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NaCl enhanced the vegetative growth of halophyte *Suaeda salsa* by improving the ability of antioxidant and osmotic adjustment

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Abstract. To evaluate the effect of a long-term NaCl supply on the vegetative growth of *Suaeda salsa*, the plant growth indicators and resistance indicators were investigated. Results showed that compared with control, 200 mM NaCl in the growth medium significantly increased the plant height, branch number, fresh and dry weight, the content of soluble sugar and protine, and reduced the membrane relative conductivity and MDA content of *S. salsa* plants. While the higher concentration (400 mM) NaCl, supplied in the growth medium, significantly increased the MDA content and the membrane relative conductivity of *S. salsa*, as well as the soluble sugar and protine content. However, the plant height, branch number, fresh and dry weight of *S. salsa* treated with 400 mM NaCl was decreased when compared with the treatment of 200 mM NaCl. It indicated that a certain concentration of NaCl could significantly increase the vegetative growth process of halophyte *S. salsa*, long-term treatment with lower or higher concentration of NaCl may inhibit the growth of *S. salsa*, the plant height and weight was lower than plants from 200 mM NaCl treatment.

1. Introduction

Abiotic stress can significantly reduce the biomass and yield of plants. These abiotic stresses mainly include extreme temperature, light, non-optimal mineral nutrition, water deficit, and so on. Among them, the serious damage to the yield of plants is the arid and saline conditions. Soil salinization is a worldwide problem, it greatly reduced the growth of non-halophyte, by limiting water absorption and leading to physiological drought ^[1], and caused nutritional deficits by disturbing nutrient absorption and utilization. At the same time, a relative amount of Na⁺ and Cl⁻ ions will be accumulated in plant cells, disturbed the intercellular ion balance, thus resulting in ionic stress ^[2]. In addition, a series of secondary stresses, such as oxidative stress will also be caused by the saline environment in non-halophyte. For example, high concentration of NaCl treatment inhibited the antioxidant response of rice, and resulted in the accumulation of large amount of active oxygen, and then inhibited the growth of rice ^[3]. While, for halophyte, it could grow well in thus high concentration salinity environment ^[4, 5, 6]. Therefore, halophytes can be used as resources in the aspect of in environmental protection and ecosystem protection. For example, *Salicornia*, a kind of succulent plant that grows in a certain concentration of salt, and could have similar yield and seed quality as soybean ^[7].

Grown in saline environment, all halophytes would face the adjustment of osmotic potential to adapt to the low external water potential, too. Halophytes could reduce the osmotic potential of the cells by means of accumulating inorganic ions and small organic molecules ^[6, 8]. Among them, proline



and betaine were two major organic osmotic regulators that involved in the osmotic regulation of some halophytes^[9]. Studies showed that some of the plants can accumulate betaine in cells, and some other plants may accumulate proline in cells, and participated in plant osmotic regulation in saline environment^[10]. Proline and Betaine also play an important role in the stability maintenance of enzymes and membrane systems, as well as the removal of reactive oxygen species (ROS)^[11], and the prevention K⁺ efflux from cells^[12]. In addition, the accumulation of soluble sugars in halophytes can also play a role in osmotic adjustment under saline conditions^[13]. Therefore, it is needed to investigate the mechanism by which that halophyte *Suaeda salsa* used for osmotic adjustment when grown in saline environment.

Suaeda salsa is a typical leaf succulent annual herb halophyte. It could grow and reproduce in saline environment. Previous studies had shown that a short-term treatment of NaCl at an appropriate concentration (for example, 7 days) promotes vegetative growth of the halophyte *S. salsa*^[14, 15]. However, it remains to be elucidated that the changes during the vegetative growth of *S. salsa*, under NaCl treatment conditions for a long-term treatment (from seed sowing to flowering). In the saline conditions, halophyte could grow and development only by adjusting one's own adaptation to the saline environment, and it remains unknown that whether the adaptation mechanism of *S. salsa* is same as the other halophytes. Therefore, in the present study, the growth indicators of *S. salsa* related to salt stress and tolerance, such as plant height, plant weight and resistance index, at the end of vegetative growth period with the NaCl treatment for 103 days, were mainly analyzed. The indicators of osmotic adjustment substances and membrane peroxidation degree laid a certain foundation for further analysis of the reproductive growth process of *S. salsa* in the medium containing different concentrations of NaCl.

2. Material and Methods

2.1 Plant material and growth conditions

S. salsa seeds were collected from the saline inland of Yellow River Delta (N37°20'; E118°36') in Shandong, after a 6-months storage in refrigeration (<4°C), seeds were sown in plastic buckets (26 cm diameter, 30 cm height) with drainage holes and filled with rinsed river sand. The growth and culture condition were same as described by Guo et al.^[16]. And the treatment continued until to the beginning of flowering stage.

2.2 Vegetative growth determination of *S. salsa*

2.2.1. Determination of plant height and branch number of *S. salsa*

The plants were irrigated with Hoagland nutrient solution containing 0, 200 and 400 mM NaCl, respectively, until the flower buds emergence (103 days after sown). The height of *S. salsa* seedlings, treated with different concentrations of NaCl, above the sand surface, was measured with a ruler (above 1 cm of the sand surface), respectively. At the same time, the branch number of *S. salsa* treated with different concentrations of NaCl was counted, the first branch was the branch produced by the main stem.

2.2.2 Determination of fresh weight and dry weight of *S. salsa*

The plants were irrigated with Hoagland nutrient solution containing 0, 200 and 400 mM NaCl, respectively, until the flower buds emergence (103 days after sown). The seedlings of *S. salsa* were cut along the sand surface, washed using distilled water and dried by filter paper quickly. The fresh weight (FW) of *S. salsa* plant was determined and the dry weight (DW) was measured after thorough dryness (105°C for 10 min, followed by 80°C for 72 h).

2.3 Determination of the resistance indicators of *S. salsa*

2.3.1 Determination of MDA content in leaves and stems of *S. salsa*

At the end of the vegetative growth of *S. salsa*, the leaves and stems from different concentration of NaCl treatment were harvested, and the MDA contents were determined using the thiobarbituric acid (TBA) method according to Heath and Packer ^[17].

2.3.2 Determination of the relative membrane permeability in leaves of *S. salsa*

At the end of the vegetative growth of *S. salsa*, the leaves from different treatment were collected. The relative membrane permeability was carried out by means of relative conductivity.

2.3.3 Determination of soluble sugar content in leaves and stems of *S. salsa*

At the end of the vegetative growth of *S. salsa*, the leaves and stems from different concentration of NaCl treatment were collected (0.2 g for each subsample), and the soluble sugar contents were determined following the method of Yemm and Willis ^[18].

2.3.4 Determination of proline content in leaves of *S. salsa*

At the end of the vegetative growth of *S. salsa*, the leaves from different concentration of NaCl treatment were collected, and the proline content in the leaves were determined ^[19].

2.4 Statistical analysis

The statistical results are described as means \pm s.d., where n is the number of replicates. The data were analyzed using the statistical software SPSS ver. 17.0 (SPSS Inc.) and one-way ANOVA software packages. Different letters in the figures indicate significant difference among the means (at 0.05) according to Duncan's test.

3. Results

3.1 Vegetative growth determination of *S. salsa*

3.1.1. The plant height and branch number of *S. salsa*

The presence of certain concentration of NaCl in the culture medium had a stimulated effect on the growth of *S. salsa*. The plant height of *S. salsa* treated with 200 mM NaCl displayed an increase (1.13 times of the control) in plant height than the plans from control. While the plant height decreased (89.0% of that in control plant) when treated with higher concentration (400 mM) NaCl (Fig. 1A). The number of first branches of *S. salsa* displayed the same trend as the plant height, it was significantly increased (1.08 times of that in control plants) in *S. salsa* treated with 200 mM NaCl, while higher concentration (400 mM NaCl) treatment reduced the branch number of *S. salsa*, it was only 85.4% of that in control plants (Figure 1B).

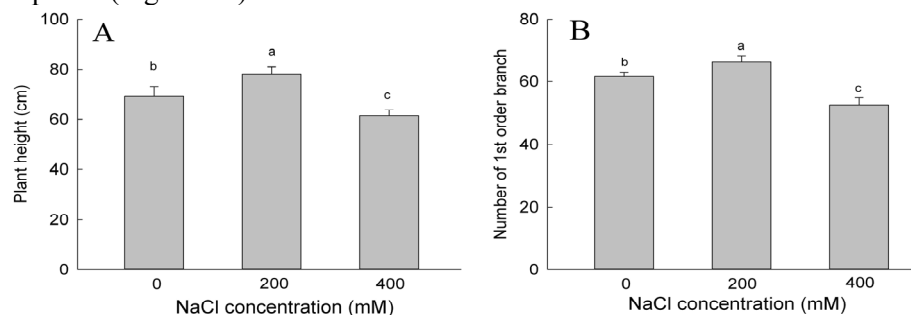


Figure 1 Plant height (A) and first branch number (B) of *S. salsa* at the end of vegetative growth under different concentration NaCl treatments. Values are means \pm SD ($n=10$).

3.1.2. The fresh weight and dry weight of *S. salsa*

NaCl treatment significantly increased the fresh weight and dry weight of *S. salsa*, the fresh weight of *S. salsa* treated with 200 and 400 mM NaCl were 1.77 and 1.52 times of that in control plants, respectively (Figure 2A), and the dry weight were 1.47 and 1.08 times of that in control plants, respectively (Figure 2B).

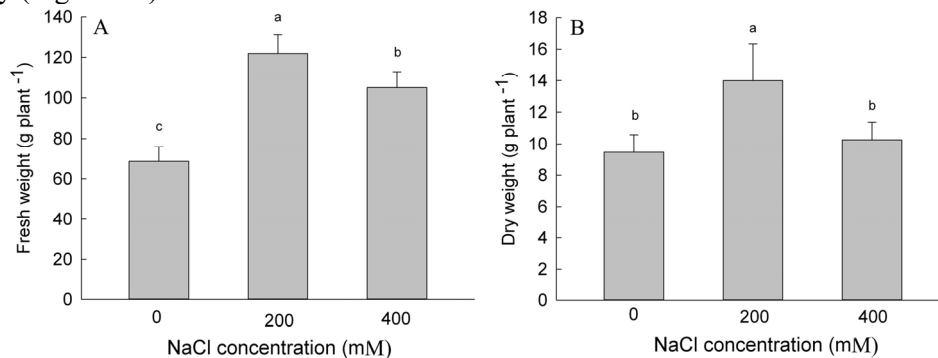


Figure 2 The fresh weight (A) and dry weight (B) of individual plant of *S. salsa* at the end of vegetative growth under different concentration NaCl treatments. Values are means \pm SD ($n=5$).

3.2 The resistance indicators determination of *S. salsa*

3.2.1 MDA content in leaves and stems of *S. salsa*

Membrane lipid peroxidation could result in membrane damage and increase membrane permeability. The amount of MDA could reflect the degree of membrane damage. The MDA content in the leaves and stems of *S. salsa* treated with 400 mM NaCl were significantly higher than that in control plant (Figure 3). There was no significant change in the leaves of *S. salsa* treated with 200 mM NaCl, while the MDA content in the stems was significantly lower than the control (Figure 3), which was 64.5% of the control.

3.2.2 The relative membrane permeability in leaves of *S. salsa*

Relative conductivity is the basic physiological indicator that reflecting the permeability of plasma membrane. The relative conductivity was decreased in the leaves of *S. salsa* treated with 200 mM NaCl, but increased in the leaves of *S. salsa* treated with 400 mM NaCl.

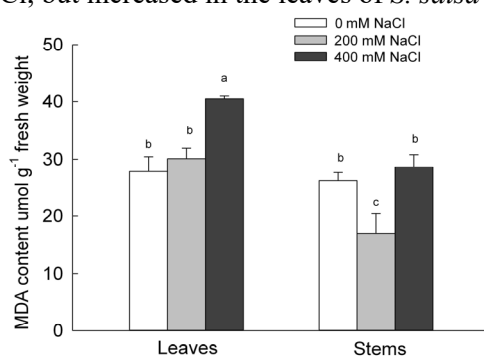


Figure 3 MDA content in the leaves and stems of *S. salsa* at the end of vegetative growth under different concentration NaCl treatment. Values are means \pm SD ($n=4$).

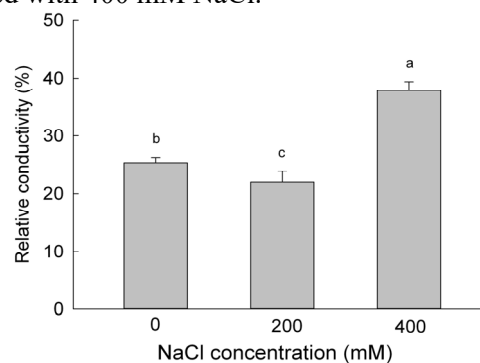


Figure 4 The relative conductivity in leaves of *S. salsa* at the end of vegetative growth under different concentration NaCl treatment. Values are means \pm SD ($n=4$).

3.2.3 Soluble sugar content in leaves and stems of *S. salsa*

Soluble sugar is a major osmotic regulator produced by plants in stress response. 200 mM NaCl treatment significantly increased the soluble sugar content in leaves and stems of *S. salsa*, and it was

1.83 and 1.12 times of that in the control, respectively. While the soluble sugar content in leaves and stems were 1.93 and 1.24 times of the control when treated with higher concentration (400 mM) NaCl (Figure 5).

3.2.4 Proline content in leaves of *S. salsa*

In unfavourable environments, proline (Pro) will be accumulated in plant to response the stresses. In 200 and 400 mM NaCl treated-leaves, the proline content was significantly higher than that in control leaves, which was 1.34 and 1.40 times of the control, respectively (Figure 6).

4. Discussion

In salinity environment, the water potential was reduced. Plants may through accumulating inorganic ions and organics to reduce the osmotic potential in cells to absorb water from the lower water potential conditions [6]. At the same time, a large amount of inorganic salt ions, such as Na^+ and Cl^- , will accumulate in plants, thus greatly reduced the growth of non-halophytes. And will cause a series of secondary stresses, for example, NaCl stress inhibited the antioxidant response, resulted in the accumulation of large amount of active oxygen [3]. The lipid peroxidation of membrane will be occurred with the high accumulation of active oxygen, increased the membrane permeability and inhibited the plant growth. However, the growth of some halophytes could be promoted by certain concentration of NaCl. For example, treatment with 200 mM NaCl for a short-trem can significantly promote the growth of halophyte *Sesuvium portulacastrum* [20]. For halophyte *S. salsa*, long-term (from the seed sowing to the end of vegetative growth stage) of NaCl treatment significantly promoted the plant growth, the plant height and first branch number were significantly increased when treated with 200 mM NaCl (Figure 1), as well as the fresh weight and dry weight (Figure 2). But the promoting effect in plant height, branch number and dry weight of *S. salsa* was not displayed in higher concentration (400 mM) NaCl treated-plants except the fresh weight (Figure 1, 2), may be treated with 400 mM NaCl significantly increased the water content, rather than the dry matter.

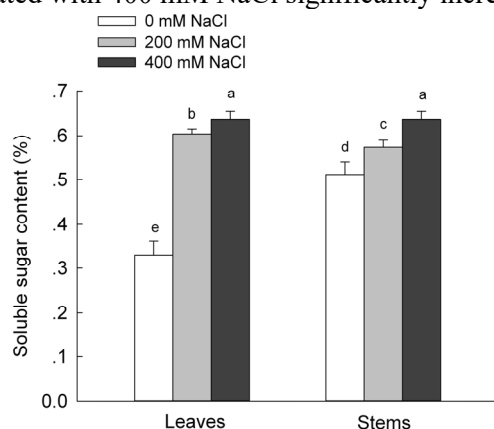


Figure 5 Soluble sugar content in leaves and stems of *S. salsa* at the end of vegetative growth under different concentration NaCl treatment. Values are means \pm SD ($n=4$).

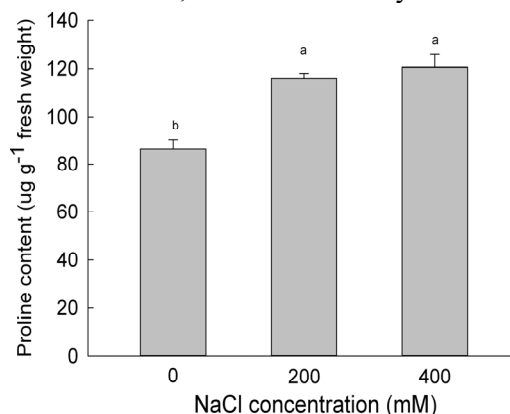


Figure 6 Proline content in leaves of *S. salsa* at the end of vegetative growth under different concentration NaCl treatment. Values are means \pm SD ($n=4$).

In addition to accumulating salt ions in cells, halophyte would accumulate small organic matter, such as soluble sugar, proline and betaine, to reduce the osmotic potential. NaCl treatment significantly increased the total soluble sugar content in the leaves and stems of *S. salsa*, when compared with the control plants (Figure 5). Furthermore, the soluble sugar content in the leaves and stems displayed a very significantly increase with the increase of NaCl concentration (400 mM). As well as the protine content in the leaves of *S. salsa* treated with NaCl (Figure 6). The high accumulation of protine plays an important role in the process of osmotic adjustment, protection of

membrane system stability and removal of reactive oxygen species (ROS) ^[11]. The membrane system of *S. salsa* was not damaged when treated with 200 mM NaCl, the cell membrane system was better than the control, the relative conductivity in leaves of *S. salsa* decreased when compared with control plants (Figure 4). At the same time, the MDA content in the leaves of *S. salsa* treated with 200 mM NaCl was significantly decreased when compared with the control (Figure 3), but increased in 400 mM NaCl treated-plants. Under NaCl treatment, the significant increase of the soluble sugar and proline content was beneficial to the osmotic adjustment of in *S. salsa*, to adapt to the low external water potential in growth medium, and to ensure to absorb the nutrient ions that needed for normal growth and development.

Vegetative growth can provide basic conditions for the reproductive growth of plants. The plant height and branch number of *S. salsa* were significantly increased in 200 mM NaCl-treated plants when compared with control plants (Figure 1). The increase in the plant height and branch number of *S. salsa* treated with 200 mM NaCl will undoubtedly lay a good foundation for the reproductive growth. But the growth promotion effect of the higher concentration (400 mM) of NaCl was not significant. It indicated that a certain concentration of NaCl could significantly increase the vegetative growth process of halophyte *S. salsa*, such as 200 mM. While a lower or higher concentration of NaCl may inhibit the growth of *S. salsa*, the plant height and weight was lower than plants from 200 mM NaCl treatment.

In conclusion, 200 mM NaCl, supplied from seed sowing to the end of vegetative growth, significantly increased the plant growth of halophyte *S. salsa*, and the enhanced growth indicators was associated with the ability of antioxidant and osmotic adjustment.

Acknowledgments

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