Effects of intercropping with \textit{Vigna radiata} and \textit{Vigna umbellata} on antioxidant enzyme activity of \textit{Cyphomandra betacea} seedlings under cadmium stress

Caifang Wu ¹, Ji Liu², Jing Sun¹, Liu Yang³ and Lijin Lin¹*

¹Institute of Pomology and Olericulture, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China
²Chengdu Academy of Agriculture and Forestry Sciences, Chengdu, Sichuan, 611130, China
³College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China
*Corresponding author’s e-mail: llj800924@qq.com

Abstract. A pot experiment was conducted to study the effects of intercropping with leguminous plants: \textit{vigna umbellata} and \textit{vigna radiate} on antioxidant enzymes of \textit{Cyphomandra betacea} seedlings under cadmium (Cd) stress. The result showed that: under Cd stress, intercropping with leguminous plants, antioxidant enzyme activity and soluble protein content of \textit{C. betacea} all increased compared to \textit{C. betacea} monoculture. Intercropping with \textit{V. radiata}, SOD and CAT activity significantly increased. Intercropping with \textit{V. umbellata}, SOD, CAT activity and soluble protein content reached the peak, and increased by 62.14%, 94.61% and 57.94%, respectively compared to \textit{C. betacea} monoculture. Under \textit{C. betacea} - \textit{V. radiata} - \textit{V. umbellata} intercropping mode, antioxidant enzyme activity of \textit{C. betacea} significantly increased. In conclusion, under Cd stress, intercropping with leguminous plants could increase antioxidant enzyme activity of \textit{C. betacea} to reduce Cd toxicity, especially intercropping with \textit{V. umbellata}.

1. Introduction

Cadmium (Cd) is a toxic heavy metal with distinctly toxic effects on plants, and can be absorbed by plants and cause oxidative damage [1]. The up-regulation of the antioxidant enzymes activities induced by intercropping alleviates the adverse impact of oxidative stress in plant [2]. \textit{Cyphomandra betacea} is a tropical fruit with nutrient-rich, which has a bright future [3]. However Cd pollution is the most serious in the orchard soil, which inhibits the growth of fruit trees and threatens the quality and safety of fruit [4]. \textit{Vigna umbellata} [5] and \textit{Vigna radiata} [6] are leguminous plants with a certain resistance to Cd [5-6]. Proper intercropping is a feasible way to phytoremediate Cd polluted soils [7-8], so that it may reduce Cd accumulation of plants and to alleviate the damaging effects of oxidative stress. Therefore, the objective of this study was to evaluate the effect of intercropping with \textit{V. umbellata} and tolerant \textit{V. radiata} on antioxidant enzymes of \textit{C. betacea} seedlings under Cd stress, then to screen out the most suitable one that can improve the antioxidant enzymes activities alleviates the adverse impact of oxidative stress in \textit{C. betacea} seedlings.
2. Materials and methods

2.1. Materials
In April 2017, the seeds of C. betacea were collected from the surrounding farmland at Chengdu Campus of Sichuan Agricultural University and the seeds of V. umbellate and V. radiata were purchased from market. The soil for the experiment was collected from the surrounding farmland at Chengdu Campus of Sichuan Agricultural University in March 2017.

2.2. Experimental design
The experiment was conducted in Chengdu Campus of Sichuan Agricultural University from March to May 2017. In March 2017, the soil was air-dried and passed through a 5-mm sieve. Each plastic pot (15 cm high, 18 cm in diameter) was filled with 3 kg air-dried soil with 10 mg/kg Cd (in the analysis pure form of CdCl$_2$·2.5H$_2$O for 4 weeks [9]. All pots were watered every day to keep the soil moist, and dug aperiodically to make soil mixed fully. In April 2017, the seeds of C. betacea were sown in the farmland. When three true leaves of C. betacea seedlings expanded, the uniform seedlings were transplanted into the previously prepared plastic pots. At the same time, the seeds of V. umbellata and V. radiata were sown in the plastic pots. There were four treatments: monoculture of C. betacea (M), C. betacea intercropping with V. umbellata (IVU), C. betacea intercropping with V. radiata (IVR), and three plants intercropping (IVUR). Three plants seedlings were planted in each pot. For intercropping of paired species, C. betacea seedlings with one V. umbellata or V. radiata seedlings were planted in each pot; for intercropping of three species, one seedling per species were planted in each pot. For each treatment with six replicates and pots placed completely random, exchanged periodically to weaken the impact of the marginal effects, the distance between pots was 15 cm. The soil moisture content was maintained at 80% of field capacity until the plants were harvested. After 1 months (May 2017), the young leaves at the top of grape seedling were selected to measure SOD, POD and CAT activity, and soluble protein content [10].

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. SOD activity of C. betacea
Intercropping with leguminous plants, SOD activity of C. betacea increased remarkably compared to C. betacea monoculture, under Cd stress (Figure 1, $p < 0.05$). Intercropping with V. umbellata, SOD activity of C. betacea increased by 62.14% compared with the monoculture. C. betacea intercropping with V. radiata or three plants intercropping, SOD activity also significantly increased compared to C. betacea monoculture, however, differences among the two intercropping treatments were not significant.

3.2. POD activity of C. betacea
Under Cd stress, intercropping significantly increased POD activity of C. betacea all increased compared to C. betacea monoculture (Figure 2). All intercropping treatments had significant effect on POD activity of C. betacea, except for intercropping with V. radiata. POD activity of C. betacea could be arranged in the following order: intercropping with two leguminous plants > intercropping with V. umbellata > intercropping with V. radiata > C. betacea monoculture.

3.3. CAT activity of C. betacea
Under Cd stress, intercropping significantly increased CAT activity of C. betacea (Figure 3, $p < 0.05$). Intercropping with V. umbellata, CAT activity of C. betacea reached the peak, significantly increased
by 94.61% compared to *C. betacea* monoculture. CAT activity of *C. betacea* under *C. betacea* - *V. radiata* intercropping mode and *C. betacea* - *V. radiata* - *V. umbellata* intercropping mode also significantly increased by 12.88% and 11.91%, respectively.

Figure 1. SOD activity of tamarillo. The same letters within each column are not significantly different at \( p < 0.05 \). M = monoculture of *C. betacea*, IVR = *C. betacea* intercropping with *V. radiata*, IVU = *C. betacea* intercropping with *V. umbellata*, IVUR = three intercropping.

Figure 2. POD activity of tamarillo. The same letters within each column are not significantly different at \( p < 0.05 \). M = monoculture of *C. betacea*, IVR = *C. betacea* intercropping with *V. radiata*, IVU = *C. betacea* intercropping with *V. umbellata*, IVUR = three intercropping.

Figure 3. CAT activity of tamarillo. The same letters within each column are not significantly different at \( p < 0.05 \). M = monoculture of *C. betacea*, IVR = *C. betacea* intercropping with *V. radiata*, IVU = *C. betacea* intercropping with *V. umbellata*, IVUR = three intercropping.

Figure 4. Soluble protein content of tamarillo. The same letters within each column are not significantly different at \( p < 0.05 \). M = monoculture of *C. betacea*, IVR = *C. betacea* intercropping with *V. radiata*, IVU = *C. betacea* intercropping with *V. umbellata*, IVUR = three intercropping.

3.4. Soluble protein content of *C. betacea*

Under Cd stress, with the exception of intercropping with *V. umbellata*, other intercropping treatments did not respond significantly to soluble protein content of *C. betacea* (Figure 4). Intercropping with *V. umbellata*, soluble protein content of *C. betacea* significantly increased by 57.94% compared to *C. betacea* monoculture \( (p < 0.05) \). Soluble protein content of *C. betacea* could be arranged in the following order: intercropping with *V. umbellata* > intercropping with *V. radiata* > intercropping with two leguminous plants > *C. betacea* monoculture.
4. Conclusions
The antioxidant system is essential for improving plant resistance to Cd toxicity. Under Cd stress, intercropping with leguminous plants, antioxidant enzyme activity of *C. betacea* all increased compared to *C. betacea* monoculture. SOD and CAT activity of *C. betacea* significantly increased under *C. betacea* - *V. radiata* intercropping mode. Intercropping with *V. umbellata*, antioxidant enzyme activity of *C. betacea* all significantly increased, and particularly SOD, CAT activity and soluble protein content increased most. Under *C. betacea* - *V. radiata* - *V. umbellata* intercropping mode, antioxidant enzyme activity of *C. betacea* significantly increased, except for soluble protein content.

In conclusion, under Cd stress, intercropping with leguminous plants could increase antioxidant enzyme activity of *C. betacea* to reduce Cd toxicity. Especially intercropping with *V. umbellata* had significant effect on antioxidant enzyme activity of *C. betacea*, which was a feasible way to alleviate the damaging effects of oxidative stress of *C. betacea* under Cd stress.

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