Salinity-alkalinity tolerance in wheat: Seed germination, early seedling growth, ion relations and solute accumulation

Jixiang Lin1,2, Xiaoyu Li2, Zhaojun Zhang1, Xingyang Yu2, Zhanwu Gao1,3, Ying Wang2, Junfeng Wang1, Zhuolin Li1 and Chunsheng Mu1*

1Key Laboratory of Vegetation Ecology of Ministry of Education, Institute of Grassland Science, Northeast Normal University, Changchun, 130024, China.
2Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, 130012, China.
3Department of geography, Baicheng Normal College, Baicheng, 137000, China.

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Germination and early seedling growth are the most critical stages for plant establishment in saline-alkaline soil. To gain a better understanding of relations between germination and seedling establishment, and the physiological adaptive mechanisms of the two stages under salt and alkali environments, wheat was tested by mixing two neutral salts (NaCl:Na2SO4) and two alkaline salts (NaHCO3:Na2CO3). Results showed that reductions of germination percentage and seedling growth were greater under alkali stress. Nongerminated seeds germinated well after being transferred from higher salinity and lower alkalinity to distilled water. The Na+ concentration and Na+/K+ ratio increased much greater under alkali stress, especially in root. The K+ concentrations in both shoot and root were not affected under salt stress, but decreased under alkali stress. Proline and soluble sugars were the primary organic osmolytes under the two stresses. Our results suggest that the deleterious effects of alkali stress are more severe than salt stress. Higher Na+ concentration in root is an important feature of wheat tolerance of salinity-alkalinity at early seedling stage. The different saline-alkaline tolerant abilities between germination and early seedling stage indicate that for wheat, successful germination does not ensure seedlings can survive in saline-alkaline soil of Northeast China.

Key words: Alkali stress, germination recovery, osmotic adjustment, physiological change, salt stress.

INTRODUCTION

Saline-alkaline soil covers 25% of soil area over the world. Excessive soil salinity and alkalinity become widespread increasing environmental problem, which causes growth inhibition and production decrease for most plants. Soil salinization and alkalinization always happen simultaneously, such as in Northeast China, alkaline meadow occupies more than 70% of land area and this number is still expanding (Kawanabe and Zhu, 1991). NaCl, Na2SO4, NaHCO3 and Na2CO3 are the main harmful salts; they all come from neutral salts and alkaline salts in the soil. Previous studies have proved that alkaline salt stress is quite different from neutral salt stress and should called alkali stress and salt stress, respectively (Shi and Yin, 1993). The impact of salt stress generally contains osmotic and ionic effects. However, alkali stress added the influence of high pH, which can inhibit ion uptake and disrupt ionic balance of plant cells (Yang et al., 2007).

Seed germination and early seedling growth are considered as the most critical stages of plant under extreme conditions (Kitajima and Fenner, 2000). Germination is the initial of life cycle and always determines where and when seedling can establish.
Higher salinity induces a reduction, a delay and even a complete inhibition of germination due to an osmotic effect or/and ion toxicity (Khaje et al., 2003). So seeds under salt conditions always germinate after high precipitation where soil salinity is reduced because of leaching (Khan and Ungar, 1986). To date, majority of studies to examine salinity effects on seed germination have usually been carried out with individual salts (especially NaCl) (Keiffer and Ungar, 1997; Khan et al., 2000), but little is known on the effect of alkaline stress on seed germination.

Generally, salt-alkali tolerance of seedlings is lower than seeds (Liu et al., 2010). For most plants, especially crops (such as wheat), the energy source for older seedling growth is mainly from photosynthesis and root absorption, while early seedlings are from endosperm. So the two stages are actually greatly differed. In addition, early seedlings seem more vulnerable because of the delicate radicles. There are numerous reports on the effects of salt stress and alkali stress on plant seedlings (Yang et al., 2007, 2008; Zhang and Mu, 2009). Older seedlings of wheat have also been reported (Yang et al., 2008; Guo et al., 2009; Li et al., 2009). But to our knowledge, few studies focus on the early seedlings of this species.

Wheat is an important crop over the world. In this paper, salt stress (4:1 molar ratio of NaCl:Na$_2$SO$_4$) and alkali stress (4:1 molar ratio of NaHCO$_3$:Na$_2$CO$_3$) were used to simulate nature conditions. The aims were (1) to compare the effects of salt stress and alkali stress on seed germination, germination recovery, growth and physiological responses in shoots and roots of wheat early seedlings, and (2) to test that wheat is more tolerant to salinity and alkalinity during seed germination than early seedling stage, successful germination of this species does not ensure seedlings can survive in the saline-alkaline soil of Northeast China.

**MATERIALS AND METHODS**

**Plant material and stress treatment conditions**

The experiment material wheat is (*Triticum aestivum*) cv. Jimai 3. Seeds were surface sterilized in 0.58% sodium hypochlorite solution for 2 min and subsequently washed with distilled water and air-dried to avoid fungus attack before being used in the experiment.

Two neutral salts (NaCl:Na$_2$SO$_4$) and two alkaline salts (NaHCO$_3$:Na$_2$CO$_3$) were both mixed in a 4:1 molar ratio and applied to the salt and the alkali stress groups, respectively. In germination tests, within each group, five salt concentrations (100 to 500 mM) were applied. The pH values of treatment solutions ranged from 6.60 to 6.90 in the salt stress groups and from 9.43 to 10.05 in the alkali stress groups. In seedling growth tests, 50 to 500 mM was applied within each group. In physiological responses of early seedling test, three concentration treatments were applied within salt stress: 50, 100 and 200 mM and 50, 100 mM within alkali stress. The pH values of treatment solutions ranged from 6.55 to 6.70 in the salt stress groups and from 9.41 to 9.50 in the alkali stress groups.

**Germination test**

Seeds were sown in 11-cm Petri dishes moistened with 12 ml of treatment solution and distilled water was used as control. Four replicates with 50 wheat seeds were used for each treatment. The petri dishes were maintained at 20°C with a 12 h photoperiod (Sylvania cool white fluorescent lamps, 200 μmol m$^{-2}$s$^{-1}$, 400 to 700 nm) in the growth chambers (HPG-400, Haerbin, China). Distilled water equal to the mean loss from dishes was added twice everyday. Seeds were considered to be germinated with the emergence of the radicle. Germination percentage was recorded everyday for 7 days. Nongerminated seeds from all the treatments were then transferred to distilled water to study the recovery of germination, which was also recorded everyday for 7 days.

Germination rate was estimated by using a modified Tims index of germination velocity, $\Sigma G/t$, where $G$ is the percentage of seed germination every day, $t$ is the total germination period (Khan and Ungar, 1984). The maximum value with our date was 100 (that is, 700/7). The recovery percentage was calculated according to the number of germinated seeds after being transferred to distilled water divided by the number of nongerminated seeds under salt and alkali stresses.

**Early seedlings growth test**

For evaluation of the effects of salt and alkali stresses on early seedling growth, the seeds were incubated initially in distilled water at 20°C with a 12 h photoperiod, when the root length reached 4 cm, these germinated seeds (25 per replicate) were then incubated with salt and alkali stresses treatment solutions (uniform early seedlings), early seedling growth was ended after 4 days and mean shoot lengths and root lengths were recorded.

**Harvest and pretreatment of wheat early seedlings**

Early seedlings of wheat were then harvested and washed with deionized water three times. Roots and shoots were separated and oven-dried at 105°C for 15 min and then dried at 65°C for 48 h to a constant weight. Dry shoot and root samples (50 mg per petri dish) were treated with 10 ml deionized water at 100°C for 1 h, the homogenate was centrifuged at 3000 g for 10 min and the filtrate was used to determine the contents of Na$, ^+$, K$, ^+$ and soluble sugars. Another 50 mg dry shoot and root samples per petri dish was treated with 10 ml of 3% (w/v) aqueous sulfosalicylic acid and the extractant was used to determine the proline content.

**Determination of inorganic ions and organic solutes**

For the analysis of Na$, ^+$ and K$, ^+$, the measurements were undertaken using an atomic absorption spectrophotometer (TAS-990; Purkinje General, Beijing, China). The contents of proline and total soluble sugars were measured using ninhydrin and anthrone, respectively, according to (Zhu et al., 1983). All soluble contents were expressed in mmol g$^{-1}$ dry weight (DW).

**Data analysis**

All parameters were analyzed by one-way ANOVAS using SPSS 13.0 (SPSS Inc, Chicago, IL, USA). The means and standard errors (SE) were reported. The level of statistical significance was $P < 0.05$. 
Figure 1. Effects of salt and alkali stresses on the (a) germination percentage and (b) germination rate of wheat seeds. A. alkali stress; S, salt stress. The values are the means of four replicates. Means followed by different letters are significantly different at $P<0.05$ according to a least significant difference test.

Table 1. Effect of salt stress and alkali stress on germination percentage and recovery percentage of wheat seeds.

<table>
<thead>
<tr>
<th>Salinity (mM)</th>
<th>Salt stress</th>
<th>Alkali stress</th>
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<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>R</td>
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<tr>
<td>0</td>
<td>96.0±1.6a</td>
<td>0a</td>
</tr>
<tr>
<td>100</td>
<td>93.0±1.2ab</td>
<td>0a</td>
</tr>
<tr>
<td>200</td>
<td>89.5±2.5bc</td>
<td>0a</td>
</tr>
<tr>
<td>300</td>
<td>82.7±4.2c</td>
<td>26.9±1.8b</td>
</tr>
<tr>
<td>400</td>
<td>64.7±2.3d</td>
<td>58.3±5.1d</td>
</tr>
<tr>
<td>500</td>
<td>46.7±1.2e</td>
<td>43.7±7.5c</td>
</tr>
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</table>

Mean (±s.e.: n=4) germination (%) indicating the initial salinity effect (S); recovery (R) after 7d of transfer to distilled water; and total germination (T).

RESULTS

Effects on seed germination and germination recovery

Germination percentage decreased with increasing salinity and alkalinity, but the extent of reduction under alkali stress was much greater than that under salt stress (Figure 1A). Maximum seed germination was obtained in distilled water. Under salt stress, when salinity was below 200 mM, no significant change in germination percentage was observed. When salinity rose above 200 mM, germination percentage decreased with rising salinity ($P<0.05$). Under alkali stress, germination percentage was significantly decreased with increasing alkalinity ($P<0.01$). Under the highest salinity and alkalinity stress (500 mM), only 46.7 and 22.0% of seeds could germinate in each treatment.

Germination rate significantly decreased with increasing salinity under both stresses, and also more markedly under alkali stress ($P<0.01$, Figure 1B). Minimum value also occurred at the highest concentration of each stress treatment, which was 24.0 and 11.0, respectively.

After 7 days of stress treatment, seeds were then transferred to distilled water to determine the recovery of germination. The results presented in Table 1 showed that there was no recovery of the seed germination at lower salt concentrations (100 and 200 mM) and higher alkali concentrations (400 and 500 mM), while under higher salt stress (300, 400, and 500 mM), recovery percentages of germination were 26.9, 58.3 and 43.7%, respectively, and were 21.3 and 25.2% under lower alkali stress (100 and 200 mM). When alkali concentration was 300 mM, only 6.6% of seeds could show germinate recovery, and seeds showed no capacity for recovery with a further increase in alkalinity.
**Effects on growth of early seedlings**

With increasing salinity, the growth of shoot and root of early seedlings was inhibited significantly, more under alkali stress than that under salt stress. Root elongation was significantly decreased under both stresses ($P < 0.01$) and no root elongation occurred at 200 mM of salt stress or 100 mM of alkali stress. Shoot elongation was not so sensitive like root under both stresses, which was not affected at lower salt stress (50 and 100 mM) and alkali stress (50 mM), but was completely inhibited at higher salt (500 mM) and alkali (400 mM) concentrations (Figure 2A and B).

**Effects on the inorganic ions and organic solutes in shoots and roots**

The Na$^+$ concentrations in shoot and root increased with the increasing salinity under both stresses ($P < 0.05$). The extents of the increases were similar under both types of stress in the shoot but were much higher under alkali stress than salt stress in the root. When salinity was 100 mM, compared with controls, the Na$^+$ concentration in root increased 7.7- and 17.8-fold under salt and alkali stresses, respectively (Figure 3A and B). Na$^+$ concentration in root was also significantly higher than that in shoot at the same salinity ($P < 0.01$). At the highest salinity of salt stress (200 mM) and alkali stress (100 mM), Na$^+$ concentration in roots was about 6.5- and 7.9-fold higher than in shoots, respectively. The K$^+$ concentrations in roots were not affected under salt stress both in shoot and root, but decreased under alkali stress (Figure 3C and D). The Na$^+$/K$^+$ ratios were also much greater under alkali stress than that under salt stress in both shoots and roots ($P < 0.01$), and also higher in roots than in shoots at the same salinity ($P < 0.01$), which were about 13.5- and 32.5-fold higher than in shoot at the highest salinity of salt stress (200 mM) and alkali stress (100 mM), respectively (Figure 3E and F).

The proline concentrations and soluble sugars concentrations were both increased with the rising salinity under salt and alkali stresses, and more for alkali stress than for salt stress ($P < 0.05$, Figure 4 A to D). The proline concentrations were similar in shoot and root, but the accumulation of soluble sugars concentrations in shoot was higher than that in root.

**DISCUSSION**

Low water potential caused by salt stress is the determining factor inhibiting seed germination (Debez et al., 2004). Our results showed that alkali stress also significantly affected seed germination, and the inhibiting degree was greater than that of salt stress (Figure 1A and B). This indicated that the interactions of Na$^+$ and high pH were more harmful to wheat seeds than that with only Na$^+$, which was consistent with previous reports (Shi and Yin, 1993). Germination rate can affect the speed and quality of seedling establishment. Several reports have indicated that rate of germination is more sensitive to salinity than to germination (West and Taylor, 1981; Dudeck and Peacock, 1985). In the present study, no significant change in germination percentage was observed under low salt stress (100 mM), while germination rate was significantly decreased ($P < 0.01$, Figure 1B).

Most seeds exposed to salinities and alkalinitities that are inhibited on germination will recover and germinate...
again after being transferred to distilled water (Zhang and Mu, 2009). Our results showed that nongerminated seeds of wheat germinated well after they were transferred from higher salinity concentrations (300, 400, and 500 mM) to distilled water. This may be an adaptive strategy of seed germination to salt stress, nongerminated seeds in a state of dormancy to escape from the rigorous environment (Debez et al., 2004). This phenomenon also indicated that high salinities only delayed germination process for most wheat seeds but did not cause them lose viability. But under alkali stress, the recovery of germination was very low and only occurred after they were transferred from lower alkalinity. Seeds lost viability and could not germinate again under higher alkalinity (Table 1). This is caused mainly by the interactions of high pH and higher alkalinity in alkali stress, which not only aggravated the effects of osmotic stress and ionic toxic on seeds but also decomposed seed structure and even destroys the embryo. This also shows that different inhibition mechanisms on seed germination of wheat between salt stress and alkali stress may exist, which deserves further research.

The root and shoot length is considered to be an important index of plant responses to salt-alkali stress. In present study, shoot and root length decreased with increasing stress intensity of both salt and alkali stresses, and the injurious effect caused by alkali stress was greater than that of salt stress (Figure 2A and B). Similar
results were also found in older seedlings of wheat by other authors (Li et al., 2009; Guo et al., 2009). The different injurious effects of the two stresses on seedling growth may be due to their different mechanisms. Alkali stress not only has the same stress factors as salt stress (low water potentials and ion toxicities), but also adds the influence of high pH. The high pH directly cause ions imbalance, metabolic disorders, and also create toxicity effects on endosperm, which is the nutrition source of early seedlings. Therefore, seedlings need to consume more energy and materials to adapt to alkali stress. Our findings also showed that wheat root of early seedlings were more markedly inhibited than shoot under both stresses conditions. This may be that when germination occurs, radicle break through the seed coat and contact with the saline-alkaline environment directly. Compared with shoot, root elongation is more sensitive to the stresses and is injured more severely.

Present study also indicated that salt-alkali tolerance in seed germination was not correlated with that in early seedling stage. Even under the highest concentration (500mM), there were still some seeds germinated under both stresses, but no root elongation occurred at relatively lower stress concentrations, indicating that germination stage is more saline-alkaline tolerant, which could be result from a lower absorption of salt-alkali component by seed, and germination process is also less responsive to high tissue sodium concentrations than seedlings growth. The result agrees with that of Meloni (2008) for Schinopsis quebracho Colorado. This means that successful germination, even at lower concentration in saline-alkaline soil of Northeast China, does not ensure seedlings that can survive for wheat. Low Na⁺ and high K⁺ in the cytoplasm are essential for the maintenance of enzymatic processes (Munns and Tester, 2008). Most plants usually absorb Na⁺ and simultaneously inhibit K⁺ absorption under salt-alkali conditions (Munns, 2002; Shi and Wang, 2005). Our results showed that the Na⁺ concentrations in shoot and root both sharply increased with rising salinity. However, the increased Na⁺ concentrations did not induce the decreased K⁺ concentrations in shoot and root under salt stress, indicating that a specific ion transport mechanism may exit between Na⁺ and K⁺ absorptions. This result is in contradiction to observations reported in older seedlings of wheat (Li et al., 2009; Guo et al., 2009). Physiological responses of this species between the two stages may be different and deserves further...
But under alkali stress, the K⁺ concentrations showed a downtrend. That may be attributable to an inhibitory effect of high pH on K⁺ absorption, which relies on the transmembrane proton gradient (Munns, 2002; Munns and Tester, 2008). We also found that Na⁺ concentration in root was much higher than that in shoot. The accumulation of Na⁺ in root indicate that there exists an inhibition mechanism of Na⁺ transport to aboveground organs (Zandstra-pom et al., 1998), preventing accumulation of toxic ions in the shoot, which is an important feature of wheat tolerance of salinity and alkalinity at early seedling stage. In addition, Na⁺/K⁺ ratio can also be used as a phyto-physiological parameter for salt-alkali tress (Keutgen and Pawelzik, 2008). Higher Na⁺/K⁺ ratio can affect ions regionalization, lead to ions imbalance, and also reduce enzyme activities. In our study, although salt stress did not affect K⁺ concentration, both stresses increase of Na⁺ concentration in shoot and root greatly raised Na⁺/K⁺ ratio, and especially in root, Na⁺/K⁺ ratio was significantly higher than that in shoot, indicating that the high pH caused by alkali stress enhance interference with the selective absorption of Na⁺-K⁺ in root and increase the Na⁺ to a toxic level (Yang et al., 2008). This may also explain some damage emerged under alkali stress especially in the root.

Plant can also synthesize compatible low molecular mass organic solutes, such as proline and soluble sugars, to protect biomacromolecules (Parida and Das, 2005). Our results showed that both proline concentrations and soluble sugars concentrations increased with rising salinity under the two stresses and more for alkali stress (Figure 4A and B). This suggested that the accumulation of proline and soluble sugars, as the major organic osmolyte, correlates closely with the intensity of the osmotic stress. Synthesis of proline and soluble sugars was not only related to the Na⁺ influx but also to the high pH. The role of them in salt-alkali tolerance of plants should be further investigated.

Conclusions

In summary, our study clearly showed that the deleterious effects of alkali stress on seed germination and early seedling growth of wheat were significantly greater than that of salt stress, which was mainly due to the high pH. Most seeds could germinate under lower salinity (200 mM) and alkalinity (100 mM), but root stopped growing under such conditions, indicating that salt-alkali tolerance of seedlings is much lower than the seeds, and germination do not ensure successful seedlings establishment. Higher Na⁺ concentration in root is an important feature of wheat tolerance of salinity-alkalinity at early seedling stage. It prevents high Na⁺ influx transport to the shoot. These findings have important practical application in planting wheat in salt-alkali soils, such as in Northeast China.

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