Australian Journal of Crop Science

AJCS

AJCS 6(5):839-845 (2012) ISSN:1835-2707

Comparative investigation of single salts stresses and their mixtures on Eragrostioid (*Chloris virgata*) to demonstrate the relaxation effect of mixed anions

Changyou Li[1*], Xiaoping Wang[2], Huan Wang[2], Futai Ni[1], Decheng Shi[2]

[1]College of Life Science, Jilin Normal University, Siping 136000, China
[2]Key laboratory of Molecular Epigenetics of MOE, Northeast Normal University, Changchun 130024, China

***Corresponding author: licy858@163.com**

Abstract

An alkali-resistant halophyte, *Chloris virgata,* which naturally grows on salt-alkalinized soils in northeast China, was studied. According to the characteristics of salt-alkalinized soil, in which four harmful salts NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 coexist, the single and mixed stresses of the four salts were applied to *C. virgata*. By measuring physiological indices such as matter accumulation rate (MAR), energy accumulation rate (EAR) and net photosynthetic rate (P_N), we compared the differences among the stresses of the four single salts and their various mixtures to show a relaxation effect of mixed anions. The MAR, EAR and P_N of *C. virgata* decreased with increased intensity (electrical conductivity) of various salt stresses, but the reduction rates were different at the same salinity ($P < 0.05$). The experiment indicated that the importance of stress effects of the four single salts on *C. virgata* were Na_2CO_3 > $NaHCO_3$ > NaCl > Na_2SO_4, respectively. After mixing four single salts, the stress effects of mixtures were significantly lower than the combined effects of the single salts of the mixture, which implied an interaction among the different anions, and as a result, their stress impacts were abated. Therefore, mixed salts had a relaxation effect on plants compared to single salt stress. The relaxation effect decreased with increased salinity levels, and increased at higher alkalinity levels of the mixed salt. This study has theoretical significance in physiological ecology and agricultural research.

Keywords: Mixed anion, Mixed salt stress, Relaxation effect, Single salt stress, Na_2CO_3, $NaHCO_3$, NaCl, Na_2SO_4.
Abbreviations: ASV: Actual strain value, EAR: Energy accumulation rate, MAR: Matter accumulation rate, P_N: Net photosynthetic rate, PSV: Predictive strain value.

Introduction

Agricultural productivity is severely affected by soil salinity (Läuchli and Lüttge, 2002). Natural salt-alkalinized soils are complex and include various ions, such as Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} and NO_3^- (Läuchli and Lüttge, 2002). In different soil patches, the components, concentration and proportions of salts are very different, which makes soil salt–alkalinization complex. NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 are the main harmful salts in many inland areas such as China (Ge and Li, 1990; Kawanabe and Zhu, 1991). Alkaline salts ($NaHCO_3$ and Na_2CO_3) have been shown to be more damaging to plants than neutral salts (NaCl and Na_2SO_4) (Shi and Yin, 1993; Shi et al., 2002a; Yang et al., 2007). Salt stress in the soil generally involves osmotic stress and ion injury (Munns, 2002). The alkali stress exerts the same stress factors but with the added influence of high-pH stress. Alkali stress is combination of salt stress and high pH stress. High-pH stress is the main reason of injurious effect of alkali stress on plants, which is greater than salt stress at similar salt concentrations. The differential response of plants to salt stress and alkali stress are mainly due to high-pH stress. Therefore, comparing salt stress and alkali stress is important for understanding the high-pH or alkali tolerance. In many areas, such as in China, natural salt–alkalinized soils contain both neutral (NaCl and Na_2SO_4) and alkaline salts ($NaHCO_3$ and Na_2CO_3), so stresses are mostly mixed salt–alkaline stresses. However, research on plant salt-resistance physiology has mainly focused on the single salt stress of NaCl (Apse et al., 1999; Ghoulam et al., 2002; James et al., 2006; Khan et al., 2000; Koca et al., 2007; Munns, 2002; Parida and Das, 2005; Patel and Pandey, 2007; Shi et al., 2002b; Soussi et al., 1998; Wang et al., 2007; Charkazi et al., 2010; Jemâa et al., 2011; Ibraheem et al., 2011). There are few reports on effects of different harmful salts and their interactions on plants. Some reports have demonstrated that different single salts have different influences on growth (Mozafar and Goodin, 1985), and gene expression (Liu et al., 2006) of plants. At the same concentration, the effects on plants of mixed salt stresses in different proportions are also different (Shi and Wang, 2005; Shi and Sheng, 2005).

There are many alkali-resistant halophytes living on natural salt–alkalinized soil. This fact implies that the stress impact of mixed salts on plants might be not as severe as previously thought. These phenomena indicate that the ion species and salt composition of a stress salt are closely related to the stress impact on plants, indicating that there may be interactions among various ions (Cramer et al., 1986; Shi and Zhao, 1997). Thus, it is necessary to compare the stresses of various single salts and their mixtures on plants, which can correctly explain the natural effects of salt or salt-alkaline stress. Eragrostioid (*Chloris virgata*) grows widely in alkalinized grassland of northeast China and is a gramineous with high resistance to salt and alkali stresses. In its habitat, there are high concentrations of four sodium salts (NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3), which are the main harmful salts to *C. virgata* (Zheng and Li, 1999). Because Na^+ is the same cation for the four salts, any change in stress effects of their mixtures can be regarded as the result of anion interactions. The matter accumulation rate (MAR), energy accumulation rate (EAR) and net photosynthetic rate (P_N) from plants are ideal indices that reflect the vital status of plant. They are also considered as optimum indices for measuring degrees of stress and the plant responses to various stresses. Any change in these indices can be used to objectively evaluate the effects of various stresses on plants. Previous studies confirmed that different plants differed in effects of salt stress, but there are few reports on comparison of effects of various single salts and their mixtures on *C. virgata*. Especially, alkali stress has been neglected in the previous (Yang et al., 2007; Yang et al., 2008). In this study, we compared the effects of four single salts (NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3) and their mixtures on the MAR, EAR and P_N of *C. virgata*.

Table 1. Salt compositions and molar proportions of various mixed salt stress treatments.

Treatment groups	Salt composition and molar proportions			
	NaCI	Na_2SO_4	$NaHCO_3$	Na_2CO_3
MA	1	1	0	0
MB	1	2	1	0
MC	1	9	9	1
MD	1	1	1	1
ME	9	1	1	9

Results

In this experiment, electrical conductivity of solutions and total salt concentrations were all positively correlated (Table 3), so electrical conductivity was used to represent salt stress intensity.

Growth and photosynthesis

The changing trends of MAR, EAR and P_N were similar under single salt stresses or mixed stresses (Fig. 1). They all decreased with increased stress intensity (all tests: $P < 0.01$). Under four single salt stresses, the decreases from the most extreme (at the same stress intensity) were $Na_2CO_3 > NaHCO_3 > NaCl > Na_2SO_4$. The MAR values decreased similar to the sequence mentioned above but the reduction of different stress intensity were as flows: 85.00%, 82.65%, 85.29% and 82.35%, compared to control treatment. In the EAR values, the decreases in greatest degree were 70.17%, 69.67%, 73.33% and 70%, respectively. In addition, P_N decreased by 88.57%, 84%, 81.14% and 71.14% of the greatest degree. Under mixed salt stresses, when pH was 6.60–8.74 (MA, MB and MC treatments) the decrease of the three indices caused by pH were small (Fig. 1). In MA treatment, the decreases of MAR, EAR and P_N in greatest degree were 77.06%, 80% and 61.71%, respectively. In MB treatment, the decreases in greatest degree were as flows: 73.53%, 74.33% and 66.86%. For MC treatment, the values decreased by 75.29%, 77.33% and 69.71%, respectively. The MD and ME treatments, when the pH was > 8.74 (at the same salinity), the pH ascent induced decrease of the three indices, MAR, EAR and P_N, in which the most decreases were 79.71%, 85% and 93.14%, respectively. Even in the event of plant death occurred by ME5, the most decreases for MAR, EAR and P_N, were 82.94%, 88.33% and 89.14%, respectively (Fig. 1). The abating rate of mixed salt stress decreased with increased salinity, and increased with increased pH (Fig. 2) (all tests: $P < 0.01$).

Linear regression analysis between physiological indices and intensity of stress

To compare the stress effects of different single salts and their mixtures, it was necessary to define the mathematical relationships, firstly between stress intensities (electrical conductivity of treatment solutions) and the physiological index (i.e. strain) of the responding plant, and then to calculate the strain value caused by one unit stress intensity (i.e. strain rate). There were good linear relationships between each physiological index and stress intensity (Fig. 1). The linear regression analyses were performed between stress intensity (assigned the independent variables x) and each strain index (assigned the dependent variables y). The strain rate (equation slope) was obtained from the regression equation. The strain rate, namely the decrease value caused by one unit of stress intensity (d_y/d_x) represents the stress action degree of the salts on *C. virgata*. A larger value of d_y/d_x would indicate a stronger stress action. For linear regressions the $R^2 > 0.9$ indicated useful equations (Table 4). Based on d_y/d_x values (Table 4) the inhibiting actions of the four single salts on P_N, MAR and EAR of *C. virgata* in decreasing order were $Na_2CO_3 > NaHCO_3 > NaCl > Na_2SO_4$. Under mixed salt stresses, the strain rates of the four indices increased with increasing alkalinity (pH).

Discussion

Differences between single salt stresses and their mixed stress impacts

NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 are the main harmful salts in many inland areas. In our experiment, the stress effects of the four single salts were clearly different. Although the four single salts all inhibited photosynthesis and the accumulations of matter and energy, the degrees of inhibition were different, from strong to weak as follows: Na_2CO_3, $NaHCO_3$, NaCl and Na_2SO_4 (Fig. 1; Table 4). The explanation of different injurious effects of the four single salts may be attributed to different mechanisms of actions. Neutral salts such as NaCl and Na_2SO_4 are mainly involved in water deficiency and ion toxicity (Ghoulam et al., 2002; Khan et al., 2000; Koca et al., 2007; Munns, 2002; Parida and Das, 2005; Patel and Pandey, 2007; Soussi et al., 1998; Wang et al., 2007). However, alkaline salts such as $NaHCO_3$ and Na_2CO_3 exert the same stress factors as neutral salts, but with the added influence of high pH stress (Shi and Wang, 2005). The high pH environment surrounding the roots can directly cause some ions (e.g. Ca^{2+}, Mg^{2+} and others) to precipitate (Shi and Zhao, 1997), which may destroy the nutrient supply and ion balance around the roots. Because the four salts are all sodium salts and strong electrolytes, they exist principally as free ions in the soil environment surrounding roots. Therefore, the differences in the four single salt stresses depend on their anions, in which physicochemical properties may determine their harmful effects. The results showed that the most harmful effect on *C. virgata* was from CO_3^{2-} followed in order by HCO_3^-, Cl^- and SO_4^{2-} (Table 4). The stress effects of either various single or mixed salts in various proportions were different (Shi and Wang, 2005; Shi and Sheng, 2005), indicating that the stress impact of mixed salts on plants are closely related to their salt composition.

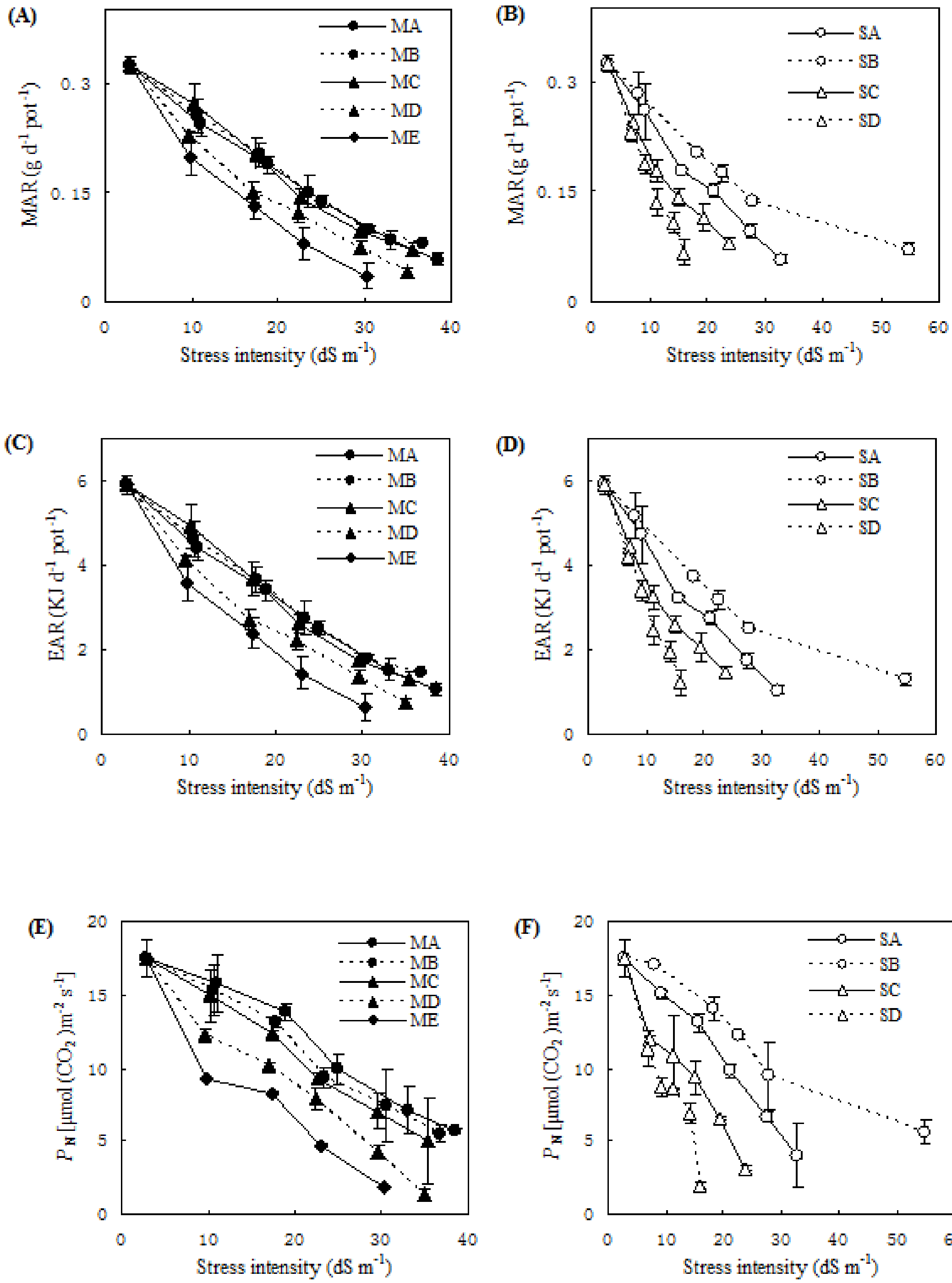

Fig 1. Effects of various single-salt (SA to SD) and mixed-salt stresses (MA to ME) on matter accumulation rate (MAR) (A, B), energy accumulation rate (EAR) (C, D) and net photosynthetic rate (P_N) (E, F) of *C. virgata*. The values are means (±S.E.) of three replicates. Treatments include: SA (NaCl); SB (Na_2SO_4); SC ($NaHCO_3$); SD (Na_2CO_3); MA ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$ =1:1:0:0; pH 6.60 − 6.85); MB ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$ =1:2:1:0; pH 8.27 − 8.46); MC ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$ =1:9:9:1; pH 8.63 − 8.74); MD ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$ =1:1:1:1; pH 9.62 − 9.74); ME ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$ =9:1:1:9; pH 10.33 – 10.69).

The more amount of a composition with a stronger stress in the mixed salts, the more stress impact they have. This conclusion was also evident in previous reports (Shi and Wang, 2005; Shi and Sheng, 2005). Under mixed salt stresses, at the same salinity, the increase of alkalinity depends on the rate of alkaline salt to neutral salt, which reportedly induces a sharp decrease of relative growth rates on different plant such as *Aneurolepidium chinense* (Shi and Wang, 2005) and sunflower (Shi and Sheng, 2005). In this paper, the response of *C. virgata* was different from the previously reported results. When pH was 6.60–8.74 (when MA, MB and MC treatments applied), increased pH or alkalinity did not significantly decrease EAR, MAR and P_N of *C. virgata*. However, only at pH above 8.74 the effects of mixed salt stresses on *C. virgata* sharply increased with the pH rise (Fig. 1). These results implied that effect of mixed salt stresses were more complex than single salts, and the stress effects on plants were not a simple combination of the effects of every single salt in the mixture.

The relaxation effect of mixed anions on the stresses

The four salts used in this study were the sodium salts and therefore, the stress impact caused by their mixtures should be due to their anion interactions. Various neutral and alkaline salts were mixed, and the stress effects of their mixtures on plants were not a simple combination of the effects from the four single salts, but were influenced by interaction of different anions. To demonstrate this, we could first suppose that there were no interactions among the salts and those mixed salt stresses were the simple combination of the separate effects of the four single salts.

The predictive strain values (PSV) of various mixed salt stress treatments were calculated based on the components, proportions and concentrations of salts in various mixtures (Table 1), the regression equation between electrical conductivity and salinity (Table 3), and the strain rates of different single salt stress (d_y/d_x) (Table 4). For example, the calculation of PSV of MC3 treatment was as follows: The total salinity of MC3 treatment is 180 mM (Table 1), this treatment included 9 mM NaCl, 81 mM Na_2SO_4, 81 mM $NaHCO_3$ and 9 mM Na_2CO_3. Based on Table 3, the electrical conductivity of the corresponding concentration was calculated for each single salt. Based on calculated electrical conductivity and d_y/d_x of the corresponding single salt (Table 4) the predictive strain value was calculated for each single salt. Thus, a sum of the predictive strain values due to the four single salts was obtained, and the sum was the PSV of the MC3 treatment. Comparing the PSV with actual strain value (ASV) of various physiological indices (Table 5), showed that PSV > ASV. Thus, the actual stress effects of mixed salts on *C. virgata* were lower than the combined effects of the separate single salts contained i.e. mixing of the different single salts abated their harmful effects. To elucidate the relation between the relaxation action and salt composition, the abating rate of mixed salt can be defined as:

$$\text{Abating rate (\%)} = (\text{PSV} - \text{ASV}) \times 100 / \text{PSV}$$

The abating rate of mixed salt stress decreased with rise of salinity, and increased with pH ascent (i.e. from MA to ME; Fig. 2). MAR, EAR and P_N are ideal indices for reflecting stress effects of different treatments on *C. virgata*. Based on the analysis of the physiological indices (Table 5, Fig. 2), it was shown that the mixing of various single salts weakened their harmful effects on *C. virgata*, i.e. a mix could abate

Table 2. pH of various mixed salt stress treatments.

	pH				
Salinity (mM)	MA	MB	MC	MD	ME
60	6.65	8.27	8.63	9.62	10.33
120	6.60	8.40	8.66	9.74	10.47
180	6.85	8.46	8.74	9.73	10.50
240	6.84	8.45	8.71	9.72	10.51
300	6.82	8.44	8.70	9.71	10.69

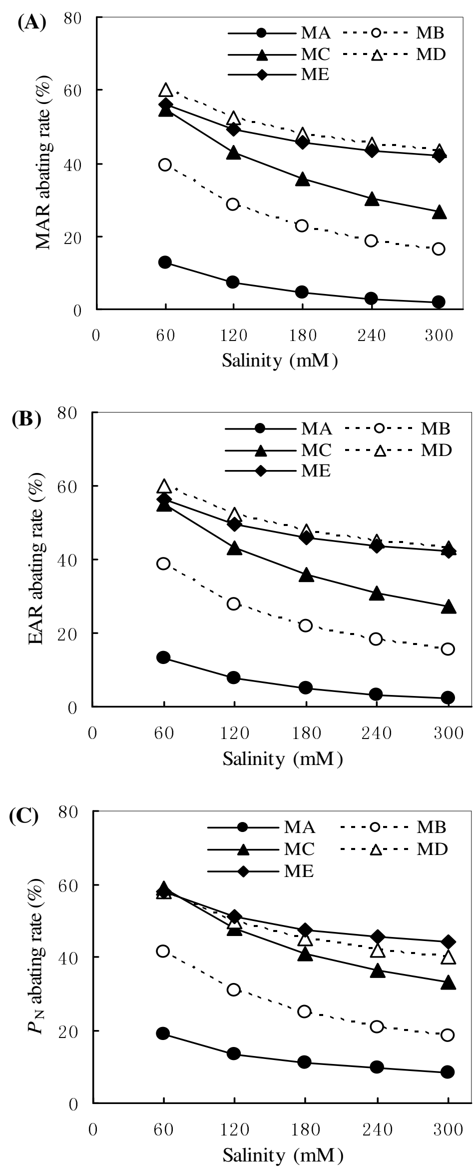

Fig 2. Effects of various mixed salt stresses on the abating rate in matter accumulation rate (MAR) (A), energy accumulation rate (EAR) (B) and net photosynthetic rate (P_N) (C) of *C. virgata*. MA ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) =1:1:0:0; pH 6.60 − 6.85); MB ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) =1:2:1:0; pH 8.27 − 8.46); MC ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) =1:9:9:1; pH 8.63 − 8.74); MD ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) =1:1:1:1; pH 9.62 − 9.74); ME ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) =9:1:1:9; pH 10.33 − 10.69);

Table 3. Regressive equation between electrical conductivity (y, dS m^{-1}) and salinity (x, mM).

Saline treatment groups	Regressive equation	R^2
MA	y=0.119x+3.68	0.996
MB	y=0.112x+3.58	0.998
MC	y=0.108x+3.54	0.998
MD	y=0.107x+3.32	0.998
ME	y=0.113x+3.09	0.999
SA	y=0.100x+3.18	0.999
SB	y=0.172x+2.64	0.999
SC	y=0.0689x+2.96	1.000
SD	y=0.149x+2.66	0.997

Note: MA-ME: mixed salt stress; SA-SD: single salt stress; R^2: the square of total correlation coefficient.

Table 4. Result of linear regression analysis between each physiological index and intensity of stresses.

Y	Saline treatment group	Regression equation	R^2	dy/dx
	MA	y=-0.355x+19.3	0.977	-0.355
	MB	y=-0.372x+18.9	0.984	-0.372
	MC	y=-0.395x+18.7	0.994	-0.395
	MD	y=-0.476x+18.1	0.985	-0.476
P_N	ME	y=-0.528x+17.1	0.921	-0.528
	SA	y=-0.456x+19.3	0.991	-0.456
	SB	y=-0.245x+18.1	0.948	-0.245
	SC	y=-0.625x+18.2	0.960	-0.625
	SD	y=-1.032x+19.5	0.933	-1.032
	MA	y=-0.136x+6.07	0.991	-0.136
	MB	y=-0.135x+6.12	0.985	-0.136
	MC	y=-0.149x+6.30	0.985	-0.149
	MD	y=-0.155x+5.85	0.962	-0.155
EAR	ME	y=-0.187x+5.91	0.953	-0.187
	SA	y=-0.162x+6.17	0.985	-0.162
	SB	y=-0.089x+5.62	0.905	-0.089
	SC	y=-0.209x+6.05	0.954	-0.209
	SD	y=-0.350x+6.73	0.986	-0.35
	MA	y=-0.0075x+0.333	0.990	-0.0075
	MB	y=-0.0074x+0.336	0.985	-0.0074
	MC	y=-0.0082x+0.346	0.985	-0.0082
	MD	y=-0.0085x+0.321	0.962	-0.008
MAR	ME	y=-0.0103x+0.325	0.953	-0.0103
	SA	y=-0.0089x+0.339	0.985	-0.0089
	SB	y=-0.0049x+0.309	0.905	-0.0049
	SC	y=-0.0115x+0.332	0.954	-0.0115
	SD	y=-0.0192x+0.370	0.986	-0.0192

Note: x = Stress intensity; y = Strain index (P_N, EAR and MAR); R^2: the square of total correlation coefficient; SA-SD: single salt stress; MA-ME: mixed salt stress. P_N: net photosynthetic rate; EAR: Energy accumulation rate; MAR: Matter accumulation rate.

single salt toxicity. In this experiment, the four salts were sodium salts, containing the same cation but different anions. Therefore, the mix of single salts can be regarded as a mix of different anions. The abating action of the mix should be the result of anion interactions. The different anions have different physicochemical properties and toxicities to plants. The abating action of their mixture on stress might be resulted from some action, such as decreasing single ion toxicity, abating the difficulty of intracellular ion balance, or other effects. The exact reason could even be very complex, and perhaps related to ion species and mechanism of plant resistance to different salts, and should be further investigated. The abating rate decreased at higher salinity levels might be related to reduced activity and interactions of ions due to increased salinity (Cramer et al., 1986; Shi and Zhao, 1997). The abating rate increased at higher alkalinity might be due to Cl^- and SO_4^{2-}, which probably abate the severe ionic imbalance caused by HCO_3^- and CO_3^{2-}. Whatever the reason is, the relaxation mechanisms should be further studied. Most of the stresses caused by natural salt–alkaline habitats are mixed salt stresses. This experiment demonstrated that mixed salt caused a relaxing effect on the stress impact, and the mixed salt stress was different from single salt stress. Thus, reconsidering the research direction of plant salt-stress physiology is recommended.

Materials and methods

Plant materials

Seeds of *C. virgata* were collected from native grassland in Changling County, Jilin Province, northeast China, and sown in 17-cm diameter plastic pots containing 2.5 kg of washed sand. Each pot contained 10 seedlings and seedlings were sufficiently watered with Hoagland nutrient solution every 2 d. Evaporation was compensated by distilled water any time required. All pots were placed outdoors and sheltered from rain. Temperatures during the experiment were 22–26°C during the day and 19–22°C at night.

Table 5. Predictive strain value and actual strain value of mixed salt stress treatments.

Treatments	P_N[μmol (CO_2) $m^{-2}s^{-1}$]		EAR(KJ pot^{-1} d^{-1})		MAR(g pot^{-1} d^{-1})	
	PSV	PSV - ASV	PSV	PSV - ASV	PSV	PSV - ASV
MA1	4.729	0.891	1.698	0.226	0.0932	0.012
MA2	7.361	0.991	2.646	0.202	0.1451	0.0104
MA3	9.994	1.092	3.594	0.178	0.1971	0.0087
MA4	12.626	1.192	4.542	0.154	0.249	0.0071
MA5	15.258	1.292	5.49	0.13	0.301	0.0055
MB1	6.541	2.717	2.287	0.888	0.1258	0.0496
MB2	9.135	2.809	3.205	0.894	0.1763	0.0504
MB3	11.728	2.9	4.123	0.9	0.2269	0.0511
MB4	14.322	2.992	5.041	0.906	0.2774	0.0519
MB5	16.916	3.084	5.959	0.912	0.3279	0.0527
MC1	9.588	5.634	3.307	1.82	0.1814	0.0992
MC2	12.486	5.976	4.315	1.868	0.2368	0.1014
MC3	15.384	6.318	5.323	1.916	0.2921	0.1037
MC4	18.282	6.66	6.331	1.964	0.3475	0.1059
MC5	21.18	7.002	7.339	2.012	0.4029	0.1081
MD1	10.958	6.324	3.769	2.258	0.2068	0.1247
MD2	15.227	7.539	5.239	2.732	0.2875	0.1508
MD3	19.495	8.753	6.709	3.206	0.3683	0.177
MD4	23.764	9.968	8.179	3.68	0.449	0.2031
MD5	28.032	11.182	9.649	4.154	0.5298	0.2293
ME1	12.329	7.115	4.231	2.387	0.2322	0.1305
ME2	17.968	9.172	6.163	3.053	0.3384	0.1669
ME3	23.606	11.228	8.095	3.719	0.4446	0.2033
ME4	29.245	13.285	10.027	4.385	0.5508	0.2396
ME5	34.884	15.342	11.959	5.051	0.6570	0.2760

Note: MA1-ME5: mixed salt stresses with different concentrations. P_N: net photosynthetic rate; EAR: Energy accumulation rate; MAR: Matter accumulation rate; PSV: Predictive strain value; ASV: Actual strain value.

Design of single salt stresses

The four single salt stress groups of NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 were denoted by SA, SB, SC and SD. Within each group five concentration treatments were utilized. From a preliminary experiment, concentrations of the four single salt stresses were determined: NaCl of 60, 120,180, 240 and 300 mM; Na_2SO_4 of 30, 90,120, 150 and 300 mM; $NaHCO_3$ of 60, 120,180, 240 and 300 mM; Na_2CO_3 of 30, 45, 60, 75 and 90 mM.

Design of salt–alkaline mixed stresses

The four salts used in single salt stresses were mixed in various proportions according to the tolerance of *C. virgata* to salt–alkaline stress and the ranges of salinity and pH in the soil. Five treatment groups (labeled MA–ME) were set with gradually increasing alkalinity. The salt compositions of the five treatment groups are shown in Table 1 and the pH in Table 2. All treatment groups had a 1:1 molar ratio of monovalent salts (NaCl + $NaHCO_3$) to divalent salts (Na_2SO_4 + Na_2CO_3); therefore, if the individual molar concentrations were the same, then the total ion concentrations were become similar throughout the treatments. Within each group, five concentration treatments were utilized, namely 60, 120, 180, 240 and 300 mM totaling 25 salt–alkaline mixed stress treatments (labeled MA1–ME5) with varying salinity and pH. This produced a wide range of salt–alkali conditions, with total salt concentrations of 60–300 mM and pH 6.60–10.69.

Stress treatment

The seedlings were subjected to stress treatment at 6-week-old. The 141 pots of uniformly growing seedlings were randomly divided into 47 sets, three pots per set. Each pot was a single replicate. One set was used as an untreated control. A second set was used for determining dry and fresh weights at the beginning of treatment and the remaining 45 sets were treated with the various stress treatments. Stress treatments were performed daily at around 17:00–18:00 by thoroughly watering treated plants with 500 mL of treatment solution per pot, in three portions. Control plants were maintained by watering with nutrient solution. The total treatment duration was 15 days.

Measurement of physiological indices

All plants were harvested at the morning after the final treatment. The plants were first washed with tap water, and then distilled water. Roots and shoots were separated and the fresh weights (FW) were determined for each plant. A portion of the fresh samples was taken to measure the physiological indices. The remainder of the samples was oven-dried at 80°C for 15 min, then vacuum-dried at 40°C to a constant weight and the dry weights (DW) were recorded. Net photosynthetic rates (P_N) of leaves were determined between 08:30 and 10:30, from fully expanded third blades, using a portable open flow gas exchange system LI-6400 (LICOR, USA). The photosynthetic active radiation (PAR) was 1200 μmol m^{-2} s^{-1}. The results were expressed as μmol CO_2 $m^{-2}s^{-1}$. Total calorific value was determined using a bomb calorimeter (PARR1281, USA), and expressed in as kJ pot^{-1}. The energy accumulation rate can be defined as follows: Energy accumulation rate (EAR) = (Total calorific value after treatment – Total calorific value before treatment)/ duration of treatment (d). The results were expressed as kJ pot^{-1} d^{-1}. Matter accumulation rate (MAR) = (Total dry weight after treatment – Total dry weight before treatment)/ duration of treatment (d). The results were expressed as g pot^{-1} d^{-1}.

Statistical data analysis

Data were analyzed by one-way analysis of variance (ANOVA) and linear regression analysis using the statistical software SPSS 13.0. All treatments were repeated three times, and the means and standard errors (SE) calculated. The level of significance was $P < 0.05$.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (No. 31072078).

References

Apse MP, Aharon G, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in Arabidopsis. Science 285 (5431): 1256–1258.

Charkazi F, Ramezanpour SS, Soltanloo H (2010) Expression pattern of two sugar transporter genes (SuT4 and SuT5) under salt stress in wheat. Plant Omics J 3:194-198

Cramer GR, Laüchli AE, Epstein E (1986) Effects of NaCl and $CaCl_2$ on ion activities in complex nutrient solutions and root growth of cotton. Plant Physiol 81: 792–797.

Ge Y, Li JD (1990) A preliminary study on the effects of halophytes on salt accumulation and desalination in the soil of Songnen Plain, northeast China. Acta Pratacult Sin 1: 70–76.

Ghoulam C, Foursy A, Fares K (2002) Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environ Exp Bot 47: 39–50.

Ibraheem O, Dealtry G, Roux S, Bradley G (2011) The effect of drought and salinity on the expressional levels of sucrose transporters in rice (*Oryza sativa* Nipponbare) cultivar plants. Plant Omics J 4:68-74.

James RA, Munns R, Caemmerer SV, Trejo C, Miller C, Condou TAG (2006) Photosynthetic capacity is related to the cellular and subcellular partitioning of Na^+, K^+ and Cl^- in salt-affected barley and durum wheat. Plant Cell Environ 29: 2185–2197.

Jemâa E, Saïda A, Sadok B (2011) Impact of indole-3-butyric acid and indole-3-acetic acid on the lateral roots growth of Arabidopsis under salt stress conditions. Aust J Agric Eng 2:18-24

Kawanabe S, Zhu TC (1991) Degeneration and conservation of *Aneurolepidium chinense* grassland in Northern China. J Jpn Grassl Sci 37: 91–99.

Khan MA, Ungar IA, Showalter AM (2000) Effects of Salinity on Growth, Water Relations and Ion Accumulation of the Subtropical Perennial Halophyte, *Atriplex griffithii* var. *stocksii.* Ann Bot 85: 225–232.

Koca H, Bor M, Özdemir F, Türkan I (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ Exp Bot 60: 344–351.

Läuchli A, Lüttge U (2002) Salinity in the soil environment. In：Tanji KK (ed) Salinity: Environment-Plants-Molecules. Boston Kluwer Academic Publishers, Boston, USA, pp 21–23.

Liu DL, Zhang XX, Cheng YX, Takano TS, Liu SK (2006) RHsp90 gene expression in response to several environmental stresses in rice (*Oryza sativa* L.). Plant Physiol Bioch 44:380–386.

Mozafar A, Goodin JR (1985) Salt tolerance of two differently drought-tolerant wheat genotypes during germination and early seedling growth. Plant Soil 96: 303–316.

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25: 239–250.

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotox Environ Safe 60: 324–349.

Patel AD, Pandey AN (2007) Effect of soil salinity on growth, water status and nutrient accumulation in seedlings of *Cassia montana* (Fabaceae). J Arid Environ 70: 174–182.

Shi D, Sheng Y (2005) Effect of various salt-alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors. Environ Exp Bot 54: 8–21.

Shi D, Wang D (2005) Effects of various salt-alkali mixed stresses on *Aneurolepidium chinense* (Trin.) Kitag. Plant Soil 271:15–26.

Shi DC, Yin LJ (1993) Difference between salt (NaCl) and alkaline (Na_2CO_3) stresses on *Puccinellia tenuiflora* (Griseb.) Scribn et Merr. plants. Acta Bot Sin 35: 144–149.

Shi DC, Yin SJ, Yang GH, Zhao KF (2002a) Citric acid accumulation in an alkali-tolerant plant *Puccinellia tenuiflora* under alkaline stress. Acta Bot Sin 44: 537–540.

Shi DC, Zhao KF (1997) Effects of NaCl and Na_2CO_3 on growth of *Puccinellia tenuiflora* and on present state of mineral elements in nutrient solution. Acta Pratacult Sin 6 (2): 51–61.

Shi H, Quintero FJ, Pardo JM, Zhu JK (2002b) The putative plasma membrane NA^+/H^+ antiporter SOS1 controls long-distance NA+ transport in plants. Plant Cell 14 (2): 465–477.

Soussi M, Ocana A, Lluch C (1998) Effects of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (*Cicer arietinum* L). J Exp Bot 49: 1329–1337.

Wang ZQ, Yuan YZ, Ou JQ, Lin QH, Zhang CF (2007) Glutamine synthetase and glutamate dehydrogenase contribute differentially to proline accumulation in leaves of wheat (*Triticum aestivum*) seedlings exposed to different salinity. J Plant Physiol 164: 695–701.

Yang C, Chong J, Kim C, Li C, Shi D, Wang D (2007) Osmotic adjustment and ion balance traits of an alkali resistant halophyte *Kochia sieversiana* during adaptation to salt and alkali conditions. Plant Soil 294: 263–276.

Yang C, Shi D, Wang D (2008) Comparative effects of salt stress and alkali stress on growth, osmotic adjustment and ionic balance of an alkali resistant halophyte *Suaeda glauca* (Bge.). Plant Growth Regul 56: 179–190

Zheng HY, Li JD (1999) Form and dynamic trait of halophyte community. In: Zheng HY, Li JD (eds) Saline Plants in Songnen Plain and Restoration of Alkaline-saline Grass. Science Press, Beijing, China, pp 137–138.