Effects of Intercropping with Post-grafting Generation of *Cosmos sulphureus* on Nitrogen Uptake of Grape under Cadmium Stress

Hongqiang Chen¹,², Lijin Lin¹, Dan Xia³, Jin Wang¹ and Xiulan Lv¹, a

¹Rural Development and Forestry Bureau of Chengdu Longquan, Chengdu, Sichuan, China
²Institute of Pomology and Olericulture, Sichuan Agricultural University, Chengdu, Sichuan, China
³College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, China

a Corresponding author: 1933063229@qq.com.

Abstract. Pot experiments were conducted to study the effects of intercropping with post-grafting generation of *Cosmos sulphureus* on nitrogen (N) uptake of grape under cadmium (Cd) stress. The results showed that intercropping with post-grafting generation of *C. sulphureus* could reduce N absorption of grape compared with grape monoculture (MG) under Cd stress. As a whole, the N content in grape were ranked in the following order: MG > intercropping with the post-grafting generation of self-rooted grafting by two same sizes seedlings of *C. sulphureus* (PSSG) > intercropping the post-grafting generation of self-rooted grafting by two different sizes seedlings of *C. sulphureus* (PSDG) > intercropping with the post-grafting generation of self-rooted grafting by the same one seedling of *C. sulphureus* (PSG) > intercropping with the generation of ungrafted *C. sulphureus* (UG). Intercropping with post-grafting generation of *C. sulphureus* had no significant effect on the alkaline N content in soil compared with MG under Cd stress (P > 0.05). Intercropping with UG, PSG and PSDG increased alkaline N content in soil, while intercropping with PSSG decreased alkaline N content in soil. In conclusion, Grape intercropping with post-grafting generation of *C. sulphureus* could improve alkaline N content in soil as a whole, but which decreased N content in grape under Cd stress.

1. Introduction

Intercropping is a cropping system that is used to improve soil quality, and may promotes the fertilizer utilization efficiencies and increases the retention of inorganic nitrogen (N) in the soil, thereby enhancing plant growth [1-2]. Cadmium (Cd) is one of the toxic heavy metals which can be absorbed by plants and have detrimental effects on plant growth and influence the absorption and utilization of nutrient by plants [3-4]. Grapes are a popular fruit in the world, but under Cd stress, Cd reduces the N absorption of grape and inhibits the growth of grape [5-6]. *Cosmos sulphureus* is a Cd-accumulator with strong remediation ability [7]. Cd-accumulator as influenced by grafting on different rootstocks, which can increase Cd accumulation of Cd-accumulator and exhibit stable inheritance [8-9]. Intercropping with Cd-accumulator can reduce the Cd uptake [10], and promote the nutrients absorption by plants under Cd stress [11]. Therefore, the objective of this study was to evaluate the effect of intercropping post-grafting generation of *C. sulphureus* on N absorption of grape under Cd
stress.

2. Materials and methods

2.1 Materials

In October 2014, the seeds of the *C. sulphureus* were collected from a same flower which was yellow and double-petaled in the surrounding farmland at Chengdu campus of Sichuan Agricultural University (30°42′N, 103°50′E). The species of the experimental cultivar of grape is Kyoho with cutting seedlings were purchased from Longquanyi area seedlings base of Chengdu in May 2015. The soil for the experiment was collected from the surrounding farmland at Chengdu campus of Sichuan Agricultural University. The basic physical and chemical characteristics of soil: pH 7.09, total N 1.50 g/kg, total phosphorus 0.76 g/kg, total potassium 18.02 g/kg, total Cd 1.96 mg/kg, alkali N 94.82 mg/kg, available phosphorus 6.30 mg/kg, available potassium 149.59 mg/kg, Cd was not detected. The basic physical and chemical properties of the soil and the determination of heavy metal Cd content were based on references [12]. Cd was used as a heavy metal for testing and it was added to the soil samples in the form of analytical pure CdCl$_2$·2.5H$_2$O solution according to design concentration.

2.2 Experimental design

In October 2014, the seeds were collected from a same flower of *C. sulphureus* which were put in the climate chamber to germinate, and the interval between two seedlings was 2 weeks. Grafting treatment was carried out when the first batch of seedlings were about 10 cm higher (the second batch of seedlings were about 5 cm higher). The grafting treatments as follow: (1) Ungrafted: the seedlings of *C. sulphureus* transplanted directly, and the seeds were collected for preservation as the generation of ungrafted *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. Rootstock leaves were retained. Scions and rootstocks were physiologically consistent, 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 retained after grafting. There was a big difference between the scion and rootstock in physiology, 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 *C. sulphureus* 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). The leaves of rootstock were retained after grafting. Scion and rootstock were different in physiology, 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 of post-grafting generation of *C. sulphureus* were put in the climate chamber to germinate and further cultivation. Then, the seedlings of *C. sulphureus* transplanted together with grape seedlings into a pot which prepared with soil by 5 mg/kg Cd when the two true leaves expanded. There were five treatments: grape monoculture, grape intercropping with UG, grape intercropping with PSG, grape intercropping with PSDG, grape intercropping with PSSG. Each *C. sulphureus* seedling of different treatments and each grape seedling were transplanted into a 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 (July 2015), grape seedlings were harvested, roots, stems and leaves were separated, 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, weighed and passed through a 100-mesh sieve, and the N content of the roots, stems and leaves were determined [13]. Then collect the soil, air dry, crush, measured the soil alkaline N [12].

2.3 Statistical analyses
Statistical analysis was carried out by 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 N content in grape roots
Under Cd stress, intercropping with post-grafting generation of C. sulphureus decreased N content in grape roots compared with MG (Figure 1). Grape intercropping with PSSG, N content in grape roots decreased (p > 0.05), which decreased by 6.08% compared with MG, while, intercropping with UG, PSG and PSDG, N content in grape roots significantly decreased (p < 0.05). N content in grape roots were ranked in the following order: MG > intercropping PSSG > intercropping PSG > intercropping PSDG > intercropping UG.

![Figure 1. N content in grape roots](image)

The same letters within each column are not significantly different at p < 0.05. MG = Grape monoculture, I. UG = intercropping with the generation of ungrafted C. sulphureus, I. PSG = intercropping with the post-grafting generation of self-rooted grafting by the same one seedling of C. sulphureus, I. PSDG = intercropping with the post-grafting generation of self-rooted grafting by two different sizes seedlings of C. sulphureus, I. PSSG = intercropping with the post-grafting generation of self-rooted grafting by two same sizes seedlings of C. sulphureus.
3.2 N content in grape stems
Under Cd stress, intercropping with post-grafting generation of *C. sulphureus* decreased N content in grape stems compared with MG (Figure. 2). Grape intercropping with PSSG, N content in grape stems decreased by 7.67% (p > 0.05), intercropping with UG, PSG and PSDG, N content in grape stems significantly decreased by 32.46%, 8.31%, 52.34% (p < 0.05) compared with MG, respectively. N content in grape stems were ranked in the following order: MG > intercropping PSSG > intercropping PSG > intercropping UG > intercropping PSDG.

The same letters within each column are not significantly different at p < 0.05. MG = Grape monoculture, I. UG = intercropping with the generation of ungrafted *C. sulphureus*, I. PSG = intercropping with the post-grafting generation of self-rooted grafting by the same one seedling of *C. sulphureus*, I. PSDG = intercropping with the post-grafting generation of self-rooted grafting by two different sizes seedlings of *C. sulphureus*, I. PSSG = intercropping with the post-grafting generation of self-rooted grafting by two same sizes seedlings of *C. sulphureus*.
The same letters within each column are not significantly different at p < 0.05. MG = Grape monoculture, I. UG = intercropping with the generation of ungrafted *C. sulphureus*, I. PSG = intercropping with the post-grafting generation of self-rooted grafting by the same one seedling of *C. sulphureus*, I. PSDG = intercropping with the post-grafting generation of self-rooted grafting by two different sizes seedlings of *C. sulphureus*, I. PSSG = intercropping with the post-grafting generation of self-rooted grafting by two same sizes seedlings of *C. sulphureus*.

### 3.3 N content in grape leaves

Under Cd stress, intercropping with post-grafting generation of *C. sulphureus* significantly decreased N content in grape leaves compared with MG (Figure. 3). Intercropping with UG, PSG, PSDG and PSSG, N content in grape leaves significantly decreased by 22.07%, 14.76%, 19.05% and 11.87% (p < 0.05) compared with MG, respectively. In addition, the difference between each treatment was extremely significant, the distribution of N content in Grape: MG > intercropping PSSG > intercropping PSG > intercropping PSDG > intercropping UG.

### 3.4 Alkaline N content in soil

Under Cd stress, Grape intercropping with UG, PSG and PSDG increased alkaline N content in soil, which increased by 4.66%, 1.19%, 0.50% compared with MG, respectively. Intercropping with SSG1 decreased alkaline N content in soil by 4.33% compared with MG (Figure. 4). The alkaline N content in soil was ranked in the following order: MG > intercropping PSSG > intercropping PSG > intercropping PSDG > intercropping UG.
Figure 4. Alkaline N content in soil

The same letters within each column are not significantly different at p < 0.05. MG = Grape monoculture, I. UG = intercropping with the generation of ungrafted C. sulphureus, I. PSG = intercropping with the post-grafting generation of self-rooted grafting by the same one seedling of C. sulphureus, I.PSDG = intercropping with the post-grafting generation of self-rooted grafting by two different sizes seedlings of C. sulphureus, I. PSSG = intercropping with the post-grafting generation of self-rooted grafting by two same sizes seedlings of C. sulphureus.

4. Conclusions
Under Cd stress, intercropping with post-grafting generation of C. sulphureus decreased N content in grape roots, stems and leaves compared with MG. In PSSG, the decrease of N content in grapes decreased less than in UG, PSG and PSDG. Grape intercropping with UG, PSG and PSDG increased alkaline N content in soil, intercropping with PSSG decreased alkaline N content in soil compared with MG under Cd stress. In conclusion, intercropping with post-grafting generation of C. sulphureus could improve alkaline N content in soil as a whole, but which decreased N content in grape under Cd stress. Therefore, under Cd stress, intercropping with PSSG had little effect on the N absorption by grape. Under Cd stress, grape intercropping with post-grafting generation of C. sulphureus was not the rational intercropping combination.

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