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Original article

Salt Effect on Biochemical Behavior Fodder Halophytes [1]

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Abstract

Atriplex halimus L., endemic to the Mediterranean region and *Atriplex canescens* (Pursh) Nutt. endemic to the American regions introduced in Algeria are two halophytes of semi-arid to arid regions. Salinity tolerance to NaCl (100, 300 and 600 mM/l) of Oran population of *halimus* L. and El Bayadh population of canescens (Pursh) Nutt. is analyzed. The parameters studied are Na++, K+, Ca++, Mg++ and Cl- . These are studied using two t methods (flame spectrophotometry and microanalysis EDX). In response to NaCl stress, the contents of Ca++ and K+ decrease. However, at low salt concentrations, Ca++ accumulates in the stems and leaves of *halimus* L. and only in the plant roots of *canescens* (Pursh) Nutt.. However, the leaves become less and less rich in K+, Mg++ under all salinity treatments in all organs of both species. Na+ accumulates in large amounts in the leaves. However, this accumulation slows down under the effect of salt beyond 300 mM/l in canescens (Pursh) Nutt. while the load in this cation increases in the stems and roots. Therefore, halimus L. is one halophyte of "includer" type whereas *canescens* (Pursh) Nutt. is "includer" one at concentrations low or equal to 300 mM/l. But at 600 mM/l, the plant changes to become an"excluder" halophyte. This change in the type can be a way to avoid the harmful effects of stress resulting from ionic salt stress in this species. On the other hand, microanalysis (EDX) shows that the Ca++ and Na+ are two essential elements of *halimus* L. roots and that only Ca++ is for *canescens* (Pursh) Nutt.. At the level of leaves, Na+ and Cl- essentially characterize halimus L. However, for plants of canescens (Pursh) Nutt., K+ and Cl- are dominant. Na+ then represents the specific component of the roots and leaves of *halimus* L. and K+ represents the specific element of canescens (Pursh) Nutt. leaves.

Keywords: Atriplex, Halophytes, Cation, Salt Stress.

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INTRODUCTION

Material and Methods

The seeds of *Atriplex. halimus* L. are native to the city of Oran. The seeds of *Atriplex canescens* (Pursh) Nutt. are taken from the atriplexaie of the El Bayadh region, located 370 km southeast of Oran and 520 km southwest of Algiers.

These are studied using two methods, flame spectrophotometer and microanalysis EDX.

Experimental Protocol

The experiment is carried out at the Botany building of the University of Poitiers (French). The seeds of both species are washed in a 1% sodium hypochlorite solution for 5 minutes and then rinsed several times with distilled water. Seeding is done on potting soil in cells placed in the greenhouse. Seed watering is carried out with tap water and executed in the form of fine droplets in order to avoid the removal of seeds. Two-week-old seedlings are transplanted into pots 15 mm in height and 17 mm in diameter filled with a mix of potting soil and sand (Fontainebleau sand) in proportions of V / 2V. Watering is 60% of the substrate retention capacity (170 ml of nutrient solution professional Peters solution) three times a week until the application of salt stress (Na Cl) four months after sowing. The photoperiod is 16 hours and the relative humidity is 60%. The temperature fluctuated between 22 and 24 ° C. Three saline treatments were retained, 100, 300 and 600 mM/l of nutrient solution. Stress is applied gradually by increasing the saline concentration by 50 mM-1 per day. Once the salt concentration is reached, the plants are watered for 30 days at a rate of three times per week in the saline solution. At the end of the stress, the plants are harvested. The roots are separated from the aerial parts and then all are washed under running water, packed in freezer bags and stored at -20 ° C.

Extraction and dosage of mineral salts

For extraction of the inorganic ions (K+, Na+, Ca++ and Mg++), 200 mg of the fresh samples were dried at 80 ° C for 48 h, reduced to a very fine powder and dissolved in 20 ml of 5% HCl . After centrifugation (15 min at 9000 g X), the supernatant is recovered and then filtered at 0.22 µm.

Determination by flame spectrophotometry

These mineral elements are measured at the IC2MP or Institute of Chemistry of Materials and Materials of Poitiers. The method used is that of Lambert (1976). The supernatant obtained is diluted to 1/25. For the determination of Mg++ and Ca++, it is imperative to add to the diluted supernatant 250 µl of a 1% Lanthanum solution. Na+, K+, M++ and Ca++ are determined by flame spectrophotometry at 589 nm, 680 and 6 nm, respectively. The calibration of the spectrophotometer is carried out by solutions of increasing concentrations ranging from 1 to 5 mg/l from a multi-element stock solution of concentration equal to 10 mg/l. Levels of these elements in mg/g of dry weight are calculated.

Determination of mineral salts by electron microscopy (TEM) coupled to X-rays (microanalysis EDX)

Take 1g of plant material, dry in an oven for 48 hours at 80 ° C then reduce to very fine powder and pass through a filter of 400 Mesh. X-ray spectral are use to perform a chemical microanalysis of the sample. Thus, when the electronic spot of the incident beam strikes a microsurface of the object, each atom of the underlying microvolume can emit an X-ray spectrum with its characteristic lines. The capture and the treatment of the RX allow an analysis in energy (keV) of the lines which leads to the identification of the atoms present in the zone pointed by the probe. It will therefore be possible for each dotted area to draw a dispersive spectrum with the abscissa photon energies and ordinate the number of photons received. The interpretation of the spectra is facilitated by a database which contains for each element the energies and the intensities of the lines that it produces. It is possible with complex calculations to know the respective quantities of the different elements analyzed.

The different comparisons are made at the Brown-Forsyth Test

Results

Flame spectrophotometer analysis

Sodium (Na+)

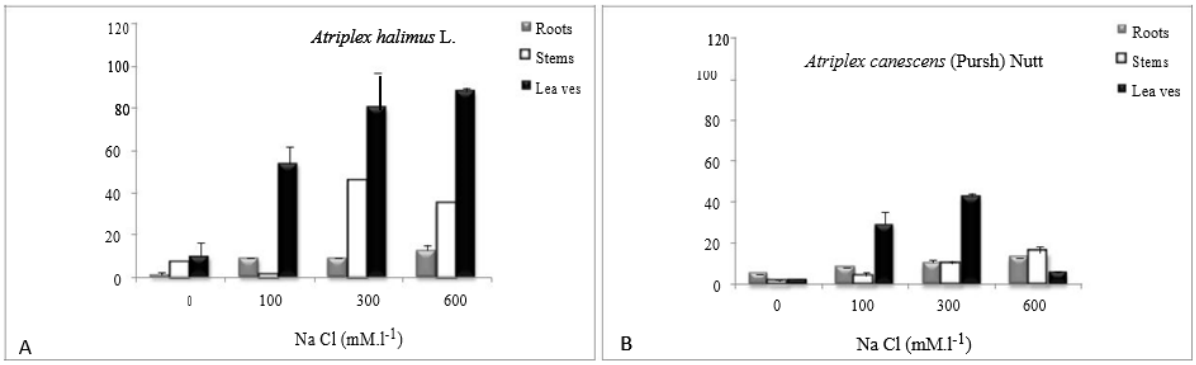


Figure 1. Sodium content (mg/g DW) of roots, stems and leaves of *Atriplex halimus* L. and *Atriplex canescens* Pursh (Nutt) under saline stress. The static test shows that the differences are significant

Figure 1 A shows that in control *Atriplex halimus* L. plants, Na^+ is contained much more in the leaves than in the roots. Indeed, the leaves show a value of about ten times more than that shown by the roots is 10.02 and 1.39 mg. The stems show intermediate values. The levels of Na^+ increase considerably in the three organs as a function of the increasing concentrations of salt except for the stems which show their highest content at 300 mM/l and their very low content (1.53 mg/g DW) at 100 mM/l. The maximum values reached by roots, stems and leaves are 12.73, 45.25 and 88.10 mg/g DW respectively. In contrast, Figure 1B shows that in *Atriplex canescens* (Pursh) Nutt. Leaves (2.57 mg/g DW) show only half the Na^+ content of the one presented by the roots (5.18 mg/g DW) and that the stems display the

lowest value (1.30 mg/g DW). In response to stress, the roots, stems and leaves show an increase in Na^+. The highest increase (42.80 mg/g DW) is recorded in leaves at the concentration of 300 mM/l. Maximum values of roots (12.90 mg/g DW) and stems (15.60 mg/g DW) are reached at 600 mM/l.

Potassium (K^{+})

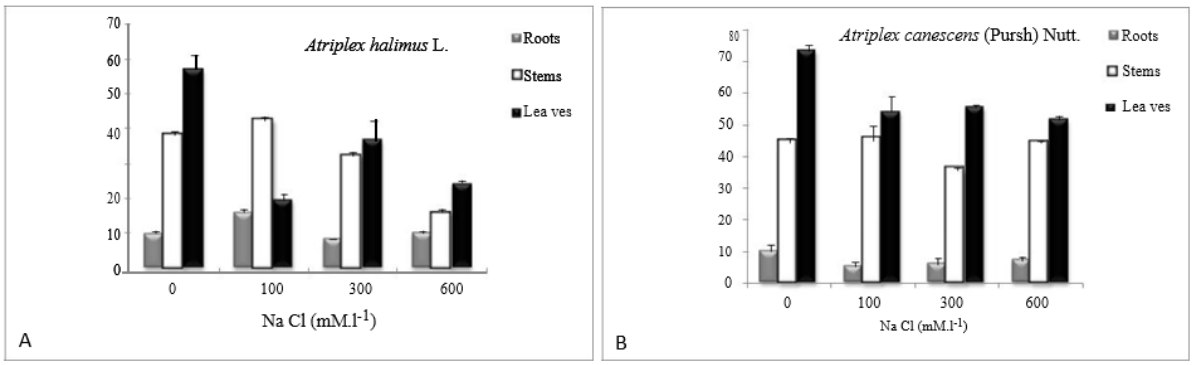


Figure 2. Potassium content (mg/g DW) of roots, stems and leaves of *Atriplex halimus* L. and *Atriplex canescens* Pursh (Nutt) under saline stress. The static test shows that the differences are significant

The results of Figure 2.A show that the leaves of *Atriplex halimus* L. are rich in K^+. Indeed, the K^+ content of the leaves (56.94 mg/g DW) represents six times the value shown by the roots (9.86 mg/g DW). The stems show an intermediate value (38.23 mg/g DW). At the root level, the K^+ content decreases regardless of the treatment with the exception of the 100 mM/l treatment, where this content increases from 9.86 to 15.80 mg/g DW. At 300 and 600 mM/l, this content is 8.29 and 10.10 mg/g DW respectively; these values remain very close to those of the control roots. At the level of the stems, the K^+ shows the same variations as for the roots but with much larger values. Indeed, at 100 mM/l, the K^+ content is 42.46mg/g DW. At 300 and 600 mM/l, this content is 32.19 and 15.94 mg/g DW. At the leaf level, the K^+ content decreases with respect to the saline concentration. The lowest content is that recorded at 100 mM/l with 19.80 mg/g DW is a decrease equal to almost three times. At 300 mM/l, the amounts of K^+ decrease by one and a half times (36.80 mg) whereas at 600 mM/l, this content drops to 24.32 mg/g DW. It should be noted that, for all the treatments (controls and stressed), the accumulation of K^+ is done first in the leaves, the stems then the roots except for the treatment 100 mM/lfor which, the stems is placed in first position followed by leaves and roots.

Figure 2.B also shows the richness of K^+ leaves of *Atriplex canescens* (Pursh) Nutt. Indeed, the leaves (73.51 mg/g DW) have contents seven times higher than those shown by the roots (10.05 mg/g DW).

In terms of salt stress, it can be seen that the K^+ content decreases, whatever the concentration at the root level. This decrease is almost 50% at 100 $mM.l^{-1}$. At the level of the stems, unlike the roots and leaves, the K^+ contents remained substantially close to the values of the controls (44.37 mg/g DW), ie

45.13 mg/g DW at 100 mM and 44, 24 mg/g DW at 600 mM/l) except at 300 mM/l where a slight decrease (35.92 mg/g DW) is recorded compared to control plants. At the leaf level, the K^+ content decreases for the three saline treatments. This decrease is of the order of 30%.

The K^+ is contained in the leaves of both species, it represents six times the root content in the leaves of *Atriplex halimus* L. and seven times the root content in the leaves of *Atriplex canescens* (Pursh) Nutt.

Calcium (Ca^{++})

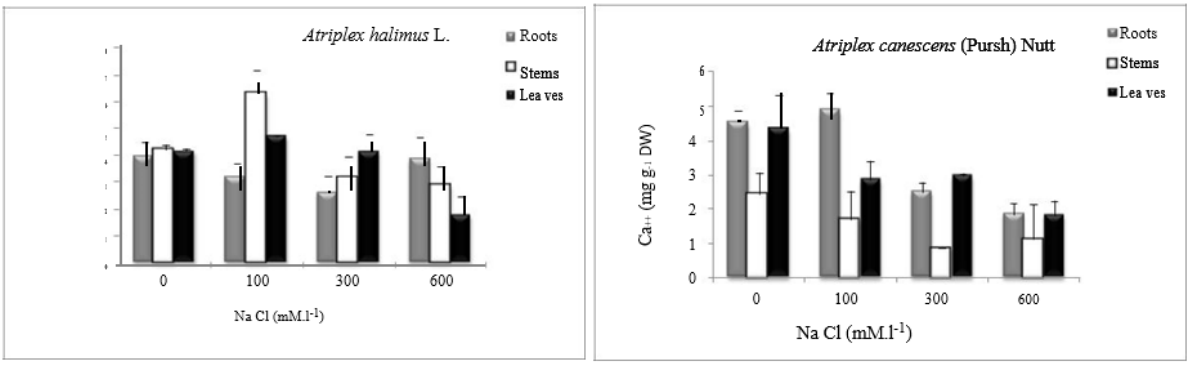


Figure 3. Calcium content (mg/g DW) of roots, stems and leaves of *Atriplex halimus* L. and *Atriplex canescens* Pursh (Nutt) under saline stress. The static test shows that the differences are significant

The results (Figure 3A) show that Ca^{++} is contained in the roots, stems and leaves of control plants at similar levels (3.94, 4.20 and 4.15 mg/g). DW). At the root level, Ca^{++} levels decrease to 100 and 300 mM/l(3.94 to 3.18 and 3.94 to 2.61 mg/g DW) while they remain almost unchanged at 600 mM/l(3.86 and 3.94 mg/g DW). At the level of the stems, this content increases under the effect of 100 mM/lNaCl (4.20 to 6.28 mg/g DW) and decreases successively to 300 (4.20 to 3.18 mg/g DW) and 600 mM/l (4.20 to 2.89 mg/g DW). At the leaf level, the results show an increase in Ca^{++} levels to 100 mM/l (4.15 to 4.69 mg/g DW) and storage of it at 300 mM/l (4.15 to 4.14 mg/gDW). On the other hand, at 600 mM/lleaves lose more than half of their Ca^{++} content (4.15 to 1.77 mg/g DW).It should be noted that the Ca^{++} contents of the roots and leaves are reversed between 300 and 600 mM/l treatments. Indeed, at 300 mM/l, the leaves show the highest levels whereas at 600 mM/l, root show the most important contents.

Figure 3.B also shows that Ca++ is contained in the roots, stems and leaves of the control plants (4.51, 2.41 and 4.34 mg/g DW) but in contrast to the organs of Atriplex halimus L., the stems show values equal to about half of the values shown by the roots and leaves. At the root level, the Ca++ content increases at 100 mM/l(4.51 to 4.87 mg/g DW) and decreases by almost half at 300 mM/l (4.51 to 2.51 mg/g DW and more at 600 mM/l (4.51 to 1.86 mg/g DW). At the stem level, the Ca++ content decreases to 100 mM/l(2.42 to 1.69 mg/g DW) and 300 mM/l (2.42 to 0.86 mg/g DW) but at 600 mM/l, the levels rise while remaining lower than controls (2.42 to 1.12 mg/g DW). At the leaf level, this content decreases

almost by half under the effect of the three treatments; the contents vary from 4.34 to 2.86 mg/g DW under 100 mM/lNa Cl, from 4.34 to 2.97 mg/g DW under 300 mM/land from 4 to 34 to 1.83 mg/g DW at 600 mM/l).

Magnesium (Mg^{++})

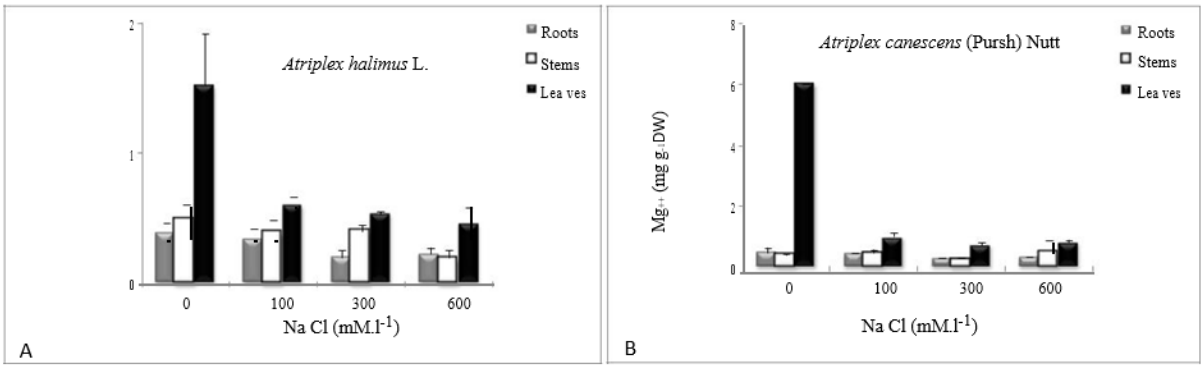


Figure 4. Magnesium content (mg/g DW) of roots, stems and leaves of *Atriplex halimus* L. and *Atriplex canescens* Pursh (Nutt) under saline stress. The static test shows that the differences are significant

The results (Figure 4A) show that the leaves of *Atriplex halimus* L. are richer in Mg^{++} than the roots of the control plants (1.52 and 0.38 mg/g DW). The stems display the intermediate value (0.48 mg/g DW). In stressed plants, the Mg^{++} content decreases regardless of concentration or organ. In fact, the content goes from 0.38 to 0.33, 0.19 and 0.21 mg/g DW at the roots and goes from 1.52 to 0.59, 0.53 and 0.45 mg/g DW at leaf level at 100, 300 and 600 mM/l, respectively. However, this decrease is more pronounced in the leaves where it represents two-thirds than at the root level where it represents half. At the stem level, the Mg content decreases slightly. It goes from 0.48 to 0.39 mg/g DW at 100 mM/l, stored (0.39 mg/g DW) at 300 mM/l and 0.48 to 0.19 mg/g DW at 600 mM/l,

The results in Figure 4.B clearly show that the leaves of *Atriplex canescens* (Pursh) Nutt. are rich in Mg^{++}. The contents of these are about 13 times that of the roots and stems. The latter have very low levels of 0.40 mg/g PS for stems and 0.47 mg/g DW for the roots.

With regard to stress, the levels decrease some salt treatment. This decrease peaks at 300 mM/lat the root level where it is over 80% (6.04 to 0.72 mg/g DW) and at the leaf level where it is about 50 % (0.47 to 0.26 mg/g DW). The contents of the stems fluctuate between 0.45 and 0.50 mg/g DW through a decrease of almost half (0.26 mg/g DW) at 300 mM/l.

Electron microscopy (TEM) coupled to X-rays (microanalysis EDX) analysis

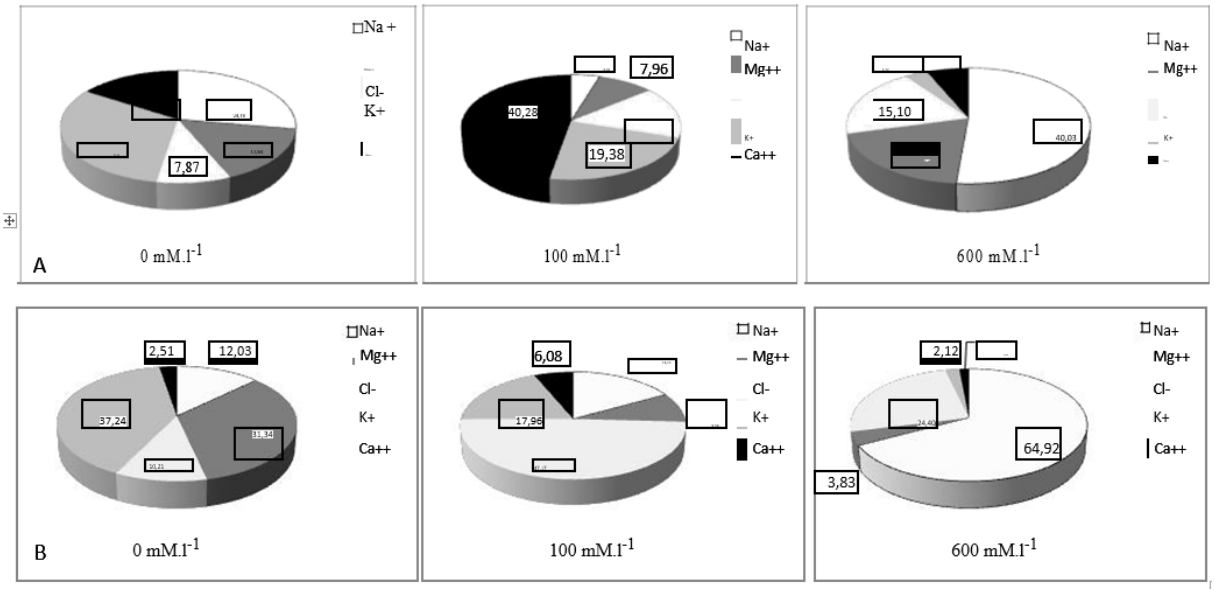
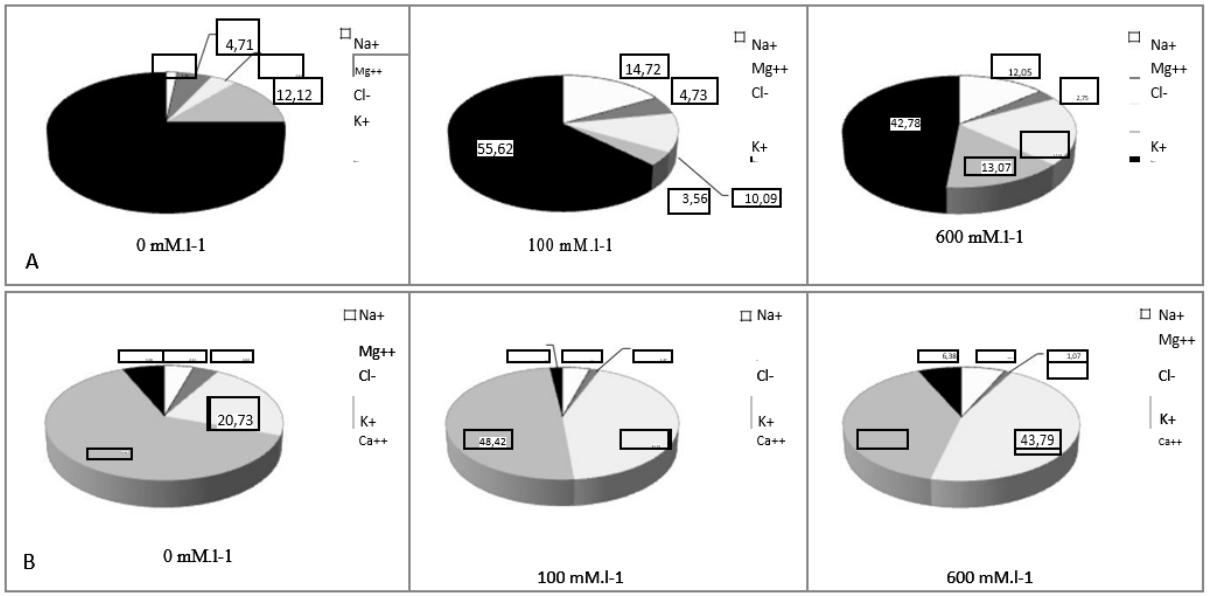


Figure 5. -Mineral salts in% D.W of roots and leaves of *Atriplex halimus* L. under salt stress.

The Figure 5.A shows that for the roots of control plants, K^+ shows the highest value (27.52%) followed by Na^+ with (24.18%). Ca^{++} and Mg^{++} are in third position with successive percentages almost equal (13.84 and 13.86%) and in the end, Cl^- shows the lowest value which is 7.87%. At 100 $mM.l^{-1}$, the order of percentages changes and Ca^{++} shows the highest value (40.28%). K^+, Cl^-, Mg^{++} and Na^+ follow one another and show the following percentages: 19.38, 13.66, 7.96 and 4.01%. At 600 $mM.l^{-1}$, the Na^+ displays the maximum value equal to 40.03%, the K^+ shows the minimum value equal to 2.72% while the Mg^{++} and Cl^- have the same percentage equal to 15.10. %. Ca^{++} shows 4.95%. At the leaves of *Atriplex halimus* L. (Figure 5.B) the control plants have the following order: K^+ (37.24%), Mg^{++} (31.34%), Na^+ (12.03%), Cl^- (10.21%) and Ca^{++} (2.51%). At 100 $mM.l^{-1}$, it is Cl^- which shows the highest percentage (47.17%), K^+ is second with 17.96% followed by Na^{++} with 16.22% Mg^{++} and Ca^{++} have the lowest percentages, successively 8.58 and 6.08%. At 600 $mM.l^{-1}$, Na^+ shows a high value (64.92%) followed by Cl^- with 24.40%. Mg^{++}, K^+ and Ca^{++} show only very low percentages and are successively: 3.83, 2.12 and 1.41%.



In *Atriplex canescens* (Pursh) Nutt., Ca^{++} shows the highest value in control roots (65.02%) followed by K^{+} with 12.12%, Mg^{++} with 4.71%, Cl^{-} with 3, 42%, and Na^{+} with 1.48% (Fig 6.A). At 100 $mM.l^{-1}$, the order of percentages does not change for Ca^{++}, which always shows the highest value (55.62%). However, Na+, Cl^{-}, Mg^{++} and K^{+} follow one another and show the following percentages: 14.72, 10.09, 7.96 and 4.73%. At 600 $mM.l^{-1}$, Ca^{++} still displays the maximum value equal to 40.03%, followed by Cl^{-} with 17.61%, K^{+} with 13.07, Na^{+} with 12.05 and finally Mg^{++} with 2.75 In conclusion, the position of the root Na^{+} in the order of the control plants changes according to the salt. In fact, the Na^{+} occupies the position 5 in the control plants, the position 2 in the plants treated at 100 mM/land the 4 position in the plants treated at 600 $mM.l^{-1}$. At the leaf level (Fig. 6.B), the control plants of *Atriplex canescens* (Pursh) Nutt. have the following order: K^{+} (59.46%), Cl^{-} (20.73%), Ca^{++} (6.06%), Na^{+} (4.22%) and Mg^{++} (3.64%). At 100 $mM.l^{-1}$, it is always K^{+} which shows the highest percentage (48.42%), Cl^{-} is second with 41.81% followed by Na^{+} with 4.19%. Ca^{++} and Mg^{++} have the lowest percentages, successively 1.91 and 1.47%. At 600 $mM.^{l-1}$, Cl^{-} shows a high value (43.79%) followed by K^{+} with 37.85%. Na^{+} and Ca^{++} show only very low percentages and are successively: 6.41, 6.38%. 1.41%. Mg^{++} displays the lowest percentage (1.07%) at control plants, position 3 in plants treated at 100 mM/land position 2 in plants treated at 600 $mM.l^{-1}$. K^{+} and Na^{+} are the major ions of the roots of *Atriplex halimus* L. In response to stress at low salt concentration, Ca^{++} and Cl^{-} are the majority. At high concentration, it is Na^{+} and Cl^{-} that accumulate. At the leaf level, K^{+} and Mg^{++} are the major ions. In response to stress at low and high concentrations, K^{+} and Mg^{++} decrease in favor of the accumulation of Cl^{-} and Na^{+}. Ca^{++} is the major ion of the roots of *Atriplex canescens* (Pursh) Nutt. It decreases with stress in favor of Na^{+} and Ca^{++} at low concentration and in favor of Cl^{-}, K^{+} and Na^{+}. The K^{+} is the major ion of the leaves; it decreases to low and high saline concentration in favor of Cl^{-}.

Discussion

Salt stress is at the origin of the reduction of the acquisition of mineral nutrients by the plant (Uddin et al., 2011). Nutritional effects of salinity include the two primary actions of salt on plants: direct toxicity due to excessive accumulation of ions in the tissues and nutritional imbalance caused by the excess of certain ions. Indeed, the accumulation of Na^+ ions in the plant limits the absorption of essential cations such as K^+ and Ca^{++} following the reduced absorption of the latter in connection with the excessive accumulation of toxic ions. According to Belkhodja (2007), the root is responsible for this sensitivity to NaCl. The osmotic effects of salt stress can also limit root growth, limiting the potential for soil nutrient uptake (Tester and Davenport, 2003). Under saline stress, the accumulation of Na^+ in plants, particularly in mesophyll, is well known (Tavakkoli et al., 2012). It is clearly shown that the ability to accumulate salt in foliar vacuoles is an important trait for Dicotyledonous halophytes, in these species this ability is coupled with other facts such as the regulation of transpiration, the synthesis of solutes compatible and the ability to function with low concentrations of cytoplasm potassium (Flowers and Dalmond, 1992).

Most halophytes use basic mechanisms of controlled accumulation and sequestration of inorganic ions by adjusting their internal osmotic balance to external salinity (Flowers and Yeo, 1986). However, halophytes are very different in their ionic absorption, not only in the amount of salt accumulated, but also in the distribution strategies (Glenn et al., 1996). Ben Ahmed et al. (2008) indicate that, in *Atriplex halimus* L. salt tolerance is acquired at an early vegetative stage of plant development, this tolerance is related to: (i) the uptake and transport to shoots of large amounts of Na^+ and Cl^- and their use in osmotic adjustment, (ii) the efficiency of vacuolar compartmentalization of these ions, which prevents ionic damage to the cytoplasm, and (iii) the ability of the whole plant to provide sufficient supply of K^+ while maintaining a high selectivity for this essential nutrient and this, despite the presence of large amounts of Na^+ in the medium. According to Belkheri and Mulas (2013), the concentration of Na^+ in plants increases with increasing salinity, it is higher in *Atriplex halimus* L. compared to *Atriplex nummularia* L., which led them to suggest that *Atriplex halimus* L. is an ion accumulator that can be used in phytoremediation. According to these authors, The accumulation of Sodium in the roots of *Atriplex halimus* L. MOR2 (clone native to Sardinia from Spain) is much larger than in its leaves. This suggests that MOR2 is an "excluder" type of Na^+, either by minimizing salt entry into the plant or by excretion mechanism via vesicular hairs that play an important role in removing salt from to the rest of the leaf, thus preventing accumulation at toxic levels in the leaves, while SOR4 acts as an "includer" to Na^+. By following the reasoning of these authors (Belkheri and Mulas, 2013), it can be deduced that *Atriplex halimus* L. (native of Oran of Algeria) is a halophyte of type "includer" since the leaves of plants accumulate much more. Na^+ that the roots. While *Atriplex canescens* (Pursh) Nutt. (originating from El Bayadh) is a "includer" type of halophyte at concentrations of less than or equal to 300 $mM.l^{-1}$. At 600

$mM.l^{-1}$, the plant changes type since the roots accumulate up to twice the value shown by the leaves to become an "excluder" type halophyte. This change of type can be considered as a means used by this plant to avoid the harmful effects of ion stress resulting from salt stress at the four-month stage of development. Nedjimi and Daoud (2009) report that the Sodium concentration increases in the stems and roots of Atriplex halimus subsp. schweinfurthii treated at 400 mM/lNaCl. This result differs from what we obtained on the same species (but not the same variety) at close concentrations (300 and 600 $mM.^{l-1}$). Indeed, at these concentrations, sodium accumulation occurred at the leaf and stem levels rather than at the root level. According to these authors, the addition of additional calcium reduces the concentration of sodium. The concentrations of Ca^{++}, K^{+} and N are at deficient levels in plants grown at high levels of NaCl and these deficiencies are corrected by the addition of Ca^{++}. The effect of Ca^{++} enhancement on growth and physiological variables could reduce the negative effect of salinity in *Atriplex halimus* plants. (Nedjimi and Daoud, 2009). Glenn et al. (2012), have shown that in saline conditions, *Atriplex canescens* (Pursh) Nutt. is slowly growing and shows no net growth above 20g l^{-1} NaCl. They also show that this species accumulates half as much Na^{++} at the stems compared to *Atriplex lentiformis* and *Atriplex hortensis*. However, all three species accumulate Na^{+} at high salt concentrations. The contents in K^{+}, Mg^{++} and Ca^{++}, are relatively constant on the salinity gradient. They concluded that drought and salinity are not additive stressors for Atriplex species, that NaCl increases their ability to extract water from the soil solution and that the accumulation of sodium in stems and type of photosynthesis (C4) are associated with severe drought and salt tolerance. The results obtained confirm the last point cited by these authors. In fact, the accumulation of sodium in the stems is noticed in *Atriplex halimus* L. at 300 mM/land in *Atriplex canescens* (Pursh) Nutt. from 100 $mM.l^{-1}$. Recently Tsutsumi et al. (2015) emphasizes the importance of leaf age. They show that Na^{+} accumulation is observed in the excretory hair cells of young leaves of *Atriplex gmelini* treated with 250 mM/lNaCl, whereas in mature leaves this accumulation is mainly found in mesophyll cells. . This, according to these authors, is due to the low presence of hair. The work of Albenisio et al (2009) provides information on the quantity ratios in organs. They show that the Na^{+} concentrations in the leaves of *Atriplex nummularia* L. are several times higher than in the roots (approximately 760 and 90 mmol g-1 DW, respectively) at 300 mM/lNaCl). Compared to our results, these Na^{++} concentrations in the leaves are multiplied by ten in *Atriplex halimus* L. (higher ratio than in *Atriplex nummularia* L.) and in four in *Atriplex canescens* (Pursh) Nutt. (lower ratio than in the two species *Atriplex halimus* L. and *Atriplex nummularia* L.) Benzarti et al. (2014), pushing salt stress to 1000 mM. 1^{-1}, also show that the content of Na^{+} but also that of Cl^{-} increases in *Atriplex portulacoid*. This increase is particularly visible in the leaves relative to the roots. In addition, no symptoms of salt-induced toxicity were observed and the leaf water content was maintained even at the highest salinity level of 1000 mM. l^{-1}. These studies show that atriplex species can withstand even higher concentrations than those tested in our experiments. This assumes that the increase of saline concentrations beyond 600 mM/lNaCl is possible in order to be able

to know the true thresholds of tolerance of the two species studied with respect to *Atriplex portulacoid*. Concerning K^+, the work done by Albenisio et al. (2009) also showed significantly higher K^+ concentrations in the leaves of *Atriplex nummularia* compared to roots, for salt concentrations ranging from 0.75 to 600 mM NaCl for 7 weeks. According to these authors, the osmotic adjustment at the leaf level of these plants is about 3 times higher than that presented by the roots. According to this reasoning, this osmotic adjustment at the leaf level will be about 5 times higher than at the root level in *Atriplex halimus* L. and about 7 times at *Atriplex canescens* (Pursh) Nutt. The accumulation of Na^+ ions affects the absorption of K^+ and this as a function of the concentration of the first element. Thus, the presence of Na^+ in low concentration may increase the absorption of K^+ (Uddin et al., 2011), such as the roots and stems of *Atriplex halimus* L. at 100 mM.l^{-1}. Indeed, the root K^+ content increases from 9.86 to 15.80 mg. g^{-1} PS. That of the stems goes from 38.23 to 42.46 mg. g^{-1} DW. This case is not verified at *Atriplex canescens* (Pursh) Nutt. Nevertheless, the K^+ content in this species is almost maintained for all three treatments. While high concentrations of Na^+ 300 and 600 mM/ldecrease the uptake of K^+ in organs in both species studied. This decrease is also noticed in *Suaeda fructicosa*, rice and sugar cane (Uddin et al., 2011). This absorption can even stop completely in beans and oleanders grown in the presence of 12 g / l NaCl (Haouala et al., 2007). Hassani et al. (2008) report that the K^+ load in the leaves of barley *Hordeum vulgare* L. remains substantially low and regresses very slowly from the control to plants treated at 250 mM. l^{-1} NaCl. In *Aster tripolium* stressed to salinity (NaCl), the osmotic adjustment is mainly due to the accumulation of sodium and chloride. However, salt has been unevenly distributed in plants. The K^+ / Na^+ selectivity is high in the lateral roots and low in the petioles, so these organs serve as "salt filters" that prevent excessive salt accumulation and ion toxicity in leaf limbs and in the main root organ of storage of organic substances (Geissler et al., 2009). According to the same authors, Despite some signs of ion toxicity and nutritional imbalance, these factors do not seem to be mainly responsible for the limited tolerance of *Aster tripolium* to salinity. In order to maintain a positive water balance, the salt-treated plants increase the resistance of the stomata. The closure of the latter leads to a significant decrease in photosynthesis. The weak assimilation rate contributes to a significant decrease in growth (reduction of the maximum yield between 50% and 75%), as well as a higher energy consumption required for the various mechanisms of tolerance to salinity such as, the synthesis of compatible solutes (proline, carbohydrates) and that of stress-induced proteins. On the other hand, an elevation of CO2 concentration in the atmosphere leads to a significant increase in photosynthesis. This indicates, with a higher water potential, that the plant water relationships have improved. By reducing the stomatal resistance the energy gain is maximum. Supplementary feeding with energy-rich organic substances is not used in biomass production, but to increase investment in salinity tolerance mechanisms as a strong synthesis of proline, carbohydrates and proteins. These mechanisms have led to a higher survival rate in saline conditions (Geissler et al., 2009). The comparison of our results with those obtained in glycophyte plants such as Alfalfa is possible thanks to the work of Mezni et al. (2010)

who report that the stems and leaves of three varieties of Alfalfa (Gabèz Hunterfield), and Hyb555) accumulate large amounts of Na^+ and small amounts of K^+ at high NaCl concentration (10 g l^{-1}). However, the Gabez variety differs from other varieties in having lower Na^+ accumulation and higher K^+ leaf accumulation at high salt concentration. This last variation of response seems to be the case of *Atriplex canescens* (Pursh) Nutt. at all salt concentrations tested (100, 300 and 600 mM.l^{-1}) where the low accumulation of Na^+ stems (3.77, 10.08 and 15.60 mg g PS) and leaves (28.99 , 42.80 and 5.51mg/g DW) led to a high accumulation of K^+ at the stem (45.13, 35.92 and 44.24 mg, g^{-1} DW) and foliar (54.20 , 55.60 and 51.76 mg, g^{-1} PS) at successive concentrations of 100, 300 and 600 mM.l^{-1}. According to Tafforeau (2002), calcium transporters were found in the plasma membrane, the endoplasmic reticulum, the plastids and the vacuole. These carriers may have important roles in the shape and structure of calcium signals. Calcium plays a key role in perception, but also in a mechanism for memorizing abiotic signals by plants (Ripoll et al., 2007). Calcium influx resulting from salt stress triggers the Salt Overly Sensitive (SOS) pathway to maintain cell homeostasis (Lamzeri, 2007).

By comparing the different salt stress responses of the two species, Ca^{++} and Na^+ are two essential elements of the roots of *Atriplex halimus* L. whereas for *Atriplex canescens*, Ca^{++} seems to be important. Ca^{++}, therefore, represents the element common to both studied species. Na^+ represents the specific element of *Atriplex halimus* L. At the leaf level, Na^+ and Cl^{-1} are two essential elements of *Atriplex halimus* L., in *Atriplex canescens* (Pursh) Nutt. and Cl^{-1} which are dominant. Cl^{-1} therefore represents the element common to both studied species. Na^+ represents the element specific to *Atriplex halimus* L. and K^+ represents the element specific to *Atriplex canescens* (Pursh) Nutt.In wheat (*Triticum turgidum* L. cv Claudio) and according to this type of analysis (EDX), concentrations of organic anions increase in the aerial parts and roots while the concentrations of inorganic anions decrease with the exception of chloride, along with the increase in Cd concentrations in the solution (Rizwan, 2012). X-ray microanalysis of elements in halophytes indicates different Na^+ exclusion strategies of photosynthetic tissues of Bassia indica, Atriplex prostrata, *Spartine maritima*, *Limonium angustifolium* and Spongy atriplex (Pongrac et al., 2013). The response of *Atriplex halimus* L. to the different stresses (KCl, NaCl, Sorbitol) is a function of the stage of development of the plants in particular, the stress of NaCl. At the germination stage, the growth reduction can be attributed to the osmotic effect and HRD may have a role in the osmotic sensitivity. At this stage, the accumulation of Na^+ in the vacuole could be a strategy for reducing the osmotic effect. At seedling emergence stage, growth inhibition could be mainly attributed to the ionic effect that may have resulted from excessive Na^+ accumulation with incompatible regulations of Na^+ manipulation genes. In the vegetative stage, *Atriplex halimus* L. is an obligate halophyte with regulated mechanisms of tolerance to two ionic and osmotic components of salt stress. Nada and Abogadallah (2015) concluded that *Atriplex halimus* L. has glycophytic characteristics at the early stages of growth. Microanalysis (scanning electron microscopy and energy dispersive X-ray spectrometry (SEM-EDS) of the mineral elements of *Atriplex canescens* (Pursh) Nutt seedlings treated

with salinity for 12, 24 and 48 hours shows that , the leaf surface is abundantly covered with trichomes with 10-70 µm vacuoles, which both prevent and delimit the formation of crystals, suggesting that these vacuoles store water and ions of orderly, the most abundant ions are Cl^-, Na^+ and K^+ Mg^{++} is the only element present at any time of exposure to salt, which suggests its importance for the function of the gland Cl^- and K^+ after 12 hours of exposure, the concentration of K + decreases after 48 hours and is gradually replaced by Na^+ (Garza Aguirre et al., 2015). Rossi et al. (2015) conclude that the different apoplastic adjustments in roots play a role in reducing the flow of ions from olive shoots (*Olea europaea* L.). This reduction is a function of the genotype and concentration of Na^+ of the culture medium.

Conclusions

The effect of salt stress on the absorption of Ca^{++} (calcium) shows that the roots of *Atriplex canescens* (Pursh) Nutt. are sensitive to salt at low concentrations leaf calcium from *Atriplex halimus* L can be used in osmotic adjustment at low and moderate saline concentrations. under normal conditions, the osmotic adjustment of the leaves by potassium is about five times higher than that of roots in *Atriplex halimus* L. and about seven times in *Atriplex canescens* (Pursh) Nutt. These two ratios are higher than that of *Atriplex nummularia* evaluated three times by Albenisio et al. (2009). The results show the richness of the leaves of *Atriplex canescens* (Pursh) Nutt. in Mg^{++} (magnesium). Indeed, this content is triple that contained in the leaves of *Atriplex halimus* L. Comparing the two species, *Atriplex halimus* L. accumulates Na^{++} in the leaves whereas *Atriplex halimus* L., originating from Oran of Algeria is a halophyte of type "includer" since the leaves of plants accumulate much more sodium than the amount accumulated by the roots. While *Atriplex canescens* (Pursh) Nutt., Native to El Bayadh is an "excluder" type halophyte at concentrations less than or equal to 300 $mM.l^{-1}$. At 600 $mM.l^{-1}$, the plant changes type since the roots accumulate up to twice the value shown by the leaves to become an "excluder" type halophyte. This change of type may constitute a way to avoid the harmful effects of ion stress resulting from salt stress for this plant. *Atriplex canescens* (Pursh) Nutt. accumulates in the roots in smaller proportions. In response to salt stress, Na^{++} accumulates largely in the leaves. Nevertheless, this leaf accumulation stops above 300 mM in *Atriplex canescens* (Pursh) Nutt., The stems and roots are then loaded with Na^{++} in this species. high concentrations of Na^+, decrease the K^+ uptake in the three organs of the two species studied. Na^{++} and Cl^{-1} are the essential elements of *Atriplex halimus* L., K^+ and Cl^{-1} are essential for *Atriplex canescens* (Pursh) Nutt., Na^{++} is therefore the specific element of *Atriplex halimus* L. and K^+ represents the specific element to *Atriplex canescens* (Pursh) Nutt. microanalysis (EDX) makes it possible to highlight that Ca^{++} is for *Atriplex canescens* (Pursh) Nutt. Thus, Na^{++} represents the specific element of the roots of *Atriplex halimus* L. At the leaf level, Na^{++} and Cl^{-1} are the essential elements of *Atriplex halimus* L., K^+ and Cl^{-1} are essential for *Atriplex canescens* (Pursh) Nutt., Na^{++} is therefore the specific element. to *Atriplex halimus* L. and K^+ represents the specific element to *Atriplex canescens* (Pursh) Nutt.

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