

Effects of Intercropping with Post-Grafting Generation of *Impatiens balsamina* on Nitrogen Uptake of Grape Seedlings under Cadmium Stress

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Abstract. A pot experiment was conducted to study the effects of intercropping with post-grafting generation of *Impatiens balsamina* on nitrogen content in each part of grape seedlings and the alkaline nitrogen content in soil. Results showed that under cadmium stress, intercropping with post-grafting generation of *I. balsamina* (intercropping with *I. balsamina* of non-grafting, intercropping with post-grafting generation of self-rooted grafting by the same one seedlings of *I. balsamina*, intercropping with post-grafting generation of self-rooted grafting by the two same sizes seedlings of *I. balsamina*, intercropping with post-grafting generation of self-rooted grafting by the two different sizes seedlings of *I. balsamina*) reduced the nitrogen content in each part of the grape seedlings compared with monoculture. But all the intercropping treatment increased the soil alkaline nitrogen content compared with monoculture. In conclusion, intercropping with post-grafting generation of *I. balsamina* reduced the nitrogen content of grape seedlings and increasing the soil alkaline nitrogen content.

1.Introduction

Cadmium is one of the most toxic heavy metal which will endanger the growth and development of plants and ultimately affect human health [1]. In agricultural production, intercropping can not only increase the yield of plants, reduce the occurrence of pests and diseases, but also improve the soil environment and increase the uptake of nutrients by plants [2-3]. Grapes are a popular fruit in the world, but under Cadmium stress, Cadmium reduces the nitrogen absorption of grape and inhibits the growth of grape [4-5]. *Impatiens balsam* is a cadmium enriched flower plant, which is weak in restoring cadmium contaminated soil [6]. Grafting is a common agronomic and studies have shown that grafting leads to changes in DNA methylation, produces reversible genetic changes in genomic function, and may have morphological, physiological, and ecological consequences [7-8]. At present, there are few reports about the influence of the post-grafting generation of *Impatiens Balsamina* on grape growth. Therefore, in this experiment, intercropping with post-grafting generations of *I. balsamina* (intercropping with *I. balsamina* of non-grafting, intercropping with post-grafting generation of self-rooted grafting by the same one seedlings of *I. balsamina*, intercropping with post-grafting generation of self-rooted grafting by the two same sizes seedlings of *I. balsamina*,



intercropping with post-grafting generation of self-rooted grafting by the two different sizes seedlings of *I. balsamina*) were used to study the effects of post-grafting generation on nitrogen content of grape under cadmium stress.

2. Materials and methods

2.1 Materials

In October 2014, the seeds of a yellow and double petal of *I. balsamina* was collected from 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 which 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, 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, alkaline nitrogen 94.82 mg/kg, available phosphorus 6.30 mg/kg, available potassium 149.59 mg/kg, cadmium not detected. The basic physical and chemical properties of the soil and the determination of heavy metal content were based on references [9]. Cadmium was used as a heavy metal for testing and it was added to the soil samples in the form of analytical pure $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$ solution according to design concentration.

2.2 Experimental design

In April 2015, the soil was air-dried, ground and passed through a 6.72 mm (3 meshes). Each plastic pot (20 cm high, 21 cm in diameter) was filled with 3 kg of ground soil. According to the designed concentration, cadmium was added to the soil in the form of $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$ analytical purity and mixed, resulting in a cadmium concentration of 5 mg/kg keep the soil moist, put it in balance naturally for 4 weeks, and mix it irregularly to ensure that the soil is fully mixed evenly.

In October 2014, the seeds were collected from the same flower of *I. balsamina* which were put in the climate chamber to germination, 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 specific grafting method is as follows: (1) Non-grafting: the seedlings of *I. balsamina* transplanted directly, and the seeds were collected for preservation as the non-grafted generation of *I. balsamina* (UG). (2) Self-rooted grafting by the same one seedling: the seedlings of *I. balsamina* seedlings were about 10 cm high, which were cut off from 6 cm above the ground and divided into two parts. Take the upper parts were scion and the lower parts were rootstock for grafting, and keeping the 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 *I. balsamina* (SG). (3) Self-rooted grafting by two same sizes seedlings: the *I. balsamina* seedlings were about 10 cm high, and cut off from 6 cm above the ground and divided into two parts, and the lower parts was taken as rootstock, scion was a upper seedling (4 cm) of another *I. balsamina* with a height of 10 cm, and the leaves of rootstock were retained after grafting. Scion and rootstocks were different in physiology, and collected seeds for preservation as the post-grafting generation of self-rooted grafting by two same sizes seedlings of *I. balsamina* (SSG). (4) Self-rooted grafting by two different sizes seedlings: the *I. balsamina* seedlings were about 10 cm high, and cut off from 6 cm above the ground and divided into two parts, and the lower parts was taken as rootstock, scion was a upper seedling (4 cm) of another *I. balsamina* with a height of 5 cm, and the leaves of rootstock were retained after grafting. Scion and rootstocks were quite different in physiology, and collected seeds for preservation as the post-grafting generation of self-rooted grafting by two same sizes seedlings of *I. balsamina* (SDG).

In May 2015, the seeds in offspring of *I. balsamina* that treated with different grafting technology were put in the climate chamber to germination and further cultivation. Then, the seedlings of *I. balsamina* transplanted together with grape seedlings into pot which prepared with soil by 5 mg/kg cadmium when the two true leaves expanded. There are five treatments: monoculture of grape (MG),

grape intercropping with UG, grape intercropping with SG, grape intercropping with SSG, grape intercropping with SDG. One *I. balsamina* 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 dug up 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, weighed, passed through a 100-mesh sieve. The nitrogen content of the leaves was determined by H₂SO₄-H₂O₂ heating digestion and Nessler calorimetry method [10], alkaline nitrogen was determined by alkali diffusion method [9].

2.3 Statistical analyses

Statistical analyses were performed with SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA). Data were analyzed with one-way analysis of variance with least significant difference at the 5% significance level.

3. Results and discussion

3.1 Nitrogen content in grape roots

Under cadmium stress, the nitrogen content in roots of grape intercropping with post-grafting generations of *I. balsamina* was significantly lower than monoculture ($p < 0.05$, Figure 1). The nitrogen content of the grape roots was ranked as following order: MG (monoculture) > intercropping with SSG > intercropping with UG > intercropping with SDG > intercropping with SG, and the nitrogen content in roots of grape intercropping with UG, SG, SSG and SDG decreased by 21.63% ($p < 0.05$), 31.74% ($p < 0.05$), 17.32% ($p < 0.05$) and 22.47% ($p < 0.05$) compared with the monoculture.

3.2 Nitrogen content in grape stems

Under cadmium stress, the nitrogen content in stems of grape intercropping with UG, SG, SSG and SDG were significantly lower than that of the monoculture ($p < 0.05$, Figure 2). The nitrogen content of the grape stems was ranked as following order: MG (monoculture) > intercropping with SDG > intercropping with SG > intercropping with UG > intercropping with SSG. And among all intercropping treatments, the highest nitrogen content in stems of grape was the treatment of intercropping with SDG.

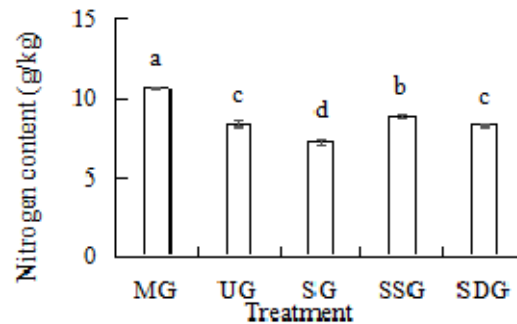


Figure 1. Nitrogen content in grape roots.

Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 22.0 followed by the least significant difference test ($p < 0.05$). MG = monoculture of grape, UG = intercropping with *I. balsamina* of non-grafting, SG = intercropping with post-grafting generation of self-rooted grafting by the same one seeding of *I. balsamina*, SSG = intercropping with post-grafting generation of self-rooted grafting by two same sizes seedlings of *I. balsamina*, SDG = intercropping with post-grafting generation of self-rooted grafting by two different sizes seedlings of *I. balsamina*. The same as below.

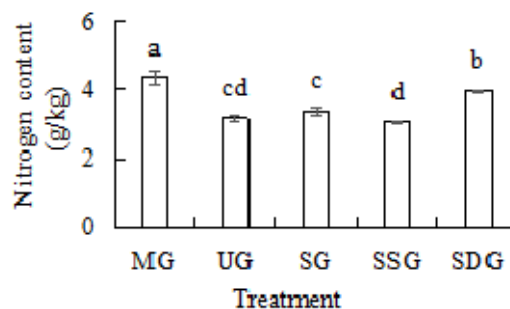


Figure 2. Nitrogen content in grape stems.

3.3 Nitrogen content in grape leaves

Intercropping with UG, SG, SSG and SDG, the nitrogen content in leaves of grape under cadmium stress was significantly lower than of monoculture, which was reduced by 27.45% ($p < 0.05$), 15.96% ($p < 0.05$), 22.43% ($p < 0.05$) and 24.30% ($p < 0.05$, Figure 3). Among all intercropping treatment, the highest nitrogen content in leaves of grape was intercropping with SG, while the lowest nitrogen content was intercropping with UG.

3.4 Alkaline nitrogen content in soil

In cadmium contaminated soil, all intercropping treatments did not significantly increase the soil alkaline nitrogen content compared with the monoculture (Figure 4). Compared with the monoculture, intercropping with SSG increased obviously, by 6.29% ($p < 0.05$), intercropped with SDG increased by 1.89% ($p < 0.05$), intercropping with UG increased by 1.71% ($p < 0.05$), intercropping with SG increased by 1.26% ($p < 0.05$).

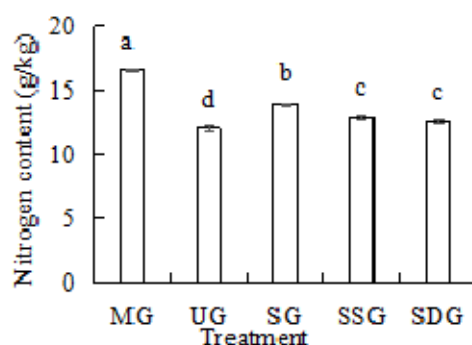


Figure 3. Nitrogen content in grape leaves.

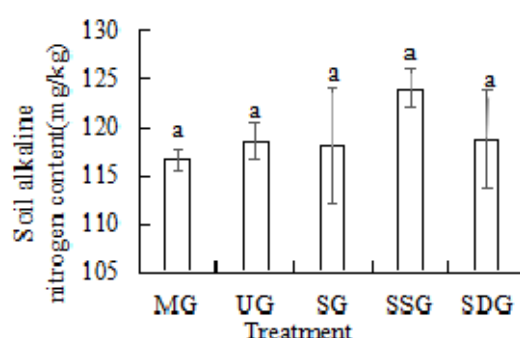


Figure 4. Alkaline nitrogen content in soil.

4. Conclusions

The experiment showed that different post-grafting generations of *I. balsamina* had different effects on nitrogen content of grape seedlings under cadmium stress. The nitrogen content in roots of grape intercropping with SSG was higher than that of other intercropping treatments. In grape stems, the nitrogen content of grape intercropping with SDG was higher than that of other intercropping treatments. In grape leaves, the nitrogen content of grape intercropping with SG was the maximum in all intercropping treatments. The content of soil alkaline nitrogen in all intercropping treatments was increased compared with monoculture, and the soil alkaline nitrogen content was maximal after intercropping with SSG. In summary, intercropping with post-grafting generations of *I. balsamina* under cadmium stress decreased the nitrogen absorption of grape and increased the content of soil alkaline nitrogen.

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