Influence of salt stress on ecophysiological parameters of *Periploca sepium* Bunge

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ABSTRACT

An experiment was carried out to investigate the effect of salt stress on *Periploca sepium* Bunge seedlings using three levels of salinity, 50 mmol/L, 100 mmol/L, and 200 mmol/L sodium chloride (NaCl) solution. The results showed that growth parameters and net photosynthetic rate (P_n), stomatal conductance (G_s) of *Periploca sepium* Bunge were enhanced under low salinity levels (50 mmol/L NaCl), which reduced strongly with increasing salinity levels. Under 100 mmol/L NaCl and 200 mmol/L NaCl stress, the decline of P_n was mainly caused by non-stomatal factors. The water use efficiency (WUE), apparent light use efficiency (LUE), carboxylation efficiency (CUE) were enhanced under low salinity levels (50 mmol/L NaCl), the maximum value of WUE was observed at 100 mmol/L NaCl, the minimum value of WUE was observed at 200 mmol/L NaCl, the LUE, CUE were reduced by 52% and 47%, at 200 mmol/L NaCl, respectively, compared to control. Activities of the antioxidative enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were enhanced by low salinity treatment (50 mmol/L NaCl), but CAT activity decreased at 200 mmol/L NaCl stress. Malondialdehyde (MDA) was non-significant compared to the control under low salinity levels (50 mmol/L NaCl), the maximum value was observed at 200 mmol/L NaCl. These results suggest a possibility to improve saline soil utilization of *Periploca sepium* Bunge in Yellow River Delta region.

Keywords: salinity; growth; gas exchange; protective enzyme; lipid peroxidation

High salinity is the most widespread abiotic stress and constitutes the most stringent factor in limiting plant distribution and productivity (Yildirim et al. 2009, Qin et al. 2010). Salt stress has various effects on plant ecophysiological processes such as changes in plant growth (Maeda and Nakazawa 2008), protective enzyme activities (Wu et al. 2010), properties of photosynthesis (Ashraf and Shahbaz 2003, Kao et al. 2003), mineral distribution (Yang et al. 2008a), membrane permeability (Dogan et al. 2010), and so on.

The Yellow River Delta differs in many respects from other major deltaic systems of the world. The tremendous annual sediment load combined with many dramatic changes in the course of the river resulted in a complex and episodic deltaic history (Bornhold et al. 1986). It is the only large delta in China to undergo extensive development which is characterized by extensive coverage of saline soils (Fang et al. 2005). Salt stress is a major abiotic stress problem in the region. Utilization of salt-tolerant plants is expected to be an effective method to improve saline soil. Hence, better understanding of the mechanisms that enable plants to adapt to salt stress is necessary for exploiting saline soil (Patel and Pandey 2008).

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Periploca sepium Bunge is a woody climbing vine belonging to the family Asclepiadaceae. It is widely distributed in northeastern area, northern area, northwestern area and southwestern area of China. The root bark of *Periploca sepium* Bunge has been used as a traditional Chinese herbal medicine for more than two thousand years in China (Ma et al. 2008). To our knowledge, no information is available on salt tolerance for *Periploca sepium* Bunge, thus it is necessary to study its mechanisms. In this research, the effect of sodium chloride on growth, photosynthetic gas exchange, and protective enzyme activities of *Periploca sepium* Bunge were studied so as to provide some fundamental bases for vegetation restoration in yellow river delta region.

MATERIALS AND METHODS

Plant materials. The seeds of *Periploca sepium* Bunge were originally collected from the National Nature Reserve of Shell Islands and Wetlands in Binzhou, P.R. China. The experiment was conducted in a greenhouse supplied with natural sunlight in the Shandong Provincial Key Laboratory of Eco-environmental Science for Yellow River Delta, Binzhou University, Binzhou, P.R. China from March to June 2008. The greenhouse temperatures were from 28°C to 12°C, relative humidity ranged from 35% to 65%. The experiment was set as four treatments including control, each treatment with three replications. The pots of 15 cm diameter lined with polythene sheets were filled with 2.0 kg of well washed pure shell sand. Thirty seeds were sown in each pot. At 15 days after germination, plants were thinned to 6 plants per pot. Full Hoagland's nutrient solution was applied to each pot every week before salt treatment.

Stress treatment. The salt stress was applied on the 30th day after germination. *Periploca sepium* Bunge seedlings uniform in size, were subjected to salt stress by adding NaCl solutions of four concentrations, 0 (control), 50, 100, and 200 mmol/L NaCl. The final salt concentration was progressively adjusted with increasing NaCl solutions (50 mmol/L per day). At the same time, the plants were irrigated with fresh water every three days to maintain sand water.

Indices measurement. Leaf gas exchange measurements were conducted on three to five medial leaves for each treatment on 61st day after salt treatment. Net photosynthetic rate (P_n, μmol/m^2/s) transpiration rate (T_r, mmol/m^2/s), stomatal conductance (G_s, mmol/m^2/s), intercellular CO_2 concentration (C_i, μmol/mol) and environmental factors such as photosynthetically active radiation (PAR, μmol/m^2/s), atmospheric CO_2 concentrations (Ca, μmol/mol), temperature (Ta, °C) and atmospheric humidity (RH, %) et al. were measured simultaneously with an LI-6400 Portable Photosynthesis System in the morning between 9:00 a.m. and 10:00 a.m. In the native environment, with the following specifications and adjustments: the photosynthetic active radiation 1000.8 ± 9.2 mmol/m^2/s, ambient CO_2 concentration 390.5 ± 5.2 μmol/mol, air temperature 24.7 ± 1.6°C, and relative humidity 32.4 ± 3.6%. The following indicators were calculated: stomatal conductance limitation (Ls) = 1 – Ci/Ca (Berry and Downton 1982), water use efficiency (WUE, μmol/mmol = P_n/T_r (Nijs et al. 1997), apparent light use efficiency (LUE) = P_n/PAR (Long et al. 1993), carboxylation efficiency (CUE, mol/m^2/s) = P_n/C_i (He and Xu 2000).

On the 62nd day after salt treatment, extraction for determination of SOD, POD, CAT activities were prepared from 0.3 g of tissues homogenized under ice-cold conditions in 3 ml of extraction buffer, containing 50 mmol/L phosphate buffer (pH 7.4) at 4°C. The homogenates were centrifuged at 12 000 g for 20 min and the supernatant fraction was used for the assays. The activities of SOD, POD, CAT were measured by the standard analytical methods of Groppa et al. (2003), and Trevor et al. (1994). Lipid peroxidation in the leaf tissue was measured as formation of malondialdehyde (MDA, a product of lipid peroxidation) determined by thiobarbituric acid reaction using the method of Selvakumar (2008).

Height growth and biomass of the seedlings were measured on the 63rd day after salt treatment. All plants at each treatment were taken for biomass measurements and were divided into roots, leaves and stems. Then dry weight (dried at 105°C for 20 min and then dried at 70°C for 24 h) were measured on each root, leaf and stem sample.

Statistical data analysis. Statistical analysis was performed using the software SPSS 13.0. The results were subjected to a one-way ANOVA using the Tukey test to check for significant differences between means (P < 0.05). Error bars in figures represent standard deviation.

RESULTS AND DISCUSSION

Effect of salt stress on seedling growth. Seedling growth is normally limited by increasing concentration of NaCl (Sreenivasulu et al. 2000). In our study, with increasing salinity levels, the plant height, root length, root fresh mass, root dry mass, stem fresh mass, stem dry mass, leaf dry mass of *Periploca sepium* Bunge were enhanced under low salinity levels (50 mmol/L NaCl), which reduced strongly with increasing salinity levels (Figure 1). Maximum reduction of all growth parameters were observed at 200 mmol/L NaCl. The plant height, root length, root fresh mass, root dry mass, stem fresh mass, stem dry mass, leaf fresh mass, dry mass were reduced by 40%, 47%, 65%, 58%, 60%, 57%, 54% and 38%, respectively, compared to control. The present results were in line with those of Parveen and Farrukh (2009), who reported decline in fresh and dry weights of shoots under high salinity stress. The stem dry mass, leaf fresh mass, dry mass were non-significant at 100 mmol/L NaCl compared to control, whereas they were significant at 50 mmol/L NaCl and at 200 mmol/L NaCl.

Effect of salt stress on seedlings photosynthetic gas exchange. Salinity reduces P_n and G_s in most plant species (Ashraf and Shahbaz 2003, Koyro 2006). In the present study, the P_n and G_s were enhanced under low salinity levels (50 mmol/L NaCl), and were reduced by 15%, 27%, at 150 mmol/L NaCl, and 50%, 47%, at 200 mmol/L NaCl, respectively, compared to control with increasing salinity levels (Figure 2). The changes are similar to the results of Yang et al. (2008b). Effect of salinity was non-significant on T_r at 50 mmol/L NaCl, but the T_r declined by 41%, 52%, at 150 mmol/L NaCl and 200 mmol/L NaCl, respectively, compared to control. With increasing salinity levels, the C_i reduced by 9%, 22% and 5% compared to control, but the L_s was enhanced by 10%, 28% and 4%. According to Farquhar and Sharkey's views (1984), at 100 mmol/L NaCl and 200 mmol/L NaCl stress, the decline of net photosynthetic rate of *Periploca sepium* Bunge was mainly caused by non-stomatal factors. The WUE, LUE, CUE were enhanced under low salinity levels (50 mmol/L NaCl) (Figure 2). With increasing salinity levels, the maximum value of WUE was observed at 100 mmol/L NaCl, the minimum value of WUE was observed at

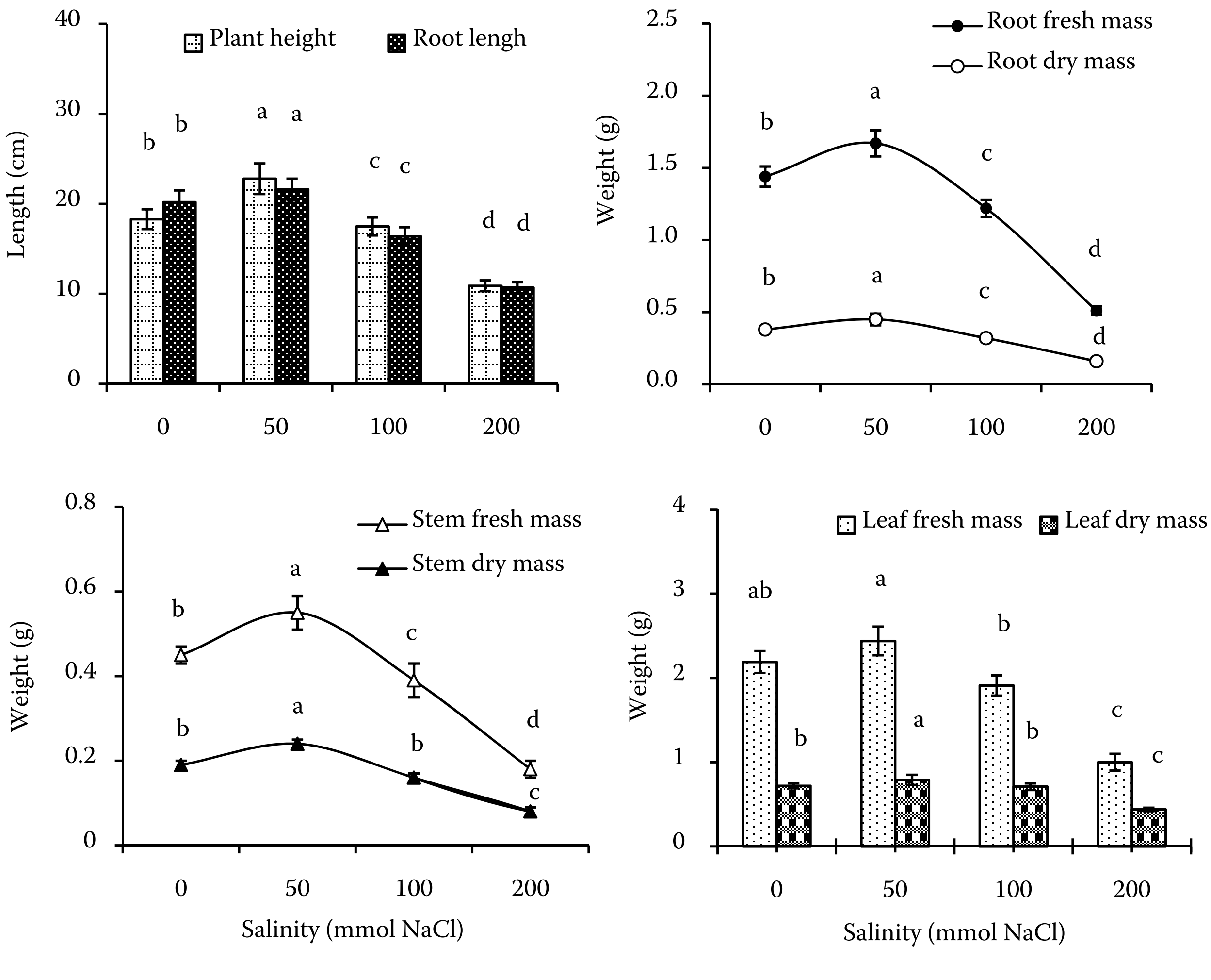

Figure 1. Response of growth of *Periploca sepium* Bunge to salt stress. The different small letters were significantly different at 0.05 according to the Duncan test, the same as followed

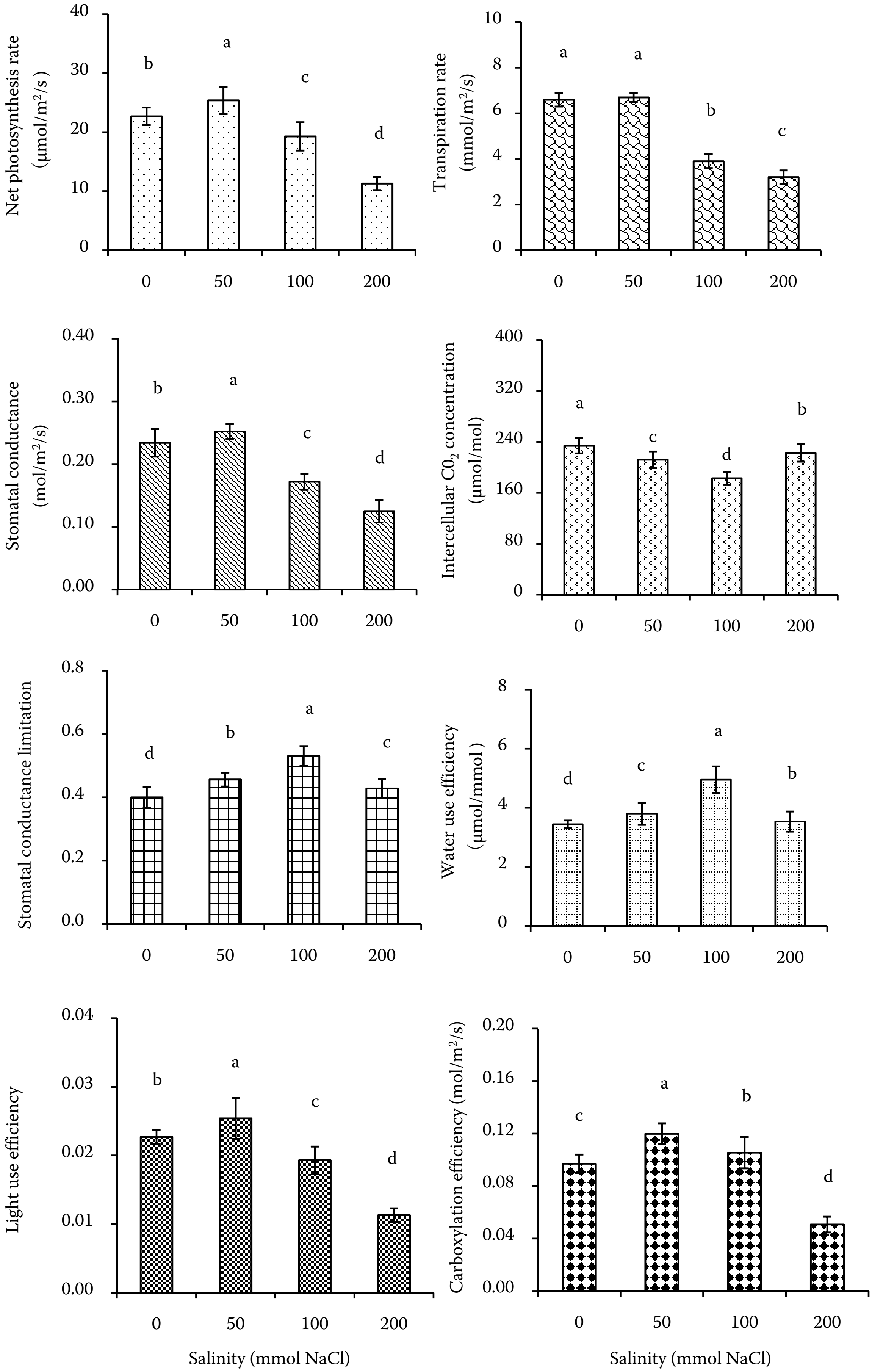

Figure 2. Effect of salt stress on photosynthetic characteristics of *Periploca sepium* Bunge seedlings

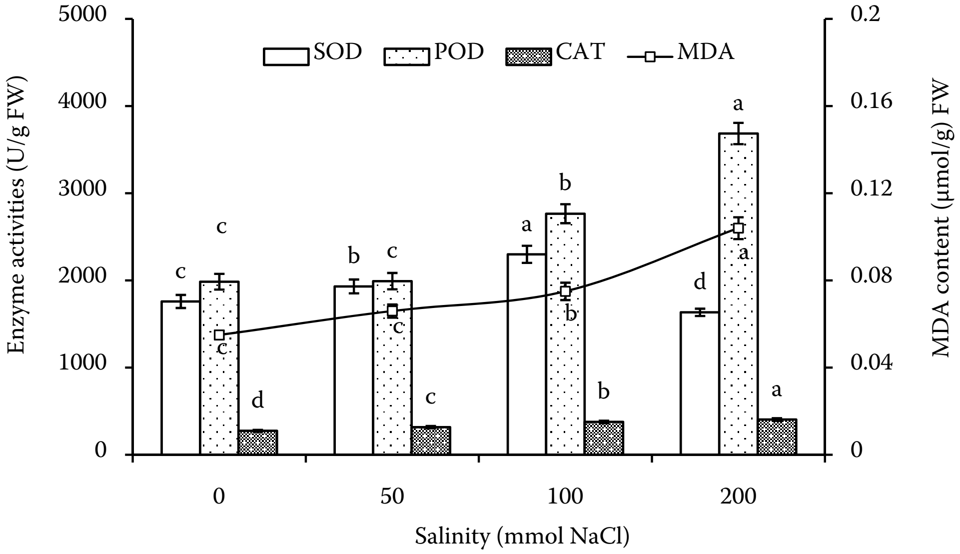

Figure 3. Response of systems of anti-oxidation of *Periploca sepium* Bunge seedlings to salt stress

200 mmol/L NaCl; the LUE reduced by 17% at 150 mmol/L NaCl, 52% at 200 mmol/L NaCl compared to control; the CUE was enhanced by 8% at 150 mmol/L NaCl, while it was reduced by 47% at 200 mmol/L NaCl.

Effect of salt stress on seedlings protective enzyme activities. The level of the antioxidant enzymes SOD, POD, and CAT may determine the sensitivity of plants to lipid peroxidation (Kanazawa et al. 2000). In our study, with increasing salinity levels, activities of the antioxidative enzymes of SOD, POD, and CAT were enhanced by salts treatment of *Periploca sepium* Bunge seedlings, but CAT activity decreased at 200 mmol/L NaCl salt stress (Figure 3), indicating that the ability of these antioxidant enzymes to eliminate ROS is limited. The activity of enzymes of POD and CAT is higher than that of SOD at 200 mmol/L NaCl, which suggests that POD and CAT provide a better defense mechanism against salt stress-induced oxidative damage in *Periploca sepium* Bunge seedlings. The results are similar to Yeonghoo et al. (2004).

Effect of salt stress on seedlings malondialdehyde. The increased accumulation of lipid peroxides is indicative of enhanced production of toxic oxygen (Markovska et al. 2009). In this study, MDA concentration was non-significant compared to the control under low salinity levels (50 mmol/L NaCl), which was enhanced with increasing salinity levels, the maximum value was observed at 200 mmol/L NaCl (Figure 3). The results indicated that salt stress (> 100 mmol/L NaCl) produced superoxide radicals, resulting in increased lipid peroxidative products and oxidative stress in *Periploca sepium* Bunge seedlings.

In conclusion, *Periploca sepium* Bunge possesses moderate salt tolerance capacity, which can help to adapt to 100 mmol/L NaCl level. These results suggest a possibility to improve saline soil by utilizing *Periploca sepium* Bunge in Yellow River Delta region, where salinity is often the common major abiotic stress for plants. This study was done in the laboratory; although it may provide some information, it may not infer the actual field condition (Hameed et al. 2006). Therefore, further study is required to obtain some meaningful conclusions.

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