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Research Article

EFFECT Of SALT STRESS ON SOME PHYSIOLOGICAL PARAMETERS IN *Atriplex halimus* L.

N. Soualmi[1]*, M. Belkhodja[1], A. Adda[2]

[1]Faculty of science, Oran University, Es senia, Algeria

[2]Department of nature and life science Faculty of science Tiaret University, Algeria

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ABSTRACT

The impact of salinity applied for five weeks followed two provenances of *Atriplex halimus* L. harvested from Oran (Algeria coast) and Ain Dheb (high plateaus west of Algeria). The analyzed parameters are physiological from the leaves of young plants grown in a greenhouse stored in air-conditioned glass. Two types of salt stress are imposed on plants from the 165th day after sowing for a batch $CaCl_2$ + NaCl to 300 meq and 600 meq.l-1and for the other batch to undiluted water sea with a frequency of watering every other two days. The results of water indicators such as the relative water content (RWC or TRE) showed some fluctuation during the five weeks under the two types of stress. Transpiration (water loss from the excised leaf or RWL) measured after 30, 60 and 120 min at the end of the period of application of stress presents a decline in both sources and types of stress. In addition, the stomatal resistance showed considerable variability for both sources and the type of stress.

Keywords: A. halimus L., salt stress NaCl + $CaCl_2$, Seawater, RWC, sweating, stomatal resistance.

Author Correspondence, e-mail: snadia04@yahoo.fr

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1. INTRODUCTION

Growth and development of plants are set harmoniously by external environmental selections and internal signals [13] However, the plants in the wild are exposed to multiple stressors [3] and their answers determine their ability to survive [8]. Algeria is among the countries affected by salinity [12], however, significant water resources are available, but they are of a poor kind (brackish) [23]. Elevated concentrations of salts in soils, explain large reductions in crop yield worldwide [5], nearly 100 million hectares of land are affected by salinity [17].

In our work we chose *halimus Atriplex* L., a halophytic plant of economic and ecological interests[20] ; [25].Our goal is to evaluate the effects of different salt concentrations of NaCl + CaCl2 and sea water on some physiological parameters of two provenances of Atriplex L. halimus.

2. MATERIALS AND METHODS

Plant material

This work focussed on two provenances of *Atriplex halimus* L. The fruits are harvested from the same mother plant on the campus of the University of Oran and Ain Dheb. The seeds are desinfected with bleach to 8% for 5 min, rinsed with distilled water three times and then placed in plastic trays filled with soil water for germination. Watering with distilled water is given every other two days. After 45 days, the seedlings are transplanted into the cylinders (1 meter high with 16 cm diameter) PVC to one plant per cylinder.

Experimental protocol

The cylinders are lined with gravel at the bottom for drainage and filled with a substrate (24.5 Kg) mixed with sand, soil and compost (8V/V/V). After transplanting seedlings into the cylinders of water additions are made twice a week to the nutrient solution of Hoagland [10] diluted to 1/1000th. On other days, watering is done daily with distilled water only for 4 months (until the application of salt stress, this water supply is determined compared with the retention capacity of the culture substrate.

From the 165 th day of seedling plants are stressed by both protocols, using saline NaCl + $CaCl_2$ and 600 to 300 meq meq.l^{-1} solution of Hoagland and not sea water diluted for five weeks at field capacity to compensate for losses through evapotranspiration. In fact, before every watering weighing cylinders are operated to determine water loss. Control plants were watered to the nutrient solution of Hoagland (1938). After five weeks of treatment the behavior of two provenances of *Atriplex halimus* L. is analyzed under salt stress.

Water parameters analyzed

- The relative water content (RWC) of method and Barrs Weatherley (1962) [4] and Scippa et *al* (2004) [21]
- The RWL (Water Loss Rate) 30 min, 60 min and 120 min according to the method of Clarke et *al*, (1989) [7].

- The stomatal resistance with a porometer.

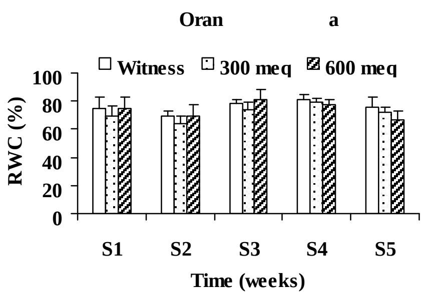
The results were analyzed using the General Linear Model (GLM) using SPSS Software. Averages are thanks to the 5% threshold.

3. RESULTS

Relative Water Content (RWC)

Under NaCl + $CaCl_2$ stress

The results (Fig. 1a) indicate that the RWC leaves of *Atriplex halimus* L. from Oran to evolves in parallel for all treated leaves 300 meq NaCl + CaCl2 compared with control plants. From the first to the second week the RWC leaves decreases in intensity respectively 75.05 and 69.67% to 69.06% and 64.33%. This parameter increases to the third and fourth weeks to 75.45% and 71.70% decrease for the fifth week to 75.70% and 71.70%. As for stress 600 meq NaCl + $CaCl_2$ plants, the fall of the RWC occurs the second week, after an increase in the third week of 81.30%, it isreported to a gradual reduction until the fifth week to 66.41%.For plant leaves from the Ain Dheb (fig.1b) RWC peaked in the third week in both salt treatments compared with control plants (79.74% to 77.87%, 77.93%). RWC is a progressive fall until the fifth week to reach respective values of 76.50%, 71.14% and 71.14%.



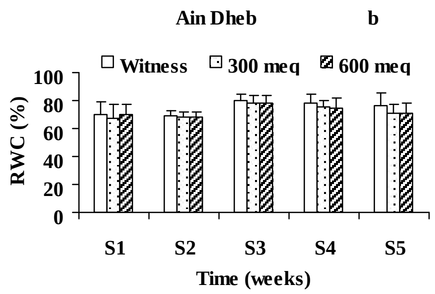


Fig.1. RWC leaves of *Atriplex halimus* L. plants the provenance of Oran (a) and Ain Dheb (b) stressed NaCl + CaCl2 for five weeks.

The statistical study shows that changes in the RWC in provenances of *Atriplex halimus* L. Oran are not dependent on salinity levels during the first four weeks. In fact, the tests indicate that the probabilities vary around 0.26, 0.19, 0.52, and 0.138. On the fifth week, the salinity effect is expressed significantly on this parameter ($p = 0.022$). Among provenances of *Atriplex halimus* L. Ain Dheb probabilities calculated from the first to the fifth week of treatment (0.71, 0.88, 0.68, 0.39, 0.20) conclude that changes in RWC observed in this case are not related to different levels of salinity.

Stress in seawater

Under the treatment in seawater, the RWC of *Atriplex halimus* plants L. the originated from Oran (Fig.2 a) is characterized by higher rates between 81.07% in the control plants and 78.55% for those treated seawater on the fourth week. A sudden drop is then observed in the fifth week respectively 75.45% and 71.34%.Among provenances Ain Dheb (Fig. 2 b), the rate of the RWC after a week of stress are 68.65% for control plants and 67.35% for stressed plants seawater These values increase the second week to record a subsequent gradual decline until the fifth week to rate 76.50% and 71.72% (FIG. 2b).Statistical analysis reveals that the rate of RWC varies independently from the salinity of the sea water irrespective of the source plants. These results are confirmed by the probabilities recorded during the period from the first to the fifth week of stress, or 0.10, 0.81, 0.23, 0.20, 0.21 for sources of Oran and 0.47, 0.52, 0.24, 0.27, 0.20 in provenances from Ain Dheb.

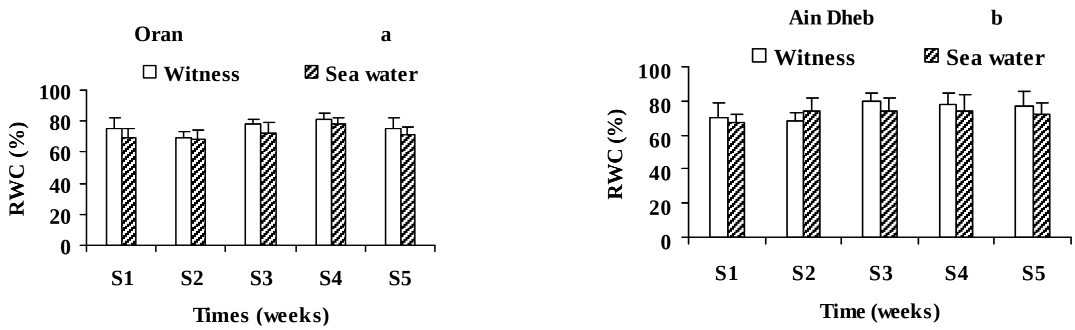


Fig.2. RWC (%) of leaves of *Atriplex halimus* plants L. provenances (Oran) (a) and Tiaret (b) stressed in seawater for five weeks.

Water loss through the excised leaf (R.W.L)

Stress with NaCl + $CaCl_2$

Measurements of transpiration (RWL) are made after the fifth week of stress after 30 min, 60 min and 120 min.Figure 3a shows that sweating in Oran provenances after 30 minutes, varies from 0.067 $mg.cm^{-2}.mn^{-1}$ for leaves of control plants at 0.057 $mg. cm^{-2}. mn^{-1}$ or those treated 300 meq and 0.06 $mg.cm^{-2}.mn^{-1}$ in treatment at 600 meq. At the 60th minute, plants respond by reducing transpiration under salt stress compared with control plants (0.055mg. $cm^{-2}. mn^{-1}$ for the treatment meq 300 and 0056 mg. $cm^{-2}. mn^{-1}$ 600 meq against 0.060 mg. $cm^{-2}. min^{-1}$ in control plants). After 120 minutes, perspiration slows to oscillate around 0.041mg.cm^{-2}.mn^{-1} for witnesses and down to 0.039 and 0.038 mg.cm^{-2}.mn^{-1} in saline treatments.

Among provenances from Ain Dheb (Fig. 3 b), salinity causes a regressive reaction of leaf transpiration during 120 min. Indeed, for witnesses, the RWL shows 0.065 mg.mn.$^{-2}$ .cm^{-1}for 30 min, this value goes down to 0.060 mg.cm^{-2}.mn^{-1} after 60min and then 0.039 mg.cm^{-2}.mn^{-1} after 120 minutes. Under stress 300 meq and 600 meq RWL takes the same pattern for 30 minutes with the respective values of 0.085 mg .mn^{-2}.cm^{-1} and 0061 min-1 mg.cm^{-2}.mn^{-1}. These values undergo significant changes after 120 minutes of action is 0.048 mg.cm^{-2}.mn^{-1} at 300 meq and 0.036 mg. cm $^{-2}$. min^{-1} at 600 meq salts.

The average comparisons performed at 5% level, show that salt stress imposed on plants does not influence the RWL among provenances of Atriplex halimus L Oran. The probabilities of the three measurements recorded at 30 min after, 60 min and 120 min are respectively 0.955, 0.317, 0.248.

Among provenances from Ain Dheb the statistical test at the 5%, shows that the NaCl + CaCl2 treatments have no influence on water loss recorded during 120 min (p = 0.311, p = 0.410 and p = 0.468).

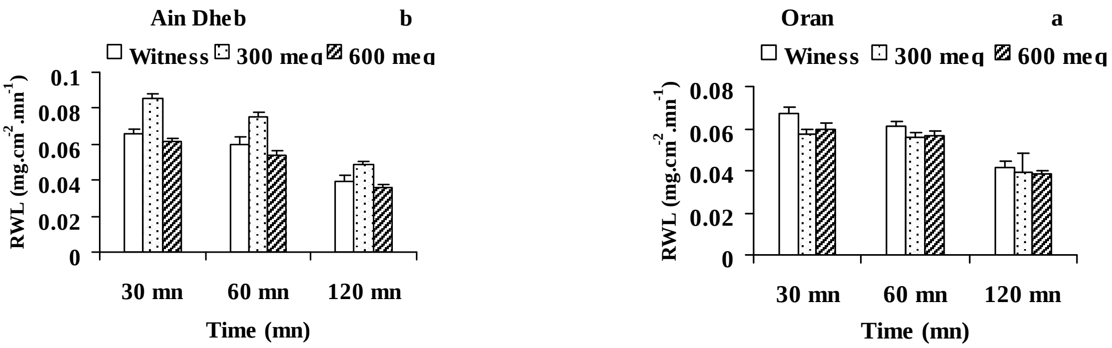


Fig.3. RWL leaves of *Atriplex halimus* plants L. provenances Oran (a) and Ain Dheb (b) for 30 min, 63 min and 12 min at the end of five weeks of stress NaCl + $CaCl_2$

Stress in seawater

Experiments which used seawater (Fig. 4 a) shows that the RWL among provenances Oran concludes that even under these conditions transpiration decreases regularly. We notice that from the beginning of the experiment and conditions without salt shows that the RWL 0.067 mg. cm $^{-2}$. min^{-1} to 30 min, 0054 mg.cm^{-2}. min^{-1} to 60 minutes and 0.041mg.cm^{-2}.mn^{-1} to 120 minutes. In plants grown under the treatment with the seawater RWL fluctuates around 0.059 mg.cm $^{-2}$.mn^{-1}, 0.054 mg.cm^{-2} min^{-1} and 0.035 mg.cm^{-2}.mn^{-1} during the respective periods.

For sources Ain Dheb (Fig. 4 b), plants fed with nutrient solution present value of 0.065 mg. .mn^{-2} cm^{-1} after 30-min. For those stressed in seawater, the RWL varies slightly to 0.062 mg. cm $^{-2}$. min^{-1}. After 60 min of water loss we point 0.060 mg. cm $^{-2}$. min^{-1} for control plants and 0.054 mg. cm^{-2}. min^{-1} for plants treated seawater At the end of the experiment we note 0.039 mg. cm $^{-2}$. min^{-1} for control plants and 0.03 mg. cm $^{-2}$. min^{-1} for the treatment of sea waterin the case of sources of Oran, statistical analysis revealed a non-significant result for the RWL. Probabilities show values of 0.28, 0.39 and 0.33.

When we compare the results from different provenances Tiaret probabilities show insignificant for the treatment of sea water compared with the control RWL, the probabilities indicate values of 0.96, 0.71 and 0.62.

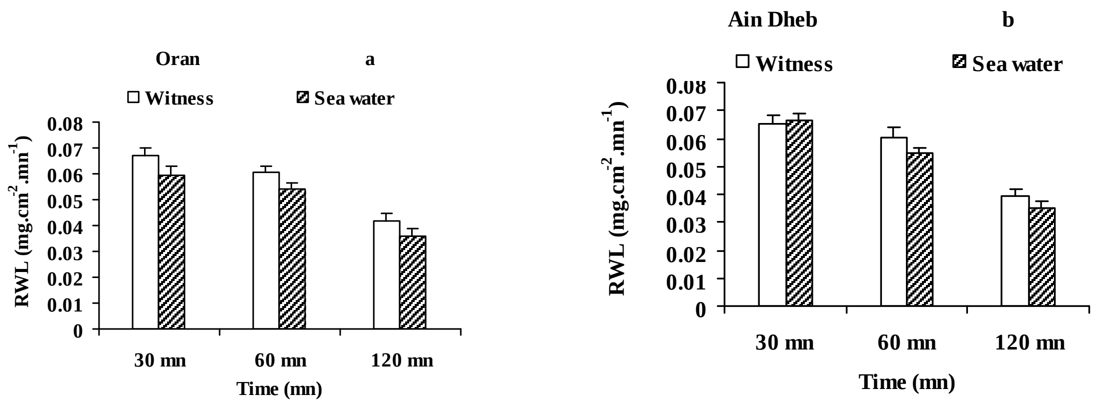


Fig.4. RWL leaves of *Atriplex halimus* L. plants provenances Oran (a) and Ain Dheb (b) during 30, 60 and 120 min at the end of five weeks of stress in seawater

Stomatal resistance

NaCl + $CaCl_2$ stress

Stomatal resistance is calculated after the fifth week of stress. Figure 5 illustrates the results of leaf stomatal resistance sources in Oran and Ain Dheb. Stomatal resistance tends to increase with the intensity of salt stress. It spends 6.25 s.cm^{-1} . Plants for witnesses to 17.04 s. cm^{-1}. 89.14 and s. cm^{-1} for those undergoing stress 300 and 600 meq meq salts.

Statistical analysis reveals in stressed plants 300 and 600 meq meq a highly significant stomatal resistance (p = 0.000).

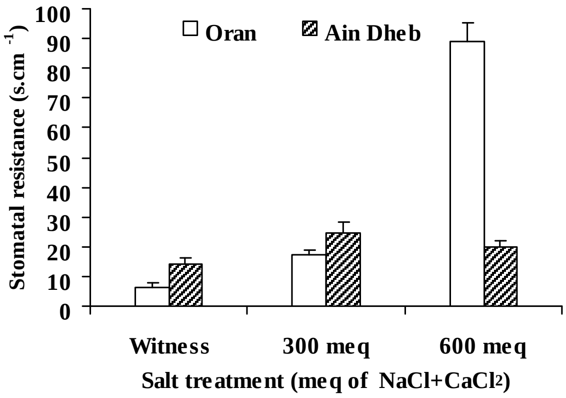


Fig.5. Stomatal resistance (s.cm^{-1}) of leaves of *Atriplex halimus* L. plants provenances of Oran and Ain Dheb stressed NaCl + $CaCl_2$ in the fifth week of stress

However, sources in Ain Dheb (Figure 5) stomatal resistance follows a lower trend in all treatments. Compared with plants provenances Oran, this parameter expresses substantially similar values for the control and treatment in 300 meq. When plants receive saline 300 meq, stomatal resistance decreases significantly compared with previous treatments when it is reduced about five times (19.74 s.cm^{-1}) Compared with the value recorded for the plants provenances Oran under treatment at 600 meq (89.14 s.cm^{-1}).

Stress in seawater

Statistical analysis reveals no significant differences between cultivated plants at different stress and control plants (p = 0.09).

In plants originated in Oran stressed in seawater (Fig. 6) we notice a stomatal resistance of 61.25 s. cm^{-1}., Or 8.5 times greater than in plants receiving nutrient solution (6.25 s.cm^{-1}).

The statistical processing of the values shows highly significant stomatal resistance (p = 0.000) in the treatment of seawater leaves compared with controls.

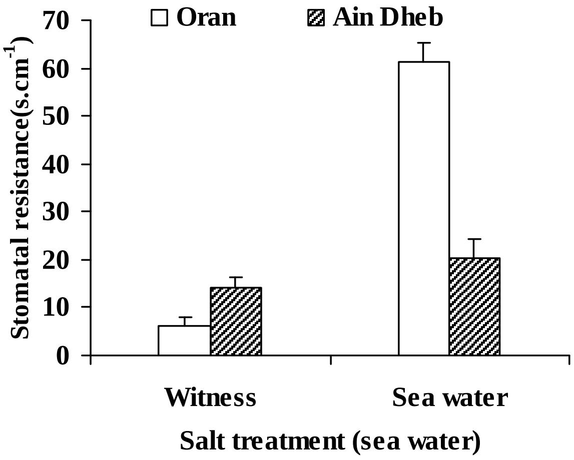


Fig.6. Stomatal resistance (s.cm^{-1}) of leaves of Atriplex plants halimus L. provenances of Oran and Ain Dheb stressed in seawater in the fifth week

For provenances of *Atriplex halimus* L. Ain Dheb treated seawater (Fig. 6), we did not notice significant differences (p = 0.125) between the control plants (14.10 s.cm^{-1}) and those watered with seawater (20.26 s.cm^{-1}).

4. DISCUSSION

The effect of the salt results in the reduction of perspiration (RWL) as the two types of stress and in all plants. However, sources in Ain Dheb treated with NaCl + $CaCl_2$, are irrigated to 300 meq displaying the most important values of transpiration plants.

In addition to the stomatal resistance, responses of *Atriplex halimus* L.plants vary from a stress to each other. It shows the origins of Oran express highly significant values (p = 0.000) in the two types of salt stress. The leaves of these plants generally have closed their stomata at the same time as the stress intensifies surely to minimize water losses.

Following this analysis, it is possible to retain the essential elements highlighting the responses of the two provenances of Atriplex L. halimus under salt stress. Thus, the analysis of the relative water content used to describe the water status of the plant permits to evaluate the ability to achieve a good osmoregulation and maintain cell turgor [16]. Our results are consistent with those reported by [15].

Work Karimi et *al*. (2007) on Atriplex verrucifera MB reported that RWC drop after three weeks of saline treatment with a significant accumulation of sugars. [9] attributed the increase in soluble sugars in a starch degradation following their rapid conversion to sucrose, fact which may be attributed to inhibition of the synthesis of starch reserves. Salt stress alters cellular compartmentalization for sugar synthesis [24].

Several studies have shown the decrease in RWC during salt stress, as in alfalfa [14], wheat [1] and Casuarina glauca [2] .

Work demonstrated in a halophyte Sesuvium portulacastrum increased sweating during salt stress [22]. [6] showed that the loss of water by transpiration increased mineral absorption in plants. [18] established the relationship between transpiration and stomatal resistance in Arabidopsis thaliana. This last parameter increases during salt stress to minimize water loss.

Stomatal resistance was more significant among provenances of Oran in the two types of stress. Plants subjected to salt stress close their stomata earlier than plants under normal conditions, it increases stomatal resistance due to the decrease in water absorption [19].

5. CONCLUSION

Our results show that the effects of the intensity and duration of salinity showed good tolerance and heterogeneity of expression from the two provenances of *Atriplex halimus* L.

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