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Effects of intercropping with *Vigna umbellata* and *Vigna radiata* on cadmium accumulation of *Cyphomandra betacea* seedlings

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Abstract. This study investigated the effect of intercropping on the accumulation of cadmium (Cd) by *Cyphomandra betacea* seedlings that was intercropped with leguminous plants: *Vigna angularis* and *Vigna radiatus*. The result showed that: under Cd stress, intercropping with leguminous plants, biomass of *C. betacea* displayed a general ascending trend compared to *C. betacea* monoculture. Intercropping with *V. umbellata*, the biomass of roots, stems, leaves and shoots significantly increased respectively by 24.79%, 49.16%, 23.93% and 30.45%. Root shoot ratio decreased. However, under Cd stress, intercropping with leguminous plants substantially enhanced Cd uptake by stems and leaves of *C. betacea*. And translocation factor increased, the capacity of Cd translocation to shoots was improved. In conclusion, under Cd stress, intercropping with leguminous plants could increase biomass of *C. betacea*. Intercropping with *V. umbellata* increased the biomass most, and the ability of Cd tolerance of *C. betacea* was improved.

1. Introduction

In recent years, heavy metal pollution is increasingly serious, especially the heavy metal cadmium (Cd) pollution, which is more and more serious [1]. Cd can be absorbed by plants and has distinctly toxic effects on plants [2]. *Cyphomandra betacea* is a tropical fruit with nutrient-rich and has a bright future [3]. According to literature, Cd pollution is the most serious in the orchard soil [4], which inhibits the growth of *C. betacea* and other fruit trees, also threatens the quality and safety of fruit. Thus effective treatment on the heavy metal Cd is imperative. Phytoremediation technology has provided a new approach for the treatment of Cd soil pollution, such as proper intercropping, which may reduce heavy metal accumulation of common plants [5-6]. *Vigna umbellata* [7] and *Vigna radiata* [8] are leguminous plants which have wide adaptability, strong stress resistance and nitrogen fixation and soil nutrient capacity. It is a suitable crop for intercropping with young fruit trees [9-10]. Therefore, the objective of this study was to evaluate the effect of intercropping with *V. umbellata* and *V. radiata* on Cd accumulation of *C. betacea* seedlings under Cd stress, then to screen out the most suitable one that can reduce Cd accumulation of *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. umbellata* 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 $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ for 4 weeks [11]. 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. Each treatment consisted of one pot with six replicates arranged in a completely randomized design with 10 cm spacing between pots. The margin effect was weakened by periodically changing the positions of the pots during the experiment. The soil moisture content was maintained at 80% of field capacity from soil preparation until the plants were harvested.

After 1 months (May 2017), *C. betacea* seedlings were harvested, roots, stems and leaves were separated, then washed with tap water firstly, followed by deionized water for three times. Finally, simmered for 15 min at 110 °C, and then the tissues of all plants were dried at 80 °C until constant weight, weighed and passed through a 100-mesh sieve, Cd content of the roots, stems and leaves were determined [12].

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. Biomass of *C. betacea* seedlings

Under Cd stress, intercropping with leguminous plants, biomass of *C. betacea* displayed a general ascending trend compared to *C. betacea* monoculture (Table 1). All roots biomass increased significantly under intercropping treatments, except for three plants intercropping. Under *C. betacea* - *V. umbellata* intercropping mode, roots biomass of *C. betacea* increased most, which increased by 24.79% ($p < 0.5$). Intercropping with leguminous plants, stems biomass, leaves biomass and shoots biomass of *C. betacea* significantly increased ($p < 0.5$). And *C. betacea* intercropping with *V. umbellata*, stems, leaves and shoots biomass of *C. betacea* reached the top point, and increased respectively by 49.16%, 23.93% and 30.45%. Root shoot ratio decreased and showed the follows order: *C. betacea* monoculture > intercropping with *V. umbellata*, three plants intercropping > intercropping with *V. radiata*.

3.2. Cd content in *C. betacea* seedlings

Under Cd stress, intercropping decreased Cd content in roots *C. betacea*, which decreased respectively by 10.22%, 2.56% and 7.64%, differences among the intercropping treatments were not significant (Table 2, $p > 0.05$). However, Cd contents in stems, leaves and shoots of *C. betacea* significantly increased. Cd content in shoots of *C. betacea* showed the follows order: intercropping with *V. radiata* > three plants intercropping > intercropping with *V. umbellata* > *C. betacea* monoculture. Under intercropping treatments, shoots Cd content increased respectively by 97.56%, 13.71% and 76.29% over that of the corresponding mono-cropping treatments ($p < 0.05$). Translocation factor was increased, Cd translocation capacity of *C. betacea* improved.

Table 1. Biomass of *C. betacea* seedlings.

Treatments	Roots (g/plant)	Stems (g/plant)	Leaves (g/plant)	Shoots (g/plant)	Root/shoot ratio
M	0.242±0.004c	0.179±0.006c	0.514±0.008c	0.693±0.014c	0.349
IVR	0.267±0.007b	0.251±0.008a	0.577±0.011b	0.828±0.020b	0.322
IVU	0.302±0.010a	0.267±0.010a	0.637±0.014a	0.904±0.024a	0.334
IVUR	0.262±0.008bc	0.211±0.007b	0.574±0.018b	0.785±0.025b	0.334

Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture of *C. betacea*, IVU = *C. betacea* intercropping with *V. umbellata*, IVR = *C. betacea* intercropping with *V. radiata*, IVUR = three intercropping. The same as following figures.

Table 2. Cd content in *C. betacea* seedlings.

Treatments	Roots (g/plant)	Stems (g/plant)	Leaves (g/plant)	Shoots (g/plant)	Translocation factor
M	115.12±3.790a	0.251±0.010d	0.594±0.007d	0.505±0.007d	0.004
IVR	103.35±2.616a	0.634±0.011a	1.157±0.016a	0.998±0.013a	0.010
IVU	112.17±6.520a	0.376±0.008c	0.658±0.013c	0.575±0.011c	0.005
IVUR	106.32±5.671a	0.491±0.006b	1.038±0.010b	0.891±0.009b	0.008

Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$).

4. Conclusions

Biomass is an important indicator of growth. Under Cd stress, intercropping with leguminous plants, biomass of *C. betacea* all increased compared to *C. betacea* monoculture. Under *C. betacea* - *V. umbellata* intercropping mode, roots and shoots biomass increased most, increased by 24.79% and 30.45% respectively. Overall comparison results illustrated that biomass in whole *C. betacea* plant follows the order: intercropping with *V. umbellata* > intercropping with *V. radiata* > three plants intercropping > *C. betacea* monoculture. The results of the increase of biomass of *C. betacea* has shown that ability of Cd tolerance of *C. betacea* was improved. Under Cd stress, intercropping decreased roots Cd content in *C. betacea* ($p > 0.05$), but significantly increased Cd content in stems, leaves and shoots ($p < 0.05$), which showed that the capacity of Cd translocation to shoots was improved. Cd content in shoots of *C. betacea* showed the follows order: intercropping with *V. radiata* > three plants intercropping > intercropping with *V. umbellata* > *C. betacea* monoculture.

In conclusion, under Cd stress, intercropping with leguminous plants could increase biomass of *C. betacea*. Intercropping with *V. umbellata* increased biomass most, and the ability of Cd tolerance of *C. betacea* was improved.

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