Effects of Cadmium Stress on Antioxidant Enzymes Activities and Soluble Protein Content in *Cyphomandra betacea* Seedlings

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Abstract: To find out the toxic mechanism of *Cyphomandra betacea* seedlings to cadmium (Cd) stress and provide reference for its reduction of Cd content, the annual *C. betacea* seedlings were treated with Cd of different concentration gradients (0-25 mg/kg) by pot experiment, the soluble protein content and the activities of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), were determined. The results showed that with the increase of Cd concentration in soil, the soluble protein content and the activity of SOD, POD and CAT in the leaves of *C. betacea* seedlings first increased and then decreased. The antioxidant enzyme activities reached their maximum when the soil Cd concentration was 10 mg/kg, and the soluble protein content reached its maximum when the soil Cd concentration was 1 mg/kg. The antioxidant enzymes might have a cooperative action with detoxification mechanism when the *C. betacea* seedlings were under Cd stress. Moreover, the *C. betacea* seedlings could reduce the Cd toxicity by increasing the soluble protein content when the Cd concentration in soil was within 1 mg/kg; when the concentration became higher, they might reduce the Cd toxicity by binding protein to Cd to form complex.

1.Introduction

The antioxidant enzyme system including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and so on, this is considered to be the most important defense system for plants under environmental stress [1]. So, study the response of antioxidant enzyme system to stress is of practical significance for its resistance. Cadmium (Cd) is one of the most toxic heavy metals, and has posed a serious threat to plant production in recent years [2]. Other researchers showed that Cd stress could change the physiological and biochemical metabolism of plants, and destroy the balance between the production and elimination of intracellular free radicals, which caused damages such as membrane lipid peroxidation, Enzyme inactivation and DNA breakage, etc. [3-5]. It was proved that the change of antioxidant enzyme activities maked plants adapt to Cd stress, but it might have a certain threshold, and the response of antioxidant enzymes was different inside and outside the threshold [5-6]. Futhermore, the antioxidant enzyme activity, its variation tendency and threshold changed from plants to plants [6]. Privious studies showed that soluble protein was an important osmotic regulator, which could maintain the osmotic pressure of plants under stress and stimulate the activity of antioxidant enzymes, and plants themselves could reduce the damage of Cd stress by regulating the proteolysis pathway [7-8]. *Cyphomandra betacea* is a perennial fruit tree of *Solanaceae* of the Solanaceae family [9]. In this study, we conducted a pot experient and analysed the variation tendency of SOD, POD, CAT and soluble protein content in the leaves to understand the defense system for Cd stess in C. *Betacea* seedlings, to provide reference for the development of the comprehensive value of C. *betacea*.

2. Material and methods

2.1 Materials

The seeds of *C. betacea* were collected from a perennial *C. betacea* at Chengdu Academy of Agriculture and Forestry (30° 42′ N, 103° 51′ E) in August 2016, air-dried and stored at 4 °C respectively.

2.2 Experimental design

The experiment was conducted at Chengdu campus of Sichuan Agricultural University (30° 42′ N, 103° 51′ E) from May to August 2017. The soil samples were air-dried and passed through a 5-mm mesh in May 2017, and then 3.0 kg of soil was weighed into each polyethylene pot (15 cm tall, 18 cm diameter). Cd was added to make a final soil Cd concentration of 0, 1, 5, 10, 15, 20 and 25 mg/kg with a saturated heavy metal solution in the form of $CdCl_2 \cdot 2.5H_2O$. The soils were mixed periodically during the next 4 weeks, and the soil moisture was kept at 80%. The seeds of *C. betacea* were sown in the farmland of the Chengdu campus in June 2017. Two weeks later, three seedlings of each treatment were transplanted into each pot, and each treatment was repeated five times. The position of the pots was randomly arranged and exchanged during the whole growth process with a 10-cm spacing between each pot to reduce the marginal effect.

When the *C. betacea* seedlings grew two months (August 2017) under Cd stress, the upper mature leaves of *C. betacea* seedlings were collected, grinded and then centrifuged, and the supernatant was used to determine the activity of antioxidant enzymes and the soluble protein content [10]. The SOD activity was determined by NBT, and 50% inhibition of the photochemical reduction of nitro-blue tetrazolium inside unit time was used as a unit of the enzyme activity, the POD activity was determined by guaiacol colorimetry, and the change value of A470 per minute showed the activity of the enzyme, and the CAT activity was determined by ultraviolet spectrophotometer, the drop of A240 per minute was used as a unit of the enzyme activity, and the soluble protein content was measured by Comus brilliant blue g250 staining method.

2.3 Statistical analyses

Statistical analyses were conducted by SPSS 17.0 statistical software (IBM, Chicago, IL, USA). Data was analysed by one-way analysis of variance with least significant difference (LSD) at the $P = 0.05$ confidence level.

3.Results and discussion

3.1 SOD activity in C. betacea seedlings

SOD is one of the main components in the antioxidant enzyme system of plants, and it is usually related to plant tolerance to most environmental stress [1]. Figure1 showed that the SOD activity in *C. betacea* under Cd stress was significantly higher than that of the control, which indicated that SOD had a positive response to it. Compared with control, the SOD activity increased by 12.58% ($P<0.05$), 15.60% ($P<0.05$), 30.06% ($P<0.05$), 24.09% ($P<0.05$), 8.85% ($P<0.05$) and 4.98% ($P<0.05$) respectively, when the *C. betacea* seedlings were under soil Cd concentration of 1, 5, 10, 15, 20 and 25 mg/kg. With the increase of Cd concentration in soil, the

SOD activity in the leaves of *C. betacea* seedlings increased firstly, and then decreased. When the Cd concentration in soil reached 10 mg/kg, the activity of SOD was 352.6 U/g, reaching its maximum. Therefore, Cd stress could invoke SOD activity markedly, but it might also damage SOD when the Cd concentration in soil was too high. Moreover, SOD played an important role in protection of *C. betacea* seedlings when under Cd stress with Cd concentration within 25 mg/kg, and the change trend of SOD activity was different within and beyond 10 mg/kg.

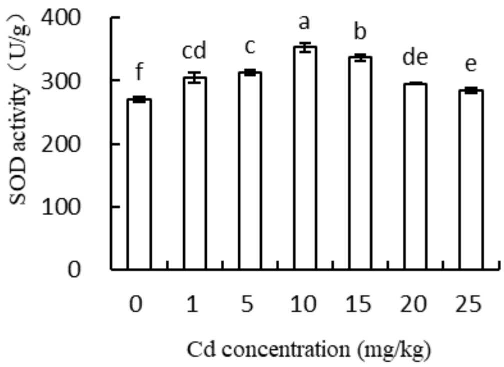

Figure 1. SOD activity in C. betacea seedlings. Values were means of five replicate pots. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test (P<0.05). 0 = Cd free , 1 = 1 mg/kg soil Cd concentration, 5 = 5 mg/kg soil Cd concentration, 10 = 10 mg/kg soil Cd concentration, 15 = 15 mg/kg soil Cd concentration, 20 = 20 mg/kg soil Cd concentration. Same as below.

3.2 POD activity in C. betacea seedlings

POD was an adaptive enzyme with high activity in *C. betacea* seedlings, and its changing trend of activity under Cd stress was similar to that of SOD's (Figure 2). When the Cd concentration in soil increased, the POD activity first increased and then decreased, but the POD activity changed more gently compared with the SOD activity. Under soil Cd concentration of 1, 5, 10, 15, 20 and 25 mg/kg, the POD activity increased by 7.07% ($P<0.05$), 9.83% ($P<0.05$), 28.38% ($P<0.05$), 14.08% ($P<0.05$), 8.78% ($P<0.05$) and 7.39% ($P<0.05$) respectively, compared with the control. The same as the SOD activity, the POD activity reached its maximum when the Cd concentration in soil was 10 mg/kg, and the maximum of POD activity in the leaves of *C. betacea* seedlings was 2325 U/g/min. Therefore, 10 mg/kg might be the optimum concentration to promote antioxidant enzymes activities. In general, the POD activity in *C. betacea* seedlings was high, and Cd stress could increase the POD activity, and POD might have a cooperative action with SOD when the *C. betacea* seedlings were in the soil with Cd concentration within 25 mg/kg.

3.3 CAT activity in C. betacea seedlings

CAT is an important enzyme in pants that is mainly used to scavenge hydrogen peroxide [1]. Figure 3 showed that with the increase of Cd concentration in soil, the trend of CAT activity increased first and then decreased, which was similar to the change trend of SOD activity and POD activity. The highest CAT activity in *C. betacea* seedlings was 2.313 mg/g/min, which occurred when they were under soil Cd concentration of 10 mg/kg. For changes, the CAT activity remained equally high when the Cd concentration in soil was 1, 5 and 10 mg/kg. When the Cd concentration in soil reached 20 mg/kg, the CAT activity in *C. betacea* seedlings became the same as that of the control, and when it reached 25 mg/kg, it even became lightly lower than the control ($P>0.05$). When the *C. betacea* seedlings were under soil Cd concentration of 1, 5, 10 and 15 mg/kg, the CAT activity increased by 16.15% ($P<0.05$), 17.46% ($P<0.05$), 21.29% (*P<0.05*) and 11.38% (*P<0.05*) respectively compared with the control. It was suggested that CAT could maintain the balance of reactive oxygen species at low soil Cd concentration to reduce Cd stress, and the action was suppressed when the Cd concentration in soil was more than 10 mg/kg, and the CAT activity decreased to the control level when the Cd concentration reached 20 mg/kg. Tt was inferred that 25 mg/kg might be the maximum Cd concentration that CAT could adapt to.

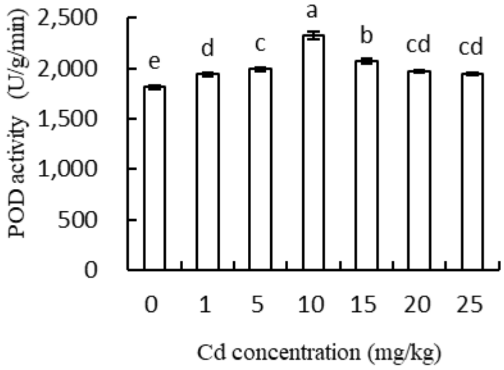

Figure 2. POD activity in C. betacea seedlings.

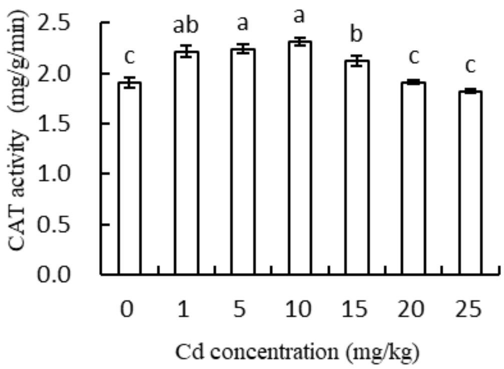

Figure 3. CAT activity in C. betacea seedlings.

3.4 Soluble protein content in C. betacea seedlings

Regulating the hydrolysis of protein in plants could reduce the damage of Cd stress [8]. Figure 4 suggested that the soluble protein content in *C. betacea* seedlings increased first and then decreased as the soil Cd concentration increased, which was consistent with the change of antioxidant enzymes activities, but their thresholds were quite different. With the increase of soil Cd concentration, the soluble protein content of *C. betacea* seedlings increased when the Cd concentration was within 1 mg/kg, and it began to decrease when the Cd concentration was more than 5 mg/kg. Under 1 mg/kg soil Cd concentration, the soluble protein content reached 12.79 mg/kg, which increased by 18.98% (*P<0.05*) compared with the control. When the *C. betacea* seedlings were under soil Cd concentration of 5, 10, 15, 20 and 25 mg/kg, the soluble protein content decrease by 6.70% (*P>0.05*), 14.23% (*P<0.05*), 15.81% (*P<0.05*), 33.02% (*P<0.05*) and 37.21% (*P<0.05*) respectively, compared with the control. We presume that when the Cd concentration in soil was within 1 mg/kg, the *C. betacea* seedlings could reduce the Cd toxicity by increasing the soluble protein content to regulate osmotic pressure and activate antioxidant enzymes, while when the concentration became higher, they might reduce the Cd toxicity by binding the protein to Cd to form complexes to avoid the toxic effect of Cd on cells.

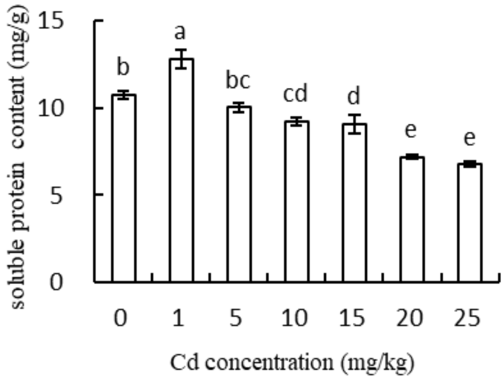

Figure 4. Soluble protein content in *C. betacea* seedlings.

4.Conclusions

With the increase of Cd concentration in soil, the SOD activity, POD activity, CAT activity and soluble protein content in the leaves of *C. betacea* seedlings increased firstly, and then they decreased. The antioxidant enzymes reached their maximum when the soil Cd concentration was 10 mg/kg, and the soluble protein content reached its maximum when the soil Cd concentration was 1 mg/kg. When the Cd concentration in soil reached 10 mg/kg, the activity of SOD was 352.6 U/g, reaching its maximum. For *C. betacea* seedlings, the POD activity changed more gently compared with the SOD activity, and the maximum of POD in the leaves of *C. betacea* seedlings was 2325 U/g/min. The CAT activity remained equally high when the Cd concentration in soil was 1, 5 and 10 mg/kg, and when the Cd concentration in soil reached 20 and 25 mg/kg, the CAT activity in *C. betacea* seedlings decreased to the control level. Under 1 mg/kg soil Cd concentration, and the soluble protein content reached 12.79 mg/kg, which was its maximum. Cd stress could invoke SOD activity, POD activity and CAT activity markedly, but it might also damage them when the Cd concentration in soil was too high, and their variation trend was highly consistent, which indicated that they might have a cooperative action with detoxification mechanism when the *C. betacea* seedlings were under Cd stress. *C. betacea* seedlings might reduce the Cd toxicity by increasing the soluble protein content to regulate osmotic pressure, activate antioxidant enzymes and so on when the Cd concentration in soil was less than 1 mg/kg, while when the concentration became higher, they might reduce the Cd toxicity by binding the protein to Cd to form complexes.

Acknowledgements

This work was financially supported by the Project of Sichuan Provincial Education Department (17ZB0342).

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