

Research Article

Growth Response to Ionic and Osmotic Stress of NaCl in Salt-tolerant and Salt-sensitive Maize

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Abstract

Salt-tolerant maize (STM) and salt-sensitive maize (SSM) were treated with 100 mM NaCl for 1, 3 and 6 d and the contents of Na⁺ and Cl⁻ (cps) of different organelles of leaf cells determined by X-ray microanalysis. The results showed that Na⁺ and Cl⁻ entered the cytoplasm, vacuole, chloroplast and apoplast simultaneously. When STM and SSM were treated in 100 mM NaCl at atmospheric pressure (–P) and with pressure equivalent to the osmotic pressure of the NaCl (+P), the dry weights of STM (+P) and SSM (+P) plants were greater than that of STM (–P) and SSM (–P) plants, showing that the inhibitory effect of salt on plant growth was at least partially due to the osmotic effect of the NaCl. When STM and SSM were treated with NaCl and iso-osmotic polyethylene glycol, the dry weights of plants given the iso-osmotic polyethylene glycol treatment were lower for both maize lines than that of the NaCl-treated plants. Our data show that under NaCl stress, both STM and SSM seedlings simultaneously suffered from osmotic and ion stresses.

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Introduction

The stresses suffered by plants under salinity include osmotic stress and specific ion stress (Waisel 1972; Flowers et al. 1977; Greenway and Munns 1980; Munns et al. 1983; Yeo and Flowers 1984; Zhao 1993). However, whether the growth reduction in saline substrates is due to osmotic stress or to salt toxicity is unclear. In 1988, Munns (1988) put forward a two-phase model of plant growth under salt stress, in which the effects of water deficit dominate in phase 1 and those of ions dominate in phase 2. Subsequently, Munns et al. (1995) reported further details of the two-phase hypothesis. In the papers they suggested that osmotic stress and ion excess are not alternatives, but arise in sequence in two distinct phases, with genotypic differences in salt tolerance being evident only in the second phase. The growth reduction in the first phase would be due to the osmotic strength of the salt solution outside the roots, and thus affect all genotypes similarly. The

second phase would start only after salt accumulated to toxic levels in enough leaves to cause injury and hence reduce the supply of assimilates to the growing regions. The first phase might be quite long (several weeks) for some species or short (several days) for other species. The hypothesis, which received support (Breckle 1995) and opposition (Neumann 1997) has little experimental proof. In the present study, we determined some key physiological data of maize seedlings, with different salt-tolerance, that is, salt-tolerant maize (STM: Ludan850) and salt-sensitive maize (SSM: Ludan961), under salt stress in order to examine the veracity of this hypothesis. Ludan 850 (STM) variety was appraised by Shandong Appraisal Committee of Crop Varieties in 1999 (No. 279) and its advance ratified by China Department of Science and Technology in 2000 (No. 01279). Wang et al. (2000) reported that production of Ludan 850 maize was 19.6% to 24.6% greater than common maize (as Ludan 961, etc.) in saline soils (salt content 3 to 5 g/L). The threshold of Ludan 850 and that of Ludan 961

were 8 and 2 to 3 ds/m as determined by Life Science College, Shandong Normal University.

Results

Dynamic changes of Na⁺ and Cl⁻ contents in different compartments of leaf cells of STM and SSM seedlings under NaCl stress

Table 1 shows the relative changes of Na⁺ relative concentration (X-ray counts per second) in the cytoplasm, vacuole, chloroplast and apoplast in the leaves of STM and SSM seedlings growing in the 100 mM NaCl treatment. Na⁺ contents were very low before NaCl was applied (0 d) in the organelles of leaves of both STM and SSM, but increased quickly once the seedlings were in 100 mM NaCl and were positively correlated with treatment time. They had the largest increase measured in vacuoles and apoplast. Na⁺ content in vacuoles of STM was higher than SSM, but the situation was reversed in cytoplasm, chloroplasts and apoplast.

The corresponding dynamic change of Cl⁻ contents in the cytoplasm, vacuole, chloroplast and apoplast in the leaves of STM and SSM seedlings under NaCl stress was very similar to that of Na⁺. Furthermore, it is evident that the Na⁺ and Cl⁻ contents in the compartments (with the exception of vacuoles) of SSM leaf cells were higher than those of STM, especially in the cytosol (**Table 1**).

Effect of NaCl on the photosynthetic rate of maize seedlings

Apart from the relative contents of Na⁺ and Cl⁻, we also determined the photosynthetic rate of the maize seedlings under salt stress. As **Figure 1** shows, photosynthetic rates were inhibited for STM and SSM after 1 d of treatment in 100 mM NaCl and the degree of inhibition was positively correlated with treatment time; the reduction percentages of photosynthetic rate of STM and SSM were 27.8% and 46.3% on the first day, 38.8% and 59.4% on the third day and 58.6% and 75.0% on the sixth day, respectively, when compared with control values. The reduction in photosynthesis of STM was less than that of SSM at all measurement times.

Dynamic changes of dry matter accumulation of STM and SSM under 100 mM NaCl with and without pressure

Table 2 showed that the dry matter accumulation of STM and SSM plants grown in the presence of NaCl with pressure (+P) was higher than that of maize grown without additional pressure (-P) after treatment for 15 d. The dry matter accumulation of both STM and SSM seedlings under NaCl (+P) and NaCl (-P) both decreased compared with controls; the growth of STM seedlings was less inhibited than SSM seedlings, both for 100 mM NaCl (+P) and 100 mM NaCl (-P). The inhibition of the growth of the plants was greater in the 100 mM NaCl

Table 1. Counts of Na⁺ and Cl⁻ (cps) in different parts of leaf cells of maize seedlings treated with 100 mM NaCl

| Parameters | Organelle | Plants | NaCl treatment times | | | |
|-----------------------|-------------|--------|----------------------|--------------|--------------|----------------|
| | | | 0 d | 1 d | 3 d | 6 d |
| Na ⁺ (cps) | Cytoplasm | STM | 3.19 ± 0.12 | 21.09 ± 2.26 | 30.63 ± 3.13 | 50.15 ± 3.68b |
| | | SSM | 3.22 ± 0.15 | 29.05 ± 3.15 | 41.96 ± 5.00 | 75.50 ± 3.58a |
| | Vacuole | STM | 4.36 ± 0.22 | 37.02 ± 3.83 | 69.46 ± 5.89 | 79.38 ± 5.77a |
| | | SSM | 2.60 ± 0.20 | 23.10 ± 1.15 | 32.05 ± 2.09 | 53.80 ± 3.59b |
| | Chloroplast | STM | 3.13 ± 0.13 | 32.71 ± 3.49 | 48.48 ± 4.05 | 55.02 ± 6.49b |
| | | SSM | 2.95 ± 0.17 | 39.19 ± 3.10 | 52.68 ± 3.11 | 70.25 ± 6.23a |
| | Apoplast | STM | 1.13 ± 0.36 | 44.12 ± 4.33 | 82.37 ± 6.45 | 82.37 ± 6.45b |
| | | SSM | 1.85 ± 0.21 | 54.99 ± 3.22 | 82.16 ± 6.90 | 92.30 ± 6.25a |
| Cl ⁻ (cps) | Cytoplasm | STM | 4.36 ± 0.17 | 15.23 ± 2.13 | 47.77 ± 2.80 | 49.02 ± 2.68b |
| | | SSM | 5.00 ± 0.17 | 20.33 ± 3.44 | 64.09 ± 3.00 | 69.92 ± 5.56a |
| | Vacuole | STM | 6.20 ± 0.31 | 40.69 ± 4.50 | 64.11 ± 3.62 | 85.68 ± 3.60a |
| | | SSM | 4.82 ± 0.31 | 17.99 ± 1.11 | 50.12 ± 3.62 | 53.08 ± 2.59b |
| | Chloroplast | STM | 5.20 ± 0.27 | 21.13 ± 3.34 | 61.38 ± 6.22 | 73.42 ± 9.95b |
| | | SSM | 3.55 ± 0.27 | 28.36 ± 4.09 | 71.38 ± 6.22 | 90.87 ± 5.97a |
| | Apoplast | STM | 8.23 ± 0.49 | 50.28 ± 5.55 | 71.35 ± 6.63 | 81.24 ± 11.32b |
| | | SSM | 6.60 ± 0.49 | 56.21 ± 6.00 | 79.35 ± 6.63 | 101.67 ± 9.12a |

Means of Na⁺ or Cl⁻ in cells of each organelle having different letter are significantly different at $P < 0.05$. Data are based on mean ± SD, $n = 7$. SSM, salt-sensitive maize; STM, salt-tolerant maize. Seedlings were treated with 100 mM NaCl for 0, 1, 3 and 6 d.

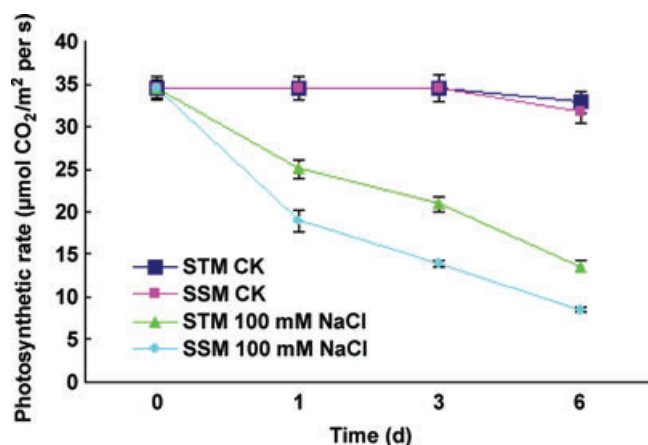


Figure 1. The effect of NaCl on the photosynthetic rate of salt-tolerant maize (STM) and salt-sensitive maize (SSM).

Seedlings were treated with 0 and 100 mM NaCl for 1, 3 and 6 d. Vertical bars indicate standard errors of means ($n = 5$).

(–P) treatment than in the 100 mM NaCl (+P); the dry matter accumulations over 15 d of STM were 65.8% and 56.7% of controls under NaCl (+P) and NaCl (–P), respectively, and 55.0% and 34.5% in SSM.

The total effect of 100 mM NaCl on STM and SSM in (+P) and (–P) were 1.0 (2.3 minus 1.3) and 1.4 (2.2 minus 0.8), respectively: clearly, the effect of NaCl on SSM was greater than on STM. Based on Table 2, we calculated the osmotic effect of 100 mM NaCl on STM and SSM in (+P) and (–P) treatments. The osmotic effects were 0.8 (2.3 minus 1.5) and 1.0 (2.2 minus 1.2), making up 79.1% and 68.8% of total NaCl effect, respectively. The ionic effects on STM and SSM were 0.21 (1.01 minus 0.80) and 0.45 (1.44 minus 0.99), making up 20.9% and 31.3% of total effect of 100 mM NaCl, respectively (Figure 2). These results showed that the osmotic effect of NaCl occurs in the first phase and is different between STM and SSM, with an effect on STM being greater than on SSM.

Table 2. Effects of 100 mM NaCl with pressure (+P) and without pressure (–P) on the dry matter accumulation of salt-tolerant maize (STM) and salt-sensitive maize (SSM)

| Plants | NaCl (mM) | Treatment time (d) | Dry weight (g/plant) | | |
|--------|-----------|--------------------|----------------------|--------------|--------------|
| | | | Control | +P | –P |
| STM | 0 | 15 | 2.33 ± 0.14a | | |
| SSM | 0 | 15 | 2.20 ± 0.17b | | |
| STM | 100 | 15 | | 1.53 ± 0.05a | 1.32 ± 0.05a |
| SSM | 100 | 15 | | 1.21 ± 0.04b | 0.76 ± 0.02b |

Means in each column having different letter are significantly different at $P < 0.05$. Data are based on mean ± SD, $n = 3$. Pressure was applied for 15 d to plants at 100 mM NaCl.

Effects of 100 mM NaCl and iso-osmotic polyethylene glycol on the dry matter accumulation of STM and SSM

Figure 3 showed the dry matter of STM and SSM gradually increased with treatment time. The control plants grew faster than the plants treated with NaCl or iso-osmotic polyethylene glycol (PEG): growth of STM was faster than SSM. These data are expressed relative to the control in Figure 3. After treatment for 30 d, the dry matter percentage of STM and SSM treated with NaCl or iso-osmotic PEG were 50.9%, 65.7% and 12.2%, 33.0% compared with the control, respectively. The dry matter percentages of STM and SSM treated with 100 mM NaCl or iso-osmotic PEG were 62.8%, 76.9%, and 38.5%, 49.5%, respectively, compared with the control after treatment for 15 d (in the first phase, based on Munns' hypothesis).

These results showed that the inhibitory effect of 100 mM NaCl on STM and SSM are stronger than iso-osmotic PEG in 30 d. This is as a result of salt stress of plants. Based on Figure 3, we may calculate the ionic effect of NaCl, which gradually increased with growth times (Figure 4). These results show that any ionic effects of NaCl continually influence plant growth as the osmotic effect of NaCl is presented in the first phase and second phase of Munns and that of NaCl on SSM is bigger than that of STM.

Effects of various organic and inorganic solutes on the plasmalemma permeability of STM and SSM leaves

Figure 5 shows the effects of various inorganic (NaCl, KCl, CaCl_2 , Mg_2SO_4 and Na_2SO_4) and organic solutes (sorbitol and PEG) on the plasmalemma permeability (relative electric conductivity). In STM and SSM, NaCl proved the most injurious to the plasmalemma permeability among the inorganic solutes. Relative to NaCl (as 100%), the effects of KCl, Na_2SO_4 , Mg_2SO_4 , and CaCl_2 of SSM and STM were 87.0%, 79.5%, 18.3%, 14.7% and 83.0%, 73.0%, 16.9%, 11.2%, respectively. As for the organic solutes, the effects of sorbitol and PEG on SSM and STM were nearly equivalent while NaCl, KCl and Na_2SO_4 were much higher than those of the inorganic solutes (PEG and sorbitol: 8.6%, 9.9%, 10.9%, and 8.2%, 9.4%,

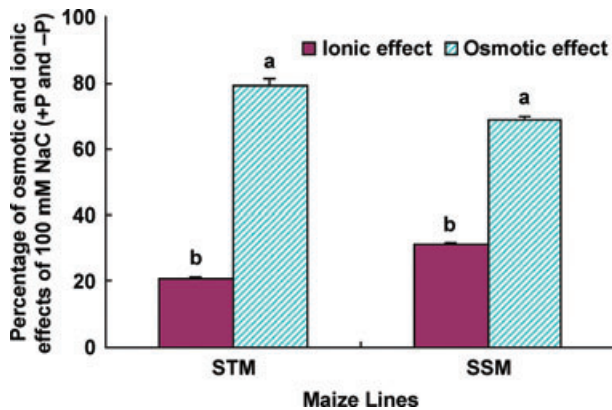


Figure 2. Effect of pressure (+P and -P) on the reduction percentage osmotic effect of total effect in salt-tolerant maize (STM) and salt-sensitive maize (SSM) after 15 d of 100 mM NaCl treatment.

Means of each line having different letter are significantly different at $P < 0.05$. Vertical bars indicate standard errors of means ($n = 3$).

10.3%, respectively). The effect of the solutes on the plasmalemma permeability of SSM was larger than those of STM for all the measurements, except for the KCl and $MgSO_4$ treatments.

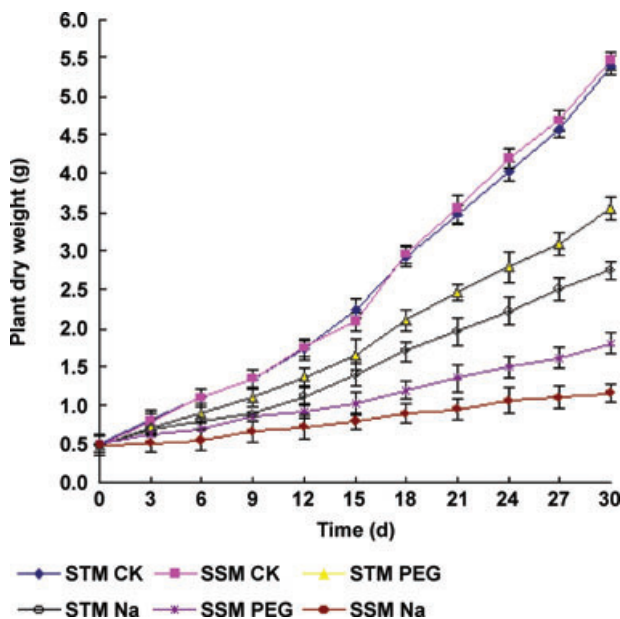


Figure 3. Effects of NaCl and iso-osmotic polyethylene glycol (PEG) on plant dry weight of salt-tolerant maize (STM) and salt-sensitive maize (SSM).

Seedlings were treated with NaCl and iso-osmotic PEG for 15 d. Vertical bars indicate standard errors of means ($n = 3$).

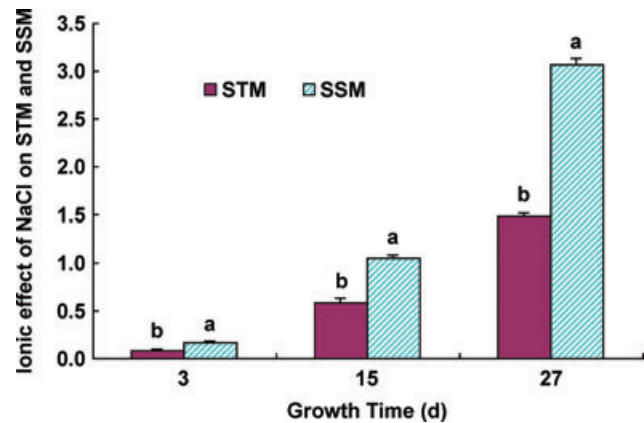


Figure 4. Ionic effect of NaCl on salt-tolerant maize (STM) and salt-sensitive maize (SSM).

The numbers calculation is according to results of **Figure 2**. Ionic effect is dry weight of control minus that of plants treated by polyethylene glycol (PEG). Means of each time course having different letter are significantly different at $P < 0.05$. Vertical bars indicate standard errors of means ($n = 3$).

Discussion

From the effect of NaCl stress on the dry matter accumulation of STM and SSM, we concluded that the responses of the two cultivars to salt stress were different. There was significantly higher dry matter accumulation by STM than SSM and the two response curves (**Figure 3**) differed from each other for the whole treatment period. Furthermore, from the responses of maize seedlings to 100 mM NaCl and iso-osmotic PEG, it was evident that NaCl had a greater effect than iso-osmotic PEG on both genotypes, suggesting that the greater effect of NaCl was due to the stress caused by the accumulation of the Na^+ and Cl^- .

The X-ray microanalysis on Na^+ and Cl^- relative contents in various compartments at different times under 100 mM NaCl showed that Na^+ and Cl^- , once applied to plants, enter apoplast, chloroplast, cytosol and vacuoles almost simultaneously (**Table 1**) and their contents are then positively correlated with the treatment time; it does not appear that ions first enter vacuoles, then other organelles only after vacuoles are saturated with Na^+ and Cl^- , as suggested by Munns (1993). Moreover, it was found that the relative contents of Na^+ and Cl^- in the apoplast were even higher than those in the vacuoles (especially for SSM) (**Table 1**), which reminds us of the argument put forward by Oertli (Oertli 1968) that salt stress on plants is mainly due to the accumulation of Na^+ and Cl^- in the apoplast where salts are easily accumulated before they can enter cells, a view supported by Flowers et al. (1991). We

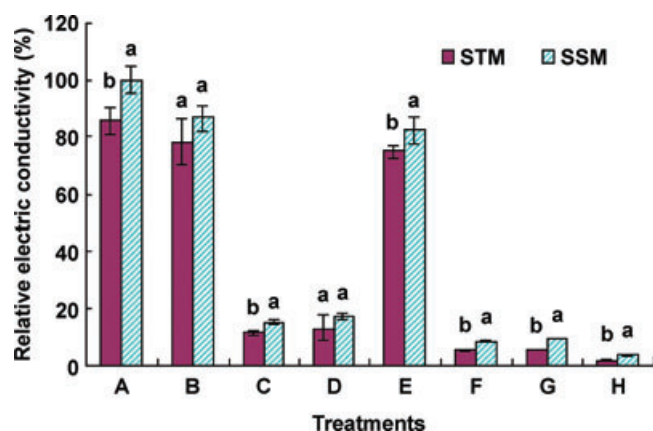


Figure 5. Effects of different inorganic and organic chemicals on the relative electric conductivity of leaves in salt-tolerant maize (STM) and salt-sensitive maize (SSM) seedlings after treating for 30 min.

- (A) NaCl.
 (B) KCl.
 (C) CaCl₂.
 (D) MgSO₄.
 (E) Na₂SO₄.
 (F) Sorbitol.
 (G) polyethylene glycol (PEG).
 (H) in ion-free water.

Means of each treatment having different letter are significantly different at $P < 0.05$. The vertical bars indicate standard errors of means ($n = 5$).

also argue that the reason the salt tolerance of STM is greater than that of SSM, is because the relative contents of Na⁺ and Cl⁻ of STM in vacuole and apoplast were higher than that of SSM. In addition, **Figure 1** shows that photosynthetic rates of maize seedlings dropped continuously, presumably mainly as a result of decreases in Rubisco activity (Zhao and Feng 1998) and glycolipin content in thylakoid membrane (Muller 1978) and the damage to the chloroplasts (Rao and Rao 1986).

Where plants were grown with additional pressure, to counteract the osmotic effect of NaCl (100 mM NaCl), the dry matter accumulation of both STM and SSM was higher than that without pressure, and the dry matter accumulation of STM was higher than that of SSM (**Table 2**). The total effect of 100 mM NaCl on STM and SSM from the results of **Table 2** were 1.01 and 1.44, respectively, after treatment for 15 d (**Figure 2**). The osmotic effect of STM and SSM were 79.1% and 68.8%, respectively, and the ionic effects of STM and SSM were 20.9% and 31.3%, respectively. The total effect of NaCl

on SSM was more than SSM. The main reason is that salt tolerance of STM is bigger than SSM. This result suggests that the osmotic effect is greater than the ionic effect of salt stress and the osmotic effect between STM and SSM is different in the first phase, which is different from Munns' two-phase hypothesis. Again, osmotic effect of NaCl on plant growth is very large in the beginning in saline stress. Otherwise, we can get similar results from **Figure 2**, where plants grown in NaCl and iso-osmotic PEG, only by osmotic stress, the dry matter accumulation of STM was higher than SSM in NaCl. These differences in growth were apparent, indicating that the salt-tolerance of the two lines were different from the beginning to the end; the ionic and osmotic stresses simultaneously inhibited the growth processes of both STM and SSM. These are different from Munns' results: the growth response to NaCl of salt-sensitive plant (SSP), medium salt-tolerant plant (MSTP) and salt-tolerant plant (STP) is the same in phase 1 (Munns 1993; Munns et al. 1995). Otherwise, we can calculate the ionic effect of NaCl on the growth of STM and SSM (dry weight of control minus dry weight in PEG) from **Figure 3**, which gradually increased with growth times (**Figure 3**). This proved that ionic effect always inhibits plant growth as osmotic effect in the first phase and ionic and osmotic effects of NaCl all inhibit plant growth simultaneously in the two phases.

Where leaf disks were exposed to a range of iso-osmotic solutes, damage to the plasmalemma permeability varied between solutes (**Figure 5**), suggesting that even when the osmotic stress was constant, specific ions differ in their toxicity. We suggest from this experiment that a basic toxic reaction to salt may be the production of lesions in membranes – generally lesions in the plasmalemma – which result in leakage of solutes from the cells. Disturbances of the membranes of chloroplasts might account for the observed damage to photosynthetic effectiveness with salt stress (**Figure 1**); a similar disturbance to mitochondria might account for any alteration to respiratory effectiveness with salt stress. Moreover, the effect of NaCl on the permeability of the plasmalemma was greater than that of PEG after just 30 min, suggesting specific ions-effects occur soon after the beginning of salt treatment.

From the above results, we may summarize that the ionic and osmotic effects of NaCl stress growth of STM and SSM are presented in phase 1 and phase 2 simultaneously. Otherwise, the osmotic effect of NaCl on different salt-tolerant plants is also different in the first phase, i.e., the ability of tolerance to osmotic stress in salt-tolerant plants is bigger than the less salt-tolerant plants in phase 1, as well as the ionic effect of NaCl on plant growth is to gradually increase from phase 1 to phase 2, as mentioned by Munns. Obviously, there is not enough evidence of the hypothetical two-phase growth response to salt. More careful experiments need to be undertaken to ensure more evidence is obtained.

We deduce that the course of ion stress is different from osmotic stress under salt stress; ion stress is at its lowest level at the beginning, and then rises continuously due to the quick entrance and accumulation of Na^+ and Cl^- in the cells (**Tables 1, 2**). Finally, new homeostasis both within the cells and between tissues and their habitats are established. The osmotic stress negative value is the highest level at the beginning, and then is reduced to a fixed level due to the permeance of outside inorganic solutes as Na^+ , Cl^- and the synthesis of organic solutes as the organic acids (e.g. proline etc). Otherwise, the ability of tolerance to osmotic stress and that of tolerance to ionic stress are produced simultaneously in plants and have relation to salt tolerance of plants.

It was more recently reported that the determinants of salt-tolerance are effector molecules and regulatory molecules that contain many known and unknown molecules, so the differentiation of plant salt tolerance is great and the factors of influence on the salt-tolerance are also significant. The salt tolerance of plants is a very complex biological characteristic, so it is difficult to explain whether to use one or several physiological and ecological changes (Hasegawa et al. 2000; Zhu 2001; Flowers and Colmer 2008).

Materials and Methods

Plant materials and culture

Salt-tolerant maize (STM) Ludan 850 and salt-sensitive maize (SSM) Ludan 961 were provided by the Maize Institute, Shandong Academy of Agriculture Science. Having been sterilized with 0.1% HgCl_2 and washed with tap water, selected caryopses were immersed in distilled water for 4 h and then sown in 10 cm-diameter plastic pots filled with sand, and kept moist by hand-watering with ion-free water until emergence. The plants in pots were used for X-ray microanalysis, the measurement of photosynthetic rate and plasmalemmal permeability. Other seeds, previously treated in the same way, were sown in a plastic container (50 × 35 × 20 cm) filled with sand, and again kept moist by hand-watering with ion-free water until emergence. When the seeds germinated and the first foliage leaf was fully expanded, seedlings were transplanted into holes in a sheet of plastic foam (50 × 20 × 2 cm), with one plant per hole; and then the plastic foam was supported over a large enamel container with full-strength Hoagland solution No. 2 supplemented with CaCl_2 to give a molar ratio of Na:Ca of 15:1, and aerated by an air pump. Once the third leaf of the seedlings was fully expanded, a solution of NaCl was added at a daily increase of 25 mM until the desired levels were reached. Alternatively a solution of PEG was added, whose daily increase was iso-osmotic to the NaCl treatment. The PEG used had a mean MW of 6000 and a concentration of

24.5 mM, and was found to exert the same osmotic pressure as 100 mM NaCl as measured with a freezing point osmometer). The plants grown in sand were used for the pressurization of iso-osmotic NaCl and PEG treatments. Each treatment solution was changed every 3 d (three plants per pot as one replicate) for each treatment. All seedlings were grown in a greenhouse (25–30°C/10–20°C day/night, 13:11 h light : dark cycle, photo-synthetic photon flux density [PPFD] 500–550 $\mu\text{mol}/\text{m}^2$ per s, relative humidity 70%).

Na^+ and Cl^- relative contents in leaves and roots by X-ray microanalysis

Seedlings were cultured in full-strength Hoagland solution for 15 d. Subsequently, seedlings were treated with 100 mM NaCl for 0, 1, 3 or 6 d, and samples taken from plants in five pots (five replicates). Sample preparation followed Fritz (1989) and Li and Fritz (1990). Pieces (5 × 5 mm) of fully-mature leaves 4 cm from the tip (where vacuoles have formed in leaves) were cut near the midrib. The tissue samples were put into small cages of aluminum foil and plunged into isopentane and propane (v/v: 1/3) cooled with liquid N_2 , for rapid freezing, and then freeze-dried (freeze-drier Japan, Yamata). Modified T-shaped valves were used for infiltration with diethyl ether under vacuum at 27 °C for 24 h after freeze-drying. The samples were then cut into small pieces suitable for sectioning with an ultra microtome and infiltrated with styrene-methacrylate, transferred into gelatin capsules and polymerized at 60 °C for 7 d. Embedded materials were cut in a dry state with an ultra microtome. Slices were coated with carbon. The sections prepared in this way were suitable for the analysis of Na^+ distribution because in the visible image the tonoplast distinctively separated the vacuole and cytoplasm.

The slices were examined with a HITACHI-H800 transmission electron microscope (Hitachi, Tokyo, Japan) assembled with an EDAX-910 energy-disperse X-ray analyzer. The accelerating voltage was 120 kV with a take-off angle of 258. The counting time for all analyses was 60 s. The data were expressed as counts per second (CPS) of an element peak after subtraction of the background. In the cells of epidermis and mesophyll, the analysis was made in the vacuoles and cytoplasm. For each micro-area seven measurements were carried out and replicated five times; the results are presented as averages.

Measurement of photosynthetic rate

Maize seedlings were transplanted to pots with sand when the third leaf was fully expanded, where they then received half-strength Hoagland solution for 3 d and full-strength Hoagland solution for 4 d, before being treated with 0 and 100 mM NaCl for 1, 3 and 6 d. The photosynthetic rate of the third leaf

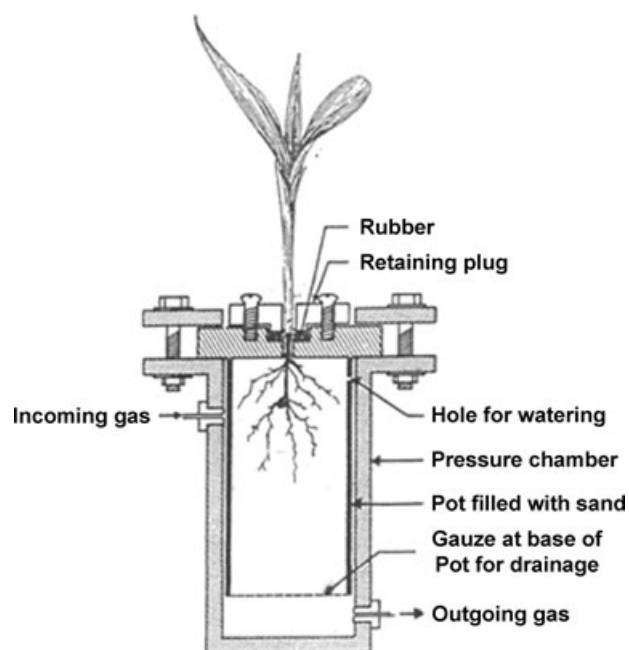


Figure 6. Section through pressure chamber to show a maize seedling sealed to the top of a pot placed inside the pressure chamber in the experiment.

Six such chambers were connected in series. An air: N_2 mixture, at a ratio that would provide a partial pressure of O_2 equal to atmospheric, which was bled continuously through the system to remove respired CO_2 .

was determined at different times with Li-6400 photosynthesis measurement system with five replicates.

Pressurization treatments

Maize seedlings, with shoots of 10–15 mm in height, were transplanted into specially made pots. All pots were packed with sand and received half-strength and full-strength Hoagland solution for several days, and then one pot was placed in a pressure chamber (see **Figure 6** and detailed information from Passioura and Munns 1984; Termaat et al. 1985; Zhao et al. 1991). Pressure was applied as an air: N_2 mixture, at a ratio that would provide a partial pressure of O_2 equal to atmospheric. The chambers were ventilated at a rate that would remove respired CO_2 . The applied pressure was of the same value as the osmotic pressure of the NaCl in the growing medium, namely 0.44 MPa, which counteracted the osmotic action of NaCl. The NaCl was increased at 25 mM per day, as was the appropriate applied pressure. Pressure was applied for 15 d to plants at 100 mM NaCl, and for control at 0 mmol/L with three replicates per treatment. This treatment ensured that leaves were always fully turgid.

Salt-tolerant maize and salt-sensitive maize treated by NaCl (100 mM) and iso-osmotic PEG

The salt-tolerant maize (STM) and salt-sensitive maize (SSM) were treated with NaCl and iso-osmotic PEG (MW 6000, concentration was 24.5 mM) and sampled after 15 d (all treatments in three replicates). Whole plants were carefully removed from the plastic plates and pots and their fresh weights measured immediately, after which the plants were dried in an oven at 105 °C for 10 min to destroy the enzyme activity, and then were kept at 80 °C for 24 h before the dry weights were determined with three replicates.

Determination of plasmalemma permeability of leaf cells in STM and SSM seedlings

Seedlings were cultured in full-strength Hoagland solution for 15 d. Leaf discs of STM and SSM were removed from the second leaves of seedlings in the control group with a punch and immersed for 30 min (Leopold and Willing 1984) in 100 mM NaCl and iso-osmotic KCl, $CaCl_2$, Na_2SO_4 , Mg_2SO_4 , Sorbitol as well as PEG. After the leaf discs were removed, the electrical conductivity of the solutions was measured with a conductivity meter (Orion 150A), then the solution with the leaf discs was boiled for 10 min and the total conductivity was measured again. The plasmalemma permeability was expressed as the relative electrical conductivity: the ratio of original conductivity to the total conductivity (Li 1999). Five replicates were used in each treatment.

Statistical analysis

Data were subjected to a One-way ANOVA using the SAS software (SAS Institute Inc. 1989).

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References

- Breckle SW** (1995) How do halophytes overcome salinity? In: Khan MA, Ungar IA, eds. *Biology of Salt Tolerance Plant*. Chelsen, Michigan, USA. pp. 199–213.

- Flowers TJ, Colmer TD** (2008) Salinity tolerance in Halophytes. *New Phytol.* **179**, 945–963.
- Flowers TJ, Hajibagheri MA, Yeo AR** (1991) Ion accumulation in the cell walls of rice plants growing under saline conditions: evidence for the Oertli hypothesis. *Plant Cell Environ.* **14**, 319–325.
- Flowers TJ, Troke PF, Yeo AR** (1977) The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.* **28**, 89–121.
- Fritz E** (1989) X-ray microanalysis of diffusible elements in plant cells after freeze-drying; pressure infiltration with ether and embedding in plastic. *Scanning Microsc.* **3**, 517–526.
- Greenway H, Munns R** (1980) Mechanisms of salt tolerance in non-halophytes. *Annu. Rev. Plant Physiol.* **31**, 149–190.
- Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ** (2000) Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51**, 463–499.
- Leopold AC, Willing RP** (1984) Evidence for toxicity effects of salt on membranes. In: Staples RC, Toenniessen GH, eds. *Salinity Tolerance in Plants: Strategies for Crop Improvement*. Wiley, New York. pp. 67–76.
- Li Q, Fritz E** (1990) X-ray microanalysis of ion content in stem tip meristem and leaves of *Populus* grown under potassium and phosphorus deficiency. *J. Plant Physiol.* **136**, 61–65.
- Li ZG** (1999) Determination of plant plasmalemma permeability. In: Institute of Plant Physiology and Shanghai Society of Plant Physiology, eds. *Modern Experimental Guide of Plant Physiology*. Science Press, Beijing. pp. 302–303 (in Chinese).
- Muller KA** (1978) Santarius: changes in chloroplast membrane lipids during adaptation of barley to extreme salinity. *Plant Physiol.* **62**, 326–333.
- Munns R** (1988) Causes of varietal differences in salt tolerance. In: Sinha SK et al., eds. *Proceedings of the International Congress of Plant Physiology*, Vol. 2. Greated Kalla New Delhi, New Delhi. pp. 960–968.
- Munns R** (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant Cell Environ.* **16**, 15–24.
- Munns R, Greenway H, Kirst GO** (1983) Halotolerant eukaryotes. In: Lange OL, Nobel PS, Osmond CB, Zeigler H, eds. *Encyclopedia of Plant Physiology*, Vol. 12c. New Series, Springer-Verlag, Berlin. pp. 59–135.
- Munns R, Schachtman DP, Condon AG** (1995) The significance of a two-phase growth response to salinity in Wheat and Barley. *Aust. J. Plant Physiol.* **22**, 561–569.
- Neumann P** (1997) Salinity resistance and plant growth revisited. *Plant Cell Environ.* **20**, 1193–1198.
- Oertli JJ** (1968) Extracellular salt accumulation, a possible mechanism of salt injury in plants. *Agrichimica* **12**, 161–169.
- Passioura JB, Munns R** (1984) Hydraulic resistance of plants, II. Effects of rooting medium and time of day in barley and lupin. *Aust. J. Plant Physiol.* **11**, 341–350.
- Rao GG, Rao GR** (1986) Pigment composition and chlorophyllase activity in pigment Pea and Gingelly under NaCl salinity. *Indian J. Exp. Biol.* **19**, 768–770.
- SAS Institute Inc** (1989) *SAS/STAT User's Guide*. SAS Institute INC., Cary, NC.
- Termaat A, Passioura JB, Munns R** (1985) Shoot turgor does not limit shoot growth of NaCl-affected wheat and barley. *Plant Physiol.* **77**, 869–872.
- Waisel Y** (1972) *Biology of Halophytes*. Academic Press, New York.
- Wang CY, Zhang XQ, Pan YS, Gong DY** (2000) Excellent salt and drought tolerant maize hybrid-Ludan 850. *Shandong Agr. Sci.* **3**, 47 (in Chinese).
- Yeo AR, Flowers TJ** (1984) Mechanisms of salinity resistance in rice and their role as physiological criteria in plant breeding. In: Staples RC, Toenniessen GH, eds. *Salinity Tolerance in Plants: Strategies for Crop Improvement*. Wiley, New York. pp. 151–170.
- Zhao KF** (1993) *Plant Salt-Resistance Physiology*. China Science and Technology Press, Beijing (in Chinese).
- Zhao KF, Feng LT** (1998) Try to review Munns' two phase hypothesis. In: Zou Q, Li D, eds. *Study on the Physiology of Culture Crops*. China Agricultural Technical Press, Beijing. pp. 69–72 (in Chinese).
- Zhao KF, Munns R, King RW** (1991) Absciscic acid level in NaCl-treated barley, cotton and salt bush. *Aust. J. Plant Physiol.* **18**, 17–24.
- Zhu JK** (2001) Plant salt tolerance. *Trends Plant Sci.* **6**, 66–71.

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