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Leaf photosynthesis, chlorophyll fluorescence, ion content and free amino acids in *Caragana korshinskii* Kom exposed to NaCl stress

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Abstract To understand the physiological response under salt stress, photosynthesis, PSII efficiency, contents of ions and free amino acids in leaves of *Caragana korshinskii* Kom (*Caragana*) exposed to three levels of salinity were investigated. Results showed that the decrease in photosynthesis of *Caragana* with salt stress was largely dependent on stomatal closure during the experimental period. In the early period of stress, due to the dissipation of excess excitation energy which occurred by the increase in non-photochemical quenching, photodamage was avoided and maximum efficiency of PSII was not affected. However, with increased salt stress, the photoprotective mechanism was not sufficient to avoid oxidative damage. Thus, damage to PSII and its resulting non-stomatal inhibition of photosynthesis may occur. At 18 days with 300 mM NaCl treatment, a non-stomatal factor was responsible for the inhibition of photosynthesis. Accumulation of Na^+ and K^+ in leaves indicated no competition between Na^+ and K^+ absorption, which suggests the potential for a unique pathway of Na^+ absorption in *Caragana.* There was a critical salinity level for the accumulation of free amino acids in salt-treated leaves of *Caragana*, i.e., free amino acids accumulated slowly below critical level, but rapidly above the critical level. In addition, proline was the most abundant among all individual free amino acids.

Keywords *Caragana korshinskii* Kom · Chlorophyll fluorescence · Free amino acids · Ion content · Photosynthesis · Salt stress

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Introduction

Salt stress that leads to both the decrease of the substrate osmotic potential and ion-specific toxicity affects almost every aspect of the physiology and biochemistry of plants (Cuartero et al. 2006). Salinity reduces stomatal conductance greatly and consequently reduces photosynthetic rate (Munns and Tester 2008). However, the inhibition of photosynthetic rate imposed by stomatal closure may promote an imbalance between photochemical activity at photosystem II (PSII) and electron requirement for photosynthesis, leading to excess excitation and subsequent photoinhibitory damage of PSII reaction centers (Krause 1988; Souza et al. 2004). Thus, non-stomatal inhibition of photosynthesis may occur when the photosynthetic apparatus is damaged. However, some studies have shown that salinity does not damage PSII (Mishra et al. 1991; Morales et al. 1992), because excess excitation energy is dissipated by certain photoprotective mechanisms and the damage to PSII is avoided (Qiu et al. 2003). Under severe stress, excess excitation of the photochemical system occurs and the capacity of the protective mechanism is exceeded (Souza et al. 2004). The damage to PSII reaction centers may then occur. Chlorophyll fluorescence analysis can give insights into the ability of a plant to tolerate environmental stresses and the extent to which stresses damage the photosynthetic apparatus (Maxwell and Johnson 2000).

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The damage caused by long-term salinity is the excessive accumulation of Na^+ (Martinez-Rodriguez et al. 2008), and the main site of Na^+ toxicity is the leaf blade, where Na^+ is accumulated after being deposited in the transpiration stream (Munns 2002). High Na^+ content in the leaf blade may disturb cellular ion homeostasis (Takahashi et al. 2007). Potassium is an activator of many enzymes which are essential for metabolic reactions (Salisbury and Ross 1992). Plant cells need to maintain high K^+ levels under salt stress to maintain normal metabolic reactions (Sairam and Tyagi 2004), and K^+ and Na^+ homeostasis in plants is important for salt tolerance (Horie et al. 2001).

To reduce the toxicity of the accumulation of Na^+ in the leaves, a mass of compatible organic solutes are accumulated in salt-stressed leaves. Free amino acids were found as important osmotically active organic solutes (Hartzendorf and Rolletschek 2001), and the compounds may be involved in osmotic adjustment, ion transport, enzyme synthesis, free radical scavenging and redox homeostasis (Yamamoto et al. 2003; Rai 2002). Accumulation of free amino acids in leaves has been interpreted as an indicator of salt tolerance (Kumar et al. 2003). Understanding the changes in free amino acid composition and content in leaves may further explain the mechanism of plant salt tolerance.

Caragana korshinskii Kom (*Caragana*), a native deciduous shrub, is mainly distributed in arid and semi-arid areas of northwest China, such as Shaanxi, Gansu and Inner Mongolia. It is commonly used to control desertification and for vegetation rehabilitation in these areas due to its high ecological values. Now soil salinity in arid and semi-arid areas of northwest China, such as Shaanxi, Gansu and Inner Mongolia, is more and more serious because of lower precipitation and greater evapotranspiration, and physiological characters of *Caragana* may be affected by salt stress. Previous studies have focused on seed germination (Li et al. 2006; Zheng et al. 2004), water consumption (Cheng et al. 2009), water metabolism (Ma et al. 2003), water use efficiency (Ma et al. 2003, 2004) or drought tolerance (Xiao et al. 2005) of *Caragana*. However, little information is available about photosynthesis, chlorophyll fluorescence, leaf ions and free amino acids of the xerophilous plants under salinity stress.

To understand the physiological response under salt stress, photosynthesis, PSII efficiency, ion content and free amino acid content in leaves of *Caragana* exposed to different levels of salinity were investigated. The objectives of this study were to investigate: (1) the major factors affecting photosynthetic gas exchange and the utilization and dissipation of excitation energy in PSII after salt stress, and the evidence for connection between photosynthesis and chlorophyll fluorescence parameters; (2) K^+ and Na^+ accumulation and homeostasis in leaves under salt stress; and (3) the response of free amino acids to salt stress and the accumulation of free amino acids in leaves, so as to provide a better understanding of the physiological response of *Caragana* and other xerophilous plants to salt stress.

Materials and methods

Plant cultivation

The study was carried out in Northwest A & F University in April and September 2010. One-year-old seedlings of *Caragana,* collected from Wuwei, Gansu Province of northwest China, were planted in pots (29 cm in diameter and 25 cm in height) containing sand. Plants were grown in a glasshouse with natural light, supplied with full strength modified Hoagland nutrient solution (6 mM KNO_3, 4 mM $Ca(NO_3)_2{\cdot}4H_2O$, 2 mM $NH_4H_2PO_4$, 1 mM $MgSO_4{\cdot}7H_2O$, 25 μM KCl, 12.5 μM H_3BO_3, 1 μM $MnSO_4{\cdot}H_2O$, 1 μM $ZnSO_4{\cdot}7H_2O$, 0.25 μM $CuSO_4{\cdot}5H_2O$, 0.25 μM Na_2MoO_4 and 26.95 g l^{-1} FeNa-EDTA) to maintain adequate nutrition and received full watering. Ninety days after growing in the glasshouse, the plants were transferred to a climate chamber (photosynthetic photon flux density of 600 μmol m^{-2} s^{-1} at the top of the plant, air temperature of 30 °C during the 14-h photoperiod and 20 °C during the 10-h darkness, and a relative humidity of 60 %).

Salt stress treatment and harvest

For salt stress treatment, the seedlings were irrigated daily with full strength modified Hoagland nutrient solution of 1.5 l containing three concentrations of NaCl, i.e., 0 (control), 100 and 300 mM. Each treatment was replicated with nine seedlings. At 1, 9 and 18 day (s) after treatments began, three seedlings of each treatment were harvested after the measurements of photosynthetic gas exchange and chlorophyll fluorescence. When harvesting, plants were divided into leaves, young branches (newly developed branches after the onset of transplantation) and old branches (older developed branches before the onset of transplantation), young roots (newly developed roots after the onset of transplantation) and old roots (older developed roots before the onset of transplantation) according to the differences in color. Dry masses of these organs were calculated from fresh masses of harvested materials and the ratio of fresh-to-dry mass determined by sampling.

Gas exchange measurements

Three mature leaves of each plant were selected for gas exchange measurements. Net photosynthetic rate (*A*),

stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (C_i) were determined using a portable photosynthesis system (LiCor-6400; LiCor Inc. Lincoln, Nebraska, USA) with an attached LED light source (6400-02B). The measurements were carried out from 8:00 to 11:00 a.m. with a light intensity of 1,000 μmol photon m^{-2} s^{-1}. The leaf area of *Caragana* in the leaf chamber was obtained by measurements with a leaf area meter and photosynthetic parameters were calculated. *Caragana* leaves dropped easily after salt stress, so we were very careful during the measurements.

Leaf chlorophyll fluorescence yield measurements

The same leaves used for gas exchange measurements were used to measure the chlorophyll fluorescence. Chlorophyll fluorescence was measured with a modulated chlorophyll fluorometer (IMAGING-PAM, Walz GmbH, Germany). A weak light which was too low to induce any significant variable fluorescence was used and the minimum fluorescence yield (F_o) was measured. Subsequently, the maximum fluorescence yield (F_m) was measured by application of a 0.8 s saturating pulse of light of 5,000 μmol photon m^{-2} s^{-1}. A continuous actinic illumination was then applied and the steady-state fluorescence yield (F_s) was recorded after a period of time. The maximum fluorescence in the light (F'_m) was measured by another saturating pulse, and the minimum fluorescence in the light (F'_o) derived from IMAGING-PAM software was calculated as $F'_o = F_o/(F_v/F_m + F_o/F'_m)$. All samples were in a dark-adapted state (30 min) to ensure all energy-dependent quenching was relaxed before the measurements.

The maximal efficiency of PSII (F_v/F_m) was calculated as $(F_m - F_o)/F_m$. Actual efficiency of PSII (Φ_{PSII}) and the efficiency of excitation capture by open PSII centers (F'_v/F'_m) were calculated as $(F'_m - F_s)/F'_m$ and $(F'_m - F'_o)/F'_m$, respectively. Photochemical quenching (qP) was calculated as $(F'_m - F_s)/(F'_m - F'_o)$. Non-photochemical quenching (NPQ) was calculated as $(F_m - F'_m)/F'_m$. The apparent electron transport rate (ETR) was expressed as $\Delta F/F'_m \times PPFD \times 0.5 \times 0.84$ (Maxwell and Johnson 2000).

Determination of sodium and potassium contents in leaves

To analyze Na^+ and K^+ concentrations in leaves, fresh leaves were milled to fine powder with a mortar and pestle in liquid nitrogen. About 250 mg of fine powder was digested in a mixture (10 ml concentrated HNO_3 and 1 ml concentrated $HClO_4$) at 100 °C. Subsequently, Na^+ and K^+ were determined by an atomic absorption spectrophotometer (ZL-5100, PerkinElmer Ltd., Waltham, MA, USA).

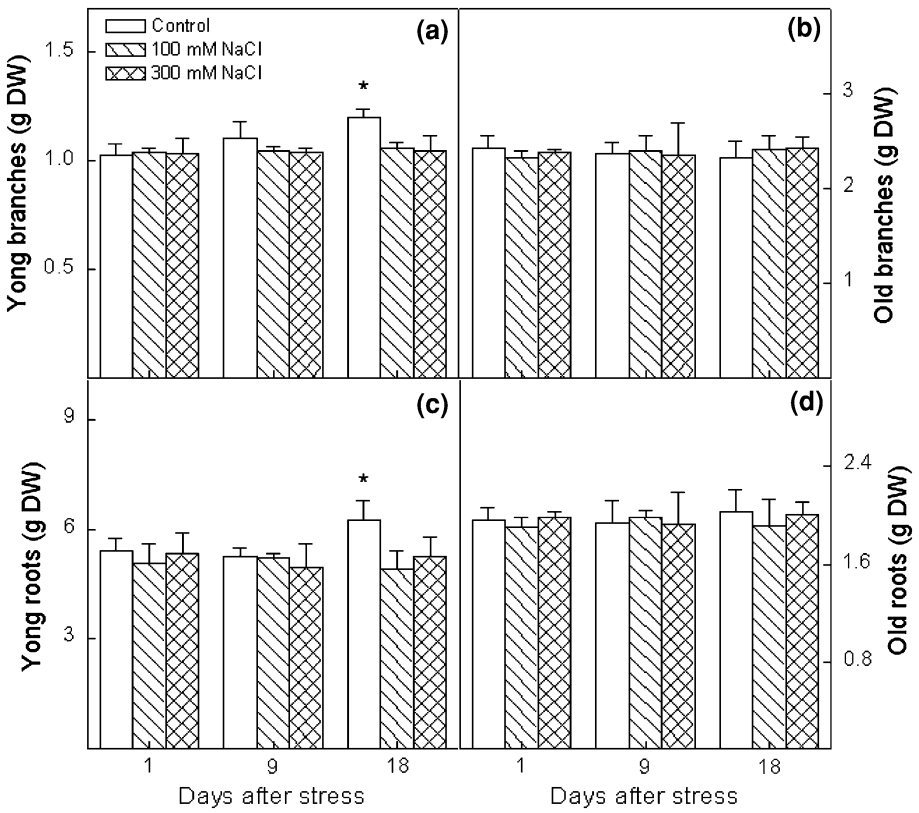

Fig. 1 Dry masses of young branches (**a**), old branches (**b**), young roots (**c**) and old roots (**d**) of *Caragana korshinskii* Kom exposed to three levels of salinity (0, 100 or 300 mM NaCl) for 1, 9 or 18 day(s). Data points are mean ± SD ($n = 3$). *Asterisk* indicates significance

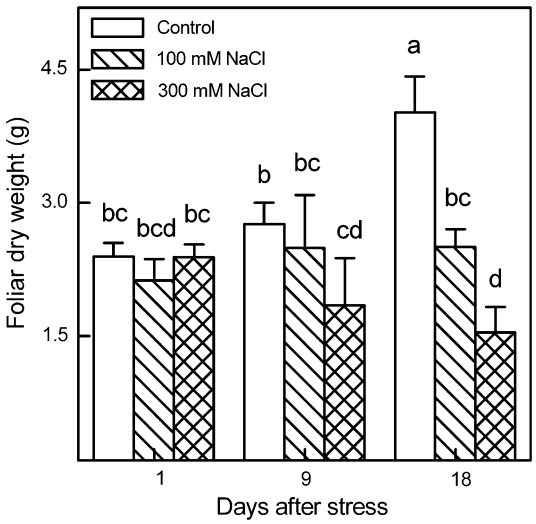

Fig. 2 Foliar dry mass of *Caragana korshinskii* Kom exposed to three levels of salinity (0, 100 or 300 mM NaCl) for 1, 9 or 18 day(s). Data points are mean ± SD ($n = 3$). *Different letters* indicate significant difference between the treatments

Determination of free amino acids in leaves

About 1 g of fresh powder was extracted in 10 ml of 80 % alcohol for 24 h. During the extraction, the solution was taken in the ultrasonic bath for 10 min every 6 h to maximize the production of free amino acids. Subsequently, the extract was filtered and evaporated in a water bath at 70 °C. Then, the extract was dissolved in 10 ml of 0.2 mol/l citric acid buffer (pH 2.2). After centrifugation (10,000*g*, 15 min, 25 °C), the supernatant was collected and subjected to an automatic amino acid analyzer (Beckmann 121 MB, Beckman Coulter Inc., Indianapolis, IN, USA).

Statistical analysis

Two-way analysis of variance (ANOVA) was performed by SPSS12.0 software to investigate salinity and sampling time on physiological parameters. Treatment means were compared for any significant differences using the Duncan's multiple range tests at a significance level of $P = 0.05$ using the SPSS12.0 for Windows software package. Variations in CO_2 assimilation rates were related to variations in fluorescence parameters using linear regression.

Results

Growth performance

The evolution of growth performance with salt treatment is shown in Fig. 1. No difference in dry masses of young branches and roots of *Caragana* exposed to three salinity levels were observed at 9 days of salinity treatment. However, after 18 days of salt treatment, the dry masses of young branches and roots exposed to NaCl were

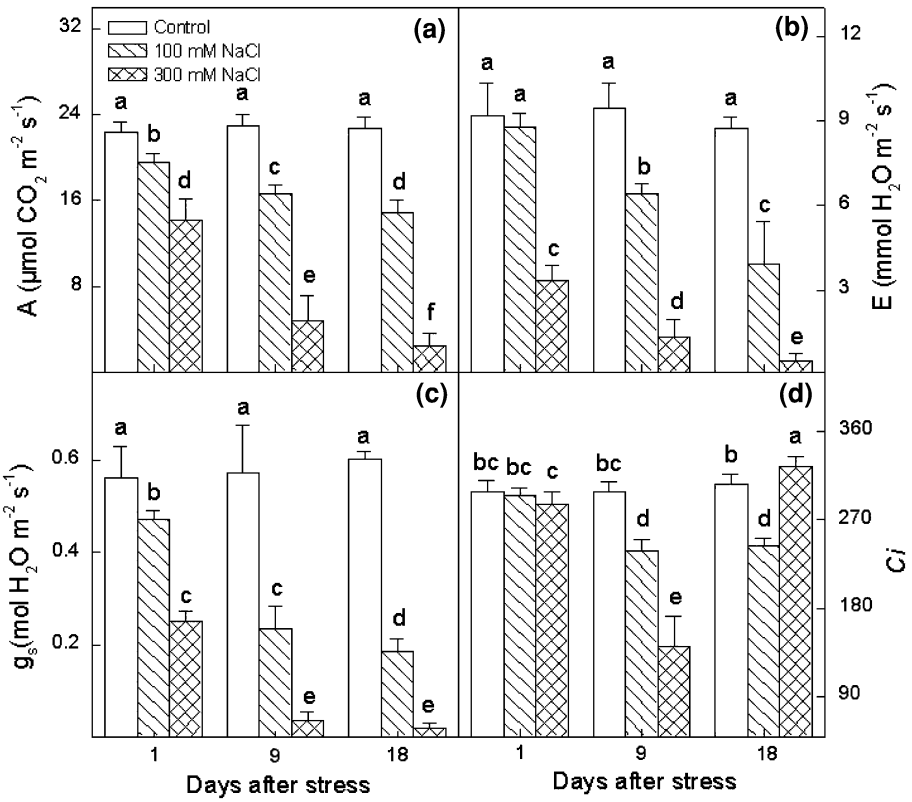

Fig. 3 Gas exchange of *Caragana korshinskii* Kom exposed to three levels of salinity (0, 100 or 300 mM NaCl) for 1, 9 or 18 day(s). Data points are mean ± SD ($n = 9$). *Different letters* indicate significant difference between the treatments. **a** Net photosynthetic rate (A), **b** transpiration rates (E), **c** stomatal conductance (g_s) and **d** internal CO_2 concentrations (C_i)

significantly decreased in comparson to the control (Fig. 1a, c). No significant differences between salt-treated plants and control plants on dry masses of old branches and roots were observed during the experiment (Fig. 1b, d).

When compared with the control plants, foliar dry mass was significantly decreased after 9 days of salt treatment, indicating that leaves were more sensitive to NaCl than branches and roots. At 18 days of salt treatment, mean foliar dry mass was 4.0, 2.5 and 1.5 g for 0, 100 and 300 mM NaCl-treated plants, respectively (Fig. 2).

Photosynthetic gas exchange

Salt treatment induced significant decreases in A of *Caragana* paralleled by the decreases in E and g_s at 1 day after the initiation of salt treatment, and decreasing continuously with the increase of treatment time and salt concentration. The minimum values of A, E and g_s were observed after 18 days of 300 mM NaCl (Fig. 3a–c).

Following the decrease in g_s, reductions in C_i were observed at 1 or 9 days after the initiation of salt treatment. However, higher C_i was observed after 18 days of 300 mM NaCl (Fig. 3d).

Chlorophyll fluorescence

The evolution of chlorophyll fluorescence of *Caragana* with salt treatment is shown in Fig. 4. F_v/F_m was almost constant at 1 day after initiation of salt treatment (Fig. 4a). However, the decrease in F'_v/F'_m was detected in light-adapted leaves at the same time (Fig. 4b). The same trend for Φ_{PSII} and ETR was observed as F'_v/F'_m, but NPQ

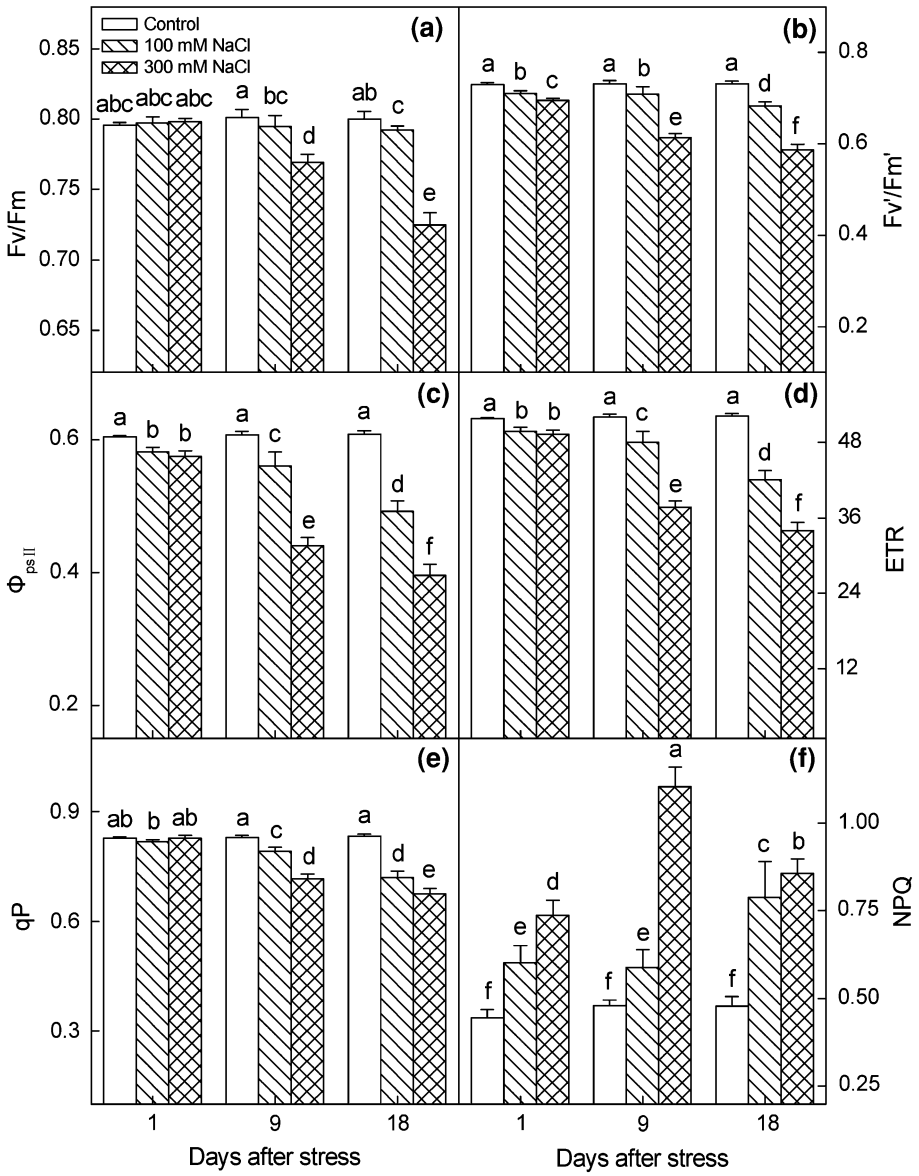

Fig. 4 Chlorophyll fluorescence of *Caragana korshinskii* Kom exposed to three levels of salinity (0, 100 or 300 mM NaCl) for 1, 9 or 18 day(s). Data points are mean ± SD ($n = 9$). *Different letters* indicate significant difference between the treatments. **a** Maximal efficiency of PSII (F_v/F_m). **b** The efficiency of excitation capture by open PSII centers (F'_v/F'_m). **c** Actual efficiency of PSII (Φ_{PSII}). **d** The apparent electron transport rate (ETR). **e** Photochemical quenching (qP). **f** Non-photochemical quenching (NPQ)

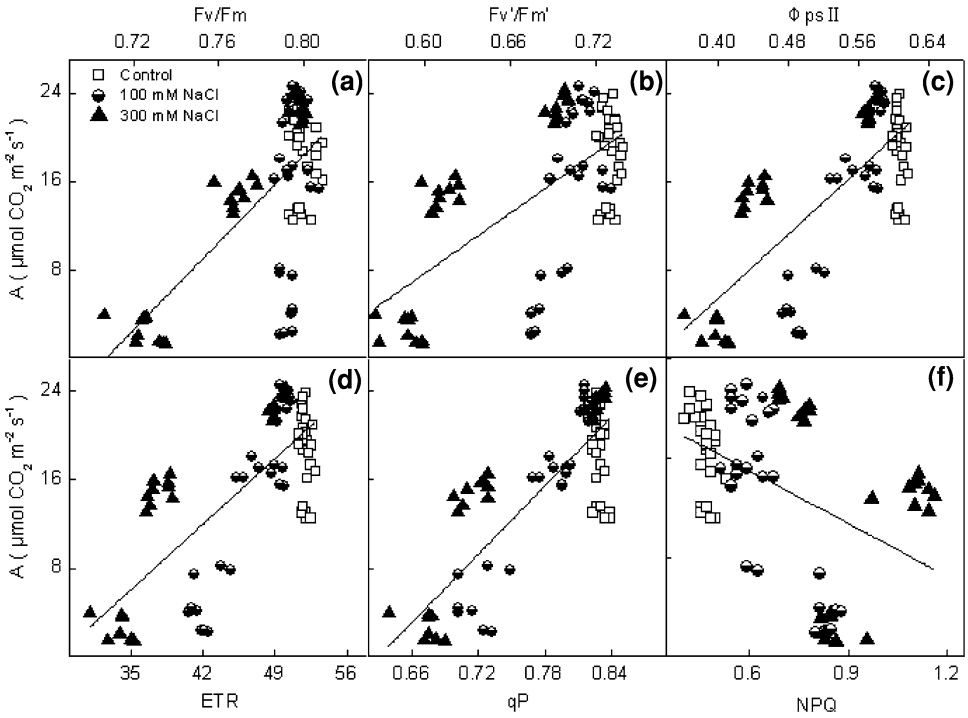

Fig. 5 The correlations between net photosynthetic rate and chlorophyll fluorescence parameters of *Caragana korshinskii* Kom exposed to three levels of salinity (0, 100 or 300 mM NaCl) for 1, 9 or 18 day(s). Correlation coefficients (R_s) were $R^2 = 0.427$ (**a**), $R^2 = 0.401$ (**b**), $R^2 = 0.564$ (**c**), $R^2 = 0.564$ (**d**), $R^2 = 0.686$ (**e**) and $R^2 = 0.214$ (**f**), respectively. All correlations were significant ($P < 0.05$)

increased significantly with the increase of salt concentration (Fig. 4c, d, f).

At 9 days with salt treatment, F_v/F_m was significantly reduced, indicating the possible occurrence of damage in PSII centers (Netondo et al. 2004). F_v/F_m continually declined with the increase of salt treatment time and concentration, and minimum F_v/F_m was observed at 18 days with 300 mM NaCl (Fig. 4a). A similar trend for F'_v/F'_m, Φ_{PSII}, ETR and qP measured in light-adapted status was observed as F_v/F_m at 9 or 18 days with salt treatment (Fig. 4b–e). However, NPQ was significantly increased at higher levels of salinity after 9 and 18 days (Fig. 4f), indicating that some excess excitation energy was dissipated thermally as a result of salt stress.

Correlation between photosynthetic parameters and chlorophyll fluorescence

A linear correlation was observed between photosynthetic gas exchange and chlorophyll fluorescence. *A* had a positive linear correlation with F_v/F_m ($R^2 = 0.427$, $P < 0.05$) (Fig. 5a), F'_v/F'_m ($R^2 = 0.401$, $P < 0.05$) (Fig. 5b), Φ_{PSII} ($R^2 = 0.564$, $P < 0.05$) (Fig. 5c), ETR ($R^2 = 0.564$, $P < 0.05$) (Fig. 5d) and qP ($R^2 = 0.686$, $P < 0.05$) (Fig. 5e). However, a negative linear correlation was observed between *A* and NPQ ($R^2 = 0.214$, $P \leq 0.05$) (Fig. 5f), which provided evidence for a link between photosynthetic and chlorophyll fluorescence parameters under salt stress.

Contents of sodium and potassium in leaves

There was significant accumulation of Na^+ in salt-treated leaves of C*aragana* at 1 day with 300 mM NaCl. The content of Na^+ in salt-treated leaves increased with the increase of treatment time. The accumulation of Na^+ in salt-treated leaves reached a maximum at 18 days with 300 mM NaCl (Fig. 6a). However, the accumulation of K^+ varied from that of Na^+ in salt-treated leaves. K^+ content in salt-treated leaves did not change during the experimental period (Fig. 6b). The ratio of Na^+ to K^+ in salt-treated leaves increased with the increase of salt treatment time and concentration. The ratio of Na^+ to K^+ with 300 mM NaCl was above 0.6, about seven times that of control at 18 days with salt treatment (Fig. 6c).

Contents of free amino acid in leaves

Seventeen free amino acids in leaves were detected in this experiment. Total and individual free amino acids did not change significantly after 1 day of salt treatment. The most abundant individual free amino acids were aspartic acid, glutamic acid, proline and serine, accounting for more than 70 % of total amino acids in both salt-treated and control leaves. However, total and individual free amino acids in salt-treated leaves increased significantly after 9 days of salt treatment, which mainly resulted from the accumulation of some individual free amino acids such as alanine, aspartic acid, glutamic acid, proline, serine and threonine.

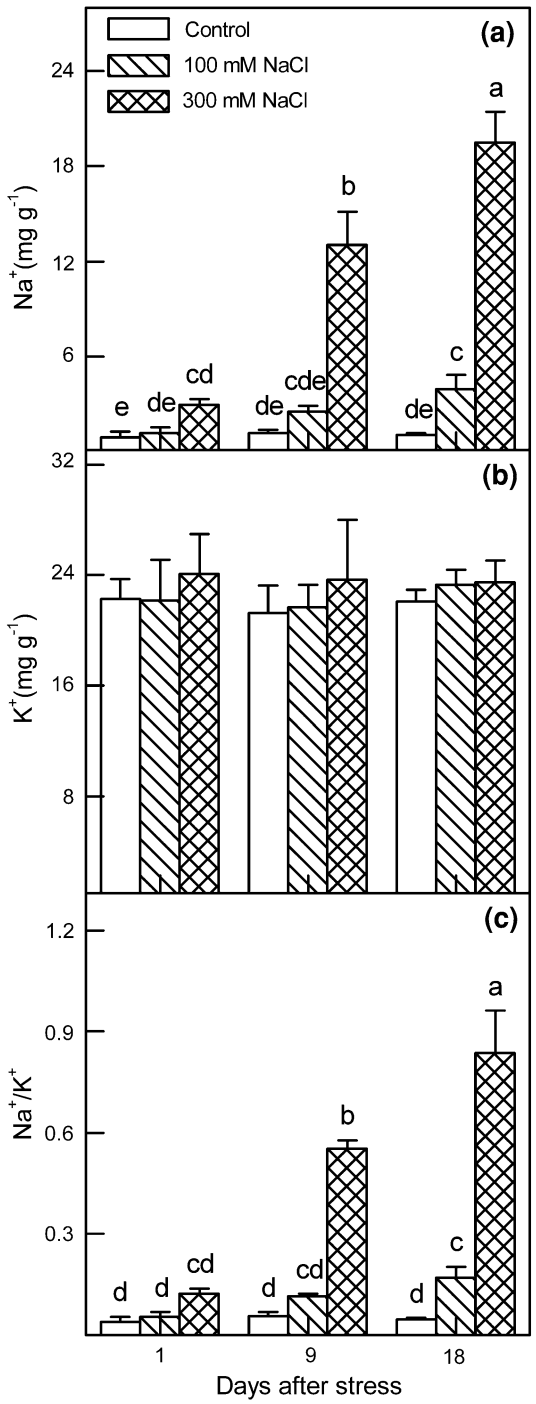

Fig. 6 Concentrations of Na^+ (**a**) and K^+ (**b**), and ratio of Na^+ to K^+ (**c**) in leaves of *Caragana korshinskii* Kom exposed to three levels of salinity (0, 100 or 300 mM NaCl) for 1, 9 or 18 day(s). Data points are mean ± SD ($n = 3$). *Different letters* indicate significant difference between the treatments

Among these free amino acids, accumulation of proline with 100 mM or 300 mM NaCl treatment was the greatest, about 6 or 12 times that of control. After 18 days of salt treatment, the contents of total and individual free amino acids in salt-treated leaves were still higher than the control, and accumulation of proline was also the greatest (Table 1).

Discussion

The effects of salt stress on different organs of *Caragana* were different. The growth of young branches and roots was susceptible to salinity, but the growth of old branches and roots was stable under salt stress. Leaf senescence and shedding led to significant reduction in leaf dry mass under salt stress.

Plants suffering from excess salinity decreased the productivity, and such reduction was often related to the decrease in photosynthetic capacity. However, the inhibition of photosynthesis may have different mechanisms (Jamil et al. 2007; Kao et al. 2006). Both stomatal and non-stomatal factors are implicated in the inhibition of photosynthesis after salt stress (Kozlowski 1997). Stomatal factors are generally significant at medium salinity but non-stomatal factors are significant at high salinity (Everard et al. 1994). In this study, the decreases in A and g_s were accompanied by the decrease in C_i at the initial and middle periods of the experiment (Fig. 3a, c, d), which indicated that the reduction in g_s was responsible for the inhibition of photosynthesis. However, the decreases in A and g_s, but higher C_i, were present at 18 days with 300 mM NaCl (Fig. 3a, c, d), suggesting that a non-stomatal factor was responsible for the inhibition of photosynthesis under severe salt stress.

Chlorophyll fluorescence analysis has proven to be a sensitive method for the detection and quantification of stress-induced changes in PSII (Mehta et al. 2010), and both light- and dark-adapted measurements can be used to determine whether photodamage has occurred in leaves or not (Naumann et al. 2008). In our study, the changes of PSII reaction centers under salt stress consisted of two different phases. At 1 day with salt treatment, the decrease in photosynthesis (Fig. 3a) coincided with the increase in NPQ and the decrease in F'_v/F'_m, ETR and Φ_{PSII} (Fig. 4b, c, d, f). The increase in NPQ has been thought to be a photoprotective mechanism to dissipate excess excitation energy (Demmig-Adams and Adams 1992), in which a higher proportion of absorbed photons are lost as thermal energy instead of being used to drive photosynthesis (Shangguan et al. 2000), leading to down-regulation of PSII to avoid over-reduction of primary electron acceptors (Epron et al. 1992; Genty et al. 1989). These changes in chlorophyll fluorescence parameters, with no significant decrease in F_v/F_m (Fig. 4a), suggest that increased photoprotection can help leaves avoid photodamage.

Table 1 Concentrations of total and individual free amino acids in leaves of *Caragana korshinskii* Kom exposed to three levels of salinity (0, 100 or 300 mM NaCl) for 1, 9 or 18 day(s)

Free amino acids	1 d			9 d			18 d		
	Control	100 mM	300 mM	Control	100 mM	300 mM	Control	100 mM	300 mM
Total free amino acid	176.6 ± 43.3d	205.5 ± 26.1cd	209.7 ± 42.6cd	173.0 ± 24.6d	629.3 ± 240.5a	777.4 ± 151.9a	174.5 ± 6.18d	399.3 ± 146.4bc	411.1 ± 28.4b
Alanine	12.6 ± 2.0bc	11.4 ± 0.5c	12.7 ± 2.4bc	9.8 ± 1.3c	22.2 ± 8.0a	25.1 ± 4.4a	11.0 ± 1.8c	21.7 ± 8.2a	20.0 ± 3.4ab
Arginine	2.6 ± 1.4ab	1.3 ± 0.5b	3.3 ± 2.7ab	1.4 ± 1.2b	6.1 ± 4.8a	5.8 ± 1.7a	2.2 ± 1.3ab	3.4 ± 1.9ab	3.4 ± 1.7ab
Aspartic acid	31.3 ± 4.9cd	35.2 ± 6.1cd	34.7 ± 9.2cd	22.3 ± 2.7d	68.2 ± 23.7a	53.0 ± 20.3abc	34.4 ± 3.3cd	59.3 ± 17.8ab	38.7 ± 2.3bcd
Cystine	1.1 ± 0.7b	2.2 ± 1.8ab	2.3 ± 0.3ab	1.3 ± 0.5b	2.4 ± 2.3ab	2.5 ± 0.6ab	2.1 ± 0.7ab	2.6 ± 0.1ab	4.2 ± 1.6a
Glutamic acid	37.8 ± 23.4ab	52.2 ± 17.0ab	52.8 ± 12.6ab	23.2 ± 4.4b	79.6 ± 38.1a	77.6 ± 33.3a	43.1 ± 4.6ab	77.3 ± 23.8a	48.2 ± 2.9ab
Glycine	1.9 ± 1.4bcd	1.6 ± 0.6cd	3.1 ± 1.6bc	1.3 ± 0.5d	2.4 ± 1.1bcd	4.9 ± 0.2a	1.2 ± 0.4d	2.3 ± 0.4bcd	3.5 ± 0.7ab
Histidine	0.5 ± 0.1a	0.8 ± 0.4a	0.8 ± 0.3a	0.3 ± 0.3a	1.5 ± 0.9a	1.5 ± 1.2a	0.8 ± 0.1a	1.0 ± 1.1a	0.9 ± 0.6a
Isoleucine	1.8 ± 0.8b	1.5 ± 0.4b	2.6 ± 1.0ab	1.2 ± 0.7b	1.8 ± 0.40b	5.8 ± 3.2a	2.2 ± 1.6b	4.1 ± 2.6ab	3.6 ± 2.6ab
Leucine	1.4 ± 0.6bcd	0.9 ± 0.5bcd	1.4 ± 0.7bcd	0.4 ± 0.4d	1.5 ± 0.3bcd	3.3 ± 0.5a	0.7 ± 0.5cd	2.0 ± 1.3b	1.7 ± 0.2bc
Lysine	2.6 ± 0.5b	2.4 ± 1.7b	2.9 ± 0.5b	0.9 ± 0.8b	4.1 ± 2.0b	9.2 ± 6.2a	2.8 ± 2.4b	2.1 ± 1.7b	1.5 ± 1.0b
Methionine	1.9 ± 1.6ab	1.9 ± 0.7ab	1.4 ± 0.4ab	1.3 ± 0.6ab	1.5 ± 0.3ab	1.6 ± 0.6ab	1.2 ± 0.9b	2.0 ± 0.6ab	3.2 ± 2.0a
Phenylalanine	3.6 ± 1.4ab	3.3 ± 1.4ab	3.9 ± 2.0ab	2.6 ± 0.3ab	6.1 ± 4.1a	2.9 ± 1.5ab	2.2 ± 1.2b	2.8 ± 1.4ab	2.9 ± 1.1ab
Proline	35.1 ± 11.5c	43.5 ± 9.5c	40.0 ± 14.3c	39.3 ± 2.9c	237.0 ± 102.5b	454.2 ± 71.6a	21.8 ± 2.0c	98.8 ± 50.5c	181.4 ± 16.7b
Serine	25.4 ± 4.7d	33.1 ± 3.5cd	31.3 ± 8.8cd	55.7 ± 12.3bcd	159.9 ± 52.4a	98.7 ± 15.7b	38.4 ± 3.1cd	96.1 ± 42.5b	74.9 ± 13.2bc
Threonine	11.6 ± 2.7b	9.8 ± 2.3b	9.9 ± 2.6b	8.8 ± 1.9b	29.4 ± 22.0a	22.6 ± 6.0ab	7.3 ± 1.6b	18.4 ± 4.7ab	15.3 ± 1.4ab
Tyrosine	2.3 ± 0.7a	1.7 ± 1.0a	1.8 ± 0.1a	1.6 ± 0.6a	3.2 ± 3.0a	1.8 ± 0.5a	1.3 ± 0.6a	1.5 ± 0.7a	2.8 ± 1.3a
Valine	3.2 ± 2.6b	2.7 ± 1.6b	4.6 ± 2.0ab	1.7 ± 0.8b	2.4 ± 1.0b	7.2 ± 1.8a	2.0 ± 1.3b	3.9 ± 1.1b	5.0 ± 2.5ab

Values indicate mean ± SD ($n = 3$). Different letters indicate significant difference between the treatments

At 9 days with NaCl treatment, along with the increase in NPQ and the decreases in F'_v/F'_m, ETR, Φ_{PSII} and qP (Fig. 4b–f), F_v/F_m significantly decreased (Fig. 4a). The increases in NPQ suggested the occurrence of photoprotection. However, protective defense is not sufficient to avoid oxidative damage (da Silva et al. 2011). Damages to the PSII reaction center and non-stomatal limitations on photosynthesis were imposed in the process. However, the reduction in stomatal conductance remained responsible for the inhibition of photosynthesis. At 18 days with 300 mM NaCl, the decrease in A and g_s was accompanied by an increase in C_i, showing that a non-stomatal factor was responsible for the inhibition of photosynthesis.

Na^+ is the main toxic ion in saline soils for most plants (Lunde et al. 2007). The influx and accumulation of Na^+ competes with K^+, and there is a decrease in K^+ uptake and an increase in Na^+ influx in plant cells during salt stress (Serrano and Rodriguez-Navarro 2001), which is confirmed by a study of wheat (Athar et al. 2008). However, K^+ content in leaves with salt stress did not significantly decrease compared with the control in this study (Fig. 6b), indicating that K^+ uptake by *Caragana* did not compete with Na^+, which is consistent with an earlier study on sea buckthorn (*Hippophae rhamnoides* L.) that is able to grow well under extreme environments, as is *Caragana*. The phenomenon of a lack of competition between Na^+ and K^+ absorption is speculated as a unique pathway for Na^+ absorption in these plants (Chen et al. 2009).

Salt stress had no effect on K^+ content in leaves, but increased the ratio of Na^+ to K^+ (Fig. 6c). The ratio of Na^+ to K^+ is also used to explain physiological parameters during salt stress, in addition to Na^+ and K^+ content, because a higher ratio of Na^+ to K^+ may cause metabolic disorders such as a reduction of protein synthesis and enzyme activities (da Silva et al. 2008). In non-halophytic plants, it is necessary to keep the ratio of Na^+ to K^+ lower than or equal to 0.6 for an optimal metabolic efficiency (Greenway and Munns 1980). However, at 18 days with 300 mM NaCl, the ratio of Na^+ to K^+ was above 0.6, suggesting that metabolic disorders may occur.

Free amino acids are directly or indirectly involved in the regulation of plant responses to environmental signals related to abiotic or biotic stress (Ashraf and Harris 2004). Our results suggest that there were no changes in total and individual free amino acids in salt-treated leaves after 1 day of salt treatment. However, total and individual free amino acids in salt-treated leaves dramatically increased at 9 days with salt treatment, suggesting the existence of a critical salinity level in *Caragana*. Total and individual free amino acids in salt-treated leaves slowly accumulated below the critical stress level, but greatly above the critical stress level, which is in agreement with the result of *phragmites australis* (Hartzendorf and Rolletschek 2001). The accumulation of free amino acids may be involved in osmotic adjustment, free radical scavenging and maintenance of protein and membrane integrity (Keutgen and Pawelzik 2008).

Among all individual free amino acids, proline content in salt-treated leaves increased significantly in this study, which agrees with a previous study (Aziz et al. 1999). The accumulation of proline during salt stress mainly results from increased synthesis and reduced degradation (Verbruggen and Hermans 2008). Proline serves as an important compatible osmolyte, and its accumulation is believed to reduce cellular water potential and avoid deleterious toxicity of high ionic strength (Hare and Cress 1997). Proline has also been proposed to serve as reactive oxygen species scavenger (Smirnoff and Cumbes 1989) and molecular chaperone (Verbruggen and Hermans 2008), and its accumulation can stabilize the structure of membranes and proteins to minimize the damage of cells under salt stress.

In summary, the reduction in g_s was responsible for the inhibition of photosynthesis at the initial and middle periods of the experiment. However, at 18 days with 300 mM NaCl treatment, a non-stomatal factor was responsible for the inhibition of photosynthesis. Salt stress did not decrease K^+ content of leaves, but increased Na^+ concentration and Na^+/K^+ ratio, indicating that unavoidable metabolic disorder occurred. As an important osmotically active organic solute, free amino acids in salt-treated leaves greatly accumulated to minimize physiological damage under salt stress when the critical stress level was exceeded.

Author contribution Prof. Xiaotao Hu made up the program of the research. Dr. Hui Yan carried out the research and wrote this manuscript under the guidance of Prof. Xiaotao Hu. Prof. Fusheng Li modified the manuscript greatly.

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