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Research Article

Physiological responses and adoptive mechanisms in oat against three levels of salt stress

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Abstract

To identify the propagation mechanisms and the adaptive strategies of oat seedlings exposed to NaCl, $NaHCO_3$ and Na_2CO_3 the main salts in the soils of the salt-alkali grasslands of the Songnen Plain of China, growth rates and physiological indices of oat seedlings were measured in plants grown in soils with different concentrations (48-144 mmol L^{-1}) of the three salts. The results demonstrated that although oat seedlings survival rates were unaffected by NaCl stress, the tiller number, plant height, and shoot and root dry weights decreased with increasing salt concentration, in the order of Na_2CO_3>$NaHCO_3$>NaCl. In addition, propagation mechanisms higher concentrations of Na^+ accumulated in the shoots and roots of oat seedlings under Na_2CO_3 stress and $NaHCO_3$ stress than in seedlings under NaCl stress. Reductions in concentrations of K^+ were also greater under both Na_2CO_3 and $NaHCO_3$ stress than NaCl, especially in the roots. Large amounts of Cl^- and proline were found to accumulate in oat seedlings, most likely as a strategy for maintaining osmotic and ionic homeostasis under NaCl stress.

Keywords: above ground parts; index; oat; physiological responses; salt stress

Introduction

Saline-alkali stress is one of the most serious factors in limiting crop production, and has brought severe harm to ecological environment and animal husbandry development, which has been clearly demonstrated by a number of studies (Manivannan *et al*., 2007; Shi and Sheng, 2005; Shi and Wang, 2005). Around the world, saline-alkali land is widely distributed in more than 100 countries, covered 4.34×10^8 hm^2 of the arable area (Wang *et al*., 2009). Covers about 10% of arable area over the world, in addition, soil salinity has adversely affected about 30% of the irrigated area and 6% of the total land area, resulted in a monetary loss of $12 billion in agricultural production (Fahad *et al*., 2015).

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Soil salinization becomes one of the environmental problems over the world. Cations of soluble salts in saline-alkaline soil mainly include Na^+, Ca^{2+}, Mg^{2+} and K^+, anions mainly include Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} and NO_3^- (Laniel *et al*., 2019). The characteristic of saline-alkaline soil in Northeast China is that Na^+ is the main cations and Cl^-, SO_4^{2-}, HCO_3^- and CO_3^{2-} are the main anions (Yang *at al*., 2008). Once soil contains alkaline salt such as $NaHCO_3$ and Na_2CO_3, pH value of the soil will increase. At this time, plants not only suffer from saline stress but also alkaline stress. The damage effect of alkaline stress caused by alkali salts such as $NaHCO_3$ and Na_2CO_3 on plant is more obvious than that of saline stress caused by neutral salts such as NaCl and Na_2SO_4 (Yang *et al*., 2010). Soil salinization and alkalization frequently co-occur such as in the west Songnen Plain of Northeast China, it becomes more serious in grassland and farmland which cause losses in agricultural productivity and pose an ecological crisis for humans. Previous studies have suggested that alkali stress results mainly from levels of the alkaline salts $NaHCO_3$ and Na_2CO_3 (Shi and Yin, 1993; Shi and Sheng, 2005; Yang *et al*., 2010; Liu *et al*., 2010). Alkali stress usually involves a combination of stresses, osmotic, ion-induced injury and high pH (Munns, 2002; Munns and Tester, 2008; Yang *et al*., 2008; Chen *et al*., 2011). The high-pH environment that surrounds the roots can greatly affect the absorption of cations and inorganic anions and also disrupt the ionic balance and pH homeostasis of the tissues (Yang *et al*., 2008; Guo *et al*., 2010). The results are decrease in photosynthesis, damage to the membrane system and finally lead a reduction in growth. Although the effects of various mixed alkali stresses have been studied extensively in a few plants (Shi and Yin, 1993; Hartung *et al*., 2002; Shi and Wang, 2005; Guo *et al*., 2010; Radi *et al*., 2012). Over the years, most studies about the effects of salt stresses on plants have mainly focused on the leaves of adult seedlings (Lourenco *et al*., 2013; Zhang and Mu, 2009). However, little information exists concerning the growth and physiological effects on shoot and root of young seedlings. Previous researches showed that the effects of salt stress on different plant parts varied greatly among different types of plant species.

Oat (*Avena sativa* L.) is an annual crop, which belongs to the family of Poaceae. It is a world-wide cultivation crop and mainly distribute in temperate latitudes of the Northern Hemisphere. Oat has no strict requirements for cultivation soil, so it can be usually planted in various kinds of soil. With the high saline-alkaline tolerance, oat grew better than other crops when planted in semiarid and saline-alkaline soils, such as wheat. Up to now, most researches focus on crops e.g., sunflower (Shi and Sheng, 2005), and grasses such as eymus chinensis and *Puccinellia distans* (Shi *et al*., 1998) and make progresses on the growth, physiological response mechanisms and adaptive strategies of these crops/grasses to saline and alkaline stresses. However, there was little research on the growth and saline-alkali of oat to saline-alkaline stresses. In order to study the saline or alkaline tolerance of oat seedlings, NaCl, $NaHCO_3$ and Na_2CO_3 stress on water potential, photosynthetic pigments, ionic balance and tissue pH and growth in the shoot and root of young oat seedlings, to enhance our understanding of the mechanism's alkali stress damage to plants and also those by which they adapt to such alkali stress.

Materials and Methods

Plant materials

Seeds were sown in 20 cm diameter plastic pots containing 3 kg of washed sand. The pots were watered daily with sufficient Hoagland nutrient solution. Each pot contained 25 seedlings. All pots were placed outdoors and protected from the rain. The experiment was carried out in an experimental area of the Northeast Normal University during the 2017 growth season.

Design of the simulated Saline-alkali conditions

Three salts, NaCl, $NaHCO_3$ and Na_2CO_3 were applied separately to create three stress groups. Within each group, six concentrations were used: 0, 48, 72, 96, 120 and 144 mmol L^{-1}. Treatments in the NaCl stress

group were labeled A_1–A_6, the $NaHCO_3$ stress group were labeled B_1–B_6 and those in the Na_2CO_3 stress group were labeled C_1–C_6. A_1 and C_5 were the controls (Once a C5 convertase (regardless of derivation) is fixed on a pathogen surface, it cleaves serum C5 to yield C5a (which diffuses away) and C5b (which remains bound to the convertase). There were three replicates per treatment.

The stress treatments were applied when the seedlings were four weeks old. This cultivar is characterized by early maturation, high salt and alkali tolerance and high disease resistance. It has a high grain yield which rises to over 2300 kg/hm^2. Its protein and fat contents are 16.6% and 5.6%, respectively. The plant can mature in live culms and provides both grain and straw. Thirty-six pots of uniform seedlings were divided randomly into 18 sets of 3 pots (seeds were sown in 20-cm diameter plastic pots containing 3 kg of washed sand. The pots were watered daily with sufficient hoagland nutrient solution. Each pot contained 25 seedlings. All pots were placed outdoors and protected from the rain. Two sets were used for the controls and the remaining 10 sets were used for the stress treatments giving three replicate pots per treatment. The treated pots were watered daily between 16.00-18.00 h with excess of a nutrient solution that include two alkaline salts, $NaHCO_3$ and Na_2CO_3 were applied separately to create two stress groups that contained the appropriate stress salts. The control plants were watered with nutrient solution at the same time. The duration of stress treatment was nine days.

Physiological indices measurements

Measurement of growth indices

All plants were harvested in the morning after the final treatment. The number of tillers per plant was recorded. The plants were first washed with tap water and then with distilled water. For each plant, the roots and shoots were separated and their fresh weights (FW) and the lengths of their shoots and the total root length per plant were determined. The samples were then oven-dried at 105 °C for 15 min before being vacuum-dried at 80 °C to constant weight. The shoots and roots dry weights (DW) were recorded. The water content (WC) of both parts was calculated using the formula WC = (FW-DW)/FW. Survival rate (SR) was expressed using the formula: SR = n/N, where n is the number of plants surviving from the total of N plants.

Measurement of proline

The dried samples were homogenized to determine free proline contents which was assayed using the acid-ninhydrin method (Shi and Sheng, 2005).

Measurement of the electrical conductivity of the leaves

Membrane permeability is reflected in a 'relative electrical conductivity', which is defined as the ratio of the electrical conductivity of leaves with intact membranes to those with membranes destroyed by a boiling water treatment. Electrolyte leakage rate (ELR) was determined as described.

Measurement of ions

Dry samples (0.1 g) of shoot and root were treated with 20 mL of deionized water at 100 °C for 1h and the resultant extract was used to determine the contents of inorganic ions and organic acids (OA). The contents of NO_3^-, Cl^-, SO_4^{2-}, $H_2PO_4^-$ were determined by ion chromatography using a DX-300 ion chromatographic system with an AS4A-SC ion-exchange column and a CDM-II electrical conductivity detector (mobile $Na_2CO_3/NaHCO_3$ = 1.7/1.8mmolL^{-1}; DIONEX, Sunnyvale, CA, USA). $Na_2CO_3/NaHCO_3$ = 1.7/1.8 mmol L^{-1}. The levels of the organic acids were also determined by ion chromatography using an DX-300 ion chromatographic system with an ICE-AS6 ion-exclusion column, CDM-II electrical conductivity detector and an AMMS-ICE II Micromembrane suppressor (mobile phase: 0.4 mmol L^{-1} heptafluorobutyric acid; DIONEX). An atomic absorption spectrophotometer (TAS-990; Purkinje General, Beijing, China) was used to determine the levels of Na^+, K^+ and Ca^{2+}.

Measurement of chlorophyll

After 9 days of stress treatment, fresh healthy leaves were cut into small segments to determine the concentrations of chlorophylls a and chlorophylls b according to Arnon (1949).

Measurement of tissue pH

To determine tissue pH, fresh shoots and roots were washed thoroughly three times with neutral deionized water, followed by surface-drying with filter paper. They were then crushed and the pH of the expressed sap measured with a digital pH meter PHS-3C; Shanghai precision & scientific instruments, Shanghai, China.

Determination of organic solute

The DX-300 ion chromatography system (DIONEX, Sunnyvale, USA) determination of organic acids, determination conditions for: ICE-AS6 analysis column, CDM-II conductance detector, AMMS-ICE II interference suppressor, perfluorinated butyric acid is 0.4 mM, mobile phase flow rate of 1.0 ml/min, the column temperature is 20 ℃, and sample volume 50 μl. The organic acid content in this experiment was the sum of all organic acid components detected, including citric acid, malic acid, formic acid, lactic acid, acetic acid, succinic acid and oxalic acid.

Statistical analyses

Statistical analysis of the data was performed using the statistical program SPSS 26.0 (SPSS, Chicago, USA). All data are represented by an average of three replicates and their standard errors (SE). Data were analyzed by one-way and two-way ANOVA. The treatment mean values for the same organ under either Saline-alkali stress were compared by post-hoc Duncan tests. The term significant indicates differences at ($P < 0.05$). Very significant indicates difference ($P < 0.01$).

Results

Growth indices analysis of oat

Under NaCl stress, the survival rate between the concentration of 0-144 mmol L^{-1} was 100%. There is no lethal limit for oats; Under $NaHCO_3$ stress, the survival rate of oat seedlings was 100% when the concentration was less than ≤120 mmol L^{-1}. The $NaHCO_3$ stress showed 100% survival rate of Oats except that 144 mmol L-1 concentration stress which decreased the survival rate by 13% compared to control group. The concentration of 144 mmol L^{-1} decreased by 13% compared with the control group (under ionic stress of Sodium carbonate. Increase in Na_2CO_3 concentration caused significant decreased in survival rate of Oat that was 100% at 48 mmol L-1, decreased by 40%, 79% at 72, 96 mmol L^{-1} respectively, and no survival rate was recorded at 120~144 mmol L^{-1} compared to control group/treatment (Figure 1A, Table 1).

Under NaCl stress, the tiller number was higher than that of the control group at a concentration of 48-96 mmol L^{-1}, indicating that low concentration of salt had a promoting effect. Under the stress of $NaHCO_3$, the number of tillers was higher than that of the control group at a concentration of 48-72 mmol L^{-1}, indicating that low alkali also promoted oat tillering ($P < 0.01$). With the continuous increase of stress intensity, tiller number of oat decreased gradually, the concentration of 120 mmol L^{-1} decreased by 60% compared with the control, and the concentration of 144 mmol L^{-1} decreased by 67% compared with the control. Under Na_2CO_3 stress, the number of tillers was lower than the control group and decreased gradually with increasing concentration. When the concentration is greater than 96 mmol L^{-1}, the number of tillers is 0 (Figure 1B, Table 1).

Under NaCl, $NaHCO_3$ and Na_2CO_3 stress, the plant length was lower than that of the control group. BY increasing solution, the length of the shoot was inhibited. The inhibitory effect was significantly increased

by raising concentration. NaCl < $NaHCO_3$ < Na_2CO_3; Under NaCl, $NaHCO_3$ and Na_2CO_3 stress (ionic concentration), the root lengths between the concentrations of 48-72 mmol L^{-1} were higher than those of the control group, which promoted root growth, while the remaining concentrations were lower than those of the control group (as shown in Figure 1C, D).

With the increase of salt concentration, shoot biomass of each treatment group was smaller than that of the control group, and the biomass under $NaHCO_3$ stress between 48-96 mmol L^{-1} was greater than NaCl, and the inhibitory effect of Na_2CO_3 on plant growth was significantly greater than that of NaCl and $NaHCO_3$ (Figure 1E, Table 1). $NaHCO_3$ concentration 48-72 mmol L^{-1} belowground biomass was higher than that of the control group, and the underground biomass under Na_2CO_3 stress kept declining (Figure 1F, Table 1).

Table 1. ANOVA tables presenting the effects of the salt concentration (CN) and salt component (CM) on the above- and underground biomass, root activity, survival rate, proline content in shoots, electrolyte leakage rate, and chlorophyll concentration in the leaves of oat

	df	Livability		Tillering amount		Root length(cm)		Plant height(cm)	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Salt component (CM)	2	1474.667	.000	14.542	.000	8.116	.001	14.718	<.0001
Salt concentration (CN)	5	217.467	.000	10.967	.000	20.089	.000	44.086	.000
CMx CN	10	183.867	.000	1.742	.109	1.104	.385	.956	.496
	df	Aenal part biomass (g)		Root biomass (g)		Shoot Proline content (μmol g^{-1})		Root Proline content (μmol g^{-1})	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Salt component (CM)	2	144.261	.000	21.997	.000	209.233	.000	185.369	.000
Salt concentration (CN)	5	172.963	.000	12.978	.000	553.221	.000	444.777	.000
CMx CN	10	9.000	.000	1.732	.111	47.058	.000	38.564	.000
	df	Electrolyte leakage rate		Shoot Moisture Content (umolg^{-1})		Root Moisture Content (umolg^{-1})		Shoot Na^+ content (umolg^{-1})	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Salt component (CM)	2	87.619	.000	82.090	.000	87.470	.000	2115.382	.000
Salt concentration (CN)	5	101.285	.000	85.136	.000	34.196	.000	542.586	.000
CMx CN	10	6.404	.000	5.426	.000	7.277	.000	201.062	.000
	df	Shoot K^+ content (umolg^{-1})		Shoot Ca^{2+} content (umolg^{-1})		Shoot Na^+ / K^+		Root Na^+ content (umolg^{-1})	
		F	*P*	*F*	*P*	*P*	*F*	*F*	*P*
Salt component (CM)	2	300.254	.000	127.715	.000	6310.965	.000	2685.294	.000
Salt concentration (CN)	5	114.581	.000	200.756	.000	1354.303	.000	680.422	.000
CMx CN	10	15.304	.000	8.443	.000	730.033	.000	244.253	.000
	df	Root K^+ content (umolg^{-1})		Root Ca^{2+} content (umolg^{-1})		Root Na^+ / K^+		Shoot Cl^- content (umolg^{-1})	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Salt component (CM)	2	694.819	.000	278.456	.000	2825.244	.000	3073.458	.000
Salt concentration (CN)	5	524.263	.000	487.236	.000	630.896	.000	75.465	.000
CMx CN	10	31.714	.000	14.519	.000	351.506	.000	538.925	.000
	df	Shoot SO_4^{2-} content (umolg^{-1})		Shoot NO_3^- content (umolg^{-1})		Shoot $H_2PO_4^-$ content (umolg^{-1})		Root Cl^- content (umolg^{-1})	

		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Salt component (CM)	2	132.437	.000	1462.176	.000	170.151	.000	4592.558	.000
Salt concentration (CN)	5	233.315	.000	119.999	.000	55.843	.000	153.736	.000
CMx CN	10	7.844	.000	37.061	.000	8.342	.000	520.620	.000
	df	Root SO_4^{2-} content ($umolg^{-1}$)		Root NO_3^- content ($umolg^{-1}$)		Root $H_2PO_4^-$ content ($umolg^{-1}$)		Shoot of pH	
		F	*P*	*F*	*p*	*F*	*P*	*F*	*P*
Salt component (CM)	2	138.370	.000	1355.839	.000	307.074	.000	.481	.622
Salt concentration (CN)	5	239.020	.000	271.457	.000	82.843	.000	.935	.470
CMx CN	10	8.211	.000	56.017	.000	14.030	.000	.394	.941
	df	Root of pH		Shoot of organic acid ($umolg^{-1}$)		Root organic acid ($umolg^{-1}$)			
		F	*P*	*F*	*P*	*F*	*P*		
Salt component (CM)	2	379.284	.000	779.940	.000	1599.005	.000		
Salt concentration (CN)	5	63.795	.000	167.848	.000	327.317	.000		
CMx CN	10	20.596	.000	64.595	.000	113.487	.000		

Water and proline contents analysis of shoot and root

Under salinity stress, the content of proline accumulation in each group was higher than the control, along with an increase in salt concentration and a significant difference (P 0.01), each group with an increase in alkali, oat proline content in leaves showed a trend of increase, alkali stress also increases, when the salt concentration increases to a certain extent, the effect of alkali rises as the leading factor, the proline accumulation is primarily in the leaves. By increasing proline content, oat adapted to saline-alkali stress. (Figure 2A and B, Table 1).

The water content of plants showed a decreasing trend overall ($P < 0.05$). When $NaHCO_3$ was at 48 mmol L^{-1}, the water content was higher than the control, and all other intensities were lower than the control. The water content decreases obviously with increasing concentration. It indicates that under the high-intensity saline-alkali stress, the water potential difference of oat seedlings in vivo and in vitro medium increases, leading to different degrees of water extravasation in vivo, so the water content decreases more significantly (such as Figure 2C and D).

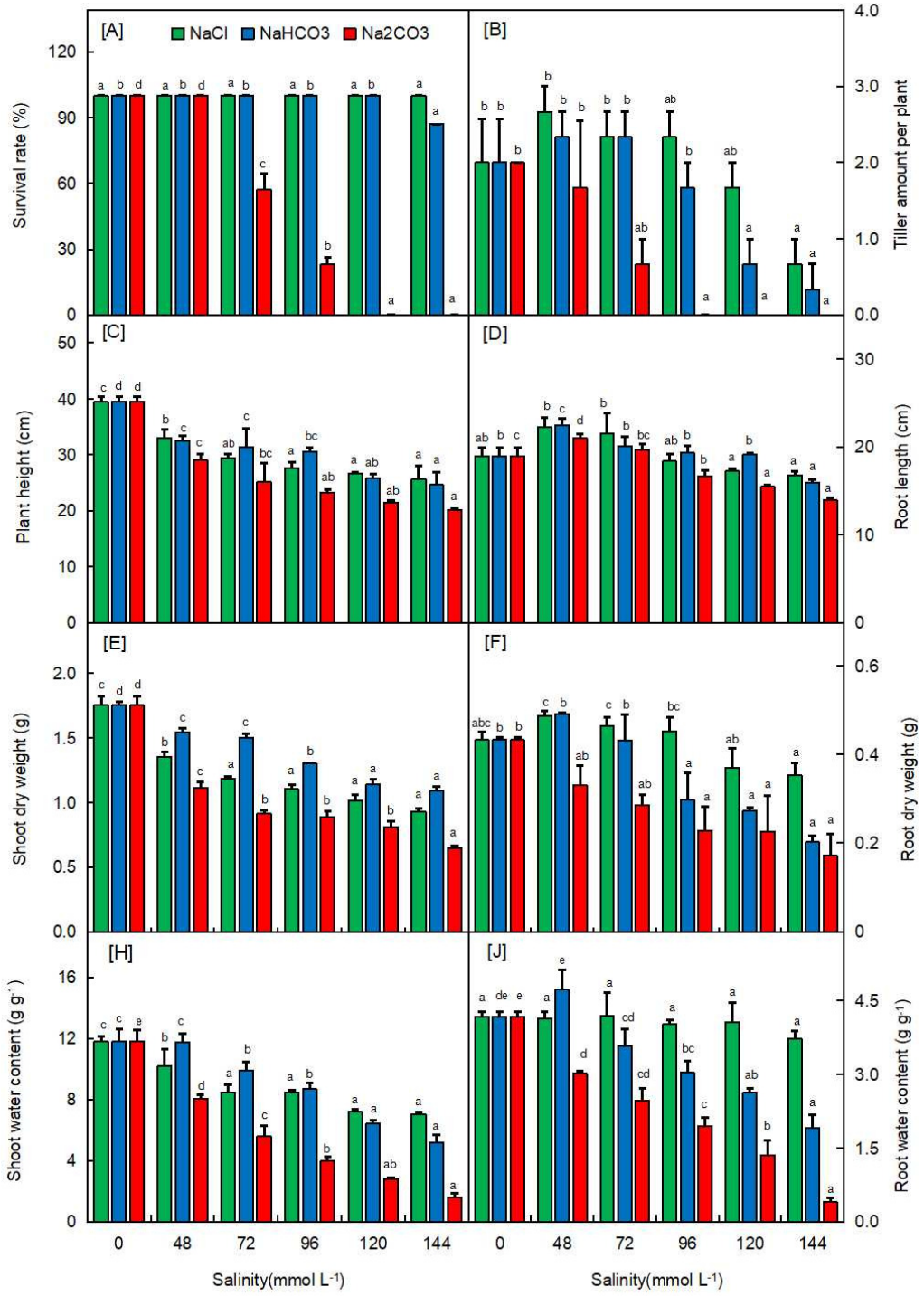

Figure 1. Effects of NaCl, $NaHCO_3$ and Na_2CO_3 stress on (A) survival rate, (B) number of tillers per plant, (C) root length, (D) plant height, (E) shoot dry weight, (F) root dry weight in oat. The 4-week-old oat seedlings were treated with NaCl stress (pH 7.01-7.13), $NaHCO_3$ stress (pH 8.03-8.26) and Na_2CO_3 stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different ($P < 0.05$) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. DW, dry weight; Shoot represents aboveground part of plant.

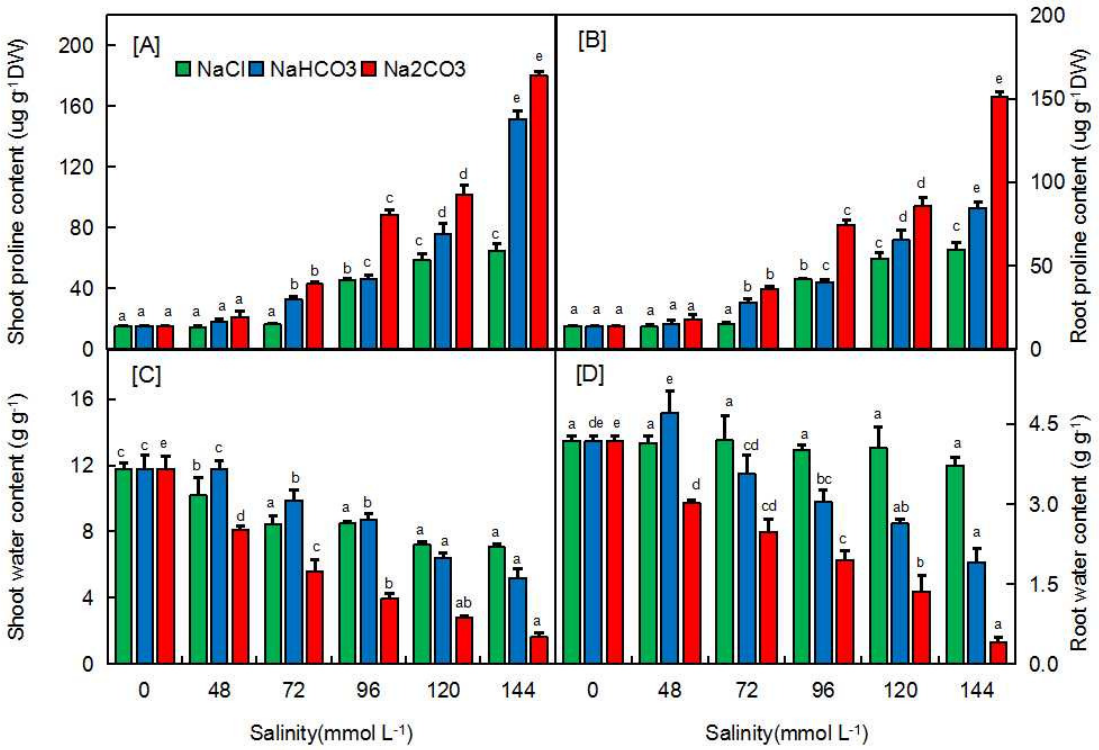

Figure 2. Effects of NaCl, $NaHCO_3$ and Na_2CO_3 stress on the (A) shoot proline content, (B) root proline content, (C) shoot water content, (D) root water content in oat. The 4-week-old oat seedlings were treated with NaCl stress (pH 7.01-7.13) $NaHCO_3$ stress (pH 8.03-8.26) and Na_2CO_3 stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different ($P < 0.05$) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. Shoot represent aboveground part of plant.

Cation's analysis of shoot and root

With the increase of NaCl, $NaHCO_3$, and Na_2CO_3 stress concentrations, Na^+ significantly increased ($P < 0.05$). In both the shoots and the roots, each of the cations showed significant differences between the $NaHCO_3$ and Na_2CO_3 stresses (Table 1). At the highest stress concentrations, the accumulation of $NaHCO_3$ stress Na^+ was 11.3 times than the control, the accumulation of Na_2CO_3 stress Na^+ was 46.6 times that of the control, and 3.9 times higher than that of $NaHCO_3$ (Figure 3). K^+ content significantly decreased ($P < 0.05$, Figure 3), Ca^{2+} decreased, and $NaHCO_3$ and Na_2CO_3 significantly decreased Ca^{2+} than NaCl salt stress (Figure 3). Na^+/K^+ increased significantly with the increase of stress concentration, and Na^+/K^+ of Na_2CO_3 at the highest stress concentration was 4.7 times than NaCl stress at the same concentration (Figure 3). The roots show the same trend.

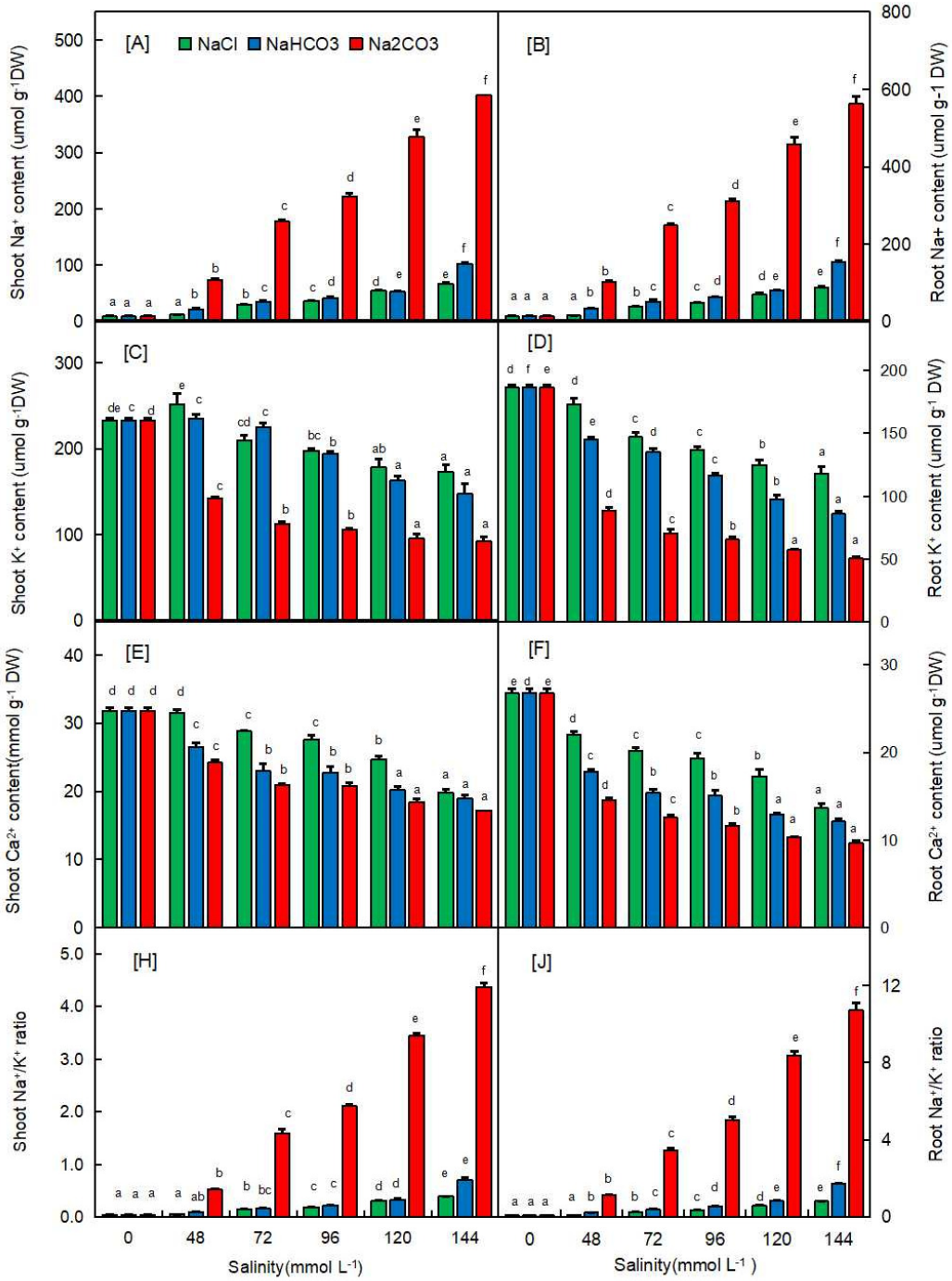

Figure 3. Effects of NaCl, $NaHCO_3$ and Na_2CO_3 stress on (A) shoot Na^+ content, (B) root Na^+ content, (C) shoot K^+ content, (D) root K^+ content, (E) shoot Ca^{2+} content, (F) root Ca^{2+} content, (G) shoot Na^+/K^+ ratio, (H) root Na^+/K^+ ratio in oat. The 4-week-old oat seedlings were treated with NaCl stress (pH 7.01-7.13), $NaHCO_3$ stress (pH 8.03-8.26) and Na_2CO_3 stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different ($P < 0.05$) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. DW, dry weight; Shoot represent aboveground part of plant.

Anions analysis of shoot and root

All the anions showed significant differences between the NaCl, $NaHCO_3$ and Na_2CO_3 stresses (Table 1). In both shoot and root, with the increase of NaCl stress concentration, the content of Cl^- significantly increased *(*$P < 0.01$), but did not change significantly under alkali stress. When the concentration of Cl^- was at the highest salt stress concentration, it was 5.5 times higher than that of the control group and 4.7 times higher than that of the highest alkali stress (Figure 4A and B). SO_4^{2-} increased under both kinds of stresses, and the accumulation of alkali stress was greater than salt stress (Figure 4C and D). The contents of NO_3^- and $H_2PO_4^-$ all decreased significantly under saline-alkali stress ($P < 0.05$), and the decrease was greater ($P < 0.05$, Figure 4E, F, G and H).

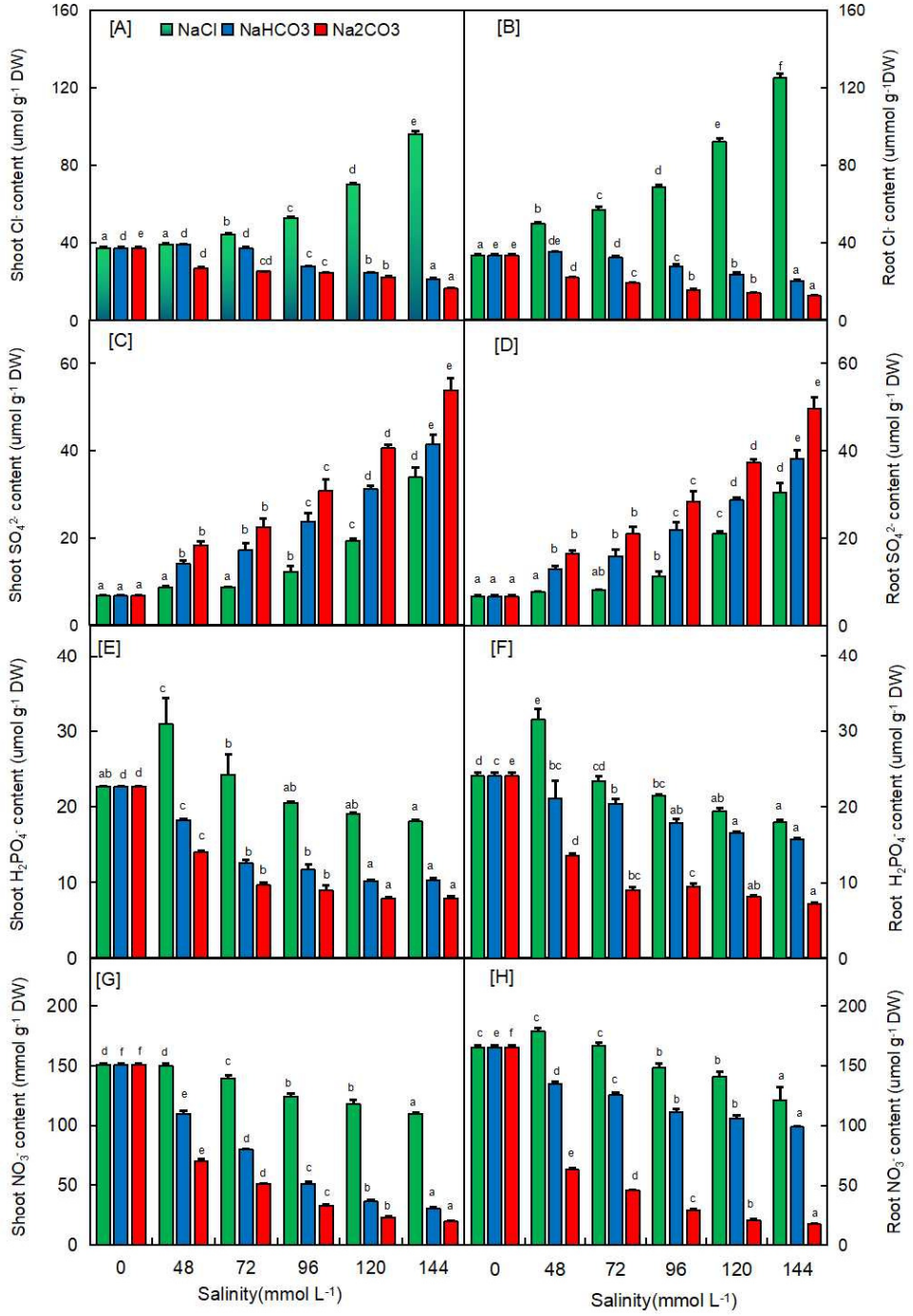

Figure 4. Effects of NaCl, $NaHCO_3$ and Na_2CO_3 stress on (A) shoot Cl- content, (B) root Cl- content, (C) shoot SO_4^{2-} content, (D) root SO_4^{2-} content, (E) shoot $H_2PO_4^-$ content, (F) root $H_2PO_4^-$ content, (G) shoot NO_3^- content, (H) root NO_3^- content, in oat. The 4-week-old oat seedlings were treated with NaCl stress (pH 7.01-7.13), $NaHCO_3$ stress (pH 8.03-8.26) and Na_2CO_3 stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different ($P < 0.05$) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. DW, dry weight; Shoot represent aboveground part of plant.

Tissue pH and ELR analysis of shoot and root

Shoot tissue pH did not show significant differences between $NaHCO_3$ and Na_2CO_3 stresses but root tissue pH and electrolyte leakage rate (ELR) were significantly higher under Na_2CO_3 stress than $NaHCO_3$ stress (Table 1). While $NaHCO_3$ stress did not affect root pH with increasing stress, middle and high Na_2CO_3 contents (≥ 72mmol L^{-1}) did cause significant increases. Both stresses increased the ELR but the extent of the reduction under Na_2CO_3 stress was much greater than under $NaHCO_3$ stress (Figure 5C). All the plants treated with 120 and 144mmol L^{-1} of Na_2CO_3 died, so their pH values were recorded.

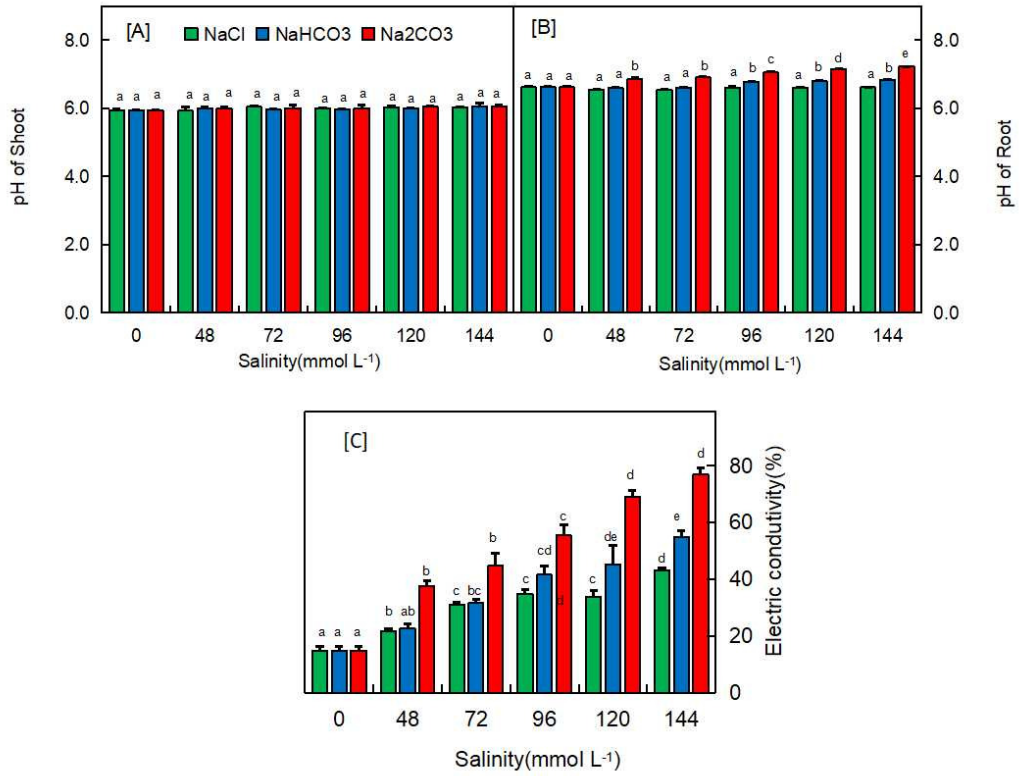

Figure 5. Effects of NaCl, $NaHCO_3$ and Na_2CO_3 stress on (A) shoot tissue pH, (B) root tissue pH, (C) ELR (electrolyte leakage rate) in oat shoots. The 4-week-old oat seedlings were treated with NaCl stress (pH 7.01-7.13), $NaHCO_3$ stress (pH 8.03-8.26) and Na_2CO_3 stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different (P < 0.05) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. Shoot represents aboveground part of plant.

Tissue organic acid analysis of shoot and root

NaCl stress had no significant effects on organic acids in stems and leaves above ground and in roots (Figure. 6 A, P>0.05). $NaHCO_3$ and Na_2CO_3 stress had significant effects on organic acids above ground and underground (Figure 6 A, P<0.05), and they increased with the increase of salt concentration. At the highest stress concentration, the organic acid content in the above-ground parts under $NaHCO_3$ stress was 1.7 times higher than that in the control, while that in the Na_2CO_3 stress was 4.1 times higher than that in the control (Figure 6B); the organic acid content in the roots under $NaHCO_3$ stress was 1.9 times higher than that in the control, while that in the roots was 4.6 times higher than that in the control, and the organic acid content in the roots was greater than that in stems and leaves.

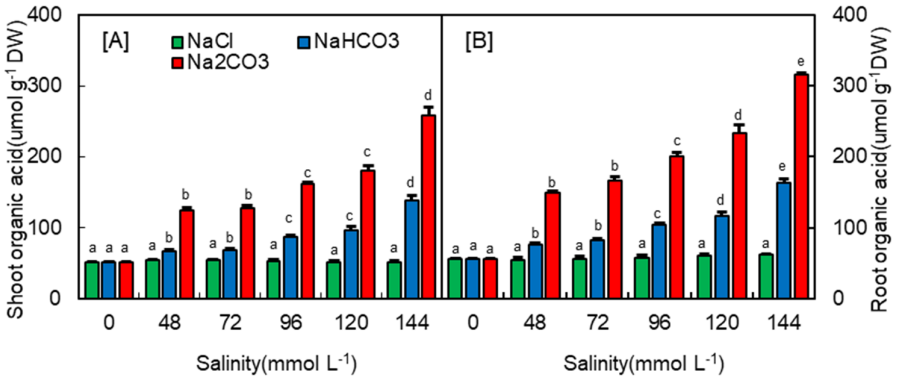

Figure 6. Effects of NaCl, $NaHCO_3$ and Na_2CO_3 stress on (A)shoot OA (organic acid) content, (B) root OA (organic acid) content in oat shoots. The 4-week-old oat seedlings were treated with NaCl stress (pH 7.01-7.13), $NaHCO_3$ stress (pH 8.03-8.26) and Na_2CO_3 stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different ($P < 0.05$) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. Shoot represents aboveground part of plant.

Photosynthetic pigments analysis of leaves

All photosynthetic pigments were significantly lower (Table 1). With the increase of salt stress concentration, the content of *Chl* a and *Chl* b in oat leaves gradually decreased, and the same concentration in each group increased with pH value above and below neutral level, and the decline trend of *Chl* a and *Chl* b was more significant. NaCl and $NaHCO_3$ had little effect on *Chl* a and *Chl* b. When Na_2CO_3 concentration was 96mmol L^{-1}, the chlorophyll a and chlorophyll b in the leaves decreased mostly. When the pH value of the treatment solution was higher than 10.00, the *Chl* a and *Chl* b content of the plant was significantly lower than the control ($P < 0.05$). With the increase of alkali strength, the content of *Chl* a and *Chl* b decreases rapidly, and the influence of alkali on the content of chlorophyll in oat is greater (Figure 7A and B).

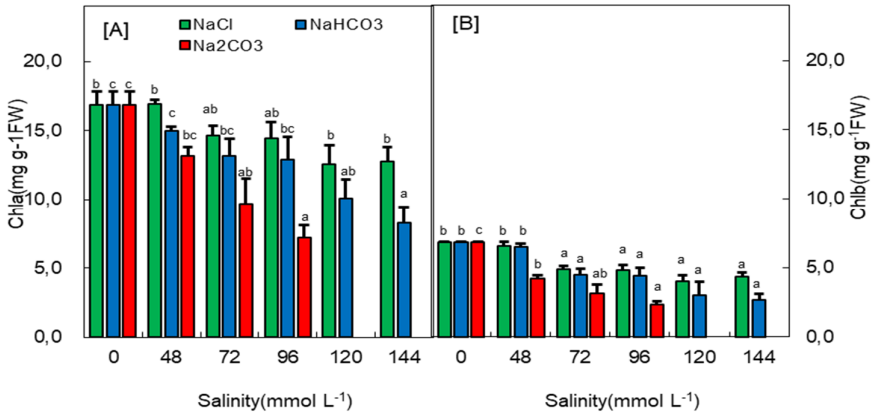

Figure 7. Effects of NaCl, $NaHCO_3$ and Na_2CO_3 stress on the (A) chlorophyll (Chl) a, (B) chlorophyll (Chl) b in *Avena sative* L. The 4-week-old *Avena sative* L. seedlings were treated with the same letters are not significantly different (P < 0.05) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. FW, fresh weight; Shoot represent aboveground part of plant.

Discussion

Differences in salt and alkali stress factors

Salty or sort is more, in soil matrix is given priority to with NaCl and Na_2SO_4 soil, called a salt, which is mainly composed of alkaline salt $NaHCO_3$ and Na_2CO_3 soil, known as the alkali soil (Shi and Zhao, 1997), including neutral salt NaCl stress effect which is the main factor of Na^+ ions poison effect and high concentration of salt to the decline in the water physiological drought brought about by the osmotic stress (Munns, 2002; Khan, 2000), alkaline salt effect is more complex than neutral salt . Among the functional factors of $NaHCO_3$ and Na_2CO_3, the double effects of high pH value should be added on the basis of salt stress (Wang *et al*., 2009). When the pH value rises in the external environment of plant roots, the physiological function of roots is destroyed by light people, and the structure of roots is destroyed by root cell dissolution by heavy people. At the same time, it also changes the existing state of various mineral ions (Taghipour and Jalali, 2019; Moynihan *et al*., 1995; Hansen and Munnes, 1988). NaCl, a neutral salt, and $NaHCO_3$, a mild salt was used in this experiment. With the same molar concentration, Na^+ and pH values of Na_2CO_3 were the highest, so it was quite heavy. NaCl of the three salts represented the effect of salt stress mainly involving osmosis and ion poisoning with Na^+ as the main stress factor (Wen and Klionsky, 2016), $NaHCO_3$ and Na_2CO_3 had both Na^+ and increased pH value. Therefore, they have different mechanism of action on plants and different degree of damage. High pH of plant root environment can not only cause severe damage to mineral nutrition and oxygen supply capacity around the root, but also directly damage the structure and function of root cells, leading to imbalance of ions around the root system and in the cell, and interfere with metabolism. When plants are under alkali stress, not only they must deal with physiological drought and ion poisoning but also high pH. Plants must expend more material and energy to adapt to alkali stress, so the inhibition of growth by alkali stress is often more serious.

Effects of salt and alkali stress on oat growth

Growth is a comprehensive reflection of plant response to saline-alkali stress and a direct evidence of plant saline-alkali resistance (Levitt, 1980). At the same time, it is also the expression of a comprehensive trait of plant anti-salinity (Pham *et al*., 2022). Tiller number can be used as a growth index to measure the alkali resistance of oat. The results of this study showed that the biomass decreased gradually with the increase of NaCl, $NaHCO_3$ and Na_2CO_3 stress concentrations, and the number of tillers and plant height also decreased, which was one of the reasons for the decline of aboveground biomass. The final composite presentation biomass declined. Survival rates reflect the selection of plants in the environment. Alkaline salt stress has an inhibitory effect on the normal growth of plants, and different alkali intensities have different effects on oat. It can endanger the survival of plants. The results of this experiment indicated that under the stress of NaCl, $NaHCO_3$ and Na_2CO_3, the survival rate decreased with the increase of salt concentration and pH value. The effect of high concentration alkali on survival rate was more obvious ($P < 0.01$).

Growth indices

Our results show that the injurious effects of Na_2CO_3 on growth were greater than those NaCl and $NaHCO_3$ for the same alkali content $NaHCO_3$ on growth were greater than NaCl for the same alkali content (Figure 1), and this is consistent with previous reports (Shi and Yin, 1993). The injurious effects of alkali are commonly thought to be due to a combination of low water potential, ion toxicity, and in particular, to high stress (Munns, 2002). Firstly, the greater alkali stress leads to severer reductions in photosynthetic pigment content due to Na_2CO_3 compared with $NaHCO_3$ (Figure 7) and a sharp increase in ELR (Figure 5C). These results indicate that high pH from Na_2CO_3 stress may damage root cell structure and function affecting such processes as the absorption of ions, damaging photosynthetic pigments and membrane systems (e.g. increases in ELR). This may be why growth under $NaHCO_3$ stress was

Tissue pH

Regardless of environmental pH, to maintain normal metabolism it is important that plants can stabilize their tissue pH (Yang *et al*., 2008). Our observations that shoot tissue pH was similar to the control values under both alkali stresses and that stress intensity increases did not have significant effects on tissue pH suggests that oat is able to maintain a stable cell pH (Figure 5A and B). However, it is surprising that when the stress level rose above certain threshold values (48 mmol L^{-1} with Na_2CO_3; 72 mmol L^{-1} with $NaHCO_3$), root pH was affected by increasing levels of stress. This may be explained as that at above 48 mmol L^{-1} in Na_2CO_3 (or 72mmol L^{-1} in $NaHCO_3$) the harmful effects of high pH were opposed by pH adjustments outside the roots (by extrusion of H^+, OA, amino acids or CO_2 produced by root respiration) with the intracellular environment being unaffected. However, when the stress intensity exceeded the capacity for root adjustment (> 48 mmol L^{-1} in Na_2CO_3 or > 72 mmol L^{-1} in $NaHCO_3$,), the result was a reduction in photosynthetic pigment content (Figure 7), and a sharp increase in ELR (Figure 5C). This suggests that the stress may have weakened the controls, leading to increases in pH. Meanwhile, compared to the shoot, it was found that the effects of both alkali stresses on root growth (i.e., root length and biomass) was reduced, indicating that the root has a higher tolerance of elevated pH. This deserves further investigation.

Water content proline content

In the saline-alkali environment, the absorption and utilization of water is a key factor for plants to adapt to adversity, which plays an important role in the growth and development of plants. Reducing the water content of plants is a rapid and economic way for plants to conduct osmotic regulation in response to osmotic stress (M Cgaughey, 2018). Oat decreased its water content under saline and alkali stress, $NaHCO_3$ and Na_2CO_3 decreased even more, and Na_2CO_3 decreased mostly under the same molar concentration (Figure 2).

Proline is most effective in the osmotic regulation of amino acids. Under stress, proline accumulation is found in both lower and higher plants, and in both salt and non-salt plants (Ejcek, 2021). Under salt stress, oat adapted to stress by increasing proline content. The more proline accumulates in plants, the stronger osmotic regulation ability of plants and the stronger resistance to adverse environment are. When treated with different concentration of Na_2CO_3 by, free proline content was higher than that of the control group, and the stress intensity was different. The content of free proline increased gradually with the seedling of *Leymus chinensis* and sunflower. Alkaline salt stress factor is unique to the high pH value, Na_2CO_3 to plants are the main stressors of high pH value instead of osmotic stress and ion poisoning (Shi *et al*., 1993; Shi *et al*., 1998), plant on the high pH environment is one of the ways of accumulation in the body has the buffer action of acid metabolites (such as organic acids, citric acid, proline, etc.) on the body pH adjustment, the adjustment process is a process of energy dissipation, although it can reduce the intracellular pH value, it also inhibits the growth of plants. The free amino acid content of star grass seedlings under Na_2CO_3 stress increases with the increase of salt concentration. Proline concentration increases with the strengthening of stress intensity, the treatment group, the proline content of oat seedlings was greater than comparison, with the increase of pH value, higher proline content, and high concentrations at high pH value, the effect of stress significantly increased and made extremely significant difference ($P < 0.01$), the seedling proline content on the degree of different combination of salt stress response. The interaction effect between salt concentration and salt combination was not significant at low salt concentration, while at medium salt concentration and high salt concentration, proline accumulation was significantly increased, indicating that the plant's osmotic regulation ability was enhanced and its resistance to adverse environment was strengthened (Shi *et al*., 1998).

Ion toxicity and ion imbalance

In salinity-alkalinity stress plants usually accumulate a large amount of Na^+ (Shi and Sheng, 2005;), and the plant will be a lot of Na^+ segregation to vacuole protect cells from poisoning (Fouilleux and Loconto, 2017), Na^+ also inhibit the absorption of K^+, Na^+ and K^+ to adapt to the saline environment and it is of great

significance to the plant, at the same time of plants under salinity stress absorption Na^+ inhibit the absorption of K^+ (Shi and Sheng, 2005; Khan, 2000), Na^+/K^+ ratio increased with the increase of stress intensity (Shi and Sheng, 2005; Khan, 2000), so did oats. Na^+ increased with the increase of salt concentration, while K^+ decreased. Na^+/K^+ ratio increases with the increase of alkalinity, and the increase of Na_2CO_3 stress is greater than NaCl and $NaHCO_3$, indicating that Na_2CO_3 is the most harmful. High pH may interfere with root selection absorption.

Ca^{2+} is not only a large number of elements of plant mineral nutrition, but also the second messenger of coupling extracellular signals and intracellular physiological and biochemical reactions. Regulation of cell division, participate in phytochrome response, the root of the growth response of ground, stomatal opening process, and inhibit the growth and development of plants (Rengel, 1992) it is generally believed, NaCI for plant growth inhibition is one of the important reasons of Na^+ instead of intracellular Ca^{2+}, and make the plant body lack of Ca^{2+}, Ca^{2+} as a phospholipid and protein in the biofilm of phosphoric acid root bridge and connection between carboxyl, can maintain the stability of the membrane structure. In the experiment, Ca^{2+} in both the aboveground and underground parts decreased more significantly. The more significant the decrease under alkali stress, the more serious the damage to biofilm. Organic acids in plants, and organic acids (mainly organic acids) in many fleshy plants have a high content, which is harmful to plants. Ca^{2+} combines with oxalic acid into calcium oxalate crystals that are not allowed to be damaged.

The adaptation strategy of oat to saline-alkali

Oat can adjust under salt stress mainly by changes of inorganic ions and proline content. High pH stress is the key to alkali stress. In order to survive in alkaline land, plants must not only adjust the pH of microenvironment outside the root, but also adjust the pH within cells to maintain normal metabolism and the relative balance of ions. The pH adjustment of the extracorporeal microenvironment may be realized by secreting H^+, organic acids and CO_2 by respiration. Intracellular pH regulation may be achieved mainly by accumulation of organic acids in vacuoles (Shi and Sheng, 2005). Under alkali stress, oat accumulates a large number of organic acids in cells, which not only makes up for inorganic anion deficiency and regulates intracellular pH, but also may be transported to the root for external pH regulation. Therefore, it can be considered that the accumulation of organic acids in the body is one of the special and positive physiological responses of oat to alkali stress. Under saline-alkali stress, plants change the distribution of ground and underground substances to adapt to the stressed environment.

Conclusions

Experiment demonstrated that, At the same molar concentration, the stress on oats was Na_2CO_3 > $NaHCO_3$ > NaCl. The stronger alkalinity, the imbalance of Na^+, K^+ and Ca^{2+}, the more serious the damage of photosynthetic pigments and membrane systems, and the slightly increased internal pH caused by severe saline-alkali. Na_2CO_3 stress may have affected cellular physiological activities. Severe damage of alkali stress to membrane system indicates that alkali stress may have affected the cytoplasm, and at the same time, alkali stress also interferes with Na^+, K^+, Ca^{2+}, Cl^-, SO_4^{2-} metabolism, resulting in significant accumulation of Cl^- and SO_4^{2-} in oat. Based on the above two analyses, it seems that alkali stress may seriously interfere the metabolism of Cl^- and SO_4^{2-}, while the special accumulation of proline and organic acids under alkali stress may be the stress response to such interference.

Authors' Contributions

ZWG conducted the research and prepared the manuscript. SP, YHC, ZZY, RLL and CSM review and edited the manuscript. AR and SH reviewed the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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