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RESEARCH ARTICLE

EFFECT OF SALINITY AND TEMPERATURE ON THE SEED GERMINATION OF *BITUMINARIA BITUMINOSA* VAR. *BITUMINOSA*

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Glossary of Abbreviations
DGS: Daily germination speed
MGT: Mean germination time
TG: Total germination

ABSTRACT

In order to rehabilitate degraded sylvo-pastoral land, we envisioned install *Bituminaria bituminosa* var. *bituminosa*, a pasture species with agronomic characters ideally suited to Mediterranean climates. The present research aims to evaluate the effect of different incubation temperatures (4, 17, 20, 28, 40, 45 °C) and different concentrations of NaCl (0, 1.5, 3, 4.5, 6 g L^{-1}) on seed germination. Total germination (TG), Mean germination time (MGT) and Daily Germination Speed (DGS), were calculated during 20 days. The results obtained from this study revelated that *Bituminaria bituminosa* var. *bituminosa* showed a high germinative potential. Is able to germinate under a wide range of temperature (4 - 40 °C) and tolerate saline stress of above 4.5 g L^{-1}.our results show that *B. bituminosa* var. *bituminosa* has promising characteristics that qualified, for rehabilitate degraded pastoral areas in Morocco.

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INTRODUCTION

Mediterranean type vegetation and ecosystems have undergone intense processes of degradation for decades, centuries, or millennia under heavy and prolonged pressure from human and livestock populations (Le Houerou, 2000).In Morocco, the arid and semi-arid areas cover 87% of arable land (27% of the territory) and are subject to degradation (Benbrahim *et al*., 2004). In our kingdom, there is a gradual degradation of the pastoral flora in all-natural ecosystems of the arid climate, hence the need for the reintroduction of pastoral plants, particularly perennial legumes, for rehabilitating the degraded herbaceous stratum.

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It is in this context that we chose *Bituminaria bituminosa* (L.) *Stirt*., as biological material for the rehabilitation of degraded sylvo-pastoral land in Morocco. *B. bituminosa*is a Mediterranean legume, commonly known as the bituminous clover, is a perennial, self-pollinating plant *(*Juan *et al*., 2004*)*, that stays green all the year round, ensuring the transition between summer and autumn forage and providing good quality of forage (Gülümser *et al*., 2010). The species of *B. bituminosa*, presents a large diversity with 3 botanical varieties, *albomarginata*, *crassiuscula* and *bituminosa* (Méndez *et al*., 1990). The third var. *bituminosa* has a wide adaptation across the Canary Islands and is the only one present in the Mediterranean basin (Méndez *et al*., 1990).The first critical phase of rehabilitation is that relating to the germination of species which will be reintroduced *(*Jordan, 1989*)*. The seedling stage is the most vulnerable in the life cycle of the plant and it is the germination that determines the time and the place suitable for growth of the seedling

However, a good course of processes leading to germination depends on the environment close to the seed that is strongly influenced by temperature, salinity, water and oxygen content, and soil structure (Benidire *et al*., 2015; Khan, 1994). In order to restore the ecological balance of degraded environments in Morocco, an understanding of seed biology and germination requirements of *B. bituminosa*, is needed to determine optimum sowing conditions and to identify any constraints to its establishment. In this study, we investigated the effects of salinity and temperature on seed germination of *B. bituminosa*, to proposed this plant like a biological material for the rehabilitation of degraded sylvo-pastoral land in Morocco.

MATERIALS AND METHODS

Germination experiment was conducted in the laboratory of Soil and Environment Microbiology, Faculty of Sciences, Moulay Ismail University, Meknes (Morocco). Seeds of *Bituminaria bituminosa* var. *bituminosa*, were obtained from the university's test fields. Seeds were separated from inflorescence, cleaned, and dry stored at room temperature. After surface sterilization with 0.1% sodium hypochlorite for 1 min, then rinsed 10 times with distilled water, germination was carried out by placing 20 seeds on Whatman filter paper in 10 cm Petri dishes containing 15 mL of distilled water or solutions of various salinity levels (0, 1.5, 3, 4.5, 6g L^{-1} of NaCl), and were transferred to germinator at 28 °C. To study the influence of the temperature on the seeds germination, the Petri dishes were transferred to 6 germinators at different temperature (4, 17, 20, 28, 40 and 45 °C).

The experiment was arranged in a completely randomized design with three replications and 20 seeds per replicate. Seed germination was recorded daily up to day 20 after the start of the experiment. Seeds with at least 2 mm radicle length were considered germinated. Parameters measured in this experiment were: Total germination (TG) measured in the twentieth day using the formula TG (%) = (total number of germinated seeds/ total seed) x 100.Mean germination time (MGT) calculated according the formula MGT= $\sum$ (ni/di). With ni: number of germinated seeds and di: day of counting. Daily Germination Speed (DGS) was calculated according to the formula: DSG= TG / D. Where TG is total germination percentage and D is number of days from start to end of the experiment. All the data were subjected to an analysis of variance (ANOVA), using IBM SPSS 20.0 software and the difference between means were compared by honesty of Tukey (HSD) tests ($p< 0.05$).

RESULTS

Bituminaria bituminosa seeds are subjected to different temperatures (4, 17, 20, 28, 40 and 45 °C) for 20 days. The results showed (Table 1) a significant effect of temperature on the final and daily germination rate of *Bituminaria bituminosa*. The optimum temperature for seed germination of *B. bituminosa* is 28 °C (Table 1). Radicle growth can begin at 4 °C with very low TG; however, at very high temperatures (45 °C) no germination was noted. The daily germination speed in relation to the different temperatures tested (Figure 1), revealed that for the temperatures of 20 °C and 28 °C the maximum germination is reached after 24 hours of incubation. At a temperature of 17 °C and 40 °C germination did not begin until after the 2nd day. While it is necessary to wait 4 days to

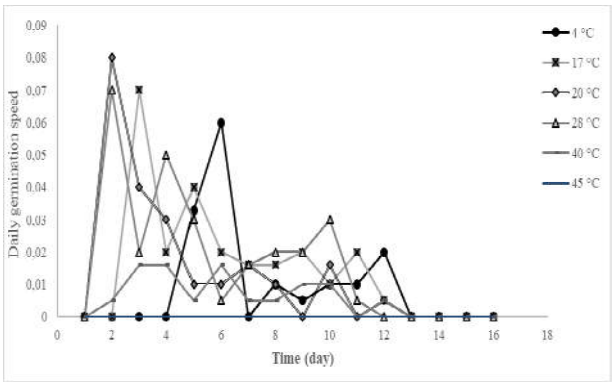

Figure 1. Effect of temperature on the daily germination speed of *B. bituminosa* seeds

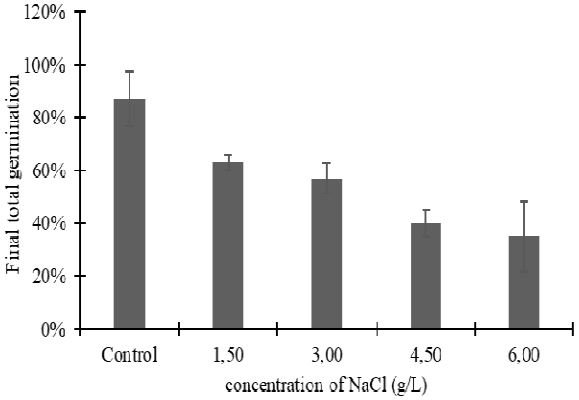

Figure 2. Effect of salinity on the final total germination of *B. bituminosa* seeds

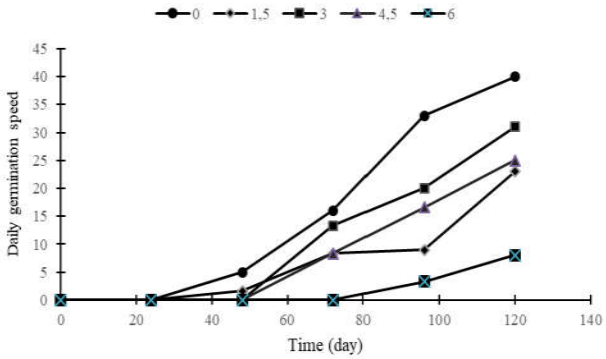

Figure 3. Effect of salinity on the Daily germination speed of *B. bituminosa*

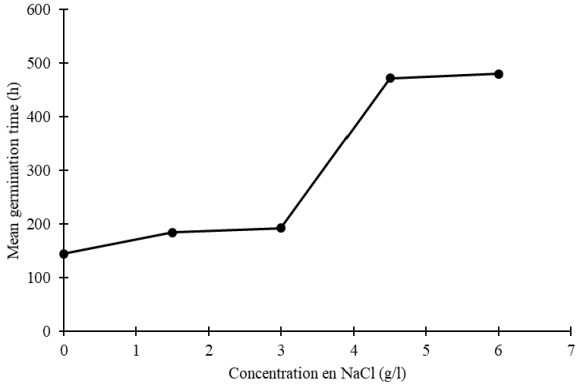

Figure 4. Effect of salinity on the Mean germination time of *B.*

Table 1. Effect of temperature on the total germination and daily germination speed

Temperature (%)	TG (%)	DSG (Day)
4	48^{a}	3^{a}
18	77^{b}	5^{b}
20	70^{c}	5^{c}
28	90^{d}	6^{d}
40	30^{e}	2^{e}
45	0^{e}	0^{f}

Values in the same column with different letters are significantly different (p <0.05). TG: Total germination; DGS: Daily Germination Speed.

Table 2. Effect the salinity in Daily germination speed of *B. bituminosa*

	Concentration of NaCl (gL^{-1})				
	Control	$1,5gL^{-1}$	$3gL^{-1}$	$4,5gL^{-1}$	$6gL^{-1}$
DGS (Day)	$4,47±0,29^{a}$	$3,80±0,23^{a}$	$3,55±0,43^{a,b}$	$2,69±0,45^{b,c}$	$1,88±0,83^{c}$

Values in the same line with different letters are significantly different (p <0.05). DGS: Daily Germination Speed

Finally, from the 13th day the germination is inhibited for all temperatures. The results of daily germination speed under salt stress were showed in Figure 1. The seed germination study of *B. bituminosa* showed no significant ($p < 0.05$) difference between the control and the seeds subjected to the different concentrations of NaCl. Also, results of effect of salinity on the final total germination of *B. bituminosa* seeds were showed in figure 2. We note that final total germination of *B. bituminosa* seeds, is decreased when salinity increases. Moreover, the evaluation of the germination power as a function of time revealed a negative effect of salinity on the latency phase, which varies considerably according to the treatments. (Figure 3).It should be noted that germination is completely inhibited at concentrations greater than $6gL^{-1}$of NaCl, hence the choice of 1.5-3-4.5 and 6 gL^{-1}NaCl in this study. Indeed, for all treatments germinations take an evolution that can be divided into two phases. A latency phase that is 24h to 48h for seeds soaked with 0 to 3 $gL^{-1}$of NaCl. This phase is even longer for 4.5 and 6 gL^{-1}treatments. The second phase corresponds to an exponential phase, between 2 to 3 days or between 2 and 4 days depending on the treatments, during which the percentages of germination increase.

The results presented in the table 2 show the average daily germination speed of *B. bituminosa* seeds subjected to different concentrations of NaCl. Daily germination decreases significantly with increasing NaCl concentration. For control and treatment of $1.5gL^{-1}$germination is significantly greater than other treatments. The seeds under a concentration of 6 gL^{-1}in NaCl daily germination have the lowest rate (1.88%). The mean germination time of the seeds exist at different saline concentration was represented in figure 4. This time varies between 6 days and 8 days for seeds subjected to a concentration ranging from 0 to 3 $gl^{-1}$of NaCl. However, it increases with increasing NaCl concentration.

DISCUSSION

In the context of climate change, the climatic conditions are very variable, especially the temperatures that have become unpredictable from one year to the next (Fabienne, 2011). Indeed, Hawker and Jenner (Hawker, 1993) concluded that high temperatures inhibit seed germination while limiting the availability of energy and hydrolysates.

This explains the inhibition of seed germination of *B. bituminosa* seeds at 45 °C. Both 4 and 18 °C were unfavorable to germination. Although a 28 °C is adequate for *B. bituminosa*seeds germination. The optimal germination temperature is between 18 and 30 °C. This behavior can then be a mechanism of adaptation to the ecological conditions of the arid environment, characterized by relatively high temperatures and essentially drought. These results are in concordance with Reyes *et al*. (2009), which showed that *B.bituminosa* germination rate is between 15-30°C. Changes in germination behavior are more important in species that usually have intermediate or high natural germination levels (Reyes, 2009).

In addition, the results of the present study confirm the effects noted by previous studies *(*Okçu, 2005; Hajlaoui, 2007*)* exerted by salinity on the germination process in other species of legumes. Under saline stress, the NaCl concentration significantly decreased the germination rate of *B. bituminosa* seeds. It is even weaker as the salt concentration increases. In 20 days of incubation, the germination percentage is 65% with 1.5 $gL^{-1}$of NaCl, this rate decreases to 35%, with 6 $gL^{-1}$of NaCl. This decrease in growth rate has been reported by other studies in several legume species *(*Bouda, 2010; Jaouadi *et al*., 2010*)*. Prado *et al*. (2000) were revealed that the process of osmotic dormancy developed under the conditions of salt stress. This stress also resulted in a two-day germination delay at 1.5 gL^{-1} of NaCl, and a three-day delay at 6 gL^{-1} of NaCl. Similar results were reported by (Bouda, 2010; Jaouadi *et al*., 2010; Radhouane , 2008; Bojović *et al*., 2010; Orlovsky *et al*., 2016). Also, Rejili *et al*. (2007) were revealed that the halophyte seeds reduce the total number of seeds germinated and delay the germination process in the presence of salt.

Conclusion

In the present study *Bituminaria bituminosa* var. *bituminosa* seeds originating from Morocco, showed variable reactions to the treatment used. The optimal seed germination temperature of *B. bituminosa* is 28 °C, although a high temperature (≥ 40 °C) significantly reduces germination. Thus, the concentration of 6 gL^{-1} NaCl inhibited the seeds germination. However, a possible introduction of *B. bituminosa* to restore degraded lands must consider the abiotic factors including salinity and temperature, which may later be a limiting factor to restore this plant.

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Conflicts of Interest: The authors declare no conflict of interest.

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