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RESEARCH ARTICLE

Study the germination of *Acacia albida* seeds under salt stress

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Abstract

Acacia plantations in Algeria are constantly subjected to constraints (drought, salinity ...) which generate their decline. In arid perimeters such as southern Algeria, the rehabilitation and conservation programs remain a priority, which is why we studied the germination behavior of this species.
In this context, we tested different physical pretreatments (scarification, boiling water) and chemical (sulfuric acid) on *Acacia albida* seeds to lift the integumentary inhibition. The test was conducted under different temperatures. Once the optimal conditions for germination are determined, we took into consideration these results, we next subjected the seeds to salt stress with NaCl concentrations ranging from 0 to 30 $g.l^{-1}$
Pretreatment of *Acacia* seeds with sulfuric acid and the use of a temperature of 25 °C have improved the rate and the average time of germination.
The results show the effect of salt influence in a highly significant way, the rate and the average time of germination. This species has a high tolerance for salt because the seeds have reached a germination rate of 21% under treatment with NaCl equal to 22 gl^{-1}. These results show the germination capacity of the *Acacia albida* under very high salt concentrations, this leads us to say that this species can be used for land reclamation and reforestation and thereby expanding its range.

Introduction

Arid and semi-arid land represent one third of the earth's surface. In these areas, the salinity of soil and irrigation water is one of limiting factors of the plant productivity and crop yield (Zid and Grignon, 1991; Baatour *et al*., 2004).
These ecosystems are characterized by low and high irregularity of precipitations (Mnif and Chaieb, 2004 ; Rezgui *et al*., 2004) associated with significant evaporation promoting the accumulation of salts in the soil (Hayek and Abdelly, 2004). This phenomenon affects nearly 7% of the overall surface area in the world (Munns, 2002). Algeria is one of the affected countries, almost 3.2 million hectares of saline surfaces (Hamdy, 1999).
The environmental and social impact of deforestation may be partly offset by the planting of native woody species, ensuring the implementation of sustainable forest system (Bonner, 1992). Among these species, leguminous trees of the *Acacia* species which play multiple and essential role : protecting and enriching the soil through the root symbiosis with Rhizobium, production of timber and air fodder, participation in human nutrition and pharmacopoeia (Kerharo and Adam, 1974; Le Houérou 1980a; Dreyfus and Dommergues, 1981; Felker, 1981; Bergeret and Ribot, 1990).
Moreover, in an arid zone, where the germination characteristics are strongly involved in the selection for plant adaptation to environmental conditions (Jordan *et al*, 1989; Koller, 1995), we can assume that the first critical phase of rehabilitation is relating to the germination of reintroducing species. For these reasons, this work has been devoted to the study of seed germination properties of native

perennial legume. *Acacia albida* (or *Faidherbia albida*) is a species of great importance. In fact, thanks to its reversed phenological cycle (leafing during the dry season), this tree is often used in agroforestry for intercropping practices which seems to favour (Charreau and Vidal, 1965; Louppe, 1990, Oliver *et al*. 1996).
The study of germination requirements of the species used in rehabilitation helps to choose the most suitable plant material for the implementation of this technique (Bell *et al*., 1992).
This work aims to study the effect of major environmental constraints affecting seed germination (Côme, 1970; Ungar, 1995), including salinity (Ennabli 1995; Hachicha, 2007) and hindering the restore trials of the endangered forest species. In this context, we focused on the study of germination under salt stress conditions and to better control the first problems encountered during the reforestation tests. Our work evaluates the germination capacity of seeds in salt stress conditions simulated by NaCl and under the effect of different temperatures. Finally, it tries to determine whether the response to osmotic constraints applied to the germination stage is a reliable early indicator of the behavior of the mature plant.
However, in the case of a legume known for its high rate of hard seeds (Behaeghe *et al.*, 1962; Clatworthy, 1984; Vora, 1989) pretreatments intended to clarify any inhibitions integument were applied to seeds of *A. albida*, which have low germination percentages (Loth *et al*., 2005), especially since this problem is an obstacle to nursery which aims to produce plants massively via synchronization and standardization of germination (Roussel, 1995).

2. Materials and methods

2.1. Presentation of *Acacia albida* Del. (*Faidherbia Albida* (Del.) A. Chev.)

This tree belongs to Sub-rule of *Tracheobionta*, the Class of *Magnolipsida*, Order *Fabales*, and Family *Mimosaceae*. *Acacia albida* is one of the largest trees of acacia type, it reaches a height of 30 m and a diameter of 1.5 m. The *A. albida* is one of the fourteen major species. The alternate bipinnate leaves have 3-9 pairs of pinnae with 10-15 pairs of leaflets. At the base of the leaves are inserted two strong straight spines or widely arched, divergent. Greyish trunk has a highly cracked or fissured bark. The flowers are whitish. The fruits are pods more or less twisted, bloated, and red-orange at maturity.
The *A. albida* is a real "miracle tree" it loses its leaves during the rainy season and turns green in dry season. So it has an opposite cycle to that of others. This tree produces leaves and pods that cattle do like. Also, in the dry season livestock congregate under its shadow where there is abundant food. By doing so, livestock enriches the bottom of the tree with its excrement. As the rainy season comes, the tree loses its leaves. Below, it thus forms a layer of leaves in addition to animal feces. Under the influence of the first rains, this mixture turns into rich humus for crops. The farmers used to sow millet under the acacia. We can see that this millet grows much better under the trees than in other fields.
The *A. albida* is a useful tree to the breeder and farmer. It provides firewood with fallen branches and it is also a good lumber in which we can prune various objects. Given its properties and use, the crop of *Acacia* should be encouraged. It is done, by seeds extracted from the pods, in several stages.

2.2. Experimental protocol

2.2.1. Effect of pretreatment on germination.

To determine the optimal conditions for germination (to be used in stress tests), we have, at first, conducted preliminary tests using different pretreatments.
The seed coat of *A. albida* have a typical anatomical structure pulses which results in a strong integumentary inhibition of the germination. This implies that natural or artificial scarification of the integument is necessary for soaking and germination of seeds. To raise the cutaneous inhibition of seed, three pretreatments were performed in comparison with the control: the first is to soak the seeds in hot water (100 °C for 1 h) and allowed to cool, the second method is immersion of seeds in concentrated sulfuric acid (H_2SO_4 96%) for an hour, the third method is the manual scarification with sandpaper (Roussel, 1984; Danthu *et al*., 1992; Ndour, 1997).
After the pretreatment, the seeds were washed with distilled water for 15 min, then treated with a fungicide (Benlate) for 1 h and then rinsed with distilled water. The seeds were germinated in the dark, because germination is indifferent to light (Danthu *et al*., 2003) in plastic Petri dishes on filter paper with four replicates per treatment, with 12 seeds per box in three incubators at three different temperatures (20, 25 and 30 °C) for the study, in parallel, the influence of the thermal factor.
The test duration was set at the germination period that spanned 30 days, the counting of sprouted seeds and for which the radicle pierced the seed coat was made every day.

2.2.2. Effect of salinity on germination. We conducted germination tests under salt stress using the optimal conditions for germination tests determined from previous pretreatment. To this end, the seeds were first soaked in sulfuric acid for 1 h, then washed with distilled water for 15 min, then

treated with a fungicide (Benlate) for 1 h and then rinsed with water distilled.
The seeds were germinated in Petri dishes on filter paper and watered daily with distilled water containing different concentrations of NaCl (0, 3, 6, 9, 12, 15 water, 18, 21, 24, 27 and 30 $g.l^{-1}$) and placed in the dark at the optimum temperature for germination identified by previous tests (25 °C) (Danthu *et al*., 1992, 1996; Ndour, 1997). The test duration was set at the germination period that spanned 30 days, the counting of sprouted seeds was daily.

2.3. Data Analysis

Each treatment was carried out with four replicates with 12 seeds per box.The data for each trial were subjected to analysis of variance classification factor and a ranking of means was performed using the Newman-Keuls test. Depending on the case, the effect of pretreatment (boiling water, manual scarification and sulfuric acid compared to the control), the temperature effect (three temperature values: 20, 25 and 30 °C), the concentration effect of NaCl with 11 concentrations (0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 $g.l^{-1}$).

3.Results

3.1. The effect of temperature and the pretreatment on the germination.

3.1.1. Effect of temperature and pretreatment on the germination capacity of the *Acacia albida.*

Figure 1, illustrating the exchange rate and average time of germination according to different pretreatments of scarification and different temperatures for a month, shows that scarification with sulfuric acid and mechanical scarification (sandpaper) allows a higher germination rate (> 95%) compared with the other pretreatments tested namely the witness and the boiling water.
The best average time of germination is noted for pretreatment with sulfuric acid: 2.5 days (**table 1**). These results are confirmed by the analysis of variance showed a very highly significant pretreatment effect on germination rate and average germination time ($p < 0.05$).
The results of the comparison of rates and average time of germination of *A. albida* at different temperatures, all pretreatments combined, using the Newman-Keuls test is shown in **table 2**. This shows that the two temperatures 20 and 25 °C have the same level of significance for the average times of germination, but differ significantly from the temperature 30 °C. However, they did not differ significantly with respect to the rate of germination.

3.1.2. Influence of temperature and scarification on the kinetic of germination

Figure 2 shows the kinetic of seed germination for each pretreatment under 25 °C which considered as the optimal temperature for germination (**table 2**). These curves represent the rates of cumulative germination for a period of 30 days. We can conclude that in all temperatures, sulfuric acid and mechanical scarification have a remarkable effect on starting seeds and germination speed. Thus, following the results obtained by these different pretreatments, we will choose in upcoming trials of salt stress, the application of pretreatment with sulfuric acid and germination temperature of 25 °C.

3.2. Effect of salinity on germination

3.2.1. Influence of NaCl on the rate and the average time of germination

Examination of **Figure 3**, illustrating the evolution of rates and average time of germination according to increasing concentrations of NaCl, shows that the increase of salt stress causes a reduction not only germination rates, but the average time germination. *A. albida* is significantly affected by NaCl from 12 $g.l^{-1}$ (41.66% germination rates) and continues to germinate even at high concentrations. (2.08 % of germination to 15 $g.l^{-1}$ of NaCl) (see the similarity of averages from the Newman-Keuls test, indicated in the **table 3)**.

3.2.2. Influence of NaCl on the kinetics of germination.

The germination kinetics of seeds as a result of increasing concentrations of NaCl **(Figure 4)** describes a sigmoidal shape comprising three phases. The analysis of this kinetic generally shows a first lag phase due to imbibition of seeds, a second exponential phase where there has been an acceleration of germination and finally a third phase characterized by a bearing which indicating the end of germination .In the control, the latency period is very short and only lasts a day, the exponential phase of germination lasts four days before reaching the stationary phase where the germination is stopped after a maximum of germination. Gradually as the salinity increases, the shape of this curve is modified in the sense that stretching, resulting in a delay and a slowdown in the rate of germination. Thus for 12 gl^{-1} NaCl, the latency and the exponential phase lasting 3 to 6 days respectively.

Table 1.— *Acacia albida* germination rates and average times comparison for each pretreatment (control, sulfuric acid, mechanical scarification, boiling water).

Pretreatment	Rates of germination (%)	Statistical similarity of the mean rates of germination	Average times of germination (day)	Statistical similarity of the mean of average times of germination
Control	2.78	A	24	C
Sulfuric acid	99.31	C	2.5	A
Mechanical scarification	100	C	2.7	A
Boiling water	25	B	06	B

Table 2. *Acacia albida* germination rates and average times comparison for each temperature (20, 25 and 30°C).

Temperature (°C)	Rates of germination (%)	Statistical similarity of the mean of rates of germination	Average time of germination (day)	Statistical similarity of the mean of average times of germination
20	55.73	A	2.6	A
25	57.81	A	2.8	A
30	56.77	A	4.0	B

Table 3. *Acacia albida* germination *rates and average times comparison for each salt treatment.*

Concentration Of NaCl ($g.l^{-1}$)	Rates of germination (%)	Statistical similarity of the averages of germination rates	Average time of germination (days)	Statistical similarity of the averages of average times of germination
0	100	A	2.4	D
3	91.67	A	2.8	D
6	91.67	A	4.2	BC
9	89.68	A	4.7	BC
12	41.66	B	5.2	AB
15	2.08	C	6.8	A
18	0	CD	0	E
21	0	CD	0	E
24	0	CD	0	E
27	0	CD	0	E
30	0	CD	0	E

Figure 01. *Acacia albida* germination rates and average times for comparison each pretreatment.
C : *control* ; BW : *boiling water* ;SC : *mechanical scarification* ; SA : *sulfuric acid*

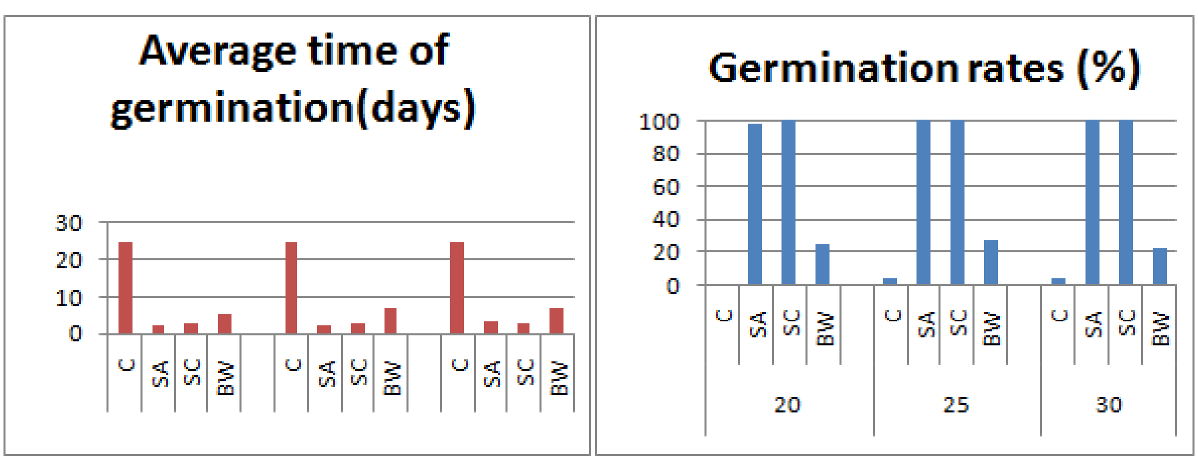


Figure 2.— *Acacia albida* germination kinetic under different pretreatments.
C : control ; BW : boiling water ;SC : mechanical scarification ; SA : sulfuric acid — Since similarities are displayed between the different temperatures, only one temperature has been shown (25 °C).

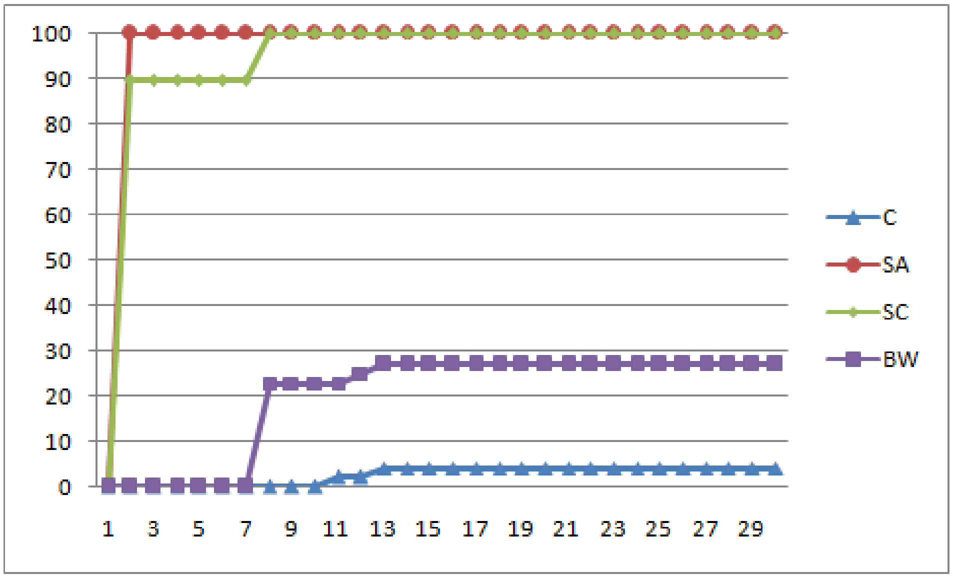


Figure. 03. *Acacia albida germination rates and average times under salt treatment Effect expressed as g.l*$^{-1}$ of NaCl.

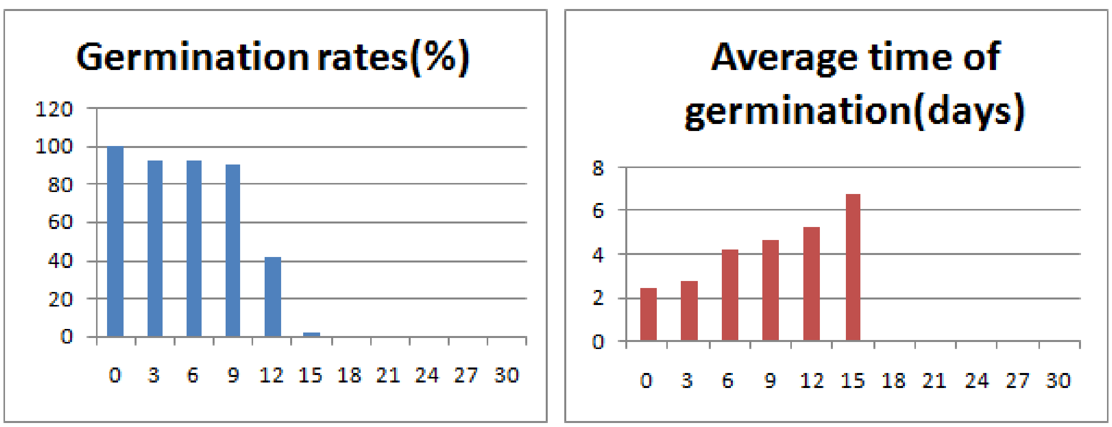
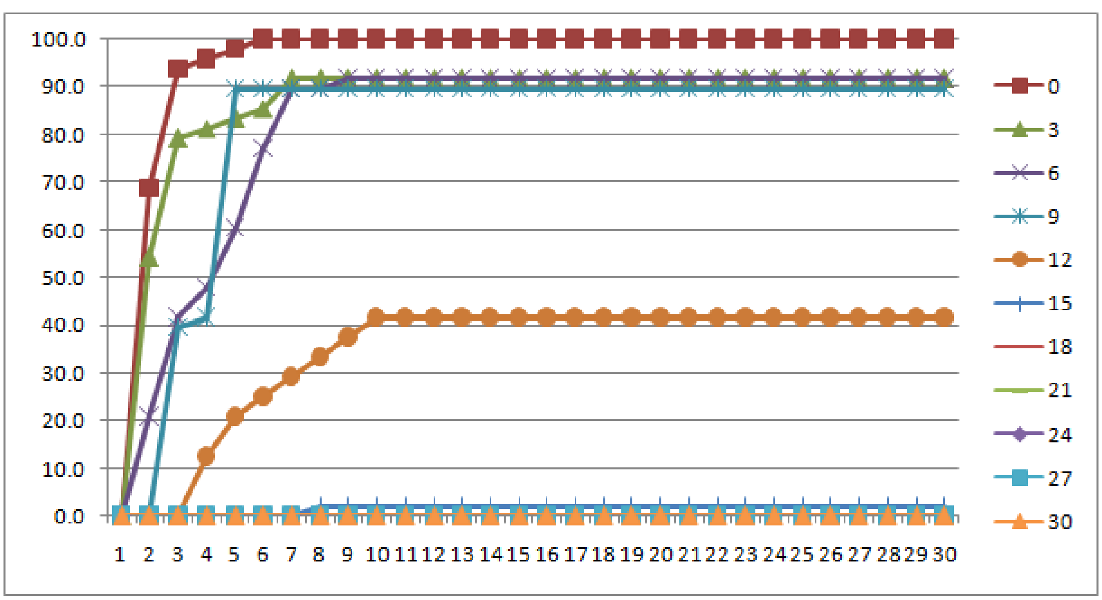


Figure 4.*Acacia albida* germination *kinetic under salt treatment effect.*



4. Discussion

Acacia albida is a crucial element in its group in the balance and preservation of many arid and desert ecosystems. The introduction of this species in reforestation programs offer a solution for sustainable reforestation in arid and semi-arid areas but also those affected by salinity [about 1.5 million hectares, or 10% of the total area (Hachicha, 2007)] and would therefore allow the diversified exploitations. However, the success of germination phases and growth of this kind involves inevitably a good knowledge of its germ and developmental characteristics and also its behavior towards environmental conditions.

A. albida seeds have various behaviors regarding the thermal factor and pretreatment time of germination; the results highlight the crucial role of sulfuric acid to remove the inhibition of seed integument. Indeed, the immersion of seed for 1 hour in pure sulfuric acid provides the highest germination rate and a decrease in mean germination time. The effectiveness of sulfuric acid to remove the cutaneous inhibition of other grassland and pasture species had been demonstrated by several authors (Behaeghe *et al.*, 1962; Clatworthy, 1984; Grouzis 1987; Vora, 1989). However, prolonged immersion of seeds in acid can damage the embryo and reduce germ performance. The optimal duration of soaking seems to be related to the hardness of the integuments (Neffati, 1994). On the other hand, our results are consistent with Neffati *et al.* (1997); reported that dryland legumes are able to germinate under a wide range of temperatures. We also confirm the findings of Teketay (1996) indicating that the majority of dryland legumes germinate at temperatures between 15 and 40 °C. About the behavior of *A. albida* seeds with regard to the salinity, our study shows that they are particularly tolerant and are able to germinate after treatment up to 12 gl^{-1} NaCl and probably an even higher concentration. Thus, the limit values shown by *A. albida* are much higher than those published by Totey *et al.* (1987) for *Acacia auriculiformis* A.Cunn. ex Benth. (germination is reduced when the salt concentration reached to 4.6 gl^{-1} NaCl) by Kayani *et al.* (1990) . For the jojoba (50% reduction of the germination capacity to 5 gl^{-1} NaCl) and by Cavalcante *et al.* (1995) for Leucaena leucocephala (Lam.) de Wit.Delay germination caused by increasing concentrations of NaCl in the environment results from a difficult of hydration of seeds due to a high osmotic potential and can be explained by the time required for the seed to establish mechanisms to adjust its internal osmotic pressure (Ben Miled *et al.*, 1986. Smaoui *et al.*, 1986). We can see a relationship between salt and tolerance during the germination and the ecology of each species. So this ability to germinate under saline conditions is an important feature for the rehabilitation and reforestation of the species, but is also interesting to use to enhance the soil marginalized and affected by salinization.

Our results suggest that after the analysis of the tolerance under salt stress, seeds of *A. albida* should not be very difficult to germinate in arid or semi-arid regions. So, in conclusion, we can say from our study, that from the moment the seeds are released from dormancy (whether by natural or artificial treatment), they are able to germinate in a wide range of temperatures and a wide range of salt stress. However, measures and techniques of restoration to undertake are still being defined through long-term studies taking into account the dynamics of development of the species from the previous stock of viable seeds in the soil and by the introduction of new seeds or seedlings to improve the regeneration process of the species.

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