Effects of long-term salinity on the growth of the halophyte *Spartina alterniflora* Loisel

Jungai Ma, Minwei Chai and Fuchen Shi*

College of Life Sciences, Nankai University, Tianjin 300071, China.

Accepted 17 October, 2011

The effects of salt stress on the growth of *Spartina alterniflora* were investigated by imposing seven levels of salt stress (0, 100, 200, 300, 400, 500, and 600 mM NaCl) on potted plants. The seedlings were grown in vermiculite in a greenhouse for six months. Optimal growth of *S. alterniflora* occurred at salinity levels of 100 and 200 mM NaCl. Seedlings grew less in freshwater conditions than in the 100 and 200 mM NaCl treatments. Higher levels of salinity (300 to 600 mM NaCl) caused a reduction in shoot number, height, biomass accumulation and leaf area. However, salt treatment resulted in only small changes in leaf water content. Although, chlorophyll concentrations were significantly higher at 100 and 200 mM NaCl than other salinity levels, photosynthetic rates were overall quite high regardless of treatment, ranging from 44.65 to 50.88 µmol m\(^{-2}\) S\(^{-1}\) of carbon gain. The results indicate that *S. alterniflora* has high tolerance to salt, and lower salt concentrations stimulate its growth.

Key words: *Spartina alterniflora*, salt stress, biomass production, chlorophyll concentration, water content, photosynthetic rates, stomatal conductance.

INTRODUCTION

Plant salt stress is an escalating global problem particularly in coastal areas which are vulnerable to the influx of sea water from natural and anthropogenic factors (Boesch et al., 1994; Rogers and McCarty, 2000). Salinity affects the distribution and abundance of rooted vascular plants in coastal wetlands (Lewis and Weber, 2002). In particular, salinity is a limiting factor for plant growth and productivity (Allakhverdiev et al., 2000). Therefore, studies of the salinity tolerance of salt marsh plants are very important and necessary for revegetating saline soils.

*Spartina alterniflora* Loisel is a rhizomatous perennial native to the Atlantic and Gulf coasts of North America (Bradley and Morris, 1991). It is recognized as one of the most important coastal grasses and commonly dominates coastal salt marshes (Pomeroy and Wiegert, 1981). *S. alterniflora* grows in essentially pure stands in the lower parts of marshes and is largely responsible for the high productivity of salt marshes (Turner, 1976). It was introduced throughout Europe, Australia and Asia to prevent shoreline erosion (Maricle and Lee, 2002). Since its introduction into China in 1979, it has expanded to approximately 50,000 hm\(^2\) along China’s east coast, due to its efficient propagation by seeds and rhizome fragments (Wan et al., 2009). It can be found in most Chinese tidal lands, from Beihai (21°36'N, 109°42'E) in the south to Tianjin (38°56'N, 121°35'E) in the north (An et al., 2007). In contrast to the ecological benefits, *S. alterniflora* has significant negative impacts on the native coastal ecosystems and many native species are threatened by this species (An et al., 2007; Wan et al., 2009). *S. alterniflora* is among the 16 most problematic exotic plants in China because it causes millions of dollars of economic loss per year (Chen et al., 2004).

*S. alterniflora* grows well in Chinese coastal wetlands because of its high salt-tolerance. *S. alterniflora* is a halophyte and can tolerate salinity as high as twice the strength of sea water. The salt tolerance of *S. alterniflora* is due to the presence of salt glands on the leaves, reduction in osmotic potential in the plant’s cells, ion...
exclusion at the root and ion partitioning in different organs (Baisakh et al., 2006). The presence of high salt levels does not seem to be required for optimal growth, and despite its comparatively high salt resistance, higher salinities may inhibit growth and productivity (Broome et al., 1975). Some studies have indicated that the optimum salinity level for *S. alterniflora* is 1 to 2% and that lower or higher salt levels inhibit its growth (Landin, 1991; Lewis and Weber, 2002; Wang et al., 2006). Other studies have concluded that increased salinity inhibits *S. alterniflora* growth (Gosselink, 1970; Smart and Barko, 1980). Therefore, the results are not completely consistent, and the effects of range of salinity levels on the development of the plant from seed to adult grass have not been described.

The objective of this work was to measure the effects of salt stress on *S. alterniflora* growth over an entire growing season. To achieve this goal, growth parameters such as shoot number, shoot height, leaf area and dry mass were monitored to determine the effects of salinity on the overall growth of *S. alterniflora*. Chlorophyll concentration, water content and stomatal conductance were also measured to better understand how salinity affects physiological processes. These results could improve methods for managing *S. alterniflora* in Chinese coastal wetlands.

**MATERIALS AND METHODS**

**Plant materials and salinity treatments**

Seeds of *S. alterniflora* were collected during autumn of 2008 from the Tianjin coastal wetland (38°33'-40°14'E, 116°42'-118°03'E) (Cao et al. 2005) in northern China. Seeds were separated from the inflorescence and stored in 4% NaCl solution at 4°C until the beginning of the experiments.

In April 2009, seeds were rinsed with fresh water to remove salts, sown into germinating trays, and watered with fresh water until germination. Once the initial shoots were about 4 cm in height, seedlings were transferred to individual plastic pots (14.8 cm diameter × 13 cm deep) filled with vermiculite. Each pot consisted of one plantlet which subsequently produced new shoots. The pots were allowed to establish with half-strength Hoagland’s nutrient solution for one week. Afterwards, seven concentrations of NaCl solutions (0, 100, 200, 300, 400, 500, and 600 mM) were employed after a preliminary test of salinity tolerance. NaCl was dissolved in Hoagland’s nutrient solution, and seedlings were watered with 0.5 L of salt solution. At the beginning of the experiment, NaCl levels were increased by 50 mM increments daily until the final level was reached. Solutions were completely replaced once a week to avoid the build-up of salinity in pots. The pots were placed on plates to prevent water and nutrient losses through leaching. The experiment was conducted with six replicates per treatment in a completely randomized design in a glass-covered greenhouse with natural temperature and light. The interior of the greenhouse ceiling was draped with 30% shade cloth to provide protection from excessive heat. The experiment was conducted over six months, and plants were harvested in November 2009.

**Growth parameters and water content**

At the end of the experiment, plants were thoroughly washed with tap water to remove the substrate and surface salt and then blotted with paper towel. The following growth parameters were recorded for plants in each pot: The number of shoots, the height of the tallest shoots, the leaf area, and the fresh weight (FW) and dry weight (DW) of above-ground and below-ground tissues. For leaf area measurements, the silhouette of each leaf was copied onto paper, which was then cut out and weighed. The relationship between the area and the weight of the paper was used to calculate leaf areas. Dry weight was obtained after oven drying at 60°C until a constant weight was reached. The leaf water content (TWC) was calculated using the following equation: TWC % = (FW-DW)/FW.

**Chlorophyll content**

After acetone extraction of the pigments from fresh leaves, the concentration of chlorophyll was determined for three replicate plants using the spectrophotometer method (Metzner et al., 1965). A leaf sample (0.1 g) was ground and extracted with 5 mL of 80% (v/v) acetone in the dark. The slurry was filtered and absorbance was measured at 645 and 663 nm.

**Photosynthetic parameters**

Before harvest, the net photosynthetic rate ($P_n$) and stomatal conductance ($g_s$) were determined during the day on the second fully expanded leaf using a LCA-4 infrared gas analyzer (Bio-Scientific, Great Amwell, Herts, UK) in the growth chamber.

**Statistical analysis**

Data were subjected to one-way analysis of variance (ANOVA) for each parameter. When the effect was significant ($P = 0.05$), differences between means were evaluated for significance using Duncan’s multiple range test ($P = 0.05$). All data were analyzed using commercially available software (SPSS version 11.0).

**RESULTS**

**Effect of salinity on plant growth**

As shown in Figure 1A, shoot number of *S. alterniflora* changed differently under various salt stresses. Though not statistical significantly, but the number of shoots increased temporarily at lower salinity (100–200 mM) with 23.33 shoots of 200 mM salinity treatment, higher than 23.17 shoots of control group. Then there was a decrease trend with increase of salinity treatment concentration. The higher salinities (300–500 mM) did not change significantly shoot number, compared to that of lower salinities, including 0, 100 and 200 mM. While the shoot number reduced to 11.83 shoots in the 600 mM NaCl treatment, significantly lower than any other treatments ($P <0.05$).

Although, there was a gradual decrease in shoot height as salinity increased, no significant differences were detected among 0, 100, 200, 300, 400 and 500 mM salt treatments. The greatest shoot growth occurred in the 100 mM treatment, and the poorest growth occurred in the 600 mM treatment. *S. alterniflora* showed a significant
reduction in shoot height at 600 mM NaCl. The height of plants growing in fresh water was shorter than that growing in the lower salinity treatments (100 to 300 mM) (Figure 1B).

**Effect of salinity on biomass production**

Increasing concentrations of salt significantly decreased the amount of above-ground (leaf and stem) biomass, below-ground (root and rhizome) biomass, and total biomass ($P<0.05$) (Figure 2). Dry weight in fresh water was lower than that growing at lower salinity levels (100 and 200 mM NaCl), although, the difference was not statistically significant.

Above-ground dry biomass was highest in plants grown at 100 mM NaCl, which was significantly higher than that of plants grown at 500 and 600 mM NaCl. There was statistically no significant difference between 100 mM NaCl and 0, 200, 300, 400 mM NaCl. Above-ground dry weight was approximately 74.44% lower in the 600 mM NaCl treatment compared to the 100 mM treatment. Compared with the 100 mM salt treatment, increasing salinity to 400 mM caused significant reductions in above-ground biomass and total biomass. Below-ground biomass of plants grown at 100 mM was significantly greater than that of any other treatment ($P<0.05$). Growth response, as measured by total dry weight, was highest at 100 mM NaCl and lowest at 600 mM NaCl.

The ratio of below-ground to above-ground biomass was highest at 100 mM NaCl but not significantly different from those at 0, 200, 400 mM NaCl. However, there was a significant decrease in the ratio from 100 to 300, 500 and 600 mM.

**Effect of salinity on leaf growth**

Salt stress significantly affected leaf area in *S. alterniflora* (Figure 3A). The leaf area values were reduced in the higher salt concentration and stimulated in the lower salt levels (100 and 200 mM). The leaf area values for the freshwater were lowered than that in 100 and 200 mM salt treatment. The highest leaf area values occurred in the 200 mM treatment. Compared to the 200 mM treatment, leaf area was reduced by 31.81% and 42.46% in the 500 and 600 mM salt treatments, respectively.

**Effect of salinity on water content**

The different treatment led to small changes in the leaf water content. The leaf water content of *S. alterniflora* was highest in plants grown at 100 mM NaCl, but there was no significant difference in leaf water content among the 0, 100, 200, 300, 500, 600 mM NaCl treatments (Figure 3B). The lowest leaf water content was in plants grown at 400 mM NaCl, which was significantly different from other treatments.

**Effect of salinity on leaf chlorophyll concentration, photosynthesis and stomatal conductance**

Changes in leaf chlorophyll concentration under different salinity stresses are shown in Table 1. The concentrations...
of total chlorophyll, and chlorophylls $a$ and $b$ were highest in the 200 mM salinity treatment and lowest in fresh water. Total chlorophyll concentration ranged from 0.93 mg g$^{-1}$ FW in fresh water to 2.33 mg g$^{-1}$ FW in 200 mM treatment. The total chlorophyll concentration at the highest salinity still reached 1.68 mg g$^{-1}$ FW. In addition, it was also clear that chlorophyll $a$ predominated over chlorophyll $b$. The chlorophyll $a/b$ ratio in $S. alterniflora$ decreased slightly in higher salt concentrations.

The effects of salinity on the rates of photosynthesis and stomatal conductance of fully expanded mature leaves in $S. alterniflora$ are shown in Figure 4. Overall, photosynthetic rates ($P_N$) were quite high, ranging from to 44.65 to 50.88 µmol m$^{-2}$ S$^{-1}$ of carbon gain. Maximal photosynthetic rates occurred in the freshwater conditions. $P_N$ was significantly lower in salt stressed plants than in control plants ($P<0.05$), and no significant changes were detected among different salt treatments.

Stomatal conductance rates ($g_s$) were measured simultaneously with photosynthetic rates. Stomatal conductance was generally higher under a salinity regime than in freshwater conditions, and plants growing in the 200 mM treatment had the highest value (Figure 4B). Overall, with increasing salinity (200 to 500 mM),
stomatal conductance decreased progressively.

DISCUSSION

Salinity is a primary constraint for plant growth and survival. In this study, we examined the effects of long-term salinity on the performance of *S. alterniflora* seedlings. Optimal growth occurred at salinities between 100 and 200 mM. Seedlings grew less in freshwater conditions than in the 100 and 200 mM NaCl treatments. Increasing salinity (300 to 600 mM NaCl) caused a corresponding reduction in shoot number, height, biomass accumulation and leaf area. Low salinity levels do not appear to have a deleterious effect on the growth of *S. alterniflora* and may actually stimulate growth. However, high salinity levels caused a reduction in total growth. These results are consistent with the previous studies of *S. alterniflora* by Landin (1991), Lewis and Weber (2002) and Wang et al. (2006). Similar patterns have been observed in *Halopyrum mucronatum* (Khan et al., 1999), *Sporobolus virginicus* (Bell and O’Leary, 2003), and *Cakile maritima* (Debez et al., 2004). Generally, lower salinity favors plant growth and higher salinity is inhibitory.

It is well known that the growth of many halophytes is depressed in culture medium lacking sodium chloride (Clough, 1984). Seedlings receiving low salt treatments grew much better than those grown in freshwater conditions, and further increasing in salt concentration retarded plant growth. Similar effects have been observed in other species, such as *Atriplex prostrate* (Egan and Ungar, 1998), *H. mucronatum* (Khan et al., 1999), *Salicornia rubra* (Khan et al., 2001) and *Bruguiera parviflora* (Parida et al., 2004). *S. alterniflora* showed high tolerance for salinity, although its growth was inhibited by higher salinity. In this experiment, a sharp reduction in growth was observed at higher NaCl concentrations, but plants remained alive and retained the capacity to initiate new shoots even in the 600 mM NaCl treatment. At harvest, on average, seedlings grown with 600 mM NaCl produced an average of 11.83 shoots. A rapid rate of shoot initiation is advantageous for a plant with a ruderal growth strategy, because it can cover bare ground quickly and be more competitive (Grime, 1977). Since *S. alterniflora*
Our results show that at 600 mM NaCl, the total chlorophyll concentration still reached 1.68 mg g⁻¹ FW. This result is not consistent with the frequently observed phenomenon that chlorophyll content decreases in plants under saline stress. In addition, only small differences in the photosynthetic rate were found within salt treatments in our experiments. Over all salt levels, photosynthetic rates ranged from to 44.65 to 50.88 µmol m⁻² S⁻¹ of carbon gain, which indicated that S. alterniflora retained high photosynthetic rate even under salinity stress. Similarly, Dai and Wiegert (1997) found that there was no difference in photosynthetic rates between tall and short S. alterniflora. The photosynthetic capacity of S. alterniflora is seldom altered by the routine changes in soil salinity encountered in salt marsh environments (Farnsworth and Meyerson, 2003; Vasquez et al., 2006). As a C₄ species, S. alterniflora has high photosynthetic rate and water use efficiency.

Salinity is an important factor affecting the distribution pattern of plants in coastal salt marshes. In China, S. alterniflora (alien) generally co-exists with Phragmites australis (native) in saline intertidal zones. Studies have shown that Spartina is more tolerant of salinity stress than is Phragmites (Vasquez et al., 2006). Spartina is dominant in high salinity conditions, whereas Phragmites tends to dominate over Spartina in areas with low salinity (Wang et al., 2006, 2010). In the Tianjin coastal wetland, S. alterniflora performs well in the intertidal zones and has expanded rapidly. Thus, proper management will be required to control this invasive exotic plant.

In summary, our results show that S. alterniflora is a highly salt tolerant perennial halophyte. This species can survive and produce new shoots in high saline media. Plant growth was stimulated by additions of NaCl to the growth medium at levels up to 200 mM, but higher concentrations resulted in less plant growth.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation of China (No. 30470179), and the authors gratefully acknowledge the assistance of Xinyuan Qiu and Wen Xu.

REFERENCES


