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Tolerance to saline and alkaline stress involves antioxidant enzyme activities in perennial turfgrass

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ABSTRACT

An understanding of the salt-alkali tolerance of each plant variety is required for their utilization and popularization. Perennial ryegrass is a vital turfgrass and forage species worldwide. To understand the stress tolerance and physiological mechanisms of the variety Weinasi, we examined the effects on germination, plant growth, and antioxidant enzyme activity of this variety under different salt-alkali stresses. The results suggested that Weinasi had specific adaptability to saline stress equivalent to less than 100 mM NaCl but high sensitivity to alkaline stress equivalent to 20 mM NaHCO3, expressed on repressed germination rate and inhibited plant growth. The adverse effects of alkaline stress on the underground parts were more evident than those on the aerial parts. This variety could resist salt-alkali stress by increasing SOD activity when growing well, but POD and CAT activities decreased gradually after different salt-alkali treatments. This result could help guide precise utilization in particular areas, artificial breeding and variety promotion of perennial ryegrass.

Keywords: *Lolium perenne*, Salt-alkali stress, Perennial ryegrass, Soil salinization, Stress adaptability.

INTRODUCTION

Soil salinization, considered as one of the key problems in the world, restricts plant growth and badly reduces crop production (Boyer, 1). In China, nearly one million square kilometers of soil area, distributed in Xibei, Dongbei, Huabei, and some coastal areas, is facing the problem of soil salinization. And such a large area of soil salinization has seriously threatened China's ecological environment and food security. Currently, Chinese government has stepped up our attention to harness the ecological environment and in-depth the researches on the mechanism of salt tolerance of plants. Because there are no economical and feasible means to improve crop yields on salinized lands at present, plants with high tolerance to soil salinization seems to be one feasible way to cope up with this challenge (Wu, 14). The understanding of the salt tolerance of plant species, their physiological mechanisms, and genetic regulations is considered as the first step of breeding of robust salt-tolerant plant species able to adapt on saline-alkali soils (Hamdia and Shaddad, 9). It could provide an important basis for promoting further agricultural production and increasing income.

Lolium perenne L. is a perennial Gramineae grass, mostly cultivated in Sichuan, Guizhou, Yunnan, Jiangsu, Zhejiang, and other southern provinces. Due to having high yield, long-season production, low fibre concentrations, and rapid regrowth (He *et al.*, 10), *L. perenne* is not only a perfect turfgrass, but also an ideal green forage and fodder in winter for fowl and livestock. In addition, this turfgrass has relative strong tolerance to soil infertility, sewage, and soil micro organism and other heavy metal polluted environments (Wu *et al.*, 15), showing high application probability and perspective. Because different varieties or populations usually exhibit various biological characteristics and stress tolerance, to better utilize them, clear understanding of their stress tolerances becomes important.

With regard to this scope, scientists have done many valuable works, such as studying the physiological responses to $NaHCO_3$ and Na_2SO_4 (Gao *et al.*, 6), sodium carbonate decahydrate stress (Guo *et al.*, 8), additional NO and $NaHCO_3$ (Liu *et al.*, 11), and other environmental stresses, such as low-temperature, heavy metal contamination (Zhai *et al.*, 16; Feng *et al.*, 4). In this study, we selected ryegrass variety Weinasi, as one mature commercial turfgrass variety, having the characteristics of developed root systems and fast growing, as the object material. We aimed to study the effects of different saline and salt-alkali stresses (including the sea water-simulated saline stress, NaCl-induced saline stress, $NaHCO_3$-induced alkaline stress, and combined salt-alkali

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stress) on seed germination rate, plant development and antioxidant enzyme activities of this ryegrass variety. Estimating and further understanding of the tolerance degrees of saline stress, alkalinity stress, and combined salt-alkali stress of this ryegrass variety would be conducive to better commercial application.

MATERIALS AND METHODS

The seeds of *L. perenne* variety Weinasi, were provided from Prof. Fu Jin-Min, School of Resources and Environmental Engineering, Ludong University, Yantai, China. Before germination, the ryegrass seeds were surface-sterilized with 70% ethanol for 30-60s, and NaClO solution (5%) for 15-20 min, and then rinsed by sterile water for several times. Twenty seeds dried on the sterilized filters were put in a dish. Nine dishes were repeated for each treatment. From the beginning of germination, the seeds and subsequent seedlings were grown at a 25°C incubator with a 16 h-day photoperiod, a 60% relative humidity.

Saline and salt-alkali stress treatments were given from the germination. The 10 ml treatment solutions were added to the Petri dishes in the beginning of treatment, and then supplemented the natural water evaporation loss with sterile water every two days. The ryegrass seeds were treated with different concentrations of four stresses, including sea water-stimulated saline stress, NaCl induced saline stress, $NaHCO_3$-induced alkaline stress, and combined salt-alkali stress. Sea water was selected from the coastal area near Yantai University, with the geographic coordinate of 121°48'N, 37°48'E. In this area, the general salt concentration is 3.3-3.5%. According to this value, the sea water was considered as approximately 600 mM NaCl solution in this study. The concentration gradient of different treatments was set as 20 mM, 50 mM, 100 mM, 200 mM, 400 mM, and 600 mM. Seeds and seedlings were treated by sterile water as the control. The concentrations of combined salt-alkali stress were set by NaCl and $NaHCO_3$ of 1:1 in concentration, e.g. 10 ml 200 mM combined salt-alkali stress treatment solution mixed by 5 ml 200 mM NaCl and 5 ml 200 mM $NaHCO_3$.

Germination rates were estimated after 16 d of different salt-alkali stress treatments. Germination rate was calculated by the ratio of all germinated seed numbers to all seed number used to germinate in the beginning of every experiment. Each dish calculated one germination rate, and each treatment was repeated in 9 dishes.

The seedlings after 16 d of different treatments were carefully removed from the Petri dishes, and put onto filters to keep dry. And then, the seedlings were divided into aerial parts and underground parts. The heights of the aerial parts and the lengths of the underground parts of treated seedlings were measured from each treated seedlings. At the same time, the fresh weights of aerial parts and underground parts were also measured from every seedling. All seedlings from each treatment were measured, and used to calculate the mean values and the mean absolute deviations.

Fresh functional leaf tissues (approximately 10 g) were sampled and weighted as the fresh weight (FW) values. And then the leaf tissues were kept at 80°C in a hot air oven to dry for 12 h until reaching the constant weight (DW) values. Relative water content (RWC, %) of leaves was calculated using the following formula: RWC = (FW-DW)/DW × 100%.

Antioxidant enzyme activities, including superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), and catalase (CAT, EC 1.11.1.6), were measured according to the methods by Chance and Maehly (2), Fu and Huang (5), Giannopolitis and Ries (7), and Rao *et al*. (13).

All data were subjected to one-way analysis of variance (ANOVA) using Duncan's multiple-range test in the software SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The time of beginning to germinate varied with different salt-alkali stress treatments. Under the non-stressed treatments (0 mM), low (20-50 mM), and medium salt-alkali stress treatments (100-200 mM), the ryegrass seeds began to germinate after 4 d. Under the high salt-alkali stress treatments (400-600 mM), the germination was observed after 13 d. Among all treatments, the sea water-stimulated saline stress treatment had the weakest effect on seed germination, resulted from the highest germination rate and the latest germination tendency as treatment concentrations increased (Fig. 1). In 600 mM sea water-stimulated saline stress treatment, no seed germination was occurred (Fig. 1) followed by the NaCl-induced saline stress treatment; no seed germination phenomenon was found when treated with higher than 400 mM. Once alkali stress existed, the seed germination was greatly affected. Under $NaHCO_3$-induced alkaline stress treatment, no seed germination phenomenon was found when the concentrations were higher than 100 mM, while under combined salt-alkali stress treatment, no seed germination was found when higher than 200 mM. When treated with 0-100 mM sea water-stimulated and NaCl-induced saline stress treatments, the germination rates showed a slight decline, until 200 mM, the germination rates showed a significant decline (Fig. 1). For the sea water-stimulated saline stress treatment, a continuous significant reduction was not seen between 200 mM and 400 mM treatments.

Under combined salt-alkali stress treatment, there was a significant decline on germination rate until treated with 100 mM. And the simple alkaline stress treatment induced by $NaHCO_3$ began to cause a significant decline when treated with 50 mM. This result indicated that alkaline stress had a more serious effect on germination rate of ryegrass. This characteristic was also mentioned by Gao *et al.* (6) when five grass species were treated with different concentrations of $NaHCO_3$ and Na_2SO_4-induced salt-alkali stress.

Data on the effects of different stress treatments on plant biomass, the heights of the aerial parts of treated seedlings, the lengths of their underground parts, and the weights of the aerial parts and underground parts are presented in Table 1.

Under the sea water-stimulated saline stress treatments, the heights of the aerial parts showed a significant decline when treated with 100 mM (Table 1). The effects of the NaCl-induced saline stress and combined stress treatment on aerial part heights were the similar to those of the sea water-stimulated saline stress treatment. All of these treatments showed a significant decline of the aerial part heights in the 100 mM level. However, the height values among different treatment patterns were different: the values under the combined stress treatment were significantly lower than those under sea water-stimulated and NaCl-induced saline stress treatments (Table 1). The aerial part heights were extremely sensitive to the $NaHCO_3$-induced alkaline stress treatment (Table 1), when the stress concentration reached 20 mM, the height values began to significantly decrease compared to the control. And the height values under the $NaHCO_3$-induced alkaline stress treatment were also significantly lower than those under the other stress treatments. When the stress concentrations reached 50 mM, the values were further falling, but the decreasing extent was not significant. This result suggested that even very low alkaline stress could affect the growth of aerial parts of plants, while the saline stress higher than 100 mM could exert an influence. The changes in the weights of aerial parts of seedlings under different stresses just coincided with those of their heights.

The effects on the lengths of the underground parts varied treated individual seedlings, which could be seen from the relative higher mean absolute deviations (Table 1). Under the sea water-stimulated saline stress treatment, 100 mM treatment solution could induce a significant decline on root length, however, there was a large increase of the length values of seedlings treated with 400 mM. Although the germination rate had been seriously affected (Table 1), the average root length per one seedling treated by 400 mM could still be at the same level when treated by 20 mM (Table 1). This seemingly strange phenomenon could be explained as the variation and difference of salt tolerance of the individual. Under the NaCl-induced saline stress treatment, the same as the sea water-stimulated saline stress treatment, 100 mM treatment solution could also induce a significant decline on root length. However, until treated with 100-200 mM, there was almost no root of treated seedlings. Under the $NaHCO_3$-induced alkaline stress and combined salt-alkali stress treatments, 20 mM treatment solution could induce a significant reduction in root length, and 50 mM treatment solution caused a significant change again. In addition, in the 20 mM level, the

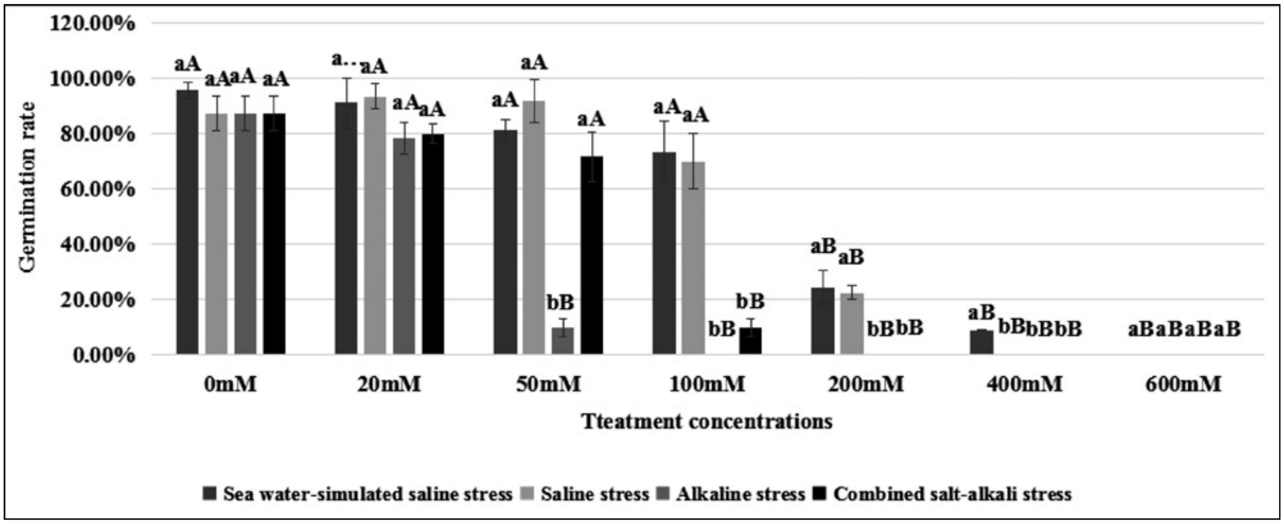

Fig. 1. Germination rates under different stress treatments. The lowercase letters denote significant difference among different stress treatments, while the capital letters indicate that among different concentrations

$NaHCO_3$-induced alkaline stress caused a significant reduction in root length among four kinds of stress treatments. And in the 50 mM level, both alkaline stress treatments induced remarkable reduction in root length compared to both saline stress treatments (Table 1). The effects on root length were similar to those on the heights of aerial parts, suggested that alkaline stress could seriously affect the root length, and the saline stress higher than 100 mM could cause a significant reduction. As for the weights of the underground parts, we found the similar change pattern as their lengths, but effects of salt-alkali stress on root weight were more serious influence than root length.

Comprehensively analyzed the effects of saline and salt-alkali stress on the heights of aerial parts and the lengths of underground parts, the result suggested that the effect on root length were greater than that of the heights of aerial parts. Under even saline stress, there was no root growing when treated with higher than 100 mM treatment (Table 1), while there still were certain amounts of plant height when treated by the same concentration treatment (Table 1). Under

Table 1. The length and weight of aerial parts and underground parts of seedlings under different saline and salt-alkali stress treatments.

	Stress treatment mode	Concentrations of stress solution						
		0 mM	20 mM	50 mM	100 mM	200 mM	400 mM	600 mM
Height of aerial parts	Sea water-simulated saline stress	102.36 ± 14.93aA	98.62 ± 21.64aA	76.65 ± 16.67aAB	50.36 ± 23.59aBC	23.47 ± 10.35aCD	22.50 ± 10.50aCD	0.00 ± 0.00aD
	Saline stress	93.10 ± 1.40aA	97.80 ± 15.80aA	81.52 ± 14.52aAB	42.35 ± 11.52aBC	20.63 ± 6.00aC	0.00 ± 0.00bC	0.00 ± 0.00aC
	Alkaline stress	93.12 ± 1.42aA	58.92 ± 16.45aB	32.94 ± 6.93bB	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00aC
	Saline-alkaline mixed stress	93.14 ± 1.41aA	66.10 ± 21.31aA	66.34 ± 29.32abA	14.16 ± 2.60bB	0.00 ± 0.00bB	0.00 ± 0.00bB	0.00 ± 0.00aB
Length of underground parts	Sea water-simulated saline stress	77.59 ± 31.49aA	62.95 ± 21.85aAB	50.24 ± 6.53aABC	27.12 ± 17.31aCDE	13.76 ± 6.09aDE	37.95 ± 16.75aBCD	0.00 ± 0.00aE
	Saline stress	65.41 ± 2.50aA	55.42 ± 23.50aA	41.00 ± 18.20aA	6.00 ± 2.90aB	3.60 ± 1.40bB	0.00 ± 0.00bB	0.00 ± 0.00aB
	Alkaline stress	65.42 ± 2.48aA	31.50 ± 9.70bB	5.72 ± 0.34bC	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00aC
	Saline-alkaline mixed stress	65.42 ± 2.50aA	36.70 ± 11.00aB	9.04 ± 3.90bC	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00aB
Weight of aerial parts	Sea water-simulated saline stress	23.90 ± 0.57aA	25.80 ± 0.69aA	16.95 ± 1.70aAB	8.40 ± 0.17aBC	2.60 ± 1.16aC	2.90 ± 0.75aC	0.00 ± 0.00aC
	Saline stress	25.04 ± 1.23aA	25.82 ± 4.32aA	22.41 ± 4.11aAB	11.51 ± 0.97aBC	6.17 ± 1.99aC	0.00 ± 0.00bC	0.00 ± 0.00aC
	Alkaline stress	25.04 ± 1.25aA	16.94 ± 3.97aB	4.94 ± 1.78bC	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00aC
	Saline-alkaline mixed stress	25.04 ± 1.23aA	21.32 ± 4.42aA	15.83 ± 2.65aA	2.33 ± 0.68bB	0.00 ± 0.00bB	0.00 ± 0.00bB	0.00 ± 0.00aB
Weight of underground parts	Sea water-simulated saline stress	18.40 ± 1.44aA	17.80 ± 1.73aA	11.10 ± 1.58aAB	4.80 ± 0.99aBC	0.70 ± 0.45aC	1.30 ± 0.60aC	0.00 ± 0.00aC
	Saline stress	15.14 ± 1.32aA	8.22 ± 1.48bB	9.60 ± 2.60abB	1.78 ± 0.82bC	1.54 ± 0.86aC	0.00 ± 0.00bC	0.00 ± 0.00aC
	Alkaline stress	15.14 ± 1.32aA	8.11 ± 3.55bB	0.37 ± 0.03cC	0.00 ± 0.00cC	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00aC
	Saline-alkaline mixed stress	15.14 ± 1.32aA	5.98 ± 2.27bB	4.95 ± 0.98bcB	0.00 ± 0.00cC	0.00 ± 0.00bC	0.00 ± 0.00bC	0.00 ± 0.00aC

The lowercase letters denote significant difference among different stress treatments, while the capital letters indicate that among different concentrations.

even the 20 mM alkaline stress, the root length had been significantly lower than that under saline stress, while for the plant height, a significant change was found in the 50 mM treatment (Table 1). Seen from the fresh weights, the effects of salt-alkali stress on the underground parts were more serious than those on the aerial parts. Because the roots were directly exposed to stress treatment solutions, the aerial parts were indirectly influenced. This could be found in the seedlings treated by the 100 mM combined stress, only the aerial parts grow normally (the nutrients for germination were mainly supplied by the endosperm of seed), but not the underground parts.

The exoteric saline or/and alkaline stress could induce the changes of RWC in leaves of seedlings. The effect degrees of each stress treatment varied markedly: 100 mM sea water-stimulated saline stress could cause a significant decrease in RWC in leaves of treated seedlings, 50 mM NaCl-induced saline stress could cause the same decrease, and only 20 mM alkaline stress had already caused significant changes in RWC (Fig. 2). However, there was no significant change in RWCs of seedlings between under the same treatment level of two different saline stresses, and two different alkaline stresses, respectively. For instance, the RWC of seedlings under 50 mM NaCl-induced saline stress was lower than that under sea water-stimulated saline stress in the same treatment concentration, however, the reduction degree was not remarkable (Fig. 2). Of course, the absence of seedlings could not be used for comparison. The result from RWCs proved that alkaline stress effect on plants is more serious than that of saline stress.

To resist exogenous stress, the antioxidant enzyme activities are usually induced to change for the more rapid removal of peroxides. When the seedlings were exposed to 100 mM saline stress, the SOD activity began to significantly increase, and reached the highest level in 100-200 mM treated seedlings. Under higher than 400 mM saline stress, the SOD activity was decreased to the level same as the control again (Fig. 3A). When the seedlings were exposed to 20 mM alkaline stress, the SOD activity was induced to significantly increase relative to the control. However, until under 50 mM alkaline stress, the SOD activity recovered to the control level (Fig. 3A). Under combined salt-alkali stress, the SOD activity was significantly higher in 50 mM and 100 mM-treated seedlings than the control value (Fig. 3A). In this study, the SOD activity was induced to increase due to stress treatments. This is also agreed by the previous study in cotton seedlings (Meloni *et al.*, 12). Combined with growth status of stressed seedlings, we found that the SOD activity significantly increased to resist the saline or/and alkaline stress when the growth status seemed well and seedlings had the capacity to respond and resist the injury from stress.

The POD activity decreased as the degrees of stress treatment strengthened. The sea water-stimulated saline stress caused to significantly decrease in POD activity until reaching 100 mM, and under the 200 mM stress treatment, the POD activity was continued to significantly decrease compared to that of 100 mM. However, until the stress treatment concentration reached 400 mM, the POD activity value recovered to increase to that in the 100 mM (Fig. 3B). When the seedlings were exposed to NaCl-induced saline stress, the POD activity began to significantly decrease from in the 50 mM, and the POD activity in the 200 mM stress treatment was remarkably lower than that in the 50 mM (Fig. 3B). Similar to SOD activity, the POD activity began to significantly decrease from

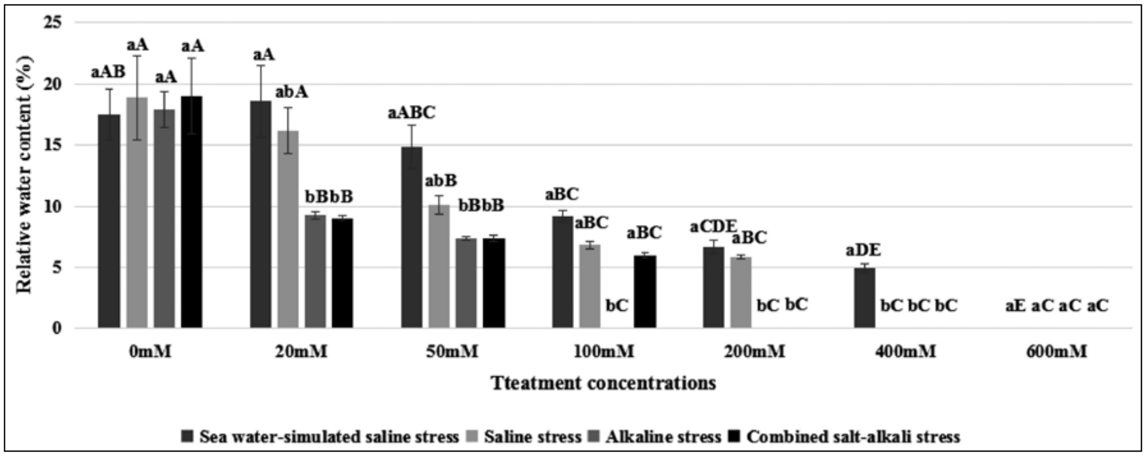

Fig. 2. Relative water contents (%) in leaves of seedlings under different stress treatments. The significant difference is expressed as above.

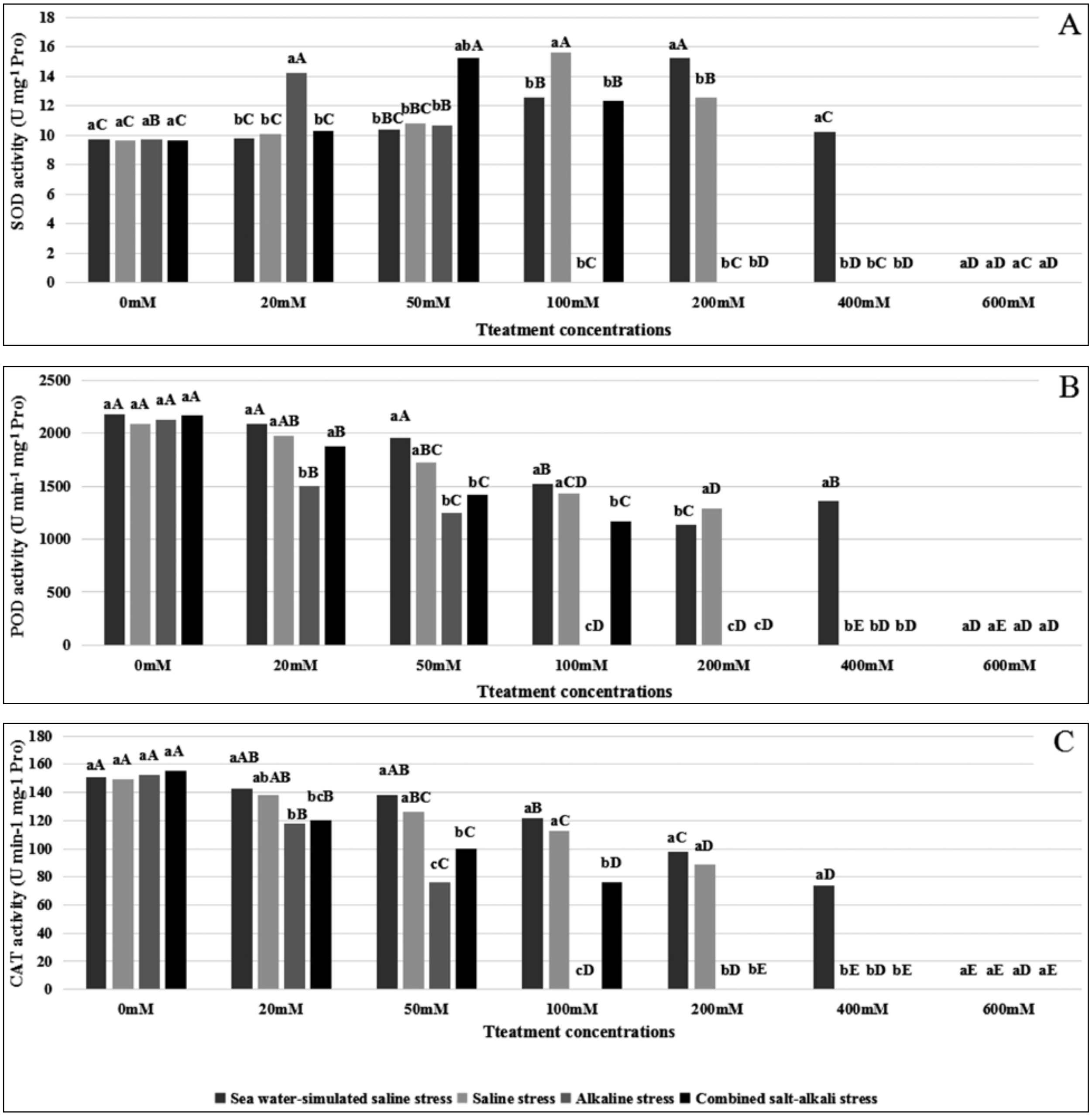

Fig. 3. SOD (A), POD (B), and CAT (C) activities of seedlings under different stress treatments. The significant difference is expressed as above.

reaching 20 mM, and until reaching 50 mM, the POD activity level showed a continuous downward trend (Fig. 3B). When the seedlings were exposed to combined salt-alkali stress, the 20 mM level had already been reduced significantly, and reaching 50 mM, the POD value continued to significantly decrease (Fig. 3B).

The change of CAT activity was almost the same as that of POD activity (Fig. 3C). Different stress treatments, sea water-stimulated saline stress, NaCl-induced saline stress, $NaHCO_3$-induced alkaline stress, and combined salt-alkali stress, could cause a significant decline of CAT activity in the 100 mM, 50 mM, 20 mM, and 20 mM, respectively (Fig. 3C). In the range of stress treatment investigated in this study, the CAT activity showed to continuously decrease. In this study, the saline or/and alkaline stress reduced POD and CAT activities, which was similar to the previous study with soybean roots by Comba *et al.* (3).

In conclusion, according to the effects of different stresses on plant growth, we concluded that *L.*

perenne variety Weinasi had certain adaptability to saline stress, since lower than 100 mM did not affect seed germination, lengths and weights of aerial parts and underground parts, and antioxidant enzyme activities, but it is extremely sensitivity to alkaline stress. Despite seed germination could be occurred but substantially reduced until 50-100 mM alkaline stress or salt-alkali stress, at the 20 mM level, it had caused a significant effect on the lengths and weights of aerial parts and underground parts, and antioxidant enzyme activities. This paper fist reported the effects of salt-alkali stress on seed germination, plant growth, and antioxidant enzyme activities of *L. perenne* variety Weinasi. Compared to other salt-alkali stresses, as the sea water-stimulated saline stress caused a relatively small effect on the seed germination rate, plant development, and antioxidant enzyme activities of this species, this species could be planted on some coastal saline soils with reasonable irrigation to reduce the salt concentration in soils, but not on soils with strong salt-alkali contamination.

AUTHORS' CONTRIBUTION

Conceptualization of research (Sun Y.L.); Designing of the experiments (Sun Y.L. and Hong S.K.); Contribution of experimental materials (Hong S.K. and Park Y.C.); Execution of field/lab experiments and data collection (Deng H.W. and Cui L.Q.); Analysis of data and interpretation (Cui L.Q. and Sun Y.J.); Preparation of the manuscript (Deng H.W., Cui L.Q. and Sun Y.L.).

DECLARATION

The authors declare no conflict of interest.

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