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Effects of grape seedlings intercropping with post-Grafting generation of *Cosmos sulphureus* on different fractions of cadmium content in soil

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Abstract. A pot experiment was conducted to study the effects of grape seedlings intercropping with post-grafting generation of *Cosmos sulphureus* on different fractions of cadmium (Cd) in soil. The results showed that when grape seedlings intercropped with the generation of ungrafted *C. sulphureus* (I CK), the content of exchangeable Cd increased by 25%, and the content of residual Cd decreased by 17% compared to the monoculture, respectively. Compared to the monoculture, grape seedlings intercropping with the post-grafting generation of self-rooted grafting by two uniform plant seedlings of *C. sulphureus* (I UG) significantly increased the residual Cd content (37%) while the exchangeable Cd content increased not significantly (11%). In conclusion, the treatment of I CK was not conducive to the reduction of the bioavailability of Cd in soil, while the treatment of I UG was better.

1. Introduction

Cadmium (Cd) is a non-essential element and has negatively effects on plants, animals and humans. There are five fractions of Cd (exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter and residual) in soil according to Tessier [1]. Different fractions of Cd have different bioavailability and the degree of damage to plants is different [2]. Exchangeable Cd is the most active fraction and easily absorbed by plant, and residual Cd is inert fraction with minimal damage to plants [3]. Cd bound to carbonates and Cd bound to Fe-Mn oxides are sensible to the varieties of soil like pH, Eh, and easily converted to exchangeable Cd. Cd bound to organic matter is relatively stable and can effectively reduce the bioavailability of Cd [4-5]. Therefore, the bioavailability of Cd can be reduced by transforming the fraction of Cd. Studies have shown that intercropping with Cd accumulators can affect the absorption of Cd by plants [6]. We guessed whether intercropping affect the absorption of Cd by plants via changing the fractions of Cd in soil, so it is necessary to study the effects on fractions of Cd in soil in intercropping mode. Therefore, the objective of this study was to evaluate the effect of grape seedlings intercropping with post-grafting generation of *Cosmos sulphureus* on different



fractions of Cd in soil under Cd stress, and to find the optimal intercropping combination which can decrease the bioavailability of Cd in soil.

2. Materials and methods

2.1. Materials

The seeds of *C. sulphureus* were collected from the surrounding farmland at Chengdu Campus of Sichuan Agricultural University (30°42' N, 103°50' E). The cultivar of grape in the experiment was Kyoho with cutting seedlings. The soil for the experiment was collected from the Chengdu campus farm of the Sichuan Agricultural University. Cd was added to the soil sample in the form of analytical pure $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ solution at the concentration of 5 mg/kg, and then the soil was thoroughly mixed. Natural balance for 4 weeks, occasionally turning soil mixing, so that the soil was fully mixed.

2.2. Experimental design

In October 2014, the seeds of *C. sulphureus* were collected and sowed. Plants were grafted when the first batch of seedlings was about 10 cm high (and the second batch of seedlings was about 5 cm high). The grafting treatments as follow [8]: (1) Ungrafted: the seedlings transplanted directly, collected the seeds for preservation as the generation of ungrafted *C. sulphureus* (CK). (2) Self-rooted grafting by the same one seedling: the seedlings were cut off from 6 cm above the ground. The upper parts were scion and the lower parts were rootstock. Rootstock leaves were retained. The seeds were collected for preservation as the post-grafting generation of self-rooted grafting by the same one seedling of *C. sulphureus* (SG). (3) Self-rooted grafting by two uniform plant seedlings: seedlings were about 10 cm high and divided into two parts. One was cut off from 6 cm above the ground, kept the lower parts as rootstock; one was cut off from 6 cm above the ground, kept the upper parts as scion (4 cm). Rootstock leaves were retained. The seeds were collected for preservation as the post-grafting generation of self-rooted grafting by two uniform seedlings of *C. sulphureus* (UG). (4) Self-rooted grafting by two different sizes seedlings: seedlings were about 10 cm high, cut off from 6 cm above the ground, the lower parts were rootstock. Seedlings were about 5 cm high, cut the upper seedling (4 cm) as scions. Rootstock leaves were retained. The seeds were collected for preservation as the post-grafting generation of self-rooted grafting by two different sizes seedlings of *C. sulphureus* (DG). The soil used in grafting experiments was Cd free.

In May 2015, the seeds of post-grafting generation of *C. sulphureus* were collected and sowed. When the seedlings expanded two true leaves, each type plant seedling and each grape seedling were transplanted into a pot respectively which prepared with soil by 5 mg/kg Cd. There were five treatments: grape monoculture (M), grape intercropping with CK (I CK), grape intercropping with SG (I SG), grape intercropping with UG (I UG), grape intercropping with DG (I DG). Six replicates were run for each treatment, and pots placed completely randomly. The distance between pots was 15 cm, and pots places were exchanged periodically to weaken the impact of marginal effects, keeping the soil moisture about 80% until the plants were harvested.

After 60 days, the soil from the rhizosphere in the corresponding pot was collected when the plants were collected. All the soil samples were air-dried at room temperature then ground to pass through a 1-mm nylon sieve for analysis of five fractions of Cd. Using modified continuous extraction method to extract different fractions of Cd in soil [1,9], and the Cd content of each form was determined by novAA 400P flame atomic absorption spectrophotometer (Analytik Jena, Germany).

2.3. Statistical analyses

Statistical analyses were conducted using statistical software of SPSS 17.0. Data were analyzed by one-way ANOVA with least significant difference at 5% confidence level.

3. Results and discussion

3.1. The content of exchangeable cadmium

Intercropping increased the content of exchangeable Cd in soil in general (Figure 1). The content of exchangeable Cd for the treatment of I CK was significantly higher than that of monoculture, which was 25% ($p < 0.05$) higher than the monoculture. The content of exchangeable Cd for other intercropping treatments was not significantly different from that for the monoculture. The contents of exchangeable Cd for the treatments of I SG, I UG and I DG increased by 9%, 11% and 9%, respectively, compared to the monoculture ($p > 0.05$).

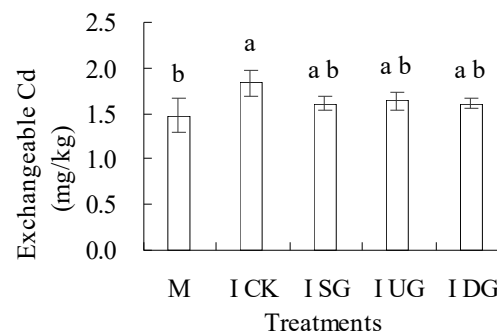


Figure 1 The content of exchangeable Cd. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = grape monoculture, I CK = intercropping with the generation of ungrafted *C. sulphureus*, I SG = intercropping with the post-grafting generation of self-rooted grafting by the same one seedling of *C. sulphureus*, I UG = intercropping with the post-grafting generation of self-rooted grafting by two uniform plant seedlings of *C. sulphureus*, I DG = intercropping with the post-grafting generation of self-rooted grafting by two different sizes seedlings of *C. sulphureus*. The same as follows.

3.2. The content of cadmium bound to carbonates

Compared to the monoculture, all the intercropping treatments decreased the content of Cd bound to carbonates in soil except the treatment of I SG (Figure 2). The content of Cd bound to carbonates for the treatment of I CK was lowest, decreased by 22% ($p < 0.05$) compared to the monoculture. The content of Cd bound to carbonates for the treatment of I SG was a little higher than that of monoculture, which was 8% ($p > 0.05$) higher than the monoculture. The content of Cd bound to carbonates for the treatments of I UG and I DG was lower than which of monoculture, and decreased by 8% ($p > 0.05$) and 13% ($p > 0.05$) compared to the monoculture, respectively.

3.3. The content of cadmium bound to Fe- Mn oxides

Intercropping decreased the content of Cd bound to Fe-Mn oxides in soil compared to the monoculture (Figure 3). The treatment of I SG decreased the content of Cd bound to Fe-Mn oxides significantly, and it decreased by 29% ($p < 0.05$) compared to the monoculture. There was no significant difference between the content of Cd bound to Fe-Mn oxides for monoculture and that for other intercropping treatments. The content of Cd bound to Fe-Mn oxides for the treatments of I CK, I UG and I DG decreased by 13%, 21% and 18% compared to the monoculture, respectively ($p > 0.05$).

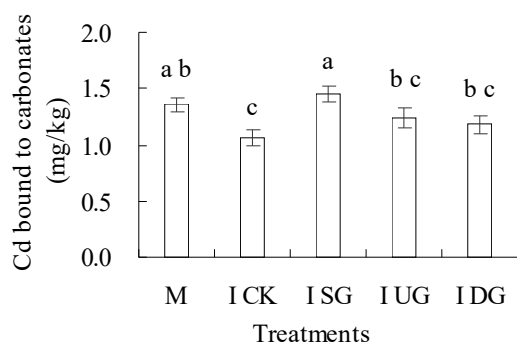


Figure 2 The content of Cd bound to carbonates

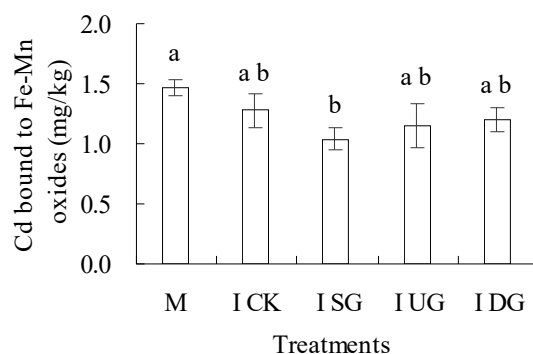


Figure 3 The content of Cd bound to Fe-Mn oxides

3.4. The content of cadmium bound to organic matter

The treatment of I UG increased the content of Cd bound to organic matter in soil compared to the monoculture, and other intercropping treatments decreased it (Figure 4). The content of Cd bound to organic matter for the treatment of I UG was highest, increased by 4% ($p > 0.05$) compared to the monoculture. The content of Cd bound to organic matter for the treatment of I DG was lowest, decreased by 8% ($p > 0.05$) compared to the monoculture. The differences between the content of Cd bound to organic matter for the monoculture and that for intercropping treatments was not significant ($p > 0.05$).

3.5. The content of residual cadmium

The content of residual Cd in soil decreased for the treatments of I CK and I SG, and it increased for the treatments of I UG and I DG, compared to the monoculture (Figure 5). The content of residual Cd for the treatments of I CK and I SG decreased by 17% ($p > 0.05$) and 4% ($p > 0.05$) compared to the monoculture. The content of residual Cd for the treatments of I UG and I DG increased significantly compared to the monoculture, and increased by 37% ($p < 0.05$) and 31% ($p < 0.05$) compared to the monoculture.

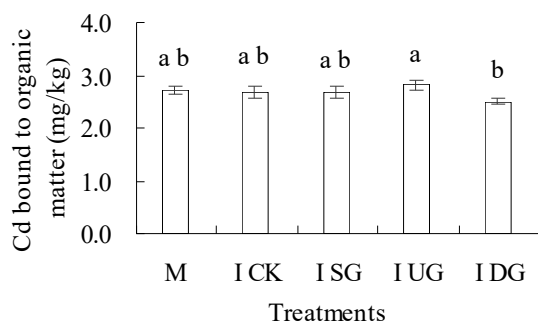


Figure 4 The content of Cd bound to organic matter

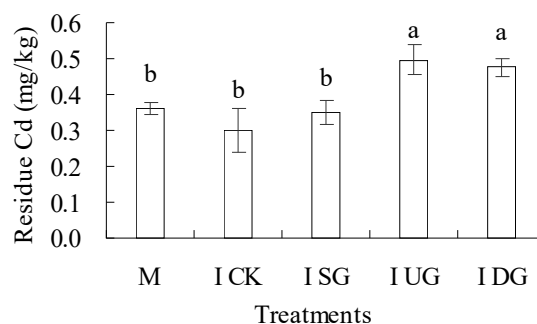


Figure 5 The content of residual Cd

4. Conclusions

In the experiment, grape seedlings intercropped with the post-grafting generation of *C. sulphureus* under Cd stress. The results showed that the treatment of I CK increased the content of exchangeable Cd (25%, $p < 0.05$) and decreased the content of residual Cd (17%, $p > 0.05$) in soil. Compared to the monoculture, the content of residual Cd for the treatments of I UG and I DG increased by 37% ($p < 0.05$) and 31% ($p < 0.05$), and the content of exchangeable Cd increased by 11% ($p > 0.05$) and 9% ($p > 0.05$) respectively. The treatment of I UG increased the content of Cd bound to organic matter (4%, $p > 0.05$) while the treatment of I DG decreased it (8%, $p > 0.05$). In conclusion, the treatment of I CK increased the content of exchangeable Cd in soil and decreased the content of residual Cd. The

treatment of I UG increased the content of Cd bound to organic matter and residual Cd while the content of exchangeable Cd did not increase significantly. Therefore, the treatment of I CK was not conducive to the reduction of the bioavailability of Cd in soil, while the treatment of I UG was better.

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