



Research Article

Comparative Study of Germinative and Recovery Behavior of Two Species of *Atriplex* (*Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L.) under Salt Stress

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ABSTRACT

This work is a comparative study of germination under salt stress conditions on two halophilic species of the Amaranthaceae family; *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. Seeds were treated with NaCl at 600- and 900-mM concentrations and stored in an oven at 25 °C for 10 days. Observations focused mainly on the germination capacity, the velocity coefficient, the latency, the average germination time and the length of the seedlings. After the reversibility test, we recovered the seeds not germinated under saline stress in order to submit them to an environment without NaCl. Results show that increasing the NaCl concentration reduces significantly the germination capacity of the seeds. Without NaCl, the final germination rate of the seeds of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. varies from 100 to 95% respectively; while this rate drops with salinity for the seeds of *Atriplex canescens* (Pursh) Nutt and becomes zero for *Atriplex halimus* L. The capacity and the average speed of germination decrease as the salinity increases. In addition, salinity negatively influences the emergence of the vegetative system of the two species of *Atriplex*. Regarding the reversibility test, the results show that the two species of *Atriplex* are affected by osmotic and toxic depressions.

Key words: *Atriplex canescens* (Pursh) Nutt; *Atriplex halimus* L.; germination; halophyte; NaCl; reversibility; stress.

INTRODUCTION

The arid and semi-arid areas constitute about two-thirds of the earth's surface (Benbrahim *et al.*, 2004; Bouda and Haddioui, 2011; Benidire *et al.*, 2015). In these areas, soil salinization is considered to be one of the main factors limiting plant productivity and agricultural yield (Flowers *et al.*, 2010; Qadir *et al.*, 2014). More than 800 million hectares of land worldwide are affected by salinity, which represents more than 6% of the earth's surface (Rengasamy, 2010; Geissler *et al.*, 2015) of which 3.8% are located in Africa (Eynard *et al.*, 2006). Algeria, with a large part of the agricultural regions, characterized by an arid and semi-arid climate, is affected by salinity. Indeed, almost 3.2 million hectares are threatened by this phenomenon (Szabolcs, 1989; Belkhodja and Bidai, 2004; Benmahioul *et al.*, 2009).

Salt stress is one of the most serious environmental factors limiting seed germination and plant growth (Mohamdi *et al.*, 2011; Hajlaoui *et al.*, 2016; Achour *et al.*, 2020) due to the modification of the water and mineral state of the seeds and / or plants, which leads to osmotic stress and an excess of sodium ions (Hajlaoui *et al.*, 2016; Gull *et al.*, 2019).

Halophytes, which represent about 1% of the world's flora, survive naturally to reproduce in saline soils. These plants developed various mechanisms to adapt to the salted soils such as osmotic adjustment, regulation of absorption and transport of minerals (Wang *et al.*, 2015).

The genus *Atriplex* of the family Amaranthaceae are halophyte plants with a series of ecological and physiological characters allowing growth and reproduction in a saline environment (Djerroudi, 2017; Attia-Ismaïl, 2018). These species can be used in plant material selection programs that can be used in pastoral improvement projects (Bouda and Haddioui, 2011; Kaci *et al.*, 2012; Piovan *et al.*, 2014), rehabilitation of degraded soils and fight against erosion, salinity (Kaci *et al.*, 2012; Piovan *et al.*, 2014) and desertification of soils (Kaci *et al.*, 2012; Akgün *et al.*, 2018).

Atriplex species respond differently to salinity according to the stages of plant development (Bouda and Haddioui, 2011; Djerroudi, 2017). Given the constraints of salinity in arid and semi-arid areas, and to enhance these areas, our objective is to assess the variability of responses at the germination stage of the two halophyte species *Atriplex canescens* (Pursh) Nutt "introduced" and *Atriplex*

halimus L., "native", exposed to salinity stress (NaCl). Therefore, we aimed to examine the effects of various levels of salinity on Germination Capacity (GC %), Velocity Coefficient (VC), Latency (L), the Average Germination Time (AGT), the Length of the seedlings, and the Reversibility test (R).

MATERIALS AND METHODS

Preparation of seeds: The selected seeds are disinfected with 5% sodium hypochlorite during 3 min, then rinsed with distilled water, then placed in petri dishes lined with filter paper. The germination tests are carried out in the dark, in oven at continuous temperature of 25 °C. For each test, we used 40 seeds divided into four lots (repetitions) of 10 seeds each. The salt stress is induced by two concentrations of NaCl (600, 900 mM) while the control was a distilled water.

Data analysis

Kinetics of Germination: The kinetics of germination is illustrated by a curve representing the evolution of cumulative germination rates over time.

Germination Capacity (GC): It represents the maximum germination percentage or the germination capacity, obtained under our experimental conditions (Côme, 1970).

$$\text{The germination percentage} = \frac{N_i}{N_t} \times 100 \quad (1)$$

N_i : Number of germinated seeds

N_t : Total number of seeds

Velocity Coefficient (VC)

The velocity coefficient or speed of germination is determined by the following formula (Heller, 1978):

$$\text{Velocity coefficient (Vc)} = \frac{\sum n}{\sum (n \cdot T_n)} \times 100 \quad (2)$$

n : number of seeds germinated during time T .

Average Germination Time (AGT): The average germination time is evaluated as follows (Heller, 1978):

$$\text{AGT} = \frac{\sum (n \cdot T_n)}{\sum n} \times 100 = \frac{1}{V_c} \times 100 \quad (3)$$

Latency (L)

The latency time is the time necessary for the germination.

The Length of the Seedlings: The length of the hypocotyl and the radical are measured after 10 days.

Reversibility of the action of NaCl: The seeds, which could not germinate at concentrations of 600 and 900 mM NaCl, were put in distilled water (Hajlaoui *et al.*, 2007).

Data Treatments: The comparison of the means of the cited parameters was carried out with ANOVA and Tukey test for pairwise comparison using SPSS software (V17).

RESULTS

Kinetics of Germination: The germination curves (Figure 1) show that the number of germinated control seeds of *Atriplex canescens* (Pursh) Nutt gradually increases in time (days) to reach a rate of 100% of germinated seeds representing the maximum capacity for germination after the 7th day of sowing.

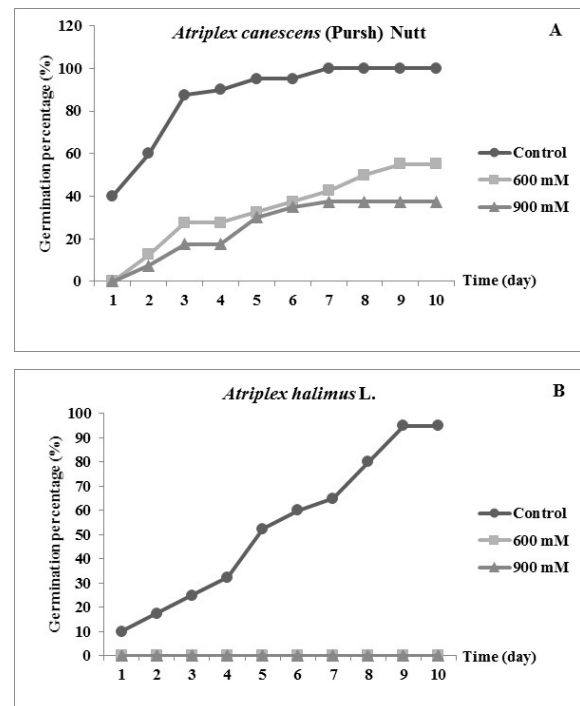


Fig. 1: Kinetics of germination of *Atriplex canescens* (Pursh) Nutt (A) and *Atriplex halimus* L. (B) under salted stress

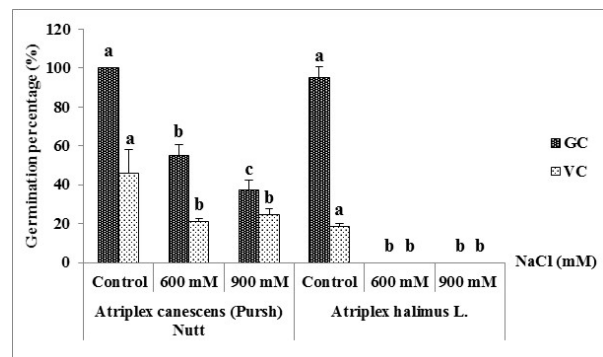


Fig. 2: Variation in the germination capacity (GC) and the velocity coefficient (VC) of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. seeds under salted stress

On the other hand, a slight delay in seed germination for a day is noticeable under 600 and 900 mM NaCl. Indeed, the seeds start the germination on the 2nd day of sowing with rates 12.5 and 7.5% respectively to reach the 8th day at 55 and 37.5% under these two salt concentrations (Figure 1 "A").

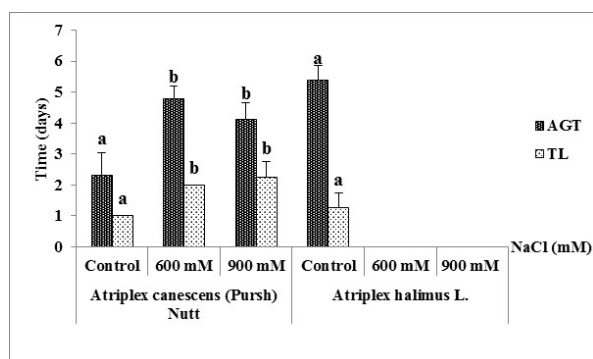
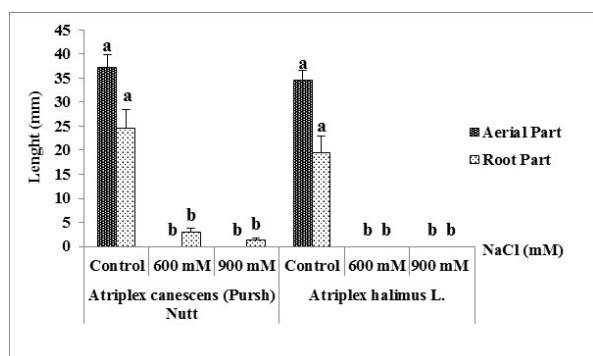
For *Atriplex halimus* L., the control seeds germinate from the first day of sowing only with 10% to arrive at a final rate at 95% of the seeds germinated at the 9th day of sowing. NaCl effect prevents germination of *Atriplex canescens* (Pursh) Nutt seeds under both, while the germination of the control seeds progresses normally for all the seeds (Figure 1 "B").

Germination Capacity and Velocity Coefficient or Speed of Germination: Results obtained show that the variation of the NaCl concentration has a highly significant effect on the capacity and speed of germination of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. compared to the control group ($p < 0.05$).

Table 1: Variation in the characteristics of seed germination of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. in response to NaCl stress (mean $\pm$ standard deviation, n = 4)

Treatments	<i>Atriplex canescens</i> (Pursh) Nutt			<i>Atriplex halimus</i> L.		
	Control	600 mM	900 mM	Control	600 mM	900 mM
Germination Capacity (%)	100 $\pm$ 0 ^a	55 $\pm$ 5,77 ^b	37,5 $\pm$ 5 ^c	95 $\pm$ 5,77 ^a	0 $\pm$ 0 ^b	0 $\pm$ 0 ^b
Velocity Coefficient (%)	45,77 $\pm$ 12,21 ^a	20,98 $\pm$ 1,70 ^b	24,54 $\pm$ 3,14 ^b	18,69 $\pm$ 1,58 ^a	0 $\pm$ 0 ^b	0 $\pm$ 0 ^b
Average Germination Time (days)	2,32 $\pm$ 0,71 ^a	4,79 $\pm$ 0,40 ^b	4,13 $\pm$ 0,52 ^b	5,38 $\pm$ 0,49 ^a	--	--
Latency (days)	1 $\pm$ 0 ^a	2 $\pm$ 0 ^b	2,25 $\pm$ 0,5 ^b	1,25 $\pm$ 0,5 ^a	--	--
Length of Aerial Part (mm)	37,25 $\pm$ 2,63 ^a	0 $\pm$ 0 ^b	0 $\pm$ 0 ^b	34,5 $\pm$ 2,08 ^a	0 $\pm$ 0 ^b	0 $\pm$ 0 ^b
Length of Root Part (mm)	24,5 $\pm$ 3,87 ^a	3 $\pm$ 0,82 ^b	1,25 $\pm$ 0,5 ^b	19,5 $\pm$ 3,42 ^a	0 $\pm$ 0 ^b	0 $\pm$ 0 ^b
Reversibility (%)	100 $\pm$ 0 ^a	55 $\pm$ 5,77 ^b	37,5 $\pm$ 5 ^c	95 $\pm$ 5,77 ^a	60 $\pm$ 8,16 ^b	45 $\pm$ 12,91 ^{ab}

Note. The different letters indicate significant differences at 5% level (P < 0.05)

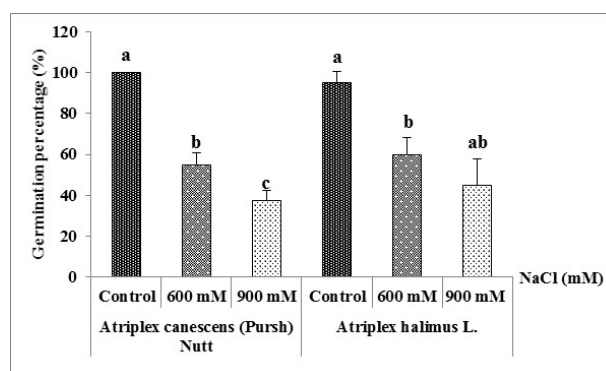
**Fig. 3:** Variation on average germination time (AGT) and latency (L) of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. seeds under salted stress**Fig. 4:** NaCl effect on the variation of the lengths of the seedlings of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L.

The control seeds of *Atriplex canescens* (Pursh) Nutt recorded an average germination capacity of 100%. Indeed, the seeds receiving NaCl at 600- and 900-mM record rates of 55 and 37% respectively. The highest velocity coefficient is recorded in the control group (45.77%) while it decreases in the seeds with 600- and 900-mM concentration (20.97 and 24.53%) (Figure 2).

In *Atriplex halimus* L., the maximum capacity and speed of germination are recorded in the controls with 95 and 18.69% respectively, while they become zero at 600 and 900 mM (Figure 2).

Average Germination Time and Latency: Statistical analysis indicates that NaCl has a highly significant effect on the average latency and the average germination time of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. seeds (p < 0.05).

The shortest average germination time in *Atriplex canescens* (Pursh) Nutt seeds is around 2.32 days in the

**Fig. 5:** The reversibility effect of the NaCl on the final germination rate of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. seeds

control group. This parameter reaches a maximum of 4.79 and 4.13 days under 600 and 900 mM NaCl. While, the shortest latency is recorded in the control seeds (1 day); this latency increases significantly with the increase of the NaCl concentration i.e. 2 and 2.25 days respectively (Figure 3).

Furthermore, the highest average germination time and latency are equivalent to 5.38 and 1 day respectively noted in the control seeds of *Atriplex halimus* L., while they become zero under 600 and 900 mM NaCl (Figure 3).

The Lengths of the seedlings: Statistical study reveals that NaCl has a highly significant effect on the lengths of the aerial and roots parts Tukey (p < 0.05).

For *Atriplex canescens* (Pursh) Nutt, the length of the aerial and the root part are maximum in the controls group with lengths of 37.25 mm and 24.5 mm respectively. On the contrary, the shortest route lengths are recorded in the seedlings treated with 600 and 900 mM NaCl, with lengths of 3 mm and 1.25 mm respectively, while the hypocotyls did not grow (Figure 4).

In addition, the maximum aerial and root lengths of *Atriplex halimus* L. were recorded in the control group 34.5 and 19.5 mm respectively. While for the seeds receiving the salt at 600 and 900 mM, no growth or development of young organs is expressed (Figure 4).

Reversibility of the NaCl effect: The reversibility test of the NaCl effect was studied at the concentration of 600 and 900 mM. These results show that the transfer of seeds into distilled water is followed by a resumption of germination.

With distilled water, *Atriplex halimus* L seeds germination is restored after the 2nd day, reaching 55 and 37.5% under 600 and 900 mM NaCl respectively.

Indeed, the germination reversibility of seeds treated with NaCl influences significantly the elongation of *Atriplex canescens* (Pursh) Nutt seeds, while the final germination rates are those of saline treatments (Figure 5).

Response of Seeds to Germination: The results presented in table 1 correspond to the average values of the different parameters of seeds germination of two *Atriplex* species for 10 days under salt stress.

DISCUSSION

The germination tests show significant differences concerning the behavior of seeds of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. exposed to 600 and 900 mM NaCl.

Maximum germination is recorded with distilled water in both species. The high NaCl concentrations (600 and 900 mM) caused a slight delay in seed germination followed by a drop in their final germination rate for *Atriplex canescens* (Pursh) Nutt. Furthermore, for *Atriplex halimus* L. germination is inhibited at concentrations of 600 and 900 mM NaCl.

These results corroborate the work of Belkhodja and Bidai (2004) in which it is noted that the seeds of *Atriplex halimus* L. germinate in environment without NaCl or in the presence of NaCl at low concentration (100 mM); a reduction in germinated seed rates occurs in intermediate concentrations (350 mM of NaCl), followed by inhibition of germination for seeds exposed to high salt concentrations (600 mM of NaCl).

Seed germination of *Atriplex canescens* (Pursh) Nutt is more tolerant to salinity than *Atriplex halimus* L., while Souhail and Chaâbane (2009) found that seed germination of *Atriplex halimus* L. is more tolerant to salinity than *Atriplex canescens* (Pursh) Nutt. On the other hand, it is shown that the germination is either greatly reduced or completely inhibited under high salt concentrations (Bouda and Haddioui, 2011; Bhatt and Santo, 2016; Djerroudi, 2017; Nisar *et al.*, 2019).

Results indicate a significant decrease in germination capacity as the concentration of NaCl increases. This is also the case for the velocity coefficient which decreases as the salt content increases in the environment, with longer latency times and average germination times.

Several studies show that the stress leads a decrease in the germination kinetics and the velocity coefficient with an increase of the average germination time and the latency for *Phaseolus* (Bayuelo-Jiménez *et al.*, 2002), *Pisum sativum* L. (Okçu *et al.*, 2005), *Vicia faba* (Benidire *et al.*, 2015) and *Retama retam* (Mehdadi *et al.*, 2017).

Results also reveal that salinity negatively affects the growth of radicles. For *Atriplex canescens* (Pursh) Nutt, 600 and 900 mM NaCl concentration slowed down the growth of the radicles, an 80% of decrease compared to control. Similar results have been noted on the slowing of the growth of seedlings of Pistachia and those of dill (*Anethum graveolens* L.) under salt stress (Benmahiouel *et al.*, 2009; Setayesh, 2013).

Alaoui *et al.* (2013) found that the growth of the aerial and root part of wheat is affected by salinity depending on the varieties and the nature of the stress applied.

This reduction in growth is mainly due to the creation of an extreme osmotic potential which prevents the absorption of water and induces the excessive accumulation of Na⁺ and Cl⁻ ions as well as a deficiency of K⁺ in the embryo (Hajlaoui *et al.*, 2007; Mohamdi *et al.*, 2011). This ionic imbalance decreases the ability of the embryo to trigger the metabolic functions useful in the hydrolysis of reserves (Abdelkader *et al.*, 2015). Panuccio *et al.* (2014) note that this decrease in growth is also due to a decrease in the number of cell division following abiotic stress.

Salinity can induce two effects, osmotic which is reversible or toxic which is irreversible (Hajlaoui *et al.*, 2007; Benidire *et al.*, 2015). Indeed, if it's an osmotic origin, we performed a resumption of germination, furthermore, no resumption of germination is recorded, when it's a toxic origin (Mehdadi *et al.*, 2017).

The reversibility of the NaCl effect is a parameter that can help to determine the origin of the depressive effect of salinity on germination. The transfer of *Atriplex* seeds into distilled water has shown some irreversibility of the repressive effect of NaCl, which probably indicates osmotic and toxic effects at the cellular level. Our results are similar to those of Benidire *et al.* (2015) who show that pretreatment with a high dose of NaCl (200 mM) and the transfer of bean seeds into distilled water is followed by a resumption of germination. However, the germination capacity remains lower than that obtained in seeds placed directly in distilled water.

Our results also corroborate with Hameed *et al.* (2013) for *Suaeda heterophylla*, Strazisar *et al.* (2013) for *Ruppia maritima*, Mehdadi *et al.* (2017) for *Retama retam*, and Haraguchi and Matsuda (2018) for *Suaeda japonica* who reported poor seed germination after transfer to fresh water.

However, Nichols *et al.* (2009) found that there is an irreversible total inhibition of the germination of the *Medicago polymorpha* and *Trifolium subterraneum* seeds at 600 mM NaCl even after transfer of these in non-saline environment; this is explained by the toxic effect of salt on the embryo.

Conclusion

Salinity affects the germination parameters examined in the two *Atriplex* species. High concentrations of salinity negatively affect the growth and length of seedlings. Seed germination of *Atriplex canescens* (Pursh) Nutt is more tolerant to salinity than *Atriplex halimus* L. Seeds of *Atriplex canescens* (Pursh) Nutt and *Atriplex halimus* L. are affected by osmotic and toxic depressions.

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