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Effects of applying hyperaccumulator straw after planting grape seedlings on soil enzyme activity under cadmium stress

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Abstract. The effects of mulching with four hyperaccumulator plants *Crassocephalum crepidioides*, *Galinsoga parviflora*, *Youngia japonica*, and *Gnaphalium affine* straws on soil enzyme activity of grape seedlings under cadmium (Cd) stress were studied by a pot experiment. The results showed that mulching with four kinds of hyperaccumulators straws, the soil catalase, phosphatase and sucrase activity were increased compared with the monoculture. Only the straw-mulch *Y. japonica* decreased the soil urease activity, which decreased by 0.10%, and others treatments all increased the soil urease activity. In conclusion, the treatment of *G. affine* is the best, *C. crepidioides* followed it.

1. Introduction

Soil enzyme activity is susceptible to physical, chemical and biological effects in the environment, and can directly reflect the soil biological activity and the soil biochemical reaction intensity [1-3]. It plays an important role in nutrient circulation, energy metabolism and pollutant monitoring [4]. Some pollutants can affect the soil enzyme activity. For example, cadmium is a heavy metal with strong toxicity, and it is a non-essential element, which is difficult to degrade. The Cd enters the soil is continuously accumulated and absorbed by plants, and entered into the human body through the food chain and endangers human health [5]. In the study, heavy metal elements Cu, Zn and Cd all reduce the activity of urease, phosphatase and sucrase in soil [6]. Straw is an important biological resource and when straw enters the soil, it will be related to soil enzyme [3]. For example, the application of *Conyza canadensis* and *Cardamine hirsuta* straws increased soil catalase activity, soil urease activity and soil sucrase activity, while application of *Eclipta prostrata* and *Nasturtium officinale* straws reduced soil enzyme activity [7]. With the soil pollution increasing, soil enzymes can reflect environmental conditions to a certain extent due to the stable and sensitive characteristics of soil enzymes [8]. The grape is a perennial fruit tree, which has a stronger enrichment ability of cadmium. Therefore, in this study, we used four Cd hyperaccumulator plants *Crassocephalum crepidioides* [9], *Galinsoga parviflora* [10], *Youngia japonica* [11], and *Gnaphalium affine* [12] straws mulching with grape seedlings of Kyoho under Cd stress by a pot experiment to study the effects on soil enzyme activity, in order to screen out which straw treatment significantly increase the soil enzyme activity, and provide reference for the grape production in Cd-contaminated area.



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 cadmium pollution) of Chengdu Campus of Sichuan Agricultural University in April 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 “Kyoho” 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 April to August 2018. In April 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 cadmium 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 May 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 keep 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 60 days, the soil in the 0 – 5 cm layer of each pot was taken out, mixed and packaged in plastic bags and brought back to the laboratory and stored in a refrigerator at 4 degrees centigrade. Soil enzyme activity was determined by soil enzyme method [13] after soil 1mm screening. The activity of catalase was expressed as the volume of 0.02 mol/L KMnO_4 per gram of soil at room temperature in 30 minutes. Soil urease activity was expressed in grams (mg) of $\text{NH}^{+3}\text{-N}$ per gram of soil at 37 °C for 24 hours. Soil invertase activity was expressed in the amount of glucose released per gram of soil at 37 °C for 24 hours. Soil phosphatase was expressed in grams of phenol per gram of soil.

2.3. Statistical Analyses

Statistical analyses were performed using 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. Soil catalase activity of grape

The soil catalase activity of grape seedlings covered with four straws of cadmium hyperaccumulators was higher than monoculture. Therefore, the soil catalase activity of grape seedling was increased after covering the straw (Figure 1). Compared to the monoculture, the treatments were improved by 14.76% ($p > 0.05$), 14.03% ($p > 0.05$), 1.84% ($p < 0.05$) and 24.93% ($p > 0.05$) respectively. When covered with straw of *G. affine*, soil catalase activity of grape seedlings was the highest, mulching with straw of *C. crepidioides* followed, but there was no significant difference between mulching with straw of *Y. japonica* and monoculture.

3.2. Soil urease activity of grape

Compared with the monoculture, the straw-mulch *C. crepidioides*, *G. parviflora* and *G. affine*

treatments increased the soil urease activity of the seedlings, but the straw-mulch *Y. japonica* decreased the soil urease activity (Figure 2). Mulching with straw of *G. affine* treatment was the highest, the straw of *C. crepidioides* followed it, which increased by 93.66% ($p > 0.05$) and 71.41% ($p > 0.05$), respectively, which showing a significant difference from monoculture. Mulching with the straw of *G. parviflora* and *Y. japonica* were not significantly different from monoculture.

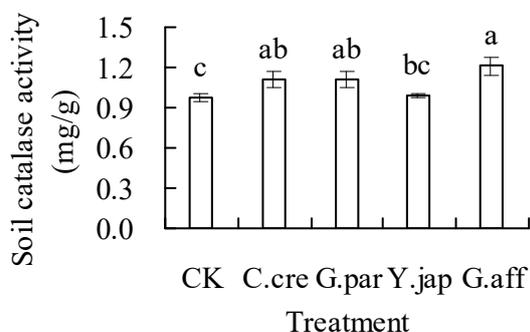


Figure 1. Soil catalase activity of grape.

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*.

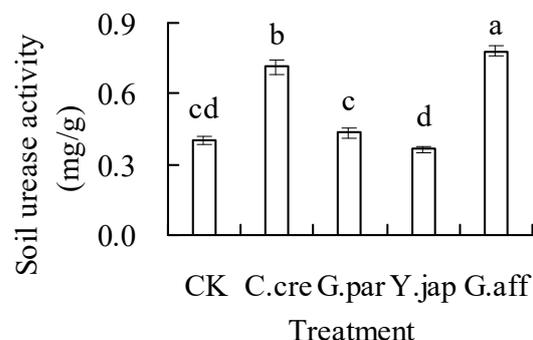


Figure 2. Soil urease activity of grape.

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*.

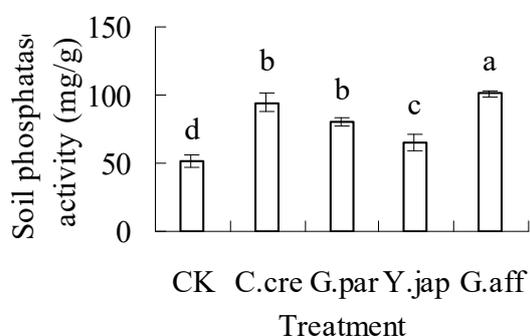


Figure 3. Soil phosphatase activity of grape.

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*.

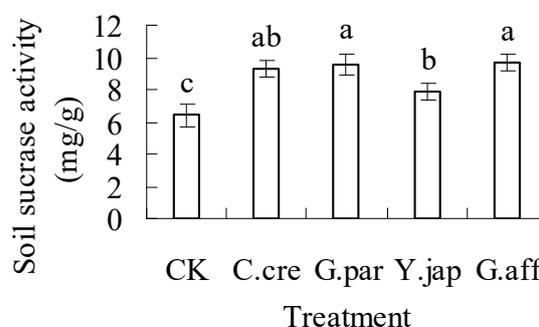


Figure 4. Soil sucrase activity of grape.

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*.

3.3. Soil phosphatase activity of grape

Compared with monoculture, all the straw-mulch treatments increased the soil phosphatase activity of the seedlings (Figure 3). The soil phosphatase activity of the seedlings was ranked in the following

order: mulching with straw of *G. affine* > mulching with straw of *C. crepidioides* > mulching with straw of *G. parviflora* > mulching with straw of *Y. japonica* > monoculture. In this order, they were 96.74% ($p > 0.05$), 84.42% ($p > 0.05$), 56.00% ($p < 0.05$), and 26.73% ($p < 0.05$) higher than the monoculture, respectively.

3.4. Soil sucrose activity of grape

Compared with the monoculture, all the straw-mulch treatments increased the soil sucrose activity of the seedlings (Figure 4). The soil sucrose activity of the seedlings was ranked in the following order: mulching with straw of *G. affine* > mulching with straw of *G. parviflora* > mulching with straw of *C. crepidioides* > mulching with straw of *Y. japonica* > monoculture. In this order, they were 51.25% ($p > 0.05$), 50.00% ($p > 0.05$), 45.00% ($p < 0.05$), and 22.81% ($p < 0.05$) higher than monoculture.

4. Conclusions

The experiment showed that mulching with different kinds of Cd hyperaccumulators straws had different effects on soil enzyme activity of grape seedlings. After covering four kinds of hyperaccumulators straws, the soil catalase, phosphatase and sucrose activity were increased compared with the monoculture. Only the straw-mulch *Y. japonica* decreased the soil urease activity, others treatments all increased the soil urease activity. In conclusion, straw-mulch treatments had improvement effects on soil enzyme activity and the treatment of *G. affine* is the best.

Acknowledgements

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