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Full Length Research Paper

Germination strategies of *Suaeda* species for saline-alkaline environments

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The Songnen Plain in northeastern China has been experiencing severe problems with both salinity and alkalinity. Investigation on seed germination and seedling growth of typical halophytes in Songnen Plain under different saline-alkaline stresses is critical for evaluating the potential of a halophyte for restoration. The interactive effects of salt (NaCl and Na_2SO_4) and alkali ($NaHCO_3$ and Na_2CO_3) stresses on seed germination, germination recovery and seedling growth of a halophyte *Suaeda glauca* (Bunge) Bunge were studied. The results showed that seed germination were not significantly different between control and 100 mM salinity at all pH levels ranging from pH 6.8 to 9.8. However, high pH in combination with salinity sharply reduced germination percentage and germination rate. However, the ungerminated seeds germinated well when the high saline-alkaline stresses were removed. Root length decreased significantly with the increasing salinity or pH, but the seedling biomass showed no significant difference at low salinity (≤100 mM) at all pH levels. These results suggested that *S. glauca* could be used as pioneer plants for ecological recovery of saline and sodic soils.

Key words: Salinity, alkalinity, germination, recovery, seedling growth.

INTRODUCTION

More than 900 million hectares of land, comprising more than 6% of the world's total land area, are affected by salt (FAO, 2007), and the salt affected areas are still increasing. Of the cultivated lands, about 0.34×10^9 ha (23%) are saline and another 0.56×10^9ha (37%) are sodic (Läuchli and Lüttge, 2002). The Songnen Plain, located in northeast China (43°30′ to 48°40′N, 121°30′ to 127°00′E), is currently experiencing severe problems with both salinity and alkalinity, resulting primarily from human activities (Zhou and Ripley, 1997). In the saline and sodic soils of Songnen Plain, Na^+ was reported as dominant cation, with Cl^-, SO_4^{2-}, HCO_3^- and CO_3^{2-}as the main corresponding anions (Yang et al., 2007). Infact, soil salinization and alkalization frequently co-occur in nature, the conditions in natural salt-alkaline soil are very complex (Yang et al., 2007; Li et al., 2010).

Seed germination is the initial and most crucial stage in the lifecycle of plants (Grime and Campbell, 1991). There are appreciable number of literatures focused on the responses of seed germination to NaCl, temperature and osmotic stress (Khan and Ungar, 1996; Khan et al., 2001, 2006; Duan et al., 2004; Zhang et al., 2010). In addition, some reports indicated that alkaline salts ($NaHCO_3$ and Na_2CO_3) caused much stronger destructive effects on plant seedling responses than neutral salts (NaCl and Na_2SO_4) (Shi and Yin, 1993; Yang et al., 2007). However, very few studies have focused on seed germination response to salt–alkaline stress, especially for *Suaeda glauca*.

S. glauca, an annual halophyte that formed a large scale community around the saline–alkali lakes and

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heavily degraded environments, is one of the 12 typical halophytes growing in Songnen grassland (Li et al., 2002; Li and Yang, 2004). Sun et al. (2008) reported that the *in vitro* dry matter digestibility of *S. glauca* was about 71%, higher than other forages collected from Songnen grassland. The enriched oil content, such as linoleic acid and linolenic acid in the seed and plant, which made it an important economic plant, is also an important character for this halophyte (Sun and Zhou, 2008; Yu et al., 2005). Several studies have reported that *S. salsa* (L.) Pall. could be affected by high NaCl stress, seawater stress and extreme temperature (Liu et al., 2004; Li et al., 2005; Song et al., 2008), but different ecotypes of the same species occurring in different habitats may have different tolerance to special environmental conditions (Bazzaz, 1973). However, understanding of effects of saline-alkaline stresses on *S. glauca*, as well as its level of tolerance, is a critical first step in evaluating the potential value of *S. glauca* for restoration of degraded saline and sodic lands.

The objectives of this study were (1) to investigate the germination responses of *S. glauca* to different salt and alkali stresses; (2) to evaluate the interactive effects of salt and alkali stresses on early seedling growth and biomass accumulation of this species.

MATERIALS AND METHODS

Seed collection site

Seeds of *S. glauca* were collected from the Songnen Plain (44°30′N, 124°30′E) in 2010. Two-thirds of the areas have been salinized and alkalinized; mild, moderate and heavy saline-alkali soil accounting for 31.69, 27.06 and 41.25%, respectively (Li and Zheng, 1997). Annual mean rainfall is 350 to 450 mm and annual mean evaporation is 1600 to 1800 mm; annual mean temperature is 2.5°C and the soil pH values are 7.5 to 9 (Yu et al., 2010). The collected seeds were stored at 4°C until the experiment in January 2011.

Germination experiments

This study was carried out at the Laboratory of Coastal Wetland Ecology, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences. Seeds were surface-sterilized in 1% sodium hypochlorite solution for 15 min, rinsed in distilled water and dried before the experiment. Twenty five seeds of *S. glauca* were placed on two layers of filter paper in 10 cm Petri dishes. The filter paper was moistened with distilled water or salt solutions. Germination tests were carried out in growth chambers (BSG-800, Shanghai, China) with a 12 h photoperiod (Sylvania cool white fluorescent lamps, 200 µmolm^{-2} s^{-1}, 400 to 700 nm, 25/15°C). To examine the effects of different saline and alkaline stresses, two neutral salts (NaCl and Na_2SO_4) and two alkali salts ($NaHCO_3$ and Na_2CO_3) were mixed in various proportions as described by Shi and Wang (2005) to establish different treatments. Treatments consisted of five levels of salinity (100, 200, 300, 400, and 500 mM) in each of five pH levels: A. pH 6.9 ± 0.14 (NaCl:Na_2SO_4:$NaHCO_3$:Na_2CO_3= 1:1:0:0), B. pH 7.7 ± 0.12 (NaCl: Na_2SO_4:$NaHCO_3$:Na_2CO_3= 1:2:1:0), C. pH 8.5 ±0.13 (NaCl: Na_2SO_4:$NaHCO_3$:Na_2CO_3= 1:9:9:1), D. pH 9.8 ± 0.10 (NaCl: Na_2SO_4:$NaHCO_3$:Na_2CO_3=1:1:1:1), and E. pH 10.5 ± 0.10 (NaCl: Na_2SO_4:$NaHCO_3$:Na_2CO_3= 9:1:1:9). In total, there were 25 stress treatments labeled as A1, …, A5, B1, …, E5, respectively. A completely randomized design was used in the germination tests. Four replicates of 25 seeds each were used for each treatment. The seeds were considered to have germinated after radicle emergence. Germination was recorded daily for 10 days.

Methods of germination expression

The rate of germination was estimated using a modified Timson's index of germination velocity = $\sum G/t$, where G is the percentage of seed germination at one-day intervals and t is the total germination period (Khan and Ungar, 1984). The maximum value possible for our data using this index was 100 (that is, 1000/10). The greater the value, the more rapid the rate of germination. All seeds that did not germinate after 10 days in different salt treatments were placed in new Petri dishes with filter paper moistened with distilled water, and incubated under the same conditions for an additional 10 days to study the recovery of germination. The recovery percentage was determined by the following formula: $a/(c–b)\times100$, and final germination percentage was determined by formula: $(a+b)/c\times100$, where a is the total number of seeds germinated after being transferred to distilled water, b is the total number of seeds germinated in saline solution, and c is the total number of seeds used (modified from Khan and Gulzar, 2003). After 10 days of germination, root length and dry weight of 20 seedlings in each treatment were measured.

Data analysis

Germination data were arcsine transformed before the analysis of variance (ANOVA). The data were analyzed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Experimental data were subjected to one-way analysis of variance and the means were separated by the least significant difference (LSD). A two-way ANOVA was used to test the significance of main effects (salinity and pH) and their interaction on seed germination and seedling growth. Tukey's HSD test and paired two-tailed tests were performed for multiple comparisons to determine significant (P<0.05) differences between individual treatments.

RESULTS AND DISCUSSION

The initial seed germination percentage of *S. glauca* was significantly affected by salinity (F=29.934, P<0.001) and pH (F=6.309, P=0.003) and the interactions of the two factors (F=7.314, P<0.001). Seeds of *S. glauca* showed maximum germination in the non-saline control treatment. The initial germination percentage was not significantly different among all pH levels when salt levels were ≤ 100 mM. When the pH was ≤7.63, the percentage germination of seeds at 200 mM salt levels was relatively high, and not significantly different from that of seeds in distilled water (Table 1). However, as the pH increased, the germination percentage significantly decreased with increasing salt concentrations. Similar effects have been observed in other halophytes such as *Puccinellia tenuiflora* (Griseb.) Scribn. & Merr. (Yang et al., 2006) and *Spartina alterniflora* Loisel. seeds (Li et al., 2010). When the pH was higher than 7.72, no seeds germinate

Table 1. The concentrations [mM], pH and molar proportions of various salt anions (Cl-,SO_4^{2-}, HCO_3^-, andCO_3^{2-}), and seed germination in different saline-alkaline treatments.

Treatments	Stress factors						Seed germination (%)		
	Concentrations (mM)	pH	Cl^- (mM)	SO_4^{2-} (mM)	HCO_3^- (mM)	CO_3^{2-} (mM)	Initial	Recovery	Final
0	0	6.8	0	0	0	0	92.5 ± 2.36[a]	0 ± 0[a]	92.5± 2.36[a]
A1	100	6.76	50	50	0	0	94 ± 3.46[a]	0 ± 0[a]	94 ± 3.46[a]
A2	200	6.81	100	100	0	0	88 ± 3.27[a]	0 ± 0[a]	88 ± 3.27[a]
A3	300	6.93	150	150	0	0	83 ± 4.43[a]	33.33 ± 23.57[a]	86 ± 6.22[a]
A4	400	6.99	200	200	0	0	56 ± 5.42[b]	16.94 ± 11.25[a]	63 ± 7.55[b]
A5	500	7.04	250	250	0	0	9 ± 3.42[c]	86.41 ± 5.00[b]	88 ± 4.00[a]
B1	100	7.58	25	50	25	0	88 ± 1.63[a]	0 ± 0[a]	88 ± 1.63[a]
B2	200	7.63	50	100	50	0	77 ± 8.54[a]	13.33 ± 8.16[a]	81 ± 7.55[a]
B3	300	7.7	75	150	75	0	46 ± 10.52[b]	43.73 ± 17.43[b]	74 ± 6.22[a]
B4	400	7.72	100	200	100	0	1 ± 0[c]	78.63 ± 6.07b[c]	79 ± 5.74[a]
B5	500	7.82	125	250	125	0	0 ± 0[c]	84 ± 4.32[c]	84 ± 4.32[a]
C1	100	8.47	5	45	45	5	82 ± 3.46[a]	0 ± 0[a]	82 ± 3.46[a]
C2	200	8.5	10	90	90	10	55 ± 4.73[b]	51.84 ± 13.14[b]	78 ± 7.39[a]
C3	300	8.53	15	135	135	15	4 ± 0[c]	65.63 ± 65.63[b]	77 ± 5.97[a]
C4	400	8.58	20	180	180	20	0 ± 0[c]	85 ± 3.79[b]	85 ± 3.79[a]
C5	500	8.63	25	225	225	25	0 ± 0[c]	85 ± 3.42[b]	85 ± 3.42[a]
D1	100	9.7	25	25	25	25	89 ± 1.91[a]	0 ± 0[a]	89 ± 1.91[a]
D2	200	9.74	50	50	50	50	44 ± 10.95[b]	59.72 ± 5.69[b]	78 ± 3.83[b]
D3	300	9.79	75	75	75	75	2 ± 1.15[c]	88.15 ± 2.02[c]	89 ± 1.91[a]
D4	400	9.81	100	100	100	100	0 ± 0[c]	91 ± 3.42[c]	91 ± 3.42[a]
D5	500	9.9	125	125	125	125	0 ± 0[c]	93 ± 1.00[c]	93 ± 1.00[a]
E1	100	10.41	45	5	5	45	86 ± 2.58[a]	0 ± 0[a]	86 ± 2.58[a]
E2	200	10.46	90	10	10	90	49 ± 12.37[b]	46.13 ± 6.96[b]	75 ± 3.00[b]
E3	300	10.51	135	15	15	135	2 ± 2[c]	92.91 ± 3.39[c]	93 ± 3.42[a]
E4	400	10.53	180	20	20	180	0 ± 0[c]	87 ± 1.91[c]	87 ± 1.91[a]
E5	500	10.61	225	25	25	225	0 ± 0[c]	91 ± 1.91[c]	91 ± 1.91[a]

Different letters in seed germination percentages indicate significant differences from different salt levels (P<0.05).

at 400 and 500 mM salt concentrations. *S.* glauca is annual plant in Songnen grassland, seed germination is the only way to reproduce. These results indicated that *S. glauca* might adapt high saline stress at low alkaline stress.

Seeds of most halophytes exposed to high saline or pH conditions could maintain viablility for extended periods, and germinate when salinity or pH value decrease to certain degrees (Song et al., 2006; Li et al., 2010). This is also a criterion for salt tolerance that distinguishes them from glycophytes (Khan and Gul, 2006). The results of the germination recovery study showed that un-germinated seeds in high concentrations of salt solutions germinated well after transferring to distilled water (Table 1), and the seeds in higher concentrations of salt solutions showed a higher germination recovery. Under high saline or alkaline conditions, seed survival rather than germination could be a protective mechanism for plants to establish successfully when environmental conditions become appropriate; the germination recovery of halophytes could occur after rains or floods that provide moisture and leach the soil salt (Khan and Ungar, 1996). Final germination percentages in different salt solutions were similar to controls except A4, D2 and E2 treatments (Table 1). Final germination percentage, an indicator of total seed germination capability during a set of favorite conditions, appears more appropriate in evaluating the level of plant tolerance to salt. These results indicated that seed of *S. glauca* plant can adapt to high saline-alkaline environments through high germination percentage in saline conditions and high capacity for germination recovery.

Initial germination rate of *S. glauca* seeds decreased significantly with increasing salt stress and pH. The two-way ANOVA showed that germination rate (Figure 1) was significantly affected by salinity (F=44.913, P<0.001) and pH (F=6.002, P=0.004) and the interactions of the two factors (F=7.798, P<0.001). Un-germinated seeds in high salt solutions germinated well and the mean germination time was shortened after transferring to distilled water;

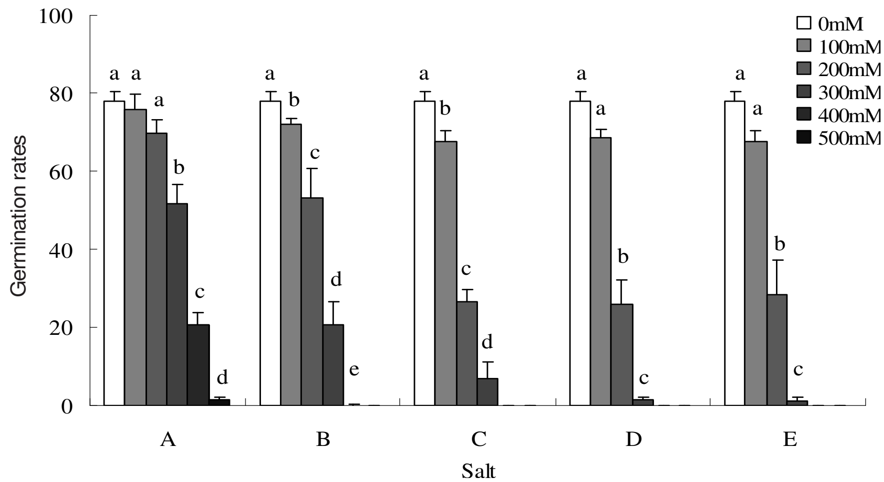

Figure 1. Germination rates (mean±s.e., n=4) of *S. glauca* seeds in different saline-alkaline solutions. Different letters indicate significant differences from different salt levels (P<0.05).

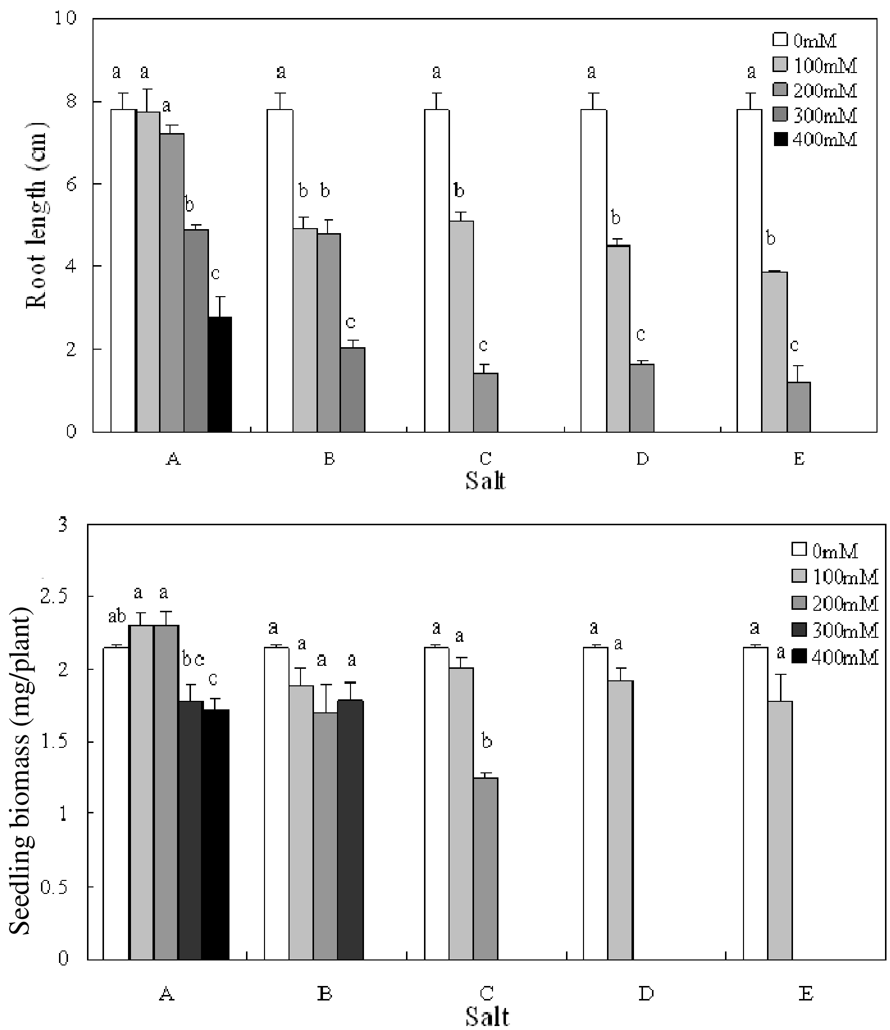

Figure 2. Root length and seedling biomass (mean ± s.e., n=20) of *S. glauca* seeds in different saline-alkaline solutions. Different letters indicate significant differences from different salt levels (P<0.05).

they all germinated in two days (data not shown). The 10 days in high salt solutions could be deemed to the process of seed priming. Several reports have demonstrated that NaCl priming could improve seed performance and provide faster and synchronized germination (Sivritepe and Dourado, 1995). These results also proved that seeds of *S. glauca* could remain viable in high saline-alkaline environments such as Songnen Plain, and germinate rapidly during rainy periods. This could be a crucial seed germination strategy for *S. glauca* seeds to survive in high saline-alkaline environments, and germinate rapidly in rainy season.

Root length of *S. glauca* seedlings was significantly decreased by salt stress except for the treatment A1 salinity 100 mM, pH 6.76) and A2 (salinity 200 mM, pH 6.81). This result indicated that *S. glauca* seedlings could adapt to low salt stress and high alkali stress. At 100 mM salt treatment, the biomass of *S. glauca* seedlings was not significantly affected by pH (Figure 2). However, the (biomass of *S. glauca* seedlings was significantly decreased by both salt and pH stress. The present germination and seedling experiment suggests that *S. glauca,* with a high ability of saline-alkaline tolerance, and high germination recovery capability, coupled with the performance of its seedlings in saline and alkaline conditions, could be used to recover and restore degraded saline-alkaline grasslands.

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