Irrigation restriction effects on water use efficiency and osmotic adjustment in Aloe Vera plants (*Aloe barbadensis* Miller)

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ARTICLE INFO

ABSTRACT

Aloe barbadensis Miller, known as Aloe Vera, requires limited irrigation depending on the capacity of the soil to retain humidity, since it is a CAM species and thus naturally adapted to conditions of dryness and high temperatures. Therefore, we postulated that plants of Aloe Vera plants under conditions of water deficit should improve their water use efficiency (WUE) by performing osmotic adjustment (OA) with a temporal correlation between WUE and OA. The objective of the investigation was to determine the effect of water restriction on the WUE and OA of *A. barbadensis* under different water treatments. 18-month old Aloe Vera plants were cultivated in pots with a soil substrate that was a mixture of equal parts of sand and organic matter with 18% of FC and 9% of permanent wilting point. To determine the effects of the soil humidity on plant WUE and OA, four treatments were arranged in a complete random design with four repetitions; these were 100%, 75%, 50% and 25% of FC, which correspond to an evatranspiration of 11.4, 9.6, 4.0 and 1.7 L per plant, respectively. The water treatments were maintained by frequent irrigation. The following variables were determined: dry matter, leaf water potential, relative water content (RWC), amount of gel produced, sap flow, proline content, soluble and total sugars and oligo and polyfructans. Aloe Vera increased WUE with increasing water deficit; the sap flow rate decreased with water restrictions, and the plants performed osmotic adjustment by increasing the synthesis of proline, soluble and total sugars as well as the amounts of oligo and polyfructans, mainly polymers of β-$(2 \rightarrow 6)$ kestotriose, changing from the inulin type to the neofructan type. The plants most and less irrigated (100% and 25% of FC) were the groups with lowest WUE. The plants irrigated with 75% of FC presented the best WUE in terms of dry mass and amount of gel produced by a litre of supplied water.

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1. Introduction

CAM (crassulacean acid metabolism) plants such as *Opuntia* spp. and Aloe Vera (*Aloe barbadensis* Miller) are known as "succulent" because they accumulate water in their leaves and stems. They are also characterized by nocturnal CO_2 assimilation and nocturnal stomatal aperture; the stomata are kept closed during the day to avoid water loss. Due to these physiological characteristics, these plants increase water use efficiency (WUE) (Winter et al., 2005) respect to C3 and C4 plants. Geerts and Raes (2009) indicate that the WUE is the relation between produced biomass and evapotranspired water or used water by the plant (ETa). The high WUE of CAM plants is related to the reduced difference of vapour concentration between the plant and the atmosphere during the period of maximum stomata opening; the water vapour content in the leaves and in the stems is around 1% of the value of air saturation (Nobel and Zhang, 1997; Nobel and Zutta, 2007).

CAM plants have other adaptations that aid in water conservation, such as low stomatal density, generally 20–30 stomata per square millimetre, and the water accumulation characteristic due to biosynthesis of polysaccharides which can retain water. The roots tend to be superficial, to an average depth of 15 cm, providing a rapid response to occasional rains (Nobel, 1997). Also, under conditions of water stress CAM plants have the capacity to synthesize secondary metabolites such as sugars, glycinebetaine, proline, serine and fructans, all molecules known as osmolytes that allow these plants to perform osmotic adjustment (Joyce et al., 1992; Kerepesi and Galiba, 2000).

In the case of Aloe Vera, there is additionally synthesis of specific proteins that protect the plant in conditions of water deficit, such as the heat shock proteins HSP70 and HSP100 and ubiquitin (Huerta et al., 2008), and the enzyme superoxide dismutase (SOD) which

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protects the plant from the oxidative stress induced by the impairment of photosynthesis (Salinas et al., 2007; Ramírez et al., 2007, 2008). Furthermore, water-stressed plants synthesize other protector molecules such as fructans which mitigate the detrimental effects induced by water deficit. These polysaccharide molecules are synthesized in plants exposed to very dry and/or very cold environments, increasing the cellular osmotic pressure by rapidly releasing oligofructans from the polyfructans (Van Den Ende et al., 2004). Perhaps due to this protective role, fructans are not present in tropical or aquatic plants. Besides increasing the osmotic pressure, fructans also contribute to membrane stabilization, protecting the plants against freezing, by binding membrane lipids (Valluru and Van Den Ende, 2008; Del Viso et al., 2009; Lammens et al., 2009). The physiological roles of fructans have been reinforced by the demonstration that transgenic plants able to synthesize fructans after transgenesis became more tolerant to cold, drought and freezing (Kawakami et al., 2008; Li et al., 2007). Probably like other osmolyte molecules, fructans contribute to maintain a high WUE in CAM plants. In spite that a lot of research has been performed on *Opuntia* spp., *Agave* spp., *Euphorbia* spp., *Aloe* spp. and all xerophytes of the desert (Zotz and Hietz, 2001; Lutgge, 2004; Winter et al., 2008), no one has studied these molecules on CAM plants. Most of the research about fructans has been done on monocots as cereals, Asparagales and Liliales (Del Viso et al., 2009).

A. barbadensis Miller (Aloe Vera) is a CAM plant with commercial value for its medical, nutritional and cosmetic uses (Ni et al., 2004; Eshun and He, 2008). Aloe Vera requires limited irrigation depending on the humidity retention capacity of the soil. Although Aloe Vera is a CAM plant and these plants have been intensively studied with respect to their adaptations to arid environments (Nobel, 2006; Winter et al., 2005, 2008; Ceusters et al., 2009; Borland et al., 2009), we formulated several questions concerning the adaptation of the species to be cultivated in the Atacama Desert of Chile, which is one of the most arid regions of the world: What is the limit of water restriction for Aloe Vera plants without affecting WUE? What physiological changes of Aloe Vera plants are expressed under a severe water deficit? Are plants of Aloe Vera able to maintain water stored in the leaves under severe water deficit? We postulated that the plants of *A. barbadensis* will increase their WUE under water restrictions as a consequence of the maintenance of the water stored in the leaves, by performing an efficient osmotic adjustment. Thus the objectives of this investigation were to determine the effect of water restriction on WUE and osmolyte synthesis of Aloe Vera under different irrigation treatments and to assess the influence of water restriction on the osmolytes synthesized by the plants to perform osmotic adjustment: total sugars, oligo and polyfructans, proline and glycinebetaine.

2. Methodology

2.1. Experimental setup

18-month old Aloe Vera plants of the Canchones Experimental Station, Universidad Arturo Prat, were collected and transplanted to 7.600 cm^3 plastic pots with free drainage. The experiments were performed in a greenhouse with controlled environmental conditions; the average temperature was 25 °C and the relative humidity was 50%. The water treatments were continued for 5 months, including a pre-experimental period of adaptation of the plants and 3 months of water relationship determinations.

A mixture of sand with organic matter in equal proportions was the substrate for all groups of plants. The substrate had a FC of 18% and a permanent wilting point (PWP) of 9%. The FC and the PWP were determined by the pressure plate method (Manifold, UK). To determine the effect of soil humidity on WUE, four irrigation treatments were established in a completely randomized design with three repetitions for each treatment. The irrigation treatments were 100%, 75%, 50% and 25% of FC for 3 months. 100% FC corresponds to the total water available determined by the difference between FC and the PWP. Previous to the experimental period, plants were acclimated for a period of 2 months to the environmental conditions and irrigated with 100% FC.

2.2. Soil water content determinations

The soil water content was measured every day in each pot. Determinations were taken at 15-cm depth using the Thetra Probe Sensor of soil moisture (Delta-T-Devices, Model ML2x). This method was checked gravimetrically and converted into volumetric content by the following equation

$$\Theta_V = \Theta_G \times \frac{P_S}{P_W} \tag{1}$$

where Θ_G is the gravimetric water content; Θ_V is the volumetric soil moisture; P_w is the water density; P_s is the density of soil.

The adjustment curve between the water content determined by the gravimetric method (transformed to volumetric) with that measured by the Thetra Probe Sensor had a R^2 of 0.996, as represented by the equation

$$\Theta_V = 0.9481 \times \Theta_{V\,\text{Thetra probe}} \tag{2}$$

The available water of the pot was 2482 cm^3. The water was replaced in the pot each time the soil water content indicated a water loss of 10%. After water application, the pots were covered with aluminum foil to avoid evaporation. The percolated water was collected and measured.

2.3. Evapotranspiration determination (Eta)

This was determined by the water balance between the cumulative water supply and the percolated water. The difference between the accumulated water supply in and the water loss by percolation was the water transpired by the plant (Eta).

2.4. Water parameters measurements

All determinations were performed once a month during a period of the 3 months. The volume was determined in medium-sized leaves of similar length. The leaves were cut from the base and the volume was determined according to Eq. (3) (Veliz et al., 2007)

$$V = \left[\frac{L}{12}\right] \pi EA \tag{3}$$

where V is the leaf volume (cm^3); L is the length of the leaf (cm); $\pi = 3.1416$; E is the average thickness of the leaf (cm); A is the width of the leaf (cm).

Water potential was determined in medium-sized leaves with the use of a Schollander pressure chamber. For the measurement, the leaf was removed from the plant at the base and the bottom of the base was covered with a plastic lamina and introduced immediately in the Schollander's chamber. The apex of the leaf was cut and the water potential was determined according to Turner (1981). The volume of the extracted leaf was determined before the measurement.

Leaf relative water content (RWC) was determined using the methodology of Muy-Rangel et al. (2004). Fresh leaves of Aloe Vera were weighed and then placed in distilled water for 2 hours until the weight of the turgid leaf was constant. After this, the leaf was weighed again. Dry weight was determined by drying the leaf in a

Table 1
Effect of four different water treatments on the leaf osmotic potential (Ψ_s) of Aloe Vera plants. The leaf Ψ_s was determined in the plants 3 months of the water treatment. The figures are the average of three independent determinations. Different letters represent significant differences among water treatments (Tukey's test, $P < 0.05$). $n = 3$; $P < 0.05$.

Water treatments (% FC)	Leaf Ψ_w (MPa)	% of difference respect to control	Leaf Ψ_s (MPa)	% of difference respect to control
100	−0.21[a]	100	−0.67[a]	100
75	−0.27[b]	128	−0.75[ab]	112
50	−0.29[b]	138	−0.81[b]	121
25	−0.35[c]	166	−0.91[c]	136

stove at 65 °C for 48 h or until a constant weight was obtained. The RWC was determined by means of Eq. (4)

$$\text{RWC} = \left[\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \right] \times 100 \qquad (4)$$

where FW is the fresh weight; DW is the dry weight; TW is the turgid weight.

To measure the sap flow of the Aloe Vera plants, a Dinamax flowmeter equipped with thermocouples of 30 mm was used. The thermocouples were inserted from the border of the leaf towards its centre until the thermocouple was introduced completely inside the leaf. The sap flow was monitored for 5 days. The first 2 days were for flow stabilization and the last 3 days were used for the determinations, after the flow became normal.

The osmolality was measured with an Advanced Osmometer Inc., Model 3320 as indicated by Ghneim-Herrera et al. (2006). For this, 100 mg of fresh leaves was macerated with 1 mL of distilled water and the homogenate was introduced in the condensation camera of the osmometer. Values were transformed to solute potential or osmotic potential by Eq. (5)

$$\Psi_S = CTR \qquad (5)$$

in which Ψ_s is the osmotic potential; C is the concentration (mOs mol kg^{-1}); T is the absolute temperature; R is the constant of gases [0.00831 MPa mol^{-1} (K)$^{-1}$].

WUE was determined using the relation between the dry biomass produced by plant leaves versus the quantity of water applied, based on Medrano et al. (2007) and Howell (2001). Also determined by the relation between the produced dry matter and the ETa (Geerts and Raes, 2009).

2.5. Osmolytes determinations

Glycinebetaine was quantified according to the method of Grieve and Grattan (1983) and the proline content was quantified according to Bates et al. (1973).

Soluble sugars were extracted from the leaves of plants subjected to the four water treatments with ethanol 80% at 70 °C for 10 min. Total sugars were extracted from the leaves with deionised boiling water for 5 min. The extracts containing the soluble and total sugars were centrifuged at 15,000 rpm for 15 min. In both cases, the supernatant was collected and the pellet discarded after centrifugation. Soluble and total sugars were quantified by the colour reaction of the Anthrone method read at 620 nm according to Dische (1962).

Oligofructans and polyfructans were extracted from homogenized leaves treated with boiling ethanol 80% for 5 min. The extract was centrifuged at 5000 rpm for 5 min and the supernatant collected. The extraction was performed twice. The polyfructans of a higher degree of polymerization will remain in the pellet.

The pellet was resuspended with deionised water and warmed to 60 °C for 15 min, and then centrifuged at 5000 rpm for 5 min. The polyfructans remain in the supernatant. The oligofructans and polyfructans were further purified from other contaminant polysaccharides such as acemannan by ion exchange chromatography using a Dowex column. The oligo and polyfructans were quantified by a modified Antrona test (Jermyn, 1956) measuring

the colour reaction at 618 nm. The concentration was compared with a fructose standard. The degree of polymerization of the oligo and polyfructans was determined by thin layer plate chromatography (TLC). The TLC for fructan polymers were run using as solvent 1-butanol:acetic acid:water (55:30:15) according to Vieira and Figueiredo-Ribeiro (1993). The plates were developed according to Wise et al. (1955) using a mixture of phosphoric acid and butanol plus 3 g of urea dissolved in 5 mL of ethanol.

2.6. Statistical analyses

Data were analyzed with the software package INFOSTAT V 2007, by means of a variance analysis (ANOVA) and Tukey's test, with a significant level of $P \leq 0.05$.

3. Results

3.1. Water potentials (Ψ_w)

During the 3-month experimental period, Ψ_w decreased with the water restrictions in the plants under different water treatments, from those with 100% to those treated with 25% of FC; the difference between the Ψ_w of plants under 50% and 25% of FC was not significant (Table 1).

3.2. Ψ_w, osmotic potential (Ψ_s) and relative water content (RWC)

The osmotic potential (Ψ_s) decreased with increasing water deficit, and therefore with decreasing Ψ_w, but the decrease was moderate, since Ψ_s changed from −0.67 MPa in the control plants to −0.91 MPa in plants treated with 25% FC, which means a decrease in the Ψ_s of 36% (Table 1), while the Ψ_w decreased by 66%.

3.3. Ψ_w effects on the leaf volume of Aloe Vera plants

The control plants (100% FC) had an average leaf volume of 505 cm^3 with a Ψ_w of −0.21 MPa, whereas the plants irrigated with 25% of FC had an average leaf volume of 383.2 cm^3 with a Ψ_w of −0.35 MPa (Table 2). This implies a decrease of only a 24.2% of the leaf volume for plants irrigated with 25% of FC for 3 months with a decrease in the Ψ_w of 40% compared to the control plants. There was also a significant difference between the leaf volume of control plants and those treated with 75% of FC, and between the leaf volume of plants under this treatment and the leaf volume of plants treated with 50% and 25% FC ($P \leq 0.05$); the volume of these two

Table 2
Effect of four different water treatments (% FC) on the leaf volume and on the leaf water potentials (Ψ_w) in Aloe Vera plants after 3 months of the water treatment. The figures are the average of three independent determinations with the standard deviations. Different letters represent significant differences among water treatments (Tukey's test, $P < 0.05$). $n = 3$; $P < 0.05$.

Water treatments (% FC)	Leaf volume (cm^3) ± DE	Ψ_w (MPa) ± SD
100	505.3 ± 13.06[a]	−0.21[a]
75	417.8 ± 7.56 [b]	−0.27[a]
50	384.0 ± 10.22[c]	−0.29[a]
25	383.2 ± 20.33[c]	−0.35[a]

Table 3
Maximum sap flow rate in Aloe Vera plants subjected to four different water treatments. The table shows the maximum flow rate expressed in liters per squared diameter per hour ($L\,dm^{-2}\,h^{-1}$) and the hour of the night when these maximum rates occurred. The figures correspond to the average of three independent determinations. Different letters represent significant differences among water treatments (Tukey's test, $P < 0.05$). $n = 3$; $P < 0.05$.

Water treatment (% FC)	Maximum sap flow rate ($L\,dm^{-2}\,h^{-1}$)	Hour of occurrence
100	2.25[a]	1:58
75	1.65[b]	2:01
50	0.54[c]	2:26
25	0.37[d]	2:34

Table 4
Increment of the leaf fresh weight respect to control (LFW) of Aloe Vera plants after 3 months of four different water treatments (% FC). The figures are the average of three independent determinations. Different letters represent significant differences among water treatments (Tukey's test, $P < 0.05$). $n = 3$; $P < 0.05$.

Water treatment (% FC)	Increment of LFW 3 months of treatment (g)
100	536.0[b]
75	707.0[a]
50	174.6[c]
25	33.8[c]

last groups of plants was not significantly different. Therefore, the leaf volume decreases with increasing water deficit.

3.4. The water deficit slows down the water flow rate

Table 3 shows the maximum rate of the sap flow in plants subjected to the four water treatments and the time of the day when this maximum occurred. The minimum flow rates for all groups of plants took place around 4:00 PM every day (data not shown) and the maximum was between 2:00 and 2:35 AM, which is characteristic of a CAM plant. The flow rate decreased with increasing water deficit from 2.25 $L\,dm^{-2}\,h^{-1}$ in plants with 11.4 L per plant (100% of FC) to 0.37 $L\,dm^{-2}\,h^{-1}$ in plants with 1.7 L per plant (25% of FC). This rate is only 16.5% of the flow rate of the plants with normal irrigation. The hour of occurrence for the maximum flow rate was somewhat variable; the maximum flow rate of plants with 25% of FC took place 35 min later than in plants with 100% of FC. The flow rate of these plants clearly shows they are under water deficit.

3.5. Water deficit increases water use efficiency (WUE) in Aloe Vera plants

The plants with 75% of FC increased production of leaf fresh weight (LFW) by 132% compared to the control plants (Table 4). The average increment of LFW for this group was 707 g per leaf, compared to control plants with an average increment of 536 g per leaf after 3 months of treatment. The average increment of LFW in the control plants was less than in plants irrigated with 75% of FC, indicating that 100% FC irrigation is not the best treatment for increasing LFW, and is less favorable for leaf growth than a moder-

ate water deficit of 75% FC. On the other hand, the plants with higher water restrictions, 50% and 25% of FC, had smaller increments of LFW, 174.6 and 33.8 g per leaf, respectively, which corresponds to 33.6% and 6.3% of the control plant increment. Therefore, there was an increase in the WUE in Aloe Vera plants under moderate water restrictions.

Similar results were obtained with the increment in the dry weight (DW) of the plants and the increment of dry matter (DM) of the leaves in Aloe Vera plants subjected to moderate water deficit (Table 5). In this table, WUE is expressed in grams of DM per litre of water supplied to the plant. WUE was higher in all plants subjected to water restrictions than in control plants. As in the case of the increment of the LFW (Table 4), plants watered with 75% of FC had the highest WUE. Plants irrigated with 50% and 25% of FC also had higher WUE than the control plants, but less than plants watered with 75% of FC. Table 5 also shows the gel production as gram of dry gel (DG) per gram of leaf dry weight (LDW). The amount of DG decreased with water deficit, although the amount of gel produced by plants subjected to 75% of FC was not significantly different from the control plants.

3.6. The osmotic adjustment of Aloe Vera plants through osmolyte synthesis

Much of the tolerance to water stress that Aloe Vera plants have, as demonstrated by the increase in the WUE under water deficit, should be related to the capacity of this CAM species to perform osmotic adjustment (OA) through osmolyte synthesis and accumulation. This is indicated in Table 1 where the treatments with a higher water deficit reduced significantly the osmotic potential.

Quantitative determinations of quaternary ammonium compounds, also known as compatible solutes, appear to perform an efficient OA in many plants. This is the case of proline and glycine-betaine (Yoshiba et al., 1997; Iba, 2002; Rontein et al., 2002). Our results indicated that proline increased in Aloe Vera plants with
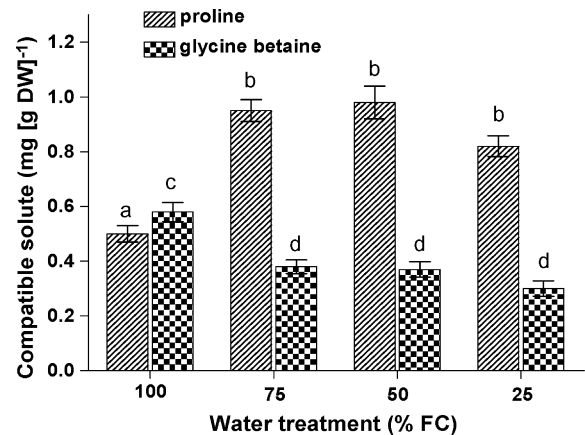

Fig. 1. Compatible solutes (proline and glycine betaine) in Aloe Vera plants subjected to different water treatments. Different letters represent significant differences among water treatments (Tukey's test, $P < 0.05$).

Table 5
Water use efficiency (WUE) in Aloe Vera plants subjected to four different water treatments. WUE was determined 3 months after the beginning of the water treatment and is expressed in grams of dry matter (DM) produced per liter of water supplied per plant (WS). The table also shows the % of DM increment per leaf (LDM), the increment of the plant dry weight (PDW), and the amount of gel expressed as grams of dry gel (DG) per gram of leaf dry weight (LDW). $n = 3$; $P < 0.05$.

Water treatments (% FC)	Amount of water supplied (WS) (L per plant)	ETa (L per plant)	Increment of LDM (%)	Increment of PDW (g)	WUE$_{ws}$ (g PDW/L)	WUE$_{ETa}$ (g PDW/L)	Dry gel (mg DG/g LDW)
100	22.5	11.4	3.81[b]	20.5[b]	0.91[a]	1.8[b]	19.6[a]
75	13.1	9.6	6.74[a]	47.7[a]	3.67[c]	4.96[d]	15.0[a]
50	6.6	4.0	5.68[c]	9.82[c]	1.48[b]	2.45[c]	9.6[b]
25	1.6	1.7	5.51[c]	1.84[d]	1.15[ab]	1.08[a]	3.1[c]

increasing water deficit (Fig. 1). The increase of proline was 74% greater in plants with 75% and 50% FC than the amount present in leaves of control plants. In plants with 25% of FC the proline accumulation was less, but not significantly different from plants subjected to 75% and 50% of FC. In the case of glycinebetaine (Fig. 1), the amount present in plants treated with 75% and 50% FC decreased to 80% of the amount present in the control group, and to 56% in plants with 25% of FC. Therefore, glycinebetaine is most probably not contributing to the osmotic adjustment of Aloe Vera.

The efficient recovery of RWC along with the WUE of Aloe Vera is not only related to the synthesis and accumulation of compatible solutes, but also to efficient CO_2 assimilation. As a consequence, it is also related to the efficient synthesis, allocation and accumulation of sugars under water deficit. Table 6 shows the amounts of total sugars, oligo and polyfructans in leaves of plants subjected to the four water treatments; all increased with increasing water deficit. The group of plants which accumulated most sugars was that treated with 25% FC; in these plants there were 2.6 times more sugars than in control plants. In CAM plants, much of the sucrose which is the final product of the Calvin Cycle is used for the synthesis of complex polysaccharides which help the plant to recover the water loss caused by dehydration by performing OA (Mohnen, 2008; Moreira and Filho, 2008; Hisano et al., 2008). This is the case of oligo and polyfructans which are polymers of fructose and use sucrose as the donor sugar for fructan biosynthesis. Oligofructans, which are fructans with a chain length between 3 and 9 fructose residues per molecule of fructan, increased 2.2 times with respect to control plants, while the polyfructans with a chain length over 9 fructose residues per molecule increased 5.3 times. Fig. 2 shows the results of a thin layer plate analysis of the oligofructans of Aloe Vera plants under different water treatments. The analysis clearly shows that the degree of polymerization of the oligofructans also increased in plants with a water treatment of 25% of FC. Interestingly, a new trisaccharide appeared in the extract of these plants. This trisaccharide was identified by its relative mobility as neo-6G-kestotriose, which is the trisaccharide characteristic of the neofructan series in which the carbon 2 of fructose binds to the carbon 6 of the glucose residue of the sucrose. This result seems to indicate that water deficit not only induces an increase in the amount and the degree of polymerization of fructans, but severe water deficit also changes the structure of the oligofructans. The structure appears to change from an inulin-type to a neofructan type in plants of Aloe Vera subjected to a severe water deficit.

4. Discussion

Although Aloe Vera is a plant adapted to arid environments, water deficit had an effect on the physiology of the plant as determined by the water status of the plant, detected by changes in the Ψ_w, Ψ_s, volume of the leaves, yield of the gel produced in the leaves, RWC, and sap flow rate. The physiological changes of Aloe Vera plants induced by water restrictions, however, seem to occur slowly during water restriction, probably due to the amount of water stored in the leaves of the plants. In a similar experimental design with Aloe Vera plants grown in a greenhouse, Rodríguez-García et al. (2007) found that under water restrictions the stomata of Aloe Vera leaves were initially open, probably because the water accumulated in the gel of the leaves maintains the stomatal conductance high, in spite of a water stress revealed by a negative Ψ_w. However, as time passed, the stomatal resistance increased under water stress. Interesting, Silva et al. (2010) found that the guard cells became smaller over time with water restriction, although the density of the stomata significantly increased in the leaves with water deficit.

Since plants treated with 25% of FC retain 50% of the water, Aloe Vera plants must have physiological mechanisms for maintaining internal water under severe water deficit. The qualitative and quantitative composition of the gel of the leaves surely plays a very important physiological role in the retention of water, and therefore, in the maintenance of the water supply for cell synthesis and leaf growth. Since the decrease in Ψ_w caused by the water restriction correlated with a decrease in the Ψ_s, osmolytes such as quaternary ammonium compounds, sugars, oligo and polyfructans should increase to produce the OA (Iba, 2002; Van Den Ende et al., 2004; Del Viso et al., 2009).

In spite of the tolerance that Aloe Vera plants seem to have to water stress, plants after 3 months of water restrictions were stressed, as demonstrated by the slower sap flow rate in plants treated with 50% and 25% of FC. At night when the maximum flow rate takes place, evatranspiration occurs due to the increase in the stomatal conductance. The sap flow rate depends directly on the stomatal opening (Medrano et al., 2007). In C3 mediterranean plants (*Diplotaxis ibicensis*, *Beta maritime*) it was observed that under a regime of limited irrigation the plants showed a decrease in the sap flow rate between 70% and 90%, compared to plants under optimal irrigation (Galmés et al., 2007). These values are similar to the Aloe Vera flow rate values. Since there is still sap flow Aloe Vera plants after 3 months of severe water deficit (25% of FC), these plants still open their stomata at night.

The increase of WUE under water restriccion seems to be a common feature for CAM plants, even for those plants which are facultative CAM (Herrera, 2009). Thus our results demonstrate that 100% of FC is not the appropriate irrigation for the plants. The WUE of Aloe Vera plants increased with water restrictions, even though the soil used in these experiments was 50% sand and had low capacity for water retention. Our results also show that 50% and 25% of FC decreased the dry weight of the leaves and gel production compared to control plants and with plants irrigated with 75% of FC (Tables 4 and 5). Howell (2001) indicated that an optimal irrigation in corn decreases the yield with respect to plants irrigated with 80% of FC. In general deficit irrigation seems to increase water productivity in several crops maintaining the plant yield if a minimal
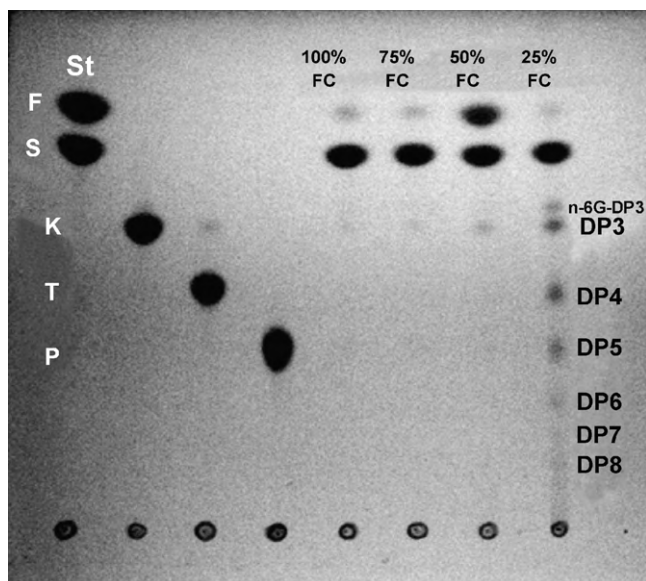

Fig. 2. Thin layer chromatography analysis of oligofructans present in Aloe Vera plants subjected to different water treatments. St, standard sugars: F (fructose), S (sucrose), K (standard kestose trisaccharide), T (standard tetrasaccharide), P (standard pentasaccharide). DP, degree of polymerization, DP3, DP4, DP5, etc means degree of polymerization of 3 sugars, 4 sugars, 5 sugars, etc. n-6G-DP3 is the neo-6G-kestotriose, trisaccharide of the neofructan series.

Table 6
Carbohydrates quantities in the leaves of Aloe Vera plants subjected to four different water treatments (% FC). The table shows the amounts of total sugars, oligofructans and polyfructans, all expressed in mg per gram of leaf dry weight (LDW). The table also shows the % of increment compared to the control. Different letters represent significant differences among treatments (Tukey's test, $P < 0.05$). $n = 3$; $P < 0.05$.

Water treatment (% FC)	Total sugars (mg/g DW)	Oligofructans (mg/g DW)	Polyfructans (mg/g DW)
100%	62.40[a]	29.66[a]	13.63[a]
75%	77.20[a]	42.86[ab]	23.20[ab]
50%	63.43[a]	29.23[a]	25.33[ab]
25%	165.2[b]	66.12[b]	72.05[b]

seasonal moisture is present in the field (Geerts and Raes, 2009). This seems to be the case for wheat, cotton and corn (Zwart and Bastiaanssen, 2004).

However, C4 plants such as corn lack the high water-storage capacity of CAM plants, which is translated into ability to buffer fluctuations in environmental water availability. Aloe Vera combines this ability of great water storage capacity with the efficient CO_2 fixation of the C4 plants by a pathway where the first enzyme for CO_2 fixation is phosphoenolpyruvate-carboxylase (PEP-carboxylase) and not RUBISCO. PEP-carboxylase has a CO_2 affinity an order of magnitude greater than RUBISCO (Cardemil, 2007; Cousins et al., 2007; Ceusters et al., 2009; Borland et al., 2009).

The efficient CO_2 assimilation of CAM plants is related to their capacity for osmolyte synthesis, mainly those derived directly from sugar synthesis. Accumulation of osmolytes in the cytoplasm is considered a mechanism which contributes to water deficit tolerance, since these osmolytes counteract the effects of drought. The physiological role of osmolytes in counteracting water deficit has been demonstrated in plants sensitive to drought, which become more tolerant to salinity, cold and drought when they were made transgenic for overexpression genes related to proline and glycine-betaine synthesis (Rontein et al., 2002; Chen and Murata, 2002). Osmolytes appear to protect the quaternary structure of proteins, maintaining enzyme activity and the highly ordered structure of membranes from the damaging effects of environmental stresses (Yoshiba et al., 1997; Sakamoto and Murata, 2001; Chen and Murata, 2008). Additionally, during salt or drought stress synthesis of proteins involved in PSII repair is affected, leading to photoinhibition. The presence of osmolytes restitutes protein synthesis, enhancing PSII repair.

Aloe Vera plants under water restriction increased the levels of proline but not the levels of glycinebetaine. According to Balibrea et al. (1999) proline is one of the most stable amino acids, and, therefore, this amino acid probably accumulates in water-stressed Aloe Vera plants due to its stability and low turnover. The increase of proline in Aloe Vera plants, however, is discrete compared to other plants. In Aloe Vera proline increased 75% over the control value, while in water-stressed soybean plants proline increased 4 times the initial level (Lobato et al., 2008). Since the physiological roles of these compatible solutes are quite similar, probably only proline is supplying the physiological needs to counteract water stress damage Aloe Vera. On the other hand, Aloe Vera as a CAM plant has plenty of the most important osmolytes, the sugars, to perform osmotic adjustment. Due to their efficient CO_2 assimilation, Aloe Vera may not need high amounts of proline to perform OA.

In CAM plants, much of the sucrose, which is the final product of CO_2 assimilation, is used for the synthesis of complex polysaccharides which help the plant to accumulate water in the leaves and stems (Mohnen, 2008; Moreira and Filho, 2008; Hisano et al., 2008). These polysaccharides have a polymer structure which traps and retains water. In this way succulent plants like Aloe Vera accumulate water. The water affinity itself is due to the exposed hydroxyl groups of the sugar residues of the polysaccharide which bind water molecules through hydrogen bonds. This binding distorts the angle of the water molecule in such a way that the water cannot form ice crystals. For this reason these polysaccharides not only pro-

tect plants against water deficit but also protect the plant from ice formation when the temperature falls below freezing (Del Viso et al., 2009). There are several plant polysaccharides with water retention properties, including the pectins, the hemicelluloses such as the galactoglucomannans also present in the gel of Aloe Vera leaves, and the fructose polymers. Fructans are reserve carbohydrates made up of fructose, forming oligomers and polymers. They are only synthesized by some plants (15% of the flowering plants, Hendry, 1993; Del Viso et al., 2009) and bacteria. These polymers of fructose are synthesized by adding the β-$(2 \rightarrow 1)$ or the β-$(2 \rightarrow 6)$ linked fructofuranosyl units to 1-kestose, 6-kestose or to neokestotriose; all three are fructan trisaccharides. The degree of polymerization as well as the types of linkage which predominate in the fructan molecules will depend on the type of fructosyl transferases present in the plant. The linear type of fructan or inulin series of fructans is present in the Astereaceae family, while the neofructan type of fructans is found in Liliaceae and in Asparagales (monocots) to which Aloe Vera belongs and the graminan series is present in Poaceae and, therefore in cereals (Kawakami and Yoshida, 2005). The fructans are also found in bacteria (Van Den Ende et al., 2004; Del Viso et al., 2009). Our results indicate that the trisaccharide 6G-kestotriose, which is characteristic of the neofructan series, appears in Aloe Vera plants subjected to most strict water deficit and probably increases the neofructan type oligosaccharide over the inulin type. The degree of polymerization also appeared to increase in Aloe Vera plants treated with 25% of FC. It is probable that the increase in soluble and total sugars, the increase in the degree of polymerization of oligo and polyfructans, the change in structure from inulin type to neofructan type fructans and the moderate increase in proline will provide the osmotic adjustment for Aloe Vera plants when there is a water stress.

5. Conclusions

As a consequence, the increase in the osmolyte biosynthesis will provide the necessary metabolic cellular protection to the plant and will cause the water retention in the leaves of Aloe Vera to buffer the water loss caused by water deficit. Ultimately this water retention increases the WUE of Aloe Vera plants under water stress, making this species suitable to be cultivated in the arid region of Northern Chile.

Acknowledgements

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