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Influence of Salinity and Temperature on the Germination of *Haloxylon ammodendron* and *Ceratoides arborescens*

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Abstract: The effects of salinity and temperature on germination of *Haloxylon ammodendron* and *Ceratoides arborescens* were investigated in arid, saline areas in the North West of China. Seeds were exposed to salt solutions containing 0-700 mM (mmol L^{-1}) of NaCl and at constant temperatures of 10, 15, 25 and 35°C (in the dark). After seeds had been thus treated for 7 days, any ungerminated seeds were transferred to distilled water for a further 7 days to investigate the recovery of germinability. The data indicate that the germination percentage of two species was not affected by relatively low salinities at 10, 15, 25 and 35°C but that it was severely inhibited at high salinity; the optimum temperature for germination was 15°C for two species; recovery germination was significantly higher from high salinity than from low salinity for *H. ammodendron* and *C. arborescens* at 10-25°C, *H. ammodendron* seeds are able to maintain their viability during exposure to high NaCl (700 mM) and high temperatures (35°C) and to recover their germination after transfer to distilled water; germination of *C. arborescens* seeds is permanently inhibited after exposure to high NaCl and to high temperature.

Key words: Xerophyte, salt stress, NaCl, recovery germination, desert, China

INTRODUCTION

Arid and semiarid regions (deserts) cover about 30% of the world's continents (except Europe) (Meigs, 1953) and are essentially regions of low and irregular rainfall in which evapotranspiration greatly exceeds precipitation (Kigel, 1995). In these areas, salinization is a serious problem. Germination is a crucial stage in the life cycle of individuals inhabiting in arid, saline environments as it determines whether or not the populations could establish successfully (Ungar, 1995; Khan and Gulzar, 2003; Mokhberdoran *et al*., 2009; Zafar *et al*., 2005).

Soil temperature and salinity are two especially important factors that control when and where seeds can germinate in these arid, saline regions (Khan and Ungar, 1999). They can also interact in determining salinity tolerance during germination (Khan *et al*., 2000). *Haloxylon ammodendron* (C.A. Mey.) Bunge is a stem-succulent xero-halophytic shrub, it occurs in saline and non-saline soils in sandy deserts. *Ceratoides arborescens* (Losinsk.) Tsien et C.G. Ma is a xerophytic semi-shrub is a native species of China and distributed on non-saline and sandy deserts. The two species are common in the drought-prone, sandy deserts of North West China. These species have traditionally been used as feed for livestock, to maintain water and soil, for firewood and to stabilize sand dunes therefore they are crucial species for protecting desert regions. They are of high economic and ecological value (Li *et al*., 2006; Zhao and Li, 1999; Jia, 1987).

This study was conducted to better understand the seed germination requirements of the two species. There is some information on the effect of salinity and temperature on the germination of halophytic species (Zia and Khan, 2004) but little information is available on the effect of salinity and temperature on the germination of *H. ammodendron* and *C. arborescens*. The purpose of this investigation was to determine how temperature and salinity interact to effect germination in these two xerophytic species.

MATERIALS AND METHODS

Seed collection: Seeds of *H. ammodendron* (seed weight: 3.826±0.070 g/1000 seeds; n = 5) and *C. arborescens* (seed weight: 1.880±0.043 g/1000 seeds; n = 5) (located at

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40°49′N; 110°49′E) were collected from plants growing in sandy deserts in Inner Mongolia and Gansu, the Northwestern provinces of China in October 2006. Dry seeds were stored at 4°C before being used in the germination experiments.

Effects of temperature and salinity on germination: To avoid fungal attack, seeds were surface sterilised in 0.58% sodium hypochlorite solution for 1 min then washed with distilled water and air dried before being used (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. The constant temperatures were 10, 15, 25 or 35°C. 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 containing 0 (control), 100, 300, 500 and 700 mM of NaCl under these temperatures. For each treatment, four replicates of 50 seeds each were used for *H. ammodendron* and *C. arborescens*. During the 7 days (because seeds germinated rapidly in distilled water and the germination percentage approached 85% during the 3 days) treatments, the solution in each petri dish was renewed every 2 days and any germinated seeds were recorded and removed every days. Seeds were considered to have germinated when the radicle had emerged (Song *et al*., 2006).

After 7 days, any ungerminated seeds from the NaCl treatments were transferred to distilled water to study the recovery of germination with germination being recorded every day for 7 days. Recovery germination was determined after 7 days of incubation using the equation:

$$\left(\frac{a-b}{c-b}\right)\times 100$$

Where:

a = The number of seeds germinating in salt solution plus those that recovered germination in distilled water

b = The total number of seeds germinating in salt solution

c = The total number of seeds tested

Total germination (%) was recorded as a/c×100 (Qu *et al*., 2008). The experimental conditions were described earlier.

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 analysed using SPSS for Windows, Version 11.5 (SPSS, 2002). One-way or two-way Analysis of Variance (ANOVA; $p<0.01$) was used to demonstrate the significance of salinity and temperature effects and their interaction in determining the germination percentage. If ANOVA showed a significant effect, the Duncan test was used to determine differences among treatments.

RESULTS AND DISCUSSION

Effects of temperature and salinity on germination: Germination for *H. ammodendron* and *C. arborescens* seed was significantly affected by salinity, temperature and their interaction ($p<0.01$) (Fig. 1a and b and Table 1).

The optimal temperature for germination was 15°C for two species in all NaCl concentrations. There were no significant differences in germination of *H. ammodendron* in the low-salinity treatments (0, 100, 300 and 500 mM NaCl) and all attained significantly greater values than the higher concentration ones (700 mM NaCl) at 10-25°C. At 15°C, the germination at the higher salinity (700 mM NaCl) was significantly greater than at other temperatures (10, 25 and 35°C). At 700 mM NaCl, germination was significantly lower than those at other salinities (0, 100, 300 and 500 mM NaCl) (Fig. 1a).

Seed of *C. arborescens* was able to germinate at temperatures between 10 and 35°C. There were no significant differences in germination at the low salinities (0, 100 and 300 mM NaCl) with all low-salinity treatments attaining significantly higher germination percentages than the higher ones (500 and 700 mM NaCl) at 10-25°C. At 15°C, germination in the higher salinity treatment (700 mM NaCl) was significantly higher than at other temperatures (10, 25 and 35°C). At 35°C, germination in the higher salinity treatments (500 and 700 mM NaCl) was significantly lower than at other temperatures (10, 15 and 25°C) (Fig. 1b; Table 1).

Recovery germination after ungerminated seeds were transferred to distilled water: Recovery germination after transfer of ungerminated seeds from solutions of different salinities to distilled water is shown in Fig. 1c and d for *H. ammodendron* and *C. arborescens*. Temperature and salinity has significant ($p<0.01$) effect on the recovery of seed germination (Table 1). Recovery germination was significantly higher at 15°C and significantly lower at 35°C than at other temperatures in 500 mM NaCl for *C. arborescens* and there was no recovery of germination in the lower NaCl concentrations at any temperature and there was recovery of germination from the higher NaCl concentrations for *H. ammodendron* or for *C. arborescens*

Table 1: Results of two-way ANOVA of interaction characteristics (F-values) for germination of seeds of *C. arborescens* and *H. ammodendron* in saline solutions of different concentrations over 7 days. Also, for recovery germination and for total germination after ungerminated seeds from the saline solutions were transferred to Distilled Water (DW) for 7 days

Species	Independent variable	Salinity[a]	Temperature[b]	Salinity x Temperature
C. arborescens	Germination (saline)	854.7**	49.9**	27.7**
	Recovery germination (DW)	1228.9**	686.9**	264.7**
	Total germination	335.0**	246.3**	95.6**
H. ammodendron	Germination (saline)	272.3**	144.5**	34.8**
	Recovery germination (DW)	7884.6**	47.5**	40.0**
	Total germination	16.8**	95.6**	21.7**

**Significant difference at $p<0.01$. [a]Germination salinity at 0, 100, 300, 500, 700 mM. [b]Germination test at 10, 15, 25 or 35°C (in dark)

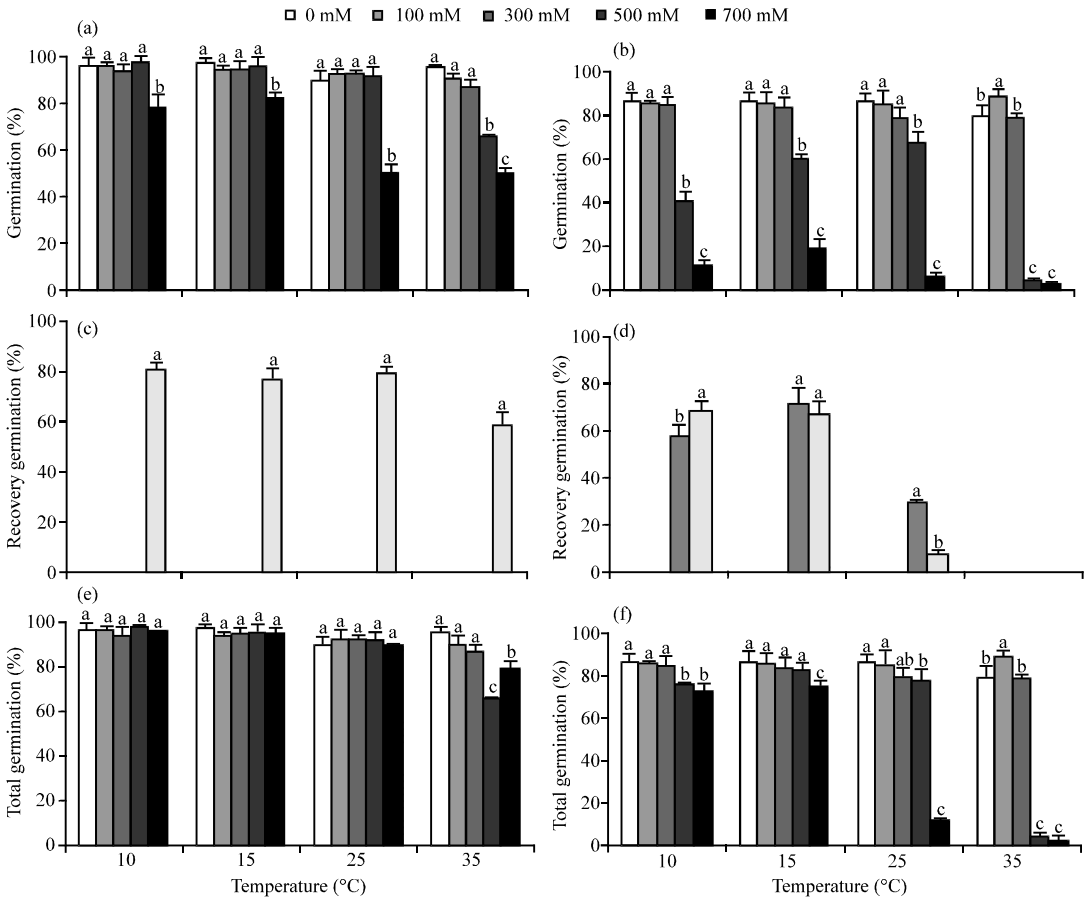

Fig. 1: a, b) Seed germination when seeds of *H. ammodendron* and *C. arborescens* were moistened with 0, 100, 300, 500 and 700 mM NaCl concentrations at constant 10, 15, 25 and 35°C in continuous darkness for 7 days; c, d) recovery germination of *H. ammodendron* and *C. arborescens* after ungerminated seeds moistened in NaCl solution at constant 10, 15, 25 and 35°C in continuous darkness for 7 days were transferred to distilled water for 7 days and e, f) total germination of *H. ammodendron* and *C. arborescens* seeds in 0, 100, 300, 500 and 700 mM NaCl at constant 10, 15, 25 and 35°C in continuous darkness. Means at the same temperature that have the same letters are not significantly different at $p<0.01$

(Fig. 1c and d). For *H. ammodendron* there was relatively a higher recovery in the high-salinity treatments (700 mM NaCl) at all temperatures (Fig. 1c).

Total germination: Two-way ANOVA showed significant effects for temperature, salinity and their interaction on total germination (i.e., summation of germination in salts and after recovery) ($p<0.01$) (Table 1). For *H. ammodendron* and *C. arborescens* the total germination for the low NaCl concentration treatments was significantly higher than for the high NaCl concentration treatments (Fig. 1e and f). For *H. ammodendron* and

C. arborescens in 500 and 700 mM NaCl solutions, total germination was significantly lower at 35°C than for the other salinities (Fig. 1e and f and Table 1). There were no significant differences between total germination at 10, 15 and 25°C and all germination percentages attained significantly greater values than at the highest temperature (35°C) for *H. ammodendron* (Fig. 1e).

In desert habitats, favourable conditions for germination tend to be highly unpredictable over space and time (Rubio-Casal *et al*., 2003). Xerophytic species can differ in their life cycles in responses to drought in seed dispersal and in germination behavior (Kigel, 1995). In the life cycle of most plants, it is the seeds that have the highest resistance to extreme environmental stress and this is especially true for xerophytic species (Gutterman, 1993). Therefore, successful establishment of a plant population is heavily dependent on the adaptability of seed germination (Qu *et al*., 2008).

The results show that the optimal temperature for germination of *H. ammodendron* is 15°C in Wuwei, Gansu (Fig. 1a) but the results are not consistent with Huang *et al*. (2003) because the seeds experienced different environments, the range of temperatures is 3.2-15.7°C in Spring in Wuwei, Gansu province (Gansu Meteorological Bureau, 1965), germination starts from late spring when the snow starts to melt. Optimal germination for *C. arborescens* occurred in 15°C (Fig. 1b) and similar results were reported for C. laten and C. compact (Wang and Yi, 2003). Perhaps this behavior is related to environment. *C. arborescens*, germinates in early spring in Inner Mongolia. *Ceratoides lanata* is adapted to germinate under the subzero temperature conditions of early Spring in the Northern Great Plains of North America. In this area, a 10-15°C diurnal fluctuation is common causing snow melt by day and refreezing at night (Wang *et al*., 2006; Bai *et al*., 1998).

Seeds of some halophytes have been found to retain their ability to germinate under high salinities and germinate when soil salinity levels are reduced (Gul and Weber, 1999). The seeds of *H. ammodendron* exposed to lower salinity concentrations (100-500 mM NaCl) did not exhibit recovery germination but recovery germination was >58-80% after exposure to high salinities (Fig. 1c). Similar results were reported by Huang *et al*. (2003) and Khan and Ungar (1996). Recovery germination was significantly higher from high salinity solutions than from low salinity solutions for *C. arborescens* (Fig. 1d). However, germination of some species is permanently in inhibited by high salinity (Khan and Ungar, 1997). This indicates that in natural habitats and at moderate temperatures, seeds of *H. ammodendron* and *C. arborescens* can remain in the soil under field conditions when salinity concentrations are beyond their tolerance limits but still germinate during the snow-melt period when salt levels decrease.

Several factors such as salinity, temperature, water, light and their interactions regulate germination at the soil interface (Ungar, 1995). The results indicate that interactions between salinity and temperature affect germination for *H. ammodendron* and *C. arborescens* (Table 1). Song *et al*. (2006) reported that germination of *Kalidium foliatum* and *Halocnemum strobilaceum* was significantly inhibited at high salinity (500 mM NaCl) and high temperature (30 and 35°C). Germination of *H. ammodendron* and *C. arborescens* was significantly inhibited at high temperatures (35°C) and high salinity (500 and 700 mM NaCl). The seeds of *H. ammodendron* clearly did not lose viability under high NaCl and high temperature, as these had high total germination at 700 mM NaCl and 35°C (Fig. 1e). Whereas seeds of *C. arborescens* lost viability under high NaCl and high temperatures because these had much lower germinations (3%) (Fig. 1f). Therefore, high salinity and high temperature inhibits germination of *H. ammodendron* but seeds can germinate when salinity concentrations are reduced. Meanwhile germination of *C. arborescens* was inhibited permanently and these clearly have different germination strategies in arid and saline environments.

The seeds of *Haloxylon ammodendron* and *Ceratoides arborescens* growing in arid, saline deserts in the northwest of China usually mature in October or November (Jia, 1987; Huang *et al*., 2001) when temperatures are below 0°C and thus too low for germination. In the arid region of Northwest of China, low soil salinity usually occurs in spring due to snow melt and low evaporation whereas in summer strong irradiance and low humidity results in rapid evaporative loss from the surface, upward capillary movement of soil water and thus salt accumulation at or near the soil surface (Kigel, 1995).

CONCLUSION

This study shows that the germination of seeds lying in the upper soil layers after rain events is frequently limited in desert environments by rapid desiccation and high salinity.

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