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# Effect of Salinity and water Stress on the Germination of *Medicago arborea* L. Seeds

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## ABSTRACT

In this paper we aim to study the viability of *Medicago arborea* L. seeds through the evaluation of their germination behavior under the effect of salt and water stress. For this, a germination tests were carried out in the darkness within an incubator using a continuous temperature of 20°C. For the salt stress, the seeds were submitted under different concentrations of NaCl (34mM, 68 mM, 102 mM, 136 mM, 170mM, 204 mM, 238 mM, 272mM) and for the water stress, different osmotic pressures were used(-0.01 Mpa, -0.02Mpa, -0.05 Mpa,-0.1Mpa, -0.2 Mpa), induced by polyethylene glycol 6000($PEG_{6000}$).

The results showed that the germination was significantly affected by high salt stress ($P < 0.05$). The increase of salinity decreased the germination capacity and the index of germination velocity, as well as it lengthened the latency time and the mean time of germination.

The seeds behaved similarly face to the water stress, since their germination rate decreased according to the increase of the concentration of $PEG_{6000}$ in the environment.

It was revealed that the concentration 204mM of NaCl and the osmotic pressure -0.05 Mpa constituted the physiological limits (threshold) for the germination of the studied seeds.

On the other hand, when ungerminated seeds were transferred from NaCl treatments to distilled water, they recovered largely their germination after only one day. This indicated that the germination inhibition was related to osmotic stress, but either to an ion toxicity since the germination capacity decreased comparing to the control test.

**KEY WORDS:** *Medicago arborea*, viability, germination, salt stress, water stress.

## I. INTRODUCTION

Arid and semi-arid lands of the Mediterranean area are characterized by the climatic variability mainly the irregularity of precipitation, drought and thermal variations associated to an important evaporation, encouraging the accumulation of salt in the soil and leading to the salinization of soil and irrigation water. Such environmental conditions constitute a limiting factors and a challenge to the regeneration of vegetal species **[20]**.

In order to deal with such environmental conditions, different strategies could be adopted, for instance, the perfect choice of the appropriate vegetation for the previous conditions which constitute one of the most convenient approaches might solves the problems of damaged soils rehabilitation in the Mediterranean area **[37]**. For that, many researches were orientated to the introduction of woody leguminous species; because of the huge potentials they could offer mainly the tolerance to abiotic stresses, socio-economic value such as fodder production, human nutrition, damaged soil protection and the maintenance of their fertility **[28]**.

*Medicago arborea* L. or alfalfa arborescence is a leguminous fodder species among the Mediterranean flora which has been adapted to periodic drought and all kinds of soil, and was used in many valorization and restoration programs of damaged steppic areas **[22]**. It I sa woody shrub of 1-4 m high, naturalized in the Mediterranean basin for its fodder, characterized by a dense foliage, trifoliate, a yellow zygomorph flowers, assembled in clusters, and fruits as thin rolled pods containing 2 to 4 smooth seeds having a bean form**[2, 10]**.

Using *M. arborea* effectively in the rehabilitation of the Algerian steppic areas require an adequate knowledge of the germinative characteristics of its seeds, so as to clarify its behavior face to the environment conditions.

Sure enough, germination is a critical initial stage of the plants development cycle insofar as it determines the establishment of the seedling, its acclimatization in the environment and probably its later productivity **[36]**.

This study aims to understand further the natural regeneration mechanism by sow of *M. arborea* and to focus on the knowledge of its seeds viability by the evaluation of their germination behavior in controlled conditions of salt and water stress.

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## II. MATERIALS AND METHODS

### II. 1. Biological material

The germination tests rest on *Medicago arborea* seeds, provided by the station of INRF (National Institute of Forestry Research) of the province of Djelfa, region of high plateau of the center of Algeria, located at 346.66m of altitude and at 34°40′ North and 3°54′ East Lambert coordinates. Seeds were collected in June 2011 and stored in paper bags at ambient laboratory temperature until their utilization in March 2013.

### II. 2. Methods

#### II. 2. 1. Selection and preparation of seeds

Only healthy, complete and mature seeds were selected, disinfected by soaking in hypochlorite of sodium 5% for 10 mn then rinsed many times with distilled water.

#### II. 2. 2. Germination

Five replicates of 25 seeds were carried out in sterilized, plastic Petri dishes of 90 mm of diameter, on two layers of Wattman filter paper.

Salt stress was induced using different concentrations of NaCl: 34 mM, 68 mM, 102 mM, 136 mM, 170 mM, 204 mM, 238 mM and 272 mM.

Water stress was induced using different concentrations of polyethylene glycol 6000 ($PEG_{6000}$), corresponding to the following osmotic pressures: -0.01 Mpa, -0.02 Mpa, -0.05 Mpa, -0.1 Mpa and -0.2 Mpa. In parallel, controlled test using distilled water, was conducted. All the tests were carried out in the darkness, in an incubator, type **Memmert**, maintained at a continuous temperature of 20°C.

A seed is considered as to have germinated when the radical breaks the coats. The germination percentage was daily recorded during the whole period of the process.

In order to test the germination recovery performance of seeds after exposure to salt stress, seeds which germination was inhibited in severe salt stress (238mM, 272mM of NaCl), were transferred to distilled water and incubated for 4 days, at 20°C **[13]**.

#### II. 1. 3. Estimation and statistical Analysis

The rate of germination was estimated by: germination capacity (GC %), index of germination velocity (IGV) or germination speed, mean time of germination (MTG) and latency time (LT) **[5, 27]**.

The means of the different parameters were compared by the analysis of variance (ANOVAI) using XL Stat software version 2012.

## III.RESULTS

### III. 1. Effect of Salt stress

#### III. 1. 1. Germination kinetics

The figure 1 shows the effect of the different NaCl concentrations on the evolution of the germination percentage of *M. arborea* seeds and permits to distinguish three phases:

-Phase of latency, required to obtain the first germination, in which the percentage remains in the average, its duration is variable according to NaCl concentrations. It is short, in the order of one day for the controlled seeds and for the seeds submitted to the concentrations of 34and 68 mM. It extended with the increase of salinity, until10 days at 204mM of NaCl.

- An exponential phase, corresponding to a rapid increase of the germination percentage, which developed for the whole tests proportionally to time.

- A third phase, appreciably linear representing the final germination percentage, otherwise, the germination capacity of the seeds, becoming then weaker with the increase of salt stress.

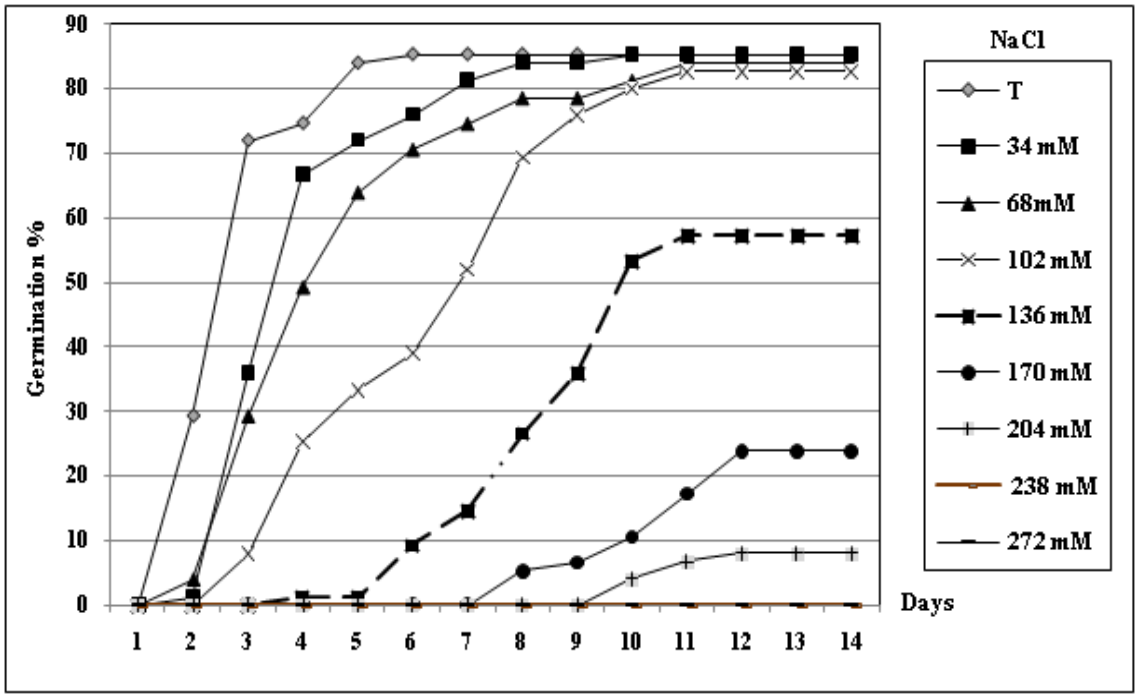


**Figure 1:**NaCl effect on the germination kinetics

**III. 1. 2. Germination Capacity and the index of germination velocity**

The means of germination capacities and the means of the indexes of germination velocity, evaluated at the different saline concentrations, are illustrated in the figure 2.

At34 mM and 68mM of NaCl, the capacity of germination is not affected and still close to the controls'. From 136 mM it declined significantly and then it is inhibited at 238mM. The germination speed decreases according to the concentration of NaCl, then to be canceled at 238mM and 272mM.

The depressive effect of NaCl on the capacity of germination and the index of germination velocity was confirmed by the analysis of variance ($P > 0.05$).

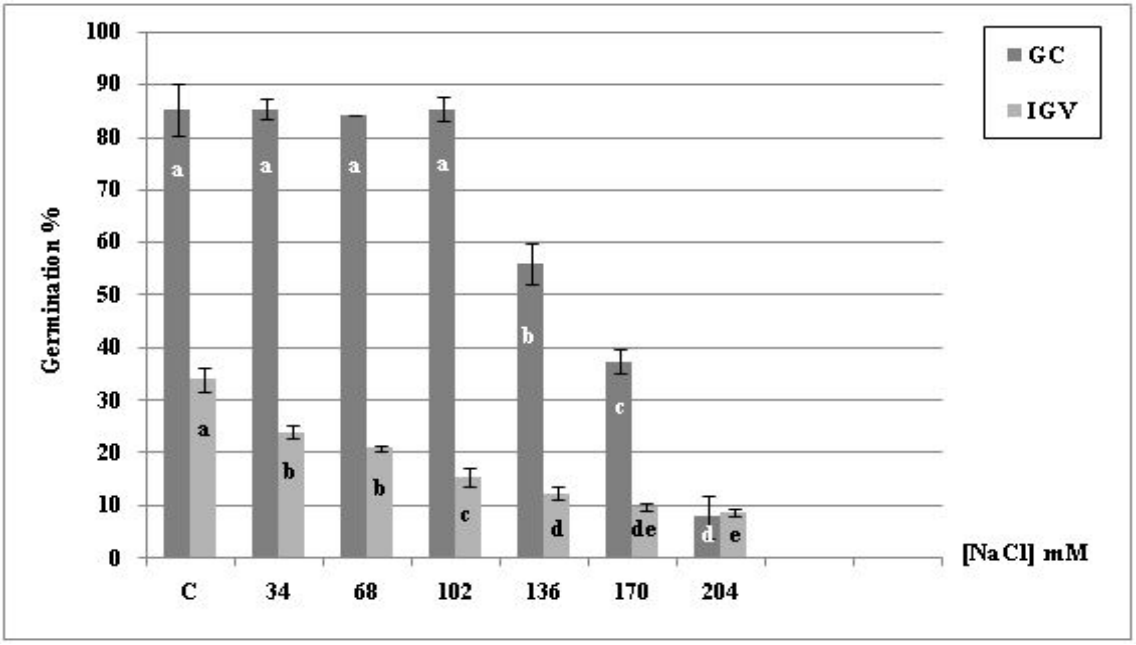


**Figure 2:** NaCl effect on the germination capacity and the index of germination velocity.
*the different letters indicate a significant difference between the means (P>0.05).

### III. 1. 3. Mean time of germination and latency time

The figure 3 shows the effect of NaCl on the mean time of germination and latency time. The mean time of germination is proportional to the saline concentrations. The germination started from the second day for the control seeds and for those submitted to low saline concentrations (34 and 68mM). More the saline concentration increase, the most the latency time amplifies, reaching a length of 10 days at 204 mM. The analysis of variance confirmed this variability (P>0.05).

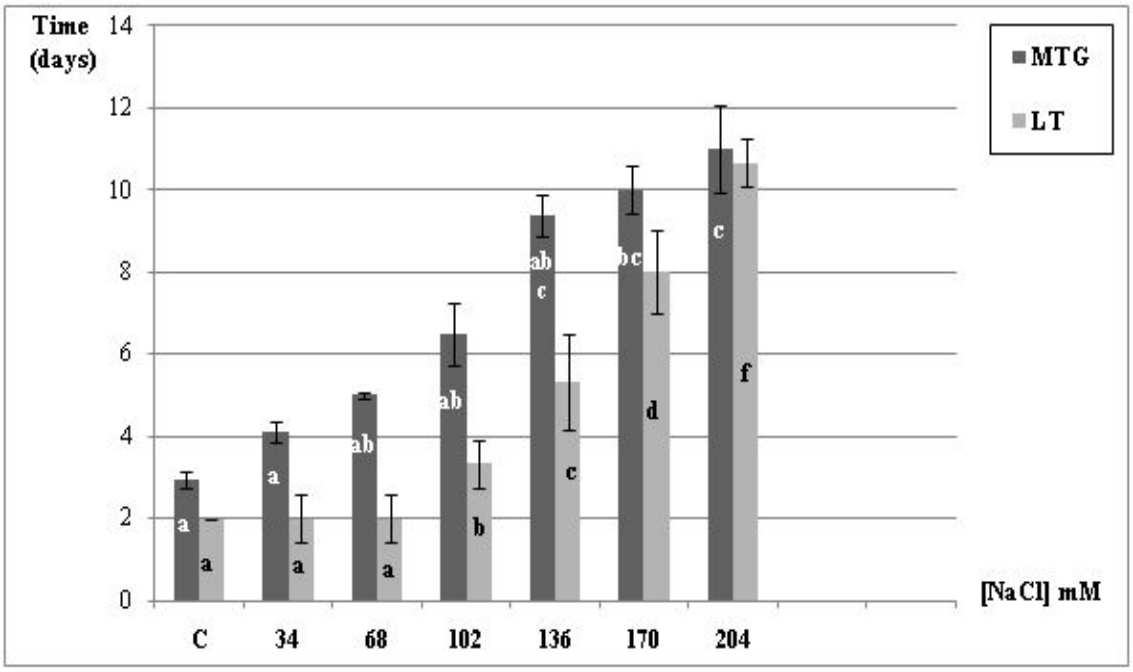


**Figure 3:** NaCl effect on the mean time of germination and latency time.
*the different letters indicate a significant difference between the means (P>0.05).

### III. 2. Recovery of germination

Ungerminated seeds under high concentrations of salt (238 Mm and 272mM) recovered their aptitude of germination once they were transferred to distilled water.

The germination was early and started from the first day after the transfer to distilled water; the germination rate increased rapidly reaching 81.33±4.62 % at 238mM, and 66.67±9.24% at 272mM of NaCl (Fig. 4).

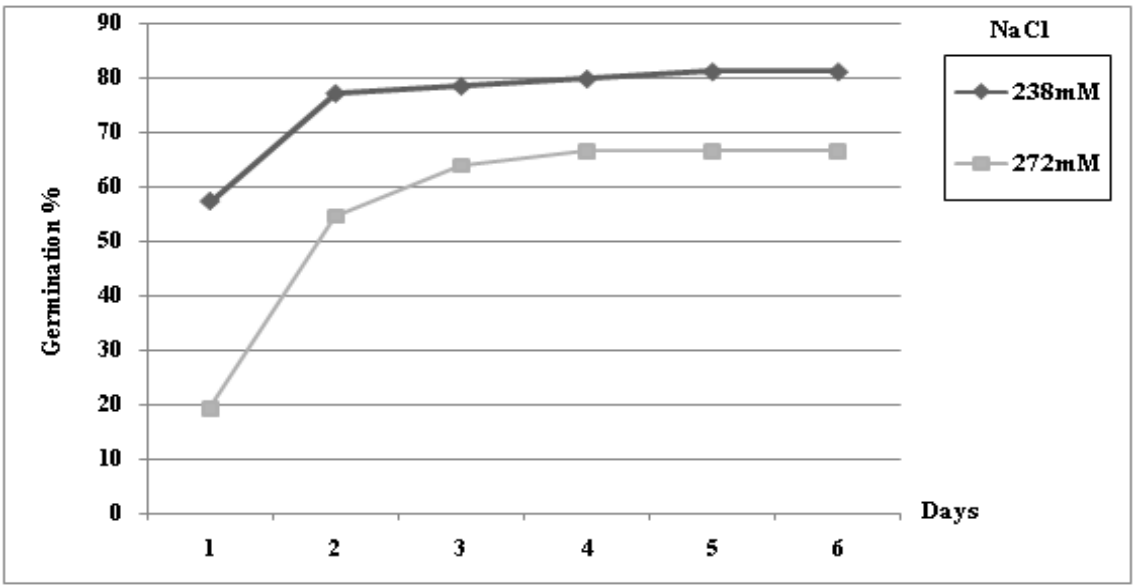


**Figure 4:** The reversible effect of NaCl on the germination kinetics**.**

### III. 3. Effect of water stress

#### III. 3. 1. Germination kinetics

The results of the evolution of the germination rate under water stress were illustrated in the figure 5. Three germination phases were distinguished:

- A latency phase of 2 to 3 days for the whole tests,
- A second phase related to an acceleration of germination mainly for the control seeds and for those exposed to low osmotic pressures (-0.01 and -0.02 Mpa),
- A third phase related to a stability of germination, indicating the capacity of germination.

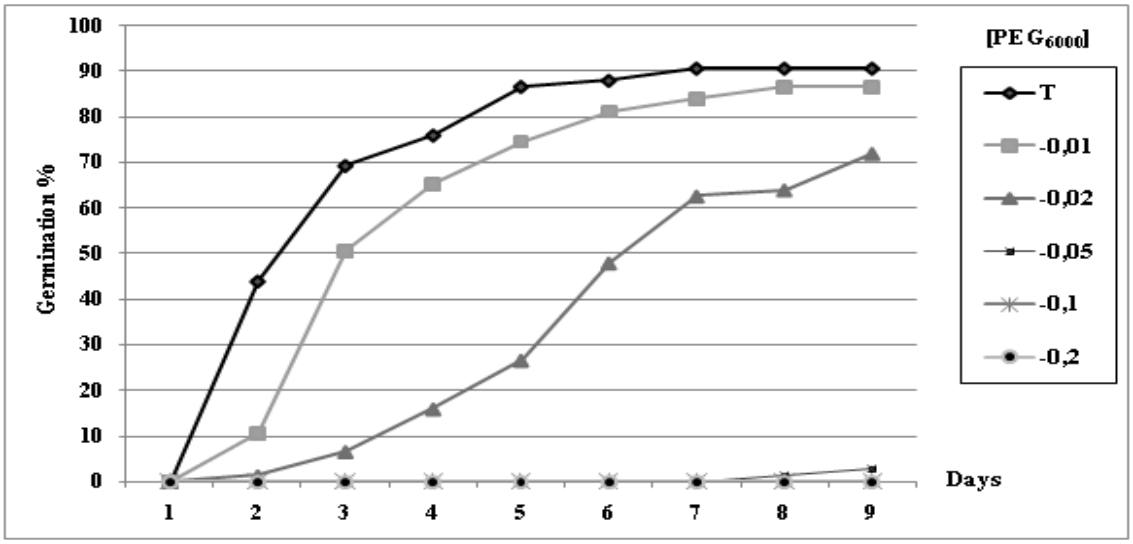


**Figure 5:** Effect of the different $PEG_{6000}$ concentrations on the germination kinetics

#### III. 3. 2. Germination Capacity and the index of germination velocity

The results revealed that the seeds are able to maintain their capacity of germination under the following osmotic pressures: -0.01 Mpa (86.67 %), -0.02Mpa (72 %) and -0.05 Mpa (12 %). However, these germination capacities remained significantly lower than those of the control seeds (90.67 %). Then, the germination was canceled at -0.1Mpa and -0.2 Mpa (fig. 6).

It is reported also in our findings that the different levels of the osmotic pressure have caused a significant decrease of the germination speed ($P > 0.05$).

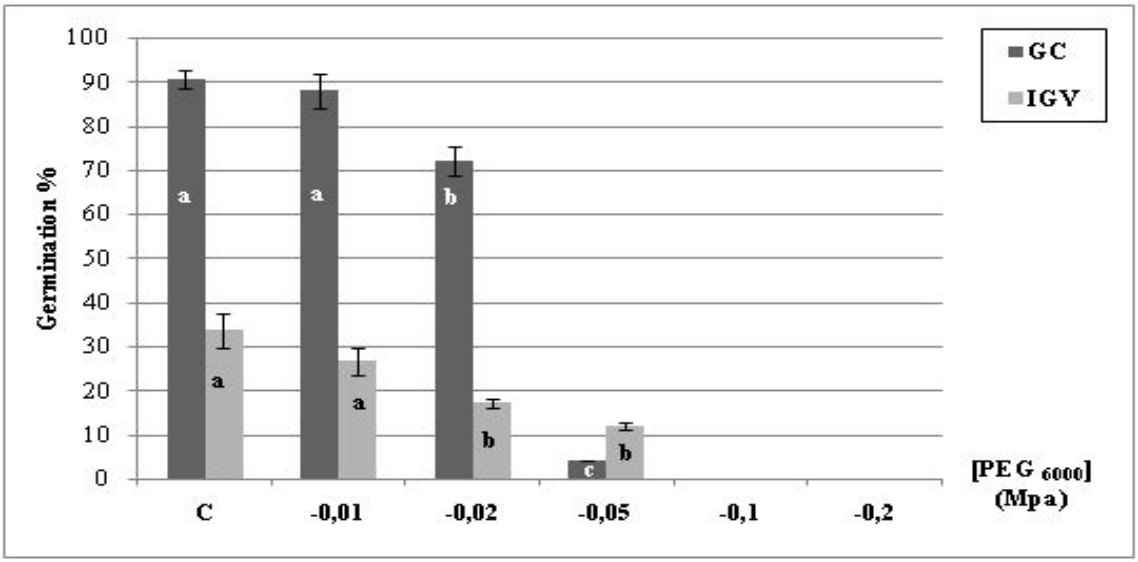


**Figure 6:** Effect of the different $PEG_{6000}$ concentrations on the capacity of germination and the index of germination velocity

*The different letters indicate a significant difference between the means (P>0.05).

### III. 3. 3. Mean time of germination and latency time

The figure 7 shows a lengthening of the mean time of germination and latency time according to the increase of the water stress. These observations were checked by the analysis of variance (P>0.05).

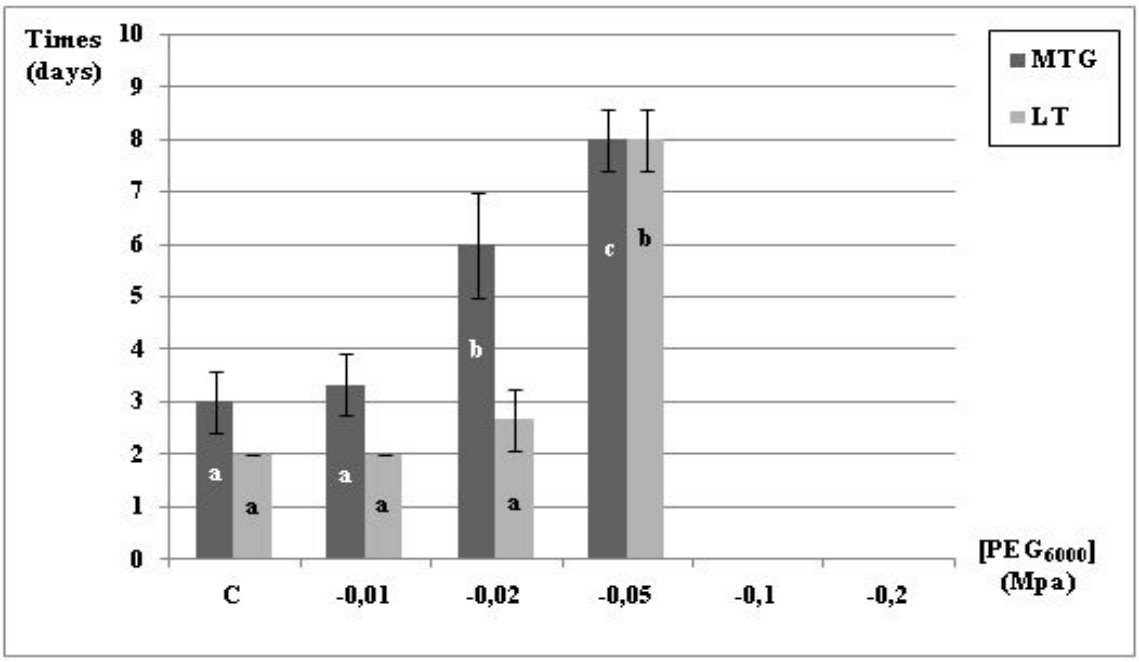


**Figure 7:** Effect of the different $PEG_{6000}$ concentrations on the mean time of germination and latency time
*the different letters indicate a significant difference between the means (P>0.05).

## IV. DISCUSSION

*Medicago arborea* constitutes an essential vegetal potential to maintain the balance of the Mediterranean region. The introduction of this species within a rehabilitation program offers a solution of durable development in many arid and semi-arid ecosystems where it can act as a strategic fodder species supporting the natural resources in grazing systems of arid environments **[35]**.

The present paper consists on exploiting this vegetal potential through the evaluation of its seeds' germinative responses under different salt and water conditions.

The results show that the germination of *M. arborea* seeds is mostly affected by the high concentrations of NaCl, at a continuous temperature of 20°C, which is considered as an optimal temperature for the germination of most dicotyledonous seeds **[27]**; seeing that at low concentrations (from 34 mM to 136 mM) of NaCl, high germination capacities are recorded comparable to the control tests. Besides, at high concentrations of NaCl (<136 mM) the germination capacity decreased gradually until it is inhibited. As Well, our results revealed that the increase of NaCl in the environment slows down the germination speed and induce a lengthening of latency time and the mean time of germination.

The level 204 mM of NaCl constitutes a tolerance limit for seeds since that their germination is maintained until this concentration, beyond, it is inhibited.

The increase of the environment's osmotic pressure in the presence of NaCl provokes a delay of seeds soaking by limiting the water absorption required for the starting of the metabolic processes involved during the germination phenomena, which explain the decrease of the rate of germination in the presence of salt. In fact, it is reported in **[33, 3]** that salinity delayed the germination, disturbing the enzymatic systems involved in the different physiologic functions of the seed during germination such as the decrease of polyphenol oxydase and amylase activities.

Our results join those of Guan *et al* **[12]** concerning *Medicago ruthenica*, of Chérifi *et al* **[6]** about *Medicago polymorpha* and *M. ciliaris*, and of Lachhab *et al*. **[20]** about *Medicago sativa*.

The concentration 200 mM of NaCl constituted a physiologic limit of germination for the following species: *Ammophila areneria*, *Corynephorus articulatus*, *Koeleria phleoïdes* and *Aeluropus littoralic* **[21]**.

For *M. arborea* seeds germinated at alternate temperatures (10-20°C, 15-25°C and 20-30°C), the limit of tolerance to salinity is 150 mM of NaCl ; although, in our tests the limit reached 204 mM using a continuous

temperature of 20°C. These observations will be possibly considered as a sign of the existence of a relationship between the effect of temperature and the germinative response of our species to salinity **[29]**.

The results of Bouda & Haddioui **[4]** are close to ours, insofar as they indicate a limit of 170mM of NaCl for *Atriplex halimus* (halophyte) seeds. It emerges from this case that glycophytes and halophytes seeds respond alike to salt stress, by reducing the total number of germinated seeds and delaying the starting of germination process **[15]**.

However, some species from *Fabaceae* family are clearly influenced by the low concentrations of NaCl which decrease respectively the speed and capacity of germination from 51 mM of NaCl ; case of *Glycine max* and *Phaseolus vulgaris* seeds **[34]** as well as the seeds of *Spartidium saharae* **[7]**.

The germinative responses of seeds to saline constraint should be cautiously interpreted since that a high rate of germination under NaCl effect is not inevitably related to salinity tolerance at late development stages **[1]**.

Yet, they are revealing of a genetic potential of tolerance of species and varieties, at least at the germination stage **[39, 23]**.

The transfer tests of seeds to control environment after treatment with NaCl are conducted to clarify the nature of action of salt on germination. The results show that the effects are first osmotic, due to the recovery of germination after removing the salt stress; the access of water into the seeds permits to adjust the osmotic pressure and to maintain an osmotic equilibrium; nevertheless a toxicity phenomena also occurred due to the accumulation of $Na^+$ and $Cl^-$ ions, since that the germination capacity decreased comparing to the control, even after being transferred to non saline environment.

Those observations have been demonstrated in the works of Hajlaoui **[13]**, about *Cicer arietinum*, who noted that when the salt has an osmotic effect on germination, the water absorption by seeds is then affected and its access to embryos is limited. When the salt exerts a toxic effect it is explained as an accumulation of ions inside the embryo which leads to an alteration of the metabolic processes and in the extreme case to the death of the embryo.

According to El-Keblawy and Al-Shamsi**[11]**, most of the seeds can stay viable and maintain their aptitude to germinate when the saline constraint is removed.

The reversibility of the salt effect has been demonstrated as well in other species of the gender of *Medicago*: *Medicago sativa* **[8]**, *Medicago ruthenica* **[12]**, *Medicago ciliaris* and *Medicago polymorpha* **[6]**.

Khan and Ungar **[19]** have reported that the recovery of germination is not a criterion of salt tolerance which distinguishes halophytes from glycophytes. In this sense Neffati **[30]** has noted that the knowledge of tolerance to salinity during germination is a useful information but insufficient to explain the distribution of species and their development in salty environments.

Our results show also that the germination of *M. arborea* seeds is significantly affected by water stress. In fact, the germination rate decreases considerably with the increase of the osmotic pressure induced by PEG. Sure enough, PEG is a molecule of a big molecular weight, relatively stable, inert, very soluble in water and not toxic even in high concentrations. It is used as an osmotic agent decreasing the water potential in order to simulate the water stress effect on plants the same way as soil desiccation **[38, 32]**.

The limit value of the osmotic pressure in which seeds germination becomes sensitive situate at -0.05Mpa, it cancels under a severe stress (-0.1 and -0.2Mpa). This explains that the ability to maintain a high water potential is considered as a mechanism allowing the plant to escape from dehydration **[25]**. Similar correlations have been highlighted by Jaouadi *et al* **[17]** on *Acacia tortilis* seeds.

Water stress can reduce the germination rate through limiting water absorption by seeds **[9]**, and by affecting reserves mobilization **[26]**, or directly by affecting the structure and proteins synthesis within the embryo **[31]**.

According to Lapeyronie **[22]**, Lucerne shrubs accommodate well to periodic droughts and they are adapted to all the types of soil which are not very humid.

In other works, it has been mentioned too that the water stress engender a decrease of germination rate. This is the case of many species of the gender of *Acacia* (*A. salicina*, *A. pendula*, *A. cyanophylla*, *A. floribunda*, *A. tortilis and A. raddiana*) **[16]**.

Unlike our results, other *Fabaceae* have held a good aptitude to germinate under severe conditions of water deficit; this is the case of six cultivars of *Medicago sativa* L. **[14]**, of *Eremosparton songoricum* (Litv.) Vass., an endemic species of Chinese desert **[25]**, and of *Spartidium saharae***[7]**.

The obtained results have emphasized that *M. arborea* seeds has a medium water demands in the germination stage, although this doesn't signify that the tolerant species to water stress during germination are necessarily adapted to drought at mature stage **[18].** Thus, in order to explain the adaptation of this species to drought, it is important to study fully the effect of water stress in the other development stages that succeed germination.

## V. Conclusion

In the light of the obtained results, it emerges that *M. arborea* seeds can maintain their aptitude to germinate until a concentration of 204mM of NaCl. The recovery of germination after removing the saline constraint indicates that Sodium Chloride exerts a temporary inhibition from an osmotic nature which is eliminated with the removal of the constraint. Therefore, *M. arborea* seeds can be classified as moderately tolerant to salinity during germination.

The effect of water stress has revealed that the studied seeds are moderately tolerant to water stress by holding their aptitude to germination until an osmotic pressure of -0.05Mpa.

The attained data have permitted to accomplish the knowledge of *M. arborea* seeds viability. They will certainly serve within the framework of the preservation of the studied species and in the production of seedlings *"in situ*" for the rehabilitation of damaged arid zones.

In order to complete this work, we recommend studying further "*in situ*" the behavior of our species and clarify its responses to the environmental constraints at the next stages of its development cycle. This will permit to decide about the possibilities to use this natural resource within restoration and preservation projects in damaged steppic areas in Algeria.

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