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ANTIOXIDANT ENZYMES RESPONSES OF DIFFERENT GENOTYPES OF *LEYMUS CHINENSIS* TO SALINE-ALKALI STRESS AND COMPREHENSIVE EVALUATION OF SALINE-ALKALI TOLERANCE

ZHUO ZHANG [1], YUHUI LI[2], SONG LIANG[2], YINGYING YAN[2] AND CHAN ZHOU[2*]

[1]College of Life Science and Bioengineering, Shenyang University, Shenyang, China
[2]School of Life Science, Liaoning University, Shenyang, China
**Corresponding Author's email: zhou_yanhan@163.com*

Abstract

We conducted pot experiment with four genotypes of *Leymus chinensis* at different concentrations of saline-alkali treatment. The saline-alkali tolerance of different genotypes of *L. chinensis* were evaluated comprehensively by *PCA*, *MF* and comprehensive evaluation value (*D* value). The results indicated that with the increase of intensity of saline- alkali stress, the content of soluble protein and the activities of CAT, POD, APX, GR, DHAR and MDHAR in the leaves of four genotypes of *L. chinensis*, ZS, MZ, DQ and CM, were increased compared with the control. And the activities of APX, DHAR and the content of soluble protein of ZS were significantly higher than those of other three genotypes. *PCA* was used to convert 8 single indexes into 3 comprehensive indexes under saline-alkali stress, and the cumulative contribution rate reached 100%. The *D* value of saline-alkali tolerance of four genotypes of *L. chinensis* were 0.167, 0.160, 0.158 and 0.149 respectively. Therefore, the four genotypes of *L. chinensis* have a certain degree of tolerance to saline-alkali stress, and their comprehensive saline-alkali tolerance of four genotypes were ZS > MZ > DQ > CM. This finding provides a certain method and material basis for further study on breeding of new varieties and antioxidant enzyme system of saline-alkali stress of *L. chinensis.*

Key words: *Leymus chinensis;* Genotypes; Saline-alkali stress; Physiological response; Comprehensive evaluation.

Introduction

In recent years, soil salt-alkalization as an abiotic stress has gradually become a global ecological problem (Rengasamy, 2010; Chai *et al*., 2013). Effects of salt and alkaline stress on plants shown in many ways, such as plant morphology, photosynthesis and cell membrane permeability (Qureshi *et al*., 2013). Researchers found that the number of *Triticum aestivum* leaves decreased and the maturity period was advanced when plant suffered from salt stress (Zhang *et al*., 2016). And Mitsuya *et al*., conducted the experiment on *Ipomoea batatas* showed that salt and alkali stress affected the plant cell structure, destroyed the chloroplast structure and affected the photosynthesis (Mitsuya *et al*., 2000; Yang *et al*., 2011; Cheng *et al*., 2015). Meanwhile, soil salt-alkalization will interfere with the dynamic balance of ions of plant cell (Ruiz *et al*., 2016; Zhang *et al*., 2020), and increase the permeability of cell membrane (Dinneny, 2015). Under the condition of saline and alkali, the researchers found that Na^+ content of plant leaves increased, while the content of K^+ decreased (Dai *et al*., 2014; Latef & Tran, 2016). ROS is a critical production to destroy plant cell normal physiological metabolism (Khan *et al*., 2014; Singh & Bhatla, 2016).

The main ways of plants response to salt-alkali stress are to synthesize osmoregulation substances (Chen & Wang, 2009) and improve the antioxidant capacity of enzymes, in order to decrease the effects of growth induced by saline-alkali environment. Proline (Yang *et al*., 2007), soluble sugar (Khan *et al*., 2017) and soluble protein (Xia *et al*., 2010) have been found as osmoregulation substances. Soluble protein as osmotic substance involved in metabolic regulation, with the increase of saline-alkali stress, the trend of soluble protein increased first and then decreased in *Saussurea runcinata* and *Chenopodium quinoa* (Xia *et al*., 2010; Yang *et al*., 2017). The soluble protein content of saline-alkali tolerant plants still showed an increasing trend at higher salt-alkali concentration. In order to maintain normal growth, plants scavenge ROS through antioxidant enzymes (Rangani *et al*., 2016). Some main antioxidant enzymes effects together to remove oxygen radicals and protect antioxidant enzyme system (Wang *et al*., 2007; Zheng *et al*., 2009). Therefore, the content of soluble protein and the activities of antioxidant enzymes can reflect the stress resistance of plants.

Leymus chinensis is high quality forage of gramineae. It is distributed in outer Baikal of Russia, the northeast of Mongolia and the west of Northeast Plain of China widely (Yang *et al*., 2019; Tong *et al*., 2019), and it has high salt and alkaline resistance (Liang *et al*., 2019). As a dominant species of Songnen grassland in Northeast China, *L. chinensis* is often used to study the salt and alkaline tolerance of plants (Cao *et al*., 2020). Previous studies have found that height, leaf number, stem length and aboveground biomass of *L. chinensis* decreased when it was in salt and alkali environments (Liu *et al*., 2015). In terms of ecological types, the salt and alkali tolerance of grey green *L. chinensis* was stronger than that of yellow green (Zhou *et al*., 2003; Yao *et al*., 2020). However, there is no systematic comparative study on the antioxidant enzyme system similarities and differences of different genotypes of *L. chinensis* under the same salt-alkali stress.

Different morphological and antioxidant enzyme indicators can reflect the salt and alkaline resistance of *L. chinensis* to a certain extent, but the adaptation to soil salt-alkalization is a holistic and complex mechanism. Therefore, single index evaluation is relatively one-sided and cannot accurately evaluate its saline-alkali tolerance. It is more reasonable to integrate and simplify multiple indexes into comprehensive evaluation indexes and use the results of comprehensive indexes to reflect the saline-

alkali resistance of *L. chinensis* (Yu *et al*., 2017). In recent years, correlation analysis, principal component analysis, membership function method and *D* value method is widely used to describe comprehensive evaluation of salt and alkaline resistance of plants. For example, some researchers have been applied in the identification of salt and alkaline tolerance of *Phaseolus vulgaris* and in the comprehensive evaluation of salt tolerance of *Zea mays* (Li *et al*., 2016; Wang *et al*., 2017). It is more scientific and reasonable to use *D* value to evaluate the saline-alkali resistance of *L. chinensis*.

Four genotypes of *L. chinensis* from different geographical sources were treated with three concentration gradients of saline-alkali stress, and eight physiological indexes were measured. Correlation analysis, principal component analysis and membership function method were used to comprehensively analyze the different traits of different genotypes of *L. chinensis* to determine the saline-alkali resistance of different genotypes of *L. chinensis*. The purpose of this experiment is to verify the following two scientific hypotheses: 1) Different genotypes of *L. chinensis* have significantly different resistance to salt and alkali stress; 2) The saline-alkali tolerance of different genotypes of *L. chinensis* can be evaluated by membership function method. It can provide scientific reference for accurate evaluation and variety breeding of *L. chinensis*, and give full play to the positive role of *L. chinensis* in improving saline-alkali land.

Materials and Methods

Material cultivation: The widely distributed *L. chinensis* is used as our experimental material, which is common in the eastern Inner Mongolia and western Northeast China. Under different habitats, different genotypes of *L. chinensis* have been formed due to adaptation and evolution. In 2012, we collected *L. chinensis* seeds from individual plants in 4 distinct populations in Daqinggou (DQ; 49.1002 °N, 121.6461 °E), Caimushan (CM; 42.4021 °N, 116.8019 °E), Zhaosu (ZS; 43.1641 °N, 81.1676 °E) and Manzhouli (MZ; 49.5978 °N, 117.3787°E). In April 2013, the *L. chinensis* seeds from the four regions were respectively placed in the light incubator at 16℃ / 28℃ (12h / 12h) for sand culture. Then we selected one of the seedlings from each region for Simple Sequence Repeats (SSR) markers to determine that each genotype used in this experiment was unique. In order to obtain a large number of tillers of the same genotype, we established four 5 m × 5 m asexual propagation experimental plots in Liaoning Provincial Sandy Land Improvement and Utilization Research Institute (Zhanggutai, Fuxin city, Liaoning province, China). When the leaf age was 2 and the plant height was 6 to 8 cm, the *L. chinensis* seedling of each genotype that marked by SSR were transplanted into the four plots to reduce the maternal effect. Through asexual propagation by a seedling, a large number of tillers of the same genotype were obtained.

Experimental methods: From April 2016 to October 2016, we conducted pot experiment in the Botanical Garden of Liaoning University. Sand-culture method was used in our experiment (Li *et al*., 2018). We put the same quality dry sand in plastic pots of 38 cm (diameter) × 40 cm (height) to avert soil amount influence the growth of *L. chinensis*.

The pot experiment used a two-factorial design involving saline-alkali treatment and genotype. We mixed NaCl, Na_2SO_4, $NaHCO_3$, and Na_2CO_3 at a molar ratio of 1: 9: 9: 1 to prepare salt-alkali mixed solution (Shi *et al*., 1998). Under this condition, four gradients of saline-alkali treatment concentration were applied: 0, 50, 200, 350 mmol·L^{-1}, and the pH is presented in Table 1. We used four identified genotypes of *L. chinensis*, DQ, CM, ZS and MZ, to explore the antioxidant enzymes response of different genotypes to saline-alkali stress. We had 16 treatment combinations with 5 replicates in this experiment, for a total of 80 pots (4 saline-alkali stress treatments × 4 genotypes × 5 replicates).

Table 1. Saline-alkali treatment concentration and corresponding pH value.

Solution concentration (mmol·L^{-1})	pH
0	7.00
50	8.52
200	8.76
350	8.91

In April 2016, tillers were transplanted into pots at a density of 25 ramets per pot. Similar size tillers were used in order to prevent the effect of tillers size when transplanting. The position of the pots is placed in a completely random principle to avoid the influence of the microenvironment such as sunlight and moisture on this experiment. We watered the tillers with Hoagland nutrient solution at 2-day intervals throughout the whole experiment.

When *L. chinensis* ramets were 8 weeks old, they were treated with saline-alkali stress. Saline-alkali mixed solution were added to the ramets on June 12 and 16, 2016, respectively. Stress treatments were performed at 5-8 p.m. The saline-alkali mixed solution of 50, 200, 350 mmol·L^{-1} was respectively used as the treatment liquid, and divided into 3 times added to the ramets. The control group was given the same amount of Hoagland complete nutrient solution. The total treatment lasted 7 days. On the morning of June 19, 2016, a total of 30 fully expanded leaves on the upper (the leaves should be at the same position) were harvested of each pot, and putted into liquid nitrogen immediately, then stored in minus 80℃ refrigerator. These leaves were used to determinate the relevant indicators.

Determination of soluble protein content and SOD, POD, CAT activities: To measure the soluble protein content, the Coomassie Brilliant Blue G-250 method was used (Bradford, 1976). The activity of SOD was measured by the method with Kono (Kono, 1978). This study used guaiacol method to determine POD activity (Bergmeyer, 1965). The activity of CAT was detected by measuring H_2O_2 decomposition at 240 nm (Aebi, 1984).

Determination of APX, GR, DHAR and MDHAR activities: The activity of APX and GR were measured according to Nakano, Asada and Halliwell *et al.* (Nakano & Asada, 1981; Halliwell & Foyer, 1978). The measurements of DHAR and MDHAR activity in this study were line with Hossain *et al.* (Hossain & Asada, 1984; Hossain *et al*., 1984).

Calculation of relevant indicators: The saline-alkali tolerance of different genotypes of *L. chinensis* were evaluated comprehensively by *SATC*, *PCA* and *MF*. *SATC* was used to determine salt-alkali resistance of different genotypes of *L. chinensis* (Yu *et al*., 2017). *PCA* can be used for analyze the *SATC* of each single index, and the primitive parameters were transformed into new comprehensive indexes. *MF* method was further used for evaluate the comprehensive indexes. Reference to the method of others (Wang *et al*., 2007; Zhang *et al*., 2016) to calculate the following parameters:

$$U(X_j) = (X_j - X_{\min}) / (X_{\max} - X_{\min}) \qquad j = 1,2,...,n \tag{1}$$

where X_j is the j_{th} comprehensive index, $U(X_j)$ is the *MF* value of the j_{th} comprehensive index, X_{min} and X_{max} is the minimum and maximum values of j_{th} comprehensive index respectively.

The weight of each comprehensive index is calculated by the contribution rate:

$$W_j = \sum_{j=1}^{n} \left| P_j \right| \qquad j = 1,2,...,n \tag{2}$$

where W_j is the weight of j_{th} comprehensive index in all comprehensive indicators, and P_j is the contribution rate of the j_{th} comprehensive index.

$$D = \sum_{j=1}^{n} \left[U(X_j) \times W_j \right] \quad j = 1,2,3,...,n \tag{3}$$

In the formula (3), D is the comprehensive evaluation value of saline alkali resistance of *L. chinensis* genotypes under saline-alkali stress.

Statistical analysis: Means and standard deviation of all data were showed. Two-factors ANOVA analysis was used to test the significance of genotype of *L. chinensis*, saline-alkali treatment and their interaction on antioxidant enzyme indicators. In addition, multiple comparisons were made at the 0.05 level to determine if there were significant differences among the four genotypes. All data were calculated using IBM SPSS statistical software 22.0 (SPSS, Inc. Chicago, IL, USA). All data processing is done using Origin 8.

Results

Soluble protein content: Two-way ANOVA showed that not only genotype but also saline-alkali treatment had significant effects on soluble protein content in leaves of *L. chinensis* (p<0.01; Table 2). When concentration of saline alkali treatment increased, the soluble protein content of DQ, CM, ZS and MZ were increased by 1.84%-11.73%, 2.02%-21.52%, 5.93%-15.04% and 3.05%-21.02% compared with 0 mmol·L^{-1} of saline-alkali treatment. And the soluble protein content of each genotype reached a maximum in 350 mmol·L^{-1} of saline-alkali treatment in our experiment. In each gradient of saline and alkali treatment, the soluble protein content of ZS and MZ were significantly higher than DQ and CM (p<0.01; Fig. 1A).

The activities of SOD, CAT and POD: Genotype played significant effect on the activities of SOD and POD (p<0.01) but not on CAT (Table 2). Saline-alkali treatment had significant effect on CAT and POD activities (p<0.01) but not on SOD (Table 2).

The increase of SOD activity of CM was 7.81%-27.60% more than those in 0 mmol·L^{-1} of saline-alkali treatment. The SOD activity of MZ increased by 22.63% at the saline-alkali concentration of 350 mmol·L^{-1}. It can be seen from Fig. 1B that the SOD activity of ZS was always the highest in different saline-alkali stress treatment gradients. As shown in Fig. 1C and Fig. 1D, the CAT activity and POD activity of DQ, CM, ZS and MZ increased when the intensity of saline alkali treatment increased. Compared with the saline-alkali concentration of mmol·L^{-1}, the CAT activities of DQ, CM and ZS were increased by 19.68% -55.48%, 15.47% -163.35% and 2.92% -167.35% respectively (Fig. 1C). The POD activity of DQ, CM, ZS and MZ reached the maximum at 350 mmol·L^{-1}, and increased by 9.45, 3.62, 10.19, and 2.41 times at 0 mmol·L^{-1} (Fig. 1D). It indicated that SOD, CAT and POD activities of CM, ZS and MZ were stronger than those of DQ.

Table 2. Two-way ANOVA on soluble protein content and antioxidant enzyme indexes of different genotypes of *Leymus chinensis* under saline-alkali stress.

Index	G		S		G×S	
	F	P	F	P	F	P
Soluble protein	23.261	**0.000**	10.791	**0.000**	0.460	0.896
SOD	10.336	**0.000**	0.920	0.436	1.566	0.148
CAT	1.790	0.158	15.089	**0.000**	1.148	0.343
POD	31.820	**0.000**	11.617	**0.000**	0.754	0.659
APX	17.987	**0.000**	2.340	0.082	0.859	0.565
GR	5.959	**0.001**	19.143	**0.000**	0.936	0.500
DHAR	10.004	**0.000**	7.754	**0.000**	0.418	0.921
MDHAR	2.400	0.076	1.234	0.305	0.133	0.999

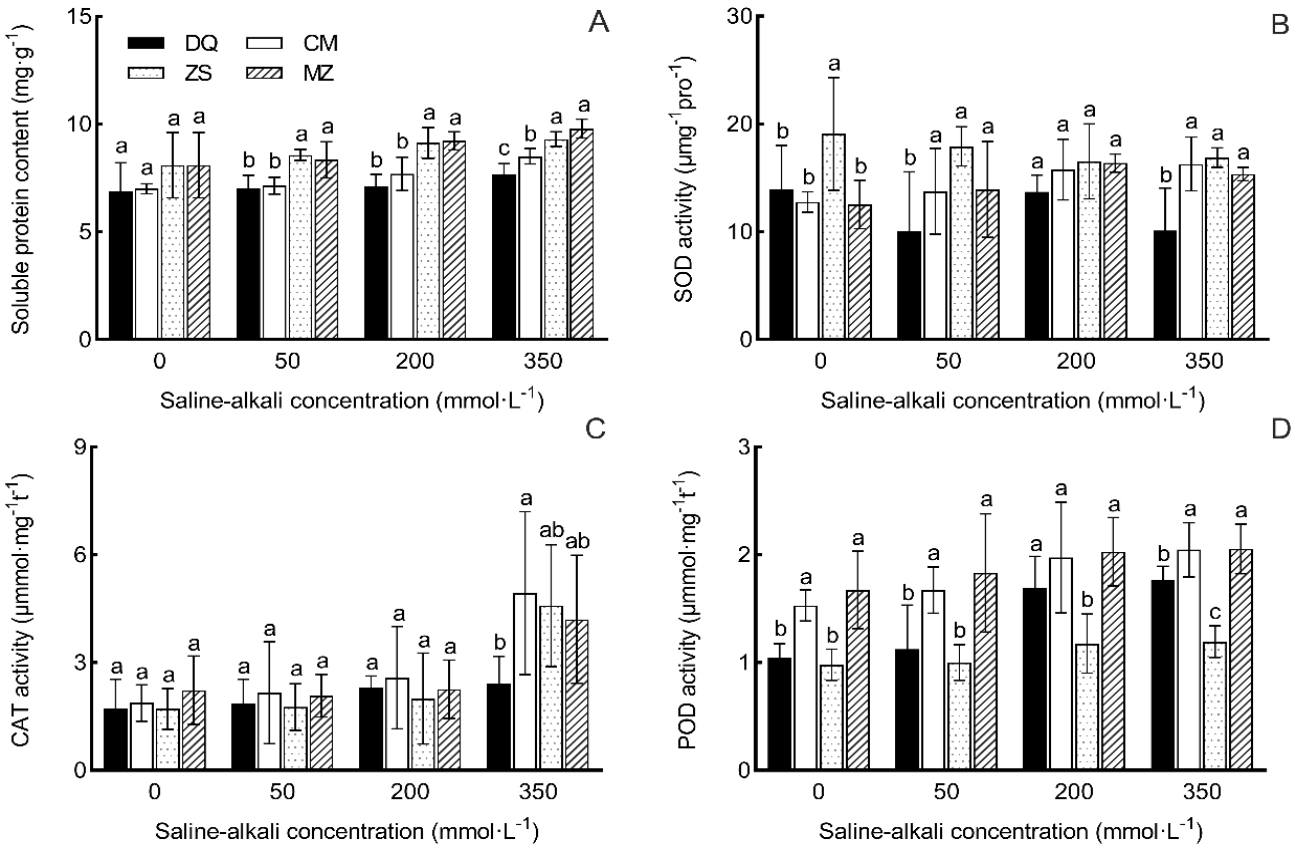

Fig. 1. The content of soluble protein, the activities of SOD, CAT and POD of different genotypes of *Leymus chinensis* at different concentration gradient of saline-alkali treatment.

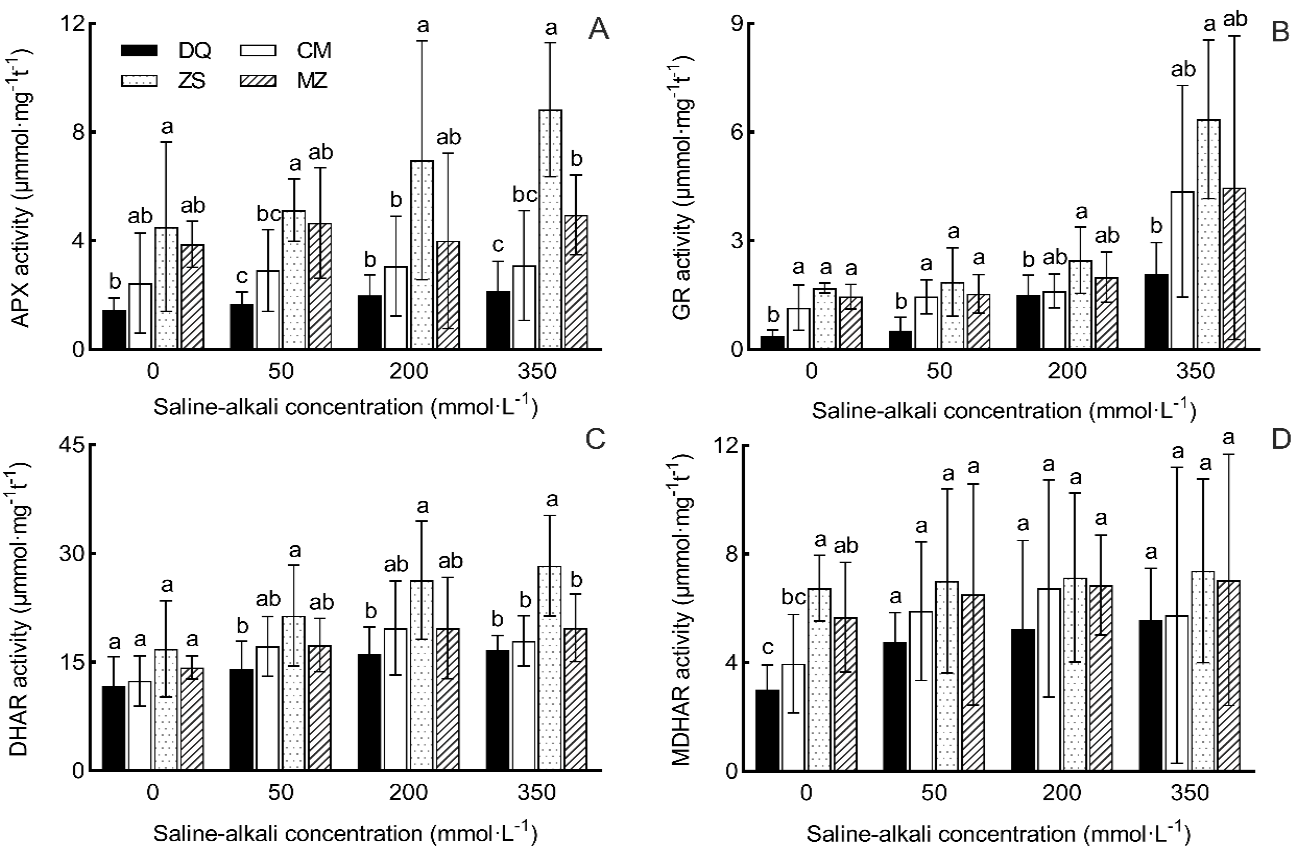

Fig. 2. The activities of APX, GR, DHAR and MDHAR of different genotypes of *Leymus chinensis* at different concentration gradient of saline-alkali treatment.

Table 3. Saline-alkaline tolerance coefficient of each single index in different *L.chinensis* genotypes.

Genotype	Soluble protein	SOD activity	CAT activity	POD activity	APX activity	GR activity	DHAR activity	MDHAR activity
DQ	1.056	0.812	1.414	1.456	1.323	1.616	1.335	1.720
CM	1.111	1.196	1.722	1.240	1.237	2.152	1.471	1.547
ZS	1.112	0.897	1.623	1.146	1.545	2.099	1.505	1.064
MZ	1.127	1.216	1.277	1.179	1.171	1.831	1.327	1.200

Table 4. Coefficients of comprehensive indexes and proportion.

	Soluble protein	SOD activity	CAT activity	POD activity	APX activity	GR activity	DHAR activity	MDHAR activity	Proportion
CI_1	**-0.971**	**-0.630**	-0.272	**0.988**	-0.072	**0.983**	**-0.542**	**0.822**	0.542
CI_2	-0.237	**-0.648**	**0.716**	-0.039	**0.920**	0.185	**0.778**	-0.120	0.311
CI_3	-0.002	0.428	**0.643**	0.148	-0.386	-0.017	0.318	**0.557**	0.147

Table 5. The comprehensive indicator values, indicator weight (*IW*), *U(Xj)*, *D* value of different *L.chinensis* genotypes.

Varieties	Comprehensive index			Membership function value			*D*	Salt-alkali tolerance
	CI_1	CI_2	CI_3	$U(X_1)$	$U(X_2)$	$U(X_3)$		
DQ	1.487	0.068	-0.183	0.491	0.458	0.434	0.158	3
CM	-0.327	0.041	1.463	0.445	0.463	0.430	0.149	4
ZS	-0.642	1.168	-0.688	0.519	0.486	0.459	0.167	1
MZ	-0.519	-1.277	-0.593	0.501	0.459	0.444	0.160	2
IW				0.542	0.311	0.147		

The activities of APX, GR, DHAR and MDHAR: Genotype had a significant effect on the activities of APX, GR and DHAR ($p<0.01$), but had no significant effect on MDHAR activity (Table 2). The GR and DHAR activities were significantly affected by saline-alkali. ($p<0.01$; Table 2). However, APX activity and MDHAR activity were not significantly affected by that (Table 2). With the increase of saline alkali treatment gradients, APX, GR, DHAR and MDHAR activities of almost all the genotypes increased in varying degrees, and reached maximum when the saline-alkali concentration was 350 mmol·L^{-1} (Fig. 2A-D). The APX activities of DQ, CM, ZS and MZ were increased by 14.02%-46.95%, 18.98%-26.24%, 13.64%-95.45% and 3.23%-27.88% higher than control group (Fig. 2A). The GR activity of each genotype were 4.5, 2.8, 2.7 and 2.1 times higher than the control group at 350 mmol·L^{-1} (Fig. 2B). The DHAR activity of DQ, CM, ZS and MZ increased by 42.28%, 44.44%, 68.08% and 38.33% compared with the control group at 350 mmol·L^{-1} respectively (Fig. 2C). Although the saline-alkali stress did not have a significant effect on the activity of MDHAR, the saline-alkali treatment still promoted the synthesis of MDHAR. The MDHAR activity of DQ, CM, ZS and MZ were increased by 84.19%, 45.07%, 9.47% and 24.26% at 350 mmol·L^{-1} (Fig. 2D).

The activities of APX, GR, DHAR and MDHAR of ZS and MZ are higher than those of DQ and CM at any concentration gradients of saline-alkali treatment. At the same time, the APX activity of ZS was obvious higher than DQ and CM when the saline-alkali concentration was 50 mmol·L^{-1} and 200 mmol·L^{-1} (Fig. 2A). The APX and DHAR activities of ZS were significantly higher than those of the other three genotypes at saline-alkali concentration of 350 mmol·L^{-1} (Fig. 2B-C). The results of four main enzymes in Ascorbic Glutathione Cycle showed that the adaptability of ZS and MZ to saline-alkali stress is stronger than that of DQ and CM, and the adaptability of ZS to saline-alkali stress was stronger than that of other genotypes.

Principal component analysis and saline-alkali resistance evaluation: The saline-alkali tolerance coefficient of each index is shown in Table 3. Principal component analysis was carried out on the saline-alkali tolerance coefficients of the 8 individual indicators obtained, and it was found that the primitive parameters can be transformed into 3 new comprehensive indexes. The contribution rates (CI_1, CI_2, CI_3) were 54.20%, 31.10% and 14.70% respectively, and the cumulative contribution rate reached 100.00%. It can be seen from Table 4 that the coefficients of soluble protein content, POD, GR and MDHAR in CI_1 were larger, the coefficients of SOD, CAT, APX and DHAR in CI_2 were larger, and the coefficient of CAT in CI_3 was larger.

The *MF* analysis of the comprehensive indicators of *L. chinensis* genotypes showed that the greater the *U* value, the greater the saline-alkali resistance of the *L. chinensis*. Taking CI_1 for example, the $U(X_1)$ value of ZS was the highest, showing that ZS had the strongest saline-alkali tolerance under saline-alkali stress, while the smallest $U(X_1)$ value of CM indicates that CM had a weak saline-alkali tolerance. The *D* value is used to estimate saline-alkali tolerance of the genotypes of *L. chinensis*. Among four genotypes of *L. chinensis*, the *D* value of ZS was the largest, revealing that it had the strongest saline-alkali tolerance, and CM had the smallest *D* value, indicating that it had the weakest saline-alkali tolerance (Table 5). The comparison results of the comprehensive saline-alkali tolerance of the four genotypes *L. chinensis* were as follows: ZS > MZ > DQ > CM.

Discussion

Effect of saline-alkali treatment on soluble protein content and antioxidant enzyme activity of different genotypes of *Leymus chinensis*: Saline-alkali stress can cause dehydration of plant cell membranes (Premachandra *et al*., 1992) and affect the osmotic regulation of plants (Wang *et al*., 2017). Soluble protein is one of the important osmotic protective substances in plants, and its content can reflect the change of plant metabolic level (Liu *et al*., 2007). Our research found that the content of soluble protein increased, which was line with the increasing of the concentration of saline alkali treatment. The reasons for the above results may be: different genotypes of *L. chinensis* have a certain degree resistance to saline alkali stress. When *L. chinensis* grow in saline alkali treatment, it accumulates a large quantity of soluble protein to improve the water-holding capacity of the cell and maintain the cell's osmotic balance to increase its adaptability to saline alkali stress. The similar results were found in other researchers (Li *et al*., 2017).

When plant suffered from high-concentration of saline alkali environment, the generation and accumulation of active oxygen usually occurs in plants (Alscher *et al*., 1997; Pancha *et al*., 2015). Plants rely on antioxidant enzymes to scavenge active oxygen and reduce their damage to cells (Apel & Hirt, 2004; Mishra *et al*., 2013; Gong *et al*., 2013). For CM and MZ, saline alkali treatment increased the SOD activity, which was in line with our expectations. The SOD can remove O_2^- and relieve the damage of CM and MZ caused by saline-alkali stress. However, the SOD activity of DQ and ZS showed a decreased trend (Fig. 1B). This result is partially contrary to the previous experimental results in *Zea mays* (Tuna *et al*., 2008) and *Oryza sativa* (Khan & Panda, 2008). The possible reason is that the regulation ability of the SOD is limited. Moreover, when SOD scavenges O_2^-, the activity of SOD decreased with the increase of H_2O_2 generated, so the enzyme activity decreases (Bray *et al*., 1974). In addition, it can be shown that the removal mechanism of O_2^- plays a smaller role in DQ and ZS. H_2O_2 is the product of the SOD scavenging O_2^-, it is still toxic, and plants can eliminate it by CAT, POD and APX in the subsequent reaction.

The results showed that saline-alkali stress increased CAT, POD and APX activities of various genotypes of *L. chinensis* (Figs. 1-2). This indicated that the scavenging mechanism of H_2O_2 played an important role in both DQ, CM, ZS and MZ. When comparing the activity of hydrogen peroxide scavenging enzymes, the scavenging activity of CAT on H_2O_2 is higher than that of POD. Our experimental results are consistent with those of others (Rout & Shaw, 2001), and we believe that CAT, as H_2O_2 scavenging enzyme, leads to plant salt tolerance. Some researchers believe that salt tolerance characteristics of different varieties are associated by changing GR activity of salt-tolerant varieties (Hernández *et al*., 2000). With the increase of the intensity of saline-alkali stress, the results of our experimental indicated that the saline-alkali stress significantly improved the activity of GR of *L. chinensis*. The increase of GR activity indicates an increase in GSH turnover rate. It showed that scavenging ability of reactive oxygen of *L. chinensis In vivo* was enhanced to improving its stress resistance. We also observed the increase of DHAR and MDHAR activities in Fig. 2, indicating that *L. chinensis* can effectively remove active oxygen through the combined action of the antioxidant enzyme system under saline-alkali stress, and resist the damage caused by saline-alkali stress.

Comprehensive evaluation of saline-alkali tolerance of different genotypes of *Leymus chinensis*: The salt-alkali tolerance of different genotypes of *L. chinensis* was obtained by *PCA* and *MF* calculation. By comparing the *D* value, the salt-alkaline tolerance of various genotypes of *L. chinensis* were ZS > MZ > DQ > CM. Our research found that the results of the comprehensive evaluation are consistent with the results of single index evaluation, and a more accurate sequence of saline alkali resistance of *L. chinensis* was obtained, indicating that the comprehensive evaluation method can be used to evaluate the saline alkali tolerance of *L. chinensis* genotypes. The comprehensive evaluation method takes into account not only the correlation between various indicators, but also the difference in importance between different indicators, which can more scientifically and accurately reflect the resistance of different genotypes of *L. chinensis* to saline-alkali stress. The research on *L. chinensis* saline-alkali tolerance can provide information for the selection and breeding of excellent new varieties of salt and alkali resistance.

Conclusion

There are intraspecies difference in saline-alkali resistance of *L. chinensis*, and the study of individual indicators shows that different genotypes of *L. chinensis* have significantly different resistance to saline and alkali. The adaptability of ZS to saline alkali treatment is significantly stronger than that of MZ, DQ and CM. The salt-alkaline tolerance of various genotypes of *L. chinensis* was evaluated by comprehensive evaluation method, the results were ZS > MZ > DQ > CM. However, due to the wide range of distribution of *L. chinensis*, many different genotypes of *L. chinensis* have formed in the process of adapting to the environment, so more genotypes of *L. chinensis* can be compared in future studies. At the same time, gene level can be added for analysis during comparison to make the results more accurate.

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