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EFFECT OF SALINITY AND DROUGHT ON THE GERMINATION OF *Lygeum spartum* L. IN THE REGION OF SAÏDA (WESTERN ALGERIAN STEPPE)

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Abstract

Zouidi M., Hachem K., Terras I., Allam A., Hadjout S., Mazari F., Aouadj S.A., Djebbouri M.: Effect of salinity and drought on the germination of *Lygeum spartum* L. in the region of Saïda (Western Algerian steppe). Ekológia (Bratislava), Vol. 42, No. 2, p. 159–164, 2023.

Albardine (*Lygeum spartum* L.) is one of the major native grass species of the semi-arid and arid regions of the Mediterranean basin. In Algeria, it is much more widespread on the high plateaus of southern Oranais. This species occupies an important place in the steppe region because it has many ecological, economic, and fodder interests. The present work aims to study the tolerance of *L. spartum* seeds to water and salt stress, two abiotic factors that affect the physiology of the plant during the germinal stage. The methodology adopted consists of using increasing concentrations under a controlled temperature (15°C) for 21 days of germination. The germination responses of the seeds to different degrees of salt stress induced by NaCl (2, 4, 6, 8, 10 g/l) and water stress induced by polyethylene glycol (PEG;−2, -4, -6, -8, −10 bar), showed that the salt and water stresses retarded the germination rate of *L. spartum* L. seeds and also decreased their percentage during the time of the experiment. However, seeds soaked in distilled water (control) recorded a maximum germination rate of 80%. The results of this study show that *L. spartum* seeds are moderately salt and drought tolerant with a depressive effect on germination rate at a salt concentration of 10g/l and an osmotic pressure of −10 bar.

Key words: *Lygeum spartum* L., germination, salt stress, water stress, steppe, Algeria.

Introduction

The Algerian steppe has become a sign of ecological and climatic imbalance recently. The intense degradation of this fragile environment is due to silting, wind erosion, overgrazing, land clearing, drought, and salinization. These factors lead to desertification, which requires a better understanding of how to combat this scourge and adapt appropriate management strategies (Haddouche et al., 2011; Nedjimi, Brahim, 2012). During the last decades, the steppe rangelands of the Algerian high plains have been marked by intense degradation affecting the vegetation cover and biodiversity and altering the soil quality (Borsali et al., 2018; Zouidi et al., 2018; Djebbouri et al., 2022). At the beginning of this degradation, the most perceptible changes are those that affect certain dominant perennial plants ensuring the physiognomy of these rangelands, such as the four steppe formations (Alfa, Armoise, Sparte and Remth).

Lygeum is a monospecific genus of the Poaceae family Willis (1980), represented by the perennial species *Lygeum spartum* L., which was described for the first time by Battandier and Trabut (1895). In Algeria, this species constitutes a dominant element of the Algerian steppe, which occupies the second place after the Alfa (Quezel, Santa, 1962), with an extended area estimated at 3 million ha. It grows on sandy soils and saline soils in arid and semi-arid bioclimatic stages (Le Houérou, 2001; Nedjimi, Beladel, 2015; Nedjimi, 2016). As such, it is an important element in the equilibrium of the environment and the fight against desertification (Le Houérou, 1995; Hourizi, 2017; Adjabi et al., 2019).

Seed germination and early growth of emerged plants represent the most fragile and vulnerable phases of the plant life cycle that are intimately connected and closely affected by various overlapping environmental factors such as temperature, water availability, salinity, light, seedling establishment requirements, and germinated seed establishment requirements. All of

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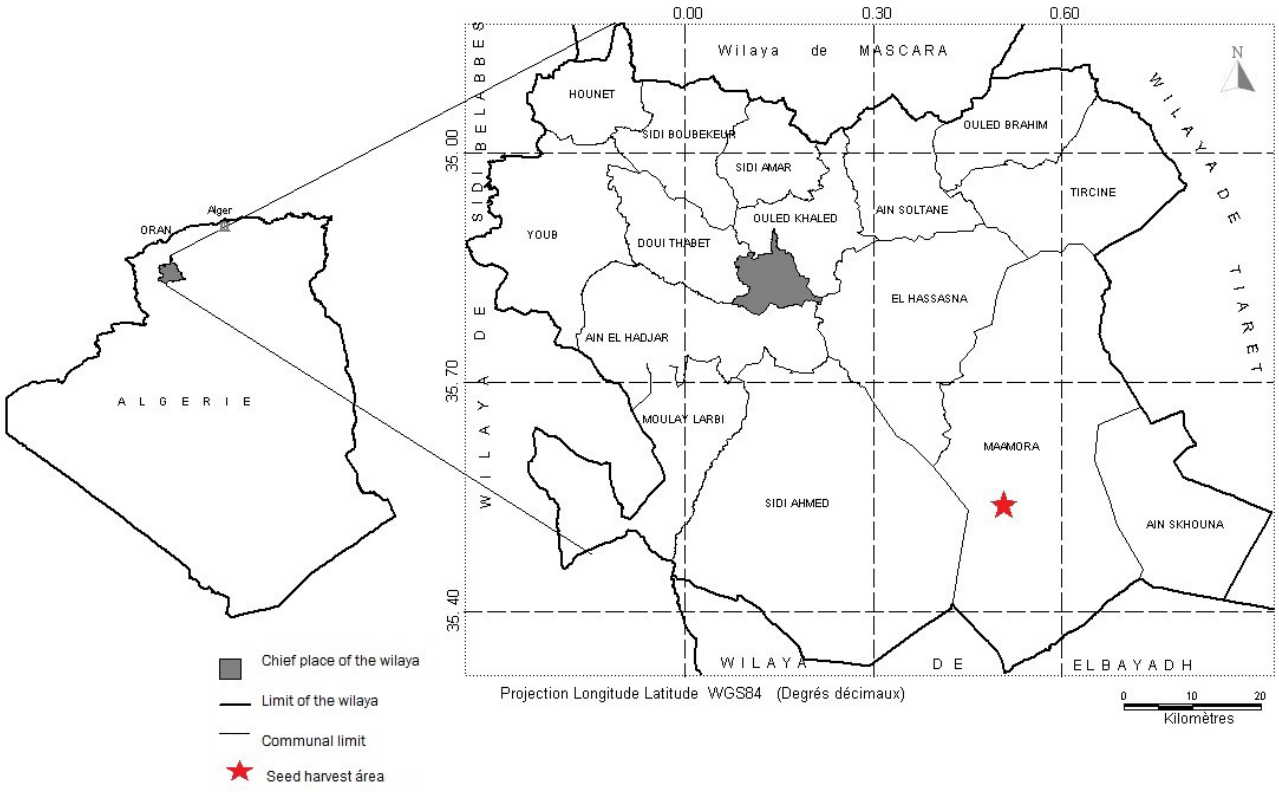


Fig. 1. Location of the harvest area of *Lygeum spartum* seeds.

these factors represent the bottleneck that should be established, whether for crop production or for land restoration (Zhang et al., 2018; Paraskevopoulou et al., 2020).

Morphologically, germination is characterized by the emergence of the radicle and is, in fact, only a process of growth of the root meristematic cells, where turgidity constitutes the essential element of its triggering (Schiefelbein et al., 1997). Physiologically, water deficit greatly disrupts protein synthesis and consequently limits the remobilization of carbohydrate reserves and energy substances stored as starch in the dried seeds (Bewley et al., 2013). The selection of parameters limiting the impact of water deficit on the progress of different phases of germination constitutes an essential objective in any work to improve the aptitudes of tolerance to this type of stress.

Soil salinity is one of the main abiotic stresses that results in water deficits limiting germination and plant growth (Munns, Tester, 2008; Abdelly et al., 2008). Salinity risks are more important in arid and semi-arid areas characterized by low rainfall, high evapotranspiration, and the use of highly mineralized irrigation water (Shannon, 1986; Benidire et al., 2015).

The objective of this work is to study the effect of osmotic stress caused by polyethylene glycol (PEG_{6000}) and salt stress caused by NaCl on the germination capacity of *L. spartum* seeds to determine the concentrations that inhibit germination. In this study, the seeds of *L. spartum* were chosen because it is a predominant species in the Algerian steppes, especially in arid and semi-arid areas, and a species that fixes the soil against desertification.

Material and methods

Plant materials andseed collection region

Fully mature *L. spartum* grains were harvested in June 2020 from the south-western area of Maamoura commune, located in the south-east of the provinceof Saïda (35°49′43″ N longitude, 00°50′18″ W latitude, and 1094 m elevation). The study area covers an area of 127,100 ha (one-fifth of the wilaya's surface area) and is part of the daïra of El Hassasna, which is one of the most important daïras of the wilaya in terms of agricultural, forestry, and steppe potential (Fig.1). It is considered an agro-pastoral area (D.P.A.T, 2011).

Seed germination experiment

Before their use in the germination tests, the healthy and intact seeds were sterilized on the surface with a hypochlorite solution (8% NaOCl) for 5 min and rinsed thoroughly with distilled water.

To assess the impact of drought on the germination of *L. spartum* seeds, germination tests were performed under different levels of water potential through the use of PEG_{6000}, which forms a non permeable, water-soluble, non ionic, and polymeric solution for the cells (Zouidi et al., 2019a). PEG_{6000} is used to induce water deficiency by reducing water availability without causing physical damage to the plant (Romo et al., 2001). According to the equation established by Michel and Kaufmann

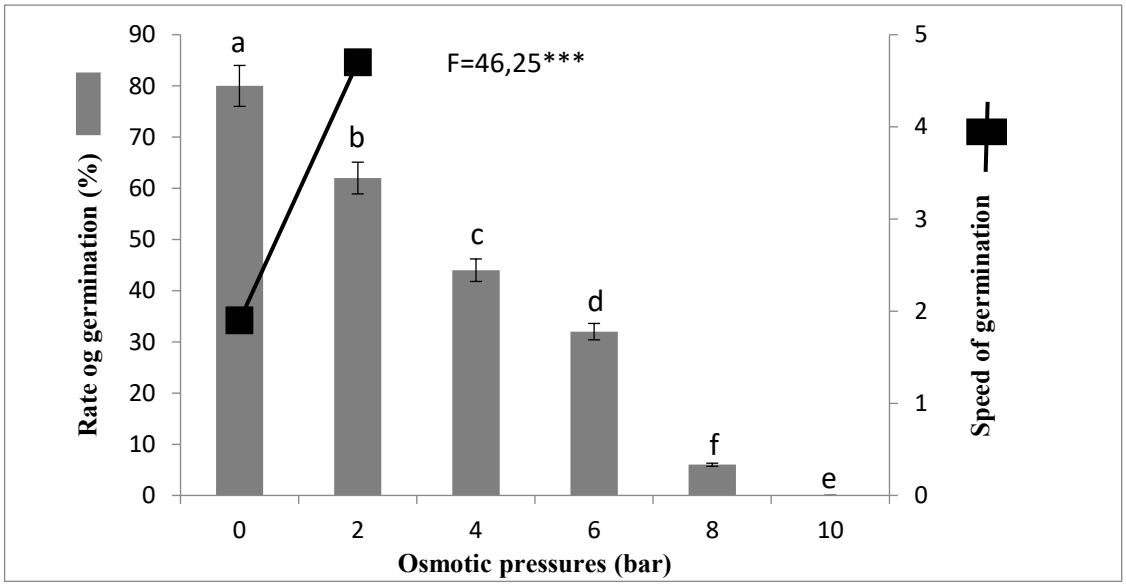


Fig. 2. Effect of different osmotic pressures on the rate and velocity of germination of *Lygeum spartum* seeds.
Notes: The bars and whiskers represent the mean ± standard deviation (n=5 repetitions). Significance levels: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; NS – not significant.

(1973), increasing concentrations of PEG_{6000} induce equally increasing water potential (0,−2,-4,-6,-8, and −10 bar). This equation was used to induce the different levels of water stress tested.

The equation linking the different parameters is as follows:

$$\Psi h = -(1.18\times10^{-2})\ C - (1.118\times10^{-4})\ C^2 + (2.67\times10^{-4})\ C\cdot\times\ T + (8.39\times10^{-7})\ \times C^2\times T, \quad (1)$$

where **Ψh is the water potential in bar, T is incubation temperature** in °C, and C is the concentration of PEG_{6000} in g/l.

To study the effects of salinity on germination, we conducted germination tests under salt stress using the optimal germination conditions determined in the experiments.

In order to do statistical analysis, five replicates were used for each treatment. Inside each plastic Petri dish (9 cm in diameter). Twenty seeds were germinated on two folds of filter paper which they soaked daily with solutions at different concentrations of NaCl solutions (0, 2, 4, 6, 8, and 10g/l) and PEG_{6000} (0, −2, -4, -6, -8, and −10 bar). The seeds were then placed at an optimal germination temperature of 15°C; the duration of the test was determined by the germination period of 21 days. The germinated seeds were counted daily and removed from the Petri dishes.

The time taken for the percentage of germination of all replicates to reach 50% was recorded as TG50.

The germination parameters measured

The germination rate (TG%) is the best parameter that identifies the concentration of PEG and NaCl. It presents the physiological limit of seed germination. It is calculated using the ratio of the number of germinated seeds and the total number of seeds according to the formula described by AgroBio (2013) as follows:

$$TG\ (\%) = (\text{number of germinated seeds/total number of seeds}) \times 100 \quad (2)$$

The germination speed allows expressing the germination energy responsible for the depletion of the seed reserves. The germination speed is estimated by the average time (T50) which corresponds to the germination of 50% of the seeds (Lang, 1965).

$$T50 = T1 + (0.5\text{-}G1/G2\text{-}G1) \times (T2 - T1), \quad (3)$$

where G1 is the cumulative % of germinated seeds with a value closer to 50% (lower) and G2 is the cumulative % of sprouted seeds with a value closer to 50% (higher).

Statistical analysis

The results were subjected to analysis of variance (ANOVA) with a single factor of variation at 5% probability level (P=0.05) after controlling normality distribution for comparing the averages of germination rates with stress. The posthoc Fisher's least significant difference (LSD) test was applied for multiple comparisons of means. We used Minitab software package version 17.

Results

The average germination capacity of the control treatment (0 bar) is 80%. According to the tests carried out at the level of our laboratory, the average rate of germination for *L. spartum* was 37.33%, which was due to the fragility of the seeds and the existence of parasitized and empty seeds as they have low storage capacity. In general, the germination rate decreases considerably with increasing water stress of the substrate.

According to the results obtained in this study, the germination rate decreased on average 0% at −10 bar, -6% at -8 bar, and 32% at -6 bar, compared to the control treatment (0 bar).

Statistically, the above results were confirmed by ANOVA, which showed a highly significant effect (P< 0.001) of the dif-

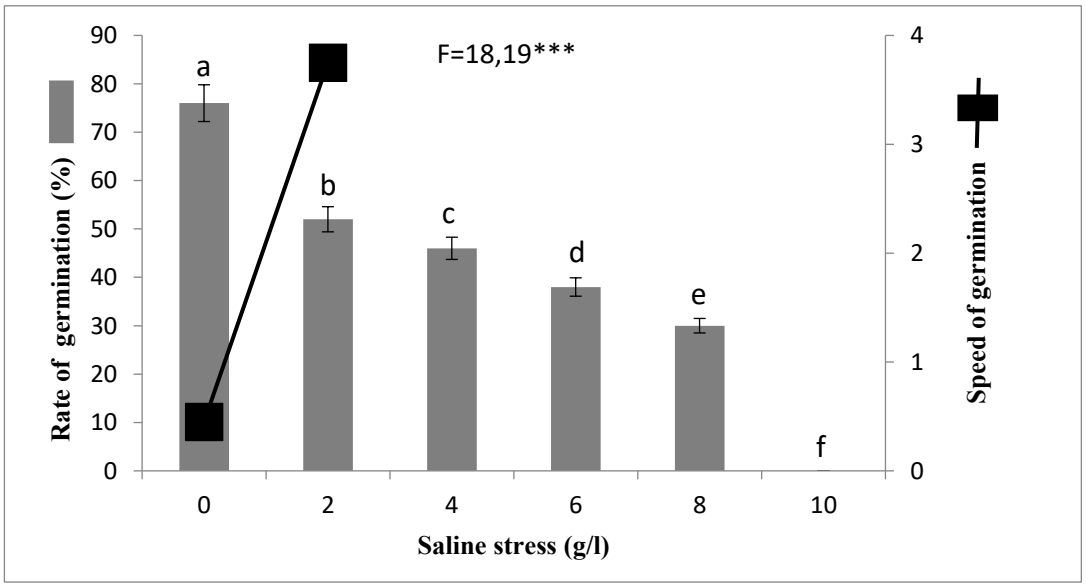


Fig. 3. Effect of different saline stress levels on the rate and speed of germination of *Lygeum spartum* seeds.
Notes: The bars and whiskers represent the mean ± standard deviation (n=5 repetitions). Significance levels: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; NS – not significant.

ferent glycol concentrations (PEG_{6000}) on the germination rate (Fig. 2).

The germination rate or the average time to germination of 50% seeds is as follows: 2 days for seeds with an osmotic pressure of 0 bar (soaked in distilled water) and 5 days for germination of seeds with an osmotic pressure of –2 bar.

Indeed, the more the concentration of PEG_{6000} of the substrate increases, the more the germinal capacity decreases while slowing down the speed of germination.

The results shown in Figure 3 indicate that the final germination rate is maximum (75%) for the seeds not stressed by NaCl (controls). Then, this value begins to decrease with the increase in NaCl concentration and finally drops to 31% for the highest salt stress (8 g/l).

The concentration of 10 g/l completely inhibits the germination of *L. spartum* grains. The results of ANOVA show that there is a significant difference in the germination rate of *L. spartum* seeds ($P< 0.001$) soaked in solutions of different salt concentrations.

In general, the germination rate decreases considerably with increasing salt stress of the substrate.

Discussion

Drought and salinity are the environmental constraints that cause the most damage to agro-pastoral production. Indeed, each year, the lost surfaces caused by these two stresses are considerable. One billion hectares are threatened worldwide, including 3.2 million in Algeria (Toumi et al., 2014). This degradation of plant cover is especially valid for arid and semi-arid areas where the climate change is becoming more and more restrictive for the growth and development of plants (Zouidi et al., 2019a),which is associated with significant evaporation favoring the accumulation of salts near the soil surface (Hayek, Abdelly, 2004; Daoudi, Sitayeb, 2020). Plant biotechnology techniques have proven to be effective on the one hand in understanding these adaptation mechanisms and on the other hand in creating plant material that is tolerant to different forms of environmental constraints (drought and salinity).

Effect of osmotic stress on germination

The exceptional germinative capacity of Albardine compared to that of Alfa gives it a role of colonizer of many spaces in semi-arid and arid zones. In North Africa, spartum is a mechanism to protect soils against erosion and also an economic source for traditional artisans. However, the combined actions of overgrazing associated with a prolonged cycle of aridity, the uprooting of tufts for basket making, and the knowledge of the techniques of exploitation of this plant are often erroneous. thus causing a degradation of the steppes with *L. spartum* (Nedjimi, 2014, 2016).

Water is an essential factor in germination and plant production. It has several functions within the plant. In particular, water within the tissues is important as a transport vector of all substances necessary for plant functioning (Heler et al., 2000; Zouidi et al., 2019b).

The results of this experiment show that the seeds of Albardine harvested in the steppe zone of western Algeria are moderately tolerant to water stress. This result confirms previous reports on the germination of other *L. spartum* species, including those of Boudjada et al. (2009) who studied variability in Albardine from seven provenances in Algeria. In the absence of sufficient moisture, the seed, even if correctly placed in the soil, does not develop, thus delaying the emergence of the crop and in case of persistent drought, the situation may result in no emergence (Feliachi et al., 2001). Drought is one of the main environmental

factors that greatly affects the germination of species and reduces their survival during the early stages of development. Our results confirm the impact of water stress on the germination of Albardine. Moreover, the germination rate decreased considerably with increasing osmotic water stress of the substrate. Water scarcity is a determining factor for plant germination and growth, particularly in arid and semi-arid regions. It induces a decrease in mean water content in stressed plants, and a significant reduction in total biomass production (Albouchi et al., 2000).

According to Rollin (2017), the speed of germination can also be taken as a criterion. It can be quantified either by the time required to obtain the germination of 50% of the seeds or by the value of the slope of the curve representing the percentage of germination as a function of time. Xerophilous species (C3) such as *L. spartum* have certain morphological and physiological adaptations to water stress; they are efficient in terms of water use and are, therefore, adapted to dry climates. After rain, *L. spartum* can react quickly to small variations in soil water content (Aidoud, 1989; Nedjimi, 2016).

Effect of salinity on germination of Lygeum spartum

Algeria is among the countries at risk, with 3.2 million ha affected by salinity (Belkhodja, Bidai, 2004), particularly in arid and semi-arid regions which are characterized by biotopes affected by salinization (Zouidi et al., 2018; Borsali et al., 2019). Indeed, this constraint constitutes a limiting factor for seed germination and productivity (Ashraf, Harris, 2004; Abdel Latef, 2010) by causing osmotic, toxic, or nutritional effects within the plant (Hanana et al., 2011). Thus, varietal selection requires knowledge of the mechanisms responsible for plant tolerance to salinity (Arbaoui et al., 2000). The study of the effects of different concentrations of a salt solution on the germination of *L. spartum* seeds showed that the germination capacity is affected by increasing the salt concentration. The influence of salinity on the germination capacity of *L. spartum* was manifested by a reduction in the rate of germination compared to controls. This reduction is more important as the concentration of salts is high.

The decrease in the final germination rate corresponds to either an increase in the external osmotic pressure which affects the absorption of water by the seeds or an accumulation of Na^+ and Cl^- ions in the embryo (Groome et al., 1991). This toxic effect can lead to alteration of the metabolic processes of germination and, in the extreme case, to the death of the embryo by excess ions. The emergence of the radicle is controlled by the osmolarity of the environment (Brueggeman et al., 2002). According to Slama (2004), salinity also acts on germination by slowing down its speed, which exposes the seeds to greater risks of germination inhibition.

Nedjimi et al. (2010) classified this grass as a moderately salt-tolerant plant that is also able to tolerate soil salinity levels equivalent to electrical conductivity (EC) values of 4–5 dS/m. At the germination stage, *L. spartum* seems to tolerate salinity at a temperature between 10 and 20 °C (Nedjimi, 2013). The highest levels of around 10g/l proved to be inhibitory.

Under the highest saline conditions, seed survival rather than germination capacity may be an appropriate criterion for success, since resumption of germination occurs in the seeds of *L. spartum* and other halophyte grasses when hypersaline conditions are attenuated. Dormancy reduces the risk of seedling mortality when moisture is limited and salinity is increased. This is most favorable under hypersaline conditions (Khan, Gulzar, 2003; Nedjimi, 2013).

Conclusion

The current situation of the Algerian steppe is alarming and is undergoing a process of degradation which requires appropriate development to protect the steppe formations, particularly the *L. spartum* formations.

Water deficit is the main environmental factor responsible for low regeneration, low yield, and other irregularities in *L. spartum*. However, the impact of this abiotic stress on the productivity of this species is dependent on its intensity and time of persistence. The work carried out in this study focused on the adaptation of *L. spartum* sown at different levels of water stress by the addition of PEG.

The results reported in this study also show that *L. spartum* is a plant moderately sensitive to the action of NaCl. The germination capacity and the germination rate are indeed moderately affected, and they decrease with an increase in the concentration of the added NaCl. The tests carried out during this experiment demonstrate that the adaptation of the species is closely dependent on and favored by the different physiological, morphological, and biochemical responses under stress conditions. All these factors influence the cultivation of this species, which could, therefore, be established in unproductive low-salt areas, thus providing a significant source of fodder. It is also likely to limit desertification in these arid zones characterized by great ecological fragility.

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