# Comparative effects of two alkali stresses, Na$_2$CO$_3$ and NaHCO$_3$ on cell ionic balance, osmotic adjustment, pH, photosynthetic pigments and growth in oat (*Avena sativa* L.)

## Zhan-Wu Gao[1], Ji-Tao Zhang[2], Zhuang Liu[3], Qing-Tao Xu[1], Xiu-Jun Li[2] and Chun-Sheng Mu[4*]

[1]Baicheng Normal University, Department of Geography, Baicheng 137000, China
[2]Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun 130012, China
[3]Baicheng City Academy of Agriculture Sciences, Ji Lin Province Research Institute of Sunflower, 137000, China
[4]Institute of Grassland Science, Northeast Normal University, Key Laboratory of Vegetation Ecology of Ministry of Education, Changchun 130024, China

*Corresponding author: mucs821@yahoo.com.cn

## Abstract

This study examines the comparative effects of NaHCO$_3$ and Na$_2$CO$_3$ on young oat (*Avena sativa* L.) plants to elucidate the species' physiological adaptive mechanisms to alkali stress. Factors considered are the intracellular influx and efflux of ions, ionic balance, osmotic adjustment, pH homeostasis, photosynthetic pigments and growth. Results show that, Na$_2$CO$_3$ had stronger effects than NaHCO$_3$, and that with increasing concentrations of both stresses the plant showed rising Na$^+$ influxes into the shoot resulting in Na$^+$ ion toxicity. This is tolerated by Na$^+$ sequestration in the vacuole; the accumulation mainly of Cl$^-$, SO$_4$$^{2-}$ and the synthesis of high concentrations of organic anions to maintain vacuolar ionic balance and, lastly by the synthesis of proline in the cytoplasm to avoid dehydration. Moreover, Na$_2$CO$_3$ stress inhibits growth more strongly, compared to NaHCO$_3$, because of the higher energy costs associated with Na$^+$ exclusion and compartmentalisation，the syntheses of organic anions, the syntheses of proline in the cytoplasm, reduced photosynthetic capacity and increased membrane permeability. Compared to the shoot, although the root had a similar response to both stresses, it showed a higher tolerance because high Na$_2$CO$_3$ stresses (>48 mmol L$^{-1}$) resulted in significant increases in root tissue pH, but did not affect the pH homeostasis of the shoot. Additionally, while both stresses decreased root dry weight, they did not significantly affect root extension growth. This indicates that oat adopts an opportunistic guerrilla strategy by which it avoids resource-poor patches of soil (e.g. high alkali) while preferentially exploiting more favorable habitats by maintaining root extension.

Keywords: alkali; ionic balance; Na$_2$CO$_3$ and NaHCO$_3$ stresses; oat; shoot and root growth.
Abbreviations: ELR, electrolyte leakage rate; OA, organic acid.

## Introduction

Alkali stress has been demonstrated in a number of reports (Kawanabe and Zhu, 1991; El-Samad and Shaddad, 1996; Campbell and Nishio, 2000; Hartung et al., 2002; Rao et al., 2008; Wang et al., 2011; Ma et al., 2011). Previous studies have suggested that alkali stress results mainly from levels of the alkaline salts NaHCO$_3$ and Na$_2$CO$_3$ (Shi and Yin, 1993; Shi and Sheng, 2005; Yang et al., 2010; Liu et al., 2010). In some areas, soil alkalisation is a severe problem. For example, approximately 7% (6.7×10$^6$ hm$^2$) of the cultivated land in northeast China has alkaline soils with only a few alkali-tolerant halophytes being able to survive there (Kawanabe and Zhu, 1991). Alkali stress usually involves a combination of stresses, osmotic, ion-induced injury and high pH (Munns, 2002; yang et al., 2008a; Chen et al., 2011). The high-pH environment that surrounds the roots can greatly affect the absorption of cations and inorganic anions and can also disrupt the ionic balance and pH homeostasis of the tissues (Yang et al., 2007, 2008b; Guo et al., 2010). The results are a decrease in photosynthesis, damage to the membrane system and finally a reduction in growth. Although the effects of various mixed alkali stresses have been studied extensively in a few plants (Shi and Yin, 1993; Hartung et al., 2002; Shi and Wang, 2005; Yan et al., 2006; Guo et al., 2010; Radi et al., 2012), these studies do not differentiate between the effects of NaHCO$_3$ and of Na$_2$CO$_3$ in isolation. Although these studies provide a better understanding of the adaptive responses of

plants under mixed alkaline stress, analysis of the stresses caused by the single alkali have not been adequately researched. Examination of the effects of NaHCO$_3$ alone, or of Na$_2$CO$_3$ alone, would seem to be a logical starting point for resolving the demographic mechanisms underlying these complex responses. Therefore, stress effects associated with NaHCO$_3$ on its own or with Na$_2$CO$_3$ on its own should be investigated as thoroughly as those of mixed alkali stress, especially, in regard to the responses of the major plant organs, the shoot and root. The present study was conducted in an experimental area of Northeast Normal University during the 2008 season. We compare the separate effects of alkali stresses, NaHCO$_3$ stress and Na$_2$CO$_3$ stress on water potential, photosynthetic pigments, ionic balance and tissue pH and growth in the shoot and root of young oat seedlings, to enhance our understanding of the mechanisms of alkali stress damage to plants and also those by which they adapt to such alkali stress.

## Results

### Tissue pH and ELR analysis of shoot and root

Shoot tissue pH did not show significant differences between NaHCO$_3$ and Na$_2$CO$_3$ stresses but root tissue pH and electrolyte leakage rate (ELR) were significantly higher under Na$_2$CO$_3$ stress than under NaHCO$_3$ stress (Table 1). The shoot

tissue pH under both stresses was closely similar to the controls (Fig. 1A, B) suggesting that these stress intensities did not affect the aboveground tissue pH. However, the root pH showed a different behaviour. While $NaHCO_3$ stress did not affect root pH with increasing stress, middle and high $Na_2CO_3$ contents ($\geq 72$mmol $L^{-1}$) did cause significant increases. Both stresses increased the ELR but the extent of the increment under $Na_2CO_3$ stress was much greater than under $NaHCO_3$ stress (Fig. 1C). All the plants treated with 120 and 144 mmol $L^{-1}$ of $Na_2CO_3$ died so their pH values were not recorded.

### Growth indices analysis of oat

With increasing alkali stress, the shoot survival rate, number of tillers per plant, root length, plant height, shoot dry weight and root dry weight all decreased (Fig. 2A-F), but the extents of the reductions were different. The effect on root length was especially slight with the effect of $NaHCO_3$ being not significant and only the most concentrated $Na_2CO_3$ (144mmol $L^{-1}$) caused a significant decrease (Fig. 2C, table 1). Furthermore, the reductions under $Na_2CO_3$ stress were much greater than those under $NaHCO_3$ stress (table 1).

### Photosynthetic pigments analysis of leaves

All photosynthetic pigments were significantly lower under $Na_2CO_3$ than under $NaHCO_3$ (Table 1). The effects of $NaHCO_3$ stress on Chl a, Chl b and carotenoid were similar with all three parameters decreasing but the extents of the reductions under $Na_2CO_3$ stress were greater than those under $NaHCO_3$. When $Na_2CO_3$ stress was $\geq 120$ mmol$L^{-1}$ the plants died and the contents of Chla, Chlb and Carotenoid were not recorded. The same concentrations of $NaHCO_3$ did not result in plant death (Fig. 3).

### Cations analysis of shoot and root

Each of the cations showed significant differences between the $NaHCO_3$ and $Na_2CO_3$ stresses (Table 1). In both the shoots and the roots, the concentration ratios of $Na^+$ and $Na^+ / K^+$ in the $Na_2CO_3$ stressed plants compared to the $NaHCO_3$ stressed plants were significantly higher, while for $K^+$ and $Ca^{2+}$ the concentration ratios were significantly lower (Table 1). Both shoots and roots showed similar trends for these cations (Fig. 4A, B, C, D, G, H). With increasing stress, both the $NaHCO_3$ and $Na_2CO_3$ stresses resulted in increased $Na^+$ contents and a decreased $K^+$ contents, and finally in an increased $Na^+ / K^+$ ratio (Fig. 4A, B, C, D, G, H). When $NaHCO_3$ and $Na_2CO_3$ stresses were compared, the extents not only of the increases in $Na^+$ but also of the reductions in $K^+$ were much greater under $Na_2CO_3$ stress than under $NaHCO_3$ stress (Fig. 4A, B, C, D, G, H). In both shoot and root, although the $Ca^{2+}$ content range was far less than for either $Na^+$ or $K^+$, it showed a similar trend for decrease compared to $K^+$ with increasing alkali stress, also, the extent of the $Ca^{2+}$ decrease for $Na_2CO_3$ stress was greater than for the $NaHCO_3$ stress (Fig. 4E, F).

### Anions analysis of shoot and root

All the anions showed significant differences between the $NaHCO_3$ and $Na_2CO_3$ stresses (Table 1). In both shoot and root, compared to the $NaHCO_3$ stress, the $Na_2CO_3$ stress showed significantly higher concentrations of $SO_4^{2-}$ and organic acids, while the $Cl^-$, $NO_3^-$ and $H_2PO_4^-$ concentrations were significantly lower (Table 1). In both shoot and root $Cl^-$ and $SO_4^{2-}$ increased with increasing stress intensity for both stressors but compared with the $NaHCO_3$ stress, the extent of

increase in $Cl^-$ in the $Na_2CO_3$ was higher than in $SO_4^{2-}$ (Fig. 5A, B, C, D). Both $NO_3^-$ and $H_2PO_4^-$ decreased with increasing stress but the reductions with $Na_2CO_3$ were much greater than with $NaHCO_3$ (Fig. 5E, F, G, H). With increasing stress, both low $NaHCO_3$ ($\leq 72$mmol$L^{-1}$) stresses and all the $Na_2CO_3$ stresses caused increases in organic acid. However, when the $NaHCO_3$ stresses were higher than 72 mmol $L^{-1}$, OA showed decreases (Fig. 5I, J).

### Water and proline contents analysis of shoot and root

In both shoot and root, proline concentrations were significantly higher under $Na_2CO_3$ stress than under $NaHCO_3$ stress while water contents were significantly lower (Table 1). Both $NaHCO_3$ and $Na_2CO_3$ stress decreased the water contents of shoots and roots (Fig.6A, B) but the extent of the reductions with increasing $Na_2CO_3$ stress were greater than with increasing $NaHCO_3$ stress. For shoot and roots, the changes in water content were in opposite directions with proline being increased by strong alkaline stress (>120mmol$L^{-1}$in $NaHCO_3$ and $\geq 96$ mmol$L^{-1}$ in $Na_2CO_3$) (Fig. 6C, D).

## Discussion

### Tissue pH

Regardless of environmental pH, to maintain normal metabolism it is important that plants can stabilise their tissue pH (Yang et al., 2007). Our observations that shoot tissue pH was similar to the control values under both alkali stresses and that stress intensity increases did not have significant affects on tissue pH suggests that oat is able to maintain a stable cell pH (Fig.1A, B). However, it is surprising that when the stress level rose above certain threshold values (48 mmol $L^{-1}$ with $Na_2CO_3$; 72 mmol $L^{-1}$ with $NaHCO_3$), root pH was affected by increasing levels of stress. This may be explained as that at above 48 mmol $L^{-1}$ in $Na_2CO_3$ (or 72 mmol $L^{-1}$in $NaHCO_3$) the harmful effects of high pH were opposed by pH adjustments outside the roots (by extrusion of $H^+$, OA, amino acids or $CO_2$ produced by root respiration) with the intracellular environment being unaffected. However, when the stress intensity exceeded the capacity for root adjustment (>48 mmol $L^{-1}$ in $Na_2CO_3$ or >72 mmol $L^{-1}$in $NaHCO_3$,), the result was a reduction in photosynthetic pigment content (Fig. 3), and a sharp increase in ELR (Fig. 1C). This suggests that the stress may have weakened the controls, leading to increases in pH. Meanwhile, compared to the shoot, it was found that the effects of both alkali stresses on root growth (i.e. root length and biomass) was reduced, indicating that the root has a higher tolerance of elevated pH. This deserves further investigation.

### Growth indices

Our results show that the injurious effects of $Na_2CO_3$ on growth were greater than those of $NaHCO_3$ for the same alkali content (Fig. 2), and this is consistent with previous reports (Shi and Yin, 1993; Yang et al., 2007). The injurious effects of alkali are commonly thought to be due to a combination of low water potential, ion toxicity, and, in particular, to high-pH stress (Munns, 2002). Firstly, the greater alkali stress due to $Na_2CO_3$ compared with $NaHCO_3$ leads to severer reductions in photosynthetic pigment content (Fig. 3) and a sharp increase in ELR (Fig. 1C). These results indicate that high pH from $Na_2CO_3$ stress may damage root cell structure and function affecting such processes as the absorption of ions, damaging photosynthetic pigments and membrane systems (e.g. increases in ELR). This may be why growth under $NaHCO_3$ stress was

**Table 1**. Results of a two-way ANOVA of plant characteristics by stress category, and by stress gradient and their interaction.

| Dependent variable | Stress category | Stress gradient | Category×Gradient |
|---|---|---|---|
| **(A) ions and organic acid** | | | |
| shoot $Na^+$ content | 2053.806*** | 441.723*** | 194.087*** |
| root $Na^+$ content | 1962.682*** | 448.914*** | 185.606*** |
| shoot $K^+$ content | 594.499*** | 144.279*** | 32.183*** |
| root $K^+$ content | 617.148*** | 151.240*** | 33.286*** |
| shoot $Ca^{2+}$ content | 237.577*** | 75.229*** | 10.253*** |
| root $Ca^{2+}$ content | 92.747*** | 135.584*** | 10.522*** |
| shoot $Na^+/K^+$ ratio | 6103.667*** | 1186.216*** | 693.524*** |
| root $Na^+/K^+$ ratio | 5843.242*** | 1178.227*** | 663.099*** |
| shoot $Cl^-$ content | 8730.910*** | 2111.533*** | 1501.335*** |
| root $Cl^-$ content, | 130.938*** | 186.921*** | 7.554*** |
| shoot $SO_4^{2-}$ content | 252.528*** | 155.270*** | 14.193*** |
| root $SO_4^{2-}$ content | 88.950*** | 73.563*** | 5.714** |
| shoot $NO_3^-$ content | 5849.245*** | 510.064*** | 121.803*** |
| root $NO_3^-$ content | 5066.797*** | 1204.266*** | 206.504*** |
| shoot $H_2PO_4^-$ content | 216.028** | 23.991*** | 10.599*** |
| root $H_2PO_4^-$ content | 174.224*** | 45.478*** | 7.879*** |
| shoot organic acid content | 1201.624*** | 73.181*** | 129.200*** |
| root organic acid content | 140.200*** | 26.039*** | 10.502*** |
| **(B) water and proline** | | | |
| shoot water content | 29.525*** | 12.718*** | 2.522 n.s. |
| root water content | 16.613*** | 4.594** | 1.929 n.s. |
| shoot proline content | 15.242** | 62.169*** | 3.025* |
| root proline content | 25.284*** | 142.933*** | 5.750** |
| **(C) tissue pH, ELR , *Chl*, *Car*** | | | |
| shoot tissue pH | 0.410 n.s. | 0.105 n.s. | 0.164 n.s. |
| root tissue pH | 112.706*** | 22.139*** | 19.165*** |
| electrolyte leakage rate (ELR) | 72.665*** | 71.086*** | 4.010** |
| chlorophyll (*Chl*) *a* | 84.845*** | 23.822*** | 8.851*** |
| chlorophyll (*Chl*) *b* | 86.057*** | 34.020*** | 8.783*** |
| Carotenoid (*Car*) | 142.607*** | 43.361*** | 21.962*** |
| **(D) growth index** | | | |
| survival rate | 1768.559*** | 303.930*** | 215.895*** |
| number of tillers per plant | 24.523*** | 12.629*** | 3.669** |
| root length | 3.679 n.s. | 2.230 n.s. | 0.315 n.s. |
| plant height | 46.796*** | 30.127*** | 1.948 n.s. |
| shoot dry weight | 26.597*** | 13.593*** | 3.485 n.s. |
| root dry weight | 13.669** | 13.946*** | 0.839 n.s. |

Note: Numbers represent $F$ values: * $P \leq 0.1$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s., no significant.

less than under $Na_2CO_3$ stress. Next, to tolerate ion toxicity, plants can exclude $Na^+$ depending on a $Na^+/H^+$ antiport, synthesise organic anions to maintain ionic balance or synthesise compatible solutes in the cytoplasm to prevent dehydration. All of these cost energy and hence involve a growth penalty. Thus, the higher $Na^+$ toxicity under $Na_2CO_3$ stress than under $NaHCO_3$ likely results in higher energy costs, which may be another reason for the reduced growth under $NaHCO_3$ stress. Additionally, it is interesting that, compared to the control, root length growth did not change significantly for any $NaHCO_3$ concentrations or for the low and medium concentrations of $Na_2CO_3$ (<144mmolL$^{-1}$). However, root dry weight growth did decrease significantly under >96mmolL$^{-1}$. It is suggested that, under natural conditions, oat appears to use an opportunistic guerrilla strategy whereby resources are allocated in the direction of a more favourable microsite by maintaining root extension rates even though root dry weight is significantly decreased as a result of damage to the photosynthetic pigments and the membrane systems (e.g. increases of ELR). By this strategy, oat can avoid resource-poor (e.g. high alkali patches of soil) while preferentially exploiting more favorable habitats.

### Ion toxicity and ion imbalance

Under high concentrations of either alkali, the first effect on the plant is likely to be in the root due to high concentrations of $Na^+$ in the soil. The $Na^+$ enters the roots passively via voltage independent nonselective cation channels and possibly via other $Na^+$ transporters such as some members of the high-affinity $K^+$ transporter (HKT) family (Munns and Tester, 2008), resulting in $Na^+$ ion toxicity in shoot. The similar radii of the hydrated ions of $Na^+$ and $K^+$ makes them difficult to discriminate, and this is the basis of $Na^+$ toxicity (Blumwald, 2000). Under both alkali stresses, $Na^+$ competes with $K^+$ for uptake into the roots (Munns, 2002; Munns and Tester, 2008). Compared to the same stress concentrations of $NaHCO_3$ and $Na_2CO_3$, the latter has the higher concentration of $Na^+$ resulting in increased $Na^+$ accumulation (associated with decreased $K^+$ accumulation) (Fig. 4ABCD). Also, the increased $Na^+$ in the stressed plant may induce a decrease in $Na^+$ exclusion, which normally enables the plant to avoid or defer ion toxicity problems. Many plant species have a $Na^+$ exclusion mechanism that depends on $Na^+/H^+$ antiport, such as salt overly sensitive 1
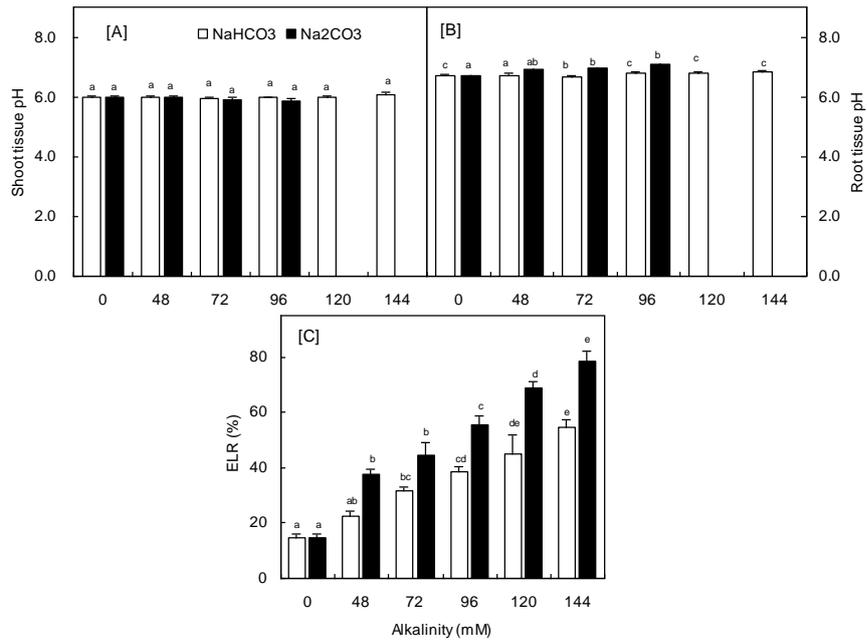
**Fig 1.** Effects of NaHCO$_3$ and Na$_2$CO$_3$ stress on (A) shoot tissue pH, (B) root tissue pH, (C) ELR (electrolyte leakage rate) in oat shoots. The 4-week-old oat seedlings were treated with NaHCO$_3$ stress (pH 8.03-8.26) and Na$_2$CO$_3$ stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different (P < 0.05) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. Shoot represents aboveground part of plant.
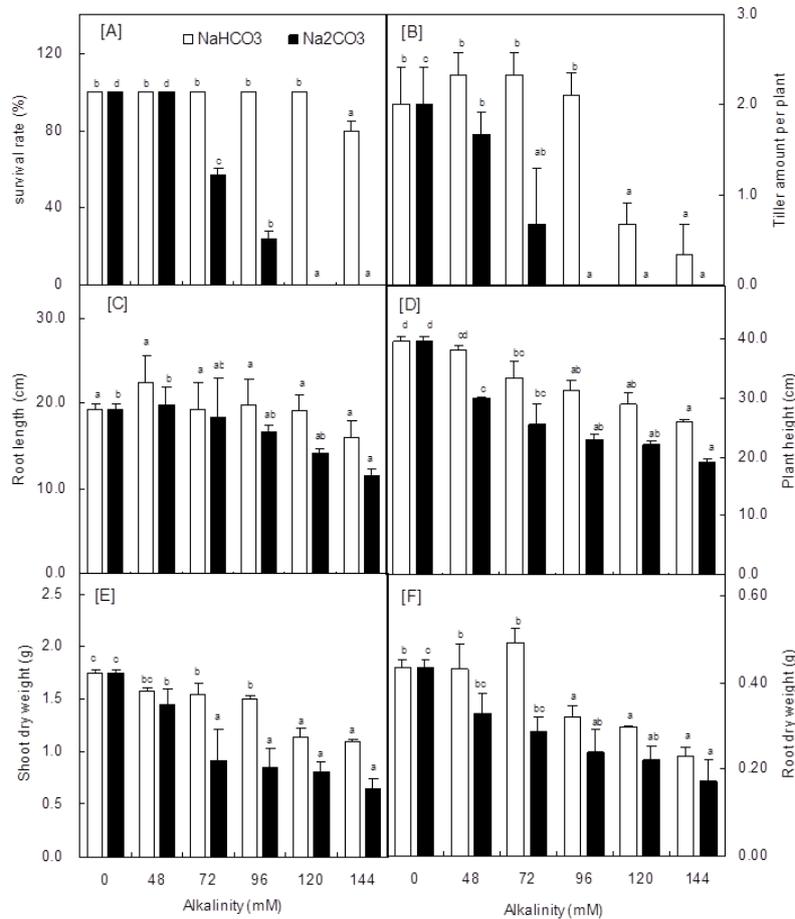


**Fig 2.** Effects of NaHCO$_3$ and Na$_2$CO$_3$ stress on (A) survival rate, (B) number of tillers per plant, (C) root length, (D) plant height, (E) shoot dry weight, (F) root dry weight in oat. The 4-week-old oat seedlings were treated with NaHCO$_3$ stress (pH 8.03-8.26) and Na$_2$CO$_3$ stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different (P < 0.05) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. DW, dry weight; Shoot represents aboveground part of plant.

(SOS1), which exchanges cytoplasmic $Na^+$ with external $H^+$ (Zhu, 2003; Munns and Tester, 2008). The exchange activity relies on the transmembrane proton gradient achieved by $H^+$-ATPase (Zhu, 2003). Compared to $NaHCO_3$, the same concentration of $Na_2CO_3$ has a higher pH, and its relatively lack of external protons may weaken the exchange activity of the $Na^+ / H^+$ antiport on the root plasma membrane (Munns and Tester, 2008), possibly reducing the exclusion of $Na^+$ from the rhizosphere and enhancing plant accumulation of $Na^+$ (Fig. 4). In addition, compared to $Na^+$ and $K^+$, other ions, especially $Ca^{2+}$ can also be influenced by both alkali stresses each of which exhibits decreasing trends with increasing concentrations. However, the extent of this effect is significantly higher in $Na_2CO_3$ than in $NaHCO_3$. This is firstly because, higher $Na^+$ in the shoot is influenced by $Na_2CO_3$ replacement of more intra-cellular $Ca^{2+}$ which results in substantial $Ca^{2+}$ loss (Fig. 4EF ); secondly, the high-pH environment of the roots with $Na_2CO_3$ can cause some ions, such as $Ca^{2+}$ to precipitate directly (Shi and Zhao, 1997); thirdly, $Ca^{2+}$ can be influenced in the plant by higher contents of organic acid (mainly oxalic acid) (Fig. 5IJ) through the formation of calcium oxalate (an insoluble crystal) from oxalic acid and $Ca^{2+}$. In the root, the high concentration of $Na^+$ causes this ion to move in the symplast across the endodermis where it is released from the stelar cells to the stelar apoplast, from where it moves through the xylem in the transpiration stream, finally reaching the shoot where the $Na^+$ toxicity occurs (Munns and Tester, 2008). The main site of $Na^+$ toxicity for most plants is in the leaf blade. Here, it can be tolerated by anatomical adaptations and by intracellular partitioning with the $Na^+$ remaining in the cell being sequestered in the vacuoles to avoid $Na^+$ toxicity in the cytosol (Serrano and Rodriguez-Navarro, 2001; Munns, 2002; Zhu, 2003).

### Ion balance

When compartmentalisation of $Na^+$ in the vacuoles takes place, plants usually accumulate inorganic anions, such as $Cl^-$, and $SO4^{2-}$, or they synthesise organic anions to maintain the ionic balance (Yang et al., 2007). This fits with our study (Fig. 5) where the concentrations of inorganic anions under $NaHCO_3$ stress were significantly lower than those under $Na_2CO_3$ stress of the same intensity. This suggests that the relatively high pH caused by $Na_2CO_3$ stress may inhibit the uptake of anions such as $NO_3^-$ and $H_2PO_4^-$ (Fig. 5EFGH). Meanwhile, $Cl^-$ and $SO_4^{2-}$ in relatively high concentrations in both alkalis make higher contributions to the ion balance. Additionally, we find that, with stress concentration increases in the plant, OA is strongly increased only with $Na_2CO_3$ whereas with $NaHCO_3$ it is decreased. This suggests that under $Na_2CO_3$ stress, OA is the dominant ion in the maintenance of equilibrium. This may be an alkali resistance mechanism involving two components, intracellular pH adjustment and extracellular pH adjustment (also possibly, pH adjustment in the root-external microenvironment). The OA synthesised might also be transported to roots for pH regulation. The process of pH adjustment may 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 may involve the exudation of a buffer compound, such as $H^+$, OA, amino acids or even $CO_2$ produced by root respiration, or other factors not considered above.

### Osmotic adjustment

When plants compartmentalise $Na^+$ into the vacuoles to avoid $Na^+$ toxicity in the cytosol (Serrano and Rodriguez-Navarro, 2001; Zhu, 2003) and accumulate inorganic anions and synthesise organic anions to maintain vacuolar ionic balance (Yang et al., 2007), they may also synthesise compatible low molecular weight organic solutes (compatible solutes) in the cytoplasm to maintain osmotic equilibrium and so prevent dehydration and protect biomacromolecules (Parida and Das, 2005). The amino acid proline is one of the most widely distributed compatible solutes occurring in many organisms from bacteria to higher plants (Flowers et al., 1977). In many halophytes, proline occurs at sufficiently high concentrations in their leaves (over 40 mM on a tissue water basis) to contribute significantly (over 0.1 MPa) to cell osmotic potential (Fricke, 2004). Thus, in our study it is clear that $Na^+$ concentration increases in the vacuoles (which also accumulate inorganic and organic anions to maintain ionic balance) and as alkali stress increases, proline concentration increases to prevent cytoplasmic dehydration (Fig. 6). Furthermore, for the same intensity of the two alkali stresses the especially high concentrations of $Na^+$ with $Na_2CO_3$ lead to relatively high concentrations of proline (Fig. 6). In particular, the induction of proline synthesis is also related to the high pH value associated with alkalinity. The involvement of proline accumulation in the damage caused by alkali stress is another aspect that requires further investigation. Apart from the above-mentioned osmotic adjustment which comes with an energy cost, a quick and energetically economical way of reducing cell water potential to avoid osmotic stress is to allow decreases in water content (Fig. 6AB). In summary, the key features pertaining to osmotic adjustment are an ability to accumulate $Na^+$ and organic acids in the vacuoles and to accumulate large amounts of proline in the cytoplasm.

### Materials and methods

### Plant materials

Oat (*Avena sativa* L.) is an annual, graminaceous plant which is very tolerant of drought, cold, saline and alkali stress and mineral deficiency. The oat cultivar No.2 baiyanmai (numbered species is B07046-2-4-1-6 in Baicheng City Academy of Agriculture Sciences, China) was used. This cultivar is characterised by early maturation, high salt and alkali tolerance and high disease resistance (Wei et al., 2007). It has a high grain yield which rises to over 2300 kg/hm². Its protein and fat contents are 16.6% and 5.6%, respectively (Wei et al., 2007). The plant can mature in live culms and provides both grain and straw.

### Cultivation

Seeds were sown in 20-cm diameter plastic pots containing 3 kg of washed sand. The pots were watered daily with sufficient Hoagland nutrient solution. Each pot contained 25 seedlings. All pots were placed outdoors and protected from the rain. The experiment was carried out in an experimental area of the Northeast Normal University during the 2008 growth season.

### Design of the simulated alkaline conditions

Two alkaline salts, $NaHCO_3$ and $Na_2CO_3$ were applied separately to create two stress groups. Within each group, six concentrations were used: 0, 48, 72, 96, 120 and 144 mmol $L^{-1}$. Treatments in the $NaHCO_3$ stress group were labeled $A_1$–$A_6$ and those in the $Na_2CO_3$ stress group were labeled $B_1$–$B_6$. A1 and B1 were the controls. There were three replicates per treatment.
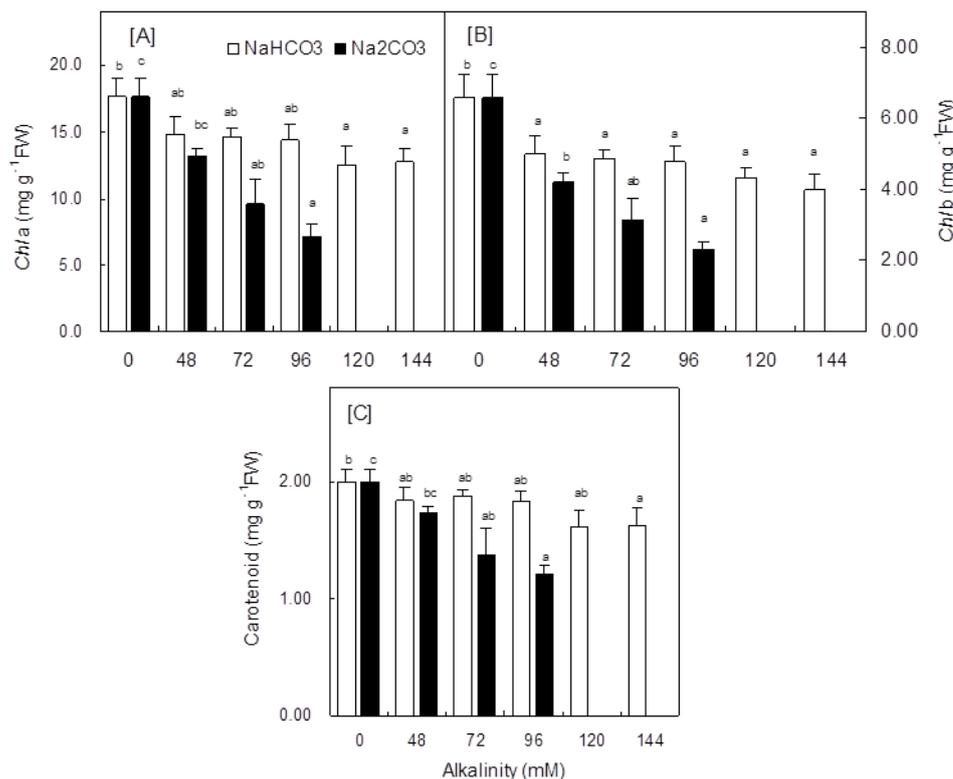
**Fig 3.** Effects of NaHCO$_3$ and Na$_2$CO$_3$ stress on the (A) chlorophyll (*Chl*) a, (B) chlorophyll (*Chl*) b in oat. The 4-week-old oat seedlings were treated with NaHCO$_3$ stress (pH 8.03-8.26) and Na$_2$CO$_3$ stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different (P < 0.05) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. FW, fresh weight; Shoot represent aboveground part of plant.

### *Stress treatments*

The stress treatments were applied when the seedlings were four weeks old. Thirty-six pots of uniform seedlings were divided randomly into 12 sets of 3 pots. Two sets were used for the controls and the remaining 10 sets were used for the stress treatments giving three replicate pots per treatment. The treated pots were watered daily between 16.00–18.00 h with excess of a nutrient solution that contained the appropriate stress salts. The control plants were watered with nutrient solution at the same time. The duration of stress treatment was nine days.

### *Physiological indices measurements*

### *Measurement of tissue pH*

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

### *Measurement of the electrical conductivity of the leaves*

Membrane permeability is reflected in a 'relative electrical conductivity', which is defined as the ratio of the electrical conductivity of leaves with intact membranes to those with membranes destroyed by a boiling water treatment. Electrolyte leakage rate (ELR) was determined as described by Lutts et al., (1996).

### *Measurement of chlorophyll*

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

### *Measurement of growth indices*

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

### *Measurement of ions*

Dry samples (0.1 g) of shoot and root were treated with 20 mL of deionised water at 100℃ for 1 h and the resultant extract was used to determine the contents of inorganic ions and organic acids (OA).The contents of NO$_3^-$, Cl$^-$ , SO$_4^{2-}$, H$_2$PO$_4^-$ were determined by ion chromatography using a DX-300 ion chromatographic system with an AS$_4$A-SC ion-exchange column and a CDM-II electrical conductivity detector (mobile
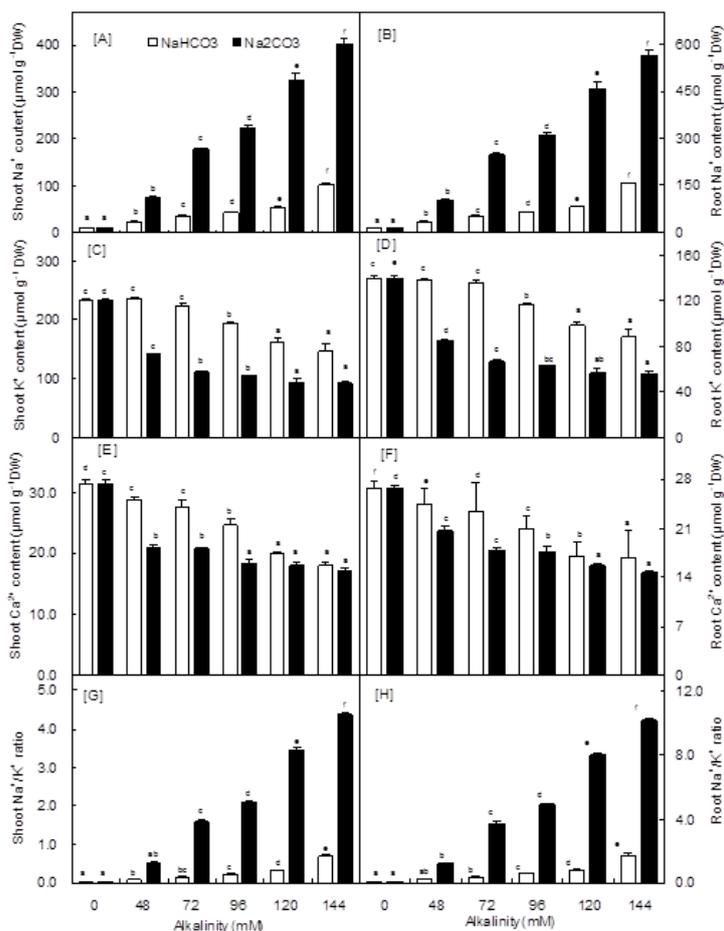
**Fig 4.** Effects of NaHCO$_3$ and Na$_2$CO$_3$ stress on (A) shoot Na$^+$ content, (B) root Na$^+$ content, (C) shoot K$^+$ content, (D) root K$^+$ content, (E) shoot Ca$^{2+}$ content, (F) root Ca$^{2+}$ content, (G) shoot Na$^+$ / K$^+$ ratio, (H) root Na$^+$ / K$^+$ ratio in oat. The 4-week-old oat seedlings were treated with NaHCO$_3$ stress (pH 8.03-8.26) and Na$_2$CO$_3$ stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different (P < 0.05) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. DW, dry weight; Shoot represent aboveground part of plant.

phase: Na$_2$CO$_3$ / NaHCO$_3$ = 1.7 / 1.8 mmol L$^{-1}$; DIONEX, Sunnyvale, CA, USA). Na$_2$CO$_3$/NaHCO3=1.7 / 1.8 mmol L$^{-1}$. The levels of the organic acids were also determined by ion chromatography using an DX-300 ion chromatographic system with an ICE-AS6 ion-exclusion column, CDM-II electrical conductivity detector and an AMMS-ICE II MicroMembrane suppressor (mobile phase: 0.4 mmol L$^{-1}$ heptafluorobutyric acid; DIONEX). An atomic absorption spectrophotometer (TAS-990; Purkinje General, Beijing, China) was used to determine the levels of Na$^+$, K$^+$ and Ca$^{2+}$.

### Measurement of proline

The dried samples were homogenised to determine free proline contents which was assayed using the acid-ninhydrin method (Zhu et al. 1983).
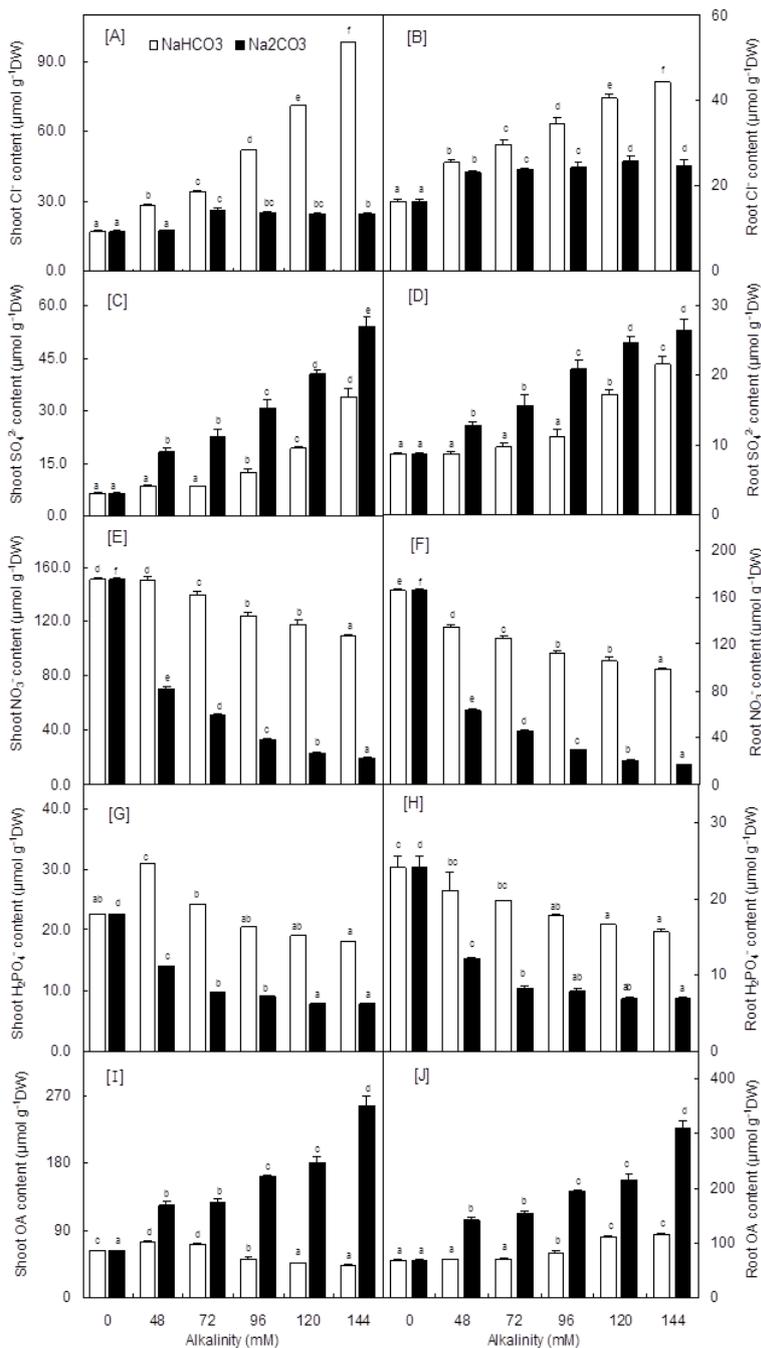


**Fig 5.** Effects of NaHCO$_3$ and Na$_2$CO$_3$ stress on (A) shoot Cl$^-$ content, (B) root Cl$^-$ content, (C) shoot SO$_4^{2-}$ content, (D) root SO$_4^{2-}$ content, (E) shoot NO$_3^-$ content, (F) root NO$_3^-$ content, (G) shoot H$_2$PO$_4^-$ content, (H) root H$_2$PO$_4^-$ content, (I)shoot OA (organic acid) content, (J) root OA (organic acid) content in oat. The 4-week-old oat seedlings were treated with NaHCO$_3$ stress (pH 8.03-8.26) and Na$_2$CO$_3$ stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different (P < 0.05) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. DW, dry weight; Shoot represent aboveground part of plant.
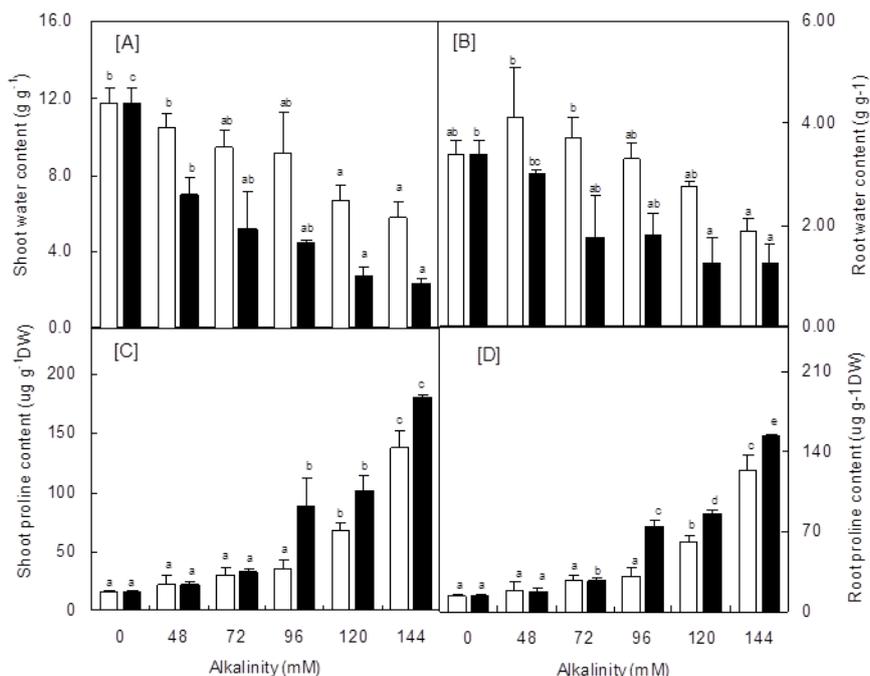
**Fig 6.** Effects of $NaHCO_3$ and $Na_2CO_3$ stress on the (A) shoot water content, (B) root water content, (C) shoot proline content, (D) root proline content in oat. The 4-week-old oat seedlings were treated with $NaHCO_3$ stress (pH 8.03-8.26) and $Na_2CO_3$ stress (pH 9.91-11.27) for 9 days. In each column, the data markers identified with the same letters are not significantly different (P < 0.05) according to a Duncan test. The error bars represent ± standard error (n = 3) of three replicates. Shoot represent aboveground part of plant.

### Statistical analyses

Statistical analysis of the data was performed using the statistical program SPSS 13.0 (SPSS, Chicago, USA). All data are represented by an average of three replicates and their standard errors (SE). Data were analysed by one-way and two-way ANOVA. The treatment mean values for the same organ under either alkali stress were compared by post-hoc Duncan tests. The term significant indicates differences at P < 0.05.

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