

Effects of buffer capacity on growth, photosynthesis, and solute accumulation of a glycophyte (wheat) and a halophyte (*Chloris virgata*)

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Abstract

Two species with different resistances to alkaline pH, the glycophytic *Triticum aestivum* (wheat) and the halophilic *Chloris virgata*, were chosen as test organisms. The salt-alkaline (SA) mixed stress conditions with different buffer capacities (BC) but with the same salt molarities and pH were established by mixing neutral (NaCl, Na₂SO₄), and alkaline salts (NaHCO₃ and Na₂CO₃) in various proportions. Growth, photosynthetic characteristics, and solute accumulation of the seedlings were monitored to test the validity of BC as a decisive index of alkali-stress (AS) intensity in SA mixed stress. At the same salinities and pHs, the relative growth rate, the content of photosynthetic pigments, and net photosynthetic rates of wheat and *C. virgata* decreased, while Na⁺ content and Na⁺/K⁺ ratios in shoots increased with increasing BC. Hence BC was a true measure of AS intensity at mixed SA stress and the alkali-resistance mechanism of plants was easy to interpret. BC of soil solution is an important parameter for estimating the alkalization degree of salt-alkalized soil.

Additional key words: alkali-stress; carotenoids; *Chloris virgata*; chlorophyll; K⁺; Na⁺; organic acids; proline; salt stress; stomatal conductance; transpiration rate; wheat.

Introduction

NaCl, Na₂SO₄, NaHCO₃, and Na₂CO₃ are the main harmful salts in many inland areas, such as in China (Ge and Li 1990, Kawanabe and Zhu 1991). Adaptation to salinity differs even in ecotypes (*Cakile maritima*; Megdiche *et al.* 2008). Alkaline salts (NaHCO₃ and Na₂CO₃) are more destructive to plants than neutral salts (NaCl and Na₂SO₄) (Shi and Yin 1993, Yang *et al.* 2007, 2008b). In spite of considerable study of NaCl stress, two very important points have been neglected, that is, alkali-stress (AS) and salt-alkaline (SA) mixed stress. Soil salinization and alkalization frequently co-occur, and cause severe problems in some areas. For example, in northeast China, salt-alkalinized grassland covers >70 % of land area, and is expanding (Kawanabe and Zhu 1991). Natural salt-stresses are mostly mixed-salt stresses, and most contain both neutral and alkaline salts. The total composition of salts and the proportion of neutral to alkaline salts may vary in different soils. Thus, the

stresses imposed by these soil media on plants could be very complex and difficult to approach experimentally, and no scientific index has yet been developed for measuring AS intensity in salt-alkalinized soils. However, our previous reports have demonstrated that buffer capacity (the amount of H⁺ titrated as HCl needed to lower pH of salt-containing treatment solution to the same pH as the nutrient solution) and salinity were dominant factors in SA stress (Shi and Sheng 2005, Shi and Wang 2005). An experiment specially aimed at buffer capacity (BC) has not yet been done. Therefore, stress treatments with different BC but the same salinity and pH were performed. We expected that the BC would be a decisive index of AS intensity in SA mixed stress. Two species with different alkali tolerances, the glycophyte wheat and the halophyte *Chloris virgata* (cf. Yang *et al.* 2008a), were compared.

Received 4 June 2008, accepted 14 October 2008.

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Abbreviations: AS – alkali-stress; BC – buffer capacity; DM – dry mass; *E* – transpiration rate; FM – fresh mass; *g_s* – stomatal conductance; OA – organic acid(s); *P_N* – net photosynthetic rate; RGR – relative growth rate; SA – salt-alkaline stress; SS – salt stress.

Acknowledgments: This work was supported by grants from the National Natural Science Foundation of China (No. 30671491 and No. 30571318) and the National Key Basic Research Program of China (No. 2007CB106800).

Materials and methods

The SA stress conditions with the same salinity and pH, but different BC, were established by mixing NaCl, Na₂SO₄, NaHCO₃, and Na₂CO₃ in various proportions (Table 1). The BC was defined (Shi and Sheng 2005) by titration with HCl as the amount of H⁺ needed to lower pH of 1 000 cm³ of salt-containing treatment solution to the same pH as the nutrient solution. For wheat, six

treatments (labelled W0–W5) were utilized (Table 1), the salinities were all 100 mM, the pH was 9.30–9.62, and the BC was 16–98 mmol H⁺. For *C. virgata*, seven treatments (labelled C0–C6) were utilized (Table 1), with all salinities being 250 mM, pH 9.57–9.70, and the BC 44–316 mmol H⁺.

Table 1. The concentrations [mM] and molar proportions of various salts (NaCl, Na₂SO₄, NaHCO₃, and Na₂CO₃) in six treatments for wheat and seven treatments for *C. virgata*. Values for salinity were 100 mM (wheat) and 250 (*C. virgata*), for Na⁺ content 150 mM (wheat) or 375 mM (*C. virgata*).

.Plant	Treatment	NaCl	Na ₂ SO ₄	NaHCO ₃	Na ₂ CO ₃	pH	BC
Wheat	W0	50.00	50.00	0	0	6.51	0
	W1	42.50	42.50	7.50	7.50	9.30	16
	W2	35.00	35.00	15.00	15.00	9.55	35
	W3	27.50	27.50	22.50	22.50	9.60	56
	W4	20.00	20.00	30.00	30.00	9.62	75
	W5	12.50	12.50	37.50	37.50	9.60	98
<i>C. virgata</i>	C0	125.00	125.00	0	0	6.52	0
	C1	106.25	106.25	18.75	18.75	9.57	44
	C2	87.50	87.50	37.50	37.50	9.70	98
	C3	68.75	68.75	56.25	56.25	9.63	153
	C4	50.00	50.00	75.00	75.00	9.64	209
	C5	31.25	31.25	93.75	93.75	9.67	258
	C6	12.50	12.50	112.50	112.50	9.67	316

Seeds of wheat (*Triticum aestivum*) cv. Xiaobingmai 33 were provided by the Institute of Genetics and Cytology of Northeast Normal University, China, seeds of *C. virgata* were collected from native grassland in Changling County, Jilin Province, China. Seeds were sown in 17-cm diameter plastic pots containing 2.5 kg of washed sand. Each pot contained 10 seedlings which were sufficiently watered with Hoagland nutrient solution every 2 d. Evaporation was compensated by distilled water at other times. All pots were placed outdoors and sheltered from rain. Temperatures during the experiment were 22–26/ 19–22 °C day/night. The nutrient solution used in this work contained 5.00 mM Ca(NO₃)₂, 5.00 mM KNO₃, 2.00 mM MgSO₄, 1.00 mM KH₂PO₄, 0.22 mM FeSO₄, 0.22 mM EDTA-Na₂, 6.72 µM MnSO₄, 3.16 µM CuSO₄, 7.65 µM ZnSO₄, 46.3 µM H₃BO₃, and 5.56 µM H₂MoO₄ (pH, 6.47).

Stress treatments were applied to 6-week-old seedlings of *C. virgata* seedlings, and to 2-week-old wheat seedlings. The pots with uniformly growing seedlings were randomly divided, three pots per set. Each pot was considered a single replicate; therefore there were three replicates per set (treatment). One set was used as an untreated control (C). Another set was used for determining dry (DM) and fresh (FM) masses at the beginning of each treatment. Stress treatments were performed once each day around 17:00–18:00 by the application of nutrient

solutions. All pots were watered thoroughly with 500 cm³ of treatment solution applied in three portions. C-plants were watered with the nutrient solution. The entire treatment duration was 10 d.

Net photosynthetic (P_N) and transpiration (E) rates and stomatal conductance (g_s) were determined at 08:30–10:30, from fully expanded third blades, using a portable open flow gas exchange system LI-6400 (LI-COR, Lincoln, USA). The photosynthetically active radiation (PAR) was 1 200 µmol m⁻² s⁻¹. The ambient CO₂ concentration was set at 360 µmol mol⁻¹. All plants were harvested in the morning after the final treatment. The plants were first washed with tap water, and then distilled water. Roots and shoots were separated and FM was determined for each pot. A portion of the fresh samples was taken to measure the amounts of photosynthetic pigments. To compute relative growth rate (RGR), the FM of fresh samples were determined and converted to dry masses (DM) according to the water content of the remaining fresh sample. Then the DMs were recorded. The remainder of the samples was oven-dried at 80 °C for 15 min, then vacuum-dried at 40 °C to constant mass, and the DM were recorded. All dry samples of shoots in each pot were comminuted to determine the contents of solutes. RGR was determined, using the formula of Kingsbury *et al.* (1984): $RGR = (\ln DM \text{ at end of treatment} - \ln DM \text{ at start of treatment}) / \text{duration of treatment}$, the DM

values at end of treatment were the sums of all the material in a pot.

Carotenoids (Car), chlorophyll (Chl) *a* and *b* were extracted with acetone, spectrophotometric determination at 440, 645 and 663 nm of each sample was done three times (SP-756, spectral slit-width 2nm, Beijing, China, and the amounts were calculated using the equations of Arnon (1949). One hundred mg of dry shoot samples were taken to determine organic acid (OA) content by complexometry (Jing and Ding 1981). A flame photo-

Results

Wheat seedlings of W5 treatment all died by the end of the treatment. SS (0 mmol H⁺ BC) and increased BC both obviously decreased RGR in wheat and *C. virgata* (Fig. 1A,B; $p < 0.05$). The effects of SS on the contents of photosynthetic pigments in wheat and *C. virgata* were small, but decreased with increased BC (Fig. 1C–H; $p < 0.05$). The SS and increased BC both obviously decreased P_N in wheat and *C. virgata* (Fig. 1I–J; $p < 0.05$). The g_s and E of salt-stressed plants were lower than those in C (without SS) for both species (Fig. 1K–N). Increased BC decreased g_s and E in both wheat and *C. virgata*.

The Na⁺ content and Na⁺/K⁺ ratio of SS plants were

meter was used for the measurement of K⁺ and Na⁺ (Wang and Zhao 1995). The proline content was measured using the ninhydrin method (Zhu *et al.* 1983).

Statistical analyses of variance and correlation were performed using the statistical program SPSS 13.0. All of the treatments were repeated three times, and the means and calculated standard errors (S.E.) are reported. The treatment mean values were compared by post hoc least significant difference (LSD) test at the 5 % level.

both higher than those in C plants for both species, and both increased significantly with increased BC (Fig. 2A,B,E,F; $p < 0.05$). The K⁺ contents of SS plants in both species were lower than those in C (Fig. 2C,D) and remained relatively unchanged with increased BC. The proline contents of SS plants were significantly higher than those in C-plants of wheat (Fig. 2G,H). With increased BC, the proline content in *C. virgata* was unchanged, but in wheat it first increased and then remained unchanged when BC > 16 mmol H⁺. OA contents in both species increased with increased BC (Fig. 2I,J; $p < 0.05$).

Discussion

The stress in a natural salt-alkalized soil is actually SA mixed stress. SS mainly causes water deficiency and ion toxicities (Munns 2002, Parida and Das 2005). AS is induced by the same stress factors as SS, but the influence of high pH stress is added. The high pH environment surrounding the roots can directly cause some ions (e.g. Ca²⁺, Mg²⁺, and others) to precipitate (Shi and Zhao 1997). The high pH may also lead to a lack of protons, the destruction or inhibition of trans-membrane electrochemical-potential gradients in root cells, and the loss of normal physiological root functions such as absorption of ions and water (Yang *et al.* 2007, 2008b). However, mixed SA stress is more complex than SS or AS. Under SA stress, plant survival depends not only on the ability to cope with water stress and ion toxicity, but also on tolerance of high pH.

The salt concentration (or salt content) or conductance are commonly used as intensity indexes of SS, and pH as the intensity index of AS, but the latter may be inappropriate in some cases. Considering that it is essential for plants to resist high pH of AS by plant pH adjustment, and the critical difficulty of pH adjustment is BC and not pH itself, we suggest that BC may be an optimum index of AS intensity in SA mixed stress.

As expected, BC is an optimum index of AS intensity. At the same salinity and pH (Table 2), the RGR, P_N , and photosynthetic pigment contents all decreased with increased BC in wheat and *C. virgata*. The reduction of plant P_N under high SS may be due to reduced intra-

cellular CO₂ partial pressure caused by stomatal closure, or to non-stomatal factors. The non-stomatal factors mainly depend on the combined effects of leaf water and osmotic potentials, biochemical constituents, contents of photosynthetic pigments (Yang *et al.* 2008a,b), etc. We found that P_N decreased with increased BC at the same salinity and pH in both species. This indicated that BC was a dominant factor inhibiting photosynthesis. The visible reduction of the P_N of wheat and *C. virgata* was related not only to the destruction of photosynthetic pigments and decreased g_s , but also to the imbalance in intracellular Na⁺/K⁺ due to high BC.

Low Na⁺/K⁺ ratio in the cytoplasm is essential for the maintenance of a number of enzymatic processes (James *et al.* 2006). Under SS and AS, plants usually accumulate large amounts of Na⁺ in vacuoles for osmoregulation (Munns 2002, Parida and Das 2005). Na⁺ enters plant cells principally through K⁺ pathways (Blumwald 2000). We found that at the same Na⁺ concentration and pH, the Na⁺ contents and Na⁺/K⁺ ratio in shoots of *C. virgata* and wheat both increased significantly with increasing BC. This indicated that high pH in SA mixed stress might interfere with the control of Na⁺ uptake in *C. virgata* and wheat roots. This was the cause of the increase of intracellular Na⁺ to a toxic level, possibly the main reason for some damage such as inhibition of P_N and decreased pigment contents, g_s , and E ; while BC could enhance injury to roots from high pH. The decrease of g_s might be closely correlated with the K⁺ transport inhibited by high

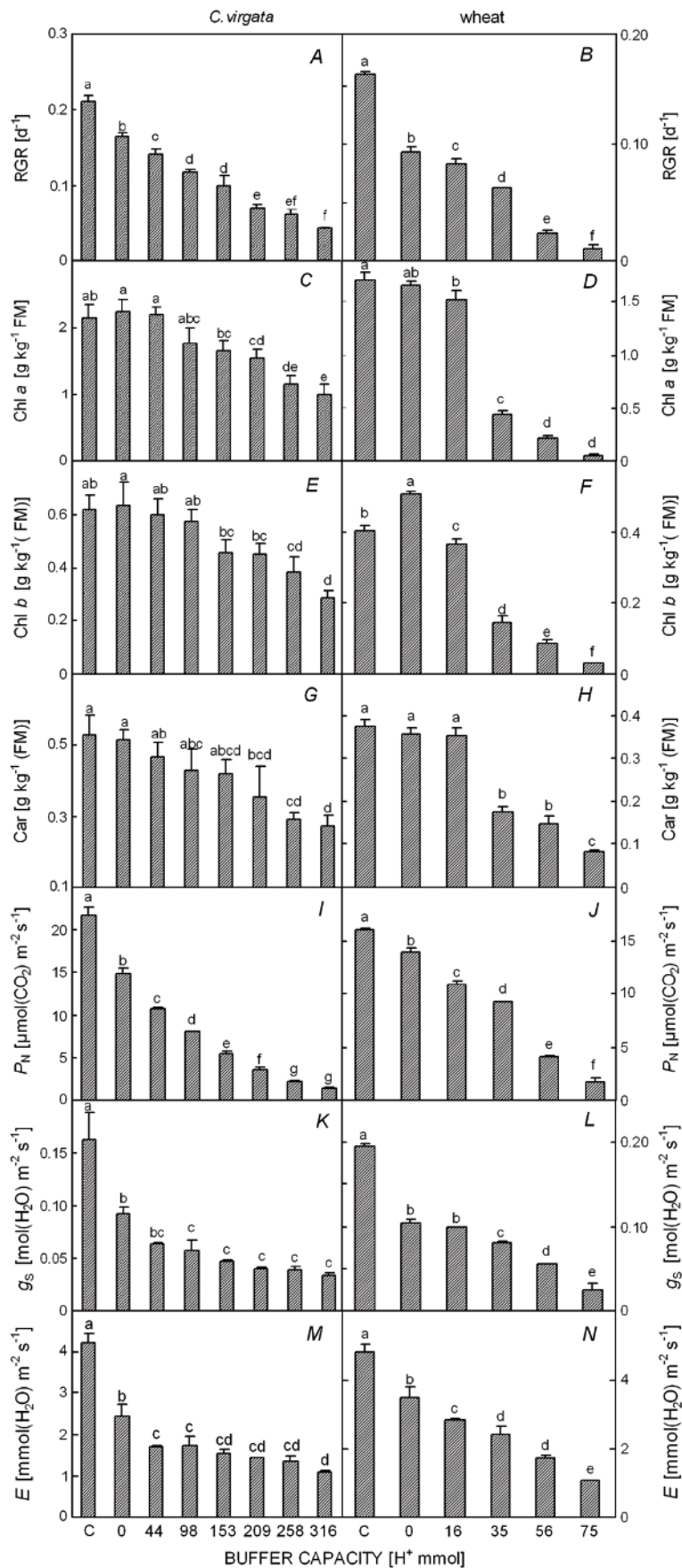


Fig. 1. Effects of buffer capacity on (A, B) relative growth rate (RGR), (C–H) contents of chlorophyll (Chl) a and b and carotenoids (Car), (I, J) net photosynthetic rate (P_N), (K, L) stomatal conductance (g_s), and (M, N) transpiration rate (E) in wheat and *C. virgata* seedlings. Means ($\pm$ S.E.) of three replicates. Different letters represent significant differences among treatments, at the 5 % level, according to least significant difference (LSD) test. Buffer capacity: defined as the amount of H^+ needed to reduce pH of 1 000 cm³ of salt-containing treatment solution to the same pH as the nutrient solution by titration with HCl; C: control treatment (without salt-stress).

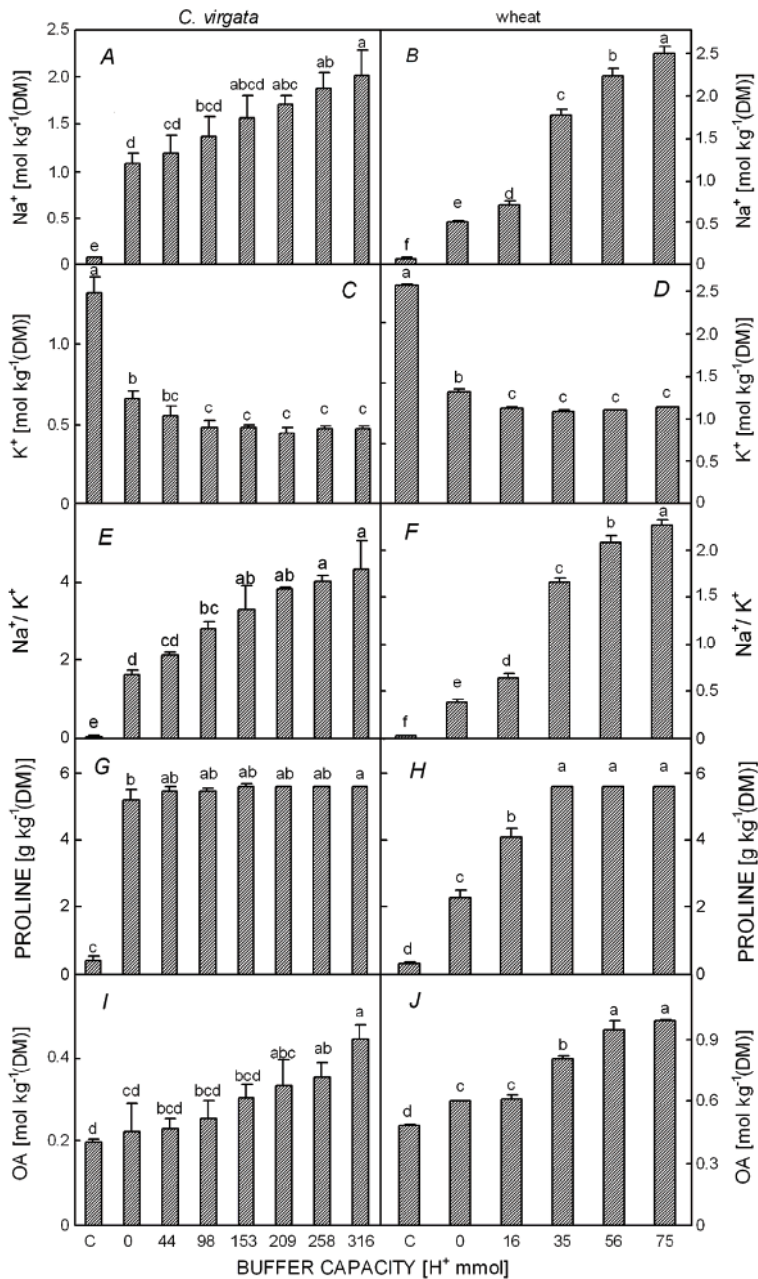


Fig. 2. Effects of buffer capacity on contents of (A, B) Na^+ and (C, D) K^+ , (E, F) Na^+/K^+ ratio, and contents of (G, H) proline and (I, J) organic acids (OA) in wheat and *C. virgata* seedlings. Means ($\pm$ S.E.) of three replicates. Different letters represent significant differences among treatments at the 5 % level, according to least significant difference (LSD) test. Buffer capacity: defined as the amount of H^+ needed to reduce pH of 1 000 cm^3 of salt-containing treatment solution to the same pH as the nutrient solution by titration with HCl; C: control treatment (without salt-stress).

Na^+ content in guard cells, or might be induced by physical or chemical signals from roots stimulated by AS. However, at the same salinity and pH, BC did not affect K^+ content. The K^+ uptake may only depend on the Na^+/K^+ ratio in the treatment solution, and not be related to high pH or BC; this should be researched in depth. Moreover, the OA contents of *C. virgata* and wheat both increased significantly with increased BC, indicating that high pH in SA mixed stress might interfere with OA accumulation. *C. virgata* and wheat might enhance OA synthesis to balance Na^+ excess and maintain intracellular pH homeostasis (Yang *et al.* 2007). The synthesized OA might also be transported to roots for pH regulation.

The accumulation of proline is generally considered

a response to osmotic stress induced by salt concentration. The accumulated proline may distribute in the cytoplasm to balance the osmotic pressure from vacuoles and to protect biomacromolecules. Under AS, proline accumulation depends on the alkali-resistant traits of plant (Shi and Sheng 2005, Shi and Wang 2005, Yang *et al.* 2007). In highly alkali-resistant *C. virgata*, proline accumulation was correlated with salinity, and not with BC. For less alkali-resistant wheat, proline accumulation was correlated with salinity and also with BC. This supports our previous conclusion that the pH adjustment outside roots was the key mechanism of plants that resist AS. This experiment demonstrated that at low BC, the stress effect of high pH on the plants was small. The

greater the BC, the more significant was the effect of high pH within the SA mixed stress. BC could enhance high-pH injury and was an optimum index for measuring AS intensity in SA mixed stress. We propose that pH alone cannot reflect the AS quality of salt-alkalized soil, and that the BC of soil solution is an important parameter for such estimation. This is a valuable new criterion that could aid research in ecology, ecophysiology, and agronomy.

Although high pH is an important stress factor of salt-alkalized soil, the pH does not reflect the intensity of stress on plants, and BC is essentially a measure of action of high pH on plant. BC is closely correlated with the mechanism of plant resistance to AS. The pH adjustment outside the roots is a key physiological mechanism for plants resisting AS (Yang *et al.* 2007, 2008b). By the pH adjustment of plants, a high pH accompanied by low BC is easily lowered, but that with high BC is more difficult to lower. Our experiment also showed that *C. virgata* as an alkali-resistant halophyte can grow well under high

BC (316 mmol H⁺) and high pH (pH 9.67), while wheat seedlings all died at low BC (98 mmol H⁺) and pH 9.60. Therefore, we propose that the alkali-resistance of plants depends on the pH adjustment ability of their roots. The mechanism of pH adjustment might be the exudation of a buffer compound, such as H⁺, OA, or amino acids, or CO₂ produced by root respiration (Yang *et al.* 2008b). To adapt to SA stress, plants need to expend more material and energy than adaptation to SS because of the additional pH adjustment required. Under high pH and low BC, the high pH was easily adjusted by plants, which grew well because of the low demand for material and energy. Contrarily, under AS with high BC, plants expend more material and energy and are also more damaged by high pH, because of little effective pH adjustment. The decreased RGR in wheat and *C. virgata* with increased BC might be attributed to increased energy demand for pH adjustment.

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