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Effects of grape seedlings intercropping with post-grafting generation of *Impatiens balsamina* on different fractions of cadmium content in soil

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Abstract. To study the effects of grape seedlings intercropping with post-grafting generation of *Impatiens balsamina* on different fractions of cadmium (Cd) in soil, a pot experiment was conducted. The results showed that grape seedlings intercropping with post-grafting generations of *I. balsamina* significantly increased the content of exchangeable Cd in soil. Grape seedlings intercropping with the post-grafting generation of self-rooted grafting by two different sizes seedlings of *I. balsamina* significantly increased the content of residual Cd (52%), and increased the exchangeable Cd content (19%). In conclusion, grape seedlings were not suitable for intercropping with post-grafting generations of *I. balsamina*, although some intercropping treatments increased the content of residual Cd.

1. Introduction

The pollution of cadmium (Cd) in agricultural environment increased significantly due to mining, military, industrial activities and other anthropogenic sources, which seriously threatens the growth of plants [1]. Cd in soil can be divided into five fractions normally: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter and residual [2]. Exchangeable Cd is the most active fraction and easily absorbed by plant, and residual Cd is inert fraction with minimal damage to plants, the bioavailability of Cd bound to carbonates, Cd bound to Fe-Mn oxides and Cd bound to organic matter is between that of exchangeable Cd and residual Cd [3-4]. Studies have shown that grafting increased Cd content of post-grafting generation of *Impatiens balsamina*, and intercropping with inter-species post-grafting generation of *Solanum photeinocarpum* increased the Cd contents of *Brassica rapa chinensis* [5-6]. It inferred that intercropping affect the absorption of Cd by plants via changing the fractions of Cd in soil, so it is vital to study the effects on fractions of Cd in soil in intercropping mode. Therefore, in the study, four post-grafting generations of *I. balsamina* were used to intercrop with grape seedlings under Cd stress and to study grape seedlings intercropping with post-grafting generation of *I. balsamina* on different fractions of Cd in soil under Cd stress.



2. Materials and methods

2.1. Materials

The cultivar of grape was Kyoho with cutting seedlings. The seeds of *I. balsamina* were collected from the surrounding farmland at Chengdu campus of Sichuan Agricultural University (30°42'N, 103°50'E). The soil for the experiment was collected from the Chengdu campus farm of the Sichuan Agricultural University. The soil was air-dried, ground and passed through a 6.72-mm sieve, then soaked it in the solution with 5 mg/kg Cd (in the form of $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$), and keeping the soil in the stage for 4 weeks to make the soil mixed well with Cd.

2.2. Experimental design

In October 2014, the seeds of *I. balsamina* 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 [5]: (1) Ungrafted: the seedlings transplanted directly, collected the seeds for preservation as the generation of ungrafted *I. balsamina* (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 *I. balsamina* (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 *I. balsamina* (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 *I. balsamina* (DG). The soil used in grafting experiments was Cd free.

In May 2015, the seeds of the post-grafting generation of *I. balsamina* were sowed at artificial climate chamber. When the seedlings expanded two true leaves, one grape seedling and one post-grafting generation of *I. balsamina* were transplanted into each pot (one grape seedling for grape monoculture). 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 fractions of Cd analysis. Using modified Tessier continuous extraction method to extract different forms of Cd in soil [2, 7], 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

The content of exchangeable Cd in soil for all the intercropping treatments significantly increased compared to the monoculture (Figure 1). The exchangeable Cd content could be arranged in the

following order: I UG > I DG > I CK > I SG > M, and increased by 29% ($p < 0.05$), 19% ($p < 0.05$), 18% ($p < 0.05$) and 12% ($p < 0.05$) compared to the monoculture.

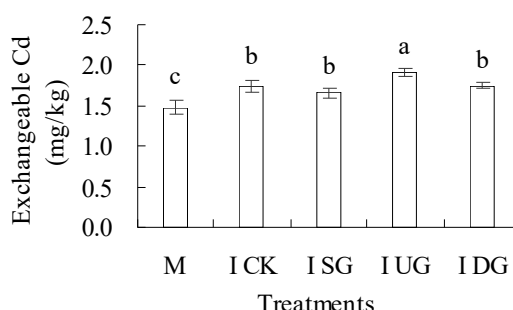


Figure 1 The content of exchangeable Cd. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = grape monoculture, I CK = intercropping with the generation of ungrafted *I. balsamina*,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

3.2. The content of cadmium bound to carbonates

Compared to the monoculture, the content of Cd bound to carbonates in soil for the treatments of I CK and I DG increased, while that for the other two treatments decreased (Figure 2). The content of Cd bound to carbonates for the treatment of I DG was highest, increased by 9% ($p < 0.05$) compared to the monoculture. The content of Cd bound to carbonates for the treatment of I SG was lowest, which was 11% ($p < 0.05$) lower than the monoculture.

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

Intercropping significantly decreased the content of Cd bound to Fe-Mn oxides in soil compared to the monoculture, and the difference between the intercropping treatments was not significant (Figure 3). The content of Cd bound to Fe-Mn oxides for the treatments of I CK, I SG, I UG and I DG decreased by 27%, 29%, 28% and 28% compared to the monoculture, respectively ($p < 0.05$).

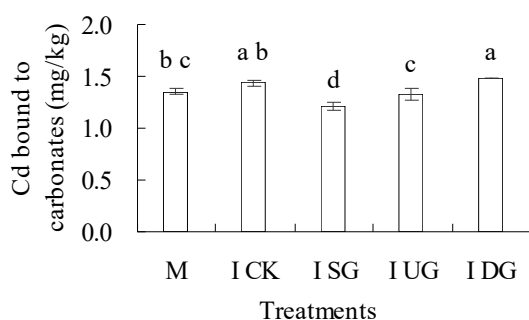


Figure 2 The content of Cd bound to carbonates

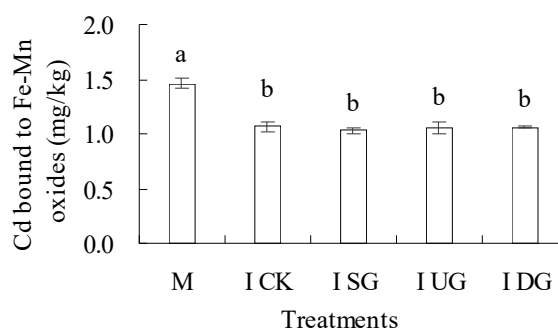


Figure 3 The content of Cd bound to Fe-Mn oxides

3.4. The content of cadmium bound to organic matter

The content of Cd bound to organic matter in soil for the treatments of I CK and I SG decreased, and it increased for the treatments of I UG and I DG, compared to the monoculture (Figure 4). The content of Cd bound to organic matter for all the intercropping treatments was not significantly different from the monoculture except the treatment of I DG. The content of Cd bound to organic matter for the treatment of I DG increased by 18% ($p < 0.05$) compared to the monoculture.

3.5. The content of residual cadmium

Compared to the monoculture, the treatments of I CK and I DG increased the content of residual Cd in soil, and the treatments of I SG and I UG decreased the content of residual Cd (Figure 5). The content of residual Cd for the treatments of I CK and I DG increased by 16% ($p > 0.05$) and 52% ($p < 0.05$) compared to the monoculture. The content of residual Cd for the treatments of I SG and I UG decreased by 4% ($p > 0.05$) and 7% ($p > 0.05$) compared to the monoculture.

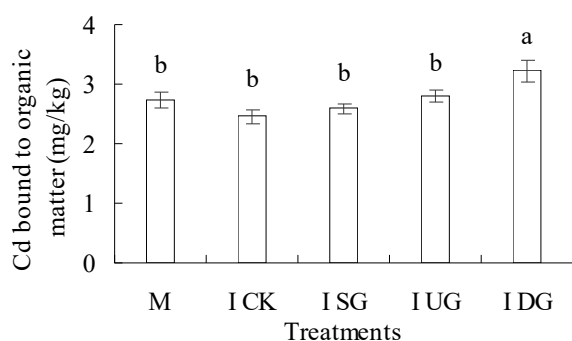


Figure 4 The content of Cd bound to organic matter

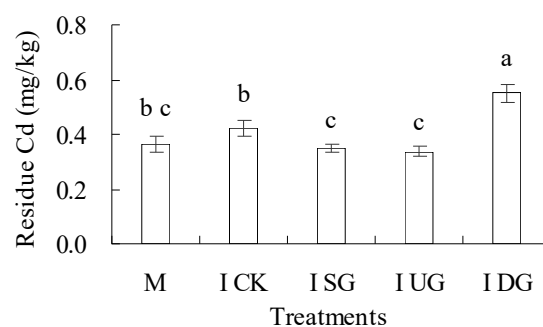


Figure 5 The content of residual Cd

4. Conclusions

In the experiment, intercropping with different post-grafting generations of *I. balsamina* had different effects on the fractions of Cd content in soil. Intercropping increased the content of exchangeable Cd significantly and the exchangeable Cd content for the treatment of I CK, I SG, I UG and I DG increased by 18% ($p < 0.05$), 12% ($p < 0.05$), 29% ($p < 0.05$) and 19% ($p < 0.05$), compared to the monoculture, respectively. The treatment of I DG significantly increased the content of Cd bound to carbonates (9%, $p < 0.05$), and the treatment of I SG significantly decreased it (11%, $p < 0.05$). The contents of Cd bound to Fe-Mn oxides for all the intercropping treatments decreased and that for the treatment of I SG reached lowest, with 29% ($p < 0.05$) lower than the monoculture. The content of Cd bound to organic matter and residual Cd for the treatment of I DG was 18% ($p < 0.05$) and 52% ($p < 0.05$) higher than the monoculture. In conclusion, although some intercropping treatments increased the content of residual Cd, it also significantly increased the content of exchangeable Cd in soil. Therefore, grape seedlings were not suitable for intercropping with post-grafting generations of *I. balsamina*.

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