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Comparative study of seed germination and growth of *Kochia prostrata* and *Kochia scoparia* (Chenopodiaceae) under salinity

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ABSTRACT

Soils of arid regions of Central Asia contain salts of different types that may differentially affect seed germination and plant development. We studied effect of NaCl, Na_2SO_4, 2NaCl + KCl + $CaCl_2$ and $2Na_2SO_4$+K_2SO_4+$MgSO_4$ on germination of *Kochia prostrata* and *Kochia scoparia* seeds under a range of concentrations from 0.5 to 5% and at two constant temperature regimes +22 °C and +6 °C. The observed salt tolerance limit of germination at constant temperature +22 °C for both species was 5–6%, while at low temperature (+6 °C) this limit was 2%. The salt tolerance of young plants (before flowering) was 3% for NaCl. Low concentrations of sulfuric and mixed salts had a stimulating effect on seed germination in *K. prostrata*. Despite similarity of salt-tolerance limits the studied species showed a significant difference in seed recovery ability, i.e. the ability of ungerminated, salt-soaked seeds to germinate after transfer to fresh water. *K. scoparia* demonstrated a full germination recovery after seed transfer to distilled water while *K. prostrata* showed only a partial recovery.

1. Introduction

Kochia prostrata (L.) Schrad. and *Kochia scoparia* (L.) Schrad. are common and widespread forage species of arid and semiarid zones of Eurasia (Dzubenko et al., 2009; Gintzburger et al., 2003) and the Mediterranean (Le Houerou, 1995) with a potential for reclamation of degraded salt-affected soils (Shamsutdinov and Shamsutdinov, 2005). *K. scoparia* was introduced from Europe to North America (Young et al., 1981; Yensen et al., 1981; Zahran, 1993) where it is used for reclamation of pastures.

K. prostrata and *K. scoparia* are salt tolerant plants (Al-Ahmadi and Kafi, 2007; Everitt et al., 1983; Francois, 1986; Khan et al., 2001). The salinity tolerance limit of seeds of *K. scoparia* soaked in salt solution is 1.0 M NaCl (Khan et al., 2001; Khan and Gul, 2006), but little comparable data is available for *K. prostrata*. Karimi et al. (2005) reported that the optimal concentration for *K. prostrata* is 150 mM NaCl, and significant decrease of growth was observed at 200 mM NaCl. Romo and Haferkampf (1987) classified this species as moderately tolerant to NaCl and KCl during germination and growth.

Kochia species exhibit a wide variability in both inter- and intraspecific salt tolerance (Al-Ahmadi and Kafi, 2007; Dzubenko et al., 2009; Everitt et al., 1983; Francois, 1986; Khan et al., 2001; Khan and Gul, 2006). Different ecotypes of *Kochia* species were found to vary in their salt tolerance limits under greenhouse and field conditions (Al-Ahmadi and Kafi, 2007; Francois, 1976; Semushina and Morozova, 1979). Seeds of *K. scoparia* can germinate in high salinity levels and serve as an example of adaptation of halophytes to high saline soils (Al-Ahmadi and Kafi, 2007). Al-Ahmadi and Kafi (2007) found that seeds of *K. scoparia* could germinate over a wide range of temperature (8-40 °C).

Our knowledge of the halophyte seeds germination is far from complete (Khan, 1999; Khan and Gul, 2006). From a total of about 2400 halophyte species (Menzel and Lieth, 1999), data on seed germination is available for only a few hundred species of subtropical halophytes (Baskin and Baskin, 2001; Ungar, 1995). During germination, salinity and temperature regimes have a synergetic effect on many halophyte species (Ungar, 1978; Khan and Gul, 2006). In *K. scoparia*, Khan et al. (2001) studied interaction of temperature with concentrations of NaCl from 200 to 1000 mM on seed germination and observed increase in salt tolerance with temperature.

An important halophyte feature is its ability to germinate after being exposed to high salinity (Ungar, 1978), with a substantial

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variability among different halophyte species in recover ability (Khan and Gul, 2006). Seeds of *K. scoparia* possess high recovery ability (Khan et al., 2001), while no comparative data are available on seed recovery in *K. prostrata*.

Central Asia harbors populations of both species, *K. scoparia* and *K. prostrata*. In this region, two *Kochia* species are found in the most arid areas on a variety of soils. Salinized soils of Central Asia are often of mixed type: chloride (chlorides of Na, Ca and Mg prevail), sulfuric, chloride-sulfuric and other (Lobova, 1967). Everitt et al. (1983) showed that germination of seeds of *K. scoparia* was close to 100% under low salinity of KCl, $MgCl_2$, Na_2SO_4 и $MgSO_4$ (below 150 mM), but for $CaCl_2$ and NaCl salinity germination was gradually decreasing from 20 to 40 mmhos/cm NaCl (equivalent to 200 and 400 mM, respectively). However, it is not clear how seeds of *K. scoparia* and *K. prostrata* respond to higher salinity. Most importantly, different ecotypes of the same species occurring in different habitats may possess different strategies to cope with the special environmental conditions during seed germination in their local site (Batanouny, 1995). As *K. scoparia* and *K. prostrata* are important fodder plants that require agricultural zoning, detailed knowledge of their response to salt tolerance at seed and seedling stages is warranted.

The aim of this study was a comparative assessment of *K. scoparia* and *K. prostrata* salt tolerance during germination and development. Specifically, we tested (i) the effects of pure vs. mixed salts and an interaction between salinity and constant temperature regimes on germination and germination recovery in these species, (ii) plant growth of *K. scoparia* and *K. prostrata* under different concentration of NaCl salinity.

2. Materials and methods

Seeds *K. prostrata* and *K. scoparia* were collected from natural populations in two desert areas, Turkmenistan (*K. scoparia*) and Uzbekistan (*K. prostrata*) in October–November 2004. The seeds were stored in paper bags at +6 °C to prevent loss of seed viability (Kitchen and Monsen, 2001).

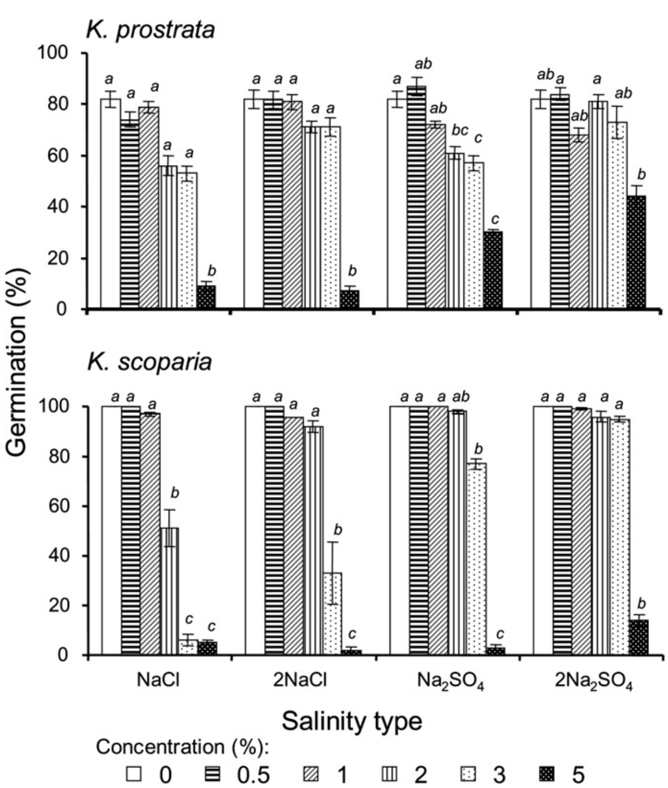

Fig. 1. Effect of different salts and their concentrations (%) on germination percentage (mean ± S.E.) of *K. prostrata* and *K. scoparia* seeds at +22 °C.

Table 1
Results of two-way ANOVA on effect of salt type and concentration on percent germination at two temperature regimes.

Source	*K. prostrata*			*K. scoparia*		
	df	F	Sig.	df	F	Sig.
Temperature +22 °C						
Salt	1	2.0	N.S.	3	43.5	<0.001
Concentration	4	43.3	<0.001	4	306.6	<0.001
Salt*Concentration	4	0.6	N.S.	12	14.3	<0.001
Error	30			60		
Temperature +6 °C						
Salt	3	4.9	<0.01	1	9.4	<0.01
Concentration	4	45.2	<0.001	4	161.2	<0.001
Salt*Concentration	12	2.6	<0.01	4	7.9	<0.001
Error	60			30		

Experiments were conducted during February–April 2005. In germination experiment we used four replicates of 25 seeds per treatment placed on Whatman filter papers in 50 mm diameter Petri dishes with added 7 ml of test solution. Solutions included distilled water, 0.5, 1, 2, 3 and 5% concentrations for NaCl and Na_2SO_4 at constant +22 °C and +6 °C, and for 2NaCl + KCl + $CaCl_2$ and $2Na_2SO_4+K_2SO_4+MgSO_4$ at constant +22 °C. The results of germination were recorded daily during 20 days.

Rate of germination was estimated using a modified Timson's index of germination velocity:

$$G = \frac{\sum G_i}{t}$$

where G_i is the percentage of seed germination at two-day intervals and t is the total germination period (Khan and Ungar, 1984). The maximum value possible using this index was 50.

In recovery experiments seeds that did not germinate under high salt concentrations were transferred to distilled water to study germination recovery. Percentage recovery was calculated by the formula $[(a-b)/(c-b)] \times 100$, where a is the number of seeds germinated in salt solutions plus those that recovered to germination in the fresh water, b is the number of seeds germinated in saline solution, and c is the total number of seeds tested (Khan and Ungar, 1984).

Germinated seeds were transplanted to pots filled with peat to study effect of salinity on plant growth. Eight two-week old seedlings were transferred to 4 L plastic boxes filled with Hoagland solution (Resh, 2001). Plants were treated with 5 NaCl solutions (0.5, 1, 2, 3 and 5%) and a control. To exclude the carryover effect, the seeds germinated under different salinities were randomly distributed among the treatments. Every seven days the solutions were renewed and plants measured for height and root crown diameter. In addition, plants were weighed after completion of the experiment.

Table 2
Results of three-way ANOVA on effect of salt type, concentration and temperature on percent germination.

Source	*K. prostrata*			*K. scoparia*		
	df	F	Sig.	df	F	Sig.
Salt	1	5.0	<0.05	1	82.6	<0.001
Temperature	1	140.9	<0.001	4	392.3	<0.001
Concentration	5	58.7	<0.001	1	320.3	<0.001
Salt * Temperature	1	0.1	N.S.	4	22.2	<0.001
Salt * Concentration	5	1.2	N.S.	1	18.0	<0.001
Temp * Concentration	5	5.7	<0.001	4	21.5	<0.001
Salt * Temp * Concentration	5	0.8	N.S.	4	18.3	<0.001
Error	72			60		

Table 3
The rate of germination at two constant temperature regimes.

Concentration, %	*K. prostrata*				K. scoparia			
	NaCl	Na_2SO_4	2NaCl + KCl + $CaCl_2$	$2Na_2SO_4$+K_2SO_4+$MgSO_4$	NaCl	Na_2SO_4	2NaCl + KCl + $CaCl_2$	$2Na_2SO_4$+K_2SO_4+$MgSO_4$
Temperature +22C								
0	20.5 ± 3.1	20.5 ± 3.1	20.5 ± 3.1	20.5 ± 3.1	48.8 ± 2.5	48.8 ± 2.5	48.8 ± 2.5	48.8 ± 2.5
0.5	25.7 ± 4.5	26.1 ± 4.6	29.5 ± 6.5	26.1 ± 4.1	42.5 ± 5.0	47.5 ± 5.0	50.0 ± 0	50.0 ± 0
1	20.5 ± 6.4	20.9 ± 1.0	24.2 ± 6.6	26.5 ± 5.8	40.0 ± 2.3	45.0 ± 5.8	48.0 ± 0	49.5 ± 1.0
2	14.0 ± 3.7	19.6 ± 2.2	20.4 ± 4.3	24.4 ± 5.6	10.2 ± 3.0	39.2 ± 0.9	41.4 ± 6.1	48.0 ± 4.0
3	6.2 ± 2.8	16.3 ± 3.5	20.6 ± 5.2	20.7 ± 7.0	1.8 ± 1.7	30.8 ± 3.5	11.2 ± 1.6	47.5 ± 2.5
5	3.8 ± 1.4	7.2 ± 2.8	2.8 ± 1.5	11.6 ± 1.4	1.6 ± 0.4	1.4 ± 0.9	0.8 ± 0.4	5.2 ± 1.9
Temperature +6C								
0	6.7 ± 2.4	6.7 ± 2.4			27.3 ± 5.4	27.3 ± 5.4		
0,5	3.4 ± 0.5	3.2 ± 1.1			10.6 ± 5.8	18.4 ± 1.4		
1	1.6 ± 0.6	1.9 ± 0.9			4.9 ± 1.5	11.6 ± 7.4		
2	1.8 ± 0.9	3.6 ± 2.5			0.6 ± 0.1	6.0 ± 3.0		
3	0.7 ± 0.2	1.5 ± 0.8			0.8 ± 0.2	0.3 ± 0.1		
5	0	1.2 ± 1.0			0	0.6 ± 0.1		

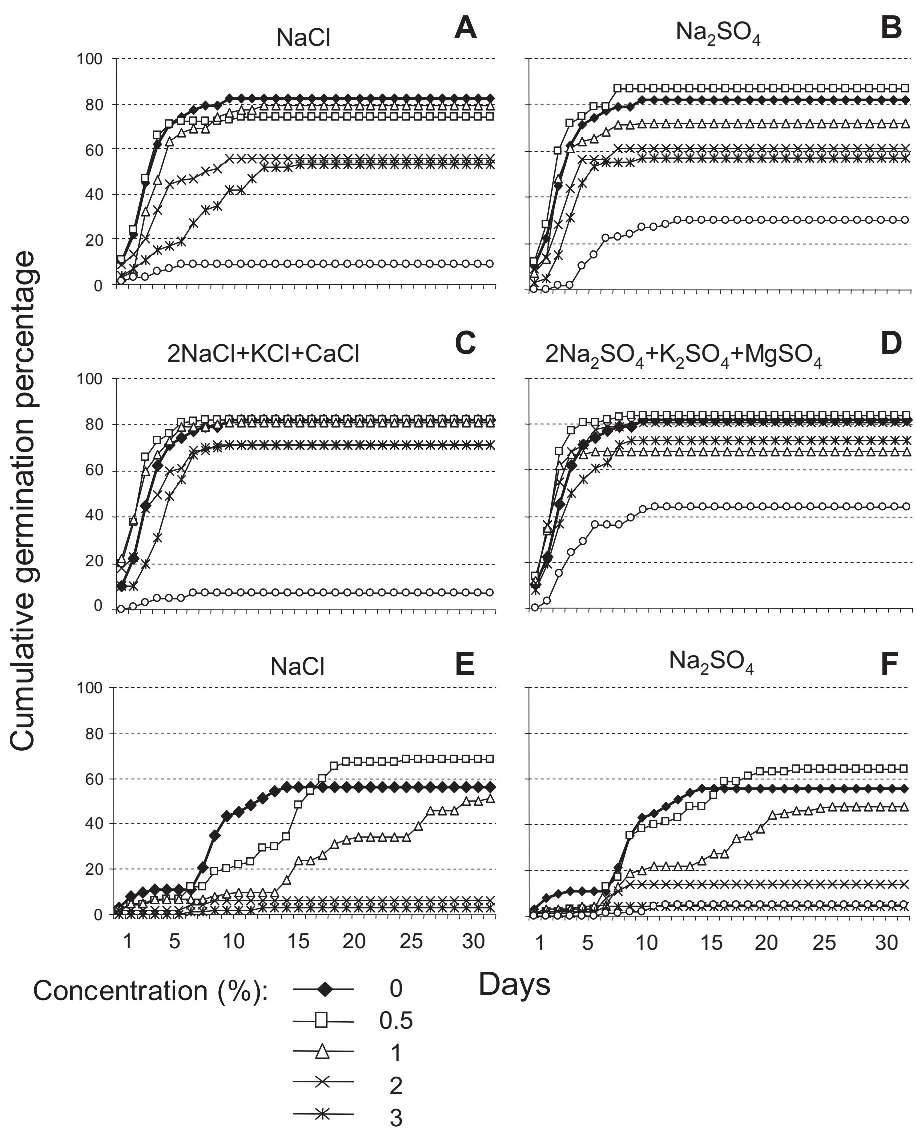

Fig. 2. Effect of different salts and their concentrations on seed germination in *K. prostrata* at two constant thermoregimes, +22 °C (A,B,C,D) and +6 °C (E,F).

Results were analyzed by ANOVA using SPSS version 13.0 (SPSS, Inc., ChicagoIL).

3. Results

Seeds of *K. prostrata* and *K. scoparia* had high percentages of germination in non-saline controls (Fig. 1). *K. scoparia* had higher percentages of germination compared to *K. prostrata* in the control and from 0.5 to 3% concentrations of different salts. Temperature, type of salinity and concentration, as well as their interactions had significant effect on final seed germination in *K. scoparia* (Tables 1 and 2). However, a response of *K. prostrata* seeds to these factors and their interactions was different from *K. scoparia*. For this species germination was affected by salt concentration under both temperature regimes, however salt type was important only at 6 °C (Table 2). A significant interaction was found between temperature and concentration but other interactions were not significant (Table 2). The germination percentage of *K. prostrata* did not differ from control for either soil type under salinity below 3%. For *K. scoparia* germination percentage did not differ from control for NaCl under salinity below 2% and for other salts below 3%. Germination rates as indicated by the index of germination velocity were much higher for *K. scoparia* than for *K. prostrata* (Table 3). Germination rates were decreasing with increasing salt concentration (Fig. 1).

In both species seeds started to germinate in the first day after sowing irrespective of salinity type and tested temperature (Figs. 2 and 3). However, high salt concentrations delayed start of germination. At room temperature (+22 °C) seeds of *K. scoparia* were rapidly germinating under low concentrations (during 3 days), but under high salinity germination period expanded to two weeks (Fig. 2). Seeds of *K. prostrata* at +22 °C were germinating more gradually (during two weeks) with almost no effect of salt concentration (Fig. 3).

Seed germination in both species was affected by temperature and the two species differed in their temperature responses (Figs. 2 and 3). Low temperature caused both reduction and delay in germination in both species under all salt types, especially for concentrations above 2%. Under non-saline control the germination at low temperature in *K. scoparia* was as high as at 22 °C, while in *K. prostrata* low temperature decreased germination from 82 to 56% (Fig. 3). Germination rate at 6 °C was very low and was decreasing with increase in salt concentration (Table 3).

There was a full germination recovery in *K. scoparia* but only a partial recovery in *K. prostrata* (Fig. 4). A one-way ANOVA done on recovery germination showed significant effect ($p < 0.05$) of salt type at 22 °C and 6 °C for both *K. scoparia* and *K. prostrata*. In both species the highest recovery was observed under 3% NaCl at +6°C. The same percentage recovery *K. scoparia* showed also at +22°C.

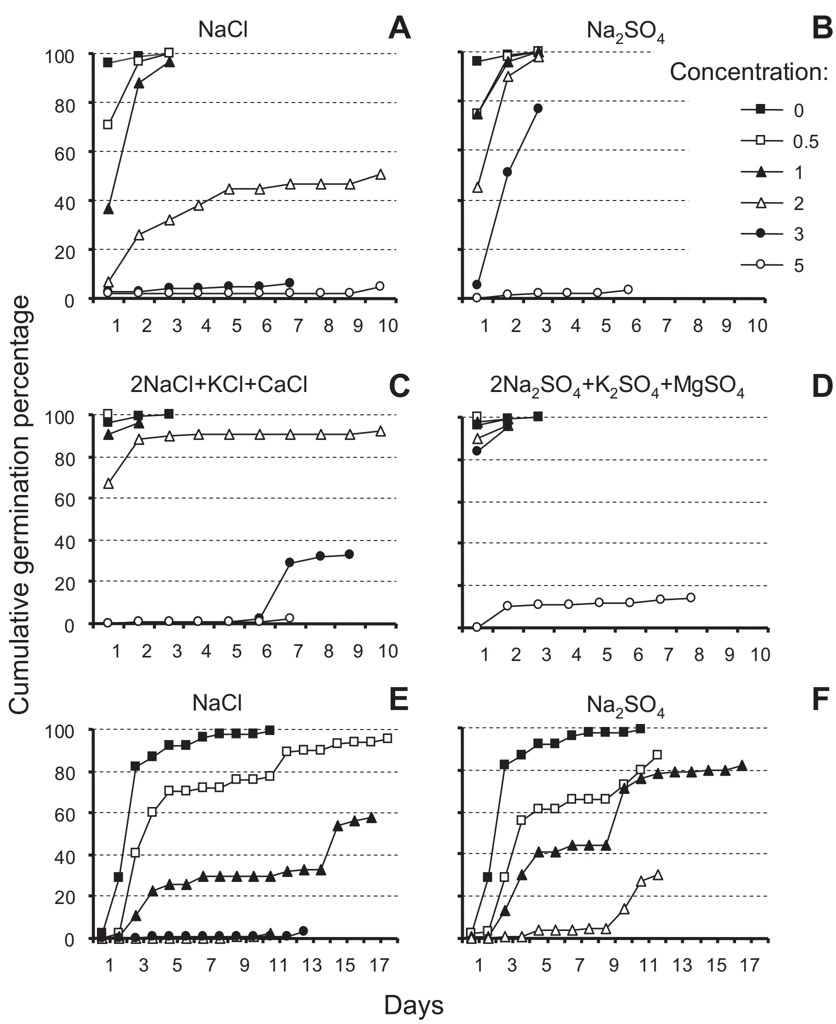

Fig. 3. Effect of different salts and their concentrations on seed germination in *K. scoparia* at two constant thermoregimes, +22 °C (A,B,C,D) and +6 °C (E,F).

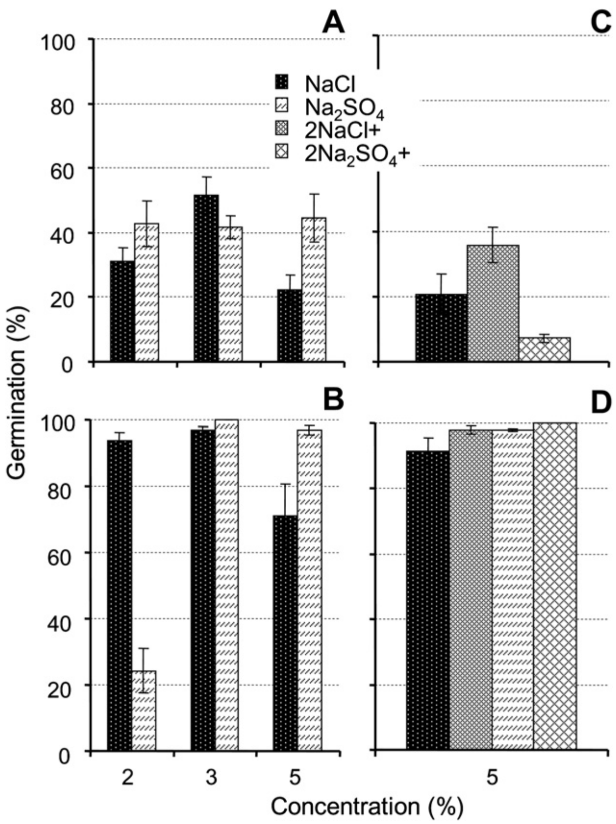

Fig. 4. Percent of recovery germination of *Kochia prostrata* (A, B) and *Kochia scoparia* (C,D) under different salt concentrations at constant temperature 22 °C (A,C) and 6 °C (B,D).

Sulfuric salt showed stimulating effect on recovery after seed exposure to 5% NaCl.

Greenhouse experiments revealed a negative effect of salinity on plant growth and survival. Plants survived a salinity of 3% but died under 5% NaCl. Plant height and weight of plants were negatively related to salinity percentage in both species (Table 4).

4. Discussion

Diversity of halophytes, at least partly, is a result of different susceptibility to salt stress at different life cycle stages. Levels of salt tolerance during germination and at the later stages of development in some species are markedly different (Ungar, 1978). Usually, however, species that are highly salt tolerant during germination have high tolerance at later developmental stages as well. Khan (1999) listed three categories of halophytes according to their salt tolerance during germination: marginally tolerant (with low germination percentage under 125 mM NaCl), moderately tolerant (germinating under up to 500 mM) and highly-tolerant (germinating under 800 mM and higher). According to this classification, *K. prostrata* and *K. scoparia* fall into the last group because their germination under 850 mM NaCl at 22 °C was 9% and 5%, respectively.

Undoubtedly, salt tolerance during germination is affected by temperature. Under low temperature (+6 °C) germination decreased to 2% in both species. Khan et al. (2001) showed that *K. scoparia* had poor germination under 1000 mM NaCl and low temperature regime (5–15 °C), but germination progressively increased with an increase in temperature to 25°C/35 °C. Such high salt tolerance can be specific for a particular accession as a result of environmentally specific local selection in Utah (Khan at al., 2001).

Responses of *K. prostrata* and *K. scoparia* seeds to application of different salts varied. In both species maximum germination was obtained in the non-saline control and under 0.5–1% NaCl, and was gradually decreasing with increase in salinity up to 5%. Similar results were reported for *Atriplex triangularis* (Khan and Ungar, 1984), *Cressa cretica* (Khan, 1991), *Sporobolus ioclados* (Khan and Gulzar, 2003), *Suaeda fruticosa* (Khan and Ungar, 1998), *Salicornia pacifica* (Khan and Weber, 1986), *S. rubra* (Khan et al., 2000), *Haloxylon ammodendron* (Huang et al., 2003), *K. scoparia* (Khan et al., 2009).

In the range of concentrations 0.5–2% rather high germination of *K. prostrata* and *K. scoparia* was observed in sulfuric Na_2SO_4 and mixed chloride (2NaCl + KCl + $CaCl_2$) and sulfuric ($2Na_2SO_4$+K_2SO_4+$MgSO_4$) salts. In mixed chloride salts ions of Ca^{2+} stimulated germination in both species. A similar effect was observed after applying $CaCl_2$ together with NaCl for *Haloxylon ammodendron* (Tobe et al., 2004), *Arthrocnemum indicum*, *A. macrostachyum*, *Desmostachya bipinnata*, *Halopyrum mucronatum* и *Urochondra setulosa* (Khan and Gul, 2006). It is possible that in mixed salts containing $MgSO_4$, magnesium has an effect on germination analogous to calcium. Everitt et al. (1983) showed that low concentrations of Na_2SO_4 and $MgSO_4$ stimulated germination of *K. scoparia* seeds. Our results show that the same salts that demonstrate stimulating effect under low concentrations (0.5–2%), with further increase in concentration (from 2 to 5%) have inhibiting effect similarly to chloride salt.

Many halophytes, e.g., *Salicornia europaea* and *Suaeda calceoliformis* (Keiffer and Ungar, 1997), *Atriplex griffithii, Cressa cretica, Haloxylon recurvum*, and *Suaeda fruticosa* (Khan, 1999), *Salsola*

Table 4
Effect of different concentrations of NaCl (in %) on the fresh weight (gr±S.E) and height (cm±S.E) of *K. prostrate* and *K. scoparia.*

Concentration (%)	Weight (gr)	Height (cm)								
		Time (day)								
		1	8	15	22	29	36	43	50	57
K. prostrata										
0	23.9 ± 6.6	2.9 ± 0.8	4.2 ± 2.0	6.6 ± 2.3	10.2 ± 3.3	15.3 ± 4.9	22.2 ± 7.0	31.9 ± 10.1	41.0 ± 14.2	55.5 ± 15.4
0.5	22.9 ± 4.5	2.1 ± 0.5	4.9 ± 1.3	7.2 ± 1.9	11.6 ± 2.4	17.3 ± 3.1	24.2 ± 4.5	35.5 ± 8.1	44.4 ± 8.1	48.2 ± 7.5
1	22.2 ± 8.4	2.3 ± 0.7	4.7 ± 1.8	5.9 ± 2.2	9.4 ± 2.5	13.1 ± 3.5	17.3 ± 3.8	22.1 ± 5.4	32.5 ± 6.0	42.1 ± 8.8
2	14.1 ± 3.4	2.0 ± 0.6	4.1 ± 0.9	4.3 ± 1.1	6.0 ± 1.5	7.4 ± 1.7	9.5 ± 2.7	13.2 ± 4.8	19.1 ± 5.7	27.8 ± 9.0
3	2.2 ± 0.4	2.1 ± 0.5	3.5 ± 1.2	3.5 ± 1.3	3.8 ± 1.9	4.0 ± 1.8	4.3 ± 1.6	5.6 ± 1.7	7.0 ± 1.9	9.3 ± 5.5
K. scoparia										
0	16.7 ± 1.8	3.4 ± 0.8	4.5 ± 0.8	7.6 ± 0.6	14.5 ± 1.6	22.4 ± 2.9	32.3 ± 3.8	–	–	–
0.5	16.0 ± 1.2	3.2 ± 0.6	4.2 ± 0.7	7.2 ± 0.7	12.6 ± 1.6	19.5 ± 2.6	30.1 ± 2.9	–	–	–
1	12.5 ± 1.2	3.2 ± 0.6	4.0 ± 0.7	6.1 ± 0.6	9.8 ± 1.1	15.0 ± 1.6	22.4 ± 2.6	–	–	–
2	1.4 ± 0.3	2.5 ± 0.8	3.3 ± 0.7	4.0 ± 0.6	5.3 ± 0.7	6.7 ± 1.1	8.9 ± 1.9	–	–	–
3	0.8 ± 0.1	2.9 ± 0.6	4.0 ± 0.6	4.6 ± 0.4	5.2 ± 0.5	5.8 ± 0.7	7.0 ± 0.7	–	–	–

iberica (Khan et al., 2002), and *Salsola imbricata* (El-Keblawy et al., 2007), showed high recovery of germination when salt stress was alleviated. Recovery of seeds of *K. prostrata* transferred to distilled water after exposure for 20 days to 5% NaCl, was gradual and moderate. These results allow us to characterize *K. prostrata* as moderate salt-tolerant species. Recovery germination of *K. prostrata* was much lower than germination of untreated seeds in distilled water and final germination percentages did not differ between the two constant temperature regimes of +22C and +6C. *K. scoparia* had better recovery ability than *K. prostrata*. Seeds of *K. scoparia* were readily germinating (in 1–2 days) after transfer to distilled water and final germination percentages were close to the control. In contrast to *K. prostrata*, recovery of *K. scoparia* varied between temperature regimes and was lower under +6 °C.

According to Shamsutdinov et al. (2005), exceeding a threshold of salt concentration in soil leads to increase in plant content of Na^+ and Cl^- and reflects the efficiency of ion balance. These authors suggested that salinity threshold for *K. prostrata* is higher than for *K. scoparia* (500 vs. 400 mM, respectively). Indeed, in our study *K. prostrata* was found to be more salt tolerant than *K. scoparia* in plant growth. The growth rate and the fresh mass of *K. prostrata* under different concentrations of NaCl were significantly higher as compared with *K. scoparia*. In both species, however, NaCl concentration up to 1% (170 mM) had no effect on these two characters, and had a negative effect at 5% (340 mM) NaCl. These results are similar to those of Ungar (1996) on *Atriplex patula*, Karimi et al. (2005) on *Kohia prostrata*, Khan et al. (2000) on *Atriplex griffithii*.

The salt-tolerance limits of *K. prostrata* and *K. scoparia* from Central Asia during germination in pure (NaCl, Na_2SO_4) and mixed ($2NaCl + KCl + CaCl_2$ and $2Na_2SO_4+K_2SO_4+MgSO_4$) salts at permanent +22 °C was 5%. Sulfuric Na_2SO_4, mixed chloride $2NaCl + KCl + CaCl_2$ and sulfuric $2Na_2SO_4 + K_2SO_4 + MgSO_4$ salts (in concentrations ranging 0.5–3%) alleviated Na and Cl effects and under low concentration stimulated germination. In both species salinity and temperature had synergetic effect, decreasing salt tolerance limit down to 2% NaCl under constant low temperature (+6 °C). Thus, salt tolerance of both studied species is much higher during seed germination than at early growth developmental stage.

Despite similarity of salt-tolerance limits the studied species showed significant difference in seed recovery ability. Negative impact of high salt concentrations on germination in *K. prostrata* appears to be more related to ion-toxity than to osmotic effect. In contrast, high seed recovery ability of *K. scoparia* appears to be due to osmotic effect. Such phenomena should be taken into consideration during phytoremidation of salt-affected lands.

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