Effects of intercropping with post-grafting generation of *Cosmos sulphureus* on cadmium accumulation of grape seedlings

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Abstract. The pot experiment was carried out to study the effects of intercropping with post-grafting generation of *Cosmos sulphureus* on the cadmium accumulation of grape seedlings under cadmium stress. *C. sulphureus* which non-grafted (the offspring is named *C. sulphureus* S), self-rooted grafting by the same plant seedlings (the offspring is named *C. sulphureus* D), self-rooted grafting by two uniform plant seedlings (the offspring is named *C. sulphureus* T), and self-rooted grafting by two different development stages of plant seedlings (the offspring is named *C. sulphureus* Y). The results showed that intercropping significantly reduced the soil pH and increased the available cadmium content of the soil. Compared with grape monoculture, intercropping significantly reduced the cadmium content of grape seedlings roots. Among all treatments, grape intercropping *C. sulphureus* Y maximized the biomass of grape seedlings and the cadmium accumulation of the post-grafting generation of *C. sulphureus*, and the cadmium content of the grape seedlings shoots was significantly lower than other treatments.

1. Introduction

Grape (*Vitis vinifera* L.) is one of the most important fruit trees in China and the development of fruit industry is fast in recently [1]. Cadmium pollution is increasingly serious and greatly threatens the crop and quality of grape in some vineyards [2]. Cadmium accumulator and hyperaccumulator can be used for remediation of heavy metals-contaminated soil [3], under cadmium stress, grape intercropping with accumulator and hyper-accumulator can effectively reduce the cadmium content of grape seedlings, which provides a reference for the safe cultivation of vineyards [4]. However, the majority of the known hyperaccumulators of heavy metals or metalloids are low-biomass plants [5].

Grafting may play in further improving cadmium phytoextraction, because grafting significantly affects the growth and development of hyperaccumulator and their offspring, as well as the ability of heavy metal accumulation [6-7]. *Cosmos sulphureus* is a accumulator with strong cadmium absorption [8], our previous studies found that different grafting with different rootstocks promoted the growth and stress resistance of the offspring of *C. sulphureus* [9], and these offspring of *C. sulphureus* which intercropping with grape may affect the cadmium accumulation ability of grape. Therefore, the effects of intercropping with post-grafting generation of *C. sulphureus* on the cadmium accumulation of grape seedlings under cadmium stress were studied.
2. Materials and methods

2.1 Materials
In December 2015, one-year-old grape cutting seedlings (Kyoho) were collected in Chongzhou Modern Agricultural Research & Development Base of Sichuan Agricultural University (30°55′N, 103°49′E), and stored by sand for reserve. The *C. sulphureus* seeds were collected from a same flower with yellow and double-petaled. The soil for the experiment is fluvo-aquic soil, no cadmium was detected. And the *C. sulphureus* seeds and soil all collected from the surrounding farmland at Chengdu campus of Sichuan Agricultural University (30°42′N, 103°50′E). The soil was air-dried and crushed, weigh 2.5kg soil and loaded into pots of 21 cm x 20 cm (height × diameter). Add analytical pure CdCl2·2.5H2O to the soil, and make its cadmium concentration 5 mg kg⁻¹.

2.2 Experimental design
Grafting treatment: in April 2015, *C. sulphureus* seeds were germinated at twice (2 weeks between the first germination and the secondgermination), when the seedling height was about 3 cm, 2 true leaves were expanded, which were transplanted to the cadmium-free soil for growth. When the first batch of seedlings were about 10 cm high (the second batch of seedlings were about 5 cm higher), grafting treatment was carried out, reference [9], treatments as follow: *C. sulphureus* which non-grafted (the offspring is named *C. sulphureus* S), self-rooted grafting by the same plant seedlings (the offspring is named *C. sulphureus* D), self-rooted grafting by two uniform plant seedlings (the offspring is named *C. sulphureus* T), and self-rooted grafting by two different development stages of plant seedlings (the offspring is named *C. sulphureus* Y). Then, the seeds of post-grafting generation of *C. sulphureous* were collected for preservation.

Intercropping treatments: in February 2016, the grape seedlings were raised in the greenhouse. After grape seedlings put forth new roots, they were transplanted to the cadmium-free soil for growth. In April 2016, the seeds of post-grafting generation of *C. sulphureous* were seeded in perlite to promote germination, when the seedlings of *C. sulphureous* were about 3 cm and 2 true leaves were expanded, intercropping with grape seedlings under cadmium stress (cadmium concentration 5 mg kg⁻¹ [10]). Intercropping treatments as follow: grape monoculture (MG), grape intercropping with *C. sulphureus* S (grape/ *C. sulphureus* S), grape intercropping with self-rooted grafting by the same plant seedlings (grape/ *C. sulphureus* D), grape intercropping with self-rooted grafting by two uniform plant seedlings (grape/ *C. sulphureus* T), and grape intercropping with self-rooted grafting by two different development stages of plant seedlings (grape/ *C. sulphureus* Y). Repeat 3 times for each process treatment, and before harvest, soil moisture content is maintained at 80% of field water capacity.

In June 2016, grape and *C. sulphureus* was harvested. After washing with tap water, the plants were rinsed repeatedly with deionized water, blanched at 110 °C for 15 min, dried at 75 °C until reaching a constant weight, and weighed. The content of cadmium in root, stem, leaf, root and aboveground part of grape and *C. sulphureous* seedlings was determined [11]. The soil at the contact site of the roots of the two plants was collected, dried, crushed and mixed, passed through a 1-mm sieve, and the pH value was measured, soil available cadmium content[11].

2.3 Statistical analysis
Use Microsoft Excel 2010 and SPSS 13.0 software for statistical analysis, data analysis by one-way ANOVA with least significant difference at 5% confidence level. Translocation factor = the cadmium content of shoots/ the cadmium content of roots [9].

3. Results and discussion

3.1 Cadmium content of grape seedlings
Under cadmium stress, intercropping decreased cadmium content in grape seedlings compared to MG (Table 1). Intercropping with *C. sulphureus* T and *C. sulphureus* Y, cadmium content in grape roots decreased by 27.82% (*P < 0.05*) and 31.99% (*P < 0.05*) compared to MG, respectively.
Intercropping with \textit{C. sulphureus} \textit{Y}, cadmium content in grape stems, leaves and shoots are the lowest, decreased by 73.49\% \textit{(P < 0.05)}, 81.74\% \textit{(P < 0.05)} and 78.92\% \textit{(P < 0.05)} compared to MG, respectively. In addition, intercropping with post-grafting generation of \textit{C. sulphureus} also decreased the translocation factor of grape seedlings, and intercropping with \textit{C. sulphureus} \textit{Y}, which got the lowest.

### Table 1. Cadmium content of grape seedlings.

| Treatments            | Roots (mg kg\(^{-1}\)) | Stems (mg kg\(^{-1}\)) | Leaves (mg kg\(^{-1}\)) | Shoots (mg kg\(^{-1}\)) | Translocation factor |
|-----------------------|-------------------------|-------------------------|--------------------------|--------------------------|----------------------|
| MG                    | 44.76±7.21a             | 0.464±1.15a             | 0.438±1.14a              | 0.446±0.88a              | 0.010                |
| grape/C. sulphurous S | 36.72±3.34ab            | 0.358±0.45b             | 0.313±1.02b              | 0.326±0.46b              | 0.009                |
| grape/C. sulphurous D | 36.17±4.48ab            | 0.276±0.24c             | 0.236±0.61c              | 0.248±0.39c              | 0.007                |
| grape/C. sulphurous T | 32.31±4.20b             | 0.173±0.44d             | 0.140±0.28d              | 0.149±0.27d              | 0.005                |
| grape/C. sulphurous Y | 30.44±3.47b             | 0.123±0.18d             | 0.080±0.15c              | 0.094±0.16e              | 0.003                |

Values are mean ± SD \textit{(n = 3)}. Different lowercase letters indicated significant differences among treatments at 0.05 levels, and the same below.

#### 3.2 Biomass and cadmium content on post-grafting generation of \textit{C. sulphureus}

The effect of intercropping on post-grafting generation of \textit{C. sulphureus} biomass and cadmium content were different (Table 2). The roots biomass of \textit{C. sulphureus} \textit{Y} and \textit{C. sulphureus} \textit{T} were significant higher than of \textit{C. sulphureus} \textit{D} and \textit{C. sulphureus} \textit{S}, and shoots biomass of \textit{C. sulphureus} \textit{Y} was higher than others \textit{(P < 0.05)}. \textit{C. sulphureus} \textit{Y} roots cadmium content obviously was higher than others \textit{(P < 0.05)}. Also, \textit{C. sulphureus} \textit{Y} and \textit{C. sulphureus} \textit{T} had significantly higher cadmium accumulation than \textit{C. sulphureus} \textit{D} and \textit{C. sulphureus} \textit{S}. And roots and shoots cadmium accumulation reached the maximum in \textit{C. sulphureus} \textit{Y}, were 458.5 and 513.1 \textit{μg} plant\(^{-1}\), respectively.

### Table 2. Biomass and cadmium content on post-grafting generation of \textit{C. sulphureus}.

| Treatments            | Biomass \textit{(g plant\(^{-1}\))} | Cadmium content \textit{(mg kg\(^{-1}\))} | Cadmium accumulation \textit{(μg plant\(^{-1}\))} |
|-----------------------|-------------------------------------|------------------------------------------|-----------------------------------------------|
|                       | Roots | Shoots | Roots | Shoots | Roots | Shoots |
| grape/C. sulphureus S  | 3.32±0.30b       | 13.04±0.86b     | 32.09±3.36c       | 25.30±2.79a     | 106.1±11.4c   | 330.3±31.8bc |
| grape/C. sulphureus D  | 3.51±0.35b       | 10.47±0.62c     | 40.91±4.75b       | 27.14±4.33a     | 142.9±23.7c   | 284.6±28.4c  |
| grape/C. sulphureus T  | 7.66±0.41a       | 14.22±1.07b     | 42.08±3.92b       | 28.88±3.64a     | 323.1±26.9b   | 413.0±42.0b  |
| grape/C. sulphureus Y  | 8.33±0.62a       | 17.22±1.73a     | 55.26±6.54a       | 29.75±3.71a     | 458.5±37.5a   | 513.1±37.9a  |

#### 3.3 Soil pH and available cadmium content

Under cadmium stress, intercropping significantly lowered soil pH compared to MG (Table 3). Contrarily, intercropping significantly increased soil available cadmium content compared to MG, which was ranked in the following order: intercropping with \textit{C. sulphureus} \textit{Y} > intercropping with \textit{C. sulphureus} \textit{T} > intercropping with \textit{C. sulphureus} \textit{D} > intercropping with \textit{C. sulphureus} \textit{S} > MG.

### 4. Conclusions

In summary, grape intercropping with post-grafting generation of \textit{C. sulphureus} which can provide theoretical basis for reducing the heavy metal content of cash crops and enhancing the restoration efficiency of heavy metal enriched plant/common plant intercropping model.
Table 3. Soil pH and available cadmium content.

| Treatments       | pH      | Soil available cadmium (mg kg⁻¹) |
|------------------|---------|---------------------------------|
| MG               | 7.19±0.038a | 2.763±0.012d                   |
| grape/C. sulphurous S | 6.91±0.012b | 3.937±0.006c                   |
| grape/C. sulphurous D | 6.86±0.012b | 4.093±0.029b                   |
| grape/C. sulphurous T | 6.85±0.006b | 4.350±0.012a                   |
| grape/C. sulphurous Y | 6.84±0.017b | 4.405±0.012a                   |

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