

Effects of Mulching with Hyperaccumulator Straw on Antioxidant Enzyme Activity of Grape Seedlings under Cadmium Stress

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Abstract. A pot experiment was conducted to study the effects of mulching with straw of different cadmium hyperaccumulators (*Crassocephalum crepidioides*, *Galinsoga parviflora*, *Youngia japonica* and *Gnaphalium affine*) on antioxidant enzyme systems of grape seedlings under cadmium stress. The results showed that all the straw-mulch treatments increased the peroxidase (POD) activity, superoxide dismutase (SOD) activity and soluble protein content of grape seedlings, but decreased the catalase (CAT) activity and malonic dialdehyde (MDA) content of grape seedlings. Mulching with straw of *Y. japonica*, the POD activity and SOD activity reached the highest level, which was 35.81% and 19.63% higher than monoculture respectively. Mulching with straw of *Galinsoga parviflora*, the soluble protein content reached the highest level, which was 29.23% higher than monoculture. However, all the straw-mulch treatments decreased CAT activity and MDA content compared with monoculture, and mulching with *Y. japonica* straw was the lowest, which was 85.49% and 68.49% lower than monoculture. In conclusion, covering different cadmium hyperaccumulator straws under cadmium stress can promote the growth of grape seedlings.

1. Introduction

In recent years, with the overexploitation of mineral resources and the extensive use of chemical fertilizers in agriculture, soil cadmium (Cd) pollution has become more and more serious, which has become an important factor affecting the quality of China's sustainable agriculture and ecological environment. Moreover, the accumulation of Cd in crops through the food chain can cause teratogenic, carcinogenic, and mutagenic and bone pain diseases [1]. The activity or content of antioxidant enzymes, which play an important protective role in plants, may be altered by the stress of pollutants [2]. The results showed that with the increase of Cd²⁺ concentration and the prolongation of Cd²⁺ toxicity time, SOD and CAT activities of maize seedlings decreased, POD activity increased, protein content decreased, membrane lipid peroxidation increased (MDA content increased), plasma membrane permeability increased, and the growth of maize seedlings was inhibited [3]. Under Cd stress, the accumulation of superoxide anion free radicals in wheat leaves increased significantly, and MDA content increased, showing the occurrence of membrane lipid peroxidation. Cadmium stress disordered the function of active oxygen scavenger system, in which SOD activity decreased and POD activity increased, while CAT activity did not change significantly [4]. Straw mulching is a common



production technology in agricultural production. The main purpose of straw mulching is to transform organic compound into organic matter and other nutrients, so as to achieve the purpose of fertilizing soil and improving soil texture [5]. Studies have showed that under the condition of Cd pollution, the Cd tolerant plants covered by soil increased the activity of the antioxidant enzyme (SOD, POD and CAT) of *Cyphomandra betacea* [6], and the application of the enriched plant straw increased the antioxidant enzyme activity of watercress leaves and increased the soluble protein content of watercress leaves [7]. Therefore, mulching with straw of Cd hyperaccumulators could have effects on antioxidant enzyme systems of other plants. In the study, four kinds of Cd hyperaccumulators (*Crassocephalum crepidioides* [8], *Galinsoga parviflora* [9], *Youngia japonica* [10] and *Gnaphalium affine* [11]) straws were mulched on the surface of Cd contaminated soil, then cultivated grape seedlings, and study the effects of mulching with straw of different Cd hyperaccumulators on antioxidant enzyme systems of grape seedlings under Cd stress.

2. Materials and Methods

2.1. Materials collection

The materials used in the experiment were *C. crepidioides*, *G. parviflora*, *Y. japonica* and *G. affine*. The straws were collected from the farmland (without Cd pollution) of Chengdu Campus of Sichuan Agricultural University in March 2018. The shoot of plants was collected and washed with tap water, then washed three times with deionized water, and dried at 110 °C for 15 min, then dried to constant weight at 75 °C. The straw was cut into small sections of less than 1 cm and set aside. The cultivar of grape is “Xiahei” with cutting seedlings. The fluvo-aquic soil was collected from the farmland of Chengdu Academy of Agriculture and Forestry Sciences.

2.2. Experimental Design

The experiment was conducted in Chengdu Campus of Sichuan Agricultural University from March to May 2018. In March 2018, the soil was air-dried, ground and passed through a 5-mm sieve. Each plastic pot (15 cm high, 18 cm in diameter) was filled with 3 kg of ground soil and soaking uniformly Cd solution with 5 mg/kg Cd (in the form of $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$) for 4 weeks. All pots were watered every day to keep the soil moisture about 80%, and dug aperiodically to make soil mixed fully. In April 2018, the prepared Cd hyperaccumulators straw was respectively mulched in the prepared Cd contaminated soil, so that it was covered in the soil surface. Each kilogram of soil covered 2 g straw (6 g straw in each pot), and kept the soil moist and balanced for 1 weeks. Then three uniform grape seedlings (the shoots were about 15 cm) were transplanted into each pot. The experiment consists of 5 treatments: monoculture of grape, mulching with straw of *C. crepidioides*, mulching with straw of *G. parviflora*, mulching with straw of *Y. japonica* and mulching with straw of *G. affine*. Three replicates were run for each treatment and the distance between pots was 15 cm. All pots were watered every day to keep soil moisture at 80% and exchanged the pot position aperiodically to weaken the impact of marginal effects until the plants were harvested. After 40 days, the upper young leaves of grape seedlings were collected to determine the enzymatic activity (SOD, POD and CAT), soluble protein and MDA content.

2.3. Statistical Analyses

Statistical analyses were performed with SPSS 20.0 statistical software. Data were analyzed with one-way analysis of variance with least significant difference at the 5% significance level.

3. Results and Discussion

3.1. POD activity of grape seedlings

The POD activity of grape seedlings covered with four straws of Cd hyperaccumulators was higher than monoculture. Therefore, the POD activity of grape seedling was increased after covering the

straw (Figure 1). Compared to monoculture, the treatments were improved by 15.64% ($p > 0.05$), 6.32% ($p > 0.05$), 35.81% ($p > 0.05$) and 31.50% ($p > 0.05$) respectively. When covered with straw of *Y. japonica*, POD activity of grape seedlings was the highest, mulching with straw of *G. affine* followed, but there was no significant difference between mulching with straw of *G. parviflora* and monoculture.

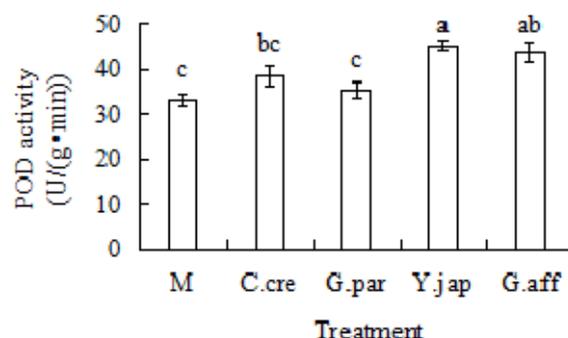


Figure 1. POD activity of grape seedlings
Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 20.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, C.cre = mulching with straw of *C. crepidioides*, G.par = mulching with straw of *G. parviflora*, Y.jap = mulching with straw of *Y. japonica*, G.aff = mulching with straw of *G. affine*. The same as below.

3.2. SOD activity of grape seedlings

Compared with monoculture, all the straw-mulch treatments slightly increased the SOD activity of the seedlings (Figure 2). Mulching with straw of *Y. japonica* treatment was the highest, the straw of *G. affine* followed it, which increased by 19.63% ($p > 0.05$) and 7.15% ($p > 0.05$) respectively, which showing a significant difference from monoculture. Mulching with the straw of *C. crepidioides* and mulching with the straw of *G. parviflora* were not significantly different from monoculture, which increased by 2.82% ($p > 0.05$) and 5.28% ($p > 0.05$) respectively.

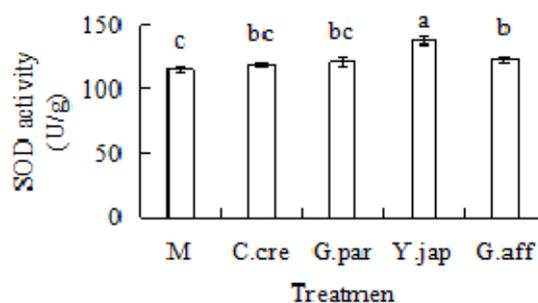


Figure 2. SOD activity of grape seedlings

3.3. CAT activity of grape seedlings

Compared with monoculture, all the straw-mulch treatments slightly increased the CAT activity of the

seedlings (Figure 3). The CAT activity of the seedlings was ranked in the following order: monoculture > mulching with straw of *C. crepidioides* > mulching with straw of *G. parviflora* > mulching with straw of *G. affine* > mulching with straw of *Y. japonica*. In this order, they were 22.82% ($p < 0.05$), 33.05% ($p < 0.05$), 60.01% ($p < 0.05$), and 85.49% ($p < 0.05$) lower than monoculture.

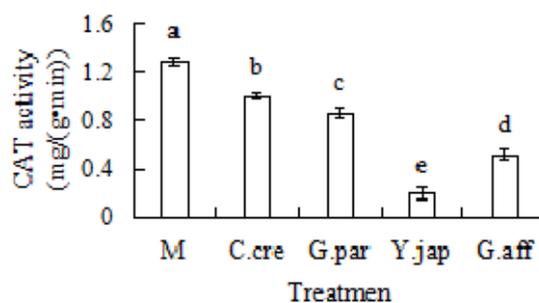


Figure 3. CAT activity of grape seedlings under Cd stress

3.4. Soluble protein content of grape seedlings

The soluble protein content of all the straw-mulch treatments increased, comparing with monoculture (Figure 4). When mulching with straw of *G. parviflora*, the soluble protein content reached maximum, which increased by 29.23% ($p > 0.05$) compared with monoculture. There were no significant difference in soluble protein content among the treatments of mulching with straw of *C. crepidioides*, mulching with straw of *Y. japonica* and mulching with straw of *G. affine*. And the three treatments slightly increased the soluble protein content of the seedlings compared with monoculture.

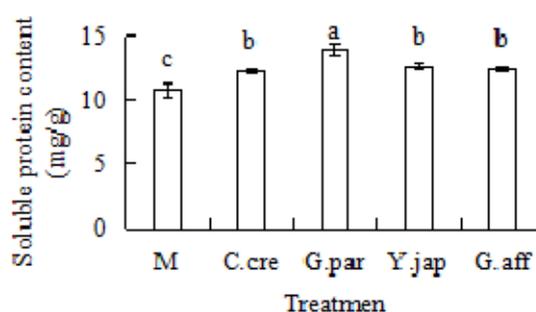


Figure 4. Soluble protein content of grape seedlings

3.5. MDA content of grape seedlings

The MDA content of grape seedlings is similar to CAT activity. All treatments reduced the MDA content of grape seedlings compared with monoculture (Figure 5). The content of MDA was ranked in the following order: monoculture > mulching with straw of *C. crepidioides* > mulching with straw of *G. parviflora* > mulching with straw of *G. affine* > mulching with straw of *Y. japonica*. Therefore, covering the straw of different Cd hyperaccumulators can alleviate the accumulation of MDA content of grape seedlings, and the content is significantly lower than monoculture. Mulching with straw of *Y. japonica* is the lowest, and the effect of alleviating Cd stress is the best, which is 68.49% lower than monoculture.

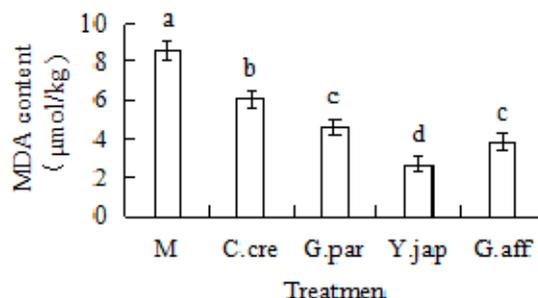


Figure 5. MDA content of grape seedlings

4. Conclusions

The experiment showed that mulching with different kinds of Cd hyperaccumulators straws had different effects on antioxidant enzyme system, soluble protein content and MDA content of grape seedlings. After covering four kinds of hyperaccumulators straws, the POD activity, SOD activity and soluble protein content were increased compared with monoculture, thereby regulating the intracellular peroxide production. POD activity and SOD activity were highest when covering *Y. japonica* straw, followed by *G. affine*, which was 35.81%, 35.81%, 19.63% and 7.15% higher than monoculture respectively. The soluble protein content reached the maximum when mulching with straw of *G. parviflora*, which was 29.23% higher than monoculture. However, compared with monoculture, all treatments reduced the CAT activity of grape seedlings, and the mulching with straw of *Y. japonica* reached the lowest, which was 85.49% lower than monoculture. The MDA content of grape seedlings was similar to that of CAT. The MDA content of all treatments was lower than monoculture, indicating that the coverage of straw reduced the accumulation of MDA in membrane peroxidation and reduced the damage to the macromolecules of seedlings. Among them, mulching with straw of *Y. japonica* reached the lowest level, and the effect of alleviating Cd stress was the best, which was 68.49% lower than monoculture. Therefore, covering different Cd hyperaccumulators straws under Cd stress can increase POD activity, SOD activity and soluble protein content, reduce MDA content, so it can promote the growth of grape seedlings under Cd stress. In general, mulching with straw of *Y. japonica* works the best.

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