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Effects of various mixed salt-alkaline stresses on growth, photosynthesis, and photosynthetic pigment concentrations of *Medicago ruthenica* seedlings

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Abstract

Soil salinization and alkalinization frequently co-occur in naturally saline and alkaline soils. To understand the characteristics of mixed salt-alkali stress and adaptive response of *Medicago ruthenica* seedlings to salt-alkali stress, water content of shoots, growth and photosynthetic characteristics of seedlings under 30 salt-alkaline combinations (salinity 24–120 mM and pH 7.03–10.32) with mixed salts (NaCl, Na_2SO_4, $NaHCO_3$, and Na_2CO_3) were examined. The indices were significantly affected by both salinity and pH. The interactive effects between salt and alkali stresses were significant, except for photosynthetic pigments. Water content of shoots, relative growth rates of shoots and roots and pigment concentrations showed decreasing trends with increasing salinity and alkalinity. The root activity under high alkalinity and salinity treatments gradually decreased, but was stimulated by the combined effects of low alkalinity and salinity. The survival rate decreased with increased salinity, except at pH 7.03–7.26 when all plants survived. Net photosynthetic rate, stomatal conductance and intercellular CO_2 concentration decreased with increased salinity and pH. *M. ruthenica* tolerated the stress of high salt concentration when alkali concentration was low, and the synergistic effects of high alkali and high salt concentrations lead to the death of some or all seedlings. *M. ruthenica* appeared to be salt-alkali tolerant. Reducing the salt concentration or pH based on the salt components in the soil may be helpful to abate damage from mixed salt-alkaline stress.

Additional key words: mixed salt-alkali stress; *Medicago ruthenica*; relative growth rate; root activity; photosynthesis; photosynthetic pigments; water content.

Introduction

Salinization and alkalination of soil is a widespread environmental problem. Of the world's currently cultivated land area (1.5×10^9 ha), about 0.34×10^9 ha (23%) is saline and another 0.56×10^9 ha (37%) is sodic (Tanji 1990). Overgrazing, harvesting for hay and intensive cultivation contribute to the large-scale development of salt-alkaline soils and substantial losses of arable lands, especially in arid and semiarid regions of most countries. The salinization and alkalination can severely affect natural grasslands and farmlands. The grasslands affected by salt and alkali constitute an area of approximately 3.7×10^6 ha in the Songnen Plains in northeast China, which represent nearly 70% of the total natural grasslands in China (Deng *et al*. 2006).

Because soil salinization and alkalinization frequently co-occur, the conditions in naturally saline and alkaline soils are very complex, the total salt concentration composition of salts and proportions of neutral salts to alkaline salts varies with different soils. In the extensive alkaline soils over much of northeast China, the neutral salts NaCl and Na_2SO_4 and alkaline salts $NaHCO_3$ and Na_2CO_3 are the main salt components (Ge and Li 1990). The problem of soil alkalinization due to $NaHCO_3$ and Na_2CO_3 may be more severe than the problem of salinization caused by neutral salts, such as NaCl and Na_2SO_4, as the alkaline salts are more destructive to plants than neutral salts (Shi and Yin 1993, Yang *et al*. 2007, 2008b). Alkaline salt stress is referred to as "alkali

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Abbreviations: C_i – intercellular CO_2 concentration; Car – carotenoid; Chl – chlorophyll; DM – dry mass; FM – fresh mass; g_s – stomatal conductance; P_N – net photosynthetic rate; PAR – photosynthetic active radiation; RGR – relative growth rate; TPF – triphenylformazan; TTC – triphenyltetrazolium chloride.
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stress", while "salt stress" refers to neutral salt stress (Shi and Sheng 2005). Some reports clearly demonstrated that alkali stress was more severe than salt stress (Campbell and Nishio 2000, Hartung *et al*. 2002, Shi and Sheng 2005, Shi and Yin 1993, Tang and Turner 1999). Generally, salt stress involves osmotic effects and specific ion effects (Munns 2002), the former mainly dependent on salt concentration. For alkali stress, in addition to these two types of effects, there is another important factor, high pH.

There are a few reports on the effects of mixed salt-alkaline stresses on plants (Shi and Sheng 2005, Shi and Wang 2005, Peng *et al*. 2008, Li *et al*. 2010). However, to our knowledge, there are no reports on the effects of mixed salt-alkaline stresses on water content of shoots, photosynthesis and photosynthetic pigments of plants, especially under the conditions of increased pH. Growth and photosynthesis are important for the development and acclimation of plants; they can directly influence the productivity and fitness of agricultural crops and grasses.

Medicago ruthenica (L.) Sojak, a perennial legume widely distributed in Siberia, Mongolia, and northern China, commonly grows on open hillsides, mixed grass steppes, and meadows (Small and Jomphe 1989, Shi 2006). Balabaev (1934) noted that it is a unique species of *Medicago* adapted to dry, stony locations with extremely low snowfall and very cold winters. Campbell *et al*. (1997) indicated that *M. ruthenica* may be superior to *Medicago sativa* in nutrient-use efficiency and thus may be more suitable for low-input cropping systems. Due to its superior cold-resistance to *M. sativa*, *M. ruthenica* is regarded as an excellent legume in highland in cold regions. Therefore, *M. ruthenica* is one of promising legume species for forage in arid and semiarid areas and as a candidate species for promoting the recovery of the Songnen Grasslands in northern China which is often affected by the mixed salt-alkali stress. However, little is known about the mixed salt-alkali stresses on the performance of *M. ruthenica*.

We used mixtures of two neutral salts, NaCl and Na_2SO_4 and two alkaline salts $NaHCO_3$ and Na_2CO_3, in various proportions, to investigate the effects of a range of mixed salt and alkaline conditions on the ecophysiological characteristics of *M. ruthenica* seedlings. Thirty treatments of mixed salt and alkaline conditions were applied to assess ecophysiological responses of *M. ruthenica* seedlings to determine the growth and photosynthetic characteristics of the seedlings with changes in conditions and to identify mechanisms of plant resistance under salt-alkaline stress.

Materials and methods

Plant material and culture condition: The *M. ruthenica* seeds were collected from the natural grasslands of the Songnen Plains. Experiments were conducted on the campus of Northeast Normal University, Changchun City (43°51′N, 125°91′E) with an annual average air temperature of 4.9°C, precipitation of 500–600 mm, 206 m above sea level on the Songnen Plains, from July 5th to September 10th in 2009. This region experiences a continental monsoon and the climate is similar to that of the region where the Grassland Research Station of Northeast Normal University is located (44°38′N, 123°41′E). The *M. ruthenica* seeds were sown in plastic pots (18 cm diameter × 13 cm depth) filled with 10 cm washed sand. All of the pots were placed outdoors but kept protected from the rain. Each pot was watered with 500 mL of Hoagland nutrient solution every two days, and evaporated water was replenished with distilled water daily after weighing. The nutrient solution used in this work contained 2 mM KH_2PO_4, 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 5 mM $MgSO_4$, 46 μM H_3BO_3, 6.7 μM $MnSO_4$, 0.77 μM $ZnSO_4·7H_2O$, 0.32 μM $CuSO_4·5H_2O$, 0.56 μM H_2MoO_4, 22 μM EDTA-Na_2, and 20 μM $FeSO_4·7H_2O$. Each pot contained 30 plants.

Design of simulated salt and alkaline conditions: Based on the composition of salt-alkaline soils in northeast China (Ge and Li 1990), two neutral salts (NaCl and Na_2SO_4) and two alkaline salts ($NaHCO_3$ and Na_2CO_3) were used in treatments. The four selected salts were mixed in various proportions based on the resistance of *M. ruthenica* against salt-alkaline stress and the ranges of salinity and pH in the Songnen Plains. Six treatment groups (A, B, C, D, E, and F) were defined with gradually increased alkalinity, and their salt composition is shown in Table 1. All treatment groups had a 1:1 molar ratio of monovalent salts (NaCl + $NaHCO_3$) to divalent salts (Na_2SO_4 + Na_2CO_3), *i.e*. monovalent salts contribute with the same molar ratio of Na^+ to treatment solution as the divalent salts. Five concentrations were used for treatments within each group, namely 24, 48, 72, 96, and 120 mM, giving 30 mixed salt-alkaline stress treatments in total (Table 2). This produced a wide range of salt-alkaline conditions, with total salt concentrations ranging from 24 to 120 mM and the pH from 7.03 to 10.32.

Salt-alkaline stress treatment: When the seedlings were six weeks old, they were subjected to a designated salt-alkaline stress treatment. Seedlings growing uniformly (in 96 pots) were selected and randomly divided into 32 sets of three pots each. Each pot was considered as one replicate. One set was used for the evaluation of a growth parameter at the beginning of the treatment. Another was used as control and watered only with nutrient solution; the remaining 30 sets were treated by the addition of 500 mL of salt-alkaline stress treatment solution per pot. The salt-alkaline stress treatment was performed at approximately 16:30 h every two days. The amount of evaporated water was determined by weighing the pots in

Table 1. Salt composition and their molar ratios within treatments. Two neutral salts (NaCl and Na_2SO_4) and two alkaline salts ($NaHCO_3$ and Na_2CO_3) were used and mixed in various proportions. All treatment groups had a 1:1 molar ratio of monovalent salts (NaCl + $NaHCO_3$) to divalent salts (Na_2SO_4 + Na_2CO_3), *i.e.* monovalent salts contribute with the same molar ratio of Na^+ to treatment solution as the divalent salts.

Treatment	NaCl	Na_2SO_4	$NaHCO_3$	Na_2CO_3
A	2	1	0	0
B	1	1	1	0
C	12	9	8	1
D	8	9	12	1
E	12	1	8	9
F	0	0	2	1

the morning and evening, and it was replenished with distilled water daily.

Sampling and measurement: After seven days of stress treatment, fresh leaves were collected from the plants and cut into small segments to determine the concentrations of chlorophyll (Chl) *a*, Chl *b* and carotenoids (Car). Chl *a*, Chl *b*, and Car were extracted with acetone and spectrophotometric determinations were made at 440, 645, and 663 nm for each of the three samples. The data were calculated using the methods as described by Zhu (1993), expressed in mg g^{-1}(FM).

Net photosynthetic rates (P_N), stomatal conductance (g_s) and intercellular CO_2 concentrations (C_i) of leaves were determined at 08:30–10:30 h on the first fully expanded blade, using a portable open flow *LI-6400* gas-exchange system (*LI-COR Biosciences,* Lincoln, USA) at 14 days after treatment. The PAR was 1,200 μmol m^{-2} s^{-1}. The ambient CO_2 concentration was set at 360 μmol mol^{-1} and the air temperature was 25°C. Measurements were repeated five times for each blade, for five blades per pot, and data were recorded as an average for each pot.

After 14 days of stress treatment, living and dead plants in each pot were counted, and the rate of seedling survival was calculated as a percentage of the number of living plants to the total number.

All plants were harvested at the end of the treatment, carefully washed with tap water and then washed three times with distilled water. The water that remained on the surface of the plants was blotted with filter paper. Roots and shoots were separated and a portion of fresh sample was taken to measure amounts of photosynthetic pigments and root activity. The fresh mass (FM) of the samples was determined and converted to dry mass (DM) based on the water content of the remaining fresh samples. The FMs were recorded and the remainder of the sample was oven-dried at 80°C for 15 min, then vacuum-dried at 40°C to a constant mass and the DM values were recorded. The water content [%] was calculated using the formula (FM – DM) × 100/FM.

The relative growth rate (RGR) was determined using the equation of Kingsbury *et al*. (1984):

RGR = (ln DM at the end of a treatment – ln DM at the beginning of a treatment)/duration of treatment.

The DM values at the beginning and end of a treatment were the sums of all the material in a pot.

The activity of the root system was determined as described by Comas *et al*. (2000). Fresh roots were incubated for 60 min at 37°C in triphenyl tetrazolium chloride (TTC) solution (0.04% in pH 7.0 phosphate buffer). The red product (TPF) in the roots was extracted using ethyl acetate. The absorbances were determined by spectrophotometry at 485 nm. The activity of the root system was expressed relative to the control value of 100%. TTC is always considered to be a sensitive indicator of changes in cellular metabolism, and in this study was used as an indicator of changes in root metabolism.

Statistical analysis: A two-way factorial *ANOVA* was used to test the effects of pH, salt concentration, and their interaction. Differences between means of treatments were performed by the *Duncan*'s multiple test at $P<0.05$. All data analysis was carried out using the *Statistical Package for Social Sciences* (*SPSS*) (version *13.0*).

Results

Salinity and pH with various mixed salt-alkaline solutions: During the experimental period, the salinity ranged from 24 to 120 mM and pH ranged from 7.03 to 10.32 with increased salt concentration (Table 2). The difference in the range of pH values was greater among groups than within a group. These simulated salt-alkaline treatments were designed to represent complex natural salt-alkaline conditions.

Water content of shoots, relative growth rate, root activity and survival rate: Salinity and alkalinity significantly affected the water content of shoots, RGR, root activity and survival rate of *M. ruthenica* seedlings under the applied mixed stresses (all $P\leq0.01$). There were significant interactive effects between the salt concentration and pH on the water content of shoots, RGR, root activity, and survival rate of seedlings ($P\leq0.01$). Under the various stress treatments, RGR of both shoots and roots showed decreasing trends with increased salinity and alkalinity (Fig. 1*A,B*). The water content of shoots decreased with increased salinity and alkalinity in present study (Fig. 2*A*) and decreased moderately with the increased salinity between pH 7.03–8.22. However, when pH was above 8.30, the water contents of shoots decreased sharply with increased salinity and alkalinity. Interactions between salt and alkali stress caused significant changes in root activity of seedlings along the salinity gradient ($P\leq0.01$) (Fig. 2*B*). Root activity of

Table 2. Stress factors for different treatments. Five concentrations (24, 48, 72, 96, and 120 mM) were used within each group, giving 30 mixed salt-alkaline stress in total and the pH ranged from 7.03 to 10.32. A: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 2:1:0:0; B: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 1:1:1:0; C: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:9:8:1; D: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 8:9:12:1; E: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:1:8:9; F: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 0:0:2:1.

Treatment	pH	Salinity [mM]	Na^+ [mM]	Cl^- [mM]	SO_4^{2-} [mM]	HCO_3^- [mM]	CO_3^{2-} [mM]
A_1	7.03	24	32	16.0	8.0	0.0	0.0
A_2	7.12	48	64	32.0	16.0	0.0	0.0
A_3	7.18	72	96	48.0	24.0	0.0	0.0
A_4	7.22	96	128	64.0	32.0	0.0	0.0
A_5	7.26	120	160	80.0	40.0	0.0	0.0
B_1	7.29	24	32	8.0	8.0	8.0	0.0
B_2	7.45	48	64	16.0	16.0	16.0	0.0
B_3	8.04	72	96	24.0	24.0	24.0	0.0
B_4	8.22	96	128	32.0	32.0	32.0	0.0
B_5	8.30	120	160	40.0	40.0	40.0	0.0
C_1	8.80	24	32	9.6	7.2	6.4	0.8
C_2	8.92	48	64	19.2	14.4	12.8	1.6
C_3	8.95	72	96	28.8	21.6	19.2	2.4
C_4	8.99	96	128	38.4	28.8	25.6	3.2
C_5	9.02	120	160	48.0	36.0	32.0	4.0
D_1	9.03	24	32	6.4	7.2	9.6	0.8
D_2	9.05	48	64	12.8	14.4	19.2	1.6
D_3	9.10	72	96	19.2	21.6	28.8	2.4
D_4	9.15	96	128	25.6	28.8	38.4	3.2
D_5	9.22	120	160	32.0	36.0	48.0	4.0
E_1	9.45	24	32	9.6	0.8	6.4	7.2
E_2	9.56	48	64	19.2	1.6	12.8	14.4
E_3	9.64	72	96	28.8	2.4	19.2	21.6
E_4	9.69	96	128	38.4	3.2	25.6	28.8
E_5	9.79	120	160	48.0	4.0	32.0	36.0
F_1	9.84	24	32	0.0	0.0	16.0	8.0
F_2	9.91	48	64	0.0	0.0	32.0	16.0
F_3	9.96	72	96	0.0	0.0	48.0	24.0
F_4	10.11	96	128	0.0	0.0	64.0	32.0
F_5	10.32	120	160	0.0	0.0	80.0	40.0

plants was stimulated by the combined effects of low alkalinity and salinity especially between pH 7.03 and 8.04 (Fig. 2*B*). The root activity of the seedlings under high-alkalinity and salinity (group D, E, F and 72–120 mM concentration of group C) gradually decreased along the salinity gradient (Fig. 2*B*). Examination of root activity in 72–120 mM salinity of group E and F was not possible because the roots were severely damaged. The survival rate of seedlings decreased with increased salinity except in group A, in which survival rate was 100% (Fig. 2*C*). This indicated that all *M. ruthenica* seedlings survived irrespective of the salinity if the pH was less than 7.26. In groups B, C and D, the survival rate was less than 100% when the salt concentration was higher than 72 mM (group B and C) and 48 mM (group D) and the pH value was 9.05. Then, an additional increase in either salinity or pH significantly reduced survival rates. When the seedlings were exposed to high alkalinity (group E and F), their survival rate decreased relatively rapidly along the salinity gradient and resulted in the death of all seedlings in groups E3, E4, E5, F3, F4, and F5 (Fig. 2*C*).

Chl *a*, Chl *b* and Car contents: Salinity and alkalinity significantly affected the Chl *a*, Chl *b* and Car content of *M. ruthenica* seedlings under the applied mixed stresses (all $P \leq 0.01$) and the concentration of these pigments all showed decreasing trends with increased salinity and alkalinity (Fig. 3*A,B,C*). In group A, Chl *a* and Chl *b* concentration did not change markedly along the salinity gradient. Interactive effects between salt concentration and pH was not significant for Chl *a*, Chl *b* and Car content ($P > 0.05$). In group B_1 and C_1, low alkalinity stimulated Chl *a*, Chl *b*, and Car content.

P_N, g_s, and C_i of *M. ruthenica* leaves were significantly influenced by salinity, alkalinity and their interactive effects in all treatments ($P \leq 0.01$). P_N, and g_s of *M. ruthenica* leaves decreased with increased salt concentration and pH, except for the values of A_1 and B_1, for which P_N and g_s were slightly higher than the control (Fig. 4*A,B,C*). The C_i of *M. ruthenica* leaves decreased with increased salinity and alkalinity.

Discussion

Evaluation of various mixed salt-alkali conditions: The 30 treatments evenly covered various salt-alkaline conditions in a range of total salt concentration from 24–120 mM and pH from 7.03 to 10.32. The mixed treatments used in the experiment reproduced complex salt-alkaline conditions and made the research of complex salt-alkaline stress possible. In natural salt-alkali soils, stress conditions and interference factors are very complex and difficult to simulate, which severely limits the investigation of plant responses to salt-alkaline stress in natural grasslands. Previous reports mainly focused on either salt or alkali stress (Bell *et al*. 1993, Ben Amor *et al*. 2005, Elmore *et al*. 2006, Jianaer *et al.* 2007) and a few have reported on mixed stress (Shi and Sheng 2005, Peng *et al.* 2008), similar to our study, which involved simultaneous salinity and alkalinity stress.

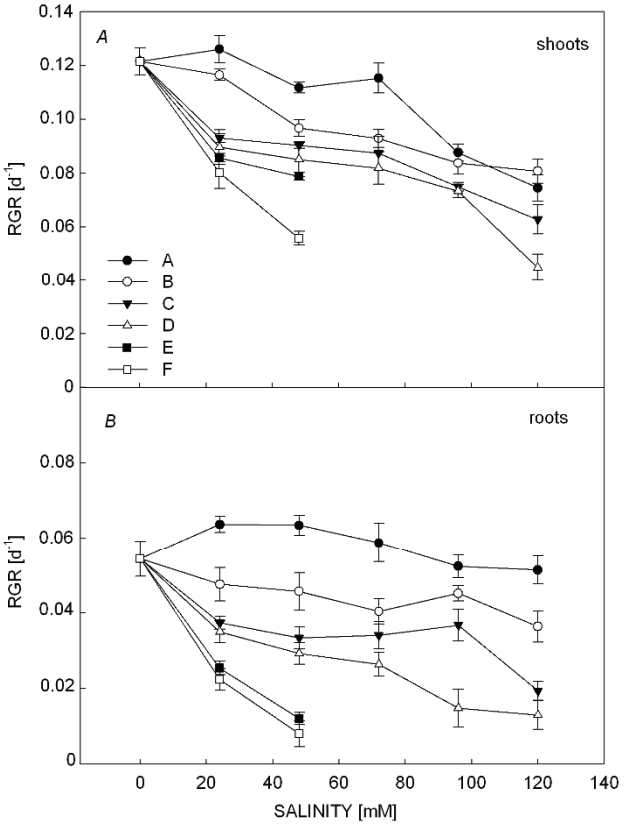

Fig. 1. Relative growth rate (RGR) of shoots (*A*) and roots (*B*) of *M. ruthenica* seedlings under various mixed salt-alkaline stress conditions. Six-week-old seedlings were subjected to treatments with mixed salts for 14 days. Means (± SE) of three replicates. A: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 2:1:0:0; pH 7.03–7.26; B: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 1:1:1:0; pH 7.29–8.30; C: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:9:8:1; pH 8.80–9.02; D: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 8:9:12:1; pH 9.03–9.22; E: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:1:8:9; pH 9.45–9.79; F: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 0:0:2:1; pH 9.84–10.32.

Ecophysiological responses of *M. ruthenica* seedlings to various mixed salt-alkaline stresses: Seedling establishment is one of the stages that is most sensitive to salinity (Jones and Jones 1989). Understanding plant responses at the seedling stage is especially important for understanding the mechanism of salt resistance, sensitivity, and survival in plants (Mayer and Poljakoff-Mayber 1963).

Plants can lose water content as a quick and economical approach to osmotic adjustment in response to osmotic stress in general conditions (Lissner *et al*. 1999). In the present study, the water content of shoots of *M. ruthenica* seedlings was both significantly affected by salinity and alkalinity and the reductions in water contents of shoots with increased alkalinity were greater than with salinity (Fig. 1*A*). The previous studies showed that the reduction of water content was more severe under alkaline stress than saline stress in some species when salt stress and alkali stress were imposed separately (Guo *et al*. 2010, Yang *et al*. 2007, 2008a, 2009). In this study, results showed that the combined stress of salinity and alkalinity had significant effects on the water content of *M. ruthenica* shoots ($P \leq 0.01$). The sharp decrease in water content of shoots with alkalinity not only contributed to the osmotic stress but also might result from the destructive effect of high pH on root function and water uptake or accumulation of solutes. Low water content might not enable *M. ruthenica* seedlings accumu late osmolytes with minimum energy consumption, like some halophytes, leading to its relative sensitivity to salt and alkali stresses than halophyte.

The RGR reflects many vital plant activities during vegetative growth and so is a good index for measuring plant responses to various stresses. In the present study, the RGR of shoots and roots were both significantly affected by salinity and alkalinity (Fig. 1*A,B*, $P \leq 0.01$). As a common phenomenon in mesophytes, plant growth is suppressed under saline conditions (Ashraf and Harris 2004). However, in the present study, the growth was not inhibited, but stimulated in shoots under low salt levels (A_1) and in roots (group A) under moderate pH levels below 7.26, indicating that *M. ruthenica* is relatively tolerant to salinity. But it might be more sensitive under alkali stress than the halophyte *Puccinellia tenuiflora* which took the stimulated growth under alkali levels below 60 mM (Guo *et al*. 2010). Generally, it is considered that salt stress inhibits plant growth by water deficiency and ion toxicity (de Lacerda *et al*. 2003, Marcum 1999, Ghoulam *et al*. 2002, Soussi *et al*. 1998), but plant growth is only moderately inhibited, or even stimulated, by salt stress for salt-tolerant species (Cramer *et al*. 1986, Marcum 1999). Similar results have been reported for several other species, which also showed optimal growth in the presence of salt (Short and Colmer 1999, Khan *et al.* 2000a, Khan *et al.* 2000b, Guo *et al.*

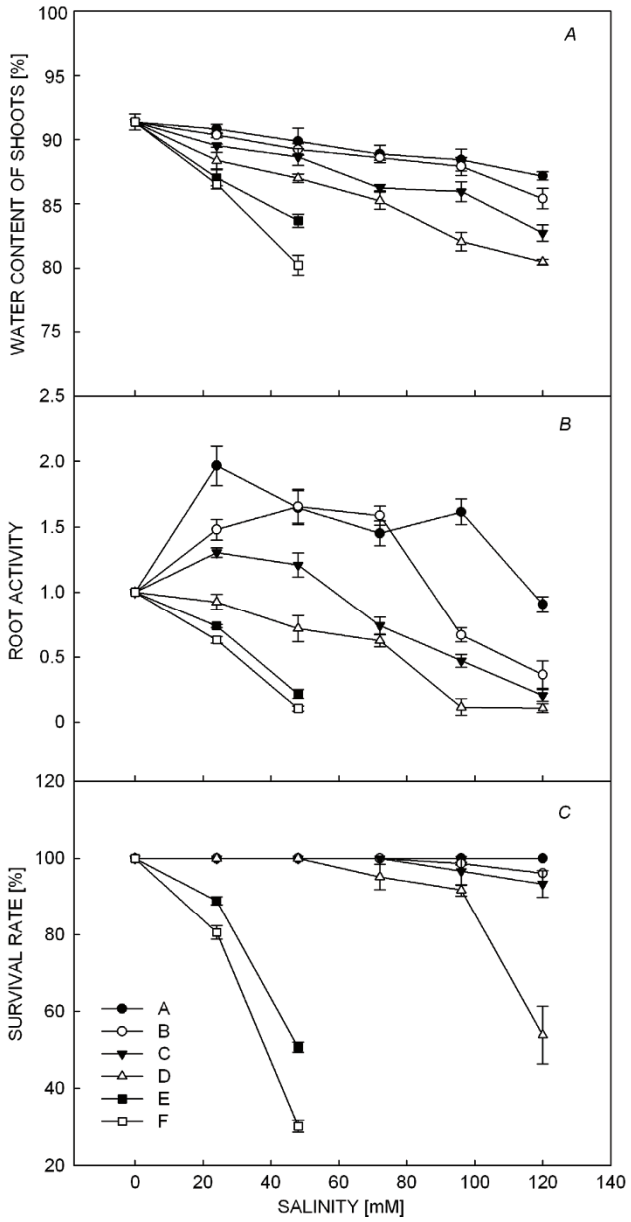

Fig. 2. Water contents of shoots (*A*), root activity (*B*) and survival rate (*C*) of *M. ruthenica* seedlings under various mixed salt-alkaline stress conditions. Six-week-old seedlings were subjected to stress treatment with mixed salts for 14 days. Means (± SE) of three replicates. A: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 2:1:0:0; pH 7.03–7.26; B: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 1:1:1:0; pH 7.29–8.30; C: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:9:8:1; pH 8.80–9.02; D: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 8:9:12:1; pH 9.03–9.22; E: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:1:8:9; pH 9.45–9.79; F: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 0:0:2:1; pH 9.84–10.32.

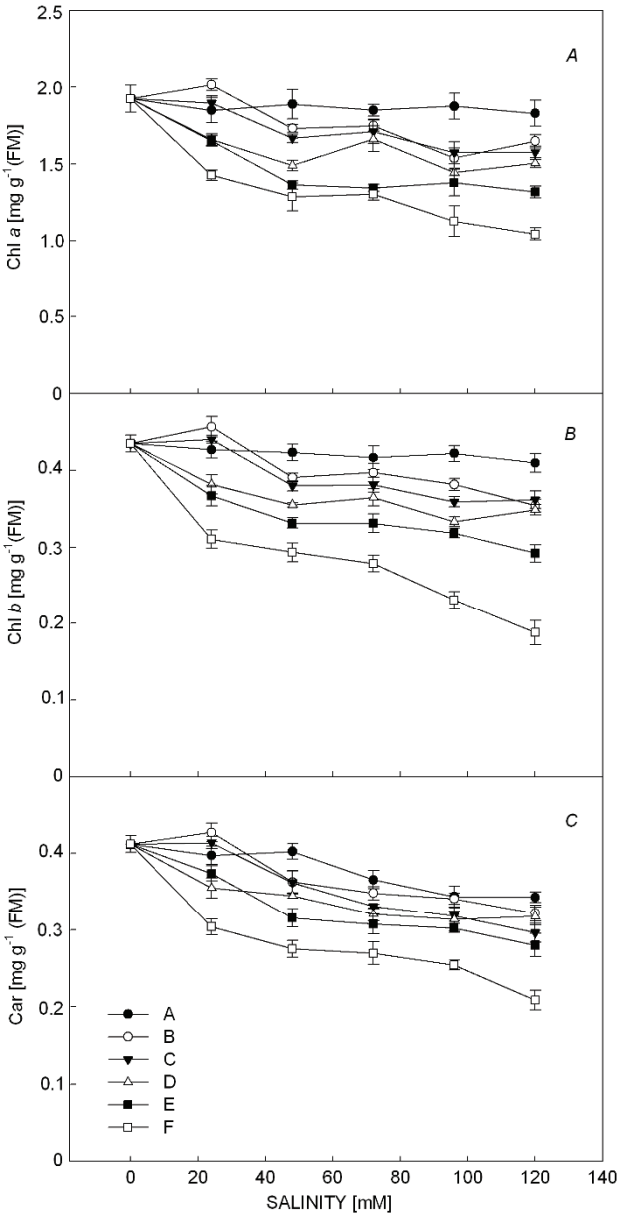

Fig. 3. Concentration of chlorophyll (Chl) *a* (*A*), Chl *b* (*B*) and carotenoid (Car) (*C*) in the leaves of *M. ruthenica* seedlings under various mixed salt-alkaline stress conditions. Six-week-old seedlings were subjected to stress treatment with mixed salts for 7 days. Means (± SE) of three replicates. A: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 2:1:0:0; pH 7.03–7.26; B: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 1:1:1:0; pH 7.29–8.30; C: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:9:8:1; pH 8.80–9.02; D: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 8:9:12:1; pH 9.03–9.22; E: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:1:8:9; pH 9.45–9.79; F: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 0:0:2:1; pH 9.84–10.32.

2010). Of course at higher levels of salinity and alkalinity, the growth of *M. ruthenica* seedlings was markedly inhibited, with the inhibition of alkaline stress being stronger than that of salt stress.

Plant roots are the key structures in contact with soils; therefore, it is the abiotic stresses (*e.g.*, salt, alkalinity, drought) in environments that primarily injure the roots. In the present study, the roots of *M. ruthenica* seedlings acclimated to salt and alkali stresses in the environment, but extreme high salinity or pH caused significant reduction of root activity which indicated the strength of roots metabolic activity directly (Fig. 2*B*). High pH in the

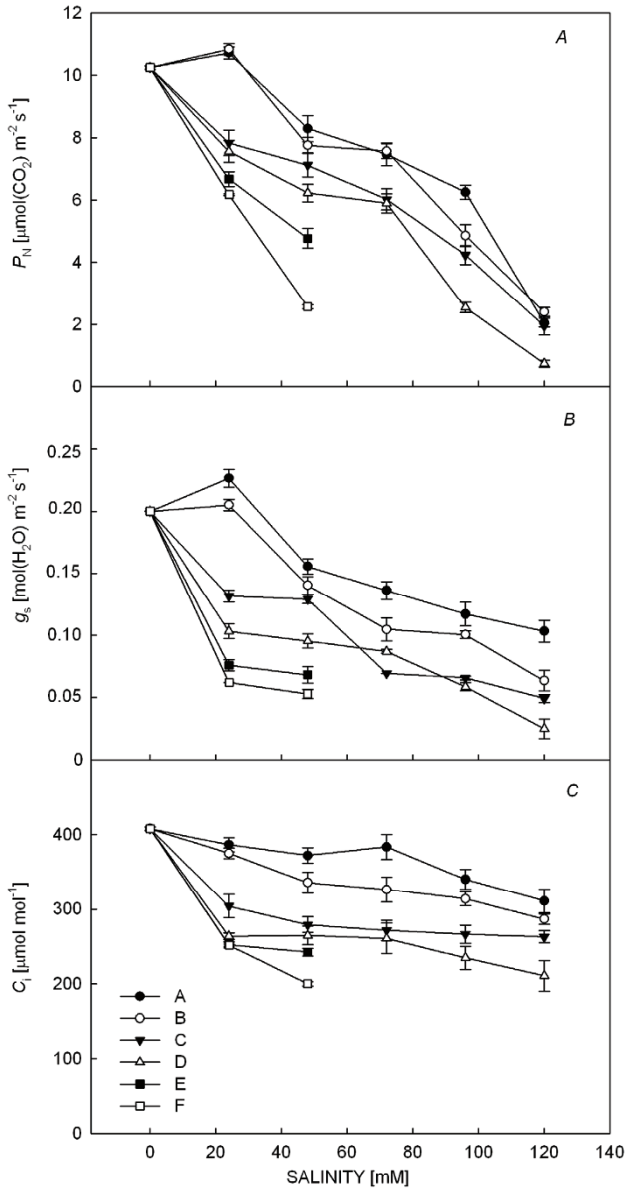

Fig. 4. Net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*) and intercellular CO_2 concentration (C_i) (*C*) of *M. ruthenica* seedlings under various mixed salt-alkaline stress conditions. Six-week-old seedlings were subjected to stress treatment with mixed salts for 14 days. Means (± SE) of five replicates. A: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 2:1:0:0; pH 7.03–7.26; B: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 1:1:1:0; pH 7.29–8.30; C: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:9:8:1; pH 8.80–9.02; D: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 8:9:12:1; pH 9.03–9.22; E: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 12:1:8:9; pH 9.45–9.79; F: ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$) = 0:0:2:1; pH 9.84–10.32.

rhizosphere appears to be one of the main factors that inhibits plant growth (Campbell and Nishio 2000) because the high pH environment surrounding the roots might damage root structure and functions such as absorption of water (Fig. 2*A*). The high pH environment can also direct some ions, *e.g.* Ca^{2+} and Mg^{2+}, to precipitate (Shi and Zhao 1997), which may destroy the nutrient supply and ion balance around the roots. Root activity was significantly stimulated under low salinity and low pH in this experiment (Fig. 2*B*), which further confirms that *M. ruthenica* is a relatively salt-tolerant species. A similar stimulation of root activity was reported for barley plants treated by salt-stress and alkalinity-stress (Yang *et al*. 2009). In terms of survival, *M. ruthenica* seedlings were more sensitive to alkaline stress because high salinity did not increase mortality (survival rate was 100% in group A5), but mortality increased sharply under high pH (D_4, D_5 and group E, F) (Fig. 2*C*).

Chl and Car are the main photosynthetic pigments of higher plants. Photosynthetic pigment concentrations were determined after seven days of treatment so the results could reflect the response of seedlings during the mid period of treatment. The concentrations of Chl and Car were significantly reduced by increased salinity and alkalinity, while Chl *a* and Chl *b* concentrations were slightly increased at low salinity and low pH, but not markedly changed between pH 7.03–7.26 (Fig. 3*A,B*). Similar results were attained for wheat when grown under salt and alkali stress (Yang *et al*. 2008b). The decreasing photosynthetic pigment concentrations with increased stress indicated that alkali salt may enhance the activity of the Chl-degrading enzyme chlorophyllase (Reddy and Vora 1986). Another possible reason might be due to the precipitation of Mg^{2+} in high pH, hence inhibiting Chl synthesis (Shi and Zhao 1997). Elstner (1982) reported that the disturbance of the balance of certain ions (*e.g.* Na^+) under saline and alkaline conditions could inhibit proteinase activity and alter the chlorophyll concentration in leaves, leading to reduced photosynthesis in the plants. Iron deficiency may be another possible reason for the reduction of Chl concentration. Iron is essential for the proper functioning of multiple metabolic and enzymatic processes such as electron transport, nitrogen fixation, Chl biosynthesis and photosynthesis (Briat 2007, Jeong and Guerinot 2009) during plant growth and development. Though there was sufficient iron in the treatment solutions, the high pH can cause chemical reactions that make iron insoluble into solid forms and unavailable to plant roots. The reduction in Chl concentration during the growing season can reduce plant growth, vigor, and tolerance to stress conditions.

There were no significant interactive effects of Salinity and alkalinity on the concentration of photosynthetic pigments at seven days after the start of the treatment ($P>0.05$). However, significant interactive effects on photosynthesis were observed at the end of treatments ($P\leq0.01$) due to synergistic effects of salt and alkali stress occurred during the mid to late period of treatments.

P_N of a plant usually decreases with increased stress intensity (Sultana *et al*. 1999, Koyro 2006, Wei *et al*. 2006). However, we found that P_N of *M. ruthenica* was higher than that of controls in 24 mM (low alkalinity

treatments, A_1 and B_1); it then decreased sharply with increased salinity (Fig. 4*A*). Resistance of *M. ruthenica* to low salinity was evidently stronger than resistance to high salinity and alkalinity. *M. ruthenica* showed similar P_N response to some glycophyte plants (*e.g*. wheat, barley, sunflower) under salt and alkali stresses (Yang *et al*. 2008b, 2009, Liu and Shi 2010). Relative to the alkali-resistant halophyte *Chloris virgata, M. ruthenica* was relatively sensitive to salt and alkali stresses. *Chloris virgata* did not change significantly the photosynthetic parameters under moderate salt- and alkali stresses (below 120 mM) (Yang *et al*. 2008a). Due to the reduction of Chl contents, iron chlorosis could reduce the leaf P_N significantly (Larbi *et al*. 2006). The decreasing of g_s (Fig. 4*B*) may be an adaptive response to the decreased water content (Fig. 2*A*) induced by the physical or chemical signal materials of the root stimulated by low water potential (Munns and Termaat 1986, Zheng *et al*. 2002). Furthermore, the reduction of photosynthetic pigments content, the drop of g_s, and imbalance of mineral elements in plant cells could all affect the leaf P_N.

Results showed that the effect of alkalinity on C_i was stronger than that of salinity (Fig. 4*C*). This may be attributed to high pH, which can stimulate roots to generate physical or chemical signals that promote stoma activity.

Synergistic effects of salt stress and alkali stress: The effect of mixed salt-alkaline stress differs greatly from solely salt- or alkali stress due to their significant interactions between salinity and pH. By considering the total salt concentration (salinity) as a measure of the strength of salt stress and the pH as the strength of alkali stress, we identified significant synergistic effects of salt stress and alkali stress on some ecophysiological characteristics of *M. ruthenica* in the present study. Similar effects of mixed salt-alkaline stress have been found in sunflower and alfalfa (Shi and Sheng 2005, Peng *et al*. 2008). The synergistic effects of salt- and alkali stress are relatively harmful to plants; this may be due to osmotic and ion effects, in addition to high pH which together can lead to severe damage of plants. The osmosis effects and ion toxicity effects depend on salt concentration, while pH effects depend on buffering capacity, which in turn is closely related to both alkalinity and concentration of the applied salt. This implies that the higher alkalinity and salt concentration, the greater the buffering capacity; therefore it is more difficult for plants to acclimate.

Concluding remarks: *M. ruthenica* can tolerate the stress of high salt concentration when pH is low, and can survive in high-alkalinity and low-salt stress. However, the synergistic effects of high alkali- and salt concentrations resulted in the death of some or all seedlings. *M. ruthenica* had a similar recovery mechanism as alfalfa after suffering from damage along the salt concentration gradient, suggesting that *M. ruthenica* is tolerant to salt-alkali stress. Decreasing the salt concentration or pH based on the salt components in the soil may be helpful to abate damage from mixed salt-alkaline stress. Furthermore, it may be possible to restore vegetation by decreasing the pH. Nevertheless, the phenomenon of salinity enhancing harmful effects of high pH is complex. Resistance mechanisms of *M. ruthenica* to alkalinity stress remain to be elucidated and deserve further investigation.

References

Ashraf, M., Harris, P.J.C.: Potential biochemical indicators of salinity tolerance in plants. – Plant Sci. **166**: 3-16, 2004.

Balabaev, G.A.: Yellow lucernes of Siberia, *Medicago ruthenica* (L.) Lebd. and *M. platycarpos* (L.) Lebd. – Bull. App. Bot. Genet. Plant Breeding Service **7**: 13-123, 1934.

Bell, D.T., Wilkins, C.F., Van der Moezel, P.G., Ward, S.C.: Alkalinity tolerance of woody species used in bauxite waste rehabilitation, Western Australia. – Restor. Ecol. **1**: 51-58, 1993.

Ben Amor, N., Ben Hamed, K., Debez, A., Grignon, C., Abdelly, C.: Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. – Plant Sci. **168**: 889-899, 2005.

Briat, J.F.: Iron dynamics in plants. – In: Delseny, M. (ed.): Incorporating Advances in Plant Pathology. Advances in Botanical Research, Vol. **46**: Pp. 138-169. Academic Press, London 2007.

Campbell, S.A., Nishio, J.N.: Iron deficiency studies of sugar beet using an improved sodium bicarbonate-buffered hydroponics growth system. – J. Plant Nutr. **23**: 741-757, 2000.

Campbell, T.A., Bao, G., Xia, Z.L.: Agronomic evaluation of *Medicago ruthenica* collected in Inner Mongolia. – Crop Sci. **37**: 599-604, 1997.

Comas, L.H., Eissenstat, D.M., Lakso, A.N.: Assessing root death and root system dynamics in a study of grape canopy pruning. – New Phytol. **147**: 171-178, 2000.

Cramer, G.R., Läuchli, A., Epstein, E.: Effects of NaCl and $CaCl_2$ on ion activities in complex nutrient solutions and root growth of cotton. – Plant Physiol. **81**: 792-797, 1986.

de Lacerda, C.F., Cambraia, J., Oliva, M.A., Ruiz, H.A., Prisco, J.T.: Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. – Environ. Exp. Bot. **49**: 107-120, 2003.

Deng, W., Qiu, S.W., Liang, Z.W.: [Background of Regional Ecoenvironment in Daan Sodic Land Experiment Station of China.] – Science & Technology Press, Beijing 2006. [In Chin.]

Elmore, A.J., Manning, S.J., Mustard, J.F., Craine, J.M.: Decline in alkali meadow vegetation cover in California: the effects of groundwater extraction and drought. – J. Appl. Ecol. **43**: 770-779, 2006.

Elstner, E.F.: Oxygen activation and oxygen toxicity. – Annu. Rev. Plant Physiol. **33**: 73-96, 1982.

Ge, Y., Li, J.D.: [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, 1990. [In Chin.]

Ghoulam, C., Foursy, A., Fares, K.: 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, 2002.

Guo, L.Q., Shi, D.C., Wang, D.L.: The key physiological response to alkali stress by the alkali-resistant halophyte *Puccinellia tenuiflora* is the accumulation of large quantities of organic acids and into the rhyzosphere. – J. Agron. Crop Sci. **196**: 123-135, 2010.

Guo, R., Shi, L.X., Ding, X.M., Hu, Y.J., Tian, S.Y., Yan, D.F., Shao, S., Gao, Y., Liu, R., Yang, Y.F.: Effect of saline and alkaline stress on germination, seedling growth, and ion balance in wheat. – Agron. J. **102**: 1252-1260, 2010.

Hartung,W., Leport, L., Ratcliffe, R.G., Sauter, A., Duda, R., Turner, N.C.: Abscisic acid concentration, root pH and anatomy do not explain growth differences of chickpea (*Cicer arietinum* L.) and lupin (*Lupinus angustifolius* L.) on acid and alkaline soils. – Plant Soil **240**: 191-199, 2002.

Jeong, J., Guerinot, M.L.: Homing in on iron homeostasis in plants. – Trends Plant Sci. **14**: 280-285, 2009.

Jianaer, A., Yang, C.U., Shi, D.C., Wang, D.L.: [Physiological response of an alkali resistant halophyte *Kochia sieversiana* to salt and alkali stresses.] – Acta Bot. Borea. –Occident. Sin. **27**: 79-84, 2007. [In Chin.]

Jones, H.G., Jones, M.B.: Introduction: Some terminology and common mechanisms. – In: Jones, H,G., Flowers, T.J., Jones, M.B. (ed.): Plant Under Stress: Biochemistry, Physiology and Ecology and Their Application to Plant Improvement. Pp. 1-10. Cambridge Univ. Press, New York 1989.

Khan, M.A., Ungar, I.A., Showalter, A.M.: The effect of salinity on the growth, water status, and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.) Forssk. – J. Arid Environ. **45**: 73-84, 2000a.

Khan, M.A., Ungar, I.A., Showalter, A.M.: Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial halophyte, *Atriplex griffithii* var. *stocksii*. – Ann. Bot. **85**: 225-232, 2000b.

Kingsbury, R.W., Epstein, E., Pearcy, R.W.: Physiological responses to salinity in selected lines of wheat. – Plant Physiol. **74**: 417-423, 1984.

Koyro, H.W.: Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). – Environ. Exp. Bot. **56**: 136-146, 2006.

Larbi, A., Abadía, A., Abadía, J., Morales, F.: Down co-regulation of light absorption, photochemistry, and carboxylation in Fe deficient plants growing in different environments. – Photosynth. Res. **89**: 113-126, 2006.

Li, R., Shi, F., Fukuda, K.: Interactive effects of various salt and alkali stresses on growth, organic solutes, and cation accumulation in a halophyte *Spartina alterniflora* (Poaceae). – Environ. Exp. Bot. **68**: 66-74, 2010.

Lissner, J., Schierup, H.H., Comın, F.A., Astorga, V.: Effect of climate on the salt tolerance of two *Phragmites australis* populations. I. Growth, inorganic solutes, nitrogen relations and osmoregulation. – Aquat. Bot. **64**: 317-333, 1999.

Liu, J., Shi, D.C.: Photosynthesis, chlorophyll fluorescence, inorganic ion and organic acid accumulations of sunflower in responses to salt and salt-alkaline mixed stresses. – Photosynthetica **48**: 127-134, 2010.

Marcum, K.B.: Salinity tolerance mechanisms of grasses in the subfamily Chloridoideae. – Crop Sci. **39**: 1153-1160, 1999.

Mayer, A.M., Poljakoff-Mayber, A.: The Germination of Seeds. – Pergamon Press, Oxford 1963.

Munns, R.: Comparative physiology of salt and water stress. – Plant Cell Environ. **25**: 239-250, 2002.

Munns, R., Termaat, A.: Whole-plant responses to salinity. – Aust. J. Plant Physiol. **13**: 143-160, 1986.

Peng, Y.L., Gao, Z.W., Gao, Y., Liu, G.F., Sheng, L.X., Wang, D.L.: Eco-physiological characteristics of alfalfa seedlings in response to various mixed salt-alkaline stresses. – J. Integr. Plant Biol. **50**: 29-39, 2008.

Reddy, M.P., Vora, A.B.: Changes in pigment composition, Hill reaction activity and saccharides metabolism in bajra (*Pennisetum typhoides* S & H) leaves under NaCl salinity. – Photosynthetica **20**: 50-55, 1986.

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

Shi, D.C., Yin, L.J.: [Difference between salt (NaCl) and alkaline (Na_2CO_3) stresses on *Puccinellia tenuiflora* (Griseb.) Scribn. et Merr. plants.] – Acta Bot. Sin. **35**: 144-149, 1993. [In Chin.]

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

Shi, D.C., Zhao, K.F.: Effects of NaCl and Na_2CO_3 on growth of *Puccinellia tenuiflora* and on present state of mineral elements in nutrient solution. – Acta Pratacu. Sin. **6**: 51-61, 1997.

Shi, F.L.: [Study on drought resistance of *Medicago ruthenica* accessions.] – Chin. J. Grassl. **28**: 39-42, 2006. [In Chin.]

Short, D.C., Colmer, T.D.: Salt tolerance in the halophyte *Halosarcia pergranulata* subsp. *pergranulata*. – Ann. Bot. **83**: 207-213, 1999.

Small, E., Jomphe, M.: A synopsis of the genus *Medicago* (Leguminosae). – Can. J. Bot. **67**: 3260-3294, 1989.

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

Sultana, N., Ikeda, T., Itoh, R.: Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. – Environ. Exp. Bot. **42**: 211-220, 1999.

Tang, C., Turner, N.C.: The influence of alkalinity and water stress on the stomatal conductance, photosynthetic rate and growth of *Lupinus angustifolius* L. and *Lupinus pilosus* Murr. – Aust. J. Exp. Agric. **39**: 457-464, 1999.

Tanji, K.K.: Nature and extent of agricultural salinity. – In: Tanji, K.K. (ed.): Agricultural Salinity Assessment and Management. Pp. 1-18. Am. Soc. Civil Eng., New York 1990.

Wei, Y., Xu, X., Tao, H., Wang, P.: Growth performance and physiological response in the halophyte *Lycium barbarum* grown at salt-affected soil. – Ann. Appl. Biol. **149**: 263-269, 2006.

Yang, C.W., Chong, J.N., Kim, C.M., Li, C.Y., Shi, D.C., Wang, D.L.: 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, 2007.

Yang, C.W., Jianaer, A., Li, C.Y., Shi, D.C., Wang, D.L.: Comparison of the effects of salt-stress and alkali-stress on photosynthesis and energy storage of an alkali-resistant halophyte *Chloris virgata*. – Photosynthetica **46**: 273-278, 2008a.

Yang, C.W., Wang, P., Li, C.Y., Shi, D.C., Wang, D.L.: Comparison of effects of salt and alkali stresses on the growth

and photosynthesis of wheat. – Photosynthetica **46**: 107-114, 2008b.

Yang, C.-W., Xu, H.-H., Wang, L.-L., Liu, J., Shi, D.-C., Wang, D.-L.: Comparative effects of salt-stress and alkali-stress on the growth, photosynthesis, solute accumulation, and ion balance of barley plants. – Photosynthetica **47**: 79-86, 2009.

Zheng, G.Q., Xu, X., Xu, Y.Z., Liu, Z.L.: [The effect of salt stress on the stomatal and non-stomatal limitation of photosynthesis of *Lycium barbarum*.] – Acta Bot. Boreal-occident Sin. 22: 1355-1359, 2002. [In Chin.]

Zhu, G. L.: Carotenoid and chlorophyll determine. – In: Zhu, G.L. (ed.): Laboratory Manual of Plant Physiology. Pp. 51-54. Beijing Univ. Press, Beijing 1993.

Saakov, V.S., Drapkin, V.Z., Krivchenko, A.I., Serdyuk, A.S., Rozengart, E.V., Bogachev, Y.V., Knyazev, M.N.: [**Derivative Spectrophotometry and EPR Spectroscopy for Solving Ecological and Biological Problems**.] – Technical Literature, St. Petersburg Electrotechnical University, St Petersburg, 2010. ISBN: 978-5-7629-1057-6. 408 pp. Euro 15.00. [In Russ.]

This book written in Russian language by competent specialists deals with the practical application of derivative spectroscopic signals in biochemical, physiological, and environmental research with a physical chemistry orientation. It is a valuable methodical and multi-disciplinary approach presented in four chapters.

Chapter I describes the basics of derivative spectrophotometry and gives the characteristics and principles of the reliable acquisition of derivative spectra of high order (DSpHO), *e.g*. 2^{nd}, 4^{th}, 8^{th}, 12^{th} , or even 20^{th} order, and their qualitative and quantitative analysis in various fields of biology and pharmacy, and in analytical, nuclear and radio chemistry. The matter is that higher derivations of spectra provide a higher discriminatory and resolution power which allows *e.g*. to better identify compounds, to check their purity and to determine their real wavelength band positions.

Chapter II is centered on methods of derivative spectrophotometry for analysis of biologically active compounds. It describes the fine resolution of DSpHO of aromatic amino acids and various proteins. Especially the effect of thermal and γ-radiation on changes of the phenylalanine and tryptophan peaks in the 2^{nd} derivative spectra of proteins are outlined as well as radiation damages detectable in the 4^{th} and 8^{th} derivative of absorption spectra with particular emphasis on albumin and the radiolysis of γ-globulin which may be associated *e.g*. with serotonin accumulations. In addition, the use of DSpHOs in the binding of Ca^{2+} by mono-, bis- and tris-substituted guanidine derivates are described and various other similar Ca^{2+} interactions with particular organic compounds.

Chapter III presents the "Applicability of methods of DSpHO (derivative spectra of high orders) for analysis of pigments in plants and animals". Thus, absorption spectra and DspHOs of isolated and higly purified plastidic carotenoids from plant extracts such as violaxanthin, lutein and neoxanthin are presented in polar and nonpolar solvents. Special consideration is given to neoxanthin as common oxidation product of α- and ß-ionone carotenoids and its metabolism in plants and insect eyes. Examples are given for the application of DspHOs in the study of metabolic conversion and oxidation of ^{14}C or ^{3}H-labeled carotene to other carotenoids in living tissues and chromoplasts. Moreover, the use of DspHOs in the study of alternative biosynthesis pathways of carotenoids in prokaryota and eukaryota is demonstrated as well as the possibility of using α-ketoglutaric acid and malic acids as source for biosynthesis of carotenoids in C_3 and C_4 plants. This chapter also reports on attempts of applying the DspHO technique to study the coupling of the xanthophyll cycle transformations (de-epoxidation and epoxidation) with the photosynthetic electron transport chain. In addition, the DspHO method has been applied to study the effect of radiation on chlorophyllprotein complexes which opens possibilities for scanning terrestrial vegetation and ocean surfaces from aircrafts and satellites by means of reflectance spectra.

Chapter IV, in turn, introduces to Electron Spin Resonance (ESR) spectroscopy for solving of some scientific and practical problems in biology, medicine and ecology. It provides the theoretical and methodological aspects of ESR spectra and gives examples for application possibilities of the ESR method in biochemical and biomedical research for health care and environment protection. In addition, the authors describe a simple, rather inexpensive set of a compact, automated ESR equipment which possesses high technical capabilities. It has successfully been applied for the determination of the dose of accidental irradiation obtained by exposed victims. Based on the detection of paramagnetic ions of 3*d*-groups in water, the ESR method has been used for studying multi-quantum processes in reactions of photosynthesis and photosensibilisations.

This highly interesting book can be recommended to graduate and Ph.D. students and in particular to analytical scientists in biology, biochemistry, medicine, pharmacy, genetics and environmental research. The book may be achieved from the Library of the Sechenov Institute of Developmental Physiology and Biochemistry, Russian Academy of Sciences (Thorez Av. 44, 194223 St. Petersburg, Russia. A translation of this valuable book into English would considerably increase its distribution.

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