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The Mediterranean Seawater: The impact on the germination and the seedlings emergence in three *Acacia* species

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Key words: *Acacia* species, germination, water content, seawater, salt stress.

Abstract

This study was conducted in order to compare the levels of tolerance to salinity in three *Acacia* species (*Acacia dealbata* Link., *Acacia ehrenbergiana* Hayne and *Acacia tortilis* (Forssk.) Hayne var. *raddiana*) during germination. We were able to determine the optimum conditions for germination to assess suitability for germination of these species under different concentrations of seawater (0: Control, 10, 30, 50 and 100%) in Petri dishes. Preliminary results showed that germination was completely inhibited from 100% of seawater for all studied species. The capacity of germination is evaluated by the cumulative rates of seeds germinated into a period of 2 weeks. In addition, length and water content were measured on seedlings aged about 2 weeks. The effect of seawater on the germination and the growth of seedlings differed significantly ($P < 0.001$) between the species. *Acacia tortilis* var. *raddiana* has proved to be the most tolerant to salinity with a very high germinability (96% at 50% of seawater concentration). *Acacia tortilis* var. *raddiana* and *Acacia ehrenbergiana* showed great tolerance to 50% of seawater in all studied parameters. The results also revealed that the effect was varied depending on the growth stage, salinity levels and species.

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Introduction

The Mediterranean Coast of Algeria is characterized by its scope, by the importance of its sandy beach and its character. Nevertheless, this coastline is undergoing constraints and transformations that could influence the present and future state of its beaches. These effects are due to the evolution of human pressure and the weather-marine dynamics of the coastline.

The natural environment in the Algerian coastline is characterized by a prolonged drought and a very reduced precipitation. In addition, the weakness of the vegetation covers the influence of soil degradation and accelerate erosion (Illou *et al*., 2012). On the other part, the limited water resources and salinity made a major constraint for agricultural and socioeconomic development (Zalidis *et al*., 2002; Hanjra and Qureshi, 2010). Indeed, in arid and semi-arid areas, salinity is one of the most pronounced problems of agricultural irrigation (Connor, 2012). In these regions, there is an urgent need to use saline water for irrigation because of limited water resources (Gaaloul, 2011; Rameshwaran *et al*., 2016). The amount and quality of irrigation water available in many of the arid and semi-arid regions of the world are the main limiting factors to the extension of agriculture, resulting from these situations problems are manifold and require solutions to short and medium term (Dai and Li, 2013). Among them, the most important and urgent is the reforestation designed to restore vegetation in areas threatened by erosions (Luzzadder-Beach *et al*., 2016).

In the choice of plant species to use for this purpose, it must take into account the following criteria: the speed of growth, the ability of the plant concerned to take root deep in the ground, its ability to contribute to the maintenance and/or the restoration of the ecological balance and the ability to serve many uses with economic potential (Schneider *et al.,* 2014). Thus, for decades, tree legumes subject to selection (origins, species), are studied for ecological adaptability, growth and production of wood and litter (Gei and Powers, 2013). The crossing of the stage of germination is decisive and crucial in any development and seedling growth (Zhang *et al*., 2010). However, the process leading to germination is strongly influenced by temperature, water and oxygen contents and the structure of the soil (Liu *et al*., 2011). The germination of seeds is usually the critical step in the establishment of seedlings and so the determination of a successful agricultural production (Kumar, 2013; Bushman *et al*., 2015).

The objective of the present investigation was to determine and compare the effects of various seawater dilutions on seed germination of three leguminous trees (*Acacia dealbata* Link., *Acacia ehrenbergiana* Hayne and *Acacia tortilis* (Forssk.) Hayne var. *raddiana*), and deduce if the seawater could be an alternative for irrigation based on the percentage tolerated by the studied species in order to generate knowledge valuable for reforestation programs under saline conditions.

Materials and methods

Seeds collection

Seeds of three leguminous trees (*Acacia dealbata*, *Acacia ehrenbergiana* and *Acacia tortilis* var. *raddiana*) were tested in the laboratory for confirming their viability before establishing the salinity tolerance test in Petri dishes in November 2015. The seeds collected in 2015 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 2016. The pods of *Acacia ehrenbergiana* and *Acacia tortilis* var. *raddiana* were collected in *Oued Tin Amezzegin* (Tamanrasset, Southern of Algeria) and those of *Acacia dealbata* in a coastal *forest of M'sila* (Oran, North-West of Algeria).

Seed germination and saline treatments

The teguments of seeds of the *Acacia* genus have a typical anatomical structure 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 60 minutes for the seeds to ensure a germination success at 100% in non-saline conditions (Roussel, 1984; 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 of acid. They will be then put to dry on absorbing paper while waiting for their immediate setting in the culture medium.

In order to study the effect of salt on germination, we used different dilutions of the Mediterranean seawater based on the percentage content in a distilled water (10, 30, 50 and 100%). For each species, the seeds are put to germinate in Petri dishes of 10 centimeters in diameter papered of two layers of paper Whatman N°1, this one is moistened with 15 ml of distilled water for the control (0%: distilled water) and with 15 ml of one of the seawater solutions.

The norm is to take care that the seeds preserve a certain moisture throughout the period of the experiment. The Petri dishes are then put in the darkness at an ambient temperature (25 ± 2°C). About two-thirds of the volume of the water or solution in each Petri dish (containing 20 seeds) were replaced daily during the fifteen days of the experiment. Each experiment was replicated five times .

Studied parameters

The Final rate of germination (FRG): 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 for the period of germination which was extended out over 15 days.

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

Emergence test: The data for the seedling length (cm), fresh (FW) and dry weight (DW) of seedling (mg) and were measured after fifteen days of seawater treatments. Dry weights were measured after drying at 80°C for 48 hours in an oven (Monneuveu 1980). The water content of seedlings was calculated using the following formula:

$$WC\ (\%) = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{DW}} x\ 100$$

The average of a water content of the plant is a very used method at present, to estimate the hydric state of the plant in the condition of hydric stress.

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 mean values of species, after seawater 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).

Results

The Final rate of germination

The effect of salinity on germination is presented in Fig. 1. The final germination percentage differed significantly between species after irrigation with seawater of different salinity levels. Germination tests showed that the seeds of the three acacia genius germinated at all levels of seawater concentration except for the treatment 100 % of seawater where no germinal activity was observed.

High significant differences ($p < 0.001$) were obtained between the treatments, the species and their interaction (Table 2). The results on one hand show that the low concentration of seawater has no effect on the seed germination for all three tested species (Table 1).

Table 1. Effect of different concentrations of Mediterranean seawater on final germination percentage (FGP), mean daily germination (MDG), seedlings length (SL), Seedlings fresh weight (FW), Seedlings dry weight (DW) and Seedlings water content (WC) (after 15 days of treatment in petri dishes). Values are means of five replicates ± SD (Different letters in the same column indicate significant difference at p ≤ 0.05, as assessed by Duncan's Multiple Range Tests).

	Seawater (%)	FGP (%)	MDG (%)	SL (cm)	FW (mg)	DW (mg)	WC (%)
	0	99a	6.6a	12.88a	162.78a	29.68a	81.8a
	10	99a	6.6a	12.72a	148.72b	23.8b	84.15a
AD	30	97a	6.47a	7.66b	112.76c	24.28ab	78.53b
	50	42b	2.8b	3.22c	81.28d	27.92ab	65.68c
	100	0c	0c	0d	0e	0c	0d
	0	100a	6.67a	17.82a	268.82b	28.31d	89.47a
	10	99a	6.60a	18.06a	273.26a	59.24b	78.34b
AE	30	99a	6.6a	14.16b	204.04c	49.09c	75.96c
	50	78b	5.2b	5.2c	163.94d	78.39a	52.21d
	100	0c	0c	0d	0e	0e	0e
	0	99a	6.6a	16.74a	1163.52	169.48a	85.48a
	10	99a	6.6a	12.9b	914.55b	177.4a	80.69b
AT	30	98a	6.53a	10.62c	795.48c	172.16a	78.4c
	50	96a	6.4a	5.62d	595.1d	156.8a	73.68d
	100	0b	0b	0e	0e	0b	0e

A. dealbata (AD), A. ehrenbergiana (AE) and *A. tortilis (AT)*

Effectively, the final rate of germination of the three studied species gave percentages very close to 100% and this to the first three seawater treatments (0, 10, and 30 %). However, at 50 % of seawater, the effect of species was exposed with different rates: 42% for *A. dealbata*; 78 % for *A. ehrenbergiana* and 96% for *A. tortilis var. raddiana* (Fig. 1).

Mean Daily Germination (MDG)

The mean daily germination is a parameter which reflects clearly the temporal behavior of the seeds and this in various concentrations of the seawater (0, 10, 30, 50, 100%) and after 15 days of treatment.

The fig. 2 reveals that the three concentrations of seawater (0, 10 and 30%) have similar values. The percentage of the MDG registers very different values involving the factor species with a highly significant effect (Table 2). Indeed, the values of the MDG decreases from the most higher obtained from the seeds of *A. tortillis* (6.4%) and *A. ehrenbergiana* (5.2%); and the lower obtained from *A. dealbata* seeds (2.8%) which remains so acceptable in a solution of seawater diluted at half (Table 1).

Seedlings lenght

The seedlings length of the investigated species are presented in Fig. 3. The obtained results show that the salt stress led by the various concentrations of the seawater (0, 10, 30, 50, 100%) influence the growth in length of the three species seedlings. Also, there is a very highly significant difference between the averages of the total length of seedlings (Table 2). The interaction of (species × salinity) for this morphological parameter was significant after ANOVA using Duncan's test at ($P < 0.001$) (Table 1, 2). Results showed that increasing salinity decreased seedlings length. Under distilled water irrigation (0% of seawater), all species showed higher lengths. However, significant differences were found in seedlings length between species irrigated with the different seawater dilutions. It is noticeable that *A. ehrenbergiana* seedlings showed a high degree of response (18.06 cm) to seawater much more than the other two species.

The water content

Fig. 4 indicates a comparison of the water content calculated with the fresh and dry weight of seedlings subjected to various concentrations of the seawater including plants controls irrigated only with the distilled water. Table 2 indicates a very highly significant ($p < 0.001$) effect concerning the interaction (Species × salinity). Indeed, even if Fig. 5 shows histograms of almost similar lengths of the three species at the level of the concentrations 10 and 30% of seawater; the concentration of 50% of seawater makes bring out the effect species and only the seedlings of *A. tortillis* were able to preserve their regime in water content which remains almost stable with 73.68% compared to a value of 85.48% obtained in control seedlings of the same species.

Table 2. Variance analysis for the traits investigated for the three *Acacia* species [(*A. dealbata (AD), A. ehrenbergiana (AE)* and *A. tortilis (AT)]* in response to salinity stress using different concentrations of Mediterranean seawater.

Parameters	Source of Variables	DF	Sum of Squares	Mean Square	F of Fisher
Final percentage of germination (FGP)	TRT (% of seawater)	4	109833.33	27458.33	2941.96***
	SP	2	1600.66	800.33	85.75***
	TRT*SP	8	5972.66	746.58	79.99***
Mean daily germination (MDG)	TRT (% of seawater)	4	488.148	122.03	2941.96***
	SP	2	7.11	3.55	85.75***
	TRT*SP	8	26.54	3.31	79.99***
Seedlings length (SL)	TRT (% of seawater)	4	2702.03	675.50	3789.31***
	SP	2	175.96	87.98	493.56***
	TRT*SP	8	105.76	13.22	74.17***
Fresh weight (FW)	TRT (% of seawater)	4	2504744.89	626186.22	3369.82***
	SP	2	5163346.69	2581673.34	13893.3***
	TRT*SP	8	1682412.25	210301.53	1131.74***
Dry weight (DW)	TRT (% of seawater)	4	84102.59	21025.64	113.15***
	SP	2	183131.31	91565.65	492.76***
	TRT*SP	8	52241.11	6530.13	35.14***
Water content (WC)	TRT (% of seawater)	4	75157.54	18789.38	5265.91***
	SP	2	254.11	127.05	35.61***
	TRT*SP	8	1176.83	147.10	41.23***

TRT (Treatment); SP (Species)

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

The values of the water content in the vegetative seedlings of the three tested *Acacia* species were found to be generally depressed with the intensification of salinization level. Moreover, results presented in table 1 shows that the fresh and the dry vegetative weight decreased in all species with salinity comparing with the control.

Discussion

The salinity was recognized as a problem since thousands of years, particularly in the arid and semi-arid regions where there is not enough rain to wash salts beyond the roots zone (Kempton and Atkins, 2000; Yadav *et al.*, 2011; Ingram, 2016). The mechanisms of adaptation to the salinity of the Acacia species were widely studied, but most of the studies concerned some species. Until now, comparative studies of the various species of *Acacia* towards this abiotic stress are rare (Niknam and McComb, 2000; Levinsh, 2006; Abbas *et al.*, 2016).

The constraint to realize this study comes from great difficulties to make these species germinate. So, the present work has for objective to compare the reaction of three species of the *Acacia* (*Acacia dealbata*, *Acacia ehrenbergiana* and *Acacia tortilis* var. *raddiana*) by following their kinetics of germination and by considering the emergence of seedlings under salt conditions from the lowest concentrations to the most highest, using the seawater as a limiting factor leading the salt stress.

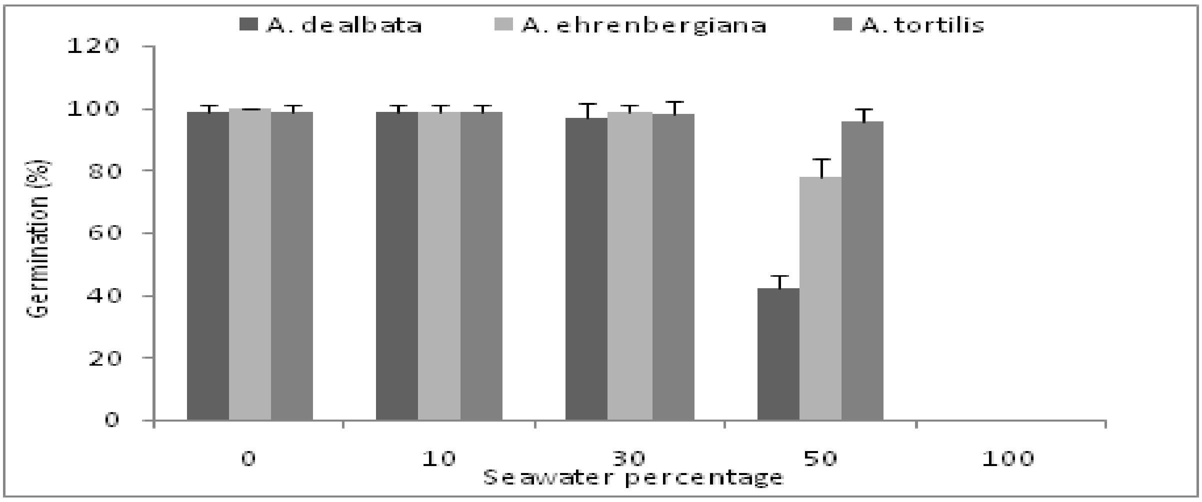

Fig. 1. Final germination percentage of Acacia species under irrigation with different salinity concentrations of seawater after 15 days of treatment.

Estrelles *et al.* (2015) declare that the salt stress is the essential factor which limits the germination of the species in the arid and semi-arid regions. Moreover, enormous progress is realized to estimate their potentialities (Hu and Schmidhalter, 2005; Estrelles *et al.,* 2015) and the use of the water highly salted as the seawater for the irrigation of various species (He and Cramer, 1992; Flowers and Colmer, 2015).

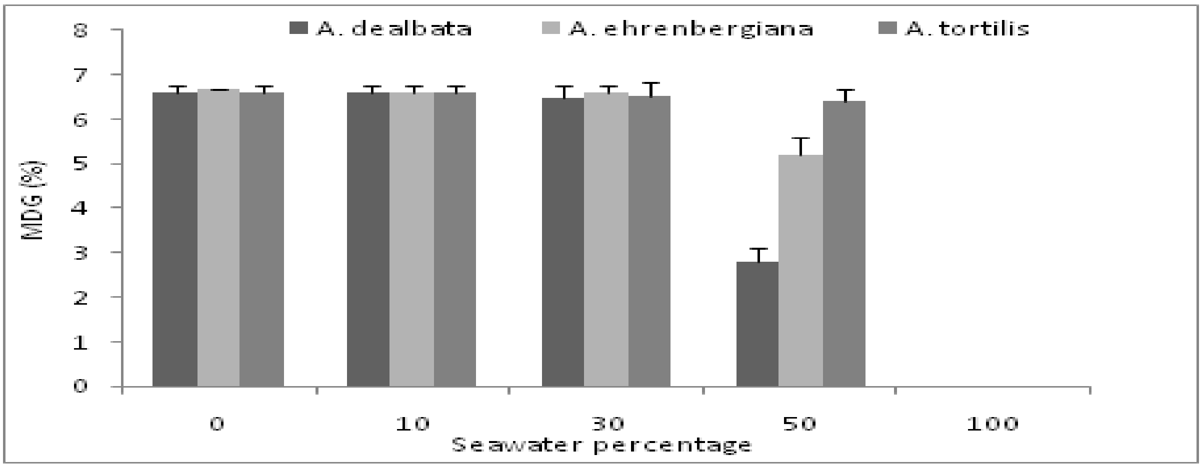

Fig. 2. Mean daily of germination (MDG) of Acacia species under irrigation with different salinity concentrations of seawater during 15 days of treatment.

Considering the importance of the germinal phase in the establishment of plants and the effect of the abiotic factors on germination behavior of these three species, we analyzed the germinal characteristics: the germination of these species was followed by making vary various concentrations of the solution of seawater.

The results indicated that the increase of seawater salinity affected seed germination of the three

Acacia species especially at the highest concentration (100%) where no germination was observed. The effect of the external salinity on the seed germination may be partially osmotic or ion toxicity which can alter physiological processes such as enzyme activation (Hsiao *et al.*, 1976; Hajer *et al.*, 2006; Nawaz *et al.*, 2013).

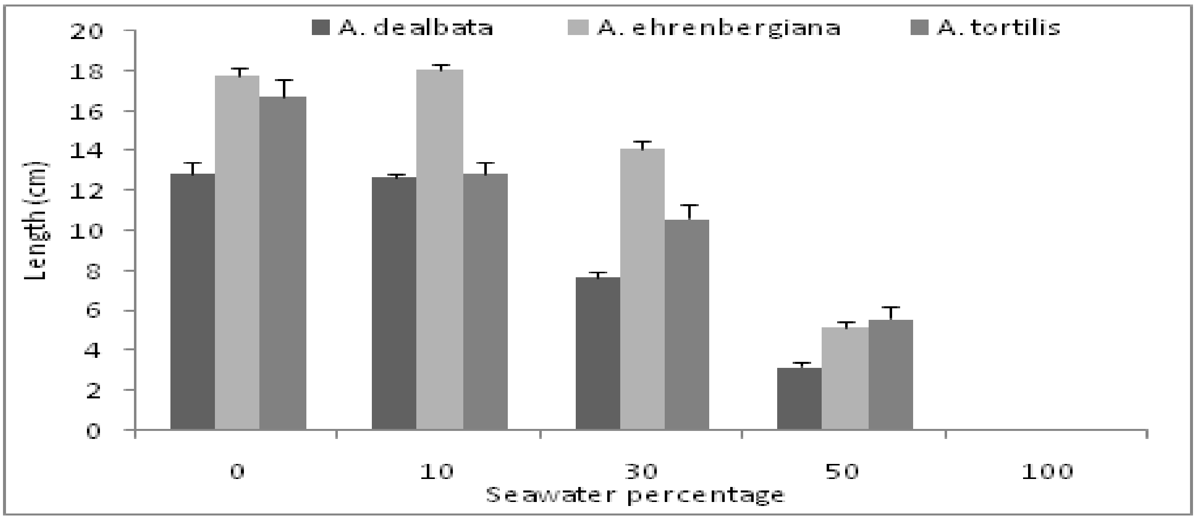

Fig. 3. Seedlings length of Acacia species under irrigation with different salinity concentrations of seawater after 15 days of treatment.

In our experiments, all *Acacia* seeds germinated best under nonsaline conditions (0% of seawater) and saline conditions (10 and 30% of seawater). However, only the seeds of *A. tortillis* and *A. ehrenbergiana* kept the germinal power very interesting in 50% of seawater with a rate of respective final germination of 96% and 78 %; against a rate which tended to decrease for the seeds of *A. dealbata* with 42% of germination. The germination speed and percentage progressively decreased with intensifying salinity. The concentration in pure solution of seawater inhibited completely the germination of the three studied species and this during all the time of the experiment which spread out until 15 days.

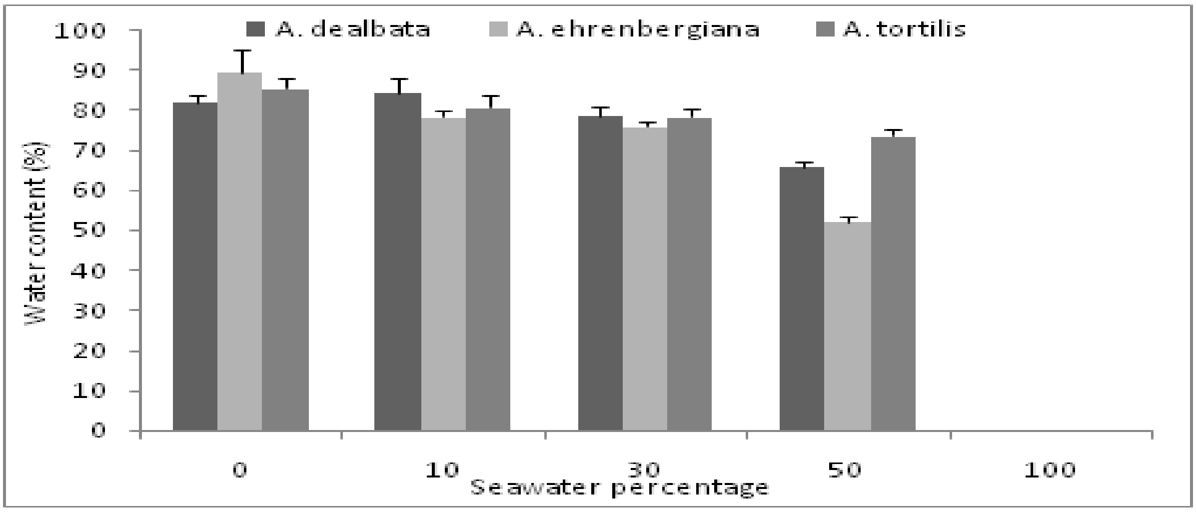

Fig. 4. Water content of the Acacia species seedlings under irrigation with different salinity concentrations of seawater after 15 days of treatment.

The salinity affects the germination by two manners: it can have enough salts in the decrease of the osmotic potential that delay or prevent the uptake of the sufficient water for the mobilization of the reserves necessary for the germination (Sidari, 2008; Mohammed *et al*., 2011). Constituents or ions of salt can be toxic in the embryo (Serrano, 1996). Although the effects of the raised content in salt on metabolic processes, the salinity could reduce the degradation of proteins (Türkan and Demiral, 2009) and led changes of the activities of many enzymes implied in the process of the reserves hydrolysis (Bewley and Black, 2012).

According to our results, the seeds of these three species germinate in the absence and/or in the presence of a low salinity, they are tolerant in the presence of salts; but this tolerance differs from a species to the other one because the salt plays a determining role grafting for the seeds germination. The works of Khan and Ungar (1991) assert that the germination of seeds in the salt environment is variable and specific in the species. Nielsen *et al.* (2003) expose that the threshold of the salinity leads a significant reduction of the germination to various species of halophytes. Indeed, the increase of the concentration of the seawater affected negatively the rates of germination, especially at the highest level (Sousa *et al*., 2007).

The seeds of the three studied species were not able to germinate at 100% seawater, an incapacity due to the osmotic or toxic effect of these salts which enters in the chemical composition of the seawater. Effectively, the salt would affect the endogenous contents in growth hormones (kinetin and gibberellic acid) implied in the process of germination (Khan and Rizvi, 1994). During the germination, the emergence of the radicle would be controlled by the osmolarity of the medium, while the later growth of the seedlings would be limited by the mobilization and the transport of the reserves towards the embryonic axis (Gomes and Sodek, 1988).

On the other hand, the respective reports of (the seedlings length, the fresh and dry weight as well as the water content) at the end of the experience vary according to the level of salt stress and the species. According to Rejili *et al.* (2010), the osmotic effects are translated by the inability of seeds to absorb sufficient amounts in water to return them to their critical threshold of moisture, necessary for the release of the process of germination; however, the toxic effects are bound to a cellular accumulation of salts which cause disturbances of enzymes involved in the physiology of seed germination, prevent the levying of dormancy of embryos and lead to a decrease in the capacity of germination.

The results indicated that all the studied parameter are decreased in saline condition due to the exposure to seawater. A similar result was obtained by (Boyko and Boyko, 1964; Ahmad and Ismail, 1993; Ashraf *et al*., 2008) studying others *Acacia* species. In our experience, the optimal growth of seedlings is near 30% of seawater for the three species. That response of growth to the moderate salinity can be largely the consequence of a higher absorption of dissolved nutriments which are essential to lead the expansion of cells, in order to maintain the hydric potential in plant tissues (Munns, 2002). This variation is characterized by a strong absorption capacity and a preferential accumulation of the chlorine and the sodium in the seedlings (Wakeel, 2013).

The water content in seedlings is a good indicator of the hydric state, it decreases slightly at plants put under salinity stress (Verslues *et al*., 2006). It is particularly noticeable when the vegetal is exposed to 50% of seawater, what seems to be a behavior of tolerance in the salt stress. The absorption of water is maintained at a level being enough for avoiding the dehydration of tissues and being so able to dilute salts introduced into cells (Boyer *et al*., 2008). These observations are confirmed by the works of Guja *et al.* (2010). Indeed, the salt stress, led changes at the level of the hydric status of the plant (Hasegawa *et al*., 2000), reduces the contents to water and decreases the transpiration (Jogaiah *et al*., 2014) and the hydric absorption by roots (Alharby *et al*., 2014). The analysis of the relative content in water allows to describe in a global way the hydric status of the plant and to estimate the capacity to realize the best osmoregulation and to maintain the cellular turgescence (Kholodova *et al*., 2002). Chaves *et al*. (2009) conclude that the salt stress is the result of a hydric deficit in the plant in the form of physiological drought. This osmotic stress is essentially translated by the toxic accumulation of the ions in cells and/or a nutritional disproportion due to an excess of certain ions (Aloui *et al.,* 2014).

Conclusion

The use of the seawater in the agroforestry offers an alternative of irrigation in zones suffering from a deficiency of pluviometry or from the shortage of the water of irrigation especially that these species are projected for the revegetation of the coast, a zone very close to the vast source of the seawater. In this context, we showed that irrigation with saline water did not affect germination of the three acacia species in a concentration of seawater diluted by half. However, further experiments are needed to evaluate the effect of seawater irrigation in situ with à long-term evaluation. For that reason, it is recommended to use the two leguminous tree of *Acacia* genus (*Acacia tortilis* var. *raddiana* and *Acacia ehrenbergiana*) for afforestation-reforestation programs using Mediterranean Seawater mixed fresh water or rain water in the Mediterranean coast area.

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