Interspecific Variation in Salt Tolerance of Some Acacia Species at Seed Germination Stage

Chérifi Khalil[1*], Boufous El Houssein[2], Boubaker Hassan[3], Msanda Fouad[4], El Mousadik Abdelhamid[5]

[1,3,4,5]Laboratory of Biotechnology and Valorization of Natural Resources, Faculty of sciences, Ibn Zohr University, P. O. Box 8106, 8000 Agadir, Morocco
[2]Department of Biochemistry and Microbiology, Laval University, Quebec city (Quebec), Canada

Abstract**—** *Germination of seeds from six Acacia species was evaluated under salt stresses using five treatment levels: 0, 100, 200, 300, and 400mM of NaCl. Corrected germination rate (GC), germination rate index (GRI) and mean germination time (MGT) were recorded during 10 days. The results indicate that germination was significantly reduced in all species with the increase in NaCl concentrations. However, significant interspecific variation for salt tolerance was observed. The greatest variability in tolerance was observed at moderate salt stress (200 mM of NaCl) and the decrease in germination seems to be more accentuated in A. cyanophylla and A. cyclops. Although, A. raddiana, remains the most interesting, it preserved the highest percentage (GC = 80%) and velocity of germination in all species studied in this work, even in the high salt levels. This species exhibits a particular adaptability to salt environment, at least at this stage in the life cycle, and could be recommended for plantation establishment in salt affected areas. On the other hand, when ungerminated seeds were transferred from NaCl treatments to distilled water, they recovered largely their germination without a lag period, and with high speed. This indicated that the germination inhibition was related to a reversible osmotic stress that induced dormancy rather than specific ion toxicity.*

Keywords**— *Acacia species, Osmotic stress, Germination recovery, Salt tolerance, Seed germination, Variability, Plant breeding, Rehabilitation, Salt areas.***

I. INTRODUCTION

Salinity of soils is one of the most environmental factors limiting agricultural production and has significant effects on crop productivity and biodiversity. It has more severe impact in arid and semi-arid environments and combined with the water constraint presents a serious threat to food stability in these areas [1,2,3].Indeed, salinization already has affected more than 800 million hectares throughout the world or 6% of total land [4,5]. In the North Africa and the Middle East, salinity affects 15 million hectares of arable lands and this area is in continuous progression [6,7]. In Morocco, more than 5% of areas are already affected by salinity to various degrees [8]. They correspond especially to arid and semi-arid regions where 80% of available water for irrigation contains salinity higher or equal to 5 g/l [9]. These wide geographical areas are not exploited to a great extent, except when they occasionally constitute a poor pasture land or irrigated domain with low yield.

The best economic approach for exploitation and rehabilitation of these marginal regions is selection of salt tolerant species and varieties capable of sustaining a reasonable yield within salt-affected soils [10,11,12]. The effectiveness of such approach depends on the availability of genetic variation in relation with salt tolerance and its exploitation by screening and selection of the powerful plants under saline stress [13,14,15]. For many crop species, intraspecific variability for salt tolerance has been identified among cultivars and wild species [16,17,18,19]. These rustic resources are well adapted and constitute a potential reservoir useful to provide interesting materials in order to diversify and increase the productivity, particularly in pasture land affected by salinity.

Acacia species have the ability to survive in a diverse range of habitat and environments. They are well adapted to the arid and semi-arid regions and are known for them tolerance to high pH and salinity as well [20,21]. These species are able to stabilize and fertilize soils via nitrogen fixing and mycorrhizal symbioses [22,23] and constitute sources of wood, fodder, medicine and gum [24]. *Acacia* trees may thus include ideal candidates for enabling saline land reclamation with a potential for financial returns because of their combined production and soil improvement [25,26,27].

In Morocco, the genus *Acacia* is represented by four spontaneous species including one Moroccan endemic (*Acacia* gummifera Wild.); the other three are *Acacia raddiana* Savi, *Acacia ehrenbergiana* Hayne., *Acacia albida* Del. Otherwise, about ten species are introduced for ornamentation, reforestation or the fight against desertification [28].

Successful establishment of plants often depends on germination success, especially those that grow in salt affected areas. Thus, seeds must remain viable for long period in high salinity conditions and germinate when salinity decreases [29]. Various halophytic or glycophytic species, show an important variability with their ability to germinate under different salt concentration [30,31,32]. The effects on germination depend on the concentration of NaCl and varied among the plant species [33]. To overcome salt stress effect, plants have evolved various mechanisms that help them to adapt the osmotic and ionic stress caused by high salinity [26]. Otherwise, salts can affect germination of seeds either by restricting the supply of water (osmotic effect) or by causing specific injury through ions to the metabolic machinery (ionic effect) [34].

The purpose of this study was to assess and compare the seed germination response of six *Acacia* species (*Acacia gummifera*, *Acacia raddiana*, *Acacia eburnea*, *Acacia cyanophylla*, *Acacia cyclops* and *Acacia horrida*) under different NaCl concentrations in order to explore opportunities for selection and breeding salt tolerant genotypes that can be utilized in future land reclamation projects. The study will also assess to determine whether salt stress is induced by osmotic constraints or by toxic effect of NaCl.

II. MATERIALS AND METHODS

2.1 Plant Material

Six *Acacia* species were studied: Tow spontaneous species (*Acacia gummifera and Acacia raddiana) and four* introduced species (*Acacia eburnea*, *Acacia cyanophylla*, *Acacia cyclops* and *Acacia horrida*).Mature, dry seeds were collected from trees growing under field conditions in semi-arid region of south Moroccan region. Seeds collected from pods were generously provided by the Regional Forest Seeds Station of Marrakech and stored in a cold chamber at 6°C.

Acacia gummifera is an endemic species to Morocco and *Acacia tortilis* (Forssk.) Hayne subsp. *raddiana* (Savi) Brenan, commonly named *A. raddiana*, considered as a keystone species, is more prevalent in the inland part of the ecoregion, and are widely distributed throughout the Sahara desert [35,36]. The other four species were introduced in Morocco from south-western Australian in the 18th century. These plantations were created for several purposes such as their use as ornamental plant, in the fight against desertification and for dune stabilization [37].

2.2 Germination

Seeds from different pods were manually scarified, to overcome hard seed coat dormancy, and sterilized with 0.5% sodium hypochlorite solution (NaOCl) for 10 minutes, then rinsed with sterile distilled water several times, and briefly blotted on filter paper. Three replicates of 20 seeds from each accession were placed in plastic Petri dishes (90 mm diameter) on filter paper wetted with distilled water (control) and four salinity concentrations (100, 200, 300 and 400 mM NaCl). Petri dishes were randomized in a precision incubator and maintained in the dark at 25 ±0.5°C.Seeds were considered to have germinated when their radicle reached at least 3 mm long. Germination response was recorded daily for 10 days.

Several germination parameters were calculated to characterize the salt tolerance, including the corrected germination rate (GC), germination rate index (GRI) and mean germination time (MGT):

- Corrected germination rate (GC) was expressed as the number of seeds germinated in a concentration of salt divided by the number of germinated seeds in distilled water (control) for 10 days [38].
- The germination rate index (GRI) was calculated by using the following formula:

$$GRI = (G1 / 1) + (G2 / 2) + \ldots + (Gx / x)$$

Where G is the germination percentage at each day after sowing, and 1, 2, … ,x represents the corresponding day of germination. The value of GRI was higher when seeds germinated earlier. This parameter described by [39,40] is a measure of seedling vigor and should involve not only germination but emergence characteristics.

- The Mean Germination Time (MGT) is a measure of the rate and time-spread of germination (lower values indicating faster germination). It was estimated as:

$$MGT = \sum ni\text{-}ti / N$$

where t is time from the beginning of the germination test in terms of days and n is the number of newly germinated seeds at time t [41,42].

To test germination recovery performance after salt exposure, ungerminated seeds in severe salt stress (300 and 400 mM of NaCl) were transferred to distilled water and incubated for 6 days. We calculated the recovery germination percentage (number of germinated seeds in recovery test / number of seeds transferred to distilled water) [43].

2.3 Statistical analysis

All values expressed as a percentage were arcsine square root transformed before performing statistical analysis to normalize the data and improve homogeneity of variance [44]. These measures were submitted to a two ways analysis of variance (ANOVA) with species and salinity treatments as factors followed by a Student–Newman–Keuls post hoc test. A difference was considered to be statistically significant when $P < 0.05$. All statistical analysis were performed with Statistica software Version 6.1 for Windows [45].

III. RESULTS AND DISCUSSION

3.1 Effect of salinity on seed germination

For the six species, the two-way ANOVA indicated highly significant main effect of both species and salinity regarding final germination percentage and germination rate index ($P<0.001$) (Table 1 and 2). However the existence of a significant interaction between these two effects (F = 34, 00 at (GC) and F = 6,385 at (GRI)) indicates that species studied did not similarly respond to the effect of salt at a given concentration of NaCl.

TABLE 1
TWO-WAY VARIANCE ANALYSIS (ANOVA) FOR FINAL GERMINATION PERCENTAGE (GC) OF THE SIX SPECIES UNDER DIFFERENT CONCENTRATIONS OF NaCl.

Source of variation	d. f.	MC	F
Species (Sp.)	5	0,618	270,110**
Salinity (Salt.)	4	5,113	2233,421**
Sp. X Salt	20	0,077	34,000**
Error	60	0,002	

d.f.: Degree of freedom, MC: Mean square, F: ratio of variances, **significant at 1% probability level.

TABLE 2
TWO-WAY VARIANCE ANALYSIS (ANOVA) FOR GERMINATION RATE INDEX (GRI) OF THE SIX SPECIES UNDER DIFFERENT CONCENTRATIONS OF NaCl.

Source of variation	d. f.	MC	F
Species (Sp.)	5	0,570	283,194**
Salinity (Salt.)	4	4,149	2064,370**
Sp. X Salt	20	0,040	20,010**
Error	60	0,002	

d.f.: degree of freedom, MC: Mean Square, F: ratio of variances, **significant at 1% probability level.

For each concentration of NaCl, a significant interspecific variation in both potential of seed germination (fig. 1) and seedling vigor (fig. 2) among the six examined species. Mean comparison at different salinity levels indicated that increase of salinity causes a decrease in seed germination capacity, which was higher in distilled water than in any NaCl concentration. Moreover, all the species showed an increase in MGT (Mean Germination Time), indicating that seeds germinated more slowly as salinity increased (Table 3).

TABLE 3
MEAN GERMINATION TIME (MGT) OF DIFFERENT SPECIES IN VARIOUS NaCl CONCENTRATIONS.

Species	NaCl Concentrations				
	0mM	100mM	200mM	300mM	400mM
A. cyanophylla	2,48**a**	2,98**a**	5,83**b**	NC	NC
A. cyclops	2,58**a**	3,09**a**	5,42**b**	6,67**c**	NC
A. eburnea	3,20**a**	3,80**a**	4,31**a**	5,55**b**	7,00**c**
A. gummifera	3,05**a**	3,48**a**	4,24**a**	5,66**c**	6,22**c**
A. horrida	3,03**a**	3,58**ab**	4,76**b**	6,50**c**	NC
A. raddiana	2,66**a**	2,80**a**	3,30**a**	4,86**b**	6,08**c**

Values followed by the same letter in a row are not significantly different at P<0.05. (Newman-Keuls test).
NC: MGT was not calculated because of insufficient germination

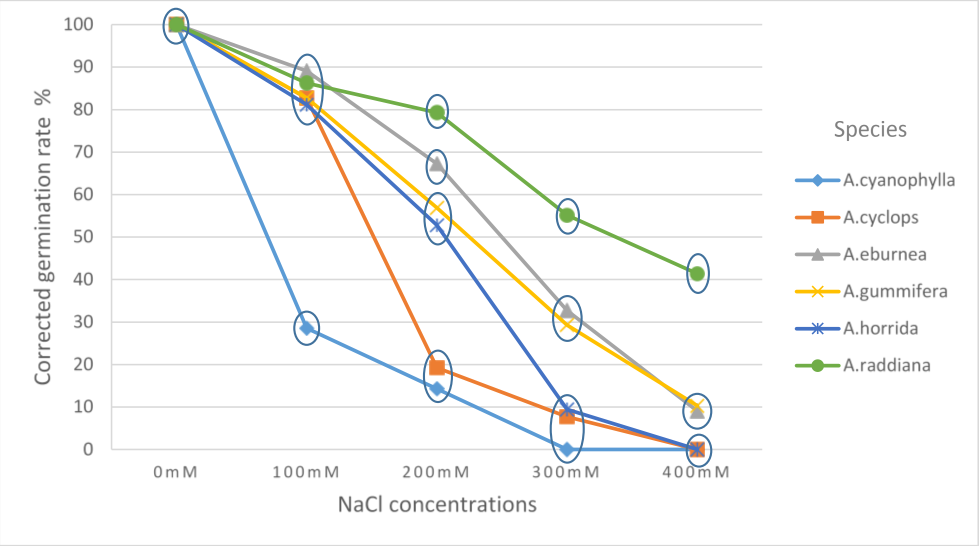

FIG. 1: CORRECTED GERMINATION RATE OF DIFFERENT SPECIES IN VARIOUS NaCl CONCENTRATIONS.
At each concentration of NaCl, values in the same ellipse are not significantly different ($p < 0.05$) (Newman-Keuls test).

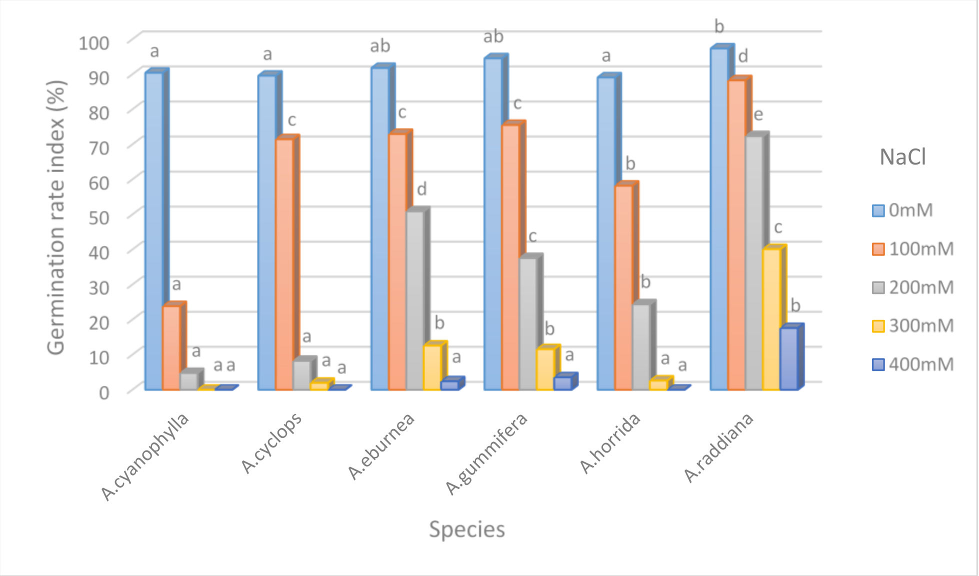

FIG. 2: GERMINATION RATE INDEX OF DIFFERENT SPECIES IN VARIOUS NaCl CONCENTRATIONS.
At each concentration of NaCl, means of populations having the same letter are not significantly different ($p < 0.05$) (Newman-Keuls test).

At the lowest stress (100 mM), the decrease in germination seems to be more accentuated in *A. cyanophylla* (GC never fell above 30%). However, all the other species are distinguished by forming a homogeneous group which is little affected by salinity compared with control (GC = 84% on average). At this saline level, seeds germinated rapidly and no significant change was noticed in germination speed (MGT not differing from that of control) (Table 3).

At 200 mM, the results revealed considerable interspecific variation in the response of seed germination to salinity among the studied species. Thus, four groups are distinguished, the first is formed by *A. raddiana* which showed very high germination percentage (GC = 80%) and revealed earlier seed germination MGT = 3,29, followed by *A. eburnea* (GC and GRI close respectively to 67% and 51%), while *A. gummifera*, and *A. horrida* occupy an intermediate position between the tolerant species and the rest of the others, considered as the most sensitive (GC = 15% in the case of *A. cyanophylla* and 19% for *A. cyclops*). Otherwise, from this level, seed germination was relatively slower in *A. cyanophylla* and *A. cyclops* (high MGT).

The elevated doses of salt (300 and 400 mM) induced significant reduction in seed germination and retarded their initiation of all the species. *A. cyanophylla*, *A. horrida*, and *A. cyclops* seem to be sharply affected in the same manner by these two concentrations, their corrected germination percentage does not exceed 6% and the time to germination gradually lengthened (MGT> 6 days) at 300 mM and no germination took place in this group at 400 mM. Seeds of *A. gummifera* and *A. eburnea* can be regarded as moderately tolerant to salt stress and react in the same way at these two concentrations (GC and GRI close respectively to 32% and 12% in the case of 300 mM). In this case germination was significantly delayed at the tow highest NaCl levels.

Whereas, *A. raddiana* continued to record highest percentage and velocity of germination in all species studied in this work, even in the high salt levels with GC reached 55% at 300 mM and 41% at 400 mM of NaCl. This species exhibits a particular adaptability to salt environment, at least at this stage in the life cycle, and could be recommended for plantation establishment in salt affected areas.

The effect of salinity on germination has been addressed by several authors and in different species [46,47,34,48,49]. This stage is very important for the development of the plants, particularly those that live in environments affected by salinity. In this study, the monitoring of germination process revealed that salinity notably affected germination in *Acacia* species but also delayed the time needed to complete germination, especially with increasing salinity level. This is consistent with our result particularly for sensitive species and is explained by the time required for the seeds to develop mechanisms allowing to adjust them internal osmotic pressure [50].

Additionally, the results showed significant interspecific variation in salt tolerance during germination of the species studied in the range of concentrations of sodium chloride from 100 to 400 mM. This variability, required to start a breeding programs for salt tolerance, have been also observed in several species including halophyte or glycophyte species [51,52,43,15,49].

The Moroccan *Acacia raddiana* particularly, tolerate salinity until 400 mM (probably also at higher levels) with a germination rate that exceeds 40%. Indeed, according to Ndour and Danthu [53] and Danthu and al. [54], *Acacia raddiana* is among the African *Acacia* species whose germination is less affected by the presence of salt. Its seed germination could be blocked only up to salt concentrations close to seawater (35 g/l). Previous studies have also reported that this species were the most tolerant and could be used in increasing forage production in salt affected areas [55,56,57,58]. Furthermore, the observed germination percentages in our study are relatively well higher than those published by Jaouadi and al. [50] in Tunisian species (maximum germination rate of 21% under 22 g/l of NaCl) and by Abari and al. [59] in Iranian taxa (germination was stopped at 300 mM). El Nour and al. [46] reported that no germination took place in four Yemeni *Acacia*s (*A. cyanophylla*, *A. seyal*, *A. tortilis*, and *A. tumida*) at salt concentration above 20 dSm^{-1} (around 200 mM of NaCl).

Among the rest of the five *Acacia* species examined in the present study, *A. gummifera* and *A. eburnea* was shown to possess the medium level of salt tolerance, whereas *A. cyanophylla*, *A. horrida*, and *A. cyclops* were categorized as the least tolerant group. Several papers that have compared salt tolerance in some *Acacia* species have suggested relatively the same ranking [60].

Moreover, previous studies have reported that salt tolerance was positively correlated with seed size [61,26]. *A. raddiana*, *A. gummifera* and *A. eburnea* seeds are larger than those of *A. cyanophylla*, *A. horrida*, and *A. cyclops*; and according to Croser and al. [62], larger seeds may contain more food reserves, which could be used to overcome osmotic effects of salts, and greater energy reserves making them less dependent on photosynthesis for early growth. Similar observation which made on *Triticum aestivum* [63,64], on *Atriplex* species [65] as well as on *Acacia longifolia* [26], which also showed that larger seeds had greater success in overcoming osmotic constraints during the initial stages of germination.

El Nour and al. [46] reported that salinity notably delayed the germination process of *Acacia* species, especially with increasing salinity level. This is consistent with our result particularly for sensitive species and explained by the time required for the seeds to develop mechanisms allowing to adjust them internal osmotic pressure [50].

3.2 Recovery of Germination

After transfer of the seeds that failed to germinate under high concentrations of salt to distilled water, they recovered relatively their aptitude of germination at all the species studied. The delay in germination speed tended to be relatively rapid than that observed in distilled water (fig. 3).

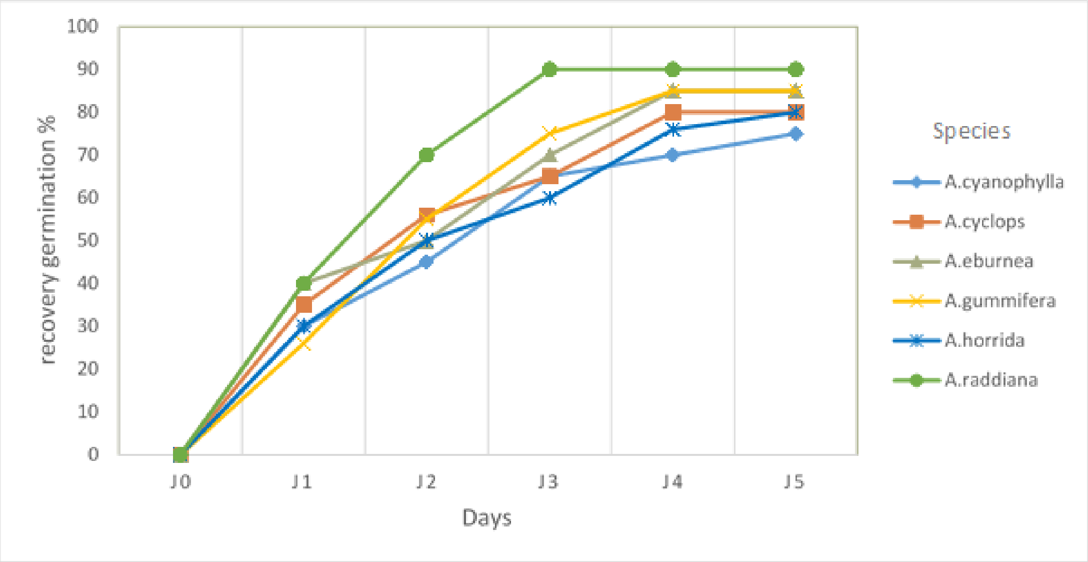

FIG. 3. THE REVERSIBLE EFFECT OF HIGH NaCl CONCENTRATION ON THE GERMINATION KINETICS IN THE STUDIED SPECIES.

This revealed that NaCl had no toxic effect, because salt stress did not damage the embryo as verified by not only the germination recovery but also pink embryos in the tetrazolium staining test. NaCl concentrations did not destroy seed germination ability, it had only repressed the germination momentarily and the viability was preserved. These results are consistent with those obtained in several other salt tolerant species including *Atriplex halimus* [38], *Medicago ruthenica* [66], *Medicago polymorpha* and *Medicago ciliaris* [67], *Panicum turgidum* [30], *Triticum aestivum* [68], *Brassica napus* [19] and *Lolium perenne* and *Bromus tomentellus* [69].

An important characteristic of salt tolerant seeds, which differentiate them from seeds of glycophytes, is their aptitude to maintain seed viability for lengthy period of time during exposure to hyper-saline conditions and then initiate germination when salinity stress is decreased [70,71,30]. The present study reveals that the recovery of germination is not a criterion of salt tolerance which distinguishes halophytes from glycophytes. It was maybe due to a reversible osmotic effect that induced dormancy, as revealed in findings of [72,68]. Consequently, a high proportion of seeds remained viable and had the ability to germinate when salinity stress was alleviated [30].

Reduction in mechanisms of germination by osmotic stress may be related to the lower diffusion of water through the seed coat [73,47] caused by the increased osmotic pressure environment, preventing the seed imbibition [65]and mobilization of reserves for embryo’s growth [74]. Thus, dormancy decrease the risk of seedling mortality when moisture is limited and salinity is augmented [75]. High recovery germination speed revealed in our studied species, particularly in *A. raddiana*, indicates that seeds have the ability to avoid deterioration caused by prolonged exposure to unfavorable biotic factors [76]. This situation constitutes an ecophysiological adaptive strategy to take full advantages of favorable conditions, available for a short time, during the germination stage [77]. It also, secure the long-term subsistence of seed bank helping the species in dispersal germination and seedling establishment over years [75]. Tilaki and al. [69] revealed that under saline environments, seed survival may be a suitable condition for success instead of germination capacity, since recovery germination does occur in the seeds when hyper-saline conditions are alleviated.

IV. CONCLUSION

The present study, indicate that salinity decreased rate and speed of germination. It reveals also significant interspecific variation in the potential for salt tolerance at germination among the six species. The Moroccan species *Acacia raddiana* remains the most interesting at this stage. It preserves a high rate of germination until a concentration 400 mM of NaCl, with a germination rate that exceeds 40%. Germination recovery after removing the saline stress indicates that sodium chloride caused a reversible osmotic effect of germination rather than ion specific toxicity and exerts a temporary inhibition of germination which is eliminated with the removal of the constraint. This ability to germinate after exposure to higher concentrations of NaCl suggest that these species, especially the most tolerant, could be able to germinate under the salt affected soils and could be utilized for the rehabilitation of damaged arid zones. Nevertheless, germination in field conditions is more difficult due to other environmental factors such as, drought, light, and temperature. A more ambitious program, including *Acacia* species, is necessary, not only at germination but also at the other stages of the life cycle. This opens the possibility to continue this work to verify correlation between salt tolerance during seed germination and early stage of plant development which will be most useful in a breeding program for selecting salt-tolerant genotypes in *Acacia* species.

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