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Germination Kinetics in Two *Acacia karroo* Hayne Ecotypes under Salinity Conditions

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Abstract

Acacia karroo Hayne is the most important woody invader of grassland in South Africa, and is one of the fastest-growing acacias, and produces high-density wood. This study aims to compare the germinative behaviour of *A. karroo* seeds collected from two ecotypes geographically and climatically different (Coastal and Steppic area) in salt stress. The seeds were exposed to saline stress conditions by increasing concentrations of sodium chloride (NaCl) varying from 0 to 50, 100, 150, 200, 250, 300, 400 and 600 mM. Germination was estimated by the daily rates and the final germinated seed rate during a period of 21 days in Petri dishes at 25˚C (5 replicates of 20 seeds). The emergence of seedlings was appreciated by their overall length. The results revealed the existence of a significant variation ($p < 0.001$) between the two seed sources. Indeed, those collected from an arid ecotype were most tolerant at higher sodium chloride concentrations. The response to salinity stress varied in time with NaCl concentration and seeds origin which could be attributed to intraspecific variations resulting from the natural selection of the same species. Under 400 mM of NaCl, 20% of germination was obtained from the coastal seeds against 66% from the steppic seeds. The repeated measures analysis of variance also revealed a significant effect of factors "Concentrations of NaCl, ecotypes, time of germination and their correlation" on the kinetics of germination.

Subject Areas

Ecology, Plant Science, Soil Science

Keywords

Acacia Trees, Seed, Salinization, Tolerance, Forestry

1. Introduction

In the world, lands salinization progresses and reaches a fifth of the irrigated grounds. Indeed, much of irrigated grounds are threatened by salinization, an accumulation of salts in the soils which decreases plant yields and can deteriorate the agricultural lands in an irrevocable way [1]. Moreover, recurring drought and increasing anthropization led to a strong degradation of ecological systems and to salinization [2]. Arid ecosystems are characterized by a specific climate with a long season of dryness (from 8 to 10 months) and with an intense evapotranspiration [3]. Otherwise, the climatic changes become increasingly constrained for the plant growth and development [4]. Algeria belongs to the Mediterranean countries where drought leads obviously to the process of salinization [5]. These two natural constraints of drought and salinity contribute to deforestation which acts negatively in biodiversity and ecosystem stability.

As much of forest trees, Acacia trees are frequently used in reforestation programs [6] [7] and in agro-sylvo-pastoral adjustment systems for arid and semi-arid regions. They are highly drought tolerant and are well-adapted to the poor soils of their environment [8] [9] [10]. These species are also well adapted to the degraded area and contrasted climatic conditions [11] [12] [13]. These properties are especially related to their capacities of symbiosis with beneficial micro-organisms of the ground: rhizobium and mycorrhizae [14]. *Acacia karroo* is one of the fastest-growing acacias and produces high-density wood (800 - 890 kg/m^3) and is very useful for the best honey production. It is named Sweet thorn and is a small to medium-sized tree, usually 4 - 8 m tall, but specimens up to 17 m tall have been found [15].

The fundamental importance of germination physiology to agriculture and horticulture is so obvious that it needs hardly to be stated, because almost all of our reliance on plants depends ultimately on the germinability of their seeds. The stage of germination is often limited by several environmental factors such as temperature, salinity, light, soil moisture, oxygen concentration and pH [16]. Thus, seed germination has a great importance for natural or artificial regeneration. Moreover, the intraspecific variations of tolerance to salinity can be remarkable [17]. In this investigation, the effects of saline stress were studied for two ecotypes of *Acacia karroo* Hayne seeds by using sodium chloride (NaCl) solutions with concentrations varying from 0 (Control) to 600 mM. The aim is to evaluate the influence of saline stress on the germination and the emergence of the vegetative apparatus and to determine if there is a significant intraspecific variation for tolerance to salinity between seeds of different bioclimatic zones (Coastal and Steppic).

2. Materials and Methods

2.1. Plant Material

We used seeds of *Acacia karroo*, one batch was collected from Msila forest, a coastal forest in the west of Oran (Algeria), area with Mediterranean climate of the North-West of Algeria (35˚38'35.3"N, 0˚51'33.2"W). The second batch was sampled from the forest of Sen El Bae, area of Djelfa (34˚40'00.3"N, 3˚09'18.8"E), a steppic area localized in the south-west of Oran (Algeria) and characterized with an arid climate. Plants were authenticated by Algerian Forest Research Institute. The mature pods were collected on 10 different trees in 2015. We have extracted the seeds from the dried pods by carefully crushing or breaking open the seedpods. Crushed debris was then separated from the seeds by sifting everything through a fine mesh screen. The flotation was also used to sort out seeds. The test of flotation aims at eliminating empty seeds, broken and insect-damaged. The clean seeds, of a weight of (21 to 23 grams/1000 seeds), were then deposited on the filter paper to dry. Once dried, they are stored in glass container 4˚C, to simulate the vernalization condition for 2 months-period, waiting their manipulation in 2016.

2.2. Germination Conditions and Salinity Treatments

Factorial combinations of two sources of *A.karroo* and nine levels were salinity test treatments. Salinity levels of 0, 50, 100, 150, 200, 250, 300, 400, 600 mM (millimolar) were created using sodium chloride (NaCl). First of all, collected seeds were cleaned then placed in boiled distilled water for three minutes, as a well-known pre-germination treatment for most Acacia seeds [19].

Twenty seeds per dish were used for each treatment. Seeds were germinated in 10 cm Petri dishes with Whatman filter papers N˚1 humidified with the appropriate solution or distilled water for 0 mM (Control) of NaCl concentration. Seeds were incubated under continuous dark at 25˚C ± 1˚C (Celsius) in controlled temperature room. The papers were changed with the same treatment each 3 days to prevent salt accumulation [18]. The seeds were moistened with the appropriate solutions of NaCl and kept wet throughout the experiment. The germination criterion was taken into account when radicle had pierced the tegument with 2 mm (millimeter) in length [20].

2.3. Studied Parameters

The studied parameters during this work were:

Final germination percentage (FGP): this parameter constitutes the best identification means of salt concentration which presents the physiological limit of germination. It is expressed by the report/ratio numbers seeds germinated on a total number of seeds [21].

Kinetics of germination: for better apprehending the physiological significance of germination behavior, the number of germinated seeds was counted every 3 days until the 21^{st} day of the experiment.

Mean Daily Germination (MDG): MDG is the percentage of final germination /number of days to final germination [22].

Lengths of the seedlings (LS): the length of seedlings in centimeter (cm) was measured using a cotton yarn because of the seedling curves in Petri dishes after 21 days of sowing.

2.4. Statistical Analysis

The experiment was made as a completely randomized design with five replicates of twenty seeds (n = 5). The variances from each Petri dish comfort the data to be reliable. The data were statistically treated by Fisher's analysis of variance (ANOVA). The Generalized Linear Model (GLM) was used in the kinetics of germination (Repeated Measures Analysis of Variance). The means values of provenances, after sodium chloride treatments and their interactions were compared by the Duncan's Multiple Range Test at $p \leq 0.05$ using SAS software version 9.0 (SAS 2002).

3. Results

The results of a two-way-ANOVA clearly indicate that the treatments operated by the various concentrations of sodium chloride exerted a very highly significant effect on the germinative capacity and seedlings length growth (Table 1 and Table 2). Overall, this germination rate decreased when the saline stress increased. However, a clear difference was observed between the two sources of seeds where the steppic seeds seemed to present a better behavior with respect to increasing in treatment levels. In addition, no germination appeared up to 600 mM of NaCl for both ecotypes (Table 2).

Table 1. Effect of NaCl (mM) on different germination parameters.

Ecotypes	NaCl (mM)	FGP (%)	MDG (%)	SL (cm)
Acacia karroo (Coastal area)	0 (Control)	96 ± 5.47a	4.57 ± 0.26a	11.3 ± 1.17a
	50	50 ± 10.00b	2.38 ± 0.47b	6.57 ± 0.78b
	100	46 ± 13.41b	2.19 ± 0.63b	5.23 ± 0.59c
	150	40 ± 15.81cb	1.90 ± 0.75cb	3.81 ± 0.36d
	200	30 ± 17.32cd	1.42 ± 0.82cd	2.78 ± 0.47e
	250	28 ± 4.47cd	1.33 ± 0.21cd	1.3 ± 0.34f
	300	22 ± 10.95d	1.04 ± 0.52d	1.16 ± 0.28f
	400	20 ± 15.81d	0.95 ± 0.75d	0.88 ± 0.25f
	600	0 e	0 e	0 g
Acacia karroo (Steppic area)	0 (Control)	100 ± 0.00a	4.76 ± 0.00a	12.75 ± 0.4a
	50	100 ± 0.00a	4.76 ± 0.00a	12.63 ± 0.54ab
	100	100 ± 0,00a	4.76 ± 0.00a	11.92 ± 0.79b
	150	98 ± 4.47a	4.66 ± 0.21a	9.52 ± 1.33c
	200	96 ± 5.47a	4.57 ± 0.26a	6.92 ± 0.96d
	250	92 ± 10.95ab	4.38 ± 0.52ab	4.07 ± 0.79e
	300	84 ± 15.16b	4 ± 0.72b	3.3 ± 0.71f
	400	66 ± 8.94c	3.1 ± 0.42c	1.07 ± 0.67g
	600	0 d	0 d	0 h

FGP: final germination percentage; MDG: mean daily germination; SL: seedlings length (SL). Values are means of five replicates of 20 seeds ± SD for the germination parameters and means of eight replicates ± SD for the length. Different letters in the same column indicate significant difference at 0.05, as assessed by Duncan's Multiple Range Tests.

Table 2. Variance analysis for the traits investigated for the two ecotypes of *Acacia karroo* Hayne seeds (Coastal and Steppic) in response to salinity stress.

Parameters	Source of Variables	DF	Sum of Squares	Mean Square	F of Fisher
FGP	[NaCl] (mM)	8	586.2	73.27	76.24***
	Ecotypes	1	453.37	453.37	471.72***
	[NaCl] × Ecotypes	8	126.82	15.85	1649***
MDG	[NaCl] (mM)	8	1.32	0.16	76.23***
	ecotypes	1	1.02	1.02	471.73***
	[NaCl] × Ecotypes	8	0.28	0.03	16.49***
SL	[NaCl] (mM)	8	2233.43	279.17	613.56***
	Ecotypes	1	377.65	377.65	829.97***
	[NaCl] × Ecotypes	8	204.88	25.61	56.29***
	Generalized Linear Model (GLM) (Repeated Measures Analysis of Variance)				
	Between Subjects Effects				
Kinetics of Germination	[NaCl] (mM)	8	4403.17	550.39	104.87***
	Ecotypes	1	2871.46	2871.46	547.11***
	[NaCl] × Ecotypes	8	704.66	88.08	16.78***
	Within Subjects Effects				
	Time (days)	6	283.75	47.29	160.98***
	Time × [NaCl]	48	242.64	5.05	17.21***
	Time × Ecotypes	6	10.87	1.81	6.17***
	Time × [NaCl] × Ecotypes	48	178.9	3.71	12.63***

*, **, ***: significant at 5%, 1% and 0.1% level, respectively; and ns: not significant. FGP: final germination percentage; MDG: mean daily germination; SL: seedlings length (SL) (cm). DF: degree of freedom.

3.1. Final Germination Percentage

Table 1 represents the response of germination to the saline stress for two batches of *A. karroo* seeds collected from two bioclimatically different areas. We clearly observe the clear effect of the seeds origin with regard to salinity tolerance. The treatment effect of NaCl with its increasing concentrations doesn't have a great influence on the germination of *A. karroo* seeds (Steppic). On the other hand, the effect of NaCl was distinctly significant for the seeds collected from the coastal area, confirming its salt sensitivity (Table 1 and Table 2). For 0 mM of NaCl, whatever the origin of the seeds, the maximum rate of germination was equal or very close to 100% from both provenances. The final rate of germination reflected clearly that the seeds of *A. karroo* (Steppic) are salt tolerant.

3.2. Kinetics of Germination

Table 2 indicates that the results of repeated measures analysis of variance were statistically significant ($p < 0.001$) between-subjects effects (NaCl concentrations × provenances) and within-subjects effects (time × NaCl concentrations × provenances). Indeed, the examination of Figure 1, representing the dynamics of the rates of germination according to the increasing NaCl concentrations illustrated three phases, a first phase of latency, due to imbibition after some time, a second exponential phase showed an acceleration of germination and finally a third phase characterized by a stage indicating a stationary phase of seed germination. In 0 mM of NaCl, the phase of latency and the exponential phase were very short and last only three days before reaching the stationary phase for the steppic seeds. In the same condition; these phases took 12 days for the coastal seeds (Figure 1). In the presence of NaCl, a reduction of the rate and the speed of seed germination were observed in the littoral seeds of *A. karroo*. Salinity levels affected also *A. karroo* seeds from collected the arid climate, except that their kinetics of germination was maintained at distinct intervals showing a good tolerance to NaCl (Figure 1).

3.3. Mean Daily Germination

For both zones, salinity effect (concentration) resulted in increasing or reducing of germination speed compared to the control. The rate of germination of *A. karroo* (Steppic) was much more significant than that observed for the coastal seeds in saline conditions. Mean daily germination tended to be completely canceled at 600 mM of NaCl for both studied ecotypes (Table 1).

3.4. Seedling Emergence

The effect of NaCl on seedlings emergence of *Acacia karroo* was evaluated by measurement of only one morphological characteristic which was the total length of the 21-days old seedlings. The two-way analysis of variance (provenances and NaCl concentrations), showed a highly significant provenance and

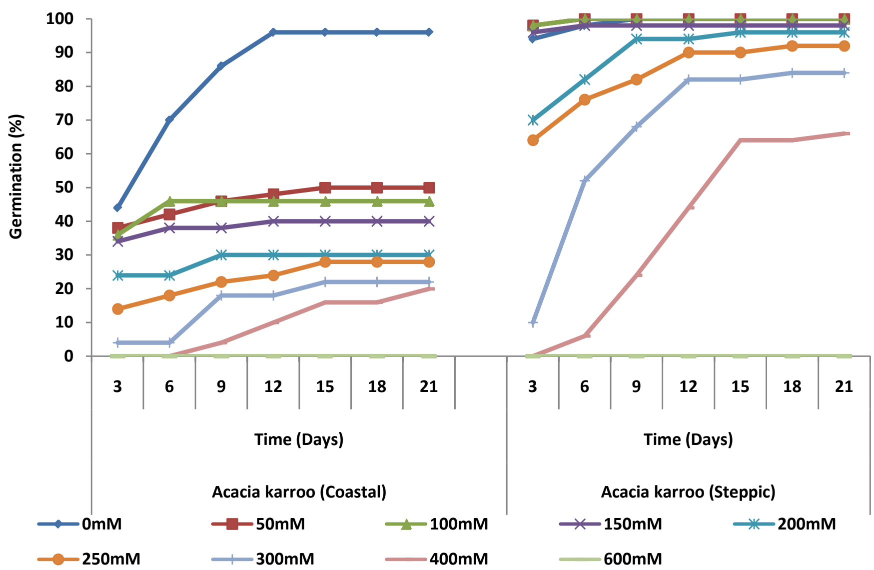

Figure 1. Effects of various NaCl concentrations (0 to 600 mM) on the kinetics of germination of two different ecotypes of *Acacia karroo* Hayne seeds (Coastal and Steppic) over a 21-day period.

concentration effect ($p < 0.001$) and also for their interaction (provenances × concentration of NaCl) for the parameter: length (**Table 2**). According to **Table 1**, we noted that for all used concentrations, the effect of NaCl on *Acacia karroo* seeds was different from one area to another and this even under 0 mM where seedlings of *A. karroo* of the arid region showed a growth in length higher than those for the coastal region. The seedlings of *A. karroo* of the steppic source appeared to be the most salt-tolerant even if their growth is reduced by half at the concentrations ranging from 100 to 200 mM of NaCl. The effect of the progressive increase in NaCl concentration in culture medium resulted in a strong reduction of the seedlings length.

4. Discussion

A large number of studies have been carried out on the effects of drought and salinity stress on the germination of forest tree species. Until now, the comparative studies of various species of Acacia under salinity stress were rare. The majority of species and ecotypes showed different sensitivity to the saline stress in regard to seed germination [23] [24] [25]. Few of these studies took into account the provenance selection based on bioclimatic classification. The germination is the first delicate stage for planting the production and the seeds are confronted with a serious problem of soil salinization [26] [27] [28] [29]. [30] has assessed that the saline stress is of paramount importance in determining seed characteristics and germination limitations in arid and semi-arid areas.

According to our results, the seeds of *Acacia karroo* germinated in the absence and/or in the presence of a low or high concentration of NaCl, are salt-tolerant, but this tolerance differs according to ecotype. For all the studied parameters, the collected seeds from the arid area showed very interesting results. This type of response has been interpreted as indicative of salt-tolerance. Research of [31] and those of [32] have affirmed that the seed germination in saline medium is related to the species. The salt stress of sodium chloride caused a decrease of final percentage of germination. Seed germination was reduced considerably in high salt level (400 mM). Indeed, high salt concentration of a germination medium may induce a reduction and delay in seed germination.

Salinity affects germination in two ways: there can be enough salts in culture medium which reduce the osmotic potential, changing the enzyme activity in the process of hydrolysis of reserves [33]. [34] showed that the osmotic effects result in the inability of seeds to absorb sufficient quantities of water to bring back their critical point of hydration to release the process of germination. However, the toxic effect is related to a cellular accumulation of salts which cause disturbances in enzymes metabolism and respiration. Many studies have shown that the effect of salt on germination could be an inhibitor (toxic) when the concentration is very high, or speed reducer (osmotic) when the concentration is lower [35] [36] [37]. Increased salinity leads to a delay in germination due by the necessary time to seeds to adjust their intern osmotic pressure [38]. [39] has also shown that the aptitude to germinate under drought or salinity conditions does not necessarily reflect the behavior of the adult plants.

By following the lengths of the plants under salt stress during the treatment periods, it appears there is a general trend for the decrease in the lengths of the seedlings using increasing sodium chloride concentrations for ecotype seeds. The reasons of this reduction can be explained by the inhibition of water uptake, caused by excessive accumulation of ions Na^+ and Cl^- [40]. These weak lengths were also in relation to the delay of seed germination. Under natural conditions, salt dependence and salt tolerance at germination stage are of great interest because successful germination in a wide range of conditions increases the probability of naturalization and soil rehabilitation. Understanding seed characteristics and seedling establishment patterns is essential for the development of effective management strategies for afforestation-reforestation [41].

In this study, it was possible to evaluate and to determine the most salt-tolerant seeds which provided from the steppic ecotype for their use in afforestation and reforestation systems in the arid and semi-arid regions where salinity does not stop progressing. The seeds of this ecotype could be the objective of future selection in order to fix the character of salt-tolerance. Moreover, as the culture is an invasive leguminous tree, it could contribute effectively and quickly to the nitrogen amendment in the degraded grounds and serve as a good conception point to restore the biodiversity.

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