Calcium supply reduced effect of salinity on growth in the Mediterranean shrub (*Atriplex halimus* L.)

Samira Soualem[1, 2], Ahmed Adda[1, 2], Moulay Belkhodja[3] and Othmane Merah[4, 5]

[1]Laboratoire d'agro-biotechnologie et de nutrition en zones semi-aride Université Ibn Khaldoun de Tiaret, Algérie
[2]Laboratoire de physiologie végétale appliquée aux cultures hors sol. Université Ibn Khaldoun de Tiaret, Algérie
[3]Département de Biologie, Laboratoire de Physiologie Végétale, Université d'Es-Sénia, Oran, Algeria.
[4]Université de Toulouse, INP-ENSIACET, LCA (Laboratoie de Chimie Agro-industrielle), F-31030 Toulouse, France
[5]INRA, UMR 1010 CAI, F-31030 Toulouse, France
samirasoualem2006@yahoo.fr

Abstract: The effect of calcium sulfate ($CaSO_4$) supply under salt stress was studied in two populations of *Atriplex halimus* from two locations (coastal western Algeria (Oran) and continental semi-arid zone (Djelfa)) contrasted for salinity gradients. The plants were grown in pots and subjected to salt stress (0, 300 or 500mM NaCl) with a supply of (5 or 10mM) of $CaSO_4$. Growth, mineral, proline and soluble sugars contents were measured. The results showed a reduction in growth with increasing NaCl concentration. The impact of salinity was more pronounced on the inland population than the coastal one. The leaves Na^+ content increased with increasing salt stress and led to reduced plant growth. In response to the intensity of salt stress and $CaSO_4$ supply, plants accumulated more soluble sugars, proline and K^+. This accumulation was more pronounced at high concentrations of NaCl and $CaSO_4$ in both populations. Our results emphasized that supply of $CaSO_4$ reduced the inhibitory effects of NaCl.

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1. Introduction

Salinity is one of the most important environmental factors limiting crop production of marginal agricultural soils in many parts of the world. Soil salinity affects plant growth in a variety of ways, reducing water uptake, causing toxic accumulation of sodium chloride and reducing nutrient availability. Salinity also induces water deficit even in well-watered soils by decreasing the osmotic potential of soil and therefore it is difficult for roots to extract water from their surrounding media (Jaleel *et al.*, 2007). In these zones and other dry lands, halophytic plants, as *Atriplex* spp., often dominate because of their tolerance to drought and salinity (Martınez *et al.*, 2003). These species are adapted to salinity by tolerating salts internally and/or by excreting salt (Tattini *et al.*, 2008). However, the presence of high salt levels does not seem to be required for optimal growth. It is reported that soil salinity as well as water deficit suppresses shoot growth more than the root growth (Sahnoune *et al.*, 2004; Adda *et al.*, 2005). Salinity have been reported to cause reduction in leaf area as well as in rate of photosynthesis, which together result in reduced crop growth and yield (Boughalleb *et al.*, 2009)

Tolerance of halophytes to the ionic and the osmotic components of salt stress is linked to their ability to synthesize osmoprotectants in order to maintain a favourable water potential gradient and to protect cellular structures (Sharma and Dietz, 2003). Osmoregulation system is one of the defensive responses to abiotic stresses particularly to salinity and drought (Ben Hassine *et al.*, 2010). Compatible solutes accumulation in the cytoplasm is considered a mechanism to impart salt tolerance (Jaleel *et al.*, 2007).

In the Chenopodiaceae species, such as those belonging to the genus *Atriplex*, osmotic adjustment is thought to be mainly performed through the accumulation of the glycinebetaine which may protect membranes and other cellular structures against destabilization induced by salinity (Shen *et al.*, 2002). In *Atriplex halimus*, both proline and soluble sugars may be accumulated in response to salinity (Martınez *et al.*, 2004). However, *A. halimus* is also characterized by a large intraspecific variability in terms of physiological strategy allowing the plant to cope with NaCl and osmotic stress affecting many traits (Ben Hassine *et al.*, 2008). The main differences are osmolites contribution in osmotic adjustment and their kinetic of accumulation (Martınez *et al.*, 2004). Recently, inland and coastal populations have been reported to be able to produce both proline and glycinebetaine (Ben Hassine *et al.*, 2010). However, their accumulation appeared to be different, with a faster accumulation of proline in comparison to glycinebetaine. It has been also demonstrated that the coastal population was more tolerant to salinity than the inland one and displayed higher ability to accumulate both Na^+ and Cl^{2-} to higher extent (Ben Hassine *et al.*, 2008). Though, there are many studies were undertaken in this respect, it becomes necessary to find more discriminating criteria, which can provide information about the different adaptive strategies to cope with salt stress and could help the selection of suitable genotypes of this species for semi-arid regions

In last decade, it has been reported that supply of calcium reduces Na^+ toxicity symptoms in different plant species and promotes plant growth (Bonilla *et al.*, 2004). Calcium is also seems to play an important role in Na^+ exclusion and retention mechanisms, which may be an important ability for survival under saline conditions (Levent *et al.*, 2007; Tattini *et al.*, 2008). Apart from effects on intracellular targets, supply Ca^{++} may provide rapid and long-term protection against salinity stress via its stabilizing effect on cell wall components, membrane lipids and proteins (Lauchli and Grattan, 2007). Moreover, elevated extracellular Ca^{++} inhibits the membrane injuries (Tattini *et al.*, 2008).

Most reports previously done on *Atriplex halimus* have focused on water stress (Martınez *et al.*, 2003; Martınez *et al.*, 2004; Sharma and Dietz, 2006) on wild ecotype from Tunisia, Morocco or both. Neveretheless, no information is available on Algerian ecotype and their response to salt stress. A better characterization of the response of populations of *Atriplex halimus* from different regions and ecosystems are needed, since this species exhibits a high level of phenotypic and genetic variability. Therefore, the present work aims to determine response of two Algerian coastal and inland populations to saline conditions and to examine the effect of Ca^{++} supply and its impact on salinity tolerance in Mediterranean Shrub (*Atriplex halimus* L.).

2. Material and methods

2.1. Plant material

The seeds of *A. halimus* were collected from the area of El Mesrane in the province of Djelfa, inland zone; 3°03'E, 34°36'N, and 830 m elevation and the area of Es-Senia in the province of Oran, a coastal site from west Algeria 35º38´N, 00º36´W,and 92m elevation. The soil salinity level was estimated on independent soil samples per site. Electrical conductivity was measured on saturated paste extracts collected at a 20cm depth using a Mobile-Cassy 524009 conductimeter. The mean electrical conductivity was 1.03±0.43 $mS.cm^{-1}$ for Djelfa and 18.2±1.03 $mS.cm^{-1}$ for Oran, confirming therefore the saline properties of the latter and the non-saline character of the former site.

2.2. Growth conditions

Experiment was carried out in greenhouse at the Laboratory of Plant Physiology of University Ibn Khaldoun of Tiaret (West of Algeria, 35.33 35° 19'N, 1.333 1° 19'E and 1075m elevation). After removal of the fruiting bracts, surface seeds were sterilized in 97% ethanol during 30s, followed by a treatment with 0.8% formaldehyde for 40 min and 5% calcium hypochlorite for 20min. They were rinsed three times with sterile deionized water. Seeds were therefore sown in washed sand trays and watered daily. Thirty days after emergence, 1-month-old similar vigorous seedlings were transplanted in pots containing sand. Along the experimentation the diurnal temperature was maintained at 30°C (±2°C) and night 15°C (±2°C). Plants were irrigated daily with full strength nutrient solution containing: 3mM KNO_3, 4mM $Ca(NO_3)_2$, 2mM H_3PO_4, 2mM $MgSO_4$, 5mM NaCl, 9.25µM H_3BO_3, 35.8µM Fe-EDDHA, 1.8µM MnSO4, 1.35µM $ZnSO_4$, 0.79µM $CuSO_4$, and 0.21µM $(NH_4)\ 6Mo_7O_{24}$, pH 5.8. After 3 months of acclimation, the salt stress was induced by applying NaCl at low (300mM) and high (500mM) concentrations, combined or not with $CaSO_4$ at 5 and 10mM as presented in **Table 1**. The experimental design was a complete randomized blocks represented by nine treatments applied to both populations. In each treatment, populations were represented by four replications.

2.3. Measurements of growth, mineral and organic solutes concentration

From each treatment, four plants were harvested. The number of nods in the principal stem was measured after 3 months of saline treatment. The dry weight (DW) of the shoot was also determined after oven drying the plants at 80°C for 48 hours.

For free proline quantification, 1g of tissue was extracted with 5mL of salicylic acid (5%); after centrifugation at 5000g, free proline was specifically quantified according to (Bates *et al.*, 1973 17).

Soluble sugars were extracted in 80% ethanol from 1g of leaf fresh tissue and quantified by the classical anthrone method using a spectrophotometer (Pharmacia Biotech. Novaspec II, Ontario, Canada). A standard curve was established using glucose and the results are therefore expressed in $µg.g^{-1}$ of fresh weight (FW).

For major cations (Na^+, Ca^{++}, K^+) quantification, tissues harvested on five plants per saline treatment were oven-dried at 80°C for 48 h, and 50mg dry weight (DW) were digested in 35% (v/v) HNO3. Analyses were performed by flame atomic absorption spectrophotometer (Perkin Elmer AA200n, California, USA).

2.4. Data analysis

Analyses of variance (Statistica 8 software package) were performed in order to assess effects of salt treatments and populations. The mean pair-wise comparisons were based on the Duncan test.

3. Results

Effects of populations, salt treatments and calcium supply on growth, organic and mineral components are displayed in **Table 2**. Mean values of each trait in each salt and Ca supply treatment for each ecotype are presented in **Table 3**.

3.1. Effect of salinity and $CaSO_4$ on growth

There were significant population effect on dry shoot weight and number of nods (**Table 2**). In contrast, the two traits in both populations were significantly reduced when NaCl substrate content was increased (**Table 2**). Nevertheless, the impact of salinity on growth was less marked in ecotype from Oran (coastal population). For example, in the plants treated with 500Mm of NaCl, the reduction of shoot dry matter was about 72.98% in Djelfa (inland population) plants and 47.67% Oran plants. Moreover, there was significant population X salt treatment interaction for both characters (**Table 2**).

Exogenous application of $CaSO_4$ clearly and significantly improved the growth in both populations. The supply of 10mM of $CaSO_4$ strongly increased growth at high NaCl concentration (**Table 3**).

3.2. Effect of salinity and $CaSO_4$ on soluble sugars and proline contents

Both NaCl and $CaSO_4$ treatments caused a significant modification of total soluble sugars and proline contents in leaves whatever the population (**Tables 2 and 3**). Effect of ecotype was significant only on proline content. Salt, ecotype and Ca supply interactions were significant for both biochemical traits (**Table 2**). As showed in Table 3, addition of 300mM of NaCl increased slightly the soluble sugars content, in coastal plants (4%) compared to inland ones (164%) at the same condition. In contrast, at higher concentration of NaCl (500mM), a marked increase of sugars content was recorded in coastal population plants. Similar variations were observed for proline content in leaves.

Total soluble sugars and proline contents in salt-treated plants increased in a similar way in response to $CaSO_4$ supply for both populations. This trend was more pronounced in the plants grown at high concentration of NaCl (500mM).

3.3. Effect of salinity and $CaSO_4$ on mineral content

Salinity reduced significantly Ca^{++} concentration in the both populations (**Tables 2 and 3**) in comparison to control plants. The highest stress intensity (500 mM NaCl) strongly decreased the Ca^{++} concentration. This decrease was more marked in coastal plants than in inland ones (**Table 3**). Supply of $CaSO_4$ induced accumulation of higher concentration of calcium in leaves whatever population and salt treatment (**Table 3**). Moreover the greatest increase of Ca^{++} concentration was obtained when higher $CaSO_4$ treatment was applied.

As expected, the rise of salinity significantly increased Na^+ concentrations in both populations (**Tables 2 and 3**). Plants from Oran accumulated more Na^+ than those from Djelfa in all treatments (**Table 3**). The Ca supply reduced significantly the accumulation of Na^+. This decrease was more important in Oran plants at the highest salt treatment.

Ecotype and salt treatment affected significantly K^+ concentration in leaves (**Tables 2 and 3**). Plants from the two origins showed contrasted responses to salt treatment increase. Indeed, concentration of K^+ in leaves of inland plants decreased with the raise of salt treatment. In contrast, coastal plants exhibited an increase of K^+ content in leaves when NaCl concentration raised (**Table 3**). Supply of $CaSO_4$ had more pronounced effect on K^+ content. The concentrations of K+ in plants of both populations increased with the augmentation of $CaSO_4$. Under saline conditions, Ca^{++} supply increased the K^+ concentration in all treated plants.

Table1. Composition of treatment solutions used in our study.

Treatment	Composition
Control	Nutrient solution
NaCl 1	300mM NaCl
NaCl 2	500mM NaCl
5Ca	5mM $CaSO_4$
10 Ca	10mM $CaSO_4$
NaCl 1+5Ca	300mM NaCl+5mM $CaSO_4$
NaCl 1+10Ca	300mM NaCl+10mM $CaSO_4$
NaCl 2+5Ca	500mM NaCl+5mM $CaSO_4$
NaCl 2+10Ca	500mM NaCl+10mM $CaSO_4$

Table3. Effects of NaCl and $CaSO_4$ supply on growth, organic compounds and mineral contents. F values are displayed

Source of variation	Shoot dry weight	Number of nods	Soluble sugars	Proline	Na^{+}	K^{+}	Ca^{++}
Ecotype effect	9.11**	29.68**	2.5ns	62.0**	102.6**	12.2**	1.0ns
NaCl effect	122.49**	388.35**	45.2**	20.1**	155.1**	5.0*	96.7**
$CaSO_4$ effect	7.26**	7.95**	35.5**	37.7**	51.8**	24.8**	261.7**
Ecotype × NaCl effect	76.43**	16.74**	0.8ns	3.3*	0.2ns	11.6**	1.2ns
Ecotype × $CaSO_4$ effect	0.27ns	1.80ns	0.8ns	3.6*	3.7*	1.6ns	4.2*
NaCl × $CaSO_4$ effect	17.14**	22.62**	2.8*	4.5**	4.1**	0.7ns	11.1**

ns : non significant, * : significant at p=0.05, ** : significant at p=0.01 level.

Table 3. Mean values±ES of growth traits and biochemical composition traits measured on plants of *Atriplex halimus* from Djelfa and Oran exposed for 90 days to different salt treatments (0, 300 or 500mM NaCl) and/or $CaSO_4$ supply (5 or 10mM).

Population	Treatment (mM)	Shoot dry matter (g)	Number of nods /plant	Soluble sugars ($\mu g.g^{-1}$ FW)	Proline ($\mu mol.g^{-1}$ FW)	Na^{+} ($\mu mol.g^{-1}$ DW)	Ca^{++} ($\mu mol.g^{-1}$ DW)	K^{+} ($\mu mol.g^{-1}$ DW)
Inland (Djelfa)	Control	12.4 ± 1.04^{k}	24.0 ± 0.40^{h}	18.3 ± 2.21^{a}	21.1 ± 0.12^{b}	743 ± 40.9^{bc}	426 ± 5.33^{d}	1195 ± 94.86^{cdef}
	NaCl-300	5.0 ± 0.16^{b}	11.0 ± 0.57^{b}	48.5 ± 0.21^{bcd}	28.5 ± 1.35^{cd}	1292 ± 14.7^{gh}	373 ± 27.05^{bcd}	1223 ± 51.15^{defg}
	NaCl-500	3.35 ± 0.42^{a}	7.5 ± 0.28^{a}	41.6 ± 0.28^{bc}	13.8 ± 1.18^{a}	1126 ± 24.0^{ef}	307 ± 6.26^{ab}	1061 ± 31.88^{abc}
	Ca-5	11.57 ± 0.50^{jk}	21.5 ± 0.50^{h}	41.8 ± 0.21^{bc}	29.0 ± 1.21^{cde}	610 ± 14.2^{ab}	703 ± 9.13^{f}	1239 ± 37.20^{defg}
	Ca-10	10.69 ± 0.44^{ij}	20.5 ± 1.32^{f}	42.2 ± 0.2^{bc}	30.0 ± 0.60^{cdef}	558 ± 94.2^{a}	717 ± 3.23^{f}	1300 ± 26.24^{efg}
	NaCl-300+Ca-5	6.7 ± 0.69^{c}	12.25 ± 0.47^{bc}	55.0 ± 0.48^{cd}	30.5 ± 1.57^{cdef}	1167 ± 107^{fg}	593 ± 52.81^{e}	1258 ± 7.42^{def}
	NaCl-300+Ca-10	7.1 ± 0.12^{cd}	13.6 ± 0.50^{cd}	65.1 ± 0.56^{efg}	32.3 ± 0.01^{defg}	972 ± 47.1^{de}	717 ± 3.23^{f}	1306 ± 7.79^{fg}
	NaCl-500+Ca-5	4.3 ± 0.07^{ab}	11.5 ± 0.28^{b}	62.3 ± 0.61^{def}	22.1 ± 0.35^{b}	1097 ± 36.7^{ef}	392 ± 6.00^{cd}	1136 ± 38.36^{bcd}
	NaCl-500+Ca-10	6.5 ± 0.25^{c}	14.67 ± 0.33^{de}	71.7 ± 0.72^{fg}	32.0 ± 0.52^{defg}	923 ± 27.6^{d}	557 ± 12.89^{e}	1375 ± 7.40^{g}
Coastal (Oran)	Control	9.67 ± 0.39^{hi}	22.5 ± 0.86^{gh}	39.3 ± 0.41^{b}	25.5 ± 2.82^{bc}	1091 ± 14.27^{ef}	354 ± 42.58^{bcd}	9233 ± 12^{a}
	NaCl-300	8.4 ± 0.38^{defgh}	14.75 ± 0.25^{de}	40.8 ± 2.46^{bc}	34.0 ± 0.02^{efg}	1520 ± 19.6^{ij}	313 ± 3.76^{abc}	1036 ± 43.15^{ab}
	NaCl-500	5.06 ± 0.21^{b}	12.0 ± 0.57^{bc}	48.1 ± 0.95^{bcd}	28.2 ± 1.08^{cd}	1543 ± 70.32^{j}	264 ± 38.5^{a}	1147 ± 74.51^{bcde}
	Ca-5	8.2 ± 0.24^{defg}	21.25 ± 0.47^{fg}	41.5 ± 0.21^{bc}	33.1 ± 0.79^{defg}	860 ± 10.71^{cd}	681 ± 3.63^{f}	102343.55^{ab}
	Ca-10	7.62 ± 0.48^{cdef}	20.0 ± 0.40^{f}	39.2 ± 0.21^{b}	34.9 ± 0.43^{fg}	682 ± 8.06^{ab}	716 ± 9.30^{f}	1192 ± 10.78^{cdef}
	NaCl-300+Ca-5	9.02 ± 0.33^{fgh}	12.25 ± 0.40^{de}	53.4 ± 1.31^{bcde}	35.0 ± 0.39^{fg}	1399 ± 44.51^{hij}	697 ± 11.9^{f}	1230 ± 75.68^{defg}
	NaCl-300+Ca-10	9.17 ± 0.32^{gh}	15.8 ± 0.58^{e}	73.0 ± 0.49^{fg}	36.1 ± 0.35^{g}	1361 ± 48.61^{hi}	711 ± 11.5^{f}	1249 ± 87.68^{defg}
	NaCl-500+Ca-5	7.47 ± 0.30^{cde}	13.5 ± 0.28^{cd}	63.7 ± 2.4^{ef}	32.8 ± 0.79^{defg}	1490 ± 32.63^{ij}	383 ± 29.9^{bcd}	1300 ± 4.47^{efg}
	NaCl-500+Ca-10	8.77 ± 0.36^{efgh}	15.67 ± 1.45^{e}	78.5 ± 0.44^{g}	33.0 ± 0.39^{defg}	904 ± 20.4^{cd}	556 ± 55.2^{e}	1304 ± 6.33^{efg}
	LSD	0.80	1.48	10.1	3.5	121.3	55.8	103.1

Mean values±ES of each trait and for both populations with same letter are not significantly different at the threshold of 5%.

4. Discussion

Effects of salinity on plants result from complex interaction between morpho-physiological and biochemical processes (Munns and Tester, 2008) allowing to show injurious indications. Our results showed that increasing salt concentration induced a strong decrease in the growth estimated on the shoot dry weight and number of nods in both coastal and inland populations (**Tables 2 and 3**). Several studies have shown that *A. halimus* responded to low salinity by increased plant growth, but higher salt levels induce morphological injuries (Ma *et al.*, 2006; Manchanda *et al.*, 2008; Boughalleb *et al.*, 2009; Reef *et al.*, 2012). It is well known that abiotic stresses reduce significantly plant growth and production (Merah, 2001; Sahnoune *et al.*, 2004; Adda *et al.*, 2005; Munns and Tester, 2008). In fact, Salt stress may inhibit cell division and elongation and therefore the plant development by reducing growing tissues in shoots (Ma *et al.*, 2006; Manchanda *et al.*, 2008; Boughalleb *et al.*, 2009; Reef *et al.*, 2012) which leads to the decrease of dry matter content and grain yield (Martinez *et al.*, 2003; Adda *et al.*, 2005). However, halophyte species may preserve adequate photosynthetic machinery under salt stress which leads to maintain sufficient metabolic activity and growth (Rabhi *et al.*, 2012).

A significant variability was found between populations for shoot growth. Moreover, results also revealed that the shoot growth is more sensitive to salinity in inland plants than in coastal population (**Table 3**). These results confirm previous reports that have shown differences in dry matter production between Coastal and Inland populations of Mediterranean Shrub (Ben Hassine *et al.*, 2008, 2010). The present investigation also demonstrated that the two populations respond differently to Ca^{++} supply. There was gradual decrease of growth in inland plants (Table 3) with an increase in NaCl concentration at low calcium supply (5mM). In contrast, supplementary Ca^{++} at the different harvests mitigated the injurious effect of salinity in coastal plants.

These results were in full agreement with those reported in pea, tomato and *Avicennia marina* plants (Bonilla *et al.*, 2004; Levent *et al.*, 2007; Reef *et al.*, 2012) and have highlighted that the presence of Ca^{+2} ions contributes to alleviate significantly the toxic effects of salinity by enhancing tissue growth (Bonilla *et al.*, 2004; Manchanda *et al.*, 2008).

In our study, carbohydrate content increased in leaves with the rise of salt concentration suggesting that this comportment has a positive impact on plant resistance to this constraint. Moreover, supply of gradual concentration of $CaSO_4$ improved shoot total soluble sugars contents in both coastal and inland plants, whatever the salt treatment (**Table 3**) as previously reported (Bonilla *et al.*, 2004). Similarly, coastal plants accumulated higher amounts of proline than those of the inland population grown under salinity (**Table 3**).

Accumulation of compatibles solutes reduces osmotic potential in the cytoplasm and contributes to maintaining water homeostasis among several cellular compartments (Reef *et al.*, 2012). Among all organic compounds, soluble carbohydrates represent about 50% of the total osmotically active organic solutes (Manchanda *et al.*, 2008; Zebib, 2012).

In the present study, $CaSO_4$ applications to salt-stressed plants resulted in significant increase of proline contents, especially at high salinity treatment (500mM NaCl) and the effect was more marked in inland plants (**Table 3**). Differences between populations of Mediterranean Shrub for osmolites may be involved in both osmotic adjustment and protection of cellular structures (Levent *et al.*, 2007; Ben Hassine *et al.*, 2008; Tattini *et al.*, 2008).

The ability of plants to control cytoplasmic Na^+ accumulation against vacuolar compartmentation is critical for salt tolerance determination and is involved in cellular process such as the sequestration of toxic Na^+ ions in vacuoles and is consistent with adaptation to soil salinity (Ma *et al.*, 2006; Munns and Tester, 2008). Moreover, physiological assays suggest a major role for shoot tissue tolerance to Na^+ ions which confirms that survival rate is more correlated with the Na^+ content of the apoplast fraction of the shoot rather than with total shoot Na^+ content (Manchanda *et al.*, 2008; Lowry *et al.*, 2009; Zebib, 2012). In our work, maximal Na^+ content in the leaves was observed in salt-stressed plants (**Table 3**). Accumulation of this ion was always higher in coastal plants than in inland ones.

Inland and coastal populations of *Atriplex halimus* have been found to differ widely in terms of physiological strategy allowing the plant to cope with NaCl and osmotic stress (Levent *et al.*, 2007; Ben Hassine *et al.*, 2008). One possible reason for this finding is that populations are adapted to osmotic stress but by different mechanisms. Inland populations may be adapted to osmotic stress from rapidly drying soils during the summer drought while costal populations are adapted to osmotic stress caused by soil salinity. This hypothesis is supported by studies in other coastal and inland ecotypes of plants such as *Atriplex halimus* (Ben Hassine *et al.*, 2008) and *Mimulus guttatus* (Lowry *et al.*, 2009). Moreover, a significant correlation was already noticed between leaf Na^+ concentrations and salt tolerance in this Mediterranean shrub (Walker *et al.*, 2008). However, Na^+ judged toxic at the high doses inducing diminished growth and an accumulation of various organic solutes characteristic of stress conditions. Involvement of Na^+ in stress metabolism is not restricted to the osmotic adjustment, especially when considered that more than 40% of the absorbed Na^+ is excreted through trichomes at the leaf surface to remove the excess of deleterious toxic ions from photosynthetically active tissues (Reef *et al.*, 2012). This may explain the high accumulation of this ion at 500mM NaCl in our study (Table 3).

Exogenous treatment with CaSO4 induced a decrease in Na content in both populations exposed to NaCl especially at higher doses. This result suggests that, sodium ions may compete with calcium ions for membrane selectivity which allowed proposing that high calcium levels can protect the cell membrane from the adverse effects of salinity (Busch, 1995).

In our study, K^+ contents increased slightly in the presence of NaCl, and were higher in leaves of inland plants than those of coastal ones (**Table 3**). It has been suggested that high-salt concentration in the root zone can induce the uptake and transport of major ions such as K^+ and Ca^{++} and that salt stress causes a drastic decrease in K^+ contents in the shoot of cereals (Shen *et al.*, 2002; Lauchli and Grattan, 2007). An opposite trend was recorded in the case of *A. halimus* which could be explained by the mineral uptake specificity in some halophytes (Levent *et al.*, 2007; Manchanda *et al.*, 2008). However, the content of K^+ was slightly enhanced by supply of $CaSO_4$. Ca^{++} have been reported to improve the K^+/Na^+

selectivity of the membrane and alleviate the toxic effects of Na+ in plants (Lauchli and Grattan, 2007).

5. Conclusion

In our study, salt stress affected plant growth and this effect was depending on stress intensity. This fact results from mineral uptake and imbalance in the availability of different ions. As result, metabolism activities were reduced which in turn induced decrease in different physiological activities leading in the depression of plant growth. The inland and coastal populations supported differently salt stress by accumulating osmolites by various ways.

The supply of $CaSO_4$ to NaCl-stressed plants had a significant role in alleviation of salinity stress. Indeed, plant growth was significantly improved in both populations by different physiological processes. This fact is probably at the origin of the natural distributions of the two populations in saline or arid environments. $CaSO_4$ supply under high salinity reduced the absorption and the accumulation of Na^+ in the leaves and increased the uptake of K^+ and improved the accumulation of organic compounds which may be participated in both osmotic adjustment and protection of cellular structures. Our result highlighted the possibility to mitigate the effect of salt stress by Ca supply and could help in this constraint management especially in coastal and saharian agricultures which use higher water quantities for irrigation.

Corresponding author:
Samira Soualem
Laboratoire d'agro-biotechnologie et de nutrition en zones semi-aride Université Ibn Khaldoun de Tiaret, Algérie
Email: samirasoualem2006@yahoo.fr

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