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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, Na_2SO_4, $NaHCO_3$ and Na_2CO_3; 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 affacted 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 1.5×10^9 ha, but 23% (0.34×10^9 ha) of the area is saline, and 37% (0.56×10^9 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, Na_2SO_4, $NaHCO_3$ and Na_2CO_3, 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, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 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 ℃. 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 Na_2SO_4) and two alkaline salts ($NaHCO_3$ and Na_2CO_3) were chose according to the salt composition of the salt-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 (A_1-F_5, 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

	Salt composition and molar proportions			
Treatments	NaCl	Na_2SO_4	$NaHCO_3$	Na_2CO_3
A	2	1	0	0
B	1	1	1	0
C	12	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	Stress factors						
	pH value	Salinity(mM)	Na^+(mM)	Cl^-(mM)	SO_4^{2-}(mM)	HCO_3^- (mM)	CO_3^{2-}(mM)
A_1	7.10	50	66.7	33.3	16.7	0.0	0.0
A_2	7.14	100	133.4	66.6	33.4	0.0	0.0
A_3	7.20	150	200	100	50	0.0	0.0
A_4	7.24	200	266.8	133.2	66.8	0.0	0.0
A_5	7.28	250	333.5	166.5	83.5	0.0	0.0
B_1	7.80	50	66.7	16.7	16.7	16.7	0.0
B_2	8.05	100	133.4	33.4	33.4	33.4	0.0
B_3	8.22	150	200	50	50	50	0.0
B_4	8.28	200	266.8	66.8	66.8	66.8	0.0
B_5	8.35	250	333.5	83.5	83.5	83.5	0.0
C_1	8.90	50	66.7	20	15	13.3	1.7
C_2	8.92	100	133.4	40	30	26.6	3.4
C_3	8.96	150	200	60	45	39.9	5.1
C_4	8.98	200	266.8	80	60	53.2	6.8
C_5	9.02	250	333.5	100	75	66.5	8.5
D_1	9.05	50	66.7	13.3	15	20	1.7
D_2	9.10	100	133.4	26.6	30	40	3.4
D_3	9.15	150	200	39.9	45	60	5.1
D_4	9.18	200	266.8	53.2	60	80	6.8
D_5	9.24	250	333.5	66.5	75	100	8.5
E_1	9.56	50	66.7	20	1.7	13.3	15
E_2	9.60	100	133.4	40	3.4	26.6	30
E_3	9.64	150	200	60	5.1	39.9	45
E_4	9.69	200	266.8	80	6.8	53.2	60
E_5	9.76	250	333.5	100	8.5	66.5	75
F_1	9.90	50	66.7	0.0	0.0	33.3	16.7
F_2	9.94	100	133.4	0.0	0.0	66.6	33.4
F_3	9.98	150	200	0.0	0.0	100	50
F_4	10.12	200	266.8	0.0	0.0	133.2	66.8
F_5	10.18	250	333.5	0.0	0.0	166.5	83.5

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 12h photoperiod (Sylvania cool white fluorescent lamps, 200 μmolm^{-2}s^{-1},400-700 nm, HPG-400, Haerbin, China). Seeds were considered germinated with the emergence of the radicel. 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, $\sum G/t$, 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 (Fig.1, 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),

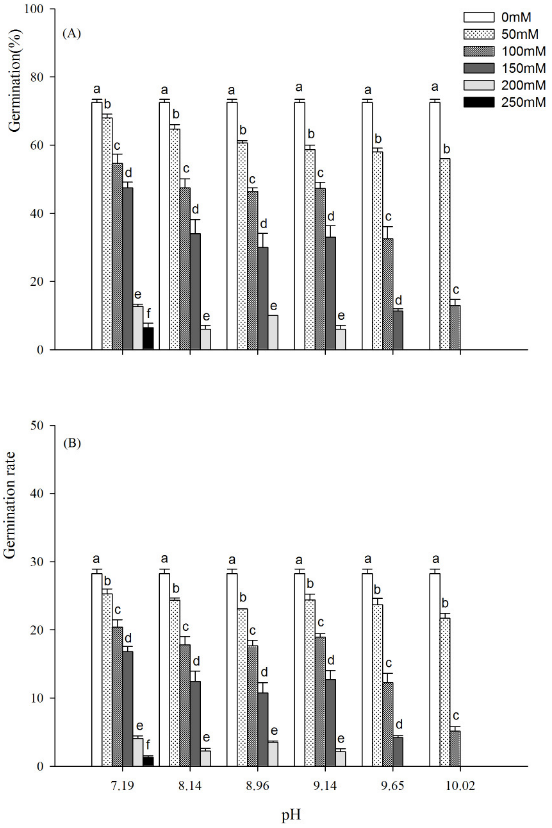

Fig. 1. Germination (A), germination rate (B) of *Leymus chinensis* in each treatment. The values are the means of four replicates. Means followed by different letters are significantly different at $p<0.05$ according to a least significant difference test

Tab. 3. Two-way ANOVA of effects of salinity, pH and their interactions on seed germination and seedling growth of *L. chinensis*

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. *** $p < 0.001$

Tab. 4. Seed germination situations under various treatments

Treatment/Average pH	Salinity (mM)	Seed germination (%, mean±s.e., *n*=4)		
		Initial germination (%)	Recovery percentage(%)	Total germination (%)
Control	0	72.5±1.0^{a}	0.0±0.0	72.5±1.0^{a}
A/ pH=7.19	50	68.0±1.2^{b}	4.9±0.6^{a}	69.6±0.8^{a}
	100	54.7±2.7^{c}	11.3±0.7^{b}	59.9±2.0^{b}
	150	47.5±1.7^{d}	19.0±1.0^{c}	56.7±0.7^{b}
	200	12.7±0.7^{e}	22.8±0.8^{d}	32.7±0.7^{c}
	250	6.5±1.3^{f}	32.8±1.3^{e}	36.0±1.2^{c}
B/ pH=8.14	50	64.7±1.3^{b}	5.0±0.6^{a}	67.5±2.3^{a}
	100	47.5±2.6^{c}	18.3±1.6^{b}	58.3±2.4^{b}
	150	34.0±4.2^{d}	37.0±1.3^{c}	58.0±2.0^{b}
	200	6.0±1.2^{e}	37.7±1.1^{c}	42.4±1.6^{c}
	250	0.0±0.0^{e}	40.7±1.3^{c}	40.7±1.3^{c}
C/ pH=8.96	50	60.7±0.7^{b}	13.6±1.8^{a}	64.7±0.7^{b}
	100	46.5±1.0^{c}	22.7±1.9^{b}	59.3±0.7^{c}
	150	30.0±4.2^{d}	35.3±0.6^{c}	54.7±2.9^{d}
	200	10.0±0.0^{e}	29.4±1.4^{cd}	36.5±1.3^{e}
	250	0.0±0.0^{f}	32.7±0.7^{d}	32.7±0.7^{e}
D/ pH=9.14	50	58.7±1.3^{b}	29.0±2.0^{a}	70.7±0.7^{a}
	100	47.3±1.8^{c}	39.1±2.0^{b}	68.0±0.0^{ab}
	150	33.0±3.4^{d}	43.4±3.3^{b}	64.0±3.8^{b}
	200	6.0±1.2^{e}	40.9±1.5^{b}	45.3±1.3^{c}
	250	0.0±0.0^{e}	44.5±1.5^{b}	44.5±1.5^{c}
E/ pH=9.65	50	58.0±1.2^{b}	38.1±2.4^{ab}	74.0±1.2^{a}
	100	32.5±3.6^{c}	35.3±1.1^{a}	53.3±1.3^{b}
	150	11.3±0.7^{d}	35.5±1.1^{a}	41.3±1.3^{c}
	200	0.0±0.0^{e}	38.0±2.6^{ab}	38.0±2.6^{c}
	250	0.0±0.0^{e}	44.0±3.0^{b}	44.0±3.0^{c}
F/ pH=10.02	50	56.0±0.1^{b}	41.9±1.8^{b}	74.4±1.3^{a}
	100	13.0±1.7^{c}	34.2±1.8^{a}	53.0±4.7^{b}
	150	0.0±0.0^{d}	32.5±1.0^{a}	32.5±1.0^{c}
	200	0.0±0.0^{d}	39.0±1.3^{b}	39.0±1.3^{c}
	250	0.0±0.0^{d}	47.5±1.7^{c}	47.5±1.7^{b}

Different letters indicate significant differences from different salinity concentrations (p < 0.05)

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.

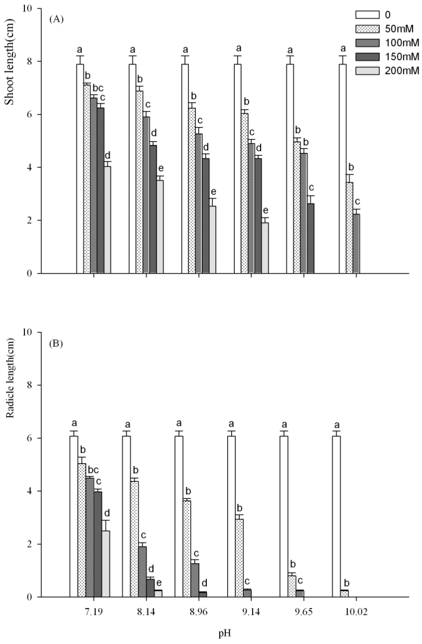

Fig. 2. Shoot length (A) and radicle length (B) of *Leymus chinensis* seedlings in each treatment. The values are the means of four replicates. Means followed by different letters are significantly different at $p<0.05$ according to a least significant difference test

Tab. 5. Results of stepwise regression between every index and stress factors

Model		R2	ANOVA test	β1	β2	β3	β4	β5	β6
Germination (%)	Y=137.766-0.299X2-7.463X1	0.888	$p < 0.001$	-0.293	-0.873				
Germination rate	Y=49.546-0.118X2-2.452X1	0.889	$p < 0.001$	-0.248	-0.887				
Shoot length (cm)	Y=17.677-0.027X2-1.161X1	0.862	$p < 0.001$	-0.442	-0.776				
Root length (cm)	Y=19.339-1.891X1-0.029X5-0.011X4	0.818	$p < 0.001$	-1.107			-0.280	-0.497	

X_1=pH ; X_2=Salinity ; X_3= Cl^- ; X_4= SO_4^{2-} ; X_5= HCO_3^- ; X_6= CO_3^{2-}. β_1-β_6: Standardize regression coefficients corresponding X_1- X_6, 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

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 (β_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 changed into the dominant factor for radicle growth, and strongly affacted seedling establishment. This means that the mixed salt-alkaline stresses have different impacts on *L. 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 *L. 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 *L. chinensis* should facilitate the effective utilization of this species under such complex environment.

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References

Debez A, Hamed BK, Grignon C, Abdelly C (2004). Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritime*. Plant Soil 262:179-189.

Grutterman, Y (1993). Seed germination in desert plants. Berlin: Springer-Verlag 169-206.

Guo R, Shi LX, Yang YF (2009). Germination, growth, osmotic adjustment and ionic balance of wheat in response to saline and alkaline stresses. Soil Sci Plant Nutri 55:667-679.

Huang ZH, Zhu JM, Mu XJ, Lin JX (2002). Advances on the mechanism of low sexual reproductivity of *Leymus chinensis*. Grassland China 24:55-60.

Li R, Shi F, Fukuda K (2010). Interactive effects of salt and alkali stresses on seed germination, germination recovery, and seedling growth of a halophyte *Spartina alterniflora* (Poaceae). South African J Bot 76:380-387.

Lin JX, Wang JF, Li XY, Zhang YT, Xu QT, Mu CS (2011). Effects of saline and alkaline stresses in varying temperature regimes on seed germination of *Leymus chinensis* from the Songnen Grassland of China. Grass For Sci 66:578-584.

Liu GS, Qi DM (2004). Research progress on the biology of *Leymus chinensis*. Acta Prataculturae Sinica 13:6-11.

Liu J, Zhu JK (1997). Proline accumulation and salt-stress-induced gene expression in a salt-hypersensitive mutant of arabidopsis. Plant Physiol 114:591-596.

Munns R (2002). Comparative physiology of salt and water stress. Plant Cell Envir 25:239-250.

Peng YL, Gao ZW, Gao Y, Liu GF, Sheng LX, Wang DL (2008). Eco-physiological characteristics of alfalfa seedlings in response to various mixed salt-alkaline stresses. J Integr Plant Biol 50: 29-39.

Qu XX, Huang ZY, Baskin JM, Baskin CC (2008). Effect of temperature, light and salinity on seed germination and radicle growth of the geographically widespread halophyte shrub *Halocnemum strobilaceum*. Ann Bot 101:293-299.

Shi DC, Wang DL (2005). Effects of various salt–alkali mixed stresses on *Aneurolepidium chinense* (Trin.) Kitag. Plant Soil 271:15-26.

Shi DC, Yin LJ (1993). Difference between salt (NaCl) and alkaline (Na_2CO_3) stresses on *Puccinellia tenuiflora* (Griseb.) Scribn.et Merr. Plants. Acta Bot Sin 35:144-149.

Shi DC, Yin SJ, Yang GH, Zhao, KF (2002). Citric acid accumulation in an alkali-tolerant plant *Puccinellia tenuiflora* under alkaline stress. Acta Bot Sin 44:537-540.

Song J, Feng G, Zhang FS (2006). Salinity and temperature effects on germination for three salt resistant euhalophytes, *Halostachys caspica*, *Kalidium foliatum* and *Halocnemum strobilaceum*. Plant Soil 279:201-207.

Ungar IA (1991). Ecophysiology of vascular halophytes. Boca Raton: CRC Press 9-48.

Wang ZW, Li LH, Han XG, Dong M (2004). Do rhizome severing and shoot defoliation affect clonal growth of *Leymus chinensis* at ramet population level? Acta Oecologica 26:255-260.

Yang CW, Chong J, Kim C, Li CY, Shi DC, Wang DL (2007). Osmotic Adjustment and ion balance traits of an alkali resistant halophyte *Kochia sieversiana* during adaptation to salt and alkali conditions. Plant Soil 294:263-276.

Yang CW, Shi DC, Wang DL (2008). Comparative effects of salt stress and alkali stress on growth, osmotic adjustment and ionic balance of an alkali-resistant halophyte *Suaeda glauca* (Bge.). Plant Growth Regul 56:179-190.

Zhang JT, Mu CS (2009). Effects of saline and alkaline stresses on the germination, growth, photosynthesis, ionic balance and anti-oxidant system in an alkali-tolerant leguminous forage *Lathyrus quinquenervius*. Soil Sci Plant Nutri 55:685-697.

Zhang JT, Mu CS, Wang DL, Wang JF, Chen GX (2009). Shoot population recruitment from a bud bank over two seasons of undisturbed growth of *Leymus chinensis*. Botany 87:1242-1249.