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Effect of salinity on the germination of three species of the *Acacia* genus (*A. karroo*, *A. saligna*, and *A. tortilis*)

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Abstract. This scientific paper explores the impact of soil salinity on plant growth, with a particular focus on the relationship between salt tolerance and seed germination. To investigate this, three species of *Acacia* (Fabaceae), namely *A. karroo*, *A. saligna*, and *A. tortilis*, were selected, and their seeds were collected from Algeria. To overcome seed coat inhibition, seeds were treated with concentrated sulphuric acid, followed by a wash with distilled water before being sown in a culture medium containing varying concentrations of salt, specifically sodium chloride (NaCl) ranging from 0 to 600 mM. The germination tests were conducted over a 21-day period, with measurements taken at intervals of three days, and both the final germination percentage (FGP) and mean germination time (MGT) were calculated. The results showed that at 600 mM NaCl concentration no germination occurred during the experiment. The germination rates exhibited three phases, an initial latency phase, a second exponential phase of accelerated germination, and a third plateau phase. *A. karroo* seeds demonstrated the highest salt tolerance, germinating under high salinity conditions of 400 mM NaCl, with a FGP of 66%. In contrast, *A. tortilis* showed significantly lower salt tolerance, with only 20% germination at the same concentration. *A. saligna* had the lowest salt tolerance, with germination only occurring under 150 mM NaCl concentration and with a FGP of only 18%. Based on these findings, the rank order of the studied species in terms of decreasing tolerance to high salinity conditions, as determined by their respective germination capacities, is as follows: *A. karroo* > *A. tortilis* > *A. saligna*. Although *A. saligna* was the most sensitive species, it was still categorized as a salt-tolerant glycophyte. Overall, this study provides valuable insights into the impact of salt stress on *Acacia* species and could contribute to the development of salt-tolerant crops in the future.

Keywords: Fabaceae, germination, salt tolerance, *Acacia*, NaCl, soil salinity

1. Introduction

The ecosystems of arid and semi-arid regions are characterized by sporadic precipitation and extended periods of drought, which contribute to soil salinization [1]. One strategy to address this issue is to investigate halophyte species that can thrive in saline environments and have the potential to restore damaged soils, thus limiting soil and irrigation water salinization [2]. Halophytes with high growth rates and the ability to store salt in their leaves are particularly suitable for rehabilitating and desalinating arid and semi-arid lands [3].

Seed germination is a critical process for agroforestry and horticulture since our reliance on plants is primarily based on their ability to germinate. However, various environmental factors, including temperature, salinity, light, soil moisture, oxygen concentration, pH, and soil structure, often limit the germination process [4]. Halophyte species that exhibit strong and rapid germination after overcoming unfavourable conditions have a significant ecological advantage in terms of seed dispersal and establishing the next generation of seedlings. This is particularly relevant for annual halophyte species that produce seeds only once during their life cycle and have developed survival mechanisms to ensure seed viability and species persistence [5].

Numerous research teams have concentrated on the detailed and overall characterization of the adaptation of specific *Acacia* species to stressful environmental conditions, whether indigenous or exotic, to incorporate them into sustainable afforestation/reforestation programmes ([6]–[8]). Algeria's soil appears to support an interesting species diversity of the *Acacia* genus with a rustic character, which can survive in extreme pedoclimatic conditions, and it would be beneficial to reintroduce them into reforestation programmes. In Algeria, Kheloufi et al. [9] identified ten *Acacia* species, including *A. albida*, *A. laeta*, *A. ehrenbergiana*, *A. nilotica*, *A. seyal*, and *A. tortilis*, classified as native species [10], as well as introduced species such as *A. farnesiana*, a native of America [11], *A. karroo*, native to South Africa [12], and *A. saligna* and *A. decurrens*, both native to Australia [13]. These species have a significant advantage due to their ability to form a symbiotic relationship with soil microorganisms such as rhizobium and mycorrhizae. This association allows them to survive in soil that is deficient in essential nutrients [14].

Salinity is a major abiotic stress that has a negative impact on plant growth and development globally. In regions with arid and semi-arid climates, the salinization process occurs due to high evaporation rates and insufficient precipitation. The aim of this study is to explore the impact of varying levels of salt stress (NaCl) on *Acacia* seed germination, with nine different levels tested, ranging from the lowest to the most severe. However, only three of the ten Acacia species found in Algeria will be examined in this study, based on their geographic distribution and

density. *A. saligna* represents the northern region, *A. tortilis* the southern region, and *A. karroo* is an overlapping species present in both areas.

2. Materials and methods

Harvest and origin of seeds

Table 1 presents the provenances of the seeds used in this study for the three *Acacia* species, *A. karroo*, *A. saligna*, and *A. tortilis*. Additionally, the table includes biometric data for each species such as the weight of 1,000 seeds and the length and width of seeds. The measurements were taken based on a sample of 100 seeds per species. The experiment took place at the Department of Biotechnology Laboratory at the University of Batna 2 in Algeria.

Table 1. Characteristics and origins of the Acacia seeds studied

	A. karroo	***A. saligna***	***A. tortilis***
1,000 seed weight (g)	44.8	14.7	53.4
Length (cm)	0.54 ± 0.05	0.49 ± 0.02	0.59 ± 0.03
Width (cm)	0.32 ± 0.02	0.28 ± 0.01	0.42 ± 0.01
Region in Algeria	Djelfa	Aïn Témouchent	Tamanrasset
GPS coordinates	34˚40’ N; 3˚09’ E	35°26’ N; 1°13’ W	22°46’ N; 5°34’ E
Altitudes (m)	1,155	2	1,409

The seeds used in the experiment were obtained by manually crushing the naturally dried pods harvested from 10 trees of each *Acacia* species. To minimize inter-genetic variation, the seeds were mixed after harvest. The mixed seeds were then stored in paper bags at a temperature of 4 °C for three months, simulating the vernalization period [15].

Figure 1. Seeds of the different studied Acacia species

Seed germination

The seed coats of *A. karroo*, *A. saligna*, and *A. tortilis* have an anatomical structure typical of the *Acacia* species, resulting in strong seed coat inhibition of germination (*Figure 1*). This implies that natural or artificial scarification of the seed coat is necessary to allow the imbibition and germination of the seeds. To overcome seed coat inhibition, immersion of the seeds in concentrated sulphuric acid (98% H_2SO_4) for 120 minutes was necessary to achieve near 100% germination success under non-saline conditions for *A. saligna* and *A. tortilis* and only 30 minutes for *A. karroo* seeds ([15], [16]). After the acid immersion, the seeds underwent washing with distilled water to eliminate any traces of acid. They were then dried on absorbent paper until immediately sowing them in a suitable culture medium containing different salt concentrations.

Experimental design and application of salt stress

In order to study the effect of salt on germination, we used sodium chloride (NaCl). Seeds (3 species × 5 Petri dishes × 10 seeds × 1 salt × 9 concentrations) were germinated in 10-cm-diameter Petri dishes lined with two layers of Whatman N°1 paper, which were moistened with 20 ml of distilled water for the control (0 mM) and with 20 ml of one of the saline solutions having the following concentrations: 50, 100, 150, 200, 250, 300, 400, and 600 mM. It was important to ensure that the seeds maintained a certain level of humidity throughout the experiment. The counting of germinated seeds with a perforated testa was carried out every 3 days during the 21-day experiment. The Petri dishes were then placed in darkness at room temperature: 25 °C (± 2 °C). The seeds were moistened every three days with 15 ml of appropriate NaCl solution. Also, the papers were replaced every three days to prevent salt accumulation [17].

In the germination tests, the final germination percentage (FGP) and the mean germination time (MGT) for each species and treatment were calculated using the following procedures and formulas:

$$\text{FGP (\%)} = \frac{\sum ni}{N} \times 100,$$

where FGP is the final germination percentage, *ni* is the number of germinated seeds on the last day of the test, and *N* is the total number of seeds incubated per test [18].

$$\text{MGT (days)} = \frac{\sum(ti.ni)}{\sum ni},$$

where MGT is the mean germination time, *ti* is the number of days since the beginning of the test, *ni* is the number of germinated seeds recorded at time *t(i)*, and *Σni* is the total number of germinated seeds [19].

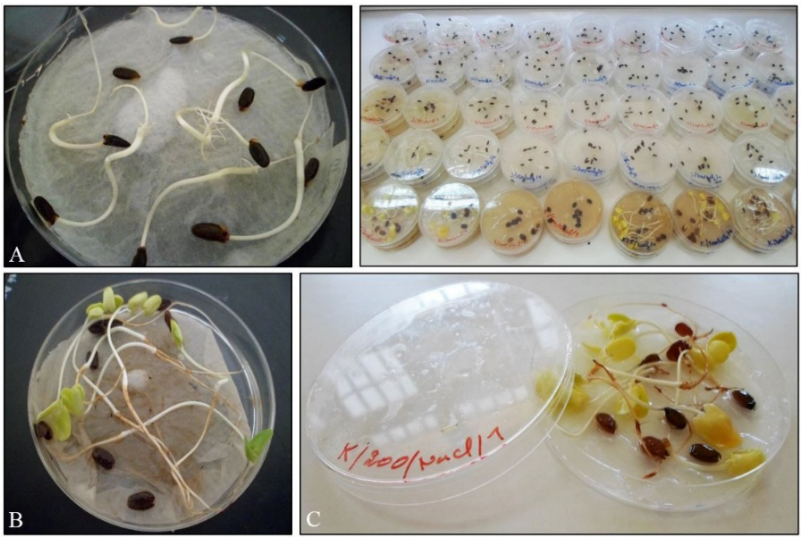

Figure 2. Experimental design and germination in Petri dishes
(A) *A. saligna*; (B) *A. tortilis*; (C) *A. karroo*

Statistical analyses

The effects of different NaCl concentrations on the two variables studied were tested by analysis of variance (ANOVA). Differences between treatments following ANOVA were made by means comparison. Multiple comparisons of means were carried out using Tukey's test ($p \leq 0.05$). Pearson's correlation coefficient was calculated for the three variables studied ($p \leq 0.05$). For germination kinetics, we applied the GLM (General Linear Model) procedure with univariate tests of hypotheses for the between-subject and within-subject effects. All statistical analyses were performed using SAS software Version 9.0 (Statistical Analysis System) (2002).

3. Results

Germination kinetics

The data presented in *Figure 3* displays the overall germination rates for seeds of three different *Acacia* species (*A. karroo*, *A. saligna*, and *A. tortilis*) over a period of 21 days as a function of increasing NaCl concentrations (mM). The figure

highlights three distinct phases: an initial phase of seed imbibition resulting in a latency period, followed by an exponential phase of rapid germination, and, finally, a plateau phase indicating a cessation in germination (stationary phase) (*Figure 3*). Notably, none of the seeds from the three species were able to germinate at a NaCl concentration of 600 mM during the 21-day experimental period.

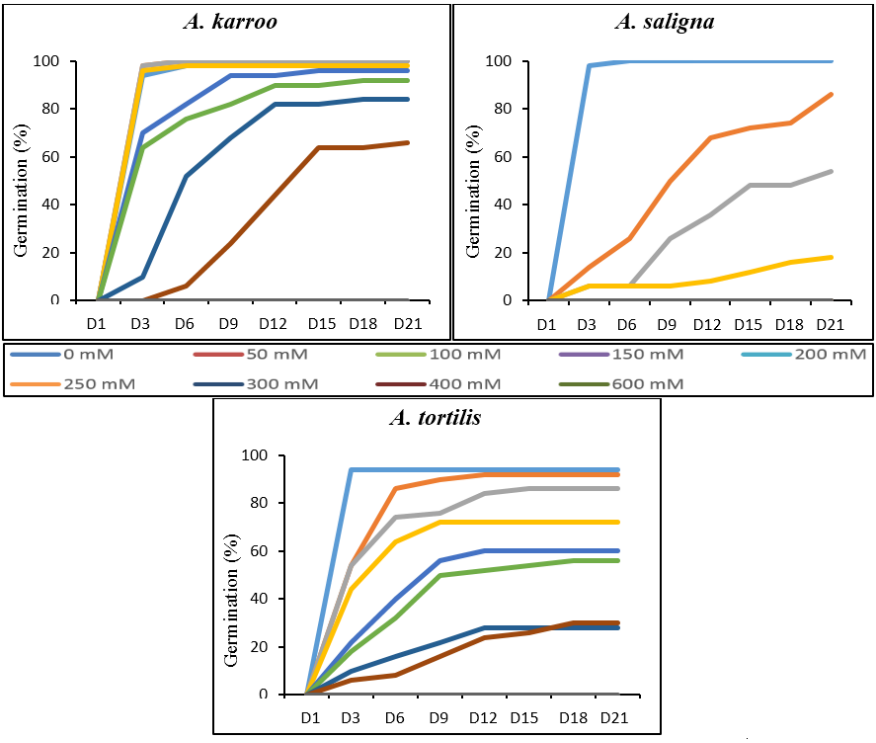

Figure 3. Effects of NaCl concentrations ranging from 0 to 600 mM on the germination kinetics of A. karroo, A. saligna, and A. tortilis seeds over 21 days

With increasing salinity, the germination curve is altered, causing a delay and reduction in the germination rate (*Figure 3*).

For *A. karroo*, the stationary phase begins on the 6th day in the control group, while seeds treated with 50, 100, and 150 mM reach the stationary phase on the 3rd day with almost 100% germination. As salinity levels rise, the stationary phase is further delayed, and under 400 mM NaCl, it exceeds 21 days.

For *A. saligna*, no germination occurs above 200 mM NaCl during the 21-day period, while the control group shows 98% germination by the 3rd day with a stationary phase starting on the 6th day. Seeds treated with 50, 100, and 150 mM have a low initial germination rate, which improves over time.

For *A. tortilis*, the stationary phase begins on the 3rd day in the control group with 94% germination. As salinity increases, the stationary phase starts at around the 15th day, and the germination rate decreases with increasing NaCl concentration. As the NaCl concentration increases by 50 mM, the exponential phase begins with a lower germination rate than the previous level, reaching 6% germination for the 400 mM treatment compared to 54% for the 50 mM treatment.

According to *Figure 3*, the germination rates of all the species examined decrease with increasing salinity stress, and NaCl has a significant impact on this reduction ($p < 0.0001$). In addition, it is evident that the length of the latency period varies among species and increases as the concentration of NaCl increases. A repeated measures analysis of variance (performed over a 21-day period with evaluations every three days) indicates that there is a highly significant effect ($p < 0.0001$) between various factors and variables, such as treatment (TRT), species (SP), and time (T), with both between-subject and within-subject effects and their correlation being observed (*Table 2*).

Table 2. Analysis of variance for the variables: final germination percentage (FGP), mean germination time (MGT) of seeds (A. karroo, A. saligna, and A. tortilis) (SP) in response to saline stress (TRT) induced by NaCl over 21 days (T)

Parameters	**Sources of variation**	**Degree of freedom**	**F of Fisher**	**P-value**
FGP	TRT	8	197.71	< 0.0001
	SP	2	427.37	< 0.0001
	TRT×SP	16	28.06	< 0.0001
MGT	TRT	7	26.49	< 0.0001
	SP	2	58.95	< 0.0001
	TRT×SP	10	18.25	< 0.0001
Germination kinetics	**GLM procedure**			
	Repeated analyses of variance measures			
	Univariate hypothesis tests for between-subject effect			
	TRT	8	273.93	< 0.0001
	SP	2	612.11	< 0.0001
	TRT×SP	16	31.75	< 0.0001
	Univariate hypothesis tests for within-subject effect			
	T	7	1513.43	< 0.0001
	T×TRT	56	66.46	< 0.0001
	T×SP	14	131.67	< 0.0001

Final germination percentage

The three *Acacia* species had a final germination rate of over 90% when grown in distilled water. However, when grown in the presence of NaCl, a highly significant effect of NaCl on germination rates was observed through analysis of variance ($p < 0.0001$) (*tables 2–3*).

Germination was delayed in most species as the concentration of NaCl increased. To determine the salt stress tolerance ranking of the species, we used 200 mM as a reference concentration. At this concentration, there was a wide range of variation among species. *A. saligna* had no germination power, while *A. tortilis* had half the germination power compared to the distilled water control. In contrast, *A. karroo* had a 96% final germination percentage, indicating a high salt stress tolerance. The ranking of the species based on decreasing tolerance is *A. karroo* > *A. tortilis* > *A. saligna*. This ranking is also supported by Tukey's test (*Table 2*).

Table 3. Effects of NaCl on the final germination percentage (FGP) and mean germination time (MGT) of seeds (A. karroo, A. saligna, and A. tortilis) after 21 days of treatment

Species	TRT [NaCl]	FGP (%)	MGT (days)
A. karroo	0 mM (Control)	100 ± 0.00^{a}	3.24 ± 0.25^{d}
	50 mM	100 ± 0.00^{a}	3.06 ± 0.13^{d}
	100 mM	100 ± 0.00^{a}	3.06 ± 0.13^{d}
	150 mM	98.0 ± 1.47^{a}	3.06 ± 0.13^{d}
	200 mM	96.0 ± 3.47^{a}	4.39 ± 0.52^{cd}
	250 mM	92.0 ± 4.9^{ab}	4.89 ± 0.58^{c}
	300 mM	84.0 ± 3.2^{b}	7.46 ± 1.69^{b}
	400 mM	66.0 ± 2.94^{c}	11.8 ± 2.01^{a}
	600 mM	0.00^{d}	--
A. tortilis	0 mM (Control)	94.0 ± 3.47^{a}	3.00 ± 0.00^{e}
	50 mM	92.0 ± 2.36^{a}	4.47 ± 0.56^{d}
	100 mM	86.0 ± 1.47^{a}	4.96 ± 1.02^{cd}
	150 mM	72.0 ± 3.36^{b}	4.48 ± 0.85^{d}
	200 mM	60.0 ± 3.8^{c}	6.09 ± 0.71^{bc}
	250 mM	56.0 ± 3.47^{c}	6.96 ± 0.96^{b}
	300 mM	30.0 ± 2.36^{d}	6.75 ± 1.27^{b}
	400 mM	20.0 ± 2.07^{d}	9.80 ± 1.48^{a}
	600 mM	0.00^{e}	--

Species	TRT [NaCl]	FGP (%)	MGT (days)
A. saligna	0 mM (Control)	100 ± 0.00^{a}	3.06 ± 0.13^{b}
	50 mM	86.0 ± 2.7^{b}	12.0 ± 2.33^{a}
	100 mM	54.0 ± 3.9^{c}	11.2 ± 1.09^{a}
	150 mM	18.0 ± 1.47^{d}	12.0 ± 2.37^{a}
	200 mM	0.00^{e}	--
	250 mM	0.00^{e}	--
	300 mM	0.00^{e}	--
	400 mM	0.00^{e}	--
	600 mM	0.00^{e}	--

Note: For each species, the different letters in the same column indicate a significant difference at $p \leq 0.05$, as evaluated by Tukey's test.

Mean germination time

Table 2 and *Table 3* demonstrate that salt has a notable impact on the germination of most *Acacia* species. As salt concentration in the culture medium increases, germination rates substantially decline, and a delay in seed germination is evident (as indicated by the mean germination time).

In addition, *Table 4* reveals a significant negative correlation between the variables under investigation (FGP and MGT). This implies that as FGP increases, MGT tends to decrease.

Table 4. Pearson's correlation coefficient between final germination percentage (FGP) and mean germination time (MGT) for different species of Acacia exposed to different NaCl concentrations (DF = 100)

	FGP	MGT
FGP	1.00000	-0,63112
P-value		< 0.0001
MGT	-0,63112	1.00000
P-value	< 0.0001	

4. Discussion

According to Ungar [5], salinity has a negative impact on the ability of both halophytes and glycophytes to germinate and can also cause a delay in the germination process. However, the way in which plants respond to salinity varies depending on the species. It has been observed that excessive salinity in soil, usually over 100 mM of sodium chloride, can lead to reduced growth in glycophytes, including important agronomic species, as reported by Sun et al. [20].

Our findings indicate that saline stress led to a decrease in germination percentage and delay across all species. According to Fernando et al. [21], metabolic imbalances within the seeds were responsible for the reduced germination under stress conditions. The extent of reduction varied for each species. *A. karroo* displayed a higher germination percentage than *A. tortilis* and *A. saligna*. *A. saligna* seeds were only able to germinate under 150 mM or lower saline stress, with germination completely inhibited at higher levels. Genetic and environmental factors both affect seed germination, and different species have developed diverse mechanisms to adapt to sodium-chloride-induced adverse conditions.

Bradford [22] reported that salinity affects seed germination, by either osmotic or toxic effect depending on the species. Osmotic effects are caused by insufficient water absorption, which prevents seeds from reaching their critical hydration level for germination. Toxic effects arise from salt accumulation in cells, which disrupts enzyme activity and prevents dormancy breaking, ultimately leading to reduced germination capacity. In severe cases, excessive ion accumulation can alter metabolic processes and cause embryo death [23]. The findings are in agreement with previous studies conducted on *A. saligna*, *A. tortilis*, and *A. karroo* by Meloni et al. [24], Jaouadi et al. [6], and Kheloufi et al. [25] respectively. These studies showed that the germination rate decreased significantly as the salt concentration in the culture medium increased.

Several researchers have shown that the imbibition and average time of germination are affected by the osmotic potential caused by NaCl, but not the final germination rate. Additionally, Tiryaki and Andrews [26] suggest that it is the germination capacity, rather than the delay caused by salt, that is most important for the final crop yield. Environmental factors, such as water availability and salt content in the soil, also play a role in regulating germination, in addition to genetic characteristics [27]. The low external potential can hinder the enzymatic activity of seeds, causing a delay in the release and growth of the radicle [28].

The toxic effects on seed germination are caused by the absorption of sodium ions, which disturb the movement of Ca^{+2} and Na^{+} ions at the cell wall level and can prevent radicle growth [29]. Sodium chloride has been found to increase the influx of external ions and cytosol efflux solutions, thereby affecting plasma membrane permeability [30]. Moreover, it stiffens the cell wall and reduces the fluidic conductance of the plasma membrane [31]. During seed germination, the degradation of reserves occurs through the action of amylases, phosphorylases, and glucosidases. The products of hydrolysis are then transported to the embryo to support its growth and development [32]. The mobilization of reserves may be slowed down by delayed activation and synthesis of hydrolases or the transfer of inhibition of hydrolysis products from the endosperm to the embryo, which can occur due to the effects of salinity [33].

The order of tolerance of the species under study based on their germination capacity can be arranged in descending order as follows: *A. karroo* > *A. tortilis* > *A. saligna*. Although *A. saligna* is classified as a salt-tolerant glycophyte, it appears to be highly sensitive compared to the other two species. Meanwhile, *A. karroo* and *A. tortilis* can be classified as halophytes at this stage.

In the Mediterranean region, high levels of salinity are reached at the surface of the sand during summer and early autumn [34]. In the case of *Acacia*, it is important to note the strong correlation between salt and temperature: germination occurs more readily at low temperatures in the presence of salt and gradually decreases as temperature increases [24]. This has significant ecological implications, as it highlights the necessity of reducing soil salinity for successful seed germination. Typically, germination in saline environments occurs in the spring when temperatures are cooler, and soil salinity decreases at the end of winter and spring [5]. This pattern is common in most halophyte seeds, which show optimal germination in freshwater ([35], [36]). Despite the differences between halophytes and glycophytes in their adaptation to salinity, both types of plants are affected by high salinity levels when it comes to seed germination [5].

5. Conclusions

The main objective of this study was to examine how NaCl-induced salinity stress affects the germination kinetics of three *Acacia* species: *A. karroo*, *A. saligna*, and *A. tortilis*. The findings indicated that as the salinity stress increased, the germination rates of all three species decreased. The germination curves of each species went through three phases: an initial latency phase due to seed imbibition, a second exponential phase characterized by rapid germination, and a final plateau phase indicating a halt in germination. The latency phase differed among the three species and increased with higher NaCl concentrations. Statistical analysis demonstrated that NaCl significantly impacted the germination rates of these species. These results suggest that all three species are sensitive to salinity stress, with varying degrees of sensitivity. *A. saligna* was the most sensitive, displaying no germination at NaCl concentrations above 150 mM, while *A. karroo* and *A. tortilis* were tolerant to salinity stress, with *A. karroo* displaying greater tolerance than *A. tortilis*.

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