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Ion Accumulation, Water Relations and Osmotic Adjustment in *Atriplex argentina*, *A. crenatifolia*, *A. lampa* and *A. nummularia* under Saline Conditions

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Abstract: Salinity is a major stress that adversely affects growth and productivity in plants. There are species that tolerate this stress within the genus *Atriplex*. Four species, *A. lampa*, *A. crenatifolia*, *A. nummularia* and *A. argentina* were compared for their ion accumulation and water relations under saline conditions. A greenhouse study was conducted by irrigating the four species with NaCl solutions at concentrations 0%, 1%, 2% and 4% starting when plants were six months old. Plants were harvested 45 d after starting the salinity treatments and analyzed for their ion contents. In the four *Atriplex* species, Na⁺ and Cl⁻ contents in plants increased, while Ca²⁺ and Mg²⁺ decreased with the increase of salinity in the irrigation solution. The results suggested that *A. argentina* and *A. nummularia* were able to maintain a higher leaf relative water content (RWC) at low leaf water potential, which was associated with a greater capacity of osmotic adjustment. *A. lampa* showed lower ion accumulation and minor osmotic adjustment than the other species. It can be concluded that the accumulation of ions favors the lower osmotic potential and contributes to osmotic adjustment in these halophytes.

Key words: Atriplex sp., salinity stress, osmotic adjustment, ion accumulation, water relations.

1. Introduction

Plants growing in arid and saline soils are exposed to wide ranges of water stress and osmotic stress [1-3]. Soil water content and salinity are both factors affecting the soil water potential which modify plant water potential [4-6]. Saline soil environments not only reduce water potential in the plants, but also cause ion imbalance and toxicity, thus decrease plant growth and productivity [7, 8].

Halophytes plants are well adapted to extreme environmental conditions. These plants are able to avoid toxicity caused by specific ions and have the ability to adjust water potential in their tissues to levels more negative than the soil water potential [9-11]. Halophytes in the *Atriplex* genus are capable to absorb ions in excess of their nutritional needs,

particularly Na⁺ and Cl⁻ [12, 13], in order to make osmotic adjustments in their vacuoles, absorb water, and keep cells turgid [14].

The osmotic adjustment is a mechanism where the plants synthesize and accumulate inorganic ions and organic metabolites of low molecular weight to make plants more tolerant to saline environments [15]. The decrease of osmotic potential in leaves is generally regarded as an indicator of osmotic adjustment. However, this decrease in osmotic potential may also be caused by dehydration of tissues and reduction of osmotic volume [16].

In *A. triangularis*, as more salts are absorbed, the osmotic potential becomes more negative [17]. An osmotic adjustment in *A. halimus* seems to allow plants to develop normally [13, 16]. In *A. lampa*, the water potential decreased progressively when salinity in the root media increased [18]. This decline in water potential could be controlled by an osmotic

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adjustment, which maintains the turgidity required for continued growth [18]. In *Atriplex* species, both K⁺ and Na⁺ are involved in osmotic adjustment in leaves, when the soil water potential is low [4]. Khan et al. [11] demonstrated that osmotic potential and water potential in *A. griffithii* became more negative with increasing salinity, because accumulation of ions in the vacuoles.

In several *Atriplex* species, it was reported that a significant increase of Na⁺ and Cl⁻ ions were accumulated in stems and leaves, when the concentration levels of NaCl in the root substrate or irrigation water increased [10, 12, 13, 17, 19-22]. The increase of Na⁺ and Cl⁻ and decrease of K⁺, Ca²⁺ and Mg²⁺ concentration in plant tissues were reported in many Chenopodiaceae species when under saline stress [23-27].

In experiments with *A. prostate*, Wang et al. [20] reported that the stem water potential decreased from -1.26 MPa in plants irrigated with pure water to -3.00 MPa in plants irrigated with 2% NaCl solution, after 30 d of imposing the salinity stress. *A. nummularia* increases its relative water content (RWC) and osmotic adjustment when external NaCl increased [11, 22]. Glenn et al. [1] reported that the osmotic adjustment was almost entirely attributed to the uptake of Na⁺ and K⁺ cations plus a balancing anion.

There is no information regarding their different tolerance to saline soil environments, in Argentinean native species of *Atriplex*, such as *A. argentina* and *A. crenatifolia*. Currently, the two species are used as natural fodder for livestock in small communities of arid and semiarid regions of Argentina, where soils with salinity problems limit the extent of land that is available for grazing. The objective of this study was

to compare the responses to saline stress in three native species—A. lampa, A. argentina and A. crenatifolia, and an introduced species—A. nummularia.

2. Materials and Methods

2.1 Plant Material and Plant Growth

Dispersal units (capsules) of native species A. argentina, A. crenatifolia and A. lampa were obtained from arid and semiarid environments of San Juan province, Argentina. While, the dispersal units of A. nummularia were obtained from a nursery in the Agricultural Experimental Station, National Institute of Agricultural Technology (INTA), San Juan. Seeds were stored at 20 °C and 20% relative humidity (RH). Because bracts inhibit germination in Atriplex species [28], the seeds were scarified mechanically using a drawer thresher prior to planting and planted in 700 cm³ polyethylene bags filled with a sterile substrate composed of peat, perlite and sand (2:1:1 in volume, respectively). After emergence, seedlings were irrigated once a week, using distilled water and Hoagland solution [29]. For a period of six months, plants grew in containers which were placed on elevated benches, under a shade screen. Fifty plants of each species were selected for uniform height and irrigated with solutions of 1%, 2% and 4% NaCl (w/v) and deionized water (control) (Table 1). To avoid osmotic shock, salinity concentrations were gradually increased every 2 d until the final concentration was reached. In all treatments, plants were irrigated with the solutions every 48 h in order to maintain the substrate at field capacity and replace the water losses caused by evapotranspiration. Plants were harvested after 45 d of starting the salinity stress.

Table 1 Osmotic potential, electric conductivity and pH at 25 °C of NaCl solutions used to irrigate the Atriplex species.

Solutions	Osmotic potential (MPa)	Electric conductivity (dS/m)	pН
Control (deionized water)	0.00	0.09	6.98
1% NaCl (170 mM)	-0.55	16.90	6.97
2% NaCl (342 mM)	-1.23	31.70	6.84
4% NaCl (684 mM)	-2.72	52.80	6.86

2.2 Ash Content and Ion Accumulation

Six plants of each treatment were sampled and dried in an oven at 70 °C for 48 h. The dried plant material was milled and a subsample of 100 mg was burnt in a muffle furnace for 3 h at 500 °C. The relative weight of ash (%) was calculated. The ashes were dissolved in 5 mL of a sulfuric acid solution (20%) and subsequently in distilled water to obtain a 100 mL solution sample. The contents of Ca²⁺, Mg²⁺, Na⁺ and K⁺ in the sample solution were measured with an atomic absorption spectrophotometer (Analyst 200; Perkin Elmer). The accumulation of Cl⁻ was evaluated by back titration technique adapted from Dewis and Freitas [30], and data were expressed in mmol/kg dry weight (DW).

2.3 Plant-Water Relationships

Six plants were selected from each treatment 45 d after starting the salinity stress. The shoot water potential (Ψst) was measured using pressure chamber technique and sampling was performed at noon. The leaf RWC was determined using four fully expanded leaves and calculated as Eq. (1):

RWC (%) =
$$[(FW - DW)/(TW - DW)] \times 100$$
 (1) where, FW: fresh weight (g);

DW: dry weight (g) determined after 48 h in an oven at 70 $^{\circ}$ C;

TW: turgid weight (g) measured after 24 h of saturation in deionized water at 4 °C in the dark.

The leaf osmotic potential (Ψ o) was measured in four fully expanded leaves, taken from the middle of the stem. The selected leaves were frozen in liquid nitrogen for 5 s. The sample was pressed and the leaf sap was measured with a C-52 psychrometric chamber connected to a thermocouple psychrometer (Wescor HR-33T, Inc., Logan, Utah, USA). The samples were kept in the chamber during 1 h before osmotic potential was measured, in order to decrease measurement variability within treatments, as suggested by Ball and Oosterhuis [31]. The osmotic

adjustment of plants was calculated as Eq. (2) according to Refs. [32, 24]:

$$OA = \Psi oc - \Psi ot \tag{2}$$

where, OA: osmotic adjustment;

 Ψoc : osmotic potential of control leaves;

Yot: osmotic potential of treated leaves.

2.4 Statistics Analysis

The experiment was arranged as a completely random design with six replications. A two-way ANOVA was used to examine effects of species, salinity treatments and species by salinity interaction on the measured variables. In cases of significant interactions, the data were analyzed separately by species and salinity treatment [33]. Tukey's test was used to separate means. The measured variables (water potential, osmotic potential, RWC and osmotic adjustment) were evaluated with a multivariate analysis of conglomerates. This was obtained following the criteria of average Euclidean distance, average linkage and standardization of data, to compare species. All data were analyzed by using the statistical software InfoStat version 1.1 [34].

3. Results

The four *Atriplex* species had significantly difference in ash contents at different salinity levels. (Table 2). The ash contents increased significantly as the salinity augmented (Fig. 1a). The ash content of all species ranged from 14.17% in control plants of *A. crenatifolia* to 30.83% in *A. argentina* plants irrigated with the 2% NaCl solution. *A. crenatifolia* presented the lowest percentage of ashes in all treatments (18.3%) compared to the other three species. Conversely, *A. argentina* recorded the highest percentages of ashes in treatments of 1% and 2% NaCl compared to the other species. In *A. nummularia*, the increase in the ash content was progressive with the increase of the saline concentration.

When the salinity increased, Na⁺ and Cl⁻ concentration also increased (Figs. 1b and 1c). The

E4-:	P value by ANOVA			
Factor	Ash content	Na ⁺	K ⁺	Cl ⁻
Specie	< 0.0001	< 0.0001	< 0.0001	0.0001
Salinity level	< 0.0001	< 0.0001	0.1266	< 0.0001
Specie × salinity level	0.0003	< 0.0001	0.0391	0.0009

Table 2 P value for ash and ions $(Na^+, K^+ \text{ and } Cl^-)$ concentration in the four Atriplex sp. evaluated after treatments.

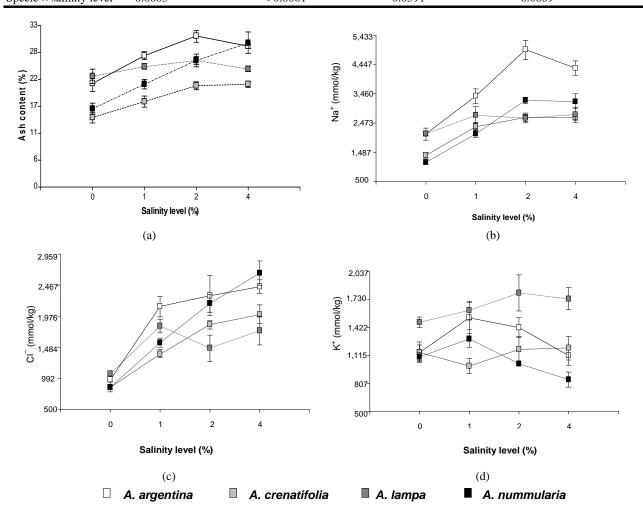


Fig. 1 Ash content (a), Na^+ (b), Cl^- (c) and K^+ (d) concentration in four *Atriplex* species after they were irrigated daily with NaCl solutions at 0% (control), 1%, 2% and 4% concentration levels after 45 d. Salinity treatments started when plants were 180 days old. Values are means of six replicates \pm standard error (SE).

concentration of Na⁺ and Cl⁻ in plants was significantly influenced by salinity level, species and its interaction (Table 2). *A. lampa* was the only specie, where the Na⁺ concentration was not significantly modified by the saline treatments (Fig. 1b). *A. argentina* presented the maximum Na⁺ concentration in all treatments compared with the other three species. When the plants were irrigated with distilled water or

1% of NaCl solution, A. crenatifolia and A. nummularia had a significant low concentration of Na⁺ compared to A. lampa and A. argentina. The Cl⁻ concentration in shoots had a similar tendency to that noticed for Na⁺, however that it was lower than Na⁺ concentration. There was significant interaction of specie \times salinity level in Cl⁻ concentration, where A. crenatifolia and A. lampa accumulated less Cl⁻ than

the others species (Fig. 1c). The introduced specie *A. nummularia* accumulated Cl⁻ rapidly, distinguishing their behavior from the rest of the species. Similarly to Na⁺ concentration, the Cl⁻ concentration in *A. lampa* increased progressively with the increase in salinity. However in *A. lampa*, there was a peak in Cl⁻ concentration for the treatment of 1% of NaCl.

The K⁺ concentration in shoots was changed significantly in response to salinity stress for species and the interaction between specie and salinity level (Fig. 1d). There was no significant difference between treatments for *A. argentina*, *A. lampa* and *A. crenatifolia*. Only in the introduced specie, *A. nummularia*, the K⁺ concentration was significantly reduced with increasing salinity, and presented the lowest concentration in 2% and 4% NaCl. *A. lampa* displayed the highest K⁺ concentration in all treatments, distinguishing it of the other three species.

The concentration of Ca²⁺ and Mg²⁺ in shoots changed significantly in response to salinity treatments (Table 3). The concentrations of these cations in the plants irrigated with saline solutions were lower than in control plants (Table 3). There was no significant interaction of salinity and specie for Ca²⁺ and Mg²⁺ concentrations in plants. The Ca²⁺ concentration was the greatest in *A. nummularia* (76.83 mmol/kg DW), while the lowest concentration

of both cations was measured in *A. crenatifolia* (Table 4).

The effect of the salinity on the plant-water relations are shown in Figs. 2-4. The shoot water potential (Ψst) of the plants decreased significantly with increasing salt concentrations in all species (Fig. 2), and there was a significant interaction between specie and salinity level. At control and 2% NaCl treatment, $A.\ lampa$ showed higher values of water potential (-2.61 MPa and -5.15 MPa, respectively) compared to the other species. The minimum shoot water potentials were registered in $A.\ crenatifolia,\ A.\ nummularia$ and $A.\ argentina$ at 2% and 4% NaCl treatments.

Similarly to water potential, the leaf osmotic potential (\$\mathcal{Y}_0\$) become more negative with the increase on salinity (Fig. 3), and also the interaction between specie and salinity level was significant. There were significant differences among species at controls and 1% NaCl treatment. However, no differences were found among species at 2% and 4% NaCl. \$A. lampa\$ presented more negative values osmotic potential (-5.27 MPa) than other species in controls and showed a less pronounced fall when increasing salt concentrations. While under irrigation with 4% NaCl, \$A. argentina\$ drastically reduced the osmotic potential (-9.53 MPa). Plants exposed to saline irrigation of 4%

Table 3 Effects of the salinity treatments on Ca²⁺ and Mg²⁺ concentrations in the four *Atriplex* species.

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Salinity level	Ca ²⁺ concentration in plants	Mg ²⁺ concentration in plants
Samily level	(mmol/kg DW)	(mmol/kg DW)
0% NaCl (control)	74.12 ^b	363.36°
1% NaCl	51.04 ^a	287.09 ^b
2% NaCl	50.32 ^a	277.32 ^{ab}
4% NaCl	53.85 ^a	250.75 ^a

Means with the same letter are not significantly different at P < 0.05 based on Tukey's mean separation test.

Table 4 Effects of the salinity treatments on Ca^{2+} and Mg^{2+} concentrations in A. argentina, A. crenatifolia, A. lampa and A. nummularia.

Species	Ca^{2+}	Mg^{2+}	
	(mmol/kg DW)	(mmol/kg DW)	
A. crenatifolia	48.44 ^a	270.29 ^a	
A. lampa	51.36 ^a	303.37 ^{ab}	
A. argentina	52.71 ^a	311.77 ^b	
A. nummularia	76.83 ^b	293.09 ^{ab}	

Means with the same letter are not significantly different at P < 0.05 based on Tukey's test.

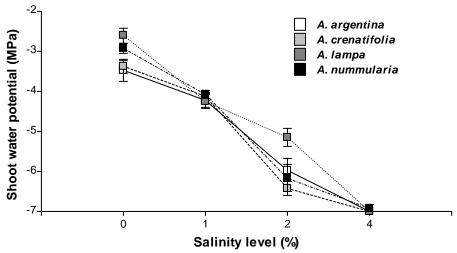


Fig. 2 Effects of NaCl on shoot water potential (MPa) occurring in *A. argentina*, *A. crenatifolia*, *A. lampa* and *A. nummularia*. Vertical lines are standard errors of means.

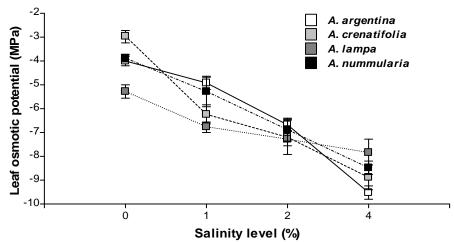


Fig. 3 Effects of NaCl on leaf osmotic potential (MPa) occurring in A. argentina, A. crenatifolia, A. lampa and A. nummularia.

Vertical lines are standard errors of means.

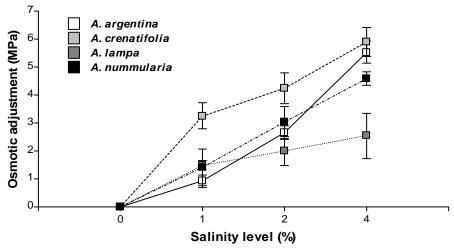


Fig. 4 Osmotic adjustment (MPa) in *Atriplex* plants irrigated with saline solutions of 0%, 1%, 2% and 4% NaCl. Vertical lines are standard errors of means.

Table 5 Average leaf relative water content (RWC) of A. argentina, A. nummularia, A. crenatifolia and A. lampa growing in saline conditions.

Species	RWC (%)
A. lampa	45.35 ^a
A. crenatifolia	49.19 ^a
A. argentina	59.46 ^b
A. nummularia	62.81 ^b

Means with the same letter are not significantly different at P < 0.05, based on Tukey's test.

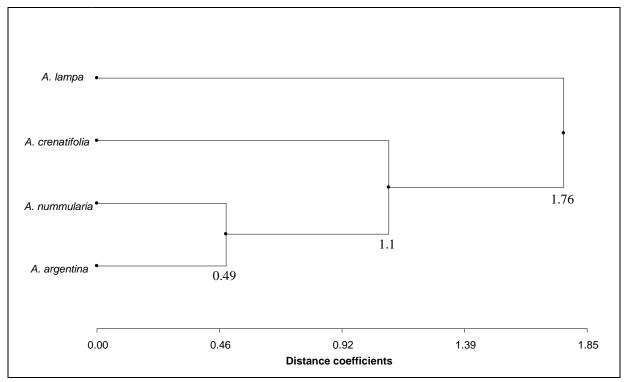


Fig. 5 Cluster analysis of four *Atriplex* species under saline conditions, using four water relations variables.

The levels of similarity between species are indicated by the distance coefficients of the lower scale (cophenetic correlation = 0.992).

NaCl showed the smallest values of osmotic potential, while controls retained the highest values (-2.98 MPa in *A. crenatifolia*).

In all the species, the osmotic adjustment increased with increasing NaCl concentration (Fig. 4). *A. crenatifolia* showed greater osmotic adjustment in all the salinity treatments. In *A. lampa*, the osmotic adjustment was significantly lower as compared to the other *Atriplex* species at the higher saline concentration.

There was no significant difference in RWC among treatments and the interaction between species and salinity level. When the species were evaluated, *A. argentina* and *A. nummularia* have the highest RWC

compared with the other two species (Table 5).

The cluster analysis differentiates two major groups in response to water variables evaluated (Fig. 5). *A. argentina* and *A. nummularia* had the lower coefficient (0.49), followed by *A. crenatifolia* (coefficient = 1.1) and *A. lampa* (coefficient = 1.76). In the group of *A. crenatifolia*, *A. argentina* and *A. nummularia*, the latter two species showed high levels of RWC in all treatments, and values are similar in osmotic adjustment, osmotic potential and water potential. However, *A. lampa* is very different from other species, as presented under settings, with low RWC and high osmotic potential in the controls and plants watered with 4% NaCl.

4. Discussion

In this study, the application of different saline treatments led to changes in the mineral portion of the four species. There was a steady increase in the percentage of ashes of plants as an increase of the inorganic ions on plants. Several authors reported similar results in other *Atriplex* species, such as *A. patula* [17], *A. prostata* [10], *A. canescens* [21, 35] and *A. griffithii* [11]. *A. lampa* was the only specie where the increase percentage of ashes was not significant, however in the other species, there was a significant increase of ashes, reaching the maximum values of 21% in *A. crenatifolia* and 29.50% in *A. nummularia* at 4% salinity level and 30.83% in *A. argentina* at 2% NaCl treatment.

It is widely accepted that when the Na⁺ and Cl⁻ are available on the ground, it can provide useful solutes to the cell to prevent harmful effects in cytosol and organelles [36]. The results in this study indicated that Na⁺ and Cl⁻ concentration in shoots increased with salinity. The pattern of Na⁺ accumulation was similar in A. crenatifolia, A. nummularia and A. argentina. In A. lampa, the concentration of Na⁺ was not modified coincident with the behavior of ashes accumulation. As well, Na⁺ and Cl⁻ concentration gradually increased as salt treatments increase in A. argentina, A. crenatifolia and A. nummularia. Similar values and pattern were reported in leaves and stems of A. nummularia [37], stems of A. prostata [12, 20], stems of A. patula [17], A. canescens [21] and others Chenopodiaceae [24, 26, 27, 38].

It was reported that the increased levels of Na⁺ and concomitant decreasing levels of K⁺ in shoots respond to the competition of Na⁺ with K⁺ for the same binding sites, and therefore interfere with the transport of potassium in the cell [26]. This study results demonstrated that the K⁺ content did not vary significantly among salt levels in *A. argentina*, *A. crenatifolia* and *A. lampa*, which agreed with results found for *A. canescens* [1, 21, 35]. Only in *A. nummularia*, the K⁺ concentration was reduced by the

increase salinity level. Similar results were reported by Ramos et al. [37] and Silveira et al. [22] in this species. Moreover, the same pattern was found in *A. halimus* [13], *A. prostata* [12, 20], *A. patula* [17] and *A. griffithii* [11].

The concentrations of Ca²⁺ and Mg²⁺ declined under saline conditions in all the evaluated species. These results also found in leaves as well as stems of *A. patula* [17] and *A. prostata* [20]. Furthermore, in other halophytes, Ca²⁺ and Mg²⁺ ions decreased with salinity increase, and these appears to be a common plant strategy in halophytes when the salinity increases [11, 24, 27].

One of the responses of halophytes to salinity stress is their capacity to adjust tissue water potentials to a level that is lower than that of the soil water potential in which they are growing [20, 39]. Shoot water potential and osmotic potential of all species become more negative with increased salinity, coinciding with other authors working in *Atriplex* species [11, 13, 18, 22]. Similar cases for water relations have also been reported for other halophytes [24, 25]. However, the values of Ψ st and Ψ o in this study were lower than values reported by Wang et al. [20] and Khan et al. [11] in similar NaCl concentrations. These species showed very low potentials, even in the plants without saline irrigation (control).

At 2% NaCl concentration, *A. lampa* diminishes the least its water potential in the four species studied, indicating that it has less ability to adjust its water potential as water potential of the medium increase. The results suggest that plants may counter the lower water potential imposed by NaCl solutions by absorbing ions from the bathing solution.

Like report of other authors [13, 16, 18, 40], all species made osmotic adjustment, when the salt concentrations increased. *A. crenatifolia* showed the highest osmotic adjustment at high salt concentration, demonstrating a greater adaptability to fluctuating saline environment. While *A. lampa*, made adjustments to a lesser extent than other species.

One characteristic of dicotyledonous halophytes is the increase in water content at moderate salt concentrations, which leads to an increase in the volume or leaf succulence [9, 23]. In this study, RWC was not significantly modified by the treatments, indicating that these species have mechanisms to maintain relatively constant RWC, even in stressful situations. These results were coincident with report in *Suaeda salsa* [27] and *Suaeda physophora* [25], where no differences in RWC with increasing concentrations of NaCl have been demonstrated. The RWC were lower in *A. lampa* and *A. crenatifolia* compared to *A. nummularia* and *A. argentina*, indicating inter-specific differences related to leaf anatomy.

The comparison between species in relation to the water balance was observed throughout the cluster. A. argentina and A. nummularia species were more similar, both maintained high values of RWC in all treatments, as well as similarities in the succulence of its leaves with a consequent greater ability to retain water. Also, the values are similar in osmotic adjustment achieved in the treatments. A. lampa, however, is very different from other species, accumulating less Na⁺ and Cl⁻, but more K⁺, and displaying less osmotic adjustment and low RWC. However, it showed similar water and osmotic potential at high saline treatment. The difference between A. lampa and the other species in the internal ion regulation may be due to different mechanisms of salt tolerance not developed in this study.

This work can help to select suitable species of *Atriplex* in order to produce forage, when one has saline grounds or waste water. Additional information on biomass production, contributing to the osmotic adjustment, will aid for more understanding of saline stress tolerance. Furthermore, the present study contributes new information about two native species of Argentina.

5. Conclusions

The application of saline solutions caused

substantial changes in the water balance of four *Atriplex* species. The four species could adjust their osmotic potential, so that they could continue absorbing water under high saline conditions.

It was showed that *A. crenatifolia*, *A. argentina* and *A. nummularia* had means to made themselves able to accumulate ions in their tissues in order to maintain osmotic adjustment. For plant survival in saline conditions, the mechanism of osmotic adjustment in these three species seemed to be more efficient than *A. lampa*.

In *A. crenatifolia*, *A. argentina* and *A. nummularia*, the absorption of ions from the irrigation solution may lead low water potential in shoots, when root environments have high NaCl concentration levels. This characteristic is critical in these three plant species, as they grow naturally in degraded soils with high salinity. Because of the limited food sources available to grazing animals in areas affected by salinity, the three *Atriplex* species should be taken in consideration when selecting species for planting.

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