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Full Length Research Paper

Comparative effects of neutral salt and alkaline salt stress on seed germination, early seedling growth and physiological response of a halophyte species *Chenopodium glaucum*

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Effects of neutral salt (NaCl) and alkaline salt ($NaHCO_3$) stress on seed germination, early seedling growth and physiological response of 15-day-old seedling of *Chenopodium glaucum*, a halophyte widely distributed in temperate saline areas of China, were determined. Results show that NaCl stress of higher concentration (≥ 300 mM) more seriously retarded and postponed seed germination of *C. glaucum*, however, the inhibitory effect of $NaHCO_3$ stress on radicle and hypocotyl elongation was greater than NaCl stress even at lower concentration. Relative water content (RWC) of *C. glaucum* remained high even under the highest salt or alkali stress. No obvious increase of osmolytes (proline, soluble sugar, betaine) was detected under lower concentration of NaCl and $NaHCO_3$ stress. The Na^+ content and Na^+/K^+ ratio increased while the K^+ content decreased under both stresses, the changing extents under $NaHCO_3$ were greater than those under NaCl stress. Moreover, seedlings exposed to lower concentration of $NaHCO_3$ generated higher levels of reactive oxygen species (ROS, O_2^-, H_2O_2), meanwhile significant increase of antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POX)] activity and non-enzymatic antioxidants [Carotenoids (Car), ascorbic acid (AsA)] content were detected in seedlings treated with lower concentration of $NaHCO_3$. Results suggest that the destructive effects of alkaline salt stress on the growth, ion balance and anti-oxidant system of seedling of *C. glaucum* were more severe than those under neutral salt stress. Different pH circumstance might be the key reason for the distinctive difference between them.

Key words: *Chenopodium glaucum*, enzymatic antioxidant, non-enzymatic antioxidant, osmolytes, oxidative stress, salt stress and alkali stress, seed germination.

INTRODUCTION

Seed germination and seedling establishment are two of the most critical stages in the life cycle of plants when confronted with adverse environments, such as salinity, drought and extreme temperatures (Ungar, 1978), among which salinity has been considered as one of the major threats for a species to successfully inhabit semi-arid and arid areas. Soil salinization and alkalization frequently co-occur, it has been shown that alkaline salts (such as $NaHCO_3$ and Na_2CO_3) are more destructive to plants at different growth stages than neutral salts (NaCl, Na_2SO_4) (Yang et al., 2008a; 2009). Considerable effort has been made to exploit the physiological and molecular mechanisms of salt resistance in halophyte, however, little attention has been paid to the response of halophyte

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Abbreviations: AsA, Ascorbic acid; **Car,** carotenoids; **CAT,** catalase; **Chl,** chlorophyll; **GSH** glutathione; **H_2O_2,** hydrogen peroxide; **MDA**, malondialdehyde; **MTG,** mean time-to-germination; **NBT,** nitroblue tetrazolium; **O_2^-,** superoxide anion radical; **POX,** peroxidase; **Pro,** proline; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase; **SS,** soluble sugar; **TBA,** thiobarbituric acid.

to alkali stress (Li et al., 2010a, b; Yang et al., 2007, 2008a).

The mechanisms for adaptation of the halophyte to salt stress involve osmotic adjustment, ions balance and antioxidant resistance mechanisms (Munns, 2005; Gill and Tuteja, 2010). Numerous study show that alkali stress cannot only exerts osmotic and ionic stresses, but with the added influence of high-pH stress. Accumulation of compatible solutes, such as betaine, proline, soluble sugar and compartmentation of Na^+ into the vacuole are considered as the basic strategies for plant re-established cellular homeostasis under alkali stress (Li et al., 2009; Yang et al., 2008b, 2009). The differences of mechanisms on antioxidant system to salt and alkali stress in halophytes have not been well-studied. Previous studies mainly emphasized the effects of salt and alkali stress on seed germination or fully-developed seedlings, while neglected one of the most sensitive stages limiting plant survival, the early seedling developmental stage.

Chenopodium glaucum is an annual halophyte widely distributed in high and temperate saline areas of China. *C. glaucum* is more salt-tolerant, however, unlike many other halophyte species, it shows no succulent leaf or assimilating branch in morphology. It can be taken as the vegetable in the folk, and also used as forage additive (Zhao and Feng, 1999) whereas, relatively little attention has been paid to the physiological mechanisms of *C. glaucum* response to neutral salt and alkaline salt stresses, especially in early seedling stage. So, the principal objectives of the present study were: (1) to test the different effects of both neutral and alkaline salts on seed germination and seedling growth of *C. glaucum* and (2) to determine the differences of physiological response of 15-day-old seedlings to neutral and alkaline salt stress.

MATERIALS AND METHODS

Seed collection

Mature seeds of *C. glaucum* were collected in August, 2009, from Shawan county of Xinjiang, China. Seeds were naturally air-dried and stored at 4°C in brown paper bag for various experiments.

Seed germination

To investigate the effect of neutral salt and alkaline salt on seed germination and early seedling growth of *C. glaucum*, different concentrations of NaCl (neutral salt) and $NaHCO_3$ (alkaline salt) solutions (0, 50, 100, 200, 300, 400 and 500 mM, respectively) were applied in the germination test, and the pH were 5.99 to 6.48 or 8.22 to 8.45 in the saline salt or alkaline salt solutions, respectively. Three replicates with 30 seeds of each were applied in various treatments. Seeds were placed in 9 cm-diameter glass Petri dishes on two layers of filter paper, onto which 5 ml of appropriate salt solutions or distilled water (control) were added. The filter paper and the remaining solution was renewed every 2 days to keep the salt concentration constantly. Seeds were set to germinate in incubator at 25 to 28°C for 16/8 h light/dark and photosynthetically active radiation (PAR) at 116 μmol $m^{-2}s^{-1}$.

Germinated seeds (with at least 2 mm of protruded radicle) were recorded every 12 h during a 15-day period. Mean time-to-germination (MTG) was calculated on the 10th day by using the following equation:

$$MTG=\sum_i(n_i \times d_i) / N,$$

Where, n is the number of seeds germinated at day i, d is the incubation period in days and N is the total number of seeds germinated in the treatment (Brenchley and Probert, 1998). The final germination percentage, and the radicle and hypocotyl lengths (15 seedlings) were calculated and measured on the 15th day.

Seedling growth and salinity treatment

Based on the result of the above experiments, further test was designed to analyze the response of seedling to different salts: (1) 50, 100 and 400 mM NaCl treatment; (2) 50 and 100 mM $NaHCO_3$ treatment (for $NaHCO_3$ treatment at 400 mM no seedling was obtained); distilled water was used as control. Seeds were placed in 18-cm-diameter glass Petri dishes on two layers of filter paper, onto which 10 ml of appropriate solutions and distilled water was added (We did not add nutrient solution to all the experiment to keep the same level between NaCl and $NaHCO_3$ treatment, for the metal ions, such as Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, in nutrient solution could form carbonate precipitation in $NaHCO_3$ solution). Seeds were set to germinate and for further growth in incubator at 25 to 28°C for 16/8 h light/dark and photosynthetically active radiation (PAR) at 116 μmol $m^{-2}s^{-1}$. The solutions were changed every 2 days to keep the salt concentration constant. Seedlings in each treatment were harvested on the 15th day, and subjected to measurement for various physiological parameters after it was rinsed with distilled water for 3 times and blotted with filter paper.

Determination of water content and osmolytes content: Proline (Pro), soluble sugar (SS) and betaine

The relative water content (RWC) was measured according to the description of Pujol et al. (2001) and calculated using the formula

RWC = (FW-DW) ×100/FW, and expressed as WC (% FW),

Where, FW is fresh weight, and DW is dry weight.

Pro was determined according to the method described by Bates et al. (1973). The mixture contained 0.2 ml seedling extract, 0.2 ml acetic acid and 0.3 ml 2.5% acid ninhydrin was incubated in a boiling water bath for 40 min and then cooled. The product was extracted with 0.5 ml toluene by vigorous shaking and the absorbance was measured at 520 nm. The Pro content was calculated based on the calibration curve and expressed as μg Pro g^{-1} FW.

SS content was determined by the classical anthrone method (Yemm and Willis, 1954). 0.20 ml seedling extract was added to 1 ml anthrone reagent, mixed and heated in boiling water for 10 min, and cooled using ice water. The absorbance was determined using glucose as the standard at 620 nm.

Betaine was determined according to the method of Cui et al. (2004), and some minor changes were made. 0.2 g seedlings were ground with 1.5 ml distilled water, after centrifugation at 7000 *g* for 15 min, 300 μl supernatant was reacted with 500 μl 15 mg ml^{-1} of saturation Reinecke salt on ice for 1 h, and then centrifuged at 1800 *g* for 15 min. The supernatant was discarded, the pellet was suspended and washed 2 to 3 times with 99% (v/v) ether, then the precipitation was dissolved in 500 μl 70% (V/V) acetone. After monitoring the absorbance at 525 nm, betaine content was calculated based on a standard curve plotted with the range of 0 to 15 mg ml^{-1} betaine.

Determination of Na^+ and K^+ content

Na^+ and K^+ contents of the whole seedling were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES). Fresh seedlings (1 g) was washed with distilled water for 3 times and then dried at 105°C to constant weight. The major cations were extracted after digestion of 0.1 g dry matter with HNO_3, and then the extract was taken to determine the free Na^+ and K^+ concentration.

Determination of H_2O_2 content and O_2^- production rate

H_2O_2 content was measured by monitoring the titanium-hydroperoxide complex at 415 nm described by Sairam et al. (2005) and made some minor changes. 0.15 g seedlings were homogenised in 1.5 ml cooled acetone, and the homogenate was centrifuged at 3000 *g* for 10 min at 4°C. 1.0 ml supernatant was transferred to a new 1.5 ml micro-tube followed by addition of 0.1 ml 5% (w/v) titanium sulfate and 0.2 ml ammonia to precipitate the titanium-hydroperoxide complex. Reaction mixture was centrifuged at 10 000 *g* for 10 min, the supernatant was discarded and the precipitation was suspended and washed three times with acetone, dissolved in 2.0 M H_2SO_4 and then centrifuged. Absorbance of the supernatant was read at 415 nm in a microplate spectrophotometer (Bio-Rad, CA, USA). Concentration of H_2O_2 was determined from a standard curve plotted with the range of 0 to 100 µM H_2O_2.

O_2^- was extracted with 50 mM PBS (pH 7.8) on ice, after being centrifuged at 10 000 *g* for 20 min at 4°C, the supernatant was used to determine the O_2^- production rate by the hydroxylamine oxidization reaction described by Li and Gong (2005). 1 ml supernatant was incubated with 1 ml 1 mM hydroxylamine at 25°C for 1 h, then 1 ml 17 mM 4-aminobenzenesulfonic acid and 1 ml 7 mM α-naphthalenamine were added, and the test tubes were incubated at 25°C for 20 min.

Assay of antioxidant enzyme activity: SOD, CAT and POX

For preparation of the crude enzyme extract, 0.2 g seedlings were homogenized in ice-cold solution containing 50 mM PBS (pH 7.8), 0.5 mM EDTA, 1% (W/V)polyvinylpyrrolidone (PVP), 1 mM AsA and 10% (v/v) and glycerol (Cui et al., 2006). The homogenates were centrifuged at 10 000 *g* at 4°C for 25 min. The supernatants were transferred onto ice and immediately used for analysis.

Activity of SOD was assayed by a test tube-reaction described by Jiang and Huang (2001). 3 ml reaction mixture in tube containing 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 mM NBT, 2 mM riboflavin and 50 µL crude enzyme extract, was incubated at 25°C under a light source (4000 lx) for 25 min. The absorbance of the reaction mixture was read at 560 nm. One unit of SOD was defined as the amount of enzyme necessary to inhibit the reduction of NBT by 50%.

CAT activity was determined by monitoring the initial rate of decomposition of H_2O_2 at 240 nm following the protocol of Aebi (1984). 0.1 ml crude enzyme extract was added to 3.0 ml reaction mixture containing 1.0 ml 0.3% H_2O_2, 1.9 ml H_2O, and mixed to initiate the reaction. POX activity was assayed by monitoring the increase in absorbance at 470 nm, 0.5 ml crude enzyme solution was added in 3.0 ml reaction mixture [containing 1.0 ml phosphate buffer (pH 7.0), 0.95 ml 0.2% guaiacol, 1.0 ml 0.3% H_2O_2] (Huang et al., 1997). The changes in absorbance at both 240 nm or 470 nm were recorded within 1 min with UV-3010 spectrophotometer (Shimadzu, Japan). One unit of CAT or POX activity was defined as every 0.01 decrement of the absorbance at 240 nm and every 0.01 increment of the absorbance at 470 nm per min.

Total antioxidant enzyme activities were expressed as unit per gram of fresh weight (U g^{-1}FW).

Determination of non-enzymatic antioxidant content: AsA, GSH and Car

0.2 g seedlings were homogenized in ice-cold 6% (w/v) TCA, AsA and GSH contents were determined in the supernatant after centrifugation at 12 000 g for 10 min at 4°C. Assay of AsA content was based on the reduction of Fe^{3+} to Fe^{2+} by AsA in acid solution and the spectrophotometric detection of Fe^{2+} complexed with 2,2-dipyridyl at 525 nm according to the method described by Hamed et al. (2007). A standard curve covering the range of 0 to 70 mM AsA was plotted. GSH was measured by the DTNB oxidation assay according to the method of Sgherri and Navari-Izzo (1995), and reaction product was spectrophotometrically evaluated by monitoring the change in absorbance at 412 nm. The total GSH was calculated from a standard curve prepared by 0 to 0.12 mM GSH solution.

Appropriate seedlings were treated with 96% (V/V) ethanol, and Car content was determined spectrophotometrically and calculated according to Lichtenthaler and Wellburn (1983).

Statistical analysis

Data were expressed as mean ± standard error (SE) obtained from four independent measurements. Statistical analyses were performed by two-way ANOVA using the software of GraphPad Prism Version 4.02 for Windows (GraphPad Software, San Diego, CA). When significant main effects existed, differences were tested by a multiple comparison Tukey test at the 0.05 significance level.

RESULTS

Seed germination and early seedling growth under NaCl and $NaHCO_3$ treatment

The effect of different concentration of NaCl and $NaHCO_3$ stress on seed germination and early seedling growth are presented in Figure 1. The final germination percentage showed no significant differences between NaCl and $NaHCO_3$ when the salt concentration was less than 400 mM (Figure 1a); it seemed that seeds were more tolerant to $NaHCO_3$ in germination. The MTG showed the same tendency, and the effect tended to be stronger with increasing salinity concentration. A two-way ANOVA analysis showed that significant differences existed between NaCl and $NaHCO_3$ treatments from 200 mM concentration (Figure 1b).

With the increase in NaCl and $NaHCO_3$ concentrations, the hypocotyl growth was promoted firstly and then inhibited. Seedlings in all treatments grew better than control when NaCl concentration was no more than 400 mM, while the hypocotyl length was significantly reduced from 100 mM under $NaHCO_3$ stress (Figures 1c and 1e). Both NaCl and $NaHCO_3$ treatments inhibited radicle growth, however, two-way ANOVA analysis showed that $NaHCO_3$ treatment had more serious effect on radicle growth (Figures 1d and e). Contrary to the germination behavior, the growth of hypocotyl and radicle was rather slower under $NaHCO_3$ treatment than that of NaCl treatment, and when $NaHCO_3$ concentration was more than 300 mM, it was almost hard to observe the growth of

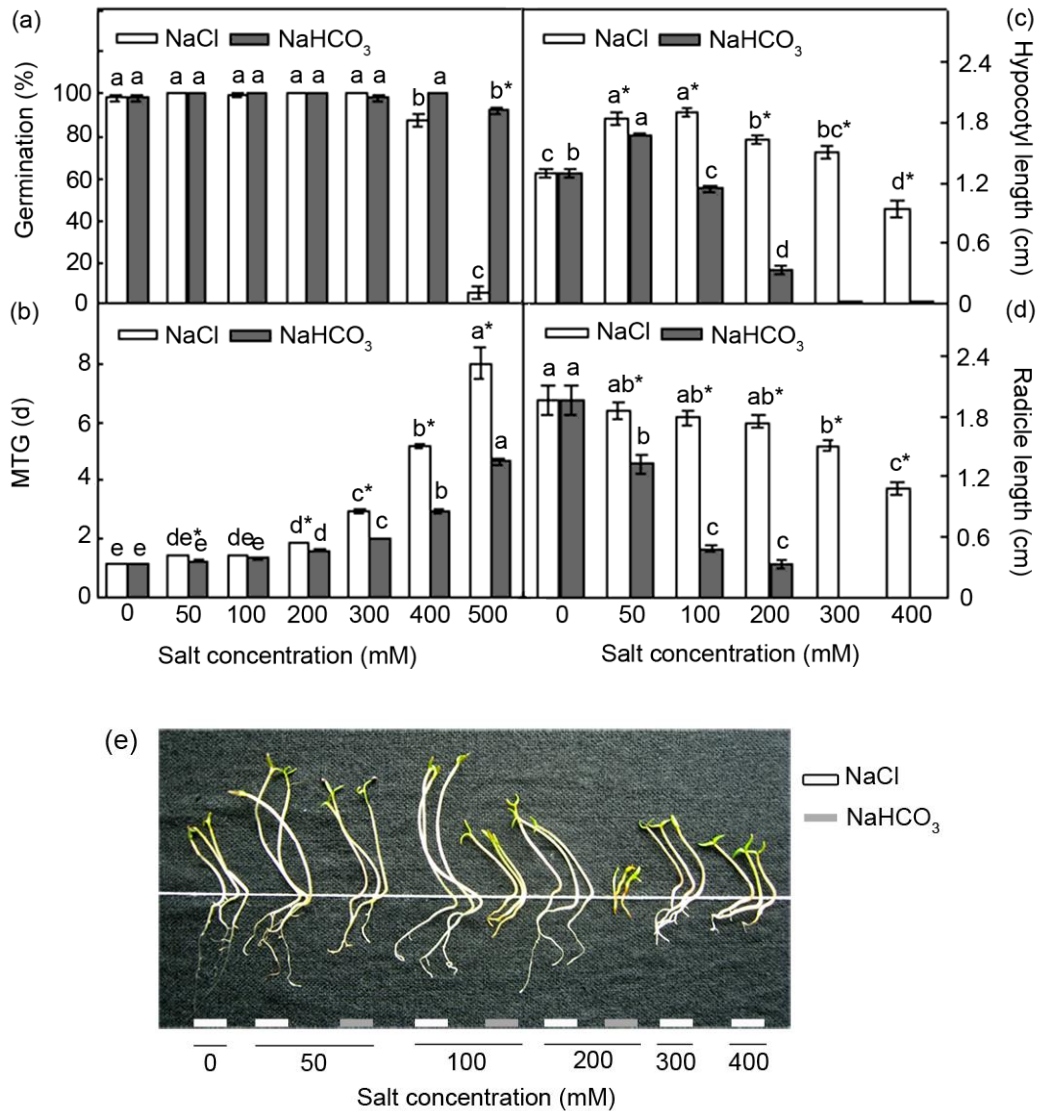

Figure 1. Responses of seed germination and early seedling growth to NaCl and $NaHCO_3$ stress. (a) Final germination percentage; (b) MTG; (c) hypocotyl length; (d) radicle length; (e) seedling morphology. Values are means ± S.E. Bars with different lowercase letters indicate significant differences among the different salinity concentrations (P<0.05). Asterisk (*) shows the significant differences (P < 0.05) between salt and alkali stresses under the same concentration.

hypocotyl and radicle.

Effects of NaCl and $NaHCO_3$ stress on osmolyte contents

With increase in the NaCl and $NaHCO_3$ concentrations, the RWC in seedlings showed a slight increase compared to the control under various NaCl and $NaHCO_3$ treatments (Figure 2a). At low saline and alkaline concentrations, osmolytes accumulation was not affected apparently, however, Pro dramatically accumulated to 3.61 times compared to the control at 400 mM NaCl stress (Figure 2b). Changes of betaine and SS content showed the same pattern with the salt concentration increase and also, both showed the lowest content at lower salt concentrations (100 mM NaCl or 50 mM $NaHCO_3$), while it significantly increased at 400 mM NaCl stress (Figure 2c and d).

Effects of NaCl and $NaHCO_3$ stress on Na^+, K^+ content

Na^+ concentration increased significantly in both NaCl and $NaHCO_3$ treated seedlings, and seedlings with NaCl treatment absorbed more Na^+ than that with $NaHCO_3$

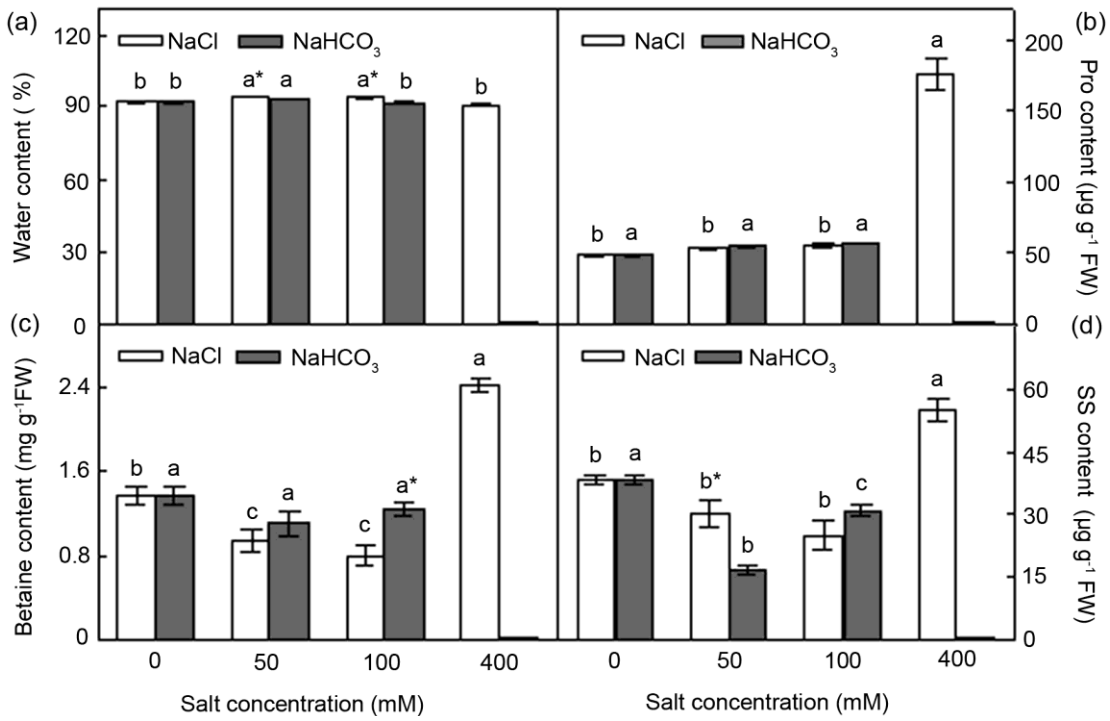

Figure 2. Changes of RWC and osmolyte contents in 15-day-old seedlings under NaCl and $NaHCO_3$ stresses. (a) RWC; (b) Pro content; (c) betaine content; (d) SS content. Values are means ± S.E. of four replicates. Bars with different lowercase letters indicate significant differences among different salinity concentrations ($P < 0.05$). Asterisk (*) shows the significant differences ($P < 0.05$) between salt and alkali stresses under the same concentration.

treatment. Na^+ accumulation under 100 mM NaCl was apparently greater than those under 100 mM $NaHCO_3$ stress (Figure 3a). For K^+ content, there was an apparent decrease at lower NaCl and $NaHCO_3$ concentration, while K^+ absorption was significantly lower in $NaHCO_3$ compared to NaCl (Figure 3b), and K^+ content remained stable at 400 mM NaCl. Na^+/K^+ ratio with lower NaCl treatment (50 or 100 mM) was significantly lower than that with $NaHCO_3$ treatment (Figure 3c).

Effects of NaCl and $NaHCO_3$ stresses on oxidative stress level

To investigate the difference on oxidative stress in seedling growth between NaCl and $NaHCO_3$ stresses, H_2O_2 content and O_2^- level were determined after salinity treatment. Different patterns were observed between NaCl and $NaHCO_3$ stresses under 50 and 100 mM concentration, H_2O_2 production increased slightly with the increase in $NaHCO_3$ concentration, while it decreased at lower NaCl concentration; H_2O_2 level was significantly induced at 400 mM NaCl stress (Figure 4a). O_2^- level in seedlings is shown in Figure 4b. No significant changes were detected in seedlings under NaCl stress compared to control, however, both 50 and 100 mM $NaHCO_3$ treatments significantly induced the production of O_2^-.

Changes in antioxidant enzyme activities

To investigate the connection between ROS accumulation and the related ROS-scavenging enzymes, activities of SOD, CAT and POX were measured (Figure 5). Activities of all the antioxidant enzymes remained unchanged under lower (50 mM and 100 mM) NaCl treatments, while it showed a significant increase at 400 mM NaCl stress while under $NaHCO_3$ treatment, the activities of three enzymes responded quickly to the rising of salt concentration, and significant increase with POX activity were observed at 50 and 100 mM, and SOD activity at 50 mM $NaHCO_3$ compared to control. In addition, all enzymes under $NaHCO_3$ treatment at lower concentrations, except for CAT at 50 mM, showed significantly higher activities than that under NaCl treatment. The increase in activities of these antioxidant enzymes seemed to correspond to the higher H_2O_2 and O_2^- accumulation (Figure 4).

Changes in non-enzymatic antioxidant content

Total Car, AsA and GSH contents were measured to investigate the antioxidant responses under different concentrations of NaCl and $NaHCO_3$ treatment. The amount of Car and AsA seemed to be unaffected at 50

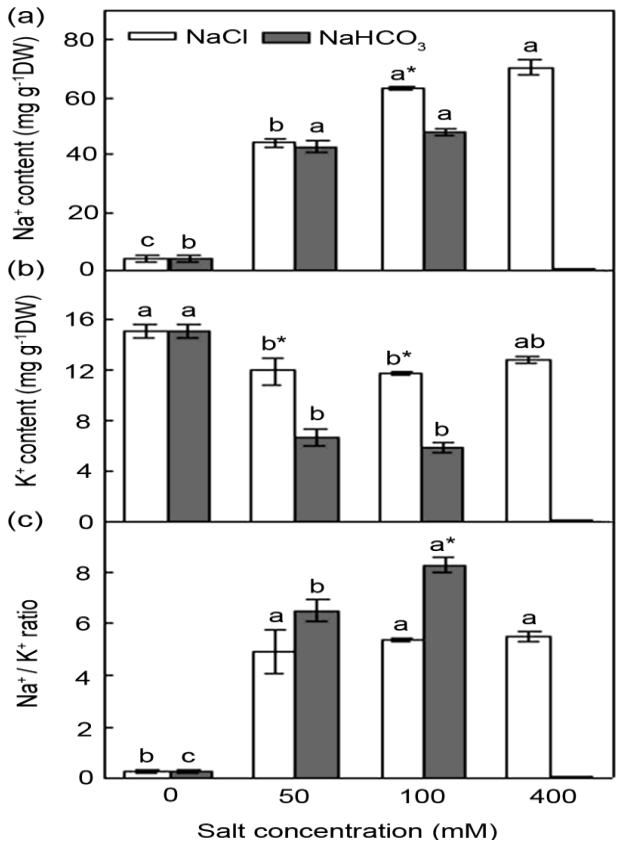

Figure 3. Changes of Na^+, K^+ content in 15-day-old seedlings under NaCl and $NaHCO_3$ stresses. (a) Na^+ content; (b) K^+ content; (c) Na^+/K^+ ratio. Values are means ± S.E. of four replicates. Bars with different lowercase letters indicate significant differences among the different salinity concentrations (P<0.05). Asterisk (*) showed significant difference (P<0.05) between salt and alkali stresses under the same concentration.

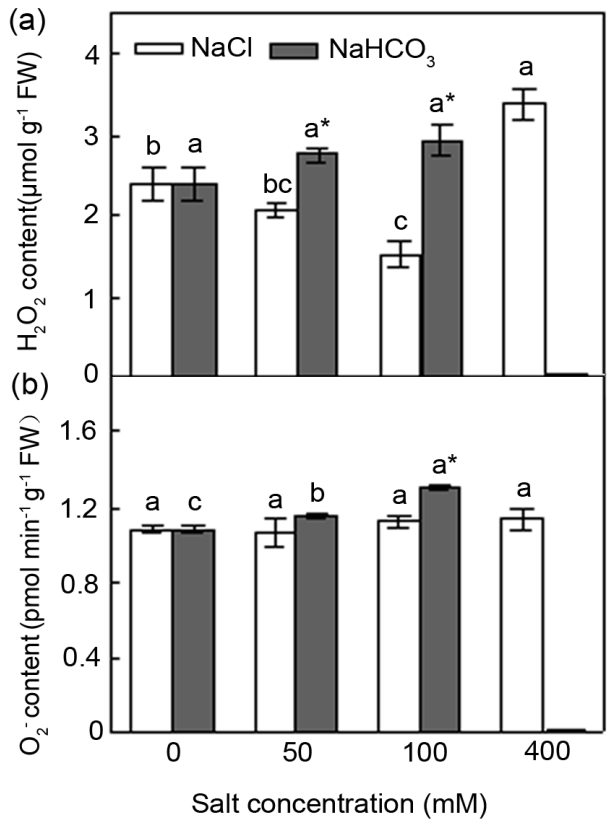

Figure 4. Changes of ROS level in 15-day-old seedlings under NaCl and $NaHCO_3$ stresses. (a) H_2O_2 content; (b) O_2^- production rate. Values are means ± s.e. of four replicates. Bars with different lowercase letters indicate significant differences among the different salinity concentrations (P<0.05). Asterisk (*) shows significant difference (P<0.05) between salt and alkali stresses under the same concentration.

and 100 mM NaCl concentrations compared to the controls, while a significant increase of Car content was observed at 400 mM NaCl treated seedlings. In contrast, the contents of Car and AsA increased significantly under all $NaHCO_3$ concentrations, except for AsA at 50 mM (Figure 6a and b). No obvious increase was observed in the amount of GSH with rising alkaline and saline concentrations, instead, significant decreases were presented under 50 and 100 mM NaCl treatments (Figure 6c).

DISCUSSION

Germination and growth

Chenopodium glaucum is a halophyte belonging to the family of Chenopodiaceae, in which many species are of salt tolerance. Hydration of cotyledons and the hypocotyl is the first step in seed germination. Salinity was reported to inhibit this process mainly by delaying germination (Promila and Kumar, 2000). In the present study, seeds of *C. glaucum* were able to germinate under higher salt concentration (both neutral and alkaline salt), while significant higher germination percentage and accelerated MTG were observed in $NaHCO_3$ compared to NaCl treatment at more than 400 mM salinity intensity. The higher pH environment of $NaHCO_3$ solution (8.22 to 8.45) may help radicle to break through seed coat in the early stage and accelerate seed germination (Joseph, 1967), however the elongation of radicle was severely restrained even at lower concentration of $NaHCO_3$ (100 mM); the seedling was yellowish, shorter and in a deteriorative growth situation at 200 mM, and the radicle did not elongated anymore at 300 mM. Similar result was reported from the salt-tolerant crops *Helianthus annuus* (Liu et al., 2010). Under alkali stress, in addition to the effects of salt stress, which generally causes osmotic stress and ion toxicity (De-Lacerda et al., 2003; Soussi

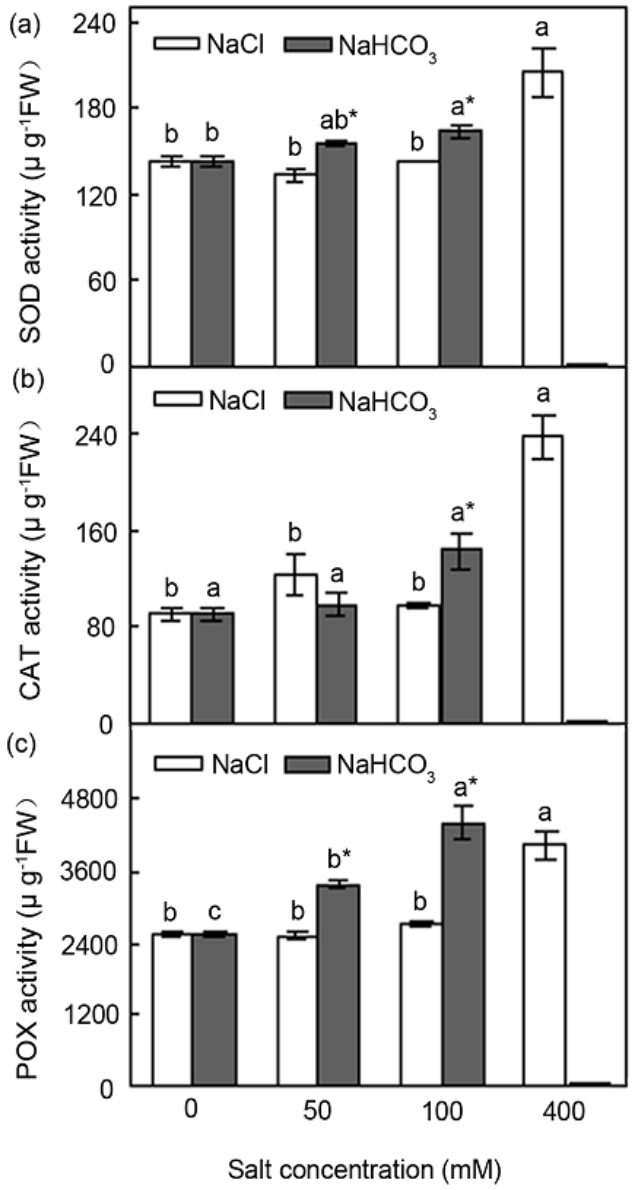

Figure 5. Changes of the activity of antioxidant enzyme in 15-day-old seedlings under NaCl and $NaHCO_3$ stresses. (**a**) SOD; (**b**) CAT; (**c**) POX. Values are means ± s.e. of four replicates. Bars with different lowercase letters indicate significant differences among different salinity concentrations (P<0.05). Asterisk (*) showed significant difference (P < 0.05) between salt and alkali stresses under the same concentration.

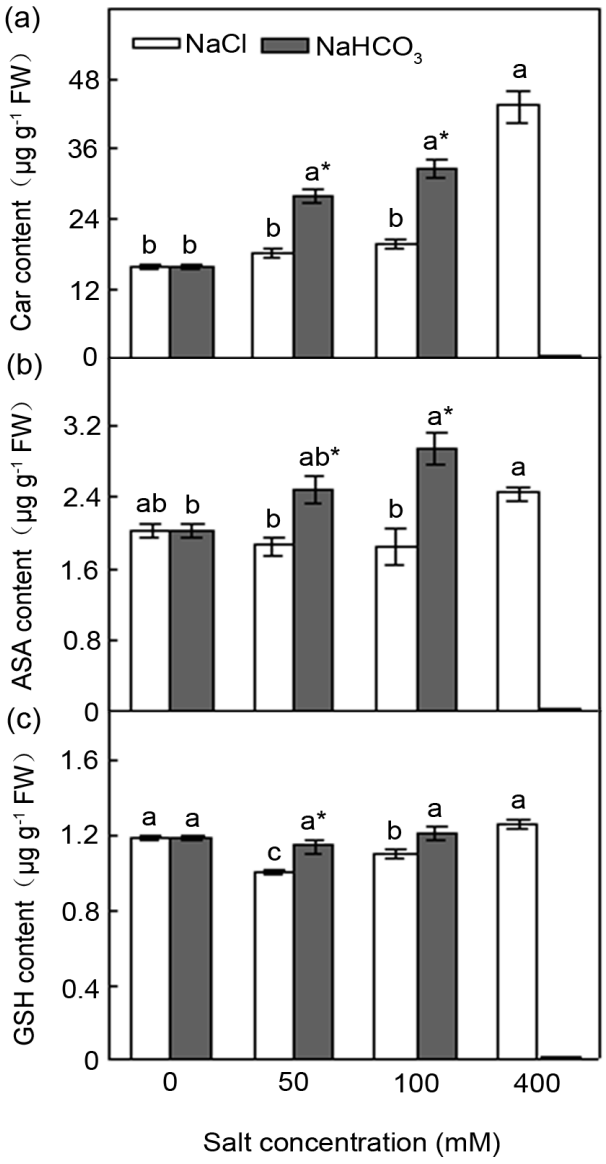

Figure 6. Changes of non-enzymatic antioxidant content in 15-day-old seedlings under NaCl and $NaHCO_3$ stresses. (a) Car; (b) AsA; (c) GSH. Values are means ± s.e. of four replicates. Bars with different lowercase letters indicate significant differences among the different salinity concentrations (P < 0.05). Asterisk (*) shows significant difference (P < 0.05) between salt and alkali stresses under the same concentration.

et al., 1998), plants also have to deal with stress of elevated pH (Yang et al., 2007). The high pH is not only disadvantageous to the supplying capacity of the root (Shi and Zhao, 1997), but directly destroys the structure and function of root cells (Shi and Yin, 2002; Shi and Sheng, 2005). Our results indicate that the radicle was more sensitive to alkaline salts, and probably due to the high-pH effect, seeds of *C. glaucum* could germinate but would have difficulty forming normal seedlings that could survive.

Osmotic adjustment

Accumulation of inorganic ions in vacuoles is an economical choice for plants in saline conditions to reduce cell water potential for the less energy consumption than synthesizing organic compounds

(Munns, 2002). Na^+ was the main inorganic osmolyte in vacuoles under either salt or alkali stress (Li et al., 2010a, 2010b; Liu et al., 2010; Shi and Sheng, 2005). In our study, Na^+ in the seedlings of *C. glaucum* increased drastically under both stresses, which showed that Na^+ served as the osmolyte in vacuoles under either salt or alkali stress. Na^+ is also 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), while plants under salt-alkaline stress usually takes up Na^+ and at the meantime inhibit K^+ absorption (Munns, 2002; Shi and Sheng, 2005; Shi and Wang, 2005). With increased salinity, K^+ content reduced slowly under NaCl stress, but sharply under $NaHCO_3$ stress. Compared to NaCl treatment, seedlings treated with $NaHCO_3$ possessed higher Na^+/ K^+ ratio, especially under 100 mM intensity. This indicates that there exist competitive inhibition between Na^+ and K^+ absorptions. The high-pH of alkaline salt disturbed the function of root cells (such as membrane selectivity), interfered the selective absorption of K^+-Na^+ in roots and resulted in imbalance of intracellular K^+-Na^+ (Shi and Wang, 2005; Shi and Sheng, 2005).

Except for inorganic ions, plants can also synthesize osmolytes to maintain the relatively high water content when confronted with salt stress (Ashraf and Foolad, 2007; Karimi et al., 2005; Moghaieb et al., 2004). Pro, SS and betaine serves as the most common organic osmolytes which were contributed to relief the low cellular water potential, and thereby maintained relatively high turgor pressure in the cell to sustain growth (Trovato et al., 2008). In the present study, seedlings were able to retain high water content without increasing the osmolytes in both salt and alkali stresses at lower salt concentration, while significant accumulations of the organic osmolytes were detected at 400 mM NaCl stress compared to the controls and the other treatments (Figures 2a, b, and c), which suggests that organic osmolytes accumulation correlates to alleviation of external osmotic stress elicited by high salinity, and the contribution of Na^+ to osmotic adjustment was greater than for osmolytes at low stress intensity.

Oxidative system regulation

Oxidative stress is a major damaging factor in plants subjected to salt and alkali stresses; changes in pH are usually accompanied by oxidative burst (Wang et al., 2008; Xu et al., 2009). Salt and alkali stresses often increase the oxygen-induced damage to cells due to the over-accumulation of ROS; O_2^- and H_2O_2 have been demonstrated to play an important role in the event of salt injury (Hernhdeza et al., 1995; Singha and Choudhuri, 1990). In the present study, enhanced generation of H_2O_2 and O_2^- was observed in seedlings of *C. glaucum* with 100 mM $NaHCO_3$ treatment, in contrast, only higher concentration of NaCl up to 400 mM treatment could be detected a significant increase of H_2O_2 content (Figure 4). Previous study showed that alkali stress engendered the intracellular acid-base disturbance, which may activate the pH-dependent cell wall peroxidases and then elicit the production of H_2O_2 (Gill and Tuteja, 2010). Thus, $NaHCO_3$ triggered an enhancement in active production of ROS in this work, which may have caused oxidative stress and resulted in poor growth, as indicated by severely restrained elongation of radicle under 100 mM $NaHCO_3$. By contrast, lower levels of H_2O_2 under 100 mM NaCl likely participated in the cellular signaling pathway as secondary messengers to induce oxidant defense under salt stress (Jiang and Zhang, 2002; Skopelitis et al., 2006).

pH stress also significantly affects the antioxidant system (Zhang and Mu, 2009). SOD has been regarded as the first defense line by catalyzing the dismutation of O_2^- to H_2O_2 (Badawi et al., 2004), H_2O_2 can be further destroyed predominantly by several classes of POX and CAT (Maribel et al., 1998; Willekens et al., 1997). In our study, significantly increased activities of SOD, CAT and POX were only detected under high concentration of neutral salt, while lower alkaline salt triggered considerable increase in the activities of the three antioxidant enzymes (Figure 5). In combination of the enhanced production of H_2O_2 and O_2^-, the data suggest that these enzymes may be involved in the metabolic regulation of ROS generated under high salt intensity and high pH stress (Gill and Tuteja, 2010).

Mounted evidence has shown that, apart from enzymatic antioxidants, a positive correlation exists between the accumulation of non-enzymatic antioxidants and the resistance to the oxidative damage caused by ROS (Athar et al., 2008). AsA and GSH serve as the most powerful antioxidants for their wide distribution and multitude functions. Both of them can minimize the oxidative damage either by directly scavenging dangerous ROS like OH, $^1O_2$ and O_2^- or via the AsA-GSH cycle (Ashraf, 2009; Foyer et al., 1997). The fully oxidized ASA has a short half-life and to avoid the lost of water, it is usually reduced. GSH plays a key role in regenerating AsA via the AsA-GSH cycle (Gill and Tuteja, 2010; Foyer and Halliwell, 1976). Car also plays an important role in protecting photosynthetic apparatus from oxidative stress by quenching $^1O_2$ and other harmful free radicals (Gill and Tuteja, 2010). Our study showed that Car, AsA and GSH levels remained unchanged or decreased in neutral salt at low concentration, while a significant accumulations of them (except for GSH unchanged) were detected with $NaHCO_3$ treatment at same concentration ($P<0.05$) (Figures 6a and b), which may reflect the high oxidative status under relatively lower concentration of $NaHCO_3$ stress.

Taking together, the destructive effects of alkaline salt stress on the growth, ion balance, osmo-homeostasis and anti-oxidant system of seedling of *C. glaucum* were more

severe than those under neutral salt stress. Different pH circumstance might be the major reason for the distinctive difference between them, because high pH value is the pivotal property of the alkaline salt solution, which is different from neutral salt solution (Yang et al., 2008b).

In conclusion, this study shows the different characteristics of seed germination and early seedling growth of halophyte *C. glaucum* under neutral and alkaline salt (NaCl and $NaHCO_3$) stress. Studies on some physiological parameters suggested that the severe cellular toxicity of $NaHCO_3$ (alkaline salt solution with pH value as 8.22 to 8.45) at lower concentration on *C. glaucum* seedlings might be mediated by intracellular acid-base disturbance, and then generate higher degree of oxidative stress and a lesser extent of osmotic stress and Na^+ toxicity. The results suggest that *C. glaucum* can tolerate higher concentration of neutral salt stress (400 mM NaCl), but cannot stand relatively lower alkaline salt stress (200 mM $NaHCO_3$) in the early seedling stage.

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