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Comparative effect of NaCl and $CaCl_2$ on seed germination of *Acacia saligna* L. and *Acacia decurrens* Willd

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Abstract

Salinity is one of the environmental factors that has a critical influence on the germination of seeds and plant establishment. Seed germination is the stage which is most susceptible to this abiotic constraint. The objective of this study was the identification of the kinetics of germination in response to salinity stress. Two experiments were separately conducted using various salinity levels of 0, 50, 100, 150, 200, 250, 300, 400 and 600 meq.L^{-1} created using NaCl as first experiment and by $CaCl_2$ at the same levels in the second experiment. The seeds of two Acacia species (*Acacia decurrens* and *Acacia saligna*) were used in each experiment. The germination was evaluated in Petri dishes based on the daily rates and the cumulated rates of germination seeds over a period of 21 days. Germination of these species decreased with increasing salinity. All *Acacia* species showed higher tolerance to increased level of $CaCl_2$ than to NaCl. The recovery of the seeds that did not germinate under salinity conditions using NaCl or $CaCl_2$ at (600 meq.L^{-1}) indicate that the sodium chloride were toxic at this concentration and thus the adverse effects of $CaCl_2$ concentration that explained as a result of lowering osmotic potential of the external solution. High significant results indicated that the NaCl presented higher toxic effects on germination parameters than the $CaCl_2$. Furthermore, *Acacia decurrens* was more tolerant than *Acacia saligna* with a rate of considerable germination of 46% with the concentration of (300 meq.L^{-1}) of NaCl.

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Introduction

The arid and semi-arid ecosystems are characterized by low and a strong irregularity of precipitations (Aguiar and Sala, 1999; Morton *et al.*, 2011) associated with a significant drought favoring the accumulation of salts in the grounds (Jaleel et al., 2008). It is possible to limit the width taken by the salinization of the grounds and water by the exploration of the saline ecosystems and the identification of the plant species with economic and/or ecological potentialities in order to use these naturally tolerant species for the rehabilitation of the damaged grounds (Le Houérou, 1992; Jordan *et al.*, 2004). Thus, several halophytic species expressing strong potentialities of growth and storage of salt in their leaves are interesting for the fixing and the desalination of the grounds in the arid and semi-arid regions (Khan and Duke, 2001).

The germination is often limited by the salinity of the environment and is shown most sensitive then the other stages (Nascimento and West, 2000). Considered by some authors as an invasive species (Low *et al.*, 2000; Pejchar and Mooney, 2009); the species of the *Acacia* genus constitute a very effective tool for a fast afforestation and a durable rehabilitation of the degraded zones (Le Houérou, 2000). Many species of this genus are used for the afforestation and for the stabilization of dunes; a dozen is regarded as good fodder crops (Le Houérou, 1980; Séhouéto, 2014). Moreover, this genus is very interesting sight its membership of the leguminous family, from their aptitude to be pushed in grounds with low in essential nutritive elements by activating a symbiosis with *rhizobia* and *mycorrhiza* (Founoune, 2001).

Algerian arid and semi-arid grounds are in urgent need to found the best species to reduce the degradation and reinstall the biodiversity. Nevertheless, the success of the phases of germination and growth of the vegetation passes ineluctably by a good knowledge of their germinative characteristics and development as well as their behaviors with the conditions of the medium. We analyzed the effect of the salinity on the germination phase of two acacia species (*Acacia saligna* L. and *Acacia decurrens* Willd), which have a very considerable density and an interesting seed production in the Algerian territory constituting the capital elements in the maintenance of many arid and desert ecosystems (Aswatappa et al., 1987; Kull et al., 2011). And as the salinity is taking an increasing and threatening scale with the NaCl as the most abundant element, we investigated its effects with various concentrations of (0 meq.L^{-1}: control) to (600 meq.L^{-1}) on the first and crucial growth stage for these acacia species. In a parallel part of this study and with the same concentrations as the NaCl, we instigated the study of the effect of another salt which is the $CaCl_2$, because a saline soil is characterized by the intervention of several salts; the most harmful is the sodium chloride (Ungar, 1996; Munns and Tester, 2008). The aim of this work is to deduce the attitude of the seeds to these two salts, and especially which of the two species would be able to contribute to a good program of remediation and rehabilitation of the degraded and saline grounds.

Materials and methods

Seeds collection

An experiment was conducted at the Department of Ecology and Environment of Batna2 University (Algeria). During this test, the choice was fixed on the seeds of two acacia species: *Acacia saligna* L. and *Acacia decurrens* Willd. The seeds collected in 2014 were cleaned and inspected very well. These seeds were stored in a dry place at a temperature of 4°C to be used in our study in 2015. The pods were collected in the National park of El Kala located in El Taref at the North-East of Algeria (classified reserve of the biosphere by UNESCO in 1990).

Seed germination

The teguments of seeds of *A. saligna* and *A. decurrens* have a typical anatomical structure of the species of the *Acacia* genus which results in a strong tegumentary inhibition of germination. That implies that a natural or artificial scarification tegument is required to allow the imbibition and the germination of seeds.

To raise the tegumentary inhibition of seeds, an immersion of seeds in the concentrated sulphuric acid (H_2SO_4 at 96%) was indispensable during 50 minutes for the seeds to ensure a germination success at 100% in non-saline conditions, following a preliminary experience testing the optimal duration of the immersion in the sulphuric acid and this guided by the works of Roussel (1984) and those of Ndour (1997). These seeds will undergo at the end of the duration of the immersion in the acid, a washing with distilled water in order to eliminate any trace from acid. They will be then put to dry on absorbing paper while waiting for their immediate setting in the culture medium.

Saline treatments

In order to study the effect of salt on germination, we used two kinds of salt: the sodium chloride (NaCl) and the calcium dichloride ($CaCl_2$). For each species, the seeds are put to germinate in Petri dishes of 10 centimeters in diameter papered of two layers of paper Whatman No1, this one is moistened with 15 ml of distilled water for the control (0 $meq.L^{-1}$) and with 15 ml of one of the salt solutions having the following concentrations: 50, 100, 150, 200, 250, 300, 400 and 600 $meq.L^{-1}$. The norm is to take care that the seeds preserve a certain moisture throughout the period of the experiment. The counting of the germinated seeds and whose radicle bored the teguments (Côme, 1970) carried out each 3 days and this during the 21 days of the experiment. The Petri dishes are then put in the darkness at an ambient temperature (25 ± 1°C). The seeds will be moistened with a three-day frequency with 15 ml of the suitable concentration of NaCl or $CaCl_2$ solution. The papers were altered ounce after every three days to prevent salt accumulation (Akbarimoghaddam *et al.*, 2011). Twenty seeds per dish were used for each treatment.

Studied parameters

The parameters studied during this work are:

The Final rate of germination: this parameter constitutes the best way of identification of the salt concentration which presents the physiological limit of germination of seeds. It is expressed by the report/ratio numbers seeds germinated on a total number of seeds (Côme, 1970). The duration of the test was fixed at the period of germination which was extent out over 21 days.

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

Mean Daily Germination (MDG): according to Osborne et al. (1993), MDG is the percentage of germination final/number of days to final germination.

Test of reversibility of the inhibitory action of salt: For the tests on the reversibility of the inhibition of germination, the test will relate to only seeds which didn't germinate after 21 days of culture. Only the high salt concentrations (600 $meq.L^{-1}$) will be retained. Thus, after soaking many times in distilled water, then placed for germination in Petri dishes in the darkness and in the absence of salt during 7 days. This parameter has the advantage to determinate the origin of the depressive effect of salt, if it is by osmotic and/or toxic nature. It is calculated by the number of germinated seeds on the total of seeds.

Statistical analysis

The experiment was made as a completely randomized design with five replicates (n=5) of twenty seeds. 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 mean values of species, after sodium chloride and calcium dichloride treatments and their interactions were compared with the Duncan's Multiple Range Test at $p \leq 0{,}05$ using SAS software version 9.0 (SAS 2002).

Table 1. Effect of NaCl (meq.L^{-1}) on kinetics of germination, final germination percentage (FGP) and mean daily germination (MDG) (after 21 days of treatment). Values are means of five replicates ± SD for the germination parameters (Different letters in the same column indicate significant difference at $p \leq 0.05$, as assessed by Duncan's Multiple Range Tests).

		Acacia saligna		*Acacia decurrens*	
		FGP (%)	MDG (%)	FGP (%)	MDG (%)
NaCl (meq.L^{-1})	Control	100±0.00a	4.46±0.00a	100±0.00a	4.46±0.00a
	50	86±20.73b	4.09±0.98b	100±0.00a	4.46±0.00a
	100	54±21.90c	2.57±1.04c	92±4.47abc	4.38±0.21abc
	150	18±4.47d	0.85±0.21d	90±10c	4.28±0.47bc
	200	0e	0e	86±5.47c	4.09±0.26c
	250	0e	0e	98±4.47ab	4.66±0.21ab
	300	0e	0e	46±13.41d	2.19±0.63d
	400	0e	0e	0e	0e
	600	0e	0e	0e	0e
$CaCl_2$ (meq.L^{-1})	Control	100±0.00a	4.76±0.00a	100±0.00a	4.76±0.00a
	50	92±10.95a	4.38±0.52a	100±0.00a	4.76±0.00a
	100	68±4.47b	3.23±0.21b	92±8.36a	4.38±0.39a
	150	66±32.09b	3.14±1.52b	96±8.94a	4.57±0.42a
	200	44±5.47c	2.09±0.26c	72±8.36b	3.42±0.39b
	250	20±10d	0.95±0.47d	54±15.16c	2.57±0.72c
	300	14±8.94de	0.66±0.42de	30±12.24d	1.42±0.58d
	400	0e	0e	14±5.47e	0.66±0.26e
	600	0e	0e	0f	0f

Results

The results of a different analysis of the variance (ANOVA) indicated clearly that the treatments operated by the various concentrations and the different kind, exerted a very highly significant effect on the germinations' capacity of the different species over a time (Table 1, Table 2).

Kinetics of germination

Acacia saligna L.

The results represented on figure 1 indicate that under the saline conditions of the two kinds of salts (NaCl and $CaCl_2$), the kinetics of germination expresses three phases, a first phase of latency, which had with the imbibition of seeds; a second exponential phase where one attends an acceleration of germination and finally a third phase characterized by a stationary stage indicating a break of germination. Completely, whatever the salt used; the germinative capacity of salt-stressed seeds is reduced and even null compared to the control and this for the eight concentrations used.

Under saline condition operated by the NaCl, the dynamics of the germinative capacity are clearly disturbed by the increase in the salt concentrations. Indeed, the curves of the NaCl treatments are very below curve control.

The seeds expressed their sensitivity starting from the lower concentration by expressing a very reduced percentages of germination with a very slow exponential phase and which lasts much longer to be stabilized at the 21st day of the experiment. It should be noted that no germinative activity appeared starting from the concentration of (200 meq.L^{-1}) of NaCl (Fig. 1).

On the other side, under condition of $CaCl_2$ treatment; evolution of germination with (50 meq.L^{-1}) reached 93,33% at the 18th day. Even if this rate is close to the control, but its acquisition took much more time than that of the control, when a maximum rate of 100% of germination had been expressed on the 3rd day. This progression tends to fall as the concentrations in $CaCl_2$ increase. With the highest levels of $CaCl_2$ (400 and 600 meq.L^{-1}), the seeds seem too affected by these conditions and show a dynamics of null germination and this throughout the experiment (Fig. 1).

Table 2. Variance analysis for the traits investigated for the two *Acacia* species (*A. saligna* and *A. decurrens*) in response to salinity stress using NaCl and $CaCl_2$.

Parameters	Source of Variables	DF	Sum of Squares	Mean Square	F of Fisher
Final Germination Percentage (FGP)	TRT (meq.L^{-1})	8	218987.77	27373.47	299.53***
	SP	1	35842.22	35842.22	392.19***
	NTR	1	1175.55	1175.55	12.86***
	TRT*SP	8	25647.77	3205.97	35.08***
	TRT*NTR	8	4854.44	606.80	6.64***
	SP*NTR	1	5555.55	5555.55	60.79***
	TRT*SP*NTR	8	7634.44	954.30	10.44***
Mean Daily Germination (MDG)	TRT (meq.L^{-1})	8	496.39	62.04	299.46***
	SP	1	81.25	81.25	392.12***
	NTR	1	2.66	2.66	12.85***
	TRT*SP	8	58.14	7.26	35.08***
	TRT*NTR	8	11.00	1.37	6.64***
	SP*NTR	1	12.59	12.59	60.78***
	TRT*SP*NTR	8	17.30	2.16	10.44***
Kinetics of Germination	Generalized Linear Model (GLM) (Repeated Measures Analysis of Variance)				
	Between Subjects Effects				
	TRT (meq.L^{-1})	8	1294202.85	161775.35	602***
	SP	1	336140.00	336140.00	1252.51***
	NTR	1	5120.31	5120.31	19.08***
	TRT*SP*NTR	25	265488.25	10619.53	39.57***
	Within Subjects Effects				
	Time (days)	6	99925.23	16654.20	481.66***
	Time × TRT	48	55070.47	1147.30	33.18***
	Time × SP	6	13318.88	2219.81	64.20***
	Time × NTR	6	8823.01	1470.50	42.53***
	Time × TRT × SP × NTR	150	92159.52	614.39	17.77***

TRT (Treatment); SP (Species); NTR (Nature of the salt).

*, **, ***: significant at 5%, 1% and 0.1% level, respectively; and ns: not significant.

Acacia decurrens Willd

Figure 2 is a synthesis of two graphs illustrating a comparison between the influence of two types of salts (NaCl and $CaCl_2$) acting under various concentrations (low and higher) on the evolution of the rate of germination of *Acacia decurrens* seeds in the course of time.

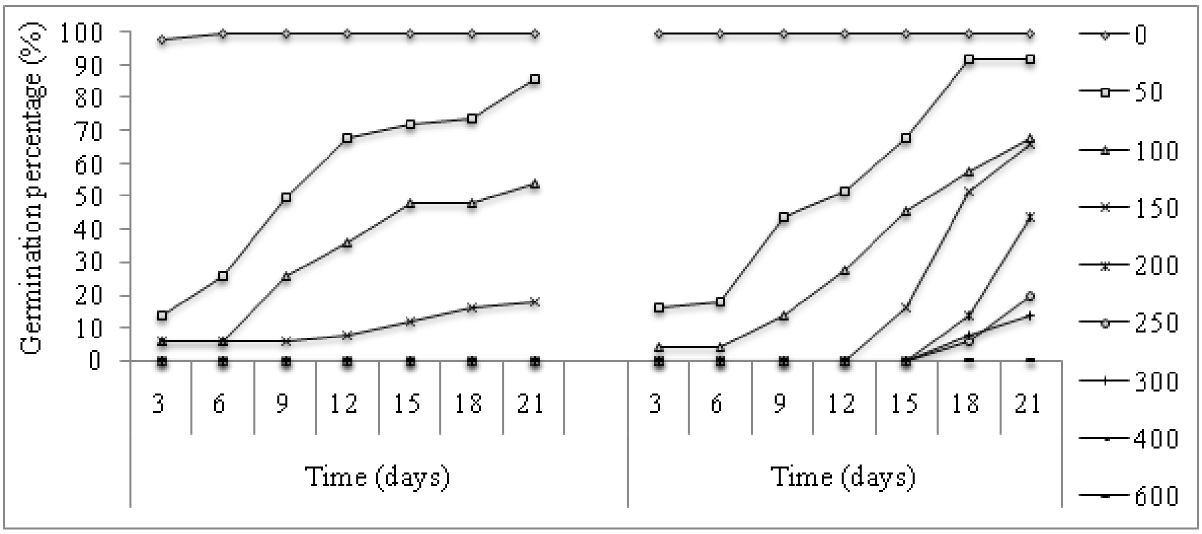

Fig. 1. Effect of the various types of salts (NaCl and $CaCl_2$) with similar concentrations of (0 to 600meq.L^{-1}) on the kinetics of germination of the seeds of *Acacia saligna* during 21 days.

For the seeds having had as solution of treatment only the distilled water (control), the percentage of germination could reach its maximum value at the 3rd day with a rate of 100% (Fig. 2).

The concentrations of (400 and 600 meq.L-1) inhibit any phenomenon of germination at the entirety of salts applied, except for the concentration (400 meq.L-1) of NaCl where germination records a value of 10% which is reached at the 9th day (Fig. 2). The seeds of this species subjected to (300 meq.L-1) of NaCl and $CaCl_2$gave interesting rates of germination, with respective maxima from 43,33 to 26,66% obtained at the 21st and the 18th day of the treatment. With a solution of medium containing a NaCl or a $CaCl_2$, the concentration of (50 meq.L-1) attribute the same rate of germination as the control (100%) (Fig. 2).

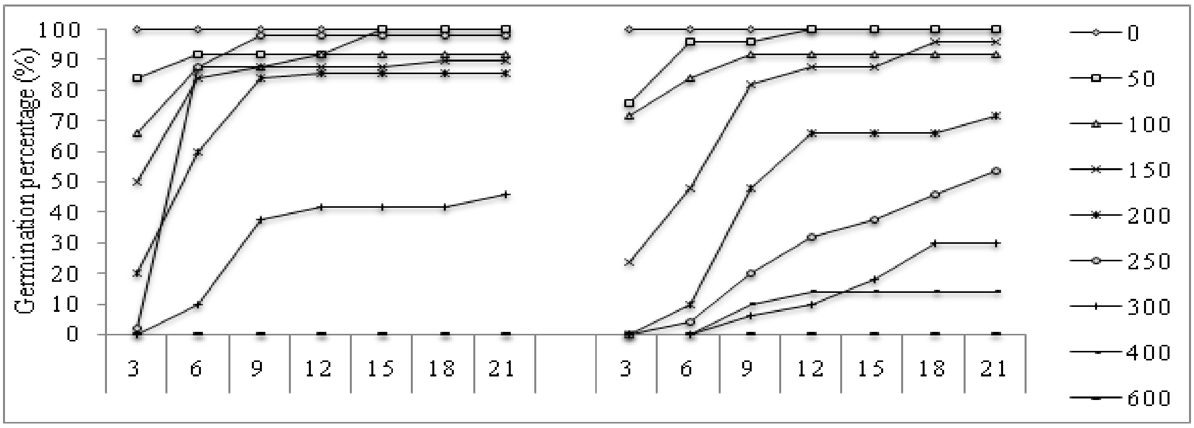

Fig. 2. Effect of the various types of salts (NaCl and $CaCl_2$) with similar concentrations of (0 to 600 meq.L-1) on the kinetics of germination of the seeds of *Acacia decurrens* during 21 days.

The final rate of germination

According to figure 3 and as a whole, the final rate of germination is a parameter which reflects clearly and distinctly that *Acacia decurrens* is more tolerant than *Acacia saligna* at the germination stage.

Indeed, the final percentage of germination of *Acacia saligna* seeds is more reduced than that recorded at *Acacia decurrens* in the same total of the treatments. The seeds of *A. decurrens* records a rate of germination under (400 meq.L-1) of calcium dichloride with 6,66% compared to a rate always null recorded at *A. saligna* under the same concentration (Fig. 3).

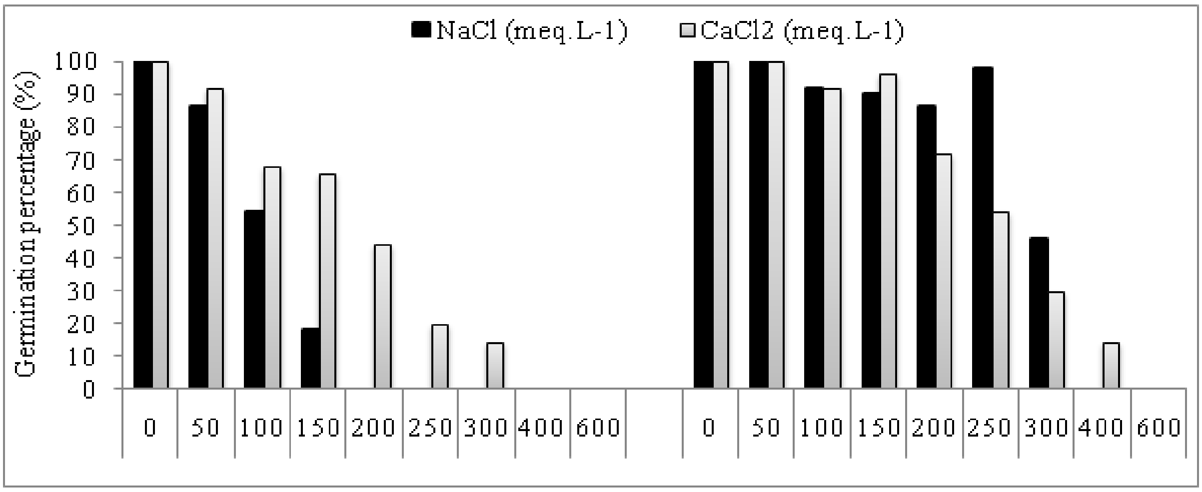

Fig. 3. Variations of the final germination percentage of two different species (*A. saligna* and *A. decurrens*), after 21 days of treatment with various salt (NaCl and $CaCl_2$) concentrations (0 to 600 meq.L-1).

Mean daily germination

Figure 4 reveals the variation in the speed of germination of two species: *A. saligna* and *A. decurrens* in the presence of various concentrations of salts. For the two species, the effect of salinity results in an increase or a reduction of the speed of germination compared to the control.

The NaCl treatment reduces gradually the speed of germination of *Acacia saligna* up to (0,85%/day) under (150 meq.L^{-1})of concentration, a speed which is cancelled with the total absence of germination starting from this concentration. For *A. decurrens*, this speed is much more remarkable even if it also decreases gradually but not also quickly compared to the control and this with the increase in the NaCl levels (Fig. 4).

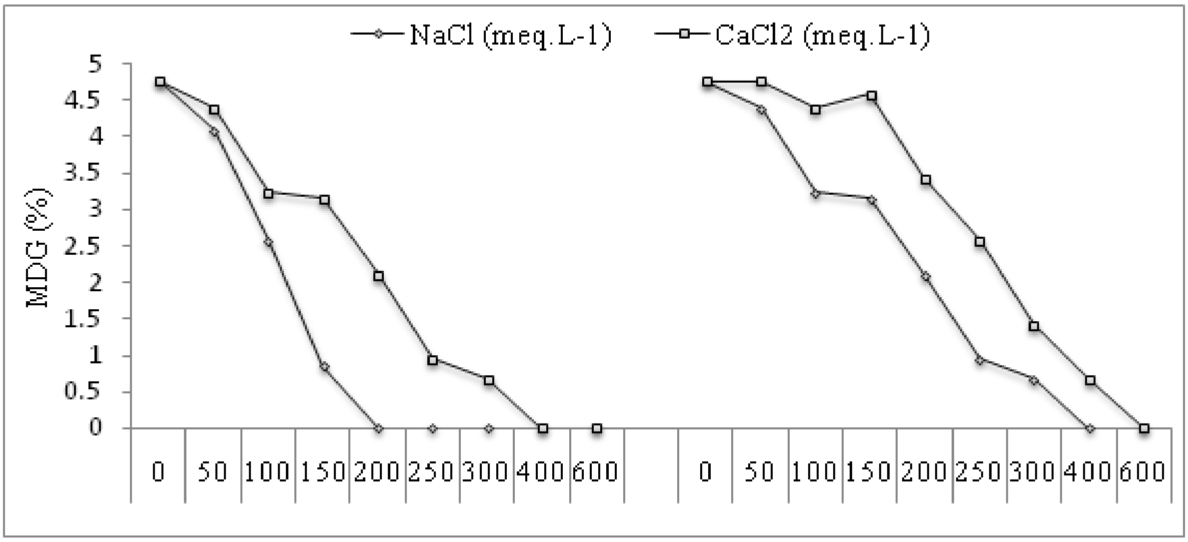

Fig. 4. Variation of the average of the mean daily germination (MDG) of two Acacia species (*A. saligna* and *A. decurrens*) in function of the sodium chloride and calcium dichloride concentrations (0 to 600meq.L^{-1}) during 21 days of treatment.

The same observation was reported on the effect of $CaCl_2$ where the speeds of germination in seeds of the two species decrease with the increasing in the concentrations. However, its effect on the MDG parameter was less marked than the effect of NaCl with a cancelled speed starting from (300 meq.L^{-1}) and this at the two studied species (Fig. 4).

Reversibility of the salt action

Figure 5 represents the response of seeds that germination cancelled at (600 meq.L^{-1}) for the two species under NaCl and $CaCl_2$ treatment, by a rate of definite germination and that after a rinsing with distilled water.

For the two species, the transfer of seeds in distilled water is followed of no resumption of germination under the salt conditions operated by sodium chloride. Indeed, the results of the reversibility of the action of salt posting nothing will confirm the toxic effect of salt in question (Fig. 5).

The germinative recovery of the two species which have, a prolonged immersion (21 days) with (600 meq.L^{-1}) of $CaCl_2$, gave a rate of considerable germination respectively (74% for *A. saligna* and 82% for *A. decurrens*), justifying the osmotic effect of this kind of salt (Fig. 5).

Discussion

The result of the analysis of the variance showed a significant effect of salinity on the stage of germination of the two species. Nevertheless, this effect differs from a kind of salt to the other. Indeed, the sodium chloride seems to be much more harmful than the calcium dichloride. It would appear that the growth response at moderate salinities may be largely the consequence of increased uptake of aqueous solutions that are required to induce cell expansion, since this maintains the presses potential in seedling tissues (Nedjimi and Daoud, 2009).

The majority of the plants are more sensitive to salinity during their phases of germination and seedling (Chiapusio *et al.*, 1997; Hilhorst and Karssen, 2000; Munzuroglu and Geckil, 2002). Among the causes of the inhibition of germination in the presence of salt, the variation of hormonal balance was evoked (Khan, 1971; Kucera *et al.*, 2005). Several authors showed a delay in germination caused by salinity at several species, even at halophytic species (Khan *et al.*, 2000; Koyro, 2006).

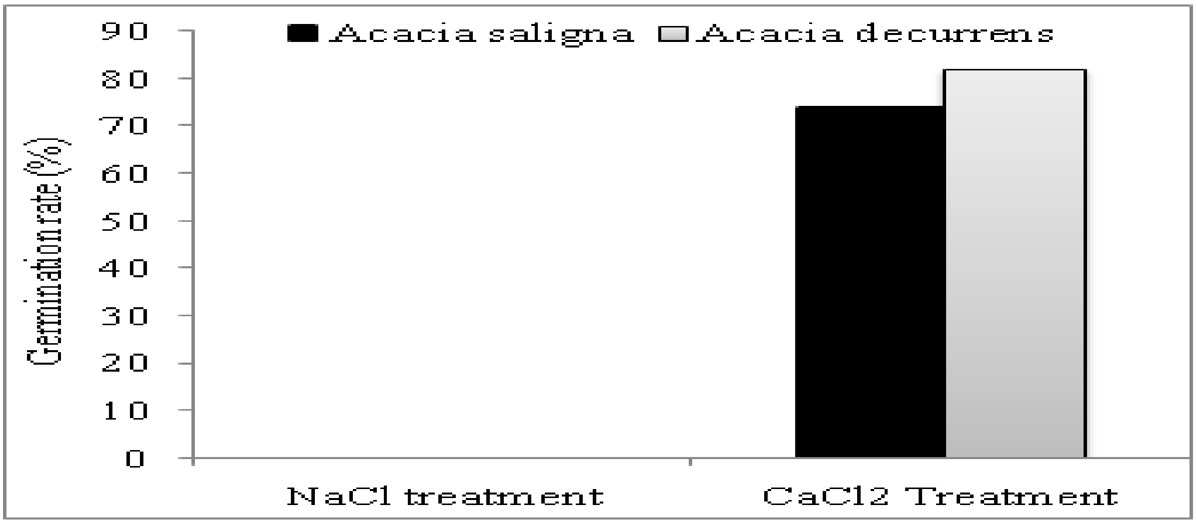

Fig. 5. Salt recovery over a 7-day period for acacia seeds treated by the high concentration of (600 meq.L-1).

Concerning the behavior of the seeds of *A. decurrens* to salinity, our study shows that they are particularly tolerant and able to germinate with an active treatment until (400 meq.L-1) of NaCl. Indeed, the germination of the species of *Acacia* can germinate under salt stress conditions close to those of sea water (35 g.L-1) as it is the case of *A. tortilis* (Jaouadi, 2010). Even if the germination of the seeds of *A. saligna* were very sensitive than those of *A. decurrens*; this germination (cancelled starting from 200 meq.L-1 of NaCl) looks appreciable compared than much forest trees; *Eucalyptus* (Pearce-Pinto et al., 1990; *Argan* (Bani-Aameur and Sipple-Michmerhuizen, 2001); *Pinus* (Sidari*et al.*, 2008).

The delay of germination generated by the increasing concentrations of the medium containing NaCl would result in a difficulty of hydration of seeds in consequence of a high osmotic potential, and can be explained by the time necessary for seeds to install mechanisms enabling an adjustment in their internal osmotic pressure (Mauromicale and Cavallaro, 1995; Bajii *et al.*, 2002; Askri *et al.*, 2007).

Increased salt concentration caused a decrease in final germination percentage. Germination was greatly reduced at the highest level of salt, especially at NaCl levels.

The increase of NaCl at the high concentration affected negatively the seed germination and harms the germination mechanism in *A. saligna seeds*. These results were in agreement with those of Guan *et al.*, (2009), founded that high levels of soil salinity can significantly inhibit seed germination. The presence of NaCl in high concentration inhibits the growth by imposition of an osmotic stress on the cell and by the toxicity of sodium in the cytoplasm (Neumann, 1997). These effects may result from changes in dry matter allocation, ion relationship, water status, physiological processes and biochemical reaction (Bernstein, 1975; Neumann *et al.*, 1994; Cramer and Quarrie, 2002).

The tolerance in the salt is not still associated with a lesser accumulation of sodium (Collins *et al.*, 2008) but rather in the capacity to maintain an ionic balance (Ghanem *et al.*, 2009). Indeed, the recovery of the seeds that did not germinate under salinity conditions using NaCl or $CaCl_2$ at (600 meq.L^{-1}) indicates that the sodium chloride were toxic at this concentration and this contrary of the adverse effects of $CaCl_2$ concentration that explained as a result in lowering osmotic potential of the external solution.

Generally and according to Ungar (1996) and Tavakkoli (2011), increasing salinity causes a decrease in both *acacia* species germination; this might be due to the toxic effects of Na^+ and in the process of germination. It alters the seeds imbibition of water due to lower osmotic potential of germination media, causes toxicity which changes the activity of enzymes of nucleic acid and protein metabolism, interrupts hormonal balance, and reduces the utilization of seed reserve (de Azevedo Neto *et al.*, 2006).

In conditions of high external concentrations of sodium, this one penetrates the root cells through canals and transporters of non-specific ions causing an electric depolarization of the membranes, a reduction of the cellular turgescence and an unsticking of the plasmic membrane of the cell wall, what engenders an activation of the calcic canals Ca^{++} (Sanders *et al.*, 1999) and consequently a modification of the concentration in cytoplasmic Ca^{++} that playing the role of first signal of stress (Monroy et Dhindsa, 1995; Saijo *et al.*, 2000; Zhang *et al.*, 2006).

Stressed seeds with $CaCl_2$ had higher germination rates than seeds treated with NaCl. However, no sign of germination appeared at (600 meq.L^{-1}) with these two types of salt. Jaleel *et al.* (2008) related in their works that the combining of the $CaCl_2$ with the NaCl treatment had varied effects on proteins, total sugars, free amino acids, proline and antioxidant enzyme activity. Supplementing the medium with Ca^{++} alleviates growth inhibition by salt in glycophytic plants (Gul and Khan, 2006). Other researchers mention that the calcium is known to increase halotolerance (Bernstein and Hayward, 1958; Bernstein, 1975; Girija*et al.*, 2002; Tuna *et al.*, 2007). Ions Ca^{++} had an ameliorative effect on the growth of NaCl-stressed plants, by modulating the overall metabolism (Shabala *et al.*, 2006).

According to the analysis of the tolerance for the saline stress, the seeds of *A. decurrens* should not have great difficulties to germinate in arid or semi-arid areas showing an increasing in NaCl/$CaCl_2$ concentrations. This physiological aptitude and functional capacity to be germinated in saline conditions represent a significant characteristic for the rehabilitation and the afforestation of the species. In conclusion, we can say according to this study, that the seeds are able to germinate in a large range of salinity stress.

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