Effects of Intercropping with Post-Grafting Generation of *Cosmos sulphureus* on Phosphorus Uptake of Grape Seedlings under Cadmium Stress

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Abstract. A pot experiment was conducted to study the effects of grape intercropping with Post-Grafting Generation of *Cosmos sulphureus* and monoculture on Total P content in grape roots, stems, leaves and available Phosphorus (P) content in soil under Cadmium (Cd) stress. The result showed that: All the intercropping patterns declined the contents of total P in grape roots and the contents of soil available P obviously. Intercropping with UG, PSG, PSDG and PSSG had different effects on the content of total P in grape stems and the contents of total P in grape leaves. Intercropping with PSSG increased the contents of total P in grape stems and leaves. Intercropping with UG and PSG decreased the contents of total P in grape stems and leaves. Therefore, intercropping with PSSG could increase grape P content under Cd stress.

1.Introduction

Grafting has important application value in overcoming soil-borne diseases, eliminating continuous cropping obstacles and improving plant yield, stress resistance and fruit quality [1]. In recent years, grafting has made some progress in the production and application of fruit trees and vegetables. Study has shown that different rootstock grafting can increase the biomass of the offspring of *Solanum nigrum* and promote the growth of the offspring of shepherd's pursel [2-3]. In agricultural production, cropping patterns such as intercropping can improve the effective use of soil nutrients, water and light by crops, thereby increasing crop yields and quality, preventing pests and diseases. Intercropping also affected the ability of heavy metals uptake and accumulation in plants [4-6]. Different intercropping combination could lead to different changes to the P uptake of plant under Cd stress. Therefore, this study investigated the effects of intercropping with Post-Grafting Generation of *C. sulphureus* on grape P then to screen post-grafting generation of *C. sulphureus* that can improve the grape P absorption.

2.Materials and methods

2.1 Plant materials
The seeds of the *C. sulphureus* used in this experiment were collected from the same yellow-flowered and double-petaled *C. sulphureus* in the farmland surrounding Chengdu Campus of Sichuan Agricultural University (30° 42′ N, 103° 50′ E) in October 2014. The cultivar of grape is Kyoho with cutting seedlings, and it was purchased from the nursery stock base in Longquanyi District, Chengdu City, Sichuan province, in May 2015.

2.2 Soil and heavy metals

The soil samples used in this experiment were collected from the farmland surrounding Chengdu Campus of Sichuan Agricultural University, pH 7.09, total nitrogen 1.50 g/kg, total phosphorus 0.76 g/kg, total potassium 18.02 g/kg, total cadmium 1.96 mg/kg, alkali nitrogen 94.82 mg/kg, available phosphorus 6.30 mg/kg, available potassium 149.59 mg/kg, Cd not detected. The basic physical and chemical properties of the soil and the determination of heavy metal Cd content are based on references [7]. Cd was used as a heavy metal for testing and it was added to the soil samples in the form of analytical pure CdCl₂·2.5H₂O solution according to design concentration.

2.3 Experimental design

In October 2014, the seeds were collected from the same flower of *C. sulphureus*, which were put in the climate chamber to germination, the interval between two grow seedlings was 2 weeks. Grafting treatment was carried out when the first of seedlings were about 10 cm higher (the second batches of seedlings were about 5 cm higher), the grafting treatment as follows: (1) Ungrafted: the seedlings of *C. sulphureus* transplanted directly, and the seeds were collected for preservation as the ungrafted generation of *C. sulphureus* (UG). (2) Self-rooted grafting by the same one seedling: the seedlings of *C. sulphureus* were cut off from 6 cm above the ground. The upper parts were scion and the lower parts were rootstock. Keep rootstock leaves. Scions and rootstocks were physiologically consistent and collected seeds for preservation as the post-grafting generation of self-rooted grafting by the same one seedling of *C. sulphureus* (PSG). (3) Self-rooted grafting by two different sizes seedlings: *C. sulphureus* seedlings were about 10 cm high, cut off from 6 cm above the ground, the lower parts were rootstock. The scions were cut the upper seedling (4 cm) from seedlings of *C. sulphureus* were about 5 cm high, and the leaves of rootstock were kept after grafting. There was a big difference between the scion and rootstock in physiology, and the seeds were collected for preservation as the post-grafting generation of self-rooted grafting by two different sizes seedlings of *C. sulphureus* (PSDG). (4) Self-rooted grafting by two same sizes seedlings: the two *C. sulphureus* seedlings were about 10 cm high, and one was cut off from 6 cm above the ground, kept the lower parts as rootstock, another was cut off from 6 cm above the ground, kept the upper parts as scion (4 cm). The leaves of rootstock were retained after grafting. Scion and rootstock were different in physiology, and collected seeds for preservation as the post-grafting generation of self-rooted grafting by two same sizes seedlings of *C. sulphureus* (PSSG).

In May 2015, the seeds in offspring of *C. sulphureus* that treated with different grafting technology were put in the climate chamber to germination and further cultivation. Then, the seedlings of *C. sulphureus* transplanted together with grape seedlings into pot which prepared with soil by 5 mg/kg Cd when the two true leaves expanded. Five treatments were applied in this experiment: grape monoculture, grape intercropping with UG, grape intercropping with PSG, grape intercropping with PSSG, grape intercropping with PSDG. One *C. sulphureus* seedling of different treatments and one grape seedling were transplanted into each pot. For each treatment with six replicates and the pots placed completely random. The distance between pots was 15 cm, and the pot position exchanged periodically to weaken the impact of the marginal effects. The soil moisture content was maintained at 80% of field capacity until the plants were harvested.

After 2 months, grape seedlings were harvested and divided into three parts of root, stem and leaf, then washed with tap water firstly, followed by deionized water for three times. Finally, weighed the fresh weight and then simmered for 15 min at 110 °C. After that, the tissues of all plants were dried at 80 °C until constant weight, and passed through a 100-mesh sieve to determine the content of
phosphorus different parts of the grape seedlings by Molybdenum antimony colorimetric method [7]. The available phosphorus content in soil by Molybdenum antimony colorimetric method [8].

2.4 Statistical analyses
Statistical analysis was carried out by using SPSS 18.0 statistical software. The data were analyzed by one-way ANOVA, with the least significant difference at the 5% confidence level.

3. Results and discussion

3.1 Total P content in grape roots
All the intercropping patterns declined the contents of total P in grape roots obviously (Figure 1). The contents of total P in grape roots of all the intercropping treatments were lower than monoculture but the difference did not reach significant levels except the treatment of intercropping with UG and PSDG, the contents of total P in roots of grape when grape intercropping with UG and PSDG were 14.97% and 27.89% (p < 0.05) lower than the monoculture, respectively.

3.2 Total P content in grape stems
Intercropping with UG, PSG, PSDG and PSSG had different effects on the contents of total P in grape stems (Figure 2). Intercropping with UG and PSG reduced the content of total P compared to monoculture of grape, and they were 35.48% and 1.61% (p < 0.05) lower than the monoculture, respectively. On the contrary, intercropping with PSDG and PSSG can increase the contents of total P. The contents of total P in the two intercropping patterns were 3.13% and 6.45% (p < 0.05) higher than the monoculture, respectively.

Figure 1. Total P content in grape roots

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). grape monoculture = MG, grape intercropping with the generation of ungrafted C. sulphureus = I.UG, grape intercropping with C. sulphureus of the post-grafting generation of self-rooted grafting by the same one seedling = I.PSG, grape intercropping with C. sulphureus of the post-grafting generation of self-rooted grafting by two different sizes seedlings = I.PSDG, grape intercropping with C. sulphureus of the post-grafting generation of self-rooted grafting by the same sizes seedlings = I.PSSG.
3.3 Total P content in grape leaves
The intercropping patterns decreased the contents of total P in grape leaves except the treatment of intercropping with PSSG (Figure 3), but the increase or decrease was not significant except the treatment of intercropping with PSDG. Sorting the contents of total P in grape leaves of each treatment from highest to lowest: I.PSSG > MG > I.UG > I.PSG > I.PSDG. In particular, the contents of total P in grape leaves for the treatment of intercropping with PSDG was 11.54% (p < 0.05) lower than the monoculture.

3.4 Available P content in soil
All the intercropping patterns declined the contents of soil available P (Figure 4) and the contents of soil available P from large to small was all ranked: MG, I.UG, I.PSG, I.PSDG, I.PSSG. The contents of soil available P for the treatments of intercropping with UG and PSG had no significance difference. On the contrary, the treatment of intercropping with PSDG and PSSG reduced the soil available P content obviously, and 16.27%, 10.74% (p < 0.05) lower than the monoculture, respectively.
Figure 3. Total P content in grape leaves
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). grape monoculture = MG, grape intercropping with the generation of ungrafted C. sulphureus = I.UG, grape intercropping with C. sulphureus of the post-grafting generation of self-rooted grafting by the same one seedling = I.PSG, grape intercropping with C. sulphureus of the post-grafting generation of self-rooted grafting by two different sizes seedlings = I.PSDG, grape intercropping with C. sulphureus of the post-grafting generation of self-rooted grafting by the same sizes seedlings = I.PSSG.

Figure 4. Soil available P content
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). grape monoculture = MG, grape intercropping with the generation of ungrafted C. sulphureus = I.UG, grape intercropping with C. sulphureus of the post-grafting generation of self-rooted grafting by the same one seedling = I.PSG, grape intercropping with C. sulphureus of the post-grafting generation of self-rooted grafting by two different sizes seedlings = I.PSDG, grape intercropping with C. sulphureus of the post-grafting generation of self-rooted grafting by the same sizes seedlings = I.PSSG.

4. Conclusions
According to the experiment, we obtained the following conclusions: All the intercropping patterns declined the contents of total P in grape roots and the contents of soil available P obviously. Intercropping with UG, PSG, PSDG and PSSG had different effects on the contents of total P in grape stems and the contents of total P in grape leaves. Intercropping with PSSG increased the contents of total P in grape stems obviously (6.45%) higher than the monoculture and the contents of total P in
grape leaves. Intercropping with UG and PSG decreased the contents of total P in grape stems and the contents of total P in grape leaves. Therefore, the treatment of intercropping with PSSG had certain effect on the increase of grape total P content under Cd stress.

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