Agricultural Water Management 97 (2010) 1564–1570

Contents lists available at ScienceDirect

Agricultural Water Management

journal homepage: www.elsevier.com/locate/agwat

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

J. Delatorre-Herrera[a,*], I. Delfino[a], C. Salinas[b], H. Silva[c], Liliana Cardemil[b]

[a] *Departamento de Agricultura del Desierto y Biotecnología, Universidad Arturo Prat, Iquique, Chile*
[b] *Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile, Chile*
[c] *Laboratorio de Relación Suelo Agua Planta, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Chile*

ARTICLE INFO

Article history:
Received 7 October 2009
Accepted 14 May 2010
Available online 18 June 2010

Keywords:
Field capacity
Water use efficiency (WUE)
Osmotic adjustment
Sap flow rate
Fructans

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.

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

* Corresponding author at: Avda. Arturo Prat 2120, Iquique, Tarapaca, Chile. Tel.: +56 57 39 45 05; fax: +56 57 44 51 90.
E-mail addresses: jose.delatorre@unap.cl, jodelato@unap.cl (J. Delatorre-Herrera).

0378-3774/$ – see front matter © Published by Elsevier B.V.
doi:10.1016/j.agwat.2010.05.008

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

$$\Theta_V = \Theta_G \times \frac{P_S}{P_W} \quad (1)$$

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

$$\Theta_V = 0.9481 \times \Theta_{V\,\text{Thetra probe}} \quad (2)$$

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)

$$V = \left[\frac{L}{12}\right] \pi E A \quad (3)$$

where V is the leaf volume (cm^3); L is the length of the leaf (cm); π = 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