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Short communication

Germination responses of *Medicago ruthenica* seeds to salinity, alkalinity, and temperature

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ABSTRACT

To determine the effects of and the interactions between salinity and temperature, and alkalinity and temperature, we conducted three germination tests of *Medicago ruthenica* (L.) Sojak cv. 'Zhilixing' seeds in growth chambers. In experiment 1, the seeds were placed at a range of temperatures (5, 10, 15, 20, 25, 30, 35, or 40 °C), and in experiments 2 and 3, we varied the salinity (0, 50, 100, 150, 200, or 250 mM NaCl) and alkalinity (1, 5, 10, 15, 20, or 25 mM Na_2CO_3), respectively, within three ranges of alternating temperatures (10–20, 15–25, and 20–30 °C). The seeds of *M. ruthenica* (L.) Sojak cv. 'Zhilixing' showed high percentage germination at all temperatures, except for 5 °C. Seeds germinated well at low NaCl and Na_2CO_3 concentrations under all three alternating temperature regimes. Approximately half the seeds germinated at high salinity and alkalinity at 15–25 °C, whereas the ungerminated seeds germinated when the high-saline and alkaline stresses were removed. These results suggest that *M. ruthenica* (L.) Sojak cv. 'Zhilixing' has potential utility as a forage legume in saline and alkaline environments.

1. Introduction

Medicago ruthenica (L.) Sojak cv. 'Zhilixing', a perennial legume species widely distributed in Siberia, Mongolia, and northern China, commonly grows on open hillsides, mixed grass steppes, and meadows (Small and Jomphe, 1989; Shi, 2006). This species is tolerant of drought, high salinity, and alkaline environments, and is therefore regarded as a promising legume for forage in arid and semi-arid areas. The Songnen Grassland, located in northeast China (44.3°N, 124.3°E), is currently experiencing severe problems with both salinity and alkalinity, resulting from human activities such as overgrazing (Zhou and Ripley, 1997). The introduction of *M. ruthenica* into this region is proposed as a strategy to improve plant and soil nitrogen levels, and to promote the restoration of regions of the Songnen Grassland. A critical first step in evaluating the potential value of this species in such restoration is to establish how its germination will be affected by probable abiotic conditions.

Seed germination is the initial and most crucial stage in the life cycle of plants (Grime and Campbell, 1991). Seed germination is affected by many biotic and abiotic factors, such as temperature, salt, light, water, oxygen concentration, and alkalinity. Temperature, salinity, and alkalinity are the main limiting factors in the germination of many species in the Songnen Grassland (Lin and Tang, 2005).

Temperature plays a major role in determining the periodicity of seed germination and the distributions of species (Baskin and Baskin, 1988). Salinity stress affects seed germination either through osmotic effects, by preventing or delaying germination (Welbaum et al., 1990), or through ion toxicity, which can render the seeds unviable (Huang and Reddman, 1995). Although high salinity may inhibit germination, the detrimental effect of salinity may itself depend upon the germination temperature (Rivers and Weber, 1971; Badger and Ungar, 1989).

High levels of alkalinity can also be a limiting factor in seed germination (Shi et al., 1998), although this has received less attention in the literature. Importantly, alkaline and neutral salts exert distinct kinds of stress on seedling growth, and plants may respond differently to each (Shi and Yin, 1993). The combined effects of temperature and increased alkalinity on germination are unknown. A better understanding of the germination processes of *M. ruthenica* should facilitate the effective utilization of this species.

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The ability to maintain seed viability during exposure to either saline or alkaline conditions, and to commence germination when such stresses are reduced or removed, is one of the mechanisms by which saline- and alkaline-tolerant species persist under extreme abiotic conditions (Ungar, 1982; Keiffer and Ungar, 1995). The germination recovery test is often used to determine whether seeds are killed or merely their germination is prevented by saline or alkaline stress.

The aims of this study were (1) to measure the responses of *M. ruthenica* to a range of temperature regimes and to determine the thermal time and minimum temperature for germination; (2) to investigate the response of seed germination to saline and alkaline stresses; and (3) to determine whether altered temperatures change the effects of saline or alkaline stress on germination.

2. Materials and methods

2.1. Seed materials and storage

Seeds of *M. ruthenica* were collected from Songnen Plain in autumn 2005 and were stored in paper bags at room temperature (20 ± 2 °C) before their use in April 2006. The seeds were soaked in sulfuric acid for 20 min to break the hard coat and then rinsed in distilled water for 4 h to wash away any remaining sulfuric acid.

2.2. Overview of the experiments

2.2.1. Experiment 1, temperature test

Seeds were germinated in distilled water under eight constant temperature regimes of 5, 10, 15, 20, 25, 30, 35, or 40 °C. The temperatures correspond to the average temperatures of the Songnen Grassland from spring to early autumn.

2.2.2. Experiment 2, salinity and temperature test

Six salinity concentrations (0, 50, 100, 150, 200, and 250 mM NaCl) were used in combination with three alternating temperature regimes (10–20, 15–25, 20–30 °C). The average salt content of the Songnen Grassland is 0.7–1% (Lu et al., 1998), which is equal to 120–171 mM NaCl.

2.2.3. Experiment 3, alkalinity and temperature test

Six alkali concentrations (1, 5, 10, 15, 20, and 25 mM Na_2CO_3) were used in combination with three alternating temperature regimes (10–20, 15–25, 20–30 °C).

2.3. Experimental methods

Germination tests were carried out in growth chambers (HPG-400, Haerbin, China) with a 16 h photoperiod (Sylvania cool white fluorescent lamps, 200 μmol m^{-2} s^{-1}, 400–700 nm). The seeds were germinated in Petri dishes (10 cm diameter) containing two layers of filter paper with 12 mL of test solution. Four replicates of 30 seeds each were used for each treatment. The seeds were considered to have germinated after radicle emergence. Germination was recorded daily for 7 d.

The rate of germination was estimated using a modified Timson index of germination velocity $= \sum G/t$, where G is the percentage seed germination at one-day intervals and t is the total germination period (Khan and Ungar, 1984). The maximum possible value of this index is 100 (i.e., 700/7). The greater the value, the more rapid the germination. After 7 d, the ungerminated seeds from the NaCl and Na_2CO_3 treatments at 15–25 °C were transferred to distilled water and exposed to 15–25 °C again to measure the recovery of seed germination, which was also recorded daily for 7 d.

2.4. Data analysis

Germination data were arcsine transformed before analysis of variance (ANOVA). The data were analysed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Two-way ANOVA was used to test the effects of the main factors (salinity, alkalinity, temperature) and their interactions (salinity and temperature, alkalinity and temperature) on the final percentage germination and the rate of germination. The thermal time and minimum temperature were calculated according to the following equation, which describes the germination times across a range of temperature regimes:

$$S = (T - T_b)t;$$

where S is the thermal time, T is the ambient temperature, T_b is the minimum temperature, and t is the germination time in days (Bierhuizen and Wagenvoort, 1974).

3. Results and discussion

Overall, *M. ruthenica* exhibited a high percentage germination from 10 to 40 °C. The rate of germination increased with increasing temperature, then decreased at temperatures above 35 °C (Fig. 1). This indicates that the seeds of *M. ruthenica* germinate under a wide range of temperatures. At 5 °C, the percentage germination and rate of germination were significantly lower than those at other

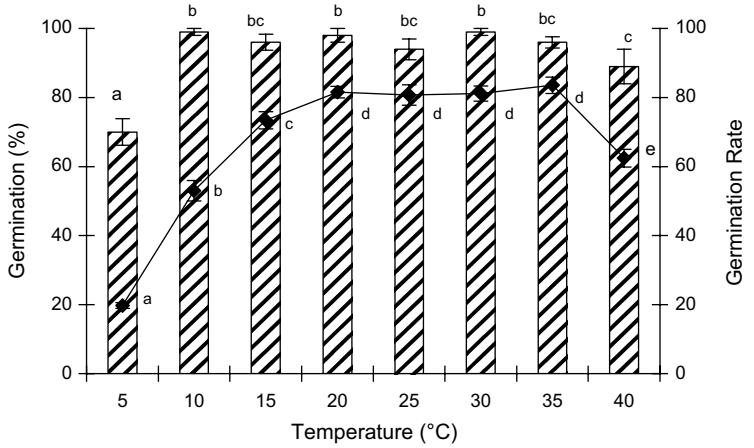

Fig. 1. Final germination percentage (columns) and germination rate (points) of *M. ruthenica* seeds at 5, 10, 15, 20, 25, 30, 35, 40 °C. Bars represent ±S.E. ($n = 4$). Different letters indicate significant differences between temperatures ($P < 0.05$).

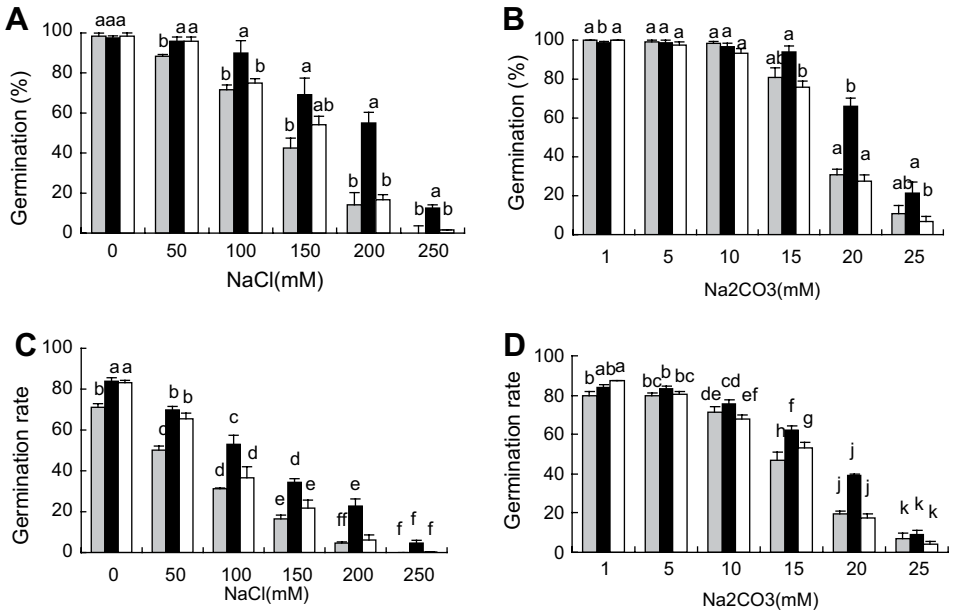

Fig. 2. Final germination percentage (A, B) and rate of germination (C, D) of *M. ruthenica* seeds in NaCl (A, C) and Na_2CO_3 (B, D) solutions at temperatures of 10–20 (■), 15–25 (■), 20–30 °C (□). Bars represent ±S.E. ($n = 4$). Different letters indicate significant differences from each other ($P < 0.05$).

temperatures. Even though the seeds imbibed water at this temperature, germination was probably prevented by chilling injury to the embryos, metabolic inactivation, or dormancy induced in the seeds (Bradbeer, 1988). The thermal time and minimum temperature required for *M. ruthenica* seeds to germinate were calculated in this study (42.76 °C. d and 0.062 °C, respectively) and could be important information to assist both farmers and researchers in making management decisions based on the relationship between germination time and temperature.

Germination was significantly affected by salinity. There was a strong interaction between salinity and temperature (Table 1, Fig. 2A and C). *M. ruthenica* showed maximum germination in the nonsaline control treatment. Seeds in lower concentrations of NaCl (50 and 100 mM) also showed a high percentage germination, similar to or slightly lower than that of seeds in distilled water (Fig. 2A). Under high salinity stress, such as 200 mM NaCl, the percentage germination reached 55% at 15–25 °C. The highest rate of germination was observed at 15–25 °C (Fig. 2C), whereas the negative effects of high NaCl concentrations were stronger at 10–20 and 20–30 °C. The recovery experiment showed that seeds exposed to a high NaCl concentration (250 mM) for a short period (7 d) germinated well after transfer to distilled water (89%). Under high-saline conditions, seed survival rather than germination is the more appropriate mechanism for plants to establish successfully, because the recovery of germination occurs when the high-saline conditions are alleviated (Khan and Ungar, 1996), as might occur during a rainy period.

A similar trend to that of salinity was observed for the alkalinity treatments. The germination of *M. ruthenica* was significantly affected by alkalinity and its interaction with temperature (Table 1, Fig. 2B and D). However, the percentage germination in NaCl was higher than that in Na_2CO_3, even though the concentrations of Na_2CO_3 were much lower than those of NaCl. Solutions of 50 mM NaCl and 25 mM Na_2CO_3 have same concentrations of Na^+, but the percentage germination in 25 mM Na_2CO_3 was significantly lower than that in 50 mM NaCl under all temperature regimes (Fig. 2A and B). This indicates that CO_3^{2-} was more harmful to *M. ruthenica* than was Cl^- (Shi and Yin, 1993). The effect of CO_3^{2-} or the pH effect could explain the damaging effect of Na_2CO_3 on seed germination (Campbell and Nishio, 2000). The pH value of the soil where *M. ruthenica* grows on the Songnen Grassland is usually more than 8.5 (unpublished data, by Song). About 66% of the seeds germinated in 20 mM Na_2CO_3 (pH 10.1) at 15–25 °C, whereas 27% of the seeds germinated at 10–20 and 20–30 °C. Because 50% of the seeds germinated after transfer from 10 mM Na_2CO_3, the seeds remained viable during their exposure to an intermediate alkaline concentration for a short period (7 d), and commenced germination when the alkalinity stress was reduced or removed. These results suggest

Table 1
Two-way ANOVA for salinity, alkalinity, temperature and their interactions

Independent variable	Germination percentage			Rate of germination		
	df	Mean-square	*F*-ratio	df	Mean-square	*F*-ratio
Experiment 2						
Salinity	5	1.68	285.63	5	1.08	428.25
Temperature	2	0.20	33.86	2	0.15	60.10
Salinity × temperature	10	0.03	5.00	10	0.01	3.20
Experiment 3						
Alkalinity	5	1.42	476.84	5	1.08	625.29
Temperature	2	0.08	27.70	2	0.04	22.65
Alkalinity × temperature	10	0.02	7.88	10	0.01	4.79

All factors are significant at $P < 0.001$ level.

that *M. ruthenica* could be recruited for the alkaline conditions in the Songnen Grassland at suitable temperatures.

We conclude that *M. ruthenica* can grow well at lower NaCl and Na_2CO_3 concentrations under all the temperature regimes tested. Approximately half the seeds also germinated under high salinity or alkalinity at 15–25 °C. The ungerminated seeds remained viable during their exposure to saline or alkaline stress for a short period. Given that *M. ruthenica* has several desirable forage characteristics, such as an ability to fix nitrogen, to increase soil fertility, and to provide nutritive feed for livestock, we suggest that *M. ruthenica* is a promising legume species for use in the recovery of the Songnen Grassland, which is under serious threat of degradation.

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