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Research Article

Effect of Salt Stress on Antioxidant Enzymes, Soluble Sugar and Yield of Oat

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Abstract: The objective of this study was to evaluate the changing mechanism of soluble sugar, antioxidantenzymes of oat at reproductive stage under salt stress. Two cultivars selected (salt-tolerant Baiyan 2, salt-sensitive. Caoyou 1) were exposed to varied salt concentrations (0, 50, 100, 150, 200 mmol/L, respectively) at seedling stage. The salt was composed by NaCl, Na_2SO_4 (1:1). Our results were that (1) salt could decrease the yield and grain weight of oat, the adverse effect of salt on tolerant cultivar was less than that of sensitive cultivar. (2) In salt stress, the soluble sugar contents were reduced at heading stage and were increased at the grain filling stage. Larger increase percentage was observed in salt tolerant cultivar Baiyan 2 than in the salt sensitive cultivar Caoyou 1 compared to control. A positive correlation was detected between the soluble sugar and MDA contents at grain filling stage. (3) Salt stress improved the MDA contents at both stages and the MDA contents were higher in grain filling stage than in heading stage. Compared with the salt sensitive cultivar, the salt tolerant cultivar had the lower MDA contents. A negative correlation was found between MDA and yield. The salt increased the SOD, POD activity and decreased the CAT activity at heading stage. The increment of SOD, CAT activity and the reduction of POD activity were found at the grain filling stage. The result suggested that oat included the salt tolerance and the soluble sugar, antioxidant enzymes could be used for discriminating oats for their potential to tolerate salt.

Keywords: Antioxidant enzyme, MDA, oat, salt stress, soluble sugar, yield

INTRODUCTION

Soil salinization is a worldwide environmental problemand influences more than 10% of the arable land(Ruili *et al*., 2010; Bray, 1997). In Inner Mongolia, more than 50% of the arable land was saline soil. Oat is the special crop in Inner Mongolia and the Chinese largest planting area of oat is in Inner Mongolia. Thus, the oat used as a biological method will contribute to the solution of soil salinization in Inner Mongolia.

In the previous studies, someone found that salt-alkali stress could affect the photosynthetic characteristic of oat at seedling stage (Wang *et al*., 2007a) someone reported that growth ofoat seedling was inhibited by salt (Wu *et al*., 2010). Some reportshave found that salt-alkali stress could improve the proline contents of oat seedling (Wang *et al*., 2007b). However, all these studies focus on the seedling stage, the salt-resistant mechanism of oat at reproductive stage has not been reported. Some researches have found that the crop mechanism at different stages in stress was obviously different (Meenakshiand Bavita, 2010; Zheng *et al*., 2009). Thusthe study on the change law at varied stages was essential.

Soluble sugar is the main organic osmolyte that could help improve crop tolerance to osmotic stress (Chen *et al*., 2011). In wheat, soluble sugar has been used as the criteria for tolerance to salt (Zheng *et al*., 2009), whereas, under salt stress, the soluble sugar of oat at reproductive stage has not been studied.

The oxidative damage led by salt is well documented in leaves (Muckenschnabel *et al*., 2002), but not much is known about the situation of oxidative damage at reproductive stage. ROS could seriously disturb the metabolism of plant by oxidative damage of lipids, proteinsand nucleic acids (Muckenschnabel *et al*., 2002). The antioxidant enzymes could decrease the oxidative damage through eliminating the ROS. So, in order to learn the salt tolerant law of oat, the studying of antioxidant enzymes is critical.

Thus, in our experiment, the salt-induced mechanism of soluble sugar, antioxidant enzymes at heading and grain filling stages was studied. The aim of the present investigation was to provide information for oat cultivating and breeding in salt soil.

MATERIALS AND METHODS

Salt tolerant cultivar Baiyan 2 and salt sensitive cultivar Caoyou 1 were used in this experiment. Two

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cultivars were obtained from Bai Cheng Agricultural Research Institute. Seeds were sown in 28 cm plastic pot. There were 90 pots in all. We put all the pots in the greenhouse of Inner Mongolia of Agricultural Science. Each pot was filled with vermiculite. The salt (NaCl: Na_2SO_4 = 1:1) were selected. When the two cultivars were at two-leaf stage, they were treated by five levels of salt concentrations (0, 50, 100, 150, 200 mmol/L) respectively. Then we took samples at heading and grain filling stages, eight oats with uniform growth vigor were chosen and these oats were put in ice-box. The top first leaf of oat was used for this experiment.

Methods: The measurement of soluble sugar was following the methods (Yemm and Willis, 1954): we put 0.05 g leaves in 6 mL de-ionized water and then the leaves were boiled for 30 min, we centrifuge it under 1200×g for 10 minus. The extracts were separated. Anthrone reaction solution was composed by 85 cm^3 vitriol oil, 15 cm^3 H_2O and 0.16 g anthrone. The absorbance of the mixed reagent (0.1 mL extracts and 3 mL anthrone reagent) was detected at 620 nm.

SOD, CAT activities were measured by the following method (Meenakshiand Bavita, 2010). Total SOD activity was measured by the method following the inhibition of the photochemic reduction of Nitrobluetetrazolium (NBT). One unit of SOD was defined as the enzyme amount that made a 50% inhibition of NBT. The reaction mixture contained phosphate buffer (50 mM, ph 7.8), 14.3 mM methionine, 82.5 µM NBT, 0.1 mM EDTA and 2.2 µM riboflavin. Then we started the reaction by putting the test tubes in 15 W fluorescent lamps. The tube was taken from the light after 10 min. We measured the solution at 560 nm. CAT activity was tested following the H_2O_2 decomposition (coefficient of absorbance = 39.4 mM/cm) in the solution including 15 mM H_2O_2, 50 mM Na-phosphate (pH 7.0) for 2 minat 240 nm. The measurement of POD activity was following the change in absorption at 470 nm led by guaiacol oxidation (Burcu *et al*., 2009). We used the 3, 3'-diaminobenzidine-tetrahy-drochloride Dihydrate (DAB) solution including 0.1% (w/v) gelatine, 0.6% H_2O_2 (pH 4.4) and 150 mM phosphate buffer.

MDA, that is a product of lipid peroxidation, the oat leaves (0.5 g) were grinded in 0.1% Trichloroacetic Acid (TCA). We centrifuged the mixture at 10,000 g. The supernatant was taken and was for the MDA test. Then we added 4 mL 0.5% Thiobarbituric Acid (TBA) in 20% Trichloroacetic Acid (TCA). We heated the solution at 95°C and then we centrifuged it at 10,000 g for 15 min. The absorbance was tested at 532 nm (Burcu *et al*., 2009).

The oat in groups of control and salt treatments were harvested from pots and then we detected the components and yield.

Analysis methods: The data are represented by an average of three replicates and are analyzed by one-way analysis of variance using SAS 9.0, ($p<0.05$) was regarded as significant difference, ($p<0.01$) was regarded as very significant difference.

RESULTS

With increasing saline, a significant decrease in the yield and grain weight of oat (Table 1) was observed, when comparing the oat treated with 50, 100, 150, 200 mmol/L salt with the control, the tolerant cultivar (Baiyan2) had lower percentage decrease inyield (7.51,16.20, 27.40, 41.56%, respectively) and grain weight (27.37, 30.80,40.97, 1.42%, respectively), while the sensitive cultivar (Caoyou 1) showed greater percentage decrease in yield (8.22, 17.51, 31.59, 51.84%, respectively) and grain weight (40.29, 45.60, 50.96, 68.38%, respectively). The yield and grain weight of Baiyan 2 were higher than that of Caoyou 1.

The grain number per spike at 100,150 mmol/L were higher than control, the increase percentage of Baiyan 2 were 29.03 and 30.24%, while that of Caoyou1 were 27.75 and 20.23%. The effect of salt on panicles was not significant.

The regression analysis between alkaline and yield was studied (Fig. 1). The data indicated that the fitting degree of regression alkaline and yield in salt tolerant cultivar Baiyan 2 was lower than in Caoyou 1.

The leaf MDA contents were increased by salt stress at both stages (Table 2), this influence was more pronounced in salt sensitive cultivar Caoyou1. Comparing the MDA contents at heading stage with the ones at grain filling stage, higher MDA contents were found at grain filling stage (75.18-127.35 nmol/g) than at heading stage (31.23-78.61 nmol/g). At heading stage, the salt tolerant cultivar under salt (Baiyan 2) showed a

Table 1: Effect of salt stress on yield andcomponents with pot experiment

	Grain weight(g)		Grain number per spike		Effective panicles		Yield (g)	
Treatment	Baiyan 2	Caoyou 1	Baiyan 2	Caoyou 1	Baiyan 2	Caoyou 1	Baiyan 2	Caoyou 1
CK	1.865±0.078a	1.875±0.049a	4.907±0.149c	2.721±0.042b	6.066±0.119a	4.641±0.369a	12.395±0.158a	13.680±0.594a
Y50	1.355±0.093a	1.119±0.098b	4.866±0.093b	2.851±0.071b	4.869±2.007a	4.956±0.062a	12.064±0.178ba	12.064±0.178b
Y100	1.291±0.127ba	1.020±0.141b	6.190±0.127a	3.476±0.602a	7.689±1.995a	4.781±0.327a	9.388±0.021b	8.635±0.177c
Y150	1.101±0.143bc	0.919±0.083b	6.391±0.399a	3.177±0.052a	7.586±0.981a	5.098±0.279a	7.699±0.692c	6.990±1.372cd
Y200	0.906±0.019c	0.591±0.102c	4.751±0.072c	2.366±0.022c	6.826±1.366a	5.001±0.140a	7.245±0.389c	5.625±0.431d

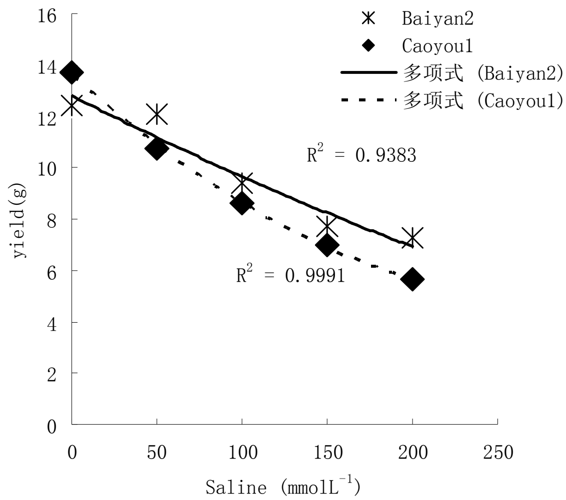

Fig. 1: Relationship between saline and yield

Table 2: Correlation analysis of physiological indexes and yield

Correlation	Heading stage	Grain filling stage
POD	0.792	0.553
SOD	0.318	-0.117
CAT	0.901**	-0.234
MDA	-0.824**	-0.688*
Soluble sugar	0.344	-0.942**

*: p<0.05; **: p<0.01

lower MDA contents (48.76-65 nmol/g) than did the sensitive cultivar Caoyou1 (51.39-78.61% nmol/g). At grain filling stage, oat treated with 200 mmol/L exhibited the highest MDA contents, the increase percentage was lower in salt tolerant cultivar Baiyan 2 (19.41%) than in salt sensitive cultivar Caoyou 1 (31.98%) compared with control. The MDA contents at both stages correlated negatively with oat yield (Table 2).

At heading stage, soluble sugar contents decreased as the salt concentration increased (Fig. 2). A lower leaf soluble sugar contents differentiated the salt tolerant cultivar Baiyan 2 from salt sensitive cultivar Caoyou 1. At 50, 100, 150, 200 mmol/L saline, respectively the decrease percentage of soluble sugar in Baiyan 2 (26.75, 39.11, 44.47 and 45.62%, respectively) were larger than in Caoyou 1 (18.53, 22.51, 28.72 and 38.32%, respectively) compared to the control treatment. At grain filling stage, the increment of soluble sugar contents was observed in both cultivars under salt stress compared to control treatment (Fig. 2). Comparing the soluble sugar contents of salt tolerant cultivar Baiyan 2 with the ones of salt sensitive cultivar Caoyou 1, the lower contents of soluble sugar were observed in Baiyan 2 (19.7-27.56 mg/g) than in Caoyou 1 (24.96-31.52 mg/g) under salt stress. Whereas, under salt stress (50, 100, 150, 200 mmol/L, respectively), the salt tolerant cultivar Baiyan 2

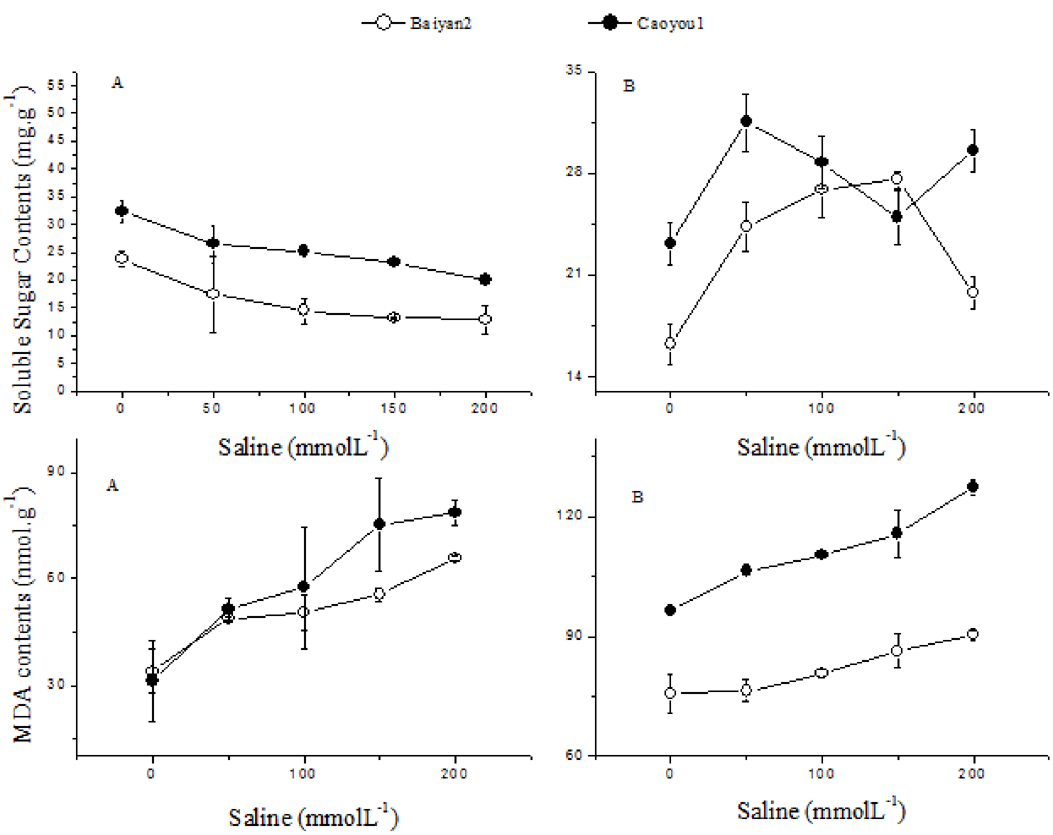

Fig. 2: Influence of saline on the soluble sugar content, MDA content in oat leaves at heading (A) and grain filling (B) stages

Table 3: Correlation analysis of MDA, POD, SOD, CAT and Soluble Sugar (SS)

	Heading stage					Grain filling stage				
Correlation	POD	SOD	CAT	SS	MDA	POD	SOD	CAT	SS	MDA
POD	1	-0.209	-0.049	-0.495	-0.495	1	0.255	0.257	-0.671*	-0.724*
SOD		1	0.194	0.276	-0.191		1	0.715*	0.037	-0.365
CAT			1	0.488	-0.933			1	0.047	-0.467
SS				1	-0.399				1	0.845**
MDA					1					1

*: p<0.05; **: p<0.01

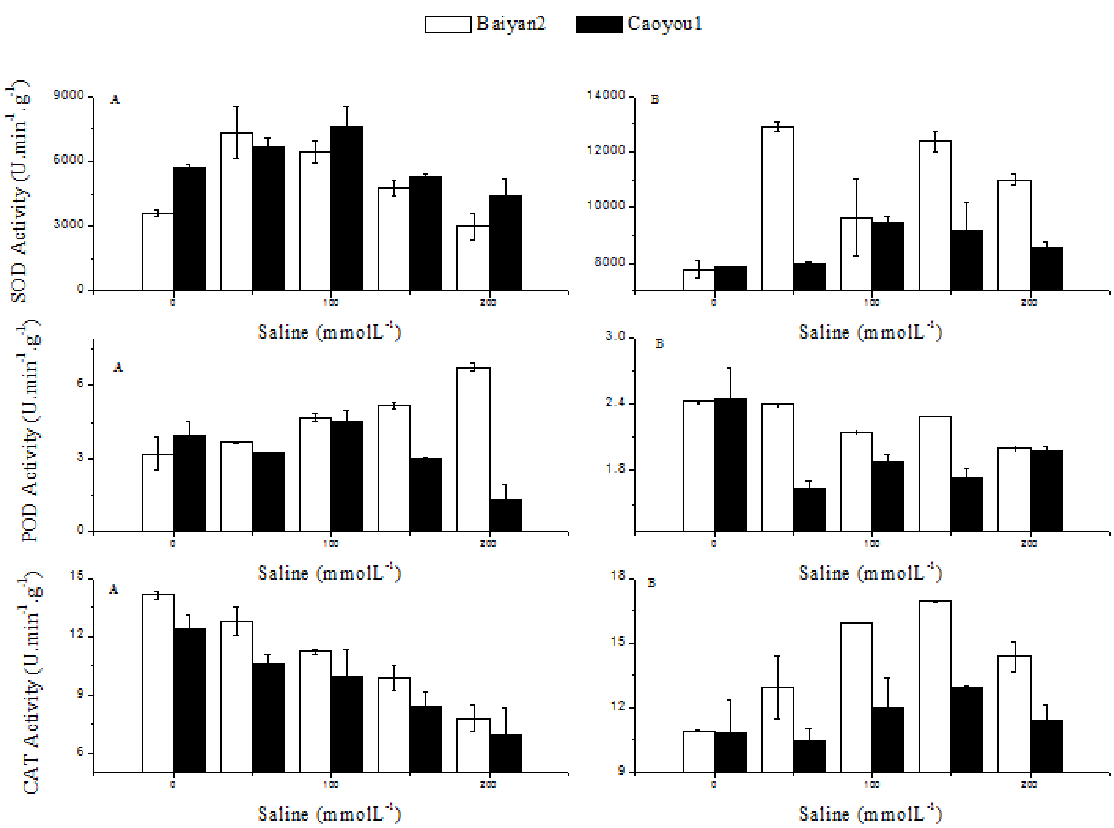

Fig. 3: Influence of saline on the SOD, POD, CAT activities in oat leaves at heading (A) and grain filling (B) stage

had the larger increase (21.97, 49.89, 65.95, 69.86%, respectively) percentage of soluble sugar than that of salt sensitive cultivar Caoyou 1 (7.73, 23.98, 27.62 and 36.03%) compared to control. A significant positive correlation between soluble sugar contentsand MDA contents at grain filling stage was found (Table 3).

At heading stage, leaf SOD was elevated with increased salt concentration (50,100 mmol/L), compared with control, the salt-tolerant cultivar (baiyan 2) showed a percentage increase of 104.84 and 80.28%, whereas salt sensitive cultivar (Caoyou 1) showed a percentage increase of 16.42 and 32.40% (Fig. 3). The saline (150 mmol/L) increased the activity of SOD in Baiyan 2, but decreased the SOD activity of Caoyou 1 compared to control. In 50 and 100 mmol/L, the increase of POD activity was observed in oat (Fig. 3), the increment over the control of 15.16%, 46.17% was found in Baiyan 2, while the POD activity of Caoyou 1 were increased by 4.36 and 15.63%. In high salt concentration (150 and 200 mmol/L), salt tolerant cultivar Baiyan 2 were decreased by 14.35, 19.54, 31.90, 39.75%, respectively a significant positive correlation between yield and CAT activity at heading stage (Table 2) and a negative correlation between CAT activity and MDA contents were found (Table 3).

At grain filling stage, salt caused increase in SOD activity (Fig. 3) and this effect was more pronounced in salt tolerant cultivar (baiyan 2). Compared to control, the SOD activity of Baiyan 2 treated with 50, 100, 150 and 200 mmol/L were increased by 65.90, 23.87, 59.20 and 41.54%, while that of salt sensitive cultivar (Caoyou 1) were improved by 1.13, 20.19, 16.79 and 8.44%, respectively.

We found the POD activity at grain filling stage was lower than the one at heading stage (Fig. 3). At grain filling stage, salt stress decreased the POD activity. When comparing the oat treated with salt with the control oat, salt tolerant cultivar (Baiyan 2) had lower percentage decrease (1.21-17.50%), the sensitive cultivar exhibited greater percentage decreases in POD activity (19.15-33.04%). A significant negative correlation between soluble sugar and POD activity and a significant negative correlation between MDA contents and POD at grain filling stage were observed (Table 3).

Salt increased the CAT activity, but the sensitive cultivar was less affected, CAT activity reached the highest in 200 mmol/L saline, the percentage increases were 31.71 and 23.67% in Baiyan 2 and Caoyou 1, respectively. CAT activity correlated positively with SOD activity(Table 3).

DISCUSSION

In this study, the reduction of yield and grain weight led by salt was observed. With regard to yield and grain weight, salt tolerant cultivar was less affected by salt compared with the sensitive cultivar, showing that oat could decrease the adverse effect of salt on the yield. A negative correlation between yield and MDA contents was observed, indicating that lipid peroxidation was an important reason that yield decreased under salt. The reduction of ROS could contribute to the yield.

Soluble sugar is the main organic osmolyte in the leaves (Wang *et al*., 2011), which plays important role in osmotic adjustment and protects against photo damage (Rodríguez-Calcerrada *et al*., 2011). In this study, the increase of soluble sugar led by salt at grain filling stage was observed, which could improve the permeability and keep a balance of water metabolism (Liao and Chen, 2007). Larger increase percentage of soluble sugar was observed in salt tolerant cultivar, indicating that oat could improve salt tolerance by increasing soluble sugar. The increment of soluble sugar contents induced by salt has been detected in wheat (Li *et al*., 2009). Myrene and Varadahally (2010) found that sugar-mediated osmotic regulation in leaves holds good only for short-term exposures, long-term salt exposure could decrease soluble sugar contents, whereas our result was that long-term salt exposure (seeding-grain filling stage) could improve the soluble sugar contents and was different from that of Myrene R.

A positive correlation between soluble sugar and MDA contents was observed in oat at grain filling stage, indicating that soluble sugar may participate in the elimination of ROS. Our result was in accordance with those of Michael *et al*. (2011), who reported that osmolyte could act as a ROS scavenger and soluble sugar could detoxify ROS in chloroplasts, vacuoles.

In this study, we found the mechanism of soluble sugar differed in two stages. At heading stage, the decrease of soluble sugar in oat was observed. Lower soluble sugar contents were detected in salt tolerant cultivar than in salt sensitive cultivar. This result was different from that of Li *et al*. (2009), who reported that salt tolerant cultivar had higher soluble sugar contents. Heading stage is an essential phase for yield and source-sink relationship is crucial for plant productivity, soluble sugar was considered as main transport form from the source to sink (Wang *et al*., 2012), thus the decrease of soluble sugar might contribute to the increment of yield by promoting the transportation of photosynthate transport from the source to sink organs.

One mechanism of the plant subjecting to biotic or abiotic stresses is the increase of Reactive Oxygen Species (ROS) (Liu and Shi, 2010). ROS could disturb the normal metabolism by lipid peroxidation. MDA is the indicator of lipid peroxidation. In the present investigation, salt increased the MDA contents of oat in each stage, the MDA of tolerant cultivar was less affected by salt compared with the sensitive cultivar, indicating that oat could decreased the damage of lipid peroxidation. Comparing the MDA contents at grain filling stage with the one at heading stage, we found the MDA contents at grain filling stage was higher, indicating that the damage of lipid peroxidation in grain filling stage was more serious and this stage should be pay more attention.

Antioxidant enzyme, that plays important role in remission of oxidative stress, has been shown to be increased in many kinds of stresses (Pooja *et al*., 2011). SOD is the enzyme which could catalyze O_2^{-} to H_2O_2, O_2. Burcu *et al*. (2009) had reported that salt could improve the SOD activity of wheat leaves. In the present study, the increment of SOD activity was observed at heading, grain filling stages compared to control, this is consistent with the result of Burcu Seckin. We found salt tolerant cultivar under salt had the larger increase percentage compared to control, which could be regarded as the reason for its better performance. This phenomenon indicated that oat could increase SOD activity to improve salt tolerance.

The POD and CAT could dismutate H_2O_2, which was the product of reaction catalyzed by SOD (Sharma *et al*., 2005). At heading stage, the increase of POD activity and the decrease of CAT activity were detected. Buecu and others had reported that salt stress could increase POD activity of wheat, which was in agreement with our result. But, Burcu Seckin also found that salt reduced the CAT activity of wheat (Burcu *et al*., 2009), which was different from our result. The POD activity was higher in salt tolerant cultivar than in salt sensitive cultivar, indicating that oat could improve POD activity to adapt to stress. In our investigation, the change law of POD, CAT at two stages was different. At grain filling stage, the decrease of POD activity was observed. This might because gain filling stage was senescence period. A negative correlation between POD and MDA was found, indicating that the reduction of POD might be led by that the damage of lipid peroxidation at grain filling stage was more serious than at heading stage. The increment of CAT activity could compensate on the reduction of POD activity.

CONCLUSION

The aim of this study was to detect the effect of salt on the soluble sugar, antioxidant enzymes of oat at reproductive stage. We choose two cultivars (salt tolerant cultivar Vao-9, salt sensitive cultivar Baiyan 5). Through comparing the difference between two cultivars, the tolerant mechanism of oat at reproductive stage was detected. Our results could be summarized as follows:

- The yield and grain weight of oat were inhibited by salt stress, the reduction percentage of salt tolerant cultivar was lower than that of salt sensitive cultivar, indicating that oat could relieve the adverse effect of salt on yield. Yield correlated with MDA contents negatively, showing that ROS was an important reason that yield decreased under salt, so the elimination of ROS could contribute to the yield.
- In this study, we found the tolerant mechanisms at different stages were varied. Oat could increase soluble sugar contents to improve the salt tolerance at grain filling stage and the soluble sugar can participate in the elimination of ROS. But, salt decreased the soluble sugar contents at heading stage.
- Comparing to the salt sensitive cultivar, lower MDA contents were found in salt tolerant cultivar, indicating that oat could decrease the damage of lipid peroxidation led by salt. The damage of lipid peroxidation at grain filling stage was more serious than at heading stage. Thus the oat supervision should be strengthened at grain filling stage.
- Oat could increase the SOD, POD activity at heading stage and improve the CAT, SOD activity at grain filling stage to adapt to the salt stress.
- Between two different resistance cultivars, the change laws of antioxidant enzymes, soluble sugar and grain weight were different, so the grain weight, MDA, SOD, POD and soluble sugar could be used as criteria of oat salt resistance

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