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Leaf anatomical alterations induced by drought stress in two avocado cultivars

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The avocado (*Persea americana* Mill.) leaf is hypostomatic with the typical anatomical pattern of dicots. The palisade parenchyma of the mesophyll is composed of an upper layer with elongated, densely arranged cells, and of a lower layer with short and loosely placed cells. Within the mesophyll, numerous idioblastic oil cells occur. In the avocado cultivars studied (‘Hass’ and ‘Fuerte’), drought stress resulted in an increase of the density of the epidermal cells and mesophyll chlorenchyma cells with a parallel decrease of their size. Mesophyll intercellular spaces increase in volume and oil cells become more numerous. The above features are much more prominent in ‘Hass’, a fact favouring the suggestion that ‘Hass’ responds to drought stress better than ‘Fuerte’.

Key words: blade structure, idioblastic oil cells, *Persea americana,* stomata, water deficit.

INTRODUCTION

Low air humidity and high temperatures result in a significant decrease of the soil water content. To survive, plants grown under such conditions, drastically reduce transpiration by closing stomata. This event reflects a decline of photosynthesis, so that plants are forced to sacrifice carbon gain for water conservation (Chartzoulakis *et al.*, 1993; Loreto *et al.*, 1995; Basu *et al.*, 1998). Adaptation to arid environments has endowed plants with specific mechanisms which allow them to successfully face reduced transpiration and photosynthesis. Such mechanisms include accumulation of active osmotic substances in cell vacuoles (maintenance of tissue turgor), increase of the number of stomata (better control of water loss), reduction of the mesophyll intercellular space volume (decrease of the amount of water vapours diffused to stomata) and increase of the number of mesophyll cells (more numerous chloroplasts and greater CO_2 uptaking cell surface) (Kyparissis & Manetas, 1993; Chartzoulakis *et al.*, 1999; Patakas & Noitsakis, 1999).

Avocado plants often suffer drought stress particularly due to inappropriate or untimely irrigation, especially during summer. The aim of the present work was to study the anatomical and morphometric alterations induced by drought stress in the leaf of two known cultivars of avocado. These data could be useful in irrigation management practices.

MATERIAL AND METHODS

Two cultivars of avocado (*Persea americana* Mill. cvs ‘Hass’ and ‘Fuerte’) were used. Ten plants (two-year-old) of each cultivar were irrigated (for two years) to keep soil matric potential at –0.03 MPa (controls). Another ten plants were water stressed (also for two years) by irrigating only when soil matric potential reached –0.5 MPa. Tensiometers and moisture sensors were placed at 20 cm depth to monitor soil matric potential.

For anatomical studies, leaves (second node from the base of annual stems) of irrigated and

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stressed plants were used. Leaf segments were prefixed for 3h with 5% glutaraldehyde in 0.025M phosphate buffer. After washing in buffer, the segments were postfixed for 5h with 1% osmium tetroxide similarly buffered. Tissue dehydration was carried out in an alcohol series followed by infiltration in Spurr's (1969) resin. Semithin sections (1 μm thick) of plastic embedded leaves were obtained in a Reichert Om U_2 ultramicrotome, stained with 1% toluidine blue O in borax and examined with a Zeiss III photomicroscope. Morphometric assessments were conducted on leaf paradermal sections. Statistical treatment was carried out by using the statistical package SPSS.

RESULTS

Normal leaves of the avocado cultivars 'Hass' and 'Fuerte' exhibit the anatomical pattern typical for the dicots (Figs 1A, B). The epidermal cells are in close contact with each other and their walls are straight (not sinuous) (Figs 2A, E; 3A, E). In the mesophyll, the palisade parenchyma is composed of two distinct successive layers. One of these is in contact with the upper leaf epidermis (palisade parenchyma I, PPI) and the other with the spongy parenchyma (palisade parenchyma II, PPII) (Figs 1A, B). The PPI cells are narrow and densely arranged, whereas the PPII cells are wider and loosely sited (Figs 2B, C; 3B, C). The spongy parenchyma consists of irregular cells forming large intercellular spaces (Figs 1A, B; 2D; 3D). Typical components of the avocado leaf are idioblastic oil cells which occur in both palisade parenchymas and in the spongy parenchyma, as well (Figs 1A; 2B-D; 3B-D; asterisks). In the latter, each oil cell is radially surrounded by a varying number of chlorenchyma cells (Fig. 2D). Oil cells are observed to occur in the highest populations within PPII. Less often are met within PPI and even less within spongy parenchyma (Table 3). In the PPII and spongy mesophyll parenchymas, oil cells are mostly globular, while in the PPI parenchyma they are ordinarily ovoid with their major axis oriented perpendicularly to the leaf epidermis. All idioblastic oil cells are observed to be isolated and not grouped.

Comparative paradermal sections serially cut from the upper to the lower leaf epidermis of 'Hass' showed that drought stress results in an increase of the cell density in all leaf histological components (Fig. 2; A-E control, F-J drought stressed; Table 1). Thus, epidermal and mesophyll cells become more numerous per mm^2 of section surface and smaller in size. The cells of the leaf upper epidermis exhibit the presence within their vacuoles of dark globular inclusions (Fig. 2F). Stomata on the lower leaf surface undergo under water deficit conditions a slight increase in density, not of statistical significance (Fig. 2E, Table 2). The upper leaf surface is devoid of stomata (Figs 2A, F).

In the case of 'Fuerte', drought stress also results in an increase of the mesophyll cell density (PPI, PPII, spongy parenchyma) which, however, is not so prominent as in 'Hass' (Figs 3B, C; G, H; Table 1). The epidermal cells, unlike the mesophyll cells, undergo a slight reduction in density. Stomata on the

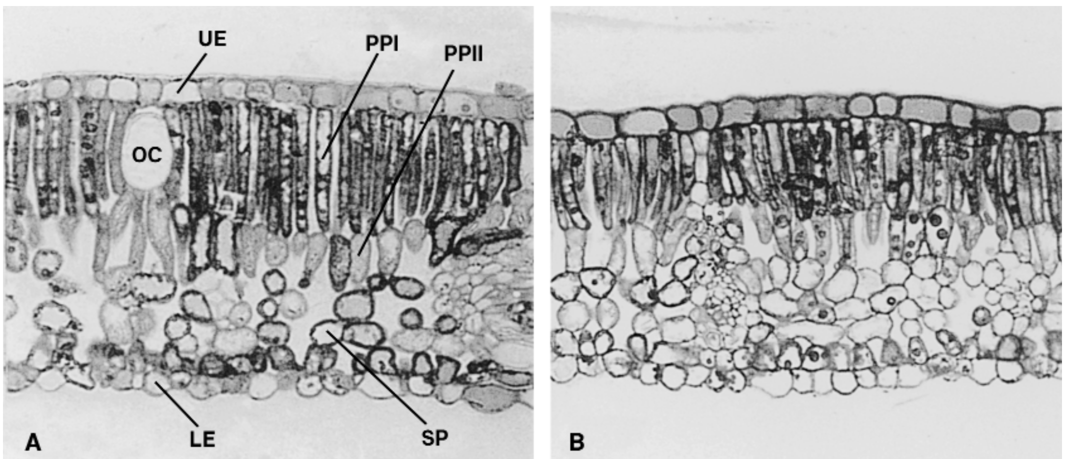

FIG. 1. Comparative leaf anatomy (leaf cross sections) of control avocado cultivars 'Hass' (FIG. 1A) and 'Fuerte' (FIG. 1B). LE = lower epidermis, OC = oil cell, PPI = palisade parenchyma I, PPII = palisade parenchyma II, SP = spongy parenchyma, UE = upper epidermis. × 250.

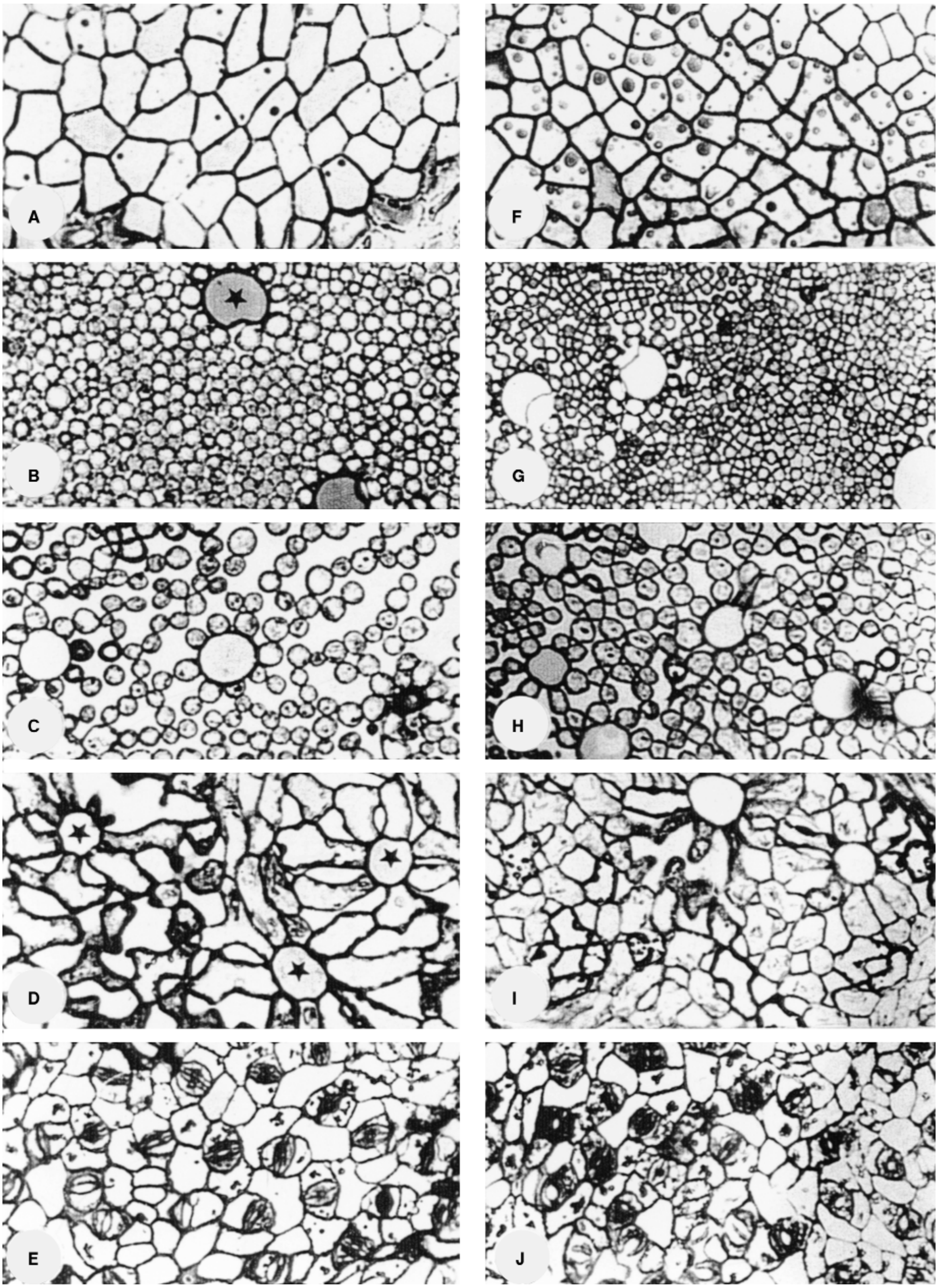

FIG. 2. A-J. Avocado cultivar 'Hass'. Comparative leaf anatomy of control (A-E) and drought stressed (F-J) plants in paradermal sections serially cut from the upper to the lower leaf epidermis. A, F, upper epidermis; B, G, palisade parenchyma I; C, H, palisade parenchyma II; D, I, spongy parenchyma; E, J, lower epidermis. Note the differences in density and size of the epidermal and mesophyll cells between the control and the drought stressed plants. Asterisks indicate idioblastic oil cells. × 280.

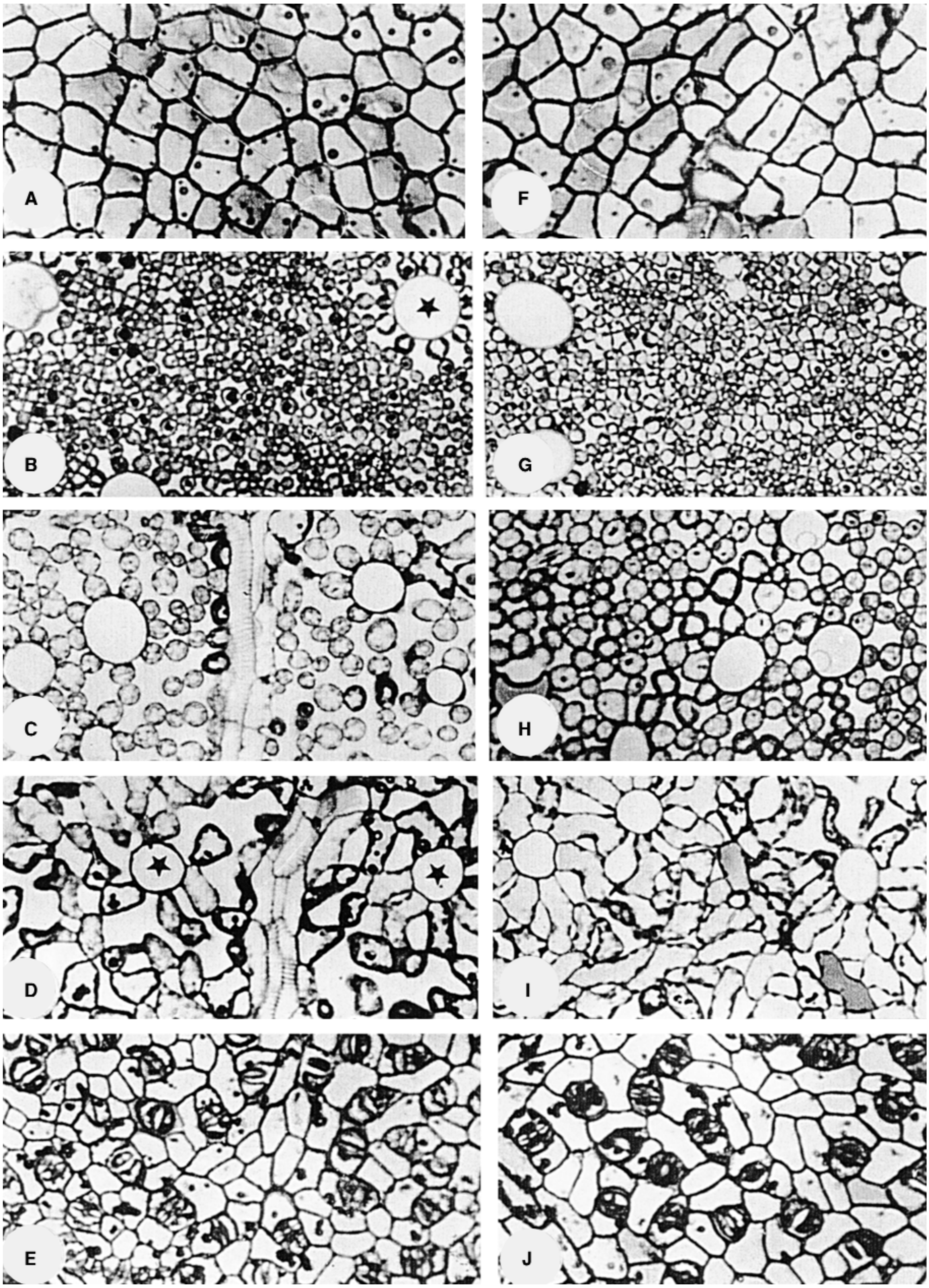

FIG. 3. A-J. Avocado cultivar 'Fuerte'. Comparative leaf anatomy of control (A-E) and drought stressed (F-J) plants in paradermal sections serially cut from the upper to the lower leaf epidermis. A, F, upper epidermis; B, G, palisade parenchyma I; C, H, palisade parenchyma II; D, I, spongy parenchyma; E, J, lower epidermis. Note the differences in density and size of the epidermal and mesophyll cells between the control and the drought stressed plants. Asterisks indicate idioblastic oil cells. × 280.

TABLE 1. Density (No/mm^2 of section) of epidermal and mesophyll cells in leaves of two avocado cultivars grown under irrigation and drought stress (paradermal sections) (n=12, ± SD,*-**-*** significantly different from the control at p<0.05, p<0.01, and p<0.001)

Histological component	cv. 'Hass'		cv. 'Fuerte'	
	Irrigated	Stressed	Irrigated	Stressed
Upper epidermis	1904 ± 147	2631 ± 103**	2386 ± 97	2128 ± 123**
Palisade parenchyma I	12044 ± 312	14626 ± 807**	13883 ± 1078	14735 ± 516
Palisade parenchyma II	5330 ± 185	6215 ± 255**	5304 ± 229	5875 ± 285*
Spongy parenchyma	1268 ± 159	1642 ± 169***	1618 ± 254	1654 ± 82
Lower epidermis	2570 ± 237	2582 ± 204	2358 ± 293	2167 ± 45

TABLE 2. Density (No/mm^2) of stomata in leaves of two avocado cultivars grown under irrigation and drought stress (n=12, ± SD, *significantly different from the control at p< 0.05)

Leaf surface	cv. 'Hass'		cv. 'Fuerte'	
	Irrigated	Stressed	Irrigated	Stressed
Upper	–	–	–	–
Lower	1004 ± 199	1033 ± 149	909 ± 69	820 ± 68*

TABLE 3. Density (No/mm^2 of section) of idioblastic oil cells in the leaf mesophyll parenchyma of two avocando cultivars grown under irrigation and drought stress (leaf paradermal sections) (n=12, ± SD, *-** significantly different from the control at p< 0.05 and p<0.01, $^{(+)}$ significantly different from stressed 'Hass' at p< 0.05)

Mesophyll parenchyma	cv. 'Hass'		cv. 'Fuerte'	
	Irrigated	Stressed	Irrigated	Stressed
Palisade parenchyma I	30 ± 4	41 ± 4**	32 ± 5	34 ± 4$^{(+)}$
Palisade parenchyma II	72 ± 6	118 ± 13**	66 ± 8	101 ± 21*$^{(+)}$
Spongy parenchyma	21 ± 6	34 ± 5**	22 ± 4	37 ± 5**

lower leaf surface become less numerous (Table 2) with no changes in their distribution. With respect to the idioblastic oil cells, they also increase in density under drought stress, but not to the extent observed in 'Hass' (Table 3).

DISCUSSION

Plants grown under drought stress face serious loss of water through transpiration. To reduce transpiration, stressed leaves of avocado undergo a reduction of their total surface area which may reach 69% in 'Hass' and 57% in 'Fuerte' compared to controls (Chartzoulakis *et al*., 2002). Leaves also undergo anatomical alterations which principally comprise an increase of the epidermal and mesophyll cell density. Densely-arranged and small-sized epidermal cells have been considered to significantly resist against cell collapsing due to arid conditions (Oertli *et al.*, 1990). On the other hand, an increased density of the mesophyll cells reflects a reduction of the intercellular space volume which entails blocking of water vapours moved to stomata.

Plants respond to drought stress by closing stomata which results in a decline of transpiration, but also of photosynthesis. Control plants of 'Fuerte' appear anatomically to be more efficient in photosynthesis than those of 'Hass', as judged by the remarkably higher number of chlorenchyma cells per mesophyll section surface in both palisade parenchymas and in the spongy parenchyma, as well. Under drought stress, the rate of cell number rise becomes higher in 'Hass' than in 'Fuerte', so that finally both cultivars obtain approx. the same density of mesophyll cells. This suggests a better adaptation to drought stress of 'Hass' than of 'Fuerte' with respect to the photosynthetic process. The effect becomes more prominent considering that under drought stress the number of stomata (and consequently the amount of CO_2 entering the mesophyll) is significantly greater in 'Hass' than in 'Fuerte'.

Drought stress in avocado did not only affect the density and size of the epidermal cells and mesophyll chlorenchyma cells, but also the density (not the size) of the mesophyll oil cells. The idioblastic oil cells of avocado have been ultrastructurally and histochemically investigated by Platt-Aloia *et al.*, (1983) and Platt & Thomson (1992). It has been found that each oil cell is bordered by three distinct layers, i.e. an external cellulosic layer (primary wall), an intermediate suberin lamella and an internal tertiary wall. The oil is constituted of terpenes and alkaloids and it becomes accumulated in a large central vacuole. Analogous ultrastructural observations have been made by Amelunxen & Gronau (1969) and Maron & Fahn (1979) in the idioblastic oil cells of *Acorus calamus* L. and *Laurus nobilis* L., respectively. The remarkable increase in the number of leaf oil cells during drought stress in both avocado cultivars might be interpreted as a contribution to maintenance of leaf turgor and to blocking of internal water vapour removal by decreasing the volume of intercellular spaces.

The anatomical observations and the morphometric assessments conducted in this study on the avocado leaf reveal that in 'Hass' (compared to 'Fuerte') drought stress resulted in 1. higher increase of the number of idioblastic oil cells 2. lower decrease of the number of stomata 3. higher rate of increase of the number of epidermal cells and mesophyll chlorenchyma cells, and 4. higher increase of the mesophyll intercellular space volume. These results favour the suggestion that as concerns transpiration and photosynthesis, 'Hass' seems to respond to drought stress better than 'Fuerte'.

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