DOI: 10.1007/s11099-015-0094-5 PHOTOSYNTHETICA 53 (1): 128-135, 2015

Physiological and biochemical responses to saline-alkaline stress in two halophytic grass species with different photosynthetic pathways

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Abstract

We examined the physiological and biochemical responses of two halophytic grasses with different photosynthetic pathways, *Puccinellia tenuiflora* (C_3) and *Chloris virgata* (C_4), to saline-alkaline stresses. Plants were grown at different Na_2CO_3 concentrations (from 0 to 200 mM). Low Na_2CO_3 (< 12.5 mM) enhanced seed germination and plant growth, whereas high Na_2CO_3 concentrations (> 100 mM) reduced seed germination by 45% in *P. tenuiflora* and by 30% in *C. virgata*. Compared to *C. virgata*, *P. tenuiflora* showed lower net photosynthesis, stomatal conductance, intercellular CO_2 concentration, and water-use efficiency under the same treatment. *C. virgata* exhibited also relatively higher ATP content, K^+ concentration, and the K^+/Na^+ ratio under the stress treatments implying that salt tolerance may be the main mechanism for salt resistance in this species. Our results demonstrated that the *C. virgata* was relatively more resistant to saline-alkaline stress than the co-occurring *P. tenuiflora*; both two species adapt to their native saline-alkaline habitat by different physiological mechanisms.

Additional key words: ATP content; gas exchange; membrane permeability; Na_2CO_3 stress; proline; salinity.

Introduction

Salt stress is common in natural as well as in agricultural ecosystems and greatly affects crop production; for example, 15% of the total agricultural land or 50 million ha was salt-affected in the most important crop-producing area of China, the Huanghuaihai Plain (Xiong and Li 1978). In the United States, high salinity limits crop production to various extents on 30% of irrigated land (Ward *et al*. 2003). Because of the widespread occurrence of salt-affected soils and its vast impact on crop production, responses to salt stress in a variety of plant species were intensively studied over the last century (Flowers and Colmer 2008).

The effects of saline-alkaline soils are becoming increasingly important (Degenhardt *et al*. 2000, Shi and Wang 2005). Salt-alkaline mixed stress ($NaHCO_3$ and Na_2CO_3) is more severe than the one of neutral salts (*e.g.*, NaCl and Na_2SO_4) in inhibiting plant growth and survival (Shi and Yin 1993). Saline-alkaline soils became a most widespread environmental stress in some regions and it reached more than 70% in grasslands, northeast China, because of overgrazing and unreasonable reclamation (Ge and Li 1992).

Previous studies on salt stress in plants have primarily focused on salt resistance mechanisms under stress of NaCl. Much less is understood about plant responses to simultaneous salinity-alkalinity stress imposed by alkaline salts. Furthermore, the physiological response to simultaneous stress of salinity-alkalinity among plant species with different photosynthetic pathways has received even less attention. Because of the pervasiveness of C_3 and C_4 species in natural and agricultural ecosystems, it is essential to improve our understanding of their divergence in response mechanisms to saline-alkaline stress and to help predict their occurrence and distribution in natural and agricultural ecosystems.

Received 7 August 2013, *accepted* 2 May 2014.
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Abbreviations: C_i – intercellular CO_2 concentration; g_s – stomatal conductance; E – transpiration rate; EC – electrical conductivity; P_N – net photosynthetic rate; RDM – relative dry mass; REL – rate of electrolyte leakage; RPH – relative plant height; SGP – seed germination percentage; WUE – water-use efficiency.
Acknowledgements: We would like to thank Y.Q. Yuan for being a help to the experiment. The work was supported by National Scientific Foundation of China (31170304, 31070228).

Because of higher water- and nitrogen-use efficiency and greater salt tolerance, C_4 halophytes are common in saline meadows and grasslands in northeast China (Wang 2004). *Chloris virgata* Sw. is one of the most widespread C_4 halophytes in these ecosystems. A co-occurring and also widespread grass species in the region is *Puccinellia tenuiflora* (Turcz.) Scrib., which is a typical C_3 species. Despite their differences in photosynthetic pathways, these two species share a number of characteristics, *e.g.*, high productivity and palatability to animals; they can form large patches of consociations in the grasslands with soil pH averaging 9.0 and reaching as high as 10.5 in early growing seasons (Li and Zheng 1997). Due to their ecological and economic importance, these two species have been previously examined for their salt resistance by Du *et al*. (1994) and Peng *et al*. (2004). However, little is known about the physiological and biochemical divergence in the mechanisms of salt resistance between these two co-occurring species. In this study, the seedlings of both species were subjected to Na_2CO_3 treatments and their physiological and growth responses to the saline-alkaline stresses were examined. We hypothesized that both species would tolerate low and medium Na_2CO_3 stresses and high Na_2CO_3 concentrations would significantly inhibit plant growth and survival. We further hypothesized that there would be differences in salt resistance between the two species under Na_2CO_3 stress, with *C. virgata* being more tolerant than *P. tenuiflora*. Results from this study would be valuable for better understanding of the physiological responses in plants to saline-alkaline stresses, as well as the physiological divergences of plants with different photosynthetic pathways.

Materials and methods

Seed germination under saline-alkaline stress: Seeds of *Chloris virgata* Sw. and *Puccinellia tenuiflora* (Turcz.) Scrib. were collected from natural saline-alkaline grassland located in the Manchuria Plain in northeast China (44°41'N, 123°44'E), in 2006. Alkaline salts (Na_2CO_3 and $NaHCO_3$) and salt (NaCl) are the main environmental stresses in the grassland with soil pH ranging from 7.2 to 10.5. The mean annual air temperature in the area is about 5ºC, varying from –18ºC in January to 23ºC in July. A more detailed description of the climate and vegetation in the region can be found in Wang (2004). Before the start of the experiment, the seeds were surface-sterilized with 0.52% sodium hypochlorite solution for 1 min and rinsed three times with distilled water to avoid fungal infection. The seeds of each species were then placed on threefold filter paper on 72 Petri dishes (90 mm diameter and 15 mm height, 100 seeds per dish) and kept at a photon flux density of 350–400 μmol m^{-2} s^{-1} with 12/12 h photoperiod at a temperature of 25/18ºC (day/night) in a phytotron (*HP 250 GS*, *Wuhan Ruihua Instrument and Equipment Co.*, Hubei, China). Petri dishes (72) were randomly divided into six groups and exposed to one of the six Na_2CO_3 concentrations: 0 (control, C), 12.5 (S12), 25 (S25), 50 (S50), 100 (S100), and 200 mM (S200).

Stress level	Na_2CO_3 [mM]	Treatment
Control	0	C
Low	12.5	S12
Moderate	25	S25
	50	S50
High	100	S100
	200	S200

The selection of Na_2CO_3 concentration range was based on soil salt components in the saline-alkalinized grassland ecosystem (0–70 mM Na_2CO_3) in Manchuria Plain. Seeds in all treatments were watered with half strength Hoagland solution to nourish the sprouting seeds about a week later. For treatments other than C, Na_2CO_3 was added accordingly in the solutions. Seed germination tests continued until germination ratios were almost steady.

Response of seedlings to saline-alkaline stress: Seeds of each species were sowed in 72 plastic pots (14 cm diameter and 25 cm height) filled with washed sand in a sunlit greenhouse. Pots were well-watered with half strength Hoagland solution every two days. Temperature was maintained at 25/18ºC (day/night) by two air conditioners with a 12/12 h photoperiod (06:00–18:00 h, controlled by using black plastic bags to darken plants) and relative humidity kept at about 65%. After germination, seedlings in each pot were thinned to 15 of a uniform size. The pots were divided into six 12-pot groups for each species. Each group of pots corresponded to one of Na_2CO_3 concentrations: C, S12, S25, S50, S100, and S200. Saline-alkaline stress was initiated 30 d after sowing (DAS), which corresponded to the third leaf stage. Plants under stress were watered with half strength Hoagland solution which differed only in the amount of Na_2CO_3 addition. Pots were arranged in a randomized complete block design with six blocks and rotated twice a week during the experiment to minimize location effects.

Gas-exchange measurements: After two weeks of salt treatment (45 DAS), measurements of net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) on fully expanded leaves were taken on the same day (1 d after watering) using *Li-6400* portable photosynthesis system (*LI-COR Inc*., Lincoln, Nebraska, USA) at about 800 μmol m^{-2} s^{-1} PPFD, provided by an external halogen lamp. Ambient temperature in the measuring cuvette was maintained at 25ºC with a relative humidity of about 65% and a CO_2 concentration of 360 μmol mol^{-1}.

Plant mass measurement and growth analysis: Plant heights were measured before they were carefully and thoroughly washed first with running water and then with distilled water after gas-exchange measurements. Plants were separated into roots and shoots. All separated samples were placed in perforated paper bags, oven dried at 80ºC for 48 h and then weighted for the dry mass (DM). Relative plant height (RPH) in the salt-treated pots was calculated as a percentage of the average plant height in the control pots. The root/shoot ratio and shoot mass allocation were calculated for each treatment. For the proline content, $[K^+]$ and $[Na^+]$ measurements, dry samples were hand-ground using mortar and pestle to a size small enough to pass through a 100-mesh screen.

Membrane permeability and ATP determination: Approximately 1.0 g of fresh leaf was taken from a plant in each treatment and cut into 1 cm long segments before being washed three times with deionized water to remove surface-adhered electrolytes. Leaf samples were divided into two equal portions and placed separately into two closed vials, each containing 20 mL of deionized water. One vial was incubated at 25ºC on a rotary shaker for 24 h before the electrical conductivity (EC_1) of the solution was determined using a conductivity gauge (*DDS-307, Leizi*, China). The other vial was autoclaved at 120ºC for 20 min and electrical conductivity (EC_2) was measured after equilibration to 25ºC. The rate of electrolyte leakage (REL [%]) was calculated as $EC_1/EC_2 \times 100$.

Hot water extraction was used for extracting ATP from leaves. Leaf ATP was determined with the ATP firefly luciferase assay method (the linear relationship between ATP concentration and densities of its fluorescence was tested) (Wang *et al*. 1999).

Biochemical determination: Ground dry leaf sample (0.1 g) was used to determine proline content according to the methods of Martínez *et al*. (2005). About 0.02 g ground samples was used to determine concentrations of K^+ ($[K^+]$) and Na^+ ($[Na^+]$) by flame photometry (*Flame Photometer 410*, *Corning*, Halstead, UK). $[K^+]$ and $[Na^+]$ were expressed as μmol g^{-1}(DM). Six measurements were made for each treatment to determine proline content, $[K^+]$, and $[Na^+]$.

Statistical analysis: Analysis of variance (*ANOVA*) was performed on data of plant growth (height and DM), seed germination, gas-exchange parameters (P_N, E, WUE, g_s, and C_i), proline content, $[K^+]$, and $[Na^+]$ to determine physiological divergence in the two grasses under different salt treatments. All statistical analyses on the data were performed using *SPSS 10.0* (*SPSS* for *Windows*, Chicago, IL, USA). A difference was considered significant if $P<0.05$.

Results

Seed germination: The two species presented a similar pattern of seed germination percentage (SGP) in response to salinity (Fig. 1). SGP at low stress increased by 19 and 22% in *P. tenuiflora* and *C. virgata* compared with C. SGP of *P. tenuiflora* decreased significantly under S25; only 25% of seeds germinated at S200. SGP of *C. virgata* seeds dropped significantly only at S50 and 33% of seeds germinated even at both highest saline-alkaline concentrations (Fig. 1). SGP of *C. virgata* were about 9, 34, and 42% higher than those of *P. tenuiflora* at low (S12), moderate (S25–S50), and high stress (S100–S200) levels, respectively.

Relative plant height, dry mass, and mass allocation: Low Na_2CO_3 concentrations showed a small effect on RPH in either *P. tenuiflora* or *C. virgata* (Fig. 2*A*). Higher salt stress, however, reduced RPH significantly. RPH in *P. tenuiflora* at S200 was 60% of that in C, while that in *C. virgata* was reduced by 26% at the same salinity level. RPH of *C. virgata* averaged about 20% higher than those of *P. tenuiflora* at salinity of S50–S200.

Low salinity stress increased RDM slightly in both *P. tenuiflora* and *C. virgata* (4 and 7%, respectively) (Fig. 2*B*). RDM of *P. tenuiflora* decreased significantly when salinity increased to S25–S200 and RDM was lowered by 36% at S200 compared with that of S12, meanwhile RDM of *C. virgata* started to decline at S50 treatment and it was reduced by 28% at S200. At salinity concentrations from S25 to S200, *C. virgata* maintained the RDM that was on average 19% higher than that of *P. tenuiflora*. Similar to RDM, shoot mass allocation was unaffected at low salinity, but started to decline at moderate stress for both species (Fig. 2*C*). Compared to *C. virgata*, average shoot mass allocation in *P. tenuiflora* dropped by 17% at moderate and high salt concentrations.

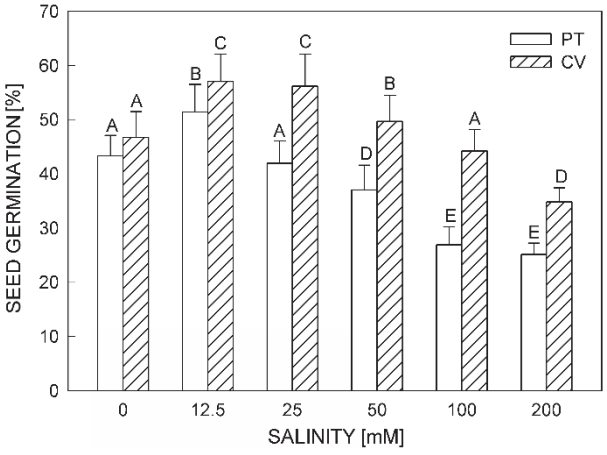

Fig. 1. Seed germination percentage (%) in *Puccinellia tenuiflora* (PT) and *Chloris virgata* (CV) under different salt stresses. Values are means ± SE (n = 12). *Uppercase letters* indicate no significant differences between treatments and species.

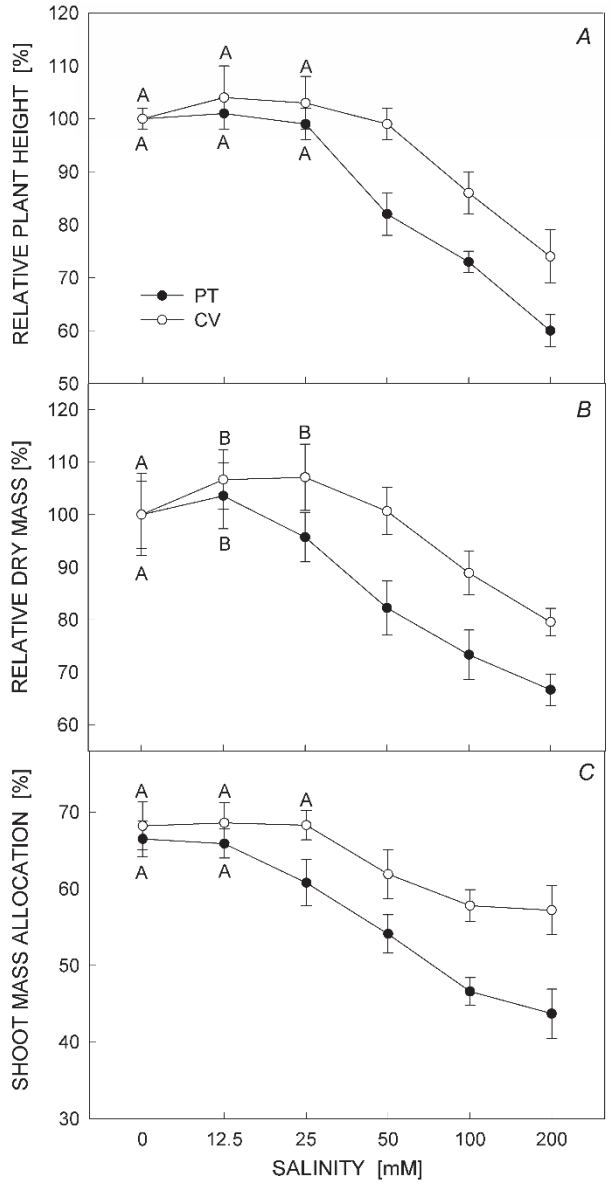

Fig. 2. Relative plant height (*A*), relative dry mass (*B*), and shoot mass allocation (*C*) in *Puccinellia tenuiflora* (PT) and *Chloris virgata* (CV) under different salt stresses. Values are means ± SE (n = 6). *Uppercase letters* indicate no significant differences between treatments and species.

Gas exchange: P_N in *C. virgata* was significantly higher than that in *P. tenuiflora* at all salt concentrations (Table 1). *C. virgata* plants at S50 and S200 treatments maintained 87.4 and 64.6% of the P_N of plants under C conditions, but those in *P. tenuiflora* at the same salinity were only 63.4 and 33.2%, respectively. P_N in *C. virgata* was only 32% higher than that of *P. tenuiflora* at two lowest salt concentrations, but it was 156% greater at S200. The other gas-exchange traits, g_s and C_i, followed the similar pattern as that of P_N (Table 1). g_s in *C. virgata* was about 3–6 times higher than that of *P. tenuiflora*, whereas C_i in *C. virgata* was only 29% higher on average.

E was higher in *P. tenuiflora* than that in *C. virgata* at low and moderate salt stress (Fig. 3*A*), however, it decreased more rapidly at high salt stress than that in *C. virgata*. WUE was consistently higher in *C. virgata* than in *P. tenuiflora* at all salt treatments (Fig. 3*B*). WUE in *C. virgata* was 52% higher than in *P. tenuiflora* at C and S12 and it reached 75% at the S200.

Membrane permeability and concentrations of Na^+ and K^+: REL differed more with the increasing salt stress in both species (Fig. 4). Low and moderate stresses had no significant effect on REL in *P. tenuiflora*. The REL maintained at about 8.5% in C, S12, S25, and S50 treatments. *P. tenuiflora* at high stress showed 29% higher REL compared with C. REL in *C. virgata* did not differ significantly from C to S12 and from moderate to high stress treatments. *C. virgata* at moderate and high stresses exhibited about 55% higher REL than those in C. Compared to *P. tenuiflora*, *C. virgata* had 66, 150, and 99% higher REL at low, moderate, and high stress treatments, respectively.

The two species exhibited similar patterns in the response of $[Na^+]$ to increasing salt stress (Fig. 5*A*,*B*). Compared with C, $[Na^+]$ in shoots and roots of *P. tenuiflora* increased by 50–76% at S25 and as high as 367–618% at S200. Similarly, shoot $[Na^+]$ and root $[Na^+]$ in *C. virgata* increased by 50–66% at S25 and 243–355% at S200. Shoot $[Na^+]$ and root $[Na^+]$ in *P. tenuiflora* were 41 and 32, 67 and 53, and 100 and 70% higher than in *C. virgata* at low, moderate and high stress levels, respectively.

Salt stress affected significantly $[K^+]$ in both species (Fig. 5*C*,*D*). Shoot $[K^+]$ and root $[K^+]$ in *P. tenuiflora* started decreasing significantly when salt stress reached S50. Shoot $[K^+]$ and root $[K^+]$ were reduced by 26 and 39% at S50, 45 and 54% at S100, and 56 and 67% at S200, compared to C. For *C. virgata*, shoot $[K^+]$ and root $[K^+]$ started to decrease at S100; it dropped by 31 and 42% at S100, and 43 and 61% at S200, respectively. *C. virgata* exhibited about 5% higher shoot $[K^+]$ and 7% higher root $[K^+]$ than *P. tenuiflora* at C, S12, and S25, but the difference in shoot $[K^+]$ between both species was much greater (35%) at S50, S100, and S200.

Proline and ATP contents: Low Na_2CO_3 stress had no effect on proline contents in the two species at the whole plant level, but proline contents increased significantly at moderate and high salinity stresses (Fig. 6*A*). The proline content in the two species at moderate and high stresses was about 2 and 5 times higher than in C. There was no significant difference in the proline content between both species at low stress, but that in *P. tenuiflora* was 50%

Table 1. Net photosynthesis (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) in leaves of *Puccinellia tenuiflora* (PT) and *Chloris virgata* (CV) under different Na_2CO_3 stresses. Values are means ± SE (n = 6). Different *lowercase letters* indicate no significant differences between treatments.

Na_2CO_3 [mM]	P_N [μmol(CO_2) m^{-2} s^{-1}]		g_s [mol(H_2O) m^{-2} s^{-1}]		C_i [μmol(CO_2) mol^{-1}]	
	PT	CV	PT	CV	PT	CV
0	8.25 ± 0.42^a	10.86 ± 0.66^b	0.244 ± 0.009^a	0.742 ± 0.051^b	278.5 ± 23.6^a	332.6 ± 20.5^c
12.5	8.25 ± 0.45^a	10.86 ± 0.59^b	0.242 ± 0.011^a	0.745 ± 0.062^b	275.4 ± 24.3^{ad}	330.8 ± 22.4^c
25.0	7.65 ± 0.36	10.76 ± 0.43^b	0.199 ± 0.009	0.723 ± 0.047^b	235.1 ± 18.9^b	317.7 ± 18.7
50.0	5.23 ± 0.41	9.49 ± 0.62	0.115 ± 0.007	0.565 ± 0.052	230.4 ± 21.1^b	297.7 ± 20.4
100.0	4.11 ± 0.38	8.82 ± 0.71	0.099 ± 0.007	0.524 ± 0.045	214.7 ± 20.2	264.3 ± 21.2^d
200.0	2.74 ± 0.39	7.02 ± 0.57	0.078 ± 0.006	0.492 ± 0.051	202.8 ± 23.1	255.9 ± 19.6

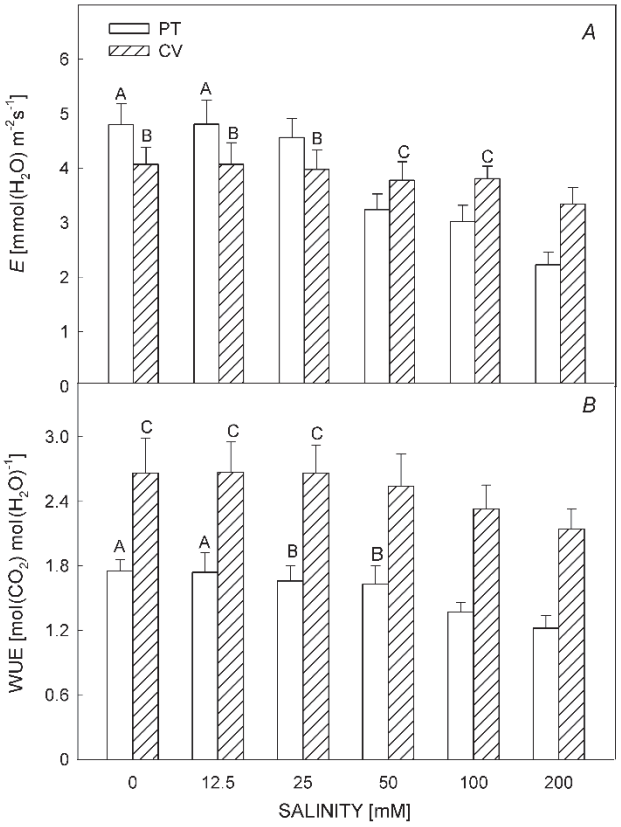

Fig. 3. Leaf transpiration (E) (A) and water-use efficiency (WUE) (B) in *Puccinellia tenuiflora* (PT) and *Chloris virgata* (CV) under different salt stresses. Values are means ± SE (n = 6). *Uppercase letters* indicate no significant differences between treatments and species.

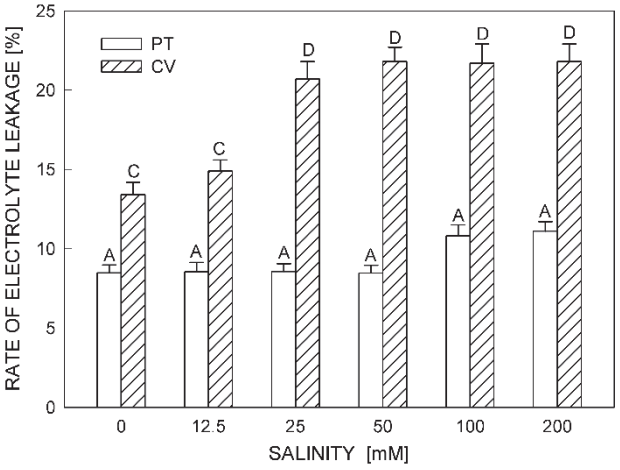

Fig. 4. Differences in rate of electrolyte leakage (membrane permeability) between *Puccinellia tenuiflora* (PT) and *Chloris virgata* (CV) under different salt stresses. Values are means ± SE (n = 6). *Uppercase letters* indicate no significant differences between treatments and species.

greater than in *C. virgata* at moderate and high stress treatments on average.

Salt stress affected significantly the ATP content in both species (Fig. 6*B*); it peaked at S12 and increased 61 and 29% compared with C in *P. tenuiflora* and *C. virgata*, respectively. ATP contents dropped by 85% in *P. tenuiflora* and 68% in *C. virgata* at S200 compared to S12. Mean ATP contents in *C. virgata* were 33, 78, and 279% higher than those in *P. tenuiflora* at low, moderate, and high Na_2CO_3 stresses, respectively.

Discussion

Results from our study demonstrated that the C_3 and C_4 halophytic grasses, co-occurring in the same saline-alkaline grassland, differed significantly in their physiological and biochemical responses to saline-alkaline stress. Low Na_2CO_3 stress was found to enhance seed germination and plant growth in both species (Fig. 1). Enhancement of plant growth at low [Na^+] seems to be common among halophytic species, as similar results have been found for other halophytes, such as *Atriplex hortensis* and *A. gmelini* under low NaCl stress (Martínez *et al*. 2005, Flowers and Colmer 2008). The enhancing effect of low [Na^+] on plant growth was primarily due to an increase in the conversion of pyruvate to phospho*enol*pyruvate under light condition, leading to enhanced photosynthesis and greater plant growth (Martínez *et al*. 2005), but it increases the risk of long-term ion toxicity, if not appropriately compartmentalized, exported, or secreted. Furthermore, high pH and ion imbalance due to alkaline salt stress were

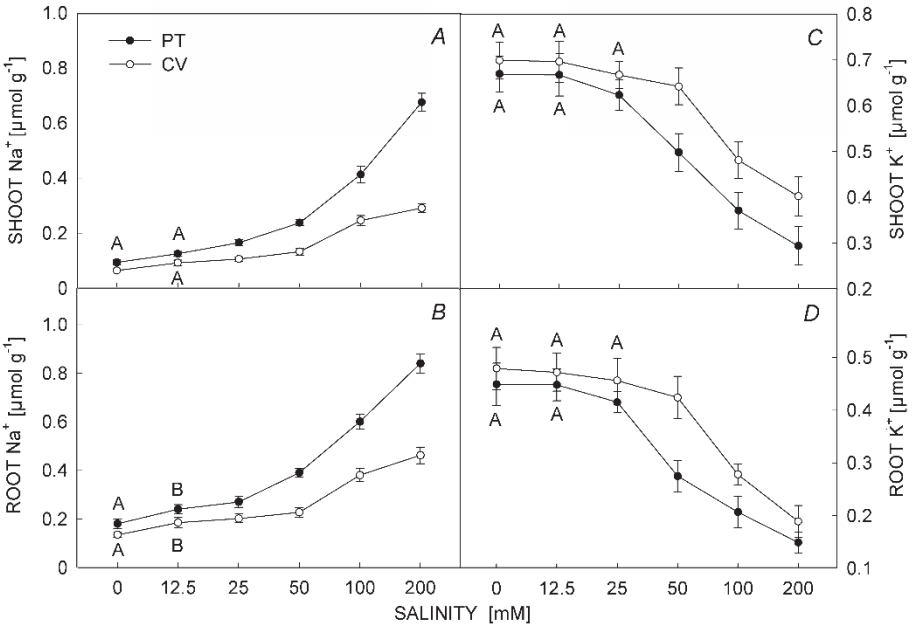

Fig. 5. Content of Na^+ in shoots (*A*) and roots (*B*), content of K^+ in shoots (*C*) and roots (*D*) in *Puccinellia tenuiflora* (PT) and *Chloris virgata* (CV) under different salt stresses. Values are means ± SE (n = 6). indicate no significant differences between treatments and species.

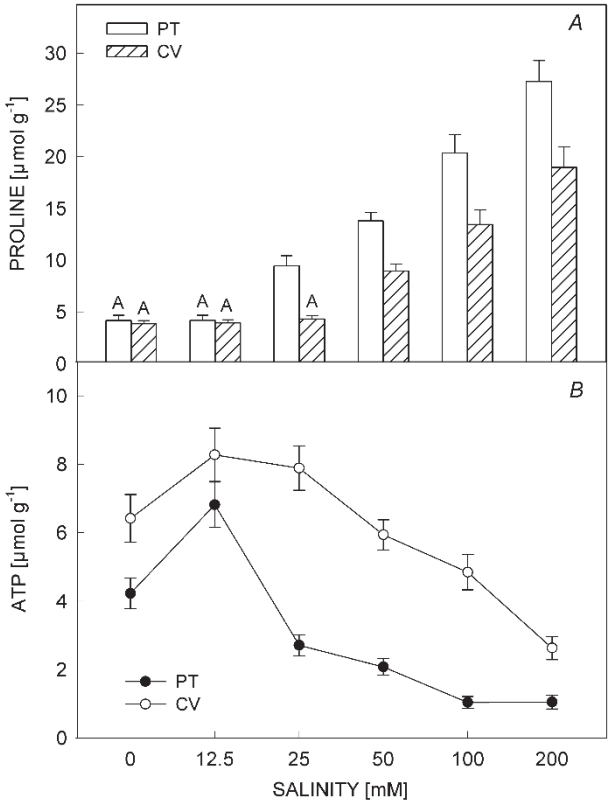

Fig. 6. Proline (*A*) and ATP (*B*) content in *Puccinellia tenuiflora* (PT) and *Chloris virgata* (CV) under different salt stresses. Values are means ± SE (n = 6). *Uppercase letters* indicate no significant differences between treatments and species.

a main factor in inhibiting plant growth and seed germination, because alkaline stress causes accumulation of abscisic acid, which restrains cell division, cell elongation, and embryo growth of plant seed (Campbell and Nishio 2000, Degenhardt *et al*. 2000). It is not surprising that moderate and high saline-alkaline stress significantly reduced seed germination and biomass production in both halophytes in our study. Relatively higher SGP, RPH, and RDM found in *C. virgata* in comparison to *P. tenuiflora* suggested that the C_4 species possess greater capacity to resist saline-alkaline stress and are better adapted to the harsh saline-alkaline environments in their native grasslands (Fig. 2). This supports our hypothesis that the two species differ in their resistance to saline-alkaline stress, although they co-occur in the same habitat.

Salt stress alters plant metabolism through changes in activation and synthesis of key enzymes (Ward *et al*. 2003). Long term salt stress can cause necrosis in leaves and reduce effective photosynthetic area, affecting plant growth (Tezara *et al*. 1999, Munns *et al*. 2006). P_N, g_s, and C_i in the two species declined with the increasing saline-alkaline stress (Table 1). Lower g_s apparently reduced the capacity of the plants to take up CO_2 from the atmosphere and subsequently reduced photosynthetic carbon assimilation (Lawlor and Cornic 2002). Relatively higher P_N and WUE in *C. virgata* than in *P. tenuiflora* at all salt treatments confirmed earlier field observations that *C. virgata* had greater capacity for maintaining high P_N and WUE in saline grasslands (Wang 2004). It suggests that C_4 species gain competitive advantage over co-occurring C_3 species in the saline-alkaline soils.

It is known that one of the most damaging consequences of salt stress in plants is the influx of Na^+ and decline in K^+ contents in tissues (Cheeseman 1988, Garthwaite *et al*. 2005). Increases in [Na^+] and decrease in [K^+] in tissues for both *P. tenuiflora* and *C. virgata* with rising salinity demonstrated that plasma membrane was severely compromised by salt stress, especially in *P. tenuiflora* at high Na_2CO_3 concentrations. Relatively higher [Na^+] in shoots and roots in *P. tenuiflora* in

comparison to *C. virgata* under salt stress showed that saline-alkaline stress had greater impact on plant growth in *P. tenuiflora* than in *C. virgata*, because the high [Na^+] in leaves can cause salt toxicity, leading to decline in growth and biomass production (Shi and Yin 1993). Relatively greater saline-alkaline tolerance in *C. virgata* might be mainly due to high [K^+] in its tissues, because [K^+] plays a major role in several physiological processes, such as osmotic regulation, protein synthesis, and enzyme activation (Garthwaite *et al*. 2005). K^+/Na^+ in roots and shoots of *C. virgata* were 40–70 and 10–200% higher than those in *P. tenuiflora* at low and high Na_2CO_3 concentrations. This may explain the fact that [Na^+] in tissues of *C. virgata* was not as high as expected, because high [K^+] can help plant cells to exclude [Na^+] through K^+/Na^+ pumps.

The increase in the proline content with rising salt concentration suggested for both species in our study that the induction of proline synthesis is related to water potential and [Na^+] in the tissues. Positive and significant associations between the proline content and [Na^+] in both shoots and roots of *P. tenuiflora* and *C. virgata* were consistent with earlier observations in other species, *e.g*., *Hordeum* (Garthwaite *et al*. 2005) and *Leymus* (Chen and Wang 2009). It has been hypothesized that proline accumulation acts as osmoprotectant in plants subjected to high salinity and other stresses, such as drought and low temperature (Irigoyen *et al*. 1992). Although the precise role of proline accumulation is still under debate for plants under stresses, there is little doubt that accumulation of free proline in plant leaves is one of the most common and direct physiological responses to environmental stress such as water deficit and salt stress (Bandurska 2000).

The significant divergence in ATP content between *P. tenuiflora* and *C. virgata* is another important indicator of their differences in saline-alkaline stress resistance. Higher ATP content in plant tissues under stresses has been known to help in osmotic adjustment, protein synthesis, enzyme activation, and ionic balance (Barkla *et al*. 1995, Martínez *et al*. 2005). Water stress during the osmotic phase decreases the amounts of ATP, leading to reduction in CO_2 assimilation (Tezara *et al*. 1999, Lawlor and Cornic 2002), but few studies have examined the effects of salt stresses on plant ATP contents (Du *et al*. 1994). Relatively higher ATP content was therefore one factor enabling *C. virgata* to maintain higher g_s and P_N under salinity stress. Our results revealed that decrease in carbon assimilation under Na_2CO_3 stress was significantly related to ATP contents, however, Cornic (2000) found that drought stress inhibits photosynthesis by decreasing stomatal aperture, not by affecting ATP synthesis. Moreover, many recent studies have shown that Na^+/H^+ antiporter is one of the most common mechanisms for halophytic plants to exclude Na^+ from cells (Ward *et al*. 2003, Flowers and Colmer 2008) and the activity of Na^+/H^+ antiporter is determined by ATP content (Barkla *et al*. 1995, Cuin 2011). Higher ATP content in *C. virgata* than that in *P. tenuiflora* could enhance the capacity of its Na^+/H^+ antiporter to exclude Na^+; it partly explained the fact that [Na^+] in *C. virgata* was lower than that in *P. tenuiflora*. Obviously high capacity in salt tolerance, as manifested by the higher K^+/Na^+ and ATP contents in *C. virgata* under all treatments, explains the observations that *C. virgata* and a great number of other C_4 species occur in the saline-alkaline grasslands (Wang 2004). Our results suggest that salt exclusion appears to be the main mechanism for salt resistance in *P. tenuiflora*, whereas salt tolerance seems to be the primary mechanism for avoiding ion toxicity in *C. virgata*.

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