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PHYSIOLOGICAL ADAPTIVE MECHANISMS OF *LEYMUS CHINENSIS* DURING GERMINATION AND EARLY SEEDLING STAGES UNDER SALINE AND ALKALINE CONDITIONS

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ABSTRACT

Germination and early seedling growth are the most critical periods for plant establishment in saline-alkaline soil, the main factor limiting plant growth and production worldwide. To explore the physiological adaptive mechanisms of the two stages under saline and alkaline conditions, *Leymus chinensis* was tested by mixing two neutral salts (NaCl: Na_2SO_4) and two alkaline salts ($NaHCO_3$: Na_2CO_3). Results showed that reductions of germination and seedling growth were greater under alkaline stress. Nongerminated seeds germinated well after transferring from higher salinity and lower alkalinity to distilled water. The Na^+ concentration, Na^+/K^+ ratio increased but K^+ concentration decreased under both stresses, and the changes were greater under alkaline stress. Under saline stress, seedlings mainly accumulated Cl^- and soluble sugars to maintain osmotic and ionic balance. But alkaline stress restrained the absorptions of Cl^-, NO_3^- and $H_2PO_4^-$, enhanced organic acids, especially malic acid and citric acid to supply the shortage of inorganic anions, and accumulated more soluble sugars to cope with osmotic stress from the high Na^+ concentration. Thus, our results suggested that the deleterious effects of alkaline stress are more severe than saline stress, high salinities only delay most seeds germination, but high alkalinities cause them lose viability. Accumulating organic acids is the primary different adaptive mechanism to saline and alkaline stresses during early seedling stage of *Leymus chinensis*. A better understanding of the germination and early seedling processes should facilitate the effective utilization of this species under salt and alkali environments.

Key words: Seed germination, Germination recovery, Organic acid, Alkaline stress, Saline stress.

INTRODUCTION

Soil salinization and alkalization is a widespread increasing environmental problem over the world, which inhibits growth and production of most plants. It has been estimated that about 932 million hectares global land have been salinized and alkalinized (Rao *et al*., 2008). Soil salinization and alkalization frequently occur simultaneously especially in the arid and semiarid regions, where evaporation is higher than rainfall. Such as in Northeast China, alkaline meadow covers more than 70% of land area and is still expanding (Yang *et al*., 2007). The existence of alkaline stress has been demonstrated that it is more severe than saline stress (Yang *et al*., 2007, 2009; Zhang and Mu, 2009; Guo *et al*., 2009). Alkaline stress has the same stress factors as saline stress but added the influence of high-pH stress, which may inhibit ion uptake and disrupt the ionic balance of plant cells (Yang *et al*., 2007).

Adaptation to saline-alkaline environment during seed germination and early seedling stages is crucial for plant establishment (Qu *et al*., 2008). Germination stage always determines when and where seedling growth starts (Tobe *et al*., 2000). Most halophytes have high salt tolerance in seed germination, whereas seedling stage is always the most fragile (Kitajima and Fenner, 2000). Early seedlings seem more vulnerable because of the weak photosynthesis and their delicate radicles. So the adaptability of the two stages (early seedling stage and adult seedling stage) to the environmental conditions may also be greatly different. A better understanding of the germination processes and early seedling physiological metabolism of *Leymus chinensis* during adaptation to the saline and alkaline conditions is crucial for its use in grassland rehabilitation and restoration.

Most seeds are located near the soil surface, where salt accumulates in salinized locations.. High salinity inhibited seed germination due to an osmotic effect and ion toxicity (Khajeh *et al*., 2003). Increasing salinity always induces both a reduction and a delay of germination. It can cause a complete inhibition of germination if the salinity concentration is beyond the tolerance limits of the seed. However, salinity concentration at the surface of soil is always changes because of the rainfall, which can reduce the salinity concentration and provide enough water for seed germinating. Thus, for halophytes, seed must remain viable under high salinity conditions and germinate until salinity decreases (Qu *et al*., 2008).

So far, most of the studies exploring the effects of saline stress on plant seedlings are focused on NaCl, and only little attention has been paid to alkaline stress. In fact, physiological responses and adaptive strategies of plant seedlings under saline and alkaline stresses are always different. For example, under saline stress, plants always synthesize great number of organic solutes such as proline and soluble carbohydrates in the cytoplasm to resist osmotic stress, and also accumulate inorganic ions such as Cl^- in order to keep ion balance (Kerepesi and Galiba, 2000; Ashraf *et al*., 2007). While under alkaline stress, accumulation of organic acids is the main way to resist high pH stress. The regulation of organic acids plays a key role when plants encounter the alkaline environments. Not only the alkali-resistant plants such as *Kochia sieversiana* and *Suaeda glauca* but also the weak alkali- resistant plants such as wheat and barley accumulate organic acids under alkaline conditions (Yang *et al*., 2007; 2008ab; 2009; Li *et al*., 2009).

Leymus chinensis (Trin.) Tzvel. is a perennial rhizomatous species of the family Poaceae, which is the dominant and most promising grass species in Songnen Grassland of Northern China. This plant grows rapidly, and is highly tolerant to arid and saline-alkaline soils conditions (Huang *et al*., 2002). In this paper, various saline (9:1 molar ratio of NaCl: Na_2SO_4) and alkaline (9:1 molar ratio of $NaHCO_3$: Na_2CO_3) conditions were simulated to natural conditions, and the aim was to investigate the physiological responses and adaptive strategies of this species under such conditions during seed germination and early seedling stages.

MATERIALS AND METHODS

Seed collection and storage: Mature *Leymus chinensis* seeds (1000 seeds weight was 2.4 g) were collected from the Grassland Ecosystem Field station, Institute of Grassland Science, Jilin province, China (123°44′E, 44°44′N, 167ma.s.l.) in July 2010. Seeds were than stored in paper bags at 4°C until further use.

Design of the simulated saline and alkaline conditions: Two neutral salts (NaCl: Na_2SO_4) and alkaline salts ($NaHCO_3$: Na_2CO_3) were both mixed in a 9:1 molar ratio, and applied to the saline stress and alkaline stress groups, respectively. In seed germination test, five concentration treatments were applied: 50, 100, 150, 200 and 250 mM within the saline and alkaline stress groups. The pH ranges in saline and alkaline stresses were 6.40-6.64 and 8.83-8.98, respectively. Osmotic potentials of the stress treatment solutions were -0.30 to -0.68 for saline stress and -0.23 to -0.61 for alkaline stress, as measured by a water potential meter (Psypro Wescor Corporation, South Logan, USA). In early seedling test, three concentration treatments were applied: 50, 100 and 150 mM within the saline and alkaline stresses. The pH ranges in saline and alkaline stresses were 6.40-6.61 and 8.83-8.90, respectively.

Experiment 1: Seed germination test: Seeds were firstly surface sterilized in 0.1% mercury chloride for 10 min, subsequently washed with distilled water and then air-dried to avoid fungus attack before being used in the experiments.

Germination test was carried out in 11-cm Petri dishes, on two layers of filter paper moistened with 12 mL of the test solution. Four replicates of 50 seeds were incubated for each treatment and the distilled water was used as a control. The Petri dishes were incubated at 20-30^{o}C with 16 h photoperiod (Sylvania cool white fluorescent lamps, 6400 lux) that coincided with the higher temperature in the growth chambers. Seeds were considered to be germinated with the emergence of the radicel. Germination percentage was recorded every 2 days for 20 d. Non-germinated seeds from all treatments were then transferred to distilled water in order 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 seed germination at one-day intervals and *t* is the total germination period (Lin *et al*., 2011). The maximum value possible using this index with our date was 50 (i.e.1000/20).

The recovery percentage was determined by the formula $(a-b)/(c-b)\times100$, where a is the total number of seeds geminated after being transferred to distilled water, b is the number of seeds germinated in each treatment solution, and c is the total number of seeds.

Experiment 2: Early seedling test

Plant materials and stress treatments: *Leymus chinensis* seeds were sown in 15 cm diameter plastic pots containing washed sands and were placed in growth chambers (HPG-400, Haerbin, China). The growth temperature regime was also 20-30℃ with 14 h photoperiod that coincided with the higher temperature. The relative humidity was 50%-60%. The pots were well drained with holes at the bottom. Each pot contained 25 seedlings and all pots were sufficiently watered with Hoagland nutrient solution daily. The Hoagland nutrition solution in our work contained 5.00 mM Ca^{2+}, 2.00 mM Mg^{2+}, 6.04 mM K^+, 22.2 ìM EDTA-Fe^{2+}, 6.72 ìM Mn^{2+}, 3.16 ìM Cu^{2+}, 0.765 ìM Zn^{2+}, 2.10 mM SO_4^{2-}, 1.00 mM

$H_2PO_4^-$, 46.3 ìM H_3BO_3, 0.556 ìM H_2MoO_4, and 15.04 mM NO_3^- (Li *et al*., 2009). After 20 days, the seedlings were subjected to stress treatments, 32 pots with uniform seedlings were divided randomly into eight sets, four pots per set. Each pot was considered a single replicate. Each set contained four replicates. One set was used as the untreated control, a second set was used to determine the mean length of the shoots at the beginning of treatment, and the remaining 6 sets were treated with various stress treatments. The pots were thoroughly watered daily with nutrient solution containing the appropriate stress salts at the same time. Control plants were maintained watered with the nutrient solution. The total duration of the treatment was 8 days.

Seedling harvest and physiological indices measurements: Harvested plants were washed with tap water and then washed with distilled water three times. The roots and shoots were separated and the shoot elongations were determined for all plants in a pot, and the FWs were determined for each plant. Then the shoots were oven-dried at 105°C for 15 min, before vacuum-drying at 65°C to a constant weight, and the DWs were determined. The water content (WC) was calculated using the formula (FW-DW)/DW. FW is short for fresh weight, DW is short for dry weight, and the result was expressed as g g^{-1}DW (Yang *et al*., 2007).

Determination of inorganic ions and organic solutes: Dry samples of plant material (25 mg) were treated with 10 mL deionized water at 100°C for 1 h and the extract was taken to determine free inorganic ions, organic acids and soluble sugars. An atomic absorption spectrophotometer (TAS-990, Purkinje General, Beijing) was used to determine the contents of Na^+, K^+, free Mg^{2+} and free Ca^{2+}. The NO_3^-, Cl^-, $H_2PO_4^-$ and SO_4^{2-} contents were determined by ion chromatography (DX-300 ion chromatographic system; AS4A-SC ion-exchange column, CDM-II electrical conductivity detector, mobile phase: $Na_2CO_3/NaHCO_3$=1.7/1.8 mM; DIONEX, Sunnyvale, USA). For the analysis of the organic acids, the measurements were also undertaken using ion chromatography (DX-300 ion chromatographic system; ICE-AS6 ion-exclusion column, CDM-II electrical conductivity detector, AMMS-ICE II suppressor, mobile phase: 0.4 mM perfluorobutyric acid; the flow rate was 1.0 mL/min; the column temperature was set at 20°C, and the injection volume was 50 ìL; DIONEX, Sunnyvale, USA). The content of proline and total soluble sugars were measured using ninhydrin and anthrone, respectively, according to Zhu *et al.* (1983).

Data analysis: All parameters were analyzed by one-way ANOVA using the statistical Software 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.

RESULTS

Effects on seed germination: Maximum seed germination of *Leymus chinensis* was obtained in distilled water. Seed germination decreased with increasing salinity and alkalinity, but the extent of reduction under alkaline stress was much greater than that under saline stress (Table1). Under saline stress, when salinity was low (50 mM), no significant change in germination percentage was observed. When salinity raised above 50 mM, germination percentage decreased with the rising salinity ($P<0.05$). But under alkaline stress, the increasing alkalinity significantly inhibited germination percentage ($P<0.01$). Germination percentage reached the minimum 19.5% when salinity was 250 mM under saline stress, while under alkaline stress, seeds could not germinate when the alkalinity was only 200 mM. Germination rate had the same downtrend with germination percentage, and also decreased more markedly under alkaline stress (Table1).

Seeds were then transferred to distilled water to determine the recovery percentage of germination. Under saline stress, with salinity≤200 mM, recovery percentages were not significant changed, however, 55.1% of the seeds recovery at 250 mM salinity and the total germination percentage of *Leymus chinensis* seeds in each treatment was similar to the control (Table1). Under alkaline stress, when the salinity was lower (50 and 100 mM), recovery percentages were higher, but decreased with the increasing alkalinity. Although the total germination percentage at 50mM alkalinity was higher than control, the higher alkalinity (>50 mM) caused a sharp decrease.

Effects on early seedling growth and water content: With the increasing salinity and alkalinity, shoot elongation and the water content of early seedlings were both inhibited significantly ($P<0.05$), and more markedly under alkaline stress (Fig.1.A B).

Effects on inorganic ions contents: With the increasing salinity and alkalinity, both of the Na^+ concentration and Na^+/K^+ ratios increased significantly ($P<0.05$, Fig.2A E), but the K^+ concentration decreased (Fig.2B). These changes were all greater under alkaline stress. Compared with the control, Na^+ concentration increased 2.1- and 4.8-fold under saline and alkaline stresses at the highest stress intensity (150 mM), and the Na^+/K^+ ratios were 0.8 and 3.3, respectively. Mg^{2+} and Ca^{2+} concentrations had a similar trend under two stresses, both decreased under saline stress but increased under alkaline stress (Fig.2C D).

The Cl^- concentration was not affected under alkaline stress, but increased under saline stress ($P<0.05$). Cl^- concentration increased 2.2-fold at the highest salinity stress compared with the control (Fig.3A). The NO_3^- concentrations decreased under both stresses, and more markedly under alkaline stress (*P*<0.05, Fig.3B). The $H_2PO_4^-$ concentration was not affected under saline stress, but decreased under alkaline stress (Fig.3C). The SO_4^{2-} concentration increased with the increasing salinity (*P*<0.05), but was not affected under alkaline stress (Fig.3D).

Effects on organic solutes contents: Malic acid, succinic acid, citric acid, acetate acid and oxalate acid were detected in the shoots of *Leymus chinensis* under saline and alkaline stresses (Fig.4). With the increasing salinity and alkalinity, changes in concentrations of all the organic acids increased under alkaline stress, especially under strong alkaline stress (150 mM) (*P*<0.05). However, saline stress did not affect citric acid concentration, and even reduced the other four organic acids and the total organic acids concentrations. Malic acid, citric acid and succinic acid were the dominant organic acids, and took up the largest proportion of the total organic acids (77.8%). The strong alkaline stress caused malic acid and citric acid to be the dominant component, and comprised 64.3% of the total organic acids. Although the acetate acid also enhanced significantly (*P*<0.05, Fig.4D), but only a small proportion of the total organic acid content and not be the dominant.

No significant change in proline concentrations were observed when salinity below 150 mM and alkalinity below 100 mM. The proline concentration sharply increased from 100 mM alkalinity (*P*<0.01, Fig.5A). The maximum proline concentration was 17.7 times that of the control under alkaline stress and only 1.1 times that of the control under saline stress. With the increasing salinity and alkalinity, the soluble sugars concentrations increased, and the increment was greater under alkaline stress (Fig.5B). Under the highest concentration of stress treatment, compared with the control, soluble sugars concentrations increased 2.2- and 2.9-fold under saline and alkaline stresses, respectively.

Table 1. Effects of saline and alkali stresses on germination percentage, germination rate, recovery percentage and total germination percentage of *Leymus chinensis* seeds.

Salinity (mM)	Saline stress				Alkaline stress			
	GS	GR	RP	TGS	GA	GR	RP	TGA
0	71.0±3.5a	25.8±2.1a	0a	71.0±3.5a	71.0±3.5a	25.8±2.1a	0a	71.0±3.5b
50	66.5±3.9a	23.8±1.5a	22.1±0.9b	72.0±3.1a	64.5±3.3b	23.9±1.5a	42.2±1.1d	78.0±2.0a
100	52.7±6.6b	16.8±2.2b	22.4±0.7b	63.3±4.8ab	30.0±0.8c	9.6±0.3b	45.0±1.7d	61.5±1.0c
150	49.5±1.7b	16.3±0.2b	26.8±3.3b	62.0±1.2ab	6.0±1.2d	1.7±0.4c	32.1±0.9c	30.0±1.2d
200	37.5±3.8c	10.7±1.3c	29.7±0.5b	57.3±3.5b	0d	0c	30.7±1.8bc	30.7±1.8d
250	19.5±2.5d	5.2±0.7d	55.1±5.4c	64.7±4.7ab	0d	0c	27.3±0.7b	27.3±0.7d

Mean (±s.e.: n=4) germination (%) indicating the initial salinity and alkalinity effects (GS and GA); recovery percentage (RP) after 20d of transfer to distilled water; germination rate (GR) and total germination under saline and alkaline stresses (TGP and TGA).

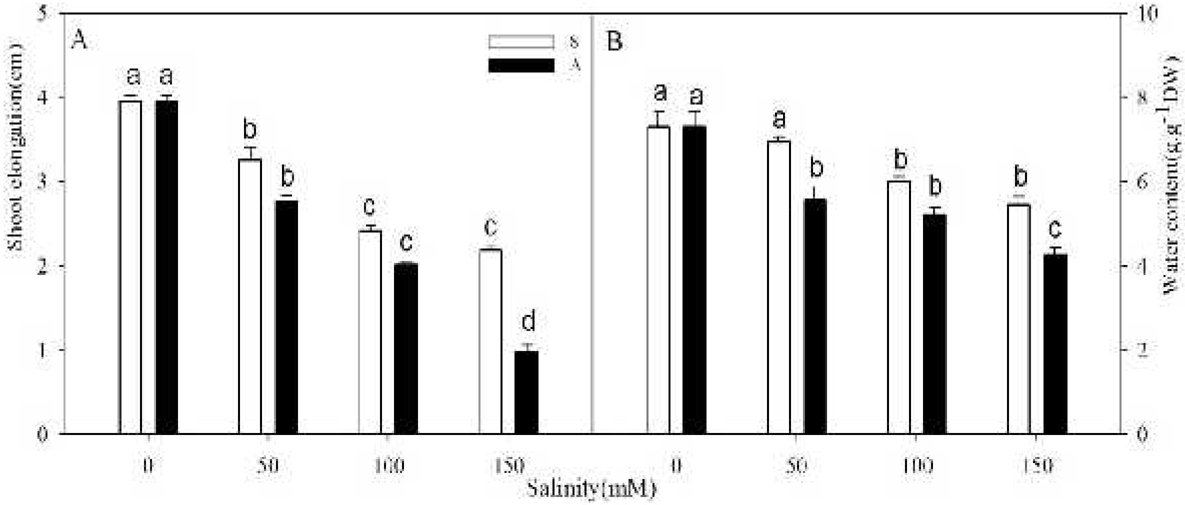

Fig.1 Effects of saline and alkaline stresses on the (A) shoots elongations and (B) water contents in shoots. S, saline stress; A, alkaline stress.DW, dry weight. The values are the means of four replicates. Means followed by different letters are significantly different at *P* < 0.05 according to a least significant difference test.

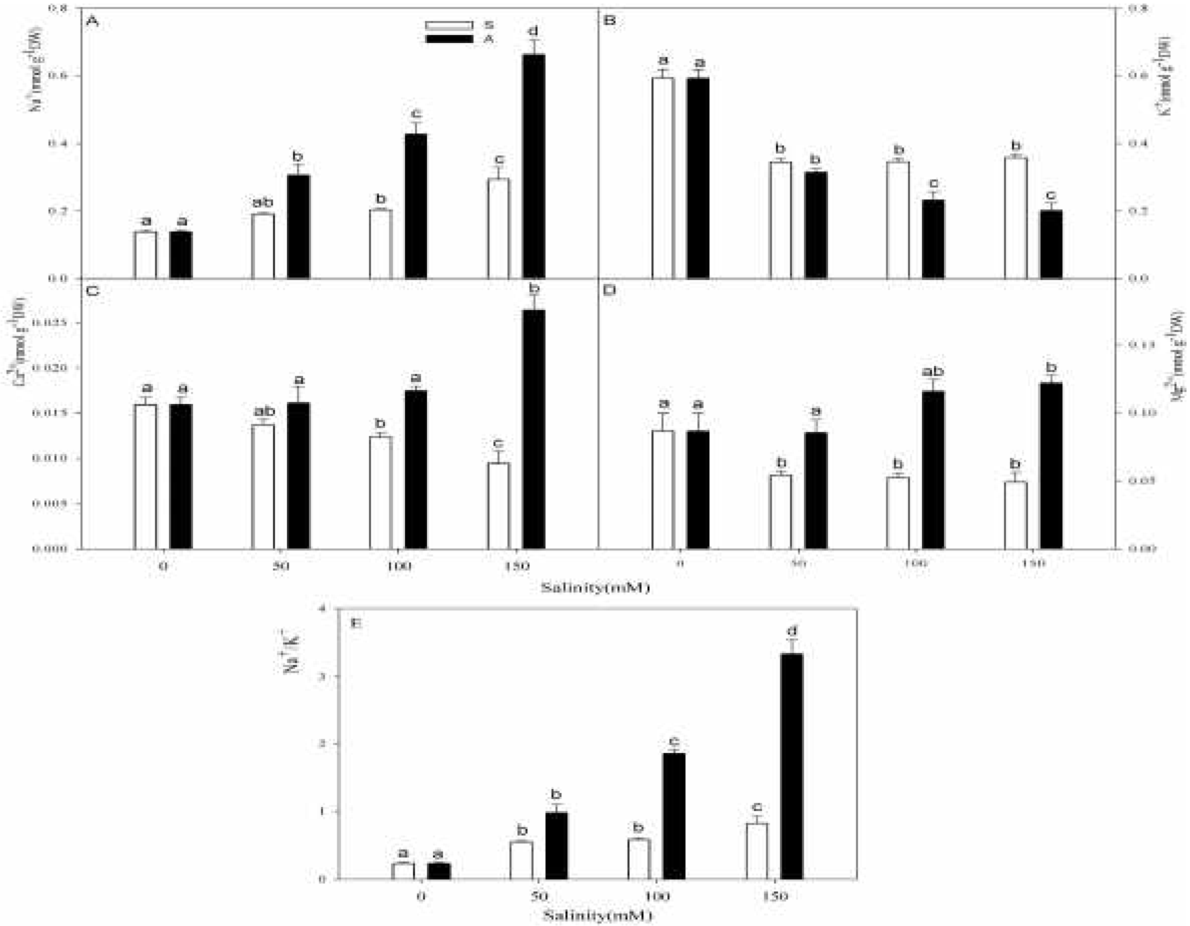

Fig.2 Effects of saline and alkaline stresses on the contents of (A) Na^+, (B) K^+, (C) free Ca^{2+}, (D) free Mg^{2+} and (E) Na^+/K^+ ratio in the shoots of *Leymus chinensis*. S, saline stress; A, alkaline stress.DW, dry weight. The values are the means of four replicates. Means followed by different letters are significantly different at *P* < 0.05 according to a least significant difference test.

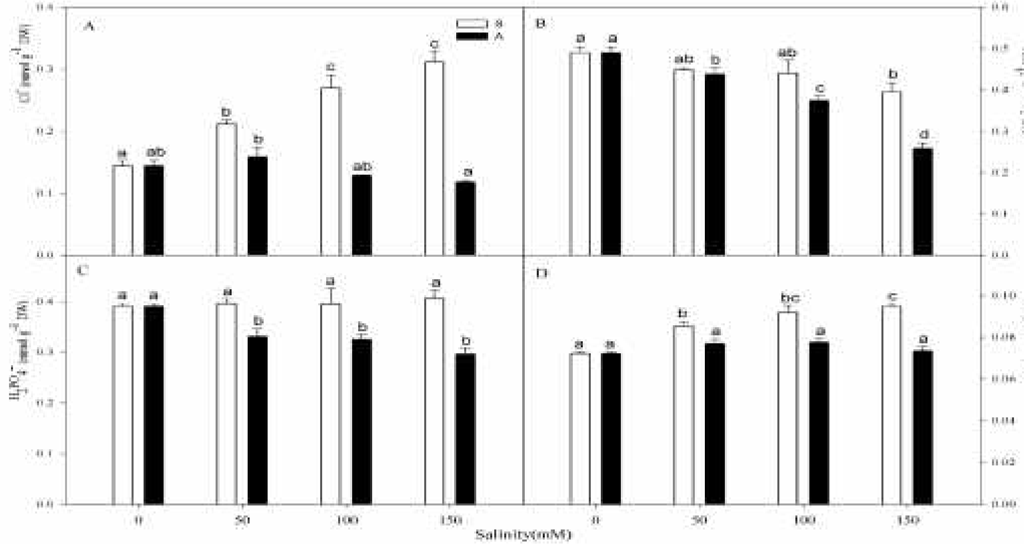

Fig.3 Effects of saline and alkaline stresses on the contents of (A) Cl^-, (B) NO_3^-, (C) $H_2PO_4^-$ and (D) SO_4^{2-} in the shoots of *Leymus chinensis*. S, saline stress; A, alkaline stress.DW, dry weight. The values are the means of four replicates. Means followed by different letters are significantly different at *P* < 0.05 according to a least significant difference test.

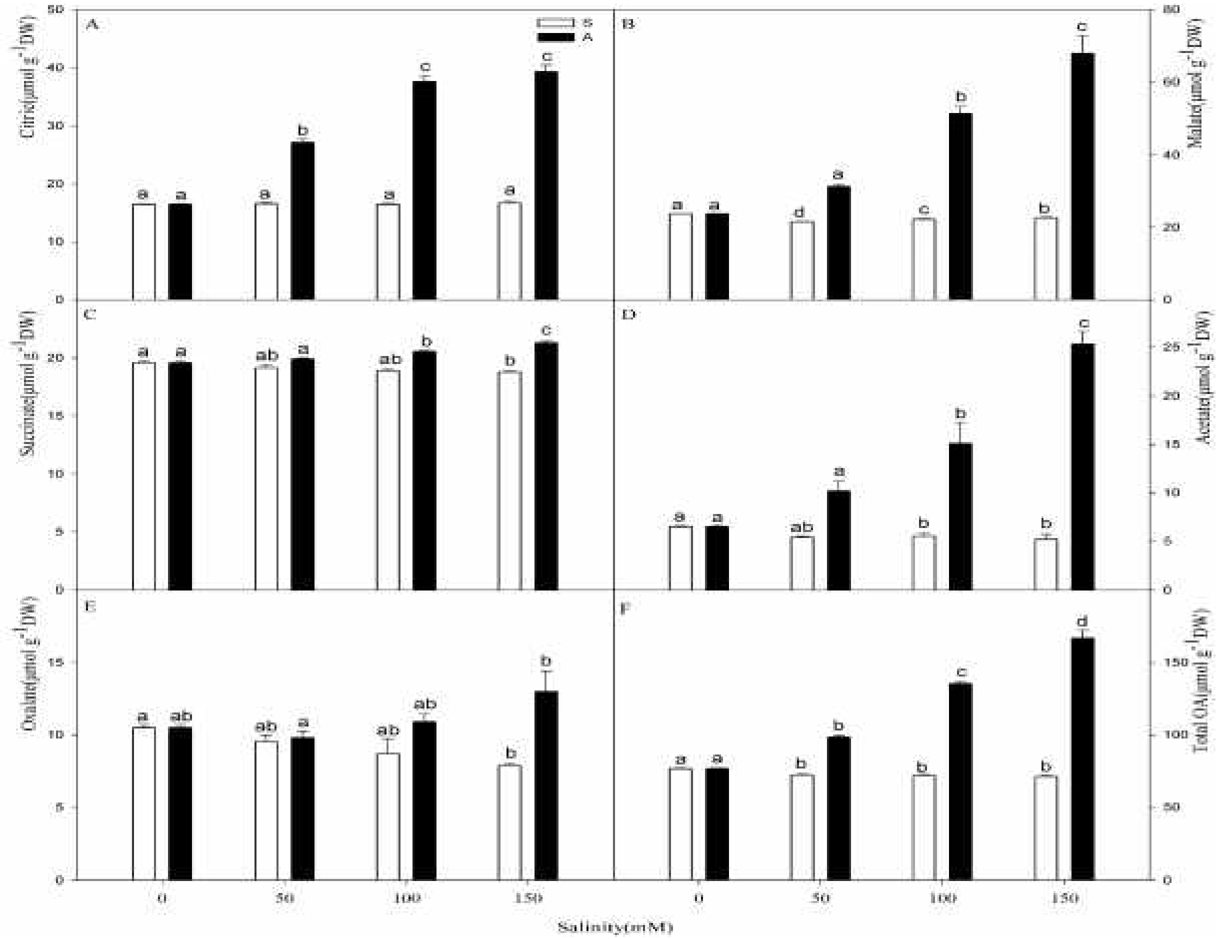

Fig.4 Effects of saline and alkaline stresses on the contents of (A) citric acid, (B) malic acid, (C) succinic acid, (D) acctate acid, (E) oxalate acid and (F) total organic acids (OA) in the shoots of *Leymus chinensis*. S, saline stress; A, alkaline stress.DW, dry weight. The values are the means of four replicates. Means followed by different letters are significantly different at $P < 0.05$ according to a least significant difference test.

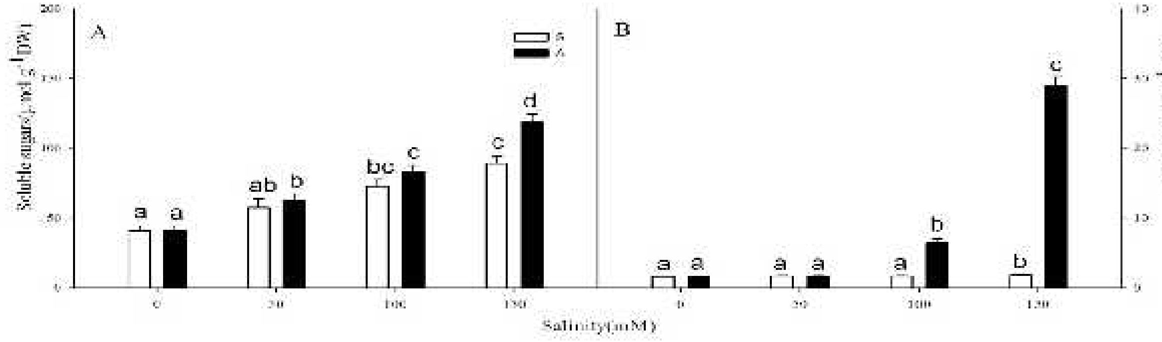

Fig.5 Effects of saline and alkaline stresses on the contents of (A) soluble sugars and (B) proline in the shoots of *Leymus chinensis*. S, saline stress; A, alkaline stress.DW, dry weight. The values are the means of four replicates. Means followed by different letters are significantly different at $P < 0.05$ according to a least significant difference test.

DISCUSSION

Salinity in the soil affects germination process by several ways such as prevention of water absorption, ionic stress and reduction of enzyme activities within the seeds. Our results showed that alkaline stress also significantly affected seed germination (germination percentage and germination rate) of *Leymus chinensis*. However, the inhibiting action of alkaline stress was greater than that of saline stress at the same stress intensity (Table1). However, at low stress intensity (50 mM), the inhibiting effects of saline stress and alkaline stress on germination percentage were similar, indicating that pH had especial adjustment function. It allowed germination occurred under lower alkalinity, but when the alkalinity was higher, high-pH not only aggravated the effects of osmotic stress and ionic toxic on seeds but also decomposed the seed structure and even destroyed the embryo, result in seed death. The reason for this may be complex, requiring further study.

After germination recovery test, we found that above 50% of nongerminated seeds of *Leymus chinensis* germinated again when they were transferred from strong saline stress (250 mM) to distilled water (Table1). This may be an adaptive strategy of *Leymus chinensis* seeds to resist high salinity stress. Nongerminated seeds in a state of dormancy to escape from the rigorous environment, and germinated again when the soil salinity decreased due to melt water or rainfall (Debez *et al*., 2004). Most other halophytes also show an important recovery of germination when stress conditions are alleviated (Qu *et al*., 2007). In our results, recovery percentage was high under high salinity stress because of the osmotic pre-treatment caused by high concentration salinity conditions, and seed germination ability is then stimulated and germinates well. However, recovery of germination decreased with increasing alkalinity in our research, which further indicates that higher alkalinities created more damage effects, caused most of the seeds lose viability, and different inhibition mechanisms on germination of *Leymus chinensis* may exist between saline stress and alkaline stress, which deserves further research.

Young seedlings of *Leymus chinensis* elongated quickly in distilled water. Elongation of shoot decreased with increasing salinity and alkalinity, but more markedly under alkaline stress (Fig.1A). This indicated that saline stress and alkaline stress had different inhibition impacts on seedling growth of *Leymus chinensis*, and the resistance of this species to saline stress is much stronger than that to alkaline stress. Similar results have also been reported for other halophytes (Yang *et al*., 2007, 2008b). The reason of this phenomenon may be due to the different mechanisms between the two stresses. Generally, the deleterious effects of saline stress on plant result from lower water potentials/ion toxicities. However, alkaline stress has the same stress factors as saline stress but added the influence of high-pH stress. The high-pH environment surrounding the roots can directly cause some ions imbalance, metabolic disorders, and may destroy the structure and function of root cells (Shi and Wang 2005). Therefore, much more material and energy must be expended for plants to survive in alkaline conditions. Thus, shoot elongation under alkaline stress was observed in present research.

Water content also decreased markedly under alkaline stress (Fig.1B). The reason may be that high-pH stress reduced the water adsorption ability of roots, which resulted lesser water contents in the shoot. Moreover, seedlings accumulated more solute contents under alkaline stress and caused more water to be taken up than that under saline stress.

Low Na^+ and high K^+ in the cytoplasm are essential for the maintenance of a number of enzymatic processes (Munns and Tester, 2008). The Na^+ entered the plant cells through the high-affinity K^+ transporter (HKT) and non-selective cation channels (Zhu, 2003). Most plants in saline-alkaline environments accumulated an abundance of Na^+ in vacuoles to decrease the cell water potential and simultaneously inhibited the K^+ absorption (Munns 2002; Shi and Wang, 2005). Our results indicated that increasing salinity and alkalinity, the Na^+ concentrations increased, while K^+ concentrations decreased, and with great change under alkaline stress (Fig.2A B). This may be attributable to an inhibitory effect of high pH on K^+ absorption, which relies on the transmembrane proton gradient (Munns, 2002). High pH weaken the control of absorption and transport of Na^+ and K^+, leading to a sharply increasing of Na^+ and reaching a toxic level. However, the result was in contradiction to observations reported in older seedlings of *Leymus chinensis* (Guo *et al*. unpublished) and some other halophytes familiar in Songnen grassland in China (Yang *et al*., 2007, 2008), which found that there was no competitive inhibition between Na^+ and K^+, suggesting that different plant species or different growth stage of a plant species might have distinct pathway of absorption mechanism of Na^+ and K^+, and deserves further investigation. In addition, Na^+/K^+ ratio can also be used as a phyto-physiological parameter for salts tress (Keutgen and Pawelzik, 2008). High Na^+ and low K^+ concentrations under alkaline stress also resulted in the Na^+/K^+ ratios reached to a high level, especially under strong alkaline (150mM) stress (3.3) (Fig.2E), and this could explained why the deleterious effect caused by alkaline stress was greater than that under saline stress.

Mg^{2+} and Ca^{2+} are both essential elements in plants. They are important components of chlorophyll and cell wall, respectively. Ca^{2+} is also a second messenger in some signal transduction pathways. Saline stress may inhibit the accumulation of Mg^{2+} and Ca^{2+} in many plants (Khan *et al*., 1999). Our results exhibited that both Mg^{2+} and Ca^{2+} concentrations decreased under saline stress but increased under alkaline stress (Fig.2C D). This may be a strain-specific physiological response of *Leymus chinensis* seedlings to alkaline stress. The result agreed with those of *Yang* (2007) for halophyte *Kochia sieversiana*. However, the contents of Mg^{2+} and Ca^{2+} were very low and their roles on osmotic adjustment were pimping.

Although a great number of inorganic ions accumulate in vacuoles, plants can also synthesize low molecular mass organic solutes in the cytoplasm, such as proline and soluble sugars, to prevent dehydration and protect biomacromolecules (Parida and Das, 2005). In the present study, soluble sugars were accumulated under both stresses and more markedly under alkaline stress in *Leymus chinensis* seedlings, which might be a correlative response to the change of Na^+ in cells to keep the balance of osmotic pressure (Fig.5A). The result also indicated that accumulation of soluble sugars might be used as a index of salt-alkali tolerance for this species. Proline accumulation is also a quick response to osmotic and ionic stress (Liu *et al*., 2010). From Fig.5B, it is evident that proline did not accumulate at low salinity, and even at 150 mM salinity the accumulation was similar to the control. Under alkaline stress, the proline concentration sharply increased at higher alkalinity (150 mM), suggesting that changes of proline content in seedlings may be only a special physiological response to high intensity pH stress, rather than a response to osmotic stress. In addition, proline concentrations under both stresses were very low compared with soluble sugars, and insignificant to the osmotic adjustment.

Organic acids also played a potential role as cell osmolytes in osmotic adjustment. Organic acids accumulated under alkaline stress but decreased under saline stress in our research (Fig.4). It is assumed that the accumulation of organic acids was a key physiological character of this species when encountered alkaline stress (high pH). Therefore, *Leymus chinensis* has different adaptive mechanisms to maintain osmotic balance during adaptation to saline and alkaline stresses. Seedlings accumulated inorganic ions such as Na^+ in vacuoles and also accumulated a large amount of soluble sugars in the protoplasm under both of the saline and alkaline stresses. The difference between the two stresses was that under saline stress, seedlings mainly accumulated an abundance of $Cl^–$, while organic acids played a key role in alkaline stress.

The influx of superfluous Na^+ under the two stresses also caused ionic imbalance in plants. Most plants always accumulate inorganic anions, such as $Cl^–$, $NO_3^–$ and $SO_4^{2–}$, and synthesize organic anions to maintain ionic balance (Yang *et al*., 2007, 2008a). In our study, *Leymus chinensis* seedlings accumulated mainly $Cl^–$ to balance the influx of Na^+ (Fig.4A). However, all the contents of inorganic anions under alkaline stress were lower than that under saline stress, indicating that high pH inhibited absorptions of Cl^-, $NO_3^–$ and $H_2PO_4^-$ (Fig.3A-C). Similar results were also reported for other plants, like wheat (Li *et al*., 2009), sunflower (Liu *et al*., 2010) and *Chloris virgata* (Yang *et al*., 2010). At this moment, seedlings always accumulated large number of organic acids to compensate the anions deficit caused by high pH. Accumulation of organic acids not only played an important osmotic role but also buffered excess toxic cations and kept ionic balance. Our results further showed that malic acid and citric acid were the dominant components of the total organic acids after strong alkaline stress. This result is different from other halophytes. For example, oxalic acid was the main metabolite of *S.glauca* and *K.sieversiana* (Yang *et al*., 2007, 2008), while *Puccinellia tenuiflora* mainly accumulated citric acid under alkaline conditions (Guo *et al*., 2010). This indicated that organic acids metabolism adjustment in different plant species might also be clearly different. In addition, synthesis of organic acids demanded much more energy than that of inorganic ions. This might also be a main reason for lesser elongations of shoots under alkaline stress.

Conclusions: In summary, this study clearly showed that the damaging effects of alkaline stress on seed germination and early seedling growth were significantly greater than that of saline stress, which might due to the negative effects of high pH. Under saline and alkaline conditions, adaptive strategies of *Leymus chinensis* during germination and early seedling stage were greatly different. Most nongerminated seeds in a state of dormancy under higher salinity, and germinated again when salinity decreased, while high alkalinity caused them lose viability.

During early seedling stage, *Leymus chinensis* mainly accumulated Cl^- and soluble sugars to maintain osmotic and ionic balance under saline stress. However, alkaline stress inhibited absorptions of Cl^-, $NO_3^–$ and $H_2PO_4^-$, enhanced organic acids to remedy the shortage of inorganic anions, and accumulated more soluble sugars to cope with osmotic stress from the high Na^+ concentration. Accumulating organic acids is the primary different adaptive mechanism to saline and alkaline stresses.

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