

**EFFECTS OF TEMPERATURE AND SALINITY ON THE SEEDS GERMINATION
OF *Retama raetam* (FORSSK.) WEBB. SCARIFIED WITH SULFURIC ACID**

Z. Mehdadi*, F. Z. Bendimered, M. Dadach and A. Aisset

University of Djillali Liabes, Faculty of natural sciences and life, Laboratory of plant
biodiversity: conservation and valorization, Sidi Bel Abbes, 22000, Algeria

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ABSTRACT

The present study consists of the elimination of tegumentary inhibition affecting seeds of *Retama raetam* by the chemical scarification. This pretreatment was carried out using pure sulfuric acid (98 %) and the seeds' germinative behavior was studied in the laboratory under controlled conditions of temperature and salinity. The results reveal that the chemical scarification by the sulfuric acid during six hours, had favored the germination of seeds which were incapable of germinating. The thermal optimum of germination expressed by the highest germination capacities and speeds as well as the shortest average times of germination and latency times corresponded to 20 °C and 25 °C. At low temperatures (0 °C and 5 °C) and high temperatures (35 °C and 40 °C), the germination was not possible. The seeds of *R. raetam* are sensitive to salinity, when the NaCl concentration increases the rate of germination decreases. The threshold of tolerance was recorded at 272 mM, from which the germination was inhibited.

Key words: *Retama raetam*; chemical scarification; germination; temperature; salinity.

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

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1. INTRODUCTION

Retama raetam is a shrub belonging to *Fabaceae* family, developing in the arid regions of the Mediterranean basin [1, 2]. The high regression of leaves' surface and of the presence of stomata in the crypts surrounded the fibers constitute morpho-anatomic characteristics permitting the species to adapt to xerophytism [3].

Thanks to its very developed root system and its characteristics to establish mycorrhizal symbiotic associations, this species ensures the stabilization of dunes, the soil fixing in the arid and semi-arid ecosystems [4, 5] and the amelioration of the fertility of damaged and eroded soils [6, 7].

Besides, *R. raetam* is capable of producing important quantities of biomasses which are used as fodder [6]. Moreover, other works denote the medicinal value of this *Fabaceae* species as diuretic and hypoglycemic [8, 9] and its anti-oxidant and anti-microbial activities [10, 11, 12]. In Algeria, this species is in progressive decline which results from a particular climate related to periods of prolonged dryness that imposes a potential but permanent risk of salinization and soil degradation [13, 14, 15, 16], of the anthropo-zoogenic action and the difficulties even the absence of its natural regeneration by the sexual way [17]. In addition, currently the lack of information about the physiology of germination of *R. raetam* seeds, the hardness of its coats and their impermeability to water and oxygen seem to be in the same case as many other *Fabaceae* [18, 19, 20], those are the essential causes of the absence of such way of regeneration.

In order to add new elements that can contribute to the regeneration and conservation of *R. raetam*, we have undertaken, in the laboratory, an experimentation that aims at removing the coats inhibition affecting the seeds by the chemical scarification using sulfuric acid, also aims to determine the thermal optimum of germination and the threshold of salt tolerance.

2. RESULTS AND DISCUSSION

2.1. Observation of the first germination stages

Figure 1 illustrates the germination evolution of *R. raetam* seeds pretreated with the sulfuric acid for 6 hours and germinated at a temperature of 25 °C. On the second day of germination,

the seed swells appreciably; then, at the end of the third day, the radicle breaks the coats and starts to lengthen from the fourth day (beginning of the growth).

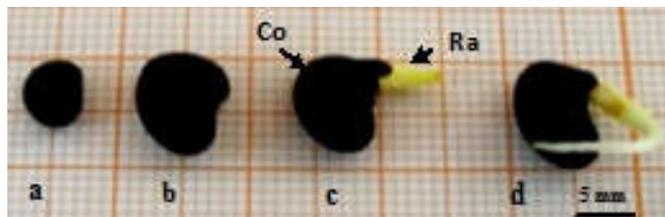


Fig.1. The different stages of seeds germination of *R. raetam*

a: *Retama raetam* seed, **b:** the swelling of the seed, **c :** the breaking of coats by the radicle, **d:** the lengthening of the root, **Co:** coat, **Ra:** radical.

3.2. The influence of sulfuric acid on germination

Figure 2 shows the effect of the pretreatment duration with sulfuric acid on the germination kinetics of seeds. The pretreated seeds with the sulfuric acid during 2 hours started to germinate on the 6th day, reaching a maximum value of 80% of germination the 9th day. However, the germination of the scarified seeds for 4 h and 6 h began the 3rd day reaching a maximum percentage of germination of 100 % respectively at the 8th and 7th day. On *R. raetam* evolving in Egypt, the best germination rate was obtained between 15 °C and 20 °C, with seeds scarified by sulfuric acid for 20 minutes [21]. The seeds which have been scarified for 8 h, started to germinate the 3rd day attaining only 60 % as a final percentage at the end of the 6th day. Similar observations were noted by Bouredja et al. [20] whose demonstrated that the chemical scarification had permitted the coats softening of *Retama monosperma* seeds which promote the germination. The same results were demonstrated in our tests after pretreatment with sulfuric acid during 4h, 6h and 8h. On the contrary, the pretreatment of 1, 2 and 3 hours in sulfuric acid did not favorise the germination. Pretreatments duration higher than 8 hours had been revealed destructive. The efficiency of this pretreatment on removing the tegumentary inhibition of leguminoseae species of Sahel, was checked in the work of Sy et al. [22]. The chemical scarification using sulfuric acid was revealed as well encouraging the germination of Alfa (*Stipa tenacissima*), a lasting poaceae of the high stepic plains, which

natural germination particularly through sexual way is difficult even absent [23, 24].

The results that follow were obtained from pretreated seeds with the sulphuric acid during 6 hours; this duration allowed the lifting of tegumentary inhibition affecting our seeds and gave the best percentages of germination.

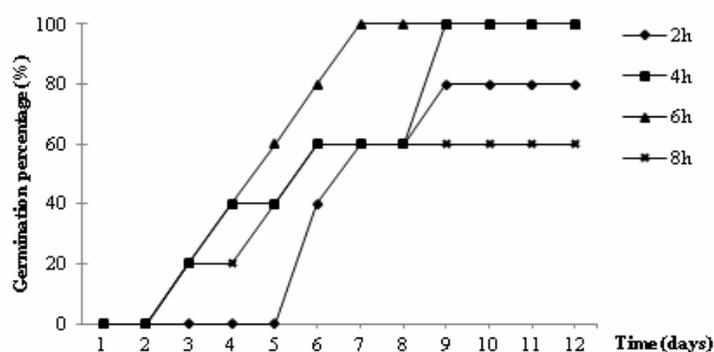


Fig.2. The effect of the different duration of chemical scarification with sulfuric acid on the germination of *R. raetam*

3.3. The influence of the thermal conditions

The germination kinetics of *R. raetam* seeds according to different temperatures is illustrated in figure 3. Three phases are distinguished in the germination curves represented in this figure.

- A latency phase, necessary for the appearance of the first germinations, in which the germination rate is weak. The duration of this phase was variable according to the temperature. This phase was short (2 to 3 days) for the temperatures 20, 25 and 30 °C, and started to get longer (4 to 5 days) for the temperatures 10, 15 and 35 °C,
- An exponential phase, corresponding to a fast increase of the germination rate which amplifies proportionally to time,
- A linear phase representing the final percentage of germination signifying the germination capacity in the experimental conditions.

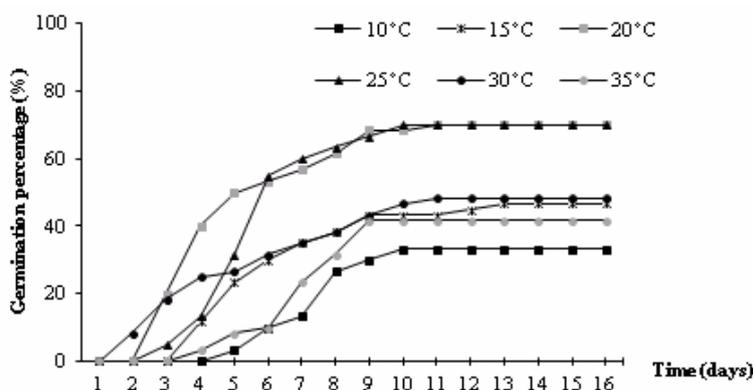


Fig.3. Germination kinetics of *R. raetam* seeds according to different temperatures

The comparison between the temperatures reveals that the percentage of germination at 20 °C and 25 °C increases rapidly reaching maximum germination capacities (70 %), germination speed (20.25 at 16.44 %) and a short latency time (3 to 4 days).

However, the slowest evolution of germination rate is observed at the temperatures 10 °C and 35 °C recording germination capacities respectively of 33.33 % and 41.67 %.

At the temperatures of 15 °C and 30 °C, middling germination capacities are noted, respectively in the order of 46.67 % and 48.33 %. At the temperatures 0, 5 and 40 °C, the germination was inhibited (Figure 4).

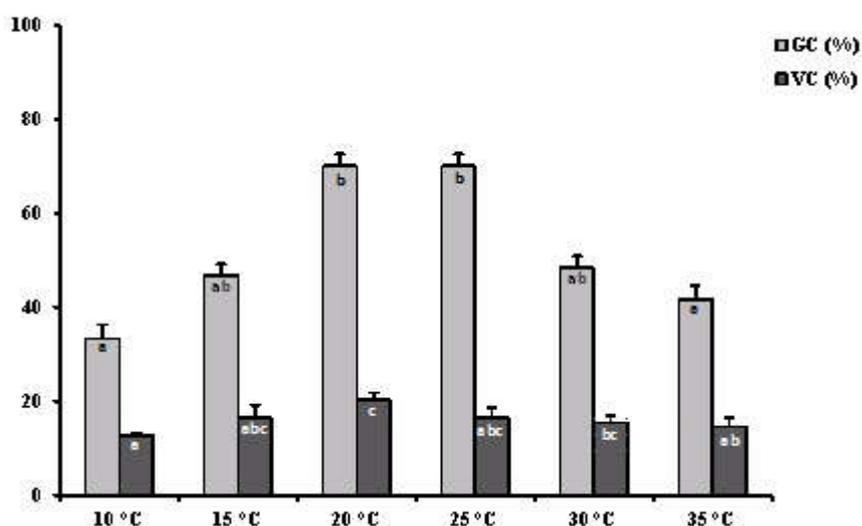


Fig.4. Temperature effect on the germination capacity (GC) and the velocity coefficient (VC)

The different letters indicate a significant difference between the means ($P < 0.05$).

The significant effect of the temperature on the germination capacity and speed is confirmed by the analysis of variance and Tuckey's test ($P < 0.05$).

At lower temperatures (0 and 5 °C) or higher (40 °C), it is possible that the embryo does not receive a sufficient amount of oxygen for germination. In fact, the embryo requires more oxygen when the temperature decreases or increases. In addition, the lower and the higher temperatures can denaturalize some enzymes which are essential for the basic metabolism of germination [25].

Our results join those of Bouredja et al. [20], which indicate that the optimal temperature of the germination of *Retama monosperma* seeds is 20 °C, while at the temperatures 5 °C, 35 °C and 40 °C, the germination weakens.

Aissat and Mehdadi [26] highlighted that the thermal optimum of the germination of *Medicago arborea* seeds, a *Fabaceae* species, is similar to *R. raetam*, is of 25 °C. The germination started at 5 °C with weak rates, decreased at a temperature higher than 30 °C and canceled out at 40 °C.

On the other hand, Berka and Harfouche [27] noted that the germination was weak even absent at low temperatures for the seeds of *Argania spinosa* L. At a temperature of 6 °C, the germination was practically inhibited. At 10-14 °C, the percentage of germination still remained weak and a maximum of 6 % was obtained at the end of 28 days. The germination starts to be consistent from 25 °C (75 % in 56 days) to reach 28 °C (80 % in 56 days).

Comparably to those findings, Mbaye et al. [25] emphasized that the optimal temperatures of germination were variable from one species to another. For *Zornia glochidiata*, the germination optimal temperature set between 25°C and 30°C, and the germination rates decreased and canceled out at 45°C.

3.4. The influence of salinity

Figure 5 shows the effect of the different NaCl concentrations on the evolution of the germination rate according to time.

The germination curves permit to distinguish three phases (Figure 5):

- A latency phase, necessary for the appearance of the first germinations, in which the

germination rate is weak. The duration of this phase varied according to the concentration of NaCl. It was short, about 3 days, for the control seeds and those submitted to 34 mM of NaCl. This phase was longer for seeds especially those subjected to 204 mM of NaCl where the value reached 9 days,

- An exponential phase, corresponding to a fast increase of the germination rate which amplifies proportionally to time, particularly for the control seeds and those submitted to the concentrations lower than 102 mM,
- A third phase appreciably linear representing the final percentage of germination signifying the germination capacity.

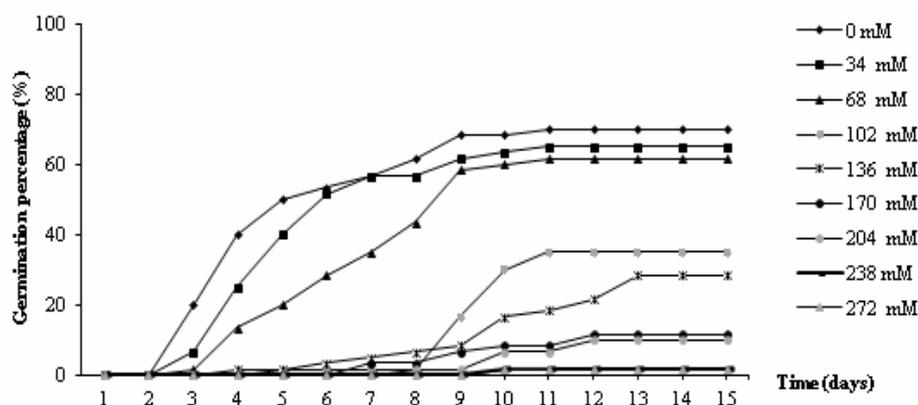


Fig.5. Germination kinetics of *R. raetam* seeds according to different saline concentrations.

Through the comparison of the germination capacities, it appears that *R. raetam* seeds tolerate saline concentrations of 34 and 68 mM, High capacities of germination were recorded but always remained lower than those obtained at control seeds with respective values of 65 and 61.17 %. From 102 mM, the capacity of germination decreased gradually until 238 and 272 mM to cancelling out at 306 mM.

The salt had the same effect on the germination speed which had decreased with the increasing of NaCl concentration. For the control test, the speed of germination was of 20.25 %, then, it decreased gradually with the increase of the salt concentration reaching 238 and 272 mM of NaCl, with a rate of 3.33% (Figure 6).

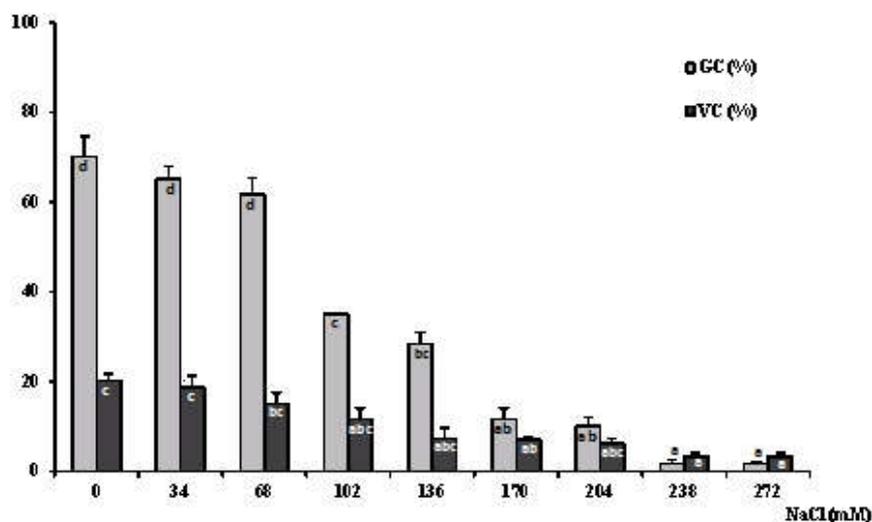


Fig.6. Effect of saline concentrations on the germination capacity and the velocity coefficient

The different letters indicate a significant difference between the means ($P < 0.05$).

Similar results were obtained on species belonging to the *Fabaceae* family such as *Cicer arietinum* [28], *Butea monosperma* [29], *Spartidium saharae* [30], *Cicer arietinum* [31], *Medicago ciliaris* and *Medicago polymorpha* [32], *Medicago sativa* [33], *Medicago arborea* [34].

3.5. The reversibility of the germination inhibition

It was observed that the salt exercises a depressive effect on the germination seeds, at high concentrations (238 and 272 mM) of NaCl.

This depressive effect can be osmotic or toxic. A recovery of germination after raising this constraint was noticed. It may be admitted that the inhibition of seeds germination of *R. raetam* is from osmotic nature. In the case of toxic effect, the recovery of germination is not possible.

The results showed that salt effects are firstly osmotic, owing to the germination recovery once the stress is removed. Nevertheless, a toxicity action could also occur due to the accumulation of Na^+ and Cl^- ion with a decrease of the germinative rate compared to the control test, even after the removal in an environment unsupplied with salt (Figure 7).

The reversibility of the response to salt was demonstrated in many works on different species [31, 35, 36].

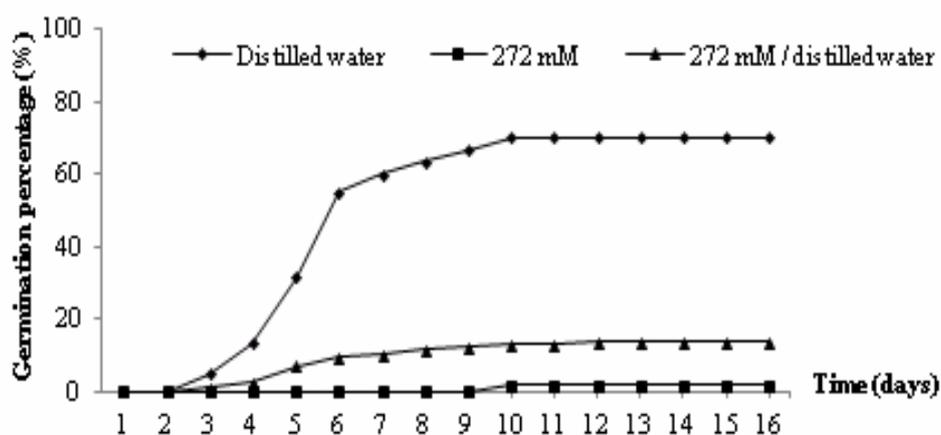


Fig.7. The reversible effect of NaCl on the germination kinetics

3. EXPERIMENTAL

3.1. Material

The studied seeds are oval, smooth, having a dark to black color, and from 3-7 mm of length (Figure 1). They have been collected in June 2010, in the high steppic plains of Djelfa (Algeria), situated at 1150 m of altitude and at 34° 41' North and 3° 15' East Lambert coordinates.

The climate of this region is continental with cold and humid winter and dry and warm summer [37].

3.2. Methods

3.2.1. Selection and pretreatments of seeds

Seeds for germination tests were selected. Only mature, uncontaminated and undeformed seeds were used. They were disinfected by sodium hypochlorite (5 %) for 10 minutes, and then rinsed with distilled water to remove all traces of chlorine.

The preliminary tests of germination have revealed the presence of tegumentary inhibition affecting *R. raetam* seeds, those, which have not been pretreated, did not germinate. Thus, we had resorted to the chemical scarification using pure sulfuric acid (98 %) in order to remove this inhibition to reach the aims of the present work [38].

Six pretreatment durations with sulfuric acid (1, 2, 3, 4, 6 and 8 hour) are applied on seeds before their germination in an oven Memmert Type, at a continuous temperature of 20 °C.

The scarified seeds are rinsed many times with distilled water then disposed in Petri dishes containing two layers of filter paper moistened by distilled water. Five replicates of 25 seeds each were used for every pretreatment's duration.

The percentage of germination is recorded daily until the stabilization of the process. The seeds were considered to have germinated as of the appearance of the radical.

3.2.2. The effect of temperatures on germination

The seeds germination previously scarified by sulfuric acid during 6 hours (the pretreatment duration giving the best germination rates) were carried-out in different temperatures: 0, 5, 15, 20, 25, 30, 35 et 40 °C.

3.2.3. The effect of salinity on germination

The germination of *R. raetam* seeds has been tested under the effect of the different saline solutions based on sodium chloride (NaCl) at different concentrations: 34, 68, 102, 136, 204, 238, 272 mM. In parallel, a control lot was conducted using distilled water.

Five replicates of 25 seeds each were used for each test of germination and for every saline solution. Seeds were germinated in Petri dishes containing two layers of filter paper wetted with distilled water (control) and with corresponding saline solutions, then randomized in an incubator (Memmert type) and maintained at continuous temperature of 25 °C. Seeds were daily moisturized in order to maintain a sufficient humidity for the germination of seeds.

3.2.4. The reversible effect of salt

This parameter allows defining the nature of the depressive effect of salt, if it is osmotic and/or toxic. Therefore, we adopted the protocol of Hajlaoui et al. [31], which consisted of testing the germination recovery performance after exposure the seeds to NaCl solution (272 mM, saline concentration that inhibited germination) at the temperature of 25 °C for four days. On the fifth day, the ungerminated seeds were rinsed three times to remove unabsorbed salt then divided into five batches of 25 seeds each in medium consisting of distilled water for four additional days.

3.2.5. Data estimation

Results were estimated using: germination capacity (GC %), velocity coefficient (VC) or germination speed and latency time (LT) [39, 25].

The average values of the different parameters were compared by the analysis of variance (ANOVA I) and the test of Tuckey using SPSS Statistics 20 software.

4. CONCLUSION

In the light of the obtained results, it revealed that *R. raetam* seeds are affected by a coat dormancy, which can be removed by a chemical scarification with sulfuric acid during 6h.

The germination tests carried out on the seeds earlier scarified with sulfuric acid showed a significant effect of the temperature and of salinity on the different measured parameters of germination.

In fact, we showed that *R. raetam seeds* could germinate at the temperatures between 10 °C and 30 °C with a recorded optimum at 20 °C and 25 °C. The low and the high temperatures, respectively lower than 5 °C and higher than 35 °C, inhibited the germination.

It is also revealed that our species tolerate the weak concentrations of NaCl (34 and 68 mM) where a maximum rate of germination compared to the control's obtained was of 65 %. This rate tends to decrease as long as the content of salt increases. The germination was no longer possible above 272 mM.

Those findings indicate that this species belong to non halophytes resistant plants, which can support weak salt concentrations [41].

The data obtained contribute to a better comprehension of regeneration mechanisms of *R. raetam* by seedling. They may be useful in the management and conservation programs of this species. This study must be supplemented by the analysis of the effect of abiotic stresses in addition to the temperature and salinity on various developmental stages.

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