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Toxicity of the Salt and Pericarp Inhibition on the Germination of Some *Atriplex* Species

[1,2]*Mâalem Souhail and* [2]*Rahmoune Chaâbane*

[1] Department of Biology, exact Sciences and life sciences and nature Faculty,
Tebessa University, 12000 Tébessa, Algeria
[2]Laboratory of Ecotoxicology and Abiotic Stresses, Sciences life and nature Department,
Sciences Faculty, Constantine Mentouri University, 25000 Constantine, Algeria

Abstract: This survey was led on the *Atriplex* that are some Xerohalophytes plants capable to evolve naturally in the salty ecosystems. However, in phase of germination, they stay sensible to salt stress as the remainder plants. During this test, we measured the answer of three different species of *Atriplex* to the salinity through certain parameters which are: rate of cumulated (RCG) germination, the middle time of germination (MTG) and the coefficient of the germination uniformity (CGU). We also tested some used treatments to break the dormancy that consisted to the imbibation or the decortication of the seminal pericarp. Results showed that the three studied species varied greatly between them, for their germinated faculty as well as to their answers to salt stress and to the treatment of the breaking dormancy. The weak values of the CGU have also marked the lots of the same seeds by a large variability during germination. Results have presented the local species *A. halimus* as the most tolerant to the salt stress and the least suffering to the problem of the pericarp inhibition. We have also noticed that the treatment of the breaking dormancy improved significantly the rate and the speed of germination. We also noted in all species, that beyond the dose of 8g/l of NaCl, the germination becomes more and slower until the total inhibition. These results can be exploited in programs of the rehabilitation of saline site

Key words: *Atriplex* · Germination · Salt stress · Pericarp inhibition · Steppe

INTRODUCTION

The arid zones occupy more than 600 000 Km² in the North of the Sahara, of which about 34% in Algeria, 31% in Libya, 19% to Morocco, 11% in Tunisia and 5% in Egypt [1]. In these zones the steppe covers 40 000 to 50 000 km²s in the North of Africa where it constitutes a fundamental resource of fodder that is essential to the pastoral activity [1]. The plant table setting in these regions don't quit degrading itself because of the natural constraints of which the most prominent are the drought, the salinity and the alkalinity of the soils. The human factor is also implied by the modification of the exploited systems of the milieu which is related to the transformation of the socioeconomic conditions and the evolution of production techniques [3]. considering the state of deterioration of the natural ecosystems and the strong human and animal pressure that they undergo, the reconstitution of the plant table setting, can no more be assured by the natural mechanisms of plants and requires the recourse to sophisticated techniques of planning and management of areas. These techniques based on the reasonable utilization of the waters of rains and the Xerohalophytes species plantation contributing to the growth of the production as well as to the protection of the soils against the erosion [4].

If would make some considerable progress in the vast saline degraded zone rehabilitation, it is necessary to select the well adapted species, to apply some suitable plantation methods and to produce plantations in tree nursery, where it will be possible to put of efficacious techniques of seeding, of scarification, of watering and of fertilization. In Algeria, an immense effort of pastoral zone rehabilitation is undertaken for more than a decade. Among the used species we find the *Atriplex* that are Xerohalophytes plants capable to push and to reproduce in the saline and marginal soils [5]. However, their seeds are sensible to the salinity in phase of germination [6-8] and they endure problems of fertility for some [9] and of mechanical dormancy for others [10] and [11] thus, induce some considerable economic losses during the plant's production in tree nursery.

Corresponding Author: Mâalem souhail, Department of Biology, exact Sciences and life sciences and nature Faculty,
Tebessa University, 12000 Tébessa, Algeria

In this context, we have led an Ecophysiologic survey, during which we have realized tests of germination in laboratory on the seeds of three different species of *Atriplex*. These last have undergone different treatment of the breaking dormancy and different saline stress level.

Our objective consisted to evaluate the impact of the saline stress and pericarp inhibition on germination. We have also studied the possibility to improve the rate of germination by breaking dormancy treatments. Thus, we have chosen germinated faculty (RCG), the speed of germination (MTG) and the coefficient of germination uniformity (CGU), as indicatory parameters on germinated activity of studied seeds.

MATERIAL AND METHODS

Germination Experiments: Seeds have been procured from the experimental station of the high commissariat to the development of the steppes, situated in Thlidjène that is in the south of the town of Tebessa (North-east of Algeria). Only intact and mature seeds have been kept.

The used Petri dishes have a diameter of 9cm and a thickness of 1.5cm. They have been covered with a double layer of filters paper.

The decorticated seeds have been removed mechanically of their pericarp, whereas those imbibed have been soaked during 24 hours in distilled water. Then they have been put to dry during 4 hours. Therefore the seeds of the species *A. canescenses* have undergone a mechanicals scarification instead of decortication because of the toughness of their pericarp that is a very hermetic and small capsule.

Each test of germination has been driven in 4 replicates of 100 seeds each. Seeds stakes to germinate have received 5ml of distilled water (control) or of saline solution (0, 4, 8, 16 and 32 g NaCl/l corresponding to 0, 68, 132, 272 and 544mM NaCl).

A seed is considered germinated, when the radical pierces teguments [12].

Measure of Germination Parameters: We have measured germinated activity of *Atriplex* seeds through the following parameters:

- Rate of cumulated germination (RCG) or germinated faculty: it is expressed by the report number of germinated seeds on the total number of seeds [12].
- *Middle time of germination* (MTG) or speed of germination: it is the time to the tip of which one gets 50% of germinated seeds: $MTM = \sum (t \times n) / \sum n$ [12].
- *Coefficient of germination uniformity* (CGU): this is an indicator on the variability of the germination of a lot seed: $CGU = \Sigma n / \Sigma [(MTG - t)^2 \times n]$ [13]. (t: Time in day, n,: number of seed germinated).

Statistical Analysis of Data: In this test, we have opted for a complete aleatory dispositive with 3 studied variables which are: Species with their three levels (A. *halimus, A. canescens* and *A. nummularia*), salinity with their 5 levels and treatment of the raised dormancy with their three levels (control, Imbibition and Decortication). The data were subject to analysis of variance (ANOVA). $P<0.05$ was used to define statistical significance. If significant difference was determinate among means a Student-Numan-Keuls'test was used to determine significant difference between pairwise comparisons among individual treatment. Only means of germinated faculty have been analyzed. Every gotten data constitutes means of 4 repetitions. Data were analysed using Statistica™ Software (Stat Soft 1998).
Breaking

RESULTS AND DISCUSSION

Rate of Cumulated Germination (RCG): The variation of the rate of cumulated germination of the three studied seeds species of *Atriplex* according to the increasing doses of NaCl and treatments of the beaking dormancy is shown in Figure 01.

Generally, the results show that *A. halimus* distinguishes itself of the two other species by the elevated germinated faculty of its seeds with an average of 46,35%, follow-up by the species *A. nummularia* with an average of 32,2% and finally the species *A. canescens* with an average of 24,25%.

These gotten results put in evidence the existence of a large specific variability between the three studied species for the RCG parameter. This last variability is also put in evidence by the analysis of the variance (ANOVA) that is marked by a very highly meaningful difference for the species factor. The test of comparison of the meanshad stand out 3 homogeneous groups thus separating the 3 species each of the other (Figure 1). This divergence intra-genre of *Atriplex*, observed in phase of germination, has also been obtained by [14] in a similar work, led on other species of *Atriplex* growing in Europe, that are: *A. sagittatas, A. hortensis, A. tatarica* and *A. rosa*.

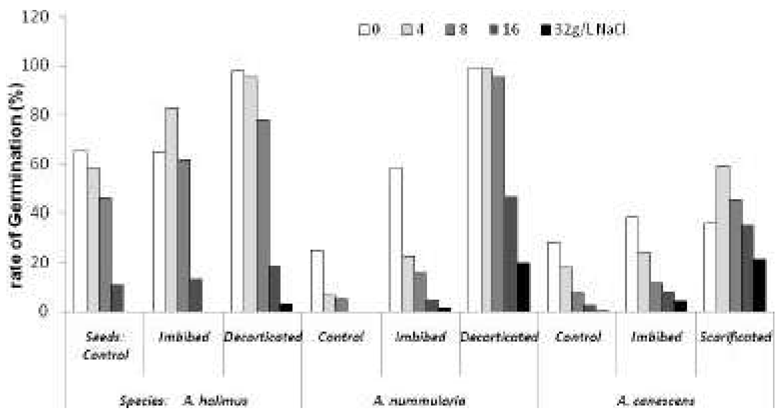

Fig. 1: The variation of Rate cumulated of germination of seeds of Three *Atriplex* species under saline and breaking dormancy treatment

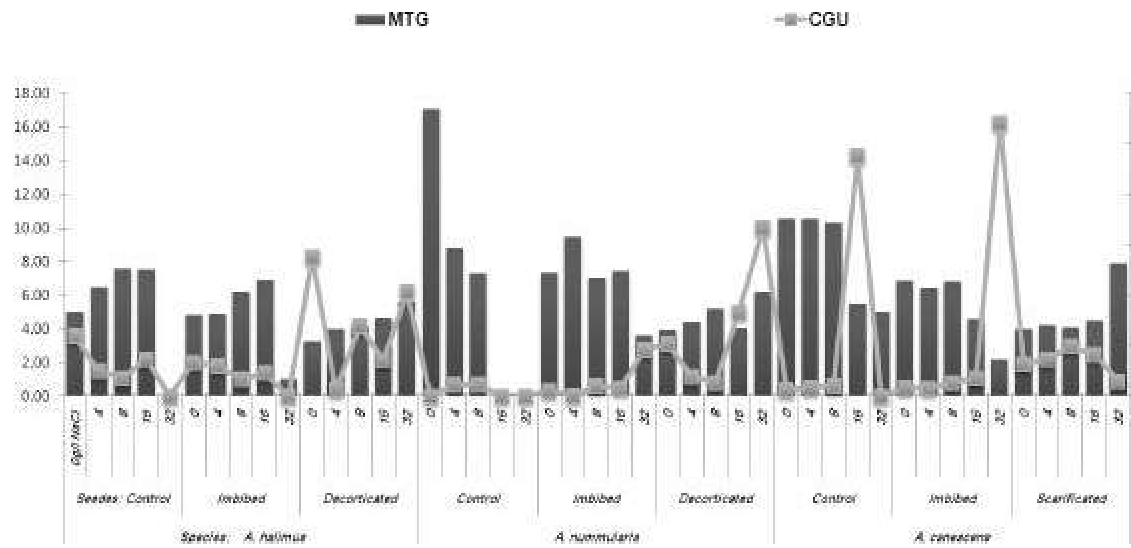

Fig. 2: The variation of Middle time of germination (MTG) and Coefficient of germination uniformity (CGU) of seeds of Three *Atriplex* species under saline and breaking dormancy treatment

Table 1: Statistical analysis

Source of variation	ANOVA (3way analyses of variance) df	Test F	Level of signification	Test of means comparison of studied variables for Rate cumulated germination (Student-Neuman-Keuls (LSD))				
Species	2	176	***	*A.halimus* **46,35[(a)]**		*A.nummularia* **33.20[(b)]**		*A.canescens* **24,25[(c)]**
Treatments	2	618	***	control 18,3[(a)]		Imbibition 27,4[(b)]		Decortication 58,1[(c)]
Salinity	4	460	***	[0] 59,55[(a)]	[4] 51,64[(b)]	[8] 40,72[(c)]	[16] 15,58[(d)]	[32 g/l NaCl] 05,5[(e)]

-df: degree of freedom.

-***: significant Probability at $p < 0,001$.

-Value followed by different letters, on the same line, differ significantly at $P<0.05$. Values are means of 4 replicates.

The results also show that the seeds of the three studied species of *Atriplex* react positively to treatments of the breaking dormancy, for all species, where a clear improvement of seed's germinated faculty has marked the lots that are submitted to the imbibition and to the decortication. Thus, the seeds treated have showed an elevated germinated faculty in comparison with a control. According to [10, 15 and 16], in several species of *Atriplex* the pericarp presents a dormancy and inhibition factor of germination.

It is to be noted also that the Australian species *A. nummularia* is the one that is the most characterized by the germinated faculty that is stimulated by treatments of the breaking dormancy. Indeed, it was distinguished from the two others by the weakest rate of germination in the lots of control seeds and the most elevated rate in the lots of decorticated seeds. Thus, we have got in this last species (*A. nummularia*) an increase of 100% of RCG in comparison with the control, at the imbibed seeds and another increase, even more important, at the decorticated seeds (400%). It is also to be signalled that germinated faculty of control seeds, in the best cases, doesn't exceed the 25% of the tested lots. [15] Have recorded during a comparable study of germination to ours, a rate of cumulated germination of 4% at control seeds and 88% at seeds ridded of their bracteoles and/or scarified. Some similar results have also been brought back by [10], which have noticed that the percentage of germination was null in control seeds and rose to 7.5% at chemically scarified seeds and to 48% in seeds without bracteoles lots. This last percentage is similar to the gotten on (46.35%) at the local species and under the same treatment.

However, in the case of local species *A. halimus*, the improvement of the RCG at the decorticated seeds didn't appear highly because the control seeds have already a good germinated faculty. But its RCG remains lower to the one of decorticated and imbibed seeds. This shows that this species didn't suffer a lot from the dormancy phenomenon practiced by bracteole as it is the case of *A. nummularia*.

So, at the American species *A. canescens*, whose seeds are only scarified (fructified capsule is very close around the seed and cannot be ridded), the improvement of germinated faculty was not very meaningful and the RCG of the treated seeds didn't pass, in the best case, the 60%.

With regard to the answer of the studied species of *Atriplex* to the saline stress, all treated plants show a reduction of germinated faculty with the growth of salinity, to some exceptions. But it is to be noted that a specific variability is observed between the three tested species. Indeed, at the local species *A. halicmus*, the rate of germination decreases progressively with doses 4 and 8g/l NaCl, then it falls excessively until the complete inhibition of germination under the dose 32g/l NaCl. According to [17], at *A. halimus,* the germination is totally inhibited beyond of the saline concentration of 40g/l of NaCl. [18] bring back also in a similar work, that the percentage of germination recorded, under the saline concentration 600meq (30g/l) NaCl, don't pass the 4%.

At the species *A. Nummularia*, the control and imbibed seeds that have been submitted to the saline treatment have recorded a severe decline, as for their percentage of germination, where total inhibitions have appeared from the dose 16g NaCl. However the decorticated seeds have reached a maximal rate of germination adjoining the 100% for cases of doses 0, 4 and 8g/l NaCl. Then, beyond these last, the rate of germination decreases proportionally with doses 16 and 32g/l NaCls corresponding respectively to values of about 45% and 20%.

Therefore, at the *A. canescens* species, the reduction of germinated faculty appears proportionally with the growth of the saline stress, for all treatments. Nevertheless, one must note that for the case of this species, we didn't achieve a complete decortication, but only a physical scarification of the pericarp. This could explain the weak germinated faculty that characterizes it.

Generally, we have noted that the treatment of the breaking dormancy have improved the germinated faculty of seeds, notably outside the saline stress. This result has conducted us to the possibility of the association of the two inhibited factors: the pericarp and saline stress which together repress the germination by an accumulated effect. This latter deduction was put in evidence by [19], at the *A. griffithii* and *A. prostrata* species evoking in addition the low temperature as a third inhibited factor of germination.

Oppositely, we have also observed, in two exceptional cases: those of imbibed seeds of *A. halimus* and decorticated of *A. canescens*, under the dose 2g/l NaCl, that the recorded RCG superior to the untreated seeds (0g/l NaCls). This has permitted us to suppose that under this moderate dose of NaCl, the seeds' germinated faculty has undergone stimulation.

Although this last observation is exceptional, it corresponds with halophile nature of the *Atriplex* species. Indeed and in a survey achieved by [12], on the halophytes grasses, they corroborate our results while bringing back that the concentration 6g/l of NaCl that has permitted a light improvement of the germinated faculty of the tested seeds.

Middle Time of Germination (MTG): Values of the middle time of germination showed in Figure 2 reveal that this parameter varies from a species to another one, from a breaking dormancy treatment to another and it also varies according to applied NaCl doses.

First, at the local *A. halimus* species, the values of this last parameter, that corresponds to the speed of germination, are almost-inversely proportional to those of the germination rate accumulated (RCT), that is to say that the middle time of germination is shorter when the germinated faculty is raised and vice versa.

However, this last behaviour wasn't the same with the two other exotic species. At *A. nummularia* the variation of the MTG deferred from a treatment to another; indeed, just as the MTG decreased with the growth of doses of NACl, at control seeds, we recorded an inverse answer in decorticated seeds. Whereas at *A. canescens*, this same parameter changed proportionally with doses of NaCl, in the occurrence with scarified seeds, but the answer was inverse for the left cases.

While taking in consideration, what we have already advanced during the discussion of the variation of the RCG parameter, as for the fact that the species *A. halimus* didn't endure too much problem of pericarp inhibition. We can say that the variation of the germination speed is governed by a very precise order. Indeed, at all the cases of the *A. halimus* species and in the case of decortication and scarification, at *A.nummularia* and *A. canescens*, respectively, the most important germination speed, that is, the middle time of germination the shorter, corresponded with the most important RCG and vice-versa. These situations also corresponded to situations where seeds are generally out pericap inhibition. This permits us to say that seeds out pericarp inhibition and saline stress are those that germinate more and more quickly.

For the left cases, even though MTG values are weak, that doesn't mean necessarily that the speed of germination is strong, but it is often related to a very low germination percentage.

According to [18], the kinetics of germination varies distinctly according to the intensity of the saline stress where a very slow progression is recorded under the elevated levels of NaCl doses and where the RCG is very low. Several studies have indicated that the seeds of glycophytes and halophytes respond to the saline stress reducing the total number of germinated seeds and while accusing a delay in the initiation of the germination process [20].

Coefficient of the Germination Uniformity (CGU): On the figure 02, we can also observe the values of the coefficient of germination uniformity (CUG) that is an indicator of the level of the germination variability. It permitted us, on the one hand, to measure germinated variability of seeds, within the same lot and on the other hand to evaluate its variation according to the different studied factor levels, to know: species, treatments of the breaking dormancy and saline stress.

Before undertaking the analysis of results, of this last parameter, it is important to insist that the majority of picks of values of the recorded RCG are insignificant, because they correspond to rates of germination, either very low or null (the RCG is informative only in the case where germination is meaningful). These last picks cases are the following: the lots of decorticated seeds under treatment 32g/l NaCl at *A. halimus* (RCG = 0.41), the same previous case, but in the lots of imbibed seeds at *A. nummularia* (RCG = 0.18) and finally at *A. canescens*, in cases of lots of control seeds (without treatment of breaking dormancy), treated with 16g/l NaCl (RCG = 0,95) and imbibed and treated with 32g/l NaCl (CUG = 1,08).

Therefore, ignoring the insane cases, previously indicated, we can say that recorded values of the RCG are generally very weak notably at the two exotic species, reflecting thus, the existence of a considerable variability, within lots of tested seeds.

Indeed, at *A. canescens*, the CUG values don't pass the 0.2 on a ladder of 1, where a light increase is observed with the first increasing doses of NaCl (0, 4 and 8g/l), then these values decrease with the dose 32g/l NaCls in control lots and under the dose 16 and 32g/l NaCl in the decorticated lots.

At *A. nummularia* and within lots of control and imbibed seeds, the CUG characterizes himself by values nearly null of all NaCl doses. But they are considerable when seeds are decorticated. These values are decreasing with the first doses of NaCl: 0, 4 and 8g/l correspondent respectively to 0.2, 0.07 and 0.05 and they are increasing with the elevated doses 8, 16 and 32g/l correspondent respectively to 0.05, 0.33 and 0.66. It is to be noted that this last value of the CUG is considered as being the most elevated in this work.

However, at the local *A. halimus* species, the gotten values of the CUG were more or less important and inversely proportional with the increasing doses of NaCl notably at the control and imbibed seeds, to the exception of the dose 16g/l NaCl where a light increase has been recorded.

However in the lots of decorticated seeds, the values of CUG level were significant but fluctuating and alternating with the successive doses of NaCl. It is to be signaled that at the local species, the most superior values have been recorded under this treatment.

Generally we can say that the most meaningful values of the CUG are those that correspond to lots of seeds that are characterized by an intense germinated faculty. This is more the case of seeds out pericarp inhibition and saline stress and those belonging to the local species; *A. halimus*.

The big variability that we have recorded has been extensively mentioned in works specialized in the genetic diversity characterizing the *Atriplex* [21-24].

CONCLUSION

The gotten results, in the context of this study, show that the *Atriplex* studied species could be promising in programs of rehabilitation of degraded pastoral zones and of the salty sites in arid regions. However, the gotten data show that the three studied species don't have the same attitude with the applied treatments. This incites us to recommend a selective utilization of species to get some best results. Indeed because we have observed that the *A. halimus* seeds are little sensitive to dormancy problem and appear very resistant to the saline stress. This character makes it a good candidate for the re-implantation of sites greatly saline, especially as it is marked by a good speed and uniformity of germination. This is also valid for the Australian species, but only in case of previous decortication of these seeds. Otherwise, in the natural state of these seeds, it can be used with the American species in sites of moderating salinity, or to produce plantations in tree-nursery.

It is also to be kept that the concentration of 8g/l of NaCl corresponds to the limit of salinity beyond of which germinated faculty of the three species decrease appreciably.

In this work, we have also noticed that salt stress and the pericarp exercised together an inhibitory and cumulated effect repressing more the germination. The exam of results permitted us also to be interested to the two other treatments of the breaking dormancy: the imbibition and the mechanical scarification of the pericarp. These two treatments are good alternatives to the decortication because they are at the same time efficient and technically more easily feasible.

We conclude that a good knowledge of the biology of *Atriplex* germination would permit a good master or to create the techniques of the improvement of seeds' germinated faculty.

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