

Comparative Effects of CaCl₂ and NaCl Salinity on Growth and Ion Partitioning of *Atriplex halimus* L.

Jamal Y. Ayad *

ABSTRACT

Atriplex halimus L. is a native persistent perennial shrub that is used for revegetation of degraded rangeland because of its ability to cope with environmental stresses. Seedlings of *Atriplex halimus* L. were grown in sand culture for four weeks and then treated with half strength Hoagland solution containing 0, 50, 100 and 300 mM of NaCl and CaCl₂ salts for twelve weeks. Results indicated that NaCl and CaCl₂ salinity caused a significant reduction in both leaf water content and relative water content by values of 72.5% and 63.7% of that in control. Reductions in photosynthetic pigments were also observed ranging from 23 to 58% for chlorophyll a and b and 45% for carotenoids at 300mM salinity. Effect of NaCl was higher than that of CaCl₂ on both water content and photosynthetic pigments. Leaves, stems, and roots dry weight and total biomass of atriplex was reduced by values ranging from 35 to 49% when treated with 300mM CaCl₂ and from 55 to 69% when treated with 300 mM NaCl. Ion partitioning between various plant parts was also affected by average salinity. Leaves accumulated higher levels of Ca, Na, Cl, and K than stems and roots. Calcium content was increased by four folds in leaves and stems and two folds in roots when treated with 300mM CaCl₂. Similarly, Na and Cl were also accumulated in leaves and stems but not in roots. Results indicated that *Atriplex halimus* L. tolerates moderate salinity up to 100mM without any significant impact on plant growth. Increased salinities greater than 300 mM starts to reduce plant growth. Presence of calcium in solution could ameliorate the problems caused by high salinity.

KEYWORDS: Atriplex Halimus, Biomass, Ion Content, Root Growth, Water Content, Salinity Stress.

1. INTRODUCTION

Drought and salinity are the major abiotic stresses limiting crop production world-wide. Salt and water stress are interrelated and coexist in natural saline environments, especially in arid and semi-arid regions. It was estimated that about 2.1% of area farmed by dryland agriculture are affected by secondary salinization to varying degrees (FAO, 2005). Plant responses to salt and water stress have much in common. Salinity reduces the ability of plants to take up water, and this quickly causes reductions in growth rate, along with a range of metabolic changes identical to those caused by water stress (Munns, 2002). The deleterious effects of salinity on plant growth are associated with osmotic stress, nutritional imbalance, ion toxicity, and or a combination of these factors (Shannon, 1998). Changes in ion and water equilibrium

associated with salinity lead to physiological, biochemical (Parida and Das, 2005) and molecular (Winicov, 1998) alterations of plant growth and development. Calcium can help to ameliorate the adverse effect of salinity on plants. It was reported to maintain membrane integrity and regulate ion transport and is essential for K/Na and Ca/Na selectivity (Maathuis and Amtmann, 1999; Renault, 2005). Increasing Ca concentration in nutrient solution was found to inhibit Na uptake (Kaya *et al.*, 2002; Nedjimi and Daoud, 2009) and reduce membrane leakage (Tuna *et al.*, 2007) and therefore reducing the adverse impacts of NaCl salinity on plants. Calcium plays a vital role in the regulation of ionic relations in plants and in improving the soil physical conditions (Qadir *et al.*, 2002).

In regions with low plant cover, it is important to consider the use of salt and drought-tolerant species for soil revegetation and preservation purposes. Revegetation of degraded rangelands using drought and saline tolerant species is an important method (Prider and Facelli, 2004; Salih, 1998). This has been revealed to be true in Jordan

* Department of Horticulture and Crop Science, Faculty of Agriculture, University of Jordan, Amman, Jordan. Received on 22/7/2010 and Accepted for Publication on 21/8/2010.

arid and semiarid areas where *Atriplex* was the major genus used usually in combination with water harvesting techniques (Al-Tabini *et al.*, 2008). Mediterranean saltbush (*A. halimus* L.) is a persistent perennial shrubs, native to Jordan, that keep their foliage throughout the year and are commonly used for extending the grazing season (Ortiz-Dorda *et al.*, 2005; Abbad *et al.*, 2004; Prider and Facelli, 2004; El-Shatnawi and Turk, 2002) and because of its tolerance to environmental stresses (Ben Hassine *et al.*, 2008; Nedjimi *et al.*, 2006). The biological approach, to overcome the salinity problems, has received considerable attention in the last few decades (Weber *et al.*, 2007). Conducting research about *Atriplex* species responses to salinity stress is becoming of great concern due to the expected increase in aridity in many areas of the globe. Moreover, quantifying the tolerance levels and identification of physiological properties used by xero-halophyte species *A. halimus* L. to cope with salt is of great interest (Chaves and Oliveira, 2004; Yamaguchi and Blumwald, 2005; Sambatti and Caylor, 2007). Therefore, this study was conducted to assess the comparative effects of CaCl_2 and NaCl salinity on leaf water status, growth and ion partitioning. The ability of *A. halimus* to maintain biomass production is an indicative of the potential to sustain productivity under salinity.

2. MATERIALS AND METHODS

Plant Materials and Growth Conditions

The experiment was carried out at the University of Jordan, Faculty of Agriculture Research Station at Jubeiha (32.02° N, 35.52° E, 980 m altitude). The seeds of *A. halimus* were obtained from the National Center for Agricultural Research and Extension (NCARE), Jordan. Seeds were surface sterilized using clorox (0.5%) for one min., followed by thorough rinsing with distilled water, and then placed in plastic pots (20 x 20 cm) containing 8 kg sand culture. There were eight treatments involved in this experiment including two salt types (NaCl and CaCl_2) and four salinity levels (0, 50, 100, and 300 mM). Plants were grown for four weeks in ambient conditions, and then thinned to one plant per pot, after which treatments were started. Plants were grown in a greenhouse at a thermoperiod of 18-25°C / 30-35°C (night/day), and an average of 14 h photoperiod (450 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 400-700 nm). Irrigation water using NaCl and CaCl_2 solutions (Table 1) prepared in half

strength Hoagland solution was started and increased by 50 mM in one-day intervals to the maximum. Irrigation with various saline solutions was made to maintain soil moisture above 80% of field capacity by weighing method for twelve weeks. The necessary leaching requirements were estimated according to Corwin *et al.* (2007) ranging from 0.01 to 0.3 of added solution. Pots were arranged in a completely random design with four replications and the position of the pots was changed weekly to avoid a position effect in the greenhouse.

Table 1. Average values of electrical conductivity (dSm^{-1}) for various NaCl and CaCl_2 solutions prepared in half strength Hoagland solution and used throughout the experiment.

Salt type	Concentration (mM)			
	0	50	100	300
NaCl	1.1	6.1	11.2	30.9
CaCl_2	1.1	8.3	14.8	43.5

Measurements

Fresh leaf samples were taken six weeks after the initiation of salinity treatments for the measurements of photosynthetic pigments. Chlorophyll a, b and carotenoid contents were estimated by Arnon method (1949) in leaf samples (0.5 g) homogenized in 10 ml 80% acetone. Absorbance was recorded at 645, 633 and 470 nm (Spectrophotometer Cary 100, Varian Australia PTY LTD., Australia). the same time, leaf relative water content (RWC) estimation was done excising five fully expanded leaves from each treatment. The fresh weight was immediately recorded after leaf excision (FW). The leaves were left in distilled water for 24 h at 25°C in darkness and the turgid weight was recorded (TW). The dry weight (DW) was then measured after 48 h at 80°C. The RWC was calculated according to Barrs and

$$\text{Watherley (1968) as: } \text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100\%$$

Twelve weeks after the initiations of treatments, plants were harvested and separated into leaves stem and roots for fresh weight measurements. Leaf area was measured using LI-3000 leaf area meter (LI-COR Inc, USA). Furthermore, roots samples were scanned to estimate the root length using a root scanner (Regent STD 1600+) and WinRhizo Pro 2005_b software (image analysis software).

Leaf, stem and root samples were oven dried at 70°C for 48 hours and weighed for dry matter determination. Leaves water content was determined from fresh and dry leaves weight per plant as

$$(LWC(\%)) = \frac{FW - DW}{FW} \times 100\%.$$

Salt tolerance was

measured as total plant dry weight at different salt concentrations compared to the total plant dry weight obtained for the controls.

Dried leaves, stems and root samples were ground into fine powder with M1F 10 basic mill (IKA-WERKE,

Germany) prepared for chemical analysis. Chlorine extraction was done by de-ionized water then titrated with 0.005 N AgNO₃ solution according to (Miller, 1998). Sodium, potassium and calcium concentration was determined after dry ashing and extraction in HCl using flame photometry for Na, K and atomic absorption spectrophotometry for Ca (Ryan *et al.*, 2003).

Data collected were subjected to analysis of variance (ANOVA) by Statistical Analysis System (SAS, 1997). Differences among treatment means were compared by using LSD test at 5% probability level.

Table 2. Interactive effects of CaCl₂ and NaCl on relative water content (RWC) and leaves water content (LWC) of *A. halimus*

		Concentration (mM)			
	Salt Type	0	50	100	300
RWC (%)	CaCl ₂	91 a	79 b	72 b	66 c
	NaCl	91 a	80 b	73 b	58 c
LWC (%)	CaCl ₂	72 a	77 ab	68 bc	57 c
	NaCl	72 a	69 ab	66 b	51 c

Means within the same row followed by the same letters are not significantly different at P<0.05.

Table 3. Interactive effects of NaCl and CaCl₂ salinity on dry matter partitioning of different plant parts and total biomass (g plant⁻¹) and root to shoot ratio of *A. halimus*

		Concentration (mM)			
Part	Salt	0	50	100	300
Leaves	CaCl ₂	12.2 a	14.1 a	11.7 ab	7.9 b
	NaCl	12.2 a	10.5 a	9.4 a	3.8 b
Stems	CaCl ₂	14.1 a	13.1 a	12.8 a	7.2 b
	NaCl	14.1 a	11.2 a	11.0 a	4.4 b
Roots	CaCl ₂	2.9 a	3.0 a	2.2 a	1.9 a
	NaCl	2.9 a	2.1 ab	2.6 a	1.2 b
Biomass	CaCl ₂	29.3 a	30.1 a	26.8 a	16.9 b
	NaCl	29.3 a	23.8 a	23.1 a	13.1 b
Salinity tolerance	CaCl ₂	100 a	103 a	93 a	58 b
	NaCl	100 a	82 a	78 a	46 b
R:S ratio*	CaCl ₂	0.108	0.111	0.091	0.122
	NaCl	0.108	0.102	0.132	0.148

Means within the same row followed by the same letters are not significantly different at P<0.05.

* No significant effect of concentration and salt type

Table 4. Interactive effects of NaCl and CaCl₂ salinity on leaf area, root length, root diameter and root length density (RLD, cm root.cm⁻³ soil) of *A. halimus*

	Salt	Concentration (mM)			
		0	50	100	300
Leaf Area (cm ² plant ⁻¹)	CaCl ₂	1561 a	1720 a	1373 a	715 b
	NaCl	1561 a	1198 ab	1055 b	445 c
Root Length (cm plant ⁻¹)	CaCl ₂	8960 a	9098 a	4807 ab	2958 b
	NaCl	8960 a	5360 a	4541 a	3654 a
Root Diameter* (mm)	CaCl ₂	1.19	1.09	1.28	1.34
	NaCl	1.19	1.10	1.28	1.37
RLD	CaCl ₂	7.1 a	7.2 a	3.8 ab	2.4 b
	NaCl	7.1 a	4.3 a	3.6 ab	2.9 b

Means within the same row followed by the same letters are not significantly different at P<0.05.

* No significant effect of concentration and salt type

3. RESULTS AND DISCUSSION

Leaf Water Content

Results indicated that NaCl and CaCl₂ salinity caused a significant reduction in both leaf water content and relative water content by values of 72.5 and 63.7% of that in control (Table 2). Reductions in leaf water content were less pronounced in response to CaCl₂ especially at high salinity level (300mM) as compared to NaCl. Reductions in leaf water content may be due to the possibility that lowered water potentials in the roots can trigger a signal from root to shoot, such as abscisic acid (Sibole *et al.*, 2003). Trends in plant water content paralleled those of fresh weight declined with increased salinity. Measurements of plant water status indicated that *Suaeda fruticosa* plants adjusted their water potential and osmotic potential to more negative levels as salinity increased. (Khan *et al.*, 2000b). In halophytes, water content and the ability to make osmotic adjustments have been seen as important determinants of growth response (Ben Amor *et al.*, 2005).

Photosynthetic Pigments

Salinity stress (100 and 300 NaCl and CaCl₂) resulted in significantly progressive decline of the photosynthetic pigments (Figure 1). Reductions in chlorophyll a and b concentration ranged from 23 to 58%, respectively at 300 mM as compared to the control. No significant differences were observed between NaCl and CaCl₂ for both chlorophyll pigments. On the other hand, NaCl

salinity caused higher reductions in carotenoids (45%) as compared to CaCl₂ (12.5%) at 300 mM level. The decreased in chlorophyll content under salinity stress could be attributed to the effect of salinity on inhibition of chlorophyll synthesis or accelerating its degradation (Reddy and Vora, 1986). Ion accumulation in leaves also adversely affected chlorophyll concentration (Yeo and Flowers, 1983). Netondo *et al.*, (2004) found that the chlorophyll content reduction of sorghum leaves started to occur in plants grown at 100 mM NaCl and higher concentrations which is consistent with the present study results. The decrease in carotenoids under salt stress leads to degradation of β -carotene and formation of zeaxanthins, which are apparently involved in protection against photoinhibition (Sharma and Hall, 1991).

Plant Growth and Dry Matter Accumulation

Dry matter partitioning of atriplex plants was only significantly affected (P<0.01) by the highest level of salinity imposed (300mM, Table 3). Leaves, stems, and roots dry weight and total biomass of atriplex was reduced by 35, 49, 34, and 42% when treated with 300 mM CaCl₂ and by 69, 69, 58 and 55% when treated with 300mM NaCl. The negative impact of NaCl was higher than that of CaCl₂ especially on leaves and stems. Salinity tolerance estimated as a percentage of control was also affected by the highest salinity level (300mM). 10% threshold reduction in salinity tolerance was obtained at 50 mM NaCl and at 100 mM CaCl₂. Root to shoot ratio was not affected by neither salt type nor concentration.

Table 5. Interactive effects of NaCl and CaCl₂ levels on Ca, Na, Cl, and K ions concentration (ppm) of various parts of *A. halimus* plants.

Plant Part	Conc. (mM)	Ca		Na		Cl		K	
		CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl
Leaves									
	0	115 c	115 a	137 b	137 c	394 c	394 c	138 a	138 a
	50	239 b	137 a	151 ab	151c	449 c	436 bc	114 b	64 b
	100	262 b	102 a	143 ab	268 b	524 b	478 ab	97 b	65 b
	300	475 a	112 a	171 a	375 a	596 a	512 a	90 b	27 c
Stem									
	0	89 b	89 a	85 b	85 c	241 c	241 b	31a	31 a
	50	171 b	117 a	88 b	100 c	314 b	272 b	26 b	18 b
	100	179 b	147 a	112 ab	180 b	325 b	269 b	24 b	14 b
	300	361 a	182 a	131 a	235 a	491 a	322 a	18 c	6 c
Roots									
	0	38 b	38 a	58	58	63	63	27 a	27 a
	50	72 a	56 a	55	58	73	74	25 ab	19 b
	100	77 a	44 a	57	62	74	79	21 bc	11 c
	300	75 a	38 a	69	71	80	84	18 c	6 d

Means within the same row followed by the same letters are not significantly different at P<0.05.
ns = non significant differences among treatments.

Leaves area per plant was also significantly affected by salt type and concentration ($p < 0.05$, Table 4). Reductions in leaves area at 300 mM reached 54% for CaCl₂ and 71% for NaCl. On the other hand, root length was affected by 300mM CaCl₂ level with no obvious effects for NaCl. Root length density measured as unit root length per unit soil volume was effected also by salt concentration. Root density was decreased by values exceeding 60% at 300mM salinity level. Although, there was no significant effect of salinity level on root thickness, there is a trend of increasing root thickness by 12-15% at 300 mM.

The reduction in biomass production could be due to the high concentration of Na and Cl ions (Zhu, 2001). Presence of calcium in the salt improved the growth of salinized plants. This beneficial effect of calcium on the growth of many species in response to saline environments has been reported (Nedjimi and Daoud, 2009). In this study, optimal growth of *Atriplex halimus*

was recorded at 50 to 100 mM CaCl₂ and at 50 mM NaCl which is in agreement with what was obtained (Nedjimi *et al.*, 2006).

Studies have shown that *Atriplex spp.*, such as *A. nummularia*, *A. griffithii* and *A. hortensis*, could survive under highly saline conditions; with optimal growth occurring at 5 to 10 g l⁻¹ NaCl (Khan *et al.*, 2000a; Ramos *et al.*, 2004; Wilson *et al.*, 2000). Low salinity levels do not appear to have a deleterious effect on the growth of *Atriplex spp.* and may actually stimulate growth which may be related to the uptake of solutes that are required to maintain water uptake by plants and therefore induce cell expansion (Sai Kachout *et al.*, 2009). In the present study, shoot dry weight was affected similarly to that in roots dry weight by salt concentration and this led to unaffected root to shoot ratio. Reduction of plant growth under saline conditions is a common phenomenon (Ashraf and Harris, 2004) but such reduction occurs differently in different plant organs. For example, in the

present experiment, root dry weight was reduced similarly to that of leaves and stems dry weight by salt stress. In contrast, Nedjimi and Daoud (2009) observed that salt stress inhibited the growth of shoot more than root in *Atriplex halimus* subsp. *Schweinfurthii* and therefore, root/shoot ratios were higher in salt treatment

compared to nonstressed control treatment. Moreover, root growth showed a substantial promotion in low salinity (90 mM NaCl) and further increases in salinity caused a progressive decline in growth (Khan *et al.*, 2000a).

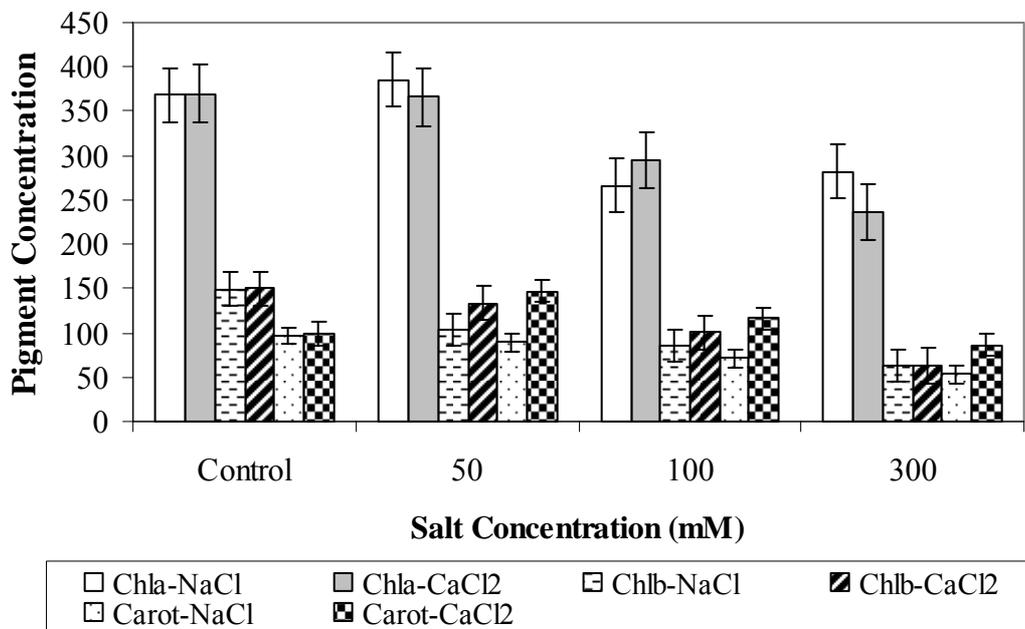


Figure 1. Effect of NaCl and CaCl₂ levels on concentration of photosynthetic pigment (µg.g⁻¹ fresh weight) of *A. halimus*. Vertical bars indicate standard error

Ion Accumulation and Partitioning

Ion partitioning between various plant parts was also affected by salt type and salinity level (Table 5). Leaves accumulated higher levels of Ca, Na, Cl and K than stems and roots. Calcium content was only affected by CaCl₂ levels in different plant parts. No effect of NaCl levels was observed on Ca contents. Calcium content was increased by four folds in both leaves and stems and two folds in roots in response to 300mM CaCl₂. Similarly Na and Cl were accumulated in the leaves, stems in response to salinity but not in roots. Use of 300mM salinity accumulated Na by values ranging from 114 to 270% for NaCl and from 120 to 150% for CaCl₂. Higher levels of Na and Cl were accumulated in leaves and stems. On the other hand, increasing salt concentration resulted in significant reductions in K levels in the three plant parts. Reductions in K content ranged from 35 to 80 in both leaves and roots and from 42 to 80% for stems when plants treated with 300 mM CaCl₂ and NaCl, respectively.

Saline tolerant plants have the ability to accumulate

ions, such as Na and Cl in their vacuole to avoid inhibition of metabolic processes (Munns, 2002). Mineral imbalances of the root environment are commonly encountered by plants grown under salinity which often affect the chemical composition and structure of root and shoot and may thereby interfere with nutrient acquisition, transport, and compartmentation (Grattan and Grieve, 1999; Grieve *et al.*, 2004; Maathuis and Amtmann, 1999; Munns, 2005). Salt tolerance of *A. halimus* is related to the absorption of high quantities of Na and Cl and their use in the osmotic adjustment (Nedjimi and Daoud, 2006). At high salinities, growth reduction might be caused by a reduced ability to make osmotic adjustments as a result of saturation of solute uptake system (Munns, 2002).

Calcium is known to have regulatory role in metabolism (Cramer *et al.*, 1986) and high Na uptake by plants may compete with Ca ions for membrane binding sites (Busch, 1995) and therefore stressed plants have lower Ca to Na ratios (Ashraf and Akhtar, 2004). Increasing NaCl in growth medium, significantly reduced

potassium concentrations. This reduction in K is well documented which may lead to stunting plant growth due to K role in controlling turgidity (Renault *et al.*, 2001). Competition between both Na and K to entry into root cells can have significant negative effects on plant growth, where concentrations of Na often exceed those of K (Tester and Davenport, 2003). This can result in high Na / K ratios that reduce plant growth and eventually become toxic (Maathuis and Amtmann, 1999).

On the other hand, presence of Ca reduces the Na/K selectivity by shifting the uptake ratio in favor of K at the expense of Na (Tuna *et al.*, 2007). At all treatments in this experiment, a large percentage of Cl was sequestered in the leaves, stems and roots of *A. halimus*, Chloride accumulation correlated negatively to growth and this could be attributed to the competition with other minerals such as NO_3^- and PO_4^{3-} (Renault, 2005; Ruiz *et al.*, 1999).

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4. CONCLUSION

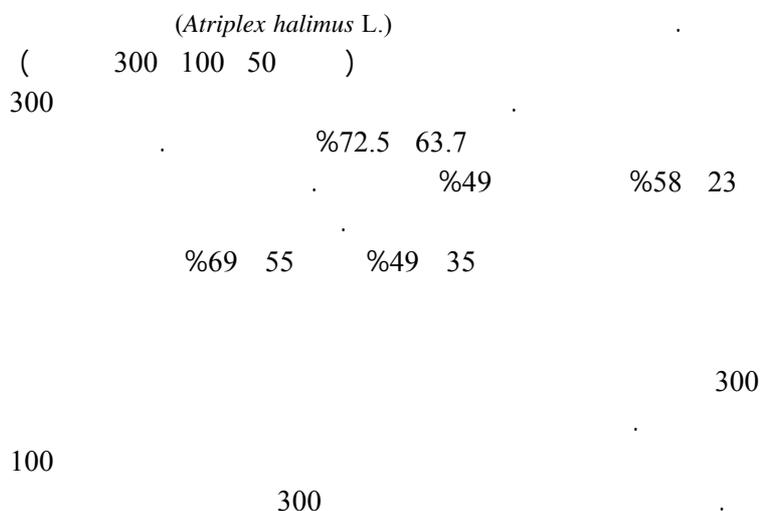
Results indicated that *Atriplex halimus* L. tolerates moderate salinity up to 100 mM without any significant impact on plant growth. Increased salinities up to 300 mM and above resulted in reduced dry matter partitioning between leaves, stems, and roots. NaCl salinity reduced K^+ and Ca^{2+} contents in shoots and roots. However, calcium chloride as a source of salinity improved the variables affected by high NaCl salinity (plant growth) and also increased K^+ and Ca^{2+} . Ion partitioning between various plant parts was also affected by various salinities. Leaves accumulated higher levels of Ca, Na, Cl, and K than stems and roots. Calcium content was increased by 4 folds in leaves and stems and 2 folds in roots when treated with 300mM CaCl_2 . Similarly, Na and Cl were also accumulated in leaves and stems but not in roots.

- Mediterranean xero-halophyte species *Atriplex halimus* L differ in their ability to accumulate proline and glycinebetaine in reponse to salinity and water stress. *Journal of Experimental Botany*, 59(6): 1315-1326.
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