Seed Germination and Radicle Growth of a Halophyte, *Kalidium caspicum* (Chenopodiaceae)

KAZUO TOBE*†, XIAOMING LI‡ and KENJI OMASA†§

†*Environmental Biology Division, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-0053, Japan and ‡Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, No. 40, South Beijing Road, Urumqi, Xinjiang 830011, People's Republic of China*

Effects of temperature, light, NaCl and polyethylene glycol (PEG)-6000 on seed germination and radicle growth in a halophytic shrub, *Kalidium caspicum* (L.) Ung.-Sternb. were investigated. When seeds were incubated in deionized water at constant temperatures between 10 and 30°C, the percentage germination in the dark exceeded 75%; light suppressed seed germination at alternating temperatures. Incubating seeds with a hypersaline solution of NaCl for 30 d had no adverse effect on their germinability. The percentage germination of seeds incubated with a -0.8 MPa NaCl solution was 73, 80 and 54% at 10, 20 and 30°C, respectively, but all radicles died before their length exceeded 5 mm. In contrast, when seeds were incubated with a -0.8 MPa PEG solution at 20°C, 68% of seeds germinated, and 95% of the emerging radicles survived beyond 5 mm. The high sensitivity of small radicles of this species to salinity indicated that salt must be removed from the soil surface for seedling establishment.

© 2000 Annals of Botany Company

Key words: Chinese desert, radicle growth, germination, halophyte, *Kalidium caspicum*, salinity.

INTRODUCTION

In saline environments, adaptation of plants to salinity during germination and early seedling stages is crucial for the establishment of species (Ungar, 1978, 1991, 1995). Seedlings are the most vulnerable stage in the life cycle of plants, and germination determines when and where seedling growth begins (Koller, 1964; Gutterman, 1993; Kigel, 1995). Therefore, appropriate germination responses of halophytic species to environmental parameters determine their distribution in saline environments.

Most seeds are located near the soil surface, where salt accumulates in salinized locations (Ungar, 1978). The salt concentration at the surface of salanized soil changes over time: continuous evaporation of ground water gradually deposits salt on the soil surface, but rainfall or melting snow can quickly leach salt from the surface and supply water to seeds. Thus, for the successful establishment of plants in saline environments, seeds must remain viable at high salinity and germinate when salinity decreases (Ungar, 1978, 1991, 1995).

Two *Haloxylon* species distributed mainly in non-saline locations in the desert region of northwestern China were found to show high adaptability to salinity in the germination and early seedling stages (Tobe *et al.*, 2000).

This suggested that adaptation to salinity in the initial life stages does not determine geographical distribution of species in this region. To further investigate this point, we investigated how a typical halophyte growing in saline locations in this region adapts to salinity in the initial life stages. We studied *Kalidium caspicum* (L.) Ung.-Sternb. (Chenopodiaceae), a stem-succulent halophytic shrub (Lanzhou Institute of Desert Research, 1985), which is restricted to highly salinized locations where the groundwater table is located about 0·5 m below the soil surface. Older seedlings survive high salinity (Natori *et al.*, 1992), but little is known about the germination and early seedling growth of this species.

We examined the effects of salinity on germination and radicle growth of *K. caspicum*. Because NaCl is the most abundant salt in many salinized locations in the Chinese desert (Fan *et al.*, 1993), we used it in our experiments. We investigated (1) whether seeds of *K. caspicum* remain viable at high salinity, and (2) whether radicles could survive salt concentrations that permit germination. To evaluate the specific effects of NaCl on seeds and radicles, we compared germination and early radicle growth in NaCl solutions with those in isotonic solutions of polyethylene glycol (PEG)-6000, which cannot permeate plant cell walls (Carpita *et al.*, 1979) and acts solely as an osmoticum (Sharma, 1973; Redmann, 1974; Hardegree and Emmerich, 1990). In addition, because temperature and light affect germination responses in many species and can alter ecological behaviour (Koller, 1964; Gutterman, 1993; Kigel, 1995), we also examined their influence on seed germination.

* For correspondence. Fax +81 298 50 2586, e-mail tobe@nies.go.jp
§ Present address: Graduate School of Agriculture and Life Sciences, University of Tokyo, Yayoi 1-1-1, Bunkyo, Tokyo 113-8657, Japan.

MATERIALS AND METHODS

Seed collection

Seeds of *K. caspicum* (mean seed weight $\pm$ s.d. 171 $\pm$ 45 µg, $n = 20$) were collected in November 1996 from plants growing on saline soil in Fukang, China (44°18′N, 87°55′E). Annual precipitation and annual mean temperature in Fukang are 172 mm and 6·1°C (means from 1958 to 1980). The monthly mean temperature is <0°C from November to March, 16–20°C from May to September, and about 10°C in April and October. The diurnal temperature difference is 10–20°C. Precipitation from November to March is about 36 mm (31% of annual precipitation) and usually falls as snow, which covers the ground during winter and melts in early spring. The seeds of *K. caspicum* ripen in November. Total salt content at a depth of 0–10 cm in a typical habitat of *K. caspicum* ranged between 16 and 54 g per kg dry soil (measurements were made in late-May, late-August, and mid-October 1992).

Seeds were initially stored at about 5°C until they were transported to Japan in March 1997, where they were stored at about −18°C. The germination experiments were carried out between April 1997 and March 1998.

Methods

In all experiments, 25 seeds were sown on three layers of filter paper (Toyo, No. 1) in a 90 mm Petri dish. The filter paper was moistened with about 10 ml of deionized water or a solution of NaCl or PEG-6000, so that about half the seed was immersed in the solution. The Petri dishes were covered with lids and kept in a controlled environment. About two-thirds of the volume of the water or solution in each Petri dish was replaced daily. Each experiment was replicated four or five times.

After each experiment, the final percentage germination (G_F) and the time taken to achieve 50% germination (TG_{50}) were calculated; TG_{50} was calculated only when the G_F of all replications exceeded 50%.

Effects of temperature and light on seed germination. To examine the effects of temperature and light on seed germination, seeds were incubated in deionized water under 12 different constant or diurnally alternating temperature regimes. For each temperature regime, germination was studied under conditions of both continuous dark and 12 h dark/12 h light, but at 5°C germination was examined in the dark only. When the temperature was alternated, seeds were exposed to the lower temperature for 12 h (dark) and the higher temperature for 12 h (light). For the light treatment, seeds were illuminated with fluorescent lamps (photon flux density at the surface of the seeds: 80–90 µmol m^{-2} s^{-1} over the wavelength 400–700 nm). Seeds were considered to have germinated when the emerging radicles were over 2 mm long. The number of germinated seeds was counted daily for 15 d.

Effects of NaCl pretreatment on seed viability. Seeds were incubated with 3·0 mol kg^{-1} NaCl solution (49% saturation at 20°C) for 30 d at 20°C in the dark. Seeds were then rinsed with deionized water and transferred to Petri dishes containing deionized water. The number of germinated seeds was counted every day for 10 d after transfer.

Effects of NaCl or PEG solution on seed germination and radicle growth. Seeds were moistened with deionized water or solutions of NaCl or PEG-6000 of known water potential (Ψ_W). The NaCl and PEG-6000 solutions were prepared according to the equations of Lang (1967) and Michel and Kaufmann (1973), but the Ψ_W of the prepared PEG solutions was finally determined with an isopiestic psychrometer (Boyer and Knipling, 1965) at 20°C. The numbers of both germinated seeds and seeds with emerging radicles longer than 5 mm were counted every day for 20 d (20 and 30°C) or 30 d (10°C). Seeds were considered to have germinated when the emerging radicles were visible. We calculated the final percentage of seeds with emerging radicles longer than 5 mm (S_F) and the time taken until the percentage of these seeds was 50% (TS_{50}). TS_{50} was calculated only when S_F of all replications exceeded 50%. ΔTS_{50}, which is a measure of the rate of radicle elongation, was calculated as TS_{50} minus TG_{50}.

Statistical analysis

The statistical significance of the difference between two values was evaluated using Student's *t*-test. The values of G_F were arcsine transformed before analysis.

RESULTS

Effects of temperature and light on seed germination

G_F was significantly ($P < 0.01$) lower in the light than in the dark at 30°C and at all alternating temperature regimes (Figs 1 and 2). In the dark, G_F exceeded 75% at constant temperatures between 10 and 30°C and at all alternating temperature regimes.
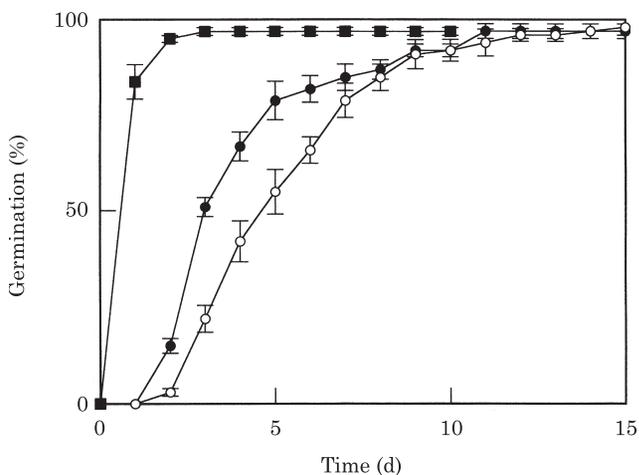
FIG. 1. Time courses of percentage germination of seeds incubated with deionized water at 20°C in the dark (●, ■) or in 12 h dark/12 h light (○). Before the experiment, seeds were either not pretreated (●, ○) or incubated with deionized water at 5°C for 15 d (■). Each point represents the mean of four replications; bars indicate s.e.
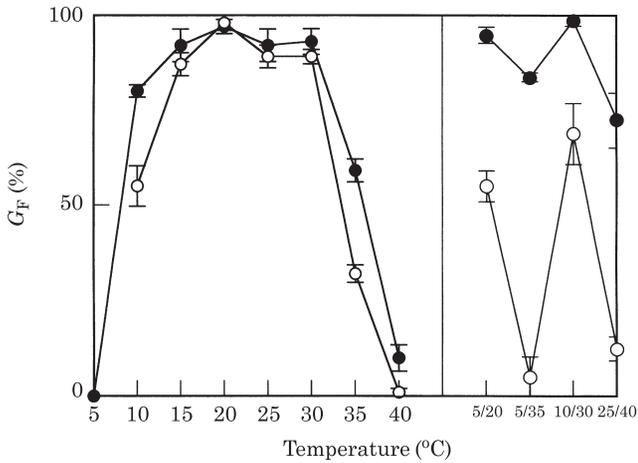
FIG. 2. Effects of temperature and light on final percentage germination (G_F). Seeds were incubated with deionized water for 15 d either in the dark (●) or in 12 h dark/12 h light (○). Each point represents the mean of four replications; bars indicate s.e.
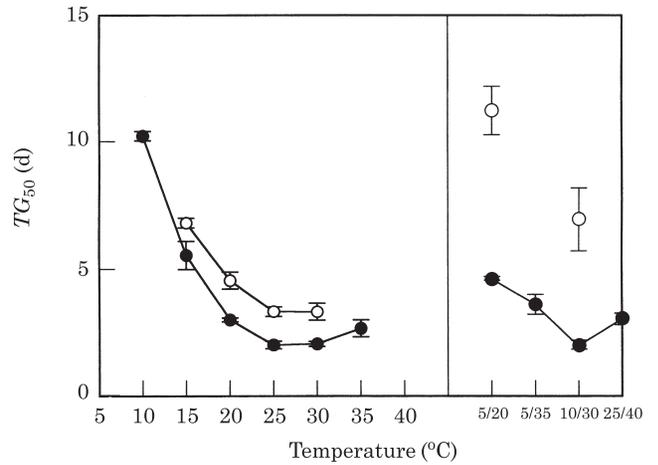
FIG. 3. Effects of temperature and light on TG_{50}. Seeds were moistened with deionized water for 15 d either in the dark (●) or in 12 h dark/12 h light (○). Each point represents the mean of four replications; bars indicate s.e. TG_{50} was calculated only when all four replications of G_F were over 50%.
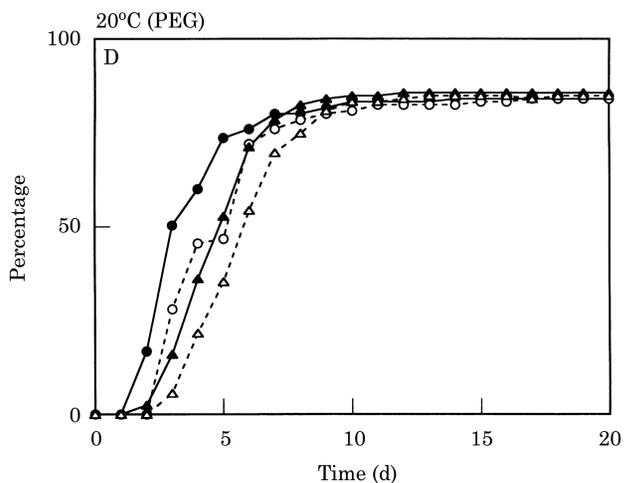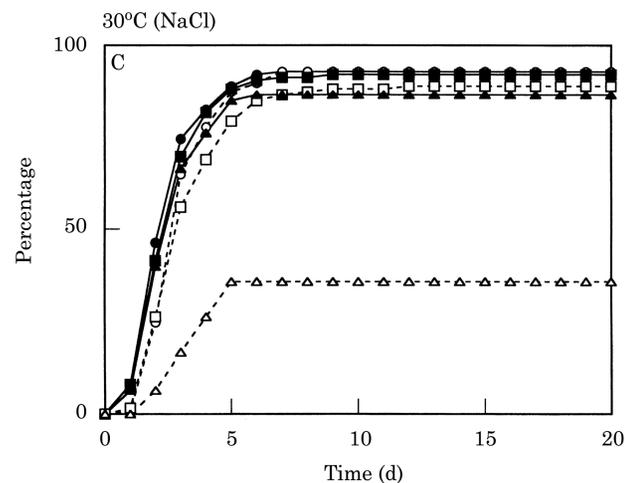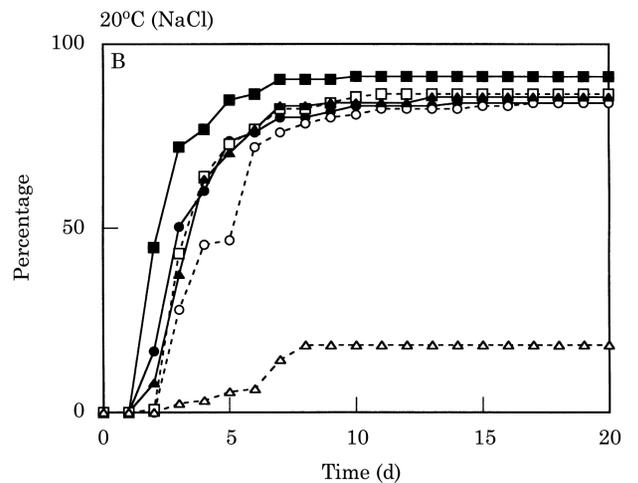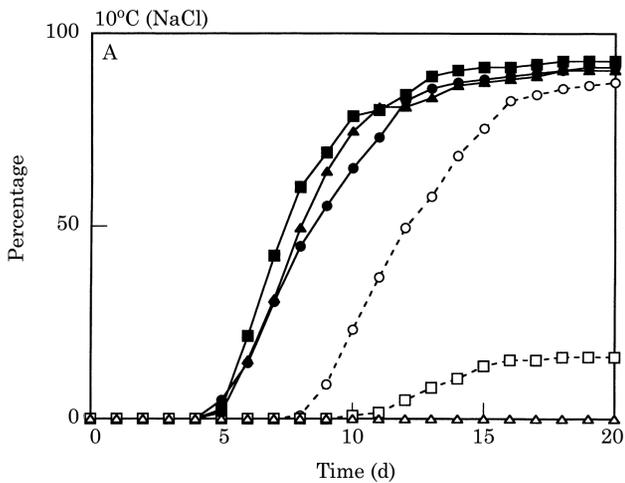
FIG. 4. Time courses of percentage germination (●, ■, ▲) and percentage of seeds with radicles over 5 mm (○, □, △). Seeds were moistened with NaCl or PEG solutions at different temperatures. Each point represents the mean of five replications. (●, ○) 0 MPa; (■, □) −0·2 MPa; (▲, △) −0·4 MPa.
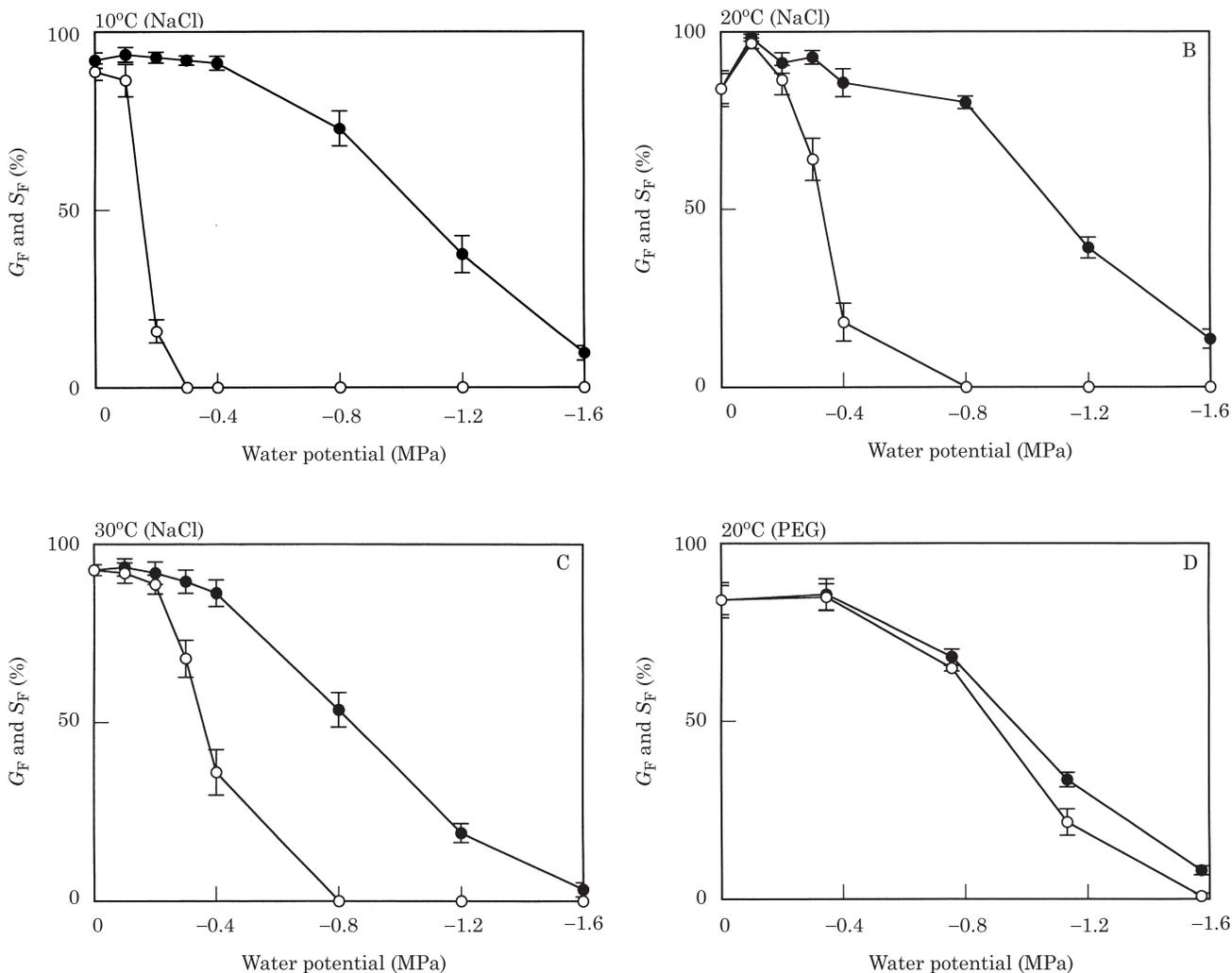
FIG. 5. Effects of NaCl or PEG treatments on final percentage germination (G_F) (●) and final percentage of seeds with radicles over 5 mm (S_F) (○) at different temperatures. Each point represents the mean of five replications; bars indicate s.e.

TG_{50} in the dark was 10·2 d at 10°C and 5·5 d at 15°C, and between 2·0 and 4·7 d at other temperature regimes (Fig. 3). TG_{50} was significantly ($P < 0.01$) lower in the light than in the dark at all temperature regimes except 15 and 30°C.

G_F was zero at 5°C (Fig. 2), but when the ungerminated seeds that had been incubated at 5°C for 15 d were transferred to a constant temperature of 20°C they germinated rapidly (Fig. 1) [TG_{50} after transfer was 0·58 ± 0·09 d (mean ± s.e.) compared with 2·99 ± 0·08 d for non-pretreated seeds].

Effects of NaCl pretreatment on seed viability

During pretreatment with 3·0 mol kg⁻¹ NaCl solution for 30 d, no seed germination was observed. The seeds germinated rapidly when transferred to deionized water. TG_{50} after transfer was 1·69 ± 0·09 d (mean ± s.e., $n = 5$), compared with 2·99 ± 0·08 d for non-pretreated seeds.

Effects of NaCl and PEG on seed germination and radicle growth

When seeds were incubated with NaCl solution of Ψ_W lower than −0·2 MPa (10°C) or −0·3 MPa (20 and 30°C), or PEG solution of −1·1 or −1·6 MPa, some or most radicles were necrotic and stopped elongating before they were over 0·5 mm long; these seedlings died eventually without further elongation (Figs 4 and 5). Consequently, in the NaCl treatment, S_F was much smaller than G_F in most of the Ψ_W and temperature conditions tested. S_F in the PEG treatment was slightly smaller than the corresponding G_F at most values of Ψ_W (Fig. 5).

Both germination percentage and radicle growth at 10°C were considerably slower than those at higher temperatures (Fig. 4). TG_{50} was significantly higher ($P < 0.01$) at 10°C than at 20 and 30°C at all Ψ_W (Fig. 6). ΔTS_{50} was significantly higher ($P < 0.01$) at 10°C than at 20 and 30°C at 0 and −0·1 MPa. Larger G_F and smaller TG_{50} at 10°C, 0 MPa (Figs 5 and 6) compared with those in the
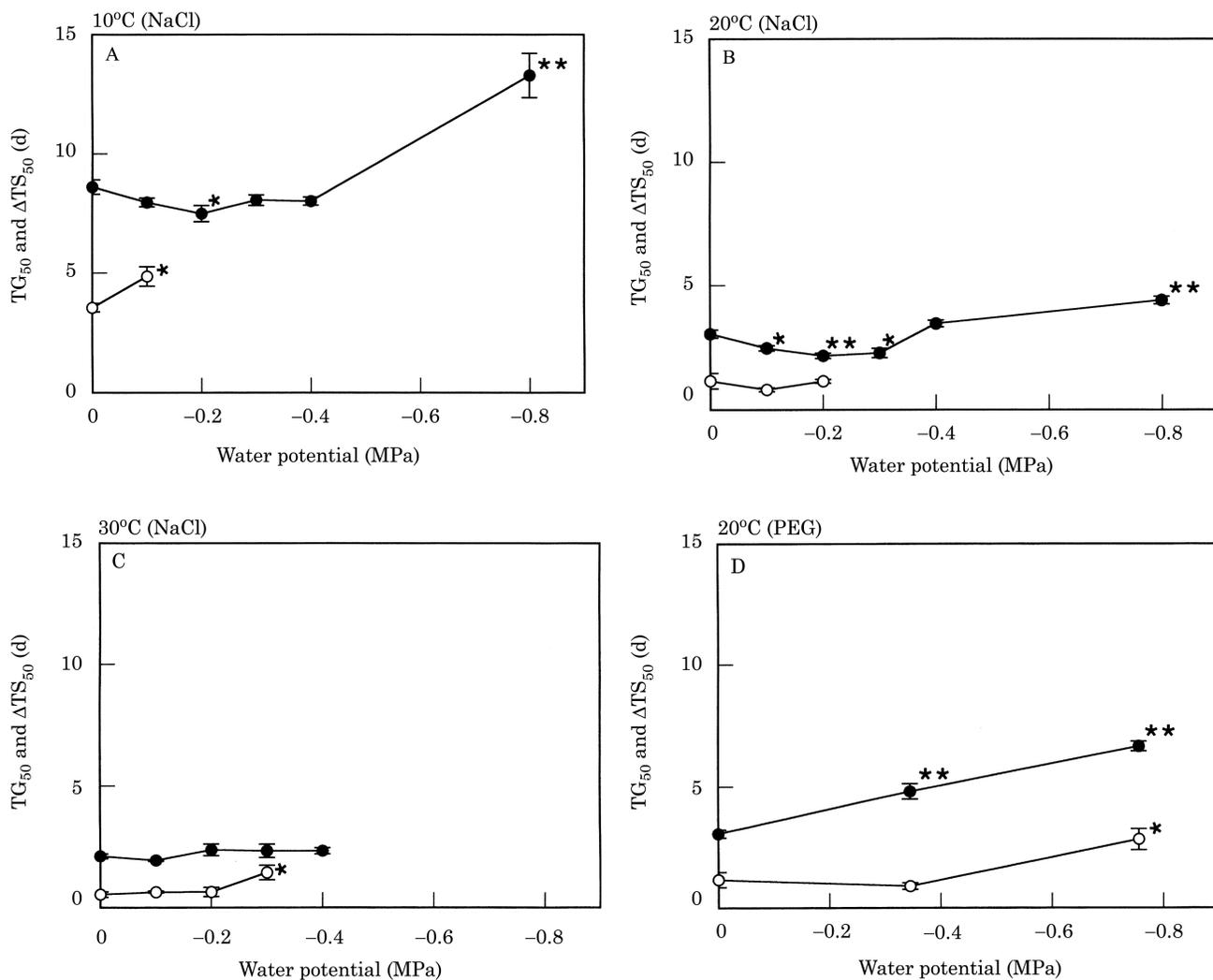
FIG. 6. Effects of NaCl or PEG treatments on TG_{50} (●) and ΔTS_{50} (○) at different temperatures. Each point represents the mean of five replications; bars indicate s.e. ★ and ★★ indicate that values are significantly different from those at 0 MPa at $P < 0.05$ and $P < 0.001$, respectively. TG_{50} was calculated only when all five replications of G_F were over 50%, and ΔTS_{50} was calculated only when all five replications of S_F were over 50%.

former experiment (Figs 2 and 3) were the result of a longer incubation time (30 d) and different germination criteria (see Materials and Methods).

G_F was significantly lower ($P < 0.01$) at 20°C in PEG than in NaCl at −0.8 MPa (Fig. 5). TG_{50} was significantly higher ($P < 0.01$) at 20°C in PEG than in NaCl at −0.4 and −0.8 MPa (Fig. 6). TG_{50} at −0.2 MPa in NaCl tended to be slightly smaller than that at 0 MPa, but TG_{50} at lower Ψ_W tended to be larger than that at 0 MPa in both NaCl and PEG treatments. ΔTS_{50} tended to be larger at lower Ψ_W (Fig. 6).

DISCUSSION

Seed germination of *K. caspicum* was relatively insensitive to temperature (Fig. 2), and we found no indication of seed dormancy. These results indicate that *K. caspicum* seeds are potentially germinable in the species' habitat at any time between April and October. The Ψ_W of the NaCl solution

that reduced G_F of *K. caspicum* was not largely different from that which reduced germination of other halophytic species (Macke and Ungar, 1971; Williams and Ungar, 1972; Khan and Ungar, 1984; Myers and Morgan, 1989; Khan and Rizvi, 1994; Ungar, 1996).

Moistening seeds with 3·0 mol kg⁻¹ NaCl solution for 30 d had no adverse effect on their germinability in deionized water. This indicates that *K. caspicum* seeds can remain ungerminated and viable on the salt-rich soil surface. In many other halophytes, seeds remain viable after moistening with hypersaline solution (Macke and Ungar, 1971; Okusanya, 1977; Ungar, 1996; Katembe, Ungar and Mitchell, 1998).

Many radicles died in NaCl solutions with Ψ_W lower than −0·2 MPa (10°C) or −0·4 MPa (20 and 30°C), although most seeds germinated (Fig. 5). This indicates that germination, even at relatively low salinity, does not ensure establishment of seedlings. In many other halophytes, most small seedlings survived and radicles

continued to elongate at salinities that allowed seeds to germinate (Khan and Ungar, 1984; Stumpf *et al*., 1986; Katembe *et al*., 1998). The radicles of *K. caspicum* are very sensitive to salinity compared to those of many other halophytic and non-halophytic species (Stumpf *et al*., 1986; Lombardi *et al*., 1998; Tobe *et al*., 1999). In contrast, most radicles continued to elongate in PEG solutions of Ψ_W higher than $-1 \cdot 1$ MPa until their length exceeded 5 mm. The marked difference in the effect of radicles of isotonic NaCl and PEG indicates that the deleterious effect of NaCl is salt-specific toxicity rather than osmotic inhibition.

Although *K. caspicum* is distributed on highly saline soil and 60-d-old seedlings can survive at a high level of NaCl (Natori *et al*., 1992), the small radicles were sensitive to NaCl. This is in marked contrast to the salinity responses of *Haloxylon persicum*, a non-halophytic shrub distributed in the same region as *K. caspicum*. *H. persicum* showed 64% germination in $-3 \cdot 0$ MPa NaCl solution, and the small seedlings continued to elongate in $-3 \cdot 0$ MPa NaCl without suffering from salt toxicity, but older seedling of *H. persicum* were sensitive to salinity (Tobe *et al*., 2000). These results indicate that salinity tolerance in the early seedling stage is not correlated with salinity tolerance in later developmental stages, and that salinity adaptability in the initial developmental stages does not determine the geographical distribution of species in this region.

Our results indicate that, although high salinity does not affect the viability of seeds, the relatively low salinity ($-0 \cdot 4$ to $-1 \cdot 2$ MPa) that causes seed germination results in high mortality of the radicles. For the establishment of *K. caspicum* in saline environments salt must be completely removed at the soil surface.

The establishment of *K. caspicum* in saline environments may depend on the leaching of salt from the soil surface by snow-melt in early spring. This study showed that although seeds do not germinate at 5°C, an increase in temperature induces very rapid germination. This indicates that seeds imbibe water at low temperatures in early spring and, as soon as the temperature rises, seeds germinate rapidly. Although radicle growth is reduced by low temperatures, low temperatures also reduce the accumulation of salt on the soil surface and thus favour seedling establishment. Precipitation in the following warmer season would also leach salt from the soil surface and allow germination; however, high temperatures would result in more evaporation and faster accumulation of salt on the soil surface, which would kill small radicles. To ascertain whether this prediction is correct, field observations will be needed.

LITERATURE CITED

Boyer JS, Knipling EB. 1965. Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. *Proceedings of the National Academy of Sciences, USA* **54**: 1044–1051.

Carpita N, Sabularse D, Montezinos D, Delmer DH. 1979. Determination of the pore size of cell walls of living plant cells. *Science* **205**: 1144–1147.

Fan Z, Chang Q, Tian C, Yabuki S, Okada A, Liu C. 1993. Genesis and characteristics of salt-affected soils in Tarim Basin. In: Ichikuni M, ed. *Proceedings of the Japan-China international symposium on the study of the mechanism of desertification*. Tokyo: Science and Technology Agency, 219–226.

Gutterman Y. 1993. *Seed germination in desert plants*. Berlin: Springer-Verlag, 169–206.

Hardegree SP, Emmerich WE. 1990. Partitioning water potential and specific salt effect on seed germination of four grasses. *Annals of Botany* **66**: 587–585.

Katembe WJ, Ungar IA, Mitchell JP. 1998. Effect of salinity on germination and seedling growth of two *Atriplex* species (Chenopodiaceae). *Annals of Botany* **82**: 167–175.

Khan MA, Rizvi Y. 1994. Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. *stocksii*. *Canadian Journal of Botany* **72**: 475–479.

Khan MA, Ungar IA. 1984. The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. *American Journal of Botany* **71**: 481–489.

Kigel J. 1995. Seed germination in arid and semiarid regions. In: Kigel J, Galili G, eds. *Seed development and germination*. New York: Marcel Dekker, 645–697.

Koller D. 1964. The survival value of germination-regulating mechanisms in the field. *Herbage Abstracts* **34**: 1–7.

Lang AGR. 1967. Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40°C. *Australian Journal of Chemistry* **20**: 2017–2023.

Lanzhou Institute of Desert Research, Chinese Academy of Sciences. 1985. *Desert flora of China*, vol. 1. Beijing: Science Press, 381–382.

Lombardi T, Fochetti T, Onnis A. 1998. Germination of *Briza maxima* L. seeds: effects of temperature, light, salinity and seed harvesting time. *Seed Science and Technology* **26**: 463–470.

Macke AJ, Ungar IA. 1971. The effects of salinity on germination and early growth of *Puccinellia nuttalliana*. *Canadian Journal of Botany* **49**: 515–520.

Michel BE, Kaufmann MR. 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiology* **51**: 914–916.

Myers BA, Morgan WC. 1989. Germination of the salt-tolerant grass *Diplachne fusca*. II. Salinity responses. *Australian Journal of Botany* **37**: 239–251.

Natori T, Mo W, Li X, Jiang S, Omasa K. 1992. The culture of Chinese desert plants in a greenhouse. *Acta Horticulturae* **319**: 453–458.

Okusanya OT. 1977. The effect of sea water and temperature on the germination behaviour of *Crithmum maritimum*. *Physiologia Plantarum* **41**: 265–267.

Redmann RE. 1974. Osmotic and specific ion effects on the germination of alfalfa. *Canadian Journal of Botany* **52**: 803–808.

Sharma ML. 1973. Simulation of drought and its effect on germination of five pasture species. *Agronomy Journal* **65**: 982–987.

Stumpf DK, Prisco JT, Weeks JR, Lindley VA, O'Leary JW. 1986. Salinity and *Salicornia bigelovii* Torr. seedling establishment. Water relations. *Journal of Experimental Botany* **37**: 160–169.

Tobe K, Li X, Omasa K. 2000. Effects of NaCl on seed germination and growth of two Chinese desert shrubs, *Haloxylon ammodendron* and *H. persicum* (Chenopodiaceae). *Australian Journal of Botany* (in press).

Tobe K, Zhang L, Omasa K. 1999. Effects of NaCl on seed germination of five nonhalophytic species from a Chinese desert environment. *Seed Science and Technology* (in press).

Ungar IA. 1978. Halophyte seed germination. *Botanical Review* **44**: 233–264.

Ungar IA. 1991. *Ecophysiology of vascular halophytes*. Boca Raton: CRC Press, 9–48.

Ungar IA. 1995. Seed germination and seed-bank ecology in halophytes. In: Kigel J, Galili G, eds. *Seed development and germination*. New York: Marcel Dekker, 599–628.

Ungar IA. 1996. Effect of salinity on seed germination, growth, and ion accumulation of *Atriplex patula* (Chenopodiaceae). *American Journal of Botany* **83**: 604–607.

Williams MD, Ungar IA. 1972. The effect of environmental parameters on the germination, growth, and development of *Suaeda depressa* (Pursh) Wats. *American Journal of Botany* **59**: 912–918.