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RESEARCH PAPER

Effects of Na_2CO_3 simulated alkali stress on tradeoff strategies of individual and clonal traits of *Leymus chinensis*

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Abstract

Alkali stress is a significant challenge across the globe which is posing serious threat to crop production and food security. This study was carried out to study the effect of different levels of alkali stress on growth and physiological traits of *Leymus chinensis*. The study was comprised of different levels of alkali stress; control (CK; 0 mmol·L^{-1}), 25 and 50 mmol·L^{-1}. The results showed that imposition of 50 mmol·L^{-1} alkali stress substantially reduced the photosynthetic capacity, relative water contents and accumulation of carbon (C), nitrogen (N) and phosphorus (P) in plant parts. Further, 50 mmol·L^{-1} alkali stress also reduced the above and below ground biomass, and severely inhibited the root growth. Moreover, increase in concentration of alkali stress inhibited clone components, tillering, tillering bud, and internode bud of *Leymus chinensis*. In conclusion the increasing concentration of alkali stress can reduce the growth and biomass production and nutrient accumulation of *Leymus chinensis*.

Keywords

Alkali stress, biomass proportion, nutrient concentration, relative height growth rate, nutrient concentration

Introduction

Soil salinization and alkalinity are serious threats around the globe negatively affecting crop productivity. Globally, 54% of saline-alkali soils contain both neutral and basic salts (Tanji 2012; Zhan-Wu et al. 2023). These salts can cause nutritional stress, damage the plant roots and inhibit the seed germination and seedling growth (Turner et al. 2020; Huang et al. 2015). Further, alkali stress also decreases plant height, biomass production, root length (Masmoudi et al. 2021) stomatal conductance, reduced transpiration, and carbon dioxide (CO_2) intake (Liu et al. 2022). The short term alkali stress can increase activities of nitrate reductase (NR) and glutamine:2-oxoglutarate aminotransferase (GOGAT), however, increasing concentration of alkaline salts can decreased NR and GOGAT activities (Zhang et al. 2014). Besides this excessive accumulation Na^+ ions in soil increased the osmotic pressure which reduce the uptake by plants and leads to reduction in plant growth and development (Xiao et al. 2018).

Leymus chinensis is a rhizome perennial grass and it has formed a reproductive strategy of weak sexual reproduction and strong asexual reproduction (Renzhong et al. 2003). It uses rhizomes for vegetative reproduction in natural vegetation to achieve population reproduction and renewal (Frankel and Galun 2012; Xiao et al. 2018; Liu et al. 2020; Li et al. 2021). When environmental conditions are good, *L. chinensis* use more energy, vegetative growth and vegetative reproduction to improve its competitiveness. However, in stress conditions *Leymus chinensis* uses more resources in reproductive tiller plants to maintain population continuation (Guo et al. 2020; Dong et al. 2023). The survival and development of *Leymus chinensis* showed that it had adopted corresponding adaptation strategies to different soil habitat conditions. Clonal plants (also known as clones) have trade-offs between sexual reproduction and asexual reproduction and between current sexual reproduction and future survival. The underground parts of cloned plants include rhizomes (clonal building blocks) and roots. The rhizome has a strong storage capacity, and the seedlings formed by the germination on the rhizome have a stronger ability to resist the stress than the seedling. Rhizomes play an important role in asexual reproduction, information exchange and material exchange among clonal strains, and prediction of patch quality (Lin et al. 2017). In high sexual reproduction resource input years, not only the number of new rhizomes and the density of rhizomes, but also the number and density of apex rhizomes and apex rhizomes significantly decreased. However, density of tillering buds and tiller nodes increased significantly, resulting in a significant decrease in total bud density and total seed density. This indicates that high reproductive distribution not only affects the number of different cloned progeny in Chinese *Leymus chinensis* population, but also seriously affects the transport distance of cloned progeny. In high reproductive years, Chinese *Leymus chinensis* adopts a relatively more intensive growth strategy (Zhang et al. 2014). Therefore, this study was performed with following objectives: 1) to study the effect of alkali stress on growth and physiological indexes of *Leymus chinensis*, 2), to study the effect of different levels of alkali stress on nutrient uptake and accumulation in *Leymus chinensis*.

Materials and methods

Test materials

Since May 2018, simulation experiments was carried out in the greenhouse of Northeast Normal University (43°51'N, 125°19'E, 236 m a. s. l.). The experimental materials were taken from the seedlings of *Leymus chinensis* growing in the wild in Changling, Songyuan City, Jilin Province. We selected fresh green and vigorous *Leymus chinensis* seedlings with uniform growth, and transplanted them into a pot with a side length of 30 cm and a height of 0.5 m filled with washed sand. We also kept an equal distance between the plants, and 20 seedlings were set in each pot.

Research methods

Stress conditions and treatment

The same treatment was carried out for each pot of seedlings at the early growth stage and watered with clean water. After that, water with 0.5 times Hoagland nutrient solution was applied every after 4 days. The nutrient solution was contained; 5.00 mmol L^{-1} Ca^{2+}, 2.00 mmol L^{-1} Mg^{2+}, 6.04 mmol L^{-1}, K^+,22.2 umol L^{-1}, EDTA-Fe^{2+}, 6.72 umol L^{-1}, Mn^{2+},3.16 umol L^{-1}, Cu^{2+},0.765 umol L^{-1} Zn^{2+}, 2.10 mmol L^{-1} SO_4^{2-},1.00 mmol L^{-1} $H_2PO_4^-$,46.3 umol L^{-1} H_3BO_3, 0.556 umol L^{-1} H_2MoO_4 and 15.04 mmol L^{-1} NO_3^-. Salt and alkali stress treatment began in July, and two concentration gradients were designed. The salt stress substances were prepared into 100 and 200 mmol L^{-1} solutions by mixing NaCl with 0.5× nutrient solution. The control group was treated with 0.5 times Hoagland nutrient solution.

15 pots of seedlings were randomly divided into 5 groups for treatment, with 3 pots in each group. Each pot was used as 1 replicate and each group had 3 replicates. Treatment was carried out from 17:00 to 18:00 every day, with a 4-day cycle. On the first day, each basin was irrigated with 1L of treatment liquid of corresponding concentration, and the remaining three days were irrigated with the same amount of water (the water amount was 0.5 L^{-1}, appropriately adjusted according to seasonal temperature and humidity). Treatment continued until the end of the growing season (for a total of 8 weeks). All flower pots were placed in the test shed to artificially protect from rain.

Plant growth index and photosynthetic index

Starting from June, 15 seedlings of *Leymus chinensis* were labeled in each pot, and their plant height and leaf length were measured once a week, and dynamic data of the whole growing season were tracked and recorded. Blackman formula was used to calculate the relative height growth rate (RHGR) of *Leymus chinensis* under different treatments by using following formula:

$$RHGR(H_i) = \ln(H_{i+1} + 1) - \ln(H_i)$$

Specifically, H_i and H_{i+1} represent plant height at Ti and T_{i+1}, respectively.

Photosynthetic indexes (net photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), intercellular carbon dioxide concentration (Ci) and water use efficiency (WUE) were measured once in the middle and late growing season (July and late August) by CIRAS-3 portable photosynthesizing apparatus (PP SYSTEMS, USA). The first fully unfolded sunny leaf above the plant was selected for measurement. Three leaves were tested in each pot, and nine leaves were tested in each treatment.

Sample collection

Leymus chinensis leaf collection

After 8 weeks of stress treatment, fresh samples of *Leymus chinensis* leaves were collected. Ten fresh leaves were collected from each pot, and a total of 30 fresh leaves were collected from each treatment. The leaves were weighed after cutting to determine fresh weight and afterwards they were oven dried to determine dry weight and leaf water contents were determined with following formula:

$$\text{leaf water content} = (\text{fresh weight} - \text{dry weight}) / \text{fresh weight} \times 100\%$$

Plant sample collection

Destructive sampling was done and samples were carefully washed to remove the sand. The number of seed plants (tillers and rhizomes), buds (tillers, internode buds and terminal buds) and the number of rhizomes were counted. Further, root length, diameter, area, volume and tip count were measured by a root system scanner (Epson 11000XL scanner).

The whole plant of *Leymus chinensis* was classified according to different organs, and the leaves, stems, rhizomes, roots and buds were separated and placed in the oven to dry at 65°C for 48 hours, and their dry weights were weighed respectively.

Determination of biomass and its chemical elements

The dry samples of various organs (leaves, stems, rhizomes and roots) of *Leymus chinensis* were grounded with a ball mill and completely formed into powder. The total carbon and total nitrogen contents were determined by element analyzer (EL-III, UK).

The total phosphorus content of various organs of *Leymus chinensis* was determined by molybdenum-antimony resistance colorimetric method (721 spectrophotometer). The standard phosphorus solutions 0, 2, 4, 6, 8, 10 mL were placed into a 50 ml volumetric bottle, and mixed with 30 ml deionized water. Then this solution was boiled with a constant volume of 100 ml, and added with dinitrophenol, sodium hydroxide, sulfuric acid solution in order to adjust pH. After adding 5 mL of molybdenum-antimony resistance, the volume was made upto 50 ml and allowed for 30 minutes to develop the color.

Data analysis

All the data were statistically analyzed by SPSS26.0 software (SPSS, Chicago, IL, USA). Before data analysis, the homogeneity of data variance was tested. The effects of alkali stress on individual traits and composition of underground bud bank of *Leymus chinensis* were examined by ANOVA, and the differences among different treatments were analyzed by Turkey's multiple comparison test

Results

Effects of alkali stress on relative height growth rate and plant height of *Leymus chinensis*

The results indicate that *L. chinensis* grew at a relatively high relative growth rate (RHGR) under the supply of nutrient solution (Fig. 1). The application of alkali stress significantly reduced the RHGR and more reduction was seen at 50 mmol·L^{-1} than 25 mmol·L^{-1} (Fig. 1).

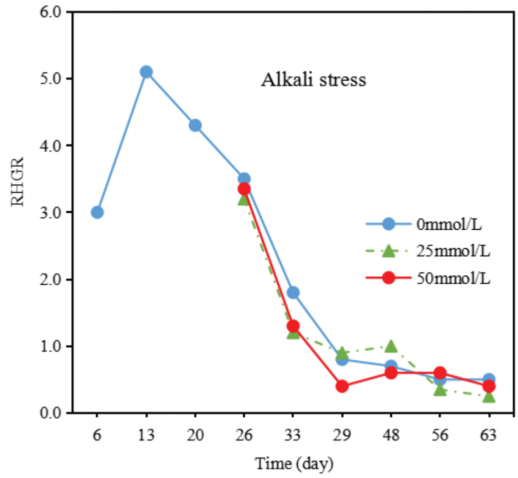

Figure 1. Effects of alkali stress on relative height growth rate of *Leymus chinensis*. Note: The first measurement from June 5 is recorded as the first day of dynamic tracking; The stress treatment began on 29 June (day 24 of tracking).

The plant height was also significantly decreased with increasing alkali stress. The plant height under 50 mmol·L^{-1} treatment was significantly lower than that under 0 mmol·L^{-1} and 25 mmol·L^{-1} treatment and plant height showed a reduction of 16.58% and 16.09% under 25 and 50 mmol·L^{-1} alkali stress (Fig. 2A).

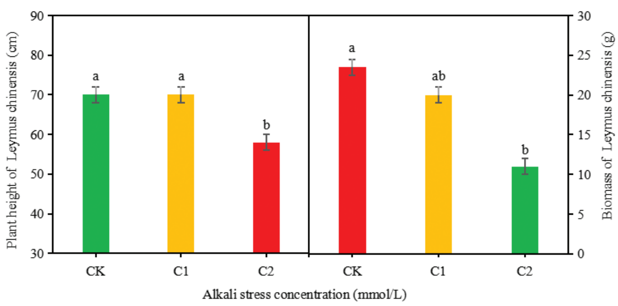

Figure 2. Effects of alkali stress on plant height and biomass of *Leymus chinensis*. Note: CK stands for 0 mmol·L^{-1}; C1 stands for alkali concentration of 25 mmol·L^{-1}; C2 stands for base concentration 50 mmol·L^{-1}. Different letters indicate significant differences between different concentrations of the same treatment ($P < 0.05$).

Plant biomass of *Leymus chinensis*

The biomass per plant of *Leymus chinensis* showed a decreasing trend with increasing alkali stress. The biomass per plant at 50 mmol·L^{-1} was decreased by 52.33% as compared to control (Fig. 2B).

The biomass of all parts showed a downward trend with the increase of alkali stress (Fig. 3 and Table 1).

The leaf biomass per plant of *Leymus chinensis* was the largest under control treatment, which was significantly higher than the other two alkali stress treatments (25 mmol·L^{-1} and 50 mmol·L^{-1}). There was significant difference between stem biomass of single plant of *Leymus chinensis* at 50 mmol·L^{-1} alkali stress. The underground biomass *of L. chinensis* at 50 mmol·L^{-1} alkali concentration was decreased by 57.87% compared with control group.

Stoichiometric indexes of various organs of Chinese *Leymus chinensis*

The increase of alkali stress concentration showed an increase in C contents. The results showed that compared with the control group, the carbon concentration was increased by 1.89% at 25 mmol·L^{-1} and 1.92% at 50 mmol·L^{-1} (Fig. 4A).

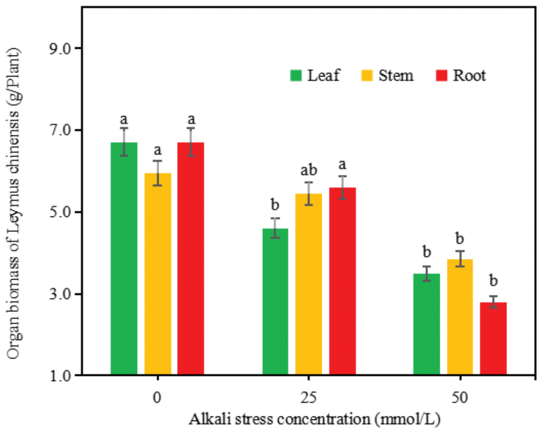

Figure 3. Effects of Alkali stress on biomass (g / L) of *Leymus chinensis*. Note: Different letters indicate significant differences between different concentrations of the same part ($P < 0.05$).

Table 1. Correlation analysis between aboveground and underground part of *L. chinensis* with alkali stress.

	Leaf	Stems	Underground Part
leaf	1	.586**	.393**
stems	.586**	1	.431**
Underground part	.393**	.431**	1
Alkali treatment	-.390**	-.232*	-.290**

Note: 1) * indicates a significant association at the 0.05 level. *Correlation is significant at the 0.05 level.2) ** indicates a significant correlation at the 0.01 level. **Correlation is significant at the 0.01 level.

There was no significant difference in N and P content between alkali stress treatment and each vegetative organ of *Leymus chinensis*. Nitrogen content in leaves and roots increased with the increase of alkali stress concentration.

Under alkali stress conditions, P content in leaves and stems of *Leymus chinensis* was lowest at 25 mmol·L^{-1} and the highest at 50 mmol·L^{-1}. Further, increasing alkali concentration showed a decreasing trend in P content in rhizome and root (Fig. 4C).

The results showed that the C/N ratio of *Leymus chinensis* leaves decreased with the increase of alkali stress concentration, and the ratio of N/P to C/P was the highest at 25 mmol·L^{-1} (Fig. 5A, B).

Under alkali stress, the C/N ratio of *L. chinensis* showed a downward trend, and the highest ratio of N/P to C/P was found at 25 mmol·L^{-1} (see Fig. 5C).

Under alkali stress, the C/N ratio of rhizome in *Leymus chinensis* firstly increased and then decreased. On the other hand change of N/P ratio was the opposite, and the C/P ratio increased with the increase of alkali treatment concentration (Fig. 5D).

The effect of saline stress on the leaf characteristics

The results showed that there was no significant change in the moisture content of the leaves under the treatment of alkali stress (Fig. 6A).

Different letters indicate significant differences between different concentrations of the same treatment ($P < 0.05$).

The leaf length was decreased, and length of leaf of blade was decreased by 1.78% at 50 mmol·L^{-1} alkali stress (Fig. 6B).

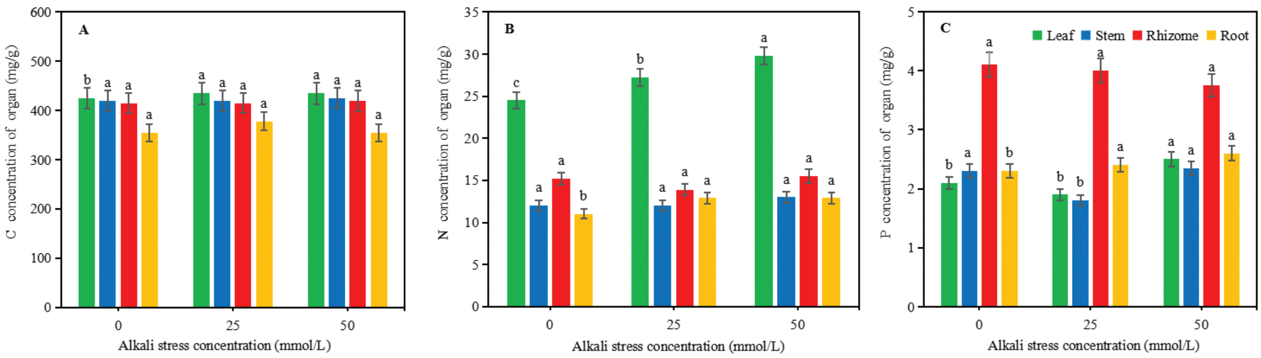

Figure 4. Effects of alkali stress on carbon content, nitrogen content and phosphorus content in organs of *Leymus chinensis*. Note: Different letters indicate significant differences between different concentrations in the same organ ($P < 0.05$).

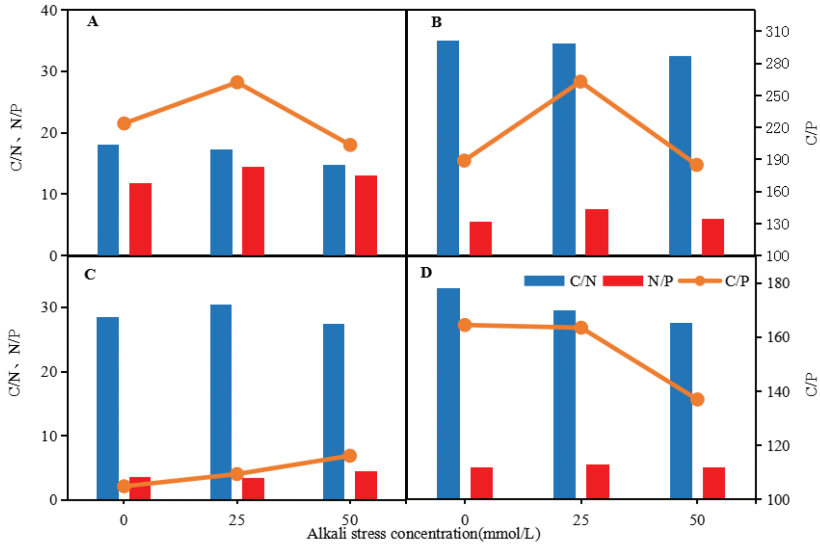

Figure 5. Effects of alkali stress on chemical metering ratio of *L. chinensis* leaves stems and rhizomes.

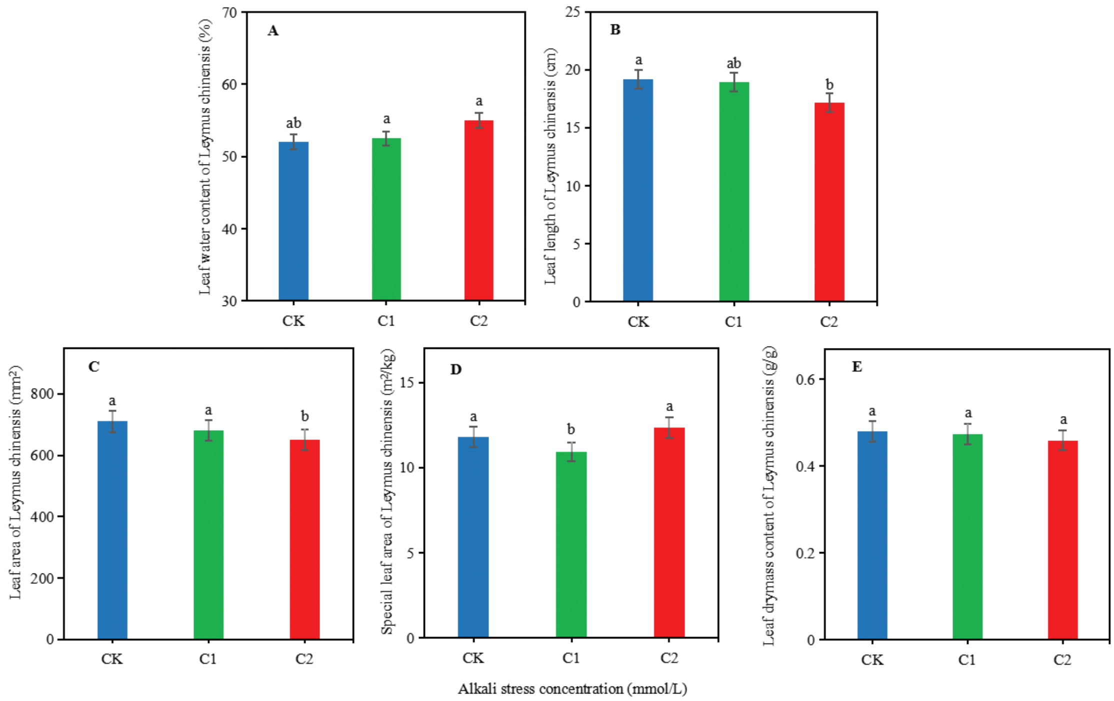

Figure 6. Effects of alkali stress on water content, leaf length, leaf area and matter content of *Leymus chinensis* leaves. Note: CK stands for 0 mmol·L^{-1}; C1 stands for alkali concentration of 25 mmol·L^{-1}; C2 stands for base concentration 50 mmol·L^{-1}.

Further, the increase of alkali stress concentration, also showed a reduction of 3.49% by 8.36% in product of the leaf surface.

The results indicated that LDMC showed a decline with increasing alkali stress and LDMC was decreased by 88% at both levels of alkali stress.

The results showed that photosynthetic rate was significantly decreased with increasing alkali stress and a reduction of 15.17% and 36.21% in photosynthetic rates was observed at 25 and 50 mmol L^{-1} alkali stress treatments. The alkali stress also decreased the leaf porosity and a reduction of 41.81% and 18.46% in leaf porosity was observed at 25 and 50 mmol L^{-1} alkali stress (Fig. 7).

The results showed that alkali stress had significant effects on photosynthetic capacity of *Leymus chinensis* (Fig. 8A). There was no significant difference between the low concentration alkali treatment and the control group, and the net photosynthetic rate of *Leymus chinensis* at 50 mmol·L^{-1} was decreased 25.75%.

Effects of alkali stress on the subsurface configuration of *Leymus chinensis*

The increase of alkali stress significantly decreased the underground length of *L. chinensis*. The underground length

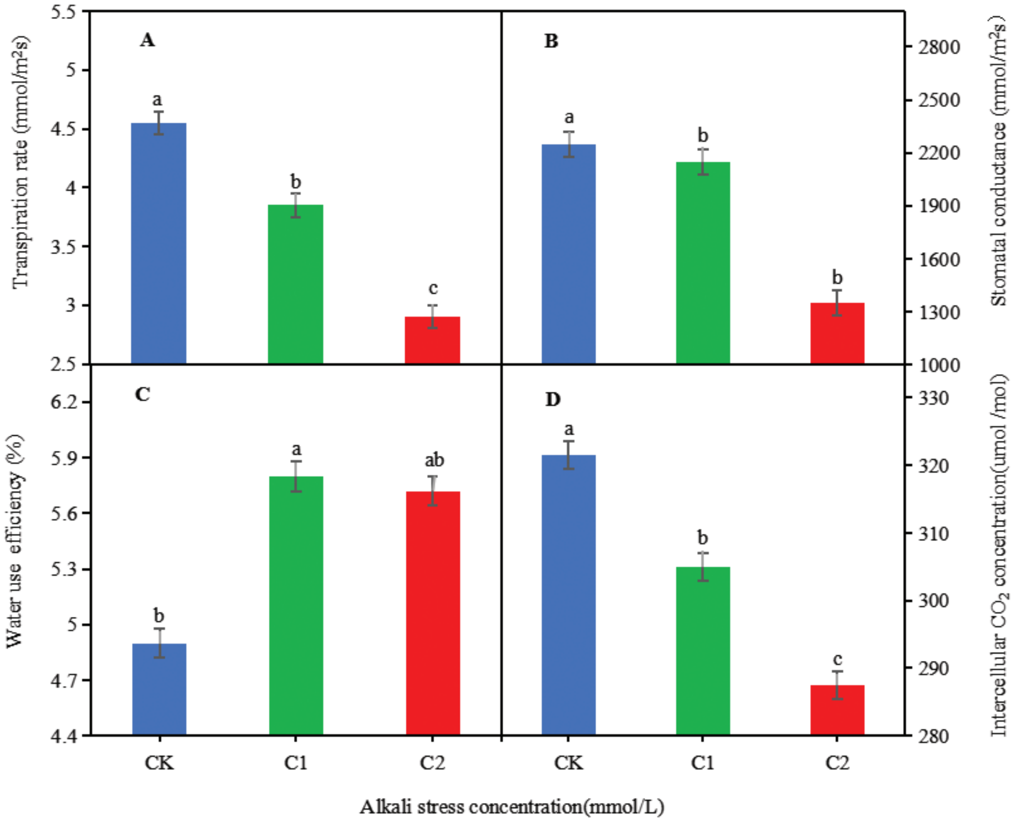

Figure 7. Effects of alkali stress on photosynthetic indexes of *Leymus chinensis*. Note: CK stands for 0 mmol·L^{-1}; C1 stands for alkali concentration of 25 mmol·L^{-1}; C2 stands for base concentration 50 mmol·L^{-1}. Different letters indicate significant differences between different concentrations of the same treatment (P < 0.05).

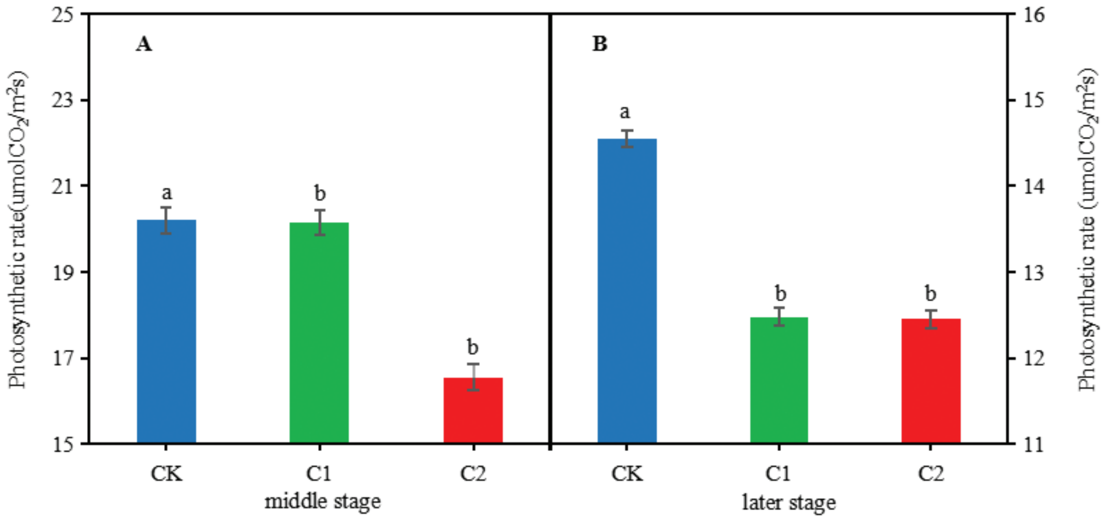

Figure 8. Effects of alkali stress on photosynthetic rate of *Leymus chinensis*. Note: CK stands for 0 mmol·L^{-1}; C1 stands for alkali concentration of 25 mmol·L^{-1}; C2 stands for base concentration 50 mmol·L^{-1}. Different letters indicate significant differences between different concentrations of the same treatment (P < 0.05).

of *L. chinensis* was decreased by 40.22% and 37.78% at 25 and 50 mmol·L^{-1} alkali stress.

With the increase of alkali stress concentration, the diameter of the underground part of *Leymus chinensis* showed a downward trend. Under alkali stress treatment, 25 mmol·L^{-1} and 50 mmol·L^{-1} diameter of underground plant parts was decreased by 6.79% and 8.22% as compared to control.

The underground area also showed a same trend and reduction of 50.14% and 43.96% in underground area was seen at 25 and 50 mmol·L^{-1} alkali stress (Fig. 9C).

The results showed that the underground area of *L. chinensis* decreased significantly with the increase of alkali stress concentration. The underground leaf area also showed a reduction of 59.1% and 49.63% at 25 and 50 mmol·L^{-1} (Fig. 9D).

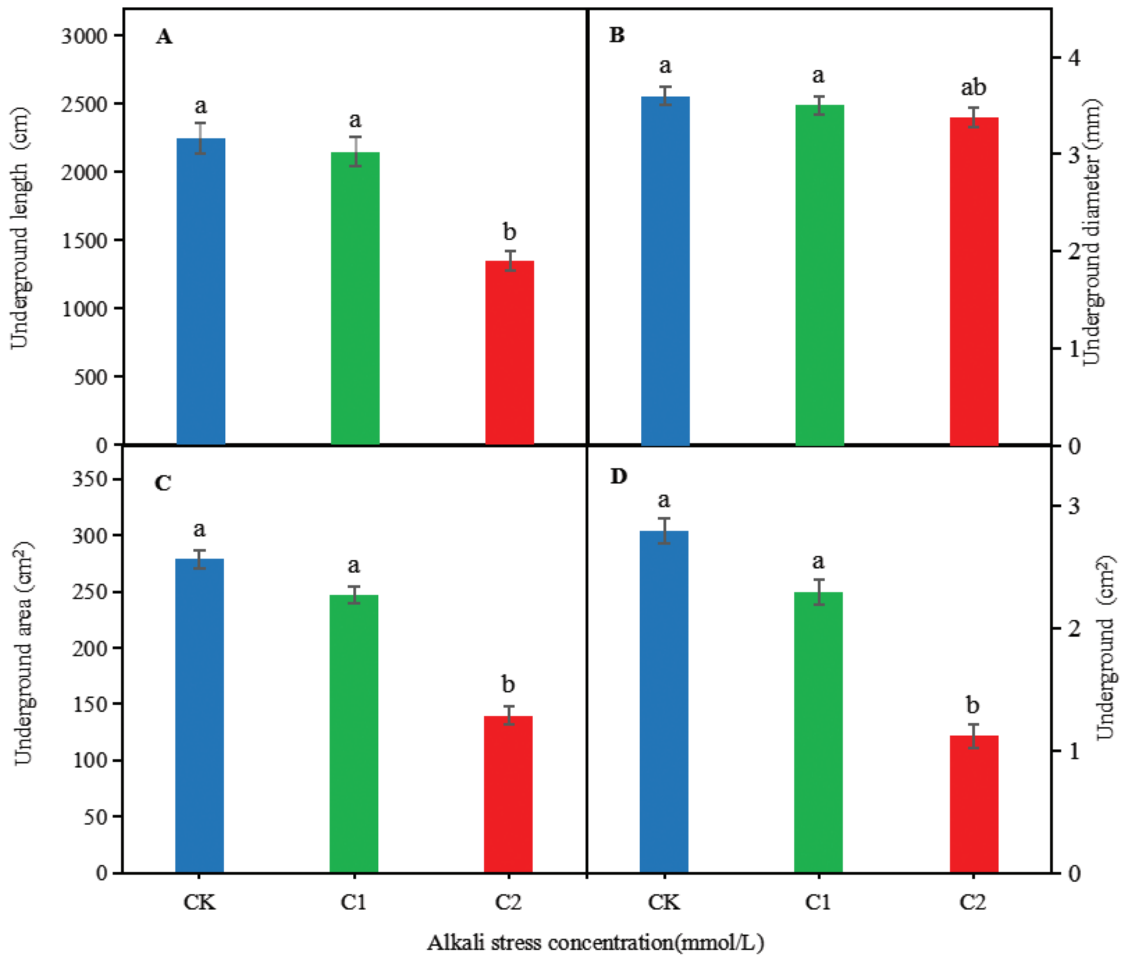

Figure 9. Effects of alkali stress on the length, diameter, and area of *Leymus chinensis* underground part. Note: CK stands for 0 mmol·L^{-1}; C1 stands for Alkali concentration of 25 mmol·L^{-1}; C2 stands for base concentration 50 mmol·L^{-1}. Different letters indicate significant differences between different concentrations of the same treatment (P < 0.05).

Correlation analysis of underground indexes

The results of correlation analysis showed that in the face of alkali stress, the changing trend of each index (diameter, length, area and volume) in the underground part of *Leymus chinensis* (Table 2). The stress effect of alkali treatment was more obvious, and the length, area and volume of the underground soil were negatively correlated with alkali treatment.

Table 2. Correlation analysis of underground part of *L. chinensis* with alkali stress.

	Diameter	Length	Area	Volume
Diameter	1	.556**	.702**	.762**
Length	.556**	1	.948**	.876**
Area	.702**	.948**	1	.979**
Volume	.762**	.876**	.979**	1
Alkali treatment	-.146	-.286**	-.326**	-.344**

Note: ** indicates a significant association at the 0.05 level. **Correlation is significant at the 0.05 level.

Effect of alkali stress on clonal growth of grass

The results showed that the total number of offspring per mother plant decreased with increasing alkali stress concentration (Fig. 10A). The alkali stress treatment differently affected the total offspring number, which was significantly lower at 50 mmol L^{-1} as compared to 25 mmol L^{-1} (Fig. 10A).

The number of total bud per plants decreased, decreasing by 25% and 60% at 25 mmol L^{-1} and 50 mmol L^{-1} compared with the control group, respectively (Fig. 10B).

With the increase of alkali stress concentration, the number of tiller plants and tillering buds decreased first and then increased. Rhizomes showed the opposite trend, which was significantly lower than control group at 50 mmol·L^{-1}, and decreased by 40.98% compared with control group (Table 3). Rhizome internode bud and rhizome terminal bud showed a decreasing trend, and the difference of rhizome internode bud was more significant, and the number of rhizome internode bud decreased by 85.06% compared with the control group at 50 mmol·L^{-1}.

Content of Na^+ and K^+ in underground bud

Under alkali stress conditions, Na^+ content at 25 mmol·L^{-1} and 50 mmol·L^{-1} was significantly increased by 127.63% and 115.22% as compared to control (Fig. 11A and Table 4).

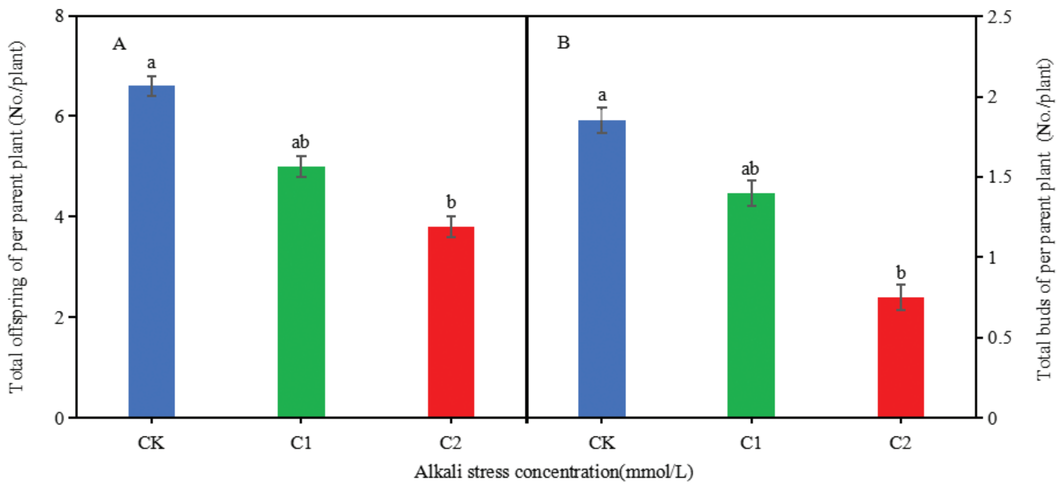

Figure 10. Effects of alkali stress on total plant number and total buds number of *Leymus chinensis*. Note: CK represents 0 mmol L^{-1}; C1 represents base concentration of 25 mmol L^{-1}; C2 represents base concentration of 50 mmol L^{-1}. Different letters indicate significant differences between different concentrations of the same treatment (P < 0.05).

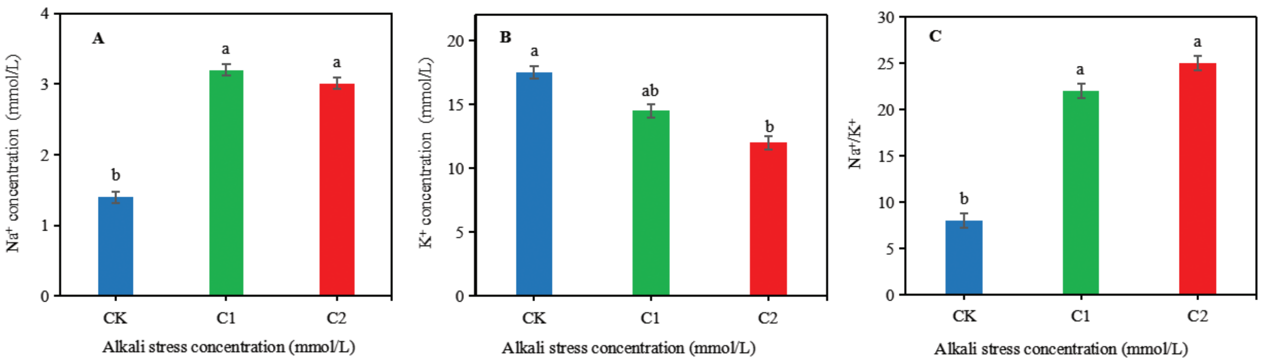

Figure 11. Effects of alkali stress on Na^+, K^+ content and Na^+/K^+ values of *L. chinensis* buds. Note: CK represents 0 mmol L^{-1}; C1 represents, base concentration 25 mmol L^{-1}; C2 represents base concentration 50 mmol L^{-1}. Different letters indicate significant differences between different concentrations of the same treatment (P < 0.05).

Table 3. Clonal growth traits of *Leymus chinensis* under alkali stress Number / mother strain.

Clonal growth	Alkali stress concentration		
	CK	C1	C2
The tillering plant	1.9722±0.19a	1.5897±0.15a	1.697±0.22a
Root stem substrain	1.6944±0.45ab	2.3077±0.45a	1.00±0.28b
tiller bud	0.1667±0.09ab	0.0513±0.036a	0.4242±0.13b
Internode buds of the rhizome	2.0278±0.34a	1.14103±0.23ab	0.303±0.12c
Root tip bud	0.5833±0.12a	0.465±0.15a	0.3939±0.14a

Note: CK represents 0 mmol L^{-1}; C1 represents base concentration of 25 mmol L^{-1}; C2 represents base concentration of 50 mmol L^{-1}. Different letters indicate significant differences between different concentrations of the same treatment (P < 0.05).

The K^+ content of underground buds showed a significant downward trend, and the K^+ content at 25 mmol·L^{-1} and 50 mmol·L^{-1} was reduced by 16.89% and 30.25% compared with the control (Fig. 11B and Table 4).

Alkali stress had a significant effect on Na^+/K^+ in underground bud of *Leymus chinensis*, and Na^+/K^+ increased with the increase of alkali stress concentration.

Underground bud anion content

The experimental results showed that there was no significant change in Cl^- content of underground buds under alkali stress, which was slightly lower than that of the control group (Fig. 12A).

Table 4. Analysis of the variance of salt treatments on the cations and anions of *L. chinensis* buds with alkali stress.

		Cation content			Anion content			
		Na^+	K^+	Na^+/K^+	Cl^-	NO_3^-	$H_2PO_4^-$	SO_4^{2-}
Alkali treatment	F	209.149	7.107	37.019	2.769	6.999	8.641	58.990
	P	0.000**	0.026*	0.000**	0.141	0.027*	0.017*	0.000**

Note: $P \leq 0.01$** The difference is extremely significant, $P \leq 0.05$* the difference is significant.

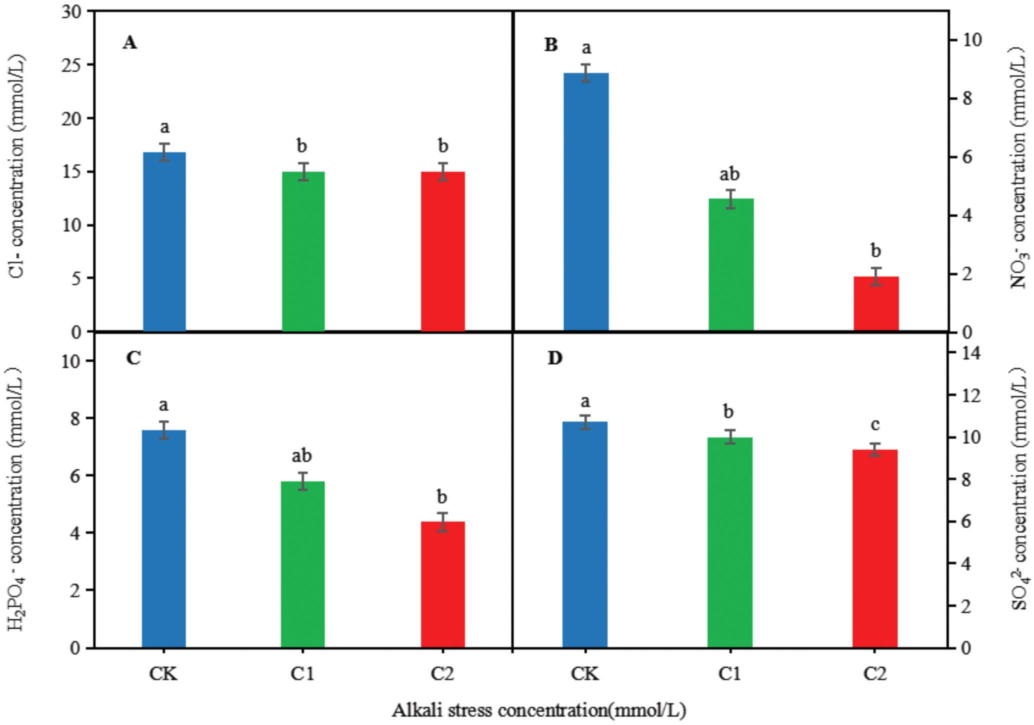

Figure 12. Effects of alkali stress on Cl^- content NO_3^- content $H_2PO_4^-$ content and SO_4^{2-}- content of *L. chinensis* buds. Note: CK stands for 0 mmol·L^{-1}; C1 stands for alkali concentration of 25 mmol·L^{-1}; C2 stands for base concentration 50 mmol·L^{-1}. Different letters indicate significant differences between different concentrations of the same treatment (P < 0.05).

With the increase of alkali stress concentration, NO_3^- and $H_2PO_4^-$ content in underground buds of *Leymus chinensis* showed a decreasing trend (Fig. 12B, C).

There were significant differences in SO_4^{2-} content in underground buds of *Leymus chinensis* at different alkali concentrations (25 and 50 mmol·L^{-1}), decreased SO_4^{2} contents by 6.82% and 11.58% as compared to control (Fig. 12 D).

Discussion

Effects of alkali stress on individual characteristics of *Leymus chinensis*

The dynamic process of relative height growth rate (RHGR) of *Leymus chinensis* showed that the RHGR response of *Leymus chinensis* was more significant under alkali stress. These results indicated that the higher the alkali stress concentration, the greater and more obvious the individual persecution of *Leymus chinensis*. The effects of alkali stress on the content of chemical characters (carbon, nitrogen and phosphorus) of various organs of *Leymus chinensis* were not significant. Under alkali stress, the carbon content distribution of each organ was as follows: leaves > stems > rhizomes > roots; nitrogen content distribution: leaf > rhizome > stem > root; the distribution of phosphorus content was the highest in rhizomes, but little difference in leaves, stems and roots. In recent years, with the development of ecological stoichiometric studies, the growth rate has become a typical theory (Danger et al. 2016; Moreno and Martiny 2018; Buchkowski et al. 2019). Biological organisms with fast growth (high growth rate) have higher N and P contents and RNA contents, while the ratio of protein to RNA and the ratio of N to P are lower (Raven 2013). The hypothesis combines the study of C:N:P values and rRNA allocation with the study of rDNA composition in different biota to closely relate nutrient allocation, organism function, and ecological dynamics. In other words, the adjustment of an organism's life history can cause changes in the C:N:P value of the organism (Sardans et al. 2012; Leal et al. 2017; Van de Waal et al. 2018). This indicates that when 25 mmol·L^{-1} alkali concentration was applied the final relative height growth rate was the lowest, and the corresponding C/P and N/P ratios in stems were the highest. Likewise, with the increase of alkali concentration, plant height and stem biomass per plant showed a downward trend, and the C/N ratio also showed a downward trend. The C/P and N/P ratios were not high at 50 mmol·L^{-1} alkali concentration this may be due to higher alkali stress which has changed the ecological stoichiometric regulation of the stems of *Leymus chinensis* (Li et al. 2020).

Effects of alkali stress on leaf traits of *Leymus chinensis*

The leaf area, dry matter content and leaf water contents were decreased with the increase of alkali concentration. The contents of carbon and nitrogen in leaves of *Leymus chinensis* showed an increasing trend while C/N value showed a decreasing trend. The response of the last relative height growth rate is opposite to the change of C/P and C/P values, which accords with the growth rate theory. Some scholars earlier proposed a determination method for growth restriction by N and P elements (Sun et al. 2013; Wang et al. 2015). These results showed that leaf stomatal conductance, transpiration rate and net photosynthetic rate of *Leymus chinensis* were decreased when treated with low concentration alkali, which was restricted by N and P elements. The plants adopted the strategy of increasing leaf length and reducing area growth to maintain normal operation (Enebe and Babalola 2018). When treated with high concentration of alkali, the water content of *Leymus chinensis* leaves was the highest, and a large amount of C, N and P elements were retained, indicating that alkali stress disrupted the water regulation of leaves, inhibited photosynthesis, and seriously reduced the synthesis of organic substances such as glucose and protein, thus affected the normal growth of plants.

Effects of alkali stress on the subsurface configuration of *Leymus chinensis*

Under alkali stress, biomass of the underground part of *Leymus chinensis* was synergistic and unified with that of the above-ground leaves and stems (Liu et al. 2015). The roots continued to extend and expand, and the underground part grew and developed well. When treated with 25 mmol·L^{-1} alkali, the stress effect caused by alkali stress gradually appeared. The ratio of C/N, C/P and N/P in roots increased, and the growth was inhibited. The diameter of the underground part decreased, and the length, area and volume were significantly reduced. The development and expansion of the roots were continuously reduced, and the stored nutrients and energy were exported to the above-ground part. When treated with 50 mmol·L^{-1} alkali, *Leymus chinensis* was severely persecuted, and its underground biomass, underground length, area and volume were significantly reduced. The ratio of N/P and C/P in rhizomes increased significantly. It is speculated that the continuous high concentration of alkali treatment has constituted a lethal stress on *Leymus chinensis*, and the root system was subjected to the double pressure of high pH value brought by alkali environment and the osmotic displacement of cation and cation ions such as Na^+ and CO_3^{2-}.

Effects of alkali stress on clonal growth of *Leymus chinensis*

The effects of alkali stress on individual characters and organs (leaf, stem, rhizome and root system) of *Leymus chinensis* were both different and uniform. Under alkali stress, the propagation of *Leymus chinensis* (especially asexual) was seriously affected (Li et al. 2014). The increasing alkali concentration, significantly decreased total bud number and total seed number (clonal reproduction capacity) of *Leymus chinensis* however, number of rhizomes was relatively increased. This indicated that *Leymus chinensis* tried to expand the underground part to escape the environmental stress in the early stage of stress treatment. The results of another study showed the same results. Under this concentration treatment, Na^+ content, K^+ content, NO_3^- and $H_2PO_4^-$ contents and SO_4^{2-} contents in underground buds of *Leymus chinensis* were significantly increased, while NO_3^- and $H_2PO_4^-$ contents were significantly decreased, and the imbalance of cations and ions seriously interfered with various enzymatic reactions, and cell metabolism was disturbed and growth and development were inhibited. The output of tiller plants was preferentially compared with rhizomes, and both tiller buds and rhizomes internode buds were significantly reduced, indicating that the reproduction and development of *Leymus chinensis* were minimized to ensure the individual growth of mother plants.

Conclusions

The increase in alkali stress significantly reduced the plant height, RHGR, biomass production, leaf area, relative water contents, and net photosynthetic rate of *Leymus chinensis*. The increasing concentration of alkali stress also led to an increase in accumulation of carbon, nitrogen, and phosphorous. Moreover, alkali stress also increased the sodium concentration which posed negative impacts on plant growth. The present study investigated the growth and physiological response of *Leymus chinensis* to alkali stress, thus more molecular, transcriptomic, and proteomics studies are needed to study this response of *Leymus chinensis* to alkali stress.

Authors Contribution

Zhan-Wu Gao, Ji-Tao Zhang and Chun-Sheng Mu participated in investigation, data collection and manuscript writing. Ge Gao,Ying-Qi Qin, Meng-Zhu Cai, Xin Li,-Li-Jie He, and Zhao-Jie Wang involved in data collection. Sulaiman Ali Alharbi, Mohammad Javed Ansari and Adnan Rasheed reviewed and improved the manuscript.

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References

Buchkowski RW, Shaw AN, Sihi D, Smith GR, Keiser AD (2019) Constraining carbon and nutrient flows in soil with ecological stoichiometry. Frontiers in Ecology and Evolution 7: 382. https://doi.org/10.3389/fevo.2019.00382

Danger M, Gessner MO, Bärlocher F (2016) Ecological stoichiometry of aquatic fungi: current knowledge and perspectives. Fungal Ecology 19: 100–111. https://doi.org/10.1016/j.funeco.2015.09.004

Dong H, Xie L, Cao H, Zhang Y, Liu Y, Xing J (2023) Propagation strategies of Deyeuxia angustifolia in heterogeneous habitats. Frontiers in Ecology and Evolution 11: 1082661. https://doi.org/10.3389/fevo.2023.1082661

Enebe MC, Babalola OO (2018) The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. Applied Microbiology and Biotechnology 102: 7821–7835. https://doi.org/10.1007/s00253-018-9214-z

Frankel R, Galun E (2012) Pollination mechanisms, reproduction and plant breeding. Springer Science & Business Media.

Guo J, Li H, Zhou C, Yang Y (2020) Effects of flag leaf and number of vegetative ramets on sexual reproductive performance in the clonal grass *Leymus chinensis*. Frontiers in Plant Science 11: 534278. https://doi.org/10.3389/fpls.2020.534278

Huang L, Liang Z, Suarez DL, Wang Z, Ma H, Wang M (2015) Continuous nitrogen application differentially affects growth, yield, and nitrogen use efficiency of *Leymus chinensis* in two saline–sodic soils of Northeastern China. Agronomy Journal 107(1): 314–322. https://doi.org/10.2134/agronj14.0250

Leal MC, Seehausen O, Matthews B (2017) The ecology and evolution of stoichiometric phenotypes. Trends in Ecology & Evolution 32(2): 108–117. https://doi.org/10.1016/j.tree.2016.11.006

Li X, Hu N, Yin J, Ren W, Fry E (2021) Historic grazing enhances root-foraging plasticity rather than nitrogen absorbability in clonal offspring of *Leymus chinensis*. Plant and Soil 466(1–2): 65–79. https://doi.org/10.1007/s11104-021-05033-5

Li X, Wang J, Lin J, Wang Y, Mu C (2014) Rhizomes help the forage grass *Leymus chinensis* to adapt to the salt and alkali stresses. The Scientific World Journal 2014: 213401. https://doi.org/10.1155/2014/213401

Li Y, Gong H, Li S, Zhang Y (2020) Ecological stoichiometry homeostasis of six microelements in *Leymus chinensis* growing in soda saline-alkali soil. Sustainability 12(10): 4226. https://doi.org/10.3390/su12104226

Lin J, Shi Y, Tao S, Yu X, Yu D, Yan X (2017) Seed-germination response of *Leymus chinensis* to cold stratification in a range of temperatures, light and low water potentials under salt and drought stresses. Crop and Pasture Science 68(2): 188–194. https://doi.org/10.1071/CP16402

Liu B, Kang C, Wang X, Bao G (2015) Tolerance mechanisms of *Leymus chinensis* to salt–alkaline stress. Acta Agriculturae Scandinavica, Section B—Soil & Plant Science 65(8): 723–734. https://doi.org/10.1080/09064710.2015.1054867

Liu B, Li M, Wang Y, Li J, Xue H (2022) Effects of saline-alkali stress on the functional traits and physiological characteristics of *Leymus chinensis* leaves. Grassland Science 68(4): 336–342. https://doi.org/10.1111/grs.12368

Liu W, Sun S, Zhang C, Lv S, Dong Q (2020) Linking plant spatial aggregation with reproductive traits and near-source seed dispersal: ecological adaptation to heavy grazing. Journal of Plant Ecology 13(4): 489–498. https://doi.org/10.1093/jpe/rtaa036

Masmoudi F, Tounsi S, Dunlap CA, Trigui M (2021) Halotolerant Bacillus spizizenii FMH45 promoting growth, physiological, and antioxidant parameters of tomato plants exposed to salt stress. Plant Cell Reports 40(7): 1199–1213. https://doi.org/10.1007/s00299-021-02702-8

Moreno AR, Martiny AC (2018) Ecological stoichiometry of ocean plankton. Annual Review of Marine Science 10: 43–69. https://doi.org/10.1146/annurev-marine-121916-063126

Raven JA (2013) RNA function and phosphorus use by photosynthetic organisms. Frontiers in Plant Science 4: 536. https://doi.org/10.3389/fpls.2013.00536

Renzhong W, Qiong G, Quansheng C (2003) Effects of climatic change on biomass and biomass allocation in *Leymus chinensis* (Poaceae) along the North-east China Transect (NECT). Journal of Arid Environments 54(4): 653–665. https://doi.org/10.1006/jare.2002.1087

Sardans J, Rivas-Ubach A, Peñuelas J (2012) The C: N: P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. Perspectives in Plant Ecology, Evolution and Systematics 14(1): 33–47. https://doi.org/10.1016/j.ppees.2011.08.002

Sun Y, Wang F, Wang N, Dong Y, Liu Q, Zhao L (2013) Transcriptome exploration in *Leymus chinensis* under saline-alkaline treatment using 454 pyrosequencing. PLOS ONE 8(1): e53632. https://doi.org/10.1371/journal.pone.0053632

Tanji KK (2012) Agricultural salinity assessment and management. Scientific Publishers.

Turner AJ, Arzola CI, Nunez GH (2020) High pH stress affects root morphology and nutritional status of hydroponically grown Rhododendron (*Rhododendron* spp.). Plants 9(8): 1019. https://doi.org/10.3390/plants9081019

Van de Waal DB, Elser JJ, Martiny AC, Sterner RW, Cotner JB (2018) Progress in ecological stoichiometry. Frontiers Media SA. 9: 1957. https://doi.org/10.3389/978-2-88945-621-5

Wang L, Fang C, Wang K (2015) Physiological responses of *Leymus chinensis* to long-term salt, alkali and mixed salt-alkali stresses. Journal of Plant Nutrition 38(4): 526–540. https://doi.org/10.1080/01904167.2014.937874

Xiao H, Peng Z, Xu CL, Zhang DG, Chai JL, Pan TT (2018) Yak and Tibetan sheep trampling inhibit reproductive and photosynthetic traits of *Medicago ruthenica* var. *inschanica*. Environmental monitoring and assessment 190: 1–16. https://doi.org/10.1007/s10661-018-6896-8

Zhan-Wu G, Peiliang S, Yan-Hui C, Hanif A, Zong-Ze Y, Rui-Li L (2023) Physiological responses and adoptive mechanisms in oat against three levels of salt stress. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 51(3): 13249–13249. https://doi.org/10.15835/nbha51313249

Zhang JT, Li XJ, Liu Z, Li XY, Gao ZW, Mu CS (2014) Growth forms of *Leymus chinesis* (Poaceae) at the different developmental stages of the natural population. Plant Species Biology 29(3): 263–271. https://doi.org/10.1111/1442-1984.12023