

## Physiological and Biochemical behaviour of *Atriplex canescens* (Pursh) Nutt. (Caryophyllales Chenopodiaceae) under salinity stress

Amir Brinis<sup>1</sup> & Moulay Belkhodja<sup>2</sup>

<sup>1</sup>Plant Breeding laboratory, Biology Sciences Department, Badji Mokhtar University, Annaba, Algeria

<sup>2</sup>Ecophysiology laboratory, Essenia University, Oran, Algeria

---

### ABSTRACT

The halophytic species *Atriplex canescens* (Pursh) Nutt. (Caryophyllales Chenopodiaceae) was submitted to increasing levels of salinity stress (NaCl) in order to understand and quantify as well some physiological and biochemical responses. The results that have been obtained showed that plant biomass production was a function of salinity gradient. As far as chlorophyll content was concerned, decreasing content was observed for chlorophyll (a) and chlorophyll (b). The energetic potential elaborated by the species was inversely proportional to salt concentration. The accumulation of osmotic substances such as proline, soluble sugars and total proteins content increased as salinity level was increasing. These results show how important is to verify specific adaptative mechanisms to salt stress. In fact, in the present study, it is worth to emphasize how osmoregulation constitutes a reliable and interesting strategy for the species to withstand salt stress. Such an analysis may also be used in plant selection at early stage as predictive indirect tests to evaluate the possible integration of plant breeding programs.

### KEY WORDS

Adaptation; *Atriplex canescens*; halophyte; osmoregulation; physiology; salinity.

Received 06.05.2017; accepted 20.12.2017; printed 30.03.2018

---

### INTRODUCTION

Salinisation is defined as an enrichment of the upper surface of the soil by soluble salts. Salinisation of the soils and water is one of the major abiotic factors that limit plant productivity and agronomic yield (Zid & Grignon, 1991). In arid and semi-arid ecosystems, it results from high evaporations of water from the soil (Munns et al., 2005) and from irregular rainfall (Mezni et al., 2002). According to Ben Naceur et al. (2001), unfair irrigation is the cause of salinity. In Algeria, almost 3.2 millions hectares are threatened by salinity (Belkhodja & Bidai, 2004). Plants respond to environmental con-

straints by numerous changes and reveal the multi-factorial character of tolerance and adaptative mechanisms of abiotic stress. The response to salt by plant species depends on the species itself, the variety, salt concentration and developing stage of plant (Ben Naceur et al., 2001). Under severe stress, plants react by several mechanisms ; physiological and biochemical ones are those which are often implicated in enzymatic activity (Stephanopoulos, 1999). Synthesis of organic compounds acting as osmoprotectors or osmotic regulators is among the very well known strategy (Ratinasabapathi et al., 2000). Meas & Nieman (1975) and Shannon (1984) have identified criteria of salt tolerance, as far as

grain yield, seed vigor, foliaire damage and height of plants were concerned.

Many other approaches, such as anatomic, ecological, physiological and molecular ones, were used in order to understand to what extent these modifications may reduce harmful effects of salt stress (Poljakoff-Mayber, 1988). As we consider salt tolerance mechanisms, according to Piri et al. (1994) resistance of plants is expressed by the capacity to survive and reproduce in presence of salt stress conditions. In plants that are sensitive to NaCl, Na<sup>+</sup> cation is accumulated in the leaves. This kind of types are called “excluders”, as compared to tolerant ones to NaCl that are called “includers”. In this last types, leaves are less Na<sup>+</sup> concentrate than roots (Haouala et al., 2007). According to Hadjadj (2009), soluble sugars accumulation in leaves is important under salt stress. In numerous species more or less resistant, it was observed an increase of total sugars (Asloum, 1990), resulting of non occurring glycolysis. Osmoregulation is one of the main adaptative strategies in response to abiotic stress, particularly salinity and drought (Benhassaine et al., 2010). Rezkallah et al. (2014) showed that accumulation of NaCl had an effect on physico-chemical characteristics of the soil. Al Satari (2014) demonstrated that an efficient use of arid soils may occur by growing several species in the same area thus having a better valorisation of these soils. On the other hand, the choice of the species to be grown in arid and semi-arid zones is determined, according to Falasca et al. (2014) by several factors, among which temperature, rainfall, type of the soil and water availability for irrigation. In salt soils, some species are threatened by extinction (Chamard, 1993); others show adaptative mechanisms (Hare & Cress, 1997). In fact, in these environments, plants adjust osmotically (Godghirs et al., 1990) their cell content by synthesizing amino acids, such as prolin (Ashraf & Mc Neily, 2004).

## MATERIAL AND METHODS

### *Plant material*

Seedling plants of *Atriplex canescens* (Pursh) Nutt. (Caryophyllales Chenopodiaceae) issued from the nursery of H.D.S.D. (High District of

Steppic Development) were grown in pots under semi-controlled conditions.

Experiment Design: four treatments were used:

T0: Control (without salinity)

T1: NaCl 50 meq

T2: NaCl 150 meq

T3: NaCl 200 meq

The size of the pots was 20 cm width and 18 cm depth. Soil composition was a mixture of organic matter and sand in the following proportions; 2/3: 1/3.

Plantules that have been transferred in pots received 250 ml each, which correspond to field capacity. Seedlings were exposed to salt stress for four (4) weeks; observations were then recorded and physiological analysis were run.

### *Analysis*

**Biomass:** it was quantified in weight of fresh and dry matter. Entire plant, shoot and root were weighted. Dry matter was obtained after 24 hours at 85°C in a laboratory oven.

**Chlorophyll content:** it was performed according to Mc Kinney & Arnon (1949).

**Prolin content:** the analysis was run according to Monneveux & Nemmar (1986).

**Soluble sugars content:** according to Shields & Burnett (1960).

**Statistical analysis:** according to Dunnett (1964)

## RESULTS AND DISCUSSION

### *Effect of salinity according to Dunett test*

As far as results are concerned (Fig. 1), it appears that plants respond positively to salt gradient. Most favorable levels seem to be T1 and T2. In a general manner, except for root biomass which remains stable, *Atriplex* L. show its ability of halophytic species. This positive responses obtained in T1 and T2 could have been a result of biochemical mechanism that increased Fresh Matter yield. The salinity level T1 and T2 could have been also considered as sort of mineral nutrient for seedlings. Beyond these levels, salinity become harmful eventough *Atriplex* seems to sustain salt concentrations.

As shown in Figure 2, dry matter weight is similar to fresh weight, as far as salinity levels are con-

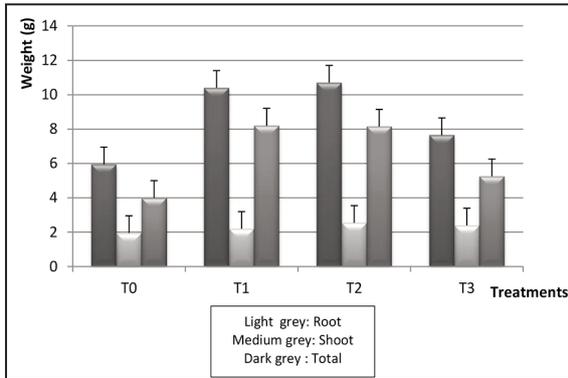


Figure 1. Fresh matter weight in *Atriplex canescens* according to salinity levels.

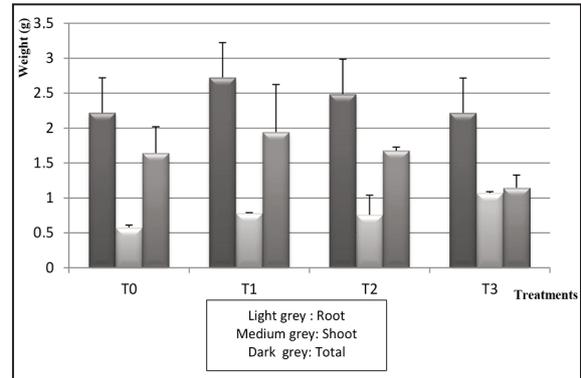


Figure 2. Dry matter weight in *Atriplex canescens* according to salinity levels.

	Total Fresh Matter		Shoot Fresh Matter		Root Fresh Matter	
	mean	probability	mean	probability	mean	probability
T <sub>0</sub>	5.90	0.104	4.000	0.098	1.950	0.149
T <sub>1</sub>	10.400		8.200		2.200	
T <sub>2</sub>	10.700		8.150		2.550	
T <sub>3</sub>	7.650	Significant	5.250	Significant	2.400	Significant

Table 1. Fresh matter weight analysed by Dunett test.

	Total Dry Matter		Shoot Dry Matter		Root Dry Matter	
	mean	probability	mean	mean	probability	mean
T <sub>0</sub>	2.218	0.468	1.641	0.184	0.576	0.017*
T <sub>1</sub>	2.723		1.942		0.783	
T <sub>2</sub>	2.486		1.676		0.759	
T <sub>3</sub>	2.217	Significant	1.145	Significant	0.070	

Table 2. Dry matter weight analysed by Dunett test.

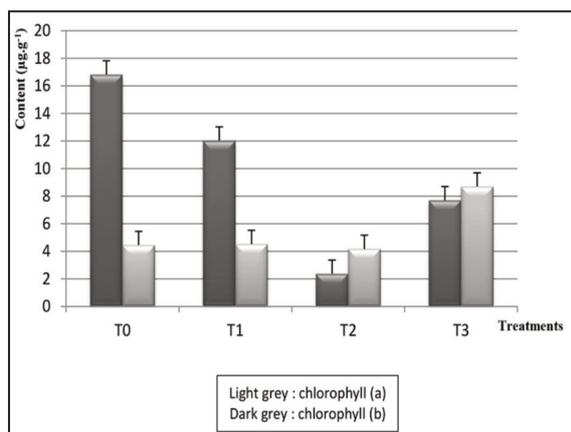


Figure 3. Chlorophyll content in *Atriplex canescens* according to a salinity gradient.

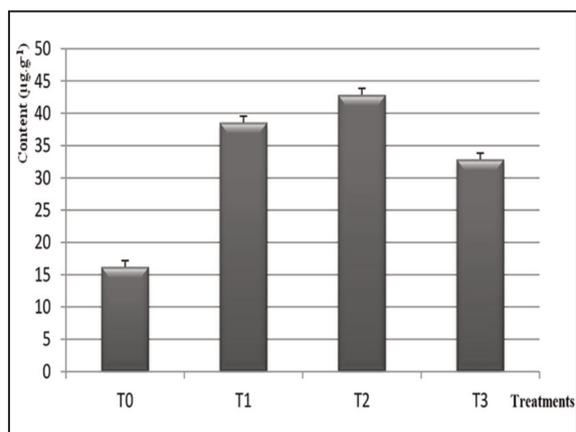


Figure 4. Proline accumulation in *Atriplex canescens* according to a salinity gradient.

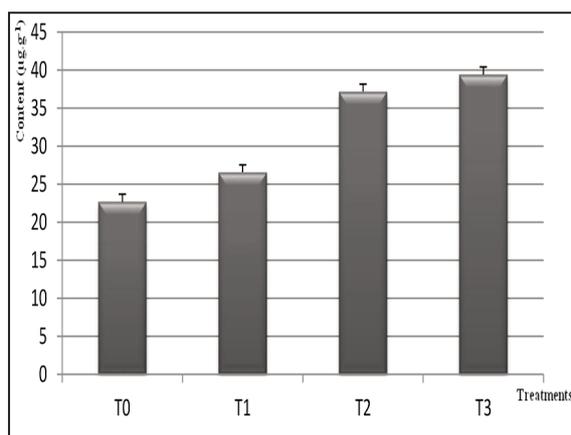


Figure 5. Total proteins accumulation in *Atriplex canescens* according to salinity levels.

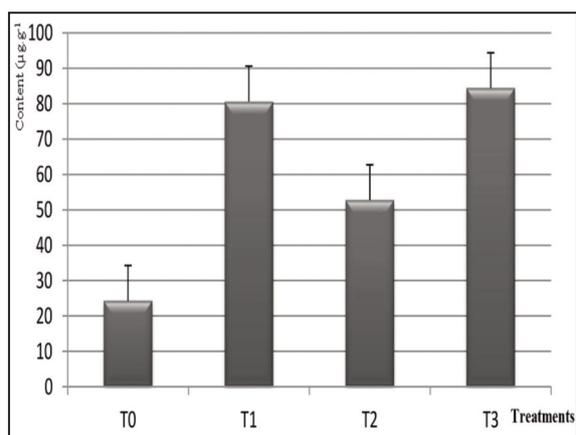


Figure 6. Soluble sugars accumulation in *Atriplex canescens* according to a salinity levels.

	Chlorophyll (a)		Chlorophyll (b)	
	Mean	Probability	Mean	Probability
T <sub>0</sub>	16.27	0.003** Highly Significant	4.44	0.002** Highly Significant
T <sub>1</sub>	12.02		4.51	
T <sub>2</sub>	2.06		4.11	
T <sub>3</sub>	7.69		8.69	

Table 3. Chlorophyll content analysed by Dunnett test.

	Mean	Probability
T <sub>0</sub>	16.15	0.000*** Very highly Significant
T <sub>1</sub>	38.5	
T <sub>2</sub>	42.82	
T <sub>3</sub>	32.85	

Table 4. Proline accumulation analysed by Dunnett test.

	Mean	Probability
T <sub>0</sub>	22.68	0.000***  Very highly  Significant
T <sub>1</sub>	26.56	
T <sub>2</sub>	37.16	
T <sub>3</sub>	39.4	

Table 5. Total Proteins accumulation analysed by Dunnett test.

	Mean	Probability
T <sub>0</sub>	24.3	0.025*
T <sub>1</sub>	80.69	
T <sub>2</sub>	52.84	
T <sub>3</sub>	84.53	

Table 6. Soluble Sugars accumulation analysed by Dunnett test.

cerned. Treatments T1 and T2 had accumulated more biomass for shoot. As far as roots are concerned, salinity did not show important incidence for biomass yield (dry matter).

### ***Chlorophyll content***

A decrease in chlorophyll content was observed except for T3 (Fig. 3). This helps us to understand that *A. canescens* uses other adaptative mechanisms to salt stress.

Table 3 shows That different treatments had high significant difference for chlorophyll (a); (0.003\*\*) and chlorophyll (b); (0.002\*\*).

### ***Osmoprotectors - Proline accumulation***

In figure 4, when salinity stress is increased, proline accumulation increased too. Because of this increase, *A. canescens* proved to behave as halophytic species. Statistical analysis shows very high significant differences between treatments. This might be due to endogenic ability of *A. canescens* to sustain high salinity level (T3).

Selection of tolerant cultivars could be based on such performance; as far as osmoregulation is concerned.

### ***Total proteins***

High NaCl concentrations stimulated protein accumulation. Very high significant differences are obtained using Dunnett test (Table 5).

### ***Soluble Sugars***

Except for treatment T2 (Fig. 6) High NaCl concentration has caused soluble sugar accumulation. Dunnett test show significant differences between treatments.

## **CONCLUSIONS**

Numerous physiological mechanisms and biochemical ones are regarded as being adaptative responses to salt and constitute specific adaptative traits.

Thus, each response is translated by physiological or biochemical marker. *Atriplex* constitute an interesting model for quantitative evaluation of different yield traits. Because of it's ability to maintain soil and preserve it against erosion that may occur under drastic environmental conditions, drought in particular, *Atriplex* may play a very important role in sustainable development areas. As to whether or not salt is to be considered one of the main abiotic constraints, much has to be done and emphasized on getting new tools of adaptative strategies. *Atriplex* is among those suitable solutions because of its capacity of covering soils, preventing them from erosion and constituting a substantial nutritional value as well, for sheep mainly.

On the basis of these indirect tests, which are physiological and biochemical ones, it appears that some of them can be of great reliable interest. The exploration of how the species behave to face to salt

stress, helps us in selecting determinant traits worth to be incorporated in breeding programs.

## REFERENCES

- Alsatari Y., 2014. Intercropping of *Atriplex halimus*, *Sal-sola* and barley for sustainable feed production under rangeland conditions in Jordan. *International Journal of Research in Applied, Natural and Social Sciences*, 2: 67–72.
- Ashraf M. & Mc Neilly T., 2004. Salinity tolerance in brassica oil seeds. *Critical Reviews in Plant Sciences*, 23: 157–174.
- Asloum H., 1990. Elaboration d'un système de production maraîchère (Tomate, *Lycopersicum esculentum* L.) en culture hors sol pour les régions sahariennes. Utilisation de substrats sableux et d'eaux saumâtres. Thèse de doctorat, développement et amélioration des végétaux, Université de Nice Sophia- Antipolis: 24–32.
- Belkhodja M. & Bidai Y., 2004. Réponse des graines d'*Atriplex halimus* L. à la salinité au stade de la germination. *Sécheresse*, 4: 331–334.
- Ben Naceur M., Rahmone C., Sdiri H., Meddahi M.L. & Selmi M., 2001. Effet du stress salin sur la germination, la croissance et la production en grains de quelques variétés maghrébines de blé. *Sécheresse*, 3: 167–174.
- Chamard P., 1993. Environment and development. Particular references to Sahel states members of CCILS. *Rev. Secheresse*, 4: 17–28.
- Falasca S.L., Pizaito M.J. & Mezher R.N., 2014. The agroecological suitability of *Atriplex numularia* and *A. halimus* for biomass production in Argentine saline drylands. *International Journal of Biometeorology*, 58: 143–144.
- Goldhirsh A.G., Hankamer B. & Lips S.H., 1990. Hydroxyproline and proline content of cell walls of sunflower, peanut, and cotton grown under salt stress. *Plant Science*, 69: 27–32.
- Hadjadj S., 2009. Contribution à l'étude de l'effet de la salinité sur des marqueurs biochimiques (proline et sucres solubles) de plantes juvéniles d'*Atriplex halimus* L. et d'*Atriplex canescens* (Pursh) Nutt. Mémoire de Magister en Biochimie et analyse des bioproduits, Université Kasdi Merbah Ouargla, 100 pp.
- Haouala F., Ferjani H. & Ben El-Hadj S., 2007. Effet de la salinité sur la plante. *Biotechnology, Agronomy, Society and Environment*, 11: 235–244
- Hare P.D. & Cress W.A., 1997. Metabolic implications of stress induced proline accumulation in plants. *Plant Growth Regulation*, 21: 79–102.
- Maas EV. & Nieman RH., 1975. Physiology tolerance to salinity. In: G.S. Jung, Group tolerance to suboptimal imand condition, 32: 277–299.
- McKinney G., 1941. Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry*, 140: 315–322.
- Mezni M., Albouchi A., Bizid E. & Hamza M., 2002. Effet de la salinité des eaux d'irrigation sur la nutrition minérale chez trois variétés de luzerne pérenne (*Medicago sativa*). *Agronomies*, 22: 283–291.
- Monneveux P. & Nemmar M., 1986. Contribution à l'étude de la résistance à la sécheresse chez le blé tendre (*T. aestivum* L.) et chez le blé dur (*T. durum* Desf). Etude de l'accumulation de la proline au cours du cycle de développement. *Agronomie*, 6: 583–590.
- Munns R., James R A. & Lauchli R., 2005. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany*, 57: 1025–1043.
- Piri K., Anceau C., El Jaafari S., Lepoivre P. & Semal J., 1994. Sélection in vitro de plantes androgénétiques de blé tendre résistantes à la salinité. L'amélioration des plantes pour l'adaptation aux milieux arides. Ed. Aupelf-Uref. John Libbey, Eurotext, Paris, 14: 311–320.
- Poljakoff-Mayber A., 1988. Ecological-physiological studies on the responses of higher plants to salinity and drought. *Arid Zone Research*, 6: 163–183.
- Rathinasabapathi B., Sigua C., Ho J. & Gage D.A., 2000. Osmoprotectant-alanine betaine synthesis in the Plumbaginaceae: S-adenosyl-L-methionine dependent N-methylation of b-alanine to its betaine is via N-methyl and N,N-dimethyl b-alanines. *Physiologia Plantarum*, 109: 225–231.
- Rezkallah C., Demai R., Malem S. & Fehdi C., 2014. Les effets d'irrigation par l'eau salée sur les propriétés physico-chimiques d'un sol planté avec *Atriplex halimus*. *Afrique Science*, 10: 139–144.
- Shields R. & Burnett W., 1960. Determination of protein bound carbohydrate in serum by a just modified anthrone method. *Analytical Chemistry*, 32: 885–886.
- Stephanopoulos G., 1999. Metabolic uxes and metabolic engineering. *Metabolic Studies. Analysis and Synthesis*, 15: 147–167.
- Zid E. & Grignon C., 1991. Les tests de sélection précoce pour la résistance des plantes aux stress. Cas des stress salin et hydrique. L'amélioration des plantes pour l'adaptation aux milieux arides. Ed. Aupelf-Uref. John Libbey, Eurotext, Paris, 14: 91–108.