

MODERATE SALT STRESS ON THE PHYSIOLOGICAL AND MORPHOMETRIC TRAITS OF *ALOE VERA* L.

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Abstract: *Aloe vera* L. is a perennial liliaceous plant with succulent green leaves, it is a xerophyte with economic and social value, but little is known about its response to salt stress and its mechanisms of tolerance, especially at moderate concentrations. The objective was to investigate the effect of salinity stress (0, 30, 60, 90 and 120 mM NaCl) on *Aloe* by measuring physiological and morphometric traits related to growth. The results of measuring physiological traits showed no significant differences compared to the control at 30 and 60 mM (chlorophylls a, b, total, relative water content, net assimilation rate, specific leaf weight, and specific leaf area), even at 90 mM or 120 mM most parameter variations were not significantly different. Similarly, for morphological traits no significant differences were found compared to the control for all 16 morphological traits at 30 mM, or even at 60 mM since at this concentration only one trait varied significantly (1 out of 16). Although the general trend of increasing NaCl was to lower leaf fresh weight as salinity increased, presumably due to a decrease in water content. However leaf dry weight was not significantly reduced at any of the salinity concentrations applied. Thus, total dry leaf biomass was basically the same or similar, this suggesting that *Aloe* has an ability to withstand these levels of salinity stress. It is noteworthy to point out, that there is a general trend of lower growth (lower parameters both physiological and morphological) that is not significant at low doses (30 and 60 mM), however becoming more significant at higher doses (90 and 120 mM). Thus, we suggest that *Aloe* has continuum responses to NaCl stress.

Keywords: biomass, growth analysis, NaCl salinity salt tolerance.

Resumen: *Aloe vera* L. es una planta liliácea perenne con hojas verdes suculentas, es una xerófila con valor económico y social; sin embargo, existe poca información disponible en torno a su respuesta a la salinidad y sus mecanismos de tolerancia, especialmente en concentraciones moderadas. El objetivo fue investigar el efecto del estrés salino (0, 30, 60, 90 y 120 mM de NaCl) en rasgos fisiológicos y morfométricos relacionados con el crecimiento de *Aloe*. Los resultados de las características fisiológicas (clorofilas a, b, total; contenido relativo de agua; tasa de asimilación neta; peso específico de la hoja y área foliar específica), no mostraron diferencias significativas en 60 y 90 mM, comparados con el control. Incluso en 90 ó 120 mM, la variación de algunos parámetros fue diferente significativamente. Igualmente, para características morfológicas no se encontraron diferencias significativas con respecto al control en las 16 características utilizadas a 30 mM. Incluso a 60 mM solamente una característica difirió significativamente. Aunque la tendencia general al incrementar la concentración de NaCl fue la disminución del peso fresco de la hoja, presumiblemente debido a la disminución del contenido de agua. Sin embargo, el peso seco de la hoja no se redujo significativamente en ninguna de las concentraciones salinas aplicadas. Así, el peso seco total de biomasa fue básicamente el mismo o similar, lo cual sugiere que *Aloe* tiene habilidad para soportar estos niveles de estrés por salinidad. Es de destacar que existe una tendencia general de menor crecimiento (parámetros más bajos tanto fisiológicos y morfológicos) que no son significativos a dosis bajas (30 y 60 mM); sin embargo, empiezan a ser más significativos a dosis más altas (90 y 120 mM). Por lo tanto, esto sugiere que *Aloe* tiene una respuesta continua al estrés por NaCl.

Palabras clave: análisis de crecimiento, biomasa, salinidad por NaCl, tolerancia a la salinidad.

Salt stress is one of the most important abiotic stresses in arid and semiarid regions (Ashraf and Harris, 2004; Hebbara *et al.*, 2003). Plants under stress have less dry matter, leaf area (Amirjani, 2011) and yield. Also it has been re-

ported that salinity changes plant morphological characteristics (Zadeh and Naeini, 2007). According to FAO (2013) increased soil salinity, aquifer depletion and land degradation reduce achievable yields, thus putting at risk farmers'

ability to bridge production gaps and improve food security. The impact of salt stress has been correlated with morphological and physiological traits like reduction in fresh and dry weight (Chartzoulakis and Klapaki, 2000). Salinity affects plant metabolism by disturbing physiological and biochemical processes of plants due to ionic and osmotic imbalances which results in the reduction of plant growth and productivity (Munns, 2005). The deleterious effects of salinity on plant growth are associated with low osmotic potential, specific ion effects, and oxidative stress, causing nutritional imbalances due nutrient or element uptake (Ashraf and Harris, 2004). Studies of plant tolerance to salt stress cover many aspects on the influences of salinity on plant response, including alterations at the morphological, physiological and molecular levels.

Aloe vera L. is a perennial liliaceous plant with succulent green leaves joined at the stem in a whorled pattern. Among all the *Aloe* species, it is highly valued due to its short growth period and high economic value, and is used in pharmaceuticals, folk medicine, healthcare, cosmetic, and food products (Reynolds and Dweck, 1999). Plants are exposed to many types of environmental stresses. Among these, salinity and drought are two of the most significant problems that limit plant growth. Plants, in turn, have evolved many different physiological and biochemical strategies for coping with water stress caused by NaCl or drought conditions. *Aloe vera* is a succulent plant with crassulacean acid metabolism (CAM) adapted to arid conditions by conserving water. The most important benefit of CAM on the plant is the ability to maintain closed most of its leaf stomata during the day. This characteristic helps CAM plants be more resistant to drought stress (Rahimi-Dehgolan *et al.*, 2012). Some experiments with *Aloe* have been carried out in different geographical locations for the assessment of growth, biomass, soluble carbohydrates, cations, gel, aloin content, and others variables or traits under saline stress (Karmarkar and Ranganathan, 1971; Mustafa, 1995; Tawfik *et al.*, 2001; Sun *et al.*, 2003; Jin *et al.*, 2004; Xu *et al.*, 2006; Zan *et al.*, 2007; Silva *et al.*, 2010; Kiran kumari *et al.*, 2012; Moghbeli *et al.*, 2012; Rahimi-Dehgolan *et al.*, 2012; Rahi *et al.*, 2013). However, the majority of these studies have used high concentrations of NaCl (up to 200 mM), high electrical conductivities, some of them up to 12 dS m⁻¹, and in some cases, those studies have used 60 % of sea water as a salinity treatment, with the consequence of negative effects and damages in growth, yield, physiology, morphology, and other traits and characteristics of *Aloe* plants. On the other hand, at present most research related to saline stress focus on glycophytes under salt stress in the laboratory, greenhouse or field; however, there are no reports about *Aloe vera*, a CAM desert plant, under relative mild or moderate NaCl stress. In general terms, when salinity is focused for selection of plants with superior tolerance to salinity, mild or moderate salinity treatments have been to use because of studies have been demonstrated that us-

ing them, the effects of salinity can be more easily evaluated because of if high salinity levels are used, the majority of the plants species died and the evaluation of yield, growth and other characteristics or variables cannot be measured. Moreover, when the studies of salt-tolerant plants use mild or moderate salinity levels, the antioxidative capacity of fruits increased (D'Amico *et al.*, 2003), with some levels of enzymes increasing such as superoxide dismutase (Keutgen and Pawelzik, 2007), causing the quality of the fruit to improve (Awang *et al.*, 1993a,b; Keutgen and Pawelzik, 2008). Other effects measurable at mild salt stress are Na or Cl exclusion (Orsini *et al.*, 2012), increase of biomass production (Adolf *et al.*, 2013). Hence, moderate salinities are probably the more suitable for measuring response to growth, yield and survival (Allen *et al.*, 1994; Munns, 2002). Also, when mild or moderate salinity levels are used, this offers the possibility of genetic improvement by selection of suitable plant material to reclaim moderately salinized lands. To improve the agricultural management of *Aloe vera* under the arid and semi-arid conditions of northwest of Mexico, it is important to investigate how crop growth and yield might be affected by the physiological and morphometric responses to saline stress, because of many farmlands around the coastal zone of this region are affected by sea water intrusion. Therefore, to explore the response of morphometric and physiological traits of *Aloe* to salt stress, plants of one cultivar of *Aloe* were subjected to four NaCl concentrations (0, 30, 60, 90, and 120 mM) to assess the salt tolerance of the species by analyzing the plant growth and other variables associated with plant physiology and to provide scientific basis for safe and productive cultivation of *Aloe* under mild saline water irrigation.

Materials and methods

Study area.- The experiment was conducted under greenhouse conditions in La Paz, located in a semiarid zone of Baja California, northwest of Mexico (24° 8' 9.73" N, 110° 25' 41.73" W), 7 m above sea level. Mean, maximum and minimum temperatures in the greenhouse were 19.2, 35.3 and 5.3 °C, with 60 % relative humidity. The meteorological observations were obtained during the study from an automated weather station located inside the greenhouse. The experimental site has a Bw (h') hw (e) climate and is considered as a semiarid climate and sustains xerophytic vegetation. The soils are characterized by good aeration and penetrability conditions for plant roots and low-medium water retention, with high content of sand, with a neutral pH at the surface but that are slightly alkaline at depths of 20-60 cm, with low organic matter content (less than 1 %).

Plant material.- Plants of uniform size, health, and color were obtained from local wild ecotype of *Aloe vera* L., with 30 cm height from the experimental field of the Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR). The plants were cultured in plastic pots of 25 cm high, 20

cm wide on the upper surface, and with holes at the bottom for drainage (one plant per pot). Containing a mixture of sand and peat-moss (1:1, v:v) (Sunshine, Sun Gro Horticulture, Canada). The pots were placed in a greenhouse and cultured with natural light. After being transplanted, 1 L of tap water was applied every three days during 51 d until plant rooting was established. Uniform plants were chosen to be treated with NaCl.

Physiological traits

Chlorophyll extraction.- Measurements of chlorophyll were performed on leaves collected from either five months old potted plant (approximately 20-30 cm length). Using a hole punch three disks were sampled from healthy, uniform in color and size leaves between 08:00 and 10:00 h (total area of 39.0 mm²). These were immediately pooled, macerated, and homogenized in 20 mL of cold, 80 % aqueous acetone, and transported in a portable icebox to the laboratory. Chlorophyll suspensions were kept in the dark for 72 h prior to measuring absorbance with a spectrophotometer (Spectronic Unicomp, Cambridge, United Kingdom). Before measuring absorbance, the pigment extracts were centrifuged for 3-5 min in glass tubes to make the extract fully transparent. Absorbance of leaf extracts was measured at 645 nm and 663 nm, and total chlorophyll content (*Chl a* + *Chl b*) was determined by the method of Arnon (1949) and expressed in terms of a leaf area basis (mg cm⁻²). This variable was measured four times during four months (one measurement per month) and an average was obtained.

Relative water content (RWC).- Measurements of RWC (%) were performed on leaves collected from five months old potted plants (approximately 20-30 cm length). Leaves were always collected from the mid-section, in order to minimize age effects. Individual leaves were selected and three disks from the same leaves (total area of 5.10 cm²) were sampled using a hole-punch. The disks were then immediately weighed (fresh mass, FM). In order to obtain the turgid mass (TM), disks were floated in distilled water inside a closed Petri dish. During the imbibition period, leaf samples were weighed periodically, after gently wiping the water from the leaf surface with tissue paper. The Petri dishes were maintained under dim light (around 20 μmol m⁻² s⁻¹) and under naturally fluctuating temperature conditions in the laboratory) which varied between 25 ± 2 °C. At the end of the imbibition period, leaf samples were placed in a pre-heated oven (Shel-Lab, model FX-5, serie-1000203, USA; Catsky, 1974; Turner, 1981), at 80 °C until constant weight (approximately 72 h), in order to obtain the dry mass (DM). All mass measurements were made using an analytical scale, with precision of 0.0001 g (Mettler Toledo, model AG204, USA). Values of FM, TM, and DM were used to calculate RWC, using the equation: $RWC (\%) = [(FM-DM)$

$/(TM-DM)] \times 100$. This variable was measured four times during four months (one measure per month) and average of the measures was obtained.

Specific leaf weight (SLW) and specific leaf area (SLA).- To calculate SLW and SLA, individual leaves were selected and three disks from the same leaves (total area of 51.0 mm²) were sampled using a hole-punch; after the disks were oven-dried at 80 °C until constant weight (approximately 72 h) to determine dry weight (mg). The SLA was calculated as the ratio of leaf area (mm²) to leaf dry weight (mg), $SLA = \text{area} / \text{dry weight}$, while SLW, the inverse of SLA was calculated as the ratio of leaf dry weight (mg) to leaf area (mm²), $SLW = \text{dry weight} / \text{area}$.

Net assimilation rate (NAR).- Once the leaves reached maturity with a size of approximately 20-30 cm length, for each treatment and replication a leaf was selected and NAR (mg cm² h⁻¹) was determined using the following procedure: at 9:00 a.m., a sample of the leaf with a punch hole of 1.7 cm in diameter (2.27 cm²) was obtained; the same day at 17:00 p.m., a second sample was obtained from the same leaf, near point close to the first sample. These samples were dried in an oven with forced air circulation at 80 °C until constant weight was obtained, to determine the dry matter production (W in mg), the difference of the final weight and the initial weight of each of the samples was calculated. This dry matter production was divided between 22 which is the number of days in which such production was also considered to be able to determine the NAR (dry matter production / unit area / unit time). From the measured leaf area (L₁) and estimated dry weight (W₁) at time t₁, the beginning of the experiment, and measured leaf area (L₂) and dry weight (W₂) at the end, time t₂, the net assimilation rate of each plant was estimated in the usual manner as $NAR (mg\ cm^2\ d^{-1}) = [(W_2 - W_1) (\log_e L_2 - \log_e L_1)] / [(L_2 - L_1)(t_2 - t_1)]$.

Morphological traits

Leaf length, width, thickness, number, fresh and dry weight.- During the experimental period, mature leaves (approximately 20-30 cm of length) were cut at the base with a sharp knife without damage the plant. For all leaves, the next characteristics were measured: leaf length (cm), width (cm) which was the average of three measures done in three places along the leaf (near the base, center and the tip), thickness (cm), which was measured in the same places where width was measured and determined using a digital caliper (General No 143, General Tools, Manufacturing Co., Inc., USA). Fresh weight (g) was determined using a conventional scale (Ohaus, model CT600-S, series 18939, USA). Each harvested leaf was placed in a pre-heated oven (Shel-Lab, model Fx-5, serie-1000203, USA), at 80 °C, until constant weight, in order to obtain the dry weight (g).

Table 1. Analysis of variance of physiological characteristics of *Aloe vera* subjected to salt stress (NaCl). RWC = relative water content, SLW = specific leaf weight, SLA = specific leaf area. Significant level of *F* statistic at ** *P* < 0.01, *** *P* < 0.001, ns= not significant *P* > 0.05.

Source	df	Chl a		Chl b		Total Chl		RWC		SLW		SLA		NAR	
		MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F
Salinity	4	0.247	ns	0.164	ns	0.464	ns	0.3	ns	0.0321	**	0.005	**	0.067	ns
Error	15	0.181		0.076		0.471		0.5		0.006		0.001		0.026	

Leaf area.- Leaf area (cm²) was measured with a Li-Cor (Li-Cor, modelo-Li-3000A, series Pam 1701, Li-Cor Lincoln, USA) portable leaf area meter. To avoid damages in the equipment due the leave thickness and weight, each leaf was drawn on a sheet paper, then cut and passed through the portable leaf area meter and leaf area registered.

Seedlings number, fresh and dry weight.- Each seedling originating from the base of the main plant was recorded, including number, their fresh and dry weight (g).

Root number, length, fresh and dry weight.- At the end of the experiment, each main plant was harvested. For all plants, the next following characteristics were measured: root number, root length (cm), fresh (g) and dry weight (g). Fresh weight was determined using a conventional scale (Ohaus, model CT600-S, series 18939, USA). Each root was placed in a pre-heated oven (Shel-Lab, model Fx-5, serie-1000203, USA), at 80 °C, until constant weight, in order to obtain the root dry weight.

Stem fresh and dry weight.- For each main plant harvested, all leaves were cut including the roots and the fresh and dry weight of each stem was measured. Stem fresh weight (g) was determined using a conventional scale (Ohaus, model CT600-S, series 18939, USA). Each stem was placed in

a pre-heated oven (Shel-Lab, model Fx-5, serie-1000203, USA) at 80 °C, until constant weight was reached, in order to obtain the root dry weight (g).

Experimental design.- The saline treatments were 0, 30, 60, 90 and 120 mM de NaCl (0.3, 2.6, 5.1, 7.6 and 10.3 dS m⁻¹, respectively). The experiment was established under a completely randomized design with four replications and each replication contained three pots with one plant per pot. The treatments were applied after 51 days of tap water irrigation. All plants were watered daily with an excess of appropriate saline solution with nutrients containing (mg L⁻¹) 220 N, 40 P, 200 K, 140 Ca, 42 Mg, 4 Fe, 1.25 Mn, 0.18 B, 0.23 Zn, and 0.25 Cu. Watered with an excess of solution (500 mL) permitted to flush the pots allowing draining the excess of solution to maintain the level of salinity. All drained solutions were collected to measure the electrical conductivity and verify that the salinity of the treatment solutions and the drained solutions were alike. The pH of all treatments solutions was maintained close to 6.5 by adding H₂SO₄ or KOH. Plants were grown on average with a 12-h photoperiod of 279 ± 23-μmol m⁻² s⁻¹ photosynthetically active radiation. The length of the experiment was 150 days.

Statistical analysis.- Data was analyzed using univariate and multivariate analysis of variance (ANOVA and MANOVA)

Table 2. Analysis of variance of morphometric characteristics of *Aloe vera* subjected to salt stress (NaCl). Significant level of *F* statistic at * *P* < 0.05; ** *P* < 0.01, *** *P* < 0.001, ns= not significant *P* > 0.05.

Source	df	Seedlings						Stems					
		Number		Fresh weight		Dry weight		Fresh weight		Dry weight			
		MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F		
Salinity	4	4.11	**	44683.1	***	19.25	***	512.3	ns	11.292	ns		
Error	15	0.872		5335.6		3.06		319.3		17.081			

Source	df	Leaf													
		Number		Fresh weight		Dry weight		Length		Area		Thickness		Width	
		MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F	MS	P > F
Salinity	4	0.252	ns	2200.8	***	0.472	ns	35.48	**	731.3	***	1.452	*	0.109	***
Error	15	0.534		323.0		0.256		9.78		144.4		0.496		0.020	

Source	df	Roots							
		Number		Length		Fresh weight		Dry weight	
		MS	P > F	MS	P > F	MS	P > F	MS	P > F
Salinity	4	11.31	ns	11.76	ns	333.0	ns	33.99	ns
Error	15	23.64		14.75		320.0		43.98	

Table 3. Effect of salt stress (NaCl) in physiological characteristics of *Aloe vera* L. Values within the same column with same letters are not significantly different at $P < 0.05$ (Tukey's HSD multiple range test).

NaCl (mM)	Chl a (mg cm ²)	Chl b (mg cm ²)	Chl total (mg cm ²)	RWC (%)	SLW (mm ² mg ⁻¹)	SLA (mg mm ⁻²)	NAR (mg cm ² d ⁻¹)
0	4.80 a	2.01 a	6.81 a	97.73 a	1.45 b	0.703 a	0.383 a
30	5.38 a	2.23 a	7.62 a	97.80 a	1.54 ab	0.680 ab	0.427 a
60	5.17 a	2.38 a	7.55 a	97.59 a	1.66 a	0.621 ab	0.593 a
90	5.98 a	2.33 a	8.31 a	97.47 a	1.66 a	0.618 b	0.607 a
120	4.89 a	2.56 a	7.45 a	97.63 a	1.59 ab	0.638 ab	0.692 a

for one way classification, being saline treatments (NaCl) the study factor. The experiment was conducted using a completely randomized design with four replications. Analysis of variance and significant differences among means were determined by one way analysis of variance (ANOVA). The least significant differences Tukey's HSD test ($P = 0.05$) were calculated for one way by ANOVA. In all cases, differences among means were considered significant at $P < 0.05$. Before ANOVA, relative water content (%) was transformed using arcsine transformation (Sokal and Rohlf, 1995). All analyses were done with Statistica software program v 10.0 for Windows.

Results

The MANOVA analysis showed significant differences between NaCl treatments (Wilks = 0.000000094, $F = 6.34$, $P = 0.01$), affecting some physiological and morphometric variables. It can be seen that the relationship of Wilks possibilities is significant at the level of 0.01. This reaffirms that there are differences between the factors in this study in some of the measured variables, and strengthens the likeli-

hood that the differences observed in the univariate analysis (ANOVA) performed on the variables, are real differences and not false positives or differences that occur simply by randomized chance (Johnson, 2000).

Physiological traits

Chlorophylls.- For Chl a, Chl b and total Chl, ANOVA showed no significant differences between NaCl treatments (Tables 1, 3).

Relative water content (RWC).- No significant differences between NaCl treatments were observed in RWC (Tables 1, 3).

Specific leaf weight (SLW) and specific leaf area (SLA).- Significant differences between NaCl treatments were observed in SLW (Table 1). Specific leaf weight was higher at 60 and 90 mM NaCl with lower values at 0 mM (Table 3). Significant differences of SLA were showed between NaCl treatments (Table 1). SLA showed higher values at 0 mM with lower values at 90 mM (Table 3).

Table 4. Effect of salt stress (NaCl) in morphometric characteristics of *Aloe vera* L. Values within the same column with same letters are not significantly different at $P < 0.05$ (Tukey's HSD multiple range test).

NaCl (mM)	Seedlings			Stems		Roots			
	Number	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Number	Length (cm)	Fresh weight (g)	Dry weight (g)
0	10.04 a	286.05 a	5.93 a	86.70 a	10.27 a	39.41 a	27.83 a	106.41 a	16.60 a
30	9.54 ab	217.79 ab	5.08 a	79.94 a	6.52 a	37.16 a	29.08 a	106.18 a	10.55 a
60	8.79 ab	182.73 ab	4.64 a	71.70 a	6.80 a	37.25 a	26.50 a	88.05 a	9.49 a
90	8.00 b	95.82 bc	2.63 ab	59.30 a	6.04 a	34.66 a	26.83 a	88.73 a	9.84 a
120	7.62 b	15.70 c	0.48 b	63.51 a	7.12 a	37.08 a	30.66 a	101.25 a	12.30 a
						Leaves			
	Number	Fresh weight (g)	Dry weight (g)	Area (cm ²)	Length (cm)	Thickness (cm)	Width (cm)		
0	10.95 a	126.88 a	3.34 a	119.24 a	39.07 a	5.88 a	3.22 a		
30	10.83 a	90.94 ab	2.65 a	96.66 ab	34.48 ab	5.34 ab	2.97 ab		
60	11.08 a	82.77 b	2.81 a	96.22 ab	34.75 ab	4.70 ab	2.96 ab		
90	10.50 a	64.82 b	2.47 a	82.65 b	31.61 b	4.30 b	2.78 b		
120	11.12 a	77.19 b	2.58 a	91.51 ab	32.00 b	5.07 ab	2.87 ab		

Net assimilation rate (NAR).- No significant differences between NaCl treatments were observed for NAR (Tables 1, 3).

Morphological traits

Leaf length, width, thickness, number, fresh and dry weight.- Salinity affected some leaf characteristics. Significant differences between NaCl treatments were observed for leaf length (Table 2). Higher values of this variable were observed at 0 mM and generally this variable decreased as NaCl increased (Table 4). Width and leaf thickness showed significant differences between NaCl treatments (Table 2). Both variables showed higher values at 0 mM and lower values at 90 mM (Table 4). No significant differences between NaCl treatments were observed for number of leaves (Tables 2 and 4). Leaf fresh weight showed significant differences between NaCl treatments (Table 2) with higher values at 0 mM and decreasing as NaCl increased (Table 4). No significant differences between NaCl treatments were observed for leaf dry weight (Tables 2 and 4).

Leaf area.- Leaf area was affected by NaCl showing significant differences between NaCl treatments (Table 2). The higher leaf area was greater at 0 mM followed by 30 and 60 mM and smallest at 90 mM (Table 4).

Seedlings number, fresh and dry weight.- The results showed that salinity stress affected all variables related to seedlings, which showed significant differences between NaCl treatments (Table 2). The seedlings number increased in the main plants treated with 0 mM and decreased as NaCl increased (Table 4). Fresh and dry weight showed higher values at 0 mM and both variables decreased as NaCl increased (Table 4).

Root number, length, fresh and dry weight.- None of the variables related to roots showed significant differences between NaCl treatments (Tables 2, 4).

Stem fresh and dry weight.- None of this variable showed significant differences between NaCl treatments (Tables 2, 4).

Discussion

The results obtained in this study are not in agreement with other studies where they have found that chlorophyll reduces as salinity stress increased (Azooz *et al.*, 2004; Dagar *et al.*, 2004; Jaleel *et al.*, 2008). The results of the present study shows that *Aloe vera* leaf chlorophyll content under NaCl stress maintain chlorophyll pigments and the stability of the pigment protein complex. Also, the salt ions do not interfere with the *de novo* synthesis of proteins, the structural component of chlorophyll, since it did not cause the breakdown of chlorophyll. The results suggest that NaCl stress has no

negative effects on the photosynthetic metabolism of *Aloe vera* plants, at least at the NaCl levels tested. Other studies showed some differences of chlorophyll between *Aloe* species under different ecological conditions including saline water where *Aloe eru* had more chlorophyll than *Aloe vera* (Tawfik *et al.*, 2001). The chlorophyll a/b ratio of the present study was 2.24 on average, which coincides with those reported by Tawfik *et al.* (2001), they reported values less than 3.0, a condition that characterizes CAM plants. On the other hand, NaCl treatments reduced the specific leaf area (SLA), which indicates that leaf expansion and carbon allocation were altered. Since plant physiologists have for many years used specific leaf area [SLA, area (mm²)/dry weight (mg)] to compare the thickness and/or density of leaves, some studies have shown that SLA is primarily influenced by plant species (Cunningham *et al.*, 1999), environment (e.g. the availability of soil moisture/nutrients and light, Li *et al.*, 2000) and changes in photosynthetic capacity [Pinkard and Beadle, 1998 (canopy size); Reich *et al.*, 1998 (between species)]. The SLA of the present study had higher values at 0 mM and lower at 90 mM, at even 120 mM the SLA was higher than at 60 mM (Table 3). These results are in agreement with those reported by Cramer *et al.* (1994), and Curtis and Lauchli (1986) where they found that leaf area ratio is significantly reduced in some species under saline stress. The inverse of SLA is namely specific leaf weight (SLW) and has been used as an indicator of leaf toughness in some herbivory studies with insects (Landsberg, 1990). In the present study, the SLW was higher at two NaCl concentration, 60 and 90 mM, being lower at 0 mM (Table 3) showing that this estimated parameter was not negatively affected by NaCl stress, contrary to that reported by Wahid (2004) who testified that in sugarcane two growth stages are affected by sodium, the leaf dry weight was more modified than leaf area, resulting in reduced SLW.

The net assimilation rate (NAR) showed no significant differences between NaCl treatments. These results are in agreement with those reported by Wignarajah (1990) who found that two major physiological traits enable the plants to tolerate salinity: (1) compensatory growth following adjustment to salinity, and (2) ability to increase both leaf area ratio (LAR) and net assimilation rate (NAR) to achieve this increased growth. In the same sense, Cramer *et al.* (1994) found in maize that relative growth rate and leaf area ratio were significantly reduced by NaCl, but net assimilation rate was unaffected. Also, Curtis and Lauchli (1986) found in kenaf that growth analysis following nine serial harvests over 40 days showed a significant decline in leaf area ratio with salt stress but no decrease in net assimilation rate. However, other species such as tomato (Alarcon *et al.*, 1994), and citrus (Ruiz *et al.*, 1997), have shown significant effects of net assimilation rate due to salinity. Relative water content of *Aloe* was unaffected by NaCl treatments. Other studies with *Aloe* have shown significant decreasing

trend in tissue water content, total soluble sugars and glucose when the plants were irrigated with 60 % of sea water concentration as compared to fresh water (Jin *et al.*, 2007). The relative water content of the present study shows values close to 98 % which demonstrate that the water content is not affected significantly in the leaf of *Aloe vera* growing under this regime of moderate salt stress, results that match with those reported by Ramírez *et al.* (2012) and Sahu *et al.* (2011) who described that *Aloe* gel in spongy form composed of parenchymatic cells containing > 98 % water concentrated with complex composition of various types of sugars, polysaccharides, phenols, and osmoprotectants. For his part, Neumann (1997) asserts that a better knowledge of the underlying physiology is required in order to understand why some species and varieties are more salt-resistant than others, which is a complex task since plant growth responses to salinity can vary with the duration and degree of stress encountered (mild, moderate, severe).

It is evident that high salinity stress could cause negative effects, which were demonstrated by Jin *et al.* (2007) studying the physiological and ecological characters on *Aloe vera* under seawater irrigation ($EC = 23.4 \text{ dS m}^{-1}$), they reported that salinity stress caused a decrease in tissue water, total soluble sugars and glucose. The salinity-tolerance and dehydration-tolerance showed by *Aloe* plants in the present study could be related with their metabolism, which is a crassulacean acid metabolism (CAM) plant with a specialized photosynthetic pathway (Cushman and Bohnert, 1997, 1999). This type of CO_2 assimilation is very efficient because it uses the same pathway of CO_2 assimilation as that found in C4 plants except that it occurs at night, meaning that stomata are closed during the day to avoid water loss and opened at night for CO_2 uptake (Borland and Taybi, 2004; Borland *et al.*, 2009). This type of assimilation contributes to strong dehydration-tolerance in *Aloe vera*, which was demonstrated by Delatorre-Herrera *et al.* (2010) and Silva *et al.* (2010) with *Aloe* plants that were capable of efficient osmotic adjustment during water deficit, increasing their water use efficiency, showing that 100 % of field capacity, equivalent to the amount of water needed to saturate the soil, is not the best condition for the growth and yield of *Aloe vera* plants, because of their water use efficiency increases considerably when water availability is at 75 % field capacity. Although water use efficient in *Aloe vera* during relatively mild water deficit, plants become stressed at severe water shortage, this species are generally considered most drought and salt-tolerant compared with other *Aloe* species such as *Aloe saponaria* (Sun *et al.*, 2003). This salt tolerance in *Aloe vera* could be related to high calcium and chloride content in their tissues (Ranganathan, 1970) or due to osmotic adjustment caused by the increase in protein, proline and PEP-case activity, as well as the absorption and accumulation of cations under NaCl stress (Murillo-Amador *et al.*, 2014). Other studies have shown that non-halophytic

succulent plants with crassulacean acid metabolism such as *Aloe vera*, shows diurnal variation in acid content and can synthesize more amino acids when they are subjected to mild salt concentration by metabolizing transaminases and inhibiting production of malate dehydrogenase (Karmarkar and Ranganathan, 1971). High concentrations of dissolved salts in the soil and root zone can cause the high osmotic pressure and, thus, water availability is reduced. These conditions affect physiological activities and subsequently crop yield since plants will need to spend more energy to obtain water (Hebbara *et al.*, 2003).

All variables measured in *Aloe vera* leaves showed significant differences between NaCl treatments exhibited lower values at 90 mM and higher values at 0 mM (Table 4). In one study, Rahi *et al.* (2013) found that number of suckers, leaf numbers, and leaf biomass increased significantly during two years at 15 and 30 exchangeable sodium percentage (ESP) as compared to control and 45 ESP. This information is in line with the present findings. In the present study, even if salinity affected the majority of *Aloe* leaf variables, the variables do not show the classical trend of decreasing as salinity increased as expected, which could be related with the moderately salt stressed which were subjected. However, other studies have revealed that salinity affects the number of leaves, plant height, number of sprouts, root weight, plant weight, leaf weight, total gel weight, and root dry weight (Moghbeli *et al.*, 2012) of *Aloe* plants subjected to 2, 4, 6 and 8 dS m^{-1} salinity. The fact that the majority of variables measured in *Aloe* leaves showed lower values at 90 mM is probably because under salt stress, energy is spent on plant homeostasis instead of growth and development (Maghsoudi and Maghsoudi, 2008). On the other hand, it has been demonstrated that osmotic pressure affects cell turgidity and development; opening and closing stomata is governed by turgidity of the guard cells whereas osmotic stress, induced by salts, suppresses cell turgidity and decreases cell division and apical meristem development (Rahimi-Dehgolan *et al.*, 2012). As *A. vera* has leaves with high percentage of water, cellular water loss or reduced cell volume leads to a decrease in growth. In the same sense, Rahimi-Dehgolan *et al.* (2012) indicated that salinity influenced to plant growth, morphological traits and biomass of *Aloe* when subjected to electrical conductivity of 3, 6, 9, 12, 15, 18, or 21 dS m^{-1} . In the current study, seedlings were more affected by salinity because the number of seedlings, fresh and dry weight decreased as salinity increased (Table 4). These results are in agreement with the classical response of the majority of non-halophytes plants where variables decrease as salinity increases and are in agreement with those results showed in *Aloe* plants by Moghbeli *et al.* (2012) and Jin *et al.* (2007). Also Pasternak *et al.* (1986), Fuentes and Rodriguez (1988) and Fuentes *et al.* (1988) reported similar results showing that the height of leaves and sprouts reduced with increasing salinity in different *Aloe* species; however, carbohydrates

and glycosides increased. Similar results have been reported in *Agave* species by De Kock (1980), Nobel and Berry (1985) and Upchurch (1981). Also Fuentes *et al.* (1988) reported two *Aloe* species that behaved favorably in soils with high salinity with little precipitation.

Results reported herein, showed that none of the variables measured in stems showed significant differences among NaCl treatments. These results are probably because of *Aloe vera* is xerophile with the characteristic that it can continue to grow and reproduce under stressed environments which is related to its adaptive physiology (Baker, 1981). Also, none of the variables measured in roots showed significant differences among NaCl treatments; curiously, root length was longer at 120 mM (Table 4) which demonstrated that this variable was not negatively affected by NaCl. Similar results were reported by Moghbeli *et al.* (2012) who found that *Aloe* plants are not able to tolerate extended periods of salinity stress while in short time spans they show some positive responses like longer root. The same authors also found that by increasing salinity stress, the plant response changed negatively, while during short periods (less than 60 days) plant respond positively to salinity stress. In other words short periods of stress are bearable, however increasing stress time eventually surpasses the degree of tolerance plant and eventually growth is reduced. This type of continuum response to increasing salt stress either by dosage or exposure was observed in this study; although the trend was visible it was not statistically significant. In addition, Mustafa (1995) suggested that in *A. vera*, weak dosages improve growth (0.1 % salinity), while 0.4 % salinity reduced growth parameters. Additionally, he demonstrated that the highest amounts of carbohydrates were obtained with 0.4 % salinity while the highest amount of crude aloin and barbaloins were obtained with 0.2 % salinity.

Conclusions

The present study reveals that *Aloe vera* has an ability to withstand salinity under moderate NaCl stress, such as that used in the present study. Although the trend was for *Aloe* fresh weight to decrease with salinity, dry weight did not significantly decrease indicating that biomass remained constant nevertheless there was a loss of water. In addition, even some of the morphometric traits of *Aloe* plants displayed higher values under NaCl treatments, which demonstrates that *Aloe* plants under moderate salt stress may respond favorably to certain dosages, similarly to what Mustafa reported in 1995. In general terms, the salinity-tolerance and dehydration-tolerance showed by *Aloe* plants in the present study could be related to the metabolism of *Aloe*, which is a crassulacean acid metabolism (CAM). This study with *Aloe* plants reveal that using moderate salt stress, instead typical high salt stress (> 200 mM) offers a new perspective in which to further study salinity-resistant

mechanisms of desert plants in a directed manner and may lead to creating new salt-resistant cultivars. This avoid the damages under NaCl treatments increasing the agronomic value of *Aloe* plants, making this species attractive for industrial production in arid or semiarid areas around the world with moderate salinity.

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