Effects of salt stress and nitrogen application on growth and ion accumulation of *Suaeda salsa* plants

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***Abstract-Suaeda salsa* is a typical pioneer species which can grow well in high salt environmental conditions. The objective of this study is to evaluate the effect of different levels of salinity (5.25, 10.5 and 21g NaCl per kg soil) and nutrient supply (0, 0.3, 0.6 and 1.2g urea per kg soil) on plant morphology, biomass, accumulation of ions and C/N ratio in leaves of *S. salsa*. The results showed that the plant height, number of branches, length of branches and diameter of shoot were significantly affected by salt stress, and the nitrogen released the negative effects of salt. The nitrogen treatment increased the biomass of leaf, shoot and root. Leaf water content was significantly affected by the interaction of salt stress and nitrogen treatment. The content of Na^+ and Cl^- increased significantly as increasing of salt, the content of K^+, Ca^{2+}, Mg^{2+} and SO_4^{2-} decreased simultaneously to keep ion balance. The C/N ratio decreased significantly as increasing of nitrogen treatment. The content of proline increased significantly with the increasing of salt and nitrogen treatments. The results together indicated that at different salt environment, different amount of nitrogen supply can be used to improve the population growth of *S. salsa* plants, and the restoration of degraded wetland could be accelerated by nutrient supply reasonably.**

Keywords-nitrogen; potassium; salt stress; seedlings; sodium; Suaeda salsa

I. INTRODUCTION

The Yellow River Delta is one of the most active regions of land-ocean interaction among large river deltas in the world. Wetlands in the Yellow River Delta support a wide variety of flora and fauna, in which there are two hundred and twenty different kinds of plant species and more than 800 animal species, including 199 kinds of bird. The Yellow River Delta has become an important over-wintering and breeding site for migrating birds in Northeast Asian Inland and Western Pacific Rim[1]. But in recent years, low flows of the Yellow River have led to a decrease in fresh water supply to the wetlands, and soluble salts in groundwater are carried to the surface in the form of capillary water, then water evaporates and salt is left in the top soil, resulting in a substantial rise in soil salinity[1], which bring out an urgent need to develop salt-tolerant plant species to overcome the soil salinity problem.

Suaeda salsa (L.), a succulent halophytic herb, widely distributed in northeast China, naturally grows on inland saline soils and coastal saline wetland[2]. Leaves of *S. salsa* can absorb many salt ions, which made them sustain high salt environment during germination and seedling stage. Fresh shoots of *S. salsa* are very valuable as a vegetable, and its seeds can produce edible oil[3]. *S. salsa* salt marsh, covering most forelands in the Yellow River Delta, is important to keep the natural succession of the wetland eco-system. The optimum soil salinity for *S. salsa* was about 12.71 g/kg[4]. In recent decades, with the exploitation of wetland resources and the rush of environmental problems, this habitat suitable to *S. salsa* is degrading.

Salt resistance in plants is usually quantified in terms of survival rates[5] and/or growing abilities under stress conditions, involving biochemical and physiological processes as well as morphological and developmental changes[6], and previous studies[7,8] have indicated that the maintenance of an adequate supply of nutrients to the growth zone should be a key component of the growth response to salinity. Nitrogen fertilization could increase crop yield[9], and the application of nitrogen fertilizer has also been reported to mitigate significantly the adverse effects caused by high salt stress on a number of plants[10,11].

Therefore, information on the biological and eco-physiological characteristics of *S. salsa* is needed to aid use in protecting the environment and developing saline agriculture. In the present study, the morphological changes and physiological processes under different NaCl and nitrogen levels were determined to investigate the adaptation mechanisms of *S. salsa* to different salt levels and how nitrogen supply can alleviate the harm effects of salt environment.

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II. MATERIALS AND METHODS

A. Plant materials

Seeds of *Suaeda salsa* (L.) were collected from Yellow River Delta in autumn 2008 before using in April 2009. Seeds were surface-sterilized with 3% sodium hypochlorite for 5 min, followed by a thorough rinsing with deionized water. Seeds were sown in 25 cm diameter plastic pots that contained 4 kg of native loamy soils in a partially shaded greenhouse under maximum photosynthetically active radiation of 1000 μmol m^{-2} s^{-1}, day/night temperature of 30/24 ± 3°C. The native loamy soils were totally mixed before used. The total nitrogen, available phosphorus, and potassium of the soil were, respectively, 1.12 g kg^{-1}, 12.9 mg kg^{-1}, and 203.6 mg kg^{-1}, and the total salt content were 2.22 g kg^{-1}.

B. Greenhouse experiments

1) The treatments combined four levels of nutrient supply with three salt levels in a factorial (nutrient × salt) design, with four replicates. The four levels of nutrient supply were 0, 1.2, 2.4 and 4.8 g slow-release urea per container (signed as NN, LN, MN and HN), and the three salt levels were 21, 42 and 84 g NaCl per container (signed as LS, MS and HS), and no salt no nitrogen treatment was used as control (C). A total of 52 pots were randomly placed in the same greenhouse as seedling culture. Slow-release urea and NaCl were thoroughly mixed into the soil. During the early growth stages (plants did not show much difference in response to salt), equal amounts of water were applied and the salt solution was maintained at 1 cm deep in the saucers. When plants started to show differences in their salt tolerance, a different amount of water was applied to each pot to make sure that the salinity level in different pots was maintained as uniformly as possible[12].

2) Seedlings were thinned to 13-15 per pot after 7 days of sowing. The treatment normally lasted for 2 months. At the end of the experiments, the plant height, total branch number, the length of each branch and diameter of shoot of 7 plants in each pot were measured. Then plants were harvested, they were first washed with tap water, then distilled water. Roots, shoots and leaves were separated and the FW was determined for each plant, and the samples were oven-dried at 105°C for 15 min, then vacuum-dried at 60°C to constant weight and the DW was recorded.

3) Dry samples of plant leaf (100 mg) were treated with 20 ml deionized water at 100°C for 20 min and the extract was taken to determine free anion contents. The contents of NO_3^-, Cl^-, and SO_4^{2-} in leaves were determined by ion chromatography (ICS-2000, Dionex, USA). A 400 mg dry sample of plant leaf was wet ashed with H_2SO_4: $HClO_4$ (10:1, v/v) digestion, and then brought to a volume of 100 ml with deionized water. Atomic emission spectrometry (AA-6800, Shimadzu, Japan) were used for the determination of sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) concentrations. Total N and C in leaves were analyzed by elemental analyzer (Elementar Vario Macro, Germany). Leaf C/N ratio was then calculated. The contents of proline were measured in dry samples. Proline was measured using ninhydrin[13].

4) Statistical analysis of the data, which involved data processing and variance analysis (ANOVA), was conducted using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Experimental data were subjected to two way analysis of variance (salt level and nitrogen supply as main factors) and the means were separated by the least significant difference (LSD). All acquired data were represented by an average of 3-4 replicate (one replicate per pot) measurements and standard deviation (S.D.). Significance was tested at the 5% level.

III. RESULTS

1) Responses of plant morphology to NaCl and Nitrogen

Plant height decreased with the increasing of salt stress, but it was not affected significantly by LS stress (Table 1). At LSMN treatment, S. salsa plants have more branches and longer shoot diameter than any other treatments. At LS treatment, the mean length of branches was significantly longer than control and all other treatments except in MSHN treatment. At MS and HS treatments, LN and MN increased the plant height, number of branches and branch length significantly compared with control (Table 1).

2) Leaf water content and plant biomass

The leaf water content (LWC) was significantly affected by the interaction of NaCl and nitrogen (Table 3). Compared with control, the leaf water content of *S. salsa* increased significantly, except at LSHN and MSLN treatment (Fig. 1, Table 3) ($P<0.05$). The LWC increased significantly with the increasing of salt stress at HN treatment (Fig. 1) ($P<0.05$). The total and leaf biomass were significantly affected by salt stress (Table 3). Compared with control, Biomass of S. salsa was significantly affected by MS and HS (Table 2). The biomass of leaf, shoot and root of *S. salsa* plants treated by LSMN were two-fold higher than those of LSNN treatment (Table 2), and at MS and HS treatments, LN and MN increased the biomass of leaf, shoot and root of *S. salsa* seedlings except the root biomass at HNHM treatment.

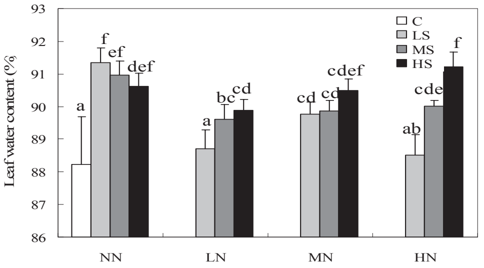

Figure 1. The effect of salt stress and nitrogen treatment on leaf water content (LWC) of *S. salsa* seedlings. The values are means (±S.D.) of three replicates. Different letters indicate significant differences from each other

TABLE1. Plant height, number of branches, length of branches and diameter of shoot of *S. salsa* seedlings under different salt and nitrogen treatments. The values are means (±S.D.) of three replicates. Different capital letters indicate significant differences from different salt levels, and different lowercase letters indicate significant differences from different nitrogen levels ($P < 0.05$).

Index	Nitrogen treatments	Salt treatments			
		C	LS	MS	HS
Plant height (cm)	C	27.0 ± 0.22 A	26.0 ± 1.68 Aa	14.5 ± 0.84 Bb	10.3 ± 0.33 Ca
	LN	–	24.1 ± 1.19 Aa	16.7 ± 0.31 Ba	12.9 ± 0.40 Ca
	MN	–	24.3 ± 2.03 Aa	15.6 ± 0.24 Bab	12.4 ± 1.33 Ba
	HN	–	24.6 ± 1.54 Aa	14.7 ± 0.75 Bb	10.7 ± 0.93 Ba
Number of branches	C	7.4 ± 0.49 A	8.6 ± 0.84 Ab	3.8 ± 0.62 Bb	3.8 ± 0.33 Ba
	LN	–	8.6 ± 0.87 Ab	5.9 ± 0.22 Ba	4.9 ± 0.36 Ba
	MN	–	12.6 ± 0.61 Aa	6.0 ± 0.58 Ba	4.9 ± 0.83 Ba
	HN	–	10.1 ± 1.12 Ab	5.1 ± 0.05 Bab	3.6 ± 0.52 Ba
Length of branches (cm)	C	6.3 ± 0.12 B	7.7 ± 0.68 Aa	3.8 ± 0.66 Cb	3.0 ± 0.07 Cb
	LN	–	7.7 ± 0.13 Aa	5.8 ± 0.18 Ba	4.8 ± 0.20 Ca
	MN	–	8.7 ± 0.33 Aa	5.5 ± 0.39 Ba	4.1 ± 0.20 Cb
	HN	–	7.6 ± 0.65 Aa	6.1 ± 0.34 Aa	3.3 ± 0.73 Bb
Diameter of shoot (mm)	C	1.82 ± 0.02 A	1.73 ± 0.08 Ab	1.11 ± 0.01 Bc	1.02 ± 0.03 Bb
	LN	–	1.84 ± 0.06 Ab	1.30 ± 0.04 Ba	1.18 ± 0.02 Ba
	MN	–	2.08 ± 0.06 Aa	1.23 ± 0.00 Bb	1.13 ± 0.07 Bab
	HN	–	1.69 ± 0.16 Ab	1.21 ± 0.01 Bb	1.02 ± 0.03 Bb

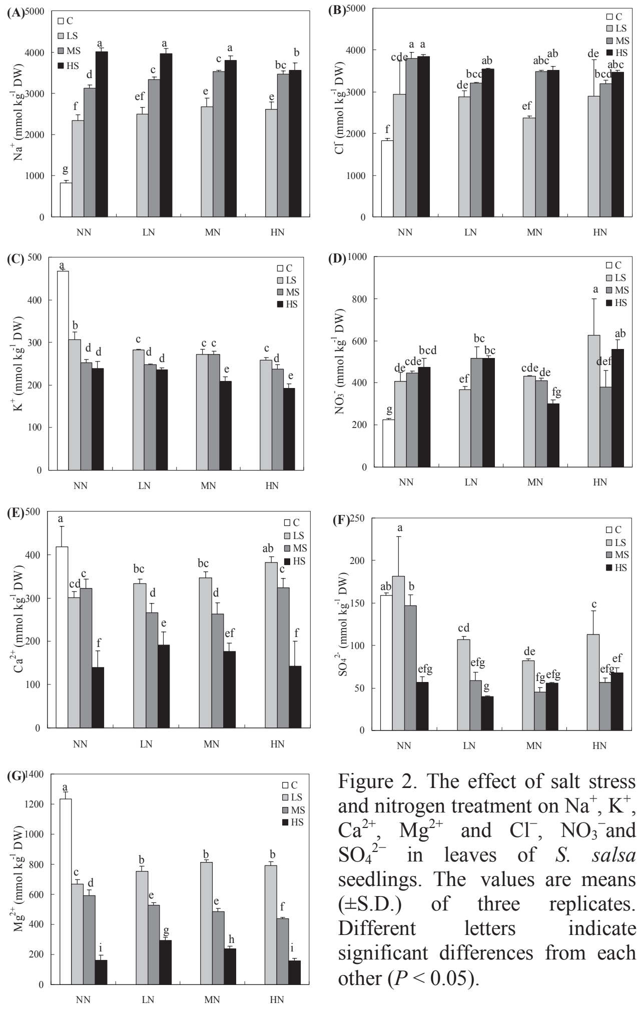

Figure 2. The effect of salt stress and nitrogen treatment on Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-, NO_3^- and SO_4^{2-} in leaves of *S. salsa* seedlings. The values are means (±S.D.) of three replicates. Different letters indicate significant differences from each other ($P < 0.05$).

3) Responses of plant Physiology to NaCl and Nitrogen

With increasing of salt stress, Na^+ content of leaves increased significantly (Fig. 2A). In contrast, the K^+ content decreased significantly compared with control, and the K^+ content was higher in LS stress than in MS and HS stress except HSMN treatment (Fig. 2C). The changes of Ca^{2+} and Mg^{2+} are similar with K^+, which decreased with the increase of salt. Ca^{2+}, Mg^{2+} and K^+ all decreased significantly compared with control resulted from the interaction effect of nitrogen and salt treatment (Fig. 2E, G).

Under salt stress, the Cl^- content in leaves increased with increasing salinity (Fig. 2B). The content of NO_3^- increased clearly as the interaction effect of nitrogen and salt treatment compared with control, except for HSMN treatment.But as the increase of nitrogen treatment, the content of Cl^- and NO_3^- remained relatively unchanged (Fig. 2B, D). The nitrogen treatment affected the content of SO_4^{2-} significantly, which were much lower in all nitrogen treatment than in NN treatment. Compared with salt stresses, the MS and HS decreased the SO_4^{2-} content more significantly than LS (Fig. 2F).

The C/N ratio in leaves was shown in Fig. 3. The control group had the highest C/N ratio. As the nitrogen content in the soils increase, the C/N ratio decreased significantly (Table 3) (P<0.001). The salt stress did not affect the C/N ratio, but the interactions of nitrogen and salt stress significantly affected the C/N ratio (Table 3) (P<0.001).

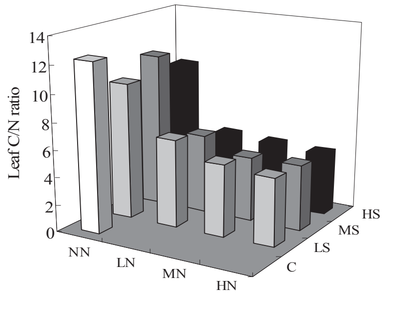

Figure 3. The effect of salt stress and nitrogen treatment on C/N ratio in leaves of *S. salsa* seedlings. Each value is means (±S.D.) of three replicates.

TABLE 2. The biomass of leaf, shoot and root of *S. salsa* seedlings under different salt and nitrogen treatments. The values are means (±S.D.) of three replicates. Different capital letters indicate significant differences from different salt levels, and different lowercase letters indicate significant differences from different nitrogen levels ($P < 0.05$).

Biomass	**Nitrogen treatments**	**Salt treatments**			
		C	LS	MS	HS
Leaf (g/plant)	C	0.311 ± 0.024 A	0.287 ± 0.028 Ab	0.093 ± 0.017 Bb	0.066 ± 0.009 Bb
	LN	–	0.449 ± 0.049 Ab	0.198 ± 0.006 Ba	0.125 ± 0.011 Ba
	MN	–	0.678 ± 0.038 Aa	0.176 ± 0.005 Ba	0.108 ± 0.024 Bab
	HN	–	0.466 ± 0.103 Ab	0.167 ± 0.006 Ba	0.069 ± 0.017 Bb
Shoot (g/plant)	C	0.191 ± 0.012 A	0.151 ± 0.018 Bc	0.039 ± 0.009 Cc	0.021 ± 0.003 Cb
	LN	–	0.203 ± 0.019 Abc	0.081 ± 0.003 Ba	0.043 ± 0.004 Ba
	MN	–	0.311 ± 0.020 Aa	0.067 ± 0.001 Bab	0.039 ± 0.010 Bab
	HN	–	0.265 ± 0.069 Aab	0.059 ± 0.001 Bbc	0.022 ± 0.007 Bb
Root (g/plant)	C	0.083 ± 0.006 A	0.047 ± 0.005 Bb	0.010 ± 0.001 Cb	0.005 ± 0.000 Ca
	LN	–	0.087 ± 0.010 Aa	0.014 ± 0.001 Ba	0.008 ± 0.000 Ba
	MN	–	0.092 ± 0.004 Aa	0.012 ± 0.000 Bab	0.008 ± 0.002 Ba
	HN	–	0.053 ± 0.013 Ab	0.011 ± 0.000 Bb	0.006 ± 0.001 Ba

TABLE 3. Two-way ANOVAs on total biomass, leaf biomass, leaf water content (%) and leaf C/N ratio of *S. Salsa* for NaCl and Nitrogen treatment and their interactions.

Independent variable		NaCl	Nitrogen	NaCl×Nitrogen
Total biomass	F	55.817	5.314	2.652
	p- values	0.000	0.005	0.035
Leaf biomass	F	61.718	7.414	3.577
	p- values	0.000	0.001	0.009
Leaf water content	F	22.060	10.587	5.392
	p- values	0.000	0.000	0.001
Leaf C/N ratio	F	925.441	15843.24	207.884
	p- values	0.000	0.000	0.000

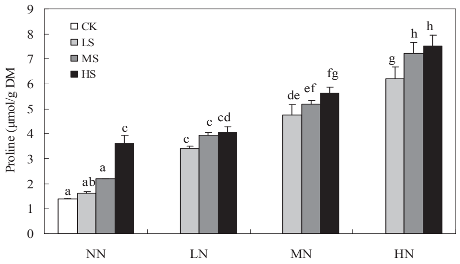

Figure 4. The effect of salt stress and nitrogen treatment on proline contents of *S. salsa* seedlings.

The responses of proline content to salt were similar to that of Na^+, which increased significantly with the increasing of salt stress, but also increased significantly with the increasing of nitrogen treatment.

IV. DISCUSSION

Water deficit and salt-specific or ion-excess affected by salinity are the main reasons which inhibit plant growth[14], but different plant species have developed different mechanisms to cope with these effects[15]. In this work, higher salt treatment significantly affected the plant height, number of branches, mean length of branches and diameter of shoot (Table 1), which were consistent with the results reported by Liu *et al.*[16]. *S. salsa* is a typical halophyte which can grow better in salt environment[4], and it needs more inorganic ions to adjust the osmotic potential and physiological needs. This is demonstrated in Table 1: there were no significant affects for plant height, number of branches and shoot diameter between LS and control treatments, however, the mean length of branches was longer in LS treatment than that of control group. The results indicated that the *S. salsa* plants needed more inorganic ions for better growth, and LS was the best level that contained suitable ions to *S. salsa* plants in this study. Such characteristics are favorable for *S. salsa* plants to grow well in coastal wetlands in Yellow River Delta and decrease the evaporation through accumulating high above-ground biomass in saline areas. Previous studies have shown that higher nutrient supply may provide more nutrient for plant growth and counteract the negative effects caused by the surrounding conditions, such as salts[16,17], and flood[18,19]. In this study, it showed that *S. salsa* plants accumulated more biomass over the same growth period in the presence of different levels of nitrogen and LS treatment (Table 2, 3). However, in MS and HS treatments, although nitrogen treatment increased the biomass of leaf, shoot and root, there were no significant differences between LN and MN treatments. Generally, plants can reduce water content as a quick and economical approach to osmotic adjustment in response to osmotic stress[20, 21]. Unexpectedly, the LWC in all salt treatments were higher than control (Fig. 1, Table 3). Being a succulent plant, maintaining high LWC can make vacuoles enlarging, which cause the cell surface to increase and make the layer of protoplasm thinner. Cell surface area and the distance between plasmalemma and vacuole membranes are key factors in deciding the speed and power of consumption of inorganic ions and organic small molecules going in and out of each cell[21]. So this might be one of the main physiological characteristics of *S. salsa* that allow it to have strong resistance to salt stress.

It has been generally observed that plants exposed to saline environment (NaCl), take up high amounts of Na^+, whereas the uptake of K^+ and Ca^{2+} is significantly reduced[15,22,23]. Plants generally compartmentalize Na^+ into vacuoles to avoid Na^+ toxicity in the cytosol[24]. Thus, Na^+ is the main inorganic osmolyte under salt stress. Ca^{2+} can maintain membrane stability, help to form cell walls and take part in signal transduction. Mg^{2+} is the key component of chlorophyll. But the Ca^{2+} and Mg^{2+} accumulation in many plants (include halophytes) are inhibited by salt stress [25, 26]. In the present study, the amount of Na^+ increased significantly as the increased of salt stress, and for maintain cations balance in plant cells, the content of K^+, Ca^{2+} and Mg^{2+} decreased simultaneously (Fig. 2). The amount of cations was not affected significantly by nitrogen. This phenomenon could be explained that cations were more sensitive to high salt stress than nitrogen treatments. Under salt stress, plants accumulate cations such as Na^+ and K^+[22], and simultaneously accumulate inorganic anions such as Cl^-[21], NO_3^- and SO_4^{2-}[27] to keep ion balance. In this study, the content of Cl^- and NO_3^- increased in salt treatments compared with control, and for the anions balance, SO_4^{2-} decreased as the increasing of salt stress.

Proline is organic osmolyte distributed principally in protoplasm. The accumulation of proline is clearly the response to osmotic stress to salt and nitrogen addition. It is possibly related to their strong resistance to salt tress.

Carbon metabolism and nitrogen metabolism are the two basic metabolism processes in plant development. They directly affect the formation and transformation of the photosynthetic product, mineral nutrition absorption, and protein synthesis. Their ratio (C/N ratio) reflects the accordant status of carbon and nitrogen metabolism[28]. In this study, with the increasing of nitrogen treatments, the nitrogen uptake increased, and the C/N ratio in leaves decreased significantly (Fig. 3, Table 3). This indicated that nitrogen supply could increase metabolic activity of nitrogen and then increase the biomass of plant[28]. This could be another important physiological characteristic for *S. salsa* to maintain normal carbon and nitrogen metabolism under high salt stress.

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