Comparative effects of salt-stress and alkali-stress on the growth, photosynthesis, solute accumulation, and ion balance of barley plants

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Abstract

We compared the effects of salt-stresses (SS, 1 : 1 molar ratio of NaCl to Na_2SO_4) and alkali-stresses (AS, 1 : 1 molar ratio of $NaHCO_3$ to Na_2CO_3) on the growth, photosynthesis, solute accumulation, and ion balance of barley seedlings, to elucidate the mechanism of AS (high-pH) damage to plants and the physiological adaptive mechanism of plants to AS. The effects of SS on the water content, root system activity, membrane permeability, and the content of photosynthetic pigments were much less than those of AS. However, AS damaged root function, photosynthetic pigments, and the membrane system, led to the severe reductions in water content, root system activity, content of photosynthetic pigments, and net photosynthetic rate, and a sharp increase in electrolyte leakage rate. Moreover, with salinity higher than 60 mM, Na^+ content increased slowly under SS and sharply under AS. This indicates that high-pH caused by AS might interfere with control of Na^+ uptake in roots and increase intracellular Na^+ to a toxic level, which may be the main cause of some damage emerging under higher AS. Under SS, barley accumulated organic acids, Cl^-, SO_4^{2-}, and NO_3^- to balance the massive influx of cations, the contribution of inorganic ions to ion balance was greater than that of organic acids. However, AS might inhibit absorptions of NO_3^- and Cl^-, enhance organic acid synthesis, and SO_4^{2-} absorption to maintain intracellular ion balance and stable pH.

Additional key words: *Hordeum*; intercellular CO_2 concentration; photosynthesis; relative growth rate; roots; shoot; solute accumulation; stomatal conductance; transpiration rate; water content.

Introduction

Agricultural productivity is severely affected by soil salinity. Natural salt-alkalinized soils are very complex, with Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, and NO_3^- as the main ions (Läuchli and Lüttge 2002). NaCl, Na_2SO_4, $NaHCO_3$, and Na_2CO_3 are the main harmful salts in many inland areas, such as in China (Ge and Li 1990, Kawanabe and Zhu 1991). Some reports have demonstrated that alkaline salts ($NaHCO_3$ and Na_2CO_3) are more destructive to plants than neutral salts (NaCl and Na_2SO_4) in various plant species (Shi and Yin 1993, Yang *et al*. 2007, 2008a,b). There has been considerable study of NaCl stress (SS), however, relatively little attention has been given to alkali-stress (AS) despite its importance. Even so, there have been reports on high-pH calcareous soils (Brand *et al*. 2002, Nuttall *et al*. 2003), alkaline soil (Hartung *et al*. 2002), AS (Shi and Yin 1993, El-Samad and Shaddad 1996, Campbell and Nishio 2000, Yang *et al*. 2007), and salt–alkaline mixed stress (Shi and Sheng 2005, Shi and Wang 2005). These reports demonstrate the existence of AS.

Soil salinization and alkalization frequently co-occur, with alkalization causing severe problems in some areas, such as northeast China (Kawanabe and Zhu 1991). Only a few alkali resistant plants can survive in these soils. SS in the soil generally involves osmotic stress and ion injury (Munns 2002), and there are similar problems for AS but with the added influence of high-pH, making AS difficult to investigate. The high-pH caused by AS may severely affect soil structure, interfere with ion uptake and intracellular ion balance in plant (Yang *et al*. 2007), inhibit growth (Shi and Yin 1993, Yang *et al*. 2007) and photosynthesis.

Barley (*Hordeum vulgare*) is an important crop, and some cultivars are tolerant to SS. We compared the

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Abbreviations: Car − carotenoids; Chl − chlorophyll; C_i − internal CO_2 concentration; DM − dry mass; E − transpiration rate; ELR − electrolyte leakage rate; FM − fresh mass; g_s − stomatal conductance; OA − organic acid; P_N − net photosynthetic rate; RGR − relative growth rate.
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effects of SS (1 : 1 molar ratio of NaCl to Na_2SO_4) and AS (1 : 1 molar ratio of $NaHCO_3$ to Na_2CO_3) on the growth, photosynthesis, solute accumulation, and ion balance of barley seedlings, to elucidate the mechanism of AS (high-pH) damage to plants, and the physiological adaptive mechanism of plants to AS.

Materials and methods

Design of simulated salt and alkali conditions: Two neutral salts were mixed in a 1 : 1 molar ratio (NaCl : Na_2SO_4) and applied to the SS group. Two alkaline salts were mixed in a 1 : 1 molar ratio ($NaHCO_3$: Na_2CO_3), and applied to the AS group. Within the SS group, five concentration treatments were applied: 40, 80, 120, 160, and 200 mM (labelled S1–S5). Within the AS group, five concentration treatments were applied: 20, 40, 60, 80, and 100 mM (labelled A1–A5). These treatment concentrations referred to the total salt concentrations of NaCl+Na_2SO_4 or $NaHCO_3$+Na_2CO_3. Therefore, in the SS solution of 200 mM, a mixture of 100 mM NaCl and 100 mM Na_2SO_4 would result in total ion concentrations of Na^+, Cl^-, and SO_4^{2-} of 300, 100, and 100 mM, respectively. The pH ranges in the SS and AS groups were 6.35–6.65 and 9.93–9.97, respectively.

Stress treatments: The seeds of *H. vulgare* L. cv. Ganpisanhao were germinated in Petri dishes for 2 d. The germinated seeds were sown in 17-cm diameter plastic pots containing 2.5 kg of washed sand. Each pot contained 15 seedlings, which were sufficiently watered with Hoagland nutrient solution daily. All pots were placed in an artificial greenhouse (24.0±1.5/19.0±1.5 °C, day/night). Plants grew in the uniform irradiance greenhouse at 15/9 h light/dark and photosynthetically active radiation (PAR) at 170 μmol m^{-2} s^{-1}. When the seedlings were 10 d-old (two leaves), 36 pots with uniform seedlings were selected and randomly divided into 12 sets, with 3 pots per set. One set was used as a control, a second set for growth index determination at the beginning of treatment, and the remaining ten sets received various stress treatments. Each pot was a single replicate with three replicates per set. Stress treatments were applied daily at 17:00–18:00 h with nutrient solutions containing the appropriate stress salts. Control plants were maintained by watering with nutrient solution. To avoid stress shock, stress salinities were increased gradually daily until the desired concentration was reached. After the highest concentration group reached the desired concentration, treatment continued for another 7 d. The duration of treatment was 12 d.

Physiological indexes: Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) of leaves were determined at 08:30–10:30 h on fully expanded third blades, using a portable open flow gas exchange system *LI-6400* (*LICOR*, USA). The PAR was 1 200 μmol m^{-2} s^{-1} (*i.e.* saturation). The ambient CO_2 concentration was set at 360 μmol mol^{-1}.The water content of shoot was calculated using dry masses (DM) and fresh masses (FM) (Yang *et al.* 2007). Relative growth rate (RGR) was determined as described in Kingsbury *et al.* (1984). Carotenoids (Car) and chlorophyll (Chl) *a* and *b* were extracted using acetone, and spectrophotometeric determination at 440, 645, and 663 nm of each sample was done three times (*SP-756*, spectral slit-width 2nm, Beijing, China). The calculation used the equations of Arnon (1949). The amount f Car was calculated as $4.7\ A_{440} - 0.27\times(20.2\ A_{645} + 8.02\ A_{663})$. One hundred mg of dry sample was taken to determine organic acid (OA) content by complexometry (Jing and Ding 1981). Dry samples of 100 mg of plant material were treated for 20 min with 20 cm^3 de-ionized water at 100 °C and the extract was used to determine free inorganic ion contents. A flame photometer was used to determine K^+ and Na^+ contents. The NO_3^-, SO_4^{2-}, and Cl^- contents were determined by ion chromatography (*DX-300* ion chromatographic system, *AS4A-SC* chromatographic column, *CDM-II* electrical conductivity detector, mobile phase: $Na_2CO_3/NaHCO_3$ = 1.7/1.8 mM, *DIONEX*, USA). The contents of proline and total soluble sugars were measured using ninhydrin and anthrone, respectively (Zhu *et al.* 1983). Membrane permeability can be reflected by the electrolyte leakage rate (ELR), which was determined with the ameliorated method of Lutts *et al.* (1996). One g of fresh leaf was taken from each pot and washed three times with de-ionized water to remove surface adhered electrolytes. The leaves were placed in a closed cuvette containing 20 cm^3 of de-ionized water. The cuvette was incubated at 25 °C on a rotary shaker for 4 h, and electrical conductivity of the solution (EC1) determined with a conductivity gauge (*DDSJ-308A*, Shanghai, China). After this the cuvette was autoclaved at 120 °C for 20 min and electrical conductivity of the solution (EC2) determined. ELR can be defined as follows: ELR [%] = (EC1/EC2)×100. The activity of the root system was determined as described by Comas *et al.* (2000). The fresh root was incubated for 60 min at 37 °C in triphenyl tetrazolium chloride (TTC) solution (0.04 % in pH 7.0 phosphate buffer). The red product in root was extracted using ethyl acetate. The absorbances were determined by spectrophotometer at 485 nm. The activity of the root system was expressed relative to the control value of 100 %. Fresh shoots were then crushed, tissue sap extruded, and the pH measured with a digital pH meter.

Statistical analyses of variance and correlation were performed using the statistical program *SPSS 13.0*. All treatments were repeated three times, and the means and standard errors (S.E.) reported, with significance tested at $p<0.05$.

Results

Growth: With increased salinity, the relative growth rate (RGR) and water content of barley decreased, with reduction under AS greater that under SS (p<0.01; Fig. 1*A,B*). Under SS, the root system activity was greater than that of controls except that of S5 (p<0.05; Fig. 1*C*). Under AS, the root system activity decreased sharply with increasing salinity (p<0.01; Fig. 1*C*). The ELR of barley increased with increased salinity under both stresses, and the increase under AS was greater than that under SS (p<0.01; Fig. 1*D*).

Photosynthetic pigments: SS did not inhibit accumulations of the three photosynthetic pigments (Table 1), but low SS stimulated their accumulation. At AS, the contents of Chl and Car all decreased sharply with increased stress (p<0.01; Table 1).

Gas exchange: The changes in P_N, E, and g_s of barley were similar (Fig. 1); all decreased with increased salinity under both stresses, with reductions under AS greater than under SS (p<0.01; Fig. 1). The effect of SS on C_i was not significant (Fig. 1*H*). The effect of low AS on C_i was also not significant (Fig. 1*H*); however, when salinity >60 mM, C_i increased sharply with increased salinity under AS (p<0.01; Fig. 1*H*).

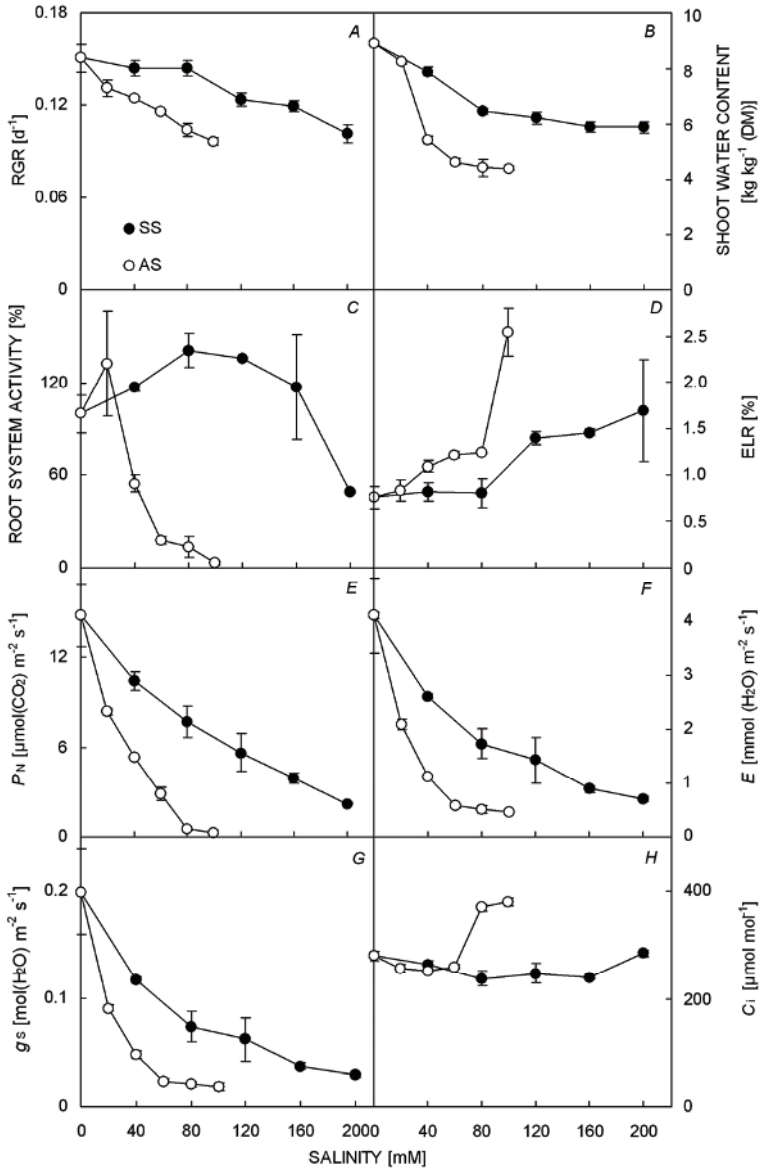

Fig. 1. Effects of salt-stress (SS) and alkali-stress (AS) on (*A*) relative growth rate (RGR), (*B*) shoot water content, (*C*) root system activity, (*D*) electrolyte leakage rate (ELR), (*E*) net photosynthetic rate (P_N), (*F*) transpiration rate (E), (*G*) stomatal conductance (g_s), and (*H*) intercellular CO_2 concentration (C_i) of barley seedlings. The 10-d-old seedlings were subjected to SS ($NaCl : Na_2SO_4$ = 1 : 1; pH 6.35–6.65) or AS ($NaHCO_3 : Na_2CO_3$ = 1 : 1; pH 9.93–9.97) for 12 d. Means (±S.E.) of three replicates.

Inorganic ion contents: The changing trends of Na^+ content and Na^+/K^+ were similar (Fig. 2). The Na^+ content and Na^+/K^+ increased with increased salinity under both stresses (p<0.01; Fig. 2*A,C*). At lower stress (salinity

<60 mM) the increments were similar for both stresses. When salinity was >60 mM, they slowly increased with increased salinity under SS, but increased sharply under AS (Fig. 2*A,C*). The effects of both stresses on the K^+ content of barley were similar. The K^+ content initially decreased gradually with increased salinity. When SS was >40 mM or AS >20 mM, there were no significant changes in K^+ content under both stresses (Fig. 2*B*). Under SS, the Cl^- content increased with increased Salinity (p<0.01; Fig. 2*D*). Under AS, the Cl^- contents were significantly lower than in control except for A1 (± S.E.) (Fig. 2*D*). With increased salinity, the NO_3^- contents decreased under both stresses (p<0.01; Fig. 2*E*), with greater reductions under AS than under SS. With increased salinity, the SO_4^{2-} contents increased, the increment under AS was greater that under SS (p<0.01; Fig. 2*F*).

Table 1. Effects of salt-stress (SS) and alkali-stress (AS) on contents of chlorophyll (Chl) *a*, *b*, and carotenoids (Car) [g kg^{-1}(FM)] in barley seedlings. The 10-d-old seedlings were subjected to SS ($NaCl : Na_2SO_4$ = 1 : 1; pH 6.35–6.65) or AS ($NaHCO_3 : Na_2CO_3$ = 1 : 1; pH 9.93–9.97) for 12 d. Means (± S.E.) of three replicates.

	Salinity [mM]	Chl *a*	Chl *b*	Car
SS	Control	1.534±0.058	0.407±0.017	0.322±0.012
	40	1.864±0.027	0.507±0.032	0.366±0.021
	80	1.870±0.076	0.519±0.026	0.389±0.0007
	120	1.645±0.075	0.417±0.009	0.269±0.010
	160	1.711±0.079	0.446±0.036	0.306±0.005
	200	1.668±0.123	0.423±0.016	0.267±0.035
AS	20	1.703±0.091	0.488±0.029	0.329±0.032
	40	0.653±0.070	0.192±0.022	0.128±0.018
	60	0.261±0.041	0.081±0.016	0.067±0.008
	80	0.111±0.013	0.033±0.006	0.055±0.011
	100	0.080±0.007	0.024±0.006	0.038±0.001

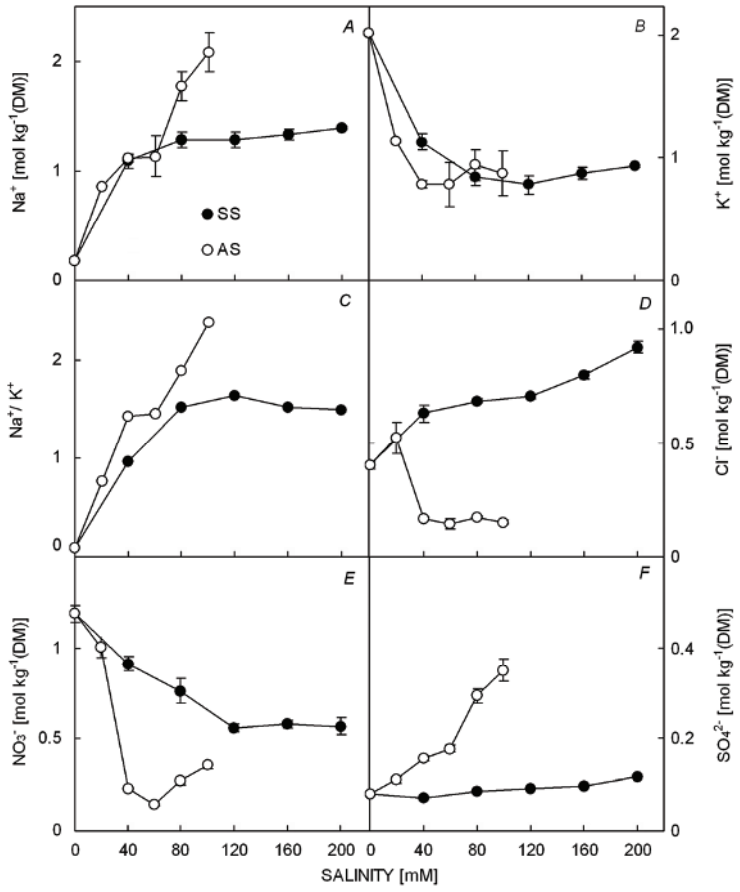

Fig. 2. Effects of salt-stress (SS) and alkali-stress (AS) on contents of (*A*) Na^+, (*B*) K^+, (*C*) Na^+/K^+, *D*) Cl^-, (*E*) NO_3^-, and (*F*) SO_4^{2-} in the shoots of barley. The 10-d-old seedlings were subjected to SS ($NaCl : Na_2SO_4$ = 1 : 1; pH 6.35–6.65) or AS ($NaHCO_3 : Na_2CO_3$ = 1 : 1; pH 9.93–9.97) for 12 d. Means (±S.E.) of three replicates.

Organic solute contents: The changing trends of OA and Na^+ contents were similar (p<0.01; Figs. 2*A* and 3*C*). Furthermore, at tissue pH >6.0, the OAs in plant cell were in the form of organic salts (Wang 2001, Yang *et al*. 2007). With increased salinity, the proline content increased; the increase under AS was greater than under SS (p<0.01; Fig. 3*B*). There was a negligible effect of SS on content of soluble sugars (Fig. 3*A*). Under AS, with increased salinity the content of soluble sugars initially increased gradually, and then declined when salinity was higher than 60 mM (p<0.01; Fig. 3*A*).

Tissue pH in barley shoots under both stresses was in close agreement with the control. The tissue pH value was 6.68 in the control and the mean tissue pH value at different stress intensities was 6.57 under SS and 6.63 under AS (Table 2). The stress intensity did not affect the tissue pH value (p>0.05).

Discussion

Growth: The RGR value reflects the life-sustaining activities of the plant, and is considered an optimum index for degrees of stress and plant responses to stresses. The RGR decrease under AS was greater than under SS (Fig. 1*A*). The injurious effect caused by AS was greater than that of SS at the same salinity, consistent with previous reports (Shi and Yin 1993, Yang *et al*. 2007). The different injurious effects of the two stresses may be due to their different mechanisms of action. The injurious effects of salinity are commonly thought to be a result of low water potentials and ion toxicities (Munns 2002). The AS exerts the same stress factors as SS but with the added influence of high-pH stress. The effects of SS on barley water content (Fig. 1*B*), root system activity (Fig. 1*C*), membrane permeability (Fig. 1*D*), and photosynthetic pigments (Table 1) were slight compared with AS. The AS induced severe reductions in water content (Fig. 1*B*), root system activity (Fig. 1*C*), and photosynthetic pigment contents, and a sharp increase in ELR. These results indicate that high-pH from AS might damage root structure and functions such as absorption of water (Fig. 1*B*) and ions (Fig. 2: Na^+, K^+, Cl^-, and NO_3^-), contents of photosynthetic pigments (Table 1), and the membrane system (Fig. 1*D*). These may be the main reasons explaining that RGR of barley under AS was less than under SS.

Table 2. Tissue pH values in the fresh shoots of barley under salt (SS) and alkali (AS) stresses. Means (± S.E.) of three replications.

	Salinity	Tissue pH
SS	0	6.68±0.01
	40	6.61±0.00
	80	6.58±0.02
	120	6.57±0.02
	160	6.55±0.08
	200	6.55±0.06
AS	0	6.68±0.01
	20	6.61±0.09
	40	6.59±0.03
	60	6.68±0.06
	80	6.64±0.07
	100	6.61±0.05

Photosynthesis: The reductions in g_s and E might be a response of plant to decreased water potential of environment (Sultana *et al*. 1999, Koyro 2006). Under both stresses, the environment water potential decreased with increased stress intensity. However, with increased salinity, the reductions of g_s and E under AS were greater than under SS (Fig. 1). This showed that changes in g_s and E might be not result from a response to decreased water potential of environment. The high-pH caused by AS may severely affect stomatal movement and gas exchange of barley leaves. The reductions of P_N under higher SS are generally considered a result of the reduction of intracellular CO_2 partial pressure caused by stomatal closure or non-stomatal factors (Bethke and Drew 1992). The non-stomatal factors mainly depend on the cumulative effects of leaf water and osmotic potential, biochemical constituents (Sultana *et al*. 1999), and

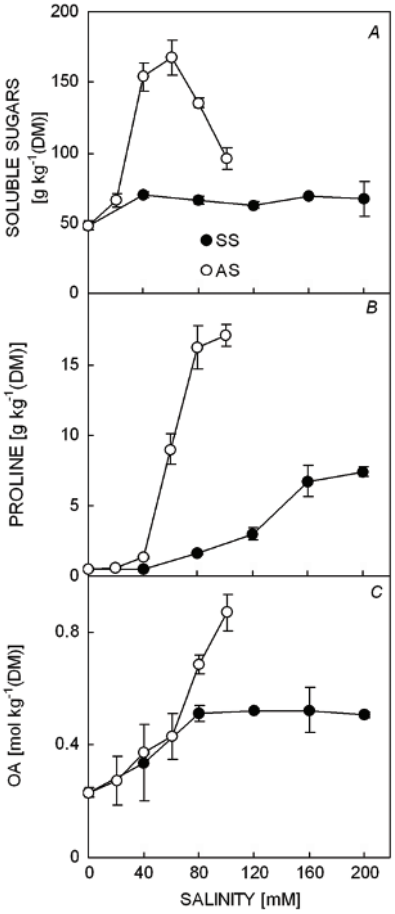

Fig. 3. Effects of salt-stress (SS) and alkali-stress (AS) on contents of (*A*) soluble sugars, (*B*) proline, and (*C*) organic acid (OA) in the shoots of barley. The 10-d-old seedlings were subjected to SS ($NaCl : Na_2SO_4$ = 1 : 1; pH 6.35–6.65) or AS ($NaHCO_3 : Na_2CO_3$ = 1 : 1; pH 9.93–9.97) for 12 d. Means (±S.E.) of three replicates.

Table 3. Percent contribution of various free anions to total negative charge in barley shoots under salt (SS) and alkali (AS) stresses. At tissue pH >6.0, the organic acids in plant cell were in the form of organic salts.

	Salinity [mM]	Cl^- [%]	NO_3^- [%]	SO_4^{2-} [%]	Organic acids [%]
	Control	18.22	53.76	7.19	20.84
SS	40	26.65	38.70	6.13	28.51
	80	25.90	28.82	6.61	38.67
	120	28.25	22.56	7.45	41.74
	160	30.47	22.21	7.41	39.92
	200	33.64	20.83	8.49	37.03
	Average	27.19	31.15	7.21	34.45
AS	20	22.74	43.73	9.69	23.83
	40	11.29	15.79	21.64	51.28
	60	9.52	9.72	23.76	57.00
	80	7.17	11.33	24.53	56.97
	100	5.14	12.24	23.77	58.85
	Average	11.17	18.56	20.68	49.59

contents of photosynthetic pigments (Ma *et al*. 1997, Koyro 2006). With increased salinity (Fig. 1), the reductions in P_N and g_s under AS were greater than under SS. The effect of SS on C_i was minor, but high AS greatly increased barley C_i (Fig. 1*H*), indicating that high-pH might affect CO_2 assimilation ability of leaf cells. These phenomena indicated that the visible reduction of P_N in barley under AS was related not only to damage to photosynthetic pigments (Table 1) and decreased g_s (Fig. 1*G*), but also to ion toxicities in the cytosol, *etc*. (Fig. 2).

Ion toxicity and compatible solutes: Plants in saline conditions usually accumulate inorganic ions in vacuoles to decrease cell water potential, as energy consumption from absorbing inorganic ions is far less than from synthesizing organic compounds. If excessive amounts of ions enter the plant, they can rise to toxic levels and inhibit photosynthesis and reduce growth rate (Munns 2002). Na^+ is the main poisonous ion in salinized soil. Low Na^+ and high K^+ in the cytoplasm are essential to maintain a number of enzymatic processes (James *et al*. 2006). At lower stress intensity, the effects of both stresses on the Na^+ content and Na^+/K^+ of barley were similar. But when salinity was >60 mM, with increased salinity Na^+ content and Na^+/K^+ increased slowly under SS, but sharply under AS (Fig. 2*A,C*). This implied that the high-pH of AS might interfere with control of Na^+ uptake in the roots and increase intracellular Na^+ to a toxic level. This may explain some damage that emerged under higher AS (Table 1, Figs. 1*D* and 2*A*). James *et al*. (2006) also reported that photosynthetic capacity was related to the cellular and subcellular partitioning of Na^+, K^+, and Cl^-. Moreover, the high pH leads to the H^+ deficit outside root and may limit the Na^+ extrusion from the root cytosol to the external environment. This may be one of the causes why the injurious effect caused by AS was greater than those of SS. However, the behaviour of barley was significantly different from that of *Kochia sieversiana,* a naturally alkali-resistant halophyte (Yang *et al*. 2007). The effects of both stresses on Na^+–K^+ selective absorption and other strain responses of *K. sieversiana* were similar. This indicates that high-pH surrounding the roots may be resisted by *K. sieversiana* root cells, and prevented from invading the intracellular environment. Therefore, we propose that pH adjustment of the roots may be a key physiological mechanism for plant resistance to AS. The process of pH adjustment might occur outside the root or in the root apoplast, or both. Therefore, the type of cells involved in pH adjustment may be epidermal, cortical, or xylem parenchyma cells. The mechanism of pH adjustment might be exudation of a buffer compound, such as H^+, OAs, amino acids, or CO_2 produced by root respiration, or by some other factors.

At the same time as inorganic ions accumulate in vacuoles, plants can also synthesize in the cytoplasm compatible low molecular mass organic solutes, such as betaine, proline, free sugars, and polyalcohols to prevent dehydration and protect biomacromolecules (Parida and Das 2005). Accumulation of proline in barley may be a response to Na^+ influx. The proline may distribute in the cytoplasm to balance the osmotic pressure from vacuoles and protect biomacromolecules (Fig. 3*B*). The role of soluble sugars might be significantly different from proline, during adapting of barley to salt and alkali conditions (Fig. 3). Under SS, increased salinity did not induce accumulation of soluble subars (Fig. 3*A*). Under AS, with increased salinity the content of soluble sugars initially increased gradually and then declined at >60 mM salinity (Fig. 3*A*). We propose that the decreased content of soluble sugars after 60 mM was not a response to osmotic stress or ion toxicity, and that the high-pH might result from abnormal metabolism caused by intercellular ion imbalance from damage to root function by high AS.

Ion balance: A stable tissue pH, as a result of intracellular ion balance, is necessary for plants to maintain normal metabolism (Yang *et al*. 2007). In a living plant, as long as the plant can adapt to the environment, the pH value in its tissue should be stable regardless of how the environmental pH value changes. The observations that the tissue pH in barley shoots under both stresses was similar to control and the stress intensity did not have any effect on tissue pH suggested that barley was able to maintain the ionic balance and stable pH in cells (Table 2), not only under SS, but also under AS even at pH >9.9. Ionic imbalance in plants is mainly caused by the influx of superfluous Na^+ (Munns 2002, Parida and Das 2005, Yang *et al*. 2007, Munns and Tester 2008). Plants usually accumulate inorganic anions, such as $Cl^–$, $NO_3^–$, and $SO_4^{2–}$, or synthesize organic anions to maintain ionic balance (Yang *et al*. 2007).

As shown in Table 3, different anions differed in their contributions to the overall negative charge and the contribution changed with stress intensity. Under SS, the percentages of various anions contributing to the total negative charge ranged from high to low as follows: OA, NO_3, $Cl^–$, $SO_4^{2–}$, but under AS the order was OA, $SO_4^{2–}$, $NO_3^–$, $Cl^–$. Moreover, the percent contributions of OA and $SO_4^{2–}$ to total negative charge were significantly higher under AS than under SS (Table 3). The percent contributions of $NO_3^–$ under AS and $Cl^–$ to total negative charge under SS were significantly higher than under AS (Table 3). In addition, the effects of stress intensity on anion contributions varied for different anions and under different type of stresses. With rising salinity, SS enhanced the contribution of $Cl^–$ and weakened the contribution of $NO_3^–$, however, AS enhanced the contributions of OA and $SO_4^{2–}$ and weakened the contributions of NO_3^- and $Cl^–$. In summary, under both stresses, barley accumulated OA, $Cl^–$, $SO_4^{2–}$, NO_3', and other inorganic anions to balance the massive influx of cations. Under SS, the contribution of inorganic ions was greater than that of OA (Table 3), while AS might damage root (Fig. 1*C*) and inhibit uptake of $NO_3^–$ and $Cl^–$ (Fig. 2). In such circumstances, barley might enhance OA synthesis and $SO_4^{2–}$ absorption to maintain intracellular ion balance and stable pH (Figs. 2*F* and 3*C*).

In summary, in barley we elucidated the damage mechanism of AS and some physiological adaptive mechanisms to it. We propose that enhancing pH adjustment capacity of roots might be effective in enhancing the alkali-tolerance of crops, and should be an important research direction of plant AS physiology in the future.

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