[a]	736.8 ±24.6[e]	1026.3 ±25.8[b]	563.9 ±12.7[f]
Xylem tissue thickness (μm)	127.3 ±3.7[b]	125.5 ±2.9[b]	148.2 ±4.3[a]	121.1 ±4.1[b]	146.7 ±5.4[a]	78.5 ±4.3[c]
Xylem vessel diameter (μm)	26.9 ±0.6[b]	26.7 ±0.8[b]	27.3 ±0.7[ab]	23.6 ±0.8[c]	28.2 ±0.6[a]	16.5 ±0.8[d]
Xylem vessel density (number/mm-2)	2086.7 ±26.5[a]	2077.2 ±20.7[a]	2029.4 ±52.1[a]	2064.4 ±55.2[a]	2087.5 ±18.4[a]	2062.9 ±45.1[a]
Vessel wall thickness (μm)	1.63 ±0.04[b]	1.59 ±0.07[b]	1.61 ±0.07[b]	1.75 ±0.04[a]	1.61 ±0.03[b]	1.77 ±0.06[a]
Phloem tissue thickness (μm)	89.5 ±2.9[a]	89.2 ±3.1[a]	87.5 ±2.0[a]	70.8 ±4.7[b]	86.4 ±2.1[a]	56.3 ±5.1[c]
Phloem sclerenchyma (μm)	43.6 ±1.7[b]	44.1 ±1.4[b]	45.8 ±2.1[ab]	37.2 ±2.8[c]	48.6 ±1.8[a]	35.7 ±1.9[c]

Data are means values ± SE of four measurements. Values in each line with the same letter are not significantly different (P = 0.05) as described by Duncan's test.

tissue thickness and xylem vessel diameter decreased by 46.5 and 41.5% as compared with the control plants, respectively (Table 2). Moderate to severe drought conditions caused increases in the vessel wall thickness and this augmentation was nearly 8.7 and 8.9% under 20 and 30 days of water deficit, respectively (Table 2). However, the xylem vessel density of *A. gombiformis* stems was unchanged by water deficit. A significant reduction in the phloem tissue and phloem sclerenchyma thickness was observed at higher drought levels (20-30 days) in *A. gombiformis* stems (Table 2, Figure 3).

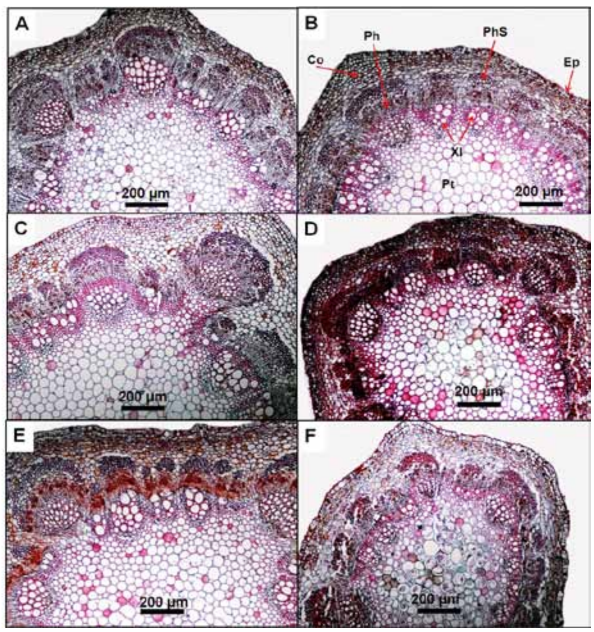

Figure 3. Stem cross-sections showing stem thickness changes in *A. gombiformis* plants grown under control (A, C and E) and drought conditions (B, D and F) at the periods 10 (A and B), 20 (C and D) and 30 (E and F) days of treatment. Bars = 200μm. Co: cortex, Ep: epidermis; Ph: phloem; PhS: phloem sclerenchyma, Pt: pith, Xl: xylem

3.4 Leaf anatomy

The structural changes in control and treated leaves of *A. gombiformis* (Table 3, Figures 4 and 5) were studied. The total thickness of lamina decreased significantly by 10.8 to 21.6% compared to the control at irrigation withheld of 20 and 30 days, respectively. Similarly, the thickness of mesophyll was reduced significantly under moderate to severe water deficit (20-30 days). The thickness of the lower epidermis increased in the plants subjected to 20 and 30 days of water deficit. In contrast, the thickness of the upper epidermis was unaffected (Table 3). Moderate to severe drought conditions (20-30 days) caused marked increases in the stomata density and this augmentation was nearly 14.5 and 31.5% under 20 and 30 days of water deficit, respectively. However, under drought conditions, the stomata did not show a highly significant difference in their size. On the other hand, the vascular bundle

Table 3. Leaf anatomical variables of *A. gombiformis* plants over the treatment periods (10, 20 and 30 days of water withheld).

Characters	10 days		20 days		30 days	
	Control	Treated	Control	Treated	Control	Treated
Leaf lamina						
Leaf thickness (µm)	116.5 ±1.9[ab]	115.8 ±2.4[b]	119.2 ±1,8[a]	106.3 ±2.1[c]	119.4 ±2.3[a]	93.6 ±1.2[d]
Upper epidermis (µm)	16.6 ±0.31[a]	16.7 ±0,23[a]	16.4 ±0.38[a]	16.9 ±0.29[a]	16.5 ±0.34[a]	16.7 ±0.33[a]
Lower epidermis (µm)	14.6 ±0.25[bc]	14.5 ±0.19[bc]	14.2 ±0.17[c]	14.9 ±0.27[b]	14.9 ±0.31[b]	16.0 ±0.35[a]
Mesophyll thickness (µm)	87.8 ±1.5[ab]	88.2 ±1.8[ab]	90.8 ±2.9[a]	75.6 ±1.9[b]	91.2 ±1.1[a]	64.3 ±1.2[c]
Vascular bundle diameter (µm)	17.8 ±0.42[a]	18.1 ±0.31[a]	17.9 ±0.22[a]	19.0 ±0.34[a]	18.7 ±0.27[a]	18.8 ±0.25[a]
Vascular bundle distance (µm)	37.8 ±1.3[a]	39.7 ±1.4[a]	37.3 ±1.1[a]	36.8 ±1.6[a]	39.1 ±2.1[a]	37.4 ±1.4[a]
Stomatal density (number mm^{-2})	34.1 ±2.5[d]	35.2 ±1.9[d]	35.8 ±2.4[d]	41.0 ±2.8[b]	38.7 ±2.9[c]	50.9 ±3.4[a]
Stomatal size (µm)	39.0 ±2.5[b]	38.3 ±1.9[b]	37.9 ±2.4[b]	39.3 ±2.8[b]	39.6 ±2.9[b]	42.1 ±3.4[ab]
Leaf mid-vein						
Mid-vein thickness (µm)	133.9 ±2.3[c]	132.8 ±2.5[c]	149.7 ±3.7[b]	128.2 ±2.2[d]	160.7 ±3.1[a]	118.6 ±1.7[e]
Width of bundle sheath (µm)	41.8 ±1.2[d]	41.5 ±1.3[d]	56.4 ±1.5[b]	47.0 ±0.9[c]	66.5 ±1.8[a]	41.2 ±1.1[d]
Length of bundle sheath (µm)	60.1 ±0.9[c]	56.5 ±1.1[d]	66.3 ±1.4[b]	54.7 ±1.1[e]	69.2 ±1.3[a]	52.6 ±1.1[f]
Xylem thickness (µm)	19.7 ±0.9[a]	19.4 ±1.1[a]	20.2 ±0.8[a]	13.2 ±0.7[b]	20.4 ±0.9[a]	12.7 ±0.5[b]
Phloem thickness (µm)	8.2 ±0.18[b]	8.1 ±0.17[b]	8.4 ±0.21[ab]	8.2 ±0.25[b]	8.6 ±0.29[a]	5.9 ±0.17[c]

Data are means values ± SE of four measurements. Values in each line with the same letter are not significantly different (P = 0.05) as described by Duncan's test.

diameter and the distance between these vascular elements were unchanged under drought conditions. For the mid-vein parameters, we found a significant reduction of 14.3 to 26.2% in the mid-vein leaf thickness compared to control plants subjected to water deficit of 20 and 30 days, respectively. The length of the bundle sheath was significantly reduced in the leaf mid-vein of *A. gombiformis* with the increasing intensity of drought while, the bundle sheath width decreased only at moderate to severe water deficit (20-30 days). For the vascular elements, we found a significant reduction of 34.6 to 37.7% of the xylem tissue compared to control plants subjected to water deficit of 20 and 30 days, respectively. The phloem thickness was reduced by 31.3% at severe water deficit (30 days), compared to control well irrigated plants (Table 3, Figure 5).

4. Discussion

Drought stress is one of the most important environmental factors limiting plant growth and development. It affects both elongation and expansion growth [11]. Results from this study indicate that that *A. gombiformis* maintain its maximum growth potential for low water deficit (10 days). Beyond this period, growth decreases with increasing water deficit. However, this species is able to maintain growth activity even at severe water deficit (30 days). This slowdown in the growth is a function of adaptation for the survival of plants under stress, reorienting redirect cells resources (energy and metabolic precursors) in the direction of defensive reactions against stress [23]. Our data are consistent with findings reported on *Eragrostis tef* and *Bituminaria bituminosa* in which water stress reduce plant growth [24]. The plant water status has been put in evidence by leaf water potential (Ψ_w) that declined significantly in *A. gombiformis* shoots which can be related to drought

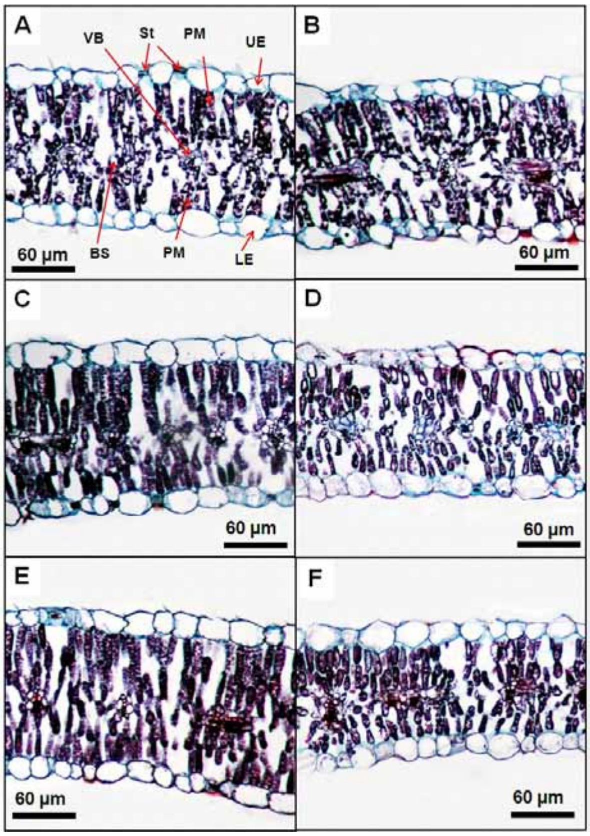

Figure 4. Leaf blade cross-sections showing leaf anatomical changes in A. gombiformis plants grown under control (A, C and E) and drought conditions (B, D and F) at the periods 10 (A and B), 20 (C and D) and 30 (E and F) days of treatment. Bars = 60μm. BS: bundle sheath, LE: lower epidermis, UP: upper epidermis, PM: Palisade mesophyll Ph: phloem, St: stomata, VB: vascular bundle, Xl: xylem

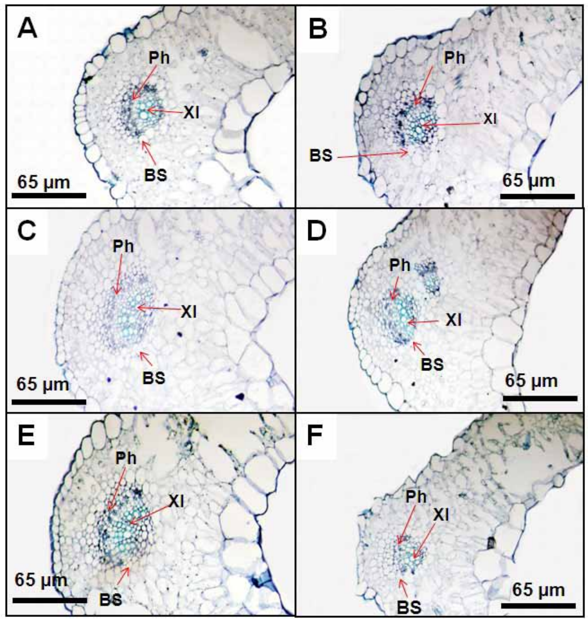

Figure 5. Mid-vein leaf changes in *A. gombiformis* plants grown under control (A, C and E) and drought conditions (B, D and F) at the periods 10 (A and B), 20 (C and D) and 30 (E and F) days of treatment. Bars = 65μm. BS: bundle sheath, Ph: phloem, Xl: xylem

tolerance and the water storage in the plant. Similar results were found in *Bituminaria bituminosa* and *Fraxinus ornus* [24-25]. Indeed, the decrease in water potential in the cytoplasm help to maintaining cellular water homeostasis [26], improving the ability of cells to maintain turgor pressure at low water potentials.

Knowledge of anatomical root modifications is essential for the explication of plants growth and hydraulic changes induced by water stress and therefore to understand the mechanisms used to confront drought conditions [11]. In our view, there is insufficient information about the changes in root anatomical as reaction to water deficit [27]. Our results showed that root cross-sectional and xylem vessel diameter decreased significantly under water deficit. In addition the cortex thickness and cortical cell size reduced significantly at moderate to severe water deficit (20-30 days) while, the xylem wall thickness and xylem vessel density were significantly increased at these drought periods (Figure 2C, D, E, and F). The stele diameter and stele/ root cross-sectional ratio showed significant increase after 20 days of irrigation withhold. Conversely, epidermis thickness was unaffected by water stress. The root and cortex thickness are generally smaller in stressed plants. A recent study showed that root cortex thickness was reduced in sensitive varieties of rice subjected to water deficit [28]. Cortical parenchyma might serve as a storage area of nutrients and water [29]. In our study, the decrease in root parenchymatic cell size, suggest an important role in determining reactions to drought in *A. gombiformis* roots. The increase of cortical parenchyma cells number in the plants subjected to severe water deficit indicated that root tissue dehydration accelerates cellular division. On the other hand, the increased stele/ root cross-section area under moderate drought stress, suggest an important role for the stele, probably in water transport by helping to retain root water under drought, rather than in water uptake under drought [28].

For stems, there was a trend for stem cross-sectional and the pith diameter to decrease with increasing water deficit. With regard to the percentage of reduction in these parameters, stems are more sensitive to water deficit than roots. In this experiment, cortex, xylem tissue, xylem vessel, phloem tissue and phloem sclerenchyma thickness were reduced significantly only at moderate to severe water deficit (20-30 days) while, xylem wall thickness was amplified (Figure 3). These results are in agreement with many investigators [13-14] indicating that *Salvia spendens* and *Triticum aestivum* subjected to water deficit showed a reduction in the stem cross-sectional, xylem tissue and phloem tissue thickness. This reduced xylem tissue area under water is due partly to a shift towards small diameter vessels [30] or sparsely distributed vessels [31]. For the reduction of the stem phloem tissue under stressful condition was probably not an adaptation to drought rather it was likely due to the fact that seedlings grown under water stress were smaller than untreated ones [13]. The reduction of the stem sclerenchyma area under drought conditions was also recorded in other species such as *Glycine max* [32].

Our results showed a narrowing xylem vessel diameter in the stems and roots of *A. gombiformis* grown under prolonged water deficit (20-30 days). Similar anatomical responses were also found in the roots and/ or stems of *Salvia spendens*, *Glycine max* and *Triticum aestivum* subjected to water stress [13-32-14]. Sibounheuang *et al*. [33] reported that xylem vessel diameter is related to the maintenance of the water conductivity. Species living in environments where water is available might only episodically have larger xylem vessels and larger diameter roots to maximize water uptake when it is available. However, large vessels may also be more prone to cavitation and embolism during water stress [34]. Indeed, plant resistance to drought is often correlated with xylem vulnerability to cavitation thus, species maintaining functional xylem conduits even under extreme drought conditions have a greater chance of survival [35]. Xylem vessel wall reinforcement is required to prevent wall implosion and cavitation, when xylem pressure is highly negative [36]. Accordingly, the prolonged water-deficient *A. gombiformis* showed thick-walled vessel elements in roots and stems. These thickenings reduce the contact angle between water and vessel wall to nearly zero, which could decrease embolism chances [37]. Previous work [38-27], showed a positive correlation between xylem density and drought acclimation of plants. In our study, *A. gombiformis* showed increased density of root xylem vessels under moderate to severe water deficit, but stem anatomical features does not present such adaptation. The decrease in xylem vessels diameter, the greater xylem wall thickness in both roots and stems added to the increased density of root xylem vessels may be beneficial under drought stress by reducing the risk of xylem vessel cavitation, suggests that *A. gombiformis* can survive under extreme water stress via various anatomical adaptation such narrowing xylem vessels with increasing density and avoiding wall implosion.

Since leaves are the main organs of internal water removal, water stressed *A. gombiformis* plants, undertake leaf anatomical alterations, in order to save water. Therefore, water deficit have a significantly impact on the most of leaf anatomical structures plants [39]. Similarly, we found that prolonged water deficit (20-30 days) caused a significant decrease in the leaf lamina, leaf mid-vein and mesophyll thickness (Table 3, Figure 4 and 5). The changes in leaf anatomy probably affect the conductance of CO_2 diffusion [40]. Thus, the reduction of mesophyll conductance was associated with thickening of the olive leaf mesophyll [41]. These results are consistent with the findings of studies of other plants as *Ctenanthe setosa* [2], and *Triticum aestivum* [14] in which water deficit caused decreased leaf thickness. Another remarkable leaf anatomical feature observed in *A. gombiformis* plants grown under severe water stress was a significant decrease of the mesophyll cells size. Thus, smaller size of mesophyll cells represents a major structural response to increased water stress. Therefore, cell size reduction in leaves occurs as a result of the role of water playing in the maintenance of turgidity being necessary for cell expansion [42], and can be interpreted as a tolerance mechanism of the leaves to maintain tissue turgor in *A. gombiformis*. Concerning epidermis size, it is common to observe a thickening of the skin under drought stress [43]. Therefore, the increase of epidermis thickness is an indication of resistance to water deficit [44-45]. Also, our results showed an increased thickness for the lower epidermis, while the upper was unaffected by increasing water deficit.

Regarding *A. gombiformis* vascular system, our results exhibited that increasing water deficit decrease the dimension of the bundle sheath in the main vein. In addition, the xylem and phloem tissue thickness reduced under prolonged water deficit. These results of leaf main-vein anatomy are consistent with the findings of studies of *Vigna unguiculata* [46]. In the present study, the vascular bundle diameter in the leaf lamina of *A. gombiformis* did not show clear trends with drought stress. In addition the distance between vascular bundles did not greatly modify under drought conditions (Table 3, Figure 4). This suggested the leaf ability to maintain comparable water transport in all treatments. Despite of this, the leaf blade thickness was affected by stress [47].

This study also revealed that stomata were evenly distributed on both leaf surfaces of *A. gombiformis*. Numerous works linked drought plant adaptation to the increase in stomatal density coupled with decrease in the stomata size [48-49]. In the present study, leaf stomatal density increased under moderate and severe drought conditions, which is consistent with the results of wheat [50] and apricot [49]. Our results show that leaf stomatal size was unaffected by drought. It is in agreement with the findings of Rodiyati *et al*. [51] reporting that stomatal size did not decrease under water stress conditions. From these results, it appears that *A. gombiformis* plant responds to drought conditions by some stomatal changes; the first increasing the density of stomata whereas the size wile probably changed when they are submitted to more severe water deficit.

In summary, we have investigated the anatomical changes in root, stem and leaf of *A. gombiformis* plants grown under increasing water deficit. Our results show drought-stressed *A. gombiformis* had reduced growth potential and this reduction was correlated with lowering leaf Ψ_w. There were several structural aspects that may have helped this species to tolerate drought conditions. Thus, under prolonged drought condition plants may be expected to develop roots and stems having narrow xylem tissue with wall-thickened vessels coupled with an increasing density of these vascular elements recorded especially in the roots. The stiffer and stronger xylem can play beneficial effect by reducing the possibility of cavitation under drought stress. On the other hand, the leaf anatomical assessment showed that there were specific adaptations. These can be summarized as the uniform mesophyll cells and the stomata evenly distributed across both leaf surfaces with increased density under water deficit are characteristic features of drought-adapted plants. In addition, the fibro-vascular system in leaf lamina was well developed in order to enhance water absorption and storage during drought conditions. All these characteristics provide the anatomical basis for drought resistant in *A. gombiformis* plants through the ability to adapt its root, stem and leaf structures therefore it could survive and grow in dry and arid conditions.

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