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Full Length Research Paper

Influence of salinity and temperature on the germination of *Hedysarum scoparium* Fisch. et Mey.

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This study was conducted to determine the effects of temperature and salinity on seed germination and their recovery of germination after being transferred from saline conditions to distilled water. The germination responses of the seeds in complete darkness were determined over a wide range temperatures (10 to 35°C) and salinities (0 to 500 mM NaCl). Germination was inhibited above or below the optimal temperature of 15°C. The highest germination percentages were under non-saline conditions, and increased NaCl concentrations progressively inhibited seed germination. Germination rate decreased with increased salinity at all temperatures, but the highest rates were at 15°C. The interaction between salinity and temperature yielded no germination at 500 mM NaCl (25 and 35°C). After 10 days, seeds were transferred from salt solution to distilled water, and germination recovered at all temperatures with low salinity. At 500 mM NaCl, there was no germination recovery at 25 and 35°C. The results showed that salt stress decreased both the percentage and the rate of germination, exposure to high concentration of NaCl permanently inhibited germination at high temperature.

Key words: Desert ecosystems, *Hedysarum scoparium*, recovery germination, salinity, temperature, seed germination.

INTRODUCTION

Hedysarum scoparium Fisch. et Mey. is an important xerophytic shrub species that is widely distributed in arid and semiarid sandy deserts. This species is a tall shrub with spring flowering, and can attain a height of 5 m. The dispersal unit of *H. scoparium* is a large pod, containing several seeds; and the species is characterized by fast growth and high drought resistance (Jia, 1987). Due to its economic and ecological value, it has been used to feed livestock, to maintain water and soil, and to stabilize sand dunes, especially by aerial seedling in arid and semiarid degraded areas of northwest China (Wu et al., 1998; Jin et al., 2007).

Germination is a crucial stage in the life cycle of individual plants inhabiting arid, saline environments as it determines whether or not the populations will establish successfully (Khan and Gulzar, 2003). In general, increasing salinity reduces and/or delays germination of both glycophyte and halophyte seeds. Most seeds are deposited near the soil surface where the concentration of salt is much higher than that below the surface (Esechie, 1995). Therefore, under field conditions, germination often occurs after rain or flooding which can provide moisture and also leach the surface salt (Khan and Ungar, 1996).

Several factors (for example, temperature, water, salinity and light) interact at the soil-atmosphere interface to regulate seed germination. Soil temperature and salinity are two important factors that control when and where seeds can germinate in arid, saline regions (Khan and Ungar, 1999). They can also interact in determining salinity tolerance during germination (Khan et al., 2000;

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Al-Khateeb, 2006). Lower temperatures can promote seed germination in *Haloxylon recurvum*, but high temperatures can significantly delay seed germination at different NaCl treatments (Khan and Ungar, 1996). Although, higher salinity usually decreases germination, the detrimental effect of salinity is less severe at optimum temperature (Al-Khateeb, 2006; Gorai and Neffati, 2007); however, little information is available on the effect of salinity and temperature on *H. scoparium* germination. The purpose of this study is to evaluate the adverse effects of salinity and temperature and their interaction on percentage of germination, germination rate and recovery responses of *H. scoparium*, grown under conditions of arid environments of China. The results obtained from this study present useful basic knowledge which is required in rehabilitating desertified lands (including salinized lands) by seed dispersal in the northwest of China.

MATERIALS AND METHODS

Seed collection

Seeds of *H. scoparium* (seed weight: 15.100 ± 0.549 g/1000 seeds; n = 5) were collected from plants growing in sandy desert (38°34´N, 102°59´E) at Gansu Province in the northwest of China in October 2006. Dry seeds were stored at 4°C before being used.

Effects of temperature and salinity on germination

To avoid fungal attack, seeds were surface sterilized in 0.58% sodium hypochlorite solution for 1 min, then washed with distilled water and air-dried before use (Gulzar et al., 2001). Germination trials were carried out in Petri dishes (9 cm in diameter) on three layers of filter paper (GB/T1914-93, Hangzhou Xinhua Paper Ltd, China) moistened with 10 ml of test solution. All Petri dishes were kept in an incubator under continuous darkness (LRH-250-G II Illuminating Incubator, Guangdong Medical Apparatus Manufacturer, Guangdong, China). Seeds were germinated in distilled water (0), 100, 200, 300 and 500 mM NaCl solutions at constant temperatures of 10, 15, 25 or 35°C. Four replicates of 25 seeds each were used per treatment. The solution in each Petri dish was renewed every 2 days, and any germinated seeds was recorded and removed daily for 10 days. Seeds were considered to have germinated when the radicle had emerged (Song et al., 2006).

After 10 days, any ungerminated seeds from the NaCl treatments were transferred to distilled water to study the recovery of germination, which was recorded daily for 7 days. The rate of germination was estimated using a modified Timson's index of germination velocity = $\sum G$ /t, where, G is the percentage of seed germination each day and t is the total germination period (Khan and Ungar, 1984). The maximum value possible using this index was 1000/10 = 100. Recovery germination was determined after 7 days of incubation using the equation: $(a - b)/(c - b) \times 100$, where, a is the number of seeds germinating in salt solution plus those that recovered germination in distilled water, b is the total number of seeds germinating in salt solution, and c is the total number of seeds tested. Total germination (%) was recorded as $a/c \times 100$ (Qu et al., 2008).

Data analysis

Germination, recovery germination and total germination data were arcsine-transformed before statistical analysis to ensure homogeneity of variance (Badger and Ungar, 1989). Data were analyzed using SPSS for Windows, version 11.5 (SPSS, 2002). A two-way analysis of variance (ANOVA) was used to demonstrate the significance ($p < 0.01$) of salinity and temperature effects and their interaction in determining the germination percentage, recovery percentage and germination rate. If ANOVA showed significant effects, Duncan's multiple range tests was used to determine differences among treatments.

RESULTS

Effects on germination (initial germination)

Germination of *H. scoparium* seed was significantly affected by salinity, temperature and their interaction ($p < 0.01$; Table 1). Seed germination was highest in distilled water and germination percentages decreased with increased salinity (Figure 1). Seeds of *H. scoparium* germinated between 15 and 35°C, with an optimum of 15°C (Figures 1 and 2). Seed germination decreased with increased NaCl concentrations at all temperatures (Figure 2). There was rapid seed germination in distilled water during the initial 3 days. Germination was increasingly delayed with increased NaCl concentrations, and completely inhibited at 500 mM NaCl (Figures 1 and 2).

Effects on germination rate

The index of germination velocity decreased with increased salinity (Figure 3). ANOVA of germination rate indicated a significant main effect of temperature, salinity and their interaction (Table 1). The highest rate of germination was at 15°C and lowest at 35°C (Figure 3).

Effect on germination recovery

ANOVA of recovery of germination indicated a significant main effect of temperature, salinity and their interaction (Table 1). The non-germinated seeds from salt treatments recovered at most temperatures. However, at 500 mM NaCl, there was no recovery of germination at 25°C; and similarly at 200 to 500 mM NaCl, there was no germination recovery at 35°C (Table 2).

Effect on total germination

ANOVA showed significant effects for temperature, salinity and their interaction on total germination (summation of germination in salts and after recovery) ($p < 0.01$; Table 1). Polynomial regression analysis was used to determine the relationships between total germination percentages and salinity at different temperatures. There was a strong negative relationship between germination and salinity, with coefficients of

Table 1. A two-way ANOVA of the effects of salinity (S), temperature (T) and their interaction on germination of *H. scoparium*.

Dependent variable	Independent variable		
	S	T	S×T
Percent germination	653.61*	135.03*	24.06*
Rate of germination	920.16*	177.20*	39.64*
Recovery of germination	399.74*	334.37*	132.87*
Total germination	522.22*	347.47*	27.52*

Data represent F values, *denotes significant difference at $p < 0.01$.

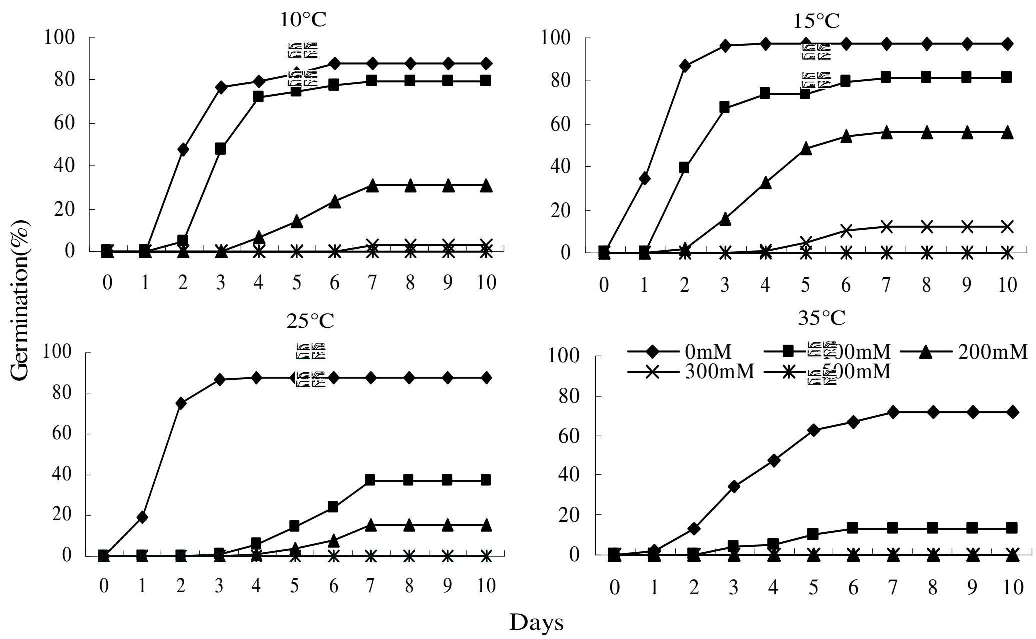

Figure 1. Cumulative germination percentage of *H. scoparium* seeds at salinity concentrations (0 to 500 mM) and incubation temperatures (10 to 35°C) over 10 days.

determination (R^2) of 0.8701 - 0.9750 (Figure 4).

Germination percentage was greatest in distilled water, decreased with increased salinity, and was severely limited at 500 mM. No seed germinated at the highest temperature (35°C) combined with 200, 300 or 500 mM NaCl (Figure 4).

DISCUSSION

Desserts are regions of irregular rainfall in which potential evapotranspiration greatly exceeds precipitation. In some regions, the water table is also high and a high rate of surface evaporation causes accumulation of salts at the soil surface (Kigel, 1995). Xerophytic species can differ in their life cycles, in responses to drought, in times of flowering, seed dispersal and germination behavior (Kigel, 1995). In the life cycle of most plants, the seeds have the highest resistance to extreme environmental stress and this is especially so for xerophytic species. Therefore, successful establishment of a plant population is highly dependent on the adaptive properties of seedgermination (Qu et al., 2008).

Our results showed that *H. scoparium* seeds were viable over a wide range of constant temperatures of 10 to 35°C in darkness. The optimal temperature for non-salt treated seeds was 15°C with a germination percentage of 97% (Figures 1 and 2). This temperature corresponds to the ecological adaptation of natural habitats of *H. scoparium*, in which seeds mature at the end of October. Seed germination starts following rains in early spring. How ever, seed does not germinate in response to rains in summer, when temperatures are at the yearly maximum. Some grasses also show similar sensitivity to

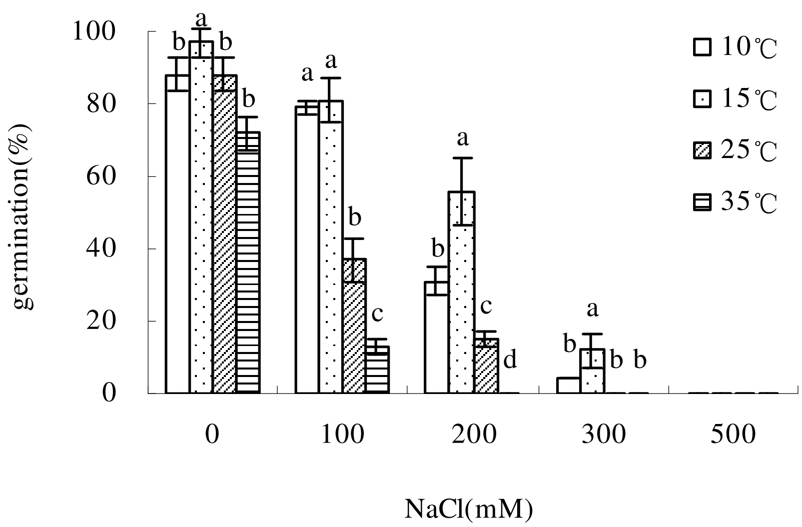

Figure 2. Mean germination percentages of *H. scoparium* seeds at salinity concentrations (0 to 500 mM) and incubation temperatures (10 to 35°C) over 10 days. Values of the germination percentages (mean ± 99% confidence limits, n = 4) with the same letter are not significantly different (p > 0.01) from the control (Duncan's test).

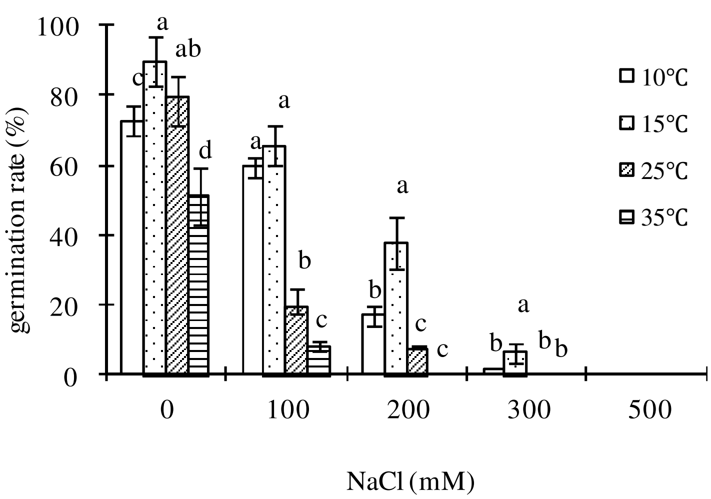

Figure 3. Mean rate of germination of *H. scoparium* seeds at salinity concentrations (0 to 500 mM) and incubation temperatures (10 to 35°C) over 10 days. Values of rate of germination (mean ± 99% confidence limits, n = 4) with the same letter are not significantly different (p > 0.01) from the control (Duncan's test).

change in temperature (Khan and Ungar, 2001; Tlig et al., 2008). Temperature shifts can affect a number of processes determining the germinability of seeds, including membrane permeability and cytosolic enzymes (Khan et al., 2002).

The highest germination percentage was in the non-saline control and variation in temperature substantially affected germination in both saline and non-saline treatments (Figure 2). Similar results were reported for *Prosopis juliflora* (El-Keblawy and Al-Rawai, 2005) and *Panicum turgidum* (Al-Khateeb, 2006). Slow seed germination under salt-stressed when compared with non-stressed conditions, however, could be due to osmotic and/or ionic effects of the saline germination medium (Ghavami and Ramin., 2007). Our results indicated that salt stress decreased the germination and delayed the emergence of *H. scoparium* seeds. Similar results were reported for *Spartidium saharea* (Zammouri

Table 2. Recovery germination percentage of *H. scoparium* seeds after transfer from 100, 200, 300 and 500 mM NaCl to distilled water at temperatures of 10, 15, 25 and 35°C.

NaCl (mM)	Temperature (°C)			
	10	15	25	35
100	58 (5.0)[b]	31 (4.2)[c]	59 (4.7)[b]	56 (3.4)[a]
200	71 (5.5)[a]	59 (3.6)[b]	67 (4.0)[a]	0 (0.0)[b]
300	64 (2.4)[ab]	72 (1.8)[a]	36 (3.3)[c]	0 (0.0)[b]
500	23 (2.0)[c]	23 (3.8)[d]	0 (0.0)[d]	0 (0.0)[b]

Values in parenthesis denote ± SD. Numbers with the same letters are not significantly different at 1%.

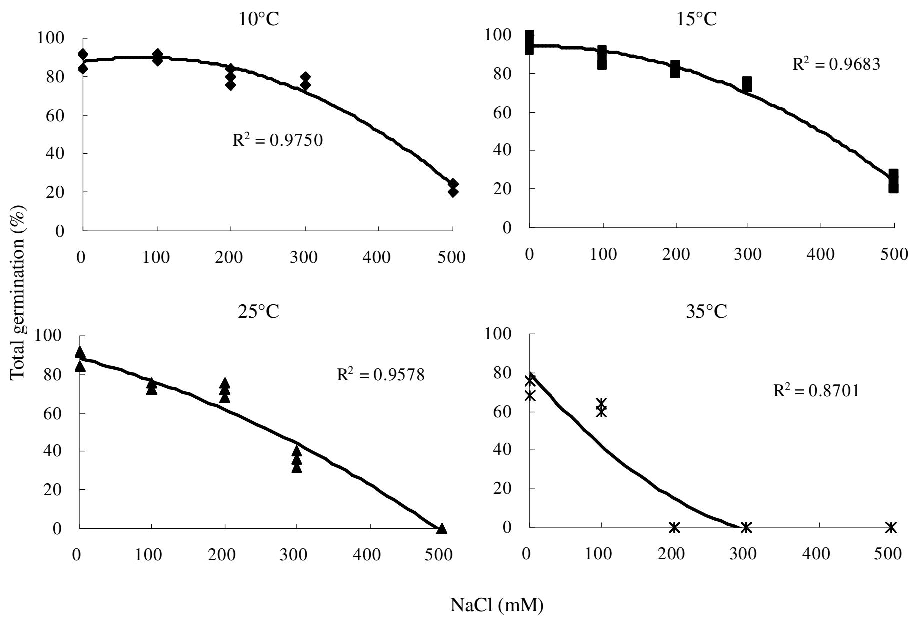

Figure 4. Regression plots for mean total germination percentages of *H. scoparium* seeds at salinity concentrations (0 to 500 mM) and incubation temperatures (10 to 35°C). Lines describing the evolution of each parameter were obtained using fitted polynomial regression. Values (n = 20) are from the five treatments with four replicates.

et al., 2010). It is also assumed that in addition to toxic effects, higher concentration of salt reduces the water potential in the medium, which hinders water absorption by germinating seeds and thus reduces germination (Zammouri et al., 2010). The initial establishment of species in deserts is related to the germination response of seeds to salinity and temperature, and early establishment usually determines whether a population will survive to maturity (Huang et al., 2003; Song et al., 2005). Seeds incubated at high temperatures with high NaCl concentrations seemed to be subjected to heavy stress, as indicated by delayed germination. Under such conditions, changes in the incubation temperature particularly under high salt concentration, may result in malfunctioning of enzyme systems and limit many physiological processes vital to seed germination (Al-Khateeb, 2006). The detrimental effect of salinity on the germination of *H. scoparium* decreased the rate and percentage of germination (Figures 2 and 3). This result substantiates several other studies that showed that halophytes and glycophytes, are especially sensitive to salt during the germination stage (Khan et al., 2002; Tobe et al., 2001).

Some halophyte seeds retain their ability to germinate while experiencing high salinities and will germinate when soil salinity levels are reduced (Gul and Weber, 1999). At 500 mM NaCl, there was no recovery of germination at 25 and 35°C, and germination was 0% (Table 2); this can be attributed to ionic and osmotic effects (Song et al., 2005). Imbibition is an essential prerequisite for germination, and its rate and extent is controlled by the surrounding soil water potential and the resistance to movement of water in the soil-seed system (Evans and Etherington, 1990). Seed imbibition rate, germination percentage and germination rate generally decrease as soil water potential decreases (Evans and Etherington, 1990), either by drought or by higher salinity. At 0 to 200 mM NaCl, the total germination of *H. scoparium* reached 72 to 97% at 10 to 25°C. This indicated that the seeds tolerated moderate salinity, and if substrate salinity was reduced at an appropriate temperature, they were able to germinate (Figure 4).

Desertification is one of the most important ecological problems in arid and semiarid areas of northwest China and results in degradation of the soil and vegetation cover. *H. scoparium* (as a legume) is a good candidate for soil-restoration projects, as it can prevent erosion, increase soil fertility by enhancing soil nitrogen content and organic matter and contribute to soil stabilization and ecosystem restoration (Jia, 1987; Wu et al., 1998). These results show that *H. scoparium* seeds had maximum germination at 15°C in darkness for all NaCl concentrations tested. Exposure to high saline concentrations (500 mM) and high temperatures (35°C) completely inhibited the germination of *H. scoparium*. Salt stress decreased both rate and percentage of germination, but recovery was possible when salinity was low. Further investigations are necessary to understand the eco-physiological strategies of plants for survival under natural environmental conditions.

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