

THE PHYSIOLOGICAL VARIATIONS OF ADAPTATION MECHANISM IN *GLYCINE SOJA* SEEDLINGS UNDER SALINE AND ALKALINE STRESSES

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Abstract

The seedlings of *Glycine soja* were treated with varying saline stress and alkaline stress. The growth, photosynthesis and concentrations of inorganic ions in tissue sap of stressed seedlings were measured to elucidate the mechanism of saline and alkaline stress (high pH) damage to *G. soja*, and the differences between physiological adaptive mechanism to alkaline stress and saline stress. Our experimental data showed alkalinity had a more severe effects on *G. soja* seedlings than salinity in the similar concentration, severely inhibited shoot and root growth, and photosynthesis. Diurnal change of p_N showed the bimodal curves getting less obvious and transformed to be single peak with increasing stress intensity which might be an efficient energy-conserving strategy for *G. soja* to adapt to saline and alkaline stress. Na^+/K^+ were all increased, with greater degrees of increasing under alkaline than under saline stress, cations and anions were almost not accumulated under high alkaline stress, while the influx of superfluous Na^+ can be balanced by the accumulation of Cl^- , SO_4^{2-} , $H_2PO_4^-$ in root under saline stress. This indicated that the roots of *G. soja* were injured so severely that couldn't absorb Na^+ and keep ion balance under high alkaline stress including high-pH stress, which might lead to greater accumulation of Na^+ in leaves under alkaline stress than that under saline stress, and then sharply reduced the growth and photosynthesis. p_N of *G. soja* seedlings was promoted under low concentration saline and alkaline stresses. Na^+/K^+ were significant lower in leaves compare with that in roots, and a large amount of Na^+ was accumulated in stems of *G. soja* seedlings under both stresses. Under alkaline stress, the K^+ , NO_3^- , Mg^{2+} and Ca^{2+} contents in leaves were increased with increasing Na^+ , and maintain high water content in root. Our results showed obvious differences between physiological adaptive mechanisms to saline stress and alkaline stress. This study would provide a theoretical basis for protection, screening and utilization of wild soybean and breeding new varieties of cultivated soybean.

Key words: *Glycine soja*, Growth, Photosynthesis, Ion homeostasis, Saline stress, Alkaline stress.

Abbreviations: AS - alkaline stress; Car - carotenoid; Chl - Chlorophyll; Chl a - Chlorophyll a; Chl a/b - Chlorophyll a/b; Chl b - Chlorophyll b; CK - Control; DM - dry mass; E - transpiration rate; FM - fresh mass; g_s - stomatal conductance; p_N - net photosynthetic rate; RGR - the relative growth rate; SS - Saline stress; WC - The water content; WUE - water use efficiency.

Introduction

Over 6% of the world's total land area and approximately 26% of the world's arable land are affected by salinity and sodicity (Munns & Tester, 2008). Soil salinization and alkalization frequently occur simultaneously. Actually, soil alkalization may be more destructive to plants than soil salinization, due to the impact of high pH that alkaline stresses included. In the northeast of China, alkalized grassland has reached more than 70% (Kawanabe & Zhu, 1991). Some reports have demonstrated that the germination, growth and photosynthesis were more severely inhibited under alkaline stress than that caused by saline stress. The mechanisms of plant response to saline stress also differ from that of alkaline stress. Alkaline stress induced several specific responses such as the inhibition of fructan synthesis, the accumulation of organic acids, and the accumulation of Ca^{2+} and the depletion of $H_2PO_4^-$ in wheat seedlings (Guo *et al.*, 2010). Species with different resistance have different response to alkaline stress, for example, wheat seedlings responded to alkaline stress by accumulating Na^+ at the expense of K^+ , and meanwhile there was no competitive inhibition between absorptions of Na^+ and K^+ in alkaline-resistant halophyte *Suaeda glauca* and *kochia sieversiana*, which can grow in extremely alkalized grasslands at pH levels of 10 or higher, the concentrations of Na^+ and K^+ both increased under saline

and alkaline stresses (Guo *et al.*, 2010; Yang *et al.*, 2008). Relatively little attention has been paid to physiological mechanisms of plant response to alkaline stresses.

Declined productivity of many plant species subjected to salt stress is often associated with a reduction in photosynthetic capacity, and extensive work has been carried out on the effects of salt stress on photosynthesis in some species. (Koyro, 2006). The reduction of photosynthetic capacity may be due either to stomatal closure or non-stomatal inhibition. Ion toxicity is related to both stomatal closure and non-stomatal factors. Both Na^+ and Cl^- are toxic to plants at high salinity concentrations (Teakle & Tyerman, 2010). The toxic effect of Na^+ or Cl^- who was more serious has been the focus of salinity research. The toxicity of Na^+ was more severe than Cl^- for *Glycine soja* (Luo *et al.*, 2005), whereas the toxicity of Cl^- was much heavier than Na^+ for *Glycine max* (Chen & Yu, 2007). The photosynthesis was inhibited by Na^+ and Cl^- though different mechanisms. High Cl^- concentration reduces the photosynthetic capacity due to non-stomatal effects: chlorophyll degradation and a reduction in the actual quantum yield of PSII electron transport (Saleem *et al.*, 2014). High Na^+ interferes with K^+ and Ca^{2+} nutrition and disturbs efficient stomatal regulation (Slabu *et al.*, 2009).

G. soja is the wild species having high protein content, dainty and conducive to the digestion and absorption for livestock that make *G. soja* become high-quality forage. It is not only has the specific chemical quality, but also has strong resistance and adaptability to biotic and abiotic stresses. *G. max* is a commercial crop, and also a salt sensitive species, introducing salt tolerance from wild relatives could be important, suggesting that genetic improvement may be possible. Moreover, *G. soja* could increase soil nitrogen content and fertilize saline-alkaline soil (Duan & Shi, 2009). The aim of this study was to analyze and compare the effects of growth, photosynthetic capacity and ion balance in *G. soja* seedlings under saline and alkaline stresses, to elucidate the mechanism of alkaline stress (high pH) damage to *G. soja*, and its physiological adaptive mechanism to alkaline stress; to provide scientific basis for soybean breeding.

Material and Methods

Seeds of *G. soja* were provided by Jilin Academy of Agriculture Science (02528), China, and were sown in 15 cm diameter plastic pots filled with 2.5 kg washed sand. Each pot contained two seedlings which were sufficiently watered with 1×Hoagland nutrient solution daily after germinated. The pots were placed outdoors and sheltered from rain. The experiment was conducted at Northeast Normal University in Changchun, Jilin province of China (43°05′~45°15′ N, 124°18′~127°05′ E) from the middle of May to the end of June, 2010. The daily average air temperature and humidity were about 23°C and 50%. The seedlings were subjected to natural irradiation.

When the seedlings were 4 weeks old, thirty-six pots of uniform seedlings were selected and then randomly divided into 12 sets (there were three replicates per set; each pot was considered as a single replicate), involving five saline stress treatments (1:1 molar ratio of NaCl to Na₂SO₄), five alkaline stress treatments (1:1 molar ratio of NaHCO₃ to Na₂CO₃), one for determining dry mass at the beginning of the stress treatment (for the calculation of relative growth rate), and the other as the control. Based on a preliminary experiment, Saline and alkaline stresses were set up five treatments. Saline treatments consisted of five concentrations (30, 60, 90, 120, and 150 mmol·L⁻¹), and alkaline treatments also consisted of five concentrations (20, 40, 60, 80, and 100 mmol·L⁻¹). The pH of treatment solutions was 6.75, 6.78, 6.79, 6.74 and 6.71 for saline stress and 9.40, 9.71, 9.77, 9.78, and 9.78 for alkaline stress, respectively. 1×Hoagland nutrient solution was taken as solvent. The seedlings were salt-treated for five days.

P_N , g_s and E of the leaves were determined using portable open flow gas exchange system LI-6400 (LICOR, USA) at one-hour interval from AM 08:00 through PM 17:00; the respective results were expressed as $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. WUE was calculated as the ratio of p_N/E . The photosynthetically active radiation (PAR) was set at $1000\pm 12 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (saturation light), CO₂ concentration $350\pm 2 \text{ cm}^3\cdot\text{m}^{-3}$, the air temperature and humidity were about 24°C and 50%. Gas exchange parameters were measured in full expanded leaves,

measurements were repeated seven times for each treatment among three replicate pots, and the averages recorded.

0.1 g dry leaf sample was dipped into 10 ml of 80% acetone/anhydrous ethanol mixture (1:1) to extract the photosynthetic pigments till the leaf became white. Spectrophotometric (SpectrUV-754, Shanghai accurate scientific instrument Co.) determination at 440, 645, and 663 nm of each sample was done 3 times. The calculation used the formulae of Holm (1954).

Plants were harvested after the determination of diurnal daily change of photosynthetic parameters. The root, stem and leaf were separated and rinsed with distilled water, and the water on the surface of the plants was blotted with a filter paper, then FM was recorded. DM was determined after incubating in a 120°C oven for 10 min and in a vacuum dryer at 80°C until the weight was constant. RGR was defined as $(\ln \text{DM at the end of stress treatment} - \ln \text{DM at the start of stress treatment}) / \text{total treatment duration}$ (Kingsbury & Epstein, 1986). WC was calculated using the formula $(\text{FM} - \text{DM}) / \text{FM} \times 100\%$.

Total soluble sugar content was measured following the method described by Yemm & Willis (1954). About 0.05 g dry shoots and roots were soaked in 4 mL deionized water. The solution was boiled (100°C) for 30 min to extract total soluble sugar and centrifuged under 4000 rpm for 10 min. The extracts were decanted and the residue was re-extracted for two more times, with extracts being completed to 15 mL. In all, 0.1 mL extracts and 3 mL anthrone reagents (0.15 g anthrone+84 mL oil of vitriol+16mL H₂O) were mixed and the absorbance of the mixture was recorded at 620 nm. The content of soluble sugar was calculated from a standard curve of glucose at 620 nm by colorimetry.

A total of 0.05 g dry samples were treated with 4mL deionized water at 100°C for 40 min, and then centrifuged at 3000 ×g for 15 min, the supernatant was collected and the course was repeated twice, with extracts being completed to 15 mL. Unified supernatants were used to determine Cl⁻, SO₄²⁻, NO₃⁻, and H₂PO₄⁻ concentrations by ion chromatography (DX-300 ion chromatographic system, AS4A-SC chromatographic column, CDM-II electrical conductivity detector, mobile phase: Na₂CO₃/NaHCO₃ = 1.7/1.8 mmol·L⁻¹, DIONEX, Sunnyvale, CA, USA). An atomic absorption spectrophotometer (Super 990F, Beijing purkinje general instrument Co., Ltd, China) was used to determine the concentrations of Na⁺, K⁺, Ca²⁺, and Mg²⁺. Selective absorption capacity of root systems for K⁺ over Na⁺ in soils = (available Na⁺/K⁺ in soils of root zone) / (Na⁺/K⁺ in whole plant) (Wang *et al.*, 2004).

Statistical analysis included one-way ANOVA in SPSS (Version13.0, SPSS, Chicago, IL) and Duncan's method to detect differences in physiological parameters of the seedlings treated by saline and alkaline stress. The means and standard errors (\pm SE) and correlation were compared using the statistical procedures of Microsoft Excel 2003. The term 'significant' indicates differences at $p \leq 0.05$.

Results

With increasing salinity, water content in the shoots was decreased under both stresses, and in particular, there was a significant change in shoot water content under alkaline stress. (Fig. 1D). Water content in root was greater than that in shoot under high concentration saline and alkaline stresses (Fig. 1C, D). The relative growth rate (RGR) of the shoots and roots were decreased with increasing salinity for both stresses (Fig. 1A, B), and the remarkable reduction in root RGR under alkaline stress. The decrements under alkaline stress were greater than that under saline stress.

The diurnal changes of p_N of *G. soja* seedlings can be expressed as bimodal curves in different salinities under both stresses (Fig. 2A, E). The first peak occurred at 12:00 or 13:00, and the second peak at 15:00. However, the bimodal curves were not obvious with increasing salinity, the change trends were almost single peak curve under high concentrations of saline and alkaline stresses. There were no differences in g_s between the seedlings of saline stress and the control at 17:00 (Fig. 2B). The E at 17:00 was the lowest for the control and saline stress, except 150 $\text{mmol}\cdot\text{L}^{-1}$ saline stress. The diurnal average value of p_N , g_s and E were all

decreased under both stresses, the reduction extent under alkaline stress was greater than that under saline stress. The p_N value of *G. soja* seedlings under 30 $\text{mmol}\cdot\text{L}^{-1}$ saline and 20 $\text{mmol}\cdot\text{L}^{-1}$ alkaline treatments were greater than the control, and the phenomenon was obvious after 13:00. The decreasing degrees of E and g_s were more significant than any other photosynthetic parameters, the diurnal mean values of E and g_s were both decreased by 80.130% under 20 $\text{mmol}\cdot\text{L}^{-1}$ alkaline treatment compared with the control that can be verified the positive relation between them again. A remarkable negative relation was found between the diurnal mean values of p_N , g_s , E and stress intensities (Table 2). The diurnal changes of WUE showed an increasing trend under all the saline stress from 08:00, but slightly reduced at 12:00 or 13:00 and then increased again; the highest value was found at 16:00 or 17:00 (Fig. 2D). The diurnal mean value of WUE was increased with increasing salinity under saline stress (Fig. 2D, Table 2) and the changing trend under saline stress was obvious at 10:00, 11:00, 12:00 and 16:00. The diurnal change of WUE under alkaline stress was so different from that under saline stress, and the diurnal mean value of WUE increased from the control to 60 $\text{mmol}\cdot\text{L}^{-1}$ alkaline treatment and then reduced (Fig. 2H).

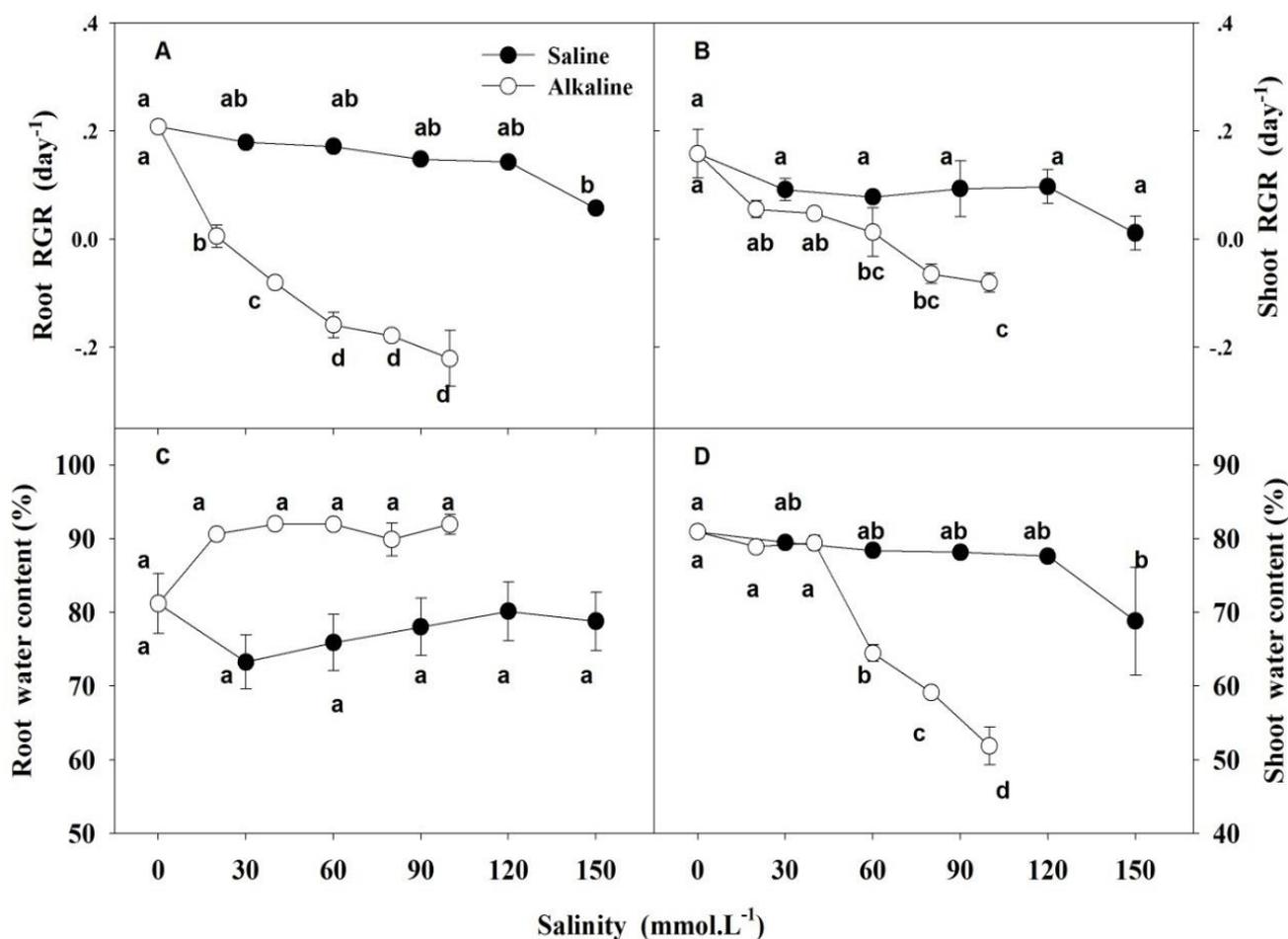


Fig. 1. Effects of saline and alkaline stresses on *G. soja* seedlings. (A) root relative growth rate, (B) shoot relative growth rate, (C) root water content and (D) shoot water content. The values are the means of three replicates. Means followed by different letters in the same stress type are significantly different at $p < 0.05$ according to Duncan's method. Saline stress: $\text{NaCl}:\text{Na}_2\text{SO}_4=1:1$; Alkaline stress: $\text{NaHCO}_3:\text{Na}_2\text{CO}_3=1:1$.

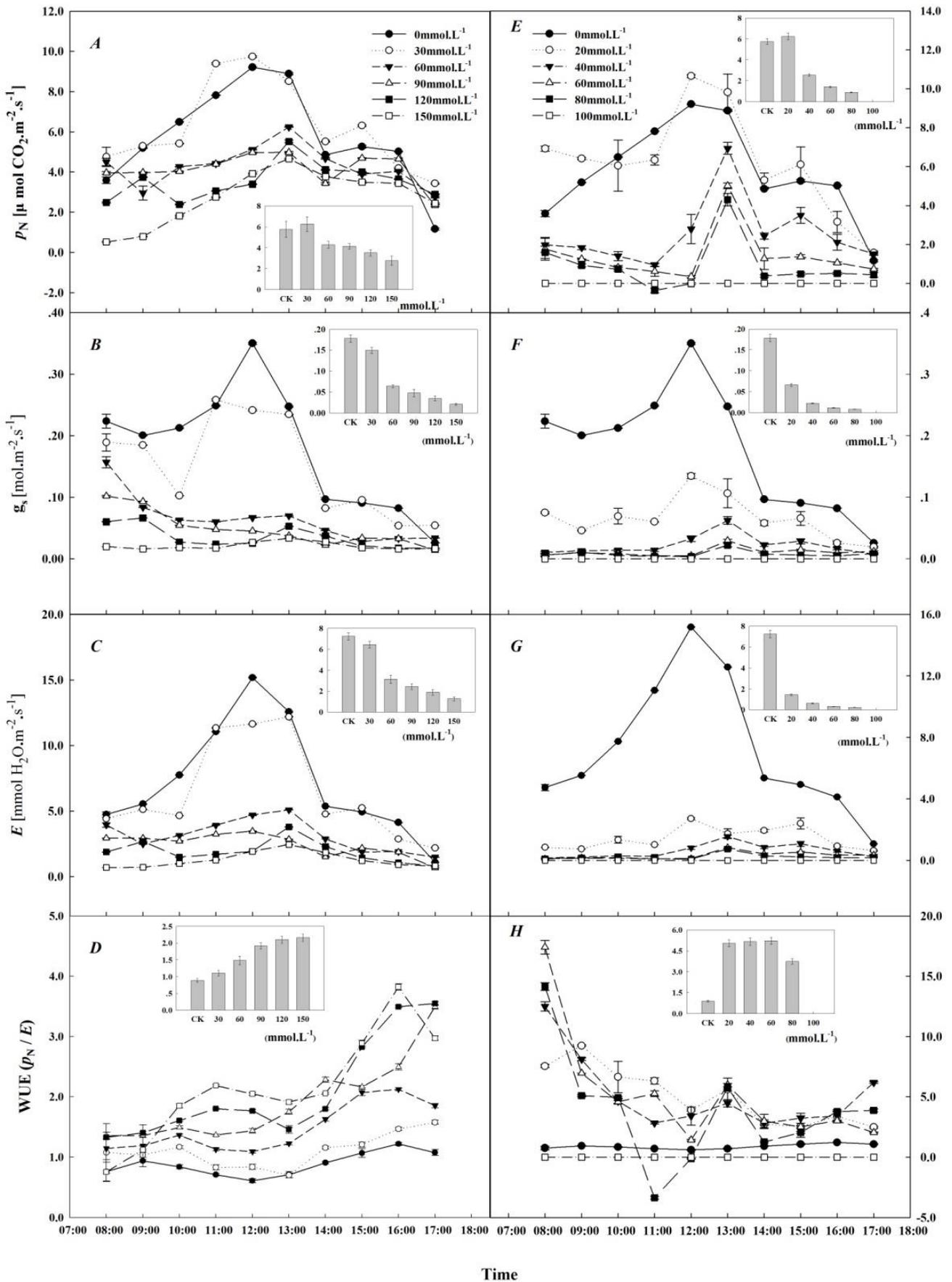


Fig. 2. Effects of saline stress and alkaline stress on diurnal changes of *G. soja* seedlings. (A, E) net photosynthetic rate, P_N , (B, F) stomatal conductance, g_s , (C, G) transpiration rate, E and (D, H) water use efficiency, WUE. The values are the means of seven replicates. The little figures are the diurnal mean value of net photosynthetic rate, P_N , stomatal conductance, g_s , transpiration rate, E and water use efficiency, WUE. Saline stress: $\text{NaCl}:\text{Na}_2\text{SO}_4=1:1$; Alkaline stress: $\text{NaHCO}_3:\text{Na}_2\text{CO}_3=1:1$.

Table 1. Effects of saline and alkaline stresses on the photosynthetic pigments of *G soja* seedlings.
Saline stress: NaCl:Na₂SO₄=1:1; Alkaline stress: NaHCO₃:Na₂CO₃=1:1.

Salinity (mmol.L ⁻¹)	Chl a (mg·g ⁻¹ DM)	Chl b (mg·g ⁻¹ DM)	Chl (a+b) (mg·g ⁻¹ DM)	Car (mg·g ⁻¹ DM)	Chl a/b	Total pigment (mg·g ⁻¹ DM)	
CK	3.172 ± 0.149b	1.044 ± 0.013b	4.216 ± 0.136b	1.134 ± 0.009bc	3.041 ± 0.181bc	5.350 ± 0.145b	
30	3.027 ± 0.061bc	0.919 ± 0.018c	3.946 ± 0.042bc	1.062 ± 0.010cd	3.296 ± 0.131ab	5.008 ± 0.052bc	
SS	60	3.127 ± 0.115b	0.918 ± 0.045c	4.046 ± 0.160b	1.161 ± 0.069bc	3.408 ± 0.041a	5.207 ± 0.229b
	90	2.867 ± 0.115c	0.853 ± 0.056c	3.721 ± 0.170c	1.026 ± 0.039d	3.365 ± 0.084a	4.747 ± 0.209cd
	120	3.104 ± 0.025bc	0.908 ± 0.035c	4.012 ± 0.060bc	1.191 ± 0.008ab	3.421 ± 0.106a	5.204 ± 0.052b
	150	2.236 ± 0.006e	0.750 ± 0.029d	2.986 ± 0.035d	1.011 ± 0.003d	2.984 ± 0.106c	3.997 ± 0.031e
AS	20	3.496 ± 0.043a	1.162 ± 0.005a	4.658 ± 0.038a	1.288 ± 0.031a	3.009 ± 0.050bc	5.946 ± 0.069a
	40	2.504 ± 0.069d	0.748 ± 0.009d	3.252 ± 0.078d	1.209 ± 0.041ab	3.344 ± 0.051a	4.462 ± 0.119d
	60	1.121 ± 0.021f	0.431 ± 0.022e	1.552 ± 0.042e	0.517 ± 0.004e	2.605 ± 0.083d	2.069 ± 0.046f
	80	0.995 ± 0.042fg	0.418 ± 0.022e	1.413 ± 0.064ef	0.478 ± 0.030e	2.383 ± 0.024d	1.891 ± 0.094fg
	100	0.802 ± 0.008g	0.431 ± 0.001e	1.233 ± 0.009f	0.293 ± 0.013f	1.860 ± 0.013e	1.526 ± 0.023g

The values are the means of three replicates. Means followed by different letters in the same line are significantly different at $P < 0.05$ according to Duncan's method

Table 2 Regression equations between the diurnal mean values of p_N , g_s , E , WUE, Chl, Chl a/b, Car, Soluble carbohydrate in leaf or root and salt concentrations.

	Regression equations	R ²	r	p
p_N	$y_{\text{saline}} = -0.665x + 6.779$	0.882	-0.939	<0.0001
	$y_{\text{alkaline}} = -1.451x + 7.723$	0.861	-0.928	<0.0001
g_s	$y_{\text{saline}} = -0.033x + 0.197$	0.884	-0.940	<0.0001
	$y_{\text{alkaline}} = -0.040x + 0.176$	0.764	-0.874	<0.0001
E	$y_{\text{saline}} = -1.260x + 8.141$	0.896	-0.947	<0.0001
	$y_{\text{alkaline}} = -1.512x + 6.500$	0.643	-0.802	<0.0001
WUE	$y_{\text{saline}} = 0.281x + 0.626$	0.956	0.978	<0.0001
	$y_{\text{alkaline}} = 0.589x + 2.240$	0.254	0.504	0.030
chl	$y_{\text{saline}} = -0.059x + 4.329$	0.582	-0.763	0.003
	$y_{\text{alkaline}} = -0.376x + 4.979$	0.858	-0.926	<0.0001
Chl a/b	$y_{\text{saline}} = 0.001x + 3.256$	0.001	0.013	0.129
	$y_{\text{alkaline}} = -0.122x + 3.438$	0.720	-0.848	<0.0001
Car	$y_{\text{saline}} = -0.004x + 1.127$	0.068	-0.261	0.035
	$y_{\text{alkaline}} = -0.105x + 1.448$	0.803	-0.896	<0.0001
Soluble carbohydrate (leaf)	$y_{\text{saline}} = 0.173x + 87.926$	0.017	0.128	0.024
	$y_{\text{alkaline}} = 3.610x + 119.24$	0.079	0.281	<0.0001
Soluble carbohydrate (root)	$y_{\text{saline}} = 0.878x + 46.743$	0.103	0.322	<0.0001
	$y_{\text{alkaline}} = -5.828x + 58.430$	0.747	-0.864	<0.0001

The chlorophyll and total photosynthetic pigments of *G. soja* leaves were decreased with increasing salinity under both stresses (Table 1). The reducing degrees of chlorophyll and total photosynthetic pigments under alkaline stress were more severe than that under saline stress. The content of Chl a and Chl b was increased by 10.21% and 11.30%, respectively, under 20 mmol·L⁻¹ alkaline stress compared to the control. Chl a/b and carotenoids in the leaves were decreased with increasing salinity under alkaline stress, showing a decreasing trend and remarkable negative relation with alkaline stress (Table 1). Carotenoids of *G. soja* seedlings were fluctuated in a range under saline stress and decreased under alkaline stress.

The soluble sugar in roots of *G. soja* seedlings increased from the control to 120 mmol·L⁻¹ saline stress, and then dropped (Fig. 3A). With increasing alkaline stress, the soluble sugar in root was decreased, and soluble sugar of root under 20 mmol·L⁻¹ alkaline stress was higher than the control (Fig. 3A). Soluble

sugar in leaves was decreased to a certain extent under saline stress (Fig. 3B). Soluble sugar in leaves was increased at low alkalinity, and highest under 60 mmol·L⁻¹ alkaline stress, it was increased by 114.62 % compared with the control.

Under saline stress, the Na⁺ content in roots and stems were increased whereas the K⁺ and Ca²⁺ contents in roots decreased (Fig. 4A, B, D, G). Compare with control, there was a significant increase under lower alkaline stress levels for Na⁺ in root, and meanwhile little accumulation under higher alkaline stress (Fig. 4A). Na⁺ content in stems and leaves were increased under alkaline stress (Fig. 4B, C). Under the same concentration stress (60 mmol·L⁻¹), Na⁺ accumulation in leaves of alkaline stressed seedlings was nearly 10 times higher than that of saline stress (Fig. 4C). The K⁺ and Mg²⁺ contents in roots were decreased under alkaline stress (Fig. 4D, J). The accumulation of Ca²⁺ and Mg²⁺ in leaves was greater than that in stems and roots under higher alkaline stress (Fig. 4G, H, I, J, K, L).

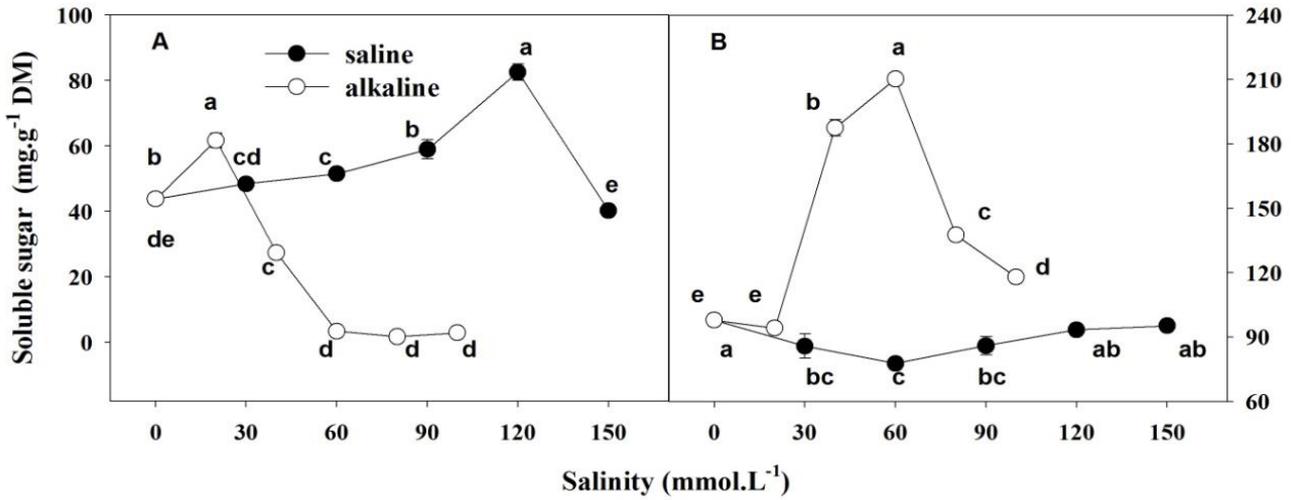


Fig. 3 Effects of saline stress and alkaline stress on soluble sugar in root (A) and leaf (B) of *G. soja* seedlings. Saline stress: NaCl:Na₂SO₄=1:1; Alkaline stress: NaHCO₃:Na₂CO₃=1:1. The values are the means of three replicates. Means followed by different letters in the same stress type are significantly different at P< 0.05 according to Duncan’s method.

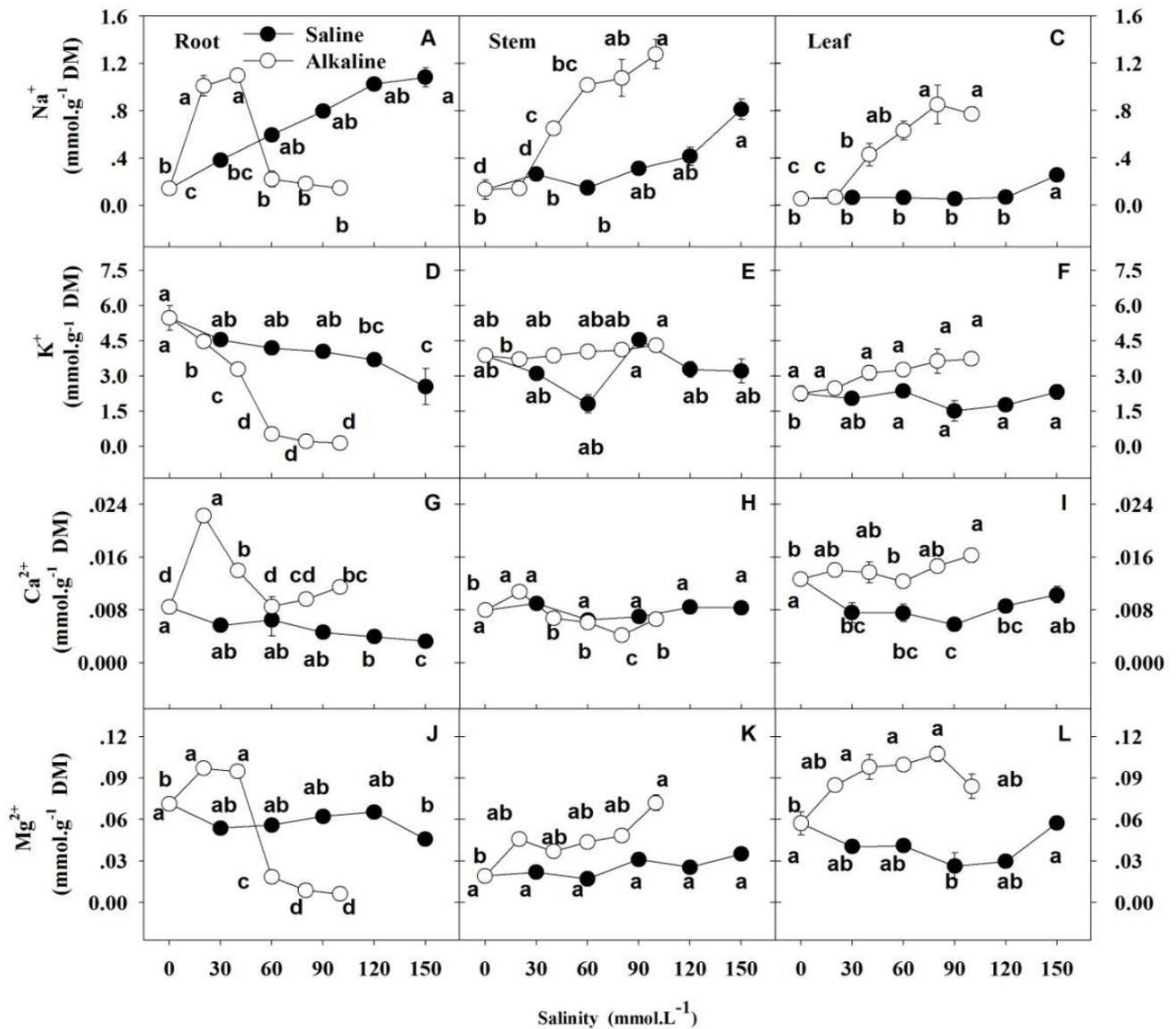


Fig. 4. Effects of saline stress on contents of Na⁺, K⁺, Ca²⁺ and Mg²⁺ in root, stem and leaf of *G. soja* seedlings. Saline stress: NaCl:Na₂SO₄=1:1; Alkaline stress: NaHCO₃:Na₂CO₃=1:1. The values are the means of three replicates. Means followed by different letters in the same stress type are significantly different at P< 0.05 according to Duncan’s method.

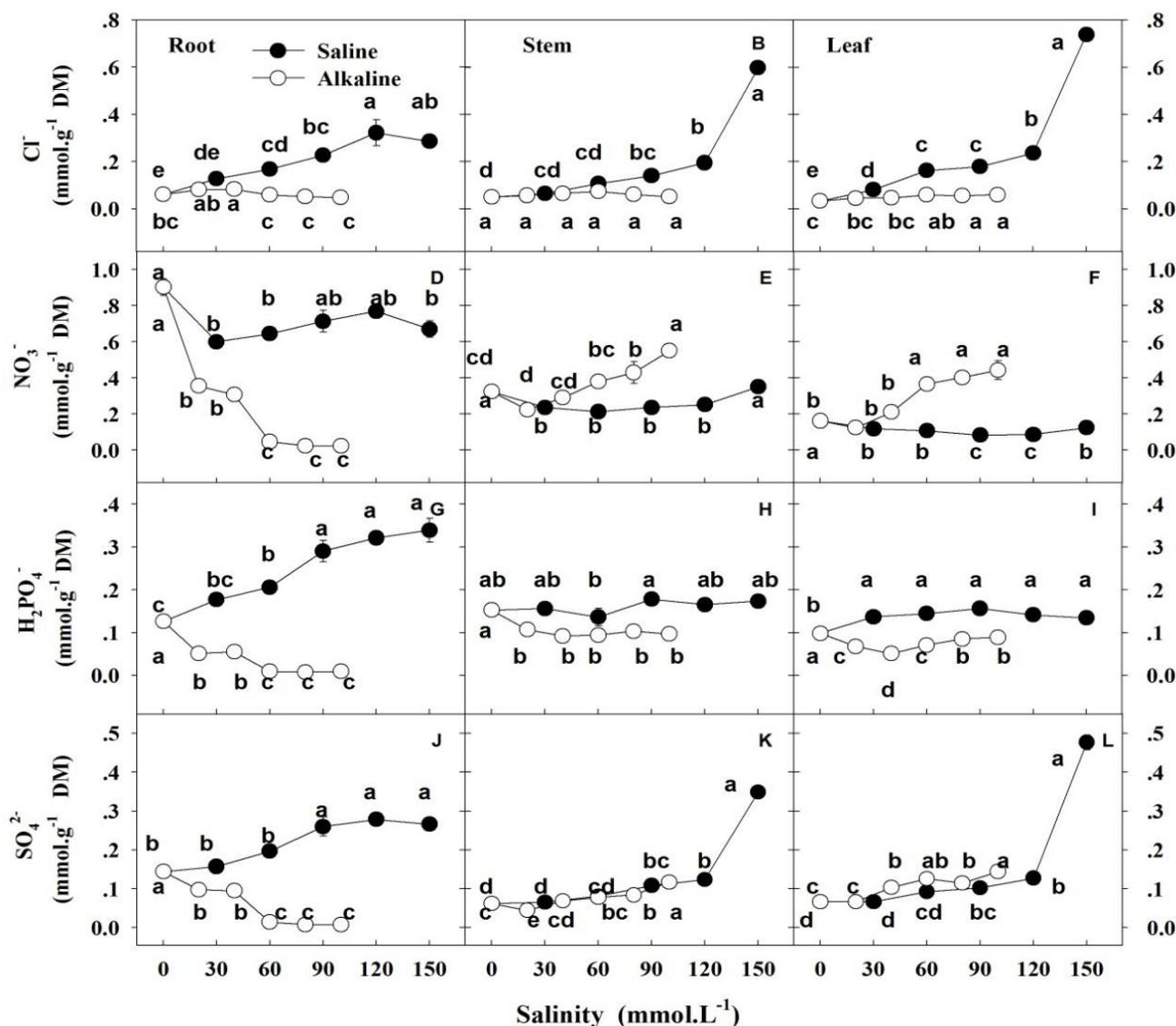


Fig. 5. Effects of saline stress on contents of Cl^- , NO_3^- , H_2PO_4^- and SO_4^{2-} in root stem and leaf of *G soja* seedlings. Saline stress: $\text{NaCl}:\text{Na}_2\text{SO}_4=1:1$; Alkaline stress: $\text{NaHCO}_3:\text{Na}_2\text{CO}_3=1:1$. The values are the means of three replicates. Means followed by different letters in the same stress type are significantly different at $p < 0.05$ according to Duncan's method.

Table 3. The ratio of Na^+/K^+ in root, stem and leaf of *G soja* seedlings under saline and alkaline stresses.
Saline stress: $\text{NaCl}:\text{Na}_2\text{SO}_4=1:1$; Alkaline stress: $\text{NaHCO}_3:\text{Na}_2\text{CO}_3=1:1$.

Salinity (mmol.L ⁻¹)	Root	Stem	Leaf	SA	
CK	0.027 ± 0.005h	0.036 ± 0.002f	0.026 ± 0.007c	0.053 ± 0.009e	
30	0.084 ± 0.007gh	0.086 ± 0.004ef	0.022 ± 0.001c	76.218 ± 6.402c	
60	0.143 ± 0.006fgh	0.092 ± 0.005ef	0.028 ± 0.002c	90.104 ± 3.989b	
SS	90	0.186 ± 0.001efg	0.068 ± 0.005ef	0.051 ± 0.025c	103.722 ± 0.636a
120	0.278 ± 0.004de	0.116 ± 0.026de	0.044 ± 0.005c	92.342 ± 1.303b	
150	0.310 ± 0.016de	0.250 ± 0.010ab	0.111 ± 0.006b	103.729 ± 5.379a	
AS	20	0.226 ± 0.006def	0.039 ± 0.009f	0.033 ± 0.002c	18.964 ± 0.484d
40	0.335 ± 0.002cd	0.168 ± 0.008cd	0.136 ± 0.004b	25.554 ± 0.177d	
60	0.450 ± 0.058c	0.245 ± 0.003ab	0.211 ± 0.006a	28.552 ± 0.725d	
80	0.903 ± 0.012b	0.267 ± 0.043bc	0.233 ± 0.012a	18.986 ± 0.256d	
100	1.120 ± 0.111a	0.296 ± 0.016a	0.207 ± 0.017a	19.133 ± 0.908d	

The values are the means of three replicates. Means followed by different letters in the same line are significantly different at $p < 0.05$ according to Duncan's method

The Cl^- and SO_4^{2-} contents in different organs were all increased under saline stress (Fig. 5A, B, C, J, K, L). Alkaline stress had a little effect on Cl^- content compare to saline stress (Fig. 5A, B, C). The SO_4^{2-} content in stems and leaves were increased under alkaline stress (Fig. 5K, L), in contrast, the SO_4^{2-} content in roots were decreased (Fig. 5J). The NO_3^- contents in roots was decreased under alkaline stress with increasing salinity, contrary to the change trend of NO_3^- content in stem and leaves (Fig. 5D, E, F). Generally, the NO_3^- content in stems and leaves were higher under alkaline stress than that under saline stress, in opposite to the roots. The H_2PO_4^- content in roots was increased under saline stress whereas decreased under alkaline stress (Fig. 5G).

The ratio of Na^+/K^+ in different organs were all increased with increasing salinity under saline and alkaline stresses. The ratio of Na^+/K^+ was decreased from the root to the shoot. The reducing degree under alkaline stress was higher than that under saline stress. The ratio of Na^+/K^+ in root was the largest under alkaline stress (Table 3). SA reflects the selective absorption of Na^+ , K^+ by the root systems (Wang *et al.*, 2002). The SA value of *G. soja* seedlings was increased under saline stress (Table 3). The SA value was increased from the control to 60 $\text{mmol}\cdot\text{L}^{-1}$ alkaline stress, and then decreased from 60 $\text{mmol}\cdot\text{L}^{-1}$ alkaline stress to 100 $\text{mmol}\cdot\text{L}^{-1}$ alkaline stress. The SA under alkaline stress was much lower than that under saline stress, and the SA value of *G. soja* seedlings under saline stress was 215.58 % as high as that under alkaline stress in the same salinity (60 $\text{mmol}\cdot\text{L}^{-1}$).

Discussion

The plant growth is always inhibited by soil salinity. The RGR is an important stress metric that allows the response of plants under stress to be calibrated in a standardized manner (Almansouri *et al.*, 1999). In terms of relative growth rate (RGR) of *G. soja* seedlings, shoot and root RGR were decreased to a much degree under alkaline stress than that equivalent plants under saline stress. It means the injurious effect of alkaline stress was severe than saline stress for *G. soja* seedlings, that may due to alkaline stress exerts the same stress factors as salt stress but with the added influence of high-pH stress (Wang *et al.*, 2011). Water content was also an important factor to evaluate survival potential for plant under stress. Water contents in root of *G. soja* seedlings were greater than that in shoots under high concentration saline and alkaline stresses. It appeared to maintain a high water content to an extent was a tolerance mechanism for *G. soja* under higher saline and alkaline stress, which might be a key characteristic of *G. soja* that allow it to accumulate osmolytes with minimum energy consumption.

G. soja, its diurnal change of p_N showed the bimodal curves under no stress; however, with increasing stress intensities, bimodal curves were getting less obvious, particularly under higher saline and alkaline stresses the diurnal change of p_N was transformed to be single peak curve. PEPCase was activated by a high concentration of Cl^- that transformed C_3 photosynthetic pathway to C_4 or CAM to adapt to salt environment. This assumption has been confirmed in some halophytes and salt-tolerant plant

species, and the photosynthetic pathway switch might be an efficient energy-conserving strategy (Niewiadomska *et al.*, 2004). In the present study, large accumulation of Cl^- in *G. soja* leaves may be an important factor for the transfer of the photosynthetic curve under saline stress. Changing mechanism of photosynthetic curve of *G. soja* seedlings under alkaline stress needs to be further study.

Salt-induced reduction in photosynthetic capacity, as salt stress impairs p_N , E , and g_s (Chaves *et al.*, 2009; Koyro, 2006). In the present research, increasing stress intensity led to marked reduction in p_N , E and g_s under alkaline compared to the same concentration saline stress. It revealed that the resistance of *G. soja* to saline conditions was stronger than alkaline ones. The drop in E and g_s of *G. soja* seedlings under both stresses indicated that decreased photosynthetic capacity has correlated with the stomatal factor. The main aim of salt tolerance is to increase water use efficiency under salinity (Parida & Das, 2005). WUE of *G. soja* seedlings was promoted by saline stress, and inhibited by high concentration alkaline stress which also can be reflected stronger resistance to saline stress for *G. soja*. In some other studies, photosynthesis was shown not to be affected by salinity and even stimulated at low salt concentrations, and such photosynthetic adaptation to salinity is observed in halophytes (Rabhi *et al.*, 2012). Meanwhile p_N of *G. soja* seedlings was promoted under low concentration saline and alkaline stresses can also be confirmed that *G. soja* was a plant with salt tolerance.

Photosynthesis limitation can be also attributed to an inhibiting feedback exerted by high sugar content in mesophyll cells often observed in leaves of salt-treated plants (Rabhi *et al.*, 2012). The soluble sugar in leaves of *G. soja* seedlings under high concentration saline and alkaline stresses was no significantly different compared to control, and insufficiently to the degree that inhibits photosynthesis. For salinity <120 $\text{mmol}\cdot\text{L}^{-1}$, increased soluble sugar in root with increasing salinity; soluble sugar in root under 20 $\text{mmol}\cdot\text{L}^{-1}$ alkaline stress higher in comparison to the control, and there was no pronounced change in leaves under saline condition. The result suggested that soluble sugar accumulation of *G. soja* seedlings played a certain role in the osmotic adjustment under both stresses.

In general, high concentration Cl^- in leaves of plant was considered as major reason which resulted in damaging chlorophyll; high pH might increase the activity of the Chl-degrading enzyme chlorophyllase (Reddy & Vora, 1986; Saleem *et al.*, 2014). Results from this study showed Cl^- contents were tiny while chlorophyll was much lower under alkaline stress. In conclusion, chlorophyll was damaged by high-pH. The Chl a/b ratio of *G. soja* seedlings maintained at 3:1 under saline stress, but it was decreased with increasing salinity under alkaline stress and the value of Chl a/b ratio was 2 approximately under higher alkaline stress which meant light-harvesting capacity of *G. soja* declined. Non-stomatal inhibition to photosynthesis of *G. soja* seedlings induced by alkaline stress was more severe than saline stress. Carotenoid stabilizes and protects the lipid phase of the thylakoid membranes, and is quenchers of the excited triplet state of chlorophyll and singlet oxygen (Bu *et al.*, 2012). In the present study, carotenoids of *G. soja*

seedlings showed no significant change under saline while inhibited by alkaline stresses indicated that carotenoids play a protective role for *G. soja* under saline stress, but limited to resist alkaline stress.

Salt stress increased Na^+ concentration in root of *G. soja* seedlings, and meanwhile decreased K^+ in the present study, except Na^+ under high alkaline stress (compared with control, Na^+ was almost not accumulated under high alkaline stress). Accumulation of Na^+ and impairment of K^+ nutrition is a major characteristic in salt-stressed plants of most species, which caused directly by interfering with K^+ -selective ion channels in the root plasma membrane (Tester & Davenport, 2003). It's not only resulted in ion toxicity but also induced nutrient imbalance. Low Na^+ and high K^+ in the cytoplasm are essential for the maintenance of many enzymatic processes, and many studies on halophytes and some tolerant glycophytes plants showed that a high K^+/Na^+ ratio was a salt tolerance criterion (James *et al.*, 2006). Our results showed Na^+/K^+ ratio in different tissues were all increased with increasing salinity under both stresses, and the extent of the increase under alkaline stress were higher than that of saline stress. It indicated that the *G. soja* seedlings had stronger selective ability for K^+ ion under saline stress than that under alkaline stress, and it also can be further supported by SA of root which showed that the capacity of selective transport by root systems was also stronger under saline stress than that under alkaline stress in the same concentration. Although Na^+/K^+ of *G. soja* seedlings increased under both stresses, it can't be implied *G. soja* was species with salt sensitive, it varied between stress extent or salt properties. High pH caused strong alkaline stress to severely affect ion accumulation and to destroy the structure of the root cells and even lead to cell death, even couldn't accumulate soluble sugar any more, ion imbalance and loss of osmotic adjustment of root under alkaline stress. The main reason for the negative impact of high pH on ions accumulation was that high pH caused a lack of protons outside the root, which impeded the transmembrane proton gradient (Zhang & Mu, 2009).

Root is the plant organ which is in direct contact with saline medium (Aroca *et al.*, 2009). The present results showed stronger selective ability of root under saline stress than that of alkaline stress, therefore, roots of *G. soja* seedlings were the principal organ for accumulating Na^+ under saline stress, and Na^+ content in leaves was much lower compared to root and stem. The effective regulation of roots inhibited Na^+ influx to leaves under saline stress, and there was no significant change of K^+ in leaves. It seemed reasonable that the Na^+/K^+ in leaves of *G. soja* seedlings was relatively lower under saline stress compare to root. Under low alkaline stress intensity, Na^+ content in root was increased which change trend was similar with saline stress, but Na^+ was almost not accumulated under high alkaline stress, it's result from that roots were injured so much by high pH that could not maintain ion balance, which result in Na^+ accumulation in leaves to toxic level. The main site of Na^+ toxicity for most plants is the leaf blade, where Na^+ accumulates after

being deposited in the transpiration stream, rather than in the roots (Munns & Tester, 2008). The harmful effect of high pH wasn't resisted by pH adjustment outside the roots under high alkaline stress intensity which the stress intensity exceeded the capacity for root adjustment. Na^+ metabolic regulation of roots was vital to maintain growth and photosynthesis of *G. soja* under both saline and alkaline stresses.

Different from saline stress, the K^+ , NO_3^- , Mg^{2+} and Ca^{2+} contents in leaves were increased with increasing Na^+ under alkaline stress, and the contents of these ions under alkaline stress were higher than that under salt stress in the same concentration. This has already been reported that the accumulation of Ca^{2+} and Mg^{2+} , might be a special adaptation of natural alkali-resistant halophytes *Suaeda glauca* and *Kochia sieversiana* to alkali stress (Yang *et al.*, 2008). This result pleaded in favour of the previous conclusion that Salt resistant plants usually show a stronger capability to maintain uptake of K^+ , NO_3^- and Ca^{2+} , and while Na^+ and Cl^- uptake must be restricted (Tavakkoli *et al.*, 2011). Compared with the saline resistant capacity of *G. soja*, alkaline resistant capacity is even worse, but also reflected the properties of stress tolerance under high alkaline stress. Calcium is required for cell-wall structure and membrane integrity and is considered one of the second messengers in plant responses to stress and other signal-transduction processes (Cui *et al.*, 2002). Our results showed that the accumulation of Ca^{2+} in roots was stimulated by low and inhibited by high alkaline stress, and salt stress had little effect on the Ca^{2+} concentration. This result was consistent with the response of tomato to alkaline stress, and accumulation of Ca^{2+} in roots may help to start the salt signal system, but it was weakened by high pH under higher alkali stress (Wang *et al.*, 2011). It must be mentioned that a large amount of Na^+ was accumulated in stems of *G. soja* seedlings under both stresses, particularly Na^+ accumulated most in stems under alkaline stress. Interestingly, *G. soja* may reduce transport of Na^+ to leaves and store it in stem, which could reduce the damaging effect of toxic ion on photosynthetic organ, and it is an another experimental evidence to verified *G. soja* with salt tolerance.

Effects of salinity on growth rates and photosynthesis are due to specific ion toxicity (Kingsbury & Epstein, 1986). Salt-induced Na^+ accumulation in leaves may decrease the stability of PSII functions and inhibit photosynthetic electron transport (Muranaka *et al.*, 2002). Our result showed that a large number of Na^+ was accumulated in leaves of *G. soja* seedlings under saline and alkaline stresses, and meanwhile Na^+ content in leaves of alkaline stressed seedlings was greater than that of saline stress in the similar concentration. Plenty of Cl^- was just accumulated under saline stress, p_N and RGR of *G. soja* seedlings decreased under both stresses. The Na^+ accumulation showed a high correlation with the progressive decrease in growth and most of the photosynthetic characteristics studied. Na^+ was the major toxic ion for *G. soja*, and this finding in agreement with

the previous study (Luo *et al.*, 2005). Both Na⁺ and Cl⁻ are toxic to plants at high concentrations, and large quantities of Cl⁻ was accumulated in leaves of *G. soja* seedlings under high saline stress, so the detrimental effect of great Cl⁻ accumulated under high saline stress can not be ignored.

From a Cl⁻ perspective, uptake of Cl⁻ could be balanced by the uptake of another cation, for example, Na⁺, or by the loss of another anion (Teakle & Tyerman, 2010). Tiny accumulation of Cl⁻ in *G. soja* seedlings under alkaline stress may be due to the existence of CO₃²⁻ and HCO₃⁻ instead of Cl⁻ to balance Na⁺. It is also related to the increasing NO₃⁻ concentration in leaves under alkaline stress, there has been also reported that many anion channels are more selective to NO₃⁻ than to Cl⁻, and Cl⁻ concentration in leaves can be reduced by the presence of NO₃⁻ in the growth medium (Teakle & Tyerman, 2010). Levels of Cl⁻ and SO₄²⁻ were significant accumulated under high saline stress (150 mmol·L⁻¹), and this could be a special response for *G. soja* to saline stress and might be related to salt-stress components (NaCl, Na₂SO₄). The special absorption mechanism of Cl⁻ in *G. soja* under alkaline stress deserves further investigation. Under saline stress, ion imbalance in root of *G. soja* caused by the influx of superfluous Na⁺, and the accumulation of Cl⁻, SO₄²⁻, H₂PO₄⁻ in root played an important role in keeping anion and cation balance and maintain tissue pH constant intracellular. The absorption of anions were inhibited, while Na⁺, Ca²⁺ and Mg²⁺ in roots were all increased under low alkaline stress, response of *G. soja* to saline stress was similar with low alkaline stress, such as photosynthetic rate was stimulated, might be other organic anion which could provide large quantities of negative charges to maintain charge balance must be synthesized. The energy consumption from absorbing inorganic ions is far less than from synthesizing organic compounds (Munns & Tester, 2008), therefore, *G. soja* needs to spend more materials and energy to adapt to alkaline stress than for saline stress, and possibly a reason for the lower RGR and photosynthesis under alkaline stress.

Conclusions

These observations imply that in *G. soja* and there are obvious differences between physiological adaptive mechanisms to saline stress and alkaline stress, which should be further investigated. Saline stress and alkaline stress are two distinct kinds of stresses also be confirmed.

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