Effects of Various Mixed Salt-Alkaline Stress Conditions on Seed Germination and Early Seedling Growth of *Leymus chinensis* from Songnen Grassland of China

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Abstract

Soil salinization and alkalization always co-occur in grassland ecosystem, but little information exists concerning the mixed effects of salt-alkaline stresses on plants. *Leymus chinensis* is considered as one of the most promising grass species in Songnen Grassland of Northern China. In this study, we investigated the effects of 30 mixed salt-alkaline conditions (NaCl, Na₂SO₄, NaHCO₃, and Na₂CO₃, pH 7.10-10.18 and salinity 50-250 mM) on seed germination and seedling growth of *L. chinensis*. The results showed that germination percentage and rate were both decreased with increasing salinity and pH. Nongerminated seeds germinated well after being transferred to distilled water from treatment groups. Shoot and radicle growth were also affected by salinity, pH and their interactions. However, radicle length decreased more markedly with increasing salinity and pH, and was strongly inhibited when pH reached 8.05. Stepwise regression analysis results showed that salinity was the dominant factor for seed germination under mixed salt-alkaline stress conditions. However, once radicle break through the seed coat, and pH changed into the dominant factor for seedling establishment. These results indicated that mixed salt-alkaline stresses had different impacts on germination and early seedling stages of *L. chinensis*. A better understanding of the germination and seedling processes should facilitate the effective utilization of this species under such complex environment.

Keywords: salt-alkaline stresses, seed germination, germination recovery, shoot and radicle growth

Introduction

Soil salinization and alkalization is a major environmental problem throughout the world, which not only limits growth and production of plant but also results in land degradation. For example, arable land acreage of the world is 1.5×10⁹ ha, but 23% (0.34×10⁹ ha) of the area is saline, and 37% (0.56×10⁹ ha) is sodic. In Songnen Plains of Northeast China, approximately 70% of the natural grassland has been seriously degraded due to the impacts of salt and alkali soils, and this trend is still increasing (Zhang and Mu, 2009).

In most salt-alkaline soil of Northeast China, the main harmful salts are NaCl, Na₂SO₄, NaHCO₃, and Na₂CO₃, coming from neutral salts and alkaline salts (Yang *et al.*, 2008). Previous studies have proved that alkaline salt stress and neutral salt stress are differed greatly, and should be called alkali stress and salt stress, respectively (Shi and Yin, 1993). The deleterious effects of alkaline stress are more severe than salt stress (Yang *et al.*, 2008; Zhang and Mu, 2009; Guo *et al.*, 2009). In general, the impact of salt stress involves osmotic and ionic effects, alkali stress has same stress factors but added the influence of high pH, which can inhibit ion uptake and disrupt ionic balance of plant cells (Munns, 2002; Yang *et al.*, 2007). However, salinization and alkalization always co-occur in nature conditions, plant survive under salt and alkaline stresses are very complex (Shi and Wang, 2005; Li *et al.*, 2009). Therefore, mixed effect of salt stress and alkali stress should be further explored, not only that of salt stress or alkali stress.

Seed germination and seedling establishment are the most crucial stages for plant survival under saline-alkaline environment (Qu *et al.*, 2008). Seed germination can determine where and when seedling growth starts (Lin *et al.*, 2011). High salinities in the soil always result in both a delay and reduction of seed germination. It can also cause a complete inhibition of germination if salinity concentration is high enough. However, due to climatic factors such as rainfall or melted snow, salinity concentration in the soil always decreases, and thus provides a suitable condition to seed for germination. Consequently, most halophytes seeds remain viable at high salinity and germinate again until salinity concentration decreases (Ungar, 1991).

*Leymus chinensis* (Trin.) Tzvel. is a perennial rhizomatous forage grass of the family Poaceae. It is widely
distributed in the eastern region of the Eurasian steppes, the Northern and Eastern parts of the People’s Republic of Mongolia, the Northeastern China Plain, and the Inner Mongolia Plateau of China (Wang et al., 2004). This plant is rich in proteins, carbohydrates, minerals and is also highly tolerant to saline-alkaline soils (Huang et al., 2002). Due to its economic values and tolerant traits towards saline-alkaline stress, Leymus chinensis is considered as one of the most promising grass species for grassland rehabilitation and restoration in Northern China (Liu and Qi, 2004). However, to our knowledge, few studies focus on this species response to mixed salt-alkaline stresses especially for seed germination and early seedling stage. In this paper, mixtures of four salts NaCl, Na2SO4, NaHCO3 and Na2CO3 were used in various proportions to simulate 30 treatments of mixed salt-alkaline conditions. The aims were (1) to test the effects of mixed salt-alkaline stresses on seed germination and early seedling growth of L. chinensis, (2) to analyze the key factor among all the stress factors for the two stages.

Materials and methods

Plant material

Mature seeds (Thousand-seed weight is 2.4 g) of L. chinensis were collected from the Grassland Ecosystem Field Station, Institute of Grassland Science, Jilin province, China (123°44′E, 44°44′N). This area is characterized by a semi-arid, continental monsoonal climate. Mean annual precipitation and temperature are 300-450 mm, and from 4.6 to 6.4 °C. The soil type is mixed salt-alkali meadow soil (Zhang et al., 2009). Seeds were then stored in paper bags at 4 °C until further use.

Mixed salt-alkaline stress conditions

To simulate mixed salt-alkali conditions, two neutral salts (NaCl and Na2SO4) and two alkaline salts (NaHCO3 and Na2CO3) were chose according to the salt composition of the saline-alkaline soil in Northeast China (Peng et al., 2008). Four salts were mixed in various proportions based on the tolerance of salt-alkaline stress and the salinity and pH in Songnen Grassland. Six treatment groups (A-F) were set with increasing alkalinity. Salt composition and molar ratio of various treatments were shown in Tab. 1. Within each group, five concentrations were used (50, 100, 150, 200 and 250 mM). Total 30 mixed stress treatments (A1-F6, pH 7.10-10.18, and salinity 50-250 mM) were shown in Tab. 2.

Tab. 1. Salt composition and molar ratio of various treatments

| Treatments | NaCl (mM) | Na2SO4 (mM) | NaHCO3 (mM) | Na2CO3 (mM) |
|------------|-----------|-------------|-------------|-------------|
| A          | 2         | 0           | 0           | 0           |
| B          | 1         | 1           | 0           | 0           |
| C          | 9         | 9           | 8           | 1           |
| D          | 8         | 9           | 12          | 1           |
| E          | 12        | 1           | 8           | 9           |
| F          | 0         | 0           | 2           | 1           |

Tab. 2. Stress factors of various treatments

| Treatment | pH value | Salinity (mM) | Na+ (mM) | Cl- (mM) | SO4^2- (mM) | HCO3^- (mM) | CO3^2- (mM) |
|-----------|----------|---------------|----------|----------|-------------|-------------|-------------|
**Seed germination test**

Seeds were surface sterilized in 0.1% mercury chloride for 10 min, and then washed with distilled water and air-dried to avoid fungus attack before being used in our experiments.

Seeds were placed in 11 cm Petri dishes on two layers of 12.5 cm filter paper moistened with 10 mL of the treatment solutions. Four replicates of 50 seeds were used for each treatment and distilled water was used as a control. The Petri dishes were placed in growth chambers and maintained at 30/20 °C with 12 h photoperiod (Sylvania cool white fluorescent lamps, 200 μmol m⁻² s⁻¹, 400-700 nm, HPG-400, Haerbin, China). Seeds were considered germinated with the emergence of the radicle. Germination percentage was recorded every 2 days for 20 d. Nongerminated seeds from all the treatments were then transferred to distilled water to study the recovery of germination, which was also recorded at 2 d intervals for 20 d.

Germination rate was estimated by using a modified Timson index of germination velocity, ∑G/t, where G is the percentage of seed germination and t is the germination time (Lin et al., 2011). The maximum value with our data was 50 (i.e.1000/20). 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 saline and alkaline stresses.

**Seedling growth test**

For evaluation of the effects of mixed salt-alkaline stresses on early seedling growth, seeds were incubated initially in distilled water at 30/20 °C with 12 h photoperiod, when the coleoptile had just emerged. 20 of the early seedlings were then incubated with treatment solutions. Early seedling growth was ended after 15 d, and shoot lengths and radicle lengths were recorded.

**Data analysis**

All data were analyzed using SPSS 13.0 (SPSS Inc, Chicago, IL, USA). Tukey’s tests were performed for multiple comparisons to determine significant (p<0.05) differences between individual treatments. A two-way ANOVA was used to test the effects of the factors (pH and salinity) and their interactions on seed germination and seedling growth. The data were expressed as mean ± S.E. Stepwise regression analysis was performed for the key factor.

**Results**

Germination of *L. chinensis* seeds was significantly affected by pH, salinity and their interactions of the two factors (p<0.001, Tab. 3). Maximum seed germination percentage was obtained in distilled water, and seed reached the highest final percentage of 72.5% under this condition. Germination decreased with increasing salinity under all treatment groups, and sharply decreased in group E and F (Fig1, Tab. 4). At highest salt concentration (250 mM), germination percentage in group A was 6.5%, while seeds showed no capacity for germination in the other five treatment groups (B-F). In group F (highest pH/alkalinity), seed could not germinate when salinity was only 150 mM. However, in the other five treatment groups (A-E),

![Fig. 1. Germination (A), germination rate (B) of *Leymus chinensis* in each treatment. 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.](image)

| Source of variance | Germination (%) | Germination rate | Recovery percentage (%) | Shoot length (cm) | Root length (cm) |
|--------------------|----------------|-----------------|------------------------|-------------------|-----------------|
| Salinity           | 1898.4***      | 1614.7***       | 104.6***               | 239.92***         | 637.48***       |
| pH                 | 113.5***       | 59.7***         | 137.9***               | 72.08***          | 589.48***       |
| Salinity×pH        | 22.6***        | 16.8***         | 14.5***                | 5.54***           | 76.70***        |

Data represent F-values at 0.05 level. *** p<0.001
Germination percentages were 47.5%, 34.0%, 30.0%, 33.0% and 11.3%, respectively.

Germination rate was also significantly affected by pH, salinity and their interactions (p<0.001, Tab. 3). The downtrend with increasing salinity and pH was similar to that of germination percentage. Germination rate in group A was higher than other groups, and group E and F had the greatest reduction, which were 4.2 and 0 at 150 mM salinity, significantly lower than the other treatment groups (A-D) (p<0.05). Once salinity concentration ≥200 mM, germination rate were 0 in group E and F.

When nongerminated seeds were transferred from treatment solutions to distilled water, most of them germinated well. Recovery percentage was also affected by pH, salinity and their interactions (p<0.001, Tab. 3). Recovery percentages increased with increasing salinity in all the treatment groups, and reached highest at 250 mM salinity (Tab. 4).

Both shoot length and radicle length were affected by pH, salinity and their interactions (p<0.001, Tab. 3, Fig. 2). There was no shoot emerged at 250 mM salinity in all the treatment groups, and only for 200 mM in group E and F. Radicle length of L. chinensis seedlings under various mixed salt-alkaline stresses was significantly less than that of controls, especially under high pH groups. For example, radicle length was only 0.2 cm in group F even though salinity concentration was 50 mM.

![Fig. 2. Shoot length (A) and radicle length (B) of Leymus chinensis seedlings in each treatment. 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](image-url)
Main stress factors of mixed salt-alkaline stresses are shown in Tab. 2. The results of stepwise regression analysis showed that there was a high linear correlation between each index and stress factors (p<0.001, Tab. 5). Among the absolute values of the regression coefficients in germination indexes, salinity ($\beta_2$) was highest for the indexes, indicating that salinity was the dominant factor among the stress factors. While among the absolute values of the regression coefficients in radicle growth, pH was highest for the indexes, indicating that pH changed into the key factor on seedling establishment of L. chinensis.

**Discussion**

Seed germination is the initial and one of the most critical stages over the life cycle of plants. Lower water potential caused by salinity is the determining factor inhibiting seed germination under saline environment (Debez, et al., 2004). Our results showed that high pH also significantly affected seeds germination. Germination percentage and rate were both affected by salinity, high pH, and their interactions (Fig. 1, Tab. 3). Furthermore, L. chinensis seeds were more tolerant to only salt stress (A group) than mixed salt-alkaline stresses (B-F groups). This mainly due to that mixed salt-alkaline stresses not only had the same stress characteristics with salt stress, but also added the impact of high pH stress, so created more harmful effects. However, higher germination percentages were observed at lower salinity (50 mM) in all the groups even though under higher pH treatments (D-F). This phenomenon indicated that germination was not inhibited by high pH when salinity concentration was low, which implied that high pH had a particular adjustment function on seeds at lower salinity. However, once salinity was higher, interactions of high pH and salinity might decrease seed germination, decompose seed structure and even result in seed death, the specific reason deserves further research. Most nongerminated halophyte seeds under high salinity environments will recover and germinate again after being transferred to distilled water (Song et al., 2006; Qu et al., 2008). Our result that recovery percentage in L. chinensis seeds showed increasing trends with salinity increment in all the treatment groups supports this phenomenon. This indicated an adaptive strategy of seed germination to high salinity 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 L. chinensis seeds but did not cause them lose viability. In addition, there are several possible causes that nongerminated seeds at higher salinity stress recovery well. First, the lower salinity conditions always suitable to L. chinensis seeds and most of them can germinate quickly in order to avoid the competition on seedling stage. Second, high salinity may enhance the priming effect on the nongerminated seeds and increase seed vigor and germination ability.

Early seedling growth is another critical stage for plants survive in salt-alkaline conditions. Our results showed that although shoot growth well at lower salinity stress, but radicle growth was greatly retarded especially at high pH groups, indicating that the radicle of L. chinensis was more sensitive to salinity and pH stresses. The results agree with Li (2010) for Spartina alterniflora. In addition, we found that although seedling lengths decreased with increasing salinity in each treatment group, the treatment with larger proportion of alkalinity resulted in lower shoot and root length. Similar results have also been reported for other plants (Li et al., 2010; Peng et al., 2008). The reason of this phenomenon may be due to the effects of high pH and its interactions with ions stress. The high-pH environment surrounding the radicles of L. chinensis especially with high salinity stress can cause ions imbalance, metabolic disorders, and also destroys the structure and function of root cells, results in lower elongations of the seedlings (Shi and Yin, 1993; Shi and Wang, 2005).

The deleterious effects of mixed salt-alkaline stresses are more complex than that of single salt or alkali stress due to the various salt components and proportions (Liu and Zhu, 1997; Shi et al., 2002). The stress characters of salt stress on plant contains osmotic and ionic effects, alkaline stress has the same characters with salt stress and exerts the high pH stress. However, when the neutral salts and alkali salts are mixed together, the negative influence on plant is much greater because of the interactions of salinity and high pH or various ions. For example, the stress on germination index and seedling growth index due to single salinity stress (from A1 to A5) or single high pH stress (A1, B1, C1, D1, E1 and F1) was smaller than that of salinity interacted with high pH (D4, D5, F4 and F5) (Figs. 1, 2). As a result, the reciprocal enhancement between salt and alkali stresses is characteristic of mixed salt-alkaline stress (Shi and Wang, 2005).

From the results of stepwise regression analysis among the stress factors, it was obvious that salinity was the dominant factor on germination stage under mixed salt-alkaline stress conditions (Tab. 5). This mainly due to that salinity concentration in the soil determines the water potential, and water absorption is the critical factor for seed germination. Therefore, with increasing salinity, the potential surrounding the seed (difference potential inside and outside of the cell) gradually decreases, and it is difficult for seeds to absorb water under such circumstances, then higher salinity strongly affects germination percentage and germination rate of L. chinensis. However, we found pH

| Model          | R2  | ANOVA test | β1     | β2     | β3     | β4     | β5     | β6     |
|----------------|-----|------------|--------|--------|--------|--------|--------|--------|
| Germination (%)| 0.888 | p < 0.001 | -0.293 | -0.873 |
| Germination rate| 0.889 | p < 0.001 | -0.248 | -0.887 |
| Shoot length (cm)| 0.862 | p < 0.001 | -0.442 | -0.776 |
| Root length (cm)| 0.818 | p < 0.001 | -1.107 | -0.280 | -0.497 |

X=pH ; X=Salinity ; X= Cl ; X= SO$_4^{2-}$ ; X= HCO$_3$ ; X= CO$_3^{2-}$ ; β: Standard regression coefficients corresponding X= Xs, the greater the absolute β value, the stronger effect of the stress factor on every index. R$:^2$ the square of total correlation coefficient, n = 30
changed into the dominant factor for radicle growth, and strongly affected seedling establishment. This means that the mixed salt-alkaline stresses have different impacts on *Leymus chinensis* during germination and early seedling stage. Compared with seed germination, seedling stage is much more complex, radicle should absorb great number of nutrient elements and also maintain osmotic and ionic balance, and high pH can strongly inhibit ion uptake and disrupt ionic balance of plant cells. Therefore, once radicles break through the seed coat, and pH changes into the dominant factor for seedling establishment.

**Conclusion**

In summary, this study clearly showed that seed germination and seedling growth of *Leymus chinensis* were affected by salinity and high pH and their interactions. Nongerminated seeds germinated well after being transferred to distilled water, indicating that seeds of this species have recovery ability under salt-alkaline environment. Although radicle and shoot length were both inhibited by mixed salt-alkaline stresses, radicle growth was more sensitive to the stress. Salinity was the key stress factor for germination, while pH was the key stress factor for seedling establishment under mixed salt-alkaline stresses. The better understanding of the germination and seedling processes of *Leymus chinensis* should facilitate the effective utilization of this species under such complex environment.

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