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Salt Tolerance in Two *Suaeda* Species: Seed Germination and Physiological Responses

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Abstract: The main aim of this study is to detect the effects of NaCl, $NaHCO_3$ and sea salt on seed germination, seedling growth and seedling cation contents of two *Suaeda* species. Three germination experiments of *S. corniculata* and *S. salsa* seeds were conducted in growth chambers. The seeds were placed at three types of salt at concentrations: 0, 25, 50, 75, 100% seawater; 0, 100, 200, 300, 400, 500 mM NaCl and 0, 100, 200, 300, 400, 500 mM $NaHCO_3$, separately. The two species varied in their salt tolerance for germination rates and percentages and showed higher germination percentage at higher salt stress (500 mM NaCl, $NaHCO_3$ and 100% seawater). Some un-germinated seeds were recovered after being transferred to distilled water. The Na^+ content in seedlings increased with the increase in stress intensity. While K^+ content and K^+/Na^+ ratio decreased under NaCl and $NaHCO_3$ stress. K^+ content increased in seawater treatment while reaching higher salt concentrations, due to the extra K^+ in seawater, but there was no significant difference among the treatments with varied seawater concentrations ($p<0.001$). These results suggested that *S. corniculata* and *S. salsa* could be used as pioneer plants for ecological recovery and exploitation of saline and sodic soils.

Key words: Salinity, germination percentage, NaCl, $NaHCO_3$, seawater

INTRODUCTION

Over 800 million hectares of land are affected by salinity throughout the world (Munns, 2005). Saline soils formed when evaporation greatly exceeds precipitation for at least part of the year and where salts are present in moderate to high amounts in the parent material of the soil or with a saline water table at shallow depth. Apart from their high salt content saline soils show a considerable diversity in their hydrological, physical and chemical properties. Saline soils may be calcium dominated, sodium dominated or magnesium dominated with a subsequent tendency towards structural degradation (depending on the presence or absence of calcium) (http://www.fao.org/AG/agL/agll/prosoil/saline.htm). Under extreme climatic conditions (low rainfall, high evaporation) salts which are present in soil solution can precipitate at the surface in various forms such as white efflorescence, salt crusts and so on (http://www.fao.org/AG/agL/agll/prosoil/saline.htm).

Salt accumulation may limit plant growth to salt tolerant plants (halophytes) only. Tolerance also varies with stages of their life cycle, which could be expressed as: (1) the ability to tolerate high salinity without loosing viability while stored in the soil (seed bank), (2) the ability to germinate at high salinities and (3) the ability to complete its life cycle at high salinities (Khan and Gul, 2002). Ravindran *et al.* (2007) have demonstrated that soil salinity levels, as measured by Electrical Conductivity (EC), can be reduced by the cultivation of halophytes which are able to accumulate salts in their plant tissues on soils affected by salinity. Among them, *Suaeda corniculata* is a halophyte which often occurs in sodic soils in plains, such as Songnen Plain, where sodium

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chloride (NaCl) and sodium bicarbonate ($NaHCO_3$) are the most abundant salts (Wang *et al*., 2007). Leaves of *S. corniculata* from saline-sodic inland are green during the whole growing period (http://www.efloras.org/florataxon.aspx?flora_id=2 and taxon_id=200006943). *S. salsa* is a typical halophyte that naturally occurs in highly saline soils in coastal saline wetland (Wang *et al*., 2004). *S. salsa* in costal wetland is usually submerged completely in seawater and the salt concentrations in the soil are always high during seed germination and at the seedling stage (Song *et al*., 2008). Leaves and stems of *S. salsa* in this area are red-violet during the whole growth period, Fresh branches of *S. salsa* are very valuable as a vegetable and the seeds can produce edible oil (Zhao *et al*., 2002). These two species (Chenopodiaceae) are common in saline soils, acquisition and maintenance of high water content may be the strategies these species use to accumulate Na^+ which may substitute for an osmoticum or regulate internal ion concentrations under saline conditions.

Seed germination is the initial and most crucial stage in the life cycle of plants (Grime and Campbell, 1991). Different abiotic factors such as temperature, soil salinity, photoperiod and soil moisture affect germination of halophytes (Noe and Zedler, 2000; Khan, 2003; Guan *et al*., 2009). However, the effect of soil salinity seems to dominate over all other factors in saline areas (Keiffer and Ungar, 1997; Heidari, 2009). The ability for seeds to recover in non-saline solution following transfer from saline solution also plays an important role in saline areas, which has been reported for several halophytes (Ungar, 1978; Khan and Ungar, 1984; Hanslin and Eggen, 2005).

High Na^+ tissue content has often been considered as the major factor responsible for salt toxicity in plants. It is conventionally assumed that an ability to exclude Na^+ correlates with plant salt tolerance (Munns and James, 2003; Garthwaite *et al*., 2005). However, the importance of maintaining an optimal K^+/Na^+ ratio for plant salt tolerance is hardly surprising and is well discussed in literatures (Cuin *et al*., 2003; Chen *et al*., 2007). It is also obvious that such an optimal ratio can be maintained by either restricting Na^+ accumulation in plant tissues or by preventing K^+ loss from the cells (Chen *et al*., 2007).

The aim of this study were (1) to investigate the seed germination of two *Suaeda* species treated with NaCl, $NaHCO_3$ and the sea salts, (2) to discuss the physiological responses of *Suaeda* species to different salts at germination stage.

MATERIALS AND METHODS

Seed collection site: Seeds of *S. corniculata* were obtained from Songnen Plain (44°3'N, 124°3'E) in 2008, where the soils are highly saline. Seeds of *S. salsa* were collected from the coastal wetland in Yellow River Delta (37°35'-38°12'N, 118°33'-119°20'E) in the autumn of 2008. The seeds were stored in paper bags at room temperature (20±3°C) until the experiment in January 2009.

Germination experiments: This study was carried out at Laboratory of Coastal Wetland Ecology, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, China from January till May in 2009. Seeds were surface-sterilized in 1% sodium hypochlorite solution for 15 min, rinsed in distilled water and dried before the experiment. The seeds were germinated in petri dishes (10 cm diameter) containing two layers of filter paper with 12 mL of test solution. Germination tests were carried out in growth chambers (BSG-800, Shanghai, China) with a 12 h photoperiod (Sylvania cool white fluorescent lamps, 200 $\mu mol\ m^{-2}\ sec^{-1}$, 400-700 nm, 25/15°C). To examine the effects of different salts, we conducted six salinity concentrations (0, 100, 200, 300, 400, 500 mM NaCl), six alkalinity concentrations (0, 100, 200, 300, 400, 500 mM $NaHCO_3$) and five sea water concentrations (0, 25, 50, 75 and 100% seawater). A completely randomized design was used in the germination tests. Four replicates of 50 seeds each were used for each treatment. The seeds were considered to have germinated after radicle emergence. Germination was recorded daily for 10 days (Mokhberdoran *et al*., 2009).

Methods of germination expression: The rate of germination was estimated using a modified Timson's index of germination velocity = $\Sigma G/t$, 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 (i.e., 1000/10). The greater the value, the more rapid is the germination. All seeds from the previous germination tests 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:

$$\frac{a}{(c-b)}\times 100$$

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).

Measurement of physiological indices: All seedlings were harvested after 10 days of salt treatment. The seedlings were first washed with distilled water three times and then dried at 60°C to constant weight. Dry samples of plant material (50 mg) were treated with 10 mL deionized water at 100°C for 1 h and the extract was taken to determine free Na^+ and K^+ concentrations. Inductively coupled plasma atomic emission spectrometry (ICP-AES) were used for the determination of sodium (Na), potassium (K) concentrations. K^+/Na^+ ratio was then calculated.

Data analysis: Germination data were arcsine transformed before the Analysis of Variance (ANOVA). The data were analysed 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). For the germination data, significance was tested at the 1% level, for the Na and K concentrations, significance was tested at the 0.1% level.

RESULTS

Final germination: The final germination of *S. corniculata* and *S. salsa* were significantly affected by NaCl, $NaHCO_3$, or seawater (Table 1) ($p<0.01$). They both remained unaffected up to 200 mM NaCl and $NaHCO_3$ treatments. In non-saline control treatment, the germination percentages were 80.5 and 91% for *S. corniculata* and *S. salsa*, respectively. At the highest NaCl concentration, the percentage of germination was no less than 40%. Moreover, the germination percentage of both *S. corniculata* and *S. salsa* were higher than 60% in all $NaHCO_3$ treatments. Only 38% seeds of *S. salsa* germinated in comparison to 57.5% germination of *S. corniculata* in 100% seawater treatment (Table 1).

Germination rate: The highest germination rate was observed in non-saline controls and it decreased with the increase of salinity. *S. salsa* seeds showed higher germination ability than *S. corniculata* seeds in all treatments except for those treated with 50, 75 and 100% seawater (Table 1).

For the two species, the rate of germination were affected significantly by NaCl treatment and all remained unaffected up to 200 mM $NaHCO_3$ treatments (Table 1) ($p<0.01$).

Germination recovery: After 10 days of salinity treatment, seeds were transferred to distilled water to determine the recovery of germination. The results presented the un-germinated seeds from all salt treatments recovered. The germination recovery ranged from 5.63% in 500 mM $NaHCO_3$ (but note the original high germination of 64.5%) to 30.43% in 400 mM NaCl for *S. corniculata* and from 16.22% in 200 mM $NaHCO_3$ to 43.55% in 100% seawater for *S. salsa* (Table 1).

Physiological indices: Na^+ concentrations of the two species increased with increasing NaCl ($p<0.001$). The overall increasing trends were similar for *S. corniculata* and *S. salsa*, except in the treatment of 300 mM NaCl, where Na^+ concentrations in *S. salsa* seedlings were higher than in *S. corniculata* seedlings (Fig. 1a-f). In $NaHCO_3$ solutions, Na^+ concentrations of *S. corniculata*

Table 1: Germination percentage, rate of germination (Mean±SE, n = 4) at different levels of different salt solutions using modified Timson's index (Khan and Ungar, 1984) and germination recovery using recovery formula (modified from Khan and Gulzar, 2003)

Test solutions and concentrations	Germination (%)		Rate of germination (%)		Germination recovery (%)	
	S. corniculata	*S. salsa*	*S. corniculata*	*S. salsa*	*S. corniculata*	*S. salsa*
NaCl						
0 mM	80.5±1.5a	91.0±1.0a	64.60±2.23a	77.55±1.39a	0	0
100 mM	78.5±0.8ab	89.0±0.9a	62.50±1.18b	73.10±1.19a	0	0
200 mM	65.0±2.3ab	87.0±1.6ab	45.95±2.61cd	70.05±3.39ab	28.57	38.46
300 mM	75.0±2.6b	79.0±0.6bc	51.50±2.98bc	61.30±1.35bc	24.00	38.10
400 mM	65.5±2.0b	70.5±0.5c	36.30±3.34d	54.20±0.42c	30.43	23.73
500 mM	44.5±3.8c	50.0±2.3d	22.60±4.23e	34.60±4.67d	28.83	24.00
$NaHCO_3$						
0 mM	80.5±1.5a	91.0±1.0a	64.60±2.23a	77.55±1.39a	0	0
100 mM	80.0±1.5ab	84.7±0.9a	64.85±2.83a	69.67±1.25ab	0	0
200 mM	72.0±1.7abc	81.5±1.7ab	59.75±2.81ab	69.55±3.98ab	7.14	16.22
300 mM	74.0±2.1abc	73.5±2.0bc	57.25±3.44ab	58.40±5.21bc	9.62	43.40
400 mM	68.5±0.6bc	64.0±1.2c	47.20±2.17bc	47.55±3.17c	19.05	41.67
500 mM	64.5±3.4c	68.0±2.6c	38.55±5.81c	50.00±4.29c	5.63	39.06
Seawater						
0%	80.5±1.5a	91.0±1.0a	64.60±2.23a	77.55±1.39a	0	0
25%	71.5±2.1ab	76.5±1.0b	51.00±2.98b	62.50±1.87b	8.77	31.91
50%	71.5±2.0ab	69.5±1.5b	53.55±2.56b	50.80±2.74b	21.05	31.15
75%	69.0±2.1b	52.0±2.7c	47.00±3.30b	35.25±4.85c	16.13	40.63
100%	57.5±1.4c	38.0±3.3d	31.95±2.35c	23.15±3.29c	25.88	43.55

Different letters indicate significant differences from different concentrations in same salt treatment ($p<0.01$)

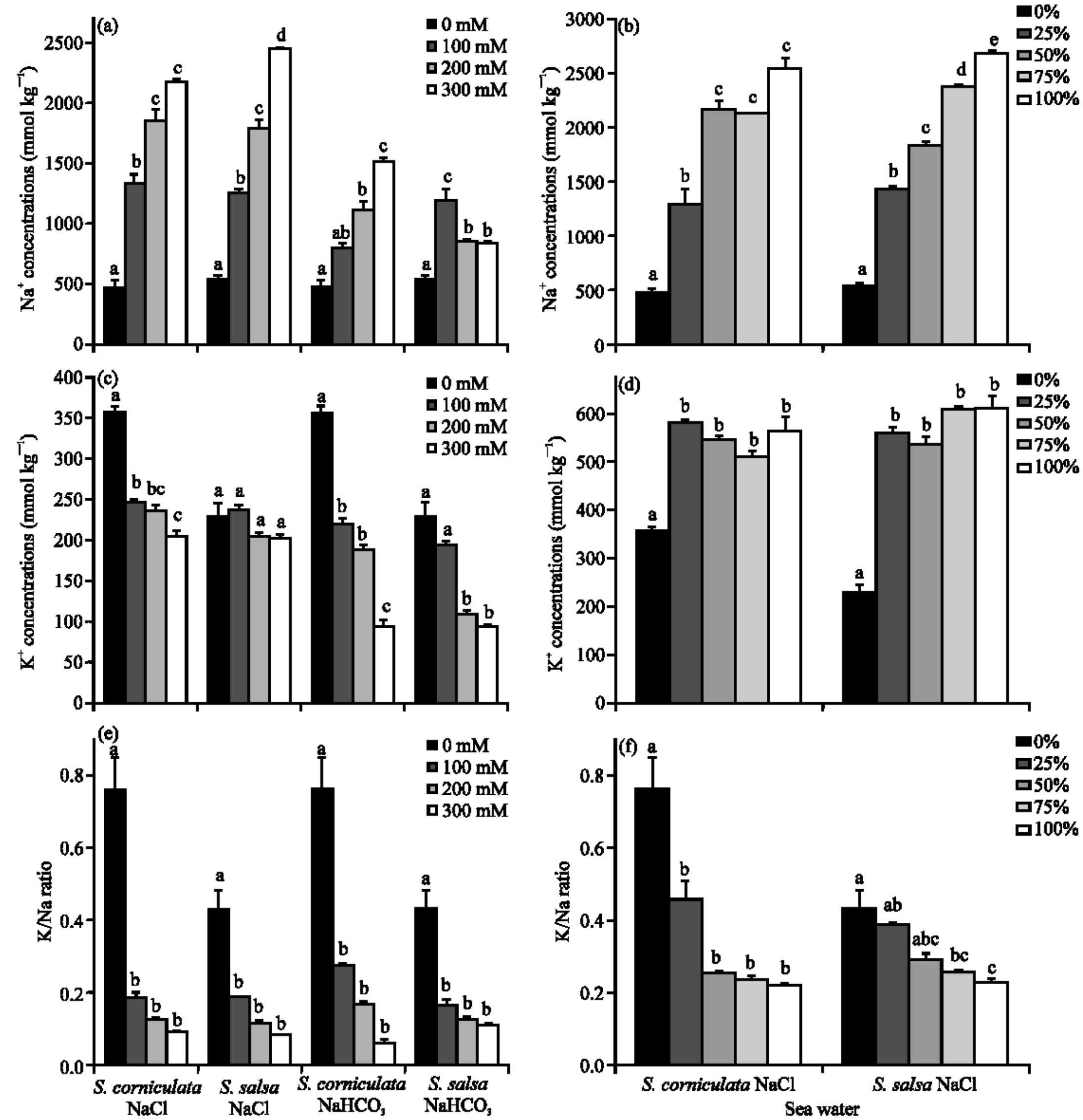

Fig. 1: Effects of NaCl, $NaHCO_3$ and seawater on concentrations of Na^+, K^+ (mmol kg^{-1} dry mass) and K^+/Na^+ ratio in the seedlings of *S. corniculata* and *S. salsa*. Bars represent±SE (n = 3). Different letters indicate significant differences from each other ($p<0.001$)

seedlings increased with increasing $NaHCO_3$ ($p<0.001$). However, in *S. salsa* seedlings the concentrations of Na^+ decreased with the salinity increased up to 200 mM (Fig. 1). In seawater treatments, Na^+ concentrations of *S. salsa* seedlings increased significantly from 0 to 100% seawater ($p<0.001$), while they were not significantly different in *S. corniculata* seedlings treated with 50, 75 and 100% seawater (Fig. 1).

K^+ concentrations of *S. corniculata* seedlings decreased significantly with increasing salinity in both NaCl ($p<0.001$ and $NaHCO_3$ ($p<0.001$) treatments (Fig. 1). For *S. salsa* seedlings, decrease in K^+ concentrations was not significant when treated with NaCl ($p<0.001$). In seawater solutions, K^+ concentrations were significantly higher than control (0% seawater), but there was no significant difference among the treatment with different seawater concentrations, which was the case for both *S. corniculata* and *S. salsa* seedlings (Fig. 1).

DISCUSSION

The effects of 3 types of salts on seed germination: *S. corniculata* and *S. salsa* are typical halophytes which naturally occur in inland saline-sodic plains and coastal saline wetland respectively. For both species, increase in salinity caused a decrease in germination percentage and delayed the germination, but they showed high salt tolerance to NaCl, $NaHCO_3$ and seawater, as quantified by the percentage of germination (Table 1). At 500 mM NaCl, the germination percentage of *S. corniculata* reached 44.5% and *S. salsa* reached 50%, this indicated that the two species can establish in most saline environments. This observation was supported by other studies on *Arthrocnemum indicum* (Khan and Gul, 1998), *Salicornia rubra* (Khan *et al*., 2000) and *Suaeda japonica* (Yokoishi and Tanimoto, 1994). These reports indicate that *S. corniculata* and *S. salsa* seeds could be classified as one of the most salt tolerant species during germination. The pH range of $NaHCO_3$ in this study was 8.4 - 9 and the germination percentages of the two species were still high: *S. corniculata* 64.5% and *S. salsa* 68% at 500 mM $NaHCO_3$ (Table 1). In sodic soils such as Songnen plain, sodium bicarbonate ($NaHCO_3$) is second most abundant salt next only to sodium chloride (NaCl), high germination percentage makes it promising to use these plants to rebuild the sodic land under serious threat of degradation. Furthermore, seeds of *S. corniculata* and *S. salsa* are highly salt tolerant and can germinate at 100% seawater salinity. This may follow the same rule as combined salt effect because in seawater a lot of ions were included (Duan *et al*., 2003).

Germination rate decreased with the increasing of salinity as expected (Table 1). When the two species were compared, the effect of salinity on *S. corniculata* was higher than that on *S. salsa* (Table 1). The recovery experiment showed that seeds exposed to different NaCl, $NaHCO_3$ and seawater concentrations for a short period (10 days) could germinate after being transferred to distilled water (Table 1). This is supported by Ungar (1995), who reported that tolerance of seeds to salinity should be interpreted at two levels: (1) the ability to germinate at saline conditions and (2) the ability to germinate at a non-saline condition after exposure to high salinity. In this study the two levels of tolerance of seeds to salinity were observed.

The effects of 3 types of salts on physiological indices: Halophytes were reported to accumulate large amounts of Na^+ under salt stress and simultaneously inhibit K^+ absorption, which results in the increase of Na^+ content and the decrease of K^+ content (Shi and Wang, 2005; Yang *et al*., 2007). Similar phenomenon is observed in this study, results shown in Fig. 1a and c indicated that with increasing stress intensity the Na^+ content in seedlings increased, while K^+ content and K^+/Na^+ ratio decreased under NaCl and $NaHCO_3$ stress, but the K^+/Na^+ ratio were not significantly different among 100, 200 and 300 mM NaCl and $NaHCO_3$ treatments (Fig. 1e). Total accumulation of Na^+ and K^+ was higher under NaCl stress than that under $NaHCO_3$ stress for both species. This indicated that the negative effects of HCO_3^- were higher than Cl^-. In seawater treatment, the K^+ content increased with the increasing of seawater (Fig. 1d), due to the presence of K^+ in seawater.

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