

Physiological responses to salinity in Silver buffaloberry (*Shepherdia argentea*) introduced to Qinghai high-cold and saline area, China

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Abstract

Since 2002, Silver buffaloberry (*Shepherdia argentea*) has been introduced from North America in order to improve the fragile ecological environment in western China. To elucidate the salt-resistance mechanism of *S. argentea*, we conducted a test with two-year-old seedlings subjected to 0, 200, 400, and 600 mM NaCl solutions for 30 d. The results showed that significant salt-induced suppression of plant fresh mass (FM) and stem height of *S. argentea* seedlings occurred only at the highest salinity level (600 mM). Leaf number, plant dry mass (DM), and chlorophyll (Chl) content declined markedly at both 400 and 600 mM. Leaf area (LA) and leaf water potential (Ψ_w) continuously declined with the increase of salinity. There was also a progressive and evident decrease in net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) with the increase of salinity and time. The correlation analysis indicated that P_N was positively correlated with g_s at all salinity levels while correlated with intercellular CO₂ concentration (C_i) only at moderate salinity levels (<600 mM). Based on the initial slope of the P_N/C_i curves, the estimated carboxylation efficiency (CE) was strongly inhibited at 600 mM. We confirm that *S. argentea* is highly tolerant to salinity. Moreover, our results show that at moderate salinity levels, salt-induced inhibition of photosynthesis is mainly attributed to the stomatal efficient closure predetermined by a low water potential in leaves; while at the high salinity levels, the inhibition is mainly due to the suppression of chloroplast capacity to fix CO₂ caused by the serious decline in both CE and Chl contents.

Additional key words: growth; net photosynthetic rate; non-stomatal limitations; P_N/C_i curves; relative water content; stomatal limitations.

Introduction

Salinity is an increasing environmental problem in the world (Debez *et al.* 2004, Marion *et al.* 2008), and the mechanisms of plant adaptation to stressor action is one of the leading topics of current biological studies (Radyukina *et al.* 2007, Zheng *et al.* 2009). The growth reduction under saline conditions is a consequence of several physiological responses, including modification of ion balance, water status, mineral nutrition, stomatal behavior, and photosynthetic efficiency (Munns 1993). Hamadia *et al.* (2004) and Suárez and Medina (2008)

found that high concentration of salt in the root zone reduces soil water potential and water availability and induces the reduction of water content leading to dehydration at the cellular level and osmotic stress. Ashraf *et al.* (2004) and Chen *et al.* (2009) showed that elevated Na⁺ and Cl⁻ contents in environment affect many indispensable nutrients uptake through competitive interactions and affecting the ion selectivity of membranes. Meanwhile, photosynthesis is the most important process affecting plant growth under saline conditions (Sudhir

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Abbreviations: CE – carboxylation efficiency; Chl – chlorophyll; C_i – intercellular CO₂ concentration; C_{ref} – reference CO₂ concentration; DM – dry mass; E – transpiration rate; FM – fresh mass; g_s – stomatal conductance; LA – leaf area; PPFD – photosynthetic photon flux density; P_N – net photosynthetic rate; RH – relative humidity; RWC – relative water content; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; SLA – specific leaf area; Ψ_w – leaf water potential.

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and Murthy 2004). Salt-induced decline of P_N is attributed to two kinds of factors: stomatal factors and nonstomatal ones. Stomatal factors include the decrease of g_s and the reduction of C_i caused by stomatal closure (Stepień and Klbus 2006, Youssef and Awad 2008). Non-stomatal factors include the decrease in chlorophyll (Chl) content (Ranjbarfordoei *et al.* 2006, Gratani *et al.* 2008), the changes in chloroplast ultrastructure (Bergmann *et al.* 2008), the depression in photosystem activity and electron transport (Bastías *et al.* 2004, Lu *et al.* 2008), and the decline in the activity and content of Rubisco (Mudalige and Longstreth 2006, Lu *et al.* 2009). However, there are still some debates on the relative contribution of stomatal and non-stomatal factors to the decline of photosynthesis under salinity. Despite an increasing number of related studies, the effect of salt stress on photosynthesis still remains poorly understood.

Qinghai Province of China is located in extremely high-cold region of western Loess Plateau, the transition zone between Tibetan Plateau and Loess Plateau, and its climate is the type of the alpine region. According to Cai (2006), Qinghai has one of the highest soil salinization degrees, with the soil salinization area of 15,720 km², 2.3% of the total area in China. Based on the geographic-ecological similarity, one effective approach to improve the fragile ecological environment in western China is to introduce and select shrub species from abroad that have excellent adaptability and characteristics under natural ecological condition (Wang 2006, Cao *et al.* 2009).

Silver buffaloberry (*Shepherdia argentea*) is a member of the Oleaster Family (Elaeagnaceae), native to Western and Central North American Great Plains (Elias

1980). *S. argentea* has edible berries traditionally used by native North Americans, and its compositional elements, biochemical activities and metabolic activities have been extensively studied (Bekker and Glushenkova 2001, Kraft *et al.* 2008). *S. argentea* adapts well to disturbed or degraded regions and it can be used for multiple shelterbelts, erosion control, wildlife habitat and mine spoil rehabilitation (Borland 1994). Since 2002, *S. argentea* has been introduced from North America to improve the ecological environment of Qinghai Province (Wang 2006, Qin *et al.* 2009a). Drought stress studies conducted by Geng and Wang (2007) show that the soil-water compensation percentage for hydration of *S. argentea* is 4.4% and the optimum soil moisture to maintain the maximum P_N is 18.5% with the highest water use efficiency (WUE) compared with *Zygophyllum xanthoxylum* and *Atraphaxis mandshurica*. Yan *et al.* (2007) found that two-year-old *S. argentea* seedlings have a high survival rate (92.9%) after transplanting and three-year-old seedlings begin flowering and fruiting. However, studies on physiological responses of *S. argentea* to salt stress are very limited but are urgently needed for a wider planting of that species.

The aim of this study was to analyze changes of water potential, growth, leaf gas exchange, and Chl content in *S. argentea* under four salinity treatments, from fresh water to hypersalinity. Our results would advance our understanding about the salt-tolerance mechanism of *S. argentea* and help identify the ability of *S. argentea* to cope with the salinity effect in high-cold and saline areas in Qinghai.

Materials and methods

Plant material and stress treatments: Seedlings of *S. argentea* were obtained from the experimental base (latitude 36°39' N, longitude 101°46' E and altitude 2 309 m) of Research Institute of Forestry, Qinghai Academy of Agriculture and Forestry, China. In April 2007, the seedlings were planted in plastic pots (one per pot), filled with 11 kg native chernozem soil (pH 7.15, organic matter 41.8 g kg⁻¹, available P 5.31 mg kg⁻¹, available K 310 mg kg⁻¹ and total N 198 mg kg⁻¹) and irrigated every 3 d, in order to maintain soil water at the field capacity.

In July 2008, the experiment was performed for 30 d under native condition in the open air. In the experiment, 20 two-year-old seedlings, uniform in size, were subjected to salt stress by adding NaCl solutions of four concentrations: 0 (control), 200, 400, and 600 mM with the calculated amount of NaCl dissolved in water. The final salt concentration was progressively adjusted with the increasing NaCl solutions (50 mM step per day). Meanwhile, the plants were irrigated with fresh water every three days to maintain soil water at the field capacity and avoid drought stress. Five replicates of each

treatment were used to measure the growth, leaf water relations and chlorophyll content of *S. argentea* at the end of the experiment before harvest, while three replicates were used to measure the characteristics of leaf gas exchange at 5, 10, 15, 20, 25, and 30 d after the salt treatment.

Growth parameters: After LA was measured, FM and DM of leaves, stems and roots of each plant were determined with an automatic leaf area meter *LI-3000* (*LICOR*, Lincoln, NE, USA). DM was determined after oven-drying at 80°C for 48 h. The root/shoot ratio and specific leaf area (SLA) were estimated based on the DM of different plant organs. Besides plant FM and DM, the stem height and leaf number were measured at the end of experiment to compare with the initial biomass of seedlings that were randomly selected with uniform size (plant FM 27.77 ± 0.70 g, DM 10.63 ± 0.68 g, stem height 44.87 ± 1.28 cm and leaf number 46 ± 6).

Leaf water relations: Predawn leaf water potential (Ψ_w) was measured on medial leaves of each plant by *HR-33T*

Dew Point Mikrovolt-meter (*WESCOR*, Logan, UT, USA). Relative water content (RWC) was determined on medial leaves of each plant and calculated as follows: $RWC [\%] = 100 \times (FM - DM) / (TM - DM)$, where FM, DM, and TM denote leaf fresh, dry, and turgid mass, respectively (Zhang *et al.* 2004, Meloni *et al.* 2008).

Gas exchange characteristics: Measurements of P_N , E , g_s and C_i were carried out on the third fully expanded leaf (from the top) of each plant (three plants for each treatment), with a portable photosynthesis system *LI-6400* (*LICOR*, Lincoln, NE, USA). All measurements were performed between 9:00–11:00 h in the native environment (Qin *et al.* 2009b), with the following specifications and adjustments: reference CO_2 concentration (C_{ref}) $391.3 \pm 6.7 \mu\text{mol mol}^{-1}$, air temperature $27.9 \pm 2.9^\circ\text{C}$, relative humidity (RH) $30.7 \pm 6.0\%$, ambient pressure $72.8 \pm 0.5 \text{ kPa}$, photosynthetic photon flux density (PPFD) at the leaf surface $1502 \pm 123 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The C_{ref} sequence for generating P_N - C_i curves was set as 400, 200, 150, 100, 50, 400, 400, 600, 800; and

$1,000 \mu\text{mol mol}^{-1}$ with the *LI-6400* CO_2 injection system at a PPFD of $1,500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Ball and Farquhar 1984, Pandurangam *et al.* 2006, Kakani *et al.* 2008).

Chl content: After harvest at the end of experiment, Chl was extracted from dry leaves with 80% acetone and the absorbance values of supernatant were determined at 645 (A_{645}) and 663 (A_{663}) nm, respectively, with a spectrophotometer (*SP-756*, spectral slit-width 2 nm, Beijing, China). Chl *a*, Chl *b*, and Chl (*a+b*) were calculated as described by Arnon (1949).

Statistical analysis: One-way *ANOVA* was performed on each measurement. Multiple comparisons of means were performed with the *Tukey's* honestly significant differences (HSD) procedure. Relationships between P_N and g_s or C_i were examined with linear regression models. $P < 0.05$ was considered statistically significant. All the statistical analyses were performed in *SPSS* software (version 15.0, *SPSS*, Chicago, IL, USA). Means and standard errors (SE) are shown in figures and tables.

Results

Growth: In this study, we observed that *S. argentea* seedlings were able to survive for at least 30 d even at the highest salinity level (600 mM NaCl) and they showed obvious salt-induced inhibition of plant FM and stem height only at this salinity level (Table 1). Plant DM and leaf number of *S. argentea* seedlings significantly declined at 400 and 600 mM NaCl, whereas LA was significantly reduced at all salinity levels (Table 1). Moreover, compared with the non-saline controls, the FM, DM, stem height, leaf number, and LA of the plants treated with 600 mM salt were reduced by 39.6%, 45.1%, 22.0%, 34.3%, and 61.4%, respectively. Compared with the controls, the root/shoot ratio of the plants treated with 400 and 600 mM was significantly increased (Table 1).

Leaf water relations and SLA: Due to salt stress, we observed a significant and continuous decrease in Ψ_w in the leaves of *S. argentea* seedlings (Table 2). The reduction was highly significant even at the lower salinity level (200 mM NaCl). Compared with the controls, Ψ_w was reduced by 11.8%, 24.9%, and 37.0% at 200, 400, and

600 mM, respectively. As shown in Table 2, salinity significantly reduced RWC in the leaves of plants treated with 400 and 600 mM. Compared with the controls, RWC was reduced by 10.2% and 15.5% in 400 and 600 mM salt-treated plants, respectively. SLA in leaves of 600 mM salt-treated plants was significantly decreased relative to the controls, but there was no obvious difference among the salt treatments of 0, 200, and 400 mM (Table 2).

Chl content: Plants treated with 200 mM maintained high values of Chl content including Chl *a*, *b*, and (*a+b*) (only 9.0%, 4.5%, and 8.3% lower than the non-saline controls, respectively), but the decrease rates of these parameters became greater at the higher salinity (Table 2). Maximum reductions in Chl *a*, *b*, and (*a+b*) (39.2%, 19.2%, and 35.9%, respectively) were at the highest salinity level (600 mM). In general, Chl *b* was less sensitive or better protected against salt stress than Chl *a* under the same salinity level, which led to an obvious drop in the Chl *a/b* ratio (data not shown).

Table 1. Plant fresh mass (FM) and dry mass (DM), stem height, root/shoot ratio, leaf number and leaf area (LA) of *S. argentea* seedlings subjected to various salinity for 30 d. Means \pm SE ($n = 5$). Different letters within the same column indicate significant differences ($P < 0.05$, *Tukey's* HSD).

Salinity [mM]	Plant FM [g]	Plant DM [g]	Stem height [cm]	Root/shoot ratio	Leaf number	LA [m ²]
0(Control)	57.15 \pm 1.36 ^a	23.59 \pm 0.87 ^a	63.68 \pm 1.34 ^a	0.52 \pm 0.02 ^c	230 \pm 6 ^a	0.117 \pm 0.005 ^a
200	57.12 \pm 2.94 ^a	23.17 \pm 1.17 ^a	61.14 \pm 1.29 ^a	0.53 \pm 0.02 ^{bc}	207 \pm 4 ^{ab}	0.090 \pm 0.005 ^b
400	50.56 \pm 1.22 ^a	19.29 \pm 0.55 ^b	59.66 \pm 1.76 ^a	0.59 \pm 0.01 ^{ab}	201 \pm 8 ^b	0.072 \pm 0.006 ^b
600	34.55 \pm 1.70 ^b	12.96 \pm 0.52 ^c	49.69 \pm 0.81 ^b	0.61 \pm 0.01 ^a	151 \pm 8 ^c	0.045 \pm 0.005 ^c

Table 2. Leaf water potential (Ψ_w), relative water content (RWC), specific leaf area (SLA) and chlorophyll (Chl) *a*, *b*, and (*a*+*b*) in the leaves of *S. argentea* seedlings subjected to varying salinity for 30 d. Means \pm SE ($n = 5$). Different letters within the same column indicate significant differences ($P < 0.05$, Tukey's HSD).

Salinity [mM]	Ψ_w [MPa]	RWC [%]	SLA [g m^{-2}]	Chl <i>a</i> [$\text{mg g}^{-1}(\text{DM})$]	Chl <i>b</i> [$\text{mg g}^{-1}(\text{DM})$]	Chl (<i>a</i> + <i>b</i>) [$\text{mg g}^{-1}(\text{DM})$]
0(Control)	-1.89 ± 0.03^a	61.61 ± 0.94^a	71.95 ± 1.05^a	2.25 ± 0.06^a	0.43 ± 0.01^a	2.68 ± 0.06^a
200	-2.12 ± 0.03^b	59.41 ± 0.87^a	70.92 ± 0.70^a	2.04 ± 0.05^a	0.41 ± 0.01^{ab}	2.46 ± 0.05^a
400	-2.38 ± 0.02^c	55.34 ± 0.83^b	69.76 ± 0.68^{ab}	1.72 ± 0.07^b	0.38 ± 0.03^{ab}	2.10 ± 0.10^b
600	-2.59 ± 0.03^d	52.07 ± 0.94^b	67.10 ± 0.47^b	1.37 ± 0.04^c	0.35 ± 0.01^b	1.72 ± 0.04^c

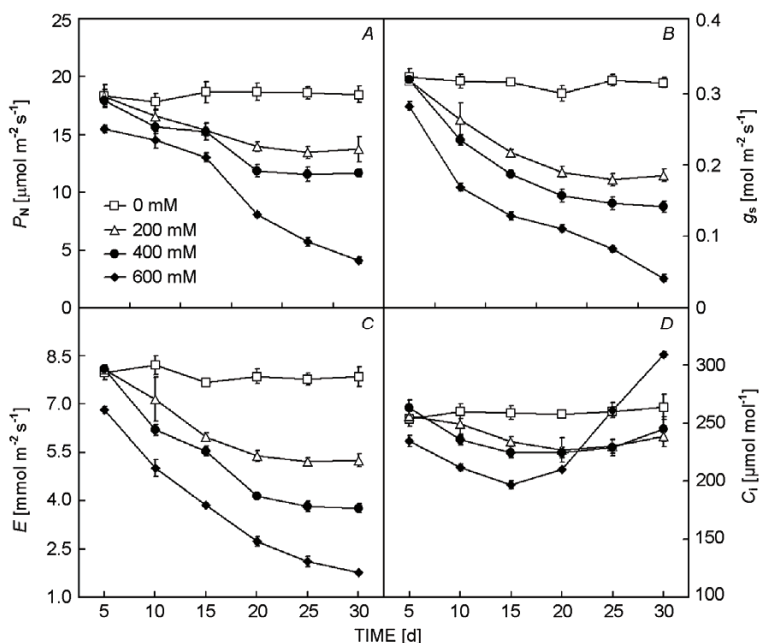


Fig. 1. Changes in (A) net photosynthetic rate (P_N), (B) stomatal conductance (g_s), (C) transpiration rate (E) and (D) intercellular CO_2 concentration (C_i) with time in the leaves of *S. argentea* seedlings subjected to all salinity levels. Means \pm SE ($n = 3$, SE is not shown when smaller than symbol).

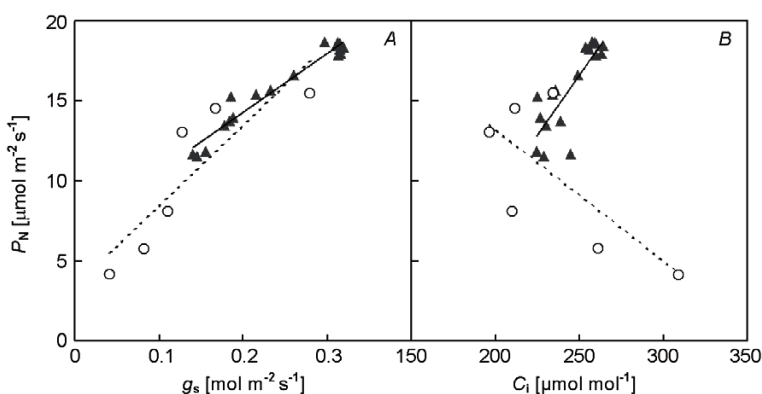


Fig. 2. Relationships between (A) net photosynthetic rate (P_N) and stomatal conductance (g_s) or (B) P_N and intercellular CO_2 concentration (C_i) in the leaves of *S. argentea* seedlings grown under moderate salinity levels (<600 mM: \blacktriangle and \blacktriangleright) or highest salinity level (600 mM: \circ and $\circ\circ$), respectively.

Photosynthesis: With the increase of salinity and time, there was a progressive and evident decrease in P_N in leaves of *S. argentea* seedlings (Fig. 1A). During the whole experiment, P_N in the leaves of plants treated with 0 mM NaCl kept relatively constant, and it was about $18.44 \mu\text{mol m}^{-2} \text{s}^{-1}$. On 20 d of 200 and 400 mM salinity, P_N was decreased to 13.73 and $11.69 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, and it was changed little further. In contrast, P_N in the leaves of plants with the treatment of 600 mM NaCl showed a sharp reduction and was reduced to $4.14 \mu\text{mol m}^{-2} \text{s}^{-1}$ on 30 d (Fig. 1A). Meanwhile, g_s values

showed a similar trend with P_N under salt stress (Fig. 1B) and the declines of g_s also resulted in the corresponding reduction in E (Fig. 1C). Nevertheless, the variation trend of C_i first dropped and then sharply increased with time at 600 mM NaCl, while C_i was slightly decreased when salinity < 600 mM. (Fig. 1D).

The correlation analysis between P_N and g_s (Fig. 2A) indicated that P_N was positively correlated with g_s when plants were treated with all salinity levels (from 0 to 400 mM NaCl: $y = 36.95x + 6.89$, $r^2 = 0.95$, $p < 0.01$; 600 mM NaCl: $y = 50.80x + 3.32$, $r^2 = 0.76$, $p < 0.01$).

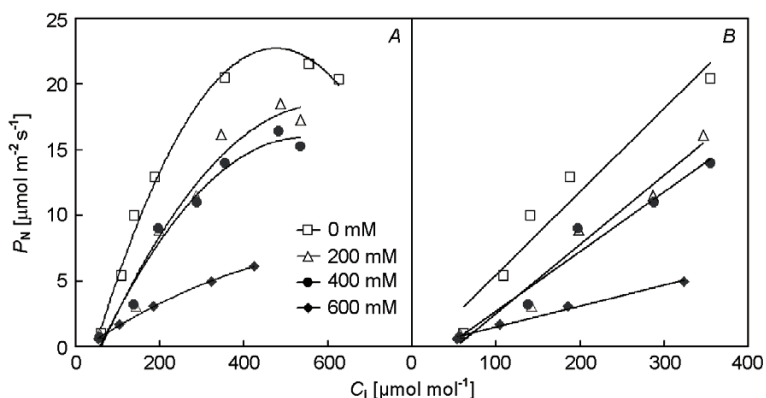


Fig. 3. Regression of (A) CO_2 response curves of photosynthesis (P_N/C_i curves) and (B) the initial slope of P_N/C_i curves in the leaves of *S. argentea* seedlings subjected to all salinity levels.

However, as shown in Fig. 2B, there was an obvious positive correlation between P_N and C_i when plants were treated with moderate salinity levels (0 to 400 mM NaCl: $y = 0.15x - 21.08$, $r^2 = 0.69$, $p < 0.05$), but there was no significant relationship between P_N and C_i at 600 mM level ($y = -0.08x + 29.69$, $r^2 = 0.52$, $p > 0.05$).

CO_2 -response curves of photosynthesis (P_N/C_i curves) were made to illustrate the effect of salt stress on carbon metabolism (Fig. 3). We found that P_N was rapidly increased with the increasing C_i under 0, 200, and 400 mM

salinity treatments but much less increment of P_N under 600 mM (Fig. 3A). The carboxylation efficiency (CE) was estimated as the initial slope of the P_N/C_i curves with linear regression models (Fig. 3B) as follows:

$$y = 0.0634x - 0.8393, r^2 = 0.94, p < 0.01 \text{ (0 mM NaCl);}$$

$$y = 0.0527x - 2.7379, r^2 = 0.96, p < 0.01 \text{ (200 mM);}$$

$$y = 0.0455x - 1.835, r^2 = 0.96, p < 0.01 \text{ (400 mM) and}$$

$$y = 0.0159x - 0.0525, r^2 = 0.99, p < 0.01 \text{ (600 mM).}$$

In particular, compared with the value of the controls ($0.0634 \text{ mol m}^{-2} \text{ s}^{-1}$), CE in the leaves of 600 mM salt-treated plants ($0.0159 \text{ mol m}^{-2} \text{ s}^{-1}$) was greatly inhibited.

Discussion

Our study provided some insights into integrated mechanisms of acclimation to salinity operating in *S. argentea* seedlings, and described the physiological manifestations of control systems at both whole plant and leaf levels. In this study, our results showed that *S. argentea* seedlings could survive at the highest salinity level (600 mM) for at least 30 d (Table 1), indicating that *S. argentea* is highly tolerant to salinity.

Biomass changes are widely used parameters to determine the plant sensitivity to salinity (Bastías *et al.* 2004, Gama *et al.* 2007). Significant salt-induced suppression of plant FM and stem height of *S. argentea* seedlings were observed only at the highest salinity level (600 mM NaCl), while plant DM and leaf number declined markedly at both 400 and 600 mM NaCl (Table 1). However, LA was significantly reduced at all the salinity levels, and the maximum reduction was at 600 mM NaCl (61.4% of the non-saline controls). All these results indicate that salinity affects the growth of *S. argentea* seedlings mainly through its adverse effects on leaf development rather than plant mass or stem height.

The change of root/shoot ratio reflects the adaptation strategies in biomass distribution pattern under stress (Zhang *et al.* 2004). In our study, since there was a greater inhibition on shoot growth than on root under saline conditions (data not shown), we observed the consequent increase of root/shoot ratio in plants treated with 400 and 600 mM at the end of experiment (Table 1).

This finding is consistent with other studies (Massai *et al.* 2004, Meloni *et al.* 2008), and strongly supports the idea of Cheeseman (1988) and Iqbal (2005) that the plant spends much photosynthetic energy on root production under salinity in searching water and/or reducing water loss in order to maintain a relatively high water relation. Several studies have suggested that the increase in root/shoot ratio due to water shortage is not only an indicator of stress tolerance, but also a good morpho-physiological marker for stress tolerance (Meloni *et al.* 2008).

Water status is highly sensitive to salinity and therefore it is dominant in determining the plant responses to stress (Patel and Pandey 2007). In our study, Ψ_w was significantly reduced with the increase of salinity level (Table 2). This result suggests that *S. argentea* seedlings can adjust water potential to more negative levels as salinity increased, which is a common reaction to salinity stress similar to those in other species (Massai *et al.* 2004, Ruggiero *et al.* 2004). In addition, salinity resulted in dehydration at the cellular level, and thus significantly reduced the RWC in the leaves of *S. argentea* (Table 2). Hladnik *et al.* (2009) reported that a fast stomatal reaction of water-deprived plants is predetermined by a low xylem water potential of the leaf. According to our results, the salinity-level increase progressively decreased g_s in the leaves of *S. argentea* seedlings (Fig. 1B). Stomatal closure is believed to be an effective mechanism for efficiently utilizing water under salinity and limiting the uptake of harmful salt ions

(Hasegawa *et al.* 2000). Moradi *et al.* (2007) found that a large reduction of g_s is considered as a major way to decrease water loss from the leaves, which can be considered as an adaptive character to salt tolerance. Thus, we concluded that the salt-induced reduction of Ψ_w and RWC in the leaves of *S. argentea* seedlings would result in stomatal closure efficiently, which in turn could be reflected by the reduction in g_s .

P_N was positively correlated with g_s under all salinity levels (Fig. 2A), which might explain why salt-induced reduction in g_s also caused a simultaneous decrease in both P_N and E (Fig. 1A,C). Such an inhibitory effect of salinity on photosynthesis has also been reported in other plants (Sharma *et al.* 2005, Sobrado 2005, Yang *et al.* 2009b). However, there was a strong positive correlation between P_N and C_i only at moderate salinity levels (0 to 400 mM) but not at 600 mM level (Fig. 2B) due to sharp increase in C_i (Fig. 1D). Similar trends of C_i under salt stress have been observed by Musyimi (2007), Lu *et al.* (2009) and Yang *et al.* (2009a). Moreover, Moutinho-Pereira *et al.* (2004) proposed that the C_i values should be treated with some caution, because of the non-uniform aperture of stomata over the surface of a vine leaf (patchiness) when environmental stresses (water stress, salinity, low humidity) are present. Hence, we made the CO₂-response curves of photosynthesis (Fig. 3, P_N/C_i curves) to analyze the effect of salinity on carbon metabolism according to Ball and Farquhar (1984). We found that the increment of P_N under 600 mM salinity level was much smaller than that when salinity <600 mM (Fig. 3A). Moreover, CE in leaves of 600 mM salt-treated plants (reflected by the initial slope of the P_N/C_i curves) was significantly inhibited compared with other saline conditions and the controls (Fig. 3B). All these results suggest that stomatal and non-stomatal limitations make obviously distinct contributions to the decline of photosynthesis with the increase of salinity. Under moderate salinity levels (<600 mM), the simultaneous reduction of g_s , C_i and P_N and the significantly positive correlation between P_N and g_s or C_i both indicate that the decline of

photosynthesis under salinity is mainly caused by stomatal limitations (restricted CO₂ availability due to stomatal closure). In contrast, at the highest salinity level (600 mM), the rapid increase of C_i , associated with serious inhibition in CE suggests that non-stomatal limitations (chloroplast capacity to fix CO₂) at the biochemical level have prevailed over stomatal or mesophyll limitations.

Our results showed that the leaves of salt-treated plants had lower Chl content at the higher salinity levels (Table 2). Youssef and Awad (2008) observed that salinity reduces assimilation rate predominantly *via* the reduction in Chl *a* content. Other studies show that environmental stress usually suppresses photosynthesis by inhibiting electron transport through photosystem II (Musyimi 2007, Pospíšil *et al.* 2007, Nauš *et al.* 2008), suggesting that a reduction in leaf Chl content under salinity is due to the destruction of Chl pigments, the instability of the pigment protein complex and structural modification of photosystem II antenna. Maximum reductions in Chl content was observed at 600 mM salinity level, which also supported the notion that nonstomatal limitations greatly inhibit photosynthesis under severe saline conditions.

In summary, *S. argentea* seedlings can maintain relatively high biomass under moderate salinity levels (< 600 mM), and the adverse effect of salinity on the plant growth is mainly through leaf development. Under moderate salinity levels, salt-induced inhibition of photosynthesis in *S. argentea* seedlings is mainly attributed to the stomatal efficient closure predetermined by a low water potential in leaves; however, under high salinity levels (*e.g.*, 600 mM), it is mainly attributed to the suppression of chloroplast capacity to fix CO₂ caused by the serious decline in both CE and Chl contents. Overall, *S. argentea* seedlings show high salt tolerance and therefore they can be widely cultivated in salt-affected areas, especially in saline and high-cold areas of Qinghai Province.

References

- Arnon, D.I.: Copper enzymes in isolated chloroplasts – polyphenoloxidase in *Beta vulgaris*. – *Plant Physiol.* **24**: 1-15, 1949.
- Ashraf, M., Mukhtar, N., Rehman, S., Rha, E.S.: Salt-induced changes in photosynthetic activity and growth in a potential medicinal plant Bishop's weed (*Ammi majus* L.). – *Photosynthetica* **42**: 543-550, 2004.
- Ball, M.C., Farquhar, G.D.: Photosynthetic and stomatal responses of the Grey Mangrove, *Avicennia marina*, to transient salinity conditions. – *Plant Physiol.* **74**: 7-11, 1984.
- Bastías, E.I., González-Moro, M.B., González-Murua, C.: *Zea mays* L. amylacea from the Lluta Valley (Arica-Chile) tolerates salinity stress when high levels of boron are available. – *Plant Soil* **267**: 73-84, 2004.
- Bekker, N.P., Glushenkova, A.I.: Components of certain species of the *Elaeagnaceae* family. – *Chem. Nat. Comp.* **37**: 97-116, 2001.
- Bergmann, I., Geiß-Brunschweiler, U., Hagemann, M., Schoor, A.: Salinity tolerance of the chlorophyll *b*-synthesizing cyanobacterium *Prochlorothrix hollandica* strain SAG 10.89. – *Microb. Ecol.* **55**: 685-696, 2008.
- Borland, J.: *Shepherdia rotundifolia*. – *Amer. Nurseryman* **179**: 106, 1994.
- Cai, D.: [The present condition cause of dysfunction and control measures of the alpine grassland ecosystem in Qinghai province.] – *Pratacultural Sci.* **23**: 7-11, 2006. [In Chin.]
- Cao, S.X., Chen, L., Yu, X.X.: Impact of China's Grain for Green Project on the landscape of vulnerable arid and semi-arid agricultural regions: a case study in northern Shaanxi Province. – *J. Appl. Ecol.* **46**: 536-543, 2009.

- Cheeseman, J.M.: Mechanisms of salinity tolerance in plants. – *Plant Physiol.* **87**: 547-550, 1988.
- Chen, W., Zhou, D., Guo, W., Xu, H., Shi, D., Yang, C.: Effects of salt stress on growth, photosynthesis and solute accumulation in three poplar cultivars. – *Photosynthetica* **47**: 415-421, 2009.
- Debez, A., Hamed, K.B., Grignon, C., Abdelly, C.: Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. – *Plant Soil* **262**: 179-189, 2004.
- Elias, T.S.: Over 2000 labeled illustrations, including range maps. – In: Elias, T.S. (ed.): *The Complete Trees of North America*. Field Guide and Natural History. – Pp. 690-691. Van Nostrand Reinhold Co., New York – 1980.
- Gama, P.B.S., Inanaga, S., Tanaka, K., Nakazawa, R.: Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. – *Afr. J. Biotechnol.* **6**: 79-88, 2007.
- Geng, S.L., Wang, Z.L.: [Study on physiological characteristics of *Zygophyllum xanthoxylum*, *Atraphaxis mandshurica*, *Shepherdia argentea* species under different soil water.] – *Shanxi Forest Sci. Technol.* **4**: 24-30, 2007. [In Chin.]
- Gratani, L., Crescente, M.F., Fabrini, G., Varone, L.: Growth pattern of *Bidens cernua* L.: relationships between relative growth rate and its physiological and morphological components. – *Photosynthetica* **46**: 179-184, 2008.
- Hamdia, A.B.E., Shaddad, M.A.K., Doaa, M.M.: Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense* inoculation on maize cultivars grown under salt stress conditions. – *Plant Growth Regul.* **44**: 165-174, 2004.
- Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J.: Plant cellular and molecular responses to high salinity. – *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51**: 463-499, 2000.
- Hladnik, J., Eler, K., Kržan, K., Pintar, M., Vodnik, D.: Short-term dynamics of stomatal response to sudden increase in CO₂ concentration in maize supplied with different amounts of water. – *Photosynthetica* **47**: 422-428, 2009.
- Iqbal, R.M.: Effect of different salinity levels on partitioning of leaf area and dry matter in wheat. – *Asian J. Plant Sci.* **4**: 244-248, 2005.
- Kakani, V.G., Surabhi, G.K., Reddy, K.R.: Photosynthesis and fluorescence responses of C₄ plant *Andropogon gerardii* acclimated to temperature and carbon dioxide. – *Photosynthetica* **46**: 420-430, 2008.
- Kraft, T.F.B., Dey, M., Rogers, R.B., Ribnicky, D.M., Gipp, D.M., Cefalu, W.T., Raskin, I., Lila, M.A.: Phytochemical composition and metabolic performance-enhancing activity of dietary berries traditionally used by native North Americans. – *J. Agri. Food Chem.* **56**: 654-660, 2008.
- Lu, K.X., Cao, B.H., Feng, X.P., He, Y., Jiang, D.A.: Photosynthetic response of salt-tolerant and sensitive soybean varieties. – *Photosynthetica* **47**: 381-387, 2009.
- Lu, K.X., Yang, Y., He, Y., Jiang, D.A.: Induction of cyclic electron flow around photosystem I and state transition are correlated with salt tolerance in soybean. – *Photosynthetica* **46**: 10-16, 2008.
- Marion, G.M., Verburg, P.S.J., McDonald, E.V., Arnone, J.A.: Modeling salt movement through a Mojave Desert soil. – *J. Arid Environ.* **72**: 1012-1033, 2008.
- Massai, R., Remorini, D., Tattini, M.: Gas exchange, water relations and osmotic adjustment in two scion/rootstock combinations of *Prunus* under various salinity concentrations. – *Plant Soil* **259**: 153-162, 2004.
- Meloni, D.A., Gulotta, M.R., Martínez, C.A.: Salinity tolerance in *Schinopsis quebracho colorado*: Seed germination, growth, ion relations and metabolic responses. – *J. Arid Environ.* **72**: 1785-1792, 2008.
- Moradi, F., Ismail, A.M.: Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. – *Ann. Bot.* **99**: 1161-1173, 2007.
- Moutinho-Pereira, J.M., Correia, C.M., Gonçalves, B.M., Bacelar, E.A., Torres-Pereira, J.M.: Leaf gas exchange and water relations of grapevines grown in three different conditions. – *Photosynthetica* **42**: 81-86, 2004.
- Mudalige, R.G., Longstreth, D.J.: Effects of salinity on photosynthetic characteristics in photomixotrophic cell-suspension cultures from *Alternanthera philoxeroides*. – *Plant Cell Tissue Organ Cult.* **84**: 301-308, 2006.
- Munns, R.: Physiological processes limiting plant-growth in saline soils: some dogmas and hypotheses. – *Plant Cell Environ.* **16**: 15-24, 1993.
- Musyimi, D.M.: Photosynthetically active radiation influence on gas exchange parameters of avocado seedlings growing under saline conditions. – *J. Biol. Sci.* **7**: 1400-1405, 2007.
- Nauš, J., Rolencová, M., Hlaváčková, V.: Is chloroplast movement in tobacco plants influenced systemically after local illumination or burning stress? – *J. Integr. Plant Biol.* **50**: 1292-1299, 2008.
- Pandurangam, V., Sharma-Natu, P., Sreekanth, B., Ghildiyal, M.C.: Photosynthetic response of wheat and sunflower cultivars to long-term exposure of elevated carbon dioxide concentration. – *Photosynthetica* **44**: 586-590, 2006.
- Patel, A.D., Pandey, A.N.: Effect of soil salinity on growth, water status and nutrient accumulation in seedlings of *Cassia montana* (Fabaceae). – *J. Arid Environ.* **70**: 174-182, 2007.
- Pospíšil, P., Šnyrychová, I., Nauš, J.: Dark production of reactive oxygen species in photosystem II membrane particles at elevated temperature: EPR spin-trapping study. – *Biochim. Biophys. Acta* **1767**: 854-859, 2007.
- Qin, J., He, K.N., Tan, G.D., Wang, Z.L., Chen, J.: [Effects of NaCl stress on growth and photosynthetic characteristics of *Hippophae rhamnoides* and *Shepherdia argentea* seedlings.] – *Chin. J. Appl. Ecol.* **20**: 791-797, 2009a. [In Chin.]
- Qin, J., He, K.N., Zhu, Y.Y.: [Relationship between photosynthetic physiological characteristics of several common shrub species and soil water content in Hobq Desert of Inner Mongolia, northern China.] – *J. Beijing For. Univ.* **31**: 39-45, 2009b. [In Chin.]
- Radyukina, N.L., Kartashov, A.V., Ivanov, Y.V., Shevyakova, N.I., Kuznetsov, V.V.: Functioning of defense systems in halophytes and glycophytes under progressing salinity. – *Russ. J. Plant Physiol.* **54**: 806-815, 2007.
- Ranjbarfordoei, A., Samson, R., Van Damme, P.: Chlorophyll fluorescence performance of sweet almond [*Prunus dulcis* (Miller) D. Webb] in response to salinity stress induced by NaCl. – *Photosynthetica* **44**: 513-522, 2006.
- Ruggiero, B., Koiwa, H., Manabe, Y., Quist, T.M., Inan, G., Saccardo, F., Joly, R.J., Hasegawa, P.M., Bressan, R.A., Maggio, A.: Uncoupling the effects of abscisic acid on plant growth and water relations. Analysis of *stol1/nced3*, an abscisic acid-deficient but salt stress-tolerant mutant in *Arabidopsis*. – *Plant Physiol.* **136**: 3134-3147, 2004.
- Sharma, N., Gupta, N.K., Gupta, S., Hasegawa, H.: Effect of NaCl salinity on photosynthetic rate, transpiration rate, and oxidative stress tolerance in contrasting wheat genotypes. – *Photosynthetica* **43**: 609-613, 2005.
- Sobrado, M.A.: Leaf characteristics and gas exchange of the

- mangrove *Laguncularia racemosa* as affected by salinity. – *Photosynthetica* **43**: 217-221, 2005.
- Stepień, P., Kłobus, G.: Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. – *Biol. Plant.* **50**: 610-616, 2006.
- Suárez, N., Medina, E.: Salinity effects on leaf ion composition and salt secretion rate in *Avicennia germinans* L. – *Braz. J. Plant Physiol.* **20**: 131-140, 2008.
- Sudhir, P., Murthy, S.D.S.: Effects of salt stress on basic processes of photosynthesis. – *Photosynthetica* **42**: 481-486, 2004.
- Wang, Z.T.: [A preliminary report on the introduction experiment of abroad shrub species.] – *Pract. Forest Technol.* **9**: 9-12, 2006. [In Chin.]
- Yan, J., Zhang, J.G., Yu, L.L., Mengsa, R.N.: [Recommendation of *Shepherdia argentea*: a new adversity-resistant plant species in sandy area.] – *Mod. Agric.* **10**: 60-61, 2007. [In Chin.]
- Yang, C.-W., Xu, H.-H., Wang, L.-L., Liu, J., Shi, D.-C., Wang, D.-L.: Comparative effects of salt-stress and alkali-stress on the growth, photosynthesis, solute accumulation, and ion balance of barley plants. – *Photosynthetica* **47**: 79-86, 2009a.
- Yang, C.-W., Zhang, M.-L., Liu, J., Shi, D.-C., Wang, D.-L.: Effects of buffer capacity on growth, photosynthesis, and solute accumulation of a glycophyte (wheat) and a halophyte (*Chloris virgata*). – *Photosynthetica* **47**: 55-60, 2009b.
- Youssef, T., Awad, M.: Mechanisms of enhancing photosynthetic gas exchange in date palm seedlings (*Phoenix dactylifera* L.) under salinity stress by a 5-aminolevulinic acid-based fertilizer. – *J. Plant Growth Regul.* **27**: 1-9, 2008.
- Zhang, Z.J., Shi, L., Zhang, J.Z., Zhang, C.Y.: Photosynthesis and growth responses of *Parthenocissus quinquefolia* (L.) Planch to soil water availability. – *Photosynthetica* **42**: 87-92, 2004.
- Zheng, Y.H., Xu, X.B., Wang, M.Y., Zheng, X.H., Li, Z.J., Jiang, G.M.: Responses of salt-tolerant and intolerant wheat genotypes to sodium chloride: Photosynthesis, antioxidants activities, and yield. – *Photosynthetica* **47**: 87-94, 2009.