Effects of *Cyphomandra betacea* Seedlings Intercropping with Hyperaccumulators on Soil Enzyme Activity under Cadmium Stress

Shuling Zheng^{1,a}, Jin Wang^{2,b} and Lijin Lin^{2,c*}

¹College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, China

²Institute of Pomology and Olericulture, Sichuan Agricultural University, Chengdu, Sichuan, China

^a630668750@qq.com, ^b251040278@qq.com, ^clj800924@qq.com

*Corresponding author. Shuling Zheng and Jin Wang contributed equally to this work.

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Abstract: The effects of *Cyphomandra betacea* seedlings intercropping with four hyperaccumulator plant species (*Bidens pilosa*, *Solanum photeinocarpum*, *Galinsoga parviflora*, and *Tagetes erecta*) on soil enzyme activity under cadmium (Cd) stress were studied by a pot experiment. The results showed that intercropping treatments could increase the soil sucrase and soil urease activities. The soil catalase and soil polyphenol oxidase activities showed different promote or inhibit effects on different hyperaccumulator plants by intercropping compared to monoculture. Only intercropping with *S. photeinocarpum*, all soil enzyme activity increased compared to *C. betacea* monoculture. Therefore, intercropping could improve the soil enzyme activity under Cd stress, and *C. betacea* intercropping with *S. photeinocarpum* was the best.

Introduction

Cadmium (Cd) is a heavy metal with the characteristics of constant accumulation, difficult to eliminate and highly toxic on plants [1]. Excessive amount of Cd inhibits the soil enzyme activity, so that soil enzyme activity can be used as an index to judge the degree of heavy metal pollution and crop damage [2-3]. *Cyphomandra betacea* is a perennial fruit tree, which has both edible and ornamental value [4], and it is also contaminated with cadmium [5]. Rational intercropping can change the effective state of heavy metals and affect the absorption by plants, and improve soil microbial diversity and soil enzyme activity [6-7]. The studies show that the heavy metal uptake in non-hyperaccumulator plants reduces and hyperaccumulator biomass and heavy metal absorption effect improves by intercropping [8-9]. Therefore, in this study, we used four Cd-hyperaccumulator plant species *Bidens pilosa* [10], *Solanum photeinocarpum* [11], *Galinsoga parviflora* [12], and *Tagetes erecta* [13] to intercrop with *C. betacea* seedlings under Cd stress to study the effects on soil enzyme activity.

Materials and Methods

Materials. In October 2013, the seeds of four hyperaccumulator plant species *B. pilosa*, *S. photeinocarpum*, *G. parviflora*, and *T. erecta* were collected from the farmland of the Ya'an campus farm of the Sichuan Agricultural University. In May 2014, the seeds were put in the climate chamber to germination and further cultivation. The seeds of *C. betacea* were harvested from 10-year-old fruit of the farmland of the Ya'an campus farm of the Sichuan Agricultural University November 2013. In April 2014, the seeds were put in the climate chamber to germination and further cultivation.

Experimental Design. In April 2014, the soil was air-dried and passed through a 6.72-mm sieve. 2.5kg air-dried soil was weighed into each plastic pot (21 cm high, 20 cm in diameter), soaking uniformly by 10 mg/kg Cd (in the form of CdCl₂·2.5H₂O) solution for 30 days. All pots were watered each day to keep the soil moisture about 80%. In June 2016, three uniform-sized seedlings (the shoots were about 10cm, three true leaves expanded) of *C. betacea* were transplanted into pot for monoculture. One uniform-sized seedling (the shoots were about 3 cm, two true leaves expanded) of each four hyperaccumulators and two *C. betacea* seedlings were transplanted into

each pot for intercropping. The monoculture of *C. betacea* was planted three seedlings for one 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 aperiodically 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 60 days, remove and blending 0-5 cm soil of every pot, encapsulated with plastic bags to be kept in laboratory at 4°C refrigerator. After soil through a 1 mm sieve, determined the soil enzyme activity [14]. Determination methods of soil catalase activity was potassium permanganate titration, soil urease activity was indigo blue colorimetry, soil sucrase activity was 3N-5-dinitrosalicylic acid colorimetry, and soil polyphenol oxidase activity was colorimetric method [15].

Statistical Analyses. Statistical analysis was carried out by using SPSS 20.0 statistical software. The data were analyzed by one-way ANOVA, with the least significant difference at the 5% confidence level.

Results and Discussion

Soil Catalase Activity. Compared with the monoculture of *C. betacea*, intercropping with *B. pilosa* and *T. erecta* decreased the soil catalase activity under Cd stress, and intercropping with *S. photeinocarpum* and *G. parviflora* increased the soil catalase activity under Cd stress (Fig. 1). The soil catalase activity by intercropping with *B. pilosa* and *T. erecta*, decreased by 6.61% ($p > 0.05$) and 0.88% ($p > 0.05$), respectively compared to monoculture. Intercropping with *S. photeinocarpum* and *G. parviflora* increased by 8.81% ($p > 0.05$), 9.69% ($p > 0.05$), respectively compared to monoculture.

Soil Polyphenol Oxidase Activity. The soil polyphenol oxidase activity was ranked in the following order: intercropping with *T. erecta* > intercropping with *S. photeinocarpum* > monoculture > intercropping with *G. parviflora* > intercropping with *B. pilosa* (Fig. 2). According to the order, they were respectively 0.53 ($p < 0.05$), 1.46 ($p < 0.05$), 0.86 ($p > 0.05$) and 1.47 ($p < 0.05$) times of monoculture.

Soil Sucrase Activity. Compared with the monoculture, except intercropping with *G. parviflora*, the soil sucrase activity increased significantly by intercropping under Cd stress (Fig. 3). The soil sucrase activity by intercropping with *G. parviflora* decreased by 9.67% ($p > 0.05$) compared to monoculture, intercropping with *B. pilosa*, *S. photeinocarpum* and *T. erecta* increased by 254.79%, 147.01%, and 34.18% respectively compared to monoculture.

Soil Urease Activity. Intercropping with four hyperaccumulators increased the soil urease activity compared to monoculture under Cd stress (Fig. 4). The soil urease activity was ranked as: intercropping with *S. photeinocarpum* > intercropping with *B. pilosa* > intercropping with *T. erecta* > intercropping with *G. parviflora* > monoculture. According to the order, they were respectively 1.43 ($p < 0.05$), 1.88 ($p < 0.05$), 1.07 ($p > 0.05$) and 1.40 ($p < 0.05$) times of monoculture.

Conclusions

Under Cd stress, intercropping treatments could increase the soil sucrase and soil urease activities. The soil catalase and soil polyphenol oxidase activities showed different promote or inhibit effects on different hyperaccumulator plants by intercropping compared to monoculture. Only intercropping with *S. photeinocarpum*, all soil enzyme activity increased compared to *C. betacea* monoculture. Therefore, intercropping could improve the soil enzyme activity under Cd stress, and *C. betacea* intercropping with *S. photeinocarpum* was the best.

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Fig. 1 Soil catalase activity. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I B.p = intercropping with *B. pilosa*, I S.p = intercropping with *Solanum photeinocarpum*, I G.p = intercropping with *Galinsoga parviflora*, I T.e = intercropping with *T. erecta*.

Fig. 2 Soil polyphenol oxidase activity. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). M=monoculture, I B.p = intercropping with *B. pilosa*, I S.p = intercropping with *Solanum photeinocarpum*, I G.p = intercropping with *Galinsoga parviflora*, I T.e = intercropping with *T. erecta*.

Fig. 3 Soil sucrase activity. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I B.p = intercropping with *B. pilosa*, I S.p = intercropping with *Solanum photeinocarpum*, I G.p = intercropping with *Galinsoga parviflora*, I T.e = intercropping with *T. erecta*.

Fig. 4 Soil urease activity. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I B.p = intercropping with *B. pilosa*, I S.p = intercropping with *Solanum photeinocarpum*, I G.p = intercropping with *Galinsoga parviflora*, I T.e = intercropping with *T. erecta*.

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