

## Seed germination and seedling growth of *Suaeda salsa* under salt stress

De-Yu Duan<sup>1,2</sup>, Wei-Qiang Li<sup>1,3</sup>, Xiao-Jing Liu<sup>1,\*</sup>, Hua Ouyang<sup>2</sup> & Ping An<sup>3</sup>

<sup>1</sup> Center for Agricultural Resources Research, Institute of Genetic and Development Biology, The Chinese Academy of Sciences, Shijiazhuang 050021, P. R. China (\*corresponding author's e-mail: xjliu@ms.sjziam.ac.cn)

<sup>2</sup> Institute of Geographical Sciences and Natural Resources Research, The Chinese Academy of Sciences, Beijing 100101, P. R. China

<sup>3</sup> Arid Land Research Center, Tottori University, Tottori 680-0001, Japan

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The effect of increasing concentrations (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.6 mol l<sup>-1</sup>) of different salts (Na<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, MgSO<sub>4</sub>, NaCl, MgCl<sub>2</sub>) in solution and soil extract solutions of comparable osmotic potentials on germination of *Suaeda salsa* seeds was studied. Seed germination decreased significantly with increased salinity. Germination inhibition was in the following order: MgCl<sub>2</sub> > Na<sub>2</sub>SO<sub>4</sub> > Na<sub>2</sub>CO<sub>3</sub> > NaCl > soil extract solution > MgSO<sub>4</sub>. Non-germinated seeds under all salt treatments recovered completely when transferred to distilled water, indicating that there was little specific ion effect on seed viability. Seedling growth was studied under different levels of NaCl salinity (0, 0.05, 0.1, 0.2, 0.4 mol l<sup>-1</sup>) stress. Low levels of salinity (0.05–0.1 mol l<sup>-1</sup>) increased seedling growth, while high levels (> 0.2 mol l<sup>-1</sup> NaCl) inhibited the growth significantly. Under salt stress, the leaves accumulated high levels of proline, whereas their soluble sugar content decreased with an increase in salinity. Na<sup>+</sup> content in leaves, stems and roots increased with salinity. Ions were mostly accumulated in the shoot, especially in leaves. Under the highest level of salinity (0.4 mol l<sup>-1</sup> NaCl), Na<sup>+</sup> accounted for more than 91.6% of the ions in leaves, 92.6% in stems and 84.1% in roots. Inorganic ions, especially Na<sup>+</sup>, were more important than organic solutes in the osmoregulation in *S. salsa* to adapt to a saline environment.

Key words: germination, halophyte, salt stress physiology, seedling growth, solutes, *Suaeda salsa*

### Introduction

Halophytes occur naturally in salt-affected lands, where the soil solution osmotic potential is smaller than –3.3 bar (Greenway & Munns 1980). They are very abundant in nature and can

be more tolerant of salt stress than glycophytes. Exploitation of halophytes has lately been receiving more attention because of increasing soil salinization and fresh water shortage globally (Khan & Weber 2006).

A plant's ability to tolerate salinity stress

varies with the stage of growth in its life cycle (Khan 2002). Germination and seedling growth are critical and salt-sensitive stages in the halophytes (Khan & Sheith 1996). It is often difficult to determine the tolerance of plants to salinity during emergence and seedling growth by growing them in salt-affected lands (Ungar 1996). Salts affect germination adversely either by restricting the supply of water (osmotic effect), or by causing specific injury to certain metabolic processes (ionic effect), or by both. However, the seeds of halophytes usually recover completely from germination inhibition, when salt stress relaxes (Hardegee & Emmerich 1990). When pretreated with salinity, seeds of some halophytes show a priming effect on germination, while others show no such effect, and there are still others that fail to germinate when exposed to high salinity (Ungar 1995, Keiffer & Ungar 1995, Khan & Ungar 1997).

The salt-affected lands in the world vary greatly in the ionic composition of the soil solution. Seeds of halophytes under natural conditions are usually subjected to salt stress dominated by NaCl. However, other chloride, sulfate and carbonate salts, singly as well as in combination, can also affect seed germination significantly (Bewley & Black 1994, Khan 2002, Duan *et al.* 2004). Potassium chloride, potassium sulfate, sodium chloride and sodium sulfate had different effects on the germinability of seeds of several halophytes (Mohammed & Sen 1990).

All plants, when grown in a saline environment, face qualitatively similar problems. High salt stress disrupts homeostasis in plant water potential and ion distribution at both cellular- and whole-plant levels (Yeo 1983). Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest, and even death. Halophytes however show dry weight (d.w.) increase in the presence of electrolytes at levels higher than those usually present in normal soils (Flowers *et al.* 1986). Osmotic adjustment is the key mechanism, by which halophytes are able to adapt themselves to high levels of salinity and continue to obtain sufficient water for growth and development (Song *et al.* 2006b). Halophytes accumulate ions such as Na<sup>+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and NO<sub>3</sub><sup>-</sup>, which leads to the increase in

the osmotic potential in their tissues that permits moisture to move from soil into tissues (Song *et al.* 2006a, 2006b). On the other hand, excess of ions can prove toxic to the plant growth. Some of the mechanisms by which halophytes overcome the potential toxic effect of high ionic concentrations involve dilution of the ions through leaf or stem succulence, elimination of high salt concentrations by excretion through salt glands and storage in salt bladder hairs, exclusion of salt by roots, and production of osmotically compatible organic solutes (Zhao & Li 1999).

*Suaeda salsa* of the family Chenopodiaceae is an annual halophyte with succulent leaves. It grows in temperate saline areas of China (Li *et al.* 2005), where the dominant ions in soil are Na<sup>+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> (Wang 1993). The seeds of *S. salsa* contain about 26% high quality oil and the young seedlings are a good source of food and fodder (Zhao & Li 1999). It therefore serves as a cash crop. For better utilization of this plant to exploit saline soils, the present study aims to investigate the influence of different levels and kind of salinity on germination of *S. salsa* and the effect of various levels of sodium chloride on its seedling growth.

## Material and methods

Seeds of *S. salsa* were collected from the coastal salt marshes in Haixing county, Hebei province, China. Seeds were separated from inflorescence and surface-sterilized with ozone gas for 30 minutes. To determine the effect of different salts on germination, solutions of NaCl, Na<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, MgSO<sub>4</sub>, and MgCl<sub>2</sub> and soil extract solutions (SES) from *S. salsa* growing area were used, containing increasing levels of salinity (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.6 mol l<sup>-1</sup>). The major ions in the soil extract collected from the *S. salsa* growing area were Cl<sup>-</sup> (53.4 % by weight) and Na<sup>+</sup> + K<sup>+</sup> (30.5%), SO<sub>4</sub><sup>2-</sup> (12.5%), Mg<sup>2+</sup> (2.3%), Ca<sup>2+</sup> (1.2%) and HCO<sub>3</sub><sup>-</sup> (0.1%). Concentration of soil extract solutions was adjusted to get osmotic potential levels equal to those in the NaCl solutions of various concentrations. Seeds were germinated on 3 sheets of 9 cm diameter filter paper in 10 cm diameter Petri dishes with 10 ml solutions. The Petri dishes were sealed with Parafilm

to prevent water loss. Forty seeds were used for each treatment with four replications.

The experiment was carried out at 15/25 °C night/day temperatures and 12 hours dark/12 hours light period in an auto-controlled growth chamber. Germination was recorded at two-day intervals and the germinated seeds were removed each time. After 9 days, ungerminated seeds were transferred to distilled water for 7 days to determine the recovery of germination. The percentage recovery was calculated as follows:

$$\text{Percentage recovery} = [(a - b)/(c - b)] \times 100 \quad (1)$$

where  $a$  = total number of seeds germinated (i.e. number of seeds germinated in saline solution plus the number germinated after being transferred to distilled water),  $b$  = number of seeds germinated in saline solution, and  $c$  = total number of seeds (40 seeds).

The ultimate relative germination percentage was calculated as follows:

$$\text{Ultimate relative germination percentage} = (U_s/U_w) \times 100 \quad (2)$$

where  $U_s$  = ultimate germination percentage after 16 days in various salt treatments, i.e.  $[(a/c) \times 100]$  and  $U_w$  = ultimate germination percentage (after 16 days) under 0 mol l<sup>-1</sup> salt stress treatment.

To investigate the effect of different levels of NaCl on the growth of *S. salsa* a hydroponic culture system was used. The seeds were sown in washed sand. After emergence, seedlings were irrigated twice a day with half-strength Hoagland solution until they reached about 5 cm in height. Then the plants to be given saline solution were pretreated with 0.05 mol l<sup>-1</sup> NaCl. The pretreatment was followed by an increment of 0.05 mol l<sup>-1</sup> until the desired concentration of salinity was reached. Then seedlings were transferred to plastic containers with 0, 0.05, 0.1, 0.2, and 0.4 mol l<sup>-1</sup> NaCl in full Hoagland solution. The solution was well-aerated using an air pump and it was replaced every week. Seedlings were harvested 30 days later.

At the end of the study the plants were divided into leaves, stem and roots and each part was oven-dried at 80 °C for 48 hours for dry

weight (d.w.) determination. The proline content in the leaves was determined by ninhydrin colorimetry (Li 2000). The soluble sugar content in the leaves was determined by sulphuric acid anthrone colorimetry (Li 2000) using an ultraviolet spectrophotometer (Hitachi-V-520). The dried plant samples were ground and were wet ashed with HNO<sub>3</sub>. The Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations in the plant tissue were assayed by an atomic absorption spectrophotometer (Hitachi-170-10, Japan).

Statistical analysis was carried out using SPSS 11.0. A one-way ANOVA was carried out to determine differences among treatment groups for germination percentage and germination recovery percentage. A Bonferroni test was also done to determine if differences between individual treatments were significant ( $p < 0.05$ ).

## Results

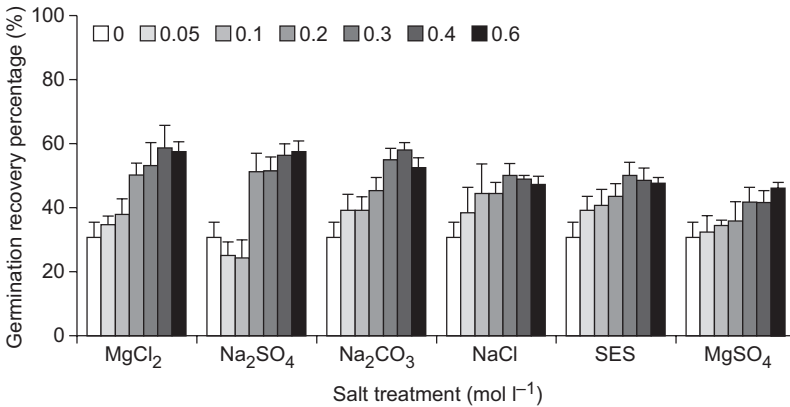
### Effect of salt stress on seed germination

The seeds germinated best in the non-saline control. The germination percentage decreased as the level of salinity increased in all the salt solutions (Table 1). Salinity inhibited seed germination significantly ( $p < 0.05$ ). The decrease in germination percentage at 0.4 mol l<sup>-1</sup> salinity ranged from 68.7% to 91.1% depending on the salt. At the highest level of salinity (0.6 mol l<sup>-1</sup> salt concentration) no seed germinated with MgCl<sub>2</sub> and only a few germinated with other salts.

A comparison of germination percentage in different salt solutions with the same concentration indicated that the differences in inhibition caused by NaCl, Na<sub>2</sub>CO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, MgSO<sub>4</sub>, MgCl<sub>2</sub> and SES were generally significant ( $p < 0.05$ ). At 0.3 mol l<sup>-1</sup> concentration, the lowest germination (only 13%) occurred under MgCl<sub>2</sub>, whereas the highest value (41%) was obtained with MgSO<sub>4</sub>.

The onset of germination was delayed by salinity, especially under strong salt stress (data not shown). In MgCl<sub>2</sub>, at 0.6 mol l<sup>-1</sup>, the first seed germinated 4–6 days later than that under control treatment.

The relative recovery in germination was better when the seeds were treated with higher



**Fig. 1.** Germination recovery percentage of *Suaeda salsa* seeds when transferred to distilled water after salt treatment. See text for the equation used.

levels (0.3 mol l<sup>-1</sup> or more) of salt stress (Fig. 1). Also, under the same level of salinity, the seeds under MgCl<sub>2</sub> recovered better than seeds under other salts. Germination recovery under NaCl, Na<sub>2</sub>CO<sub>3</sub> and MgCl<sub>2</sub> peaked at 0.4 mol l<sup>-1</sup> (Fig. 1).

The results showed that the seeds of *S. salsa* can remain viable after being subjected to salt stress. When the seeds were transferred to distilled water, the ultimate relative germination percentage was more than 80 (Table 2). At low concentrations, NaCl and Na<sub>2</sub>CO<sub>3</sub> tended to promote seed germination (Table 2). However, at

high salinity levels a negative influence on seed germination was observed.

Seed germination percentage was linearly and negatively correlated with salt stress, while germination recovery correlated positively with salt stress.

### Effect of NaCl stress on seedling growth

At low level of NaCl salinity (0.05 mol l<sup>-1</sup>) the seedling height increased by 40.4%, shoot

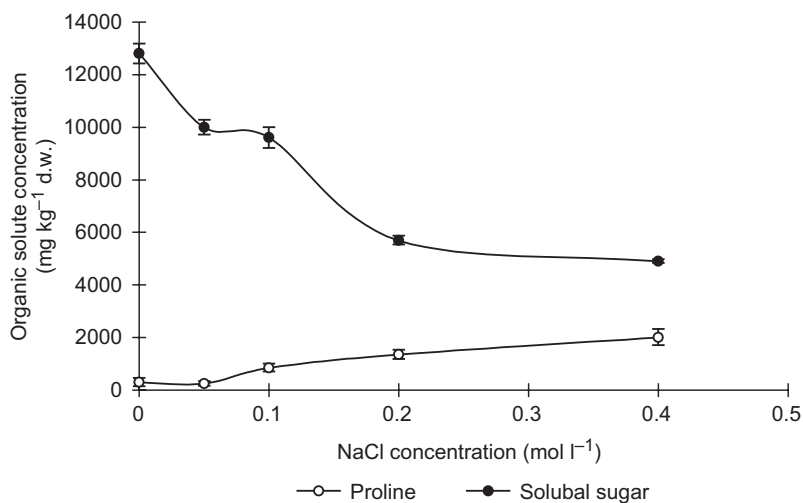
**Table 1.** Germination percentages of *Suaeda salsa* seeds treated with several salt and soil extract solution (SES). Different letters in the same column indicate significant difference at  $p = 0.05$ .

Treatment (mol l <sup>-1</sup> )	MgCl <sub>2</sub>	Na <sub>2</sub> SO <sub>4</sub>	Na <sub>2</sub> CO <sub>3</sub>	NaCl	SES	MgSO <sub>4</sub>
0	63.8 <sup>e</sup>	63.8 <sup>d</sup>	63.8 <sup>e</sup>	63.8 <sup>e</sup>	63.8 <sup>e</sup>	63.7 <sup>c</sup>
0.05	51.3 <sup>d</sup>	59.4 <sup>d</sup>	51.3 <sup>d</sup>	52.5 <sup>d</sup>	58.1 <sup>de</sup>	56.9 <sup>c</sup>
0.1	48.1 <sup>d</sup>	60.0 <sup>d</sup>	61.9 <sup>e</sup>	54.4 <sup>d</sup>	54.4 <sup>d</sup>	51.3 <sup>bc</sup>
0.2	30.6 <sup>c</sup>	36.8 <sup>c</sup>	40.6 <sup>c</sup>	39.4 <sup>c</sup>	43.8 <sup>c</sup>	51.9 <sup>bc</sup>
0.3	13.1 <sup>b</sup>	20.6 <sup>b</sup>	28.8 <sup>b</sup>	24.4 <sup>b</sup>	38.1 <sup>c</sup>	41.3 <sup>b</sup>
0.4	5.6 <sup>b</sup>	9.4 <sup>a</sup>	12.5 <sup>a</sup>	18.8 <sup>b</sup>	20.0 <sup>b</sup>	40.6 <sup>b</sup>
0.6	0	3.1 <sup>a</sup>	5.0 <sup>a</sup>	6.3 <sup>a</sup>	8.8 <sup>a</sup>	26.3 <sup>a</sup>

**Table 2.** Ultimate relative germination percentage of *Suaeda salsa* seeds when salinity stress was removed. Different letters in the same column indicate significant difference at  $p = 0.05$ .

Treatment (mol l <sup>-1</sup> )	MgCl <sub>2</sub>	Na <sub>2</sub> SO <sub>4</sub>	Na <sub>2</sub> CO <sub>3</sub>	NaCl	ESE	MgSO <sub>4</sub>
0	100.0 <sup>c</sup>	100.0 <sup>c</sup>	100.0 <sup>bc</sup>	100.0 <sup>b</sup>	100.0 <sup>c</sup>	100.0 <sup>b</sup>
0.05	94.0 <sup>c</sup>	95.7 <sup>bc</sup>	96.6 <sup>ab</sup>	101.7 <sup>b</sup>	97.2 <sup>bc</sup>	99.1 <sup>b</sup>
0.1	94.0 <sup>c</sup>	96.6 <sup>bc</sup>	110.3 <sup>c</sup>	107.8 <sup>c</sup>	96.4 <sup>bc</sup>	94.0 <sup>b</sup>
0.2	90.5 <sup>bc</sup>	100.0 <sup>c</sup>	107.8 <sup>bc</sup>	96.6 <sup>b</sup>	91.1 <sup>ab</sup>	94.8 <sup>ab</sup>
0.3	81.9 <sup>ab</sup>	84.5 <sup>ab</sup>	103.4 <sup>bc</sup>	97.4 <sup>b</sup>	93.1 <sup>ab</sup>	90.5 <sup>ab</sup>
0.4	83.6 <sup>ab</sup>	81.0 <sup>a</sup>	100.0 <sup>bc</sup>	94.0 <sup>a</sup>	89.6 <sup>a</sup>	89.7 <sup>ab</sup>
0.6	79.3 <sup>a</sup>	77.6 <sup>a</sup>	87.1 <sup>a</sup>	80.2 <sup>a</sup>	86.5 <sup>a</sup>	82.8 <sup>a</sup>

**Fig. 2.** Content of organic solutes (proline and soluble sugar) content in leaves of *Suaeda salsa* treated with NaCl. Values are given as mean  $\pm$  SE of four replicates.



dry weight by 92.3%, and root dry weight by 73.1% as compared with those in non-saline control (Table 3). Seedling growth decreased significantly ( $p < 0.05$ ) when the salinity level increased to 0.1 mol l<sup>-1</sup> level or more. As compared with the control, plant height, dry shoot weight, dry root weight and branch number at 0.4 mol l<sup>-1</sup> NaCl decreased by 37.5%, 24.1%, 41.1% and 31.9%, respectively (Table 3).

Most halophytes tend to accumulate proline and soluble sugar in their leaves at high levels of salinity to resist stress. Our study showed that proline content in leaves increased with the level of NaCl salinity (Fig. 2). As compared with the non-saline control, proline content at 0.1, 0.2 and 0.4 mol l<sup>-1</sup> NaCl increased by 181.4%, 347.6% and 565.2%, respectively (Fig. 2). In contrast, the soluble sugar content decreased by 25.0%, 55.5% and 61.7%, respectively (Fig. 2).

The Na<sup>+</sup> content in the seedlings increased with salinity while K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> content decreased (Fig. 3). At 0.4 mol l<sup>-1</sup> salt solutions,

Na<sup>+</sup> content in leaves, stems and roots was 9.3, 6.1 and 2.6 times that in the control, respectively. The content of Mg<sup>2+</sup> in leaves, stem and root decreased more sharply with salt stress than K<sup>+</sup> and Ca<sup>2+</sup> content and at 0.2 mol l<sup>-1</sup> NaCl the Mg<sup>2+</sup> was only 17.8%, 27.2% and 35.0% of that in non-saline control, respectively.

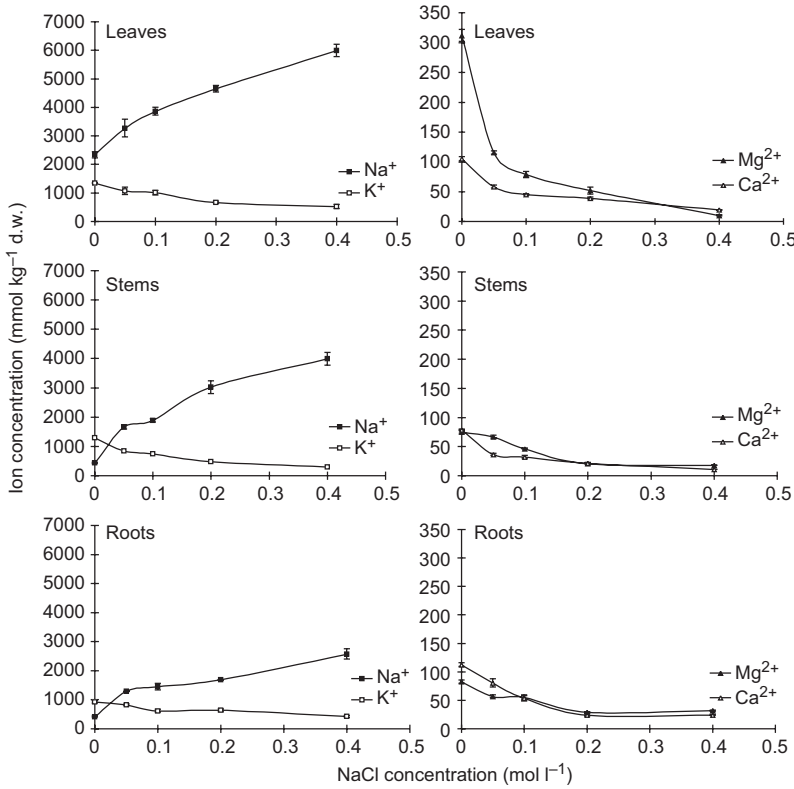
Sodium was the most dominant ion in the seedlings, especially under high salt stress. At 0.4 mol l<sup>-1</sup> NaCl, Na<sup>+</sup> accounted for 91.6% of the ions in leaves, 92.6% in stems and 84.1% in roots. Total ion concentration in the leaves was 6.539 mol kg<sup>-1</sup> d.w., while no more than 3.041 mol kg<sup>-1</sup> d.w. ions was recorded in roots (Fig. 4). The shoot biomass was over 80%–87% of the total biomass for this plant (Table 3).

## Discussion

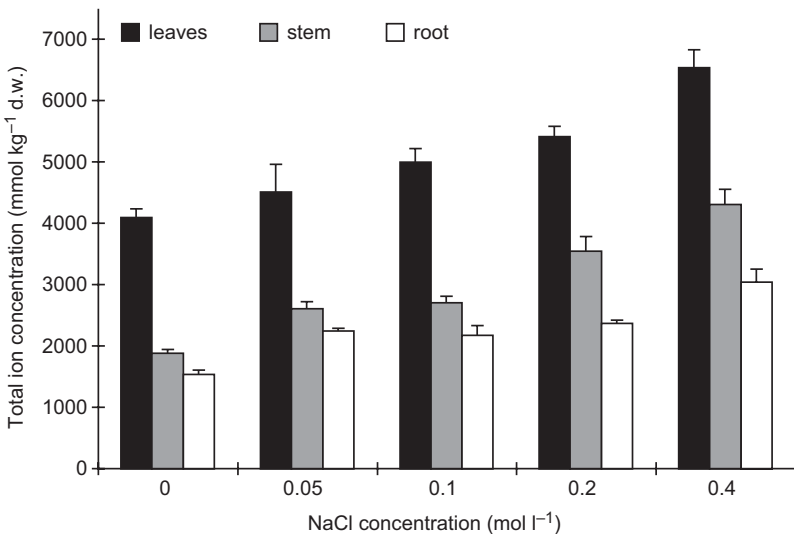
The onset of germination in *S. salsa* was delayed and germination percentage decreased because

**Table 3.** Effects of NaCl treatment on growth of seedlings of *Suaeda salsa*. All data are means of four replications  $\pm$  SE.

Treatment (mol l <sup>-1</sup> )	Index of growth			
	Plant height (cm)	Dry shoot weight (g)	Dry root weight (g)	Branch number
0	28.0 $\pm$ 0.7	1.3 $\pm$ 0.1	0.26 $\pm$ 0.0	28.3 $\pm$ 0.5
0.05	39.3 $\pm$ 2.4	2.5 $\pm$ 0.3	0.45 $\pm$ 0.0	37.8 $\pm$ 2.3
0.1	28.3 $\pm$ 3.5	1.7 $\pm$ 0.2	0.37 $\pm$ 0.1	27.8 $\pm$ 4.8
0.2	25.3 $\pm$ 2.8	1.2 $\pm$ 0.1	0.30 $\pm$ 0.0	20.3 $\pm$ 2.6
0.4	17.5 $\pm$ 0.3	1.0 $\pm$ 0.1	0.15 $\pm$ 0.0	19.3 $\pm$ 2.1



**Fig. 3.** Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> contents in leaves, stems and roots of *Suaeda salsa* treated with NaCl. Values are given as mean ± SE of four replicates.



**Fig. 4.** Total ion (Na<sup>+</sup> + K<sup>+</sup> + Ca<sup>2+</sup> + Mg<sup>2+</sup>) concentration in leaves, stems and roots of *Suaeda salsa* treated with NaCl. Values are given as mean + SE of four replicates.

of salt stress, as has also been reported by others (Breen *et al.* 1977, Zid & Boukhris 1977, Duan *et al.* 2003). Specific ion toxicity is one of the components of salt stress (Ungar 1978, Zhou *et al.* 1998). Our study showed that the level of inhibition of germination by salinity varied

greatly among different salts. The inhibition to seed germination was in the following order: MgCl<sub>2</sub> > Na<sub>2</sub>SO<sub>4</sub> > Na<sub>2</sub>CO<sub>3</sub> > NaCl > SES > MgSO<sub>4</sub>. Ungar (1996) found that germination of *Puccinellia festucaeformis* seeds, when treated with various salts, was in the order: CaCl<sub>2</sub>,

$MgCl_2 > NaCl, KCl$  and  $NaNO_3, KCl > MgSO_4$ . Our observation that single salt solutions inhibited germination more than solutions extracted from soil with multiple salts confirmed the earlier report by Shen (1999). Similar results were also reported for two turf grasses viz., *Lolium perenne* and *Festuca elata* Keng by Li *et al.* (2002).

Optimal germination for most halophytes has been reported in non-saline conditions (Khan & Weber 1986, Katembe *et al.* 1998, Gulzar & Khan 2001, Li *et al.* 2002, Khan 2002). However, for the halophyte *Chenopodium glaucum*, it was reported that seeds germinated better in low salinity stress (Duan *et al.* 2004).

Seeds buried naturally in salt-affected soils often germinate rapidly after a heavy precipitation. Most halophyte seeds maintain viable for extended period of exposure to hyper-saline conditions only to germinate when conditions are favorable (Woodell 1985, Keiffer & Ungar 1995, Khan & Ungar 1997). Strong salinity stress in our study did not devitalize seed germination ability; it only inhibited the germination temporarily and the viability was maintained. The stronger the seed germination was inhibited, the better was the germination recovery when the stress was removed.

Appropriate salinity in soil is necessary for the growth of most halophytes (Subbaraoa *et al.* 2003). Absence of some level of salinity in a halophyte's habitat can have a negative influence on plant growth even though its high levels would cause a visible stress (Gao *et al.* 2003a, 2003b). Sodium ion can be helpful in regulating osmotic pressure and water balance in plants (Nobel & Broqnell 1984). The photosynthesis of halophytes can be interrupted when the habitat is devoid of salinity (Gao *et al.* 2003a, 2003b). What constitutes the favorable level of salinity might, however, vary greatly with species due to the differences in their physiological mechanisms of resisting salt stress. Euhalophytes have greater salt-tolerance than recretohalophytes. Liu *et al.* (2006) showed that *Limonium bicolor* (a recretohalophyte) had a lower range of favorable salinity than *S. salsa* (an euhalophyte).

A lot of ions are absorbed by halophytes under salt stress and the ions get accumulated in different parts depending on the plants' salt-pro-

tection mechanisms (Breckle 1995). In our study with *S. salsa*,  $Na^+$  ions dominated and these were accumulated mostly in the shoot (leaves and stems) of seedlings. Leaves and stems of *S. salsa* are very succulent, a feature which might enable the plant to resist salt stress. Zhao *et al.* (2003) reported that ions were diluted adequately in leaves and stems of *S. salsa*.

Organic solutes have similar effects as the inorganic ions in improving tolerance to salt stress. They support the activity of enzymes and other macromolecules in addition to balancing the osmotic potential in the vacuole of the cells. Proline, one of the organic solutes, is reported to be involved in the alleviation of salinity stress in most halophytes (Xiao *et al.* 2000, Ruan & Xie 2002, Yin *et al.* 2003). Besides regulating water balance, it can protect protein from losing activity due to water deficiency (Zhao & Li 1999). However, recent experiments have shown that proline did not play an important role in osmoregulation for some halophytes (Song *et al.* 2006b). Our results however showed that proline played some role in osmoregulation in *S. salsa*.

Soluble sugars are another group of organic solutes involved in alleviating salt stress in many halophytes (Zhao *et al.* 1999, Shen & Chen 2001, Song *et al.* 2006b). However, our results showed that soluble sugar was not an important osmoregulator for *S. salsa* since its content decreased with an increase in the level of salinity. It appears that inorganic ions, especially  $Na^+$ , were more important in osmoregulation for *S. salsa* to adapt to saline environment.

## Conclusion

All salts significantly inhibited seed germination of *S. salsa*. The magnitude of inhibition of different salts was in the order:  $MgCl_2 > Na_2SO_4 > Na_2CO_3 > NaCl > Soil extract solution > MgSO_4$ . The main reason for inhibition of germination by salinity appeared to be the osmotic effect. Proline and inorganic ions were the main osmoregulation materials in the seedling, especially in leaves. Inorganic ions, especially  $Na^+$ , were more important than organic solutes in osmoregulation of the plant to adapt to saline environment.

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