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Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars

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Abstract

Two cultivars of avocado (*Persea americana* Mill., ‘Fuerte’ and ‘Hass’) plants, grown in 50 l containers, were studied under two irrigation regimes for 6 months in order to evaluate the growth response and leaf physiological and anatomical changes induced by moderate water stress. Irrigation was applied when soil water potential reached at -0.03 and -0.5 MPa for the wet and dry treatments, respectively. Leaf anatomy changed in water-stressed leaves, which could have accounted for the decreased stomatal conductance. Photosynthesis is inhibited by reducing the diffusion of CO_2 to the chloroplast, both by stomatal closure and changes in mesophyll structure, which decreases the conductance to CO_2 diffusion within the leaf. Predawn leaf water potential (Ψ) declined by 0.9 MPa for ‘Fuerte’ and 1.2 MPa for ‘Hass’ after 12 days of withholding water, whereas the turgor potential (Ψ_p) remained positive due to a decrease in the osmotic potential (Ψ_π) in both cultivars. The reduction in osmotic potential was mainly due to dehydration and only partly related to active accumulation of solutes. Tissue elasticity seems to be the predominant physiological mechanism of drought adaptation of avocado. Growth data suggests that ‘Hass’ seems to be more affected by moderate water stress. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Growth; Leaf anatomy; *Persea americana*; Photosynthesis; Water potential components; Water stress

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1. Introduction

Drought is known to limit plant productivity in many regions of the world. Recent studies have shown that growth rates of several plants are directly proportional to the availability of water in the soil (Kamel and Loser, 1995). Water deficit is also known to alter a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation (Hu and Schmidhalter, 1998). The extent to which photosynthetic capability is maintained during periods of water stress and the ability of rapid recovery of photosynthesis after rewatering may play an important role in plant adaptation to drought environments. In order to preserve photosynthesis under drought conditions, plants have evolved physiological processes to maintain to some extent tissue turgor and stomatal opening (Nunes et al., 1989). The reduction of osmotic potential (Ψ_{π}) in response to water stress is a well-established mechanism whereby many plants adjust to low soil water availability (Morgan, 1984). An increase in cell wall elasticity can also contribute to turgor maintenance under drought conditions (Patakas and Noitsakis, 1997). Furthermore, recent studies have revealed that changes in leaf anatomical characteristics can alter the CO_2 conductance diffusion components from the substomatal cavities to sites of carboxylation and thus contribute to maintenance of photosynthetic rates despite the low stomatal conductance (Evans et al., 1994).

Avocado (*Persea americana* Mill.) has recently become an important crop in northern Mediterranean countries and is successfully grown in southern Greece, especially at the island of Crete. At this island and also in many other places of the Mediterranean region, avocados often develop moderate water stress as a consequence of either inappropriate or untimely irrigation scheduling, which often results in decreased yields (Lahav and Kalmar, 1977; Levinson and Adato, 1991; Michelakis et al., 1993). However, little information exists about the influence of water stress on physiological and growth parameters of avocado as well as the mechanisms used by different avocado cultivars to withstand water stress. A better understanding of plant adaptation to water stress may help to enhance irrigation management practices.

Therefore, the objectives of this study were to investigate the effects of water stress on: (i) vegetative growth, (ii) internal water relations and gas exchange and (iii) the leaf anatomy of two avocado cultivars, 'Fuerte' and 'Hass'.

2. Materials and methods

2.1. Plant material

The experiment was carried out at the Subtropical Plants and Olive Tree Institute of Chania, Greece, during 1995. Two-year-old avocado (*P. americana* Mill.) plants, cv. 'Fuerte' and 'Hass', grafted on Mexican rootstocks were used. The plants were grown outdoors in 50 l containers filled with a free draining sandy loam soil. A meteorological station located 100 m away from the study site allowed measurements of air temperature (T) and relative humidity (RH) during the course of the experiment (Fig. 1). Tensiometers

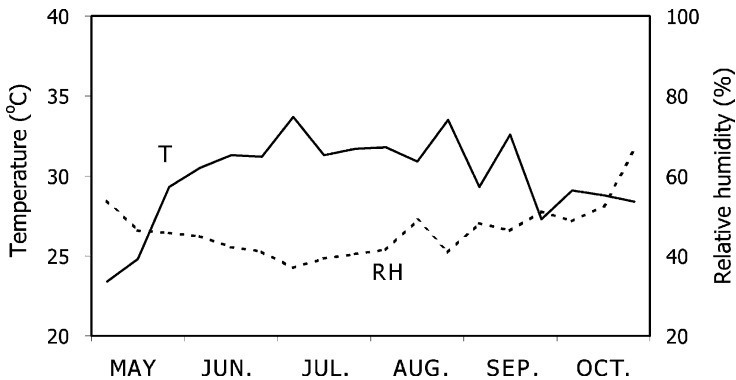


Fig. 1. Mean air temperature (T) and relative humidity (RH) at daytime during the course of the experiment.

and gypsum blocks placed at 20 cm depth were used for monitoring soil matric potential. Two soil water regimes were applied during the dry season (May–October): (1) a well irrigated treatment (control), where irrigation was applied when soil matric potential reached -0.03 MPa (average from five tensiometers); (2) a water stress treatment (stress), where irrigation was applied when the soil matric potential reached -0.5 MPa (average from five blocks). Each treatment was applied to 10 uniform plants of each avocado cultivar. To monitor plant growth, the trunk diameter was recorded every 2 weeks during the course of the experiment. At the end of the experiment, four plants of each treatment were removed and the total leaf area and the dry weight of shoots (leaves plus stems) and roots were determined. Leaf area was measured using the MK2 area meter (DELTA-T DEVICES, UK). Dry weight was determined after the material was dried in an oven at 105°C for 24 h.

2.2. Water status measurements

In order to monitor the development of water stress the predawn leaf water potential and the predawn leaf osmotic potential were measured on the same leaves at 2 day intervals as the soil dried during two stress cycles. Additionally, the diurnal changes in leaf water potential, osmotic potential and leaf conductance were measured in water-stressed and control plants on several occasions during the stress cycles. Leaf water (Ψ) and osmotic (Ψ_{π}) potentials were measured using a Wescor HR-33T microvoltmeter. Measurements (10 replicates) were made on leaf disks (0.38 cm^2) from 3rd to 4th fully expanded leaf counting from the terminal shoot apex. Turgor potential (P) was calculated by the difference $P = \Psi - \Psi_{\pi}$. The calculated net increase of solutes in cells (Ψ_{π_s}) was estimated according to Chartzoulakis et al. (1999).

Concomitant measurements of relative water content (RWC) were made on six leaf disks, 1 cm in diameter, from the same leaves used for determination of water potential components. The disks were weighed and floated on water for 6 h at a temperature of $4\text{--}6^{\circ}\text{C}$ in darkness. The disks were blotted dry and turgid weight was determined and the dry weight was measured after drying the disks for 24 h in 105°C . RWC was calculated

by the formula:

$$\text{RWC} = \left[\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \right] \times 100.$$

The bulk modulus of elasticity (ε) was calculated using the equation (Koide et al., 1991):

$$\varepsilon = \left[\frac{\Delta P}{\Delta(\text{RWC})} \right] \times 100.$$

2.3. Gas exchange measurements

Midday gas exchange measurements were made during the water stress cycle, every 2 days between 12:00 and 13:00 h. Six leaves from each treatment were used to measure the photosynthetic rate (P_n) and the leaf conductance (g_s) using a portable IRGA (LI-6200, Li-Cor). By applying relevant calculations (Von Caemmerer and Farquhar, 1981) the intercellular CO_2 concentration (C_i) was obtained. The measurements were made on the third and fourth fully expanded leaves counting from the terminal shoot apex, at the ambient CO_2 concentration.

2.4. Leaf anatomy

For anatomical studies pieces from 12 leaves per treatment, taken at the end of the experiment (October 1995), were fixed for 3 h in 5% glutaraldehyde buffered with 0.025 M sodium phosphate to pH 7.2. Samples were then washed in the respective buffer and post-fixed for 5 h in 1% osmium tetroxide similarly buffered. Tissue dehydration was carried out in an alcohol series (50% alcohol for 1/2 h, 70% alcohol for 1/2 h, 90% alcohol for 1 h, 100% for 1 h and 100% for 1 h) followed by infiltration and final embedment in Spurr's resin. Cross as well as paradermal sections for light microscopy (1 μm thick) were obtained in a Reichert Om U2 ultramicrotome, stained with 1% toluidine blue O in borax (the stain solution was 1% toluidine blue O in 1% borax solution), and examined with a Zeiss III photomicroscope.

3. Results

3.1. Plant growth

A significant reduction of trunk diameter of 'Fuerte' and 'Hass' by 34 and 39%, respectively, was observed after the 6 month period of water stress. Furthermore, moderate water stress reduced significantly the total plant leaf area by 57 and 69% and the total plant dry weight by 63 and 80% for 'Fuerte' and 'Hass', respectively (Table 1). There were also changes in the leaf morphological characteristics. Water-stressed plants had smaller leaves and lower specific leaf weight in both cultivars (Table 1). Furthermore, prolonged water stress reduced the biomass of fibrous roots in both cultivars, with more visible damage occurring with the 'Hass' than with the 'Fuerte' root system.

Table 1
Growth and morphological parameters of two avocado cultivars grown under different irrigation regimes

Parameter	Fuerte		Hass	
	Watered	Stressed	Watered	Stressed
Trunk diameter (mm)	18.2 a ^a	12.1 b	18.6 a	11.3 b
Total leaf area (m ²)	9.682 a	4.156 b	9.542 a	3.542 b
Shoot dry weight (g)	143.5 a	48.7 b	166.4 a	32.7 b
Root dry weight (g)	98.4 a	18.8 b	77.9 a	15.8 b
Root/shoot ratio	0.685 a	0.386 b	0.468 a	0.483 a
Leaf size (cm ²)	310.7 ± 19	259.1 ± 34*	280.1 ± 24	216.2 ± 31*
SLW ^b (mg cm ⁻²)	8.47 ± 0.69	6.44 ± 0.60*	8.12 ± 0.51	6.17 ± 0.79*

^a Means with different letters are significantly different at $P = 0.01$ (Duncan's test).

^b Specific leaf weight.

* Significant between treatments at $P < 0.05$ by analysis of variance (each value is the mean of 10 measurements ± S.E.).

3.2. Water relations

Predawn water potential (Ψ) in water-stressed plants remained in both cultivars at control levels (-0.6 up to -0.7 MPa) for the first 5 days after withholding water and then declined to -1.61 MPa in 'Fuerte' and -1.87 MPa in 'Hass' on day 12 (Table 2). Osmotic potential (Ψ_{π}) also decreased during the stress period resulting in positive turgor maintenance in both cultivars: 0.14 MPa in 'Fuerte' and 0.06 MPa in 'Hass' (Fig. 2). The calculated net increase of solutes in cells was 40.6% in 'Fuerte' and 29.4% in 'Hass'. After rewatering, initial values were attained in 2-day period (Table 2).

The relationship between water potential components and RWC or Hofer diagram for stressed leaves of both avocado cultivars is illustrated in Fig. 2. As expected, the initial decrease in RWC resulted in a large decrease in Ψ . A reduction of only 10% of RWC from saturation (99%) to 89% resulted in a decrease of approximately 0.9 MPa for 'Fuerte' and

Table 2
Effects of water stress on predawn leaf water potential (Ψ_1 , MPa), midday photosynthesis (P_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) in two avocado cultivars (data are mean of 10 measurements ± S.E. of means)

Cultivar	Parameter	Days after water stress				Recovery (2 days)
		0	3	7	12	
Fuerte	Ψ_1	0.69 ± 0.04	0.70 ± 0.07	1.06 ± 0.06	1.61 ± 0.09	0.84 ± 0.04
	P_n^a	6.2 ± 0.7	6.4 ± 0.9	3.8 ± 0.9	1.7 ± 0.5	7.3 ± 0.4
	g_s	0.343 ± 0.012	0.325 ± 0.017	0.105 ± 0.029	0.055 ± 0.019	0.292 ± 0.035
	Ψ_1	0.74 ± 0.06	0.78 ± 0.03	1.01 ± 0.08	1.87 ± 0.11	0.98 ± 0.07
Hass	P_n	8.2 ± 1.1	9.5 ± 0.8	5.7 ± 0.6	2.9 ± 0.6	6.7 ± 1.2
	g_s	0.380 ± 0.024	0.362 ± 0.031	0.237 ± 0.037	0.120 ± 0.031	0.219 ± 0.022

^a Conditions during measurements were: CO_2 350 ± 10 mbar, 21% (by volume) O_2 , PPF $750 \pm 100 \mu\text{mol m}^{-2} \text{s}^{-1}$, air temperature 28 ± 2 °C.

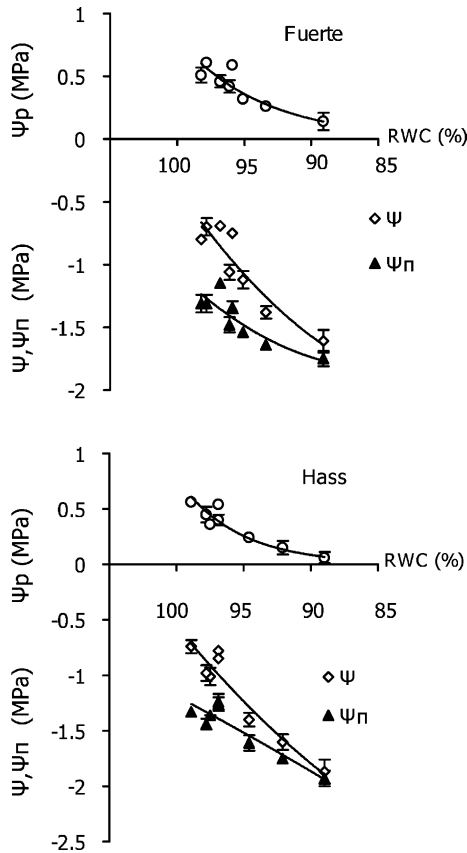


Fig. 2. A Hofler plot of changes in water (\diamond), osmotic (\blacktriangle) and turgor (\circ) potential with RWC for water-stressed avocado leaves: (A) cv. 'Fuerte'; (B) cv. 'Hass'. Data points are the mean of 10 measurements. Vertical bars represents S.E., when is bigger than symbols.

1.2 MPa for 'Hass' in Ψ . The turgor (P) component accounted for the major part of this reduction (77% for 'Fuerte' and 90% for 'Hass'), while Ψ_{π} declined by only 64% for both cultivars. Furthermore, bulk modulus of elasticity decreased during the stress period from 6.6 to 4.1 MPa and 8.3 to 5.0 MPa in 'Fuerte' and 'Hass', respectively.

3.3. Gas exchange

Midday photosynthetic rate declined progressively in both cultivars during the stress period. The decline of photosynthesis during water stress was significant from day 5 in both cultivars, being 27 and 35% of the control values by day 12 for 'Fuerte' and 'Hass', respectively (Table 2). Significant differences in stomatal conductance between watered and stressed plants were first detected at day 4 after withholding water in both cultivars. The relationship between photosynthetic rate and stomatal conductance was linear during the stress period (Fig. 3). There was also a clear correlation between leaf photosynthesis

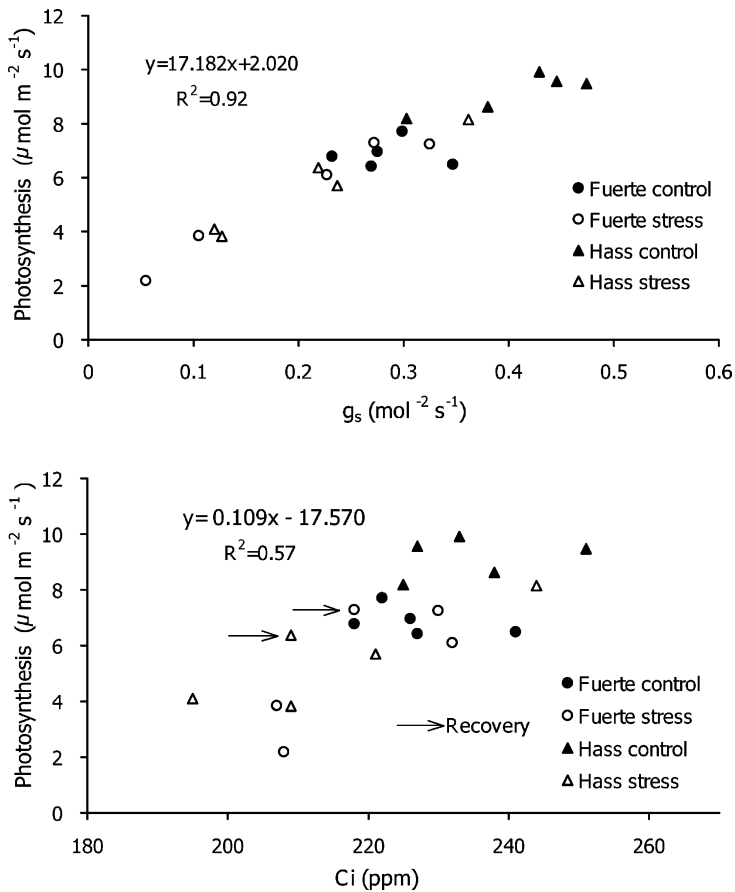


Fig. 3. Leaf photosynthesis (P_n), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of avocado leaves for control (open symbols) and water-stressed (closed symbols) plants. Conditions were: CO_2 350 ± 10 mbar, 21% (by volume) O_2 , PPFD $750 \pm 100 \mu\text{mol m}^{-2} \text{s}^{-1}$, air temperature 28 ± 2 °C. Data points are the mean of 10 measurements.

(P_n) and intercellular CO_2 concentration (C_i), but leaves recovering from water stress showed a much higher P_n rate, if compared with the stressed leaves at the same C_i . However, 'Hass' exhibited higher P_n rates at the same level of water stress than 'Fuerte' (Fig. 4). There was a strong inverse correlation ($r^2 = 0.61$) between photosynthesis of control and stressed plants and CO_2 draw-down (Fig. 5). Photosynthesis as well as stomatal conductance of 'Fuerte' fully recovered 2 days after rewatering, while in 'Hass' plants attained 20% lower values than that of the control (Table 2).

3.4. Leaf anatomy

Cross-sections of 'Fuerte' and 'Hass' leaves showed that in both cultivars the palisade parenchyma is composed of two successive and distinctive layers, the first one being in

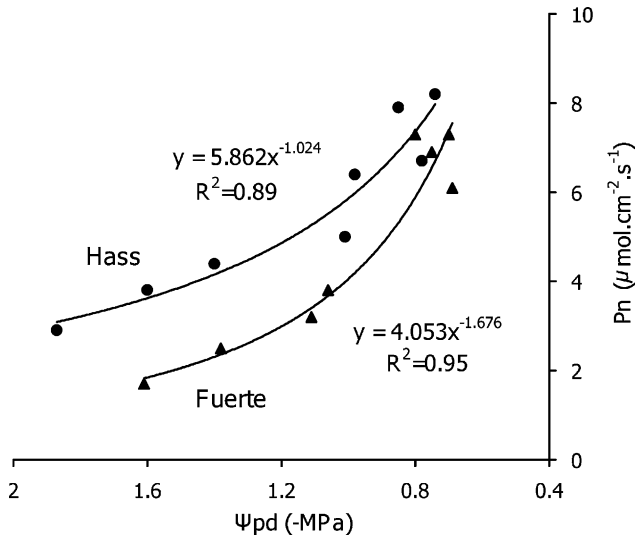


Fig. 4. Relationship between P_n and predawn leaf water potential (Ψ_{pd}) during the stress cycle for the two avocado cultivars. Data points are the mean of 10 measurements.

contact with upper epidermis (palisade parenchyma I, PPI) and the other with the spongy parenchyma (palisade parenchyma II, PPII) (Fig. 6A and C). There were significant changes in leaf anatomical characteristics induced by water stress. In particular, in both cultivars, water stress resulted in a significant decrease of the thickness of almost all histological components of the mesophyll, as well as of the entire lamina thickness (Table 3). In stressed plants of ‘Fuerte’ the chlorenchyma cells are denser than those in well irrigated ones (Fig. 6). As a consequence, the amount of intercellular spaces of water-stressed leaves was lower than in control leaves (Table 3), while the PPII cells have

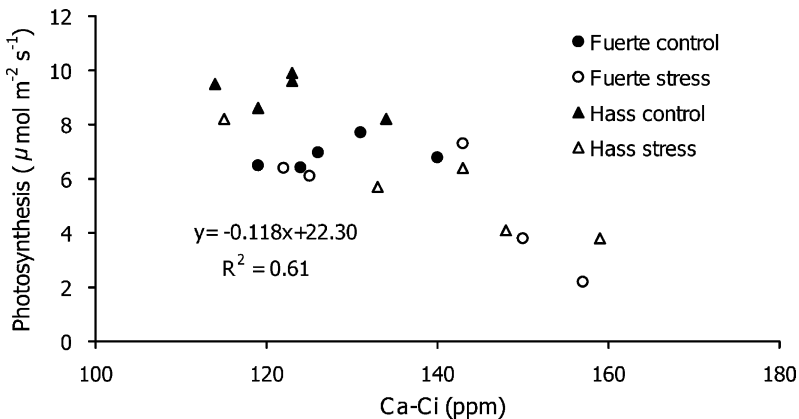


Fig. 5. Relationship between photosynthesis and CO_2 drawn-down from ambient (C_a) concentration to the intercellular (C_i) concentration. Data points are the mean of 10 measurements.

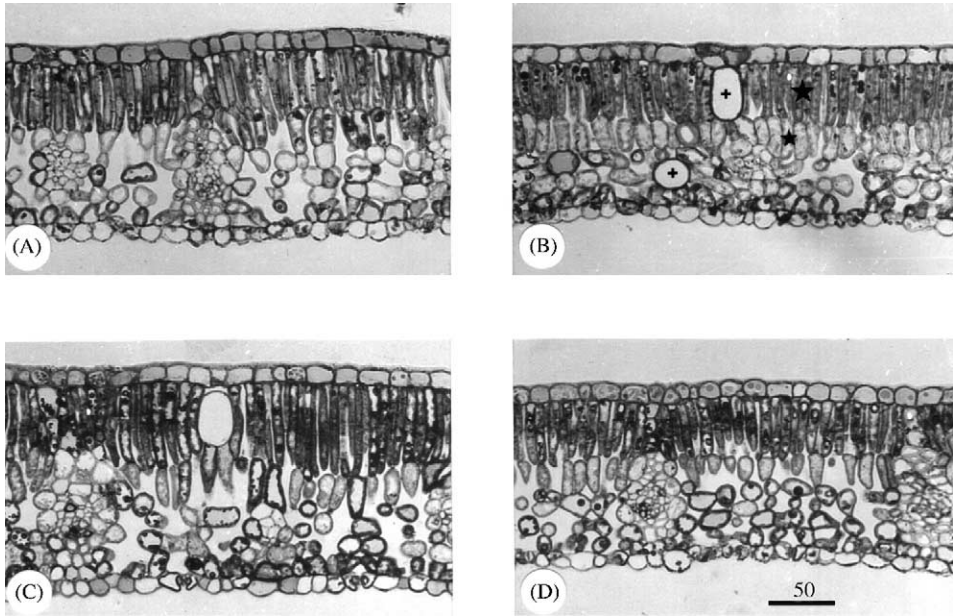


Fig. 6. Comparative leaf anatomy in blade cross-sections of 'Fuerte' (A, B) and 'Hass' (C, D) leaves grown under well irrigated (A, C) and water stress (B, D) conditions. The large asterisk indicates the PPI, the small asterisk the PPII and the cross (+) the idioblastic oil cells. Quantitative analyses of anatomical parameters are reported in Table 3. Bar in μm .

Table 3

Anatomical changes in cross-sections of fully expanded leaves of two avocado cultivars exposed to intermittent water stress

Leaf histological component	Fuerte		Hass	
	Watered	Stressed	Watered	Stressed
<i>Thickness (μm)</i>				
Upper epidermis	17.4 \pm 1.5 ^a	15.7 \pm 1.4 ^{***}	14.9 \pm 0.9	15.9 \pm 1.9
Palisade I	64.8 \pm 8.7	51.7 \pm 4.0 ^{***}	72.8 \pm 7.1	51.6 \pm 6.4 ^{***}
Palisade II	31.0 \pm 4.3	29.3 \pm 4.3	34.0 \pm 2.9	26.5 \pm 4.3 ^{***}
Spongy	60.3 \pm 6.9	53.8 \pm 6.8 [*]	65.3 \pm 8.7	55.8 \pm 7.3 ^{**}
Lower epidermis	14.6 \pm 2.5	13.6 \pm 1.9	14.0 \pm 2.3	14.6 \pm 2.5
Total thickness	193.4 \pm 16.6	167.6 \pm 7.9 [*]	203.1 \pm 8.0	168.6 \pm 10.5 ^{***}
% of total leaf area intercellular spaces	22.12	14.21 ^{**}	24.07	13.18 ^{***}

^a Data are mean \pm S.D. ($n = 12$).

^{*} Indicate significant differences at $P < 5\%$.

^{**} Indicate significant differences at $P < 1\%$.

^{***} Indicate significant differences at $P < 0.1\%$.

remarkably increased in volume and number (Fig. 6B). The PPII cells of stressed 'Hass' are peg-like and not so densely arranged as in the relevant stressed 'Fuerte' (Fig. 6D).

4. Discussion

Water stress affected avocado cultivars to a different degree. 'Hass' seems more affected by water stress, since at the same RWC turgor potential was lower than that of 'Fuerte' (Fig. 2). Nevertheless, 'Hass' had higher P_n rates than 'Fuerte' at the same leaf water potential (Fig. 4). The higher g_s exhibited by 'Hass' at the same Ψ_1 could explain the apparent contradiction. A decline in the photosynthetic rate under water stress conditions could be attributed either to a decrease in stomatal conductance and/or to non-stomatal limitations (Jones, 1992; Cornic and Massacci, 1996). The parallel changes of photosynthetic rate and stomatal conductance provides evidence that the maintenance of the photosynthetic rate could be mainly attributed to the maintenance of stomatal conductance. However, the decrease of C_i noticed the day before rewatering may indicate the occurrence of stomatal limitations. Stomatal and mesophyll resistances progressively reduce the CO_2 concentration reaching the chloroplasts. The strong inverse correlation ($r^2 = 0.61$) between photosynthesis of control and stressed plants and CO_2 draw-down caused by stomatal resistances (the difference between the ambient and intercellular CO_2 concentration, $C_a - C_i$), is a clear indication that low CO_2 concentration is the main limitation in photosynthesis of avocado (Fig. 5). The relieved mesophyll resistance when leaves are rewatered may explain the high P_n for C_i similar to those of stressed leaves during recovery.

Changes in leaf anatomy likely affect the conductance to CO_2 diffusion (Evans et al., 1994; Syvertsen et al., 1995). Reduction of mesophyll conductance was related to mesophyll thickening in salt-stressed olive leaves (Bongi and Loreto, 1989). Salt and water stress apparently reduce photosynthesis by similar mechanisms. In avocado, palisade and total thickness of water-stressed leaves were lower than in controls (Table 3). Furthermore, an increased density of spongy cells and a 35–45% decrease of intercellular spaces was also evident (Table 3, Fig. 6). The dense arrangement of spongy cells may result in reduction of the diffusion conductance in water-stressed avocado leaves. These results support the idea that direct relationship between leaf porosity and mesophyll conductance exists (Loreto et al., 1992). However, Syvertsen et al. (1995) showed that the mesophyll density is inversely correlated with the conductance through the liquid phase only.

It is well known that stomatal function is closely related to leaf water status and especially to leaf turgor. The latter could be mediated during water stress conditions by two distinct strategies: (i) by lowering the osmotic potential due to active solute accumulation and (ii) by increasing the elasticity of the cell walls. The first strategy leads to the conservation of water in the tissue, whereas the second strategy allows the maintenance of the same turgor pressure with less water. In our results drought has been shown to both decrease osmotic potential and increase tissue elasticity in both cultivars. The reduction in osmotic potential was mainly due to dehydration and only partly related to active accumulation of solutes. These results indicate that osmotic adjustment only slightly contributes to turgor maintenance. On the other hand, bulk modulus of elasticity decreased almost 50% in both cultivars providing evidence that in these cultivars tissue elasticity

seems to be the predominant physiological mechanism of drought adaptation. However, the positive role of tissue elasticity in turgor maintenance has been later questioned, as recent studies indicated that inelastic cell walls although preclude turgor maintenance to low water contents, they do have several advantages over elastic cell walls and may become together with osmotic adjustment an efficient mechanism which enable plants to sustain water stress conditions (Meier et al., 1992; Grossnickle and Russel, 1996). In contrast to the above, it has been suggested that plants with elastic cell walls have a high inherent drought tolerance (Fan et al., 1994). This may be true in the absence of osmotic adjustment and several reports indicate that in species where drought stimulates increases in tissue elasticity, there tends to be little or no osmotic adjustment with turgor maintained over a wide range of leaf water content (Nunes et al., 1989; Fan et al., 1994). This form of drought tolerance, exhibited in our results, may also correlate with a necessity to protect membranes and macro-molecules against extreme reduction in cell volume during drought.

Cell wall elasticity is known to be closely related to cell size (Steudle et al., 1977). Bulk modulus of elasticity increased with cell size and thus small cells can withstand negative turgor pressure better than large cells. This was evident in our results, where the thickness of the mesophyll decreased in the stressed plants indicating a reduction in cell size. The reduction in cell size under water stress conditions may be considered as drought adaptation mechanism (Cutler et al., 1977; Steudle et al., 1977). According to Cutler et al. (1977) reduction in cell size appears to be a major response of cells to water deficiency. Anatomical measurements also revealed two additional xeromorphic characteristics which are the appearance of palisade parenchyma in both leaf sides as well as oil cells. The former is considered as an indicator for xeromorphy and have also been reported in other species (*Olea europaea* L) grown under arid conditions.

Growth data suggests that 'Hass' is more productive under well-watered conditions, but it looks the more affected by water stress (Table 1). However, the root/shoot ratio was not affected by water stress, indicating that root growth was as sensitive as shoot growth. This result contrasts with reports in the literature of increases in root/shoot ratio with increased water stress, as was the case for 'Fuerte'. Growth is extremely sensitive to drought and is strongly influenced by the ability of the roots to grow in drying soil and maintain an optimal water status (Tyree and Alexander, 1993). Sobrado and Turner (1986) suggested that a similar degree of osmotic adjustment in root and leaf cells would help to explain the similar root/shoot ratio found in water-stressed and unstressed *Helianthus annuus*. This would explain the difference between the two cultivars in the ability to partition carbon into leaves under water stress. Our results suggest that the difference in performance observed between the two cultivars could be attributed to anatomical rather than to physiological characteristics.

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