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Comparison of the Effects of Saline and Alkaline Stress on Growth, Photosynthesis and Water-Soluble Carbohydrate of Oat Seedling (*Avena sativa L*)

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1. Introduction

High-quality arable land is necessary for human socio-economic development, but up to 60% (0.9×10^9 ha) of the world's arable land is considered too saline and/or too alkaline for agricultural use, it severely affect agricultural productivity (Richards 1990; Allakhverdiev et al., 2000; Läuchli and Lüttge 2002). The main ions in salt soil are Na^+, K^+, Cl^- and NO_3^- in the salt soil, the saline stress usually had been defined two distinct kinds of stresses: saline stress and alkaline stress (Shi and Yin 1993). Saline stress generally involves osmotic stress and ion injury, but alkaline stress not only included osmotic stress and ion injury, but also has high pH effect (Yang et al., 2009a). The high pH environment surrounding the roots can directly cause mineral elements availability significantly decreased and Ca^{2+}, Mg^{2+} and H PO_3^- to precipitate, which possible inhibit ion uptake and disrupt the ion homeostasis of plant cells (Shi and Wang, 2005; Xue and Liu, 2008).

Oat is one of the major economic and ecological food crops in the world, and it is a kind of low sugar, high protein, fat and energy foods, it's exposed to multiple environment stresses, including drought, salinity etc (Shtangeeva and Ayrault, 2007). But salinity and alkalinity are significant constraints on the productivity of oat, a major staple cereal crop with global socioeconomic importance (Gale, 2005). The tolerance of oat has been widely studied; its physiological characteristics enable it to tolerate saline stress, however there were only a few researches on alkaline stress. In this study, the aim was to analyze and compare the growth, photosynthesis and water soluble carbohydrate in oat seedlings under saline and alkaline stresses, to determine and elucidate how high pH levels cause damage to oat seedlings tissues and how oat adapt to overcome alkaline stress. The goal can be achieved either by improving the soil or planting crops that are tolerant to both saline and alkaline stress.

Abbreviations

FW – fresh weight: *DW* – dry weight; RGR − relative growth rate; *WC* – water content; *P*n – net photosynthetic rate; *g*s –stomatal conductance; *E* –transpiration rate; *WUE* –water use efficiency.

2. Materials and methods

2.1 Design of simulated saline and alkaline conditions

Saline conditions were simulated by mixing the neutral salts NaCl and Na_2SO_4 in a 9:1 molar ratio and applying them to germinating wheat plants at five concentrations: 20, 40, 60, 80 and 100 mM. Similarly, alkaline stress conditions were simulated by mixing $NaHCO_3$ and Na_2CO_3 in a 9:1 molar ratio and applying them to germinating wheat plants at the same five concentrations. The electrical conductivity (EC), pH and osmotic potential of the stress treatment solutions were measured using a conductivity meter (DDG-2080-S, Anhui, China), PSH-3C and a water potential meter (Psypro Wescor Corporation, US), respectively.

2.2 Plant materials and growth

Seedling growth was tested by sowing 20 seeds in 17-cm diameter plastic pots containing 2.5 kg of washed sand. The seedlings were watered daily with 0.5 time Hoagland nutrient solution. All pots were placed outdoors and sheltered from rain, with a day/night temperature range of 21.0–25.5°C/18.5–21.0°C. After 4 weeks, 60 pots containing uniformly growing seedlings were selected and divided randomly into 12 sets, each comprising five pots. One set was used as the untreated control (watered with Hoagland nutrient solution), a second set was used to determine growth parameters at the beginning of treatment (see below), and the 10 remaining sets were used for the stress treatments, which were applied daily for 16 days.

2.3 Measurement of physiological indices

Relative growth rate (RGR) was determined by RGR = [(ln Dry Weight (DW) at the end of stress treatment) – (ln DW at the start of stress treatment)] / total treatment duration. The water content (%WC) was calculated using the formula (FW-DW)/DW. The electrolyte leakage rate (%ELR) was determined by measuring the electrical conductivity of 1 g fresh leaf material (after washing three times with deionized water to remove surface electrolytes) which was placed in a closed cuvette containing 20 ml deionized water and incubated at 25°C on a rotary shaker for 4 h, and comparing it to the electrical conductivity of the same sample after autoclaving at 120°C for 20 min. The activity of the root system was determined by incubating fresh root tissue for 60 min at 37°C in 0.04% triphenyl tetrazolium chloride (TTC) in phosphate buffer (pH 7.0), extracting the red product in ethyl acetate and determining is absorbance at 485 nm by spectrophotometry. The leaves were measured by portable photosynthesis system (LI – 6400, USA) in their locations to determined net photosynthetic rate (P_n), stomatal conductance – (*g*s) and transpiration rate (*E*) of leaves, the water use efficiency (*WUE*) has been determined by $WUE = P_n / E$.

2.4 Measurement of carbohydrates

The shoots and roots of oat seedlings were oven-dried at 80℃ separately and then ground into small pieces. The fructan, sucrose, glucose and fructose content were determined using a high performance liquid chromatography diode (HPLC, Shimadu Class - VP, Japan), using columns (Sugar – PAK™ I) produced by the Waters company, and the concentration of the mobile phase was 0.1 mmol·L^{-1} $EDTANa_2Ca$, with the flow velocity controlled at 0.3

mL·min^{-1} at 70°C. The retention times of fructan, sucrose, glucose and fructose were 9.058, 13.545, 16.740 and 20.349 min, respectively. According to the chromatographic peak area, the carbohydrate content was determined (Rovia et al., 2008). The total carbohydrate content was determined according to the chromatographic peak area (Corradini et al., 2004; Rovio et al., 2008) using the phenol–sulfuric acid method (Somani et al., 1987).

2.5 Statistical analyses

Statistical analysis included one-way analysis of variance (ANOVA) in SPSS (Version 13.0, SPSS, Chicago, IL, USA) and Duncan's method to detect differences in physiological parameters in plants under saline and alkaline stress (P <0.01).

3. Results

3.1 Electrical conductivity (EC) and osmotic potential of stress treatment solutions

The results given in Table 1 show that, with increased salinity, the EC increased (Saline stress: 3.67 to 11.23 dS/m; Alkaline stress: 3.01 to 9.98 dS/m), while the osmotic potential of the stress treatment solution decreased (Saline stress: -0.18 to -0.59 MPa; Alkaline stress: -0.13 to -0.52 MPa). The pH value decreased slightly with increasing saline concentration (Saline stress: 6.51 to 6.40), but it increased in alkaline stress solution (Alkaline stress: 8.30 to 9.56).

Salinity (mM)		EC (dS/m)	pH	OP (MPa)
Control		1.60	6.70	-0.05
Salt-stress	20	3.67	6.51	-0.18
	40	6.10	6.55	-0.25
	60	8.30	6.46	-0.38
	80	10.68	6.41	-0.51
	100	11.23	6.40	-0.59
Alkali-stress	20	3.01	8.30	-0.13
	40	4.97	8.66	-0.21
	60	6.48	8.69	-0.30
	80	8.21	8.84	-0.47
	100	9.98	9.56	-0.52

Table 1. The electrical conductivity (EC), pH and osmotic potential (OP) of stress treatment solutions

3.2 Growth

The patterns of RGR of shoot and root decreased with increasing salinity concentrations, but the decreased trend of alkaline stress was significantly than saline stress (Fig.1 A and B). The pattern of *WC* was similar as the pattern of RGR, the extent of reduction under alkaline stress was greater than those under saline stress (Fig. 1 C and D). Saline stress caused only minor changes in ELR and RST, but alkaline stress led to a significantly increase in ELR and RST declined dramatically (Fig. 1 E and F).

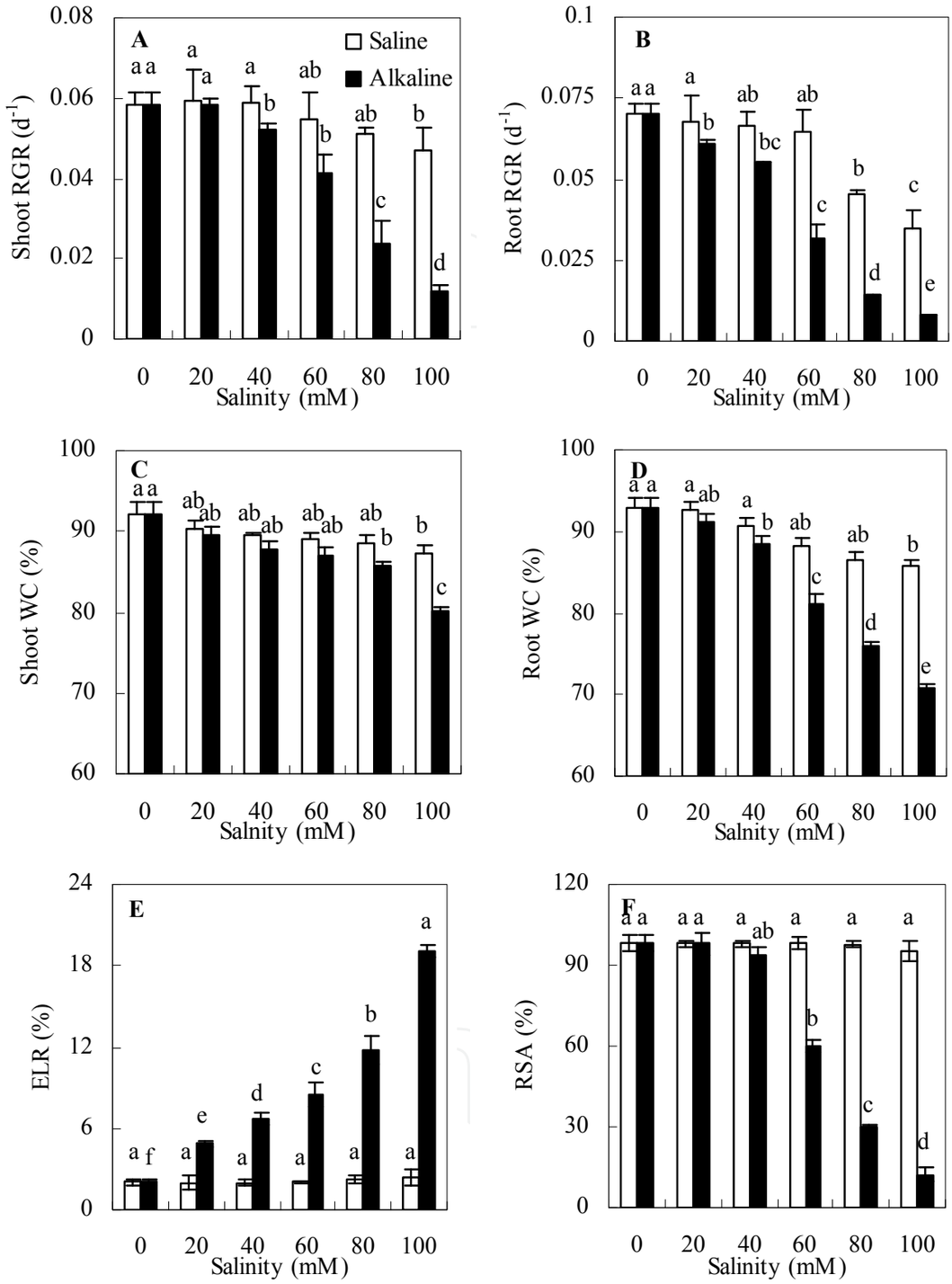


Fig. 1. Effects of salt and alkali stresses on under and up ground relative growth rate (RGR), water content (WC), Electrolyte leakage rate (ELR) and Root system activity (RSA). Salt-stress: NaCl:Na_2SO_4=9:1; Alkaline-stress: $NaHCO_3$:Na_2CO_3=9:1, the values are the means of five replicates. Means followed by different letters in the same stress type are significantly different at P <0.01 according to Duncan's methods

3.3 Photosynthesis

Under saline stress, P_n decreased slightly with increasing saline concentrations, however, under alkaline stress, the P_n value decreased dramatically with alkaline concentration increased (Fig. 2 A). The change of *g*s had a little increased at 40mM saline stress and then beginning to decreased, however, gs decreased sharply under alkaline stress (Fig. 2 B). There was no any change of *E* under saline stress, but under alkaline stress *E* decresed significantly with increasing stress, especially at 60mM (Fig. 2 C). No significant changes in *WUE* of oat seedlings was treated with saline stress, compare with saline stress, *WUE* had a decreased trend under alkaline stress (Fig. 2 D).

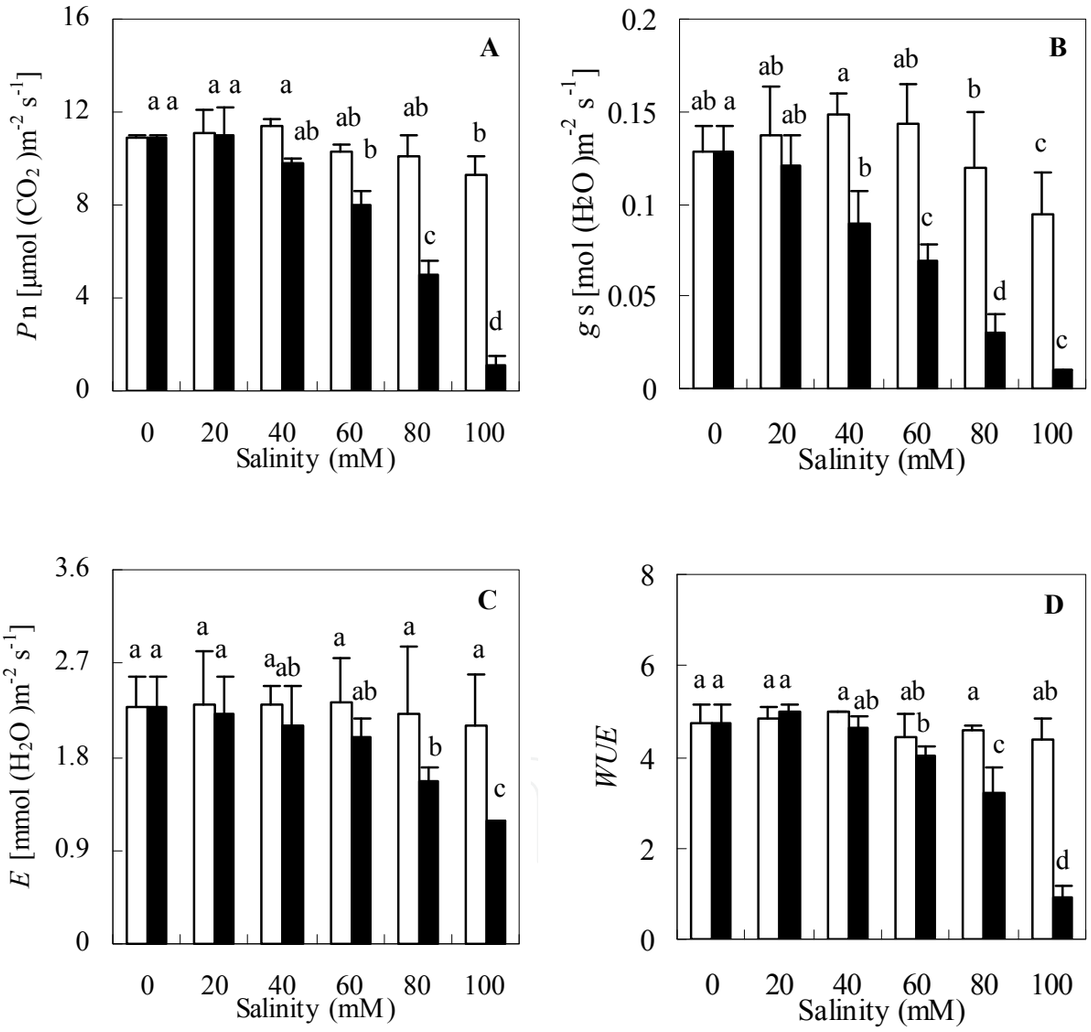
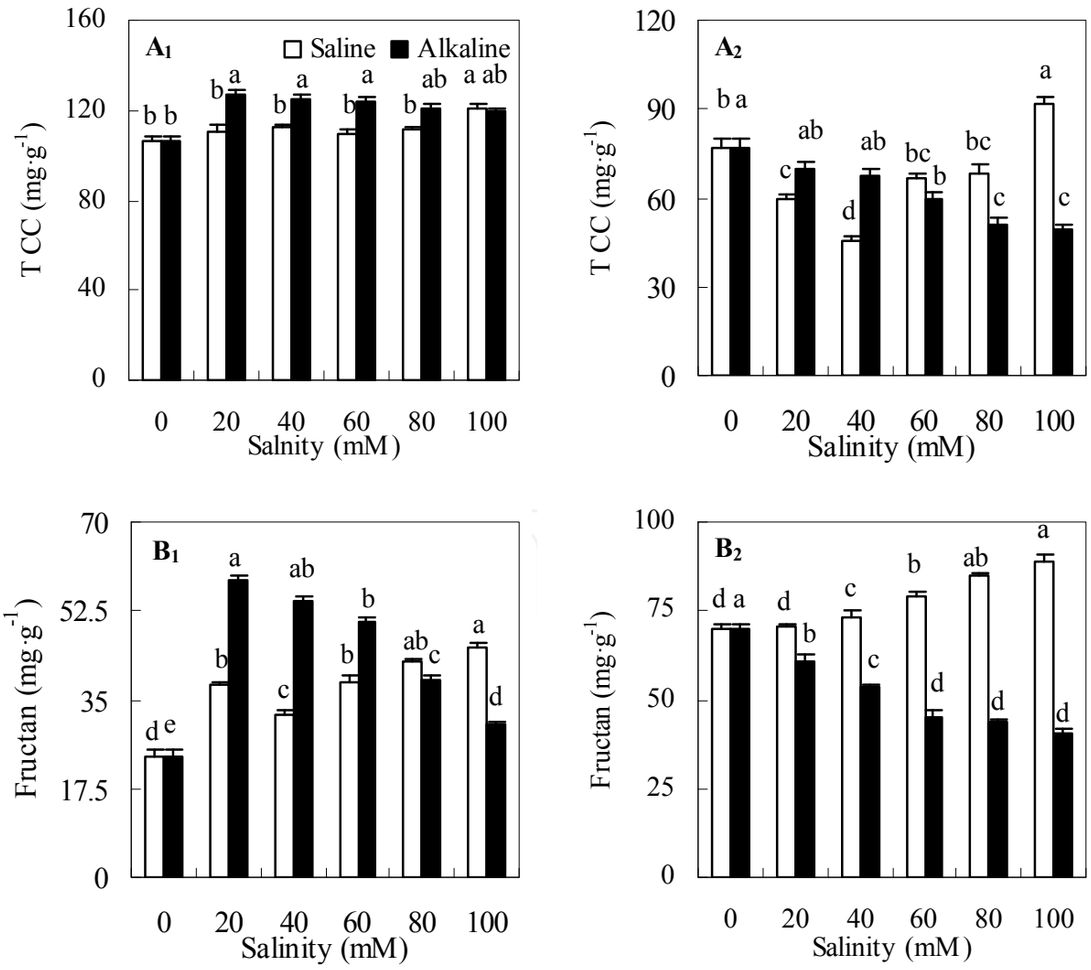


Fig. 2. Effects of salt and alkali stresses on net photosynthesis - *P*n (A), stomatal conductance - *g*s (B), transpiration rate - *E* (C) Water Use Efficiency – *WUE* (D). Salt-stress: $NaCl:Na_2SO_4$=9:1; Alkaline-stress: $NaHCO_3:Na_2CO_3$=9:1, the values are the means of five replicates. Means followed by different letters in the same stress type are significantly different at P <0.01 according to Duncan's methods

3.4 Carbohydrates

There was little change in TCC in the shoot of oat seedlings with two stresses, it increased slightly with higher salinity and alkalinity (Fig 3 A_1). The TCC of root of the control was relatively high under saline stress and it increased with increasing saline stress after 40mM; but the change in TCC declined significant reduction under alkaline stress (Fig 3 A_2). The fructan content of shoot increased under saline stress with increasing salinity, but under alkaline stress it was higher than in the control and appeared to show decline trend with salinity increasing (Fig 3 B_1). The fructan contents in root of oat seedling under saline stress showed increased change trend with increasing salinity concentration; but the fructan contents decreased with alkaline stress increased (Fig. 3 B_2). Under two stresses sucrose content of shoot and root were no significant changed compared with the control (Fig. 3 C_1 and C_2), and the trends of change in glucose were same as the patterns of the fructan (Fig. 3 D_1 and D_2), however it nearly no accumulated in root under two stresses (Fig. 3 D). Under 40mM saline stress, saline stress promoted fructose synthesis and then decreased with increasing salinity; which was higher under alkaline stress than in the control and appeared to have no significant dependence on salinity (Fig. 3 E_1); however, it nearly no accumulated in root under two stresses (Fig. 3 E_2).



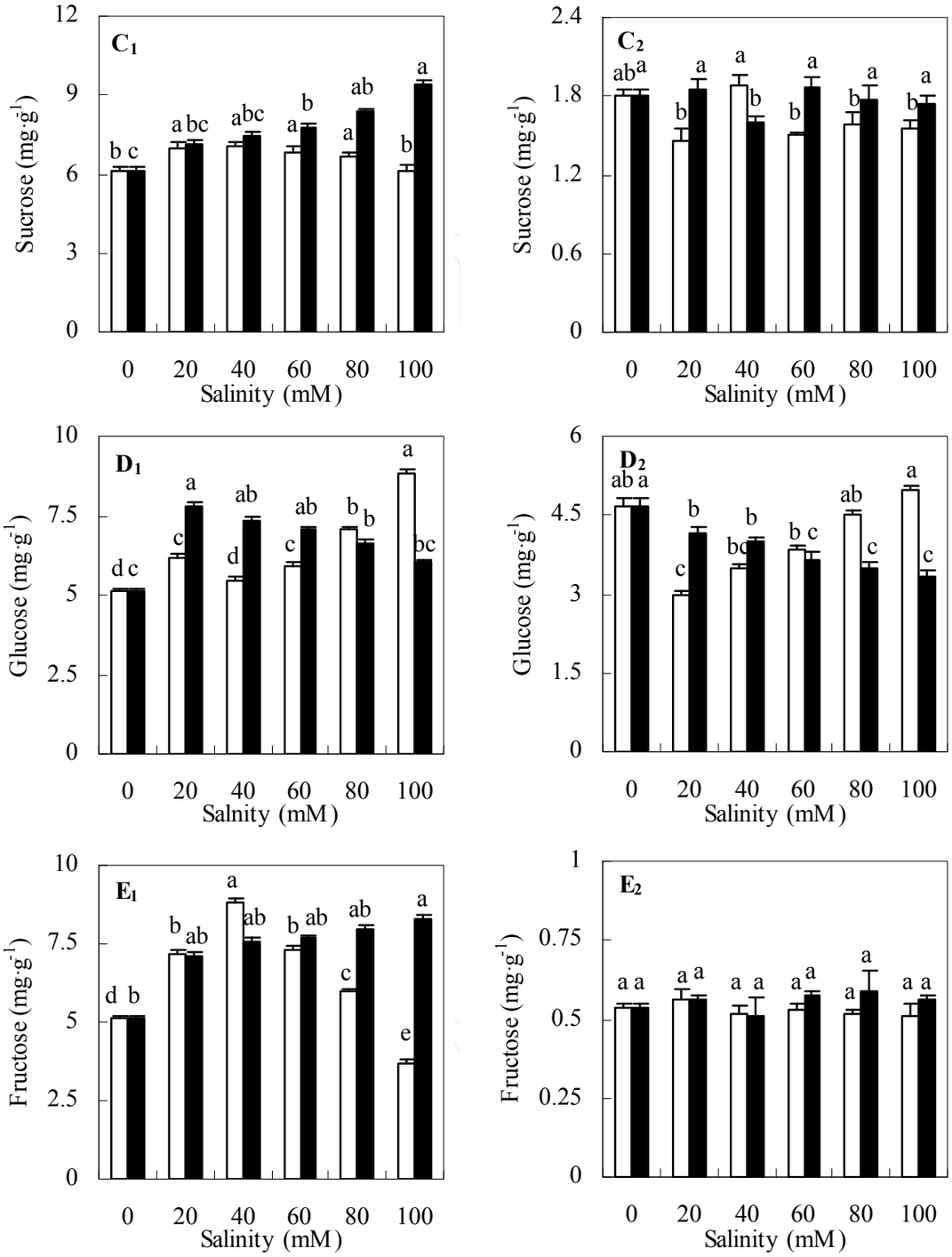


Fig. 3. Effects of salt and alkali stress on the levels of total carbohydrate content (TCC) (A1), fructan (B_1), sucrose (C_1), glucose (D_1) and fructose (E_1) in the shoots of oat seedlings, and of total carbohydrate content (TCC) (A_2), fructan (B_2), sucrose (C_2), glucose (D_2) and fructose (E_2) in the roots of oat seedlings. Salt-stress: NaCl:Na_2SO_4=9:1; Alkaline-stress: $NaHCO_3$:Na_2CO_3=9:1, the values are the means of five replicates. Means followed by different letters in the same stress type are significantly different at P <0.01 according to Duncan's methods

4. Discussion

4.1 Growth

The RGR of plant is considered to be an important index for the degree of stress, our results show that the RGR decreased in oat seedlings with increasing stress intensity for both stresses; however, the decrease for alkaline stress was greater than saline stress (Lissner et al., 1999). The different injuriousness of two stresses may result from their different mechanisms of action; the injurious effects of salinity are commonly thought to be from low water potentials and ion toxicities (Munns, 2002). Figure 1 F indicated that saline stress did not affect oat seedling root system activity, whereas alkaline stress led to a sharp decrease and even to the death of root cells. We suspect that there are the direct effect (root damage) and indirect effect (nutritional disorder) in alkaline condition; it's maybe the reasons why alkaline stress is more harmful to oat than saline stress. WC as approach to osmotic adjustment reflects osmotic stress, in this experiment compare with saline stress, alkaline stress cause WC severe reduced. Moreover, there nearly no effects of saline stress on the ELR, however, it sharply increased under alkaline stress. The results revealed that alkaline stress might damage root functions, including, the absorption of water and ions. These may be the main reasons why the oat RGR was lower for alkaline than saline stress (Yang et al., 2008).

4.2 Photosynthesis

A regression analysis between *P*n and saline concentration is shown in Table 2, the *P*n decreased by 3.525 for each 10 mM increase in saline stress and by 19.552 for each 10 mM increase in alkaline stress (Table 2). The photosynthesis of oat seedling were significantly lower under alkaline stress than that under saline stress, suggesting that alkaline stress might not only damage root functions, but also it further affects contents of photosynthetic area, photosynthetic pigments and the membrane system. Under both stresses, the environment water potential and WC of oat seedlings (Fig. 1 C and D) decreased with increasing stress intensity, the g_s was closely correlated with the change of environment water potential. The g_s of oat seedlings were lower than that of control, especially under alkaline stress. The *WUE* fell sharply under alkaline stress (Table 2), it is likely that the reduction in intracellular CO_2 partial pressure caused by stomatal closure helped to reduce net photosynthetic activity, and that the high pH in the leaf had a direct effect on the photosynthetic apparatus and on water potential, resulting in the differing results for water use efficiency (Bethke and Drew, 1992; Yang et al., 2009a,b). Our results showed clearly that saline stress had only a marginal effect on photochemical processes as previously reported (Larcher et al., 1990; Everard et al., 1994; Lu et al., 2002). In contrast, alkaline stress had a significant impact on chlorophyll a fluorescence, probably because the massive Na^+ influx and the high pH combine to encourage photoinhibition, and thus reduce the activity of *PSII*.

4.3 Carbohydrates

The accumulation and secretion of carbohydrates are a physiological response of plants to various stresses (Rosa-Ibarra and Maiti, 1995; Pan 2001). There is a different in the accumulation of carbohydrates in shoot and root respond to saline and alkaline stress, the results indicated that carbohydrates were accumulated in shoots under both saline and

alkaline stresses; however, the extent of accumulation under alkaline stress was much higher than under saline stress. In the process of adapting to two stresses in root, oat seedlings with different adaptive pathways will have different carbohydrates metabolic regulating. The research indicated that the different part of origination of oat seedlings might have different physiological pathways under saline and alkaline stress, which should be further investigated. The results of the component of carbohydrates showed that fructan was the dominant component in oat seedlings. Fructan is considered to play a key role in stress-induced metabolic processes, which appears to be advantageous for plants under low temperature, drought and anoxia (Galiba et al., 1997). Our data implied that under saline stress, oat seedling accumulated fructan to resist saline stress, however, the concentrations of fructan was decreasing with salinity concentration increase under alkaline stress, suggesting that alkaline stress was more harmful for oat seedling than saline stress. Oat seedling might enhance fructan synthesis to remedy the osmotic stress and to maintain a stable ion balance to avoid ion injury; but the high pH of alkaline stress might inhibit the fructan synthesis. The material and energy demands of fructan synthesis are much greater. Sucrose is the main carbohydrates transport form and fructose is one of monosaccharides and had a significantly high concentration in drought sensitive in plant and it participate the fructan metabolism (Martin et al., 1993, kerepesi et al., 1998). In this research, there are no significant change and differences in sucrose and fructose content under two stresses in oat seedlings root, it reveal saline and alkaline no affect they synthesis. The change trend of glucose was followed the fructan, it indicated that under saline stress glucose content increased with salinity increased, which could synthesis more glycosides, oligosaccharides and polysaccharides to increase osmotic potential in intracellular, and enhance the salt tolerance of cells (Ghasempour et al., 1998). But the alkaline stress inhibited or destroyed glucose synthesis.

	Regression equation	R^2	Decrease in *P*n and *WUE* for 10 mM increment in salinity
*P*n	Y_{Salt}= -0.3525x + 11.745	0.699	3.525
	Y_{Alkali}= -1.9552x + 14.477	0.883	19.552
WUE	Y_{Salt}= -0.0904x + 4.984	0.5445	0.904
	Y_{Alkali}= -0.7142x + 6.2631	0.7702	7.142

Table 2. Regression equations between *P*n and *WUE*

5. Conclusion

In summary, the germination of oat was inhibited by both saline and alkaline stress. The inhibit impact of alkaline stress was significantly greater than those of saline stress, reflecting the specific detrimental effects of a high pH environment. Alkaline stress might caused roots' physiological structure been destroyed, reducing root system activity, and finally inhibit oat seedlings growth. The photosynthesis of oat was not significantly lower than that of control until the intensity of saline stress reached 80 mM, but decreased sharply

under alkaline stress with increasing salinity. This implied that saline stress and alkaline stress were actually not only two distinct stresses, but also that the resistance of oat to saline stress was stronger than that to alkaline stress, indicating intracellular photosynthesis were interfered with by alkaline stress (high pH). The content of carbohydrate increased to a greater extent in response to alkaline stress than saline stress. These data suggest that plants may initially sense high pH environments as an extreme form of salinity stress, such that the response is initially similar to the response to saline stress but more pronounced. However, because these tolerance mechanisms are not sufficient to counteract the specific toxic effects of high-pH environments, additional and more specific mechanisms such as reduced fructan synthesis. These results provide useful data that will facilitate the development of strategies for the creation of engineered oat varieties that are more tolerant towards alkaline stress.

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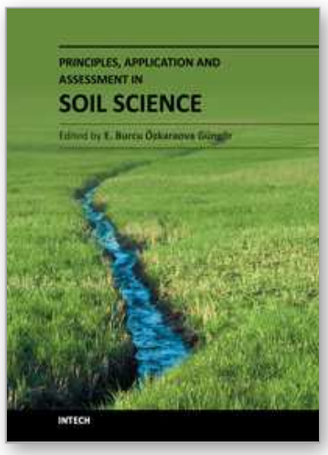

Principles, Application and Assessment in Soil Science
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Our dependence on soil, and our curiosity about it, is leading to the investigation of changes within soil processes. Furthermore, the diversity and dynamics of soil are enabling new discoveries and insights, which help us to understand the variations in soil processes. Consequently, this permits us to take the necessary measures for soil protection, thus promoting soil health. This book aims to provide an up-to-date account of the current state of knowledge in recent practices and assessments in soil science. Moreover, it presents a comprehensive evaluation of the effect of residue/waste application on soil properties and, further, on the mechanism of plant adaptation and plant growth. Interesting examples of simulation using various models dealing with carbon sequestration, ecosystem respiration, and soil landscape, etc. are demonstrated. The book also includes chapters on the analysis of areal data and geostatistics using different assessment methods. More recent developments in analytical techniques used to obtain answers to the various physical mechanisms, chemical, and biological processes in soil are also present.

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