Silva et al. Revista Chilena de Historia Natural 2014, 87:13
http://www.revchilhistnat.com/content/87/1/13

Revista Chilena de Historia Natural
a SpringerOpen Journal

RESEARCH Open Access

Relationships between leaf anatomy, morphology, and water use efficiency in *Aloe vera* (L) Burm f. as a function of water availability

Herman Silva[1*], Susana Sagardia[1], Mauricio Ortiz[1], Nicolás Franck[1], Marcela Opazo[1], Madeleine Quiroz[1], Cecilia Baginsky[1] and Cristián Tapia[2*]

Abstract

The effects of water availability were evaluated on the photosynthetic tissue anatomy in *Aloe vera* (L) Burm f. and its relationship with morphological, physiological parameters, and water use efficiency as a function of aerial biomass and gel production. Plants were subjected to four levels of water availability equivalent to 20% (T1), 15% (T2), 10% (T3), and 5% (T4) of the atmospheric evaporative demand. The plants exhibited anatomical, morphological, and physiological responses to the different watering treatments. The extreme treatments produced negative responses due to excess water in T1 and water deficit in T4. Treatments T2 and T3 elicited positive responses in cell characteristics and productivity. Anatomical and structural characteristics were closely linked to physiology. Increased stomata number was negatively related to leaf length, width, and thickness ($r = -0.85$, -0.81, and -0.59, respectively) and to biomass production ($r = -0.84$), and positively related to the increase of cuticle thickness ($r = 0.78$). Treatment T2 showed the maximum efficiency of water use for biomass production (24.6 g L^{-1}), which was closely related to cell size ($r = 0.68$) and number of stomata ($r = -0.70$).

Keywords: Anatomical adaptations; Biomass production; Growth; Stomata

Background

Some plant species possess specific pathway of carbon fixation that allow them to survive and even grow under conditions of severe water stress. The best known are the crassulacean acid metabolism (CAM) plants, particularly the species of the genera *Opuntia, Agave*, and a liliaceous species, *Aloe vera*. Most of the anatomical and morphological studies of CAM plants have been descriptive and make little mention of the water relationships and gas exchange, except for the work of Nobel (1980), Herrera et al. (2000), Nelson et al. (2005), and Herrera (2009). Although CAM species exhibit great variability in structure, adaptation to stressful environments, and in CAM metabolism expression (Borland and Taybi 2004), they share characteristics that reflect enhanced drought tolerance (Nelson et al. 2005). These plants are characterized by the maintenance of a favorable water state through their ability to minimize transpiration by closing their stomata during the day and opening them at night when the vapor pressure deficit is low (Pierce et al. 2002; Caird et al. 2007).

Another common feature of CAM plants is succulence, characterized by cells with large vacuoles, called hydrenchyma. Succulence is more frequently found in xeric than in mesic CAM species (Borland et al. 2009), which further contributes to water use efficiency (Borland et al. 2009; Herrera et al. 2008). Compared to C3 and C4 species, CAM plants characteristically have lower stomatal density which is modified by plant water availability (Silva et al. 2001). In many cases, water restrictions reportedly reduce the number of stomata (Silva and Acevedo 1984; Silva et al. 1999, 2001). The frequency is regulated by asymmetric division of epidermal cells and cell-to-cell signals that determine where the epidermal cell should undergo a new asymmetric division (Bergman and Sack 2007; Nadeau 2009). Plant water status may also be a

* Correspondence: hsilva@uchile.cl; hsilva@uchile.cl
[1]Laboratorio Relación Suelo Agua Planta, Facultad de Ciencias Agronómicas, Universidad de Chile, Santa Rosa 11.315, La Pintana, Casilla 1004, Santiago, Chile
[2]Laboratorio de Operaciones Unitarias, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Sergio Livingstone 1007, Independencia, Santiago, Chile

signal that can modify gene expression to control the frequency at which epidermal cells undergo this asymmetric cell division. Guard cell size may also be modified by water restriction (Nadeau 2009).

The leaf of CAM species may be divided into two principal parts, the grey-green external area including from the cuticle to the vascular bundles on both the abaxial and adaxial surfaces, and a clear internal zone comprising cells with large vacuoles containing the gel or hydrenchyma.

The mesophyll has chloroplasts, making it a photosynthetically active tissue, and it can directly influence CO_2 capture due to its effects on diffusive resistance (Nobel 1999). The intercellular air space (IAS) (Nelson et al. 2005) is the proportion of photosynthetic tissue that acts as the effective surface for CO_2 diffusion, and it affects photosynthesis and water use efficiency (Evans et al. 1994; Warren 2008).

In plants, water loss can be reduced by increased wax deposition in the cuticle, a reduced number of stomata per unit area, the presence of trichomes, reduction in leaf size, and by the disposition of leaves with respect to the incident radiation (Nobel 1995). In CAM plants, water loss is reduced by the nocturnal CO_2 uptake with an efficient synthesis of sugars and osmolytes which allow water retention (Borland et al. 2009). However, the cuticle may also contribute to the energy balance due to the reduction of net radiation and increase of reflectance. Variations in the anatomy of gas exchange tissues such as the thickness of the mesophyll and cuticle, cell dimensions, and the relation between the cell wall surfaces of the chlorenchyma and the external surface (A^{mes}/A) all function as adaptations to hot and dry climates (Nobel et al. 1975; Nobel 1980; Silva et al. 2001). *A. vera* L. is a perennial liliaceous plant with succulent green leaves joined at the steam in a whorled pattern. It is highly appreciated due to its short growth period and high economic value among all the aloe species and is used in pharmaceutical, folk medicine, healthcare, cosmetic products, and food products (Reynolds and Dweek 1999). This plant is different from other CAM species because it is a native of the semitropical regions of South Africa (Cowling 1982). Thus, the behavior of such plants cultivated under a semiarid environment may be different from that of the native species. We postulate that water availability induces anatomical and morphological changes that help to maintain the water use efficiency in *A. vera*. Our objective was to determine the effect of the four levels of water availability on the anatomy of the photosynthetic tissue of *A. vera* and its relation with water use efficiency.

Methods

Materials and study site

The trial was performed during the 2006 to 2007 season in the Campo Experimental Las Cardas, located in the community of Coquimbo, Region IV of Chile, at about 31° south latitude. The area has an arid Mediterranean climate, with a water deficit of 94% per year and a dry season of 10 months. The mean maximum temperature of the warmest month is 29°C; no freezes occur. The soil is Tambillo Series; the site has a slight inclination (1% to 2%) with micro-relief and abundant pebbles on the surface and in the soil (50%). The soil texture in profile is sandy loam. The N level is low, while the levels of P and K are normal for this type of soil (Aburto et al. 2008; INIA 2010).

Field experiment and experimental design

In May 2006, approximately 3-year-old plants were established at a density of 6,666 plants ha^{-1}. The border of the plantation was 1×1.5 m, giving a total area of 720 m^2.

The experimental design was completely randomized blocks with four block irrigation treatments randomized with four replications. The experimental unity was a plot of 45 m^2, with five rows and six plants per row.

Irrigation treatments

Based on previous information regarding the atmospheric evaporative demand at the experimental site, the available metabolic information about the species (Genet and Van Schooten 1992), and the water retaining characteristics of the soil, we designed four irrigation treatments representing 20% (T1), 15% (T2), 10% (T3), and 5% (T4) of the reference evapotranspiration (ET_o). An irrigation system was installed with independent electrical valves for each water treatment, along with a volumetric water meter to quantify each applied amount. Drip irrigation was used, with a double drip line for each row of plants. For treatments T1, T2, T3, and T4, respectively, an emission flow of 4 L h^{-1} was applied for 60, 45, 30, and 15 min to deliver 8, 6, 4, and 2 L h^{-1}. Irrigation was performed every 7 days during of greatest atmospheric evaporative demand (no precipitation) from October to May.

Histological studies

At the end of the experimental period, histological studies of photosynthetic tissue were performed, measuring both abaxial and adaxial tissues. Histological studies of photosynthetic tissue were performed at the end of the experimental period, measuring both abaxial and adaxial tissues. Samples from eight individuals per each treatment were taken with a 0.5-cm-diameter punch, sampling both sides of the central part of the leaf simultaneously. After elimination of the central (gel-containing) portion separating the surfaces, four sections per treatment were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for scanning electronic micrographs, and four sections per

treatment were fixed in 3% 0.1 M glutaraldehyde buffer phosphate at pH 4.3 and 1% OSO_4 for transmission electronic micrographs.

Histological slices (9 μm) were made by hand using a razor blade or using a freezing microtome ($n = 20$ per treatment). Slices were fixed in epoxy resin or paraffin, stained with toluidine blue, and used to analyze transverse and tangential sections. Slices were mounted on slides, and measurements were made using a light microscope with an ocular micrometer (Lam Research Corporation, Fremont, CA, USA; Bosch Company, Stuttgart, Germany). Tangential sections were used to measure the density ($N° \ mm^{-2}$) and dimensions (μm) of the occlusive cells. Transverse sections were used to measure the dimensions of epidermal cells, length and width of chlorenchyma cells, thickness of the mesophyll (between the limits of the epidermis and the beginning of the hydrenchyma) on both leaf surfaces, thicknesses of the cuticle and epidermis, and the distance between vascular bundles (all in μm). A succulence index was estimated as the fresh leaf weight divided by total area, expressed in grams per square centimeter. Using digitalized images, the proportion of IAS was measured in the photosynthetic tissue on both sides of the leaf as follows: Proportion of intercellular space = Intercellular area/Mesophyll area, expressed as a percentage of the photosynthetic tissue (Nelson et al. 2005).

Growth, photosynthetic area, aerial biomass production, and gel

At the end of the experimental period, we measured the length, width, and thickness of adult leaves and calculated the leaf volume according to the method of Rodríguez-García et al. (2002). In 10 leaves per treatment, photosynthetic area was measured, using a Li-6200 measurer (LI-COR Biosciences - Biotechnology, Lincoln, NE, USA) and multiplied by 2 to account for both leaf surfaces. We also measured total fresh weight, sectioned the leaves to separate, and measured the fresh weight of the gel and photosynthetic tissue.

Water use efficiency

According to Silva et al. (2010), five leaf harvests were conducted during the experiment (in February, June, July, September, and December 2007), with 10 leaves selected per treatment (one per plant) from the peripheral part of the plants. The leaves were collected by cutting the plant with a basal area of insertion, without destroying the tissue, and the collected leaves were placed in a cooler. The total fresh weight was measured. Then, the leaves were filleted to remove the gel from the photosynthetic tissue, and the samples were weighed separately on aluminum trays. Samples were then dried in an oven at 70°C to until reaching a constant weight. The photosynthetic tissue was the material remaining after gel extraction. The water use efficiency (WUE) was calculated for each harvest as the quotient of leaf biomass production or gel production and the total water applied per treatment, expressed in grams per liter. WUE was also calculated based on dry weight.

Statistical analysis

Analyses of variance (ANOVA) were performed for histological variables, after checking normality and variance homoscedasticity assumptions, with the InfoStat software (Di Rienzo et al. 2013). When significant differences among treatment means were found, these were compared with Tukey's test ($P \leq 0.05$). To explore the association between the histological observations, growth, photosynthetic area, aerial biomass and gel production, and water use efficiency variables, and their behavior in the different water treatments, a principal components analysis (PCA) was performed with the InfoStat software (Di Rienzo et al. 2013).

Results

The transversal section of an *A. vera* leaf showed well-defined limits, enabling the separation of the hydrenchyma in the median zone from the mesophyll associated with the abaxial and adaxial epidermis. The succession of tissues to the abaxial surface included an epidermis with a thick external cell wall covered by a layer of cuticle, followed by two cell layers that could be called an exodermis (Figure 1a,b) except that they contained chloroplasts and thus were considered an assimilating tissue or chlorenchyma. There was a gradual increase of the cell size, which reached a maximum in the median zone of the mesophyll and then diminished towards the interior, forming about four compact cell layers. These layers ended in the vascular bundles, which represented the limit separating the mesophyll from the hydrenchyma or gel (Figure 1a,b). Tukey's test indicated variability in all observed parameters among treatments and minor variability between abaxial and adaxial surfaces except for stomatal density and cuticle thickness (Table 1; Figure 1c,d).

Cuticle, epidermis, and stomata

Both leaf surfaces were grey-green in color, glabrous, and without trichomes. The cuticle layer thickness did not allow visualization of either the epidermic cells or the stomata (Figure 2c,d); this was achieved with successive tangential cuts that eliminated the cuticle and exposed the spatial relation of the occlusive cells with the subsidiary and epidermal cells (Figure 2e,f). Both leaf surfaces had a thick cuticle layer that increased the water loss resistance of the leaves and stomata (Figure 1c,d). Table 1 shows the cuticle thickness with slightly greater mean values for the abaxial epidermis. T4 plants had a greater cuticle thickness, with a mean of 18.7 μm compared to

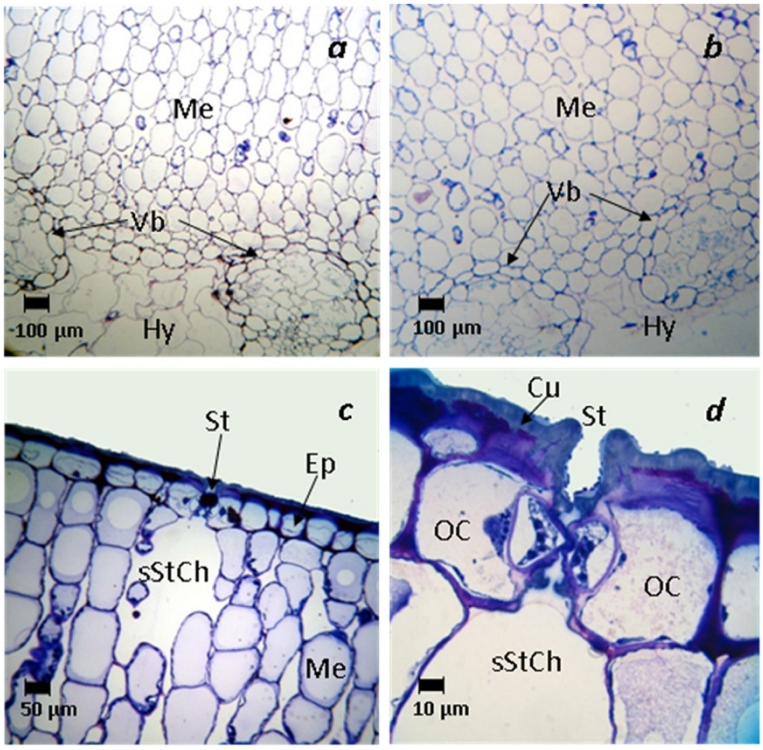

Figure 1 Optical microscopy of a cross section of *Aloe vera*. Mesophyll cells, vascular bundles, and hydrenchyma in **(a)** T1 and **(b)** T2 plants (×280). **(c)** The epidermis, a stomatal complex, the substomatal chamber, and mesophyll cells (×1,120). **(d)** The cuticle and the stomatal complex (×1,500).

13.24 μm in T1 (Table 1). This suggests that the plants reacted to the greatest water stress (5% of the atmospheric evaporative demand) by forming a thicker cuticle layer, which contributed to their survival. The cuticle layer was interrupted only at the level of stomata, and cuticular extension was observed even at the bottom of the guard cells (Figure 1c,d). The *A. vera* plants were amphistomatic (Table 1), having stomata in both the abaxial and adaxial epidermis, which is a characteristic of CAM plants.

In both abaxial and adaxial epidermis, T4 plants had the largest number of stomata per unit area: 30.8 ± 1.04 mm^{-2}, which was 42% greater than the 20.8 ± 0.44

Table 1 Average values of cuticular thickness, stomatal density, and sizes of occlusive cells and ostioles by treatment in abaxial and adaxial leaf surfaces of *A. vera* ($n = 20$)

Treatments/ surface	Cuticle thickness (μm)	Stomatal density (Nº mm^{-2})	Stomata			
			Occlusive cells		Ostioles	
			Length (μm)	Width (μm)	Length (μm)	Width (μm)
Abaxial						
T1	13.34C	21.00B	52.18AB	52.05B	39.58A	6.26A
T2	16.02B	21.10B	56.64AB	54.89A	39.55A	5.21A
T3	16.92AB	22.85B	52.77AB	51.28B	32.57B	5.38A
T4	18.70A	30.80A	55.29A	49.59B	36.93A	3.02B
Adaxial						
T1	11.74C	20.60C	57.26A	5178A	40.73B	4.61B
T2	11.76B	23.35C	57.62A	50.23B	42.02A	5.45A
T3	14.38AB	27.12B	55.89AB	48.20AB	42.06A	3.97B
T4	15.44A	30.80A	54.48B	48.18B	40.91A	2.16C
Average	*14.79*	*25.05*	*55.05*	*50.99*	*38.90*	*4.49*

Values with different letter designations in the same column and surface are significantly different ($P \leq 0.05$).

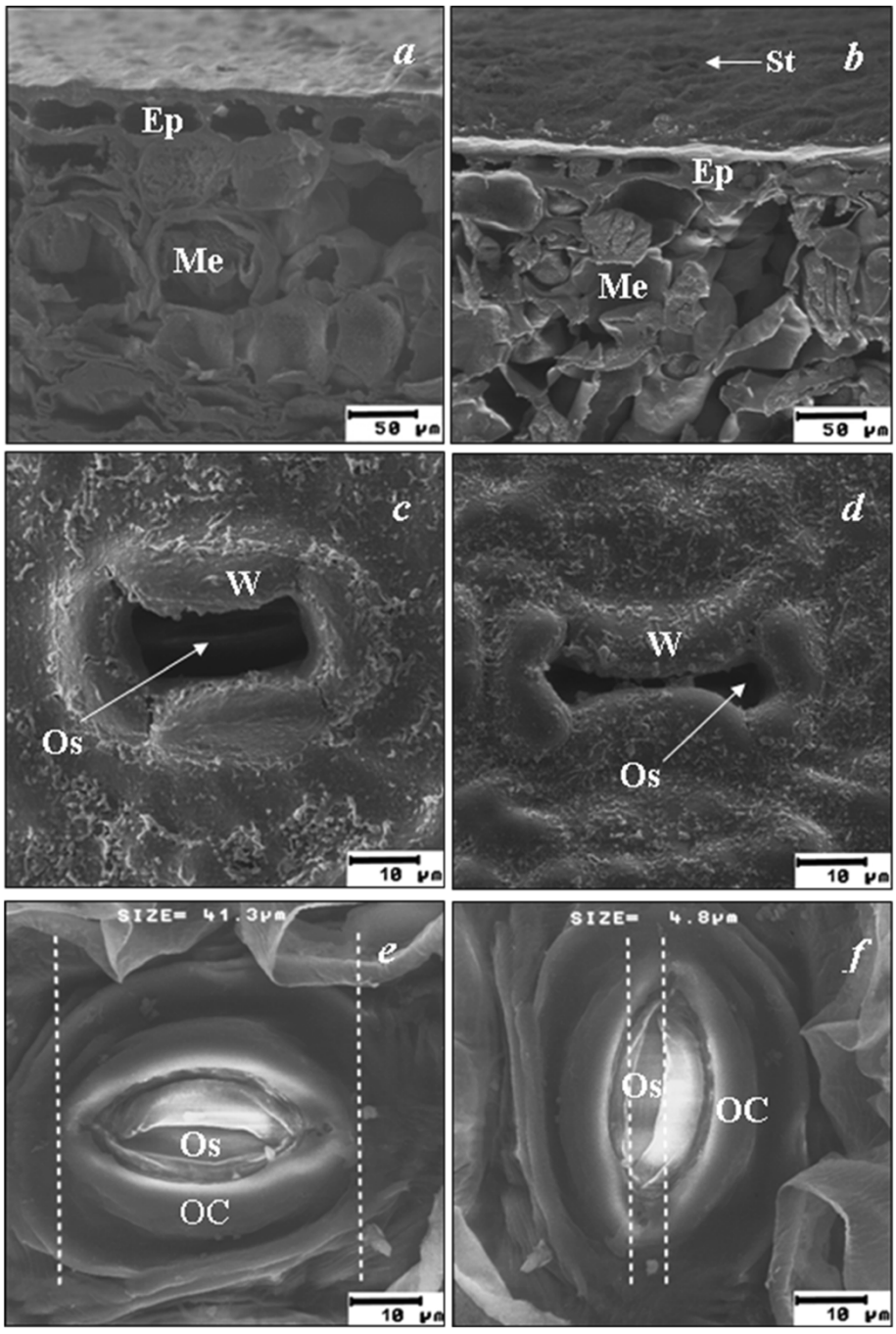

Figure 2 Scanning electron micrographs of leaves. Cross and tangential sections of the abaxial surface of leaves from **(a)** T2 and **(b)** T4 plants, with arrows indicating a stoma on the leaf surface (×300). The tangential section also shows the mesophyll tissue (Me) and epidermis-cuticle (Ep). Images of the stomata of leaves of **(c)** T2 and **(d)** T4 plants, showing the difference in the amount of epidermal wax surrounding the stomata of the two plants; arrows indicate the ostioles (OS) of the stomata (×1,500). **(e, f)** Close-up images of stomata, showing guard cells (GC) and ostioles (Os).

stomata mm^{-2} observed in T1 plants. There was a strong inverse correlation between density and water availability ($r = -0.87$). These findings may be explained by the smaller size of the epidermal cells in T4 plants, which resulted in a greater number of stomata per unit area. However, the occlusive cells increased in length and decreased in width; therefore, there was no correlation between the decrease of epidermal cell sizes and occlusive cell sizes. The mean distance between stomata was 110 ± 11.7 µm in T4 plants (data not shown) and

ranged from 130 to 154 μm in the other treatments. Therefore, the spatial distribution in T4 was 30% lower than the values for the other treatments.

The dimensions of the occlusive cells of the abaxial surface also differed among treatments; in T1 plants, these cells were 52.2 ± 1.3 by 52.1 ± 0.6 μm (an isodiametric shape), while the dimensions in T4 plants were 55.2 ± 0.72 by 49.6 ± 1.33 μm (rectangular). Similar values were found for the adaxial side (Table 1). The ostiole lengths were similar among the treatments, except for T3 abaxial side, whose mean 297 value was 32.6 μm. However, the ostiole width was significantly smaller in T4 compared to in other treatments. Ostiole width was 6.2 ± 0.25 μm in T1 and 3.2 ± 0.22 μm in T4, showing a 50% decrease and representing about 8% of the total stoma width, which seems to indicate that the stomata were nearly closed or closed in T4.

Mesophyll, cell dimensions, and intercellular spaces

The two leaf surfaces were analyzed separately for variations in the mesophyll thickness. From T2 to T4, increasing water deficit decreased the mesophyll thickness in both abaxial and adaxial surfaces, with the abaxial mesophyll being 28% less in T3 than T2 and 33% less in T4 than T2 (Tables 2 and 3). However, greater water availability did not assure larger mesophyll cell dimensions, as this thickness increased from T1 to T2 (significantly for the adaxial surface; $P \leq 0.05$) (Table 2). The maximum thickness was 1,827 μm observed in the adaxial surface of T2 plants, compared to 1,327 μm in T4 plants; a similar effect was found in the abaxial surface. The total mesophyll thickness at the leaf level was 3.4 in T2 and 2.9 mm in T4 (Table 3). All of these results are in agreement with WUE, as recently reported by Silva et al. (2010).

The IAS was significantly smaller for T4 plants, with the abaxial surface reduced by 50% and the adaxial surface reduced by about 30% compared to in T1 and T2. Table 2 presents values based on measurements of the mesophyll in the area with the largest cells, which are probably the cells with the greatest photosynthetic activity. These cell lengths varied from 111.2 to 163 μm and the widths from 65 to 76 μm. There were significant differences among treatments (Table 2); T2 plant cells were 34% and 43% larger than the cells of treatments T3 and T4, respectively. The adaxial surface mesophyll thickness was 100 μm greater in T2 plants compared to T1 due to the larger size of the photosynthetic cells. The smaller cell size in T4 plants was also responsible for the smaller intercellular spaces at the leaf level compared to in the other treatment groups (Table 2). The hydrenchyma thickness was also variable; T2 plants had a mean thickness of 18.14 mm, 32% greater than in T4 plants (12.38 mm). These treatment-related differences in *A. vera* were statistically significant ($P \leq 0.05$) (Table 3).

The vascular bundles were observed to be similar to those in C4 plants, showing a cluster of cells surrounding the xylem and phloem, with excess mechanical tissue (probably collenchyma). The outer cells formed a bundle of sheath cells, similar to those that formed the hydrenchyma (Figure 1a,b). The greatest distance between vascular bundles was observed in the most irrigated plants, with a significantly higher number of vascular bundles per unit area in the less irrigated plants. Figure 1a,b shows the effects of treatment on the number of vascular bundles, with the number of cells separating the beams ranging from 2 to 3 cells in T4 plants to 8 to 9 cells in T2 plants. The vascular bundles and the cells between vascular bundles (similar to the exodermis)

Table 2 Epidermis and mesophyll thickness, photosynthetic cell size, and percent intercellular space by treatment in abaxial and adaxial leaf surfaces of *A. vera* ($n = 20$)

Treatment/ surface	Thickness		Photosynthetic cells		Intercellular spaces (%)
	Epidermis (μm)	Mesophyll (μm)	Length (μm)	Width (μm)	
Abaxial					
T1	39.79B	1,685.00A	145.47A	76.31A	12.02A
T2	42.98B	1,737.20A	152.52A	72.93A	12.40A
T3	48.76A	1,347.10B	122.77B	74.62A	11.40AB
T4	39.93b	1,298.80c	131.22B	65.20B	8.01C
Adaxial					
T1	40.96B	1,707.70B	154.45A	69.18AB	14.35AB
T2	43.36AB	1,827.20A	163.65A	68.54B	14.80A
T3	41.87AB	1,372.50C	121.20A	66.19B	13.50B
T4	46.25A	1,329.60C	111.71B	75.91A	11.40C
Average	*42.99*	*1,563.14*	*140.37*	*71.11*	*12.23*

Values with different letter designations in the same column and surface are significantly different ($P \leq 0.05$).

Table 3 Average values of leaf thickness, mesophyll thickness, hydrenchyma width, and percent intercellular spaces by treatment ($n = 30$)

Treatments	Leaf thickness (mm)	Mesophyll thickness (mm)	Hydrenchyma width (mm)	Intercellular spaces (%)
T1	17.1A	3.390B	13.71B	13.2A
T2	21.7B	3.560A	18.14A	13.6A
T3	18.8B	2.710C	16.09A	12.4A
T4	15.2C	2.820C	12.38B	9.70B

Values with different letter designations in the same column are significantly different ($P \leq 0.05$).

represent the boundary between the mesophyll and hydrenchyma (Figure 1a,b).

Water use efficiency

T2 plants had the greatest production of total green biomass, followed by the plants of T3, T1, and T4. The slope of the biomass produced per quantity of water applied corresponded to WUE; thus, the greatest WUE values were those of the T2 and T3 plants (24.5 and 15.6 g L^{-1}, respectively) while the lowest values were those of the T4 and T1 plants (10.9 and 10.8 g L^{-1}, respectively). Similar results were found regarding gel production. As expected, lower water availability negatively influenced the WUE in T4 plants due to deficit. In contrast, water supply equalling 20% of the evaporative atmospheric demand negatively influenced the WUE value due to water excess. Watering to produce 15% of ET_o proved to be the minimum quantity of water required to obtain the maximum aerial biomass production. Similar to the above results, the linear relation between the gel produced and the water utilized during the experimental period varied as a function of water availability; the slopes represent the WUE for gel production per unit quantity of water applied by plant. The least efficient treatments were the extremes, T1 and T4. The maximum gel production was observed in the T2 plants, followed by T3 plants (17.7 and 13.1 g L^{-1}, respectively); the differences were not significant. The amounts of water applied in these cases were close to the minimum to achieve maximum gel production in these edaphoclimatic conditions (Silva et al. 2010).

Principal component analysis (Figure 3) showed the effect of water treatments on the photosynthetic tissue anatomy of the abaxial surface and its relationship with morphological and physiological parameters, which explained 86% of the total variation. Of the 25 responses, 22 were positively and only 3 negatively associated with water treatments. The analysis determined that eight anatomical parameters were associated with T2, twelve with T1, one with T3, and two with T4. The most important variables for T2 plants were mesophyll thickness (MT), length of photosynthetic cells (FCL), ostiole length (OL), succulence index (Suc index), and total biomass (Tbiomass); all with values of $r > 0.70$ (vector length Figure 3); the least important were basal leaf thickness (BT leaf) and epidermis thickness (EpT). The variables with greatest correlation for T1 and T3 were leaf basal width (BWleaf), stoma width (stmW), photosynthetic cell width (FCW), and ostiole width (OW). The effect of the low water availability in T4 was positively and significantly associated with stoma number (stmN°, $r = 0.86$), the stoma length, (StmL, $r = 0.75$), and cuticle thickness (CT, $r = 0.82$), and negatively associated with parameters of growth and productivity (Figure 3).

Discussion

Our results show that *A. vera* showed a great variability in the evaluated morphological and physiological traits in response to different irrigation levels. By considering the entire system, our results enable the establishment of relationships among drought tolerance, yield, and water use efficiency. We found that morphological and anatomical changes observed in *A. vera* plants contributed to increasing WUE. CAM plants generally exhibit improved survival under low water conditions due to increased WUE and succulence, which delay dehydration; these structural characteristics make them more adaptive to drought and, at the same time, able to produce a high yield in biomass, grains, or fruits of good quality. Drought tolerance appears to be an important characteristic of *A. vera*. The low-water treatments in this study triggered the development of the cell wall cuticle layer, which grew thicker with less water availability. Plants typically react to extreme water stress by favoring photosynthetic tissue maintenance and producing less gel to accumulate water (Silva et al. 2010). In our study, the T4 plants (irrigated with 5% of the 395 ET_o) only showed apical necrosis of the leaves.

Biomass decreased significantly under water stress ($P \leq 0.05$), tending to coincide with increased WUE. In five evaluations during the experimental period, Silva et al. (2010) reported a seasonal relationship between leaf biomass and gel produced and the amount of water used for production; their data indicated that total aerial biomass production is a function of water availability, with more water producing more aerial biomass. In each case, there was a linear relationship between the variables, but the slopes differed. In the present study,

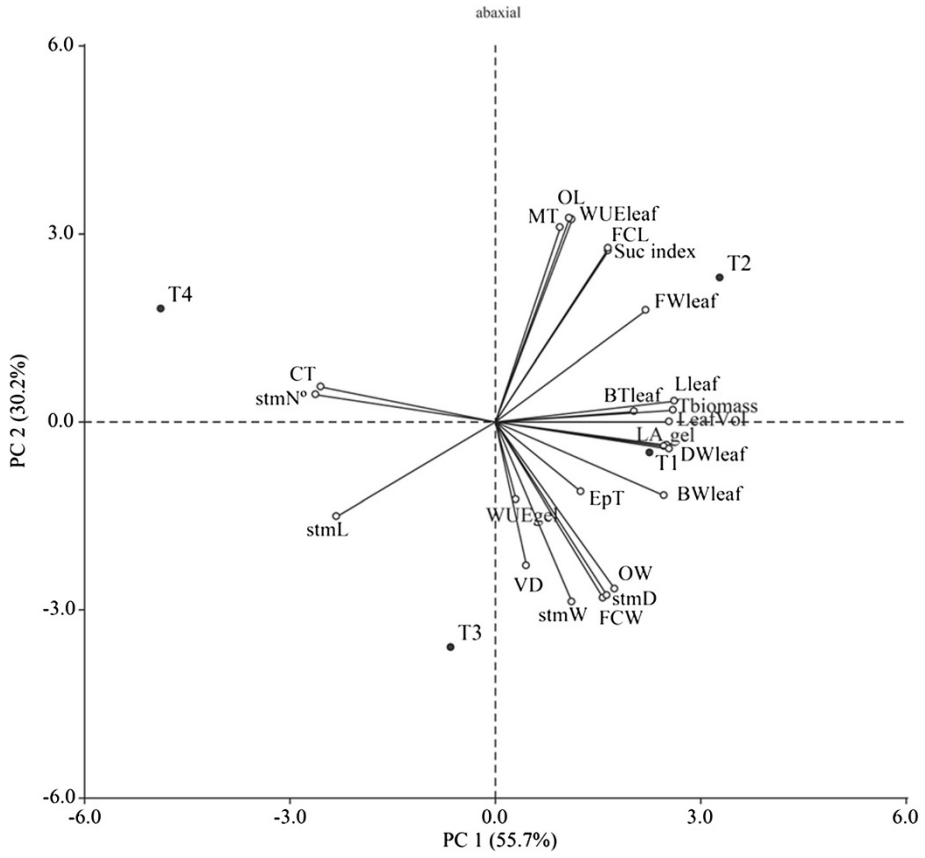

Figure 3 Principal component analysis showing the relationships between anatomy, morphology, and WUE in *A. vera*. T1 to T4, treatments; CT, cuticle thickness; stmNº, stomata number; stmL, stomata length; stmD, distance between stomata; stmW, stomata width; OW, ostiole width; OL, ostiole length; EpT, epidermis thickness; MT, mesophyll thickness; VD, distance between vascular bundles; FCW, photosynthetic cells width; FCL, photosynthetic cells length; BWleaf, leaf basal width; BTleaf, leaf basal thickness; LeafVol, leaf volume; LA, leaf area; FW, leaf fresh weight; Suc index, succulence index; DWleaf, leaf dry weight; Tbiomass, total biomass; WUE, water use efficiency.

reduced growth was observed at both extremes of the range of water treatments at T1 due to excess water and at T4 due to a lack of water which is typical of CAM plants (Silva and Acevedo 1995, Silva et al. 2010). The T2 plants had the maximum green biomass yield, corresponding with the observed larger cell size of the photosynthetic tissue in these plants. This maximum green biomass yield is also in agreement with the IAS since the intercellular spaces were larger in T2 plants than in T3 and T4 plants. This finding indicates greater CO_2 uptake in the T2 plants, favoring a higher WUE. A high WUE is often associated with a lower growth rate and yield; however, our results show that in *A. vera*, a high WUE can be associated with high green biomass productivity (Silva et al. 2010).

The stomata of *A. vera* were parallelocytic and polycyclic which is characteristic of monocot plants, such as barley (Zeiger 1972) and onion (Zeiger and Cardemil 1973). The ostiole was surrounded by two occlusive cells that were, in turn, surrounded by three layers of subsidiary cells. The stomata were at the same level as the epidermal cells. The main variation found to be associated with water deficit was that the cuticular layer, which covered the cell walls homogeneously, reaching the border of the ostiole opening. The occlusive cell length in the abaxial epidermis also changed with water treatment, increasing slightly but significantly from 52.77 μm in T1 to 55.29 μm in T4. These cells also exhibited a width reduction, suggesting that the stomata were almost closed or totally closed in T4 compared to T1. However, our group recently demonstrated that *A. vera* plants subjected to 3 months of severe water deficit still open the stomata at night because the sap of these plants flows at a rate that is 10% of the flow rate of plants under normal irrigation (Delatorre-Herrera et al. 2010). The sap flow rate directly depends on the stomatal opening (Medrano et al. 2007). The comparison of the evaluated parameters showed few differences between the two leaf surfaces, except for the occlusive cell lengths and widths; these were slightly longer and narrower on the adaxial surface and shorter and wider on the abaxial surface, indicating higher stomata resistance.

This difference probably occurs because the plants receive more solar radiation on the adaxial than the abaxial surface.

The number of stomata per unit area is a genetic characteristic, with differences associated with different metabolic pathways. CAM plants generally have a lower stomata density compared to C3 and C4 plants (Hernández et al. 2007; Silva and Acevedo 1984). In *A. vera*, several cellular mechanisms may account for changes in stomata density, including altered expansion of pavement cells, changes in the number of entry and amplifying divisions in the stomatal lineage, and the arrest or dedifferentiation of meristemoids (Bergman and Sack 2007). The partial pressure of CO_2 also reportedly regulates stoma density at the gene level (Heterington and Woodward 2003; Nadeau 2009). The present study showed that water availability was another environmental factor that regulated stoma density, which may be explained by the spatial relationships between stomata, subsidiary, and epidermal cells when cell size was reduced. Stomatal and epidermal cells are produced by a series of asymmetric divisions and cell transitions within cell lineages, with specific genes regulating the initiation of new stomata by asymmetric divisions, controlling the spacing between the asymmetric divisions, and regulating the initiation of new lineages (Nadeau 2009). In *A. vera*, water restriction probably activates the genes that control the frequency of asymmetric divisions, producing more stomata per unit area in T4 plants.

The anatomical pattern observed in *A. vera* showing tight cell packing, cell size, mesophyll thickness, stoma number, cuticle thickness, and reduced IAS to all be related to low water availability may improve water accumulation and/or water conservation (Nelson et al. 2005). Many species maximize light interception by having a high IAS ratio. However, *A. vera* possesses a low IAS, presumably because of its requirements for water economy. A low IAS ratio decreases transpirational water loss and increases water storage capacity and water use efficiency. Additionally, CAM plants have very efficient CO_2 uptake due to the C4 metabolism occurring in the same mesophyll cell, with temporal separation between the C4- and C3-CO_2 assimilation, and underpinning the WUE because of the temporal difference in activity between PEP carboxylase (night) and Rubisco (day) (Borland et al. 2009; Evans and Loreto 2000). Our results indicate that under severe water stress (T4), *A. vera* plants decreased leaf production and total growth, directly affecting biomass production (Silva et al. 2010). The strategy that *A. vera* adopts under water stress appears to involve channeling CO_2 assimilates to osmolyte synthesis and partially replacing acemannan with other polysaccharides that hold water more efficiently and better protect the plant against water stress (Delatorre et al. 2010).

Leaf length, width, and thickness were negatively associated with increased numbers of stomata; thus, these dimensions were greatest in plants that received more water (T1 and T2) and had the least number of stomata. Similar stoma number decreases have also been reported in other CAM plants, such as *Opuntia* sp. (Silva et al. 2001) and in the C3 plant *Phaseolus vulgaris* (Silva et al. 1999) probably due to decreased size of the pavement cells of the epidermis. Our allometric analysis showed a correlation between greater 470 cell size in T1 and T2 plants and greater water availability. Finally, it is important to note that cuticle thickness which protected the T3 and T4 plants against water loss that can induce plant death was negatively related to growth parameters. Overall, *A. vera* shows great plasticity to adapt to a water deficit, with close relationships between anatomical and growth parameters and physiological responses for survival under water restrictions.

Conclusions

This study of water relationships in *A. vera* showed that responses in growth and physiological/productive characteristics are highly dependent on the species anatomy. The key factor at the cell level is the stomata, the structure and physiology of which determine the crop viability. The strongest associations were found between stomatal characteristics and morpho-physiological responses. For a density of 6,666 plants ha^{-1}, the highest yield of biomass and gel was obtained by applying 15% of ET_o, suggesting that the crop coefficient (KC) would be 0.15. Water use efficiency is an indicator related to plant growth and is a function of the amount of water used to produce biomass. Knowledge of the biology of the species, the water retention characteristics of the soil, and the atmospheric evaporative demand of the experimental (or cultivation) site enabled demonstration of the close relationship between water use efficiency, anatomical parameters, and tolerance to water deficit.

Acknowledgements

This work was funded by the Vice-Rectoría de Investigación y Desarrollo (VID), Universidad de Chile (MULT 05/30-2).

Received: 12 July 2013 Accepted: 23 June 2014
Published online: 24 July 2014

References

Aburto F, Hernández C, Pfeiffer M, Casanova M, Luzio W (2008) Soil: a work of art of nature. northern field guide between 30 S° and 33 S°. In: Casanova M, Luzio W (eds) The international conference and field workshop on soil classification. Universidad de Chile, Santiago, Chile, Ovalle, Chile, p 94

Bergman DC, Sack FD (2007) Stomatal development. Annual Review Plant Biology 58:163–181

Borland A, Taybi T (2004) Synchronization of metabolic processes in plants with crassulacean acid metabolism. J Exp Bot 55:1255–1265

Borland AM, Griffith H, Hartwell H, Smith AC (2009) Exploiting the potential plants with crassulacean acid metabolism for bioenergy production on marginal land. J Exp Bot 10:2879–2896

Caird MA, Richards JH, Donovan LA (2007) Nighttime stomatal conductance and transpiration in C3 and C4 plants. Plant Physiol 143:4–10
Cowling (1982) Patterns of plant endemism in the south east Cape. The Naturalist 27:17–36
Delatorre-Herrera J, Silva H, Delfino I, Cardemil L (2010) Irrigation restriction effects on water use efficiency and osmotic adjustment in Aloe Vera plants (*Aloe barbadensis* Miller). Agric Water Manag 97:1564–1570
Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2013) InfoStat versión 2013. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina, URL http://www.infostat.com.ar
Evans JR, Loreto F (2000) Acquisition and diffusion of CO_2 in higher plants leaves. In: Leegood RC, Sharkey TD, Von Caemmerer S (eds) Photosynthesis: physiology and metabolism. Kluwer Academic, Dordrecht, pp 321–351
Evans JR, Von Caemmerer S, Setchell BA, Hudson GS (1994) The relationships between CO_2 transfer conductance and leaf anatomy in transgenic tobacco with a reduced content of Rubisco. Aust J Plant Physiol 21:475–495
Genet WBM, Van Schooten CAM (1992) Water requirement of Aloe vera in a dry Caribbean climate. Irrig Sci 13:81–85
Hernández M, Terrazas T, Delgado-Alvarado A, Cavazos ML (2007) Los estomas de Myrtillocactusgeometrizans (Mart.ex.Pfeiff.) Console (Cactaceae): Variación en su área de distribución. Rev Fitotec Mex 30:235–240
Herrera A (2009) Crassulacean acid metabolism and fitness under water deficits stress: if not for carbon gain, what is facultative CAM good for? Ann Bot 103:645–653
Herrera A, Fernández MD, Taisma MA (2000) Effects of drought on CAM water relations in plants of Peperomia carnevalii. Ann Bot 86:511–517
Heterington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. Nature 424:901–908
INFOSTAT (2009) InfoStat software version 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina
INIA (2010) Laboratorio de suelos. Interpretación de análisis químicos de suelos. Disponible en: http://www.inia.cl/link.cgi/suelo/resultados/7706
Medrano H, Galmes J, Ribas-Carbo M, Gulias J, Bota J, Pon A, Moreno M, Cifre J, Flexas J (2007) Photosynthesis and water use efficiency: improving plant productivity in semi-arid environments. Photosynth Res 91:277–277
Nadeau JA (2009) Stomatal development: new signals and fate determinants. Curr Opin Plant Biol 12:29–35
Nelson EA, Sage TL, Sage RF (2005) Functional leaf anatomy of plants with crassulacean acid metabolism. Funct Plant Biol 32:409–419
Nobel PS (1980) Leaf anatomy and water use efficiency. In: Turner NC, Kramer PJ (eds) Adaptation of plant to water and high temperature stress. Wiley, New York, pp 43–55
Nobel PS (1995) Environmental biology. In: Barbera G, Inglese P, Pimienta-Barrios E, Arias-Jiménez EJ (eds) Agroecology, cultivation and uses of cactus. Pear. Plant production and protection paper. FAO, Rome, Italy, pp 36–57
Nobel PS (1999) Physicochemical and environmental plant physiology. Academic, New York, p 608
Nobel PS, Zaragoza LJ, Smith WK (1975) Relation between mesophyll surface area, photosynthesis rate and illumination level during development for leaves of Plectanthrus parviflorus H. Plant Physiol 55:2067–2070
Pierce S, Winter K, Grifftihs H (2002) The role of CMA in high rainfall cloud forest: an in situ comparison of photosynthetic pathways in Bromeliaceae. Plant Cell and Environment 25:1181–1189
Reynolds T, Dweek AC (1999) Aloe vera leaf gel: a review update. J Ethnopharmacol 68:3–7
Rodríguez-García R, Jasso De R, Angulo-Sánchez JL (2002) Comparison between the production of leaves, gel and juice in Aloe vera grown with plastic mulch or natural conditions. In: Annual meeting association for the advancement of industrial crops and new uses council, 15–17 October 2000. St. Louis, Missouri, p 35, Abstract
Silva H, Acevedo E (1984) Adaptaciones anátomo morfológicas al déficit hídrico en Atriplex repanda Phil. Rev Chil Hist Nat 57:69–78
Silva H, Acevedo E (1995) Eficiencia en el uso del agua en diez taxa de Opuntia introducidas en la región árida mediterránea de Chile. Rev Chil Hist Nat 68:271–283
Silva H, Martínez JP, Baginsky C, Pinto M (1999) Efecto del déficit hídrico en la anatomía foliar de seis cultivares de poroto (Phaseolus vulgaris L.). Rev Chil Hist Nat 72:219–235
Silva H, Acevedo E, Silva P (2001) Anatomía del tejido fotosintético de diez taxa de Opuntia establecidos en el secano árido mediterráneo de Chile. Rev Chil Hist Nat 74:341–351
Silva H, Sagardia S, Seguel O, Torres C, Franck N, Tapia C, Cardemil L (2010) Effect of water availability on growth and water use efficiency for biomass and gel production in aloe vera (Aloe barbadensis M.). Industrial Crop Production 31:20–27
Warren CR (2008) Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO_2. J Exp Bot 59:1475–1487
Zeiger E (1972) Direct measurement of stomatal cell production in barley. Planta (Berl) 108:359–362
Zeiger E, Cardemil L (1973) Cell kinetics, stomatal differentiation and diurnal rhythm in Allium cepa. Dev Biol 32:179–188

doi:10.1186/s40693-014-0013-3
Cite this article as: Silva *et al.*: **Relationships between leaf anatomy, morphology, and water use efficiency in *Aloe vera* (L) Burm f. as a function of water availability.** *Revista Chilena de Historia Natural* 2014 **87**:13.