Cadmium stress increases antioxidant enzyme activities and decreases endogenous hormone concentrations more in Cd-tolerant than Cd-sensitive wheat varieties

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Abstract: A pot experiment was conducted to study the changes of antioxidant enzyme activities and endogenous hormones in wheat (*Triticum aestivum*) genotypes differing in cadmium (Cd) accumulation (high=Pingan 8 and low=Bainong 160) in different growth stages under Cd stress. The Cd treatment (3 mg kg-1) increased the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and concentrations of malondialdehyde (MDA) and abscisic acid (ABA); in contrast, it reduced concentration of gibberellin (GA3), auxin (IAA) and zeatin nucleoside (ZR) in wheat leaves compared with the Cd0 control. The antioxidant enzyme activities were higher in Bainong 160 than Pingan 8 under Cd stress. In addition, the changes in endogenous hormone concentration were smaller in Bainong 160 than Pingan 8 leaves. The correlation coefficients of Bainong 160 and Pingan 8 were 0.97 and 0.64, respectively. Our results suggest that low Cd accumulation (greater Cd tolerance) in Bainong 160 is associated with higher activities of antioxidant enzyme and higher concentration of hormones than Pingan 8.

Key words: Winter wheat; Cadmium; Antioxidant enzymes; Hormones

Cadmium (Cd) is a highly toxic heavy metal pollutant. In recent years, due to the discharge of industrial wastewater, the application of Cd-containing phosphate fertilizer and unreasonable sewage irrigation [1, 2], the area of farmland polluted by Cd is increasing. It is reported that there are 9.9-45 tons of cadmium discharged into soils around the world each year, and cadmium pollution is considered to be one of the most serious environmental problems in the world [3].Cd is not an essential element for plant growth. However, it is easily absorbed by roots and transported to shoots, causing chlorosis, photosynthesis inhibition, biomass reduction and plant death [4, 5]. Cadmium can impair the redox homeostasis of cells and exacerbate generation of reactive oxygen species (ROS), which leads to lipid peroxidation, membrane damage and enzyme inactivation, and ultimately affects cell viability [6]. Plants have developed a wide range of defense systems to quench ROS and reduce Cd toxicity.

Plant hormone have an important regulatory role in plant growth and development [7]. When plants are stressed by heavy metals, the hormones are involved in resisting the adverse environmental conditions [8-10].

A large difference in Cd accumulation was found in different varieties of rice [12]. Hence, gGenotype screening and usage of Cd-tolerant varieties are important measures in mitigating Cd toxicity. Enhanced activities of antioxidant enzymes increase plant Cd tolerance [11]. The activities of antioxidant enzymes were higher in aerial parts of oilseed rape cultivars with high than low Cd accumulation [13]. However, there are few studies on the dynamics of antioxidant enzymes and hormones in different varieties of wheat under Cd stress.

Wheat is the main grain crop in China. Cadmium accumulates not only in the vegetative organs of wheat, affects the growth and resulting in yield decline, but also accumulates in wheat grains, which is an important pathway of Cd entry into the human body, affecting health. In this study, two wheat varieties with high and low accumulation of cadmium were used to characterize changes in the activity of antioxidant enzymes and the concentration of endogenous hormones at different growth stages during Cd stress in order to provide a theoretical basis for the Cd tolerance mechanisms in winter wheat.

1 Materials and Methods

1.1 Experimental materials

Loamy soil (0-20 cm) was taken from the experimental field of the Scientific and Educational Park of Henan Agricultural University. The soil was air-dried, ground and sieved through a 2-mm sieve. The soil properties were as follows: organic matter 12.5 g·kg-1, alkali-hydrolyzed nitrogen 73 mg·kg-1, available potassium 170 mg·kg-1, effective phosphorus 10.8 mg·kg-1, pH 7.8. The tested wheat varieties were Pingan 8 (high Cd accumulation) and Bainong 160 (low Cd accumulation).

1.2 Experimental design

The pot experiment was conducted in the Scientific and Educational Park of Henan Agricultural University from October 15, 2016 to May 23, 2017. Each plastic pot contained 8 kg of soil. Two Cd concentrations (0 as CK and 1.5 mg·Cd kg-1 applied as CdCl2) were used in a complete factorial arrangement with two varieties. A completely randomized design was used. Each treatment was replicated three times.

After 2 weeks of Cd equilibration in soil, the fertilizers (urea, superphosphate and potassium chloride, all analytical grade) were applied in solution. Phosphate and potash fertilizers and ½ of urea were applied before sowing; the other half of urea was applied at the jointing stage. Ten seeds were sown per pot, and the pots were thinned to five seedlings after emergence. The management measures are the same as the field management measures for high yielding wheat in the local area.

1.3 Sample Collection

At jointing and grain filling stages, the wheat leaves were harvested, washed with tap water and then with deionized water, blotted dry, frozen in liquid nitrogen, and stored at -80℃ for measuring antioxidant enzymes and hormones.

1.4 Measurements and data analysis

MDA was determined by thiobarbituric acid chromogenic method. Determination of antioxidant enzyme activity: SOD was measured by nitrogen blue four azole photochemical reduction method, POD was determined by guaiacol method, and CAT was determined by ultraviolet spectrophotometry. The determination of plant hormones: The content of GA3, ZR, IAA and ABA were measured by ELISA.

1.5 Statistical analyses

[Grey correlation analysis](http://dict.cnki.net/dict_result.aspx?searchword=%e7%81%b0%e8%89%b2%e5%85%b3%e8%81%94%e5%88%86%e6%9e%90%e6%b3%95&tjType=sentence&style=&t=grey+correlation+analysis) method is calculated by the method of Deng Julong [14]. Specific steps are as follows: 1) The data in Table 1 is dimensionless. Since the dimensions of the indicators are inconsistent, the data is normalized by Xi(k)= Xi(k)/Xi(1); 2) Calculate the absolute value. The absolute value of the difference between the reference sequence X0 and the comparison sequence Xi is calculated ΔXi(k)=|X0(k)-Xi(k)|. The maximum absolute value is recorded as Δ(max), and the minimum absolute value is recorded as Δ(min); 3）Calculate the correlation degree. According to the grey system theory and method, the correlation coefficient and correlation degree between the variety and each indicator are calculated by the following formula.

Correlation coefficient ξ0i=



Resolution coefficient ρ=0.5

Correlation degree *r*i=i(k)

2 Results

2.1 Effect of cadmium stress on MDA content in winter wheat varying in Cd accumulation

In the Cd treatment (compared with the control), the MDA concentration in leaves increased significantly in Pingan 8 and Bainong 160 by, respectively, 55% and 38% in jointing stage, and 81% and 52% in grain filling stage (Fig. 1). In the Cd treatment, the MDA concentration was lower in Bainong 160 than Pingan 8.



Figure 1. MDA concentration in winter wheat with high and low accumulation of cadmium. A is the jointing stage and B is the filling stage. Means + SE, n=3.

2.2 Effect of cadmium stress on antioxidant enzyme activities in winter wheat with high and low Cd accumulation

2.2.1 Effect of cadmium stress on SOD activities

The SOD activities of Pingan 8 and Bainong 160 showed an increasing trend under cadmium stress. The increase in jointing stage was 7.74% (P < 0.05) and 4.80% (P < 0.05), and the increase in filling stage was 8.87% and 9.51% (P < 0.05), respectively (Fig. 2).



Figure 2. SOD activities of winter wheat with high and low accumulation of cadmium. A is the jointing stage and B is the filling stage.

2.2.2 Effect of cadmium stress on POD activities

The activity of POD in Pingan 8 and Bainong 160 increased significantly under Cd stress compared with the control (Fig. 3). The increase in Pingan 8 and Bainong 160 was, respectively, 24% and 14% at jointing and 12% and 31% at grain filling. There was no difference in between the two varieties in POD activity in the CD treatment at jointing and in the control at grain filling.



Figure 3. POD activities of winter wheat with high and low accumulation of cadmium. A is the jointing stage and B is the filling stage.

2.2.3 Effect of cadmium stress on CAT activities

Compared with CK, in the Cd treatment CAT activity increased significantly in Pingan 8 and Bainong 160 by, respectively 67% and 76% at jointing, and by 57% and 23% at grain filling (Fig. 4). At jointing, the two varieties did not differ in CAT activity regardless of the treatment, whereas Bainong 160 had higher CAT activity in either the control or the Cd treatment.



Figure 4. POD activities winter wheat with high and low accumulation of cadmium. A is the jointing stage and B is the filling stage.

2.3 Effects of cadmium stress on endogenous hormones in winter wheat with high and low accumulation of cadmium

It can be seen from Table 5 that compared with CK, the GA3 content in the leaves of two wheat cultivars decreased significantly under cadmium treatment at jointing stage and filling stage, and Pingan 8 decreased by 22% and ~~25.52%~~, respectively. Bainong 160 decreased by 20% and 18%, respectively.

At jointing, compared with CK, the IAA concentration in the Cd treatment significantly decreased in Pingan 8 and Bainong 160 by, respectively, 33% and 14%. At grain filling, exposure to Cd resulted in a decrease in the IAA concentration in leaves of Pingan 8 only (by 27%). The Cd treatment caused no significant change in the IAA concentration in Bainong 160. Under Cd treatment, the IAA content of Bainong 160 was 17.14% higher (P < 0.05) and 3.68% higher than that of Pingan 8 at jointing and filling stages, respectively.

Compared with CK, in the Cd treatment the ZR concentration in Pingan 8 decreased significantly at jointing (13%) and grain filling (34%). Exposure to Cd did not change the ZR concentration in Bainong 160 at jointing, but decreased it 21% (P < 0.05) at grain filling.

Compared with CK, in the Cd treatment the concentration of ABA in leaves of Pingan 8 increased by 29% at jointing and 17% at grain filling. In contrast, a corresponding increase in ABA concentration in Bainong 160 leaves was found only at jointing (by 11%).

Table 1. Endogenous hormones in winter wheat with high and low accumulation of cadmium

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Endogenous hormone content（ng·g-1FW） | Sampling period | Pingan 8 | | | Bainong 160 | |
| Control | Cd treatment | | Control | Cd treatment |
| GA3 | Jointing stage | 4.44±0.20a | | 3.49±0.12b | 4.74±0.11a | 3.77±0.27b |
| Filling stage | 6.13±0.44ab | | 4.56±0.21b | 6.73±0.24a | 5.48±0.10b |
| IAA | Jointing stage | 33.2±1.17a | | 22.3±1.08c | 30.5±1.01a | 26.1±1.23b |
| Filling stage | 54.9±2.12a | | 40.0±1.63b | 41.5±1.84b | 41.5±0.60b |
| ZR | Jointing stage | 7.26±0.19a | | 6.35±0.14b | 6.52±0.20b | 6.64±0.10b |
| Filling stage | 4.26±0.34ab | | 2.83±0.20c | 4.85±0.47a | 3.83±0.12bc |
| ABA | Jointing stage | 57.0±1.97c | | 73.5±1.32a | 57.5±0.96c | 63.8±2.13b |
| Filling stage | 104±2.9c | | 122±2.4a | 113±2.0b | 120±1.0ab |

2.4 Gray correlation analysis of antioxidant enzyme activities and hormone content in high and low accumulation cadmium varieties.

As shown in Table 4, through the gray correlation analysis, we conclude that the correlation coefficients of Bainong 160 and Pingan 8 are 0.97 and 0.64, respectively.

Table 2． Results of standardization of raw data

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cultivar | Jointing stage | | | | | | | | Filling period | | | | | | | |
| SOD | POD | CAT | MDA | GA3 | IAA | ZR | ABA | SOD | POD | CAT | MDA | GA3 | IAA | ZR | ABA |
|  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Pingan 8 | 0.89 | 0.98 | 0.87 | 0.80 | 0.93 | 0.85 | 0.96 | 1.00 | 0.84 | 0.88 | 0.83 | 0.65 | 0.83 | 0.96 | 0.74 | 1.00 |
| Bainong 160 | 1.00 | 1.00 | 1.00 | 1 | 1.00 | 1.00 | 1.00 | 0.87 | 1.00 | 1.00 | 1.00 | 1 | 1.00 | 1.00 | 1.00 | 0.98 |

Table 3. Correlation coefficient between varieties and indicators

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cultivar | Jointing stage | | | | | | | | Filling period | | | | | | | |
| SOD | POD | CAT | MDA | GA3 | IAA | ZR | ABA | SOD | POD | CAT | MDA | GA3 | IAA | ZR | ABA |
| Pingan 8 | 0.60 | 0.92 | 0.56 | 0.46 | 0.70 | 0.54 | 0.80 | 1.00 | 0.52 | 0.60 | 0.51 | 0.33 | 0.51 | 0.83 | 0.40 | 1.00 |
| Bainong 160 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.57 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 |

Table 4 Correlation degree between varieties and antioxidant enzyme activities, MDA content and hormone content

|  |  |
| --- | --- |
| Cultivar | Correlation degree |
| Pingan 8 | 0.64 |
| Bainong 160 | 0.97 |

3 Discussion

3.1 The effect of cadmium stress on the antioxidant system in winter wheat

The free radicals produced by adverse biological metabolic processes have a harmful effect on the plant membranes, but the protective enzymes system of the organism can scavenge free radicals and reduce damage [15]. The combined effect of SOD and CAT can convert ·O2- and H2O2 into H2O and O2 and inhibit the formation of high activity, such as ·OH. POD and CAT can catalyze the formation of H2O by H2O2, effectively preventing the accumulation of O2- and H2O2 and limiting the initiation of membrane lipid peroxidation by these radicals [16, 17]. POD, SOD and CAT together constitute the active oxygen scavenging system in plants, effectively eliminating free radicals and peroxides in plants [18]. Studies have shown that genotypic differences in cadmium tolerance in barley are related to antioxidant capacity [19]. It has been reported that cadmium treatment significantly increases CAT activity in hyperaccumulators, such as Sedum [20] and mustard-type rapeseed [21], while the opposite result is observed in *Brassica napus* [22] and *Arabidopsis* [23].

In our study, the SOD activity of Bainong 160 leaves was higher than that of Pingan 8 under Cd stress, indicating that the O2- concentration was lower in Bainong 160 than Pingan 8. The SOD activity of two varieties of wheat increased under Cd stress, which is consistent with the results of Ekmekçi et al. [24].

Under Cd stress, increased POD and CAT activity is associated with accelerated production of reactive oxygen species in some plant species [25]. Our study indicated that POD and CAT activities increased under Cd stress, indicating that both enzymes act simultaneously to remove H2O2 and prevent the formation of highly toxic OH. Increased CAT and POD activities indicate that accumulated H2O2 is insufficient to cause toxicity [15].

Malondialdehyde (MDA) is a product of membrane lipid peroxidation induced by oxidative stress in plant tissues [16, 26]. In the present experiment, the MDA concentration of Pingan 8 and Bainong 160 increased in the Cd treatment, indicating that Cd stress exacerbated generation of reactive oxygen radicals in wheat leaves, changing the structure and functions of the cell membranes. This is consistent with the results of Dixit et al. [27]. Shao et al. [28] showed that the antioxidant enzyme activity was significantly higher, and MDA concentration lower, in rice with strong Cd tolerance than Cd-sensitive varieties. In our study, the activities of SOD, POD and CAT were lower, and MDA concentration higher, in Pingan 8 than Bainong 160. These findings indicate that Pingan 8 is sensitive to Cd, whereas Bainong 160 has higher tolerance to Cd.

3.2 Effects of cadmium stress on endogenous hormones in winter wheat

Plants have adopted a variety of mechanisms to avoid toxicity of Cd. However, the regulation of plant hormone levels, whether physiological or molecular, in Cd tolerance is poorly understood. The study by You et al. [31] showed that Cd stress reduced the GA3 content in rice shoots. The study presented here also showed that Cd stress decreased GA3 concentration in wheat leaves, particularly in Pingan 8 because that genotype has poor to Cd.

Compared with CK, in the Cd treatment the IAA concentration in leaves of both varieties decreased at jointing (and also of Pingan 8 at grain filling), which is consistent with the results of Hu et al. [32] in Arabidopsis. These results also confirmed sensitivity of Pingan 8 to Cd.

Our study showed that Cd exposure reduces ZR concentration in wheat leaves, which is consistent with the results of Huang et al. [34] in soybean. The decrease in ZR content may be due to oxidative stress induced by Cd, resulting in oxidative degradation of cytokinins [35].

ABA may enhance or induce the transcription of the resistance genes, limiting potential Cd toxicity [36]. Sharma suggested an increase in ABA caused the stomata to close and maintain the water balance, reducing direct absorption of Cd by roots and decreasing tissue concentration of Cd and thus the toxicity [37]. Under Cd stress in the present study, the concetration of ABA in wheat leaves increased, which is consistent with the results of Wang [38] in soybean.

3.3 Gray correlation analysis

According to the results of gray correlation analysis, the performance was better in Bainong 160, the activity of antioxidant enzyme SOD, POD and CAT was higher, the content of MDA was lower, and the content of endogenous hormone GA3, IAA and ZR was higher. It showed that the antioxidant enzyme activity and the hormone content of the Bainong 160 were high, showing the high resistance to cadmium.

4. Conclusion

Bainong 160 had higher antioxidant enzyme activity and hormone concentration in leaves, and thus better Cd tolerance, compared with Pingan 8.

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