Plant Soil
https://doi.org/10.1007/s11104-018-3623-8

REGULAR ARTICLE

The best salt solution parameter to describe seed/seedling responses to saline and sodic salts

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Received: 22 January 2018 / Accepted: 6 March 2018

Abstract
Background and aims Results of studies on plant responses to salt stress often are difficult to compare because different salt parameters were used. Our aim was to compare the effects of different combinations of sodium salts on germination/seedling growth of two forage species and determine which salt solution parameter(s) was(were) most closely related to these responses.
Methods Seeds of the legume *Medicago sativa* and the grass *Elymus dahuricus* were germinated in different concentrations of saline and sodic salts. Various parameters of the salt solutions were determined, and seed germination and seedling growth metrics were measured.
Results Seeds of both species were more tolerant to saline than to sodic salts, and seedlings of *E. dahuricus* were more salt tolerant than those of *M. sativa*. Na_2SO_4 and Na_2CO_3 were more inhibitory to germination/growth of the two study species than the same concentration of NaCl and $NaHCO_3$ for saline and sodic salts, respectively. For both species, electrical conductivity, salt content (%) and Na^+ concentration best correlated with germination/growth for saline salts and Na^+ concentration for sodic salts.
Conclusions In evaluating the effects of salt on seed germination and seedling growth, both saline and sodic salts need to be considered, and Na^+ concentration is the best salt solution parameter to use in comparing and communicating the results.

Keywords EC · Na^+ · pH · Saline salts · Sodic salts · Water potential

Abbreviations

EC	Electrical conductivity
Ψ_p	Water potential
C	Salt concentration
S	Salt content
GP	Germination percentage
GT	Time to start of germination
RL	Root length
SL	Shoot length
Ch	Chlorophyll content

Responsible Editor: Jeffrey Walck.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-018-3623-8) contains supplementary material, which is available to authorized users.

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Published online: 27 March 2018

Introduction

Salinization is a serious problem that increases annually, thus affecting agricultural production and the environment (Farooq et al. 2017). Salt-affected areas cover more than 7% of the earth's land surface (Panta et al. 2014) and 20% of the global irrigated lands in at least 100 countries (Jesus et al. 2015). The global annual cost of salt-affected crop production loss in irrigated areas is estimated to be US$ 27.3 billion (Qadir et al. 2014). Given that little new productive land is available, utilization of salt-affected land and productivity enhancement of crops and forages in salt-affected soils are exclusive options for feeding the expanding world population (Qudir et al. 2008). Thus a comprehensive understanding of salt effects on growth of plant species with potential to be used for agriculture production in salt-affected soils is an important issue.

Salt sensitivity of a given plant species varies with the growth stage (Hassan et al. 2017), with seed germination and early seedling growth being the most vulnerable stages (Dodd and Donovan 1999). Thus, seedling establishment is a bottleneck that must be solved, whether for crop production or phytoremediation in salt-affected soils. Therefore, germination and seedling growth responses of glycophytes (plants that are adapted to low-Na^+ environments, Assaha et al. 2017) (Guan et al. 2009; Nichols et al. 2009; Bina and Bostani 2017) and germination tolerance mechanisms of halophytes (plants that are adapted to saline environments, Flowers et al. 1986) to salts (Ungar 1996; Li et al. 2010a; Zhang et al. 2010; Lin et al. 2016a) have attracted much research attention.

NaCl is the major component in salt-affected soils (Soriano et al. 2014) and thus has been widely used to investigate the effects of salts on germination and seedling growth (Ungar 1996; Song et al. 2005; Hanif et al. 2017). However, in addition to Na^+ and Cl^-, there are many other cations (e.g. Mg^{2+}, Ca^{2+}, K^+) and anions (e.g. SO_4^{2-}, CO_3^{2-}, HCO_3^-) in salt-affected soils (Qadir et al. 2000). The influence of single salts, other than NaCl, on plant growth has been investigated, and these studies include all the kinds of salts known to occur in salt-affected soils (Egan et al. 1997; Sosa et al. 2005; Zhang et al. 2015). Some studies on responses of seeds/seedlings to increased salinity have used mixtures of two or more kinds of salts (Yang et al. 2007; Zhang and Mu 2009; Li et al. 2010a, b; Guo et al. 2011; Lin et al. 2014; Zhao et al. 2014). However, the differences between main single salts and between single salts and mixed salts on seed germination and plant growth remain unclear. For example, what are the differences in germination and seedling growth responses of a plant species at 100 mM NaCl, Na_2SO_4, NaCl:Na_2SO_4 and NaCl:Na_2SO_4:$NaHCO_3$:Na_2CO_3? By making these comparisons, we can better understand the influences of various salts in mixture, which will allow us to extrapolate results from laboratory studies to field conditions with increased reliability.

Salt-affected soils are divided into three categories: sodic (alkaline), saline and saline-sodic soils. Sodic soils contain excess Na^+, high concentration of CO_3^{2-} or HCO_3^- with high pH (>8.5), high sodium absorption ratio (SAR, >13) or exchangeable sodium percentage (ESP, >15) and soil structural problems (Qadir et al. 2000). Saline soils also have a preponderance of Na^+ with the dominant anions being Cl^- and SO_4^{2-}, low pH and high electrical conductivities (>4 dS m^{-1}) (Flowers and Flowers 2005). The characteristics of saline-sodic soils are intermediate between the two categories. Parameters such as water potential, solution concentration, salt content, pH and electrical conductivity can be used to help characterize the three kinds of salt-affected soils. The effects of each parameter on plant growth have been studied, and two or three of them have been compared in some studies (Shi and Wang 2005; Wehr et al. 2016). However, we do not know which of these parameter(s) is(are) most closely related to seed germination and plant growth. Thus, information on plant responses to salt and the salt solution parameters that best correlate with them is needed to plan for effective restoration and utilization of salt-affected soils.

The Songnen grassland in northeast China is famous for its productive pastures, but salinization-alkalinization has become a serious problem (Zhang et al. 2013). Thus, restoration and utilization of saline-sodic Songnen grassland is an urgent ecological and economic issue. *Medicago sativa* (Fabaceae) and *Elymus dahuricus* (Poaceae) are promising perennial forage species for use in this region. *Medicago sativa* has high nutritional value and moderate salt tolerance (Farissi et al. 2011), and *E. dahuricus* is known not only for its nutritional value but also for its high tolerance to cold, drought and salt (Zhang and Nan 2007). However, information is still lacking about how the seed germination and seedling establishment phases of these two glycophytic forage species (Zhao et al. 2002) respond to saline and sodic salts (Li et al. 2010b; Sun et al. 2016).

Thus, the objectives of our study were to determine: 1) the effects of different sodium salts on germination and early seedling responses of *Medicago sativa* and *Elymus dahuricus*, 2) the effects of different combinations of sodium salts (single, two mixed, four mixed) on seeds/seedlings, and 3) which parameter(s) of salts is(are) most closely related to the seed germination and seedling growth responses to increased salinities.

Materials and methods

Seed collection and storage

Seeds of *Medicago sativa* and *Elymus dahuricus* were collected from more than 50 plants in autumn from western Jilin Province (44°33′ N, 123°31′ E) in China and stored dry in cloth bags at room temperature for 8 months to break physiological dormancy of *E. dahuricus* before the experiment was initiated.

Salt treatments

Seven different kinds/combinations of sodium salts were used: four single salts NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3; a 1:1 molar ratio mixture of NaCl:Na_2SO_4 and of $NaHCO_3$:Na_2CO_3; and a 1:1:1:1 molar ratio mixture of NaCl:Na_2SO_4:$NaHCO_3$:Na_2CO_3. Based on results of a preliminary experiment, concentrations of the saline salts (NaCl, Na_2SO_4, NaCl:Na_2SO_4) used for *M. sativa* were 40, 60, 80, 100, 120, 160, 200 and 240 mM, and concentrations of the sodic salts ($NaHCO_3$, Na_2CO_3, $NaHCO_3$:Na_2CO_3, NaCl:Na_2SO_4:$NaHCO_3$:Na_2CO_3) used were set at 10, 20, 40, 60 and 80 mM. For *E. dahuricus*, concentrations of the saline salts were set at 40, 80, 120, 160, 200, 240, 320 and 400 mM and those of the sodic salts at 20, 40, 60 80, 100, 120, 160 and 200 mM. Distilled water was used as 0 mM salt treatments. These concentrations were chosen to achieve germination from 100 to 0%. Electrical conductivity (EC) and pH of all salt solutions were measured by DDS-307 and PHS-3C instruments (INESAS Scientific Instrument Co., Ltd., Shanghai, China), respectively. Water potential of each salt solution was measured with a WP4-T Dewpoint Potentia Meter (USA). Salt content (%) and Na^+ concentration (mM) of each solution were calculated (Table 1). The salt content in the soil of Songnen grassland was reported to be 0.7–1% (Lu et al. 1998), which is equivalent to 120–171 mM NaCl.

Germination experiment

The experiment was conducted in programmed incubators with a 12-h daily photoperiod (Sylvania cool white fluorescent lamps, 100 μmol m^{-2} s^{-1}, 400–700 nm) at 25/15 °C day/night temperature. Seeds of both species were surface sterilised in 0.1% $KMnO_4$ for 10 min and rinsed with distilled water. They were then sown on two folds of filter paper placed in 9-cm-diameter new plastic Petri dishes with 10 ml of the test solution. There were three replicates with 50 seeds for each species in each salt treatment and in distilled water. The Petri dishes were sealed with Parafilm to prevent evaporation of water. The salt solutions and Parafilm were replaced as necessary. Seeds were considered to have germinated upon emergence of the radicle. Germination was recorded every four hours at the beginning and then daily when the germination speed decreased. The experimental period lasted for 14 days, at which time germination had stopped. Final germination percentage and time to start germination were calculated.

Five seedlings were sampled randomly from each Petri dish after 14 days to measure radicle and shoot length. Five hundred milligrams of fresh leaves from the remaining seedlings in each Petri dish were ground and extracted with acetone-ethanol solutions for 48 h. Absorbance (A) was measured at 663 and 645 nm with a spectrophotometer (UV-1800, SHIMADZU). The total chlorophyll content was calculated as chlorophyll a + chlorophyll b = $8.02A_{663} + 20.21A_{645}$ (Bao et al. 2012).

Data analysis

Germination data were transformed (arcsine) before statistical analysis to ensure homogeneity of variance. GLM analysis was used to analyze the effects of species, salt type, salinity and their interactions on germination percentage, germination rate, radicle length, shoot length and chlorophyll content. Multiple comparison tests were used to compare differences among treatment means at the 0.05 level. Pearson correlation between early seedling metrics and salt parameters was analyzed. Statistical analyses were carried out in SPSS (version 18.0, SPSS Inc., Chicago, Illinois, USA).

Table 1 Salt concentration (C, mM), sodium ion concentration (Na^+, mM), salt content (S, % or g/100 g), pH, water potential (Ψ_p, MPa) and electrical conductivity (EC, dS/m) of different salt solutions

Salt types	C	Na^+	S	pH	Ψ_p	EC
Control	0	0	0	6.03	0.00	0.01
NaCl	40	40	0.23	6.28	−0.14	3.36
	60	60	0.35	6.89	−0.38	4.90
	80	80	0.47	6.00	−0.46	6.46
	100	100	0.59	6.62	−0.53	7.84
	120	120	0.70	6.15	−0.59	9.25
	160	160	0.94	6.01	−0.70	12.11
	200	200	1.17	6.13	−0.84	14.65
	240	240	1.40	6.51	−1.12	17.00
	320	320	1.87	6.43	−1.39	21.90
Na_2SO_4	40	80	0.57	6.56	−0.32	5.47
	60	120	0.85	6.43	−0.38	7.66
	80	160	1.14	5.89	−0.48	9.84
	100	200	1.42	6.68	−0.53	11.82
	120	240	1.70	6.34	−0.83	13.78
	160	320	2.27	6.23	−0.94	17.27
	200	400	2.84	6.19	−1.07	20.80
	240	480	3.41	5.79	−1.31	23.50
	320	640	4.54	5.98	−1.73	29.30
$NaCl:Na_2SO_4$	40	60	0.40	6.93	−0.27	4.54
	60	90	0.60	6.64	−0.38	6.40
	80	120	0.80	6.11	−0.44	8.44
	100	150	1.00	5.91	−0.48	10.02
	120	180	1.20	6.29	−0.63	11.77
	160	240	1.60	6.00	−0.79	15.20
	200	300	2.01	5.82	−1.02	18.05
	240	360	2.41	5.81	−1.28	20.50
	320	480	3.21	6.12	−1.66	26.60
$NaHCO_3$	10	10	0.08	8.70	−0.12	0.78
	20	20	0.17	8.80	−0.15	1.35
	40	40	0.34	8.87	−0.17	2.47
	60	60	0.50	8.80	−0.32	3.62
	80	80	0.67	8.70	−0.38	4.69
	100	100	0.84	8.72	−0.50	5.67
	120	120	1.01	8.51	−0.55	6.76
	160	160	1.34	8.48	−0.71	8.69
Na_2CO_3	10	20	0.11	11.01	−0.06	1.62
	20	40	0.21	11.20	−0.14	2.91
	40	80	0.42	11.21	−0.27	5.32
	60	120	0.64	11.43	−0.37	7.50
	80	160	0.85	11.42	−0.43	9.44
	100	200	1.06	11.44	−0.53	11.41
	120	240	1.27	11.36	−0.70	13.28
	160	320	1.70	11.30	−0.96	16.66
$NaHCO_3:Na_2CO_3$	10	15	0.10	10.25	−0.10	1.12
	20	30	0.19	10.23	−0.18	2.08
	40	60	0.38	10.17	−0.27	3.88
	60	90	0.57	10.15	−0.38	5.54
	80	120	0.76	10.12	−0.42	7.08
	100	150	0.95	10.11	−0.58	8.47
	120	180	1.14	10.03	−0.66	9.93
	160	240	1.52	9.99	−0.85	12.68
$NaCl:Na_2SO_4$: $NaHCO_3:Na_2CO_3$	10	15	0.10	10.54	−0.16	1.26
	20	30	0.20	10.58	−0.19	2.26
	40	60	0.39	10.06	−0.26	4.25
	60	90	0.59	10.14	−0.34	6.06
	80	120	0.78	10.08	−0.45	7.68
	100	150	0.98	10.09	−0.62	9.34
	120	180	1.17	9.99	−0.69	10.96
	160	240	1.56	9.95	−0.89	13.98

Results

Salt type, salinity, species and their interactions had significant effects on seed germination and seedling growth whether the data for saline salts and sodic salts were analyzed together or separately ($P < 0.05$), except species × salt type effect on germination rate for sodic salts ($P = 0.189$).

As salinity increased, germination percentages of the two species decreased (Fig. 1). Seeds of the two species germinated in higher concentrations of saline than in sodic salts. Germination percentages for both species were highest in NaCl or $NaHCO_3$ and lowest in Na_2SO_4 or Na_2CO_3 solutions at the same concentration among the three saline salts and four sodic salts, respectively, with those of seeds in the mixed solutions being intermediate. Seeds of *E. dahuricus* germinated at higher salinities than those of *M. sativa* in the same salt concentration.

The time lag to start of germination increased with salinity, from 1 d in distilled water to around 4 d in 240 mM NaCl for *M. sativa* and from around 2 d in distilled water to 12 d in 100 mM Na_2CO_3 solution for *E. dahuricus* (Fig. 2). However, the time to start of germination decreased significantly at

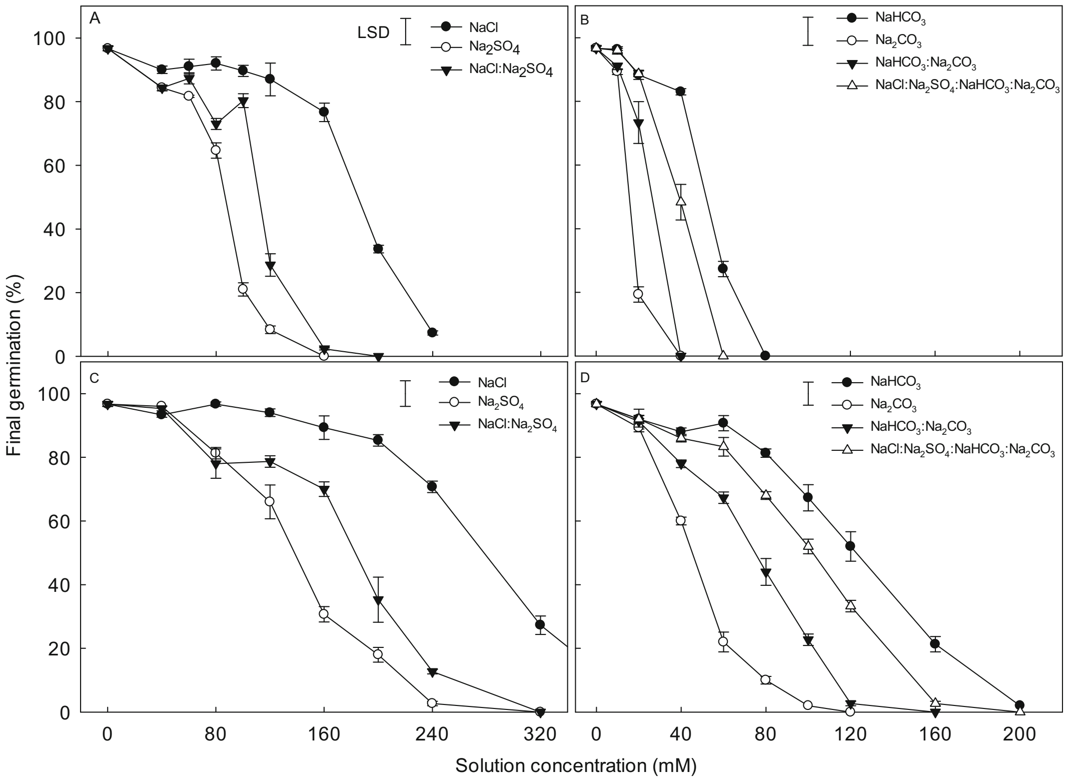

Fig. 1 Final germination percentage of *M. sativa* (**a**, **b**) and *E. dahuricus* (**c**, **d**) at different concentrations of saline (**a**, **c**) and sodic (**b**, **d**) salts

lower salinities compared with that in distilled water for *M. sativa* ($P < 0.05$). Na_2SO_4 and Na_2CO_3 had the greatest negative effects on germination time (largest increase in time to start of germination) among saline and sodic salts, respectively.

All single and combinations of salts tested greatly influenced radicle length of both species (Fig. 3). Among the saline salts, Na_2SO_4 had more inhibitory effects on radicle length than NaCl. Even 40 mM Na_2SO_4 decreased radicle length of *M. sativa* and *E. dahuricus* 70.3% and 58.6%, respectively, compared with that in distilled water. Radicle length of the two species was comparable in distilled water, but that of *E. dahuricus* was less affected by saline solutions than *M. sativa*. Among the sodic solutions, for both species, $NaHCO_3$ had less effect on radicle length than Na_2CO_3. Radicle length in mixed solutions of the four salts was longer than that in $NaHCO_3$ solution at low salinity, e.g. 10 mM for *M. sativa* and 40 mM for *E. dahuricus*. At lower salinities, $NaHCO_3$ had less or a similar effect on radicle length of *M. sativa* than on that of *E. dahuricus*. For example, 20 and 40 mM $NaHCO_3$ decreased radicle length 1.3% and 77.0%, respectively, for *M. sativa* and 51.2% and 69.3%, respectively, for *E. dahuricus* compared with distilled water. However, radicle length of *E. dahuricus* was less affected than that of *M. sativa* by sodic salts at ≥40 mM salinities.

Shoot length of *E. dahuricus* was nearly 10 times that of *M. sativa* in distilled water (Fig. 4), and for both species it was much less affected by salts than radicle length. For example, a 50% decrease in shoot length occurred at 120–160 mM NaCl, 40–60 mM Na_2SO_4, 20–40 mM $NaHCO_3$ and 10–20 mM Na_2CO_3 for *M. sativa* and at 160–200 mM NaCl, 80–120 mM Na_2SO_4, 80–100 mM $NaHCO_3$ and 20–40 mM Na_2CO_3 solutions for *E. dahuricus*. Shoot length of *E. dahuricus* was less affected than that of *M. sativa*, but low salinities of NaCl (≤100 mM) increased shoot growth compared with distilled water for *M. sativa*. The effects of salts on chlorophyll content were similar to those on shoot length (Fig. 5). Notably, salts had less influence on chlorophyll content of *E. dahuricus* than they did on *M. sativa*, especially Na_2SO_4 and Na_2CO_3.

Germination percentages, germination rates and radicle length were most strongly correlated with Na^+ concentration, EC and salt content (%) in saline salts for

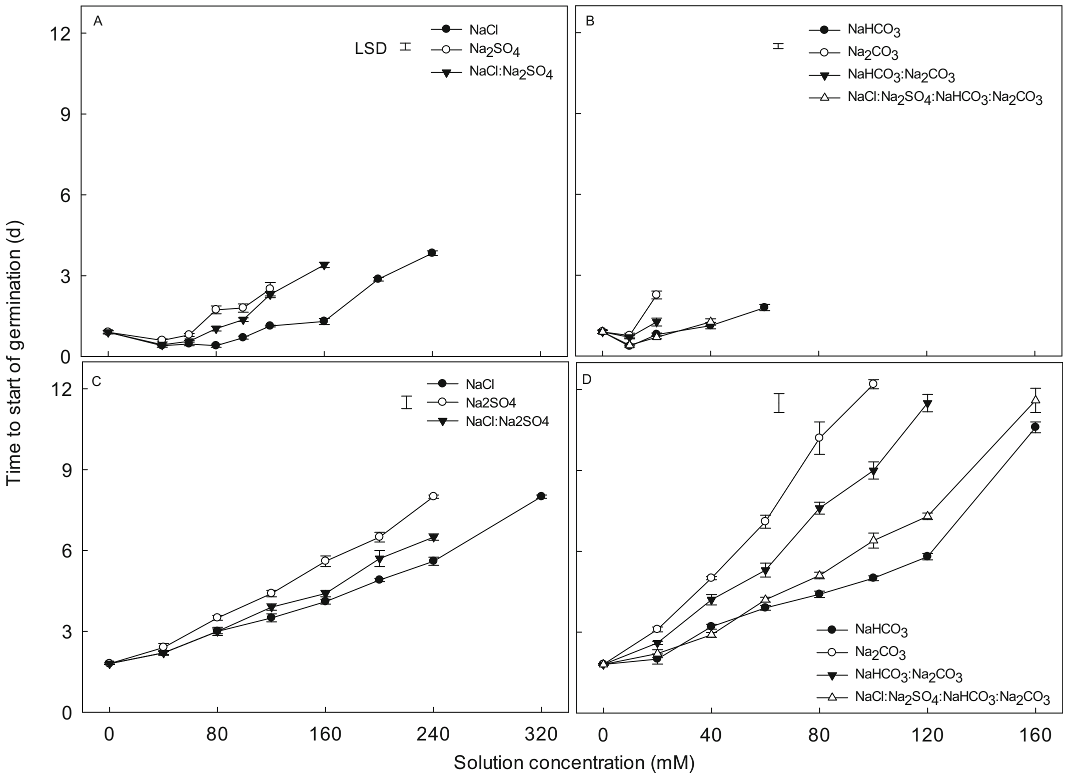

Fig. 2 Time to start of germination for *M. sativa* (**a**, **b**) and *E. dahuricus* (**c**, **d**) at different concentrations of saline (**a**, **c**) and sodic (**b**, **d**) salts

both species (Table 2). Shoot length and chlorophyll content of *E. dahuricus* were best correlated with EC, while for *M. sativa* they were best correlated with salt content (%) and Na^+ concentration. However, all germination and seedling metrics were best correlated with Na^+ concentration in sodic salts. When saline and sodic salts were analyzed together, germination and seedling metrics were best correlated with salt content (%) or Na^+ concentration for both species. Water potential and salt concentrations were significantly correlated with germination and seedling metrics ($P < 0.05$), however, pH was not significantly correlated with these growth metrics for either species in most circumstances ($P > 0.05$).

Discussion

Elymus dahuricus and *M. sativa* are excellent candidate forage species to grow in the saline-sodic Songnen grassland region of northeast China. *M. sativa* can germinate at > 240 mM NaCl and 60 mM $NaHCO_3$. The salt tolerance of *M. sativa* is comparable to that of other legume species such as *Trifolium tomentosum* (Nichols et al. 2009) and *Acacia schaffneri* (Everitt 1983). Further, seeds of *E. dahuricus* germinated to 21.3% and 2.0% in 320 mM NaCl and 200 mM $NaHCO_3$, respectively, which indicates that it is more tolerant to saline and sodic salts than the dominant grass species *Leymus chinensis* (Ma et al. 2015) and the common halophytic grass species *Chloris virgata* (Lin et al. 2016a) in the Songnen grasslands.

Effect of different kinds of salts

Among the single salts, the effects of sodic salts ($NaHCO_3$ and Na_2CO_3) were more inhibitory than those of the saline salts (NaCl and Na_2SO_4) on germination and seedling growth, as well as on chlorophyll content of the two species, which is consistent with results from studies on the glycophytes *Medicago ruthenica* (Guan et al. 2009), *C. virgata* (treated as halophyte in Lin et al. 2016a, but not in Zhao et al. 2002) and *L. chinensis* (Lin et al. 2016b). However, some halophytic species such as *Atriplex undulata* (Piovan et al. 2014) and *Borszczowia*(Suaeda) *aralocaspica* (Zhang et al. 2015) are more tolerant or equally tolerant to sodic than saline

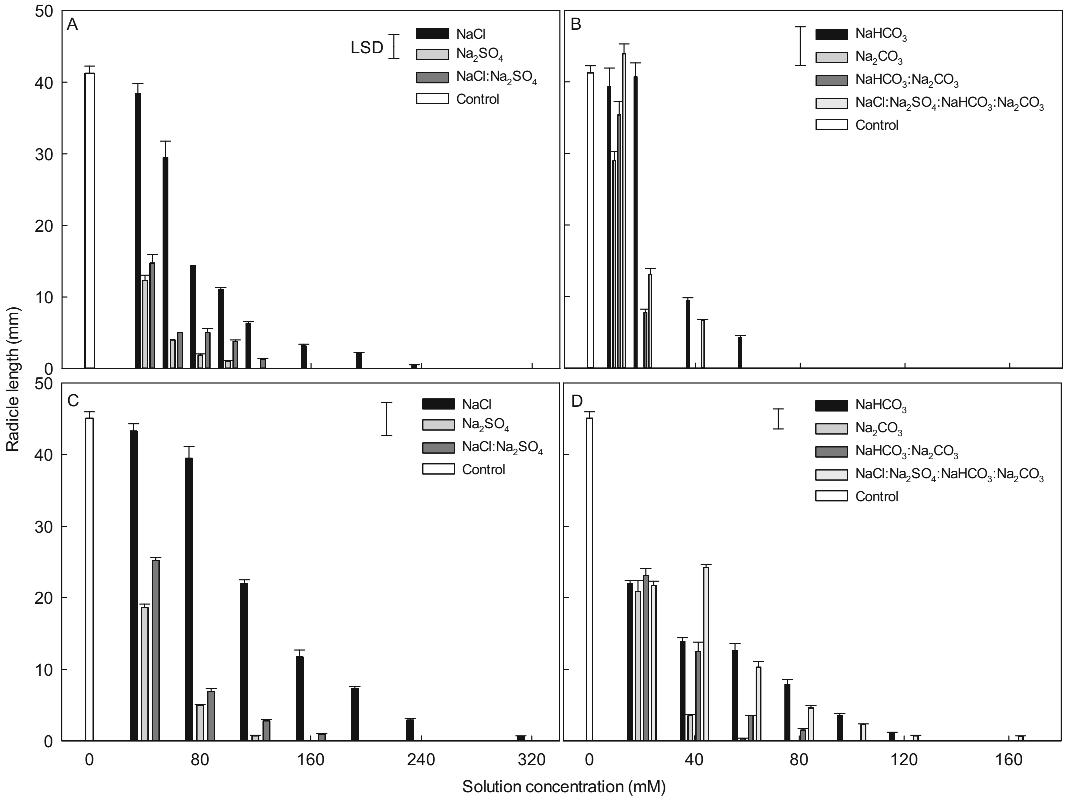

Fig. 3 Radicle length of *M. sativa* (**a**, **b**) and *E. dahuricus* (**c**, **d**) at different concentrations of saline (**a**, **c**) and sodic (**b**, **d**) salts

salts. In our study, Na_2SO_4 was more inhibitory than NaCl among saline salts and Na_2CO_3 was more inhibitory than $NaHCO_3$ among sodic salts on germination and seedling growth of both species at the same concentrations. The results from previous studies on effects of saline/sodic salts on germination are contradictory and species dependent. For example, NaCl was more inhibitory than Na_2SO_4 on germination of the halophytes *Arthrocnemum macrostachyum*, *Juncus acutus* (Vicente et al. 2009) and the glycophyte *Pinus halepensis* (Nedjimi 2017), but Na_2SO_4 was more inhibitory than NaCl on germination of the halophytes *Prosopis strombulifera* (Sosa et al. 2005) and *Ceratoides latens* (Zhang et al. 2015). Na_2CO_3 was more inhibitory than $NaHCO_3$ for germination of the glycophyte *L. chinensis* (Ma et al. 2015). However, germination percentages were comparable at the same concentrations of $NaHCO_3$ and Na_2CO_3 solutions for the glycophyte *Zea mays* (Zhang and Zhao 2011). Halophytes are usually more tolerant to salinity than glycophytes and halophytes and glycophytes use different mechanisms to cope with salt ions (Qudir et al. 2008). However, the contradictory responses to a salt are not necessarily related to whether the species is a halophyte or glycophyte.

In our study, we also included a 1:1 molar ratio mixture of $NaCl:Na_2SO_4$ and of $NaHCO_3:Na_2CO_3$ and a 1:1:1:1 molar ratio of the four mixed salts. Compared with the results for single salts, germination and seedling growth metrics of the two mixed saline and sodic salts approached those of seeds/seedlings in Na_2SO_4 and Na_2CO_3 for both species, respectively. This means the single salt with strong effect (Na_2SO_4 and Na_2CO_3) played a more important role in the two-salt mixture than the one with weak effect, regardless of saline or sodic salts. However, radicle length, shoot length and chlorophyll content of both study species in mixed salts were intermediate between those of the two single salts or approached values of NaCl or $NaHCO_3$ in some low concentration treatments (Figs. 3, 4, 5). The effect on germination traits of the four-salt mixture was intermediate between that of the $NaHCO_3:Na_2CO_3$ mixture and the $NaHCO_3$ single salt, but seedling metrics of the four-salt mixture treatment sometimes were larger than those of $NaHCO_3$. Therefore, sodic salts predominate in the four-salt mixture, with addition of saline salts mitigating the effect of the strong

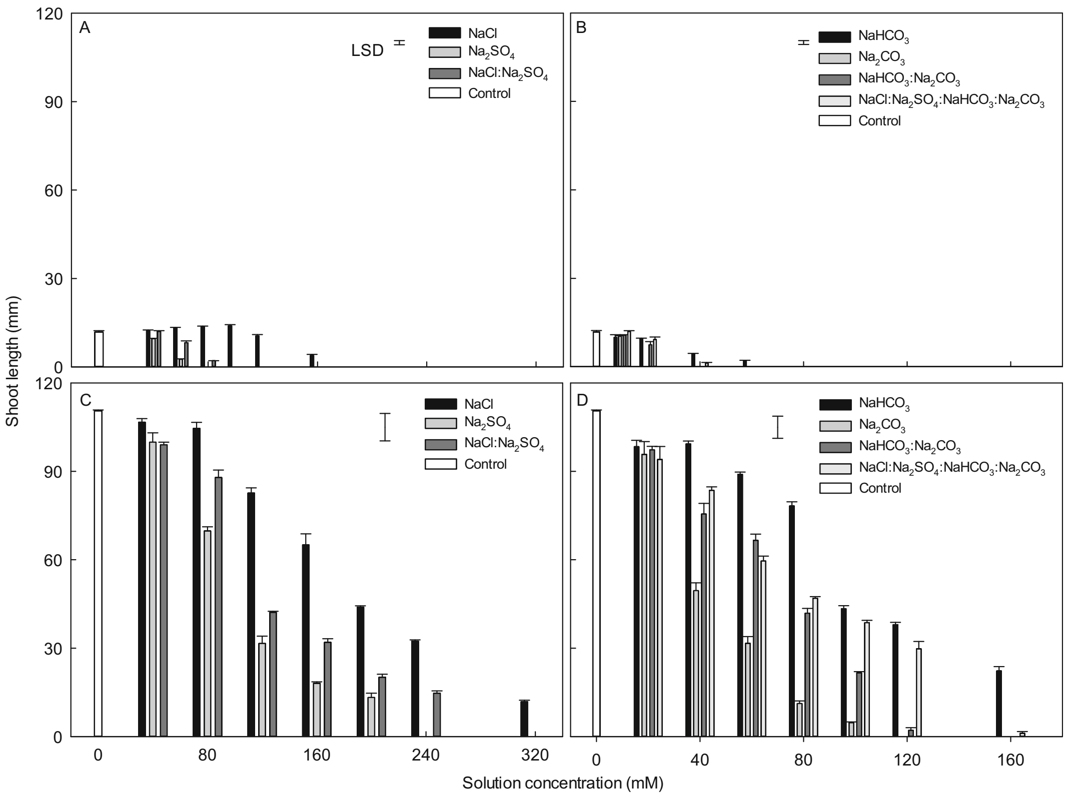

Fig. 4 Shoot length of *M. sativa* (**a**, **b**) and *E. dahuricus* (**c**, **d**) at different concentrations of saline (**a**, **c**) and sodic (**b**, **d**) salts

sodic salt (Na_2CO_3) and enhancing the effect of the weak sodic salt ($NaHCO_3$). Our results agree with those of Ryan et al. (1975) that germination is affected by both the total concentration and the type of salts involved and that extrapolation of results from monosaline solutions in the laboratory to field conditions may be at best speculative (Sosa et al. 2005). Additionally, temperature can influence germination and seedling growth responses to salinity (Zhang et al. 2013). Our study investigated germination/seedling responses to different salts under optimal temperature (25/15 °C) of the two species.

Different parameters of salt such as concentration (mM), water potential and electrical conductivity (EC) have been used in various studies on the effects of salts on germination and seedling growth according to different experimental aims (Table S1). However, lack of consistency in use of salt solution parameters often makes it difficult to compare results from different studies.

Salt concentration (mM)

Many simulation experiments have used salt concentration levels (mM) of either NaCl, other single salts or mixed salts to test the effects of salt on germination and plant growth (Gul and Weber 1999; Sosa et al. 2005; Lin et al. 2014). This approach may be used because halophytes are defined as species with the ability to complete their life cycle under salinity levels of ≥200 mM NaCl (Flowers et al. 1986; Flowers and Colmer 2008). With this salinity level in mind, a lot of work has been done to determine the range of salt tolerance for halophytes (Li et al. 2010a) as well as for glycophytes (Zhang et al. 2013). However, we found that salt concentration was not the salt parameter most closely correlated with germination and seedling growth of either study glycophytic species.

Water potential (MPa)

Saline stress usually involves osmotic effect and/or ion injury (Dodd and Donovan 1999). To distinguish the two effects, the influences of NaCl were compared with those of iso-osmotic PEG (Song et al. 2005; Zhang et al. 2010) or other salts (Egan et al. 1997; Sosa et al. 2005). The inhibitory effect of salts can be mainly an osmotic effect (Egan et al. 1997), ion toxicity (Shaygan et al.

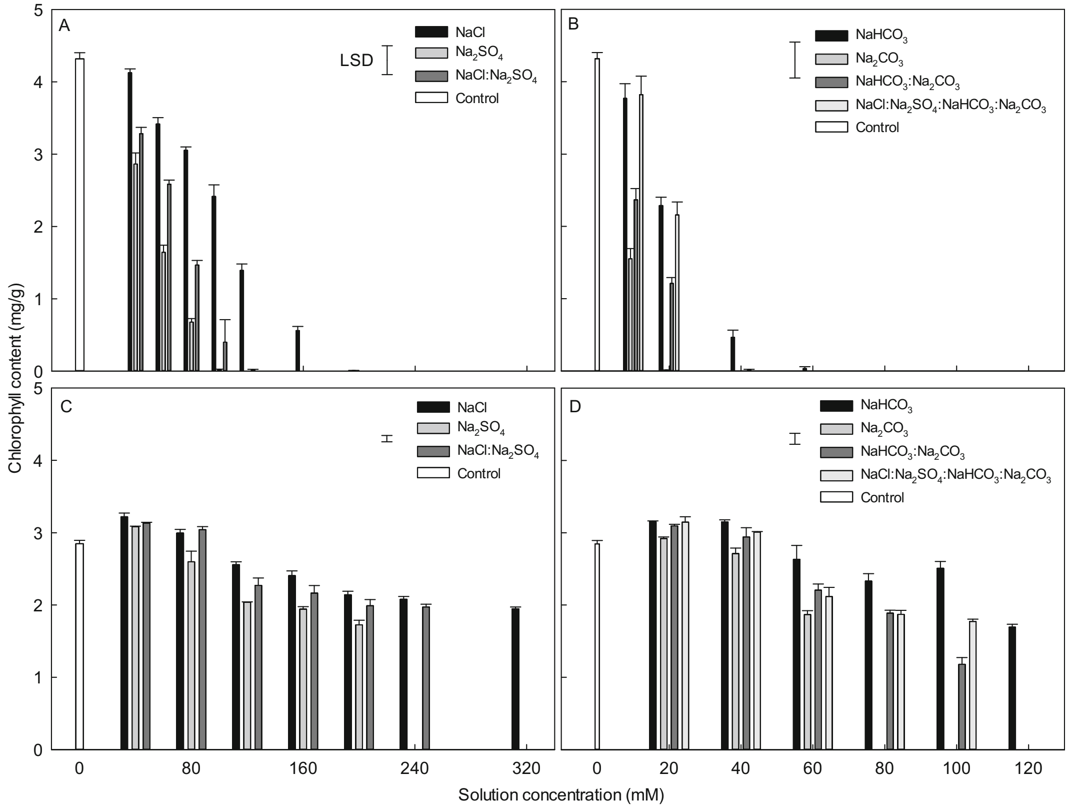

Fig. 5 Chlorophyll content of *M. sativa* (**a**, **b**) and *E. dahuricus* (**c**, **d**) at different concentrations of saline (**a**, **c**) and sodic (**b**, **d**) salts

2017) or both (Song et al. 2005), which suggests that the effect is species dependent (Dodd and Donovan 1999). Our study showed that water potential was not the salt parameter best correlated with germination and seedling growth for *M. sativa* or *E. dahuricus* in either saline or sodic conditions. However, water potential was still important, because it was significantly correlated with germination and seedling metrics in most circumstances, and the correlation coefficient was comparative to that of Na^+ concentration in certain cases, e.g. -0.976 for water potential and 0.944 for Na^+ concentration for germination rate in saline salts for *E. dahuricus*.

pH

In addition to osmotic and ion stress, sodic salts induce pH stress (Ma et al. 2015), and many researchers have emphasized the effect of pH on growth of plants exposed to sodic salts (Shi and Wang 2005; Li et al. 2010a; Basto et al. 2013; Huang et al. 2017). However, pH was not correlated to germination or seedling growth in saline and sodic conditions for either species in our study. Only root length of *M. sativa* and time to start germination of *E. dahuricus* weakly significantly correlated to pH, when analyzing with all salts (Table 2). Similarly, using mixtures of various proportions of NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 to obtain different levels of salinity (25 and 50 mM) and pH (7.2–10.8), Zhang and Rue (2014) found insignificant effects of pH on germination of seven turfgrasses. Germination of *Sorghum bicolor* also was not sensitive to pH level (7.08–9.29) produced by mixtures of NaCl, Na_2SO_4 and $NaHCO_3$ at ≤200 mM Na^+ concentrations (Zhao et al. 2014). In addition, pH was not a limiting factor for germination of *Parkinsonia aculeata*, *Acacia schaffneri* (pH 2–12, Everitt 1983), *Leymus chinensis* (pH 7.0–12.01, Ma et al. 2015) and *Salsola foetida* (pH 5–10, Hanif et al. 2017) seeds tested using buffer solutions. Therefore, pH may be not a good indicator of the negative effects of the salt-affected soils on the early stages of plant establishment, even in sodic conditions.

Table 2 Pearson correlation coefficients between early seed/seedling metrics and salt parameters

		pH	EC	Ψ_p	C	S	Na^+
	GP	0.118	−0.867**	0.779**	−0.691**	−0.917**	**−0.920****
Saline salts (3)	GT	−0.290	**0.948****	−0.895**	0.864**	0.884**	0.936**
M. sativa	RL	0.305	−0.742**	0.621**	−0.536*	**−0.812****	−0.784**
N = 19	SL	0.389	−0.578	0.276	−0.272	**−0.825****	−0.713*
	Ch	0.477	−0.954**	0.798**	−0.745**	−0.964**	**−0.982****
	GP	0.388	−0.900**	0.856**	−0.749**	−0.950**	**−0.952****
Saline salts (3)	GT	−0.349	**0.979****	−0.976**	0.945**	0.896**	0.944**
E. dahuricus	RL	0.091	−0.780**	0.692**	−0.595*	**−0.874****	−0.833**
N = 19	SL	0.260	**−0.967****	0.922**	−0.849**	−0.932**	−0.959**
	Ch	0.302	**−0.951****	0.909**	−0.841**	−0.907**	−0.936**
	GP	−0.108	−0.808**	0.591	−0.641*	−0.690*	**−0.809****
Sodic salts (4)	GT	0.143	0.733*	−0.454	0.586	0.631*	**0.745****
M. sativa	RL	0.042	−0.845**	0.690*	−0.766**	−0.807**	**−0.857****
N = 11	SL	0.379	−0.929**	0.813**	−0.930**	−0.953**	**−0.963****
	Ch	−0.068	−0.868**	0.506	−0.732*	−0.789**	**−0.879****
	GP	−0.298	−0.904**	0.797**	−0.764**	−0.835**	**−0.925****
Sodic salts (4)	GT	0.217	0.905**	−0.822**	0.809**	0.868**	**0.935****
E. dahuricus	RL	0.008	−0.782**	0.766**	−0.784**	−0.795**	**−0.800****
N = 25	SL	−0.235	−0.942**	0.862**	−0.829**	−0.891**	**−0.954****
	Ch	−0.035	−0.927**	0.890**	−0.844**	−0.903**	**−0.950****
	GP	0.153	−0.648**	0.621**	−0.587**	**−0.715****	−0.703**
All salts (7)	GT	−0.225	0.753**	−0.745**	0.751**	0.752**	**0.773****
M. sativa	RL	0.485*	−0.717**	0.660**	−0.623**	**−0.763****	−0.741**
N = 30	SL	−0.067	−0.229	0.137	−0.175	**−0.445***	−0.335
	Ch	−0.010	−0.495**	0.420*	−0.461*	**−0.566****	−0.548**
	GP	−0.176	−0.608**	0.598**	−0.536**	−0.657**	**−0.685****
All salts (7)	GT	0.307*	0.452**	−0.472**	0.454**	0.453**	**0.497****
E. dahuricus	RL	−0.150	−0.553**	0.552**	−0.485**	**−0.679****	−0.640**
N = 44	SL	−0.066	−0.743**	0.737**	−0.686**	−0.758**	**−0.792****
	Ch	0.032	−0.626**	0.648**	−0.606**	−0.651**	**−0.661****

EC, electric conductivity (dS/m); Ψ_p, water potential (MPa); C, salt concentration (mM); S, salt content (%); Na^+, sodium ion concentration (mM); GP, germination percentage; GT, time to start of germination; RL, radicle length; SL, shoot length; Ch, chlorophyll content. Correlation coefficients in bold are the highest values for each seed/seedling metric, which means the best correlated parameter for the growth metric

[*] Correlation is significant at the 0.05 level

[**] Correlation is significant at the 0.01 level

EC (dS/m) and salt content (%)

EC and salt content (%) have been relatively less frequently used than salt concentration (mM) and water potential to simulate salt effects on germination and seedling growth (Ungar 1996; Bina and Bostani 2017). However, these two salt parameters were preferred by some researchers for use in crop production and field studies in salt-affected soils (Qudir et al. 2008; Kanawapee et al. 2012; Glenn et al. 2013). The "bent stick" plant growth curve to salinity was based on EC of saline soils (Maas and Hoffman 1977; Barrett-Lennard 2002). In our study, EC was the most highly correlated salt parameter for time to start of germination for both study species and for shoot length and chlorophyll content for *E. dahuricus* in saline salts. Salt content (%) was the most highly correlated salt parameter to predict root length response for both species and to predict shoot length for *M. sativa* in saline salts. Salt content was most highly correlated with germination percentage and seedling parameters for *M. sativa* and root length for *E. dahuricus*, when all saline and sodic salts were analyzed.

Na^+ concentration

Sodium absorption ratio (SAR) or exchangeable sodium percentage (ESP) is a very important parameter to measure in salt-affected soils, especially in sodic soils (Qadir et al. 2000; Wehr et al. 2016). However, Na^+ concentration has not been emphasized enough in previous laboratory studies on salt effects. We found that Na^+ concentration was the most strongly correlated salt solution parameter in sodic salts for germination and seedling growth of both study species. In saline salts, Na^+ concentration was the most closely correlated salt solution parameter for germination percentage of both species and for chlorophyll content of *M. sativa*. Therefore, Na^+ concentration was the best indicator of seed/seedling response in all saline and sodic salts. Zhao et al. (2014) also stated that Na^+ concentration significantly influenced seed germination and seedling growth of *Sorghum bicolor* using different proportions of saline and sodic salts in mixture. Ryan et al. (1975) compared the effects of various salt types based on milliequivalents per liter (meq/l). Since Na^+ concentration was the most important salt parameter, meq/l of Na^+ may be a good unit to use in salt-effect studies with different types of salts, including monosaline and divalent salts. For example, 100 meq/l NaCl equals 100 mM NaCl, while 100 meq/l Na_2SO_4 equals 50 mM Na_2SO_4 and 100 mM of Na^+.

Conclusions

Elymus dahuricus is more salt tolerant than *M. sativa* at the seedling establishment stage, and for both species sodic salts were more inhibitory than saline salts. NaCl and $NaHCO_3$ affected germination and seedling growth less negatively than Na_2SO_4 and Na_2CO_3 at the same concentrations for saline and sodic salts, respectively, with two-salt mixtures being intermediate. The influence of sodic salts predominated in the four-salt mixture, and saline salts in the mixture alleviated the inhibitory effect of Na_2CO_3 and aggravated the relative weak effect of $NaHCO_3$. For both study species, the salt solution parameters EC, salt content (%) and Na^+ concentration were best correlated with seed/seedling metrics in saline salts and Na^+ concentration in sodic salts. Water potential and pH were not good predictors for salt effects at the seedling establishment stage. Future studies on germination and seedling growth of species in salt-affected soils should examine the effects of various saline and sodic salts and the correlation between germination/seedling responses and salt content (%) and Na^+ concentration.

Acknowledgements We thank Ms. Zongying Hu for help with the experiments and Prof. Jerry Baskin for his useful comments on the manuscript. This study was funded by the National Basic Research Program of China (2015CB150800) and the National Natural Science Foundation of China (41571055).

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