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Characteristics of seed germination in five non-halophytic Chinese desert shrub species

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The effects of temperature, NaCl, and polyethylene glycol (PEG)-6000 on the seed germination of five non-halophytic Chinese desert shrub species were investigated. The minimum temperature of germination was 10°C for all species, and the maximum temperature varied among species from 25 to 35°C. Isotonic solutions of NaCl and PEG caused different effects on seed germination in all five species. When seeds were moistened with −5·0 MPa NaCl solution for 5 days, the seeds of three species completely lost their germinability, while the seed germinability of the other two species was much less affected. These results are discussed with regard to the rehabilitation of desertified lands in China by seed dispersal.

Keywords: China; desertification; germination; non-halophyte; rehabilitation; salt; salinization; shrub

Introduction

Desertification is a serious problem in arid and semi-arid regions in China (Zha & Gao, 1997). One important strategy to rehabilitate vegetation in desertified lands is the dispersal of seeds, which is labour-saving when compared to transplanting nursery seedlings. In China, dispersal of seeds with aircraft has long been the method used to rehabilitate vegetation in desertified lands (Qi, 1998).

One important process of desertification is soil salinization. There is often a need to rehabilitate vegetation in salinazed lands by introducing agronomically important non-halophytic species (Zhou *et al.*, 1993). If seed dispersal is the method to be used, information is needed on how the seeds of these species respond to salinity and other environmental parameters.

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Water availability and temperature are important factors that determine whether or not dispersed seeds germinate. For the seeds to germinate, they must imbibe water under a favourable temperature. However, salts and other solutes in the medium cause osmotic inhibitory effects on the seed's water uptake and retard and/or suppress germination. However, the osmotic effects of solutes in the medium on seeds are mitigated when solutes permeate the seed coat and enter the seeds (Sharma, 1973; Romo & Eddleman, 1985; Tobe *et al.*, 1999, 2000*b*). However, salts entering the seeds can cause toxic effects and reduce germinability (Redmann, 1974; Bal & Chattopadhyay, 1985; Hardegree & Emmerich, 1990; Tobe *et al.*, 1999). To evaluate the specific effects of salts on seed germination, the responses of seeds to salts need to be compared with responses to isotonic high-molecular-weight polyethylene glycol (PEG) (e.g. PEG-6000), which cannot permeate plant cell walls (Carpita *et al.*, 1979) and acts solely as an osmoticum on seeds (Shama, 1973; Redmann, 1974; Hardegree & Emmerich, 1990; Bradford, 1995).

In the present study, the effects of temperature, PEG-6000 and NaCl on seed germination and seed water uptake were investigated among five non-halophytic shrub species distributed in Chinese desert regions. All five tested species serve as livestock feed and relieve sand movement (Lanzhou Institute of Desert Research, 1985). The results obtained from this study present useful basic knowledge which is required in order to rehabilitate desertified lands (including salinized lands) by seed dispersal. Because NaCl is the most abundant salt in salinized lands in China, it was selected for use in this study.

Materials and methods

Seeds of five tested species (Table 1) were collected in non-saline sand dunes in Shapotou, China (37°26′N; 104°57′E). Annual precipitation and annual mean temperature in Shapotou are 10·5°C and 188 mm (means from 1990–1995), respectively. The monthly mean temperature is lower than 5°C from November to March, and ranges between 9 and 25°C from April to October. Precipitation from April to October was 172 mm, which accounted for 91% of annual precipitation. Seeds were collected in November 1994 for *Lespedeza davurica*, June 1994 for *Caragana korshinskii*, November 1994 or 1995 for *Hedysarum scoparium*, July 1994 and September 1995 or 1997 for *Zygophyllum xanthoxylon* and June 1994 for *Atraphaxis bracteata*. Pericarps or pods of the seeds were removed, and the seeds were stored dry at 0–5°C. Experiments were carried out during the period between February 1996 and January 2000.

In all the experiments, the seeds were sown on three layers of filter paper (Toyo, No.1) in petri dishes. The filter paper was moistened with deionized water or a solution

Table 1. *Characteristics of five tested species*

Species	Family	Seed weight (mg)*
Lespedeza davurica (Laxm.) Schindl.	Leguminosae	7.07 ± 1·28
Caragana korshinskii Kom.	Leguminosae	49·9 ± 11·7
Hedysarum scoparium Fisch. et Mey.	Leguminosae	23·4 ± 4·68
Zygophyllum xanthoxylon (Bge.) Maxim.	Zygophyllaceae	13·9 ± 2·90
Atraphaxis bracteata A. Los.	Polygonaceae	5·75 ± 1·21

*Mean ± S.D. ($n = 20$).

of NaCl or PEG-6000 so that approximately two-thirds of the volume of each seed was immersed in the solution. NaCl solutions of known water potential (Ψ_W) were prepared according to Lang (1967). The Ψ_W of PEG solutions was determined by a calibration curve based on isopiestic psychrometer (Boyer & Knipling, 1965) measurements at 20°C with NaCl solutions as standards. The petri dishes were covered with lids and maintained at a constant temperature under continuous darkness in an incubator (MIR-253, Sanyo, Japan). About two-thirds of the volume of water or solution in each dish was replaced daily to avoid changes in the Ψ_W of the solution. In experiments 1–3 (see below), the seeds were considered to have germinated when the radicle length exceeded 5 mm, and germinated seeds were then discarded.

Experiment 1: effects of temperature on seed germination

Fifty (*L. davurica* and *A. bracteata*) or 25 (the other three species) seeds were sown in each petri dish (90 mm inner diameter), moistened with deionized water and incubated at a constant temperature. The experiments performed in the temperature range of 5–35°C were repeated at 5°C intervals. The number of seeds that had germinated in each dish was counted daily for 14 days. The experiment was replicated twice.

Experiment 2: effects of NaCl and PEG solutions on seed germination

The number of seeds sown in each petri dish (90 mm inner diameter) was 17 (*C. korshinskii*), 20 (*H. scoparium* and *Z. xanthoxylon*) or 25 (other two species). The seeds in each dish were moistened with NaCl or PEG-6000 solution with a Ψ_W of 0 to $-2{\cdot}0$ MPa and incubated at 20°C for 12 or 14 days. The number of germinated seeds was counted daily. The experiment was replicated four times.

Experiment 3: effects of pretreatment with an NaCl or PEG solution of low Ψ_W on seed germination

Seeds of each species were moistened with a $-5{\cdot}0$ MPa NaCl or $-5{\cdot}2$ MPa PEG solution for 5 days at 20°C in petri dishes (90 mm inner diameter: the number of seeds in each dish was equal to that in experiment 2). Thereafter, the seeds were lightly washed with deionized water and transferred to other petri dishes, in which they were moistened with deionized water at 20°C. The number of germinated seeds was counted daily for 10 days after transfer. The experiment was replicated four times.

Experiment 4: changes over time in seed water uptake

One seed was sown in each petri dish (50 mm inner diameter) and moistened with deionized water at 20°C. Changes in the weight of the same single seeds were measured for 7 days at different times after moistening. Water uptake by the seeds was evaluated in terms of relative weight gain (W_r) which was calculated as $(w - w_0)/w_0$, where w and w_0 are the seed weights at a given time and just before moistening, respectively. The seeds were regarded to have germinated when radicle protrusion was visibly detectable. The experiment was replicated six to eight times.

Experiment 5: effects of NaCl and PEG solutions of low Ψ_W on seed water uptake

One seed was sown in each dish (25 mm inner diameter) and moistened with an NaCl or PEG solution with a Ψ_W of $-2{\cdot}0$ to $-6{\cdot}0$ MPa for 7 days at 20°C. From the seed weights before and after moistening for 7 days, W_r was calculated for each seed. The experiment was replicated six or eight times for each treatment.

Statistical analysis

The statistical significance of the difference between two values was evaluated by Welch's *t*-test. The values of percentage germination were arcsine transformed before analysis.

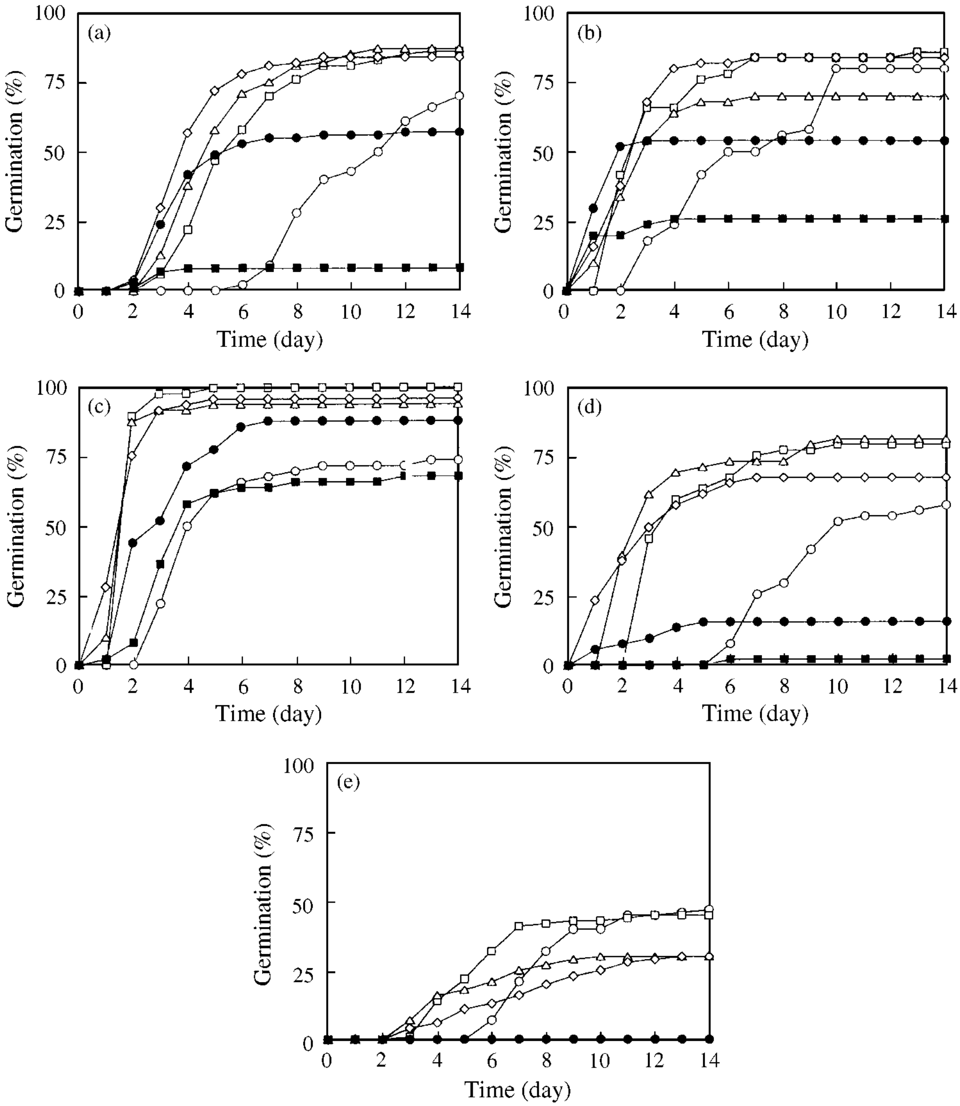

Figure 1. Changes over time in percentage germination at 10°C (○), 15°C (□), 20°C (△), 25°C (◇), 30°C (●) or 35°C (■). (a) *Lespedeza davurica*; (b) *Caragana korshinskii*; (c) *Hedysarum scoparium*; (d) *Zygophyllum xanthoxylon*; (e) *Atraphaxis bracteata*. Each point represents the mean of two replications.

Results

Experiment 1: effects of temperature on seed germination

Final percentage germination exceeded 50% in the temperature ranges of 10–30°C, 10–30°C, 10–35°C and 10–25°C for *L. davurica*, *C. korshinskii*, *H. scoparium* and *Z. xanthoxylon*, respectively (Fig. 1). The final percentage germination of *A. bracteata* (30–47% at 10–25°C and 0% at other tested temperatures) was lower than that of other species. The final percentage germination at 5°C was 4% for *H. scoparium* and 0% for the other four species.

For all five species, germination was considerably more retarded at 10°C than at higher temperatures (Fig. 1). Percentage germination of *C. korshinskii* and *H. scoparium* began to increase within a day after moistening at 20–25°C, while for *L. davurica* and *A. bracteata*, conspicuous increases in percentage germination were detected 3 days after moistening. For *Z. xanthoxylon*, the initiation of seed germination was retarded as the temperature decreased.

Because germination was favourable at 20°C for all tested species, the following experiments were carried out at this temperature.

Experiment 2: effects of NaCl and PEG solutions on seed germination

In both PEG and NaCl treatments, decreases in Ψ_W of the solutions caused retardation of germination (Fig. 2) and decreases in final percentage germination (Fig. 3) for all five tested species. The suppressive effects of PEG on final percentage of germination were greatest in *Z. xanthoxylon* (2·5% at −0·9 MPa) and least in *H. scoparium* (60% at −1·4 MPa). For *C. korshinskii* and *H. scoparium*, the final percentage of germination was larger in PEG than in the isotonic NaCl, while the germination of the other three species was generally more favoured in NaCl than in the isotonic PEG (Fig. 3). Germination was retarded more in PEG than in the isotonic NaCl in all tested species except *H. scoparium* (Fig. 2).

In all tested species, all the emerging radicles elongated until their length exceeded 5 mm, and no visible evidence was detected of any injurious or toxic effects on the radicles from the treatment with NaCl or PEG solutions.

Experiment 3: effects of pretreatment with an NaCl or PEG solution of low Ψ_W on seed germinability

During pretreatment with a −5·0 MPa NaCl or a −5·2 MPa PEG solution, seed germination was completely suppressed in all the five tested species. After PEG pretreatment, seed germinability was significantly reduced ($p = 0{\cdot}05$) only in *C. korshinskii* (Table 2). NaCl pretreatment resulted in almost complete loss of seed germinability in three species (*L. davurica*, *C. korshinskii* and *H. scoparium*), but caused less noticeable effects on the seeds of the other two species.

PEG-pretreated seeds germinated more rapidly than non-pretreated controls in *L. davurica, H. scoparium* and *Z. xanthoxylon.* NaCl pretreatment caused more rapid germination than the control in *Z. xanthoxylon.*

Experiment 4: changes over time in seed water uptake

Figure 4 shows changes over time in W_r after seeds were moistened with deionized water. For most seeds of *L. davurica*, it took more than 1 day after moistening for W_r to start to

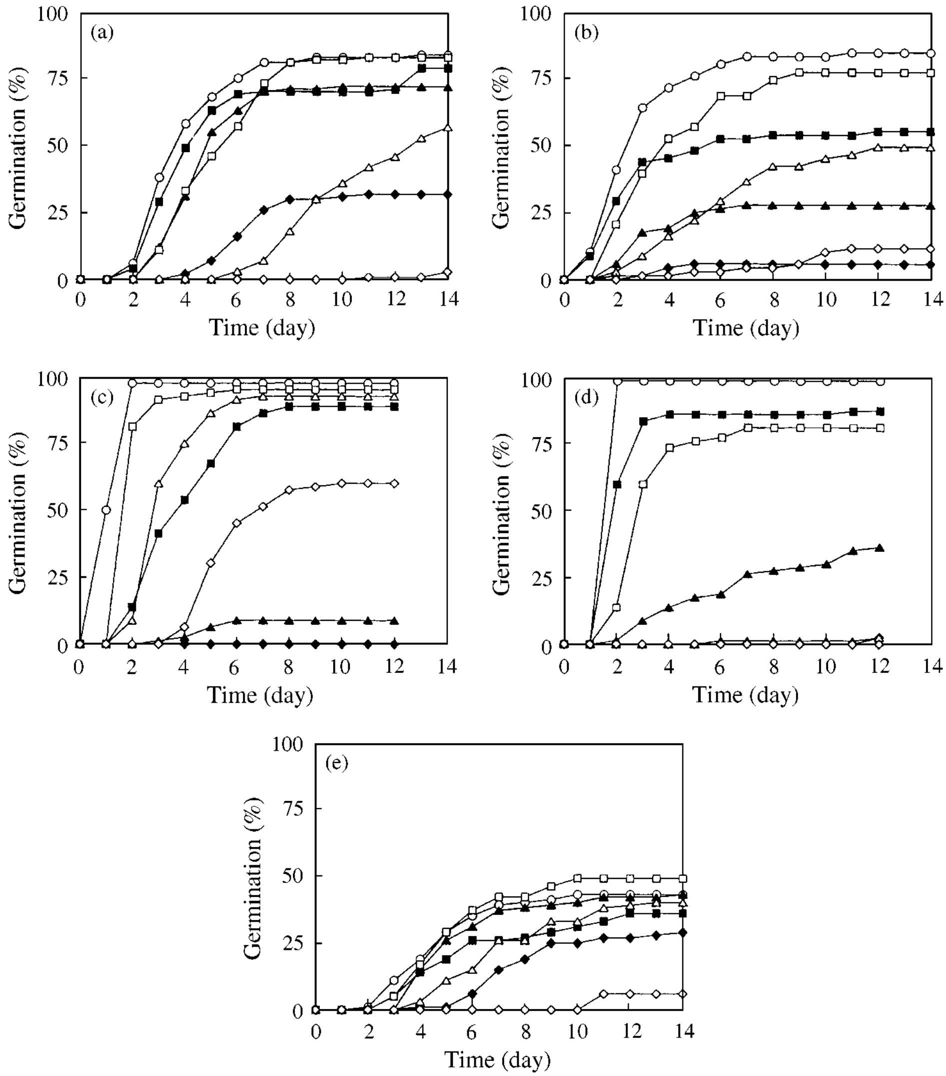

Figure 2. Changes over time in percentage germination when seeds were moistened with deionized water (○) or a solution of NaCl (■ −0·5 MPa; ▲ −1·0 MPa; ◆ −1·5 MPa) or PEG (□ −0·4 MPa; △ −0·9 MPa; ◇ −1·4 MPa). (a) *Lespedeza davurica*; (b) *Caragana korshinskii*; (c) *Hedysarum scoparium*; (d) *Zygophyllum xanthoxylon*; (e) *Atraphaxis bracteata*. Each point represents the mean of four replications.

increase, while seeds of the other four species began to imbibe water within 4 h after moistening. For all tested species, after an initial rapid increase in W_r, the increase in W_r reached a plateau and some seeds germinated. Time taken from the initial rapid imbibition to germination was short for *L. davurica* and *H. scoparium* and long for *A. bracteata*. Germinated seeds showed increases in W_r resulting from water imbibition by the elongating radicles. Some seeds remained ungerminated and W_r remained at a constant W_r similar to the W_r that germinating seeds attained just before the initiation of germination.

The W_r at which seeds began to germinate varied among individual seeds in *C. korshinskii* (1·3–1·9) and *A. bracteata* (0·7–1·3), but was relatively uniform in *L. davurica* (1·2–1·3), *H. scoparium* (1·1–1·3) and *Z. xanthoxylon* (0·9–1·0).

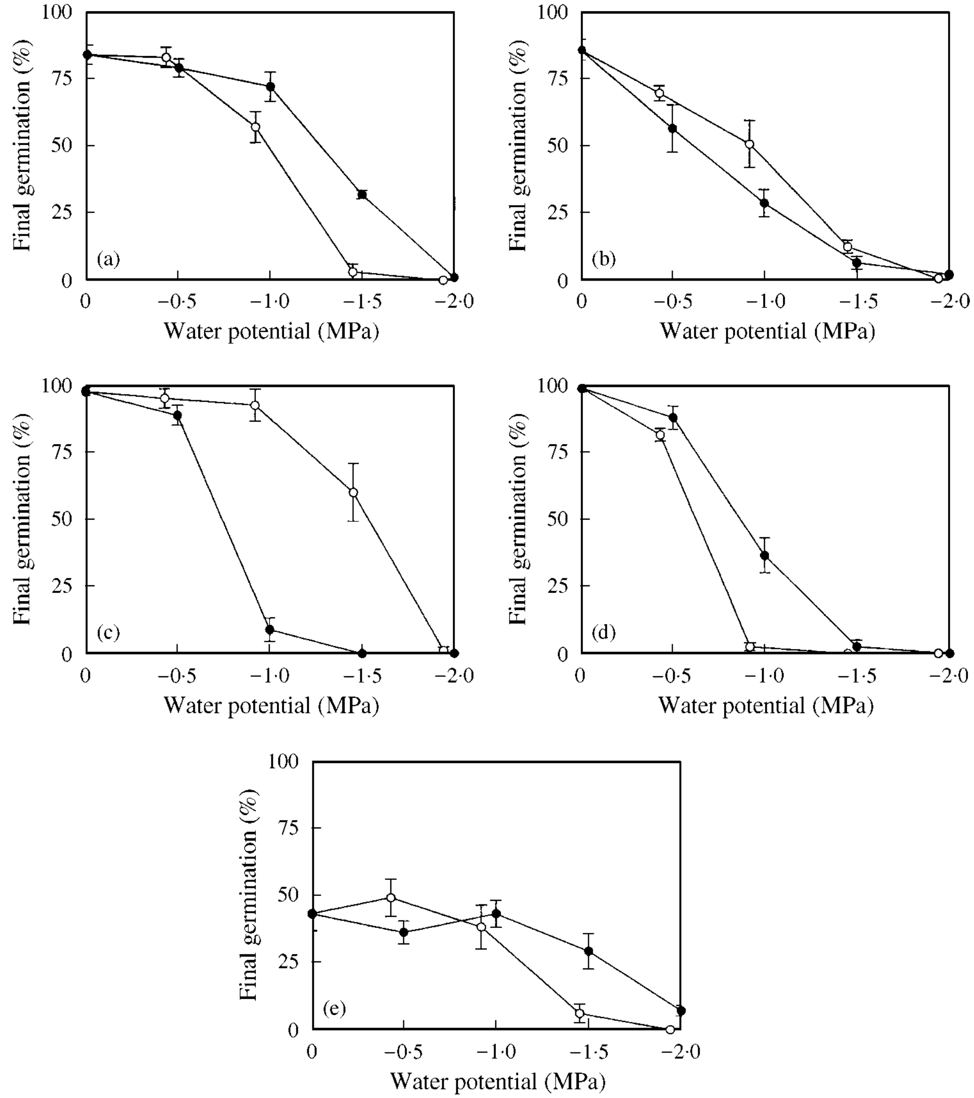

Figure 3. Changes in final percentage germination with changing water potential of NaCl (●) and PEG (○) solutions. (a) *Lespedeza davurica*; (b) *Caragana korshinskii*; (c) *Hedysarum scoparium*; (d) *Zygophyllum xanthoxylon*; (e) *Atraphaxis bracteata*. Each point represents the mean of four replications and bars indicate S.E.

Experiment 5: effects of NaCl and PEG solutions of low Ψ_W on seed water uptake

Figure 5 shows the W_r of seeds of the five species after moistening with NaCl or PEG solutions of different Ψ_W for 7 days. During the treatments, the germination of all seeds was completely suppressed. For all species, W_r was higher for NaCl than for the isotonic PEG treatment at all Ψ_W. The difference in W_r between NaCl and PEG treatments was smallest in *A. bracteata* and largest in *L. davurica*. For all species, the W_r of NaCl-treated seeds was similar to or slightly larger than the W_r at which seeds began to germinate (Fig. 4).

Table 2. *Effects of pretreatment with an NaCl or PEG solution on seed germination. Data are presented as mean of final percentage germination ± S.E. (n = 4)*

Species	No pretreatment	PEG-pretreated	NaCl-pretreated
Lespedeza davurica	83·0 ± 3·42	83·0 ± 1·92	2·0 ± 2·0**
Caragana korshinskii	83·8 ± 2·82	44·1 ± 11·39*	0 ± 0
Hedysarum scoparium	97·5 ± 1·44	92·6 ± 4·35	0 ± 0
Zygophyllum xanthoxylon	98·8 ± 1·25	96·3 ± 4·79	88·8 ± 2·50**
Atraphaxis bracteata	43·0 ± 6·61	37·0 ± 5·97	23·0 ± 5·26

* Significantly different from 'no pretreatment' at $p = 0{\cdot}05$.
** Significantly different from 'no pretreatment' at $p = 0{\cdot}01$.

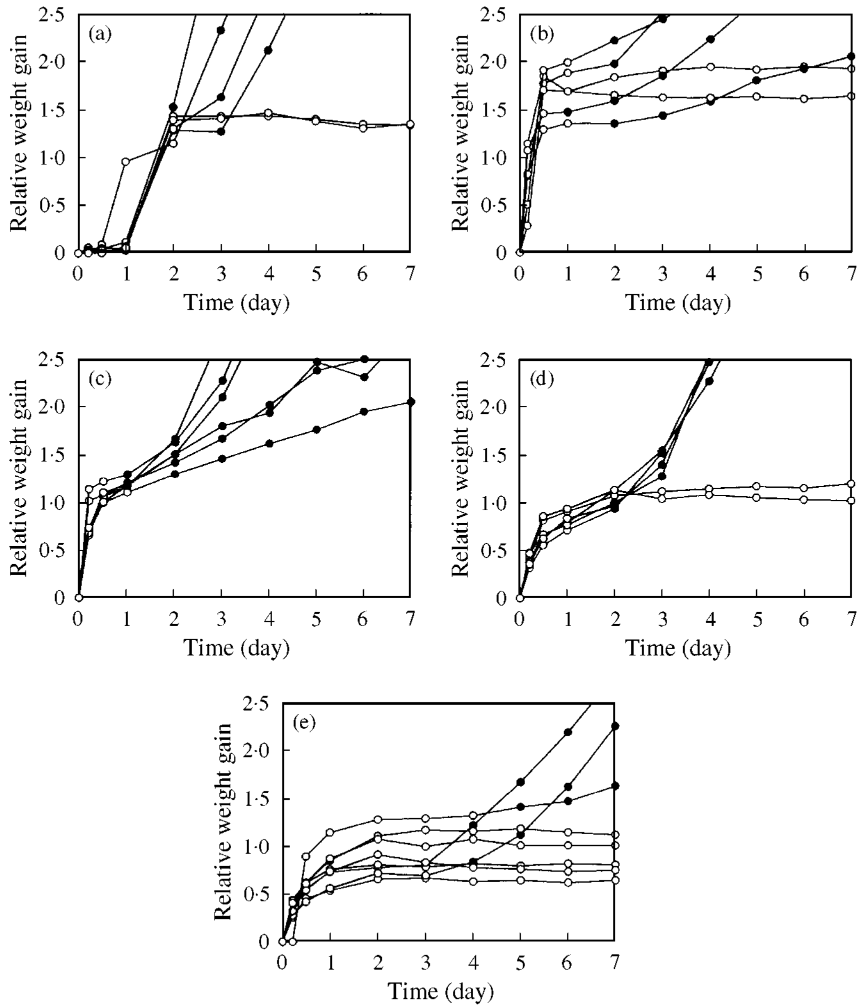

Figure 4. Changes over time in relative weight gain (W_r) of individual seeds. The symbols ○ and ● indicate ungerminated and germinated seeds, respectively. (a) *Lespedeza davurica*; (b) *Caragana korshinskii*; (c) *Hedysarum scoparium*; (d) *Zygophyllum xanthoxylon*; (e) *Atraphaxis bracteata.*

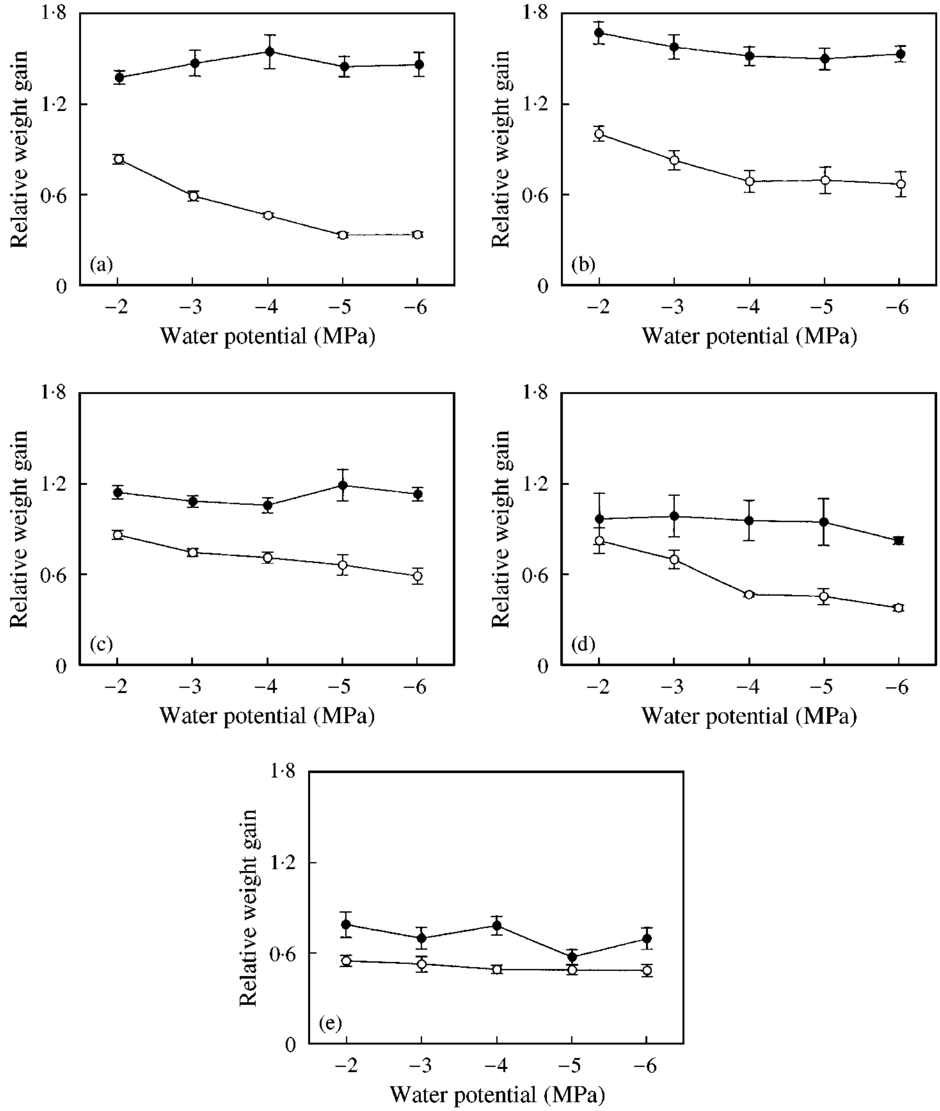

Figure 5. Relative weight gain (W_r) of individual seeds after the seeds had been moistened with NaCl (●) or PEG (○) solutions. Each point represents the mean of six to eight replications, and bars indicate S.E.

Regression analysis to test the significance of the dependence of W_r on Ψ_W showed that the W_r of NaCl-treated seeds was not significantly ($p = 0{\cdot}05$) affected by Ψ_W in any of the five species. For PEG-treated seeds, the effect of Ψ_W on W_r was insignificant ($p = 0{\cdot}05$) only in *A. bracteata* and significant ($p = 0{\cdot}01$) for the other four species.

Discussion

When seeds were moistened with deionized water, all tested species except *A. bracteata* showed a high percentage of germination over a wide temperature range. The reason for the low germination of *A. bracteata* is unclear; however, one possibility could be that some of the seeds were in dormancy. For the other four species there was no indication of seed dormancy. The lower limit of germination temperature was 5–10°C for all five species, and the upper limit differed among species: 25–30°C for *Z. xanthoxylon* and *A. bracteata*, and >30°C for the other three species. These results indicate that seeds of these species are germinable in Chinese desert regions at any time between mid-spring to mid-autumn, although the high temperature in mid-summer may reduce the germination of *Z. xanthoxylon* and *A. bracteata*.

The best season for dispersing the seeds of these species would be between late autumn and early spring: the dispersed seeds would then remain ungerminated until mid-spring because of low temperatures and low precipitation during this period; precipitation in mid-spring would cause seed germination, and precipitation from mid-spring to autumn would supply the seedlings with water required for their growth until they are mature enough to survive the dry, cold winter.

The seeds of all tested species are relatively large in size and need to imbibe a considerable amount of water before the initiation of radicle protrusion (Fig. 4). Light rainfall would not suffice to cause the germination of the dry seeds. However, the seeds of all tested species imbibed much water even at very low Ψ_W (see W_r of PEG-treated seeds in Fig. 5), and PEG pretreatment caused rapid germination in three species. This indicates that seeds can accumulate water under unfavourable water conditions and germinate rapidly with subsequent sufficient water supply. The reason why PEG pretreatment caused a decrease in the germinability of seeds of *C. korshinskii* (Table 2) is unclear; solute leakage from the seeds (Simon, 1974; Hendricks & Taylorson, 1976) or infestation of fungi may be responsible for the reduced germinability.

Isotonic solutions of NaCl and PEG caused different effects on seed germination (Figs 2 & 3), seed germinability (Table 2) and seed water uptake (Fig. 5) in all five tested species. The higher W_r of NaCl-treated seeds than of seeds treated with isotonic PEG (Fig. 5) indicates that NaCl entered the seeds of all five species and mitigated the osmotic inhibitory effect by the external medium. The more favoured germination of *L. davurica*, *Z. xanthoxylon* and *A. bracteata* in NaCl than in isotonic PEG probably resulted from the alleviatory effect of NaCl on seed water uptake. On the other hand, NaCl seems to have caused toxic effects on the seeds, but by differing degrees among different species (Table 2). The germinability of the seeds of three Leguminosae species was almost completely lost when they were treated with $-5{\cdot}0$ MPa NaCl solution, while the germinability of the other two species was affected much less. The lower percentage of germination of *C. korshinskii* and *H. scoparium* in NaCl than in the isotonic PEG (Fig. 3) could be attributed to the toxic effects of NaCl on the seeds.

In salinized lands, salts accumulate near the soil surface where many seeds are distributed. The salt concentration on the soil surface of salinized lands changes greatly over time: continuous evaporation of water gradually deposits salt on the soil surface, but occasional heavy rainfall can quickly leach salt from the surface and supply water to the seeds. Thus, for species to become established in saline environments, the seeds must be kept germinable on the salt-rich soil and germinate at a decreased salinity that allows emerging radicles to survive (Ungar, 1978, 1991). The Ψ_W of the NaCl solution that reduced the germination of the five tested species was not largely different from that which reduced the germination of many halophytic species (e.g. Williams & Ungar, 1972; Khan & Rizvi, 1994; Khan & Ungar, 1996; Tobe *et al.*, 2000*a*). Furthermore, emerging radicles showed no indication that they had suffered any injurious effects by NaCl treatment. Nevertheless, hypersalinity caused almost complete loss of seed germinability in three Leguminosae species, while many halophytes have been reported to keep their seeds germinable after moistening with hypersaline solutions (e.g. Ungar, 1996; Katembe *et al.*, 1998; Tobe *et al.*, 2000*a*). In this regard, the three Leguminosae species would not be expected to become established in saline environments after dispersal. The other two species would be better candidates for dispersal on saline lands.

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