Effects of Grape Seedlings Intercropping with Hyperaccumulators on Different Fractions of Cadmium Content in Soil

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Keywords: Intercropping; Hyperaccumulators; Cadmium fractions; Grape seedlings
Abstract: In order to study the effects of grape seedlings intercropping with hyperaccumulators on five forms of cadmium in soil, a pot experiment was conducted to to study the effects of grape seedlings intercropping with *Galinsoga parviflora*, *Siegesbeckia orientalis*, *Solanum nigrum* and *Crassocephalum crepidioides*, respectively on exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter and residual cadmium contents. The results showed that compared with monoculture, only grape intercropping with *G. parviflora* could significantly increase the content of residual Cd, which is conducive to the fixation of Cd in the soil and may reduce the plant's enrichment of Cd. Therefore, grape seedlings intercropping with *G. parviflora* could benefit for the decline of bioavailability of Cd.

Introduction

Cadmium (Cd) is a harmful heavy metal element in the environment and is a dangerous environmental pollutant [1]. Studies have shown that the content of Cd in crops is not only related to the total Cd content of the soil, but also depends on the different chemical forms of Cd in the soil [2]. Cd in soil can be divided into five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter and residual according to Tessier [3]. Exchange and residual Cd content directly determine the amount of Cd accumulation in plants. And the bioavailability of Cd bound to carbonates, Cd bound to Fe-Mn oxides and Cd bound to organic matter are in dynamic equilibrium with the exchangeable state, and continuous supply source for exchangeable Cd [4]. Therefore, different forms of Cd in soil are the key factors to explore the Cd accumulation ability of plants. The study found that intercropping Cd hyperaccumulator with cash crop can effectively prevent and control the absorption of Cd by economic crop, which can be considered as an effective measure to improve food safety under Cd stress [5]. Grape (*Vitis vinifera* L.) is a worldwide fruit rich in vitamins, amino acids, minerals and glucose, which is a fruit that people love and eat for a long time, and is also used as medicine [6]. However, due to the serious pollution of Cd in orchards in recent years, it has affected the safe production of grape to some extent. Screening suitable hyperaccumulator to intercrop with grape may affect the Cd accumulation of grape by changing the form of Cd in the soil. In this study, four hyperaccumulators (*Galinsoga parviflora* [7], *Siegesbeckia orientalis* [8], *Solanum nigrum* [9] and *Crassocephalum crepidioides* [10]) were used to intercrop with grape seedlings under Cd stress, and the fractions of Cd in soil were measured. The aim of the study was to screen the best intercropping mode for reducing the bioavailability of Cd.

Materials and Methods

Materials collection. The seeds of four hyperaccumulators (*G. parviflora, S. orientalis, S. nigrum, C. crepidioides*) and the fluvo-aquic soil samples were collected from the farmland surrounding Chengdu Campus of Sichuan Agricultural University in April, 2016. The cultivar of grape is Kyoho with cutting seedlings. And the seeds of hyperaccumulators were sown in 25°C climate chamber in April, 2016.

Experimental Design. The experiment was conducted in Chengdu Campus of Sichuan Agricultural University from April to July 2016. In April, the soil was air-dried, ground and passed through a 6.72-mm sieve. Each plastic pot (21 cm high, 20 cm in diameter) was filled with 3kg of ground soil mixed with 5 mg/kg Cd in solution (in the form of $CdCl_2 \cdot 2.5H_2O$) for 4 weeks. In May 2016, three uniform seedlings (the shoots were about 15 cm) of grape were transplanted into each pot for monoculture and two of them for intercropping, respectively. Then one uniform seedling of each hyperaccumulator with two pairs of true leaves was transplanted into each pot for intercropping. The experiment consists of 5 treatments: monoculture of grape, grape intercropping with *G. parviflora*, grape intercropping with *S. orientalis*, grape intercropping with *S. nigrum* and grape intercropping with *C. crepidioides*. Three replicates were run for each treatment, and the experiment pots were arranged in a completely randomized design. After 60 days, the soil from the rhizosphere in the corresponding pot was collected immediately 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. Soil samples (1.0 g) were digested with 5:1 (v:v) HNO_3:$HClO_4$ and measured by novAA 400P flame atomic absorption spectrophotometer (Analytik Jena, Germany) [11].

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.

Results and Discussion

The Content of Exchangeable Cadmium. For the content of exchangeable Cd in soil, compared to monoculture, only intercropping with *S. orientalis* significantly increased the content of exchangeable Cd in soil (Figure 1). Other treatments all had no significant effects on the content of exchangeable Cd ($P > 0.05$).

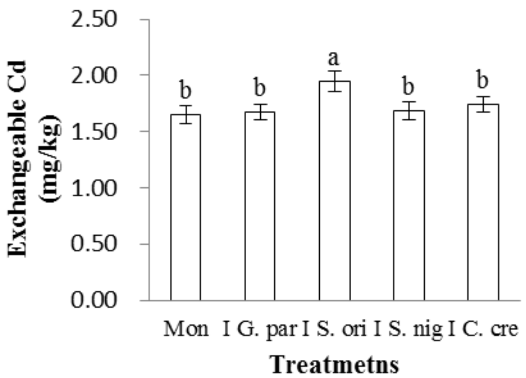

Fig. 1 The content of exchangeable Cd in soil. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I G. par = intercropping with *G. parviflora*, I S. ori = intercropping with *S. orientalis*, I S. nig = intercropping with *S. nigrum*, I C. cre = intercropping with *C. crepidioides*.

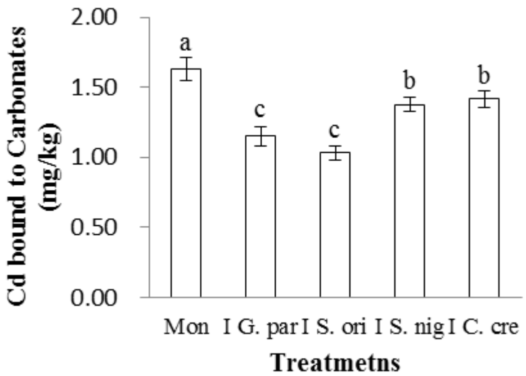

Fig. 2 The content of Cd bound to carbonates in soil. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I G. par = intercropping with *G. parviflora*, I S. ori = intercropping with *S. orientalis*, I S. nig = intercropping with *S. nigrum*, I C. cre = intercropping with *C. crepidioides*.

The Content of Cadmium Bound to Carbonates. For the content of Cd bound to carbonates in soil, compared to monoculture, intercropping with hyperaccumulators all significantly reduced the Cd bound to carbonates content in soil ($P < 0.05$). And the order of Cd bound to carbonates content from large to small was ranked: Monoculture, intercropping with *C. crepidioides*, intercropping with *S. nigrum*, intercropping with *G. parviflora*, intercropping with *S. orientalis* (Figure 2).

The Content of Cadmium Bound to Fe-Mn Oxides. For the content of Cd bound to Fe-Mn oxides in soil, compared to monoculture, only intercropping with *G. parviflora* and intercropping with *S. nigrum* significantly increased the content of Cd bound to Fe-Mn in soil (Figure 3). Other treatments all had no significant effects on the Cd bound to Fe-Mn oxides content (***P*** > 0.05).

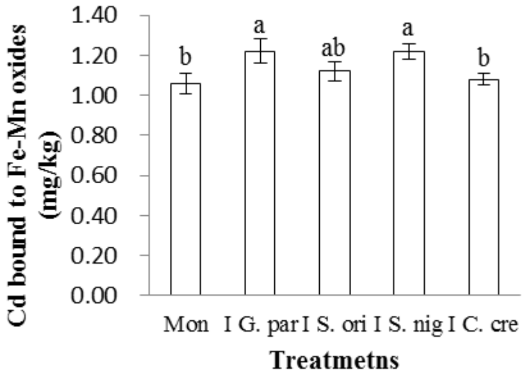

Fig. 3 The content of Cd bound to Fe-Mn oxides in soil. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I G. par = intercropping with *G. parviflora*, I S. ori = intercropping with *S. orientalis*, I S. nig = intercropping with *S. nigrum*, I C. cre = intercropping with *C. crepidioides*.

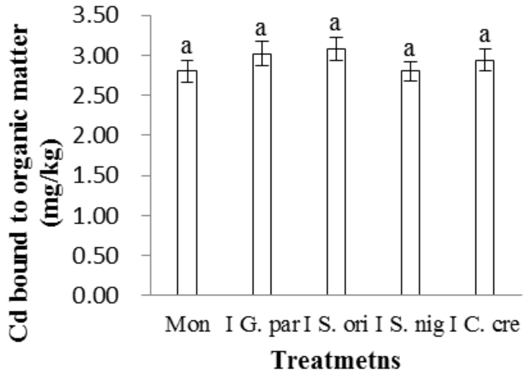

Fig. 4 The content of Cd bound to organic matter in soil. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I G. par = intercropping with *G. parviflora*, I S. ori = intercropping with *S. orientalis*, I S. nig = intercropping with *S. nigrum*, I C. cre = intercropping with *C. crepidioides*.

The Content of Cadmium Bound to Organic Matter. For the content of Cd bound to organic matter in soil, compared to monoculture, intercropping with hyperaccumulators had no significant effects on Cd bound to organic matter content in soil (Figure 4).

The Content of Residual Cadmium. For the content of residual Cd in soil, compared to monoculture, only intercropping with *G. parviflora* significantly increased the content of residual Cd in soil (Figure 5). Other treatments all significantly reduced content of residual Cd in soil (*P*< 0.05). And the order of residual Cd content from large to small was ranked: intercropping with *G. parviflora*, Monoculture, intercropping with *C. crepidioides*, intercropping with *S. orientalis*, intercropping with *S. nigrum*.

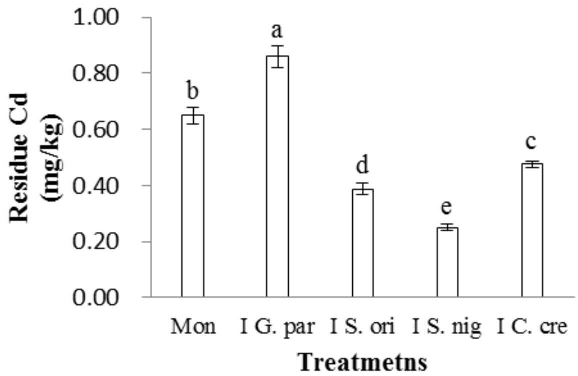

Fig. 5 The content of residual Cd in soil.Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I G. par = intercropping with *G. parviflora*, I S. ori = intercropping with *S. orientalis*, I S. nig = intercropping with *S. nigrum*, I C. cre = intercropping with *C. crepidioides*.

Conclusions

In the experiment, intercropping with different hyperaccumulators had different effects on the fractions of Cd in soil. For the content of Cd bound to organic matter in soil, intercropping with hyperaccumulators had no significant effects on it. And intercropping with hyperaccumulators all significantly reduced the content of Cd bound to carbonates in soil. Compared with monoculture, only intercropping with *S. orientalis* significantly increased the content of exchangeable Cd in soil. Other treatments had little effects on the bioavailability of soil Cd. And only intercropping with *G. parviflora* significantly increased the content of residual Cd, while other treatments significantly reduced its content. In addition, intercropping with *G. parviflora*, intercropping with *S. orientalis* and intercropping with *S. nigrum* increased the content of Cd bound to Fe-Mn oxides in soil to varying degrees. These results indicated that grape seedlings intercropping with *S. orientalis* would significantly increase the bioavailability of soil Cd, which may increase the risk of Cd uptake by grape seedlings and is not conducive to the safe production of grape. And grape seedlings intercropping with *G. parviflora* could significantly increase the content of residual Cd, which is conducive to the fixation of Cd in the soil and may reduce the plant's enrichment of Cd. Therefore, grape seedlings intercropping with *G. parviflora* could benefit for the decline of bioavailability of Cd.

Acknowledgements

This work was financially supported by the Application Infrastructure Project of Science and Technology Department of Sichuan Province (2016JY0258).

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