

Physiological response of spring soybean leaves under osmotic stress

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Received 30 December 2021; Accepted 10 July 2022

ABSTRACT

Osmotic stress has an important impact on the growth of soybean. In this study, Heinong44 (HN44) and Heinong65 (HN65) were used as the research objects and cultured in sand culture, four different stress levels were set to explore the response mechanisms of antioxidant enzymes, osmotic adjustment, membrane lipid system and agronomic traits of soybean seedlings under osmotic stress. The results showed that: (1) With the extension of osmotic stress intensity and treatment time, the antioxidant activity in soybean leaves increased first and then decreased. The antioxidant activity and osmotic adjustment substance content of HN44 were higher than HN65, and the malondialdehyde (MDA) content was lower than HN65; (2) Membrane lipid peroxidation gradually increased and reached its peak on the 7th day of severe drought treatment; (3) With the aggravation of drought and the extension of drought time, the relative water content of soybean gradually decreased, and the increase of plant height slowed down, while the change of HN65 was more obvious than that of HN44; (4) Correlation analysis showed that when osmotic stress was severe, there was a significantly positive correlation between the activities of different antioxidant enzymes, and there was a significantly positive correlation between soluble sugar and soluble protein content, and there was a significantly positive correlation between soluble sugar and MDA content; (5) Through the fitting analysis of antioxidants system, the experimental results showed that superoxide dismutase would lead to the cascade reaction of antioxidant enzyme system.

Keywords: Soybean; Osmotic stress; Antioxidants activity; Osmotic regulators

1. Introduction

Water is the source of life for crops. Drought is an important limiting factor affecting soybean growth and yield. In recent years, due to the adverse effects of drought, soybean yield decreased by 56%–77% [1]. Scholars at home and abroad extensively studied the effects of drought on crops. The results showed that with the intensification of water stress, the photosynthetic rate, stomatal conductance and chlorophyll content of crop leaves decreased, which led to serious crop yield reduction [2]. Wang et al. [3] found that the activity of antioxidant enzymes in soybean plants increased under mild drought conditions, which was used to scavenge ROS in vivo. Dong et al. [4] found that smotic stress reduced plant height and leaf area of soybean, which

had adverse effects on soybean yield. Therefore, understanding the water requirement of crops is the premise to achieve high and stable yield and reduce agricultural water consumption.

China is a big agricultural country. Soybean is one of the main crops in China, their yield and quality of soybean has always been the focus of attention. Soybean originated in China, planted more than 5,000 y. As an important cash crop, following rice, wheat and corn, soybean is important for food supply and agricultural production [5]. Soybean has high nutritional value and has an important source for human beings obtain plant protein and edible oil [6]. With the rapid development of society, the demand and consumption of soybean are increasing. In most years, there is water stress of a certain level in the environment of soybean planting in China.

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Soybean is a crop that is sensitive to water shortage is the main factor undermining the stability and yield of soybean [7]. The germination of soybean needs to absorb about 120% of its own weight of water. When the soil moisture content is above 70% of the field capacity, the emergence rate is the highest. The shortage of water seriously affects the shape of soybean and its physiological and biochemical reaction. Under the same environment, the water consumption per 1 g of soybean seed production is much higher than that of other crops such as millet, broomcorn millet, sorghum and corn [8]. Drought during the flowering period of soybean causes a large number of flowers and pods to fall, which results in a severe decline of yield.

Today, improving the drought resistance of soybean has become a key issue. Some studies showed that the accumulation of reactive oxygen species and membrane lipid peroxidation in plants increased under osmotic stress. The plants can eliminate excess active oxygen by enhancing the activity of antioxidant enzymes in the cell, thereby reducing the effect of osmotic stress on the normal metabolism of plants [9]. If plants are in a certain degree of osmotic stress, plants can increase the resistance and adaptability of plants by increasing the activity of protective enzymes and the content of osmotic adjustment substances.

At present, there are many studies on soybean drought. However, most are about the effects of continuous drought on soybean growth and stress physiology, and few focuses on periodic drought. Therefore, this experiment used sensitive soybean variety Heinong65 (HN65) and drought-tolerant variety of Heinong44 (HN44) as the experimental material, used the simulation of drought by PEG to carry out treatment of drought, researched the response of soybean leaves of osmotic regulation substances, antioxidant enzyme activity and membrane lipid peroxidation to drought. Therefore, some certain theoretical basis is provided physiological mechanism of soybean's resistance to drought.

2. Materials and methods

2.1. Experimental materials and treatment

The experiment was conducted in the raintight glass roof in Northeast Agricultural University. Materials of the experiment were drought-tolerant variety HN44 and drought-sensitive variety HN65 [10]. The experiment was carried out by sand culture, and the medium pH was about 6.8. In the experiment, the plastic bucket with the maximum diameter of 25 cm, the minimum diameter of 13 cm and the height of 16 cm was selected. The bottom of the plastic basins was covered with gauzes. After being cleaned with water, 6 kg river sand was put into the bucket. The researchers selected high-quality seeds of uniform size to seed, and kept 4 plants in a bucket. After the soybean first grew two opposite single leaves, the nutrient solution was poured with 500 mL per time once every day and night. The ingredients of nutrient solution were made by D.R. Hoagland [11]. Composition of nutrient solution (mg/L): $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.08, NH_4NO_3 142.86, CaCl_2 220.00, KH_2PO_4 136.00, Fe-EDTA (dissolve 7.45 g Na_2EDTA and 5.57 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, then the researchers ensured the volume of the nutrient solution to be 1 L respectively, and added 1 mL of stock solution to each liter

of nutrient solution during use), MgSO_4 240.00, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.22, H_3BO_3 2.86, $\text{Na}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$ 0.03, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 4.90. When the seedlings grown to V3 stage, they were treated with water stress. In this experiment, osmotic stress was divided into four groups. The concentration of PEG-6000 was determined by measuring soil water potential. The experiment was divided into control group (CK, 0% PEG-6000, water potential 0.00 MPa), mild stress group (LD, 5% PEG-6000, water potential -0.10 MPa), moderate stress group (MD, 10% PEG-6000, water potential -0.20 MPa) and severe stress group (SD, 15% PEG-6000, water potential -0.40 MPa). During the period of seedling experiment, the nutrient solution containing PEG was irrigated twice a day for 8 d. On the last day of drought simulation, the second and third leaves from bottom to top were taken from soybean main stem at 8:00–9:00 every morning. Each index was sampled five times, and five repeated tests were carried out to obtain its average value. The samples first taken out were stored in an ultra-low temperature refrigerator at -80°C , and then uniformly determined after all the samples were collected.

2.2. Determination of physiological indexes

2.2.1. Determination of superoxide dismutase activity

Fresh leaves were taken out in an ultra-low temperature refrigerator at -80°C . 0.1 g fresh leaves were weighed and placed in a pre-cooling bowl. 1 mL pre-cooling extraction medium was added to grind the homogenate on the ice bath. After centrifugation at 4°C and 10,000 rpm for 15 min, the supernatant was the crude extract of superoxide dismutase (SOD). Adding 0.05 mol/L PBS, (PH7.8) 1.5 mL, 130 mM methionine (Met) 0.3 mL, NBT 0.3 mL, EDTA- Na_2 0.3 mL, riboflavin 0.3 mL. SOD extract 0.1 mL added distilled water 0.5 mL in transparent glass tube. The test tube was placed under 4,000 LX fluorescent lamp for photochemical reaction for 20 min, and the temperature was controlled between 25°C and 35°C . Cover the tube with a cloth mask to terminate the reaction. Control tube in dark disposal zero, with distilled water instead of enzyme solution as a control. The absorbance of the reaction solution was measured at 560 nm.

$$\text{Calculating formula: SOD activity} = \frac{[(A_{\text{CK}} - A_{\text{E}}) \times V]}{(1/2 A_{\text{CK}} \times W \times V_i)} \quad (1)$$

The total SOD activity was expressed as fresh weight enzyme unit per gram (u/g FW); A_{CK} : light absorbance of the tube; A_{E} : sample tube absorbance; V : sample total volume (3.3 mL); V_i : Determination of enzyme dosage (0.1 mL); W : sample fresh weight; the protein content unit is mg/g.

2.2.2. Determination of peroxidase activity

Preparation of crude enzyme solution and SOD, reagent preparation: 0.2 mol/L phosphate buffer (pH 6.0): A mother liquor (Na_2HPO_4) 123 mL and B mother liquor (NaH_2PO_4) 877 mL were mixed. 1,000 mL PBS (0.2 M, pH 6.0);

Preparation of reaction mixture: 200 mL PBS (0.2 M, pH 6.0), 0.076 mL guaiacol (2-Methoxyphenol) was dissolved by heating and stirring, and 0.112 mL 30% H_2O_2 was added

after cooling. After mixing, it was stored in the refrigerator for further use.

Sample determination: Take 3 mL reaction solution and add 30 μ L enzyme solution, PBS as control, and then determine the OD470 value (measured for 40 s).

Calculation of enzyme activity: 1 enzyme activity unit (u) is OD value change (increase) 0.01/min.

$$\text{POD}(\text{u/g min}) = \frac{(\Delta A_{470} \times V_t)}{(W \times V_s \times 0.01 \times T)} \quad (2)$$

where ΔA_{470} : the change of absorbance in reaction time; W : is sample fresh weight (g); T : is the reaction time (min); V_t : is the total volume of the reaction solution (3.3 mL); V_s : enzymatic liquid volume (0.03 mL) for determination.

2.2.3. Determination of catalase activity

Preparation of crude enzyme solution and SOD, reagent preparation: 0.15 mol/L phosphate buffer (pH 7.0): B mother liquor (Na_2HPO_4) 457.5 mL and A mother liquor (NaH_2PO_4) 292.5 mL mixed with distilled water constant volume to 1,000 mL.

Preparation of reaction solution: Take 200 mL PBS (0.15 M, pH 7.0), add 0.3092 mL 30% H_2O_2 .

Sample determination: 3 mL reaction solution was added to 0.1 mL enzyme solution, PBS was used as control, and OD240 was measured.

Enzyme activity calculation: One enzyme activity unit (u) was set as OD value decreased by 0.01 per min.

$$\text{CAT} = \frac{[\Delta A_{240} \times V_t]}{(W \times V_s \times 0.01)(\text{u/g min})} \quad (3)$$

where $\Delta A_{240} = (A_{240i} - A_{240f}) / (t_f - t_i)$: A_{240f} is the termination value, A_{240i} initial value, t_f termination time, t_i reaction initial time; W fresh for samples weight (g); t is the reaction time (min); V_t is the total volume of the reaction solution (3.1 mL); V_s for determination with enzyme liquid volume (0.1 mL).

2.2.4. Determination of ascorbate peroxidase activity

0.10 mL enzyme solution was taken, 1.70 mL PBS (0.05 mol/L, pH 7.0) containing 0.1 mM EDTA- Na_2 was added, 0.10 mL 5 mM AsA was added, and 0.10 mL 20 mM H_2O_2 was added. The D290 value was immediately determined at 20°C within 40 s, and the reduction of AsA and enzyme activity in unit time.

$$\text{Calculation formula: APX} = \frac{[\Delta \text{OD} / \Delta t \times V_r \times V_T]}{(A \times D \times V_i \times W)} \quad (4)$$

where ΔOD is the change of absorbance in reaction time (40 s absorbance – 0 s absorbance); Δt : is reaction time (0 s); V_r : is the reaction liquid volume; V_T : extract volume; A is the extinction coefficient (2.8 mM “cm⁻¹”); D : is the thickness of the colorimetric cup; V_i : determination of liquid volume; W : is sample fresh weight.

2.2.5. Determination of soluble sugar (SSC) content

A total of 1.0 g fresh cut-and-mixed samples were weighed, ground with a small amount of quartz sand to homogenize, and diluted together with the residue to 100 mL. After filtration at room temperature, the residue was discarded.

Determination: 1 mL sample extract was added with 5 mL anthrone reagent, shaken and boiled in boiling water bath for 10 min, removed and cooled, and separated light.

3 Colorimetric measurement at 620 nm, zeroing with blank, recording absorbance value, and finding the corresponding micrograms of sugar on the standard curve.

2.2.6. Determination of soluble protein content

Determination of protein concentration in the sample Extract-Samples extract 0.2 mL (sample extraction and SOD enzyme activity determination at the same time), put into the plug scale tube, adding 1 mL of Coomassie brilliant blue G-250 reagent, fully mixed, placed 2 min after colorimetric at 595 nm, record the absorbance value, through the standard curve to find protein content.

2.2.7. Determination of proline content

Proline extraction: 0.1 g cut leaves of different treatments were placed in centrifuge tube, adding 1 mL 3% sulfosalicylic acid solution, sealing. Extracted in boiling water bath for 10 min.

Take out the test tube, cool to room temperature, absorb supernatant 0.4 mL, add 0.4 mL glacial acetic acid and 0.6 mL color liquid, heated in boiling water bath for 40 min, cool, add 1 mL Toluene fully oscillates to extract red matter, standing to be layered and absorbing the toluene layer to colorimetric at 520 nm.

2.2.8. Determination of malondialdehyde content

0.1 g of plant samples with different treatments were added with 1 mL of 5% TCA, and the homogenate obtained after grinding was centrifuged at 3,000 rpm for 10 min.

Take the supernatant 0.4 mL, add 0.67% TBA 0.4 mL, mix, boil in 100°C water bath for 30 min, cool and centrifuge again.

The absorbance values of supernatant at 450, 532 and 600 nm were measured respectively, and the concentration of malondialdehyde (MDA) was calculated according to the formula, then the MDA in fresh tissue was calculated.

$$\text{Calculation: } C_{\text{MDA}} = 6.45(A_{532} - A_{600}) - 0.56A_{450} \quad (\text{umol/L}) \quad (5)$$

2.3. Analysis software

The data were processed and analyzed by Excel 2007 and SPSS 21.0.

3. Results and analysis

3.1. Effects of osmotic stress on membrane lipid peroxidation

MDA content reflected the lipid peroxidation level of cell membrane. As shown in Fig. 1, The MDA content in the

control groups of Heinong44 and Heinong65 did not change significantly with the prolongation of treatment time at seedling stage. The MDA content of LD, MD and SD increased gradually with the extension of treatment time, and the MDA content of LD treatment was significantly lower than that of SD treatment. In the whole treatment process, the MDA content of drought-tolerant variety Heinong44 was lower than 3.8% of Heinong65. This indicated that drought-sensitive varieties had greater membrane lipid peroxidation and greater injury than the drought-sensitive variety.

3.2. Effect of osmotic stress on the contents of osmoregulation substance

The osmoregulation substances mainly include proline, soluble protein and soluble sugar. Osmoregulation can be adopted to help plants to cope with water stress. With the increase of drought degree, plant tissue will enhance the ability of plant tissue to resist dehydration by actively accumulating soluble substances. Figs. 2–4 show the content of osmoregulation substances in the control group of two varieties did not change significantly in the whole treatment. With the extension of water stress time, under mild and moderate stress, the content of osmoregulation substances in the treated leaves increased gradually. Under severe stress, the osmoregulation substance content in the treated leaves

increased first and then decreased, and reached peak on the 7th day, on the 8th day, the proline and soluble sugar of Heinong65 under severe stress were lower than the control group. During the whole treatment period, the contents of osmoregulation substances of the drought-tolerant variety Heinong44 were significantly greater than that of the sensitive variety Heinong65, the proline content was 8.9% higher, the soluble protein content was 4.4% higher, and the soluble sugar content was 7.7% higher. This indicated that drought tolerant varieties had strong ability to regulate drought.

3.3. Effect of osmotic stress on antioxidants activity

Antioxidant enzymes protect plants from reactive oxygen species. Their activities are an important index of plant drought resistance. As shown in Figs. 5–8, under osmotic stress, the activity of Heinong44 SOD is 86.7% higher than that of peroxidase (POD). POD is 53.5% higher than catalase (CAT) enzyme activity, and POD is 69.4% higher than ascorbate peroxidase (APX) enzyme activity. Heinong65 SOD is 76.3% higher than POD enzyme activity, POD is 64.3% higher than CAT enzyme activity, and POD is 85.7% higher than APX enzyme activity. The difference between the CAT and APX treatments of the two varieties was not much, nearly 10.3%. There was no significant change in the four enzyme activities of the two varieties in the control

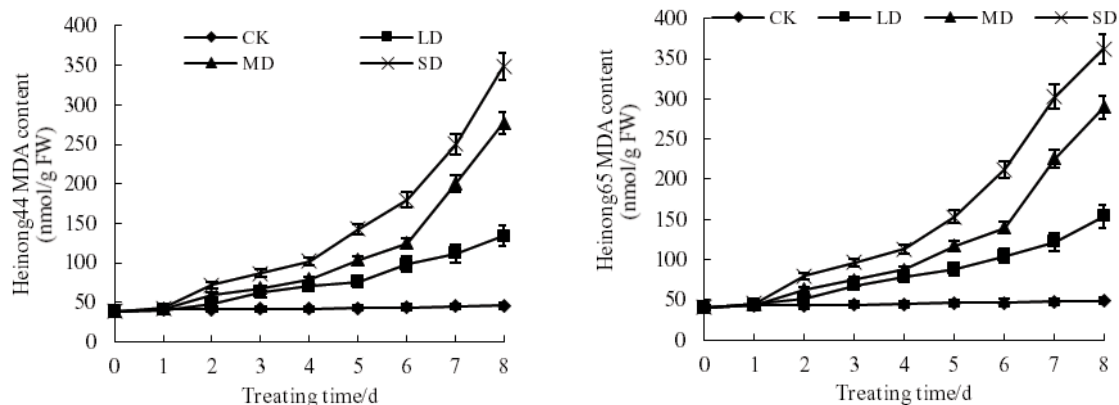


Fig. 1. Effect of osmotic stress on MDA content in leaves of two varieties of soybean.

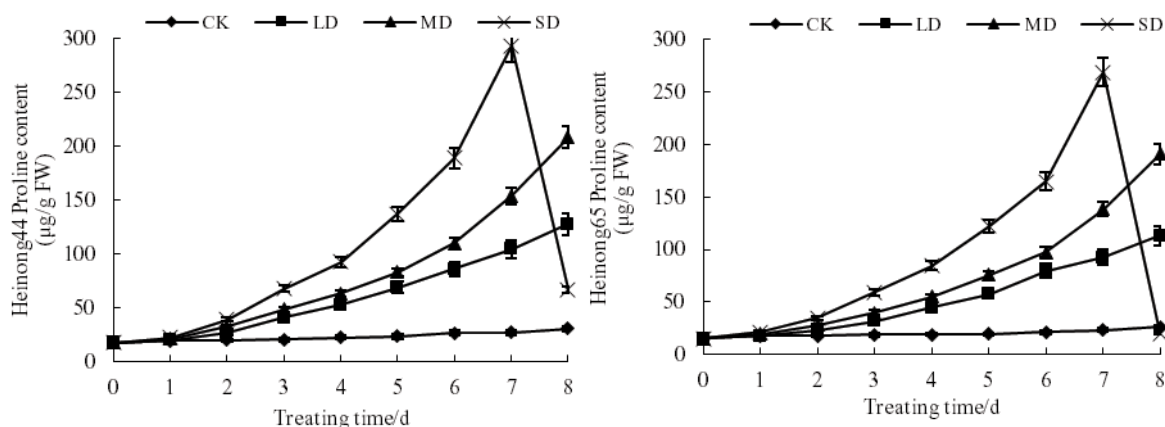


Fig. 2. Effect of osmotic stress on proline content of two varieties of soybean leaves.

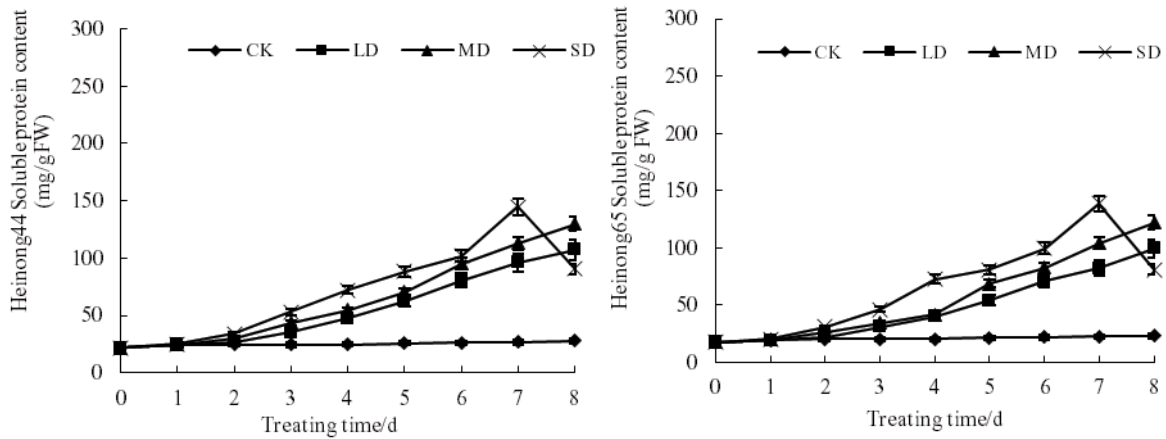


Fig. 3. Effect of osmotic stress on soluble protein content of two varieties of soybean leaves.

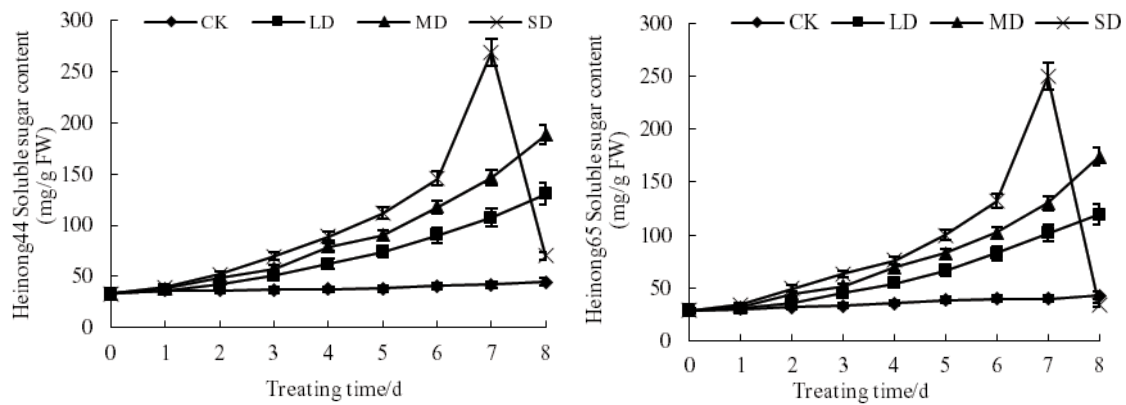


Fig. 4. Effect of osmotic stress on soluble sugar content of two varieties of soybean leaves.

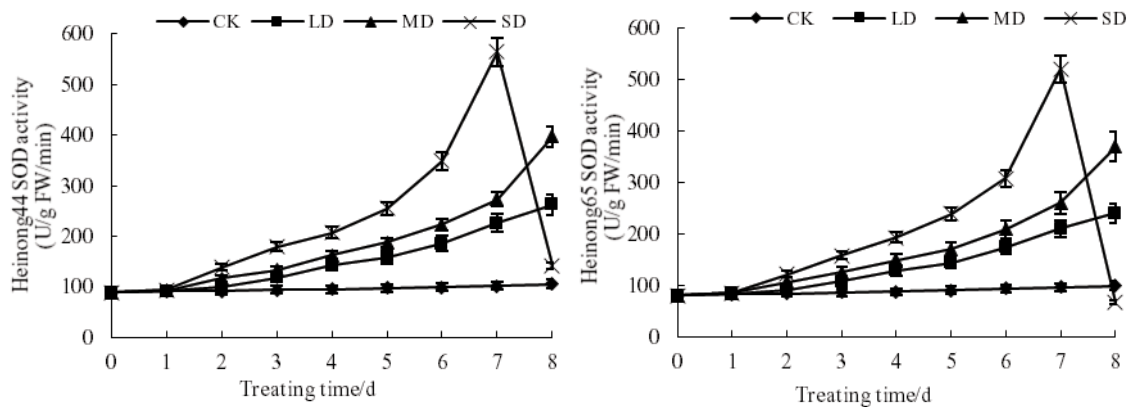


Fig. 5. Effect of osmotic stress on SOD enzyme activity in leaves of two varieties of soybean.

treatment during the whole treatment. With the increase of drought, the activities of the four enzymes in the leaves of the two varieties under light and moderate stress were continuously increasing. During the period of treatment, the activity of antioxidant enzymes of two varieties were more active than the control group, and the enzyme activity of the treatment of moderate stress were higher than

the treatment of mild stress. The antioxidant enzyme activities of the two varieties under severe stress increased first and then decreased. The activity of four antioxidant enzymes reached peak on the 7th day. This indicates that short-term water stress can induce the expression of antioxidant enzymes, but with the extension of the stress time, the increase of reactive oxygen species in leaves led

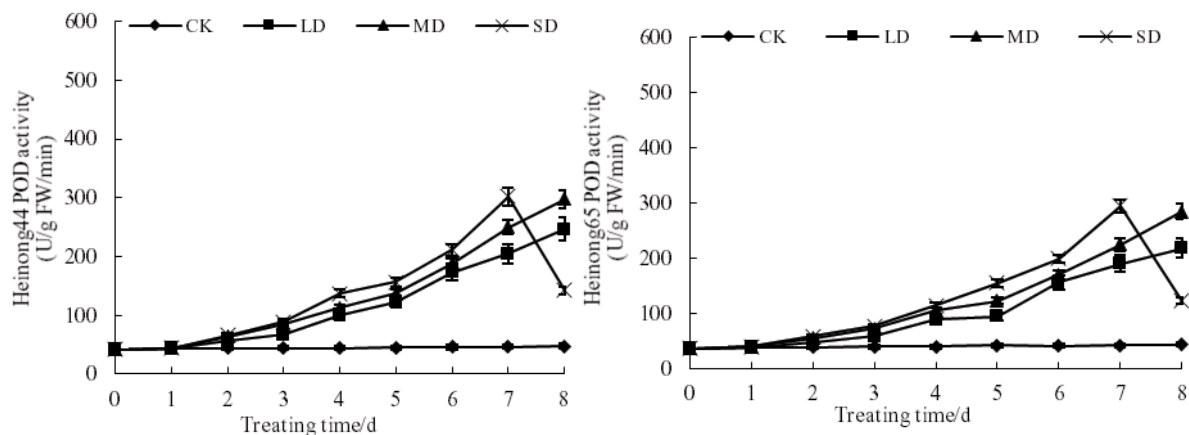


Fig. 6. Effect of osmotic stress on POD enzyme activity in leaves of two varieties of soybean.

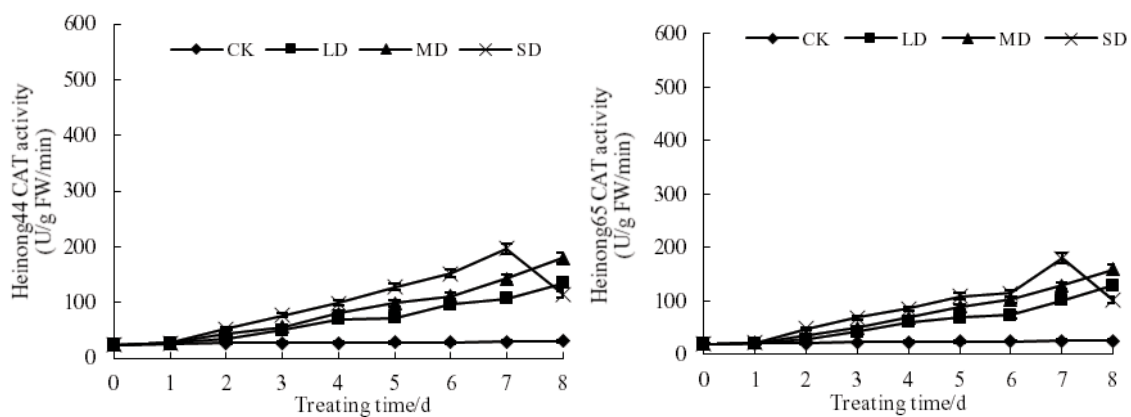


Fig. 7. Effect of osmotic stress on CAT enzyme activity in leaves of two soybean varieties.

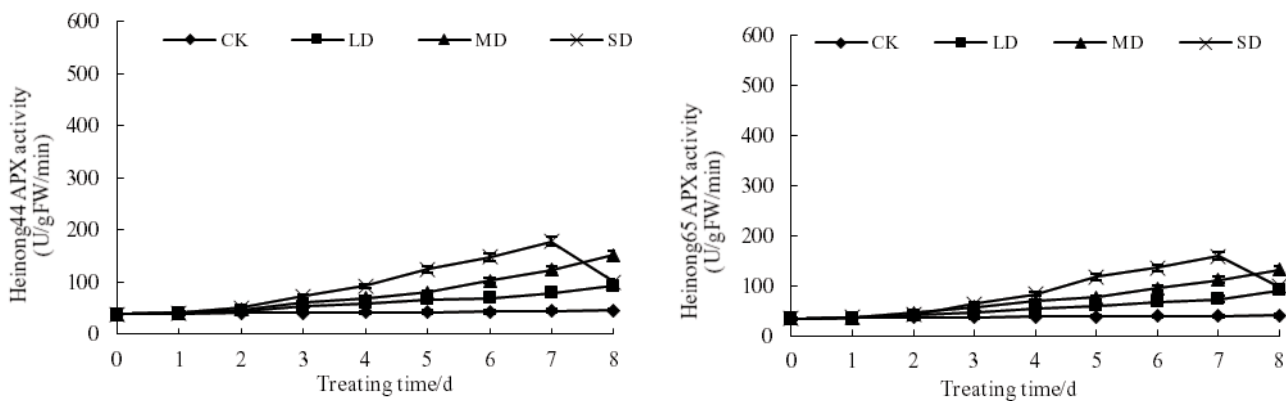


Fig. 8. Effect of osmotic stress on APX enzyme activity in leaves of two varieties of soybean.

to the destruction of leaf tissue, which in turn inhibits the activity of antioxidant enzymes. During the whole period of treatment, the activities of antioxidant enzyme of the drought-resistant variety Heinong44 were higher than that of the sensitive variety Heinong65, which indicated that the drought-resistant variety has a strong drought resistance ability.

3.4. Growth traits of spring soybean under osmotic stress

3.4.1. Effects of osmotic stress on plant height of spring soybean

Under osmotic stress, there were some differences in plant height between two soybean varieties. The plant height was the highest under CK and the lowest under SD.

As shown in Fig. 9, the plant height growth of soybean was significantly inhibited with the aggravation of osmotic stress and the prolongation of stress time. In HN44, the plant height of SD decreased by 12.2% compared with that of CK group, and in HN66, the plant height of SD decreased by 25.1% compared with that of CK group. At the same time, with the increase of osmotic stress, the plant height increased slowly. Compared with the two varieties, the plant height of HN44 was less affected by drought.

3.4.2. Relative moisture content of spring soybean under drought condition

Under osmotic stress, there was a certain difference in the change of relative water content of leaves between the two soybean varieties. The relative water content of leaves was the highest under CK condition and the lowest under SD condition. The results are shown in Fig. 10. With the increase of osmotic stress and the extension of stress time, the relative water content of soybean leaves decreased significantly. In HN44, the relative water content of SD decreased by 48.2% compared with that of CK group, and in HN66, the relative water content of SD decreased by 56.6% compared with that of CK group. Compared with HN65, the

relative water content of HN44 was stable, indicating that HN44 was less affected by drought than HN65.

3.5. Correlation analysis

3.5.1. Correlation analysis of indicators

According to Tables 1 and 2, the correlation analysis of each index under severe osmotic stress showed that the activities of any two of the four antioxidant enzymes were positively correlated. The activities of antioxidant enzymes were significantly positively correlated with the contents of proline, soluble protein and soluble sugar. The soluble protein of drought-tolerant variety Heinong44 was significantly positively correlated with soluble sugar, soluble protein and MDA content, and MDA was significantly positively correlated with POD, CAT, APX activity and soluble protein. Soluble protein and soluble sugar, soluble protein and MDA content were significantly positively correlated, and POD, CAT, and APX activities were significantly positively correlated. Soluble protein of drought-resistant variety Heinong44 was also positively correlated. However, MDA and CAT were positively correlated with POD, APX activity and soluble protein content in Heinong65. MDA was positively correlated with SOD activity, proline and soluble sugar

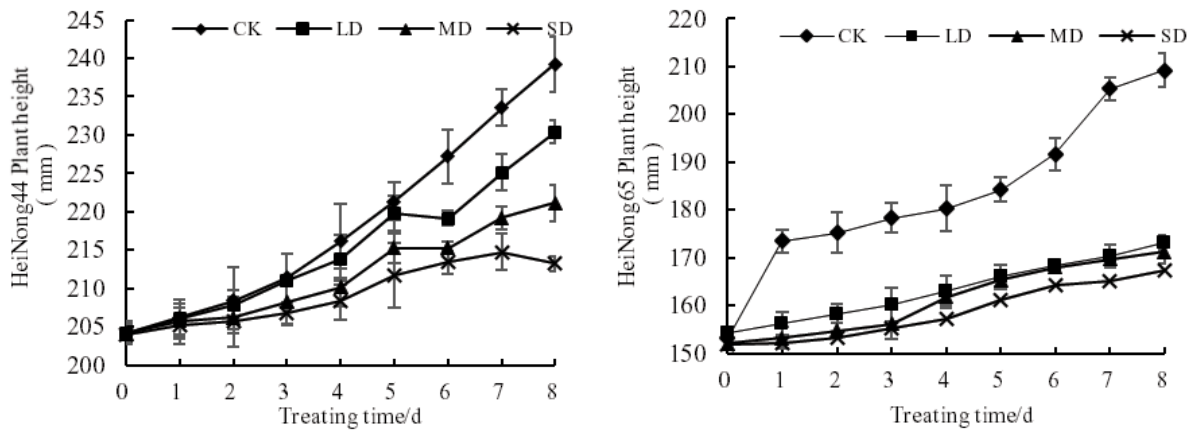


Fig. 9. Effects of osmotic stress on plant height of two soybean varieties.

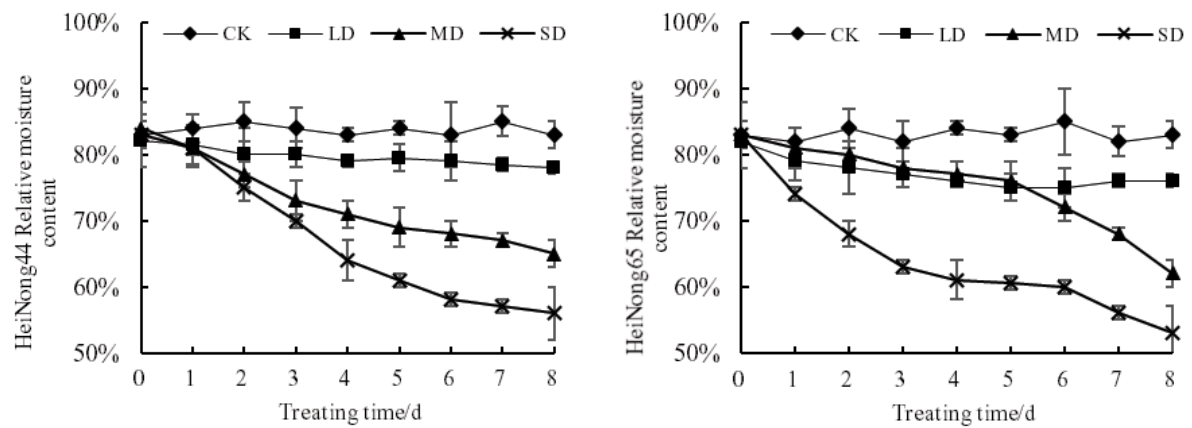


Fig. 10. Effects of osmotic stress on leaf relative water content of two soybean varieties.

content, but not significant. The correlation degree between the activity of antioxidant enzymes of the drought-tolerant variety Heinong44 was higher than that of the sensitive variety Heinong65.

3.5.2. Fitting analysis of correlation of activity of antioxidant enzymes

Excel 2013 software was used to carry out fitting analysis. Goodness of fit R was adopted to test fitting effects. Goodness of fit R is defined as a measure to test

the goodness of fit. The closer the value is to 1, the better the fitting effect is. According to some researches showed that under osmotic stress, antioxidant enzymes such as SOD could reduce the accumulation of MDA produced by osmotic stress, eliminate the active oxygen produced in interior of plants, and alleviate the damage of drought to plants. As the first defense line of plant antioxidant system, SOD plays an important role in clearing reactive oxygen species caused by stress. (Table 3). SOD catalyzes the production of O₂ and H₂O₂ by superoxide radical. H₂O₂ is catalysed by POD, cat, APX, etc. to remove the generated H₂O₂

Table 1
Correlation analysis of the indexes of Heinong44 under severe stress

	SOD	POD	CAT	APX	PRO	Soluble protein	Soluble protein	MDA
SOD	1	0.957**	0.911**	0.923**	0.993**	0.899**	0.995**	0.482
POD		1	0.983**	0.984**	0.977**	0.983**	0.962**	0.685*
CAT			1	0.993**	0.944**	0.992**	0.913**	0.732*
APX				1	0.960**	0.981**	0.921**	0.684*
PRO					1	0.932**	0.987**	0.535
Soluble protein						1	0.913**	0.781*
Soluble sugar							1	0.529
MDA								1

Note: *Significant difference at 5% level;
**Significant difference at 1% level.

Table 2
Correlation analysis of the indexes of Heinong65 under severe stress

	SOD	POD	CAT	APX	PRO	Soluble protein	Soluble protein	MDA
SOD	1	0.931**	0.862**	0.841**	0.995**	0.855**	0.996**	0.458
POD		1	0.972**	0.970**	0.951**	0.979**	0.941**	0.735*
CAT			1	0.968**	0.884**	0.989**	0.875**	0.806**
APX				1	0.882**	0.982**	0.844**	0.781*
PRO					1	0.883**	0.989**	0.495
Soluble protein						1	0.865**	0.813*
Soluble sugar							1	0.507
MDA								1

Note: *Significant difference at 5% level;
**Significant difference at 1% level.

Table 3
Estimated value and fitting degree of antioxidant enzyme activity curve

Varieties	Parameters	Independent variable	SOD	SOD	SOD	POD	POD	CAT
		Dependent variable	POD	CAT	APX	CAT	APX	APX
Heinong44	k		0.7064	0.4502	0.343	0.6104	0.4341	0.7291
	b		-9.0074	-4.8588	12.721	3.7802	22.635	18.706
	R ²		0.8486	0.8785	0.9089	0.9497	0.8562	0.9473
Heinong65	k		0.7207	0.4207	0.3206	0.5828	0.4245	0.7308
	b		-10.794	-2.3829	15.366	4.0135	22.138	19.065
	R ²		0.8592	0.8227	0.8284	0.9542	0.878	0.9263

[12]. In order to clarify the relationship between SOD and POD, CAT, APX, researchers analyzed the activity of four key antioxidant enzymes based on a linear fit curve. After testing, the fitting effects of regression equation are effective. This means that the response of SOD enzyme under osmotic stress will lead to cascade reactions, and the interaction of antioxidant enzymes will better resist stress.

4. Discussion

As researches deepen, more people discovered that the system of protective enzyme is critical for plants to resist osmotic stress, which drew the attention from agriculture researchers [13]. The results of this study showed that with the increase of drought degree, the activities of SOD, POD, CAT and APX increased first and then decreased, which may be due to the ability of plants to resist drought to a certain extent by increasing the activities of related enzymes. In this experiment, the same index in the same state enzyme content difference is not big, indicating that the test data has guiding significance. However, if the drought degree was too severe, it would lead to the disorder of enzyme system in plants and unable to carry out normal growth activities. Zhong et al. [14] carried out drought treatment on *Cinnamomum cassia* seedlings, the results showed that the activities of SOD, POD, CAT and APX also increased first and then decreased, similar to this experiment. When the stress degree and time reached a certain degree, the enzyme activity would be lower than the control group. The greater the degree of drought, the greater the increase in enzyme activity [15]. The antioxidant enzyme activity of drought resistant varieties was significantly higher than that of sensitive varieties. The results showed that the activities of SOD, POD and CAT in soybean leaves showed a unimodal curve with the increase of osmotic stress, which was similar with our experiment. Studies showed that the activities of SOD, POD and CAT in soybean leaves showed a unimodal curve with the enhance of osmotic stress, which was similar to the results of this experiment [4]. Studies have shown that osmotic stress reduces damage by increasing soluble sugar, soluble protein content and maintaining high CAT activity, which is similar to the results of this experiment [16]. Studies showed that SOD, POD and CAT activities in soybean roots increased, and then drought reached a certain degree, which was not completely consistent with our experiment [17]. The reason may be that different parts respond differently to drought. Drought can significantly increase APX activity in soybean leaves. When the drought level is in the middle level, APX activity increases most obviously. There were significant differences in the increase of APX among different varieties. The increase of APX in drought-tolerant varieties was greater than that in sensitive varieties, which was different from the research results of Zhao et al. [18], probably due to different responses of different species to osmotic stress. Drought can significantly increase APX activity in soybean leaves [8]. When the drought level reaches a moderate level, APX activity increases most significantly, and the increase of APX in different varieties is also significantly different. The increase of APX in drought-tolerant varieties was greater than that in sensitive varieties, which was different from the results of a study on rice [18],

probably due to of different species have different response to osmotic stress.

Crops produce large amounts of reactive oxygen species under adverse conditions. The accumulation of reactive oxygen species will lead to membrane lipid peroxidation, destroy cell structure, and affect the normal growth and development of crops [19]. Therefore, plants will generate obvious physiological response mechanism of osmoregulation to maintain normal physiological functions and osmotic pressure of cells. At the same time, osmoregulation substances can also slow down the damage of active oxygen produced by plants under osmotic stress. Soluble sugar is the main regulatory substance in the early stage of drought, and is the source of carbon frame and energy for the synthesis of organic solutes. It resists a certain degree of drought by increasing the concentration of cell protoplasts. Proline mainly acts at the late stage of drought, and it can change the cell osmotic potential, thereby increasing the drought resistance of plants. The results of this study showed that the Osmoregulated Substances content in soybean leaves showed a unimodal curve with the increase of drought degree and stress time, the content of Osmoregulated Substances in drought-tolerant varieties was significantly higher than sensitive varieties, which was consistent with some research results [20]. As the drought degree and stress time increased, the results of showed that MDA contents continued to increase, which was consistent with the results of a research, but was different from the results of some researches, which may be due to different responses among different species. Soybean is a crop susceptible to drought. Within a certain drought range, soybean can adapt to drought conditions by adjusting its morphology, if stress exceeds the ability of plants to adapt themselves, it can cause plant growth to be blocked or even death. In this experiment, with the increase of drought degree, the growth of soybean plant height slowed down significantly, and the relative water content of seedling leaves decreased significantly. Wang and Nia [21] analyzed the agronomic traits of barley under drought stress. The results showed that the plant height and leaf water content of barley decreased, which was consistent with the results of this experiment. Meng et al. [22] conducted drought treatment on flowering soybean, the results showed that plant height decreased with drought degree.

5. Conclusion

This experiment revealed the effects of osmotic stress on oxidase, osmotic regulator, membrane lipid system and agronomic traits of two soybean varieties with different drought tolerance. The physiological and biochemical parameters of soybean showed different characteristics under different stress levels, and the response of HN44 and HN65 to stress was also different. Under mild and moderate drought conditions, the contents of soluble sugar, soluble protein and proline in osmotic adjustment substances gradually increased with the extension of drought time, and increased first and then decreased under severe conditions. The content of MDA, the final product of membrane lipid oxidation, increased with the increase of stress and time. The contents of SOD, POD, CAT and APX increased gradually under mild and severe stress, and increased first and then

decreased under severe stress. Plant height increased slowly with the increase of drought degree and time, and leaf water content decreased with the increase of drought degree and time. The content of drought-tolerant variety HN44 changed less than sensitive variety HN65, which could basically maintain the balance of the body system.

Acknowledgements

This work was supported by the National Key R&D Program of China (No. 2018YFD1000903) and Natural Foundation of Heilongjiang Province of China (No. LH2021C023).

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