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FULL-LENGTH RESEARCH ARTICLE

Effect of Salinity and Temperature on Germination of *Lygeum spartum*

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Abstract *Lygeum spartum* L. (Poaceae) is one of the most important perennial grass species in arid steppes of Algeria. Its germination responses to environmental stresses are poorly understood. Therefore, experiments were conducted to determine the effect of salinity and temperature on the germination of seeds. Seeds were germinated at three alternating temperatures (10–20, 15–25, and 20–30 °C), with four NaCl concentrations (0, 50, 100, and 150 mM) and 12-h photoperiod. Results indicate that seeds can germinate at high salt concentrations (150 mM NaCl). However, highest germination was obtained in distilled water. Lower thermoperiods promoted germination, while high temperatures significantly inhibited the germination of seeds at all NaCl concentrations tested. Rate of germination decreased with increases in salinity. Seeds recovered after being transferred to distilled water, and recovery was higher from higher salinity concentrations and lower thermoperiods. *L. spartum* is moderately salt tolerant at germination stage, and tolerance is affected by the interaction of temperature and NaCl concentration.

Keywords *Lygeum spartum* · Germination recovery · Salt tolerance · Steppe

Introduction

Many arid and semiarid regions in the world have soils and water resources that are too saline for most of the common conventional crop system [23]. Salt-affected soils are widely spread in many arid and semiarid regions of the world and increasingly threatening agricultural expansion and productivity. Yet, in many arid environments, high-quality water is not available to support the establishment of plants for revegetation projects. The removal of sodium salts from saline soils by tolerant plants, as an alternative for costly chemical amendments, has emerged as an efficient low-cost technology [9].

Lygeum spartum L. (Poaceae) is a salt rhizomatous perennial grass ranging in distribution from Northern Africa that can tolerate extreme conditions of aridity, salinity, and high temperatures [23, 24]. The plant propagates by rhizome growth after monsoon rains, and it also produces numerous flowers and seeds from April to July [1]. The presence of fibers confers a significant role in the craft industry of the carpets, curtains, and plaits [14, 25]. The structural and architectural study of foliar fibers of this species has shown the structural diversity of this tissue. Tests of obtaining paper pulp starting from foliar tissues made it possible to confirm that the *L. spartum* is a paper plant [15].

Lygeum spartum has been especially recommended for arid zone restoration projects. In a field experiment, Rogel et al. [27] demonstrated that *L. spartum* can be used as a bio-indicator of soil salt type. *L. spartum* has been also suggested as a suitable species for the phytoremediation owing to the high translocation rates of Cd toward the aboveground tissues [4].

In arid and semiarid Mediterranean regions, salinity is one of the most detrimental factors affecting seeds

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germination and plant establishment. An increase in salinity induces both a reduction in the percentage of germinating seeds and a delay in the initiation of the germination process [22].

Germination failures on saline soils are often a result of high salt concentrations in the seed-planting zone because of the upward movement of the soil solution and subsequent evaporation at the soil surface. This has been attributed to both osmotic and toxic effects [16]. Seed germination under saline conditions occurs after high precipitation where soil salinity is usually reduced due to leaching [17].

In arid regions of Algeria, the climate is mainly characterized by low and irregular rainfall in which potential evapotranspiration exceeds precipitation. In these areas, high rate of evaporation causes an accumulation of salts on the surface of the soil. These harsh conditions have led to differential life-history strategies in perennial plants in order to maximize their fitness [13]. In such regions, germination occurs during the rainy season when soil salinity levels are usually reduced [8].

In natural field conditions, stress may be transient and the capacity of the plant to complete its life cycle is directly related to its ability to recover after exposure to stressful period [29]. Sharma and Sen [28] attributed rapid seed germination of *Haloxylon recurvum* as an adaptive strategy to the availability of water when reduced levels of NaCl content are reached in soil for short durations during the rainy season. This is because evaporation under full sunlight and higher temperatures causes an increase in the salt content by capillary movement. If a seed can take advantage of a short period of reduction in soil salinity and germinates rapidly, then seedling establishment is ensured.

Little work has been done on the germination strategies of Mediterranean steppe grasses exposed to high temperature and salinity [7]. The present research describes the ability of *L. spartum* seeds to germinate under various thermoperiod and salinity regimes, and evaluate the recovery capacity to this salinity conditions. Based on the distribution of *L. spartum*, we hypothesize that optimal germination would be maintained at moderate salinities and low temperature regimes.

Materials and Methods

Seeds of *L. spartum* were collected from the area of *Aïn Maâbed* in the province of *Djelfa* (Algeria) (2°39′E longitude, 34°50′N latitude, and 934 m elevation). Seeds were separated from each inflorescence (Fig. 1) and sterilized in 10 % sodium hypochlorite (Clorox) solution for 1 min, subsequently washed with distilled water air-dried before being used in the germination experiments. Four salinity concentrations (0, 50, 100, and 150 Mm NaCl) were used based on a preliminary test to determine the salt tolerance limits of the species.

Germination was tested in a programmed incubator at 10–20, 15–25, and 20–30 °C dark–light temperature regimes with a 12-h photoperiod.

A completely randomized design was used in the germination tests. Four replicates of 25 seeds each were germinated in twofold of filter paper placed in plastic Petri dishes (9 cm diameter) with 5 ml of test solution. A seed was considered to have germinated when the radicle emerged. After 15 days, all non-germinated seeds were placed in distilled water and under the initial temperature regimes for another 15 days to determine their ability to recover from salt treatments.

Rate of germination was calculated using a modified Timson's index [30] of germination velocity:

$$\text{Germination velocity} = \Sigma G/t$$

where $G =$ percentage of seed germinated after 2-day interval and $t =$ total time of germination.Rate of recovery germination was calculated by using the relation as follows:

$$\text{Recovery percentage} = (a - b)/(c - b) \times 100.$$

where $a =$ total number of seed germinated (the number of seeds germinated in saline solutions plus those that recovered to germination in the distilled water), $b =$ total number of seeds germinated in saline solution, and $c =$ total number of seeds [17].

Germination data were arcsine transformed before statistical analysis to ensure the homogeneity of variance. Data were analyzed using SPSS version 9.0. A two-way analysis of variance (ANOVA) was carried out to test effects of the main factors and their interaction on the rate

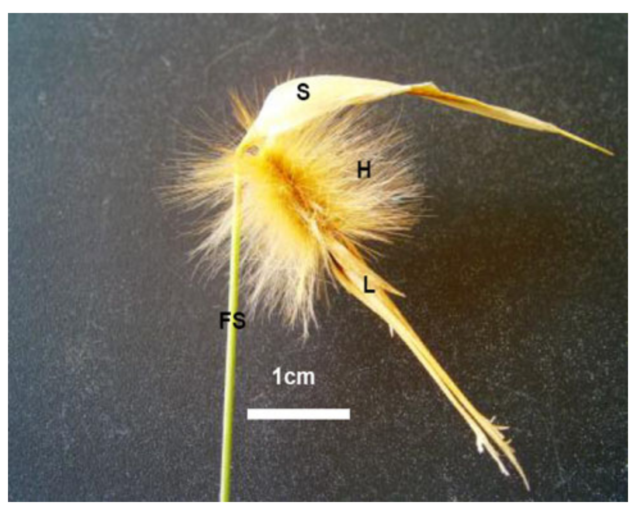

Fig. 1 Mature seed of *L. spartum* showing numerous unicellular hairs (*H*), lemma (*L*), spathe or glume (*S*), and floral stalk (*FS*)

and final percentage of germination. Tukey's test was used to estimate least significant range between means.

Results

Germination Percentage

Salinity, temperature, and their interaction significantly ($P < 0.001$) affected the germination percentage of *L. spartum* seeds (Table 1). Germination percentage decreased with increasing salinity, and the highest germination percentage was found in distilled water (Figs. 2, 3). Seeds of *L. spartum* were able to germinate at all temperatures, and the optimal temperature corresponds to 10–20 °C (Fig. 2). The delay of germination increased with increasing NaCl concentrations and was maximal at the 20–30 °C temperature than with other temperatures.

Rate of Germination

The index of germination velocity calculated by using a modified Timson's index showed that the rate germination decreased with an increase in salinity (Fig. 4). A two-way ANOVA of the rate of germination indicated a significant ($P < 0.001$) main effect of temperature, salinity, and their interaction (Table 1). The inhibitory effect of salinity on rate germination percentage was greater at 20–30 °C than at all thermoperiods. Although the increase in salinity decreases the rate germination, the detrimental effect of salinity is generally less severe at optimum germination temperature (10–20 °C).

Recovery of Germination

After 15 days of salinity treatment, seeds were transferred to distilled water to determine the recovery of germination. Germination recovery of *L. spartum* was significantly ($P < 0.001$) affected by thermoperiod, salinity, and the interaction of both factors (Table 1). Recovery percentages decreased with the increase in the temperature. Germination was higher for seeds exposed to 100 and 150 mM NaCl at 10–20 °C compared to those incubated at 20–30 °C (Table 2).

Table 1 A two-way ANOVA of the effects of salinity, temperature, and their interaction on germination of *L. spartum*

Independent variable	Salinity	Thermoperiod	Salinity × thermoperiod
Percent germination	22.31***	47.10***	4.59***
Rate of germination	52.01***	157.319***	13.85***
Percent recovery	276.97***	249.27***	47.07***

Data represent *F* values significant at $P < 0.001$(***)

Discussion

Lygeum spartum, commonly named esparto, is an important perennial grass in semiarid and arid areas in Algeria, with *Stipa tenacissima* L. and *Aristida pungens* Desf. This grass makes a natural fence against desert expansion. In addition to its ecological role, *L. spartum* has an economical interest in traditional craftsmen and paper manufacturing [6]. The conservation of *L. spartum* populations in the Algerian steppes is a very complex task as ecological conditions are not only highly variable but also extreme (low rainfall, high salinity, etc.). Overgrazing, cutting, and farming around steppe related to the increase in rural population have practically eliminated vegetation from the most arid and semiarid steppes [26]. In this context, knowledge of *L. spartum* natural regeneration is a prerequisite to limit out its potential distribution area. Native vegetation present in Algerian steppes is exposed to various stresses including salinity, drought, and extreme temperatures. The climate of the area can be characterized as arid and semiarid regions. It has an irregular rainfall of less than 300 mm per year in which potential evapotranspiration exceeds precipitation [20]. Hence, water shortage leads to the natural salinization process, resulting in soil degradation and contributing to desertification.

Germination is considered as the most sensitive stage of the plant life cycle. The first physiological disorder, which takes place during germination, is the reduction in imbibitions of water by seeds, which leads to a series of metabolic changes, including changed enzyme activities and general reduction in hydrolysis and utilization of the seed reserve [29]. Tolerance to salinity during germination is critical for the establishment of plants growing in arid saline soil regions [31]. In such regions, germination occurs during the rainy season when soil salinity levels are usually reduced [8]. Salinity inhibits germination of plant seeds in one of the two ways: (1) preventing germination without loss of viability at higher salinities; and (2) delaying germination of seeds at salinities that cause some stress to seeds but do not prevent germination.

Lygeum spartum seeds showed highest germination percentages under nonsaline conditions. An increase in salinity inhibited germination; however, about 20 % of the seeds germinated at 150 mM NaCl (Fig. 3). These results indicate that *L. spartum* is a moderately salt-tolerant grass at germination stage in comparison with other grasses found at the steppes of Algeria. Similar results were reported for other grasses: *Briza maxima* [21], *Triglochin maritima* [18], *Aeluropus lagopoides* [10], and *Sporobolus ioclados* [19].

Nedjimi [25] found that *L. spartum* was able to grow in medium containing 100 mM NaCl without displaying salt-induced toxicity symptoms, and fresh and dry weights of shoots and roots reduced significantly above 50 mM NaCl.

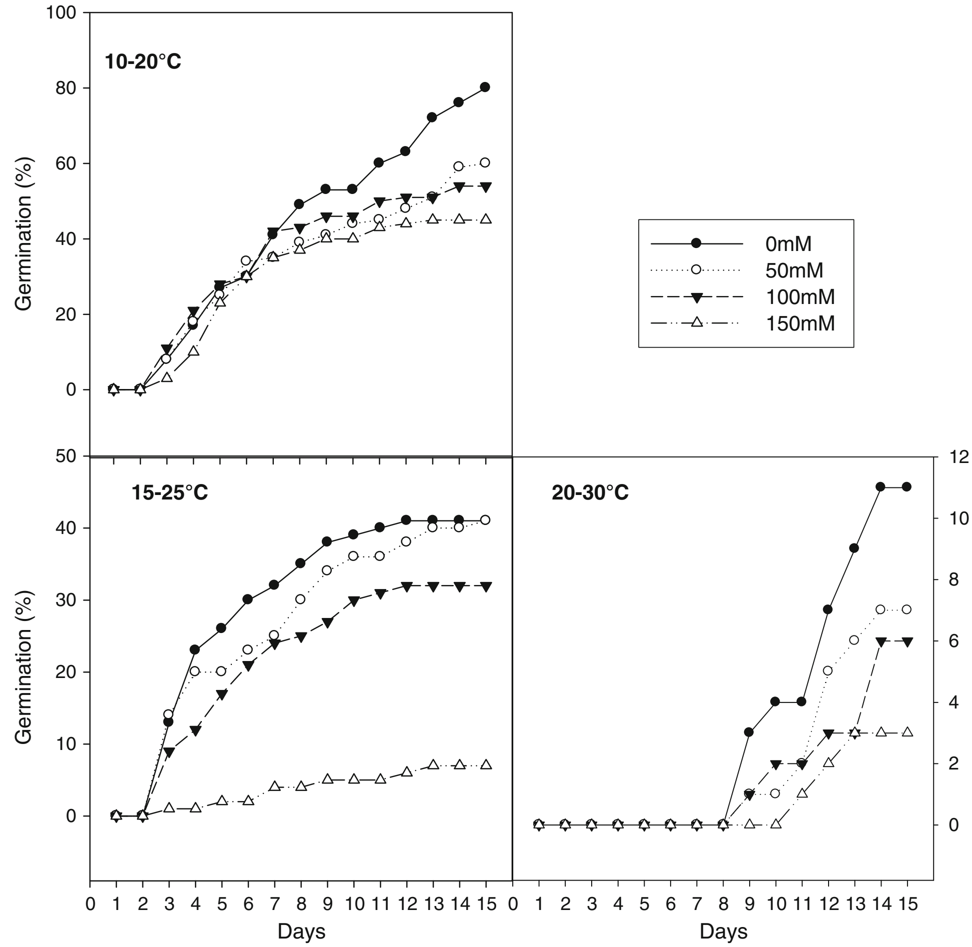

Fig. 2 Cumulative germination percentage of *L. spartum* seeds during 15 days at different temperatures as influenced by NaCl concentrations (0, 50, 100, and 150 mM) and incubation temperatures (10–20, 15–25, and 20–30 °C)

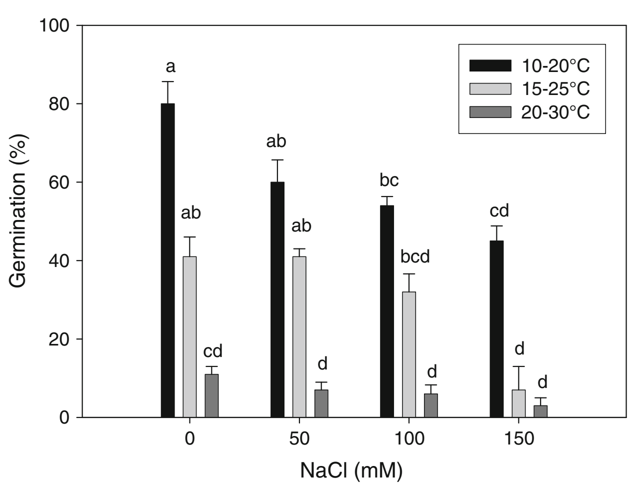

Fig. 3 Effect of NaCl (0, 50, 100, and 150 mM) and temperature (10–20, 15–25, and 20–30 °C) on final germination percentages of *L. spartum*. *Bars* represent mean ± SE ($n = 4$). *Different letters* indicate significant difference between treatments (Tukey's test $P < 0.05$)

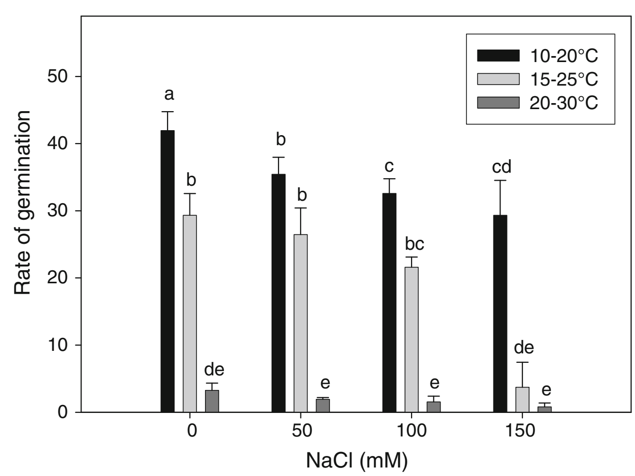

Fig. 4 Effect of NaCl (0, 50, 100, and 150 mM) and temperature (10–20, 15–25, and 20–30 °C) on rate of germination of *L. spartum*. *Bars* represent mean ± SE ($n = 4$). *Different letters* indicate significant difference between treatments (Tukey's test $P < 0.05$)

It was found that the stressed plants by NaCl accumulated more Na^+ than the control plants. The same author concluded that tolerance of *L. spartum* to salt stress was linked through a common mechanism of accumulating Na^+ for osmotic adjustment. It can tolerate low soil pH from 3 to 4 because of mycorrhizal associations, thus maximizing the use of scarce nutrients [5]. Moreover, it accumulates salt ions in tissues, which adjusts leaf water potential, enabling

Table 2 Recovery percentage (mean ± SE) of germination of *L. spartum* after they were transferred from 0, 50, 100, and 150 mM NaCl to distilled water at thermoperiods of 10–20, 15–25, and 20–30 °C

NaCl (mM)	Thermoperiod (°C)		
	10–20	15–25	20–30
0	–	–	–
50	37.5 ± 1.91b	20.5 ± 5.45cd	19.5 ± 3.42cd
100	58.25 ± 7.23a	27.25 ± 2.99c	15 ± 2.58d
150	64.25 ± 5.56a	42 ± 3.27b	12.25 ± 1.71d

Different letters in the same column indicate significant difference at $P < 0.05$ according to the Tukey's multiple range test

the plant to maintain cell turgor and limit transpiration under saline conditions. In addition, significant increase in glycine betaine and valine has been noted at 100 mM NaCl [23, 25]. In salt stress condition, the leaf water relations were characterized by low osmotic potential and low root hydraulic conductivity [24].

Sensitivity to periodic temperature and salinity fluctuations constitutes an important mechanism, which enables plants to respond to daily variations in the soil surface conditions. Temperature shifts may affect a number of processes determining the germinability of seeds, including membrane permeability, activity of membrane proteins, and cytosol enzymes [3]. Germination of seeds in salt soils usually occurs after monsoon rains when there is a reduction in temperature and soil salinity [10]. Salinity and temperature interact with their control of seed germination, and the greatest inhibition is usually found at the maximum and minimum limits of tolerance of these two environmental variables, as reported in *Hordeum jubatum* [2], and *Urochondra setulosa* [11].

The effect of salinity on germination varies considerably with temperature regimes [17]. Guma et al. [12] reported that a temperature regime of 10–20 °C is optimal for seed germination of *Salsola vermiculata*, whereas 25–30 °C was the upper threshold temperatures for seed germination. Our data showed that the 10–20 °C thermoperiod is the most favorable for germination of *L. spartum*. In the natural habitat of the population studied, such temperatures can occur after seed dispersal during late autumn and winter, in coincidence with the rainfall period (November–February). At this time of the year, seeds are exposed to cooler temperatures and low salinity in the soil and these conditions may enhance germination.

The response of seeds of *L. spartum* transferred to distilled water after 15 days at various salinities varied depending on the temperature regime. Seeds exposed to higher salinity recovered quickly at cooler temperatures (10–20 °C). Recovery of germination responses was also dependent on temperature, ranging from 12 % recovery at 20–30 °C to 64 % at 10–20 °C (Fig. 4). Under highest saline conditions, seed survival rather than germinability may be an appropriate criterion for success, since recovery of germination does occur in seeds of *L. spartum* and other halophyte grasses when hypersaline conditions are alleviated [31]. Dormancy reduces the risk of seedling mortality, when moisture is limited and salinity is increased. This is quite favorable under hypersaline conditions [19].

Conclusions

From the present results, it is evident that seeds of *L. spartum* germinate better at low temperature (10–20 °C). Salt stress decreases both rate and percentage of germination, but recovery was higher under cooler thermoperiods. In field conditions, seeds present in the soil are exposed to cooler conditions and low salinity during the late autumn and winter, and this situation may enhance the germination and recruitment earlier in the season. However, seeds would still not be able to germinate when conditions get warmer. It appears that better recovery response at cooler temperatures confers on the advantage of preempting sites before other species could germinate, and this would give *L. spartum* an excellent opportunity to successfully colonize. Therefore, to increase the probability of successful plant establishment in rehabilitating of degraded lands in Algerian steppes, germination requirements should be considered in the planning of sowing dates. According to our findings, late autumn and winter sowings would be recommended, when salinity and temperature stresses are reduced after the first rains of the season.

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