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GROWTH AND PHOTOSYNTHETIC CHARACTERISTICS OF *GLYCINE GRACILIS* SEEDINGS UNDER DIFFERENT TYPES OF SALINE STRESSES

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Abstract

Glycine gracilis was used as the experimental material. We tried to explore the difference in growth and photosynthetic characteristics of *G. gracilis* seedling under different types of salt stress, and revealed the physiological stress adaptation mechanisms for semi-wild soybean under salt stress. The results showed that low concentration salt stress increased the parameters of growth, but they were inhibited when plants were subjected to high concentration NS and AS. The inhibition effect of NS and low concentrations of AS on the total photosynthetic pigments and chlorophyll contents was not significant, and they promoted the accumulation of carotenoids and chlorophyll *a*/*b*. High concentrations of AS significantly inhibited the content of chlorophyll and carotenoids and reduced the value of chlorophyll *a*/*b*. The daily changes trend and daily means in P_{N}, E, g_s, *WUE* were significantly different under two types of salt stress. P_{Nmax} increased at the beginning and then decreased as AS rose. *LCP* of *G. gracilis* was negatively correlated with stress intensity under NS, but positively correlated with stress intensity for AS. For NS, R_{esp}, V_{cmax}, $J_{\max}$ and V_{TPU} increased at first and then decreased as the salt concentration increasing. However, they gradually declined for AS. Studies have confirmed that the range for the inhibition effect on the growth of *G. gracilis* seedlings were different for the two types salt stress. The net photosynthetic rate of *G. gracilis* also decreased for different reasons. These results also showed that *G. gracilis* could adapt to the salt stress in certain extent.

Key words: *Glycine gracilis*; Saline stresses; Growth; Photosynthetic characteristics.

Abbreviations: AS - alkali salts stress; *CE* - carboxylation efficiency calculated from CO_2 response curve; DM - dry mass; E - transpiration rate; EC - conductance; g_s - stomatal conductance; $J_{\max}$ - maximum electron transport; *LCP* - light compensation point; *LSP* - light saturation point; NS - neutral salts stress; p_{N} - net photosynthetic rate; p_{Nmax} - maximum net photosynthesis; p_{Nsat} - light-saturated rate of net photosynthesis; *QE* - apparent quantum efficiency; R_{esp} - respiration rate; RGR - relative growth rate; V_{cmax} - maximum RUBP carboxylation rate; V_{TPU} - maximum rate of triose-phosphate utilization; WC - water content; *WUE* - water use efficiency.

Introduction

The earth, especially cultivated land, has been considerably affected by the transitional use of natural resources, the rapid increase in population and the effects of global climate change. Land resources have been lost because of increasing salinization, and food security is becoming a very important issue (Turner *et al*., 1990; Pawl *et al*., 1998; Tang *et al*., 2008; Abbas *et al*., 2013). More than 100 countries around the world are affected by soil salinization. According to incomplete UNESCO statistics, the land area affected by saline-alkali salinization is more than 900 million hectares and China has more than 9000 hectares. Soil salinization has always been one of the most important problems affecting the development of farming and animal husbandry because salinity-alkalinity stress can severely affect plant and crop growth (Khan *et al*., 2000; Munns, 2002; Yang *et al*., 2007).

The effects of soil salinity on plant growth include: osmotic stress, nutritional effects, ion toxicity and physiological metabolic disorders (Wu, 2003). Neutral salt stress causes osmotic stress and ion toxicity and alkaline salt stress also causes high pH stress (Shi *et al*., 2002). Photosynthesis is an important metabolic process and if photosynthesis is damaged, then plants cannot grow normally. When plants suffer from salt stress, activity and content of RuBisco decrease and carbon assimilation declines (Sivakumar *et al*., 2000). In addition, PSII light capturing efficiency is reduced and electron transfer is inhibited, which reduces PSII activity (Yang *et al*., 2006). Therefore, studies on the effects of salt stress on photosynthesis are needed in order to improve salt stress resistance in plants and increase crop yields.

Leguminous plants are found all over the world and many leguminous crops have some tolerance to saline soils (Zheng *et al*., 2007; Zhang *et al*., 2009). Nitrogen fixation by leguminous plants can enrich the land and improve soil characteristics. Many studies have confirmed that cultivated soybean photosynthesis is damaged by high soil salt concentrations (Cao, 2007; Qu *et al*., 2009), but wild soybeans have been shown to be able to adapt to salt stress (Kao *et al*., 2003).

Therefore, this experiment selected the leguminous plant, *Glycine gracilis*, as the experimental material. Commonly known as semi-wild soybean, it is a natural hybrid species of wild soybean and cultivated soybean and is the transitional species between wild soybeans and cultivated soybean types in terms of morphological structure and physiological metabolism (Zhuang, 1999; Wang & Li, 2000). To date, research into leguminous plant photosynthesis under salinity stress has mainly focused on the cultivated soybean, rather than wild soybean and semi-wild soybean, and very few studies have attempted to distinguish between different types of salt stress.

River sand was chosen as the culture medium and the *G. gracilis* plants were grown in pots. During the seedling growth period, the plants were stress treated by gradient concentrations of neutral salts and alkali salts. We investigated the changes in the growth and photosynthetic characteristics of semi-wild soybean seedlings under different types of salt stress by measuring seedling height, biomass, photosynthetic pigment content, photosynthetic rate and other photosynthetic physiological parameters. This study showed that semi-wild soybean seedlings were able to physiologically adapt to salt stress. We provide a theoretical basis for semi-wild soybean salt stress resistance and lay a foundation for further research into the evolution of adversity physiology and of leguminous plants.

Materials and Methods

Plant materials and experimental design: *G. gracilis* seeds were provided by Jilin Academy of Agriculture Science, China. The seeds were sown in 25 cm diameter plastic pots containing washed river sand. Each pot contained eight seedlings, which were adequately watered with Hoagland nutrient solution on a daily basis. All the pots were placed outdoors in Northeast Normal University campus and sheltered from the rain. All the experiments were carried out in May and June 2012. During the experimental periods, humidity was maintained at 60% and the temperature was 24–28°C during the day and 17–20°C at night.

The stress treatments were applied when the seedlings were 6 weeks old. NaCl and Na_2SO_4 were mixed in a 1:1 molar ratio and applied to the neutral salt stress group. $NaHCO_3$ and Na_2CO_3 were mixed in a 1:1 molar ratio for the alkali salt stress group. Within each group, the total Na^+ concentrations were applied at 30, 60, 90 and 120 $mmol·L^{-1}$. The stress treatment solutions included Hoagland nutrient solution. The pH values were 6.91–6.82 and 9.53–9.85 in the neutral salt stress and alkali salt stress groups, respectively, and 1× Hoagland nutrient solution without the added salts was used as the control (Table 1). Three pots were selected before stress treatment and their biomass parameters and relative growth rates were measured. Each group was replicated three times. The stress treatments began at about 18:00, through irrigation with about 500 ml stress treatment solution.

Table1. Experimental designed.

Plots	Na^+ Concentration ($mmol·L^{-1}$)	EC ($\mu s·cm^{-1}$)	pH
Contral	0	1950	6.91
	30	4000	6.88
NS	60	4190	6.85
NaCl: Na_2SO_4=1:1	90	4930	6.83
	120	5640	6.82
	30	3350	9.53
AS	60	5080	9.84
$NaHCO_3$:Na_2CO_3=1:1	90	6880	9.85
	120	8700	9.85

Measurement of growth parameters: *G. gracilis* seedlings were harvested in the evening after the final treatment in the laboratory and were first washed with tap water and then with distilled water. Growth indices for the fresh samples, including shoot height and root length, were recorded. Then the roots, stems, leaves and petioles were separated and oven dried at 100°C for 10 min, in order to stop enzyme activity, and then vacuum-dried at 75°C to a constant weight, after which the DM was recorded.

Determination of photosynthetic pigment content: Dried samples (50 mg) were put in centrifuge tubes, together with 10 ml of 80% alcohol and anhydrous ethanol (1:1) extract. The tubes were left in the dark, until all the material had turned white. The samples were then centrifuged at 3000 × *g* for 15 min and the supernatant was collected. Each sample was replicated three times. A UV-754 spectrophotometer (Shanghai precision scientific instrument, Shanghai, China) was used at 440 nm, 645 nm and 663 nm and each experimental treatment group was replicated nine times. The photosynthetic pigment content was calculated according to Shi and Guo (2006).

Determination of soluble carbohydrate content: The alcohol extraction and anthrone colorimetric method was used to determine carbohydrate content (Shi & Guo, 2006). Each pot was replicated three times and each experimental treatment group was replicated nine times.

Measurement of the daily dynamic photosynthetic rate: The p_N, g_s and E of the leaves were determined using a LI-6400 portable open flow gas exchange system (LI-COR, Lincoln, Nebraska, USA) at 2 h intervals from 08:00 through 16:00 h. WUE was calculated as the p_N/E ratio. The photosynthetically active radiation (PAR) was 1000 ± 12 $\mu mol·m^{-2}·s^{-1}$, CO_2 concentration was 350 ± 2 $cm^3·m^{-3}$ and leaf temperature was 26.0 ± 0.8°C. Gas exchange was measured on the fifth day of stress treatment. Three plants with similar growth characteristics were selected for analysis, which was repeated three times. There were 27 replications for each treatment.

Changes in photosynthetic rate under different light intensities: After 5 days of stress treatment, the automatic curve program of the LI-6400 portable photosynthesis device (LI-COR, Lincoln, Nebraska, USA) was used to determine the p_N/Q response. The LI-6400 red and blue LED was used as the light source and the control CO_2 concentration in the LI-6400 reference room was 380 $\mu mol·mol^{-1}$. The photosynthetic active radiation flux density was set at 1500, 1200, 1000, 800, 600, 400, 200, 100 and 0 $mol·m^{-2}·s^{-1}$ in order to determine the leaf p_N of *G. gracilis* under different salinity stresses. Three plants with similar growth patterns were selected for analysis and each determination was repeated three times. There were 27 replications for each treatment. LCP, LSP, p_{Nmax} were calculated using the Olsson and Leverenz (1994) and the Prioul and Chartier (1997) equations.

Changes in photosynthetic rate under different CO_2 concentrations: After 5 days of stress treatment, the p_N/C_i curve automatic measurement procedure in the LI-6400 portable photosynthesis device (LI-COR, Lincoln, Nebraska, USA) was used to determine the photosynthetic rate under different CO_2 concentrations. The luminous flux density was provided by the red and blue LED light, which was set to 1200 $mol \cdot m^{-2} \cdot s^{-1}$ (saturated light intensity). The reference room CO_2 concentration was provided by a 'to-go' CO_2 gas source (a small cylinder of liquefied CO_2) and was regulated by an automatic program control CO_2 absorber. The CO_2 concentration was set to 50, 100, 200, 400, 600, 800, 1000 and 1200 $\mu mol \cdot mol^{-1}$. The *G. gracilis* mature leaf p_N was determined within a temperature range of 25°C ± 0.5°C. Three plants with similar growth characteristics were selected and each determination was repeated three times. There were 27 replications for each treatment. Photosynthetic parameters, such as p_{Nsat}, V_{cmax}, J_{max}, V_{TPU} and *CE,* were calculated according to Von Caemmerer and Farquhar (1981) and Farquhar *et al.* (1982).

Statistical analysis: All data were expressed as mean ± SE, and each mean value was calculated from three replicates. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 17.0 statistical software (SPSS Inc., Chicago, USA). The treatment values were compared using a F-test. The term 'significant' indicates differences for which $p \le 0.05$.

Results

Growth changes: As the Na^+ concentrations in the two types of salt stress increased, the fresh weights of the shoots and roots, the dry weight of the shoots and roots, the shoot heights and the root lengths of the *G. gracilis* seedlings all significantly decreased (Fig. 1, $p<0.05$); the AS treatment caused a larger decrease than the NS treatment. The *RGR* and *WC* values for the *G. gracilis* seedlings could be calculated from the data in Fig. 1. The *RGR* very significantly decreased as the stress levels rose and the extent of the decrease was greater under AS than under NS. The *RGR* of the aboveground part of *G. gracilis* was more significant than the belowground parts. The *WC* of the roots, stems and petioles decreased as the Na^+ concentrations in both types of salt stress increased, but this decrease was not significant. There was also no significant difference between the two types of salt at any given concentration ($p>0.05$). The *WC* of the leaves significantly decreased as stress levels rose, especially under AS ($p<0.01$). However, under AS, when the Na^+ concentration was 30 $mmol \cdot L^{-1}$, the fresh weight of the shoots, the dry weights of the shoots and roots, the shoot heights and the root lengths of the *G. gracilis* seedlings were significantly higher than the control. This suggested that *G. gracilis* was adapting to salt stress.

Photosynthetic pigments: As NS increased, the total photosynthetic pigment content, total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents in *G. gracilis* seedling leaves first increased, but then decreased, whereas chlorophyll *a*/*b* gradually declined. Each parameter, total chlorophyll and chlorophyll *a*/*b* reached a maximum when the Na^+ concentration was 90 $mmol \cdot L^{-1}$. These three factors increased plant growth by 15.67%, 14.44% and 25.00%, respectively, compared to the control. This showed that *G. gracilis* seedlings could adjust their metabolic processes and accumulate photosynthetic pigment in order to alleviate salt stress up to a certain concentration of NS. Along with AS increased, total photosynthetic pigment, total chlorophyll, chlorophyll *a*, chlorophyll *b*, chlorophyll *a*/*b* and carotenoid contents in *G. gracilis* seedling represented the declining trend and were lower than the control (Table 2). Correlation analysis showed that the total photosynthetic pigment, total chlorophyll, chlorophyll *a* and carotenoid contents had a very significant negative correlation with alkali salt concentration ($r_{Pigment} = -0.96$, $r_{Chls} = -0.95$, $r_{Chla} = -0.98$ and $r_{car} = -0.96$; $p<0.01$); chlorophyll *b* and chlorophyll *a*/*b* had a significant negative correlation ($r_{Chlb} = -0.76$ and $r_{Chla/b} = -0.78$; $p<0.01$).

Photosynthetic products: As NS increased, the *G. gracilis* seedling leaf soluble carbohydrate content rose, but this increase was not significantly different to the control ($p>0.05$). Leaf soluble carbohydrate content rose as AS increased and only fell at the highest concentration. The differences between each concentration were significant and statistically higher than the control (Fig. 2). Correlation analysis showed that leaf soluble carbohydrate content was positively related, but not significantly, to NS. However, with AS, the correlation was significantly positive ($r_{NS} = 0.81$, $p>0.05$; $r_{AS} = 0.90$, $p<0.05$). Petiole soluble carbohydrate content decreased at first and then increased as NS rose. It reached its lowest value when the Na^+ concentration was 60 $mmol \cdot L^{-1}$, which was 24.41% lower than the control. Under AS, the soluble carbohydrate content in petiole was gradually declined. The carbohydrate content was 56.37% higher than the control when the Na^+ concentration was 30 $mmol \cdot L^{-1}$, but the other salt concentration treatments were significantly lower than the control. Correlation analysis showed that petiole soluble carbohydrate content was negatively correlated with the two types of salt stress, but this was not significant ($r_{NS} = -0.12$, $p>0.05$ and $r_{AS} = -0.74$, $p>0.05$). The soluble carbohydrate contents in the roots and stems showed a consistent trend under the two types of salt treatment (Fig. 2). Under the NS treatment, it increased as the stress levels rose. It was lower than the control at low salt concentrations, but there was no significant difference between the treatments and the control in higher concentrations. Under the AS treatment, the soluble carbohydrate contents declined and all the treatments were significantly lower than the control. Correlation analysis showed that soluble carbohydrate contents in the roots and stems had a significantly negative correlation with alkaline salt stress concentration ($r_{AS,\ stem} = 0.90$ and $r_{AS,\ root} = 0.80$, $p<0.05$). At the same stress intensity, leaf total carbohydrate content under AS was significantly higher than under NS and soluble carbohydrate contents in the roots and stems under AS were significantly higher than under NS.

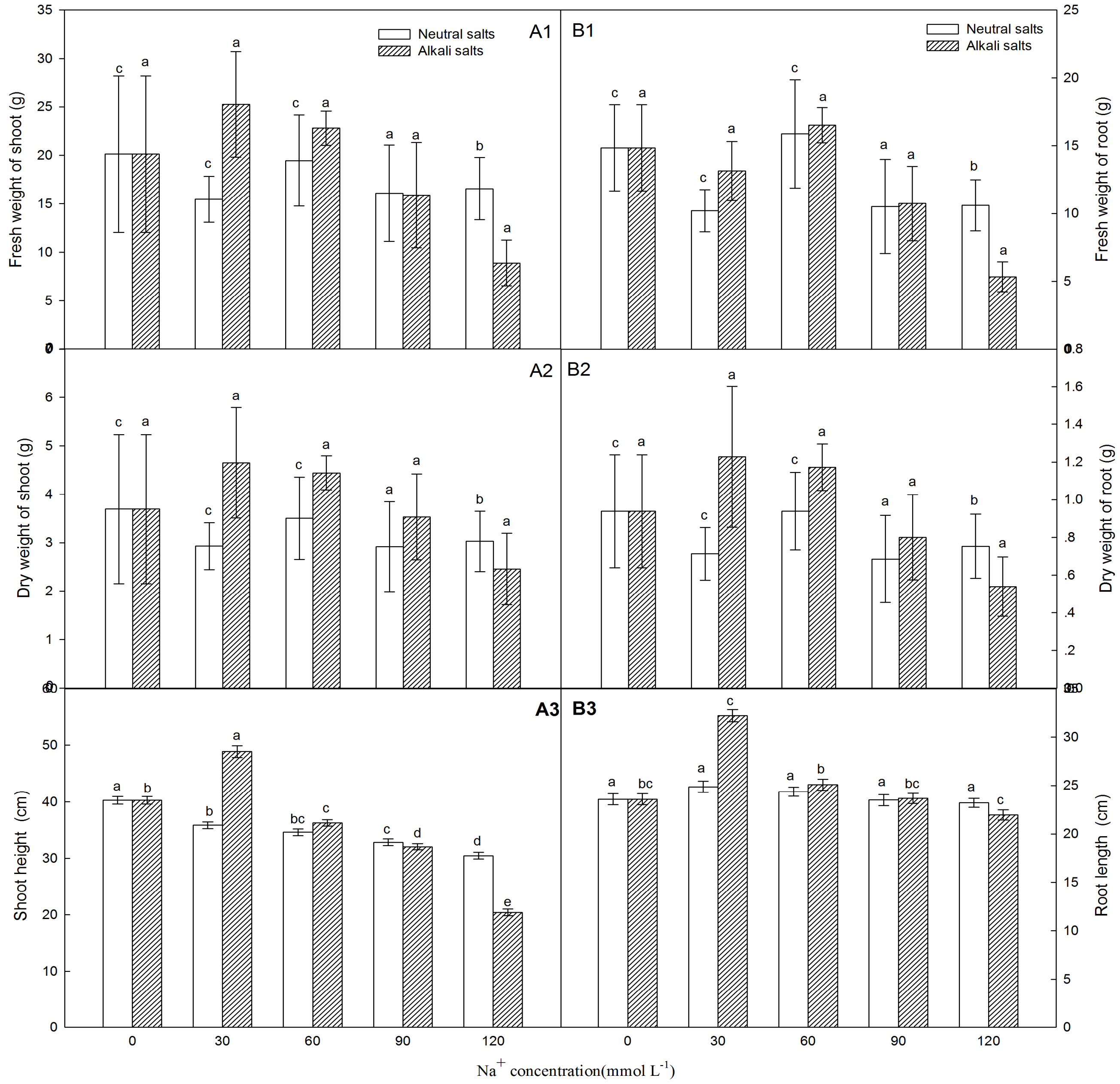

Fig. l. Effects of different type salts stresses on the fresh weight of shoot (A1), the fresh weight of root (B1), the dry weight of shoot (A2), the dry weight of root (B2),the shoot height (A3), the root length (B3) of *G. gracilis* seedlings. The values are the means of three replicates. Means followed by different letters in the same stress type are significantly different at p<0.05 according to Duncan's method. Neutral salts stress: NaCl:Na_2SO_4=1:1; Alkaline salts stress: $NaHCO_3$:Na_2CO_3=1:1.

Daily changes in the gax exchange: Daily changes in the p_N of the *G. gracilis* seeding leaves, compared to the control, showed a decline at first, but then increased gradually as stress levels rose, which produced a "bimodal curve". Under the two types of salt stress at different Na^+ concentrations, the daily changes fluctuated rather than show a unimodal or bimodal curve. The maximum p_N was at 08:00 (Fig. 3, A1, A2, $p<0.05$). The 60 mmol·L^{-1} treatment concentration produced the maximum p_N for NS and 30 mmol·L^{-1} produced the highest p_N for AS. The daily *E* changes in the leaves of *G. gracilis* seedlings, compared to the controls, declined at first, then increased and then declined again as stress levels rose. With NS, the daily changes in *E* fluctuated between the concentration treatments but, under AS the trend was similar for all treatments, compared with the control (Fig. 3, B1, B2, $p<0.05$). *E* values were highest at a treatment concentration of 60 mmol·L^{-1} at all time points for NS and 30 mmol·L^{-1} produced the maximum values for AS. The daily changes in the g_s of *G. gracilis* seeding leaves, compared to the control, declined at first and then increased gradually. Under NS, the g_s of the different Na^+ treatments declined gradually, but there were no significant difference between the treatments. Under AS, with the exception of 30 mmol·L^{-1}, there were no significant difference between the treatments (Fig. 3, C1, C2, $p<0.05$). The daily *WUE* changes in *G. gracilis* seedlings showed that all treatments and the control declined at first and then increased (Fig. 3, D1, D2, $p<0.05$). The 30 mmol·L^{-1} treatment produced the maximum *WUE* values under NS.

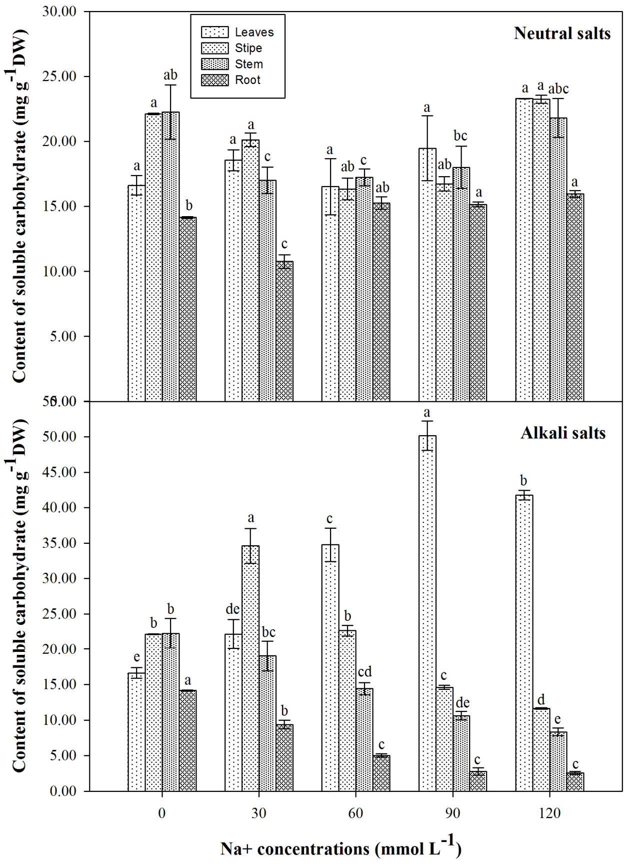

Fig. 2. Soluble carbohydrate contents in the leaves, stipe, stem and root of *G. gracilis* seedlings under different types of salt stress. The values were the means of nine replicates. Means followed by different letters in the same column were significantly different ($p<0.05$), according to the least significant difference (LSD) test. Neutral salts stress: $NaCl:Na_2SO_4=1:1$; Alkaline salts stress: $NaHCO_3:Na_2CO_3=1:1$.

Daily p_N means in the leaves of *G. gracilis* seedlings showed significant differences between the two types of salt stress. Under NS, the daily p_N means increased at first, but then declined. They reached a maximum at 60 $mmol \cdot L^{-1}$. At low stress concentrations, there were no significant difference compared to the control, but p_N significantly declined at high stress concentrations. Under AS, the daily p_N means significantly declined in a linear manner and all treatments were significantly lower than the control. The size of the decrease was also significant greater than NS (Fig. 4, $p<0.05$). Correlation analysis between the daily p_N means and stress intensity showed a significant negative correlation ($r_{NS} = -0.87$, $p<0.05$) under NS and a highly significant negative correlation ($r_{AS} = -0.97$, $p<0.01$) under AS. The daily means trend for *E* was consistent with the daily p_N means (Fig. 4). Correlation analysis between the daily means for *E* and stress intensity showed a negative correlation, but this was not significant ($r_{NS} = -0.78$, $p>0.05$) under NS, but had an extremely significant negative correlation ($r_{AS} = -0.96$, $p<0.01$) under AS. The daily g_s means were lower under NS than the than the control, but there were no significant difference between the treatments. Under AS, the daily g_s means significantly declined as stress levels increased (Fig. 4). Correlation analysis between the daily g_s means and stress intensity showed a non-significant negative correlation under NS ($r_{NS} = -0.67$, $p>0.05$) and a significant negative correlation ($r_{AS} = -0.93$, $p<0.01$) under AS. The daily *WUE* means under NS were not significantly different to the control, whereas they declined under AS, The daily *WUE* means at the low concentrations were lower than the control, but at higher concentrations they were higher than the control. However, this result was not significant (Fig. 4, $p>0.05$).

Photosynthetic rate changes due to different light intensities: Under salt stress, the changes to p_{Nmax} in the leaves of *G. gracilis* seedlings changed significantly as the stress concentration increased (Table 3). Under NS, the 90 $mmol \cdot L^{-1}$ treatment was significantly higher than the control, but the other treatments were lower than the control. However, this was not significant. Under AS, p_{Nmax} significantly declined for all treatments and was significantly lower than the control. Correlation analysis between p_{Nmax} and stress intensity showed no significant correlation ($r_{NS} = 0.30$, $p>0.05$) under NS and a significant negative correlation ($r_{AS} = -0.88$, $p<0.05$) under AS. The *QE* for photosynthesis was fixed by CO_2 mole numbers when absorbing one mole of light quantum. Under the two types of salt stress, the change in *QE* was similar to p_{Nmax} (Table 3). Correlation analysis between *QE* and stress intensity showed that there was no significant correlation ($r_{NS} = 0.33$, $p>0.05$) under NS, but was extremely significantly negatively correlated ($r_{AS} = -0.95$, $p<0.01$) under AS. *LCP* is the effective radiation value when p_N is zero. It directly reflects the ability of a plant to utilize weak light and the lower the *LCP*, the better the plant is at utilizing weak light. Under NS, the *LCP* in the leaves of *G. gracilis* seedlings increased at first and then declined as stress levels rose. The *LCP* for the 90 $mmol \cdot L^{-1}$ treatment concentration was significantly higher than the control, but the other treatment concentrations were not significantly different to the control. Under AS, the *LCP* in the leaves of *G. gracilis* seedlings declined sharply as stress levels rose. The *LCP* values for the 90 $mmol \cdot L^{-1}$ and 120 $mmol \cdot L^{-1}$ treatments were significantly lower than the control (Table 3). *LSP* is the effective light radiation value when p_N has reached a maximum. The *LSP* in the leaves were significantly different under the two types of salt stress (Table 3). Under NS, *LSP* declined gradually and the 30 $mmol \cdot L^{-1}$ treatment was significantly higher than the control. The other treatments were significantly lower than control. Under AS, the *LSP* rose at first and then declined. Again the 30 $mmol \cdot L^{-1}$ treatment was significantly higher than the control and the other treatments were significantly lower than the control. Under the same Na^+ concentration stress, the p_{Nmax}, *QE* and *LSP* in the leaves were significantly higher under NS than under AS.

Photosynthetic rate changes at different CO_2 concentrations: The CO_2 response curve reflected the relationship between p_N and CO_2 concentration. Table 4 showed the corresponding parameter changes for p_N/C_i in *G. gracilis* seedlings after treatment with the two types of salt stress.

Table 2. Effects of two types of salt stress on the photosynthetic pigments found in *G. gracilis* seedlings. The values were the means of nine replicates. Means followed by different letters in the same column were significantly different (p<0.05), according to the least significant difference (LSD) test. In each row, means with different letters were significantly different (p<0.05).

Plots	Na^+ Concentration (mmol·L^1)	Total pigments (mg·g^{-1}DM)	Total chlorophyll (mg·g^{-1}DM)	chlorophyll *a* (mg·g^{-1}DM)	chlorophyll *b* (mg·g^{-1}DM)	Carotenoids (mg·g^{-1}DM)	Chlorophyll *a*/*b*
Contral	0	1.34 ± 0.04bc	1.4 ± 0.04bc	0.90 ± 0.23bcd	0.24 ± 0.02cd	0.20 ± 0.00ab	3.74 ± 0.14ab
NS	30	1.32 ± 0.02bc	1.13 ± 0.16bc	0.89 ± 0.02bcd	0.23 ± 0.00d	0.19 ± 0.00bcd	3.91 ± 0.05a
	60	1.43 ± 0.03ab	1.23 ± 0.03ab	0.98 ± 0.01ab	0.25 ± 0.01cd	0.20 ± 0.00abc	3.88 ± 0.11a
	90	1.55 ± 0.02a	1.33 ± 0.02a	1.03 ± 0.01a	0.30 ± 0.01b	0.22 ± 0.00a	3.44 ± 0.02c
	120	1.41 ± 0.09ab	1.20 ± 0.08ab	0.92 ± 0.06bcd	0.27 ± 0.02bc	0.21 ± 0.01ab	3.37 ± 0.01c
AS	30	1.31 ± 0.03bc	1.3 ± 0.02bc	0.86 ± 0.02cd	0.27 ± 0.01bc	0.18 ± 0.00cd	3.15 ± 0.01d
	60	0.98 ± 0.11d	0.84 ± 0.09d	0.62 ± 0.07e	0.22 ± 0.03d	0.14 ± 0.02d	2.76 ± 0.03e
	90	0.85 ± 0.10d	0.71 ± 0.01d	0.50 ± 0.00f	0.21 ± 0.01d	0.14 ± 0.00e	2.39 ± 0.07f
	120	0.65 ± 0.04e	0.53 ± 0.03e	0.37 ± 0.02g	0.16 ± 0.01e	0.11 ± 0.00e	2.35 ± 0.02f

Table 3. The photosynthetic responses to light. Data shown were the means (± SD). Means followed by different letters in the same column were significantly different (p<0.05), according to the least significant difference (LSD) test. In each row, means with different letters were significantly different (p<0.05).

Plots	Na^+ Concentration (mmol·L^{-1})	p_{Nmax} [μmol·m^{-2}·s^{-1}]	*QE*	*LCP* [μmol·m^{-2}·s^{-1}]	*LSP* [μmol·m^{-2}·s^{-1}]
Contral	0	14.40 ± 1.21ab	0.05 ± 0.01b	23.60 ± 0.79b	325 ± 8.88ab
NS	30	14.81 ± 1.78ab	0.04 ± 0.42b	20.20 ± 2.72b	371 ± 27.17a
	60	12.00 ± 0.06ab	0.04 ± 0.00b	22.80 ± 2.45b	305 ± 1.01bbc
	90	17.61 ± 1.72a	0.07 ± 0.02a	38.90 ± 2.10a	286 ± 16.25bc
	120	14.32 ± 0.88ab	0.04 ± 0.01b	24.30 ± 2.60b	251 ± 12.01c
AS	30	9.17 ± 1.01bc	0.05 ± 0.09b	28.30 ± 1.10ab	229 ± 0.99c
	60	8.43 ± 1.81bc	0.03 ± 0.01bc	24.80 ± 2.65b	283 ± 19.02bc
	90	8.56 ± 1.85bc	0.03 ± 0.02bc	15.70 ± 2.15c	296 ± 4.51b
	120	1.23 ± 1.05c	0.02 ± 0.00c	15.00 ± 2.65c	195 ± 43.01c

Table 4. The photosynthetic responses to CO_2 (p_N/C_i). Data shown were the means (± SD). Means followed by different letters in the same column were significantly different (p<0.05), according to the least significant difference (LSD) test. In each row, means with different letters were significantly different (p<0.05).

Plots	Na^+ Concentration (mmol·L^{-1})	p_{Nsat} [μmol·m^{-2}·s^{-1}]	*Resp*	V_{cmax} [μmol·m^{-2}·s^{-1}]	J_{max} [μmol·m^{-2}·s^{-1}]	V_{TPU}	*CE*
Contral	0	66.19±18.61ab	33.60±5.90ab	62.70±10.30a	435.50±19.05ab	68.00±3.90ab	0.08±0.00a
NS	30	79.69±1.49a	39.00±3.00a	74.50±3.70a	489.50±70.50a	73.05±36.95a	0.10±0.00a
	60	46.66±3.61bc	21.60±7.30cd	40.80±11.25bc	175.00±51.50c	14.10±4.15b	0.05±0.01a
	90	75.26±2.84a	20.10±5.40abc	44.50±6.85ab	195.00±48.00ab	14.10±5.95b	0.06±0.22a
	120	42.04±3.69bc	19.20±1.50bcd	34.55±2.75bc	144.50±13.50bc	13.95±5.55b	0.04±0.00a
AS	30	46.41±2.58bc	16.53±9.57cd	33.50±15.30bc	231.35±15.95ab	16.55±2.76b	0.05±0.00a
	60	40.24±1.18bc	8.27±2.04d	21.15±4.25c	84.15±17.85c	6.71±1.34d	0.04±0.001a
	90	39.20±2.56bc	8.66±2.24d	21.65±4.35c	85.85±18.15c	6.88±1.41d	0.04±0.01a
	120	38.01±1.58c	8.50±1.20d	21.35±2.55c	83.50±14.50bc	1.12±1.60e	0.04±0.01a

P_{Nsat} in the leaves was significantly different for the two types of salt stress. Under NS, p_{Nsat} fluctuated as the stress levels increased and the 30 mmol·L^{-1} and 90 mmol·L^{-1} treatments were higher than the control. However the 60 mmol·L^{-1} and 120 mmol·L^{-1} treatments were lower than the control. Under AS, p_{Nsat} was declined as the stress levels increased. Under the same Na^+ concentration stress, p_{Nsat} under AS was significantly lower than under NS and the control. R_{esp} in the leaves showed the same trend under both types of salt stress. It declined gradually as stress intensity increased. Under NS, the 30 mmol·L^{-1} treatment increased by 16.07% compared to the control, but the other treatments were lower than the control. There were no significant difference between the treatment concentrations under AS and were all lower than the control. Under the same Na^+ concentration stress, R_{esp} under AS stress was significantly lower than under NS and the control. V_{cmax} is the maximum RUBP carboxylation rate and represents the number and activity of RuBisCo. J_{max} represents the maximum electron transport efficiency and reflects the maximum capacity for electron transport during plant photosynthesis. V_{TPU} represents the maximum rate of triose-phosphate utilization and reflects the transfer capacity and utilization of the photosynthetic carboxylation product. Under the two types of salt stress, the trends for $V_{cmax,}$ J_{max} and V_{TPU} were similar to R_{esp}. CO_2 carboxylation efficiency (*CE*) can be calculated from the slope when plant photosynthesis is increasing in a linear manner. It is an important index that shows how well the plant utilizes low CO_2 concentrations. *CE* in the leaves of *G. gracilis* gradually declined under the two types of salt stress, but there were no significant difference between the different concentrations and the control.

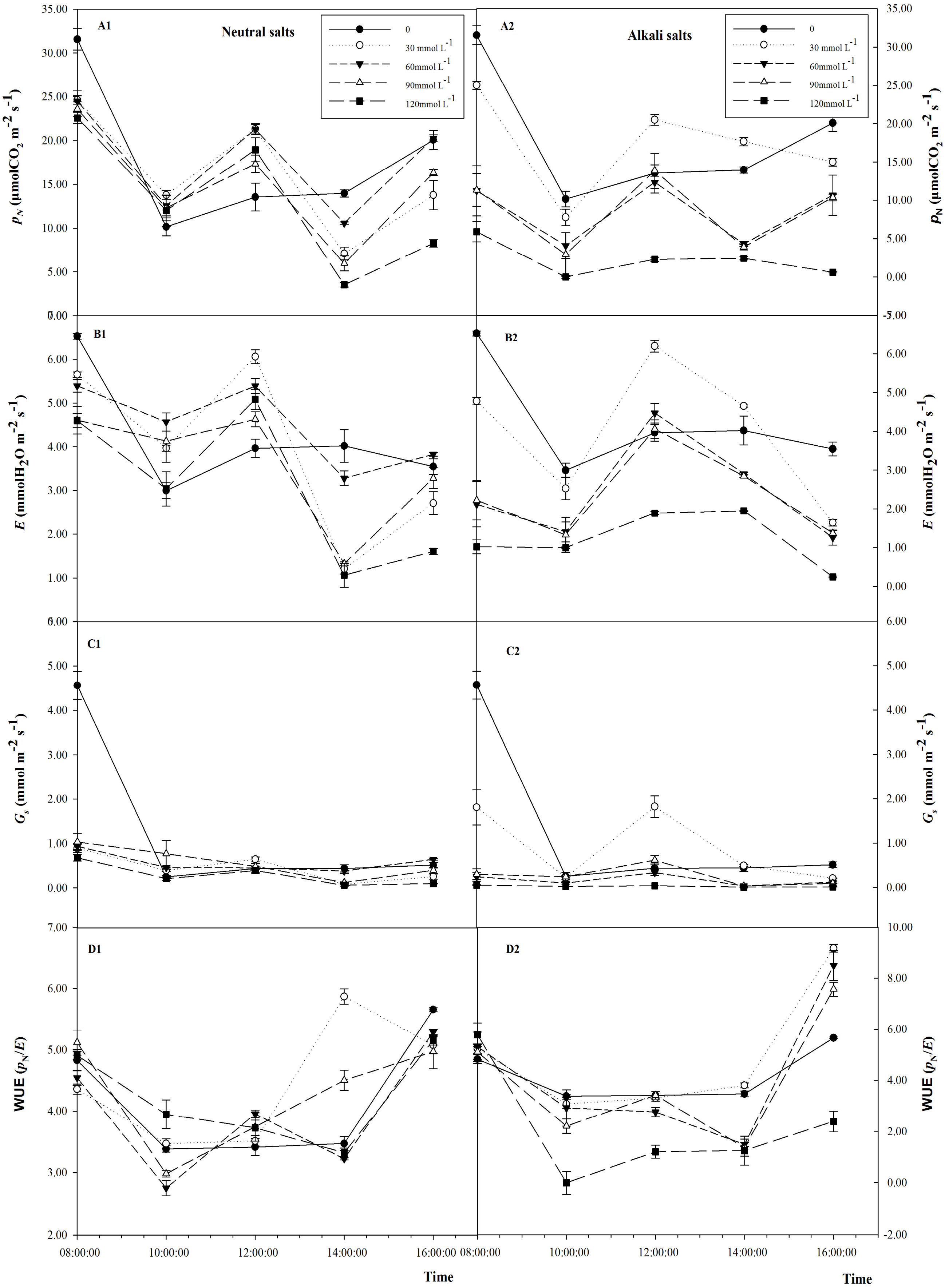

Fig. 3. Daily changes in (A) net photosynthetic rate, p_N; (B) transpiration rate, *E*; (C) stomatal conductance, g_s and (D) water use efficiency (*WUE*) of *G. gracilis* seedlings under different types of salt stress. The values were the means of 27 replicates; means followed by different letters in the same column were significantly different ($p<0.05$), according to the least significant difference (LSD) test. Neutral salts stress: NaCl:Na_2SO_4=1:1; Alkaline salts stress: $NaHCO_3$:Na_2CO_3=1:1.

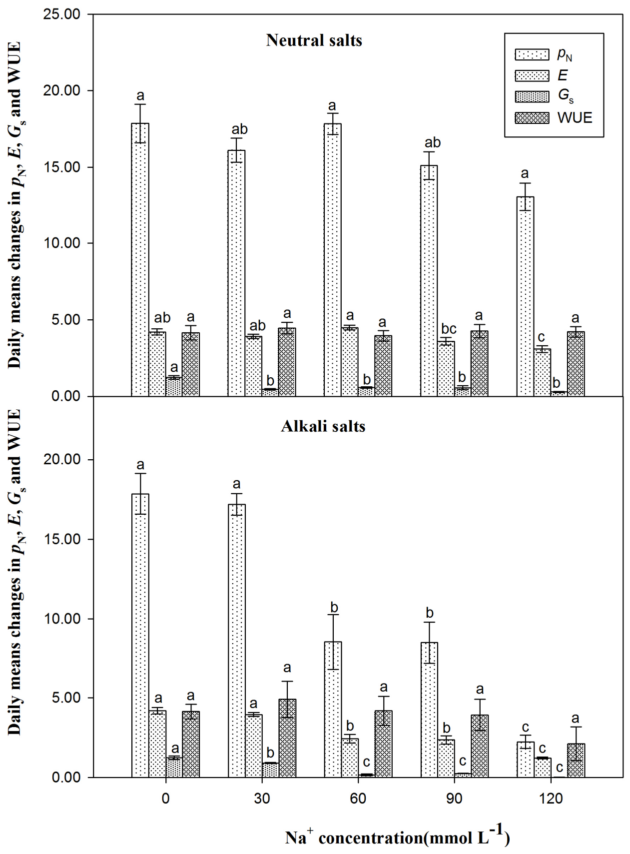

Fig. 4. Daily mean changes in net photosynthetic rate, p_N; transpiration rate, *E*; stomatal conductance, g_s and water use efficiency, *WUE*, of *G. gracilis* seedlings under different types of salt stress. The values were the means of 27 replicates; means followed by different letters in the same column were significantly different ($p<0.05$), according to the least significant difference (LSD) test. Neutral salts stress: $NaCl:Na_2SO_4=1:1$; Alkaline salts stress: $NaHCO_3:Na_2CO_3=1:1$.

Discussion

Neutral salt stress causes harm to the plant mainly through osmotic stress and ion toxicity. Alkaline salt stress can also harm the plant through inappropriately high pH levels. High pH stress can seriously affect mineral nutrition, the oxygen supply capacity of the root environment, the structure and function of root cells and cause ion imbalances between the surrounding environment and plant roots cells. All these factors can reduce plant metabolism (Yang *et al*., 2007). Our results showed that the growth parameter for *G. gracilis* seedlings increased at first and then decreased as the stress intensity rose under the two types of salt stress. The size of the increase and decrease was significantly higher under AS than under NS. Low concentrations of the two types of salt could promote the growth of *G. gracilis* as it is possible that *G. gracilis* may be able to activate an adaption mechanism at low salt concentrations. The results also showed that *G. gracilis* seedlings were able to adapt to low concentrations of salt stress, especially AS. High levels of the two types of salt stress significantly inhibited the growth of *G. gracilis* and the inhibition under AS was significantly stronger than under NS. In order to adapt to a salt stress environment, *G. gracilis* need to absorb large numbers of ions and synthesize organic osmoregulation compounds, processes that also consume large amounts of cellular energy. This energy is therefore not available for growth, which leads to the overall inhibition of *G. gracilis* growth, especially at high pH stress.

Plant photosynthetic pigments are involved in the absorption, transmission and conversion of light energy. Carotenoids are an endogenous antioxidant which accumulate under stress and have certain chlorophyll protective properties (Hui *et al*., 2012; Águila Ruiz-Sola *et al*., 2014). The effects of NS and low concentrations of AS had no significant effects on total photosynthetic pigment and chlorophyll content in *G. gracilis* seedlings leaves, but high levels of AS significantly depressed the total photosynthetic pigment and chlorophyll contents. This showed that under NS and low levels of AS, the decrease in the photosynthetic rate had nothing to do with photosynthetic pigment synthesis and damage. In fact, NS and low levels of AS could promote the accumulation of carotenoids. This showed that *G. gracilis* was able to resist salt stress. Under NS, the chlorophyll *a* and *b* ratio increased at first, but then declined as the stress levels rose. However, overall, there was no significant change in total chlorophyll content. So, it could be inferred that low levels of NS can adjust the proportion of chlorophyll *a* to chlorophyll *b* by increasing the relative content of chlorophyll *a*. However AS stress reduced the relative content of chlorophyll *a*, which might be one of the reasons for the reduction in photosynthesis. The results showed that the inhibitory effects on chlorophyll synthesis and accumulation in *G. gracilis* seedlings were greater under AS than NS stress. They also showed that *G. gracilis* seedlings were able to adapt to low levels of NS stress, with respect to photosynthetic pigment metabolism.

Carbohydrates are the most direct product of higher plant photosynthesis. They are an important structural material and an energy source in the plant and they could also play a role in signal transduction in a similar manner to primary messenger hormones (Zhao, 2006). Our experimental results showed that soluble carbohydrates accumulated in the roots under NS stress. However, under AS stress, carbohydrate contents in the roots were significantly reduced, but accumulated in the leaves. This showed that *G. gracilis* could adapt to NS stress by increasing the absorption of water by reducing root osmotic potential. Under AS, carbohydrate accumulation in the leaves may lead to an increase in the synthesis of photosynthetic product, which might lead to a further decline in the photosynthetic rate, a process also known as feedback inhibition. Substantial reductions in carbohydrate contents in the roots reduced water absorption by *G. gracilis* under AS stress, which was one of the reasons why *G. gracilis* seedling growth was inhibited.

Under NS stress, p_N daily means increased at first and then declined as the stress levels rose. g_s had an inhibitory effect at low salt concentrations, but the inhibition was reduced as the salt concentration rose. This showed that changes in p_N were not consistent with g_s. The decline in p_N was caused by non-stomatal factors when subjected to NS. Under AS, the p_N slightly increased at low stress concentrations, but gradually declined as stress levels rose. The daily g_s means gradually decreased as the salt concentration rose. This showed that under AS, the decrease in p_N was accompanied by a decrease in g_s and that this was caused by stomatal factors. At low AS concentrations, the stomatal limitation was not enough to restrict p_N, which showed that there was some resistance to salt stress. Under low NS concentrations, *E* did not show any significant difference between the stress treatments and the control at low AS concentrations. *E* values for the treatments were lower than the control, but this difference was not significant. The results showed that *G. gracilis* was able to adapt to low levels of salt stress. This adaptive ability was stronger under NS stress than under AS stress. Similarly, in terms of *WUE*, *G. gracilis* seedlings also showed certain levels of compensation and adaptability.

P_N/Q curves in plant photosynthesis describe the relationship between the effective light radiation and net photosynthetic rate (Jiang & He, 1999). Normally under low effective light radiation, light is the only limiting factor in the external environment and the net photosynthetic rate increases linearly as the effective light radiation rises. However, as the effective light radiation increases, temperature, CO_2 concentration and internal factors, such as leaves, become limiting factors and the net photosynthetic rate becomes curvilinear as the light intensity increases. When effective light radiation reaches a certain value, net photosynthetic rate no longer increases with the increase of light intensity, namely, photosynthesis has reached the saturation stage. After measuring changes in the p_N/Q curve of *G. gracilis* seedlings under different types of salt stress, the results confirmed that as the NS stress increased, A_{max} and *QE* were no longer significantly different to the control and this showed that *G. gracilis* had a certain ability to adapt to NS stress. Under AS stress, p_{Nmax} and *QE* decreased as the stress intensity rose, which suggested that photosynthetic capacity and light energy utilization efficiency in *G. gracilis* seedlings were negatively affected by AS stress. The results showed that photosynthetic inhibition in *G. gracilis* seedlings under AS was stronger than under NS. *LCP* showed a negative correlation with NS, but a positive correlation with AS, which suggested that the ability of *G. gracilis* seedlings to use weak light increased under NS, but declined under AS. This might be a *G. gracilis* adaptation mechanism to salt stress. *LSP* appeared at high NS concentrations and reached a maximum at low concentrations of AS. The maximum *LSP* for AS was higher than for NS and was higher than the control. This showed that low concentrations of both types of salt stress could improve the ability of *G. gracilis* to use bright light, which might also be a mechanism that enables it to adapt to salt stress, but this ability disappeared at higher salt concentrations.

The photosynthetic rate increased as CO_2 concentration rose. When CO_2 levels reached a dynamic equilibrium between absorption by plant photosynthesis and release by respiration, the CO_2 concentration in the environment had reached the CO_2 compensation point. When CO_2 concentration is increased further, the plant photosynthesis rate quickly increases in an almost linear manner. The slope of the straight line is called the CO_2 carboxylation efficiency and the values reflect photosynthetic efficiency at a given CO_2 concentration (He *et al.*, 2011). As the CO_2 concentration rises further then the photosynthetic rate will eventually reach a maximum and this is called the CO_2 saturation point. The p_N/Ci curve reflects the relationship between plant p_N and the CO_2 concentration in the environment. Analysis of the p_N/Ci curve showed that p_{Nsat} fluctuated as NS rose. The curve was in the form of a wave, but the values were not significantly different. This might be another adaptation by *G. gracilis* seedlings to NS stress. Under AS stress, p_{Nsat} gradually declined as stress levels rose and this showed that AS stress caused serious damage to photosynthetic capacity. The change trend in R_{esp}, V_{cmax}, J_{max} and V_{TPU} were similar under both types of salt stress.

Overall the results showed that *G. gracilis* was able to adapt to low NS stress. The adaption mechanism may improve electron transfer efficiency, promote the carboxylation efficiency of RuBisCo in the dark reaction process, increase CO_2 fixation and increase the utilization efficiency of triose phosphate; all of which would improve photosynthetic efficiency. Under high NS stress, the compensation mechanism disappeared, CO_2 utilization efficiency decreased and electron transfer efficiency and the utilization efficiency of triose phosphate were inhibited. Under AS, R_{esp}, V_{cmax}, J_{max} and V_{TPU} gradually decreased as stress levels rose. This showed that under alkaline salt stress, the amount and activity of RuBisCo decreased, which reduced CO_2 utilization efficiency and inhibited electron transfer and triose phosphate utilization.

G. gracilis seedling growth showed different degrees of inhibition under different types of salt stress. The effects of AS were significantly stronger than the effects of NS. The decline in *G. gracilis* net photosynthetic rate was caused by different factors under the two types of salt stress. NS stress affected non-stomatal factors, whereas AS stress effects were caused by stomatal factors, a decrease in photosynthetic pigment content and feedback inhibition of carbohydrate. The different types of salt stress had significantly different effects on photosynthetic physiological parameters in *G. gracilis*. However, *G. gracilis* seedlings showed a certain resistance to NS and low levels of AS. The adaptability in photosynthetic physiological parameters shown by *G. gracilis* to low levels of NS requires further research.

Conclusion

G. gracili seedling growth had different degrees of inhibitory effect under different types salts stress. AS were significantly stronger than NS. Decline of net photosynthetic rate in *G. gracili* coused by different reasons under different types salt stress. NS was affected by the non-stomatal factors; AS was reduced coused by stomatal factors, decrease of photosynthetic pigment content and feedback inhibition of carbohydrate. The effect of photosynthetic physiological parameters in *G. gracili* seedlings had significant differences under different type salt stress. At the same time, *G. gracili* seedlings showed the certain flexibility to NS and low concentration of AS. Low concentration of AS adaptability than NS in growth, but low concentration of NS performance has certain adaptability in photosynthetic physiological. It deserves further research.

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