Effects of grape seedlings intercropping with post-grafting generation of two floricultural accumulator plants on soil enzymes activity under cadmium stress

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Abstract. Pot experiments were conducted to study the effects of grape seedlings intercropping with post-grafting generation of Cosmos sulphureus and Impatiens balsamina on soil enzymes activity under cadmium (Cd) stress. The result showed that: under Cd stress, grape intercropping with the post-grafting generation of C. sulphureus had significant effect, and grape intercropping with the generation of ungrafted C. sulphureus (C.CK) significantly increased soil catalase, soil urease and soil invertase activity compared to grape monoculture (MG). Grape intercropping with the post-grafting generation of self-rooted grafting by two uniform plant seedlings of I. balsamina (I.UG), the generation of ungrafted I. balsamina (I.CK) and the post-grafting generation of self-rooted grafting by the same one seedling of I. balsamina (I.SG) also significantly increased these three enzymes activity compared to MG. Grape intercropping with I.UG was the most, and next were I.CK and I.SG. In conclusion, when grape intercropping with C.CK, I.UG, I.CK and I.SG, respectively, the enzymes activity all significantly increased.

1. Introduction
Cadmium (Cd) is a toxic substance that has obvious toxic effects on plants [1], and also decreases soil enzyme activity which is the most sensitive to the inhibition of Cd [2]. Cd-accumulator has capacity of absorption and remediation on soil Cd pollution, and intercropping with Cd-accumulator that may be a feasible way to phytoremediate Cd polluted soils [3-4]. Some soil enzyme activities in soil polluted by Cd are recovered by phytoremediation, which can be judged according to the degree of recovery of these soil enzyme activities, such as urease activity [5]. Cosmos sulphureus [6] and Impatiens balsamina [7] are floricultural accumulator plants, which have the strong accumulative ability to Cd [6-7]. We have found the absorption of Cd in the post-grafting generation of C. sulphureus and I. balsamina could significantly increase. Cd pollution is seriously in vineyard, and grape growth is inhibited [8]. Therefore, the objective of this study was to evaluate the effect of grape seedlings intercropping with the post-grafting generation of C. sulphureus and I. balsamina on soil enzymes activity under Cd stress, in order to find the optimal intercropping combination which can improve the soil enzyme activity and make reference to phytoremediate Cd polluted soils in vineyard.
2. Materials and methods

2.1. Materials
In October 2014, the seeds of C. sulphureus and I. balsamina were collected from the surrounding farmland at Chengdu Campus of Sichuan Agricultural University (30°42′ N, 103°50′ E). The cutting seedlings of Kyoho grape were purchased from Longquanyi area seedlings base of Chengdu in May 2015. The soil for the experiment was collected from the Chengdu Campus Farm of the Sichuan Agricultural University.

2.2. Experimental design
In October 2014, the seeds of C. sulphureus and I. balsamina were collected and sowed, and when seedlings was about 10 cm high and other seedlings seedlings was about 5 cm high, grafted. The grafting treatments as follow [7]: (1) Ungrafted: seedlings transplanted directly, collected the seeds and preserved respectively, as the generation of ungrafted C. sulphureus (C.CK) and I. balsamina (I.CK), (2) Self-rooted grafting by the same one seedling: seedlings were cut off from 6 cm above the ground. The upper parts were scion, the lower parts were rootstock. Collected the seeds and preserved respectively, as the post-grafting generation of self-rooted grafting by the same one seedling of C. sulphureus (C.SG) and I. balsamina (I.SG). (3) Self-rooted grafting by two different sizes seedlings: seedlings were about 10 cm high, cut off from 6 cm above the ground, the lower parts were rootstock. Seedlings were about 5 cm high, cut the upper seedling (4 cm) as scions. The seeds were collected and preserved respectively, as the post-grafting generation of self-rooted grafting by two different sizes seedlings of C. sulphureus (C.DG) and I. balsamina (I.DG). (4) Self-rooted grafting by two uniform plant seedlings: 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). Collected the seeds and preserved respectively, as the post-grafting generation of self-rooted grafting by two uniform seedlings of C. sulphureus (C.UG) and I. balsamina (I.UG). In grafting experiments, rootstock leaves were retained, the soil was Cd free.

In April 2015, Cd was added to the soil samples in the form of analytical pure CdCl₂·2.5H₂O solution at the concentration of 5 mg/kg, and then the soil was thoroughly mixed. Natural balance for 4 weeks, occasionally turning soil mixing, so that the soil is fully mixed. In May 2015 the seeds of the post-grafting generation of C. sulphureus and I. balsamina were collected and sowed. When the seedlings expanded the two true leaves, one seedling per species planted in each pot. There were nine treatments: grape monoculture (MG), intercropping with C.CK (C. CK), intercropping with C.SG (C.SG), intercropping with C.DG (C.DG), intercropping with C.UG (C. UG), intercropping with I.CK (I.CK), intercropping with I. SG (I.SG), intercropping with I.DG (I.DG), intercropping with I.UG (I.UG). Repeated 6 times for each treatment, pots placed completely random with 15-cm spacing between pots, 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, collected soil separately, measured soil invertase activity, soil urease activity, soil catalase activity [9].

2.3. Statistical analysis
Statistical analysis was conducted using SPSS 18.0 statistical software, Data analysis by one-way ANOVA with least significant difference at 5% confidence level.

3. Results and discussion

3.1. Soil catalase activity
The catalase enzymes responded differently to Cd stress under different intercropping treatments. Under Cd stress, grape intercropping with C.SG and C.UG, the inhibition on soil catalase activity was most significant, while grape intercropping with C.CK and C.DG, soil catalase activity increased compared to MG (Figure 1, p < 0.05). Grape intercropping with the post-grafting generation of I.
**3.2. Soil catalase activity**

Under Cd stress, grape intercropping with the post-grafting generation of *C. sulphureus* significantly increased soil catalase activity. Intercropping with C.CK, C.SG, C.DG and C.UG, soil catalase activity increased by 7.96%, 12.31%, 19.36% and 25.91% compared to MG, respectively (Figure 3, \( p < 0.05 \)). Grape intercropping with the post-grafting generation of *I. balsamina* have a significant effect on soil catalase activity, intercropping with I.CK, I.SG, I.DG and I.UG increased soil catalase activity by 8.90%, 33.07%, 17.99%, 45.17% compared to MG, respectively (Figure 4, \( p < 0.05 \)).
3.3. Soil invertase activity

Under Cd stress, with the exception of intercropping with C.CK significantly increased soil invertase activity, intercropping with C.SG, C.DG and C.UG all significantly decreased soil invertase activity compared to MG (Figure 5, \( p < 0.05 \)). Intercropping with the post-grafting generation of *I. balsamina*, soil invertase activity had different response. Intercropping with I.CK and I.UG all significantly increased soil invertase activity, while intercropping with I.SG and I.DG decreased soil invertase activity compared to MG (Figure 6, \( p < 0.05 \)).

![Figure 5. Soil invertase activity of *C. sulphureus* –intercrop.](image)

![Figure 6. Soil invertase activity of *I. balsamina* – intercrop.](image)

4. Conclusions

The soil catalase, soil urease and soil invertase activities responded differently to Cd stress under different intercropping treatments. Under Cd stress, grape intercropping with the post-grafting generation of *C. sulphureus* had significant effect. Grape intercropping with C.CK and C.DG, especially intercropping with C.CK, soil catalase activity increased compared to MG. Grape intercropping with C.CK, C.SG, C.DG and C.UG all significantly increased soil urease activity. Grape intercropping with C.CK significantly increased soil catalase activity, while intercropping with C.SG, C.DG and C.UG all decreased soil invertase activity compared to MG. Under Cd stress, with the exception of intercropping with I.DG significantly decreased soil invertase activity, grape intercropping with the post-grafting generation of *I. balsamina* all significantly increased the three enzymes activity compared to MG. Grape intercropping with I.UG was most significantly increased soil catalase, soil urease and soil invertase activity, next were I.CK and I.SG. In conclusion, grape intercropping with C.CK, I.UG, I.CK and I.SG, the three enzymes activity all significantly increased. Therefore, grape intercropping with C.CK, I.UG, I.CK and I.SG might be good for grape planting.

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