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Full Length Research Paper

Growth, ion content and photosynthetic responses of two *Elytrigia* Desv. species seedlings to salinity stress

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Salinity is among the major abiotic stresses limiting crop production in the world. *Elytrigia* species, the wild relatives of wheat, are extensively used as genetic resources in wheat breeding to improve its salt tolerance. The objective of this study was to examine the responses to different NaCl treatments (0, 65, 100, 135 and 170 mM) of two *Elytrigia* species (*Elytrigia intermedia* (Host.) Nevski. and *Elytrigia trichophora* (Link.) Nevski.) in terms of their growth, ion content and photosynthetic productivity during the seedling stages. For *E. intermedia*, salt treatment led to decreases in root and shoot biomass, chlorophyll content, photosynthetic rate (*A*) and stomatal conductance (g_s), and a concurrent increase in intercellular CO_2 concentration (C_i). Larger reductions in the parameters occurred in *E. trichophora*. Our results indicated that the two species differ in their sensitivity to salinity, with *E. intermedia* being classified as the more salt tolerant, and *E. trichophora* as sensitive. The two species also differed noticeably in leaf tissue concentrations of Na^+ and K^+ at various NaCl treatments, although, they both showed a trend for Na^+ content to increase and K^+ accumulation to significantly decrease in the higher salinity treatments.

Key words: *Elytrigia*, ion contents, photosynthesis, salinity.

INTRODUCTION

Salinity is one of the most important abiotic stresses limiting plant growth and productivity in arid and semi-arid regions. It affects the plant's morphological, physiological and biochemical processes, including seed germination, plant growth and water and nutrient uptake (Hasegawa et al., 2000; Munns, 2002; Zhu, 2002; Willenborg et al., 2004). Salinity stress results in stunting of plants (Takemura et al., 2000; Alshammary et al., 2004; Yamaguchi and Blumwald, 2005). Under such conditions, there is a considerable decrease in the dry weights of the shoot and roots (Lee et al., 2004; Heidari-Sharifabada and Mirzaie-Nodoushan, 2006). Zhao et al. (2007) have reported that in naked oat (*Avena nuda* L.), salt stress applied at their lowest treatment level (50 mM) reduced total leaf area (TLA) by 35% and plant dry matter (PDM) by 52%. Under a higher salinity level (250 mM), plant growth was further suppressed, causing decreases of 91 and 86% in TLA and PDM, respectively (Zhao et al., 2007).

Salinity can cause ion toxicity and ion misbalance in plants (Houshmand et al., 2005; Saleque et al., 2005). In saline soils, salinity not only causes Na^+ accumulation but also influences the uptake of other essential nutrients such as $Ca^{2+}$and K^+ due to its influence on selective ion absorption (Marschner, 1995). Consequently, plants growing in saline soils may suffer from both Na^+ toxicity and K^+ deficiency. Applying treatments of NaCl induces increases in Na^+ and Cl^-, and decreases in Ca^{2+}, K^+ and Mg^{2+} levels in many plants (Khan, 2001; Heidari-Sharifabada and Mirzaie-Nodoushan, 2006). Treating those of the species naked oat with 250 mM NaCl resulted in the accumulation of a 36-fold increase in Na^+ concentration, 79% more Ca^{2+}, and a 2.4-fold reduction in K^+ than in control plants (Zhao et al., 2007).

Plant growth in biomass is a direct reflection of net photosynthesis. Decreased photosynthetic rates may result from the closure of stoma, induced by osmotic stress, or from salt-induced damage to the photosynthetic apparatus (Paridaa and Das, 2005). Wilson et al. (2006) found a highly significant reduction in stomatal conductance (g_s), and net photosynthetic rate (*A*) in cowpea (*Vigna unguiculata* (L.) Walp.) cultivars suffering from

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salinity. Moreover, the net photosynthetic rate was found to be a more sensitive physiological indicator of the level of salinity stress than the leaf area. In rice (*Oryza sativa* L.), the *A* value decreased by about 35% at 12 ds m^{-1} under salt stress, but the reductions in g_s and transpiration rate (T_r) were 74 and 63%, respectively. Conversely, the reduction in intercellular CO_2 concentration (C_i) was much lower (15%) at the same stress level (Moradi and Ismailr, 2007).

Elytrigia species are the wild relatives of wheat, and were successfully used as a genetic resource to improve the salt tolerance of wheat (Pienaar, 1990; Mujeeb-Kazi et al., 1993; Gorham, 1994; King et al., 1997; Chen et al., 2004). Some studies have been performed on the salt-tolerance of *Elytrigia* species (Weinberg and Shannon, 1988; Zhang et al., 2005; Rogers, 2007; Zhang et al., 2007), but little information is available on the responses of *Elytrigia intermedia* and *Elytrigia trichophora* to salinity (Mao and Wang, 2004). Understanding the mechanisms underlying the growth and physiological responses to salinity would be valuable in selecting and improving *Elytrigia* strains tolerant or adapted to salt stress. Our study was designed to determine the influence of salinity stress on seedling growth, ion content, and photosynthetic productivity of two *Elytrigia* species.

MATERIALS AND METHODS

Plant materials and salt treatments

E. intermedia and *E. trichophora* were selected in this study. A 2×5 factorial experiment (two species× five NaCl concentration treatments), arranged in a completely randomized design with three replications was conducted. Twenty (20) seeds were planted in two-liter pots filled with silica sand and thinned to ten plants per pot after emergence. Pots were irrigated with distilled water for 7 days and then fertilized with a salt-free Hoagland's solution. Salinity stress was imposed on seedlings at 14 days of age by adding 65, 100, 135 or 170 mM NaCl in salt-free Hoagland's solution, while taking the salt-free Hoagland's solution itself as the control treatment. For each pot, 200 mL of salt solution was applied daily to ensure that all seedlings received an equal volume of treatment solution and to prevent additional drought stress being suffered. The experiment was performed in a greenhouse with a temperature of 25/16°C (day/night) and a 16 h photoperiod with 300 µmol m^{-2} s^{-1} illumination. The relative humidity was maintained at about 70%. The plants were then subjected to the salt treatments for 21 days before the following measurements were taken.

Plant growth measures

Plant shoots and roots were harvested after 21 days of salinity treatment, and dried at 60°C for 48 h for the determination of dry biomass and further analysis.

Ion content measures

Dried leaf samples were ground to a fine powder and approximately 0.1 g was transferred to a test tube. Ions were extracted by adding10 mL of 0.1 N acetic acid and heating in a water bath at 80°C for 2h. The extraction solution was cooled at room temperature and left overnight, and then filtered using Whitman filter paper number 40. Sodium and potassium concentrations were then determined using an atomic absorption spectrometer (Perkins Elmer, Norwalk, CT, USA).

Leaf greenness

The second from top fully expanded leaves were used for the measurement of leaf greenness with a chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Camera Co. Ltd., Japan). For each pot, 10 plants were measured at weekly intervals after stress application, and the leaf greenness was calculated as the average of the measurements made at the base, middle and tip of the leaf blade.

Photosynthetic parameters

Net photosynthetic rate (*A*), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured on the same leaf samples as the leaf greenness measurements using an infrared, open gas exchange system LI-6400 (LI-COR) following the manufacturer's instructions. The area of each leaf in the photo-synthetic meter chamber was determined manually. The measure-ments were performed under adequate light levels (300 µmol m^{-2} s^{-1}). Data were manually recorded when the gas exchange parameters became stable.

Statistical analysis

All data were subjected to analysis of variance using the general linear model procedure of SAS (SAS, 1996). Treatment mean differences were separated by the least significant difference ($LSD_{0.05}$) test if F tests were significant ($P \leq 0.05$).

RESULTS

Response of plant growth to salinity

Plant growth rate under salinity stress is a general reflection of resistance to these conditions. The root biomass of the two species was significantly reduced by an increased NaCl concentration. Compared with the control, a 47% reduction in root biomass of *E. trichophora* was observed at 65 mM NaCl and at the higher levels of salinity reduced by 83, 87 and 92% at concentrations of 100, 135 and 170 mM, respectively. At the same levels of salinity, the root biomass of *E. intermedia* was reduced by a significantly smaller amount ($P < 0.01$) than *E. trichophora* of 31, 65, 67 and 84%. No obvious genotype × salinity interaction was observed.

The shoot biomass of the two species exhibited the same trend as the root biomass (Table 1 and Figure 1B). On average, a 32% reduction in shoot biomass was found at the 65 mM salinity level compared with the control, and further decreases occurred at higher concentrations of NaCl. Under conditions of 65-170 mM NaCl stress, decreases ranged from 38 to 82% and 27 to 65% in shoot biomass in *E. trichophora* and *E. intermedia*, respectively.

Table 1. Mean squares of different sources of variation and coefficient of variation for shoot biomass, root biomass, Na^+, K^+ , chlorophyll content (SPAD), photosynthetic rates (*A*), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) determined under salinity stress.

Source	Shoot	Root	Na^+	K^+	SPAD	*A*	g_s	C_i
Genotype (G)	**	**	ns	*	**	**	ns	**
Salinity (S)	**	**	**	**	**	**	**	**
G×S	ns	ns	*	ns	ns	ns	ns	ns
CV (%)	5.4	1.3	169.1	1.5	132.1	93.2	0.02	86.3

ns = P > 0.05, * and ** represent P < 0.05 and 0.01, respectively.

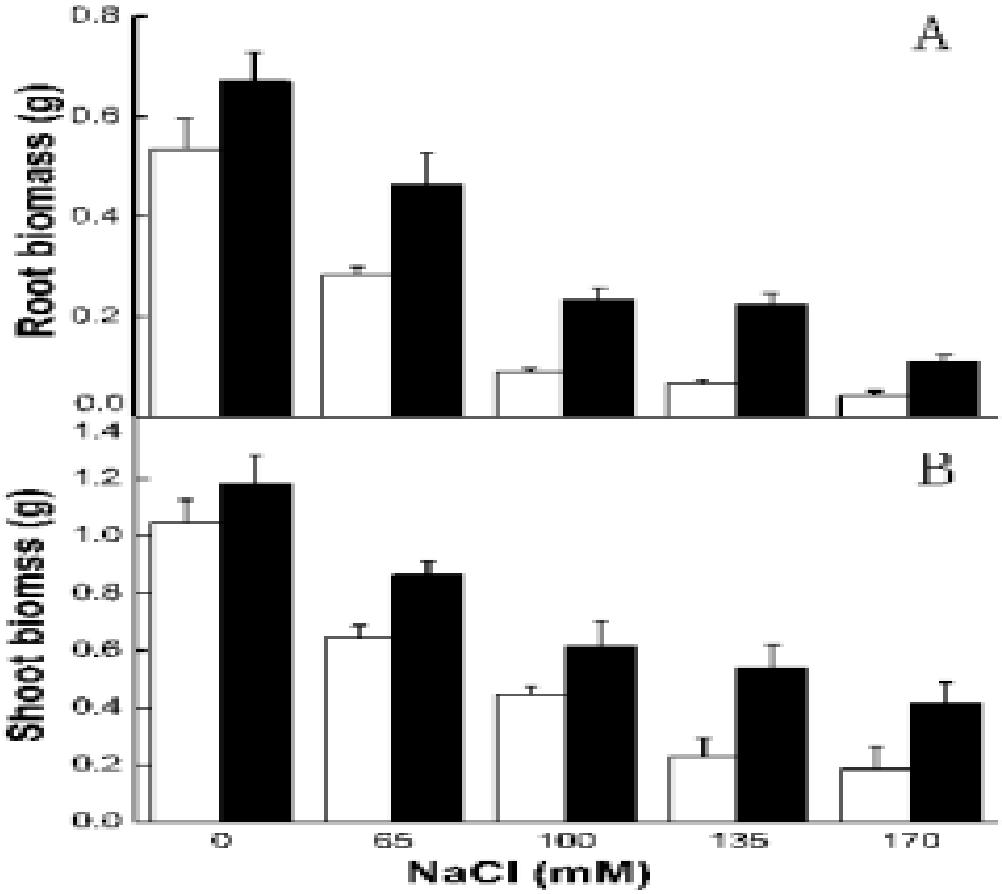

Figure 1. Accumulated root (A) and shoot (B) biomass of two *Elytrigia* species, *E. trichophora* (open columns) and *E. intermedia* (closed columns), exposed to five NaCl treatments over 21 days. Vertical bars represent mean ± S.E. (*n* = 3).

Changes in ion content under conditions of salinity

A significant salt treatment (P < 0.01) and genotype × salinity (P < 0.05) interaction occurred in leaf Na^+ content (Table 1). High Na^+ accumulation was observed in leaves and roots of *E. intermedia* and *E. trichophora*, and Na^+ content increased significantly (P < 0.01) with the increased NaCl concentration. Compared with the control, Na^+ accumulation in *E. trichophora* increased by 3, 8, 11 and 12 fold at 65, 100, 135, and 170 mM NaCl stress, respectively, whilst these values were 1.2-, 7-, 10- and 11-fold for *E. intermedia*. Moreover, in *E. trichophora*, the increase in Na^+ content was comparatively higher than in *E. intermedia* at all salt treatment levels (Figure 2A).

A significant effect of genotype (P < 0.05) and salinity treatment (P < 0.01) were also observed for K^+ (Table 1). Increasing salt levels led to a significant reduction in leaf and root K^+ concentrations (Figure 2B). The reduction in K^+ in *E. trichophora* was higher than that in *E. intermedia* at all salt levels (Figure 2B). As a result, leaf K^+ contents

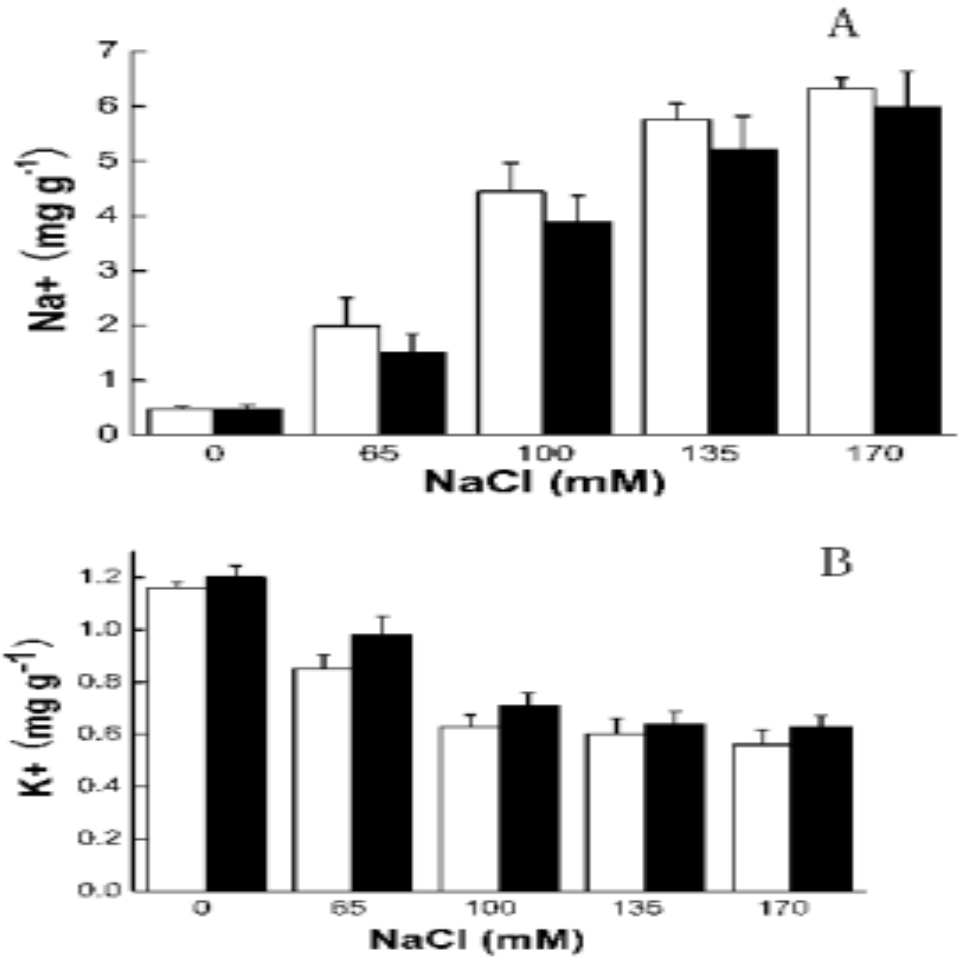

Figure 2. Effects of increasing NaCl concentration on shoot Na^+ (A) and K^+ (B) content of two *Elytrigia* species, *E. trichophora* (open columns) and *E. intermedia* (closed columns), exposed to five NaCl treatments over 21 days. Vertical bars represent mean ± S.E. (n = 3).

were slightly higher in *E. intermedia* than *E. trichophora* but the difference between the species did not reach statistical significance in all salt treatments.

Response of plant photosynthesis to salinity

Under NaCl stress, the leaf chlorophyll content of the two species was greatly reduced ($P < 0.01$) (Figure 3A). With prolonged salt stress, the differences between the two species became significant ($P < 0.01$) (Table 1). As shown in Figure 3A, leaf greenness in the 250 mM salt treatment was reduced by 41% in *E. trichophora* and by 31% in *E. intermedia* compared with the control. The results in Figure 3A clearly showed that these effects were intensified as the treatment was extended.

Under salinity stress, leaf net photosynthetic rate (A) was reduced significantly ($P < 0.01$) by an increased NaCl concentration (Table 1). A significant reduction in A was observed even at the low salinity levels (Figure 3B). The value of A decreased by approximately 21, 56, 73 and 80% when seedlings were subjected to salt stress of 65, 100, 135 and 170 mM, respectively. However, the reductions in g_s were slightly lower at 56 and 69% under 135 and 170 mM salt treatments (Figure 3C). Conversely, the C_i of both species was significantly increased by an elevated NaCl concentration, except for *E. intermedia* which showed a decrease at 65 mM. The increase of C_i reached only 33 and 47% at 135 and 170 mM of NaCl, respectively (Figure 3D). It was observed that the C_i value in *E. trichophora* was increased by 73% at 170 mM NaCl whilst in *E. intermedia* it was only elevated by 20% at the same salinity level.

DISCUSSION

Salt stress significantly reduced the growth of the two *Elytrigia* species during the seedling stage (Figure 1). The observed reduction in plant shoot and root biomass is

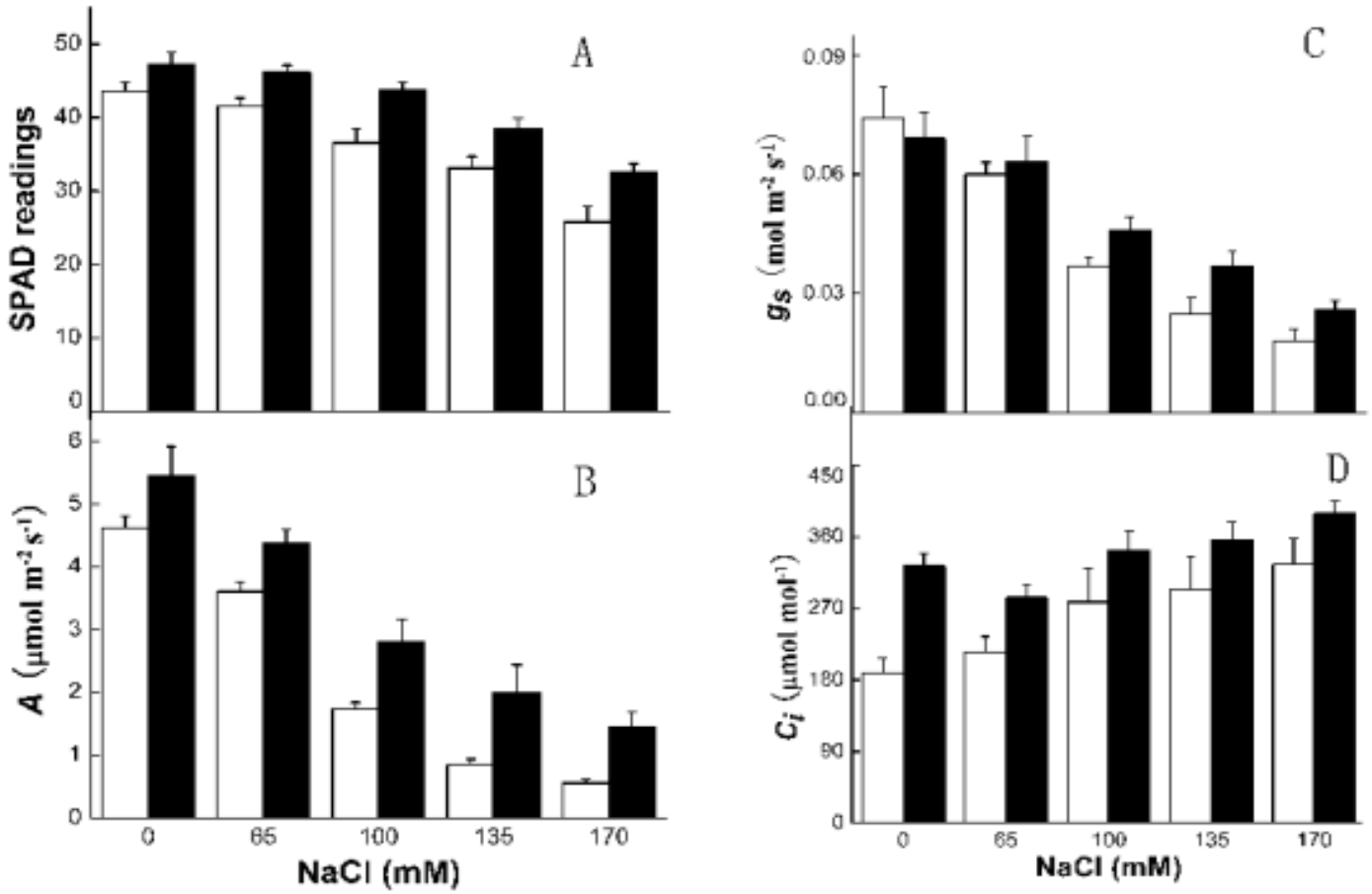

Figure 3. Effects of increasing NaCl concentration on chlorophyll content (A), photosynthetic rate (B), stomatal conductance (C) and intercellular CO_2 concentration (D) of two *Elytrigia* species, *E. trichophora* (open columns) and *E. intermedia* (closed columns), exposed to five NaCl treatments over 21 days. Vertical bars represent mean ± S.E. (n = 3).

likely to be due to a combination of slower growth and development as a result of osmotic stress (Shani and Ben-Gal, 2005) and an inhibition of photosynthesis either as a result of the direct effects of salinity on the photosynthetic apparatus or the indirect effects of a reduction in sink capacity (Kato and Takeda, 1996). *E. intermedia* was more tolerant to salt stress than *E. trichophora*, as indicated by the smaller reduction in root and shoot biomass, leaf chlorophyll content and photosynthetic productivity, and the lower Na^+/K^+ ratio in leaves under salt stress conditions.

Salinity not only caused the accumulation of Na^+, but also influenced the uptake of essential nutrients such as K^+ through the effects of ion selectivity. High Na^+ content strongly inhibited K^+ uptake and accumulation. A significant decrease in K^+ accumulation was observed even at the lowest salinity concentration applied in this experiment. As much as a 52% reduction in K^+ content was observed in the 170 mM treatment. Such a decline in K^+ accumulation due to salinity stress has been widely reported in wheat (Houshmand et al., 2005), barley (Jiang et al., 2006) and wild soybean (Kao et al., 2006).

The reduction in photosynthesis was small when plants were subjected to salt levels lower than 100 mM (Figure 3) but significant effects occurred at higher salt concentrations. This is in agreement with Netondo et al. (2004) in sorghum and Zhao et al. (2007) in rice. The change in g_s was similar to that of A in *Elytrigia*. A close relationship was found between A and shoot biomass (Figure 4A) and g_s (Figure 4B), suggesting that the severe reduction in growth under salt stress was strongly related to the reduction in leaf gas exchange properties. However, there are also reports that photosynthesis is not slowed down by salinity and is even stimulated by low salt concentrations (Rajesh et al., 1998; Kurban et al., 1999). In *Alhagi pseudoalhagi*, the leaf CO_2 assimilation rate increases under conditions of low salinity (50 mM NaCl) but is not significantly affected by 100 mM NaCl. It is, however, reduced to about 60% of the control by 200 mM NaCl. Similarly g_s values are consistent with the CO_2 assimilation rate regardless of the treatments imposed, and C_i is lower in the NaCl-treated plants than in controls (Kurban et al., 1999). The observed decrease in both g_s and transpiration rate might be among the important

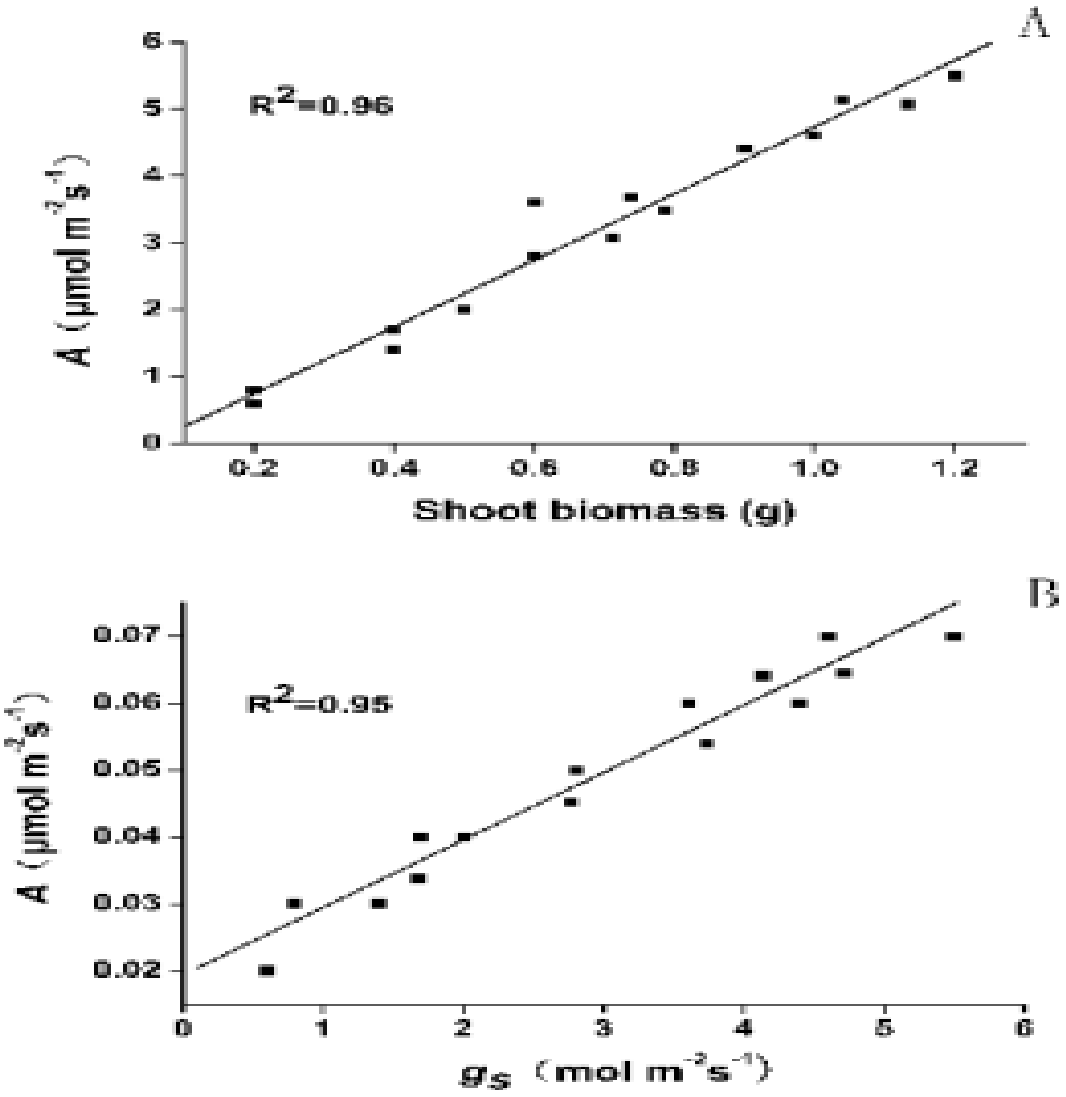

Figure 4. Relationship between photosynthetic rate and shoot biomass (A) and stomatal conductance (B) in two *Elytrigia* species exposed to five NaCl treatments over 21 days.

adaptive mechanisms conferring tolerance to salinity in rice (Robinson, 1988; Moradi and Ismailr, 2007). Decreases in the photosynthetic rate are due to several factors including the dehydration of cell membranes which reduce their permeability to CO_2, salt toxicity, the reduction of CO_2 supply because of the hydroactive closure of stomata, enhanced senescence induced by salinity, changes in enzyme activity induced by alterations in cytoplasmic structure and negative feedback by reduced sink activity (Iyengar and Reddy, 1996).

Conclusions

This study indicated that salinity stress significantly inhibited the growth of two *Elytrigia* species by reducing their shoot biomass, root biomass, chlorophyll content, *A* and g_s and resulting in an associated increase in intercellular CO_2 concentration (C_i). At the same time, their Na^+ content was significantly elevated and K^+ accumulation decreased by increases in salinity stress. The differences in salinity resistance between two species are consistent with their physiological responses measured under different salinity levels. The general growth and photosynthetic parameters of two wild *Elytrigia* species agreed with each other closely, indicating that *E. trichophora* is more sensitive to salinity than *E. intermedia*.

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