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## Morphological and Physiological Characters of *Aloe vera* Subjected to Saline Water Irrigation

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*Aloe vera* is grown in arid climates where salinity can limit plant growth and development. A study was conducted to examine the morphological and physiological characters under salt stress. Plants were cultivated in pots and irrigated with freshwater ( $EC\ 450\ \mu s\ cm^{-1}$ ) or saline lake water ( $EC\ 3, 6, 9, 12, 15, 18,$  or  $21\ dS\ m^{-1}$ ). Results indicated that salinity influenced the plant growth and morphological traits and the biomass. Glucose, xylose, and mannose concentrations in leaf gel increased with increasing salinity up to  $9\ dS\ m^{-1}$  and decreased with higher saline concentrations. Aloin concentration increased with salt stress up to  $15\ dS\ m^{-1}$  that decreased at higher salinity concentrations.

**KEYWORDS** Aloin, leaf gel, soluble sugars

### INTRODUCTION

*Aloe* sp. (Liliaceae) includes more than 500 species (5,25) and is a perennial with succulent green leaves joined at the stem in a whorled pattern (20,26), growing to 50 to 100 cm tall, and spreading by offsets. The leaves have serrated margins and are used for extraction of gel and bitter yellow sap (22,23). *A. vera* is the most commercialised *Aloe* species that is frequently cited as

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being used in herbal medicine and cosmetic products (5). The inner clear gel contains 99% water, and the rest is made of glucose, fructose, glucomannans, amino acids, lipids, sterols, minerals, and vitamins (23). *A. vera* has been used for many centuries for its curative and therapeutic properties that are due to the presence of compounds such as polysaccharides (11). The bitter yellow sap is produced from tubular cells located under upper leaf epidermis and contains anthraquinones and glycosides (23). Aloin is the most important glycoside that is produced in leaves (21).

Salt stress is one of the most important abiotic stresses in arid and semi-arid regions (4,12) that decreases plant dry matter and leaf area (2) and crop yield. Also it has been reported that salinity changes plant morphological characteristics (17). Today, millions of acres of farmland in the world are unusable due to increasing salinity levels (1), and nearly 20% of land surface and approximately half of the ground water face salinity problem (6). Only 2.5% of the total water in Earth (1,360 million km<sup>3</sup>) is fresh, and only 0.33% of fresh water is available for humans (24). Hence, use of poor-quality groundwater has become inevitable for irrigation to compensate for rapidly increasing water demands due to increasing water requirements for irrigation and the competition between human and industrial water use, especially in arid and semi-arid regions (15).

In arid and semiarid lands, where water comes at a premium, drought and salinity are most important factors that inhibit photosynthesis and increase respiration (19). *A. vera* is a succulent plant with crassulacean acid metabolism (CAM), adapted to arid conditions by conserving water. The most important benefit of CAM to the plant is the ability to leave most leaf stomata closed during the day. This characteristic makes CAM plants resistant to drought stress. In this study, effects of salinity on the morphological and physiological characters and soluble sugars and aloin content of *A. vera* were studied.

## MATERIALS AND METHODS

### Site of Study and Experimental Design

The experiment was conducted in a glasshouse at the Faculty of Agriculture, Tarbiat Modares University (longitude: 51° 43", latitude: 35° 8", and 1,215 m altitude), Tehran, Iran during the spring and summer of 2009, in a randomized complete blocks design with eight treatments and four replications.

### Plant Material and Conditions

Healthy, seven-leaf *Aloe vera* offsets were transplanted in plastic pots (22-l volume) that contained 18 kg sandy-loam soil (Table 1). The pots were placed in a glasshouse under conditions of 25/20 ± 3°C day/night temperature and supplementary photon flux density of 250 μmol m<sup>-2</sup> s<sup>-1</sup>.

**TABLE 1** Soil Analysis and Chemical peoperties

Texture	pH	EC (dS.m <sup>-1</sup> )	S (mg.kg <sup>-1</sup> )	P (mg.kg <sup>-1</sup> )	K (mg.kg <sup>-1</sup> )	Total N (%)
Sandy loam	7.5	1.68	48	17	433	0.09
Mineral N (%)	Fe (mg.kg <sup>-1</sup> )	Zn (mg.kg <sup>-1</sup> )	Mn (mg.kg <sup>-1</sup> )	Cu (mg.kg <sup>-1</sup> )	Organic matter (%)	
17	7.1	0.9	0.32	0.72	1.09	

**TABLE 2** Chemical Analysis of Soltan Lake Water in Qom

pH	EC (dS.m <sup>-1</sup> )	Carbonate (g.l <sup>-1</sup> )	Bicarbonate (g.l <sup>-1</sup> )	Chlorine (g.l <sup>-1</sup> )	Sulfate (mg.l <sup>-1</sup> )
7.25	600	0.01	8.6	161	341.5
B (mg.l <sup>-1</sup> )	Ca (g.l <sup>-1</sup> )	Mg (g.l <sup>-1</sup> )	Na (g.l <sup>-1</sup> )	Nitrate (mg.l <sup>-1</sup> )	
54.8	1.2	22.4	115	2.75	

## Salinity Treatments and Irrigation

Salinity was induced by irrigation with saline water collected from the saline Houz Soltan Lake located in Qom Province, Iran (Table 2). The lake water was diluted by adding double distilled water, and EC was adjusted to 3, 6, 9, 12, 15, 18, or 21 dS m<sup>-1</sup>. Tap water (EC 450  $\mu$ S cm<sup>-1</sup>) was used as control treatment. Field water capacity of pots was determined with a suction plate, and pots were weighed regularly and watered to approximately 0.7 of field capacity and placed in saucers so that any water that drained through was later recovered.

## Morphological Characters

Six months after salinity induction, leaf number per plant was registered, and then four mature leaves of each plant were cut at the base by knife. The leaf length and leaf fresh weights were recorded. The leaf gel was extracted manually, and the fresh and dry weights were determined. Some leaves were used to get the leaf dry weights. Finally, plants were harvested just above ground, and total biomass was measured.

## Aloin Assay

After cutting the leaf, yellow syrup that leaked from injury sites was collected carefully and stored in liquid nitrogen until chemical analysis. Samples

were freeze-dried for 24 h. Aloin was determined using high-performance liquid chromatography (Waters, USA;  $4.6 \times 250$  mm, dp 10  $\mu$ m column,  $\mu$ Bondapak C<sub>18</sub>). Aloin standard was purchased from Sigma-Aldrich, USA. Stock solution was prepared by dissolving aloin into water methanol solvent (1:1 v:v) and used to make standard solutions. Aloin concentration was calculated by using external standard and aloin standard curves.

### Soluble Sugars Assay

Soluble sugars including glucose, xylose, and mannose were estimated as described earlier (8). Leaf gel and leaf skins samples were homogenized in a mortar and pestle with 3 mL distilled water, and homogenate was filtered by filter paper; then 0.5 mL phenol (5%) and 2.5 mL sulphuric acid (98%) were added to the homogenate. After reaction, the test tubes were allowed to cool at room temperature. The amount of glucose, xylose, and mannose were determined at absorbance 480, 485, and 490 nm, respectively. The sugar concentration was calculated from a glucose, xylose, and mannose standard curves.

### Statistical Analysis

Data were analysed by analysis of variance, and significant treatment mean differences were determined by least significant difference test using the SAS software.

## RESULTS

The results showed that salinity stress affected leaf length, number of leaves, leaf fresh and dry weight, gel fresh weight, plant biomass and aloin content (Table 3). Salinity decreased leaf length, so that the highest and lowest leaf length was observed from control treatment and the highest salinity level, respectively (see Table 3). Additionally, the lowest number of leaves and leaf fresh and dry weight were recorded from those plants that were treated by 21 dS m<sup>-1</sup> saline water (see Table 3). Gel fresh and dry weight and biomass decreased due to salinity stress and, in general, there was steep fall when salinity stress increased up to 9 dS m<sup>-1</sup>. Plant biomass showed a steady rise up to 6 dS m<sup>-1</sup> and plunged gradually at higher salinity.

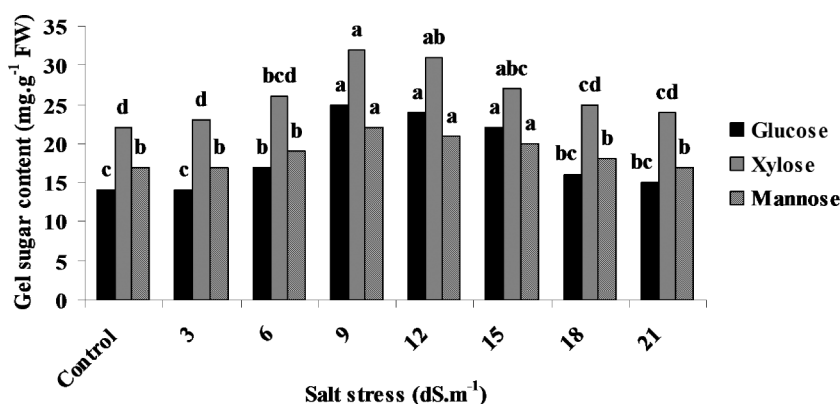
Aloin content increased dramatically because of salinity stress, but then it plummeted at 21 dS m<sup>-1</sup> salinity level. Glucose, xylose, and mannose content in gel and leaf skin were affected by salinity stress (see Table 3). Gel glucose content increased at 6 and 9 dS m<sup>-1</sup> salinity and was maintained until 18 dS m<sup>-1</sup> after which it decreased (Figure 1). A similar trend was observed with gel xylose and mannose content (see Figure 1).

**TABLE 3** *Aloe vera* Plant Characteristics and Gel and Alonin Concentrations as Affected by Salinity Levels

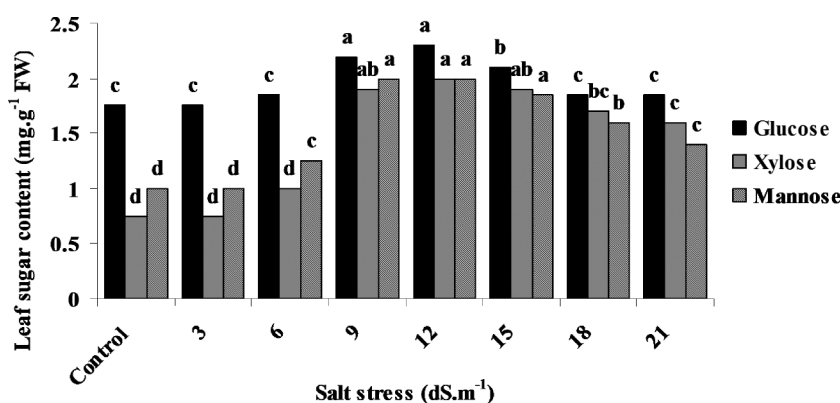
Salinity (dS.m <sup>-1</sup> )	Leaf length (cm)	Number of leaves	Leaf fresh weight (g)	Leaf dry weight (g)	Gel fresh weight (g)	Gel dry weight (g)	Biomass (g.plant <sup>-1</sup> )	Alonin concentration (%)
Control	40.62a	15a	101.29a	23.43a	58.46a	12.34a	686.2a	0.84d
3	39.93a	14.5a	99.52a	23.65a	56.16a	12.45a	713.2a	0.88cd
6	38.75a	13.25ab	91.39a	20.23b	49.98a	12.76a	720.4a	0.89cd
9	30.5b	11.5bc	38.85b	19.86b	20.56b	8.34b	225.3b	1.07c
12	24.18c	10.25cd	33.11bc	19.65b	15.15bc	6.34b	198.9bc	1.38b
15	20.43c	9cd	24.84bc	15.75c	8.79bc	3.67c	123.2bcd	1.66a
18	20.15c	9cd	19.58c	15.66c	6.33c	3.35c	86.18cd	1.64a
21	12.62d	8.5d	18.11c	12.54d	4.64c	2.18d	58.35d	1.38b

Note: Mean separation by LSD ( $p < 0.05$ ).

Means within each column followed by the same letter are not different.



**FIGURE 1** Effect of salt stress on gel glucose, xylose, and mannose concentration in *Aloe vera* leaves. Mean separation by LSD ( $p < 0.05$ ). Means followed by the same letter for each characteristic are not different.



**FIGURE 2** Effect of salt stress on leaf glucose, xylose, and mannose concentration in *Aloe vera* leaves. Mean separation by LSD ( $p < 0.05$ ). Means followed by the same letter for each characteristic are not different.

The skin glucose content increased steadily up to 12 dS m<sup>-1</sup> salinity and then decreased slowly at higher salinity levels (Figure 2). Xylose and mannose increased when plants were treated by 9 dS m<sup>-1</sup> saline water and remained stable at 9, 12, and 15 dS m<sup>-1</sup>, after which it decreased at higher salinity.

## DISCUSSION

Under salt stress, energy is spent on the maintenance of plant instead of growth and development (16). High concentrations of dissolved salts in the soil and root zone can cause the high osmotic pressure and, thus, water availability would be decreased. These conditions affect physiological activities and subsequently crop yield (12). The plants will need to spend

more energy to get water. Osmotic pressure affects cell turgidity and development (3). Opening and closing of stomata is governed by turgidity of the guard cells whereas osmotic stress, induced by salts, suppresses cell turgidity (6) and decreases cell division and apical meristem development (16). As *A. vera* has leaves with high percentage of water, cellular water loss or reduced cell volume led to decrease of growth. Additionally, high accumulation of toxic salts such as  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{Br}^-$  in apoplastic and cytoplasmic cell compartments of leaves can cause desiccation, cell plasmolysis and, finally, cell death due to the dramatic increase in osmotic pressure. Symptoms include chlorosis and necrosis of meristematic areas followed by leaf margins on older and, finally, young growth. Salt accumulation in leaves and premature leaf abscission in response to salt stress has been suggested to be a defensive mechanism used by plants to rid themselves of damaging organisms (10). The findings in this study confirm previous researches indicating that salinity decreased growth (27) leaf number (9,13), leaf length, leaf fresh weight, and soluble sugars concentration (14). The highest carbohydrates were reported as being obtained with 0.4% salinity (18).

Secondary metabolite production plays an important role in adaptation of plants to varying unfavorable environmental conditions. In this study, aloin content increased at salinity stress up to  $18 \text{ dS m}^{-1}$ . The increase of aloin content can be due to low water content in leaves. In previous studies, the highest amount of aloin in *A. vera* was obtained with 0.2% salinity (18). Soluble carbohydrate accumulation in plants as a response to drought and salinity stress has been reported (4). Under normal conditions, the concentration of dissolved substances in the roots is more than water and soil, so water can penetrate into the roots easily while under salinity stress conditions. In response to this event, osmotic adjustment in plants can occur by the accumulation of high concentrations of either inorganic ions or low-molecular-weight organic solutes such as sugars and organic acids (4). Osmotic adjustment is an important adaptation mechanism to salinity in plants because it helps maintain turgor pressure and cell structures in stressed plants. The accumulation of soluble carbohydrates as osmolytes in plants has been widely reported as a response to salinity or drought. Soluble sugars prevent protein dehydration on account of turgor pressure (7). In the current study, it was observed that leaf water content decreased due to salinity stress (data are not shown), which increased the concentration of soluble sugars in the gel in the leaves.

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