Effects of intercropping with *Vigna umbellata* and *Vigna radiata* on photosynthetic pigment contents in *Cyphomandra betacea* seedlings under cadmium stress

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**Abstract.** A pot experiment was conducted to study the effects of intercropping *Vigna umbellata* and *Vigna radiata* on photosynthetic pigment contents in *Cyphomandra betacea* seedlings under cadmium (Cd) stress. The result showed that under Cd stress, all intercropping treatments increased the chlorophyll a, chlorophyll b, carotenoid and total chlorophyll contents of *C. betacea*, especially the intercropping with *V. umbellata* which was highest in contents of chlorophyll a, chlorophyll b, carotenoid and total chlorophyll of *C. betacea*. On the contrary, all intercropping treatments decreased the chlorophyll a/b of *C. betacea* compared to the monoculture and intercropping with *V. umbellata* was lowest. Therefore, intercropping with *V. umbellata* could be the best choice to increase the photosynthetic pigment *C. betacea* under Cd stress.

1. **Introduction**

Cadmium (Cd) is one of the most toxic elements among known elements, which can inhibit cell division to prevent plant growth and affect cell structure [1]. The increase of Cd concentration can also inhibit chlorophyll synthesis and affect plant photosynthesis [2]. Cd is easily absorbed and enriched by crops in production activities, which not only seriously affects the yield and quality of crops, but also endangers human health through the accumulation of food chain in the human body [3]. Reasonable intercropping of crops can efficiently utilize light, heat, water and nutrient resources, and has the advantage of high and stable yield [5-6]. At the same time, intercropping can also effectively reduce pests and diseases, the use of chemical fertilizers and pesticides, environmental pollution, and production costs and improve yield [7-8]. Studies have shown that *Vigna umbellata* has strong tolerance to heavy metal copper, while *Vigna radiata* appears to die after thinning seedlings [4]. Therefore, this study investigated the effects of intercropping with *V. umbellata* and *V. radiata* on photosynthetic pigment content in *Cyphomandra betacea* seedlings under Cd stress then to screen out the most suitable one that can improve the absorption of photosynthetic pigment of *C. betacea*. 
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 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. 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. One month later (May 2017), the upper mature leaves of *C. betacea* seedlings were collected to determine the photosynthetic pigment (chlorophyll $a$, chlorophyll $b$, total chlorophyll and carotenoid) contents [10].

2.3. Statistical analyses
Statistical analysis was carried out by using SPSS 18.0 statistical software. The data were analyzed by one-way ANOVA, with the least significant difference at the 5% confidence level.

3. Results and discussion

3.1. Chlorophyll $a$ content in *C. betacea*
Under Cd stress, there are significant levels between the monoculture and intercropping (Figure 1). Compared to monoculture, all intercropping patterns increased the content of chlorophyll $a$ and the content of chlorophyll $a$ of intercropping with *V. radiate*, *V. umbellata* and three intercropping were 20.91% ($p < 0.05$), 28.6% ($p < 0.05$) and 18.56% ($p < 0.05$), respectively, higher than the monoculture. Sorting the content of chlorophyll $a$ of each treatment from highest to lowest: intercropping with *V. umbellata* > intercropping with *V. radiate* > Three intercropping > monoculture.
Figure 1. Chlorophyll a content. 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 $C. \text{betacea}$, IVU = $C. \text{betacea}$ intercropping with $V. \text{umbellata}$, IVR = $C. \text{betacea}$ intercropping with $V. \text{radiata}$, IVUR = three intercropping. The same as following figures.

3.2. Chlorophyll b content in $C. \text{betacea}$

All intercropping treatments increased the content of chlorophyll b than the monoculture and the differences can do reach significant levels (Figure 2). The content of chlorophyll b of intercropping with $V. \text{radiate}$, $V. \text{umbellata}$ and three intercropping were 45.53% ($p < 0.05$), 73.98% ($p < 0.05$) and 24.4% ($p < 0.05$) respectively, higher than the monoculture. For the chlorophyll b content, the order from large to small was ranked: intercropping with $V. \text{umbellata}$ > intercropping with $V. \text{radiate}$ > Three intercropping > monoculture.

Figure 2. Chlorophyll b content. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$).

3.3. Carotenoid content in $C. \text{betacea}$

Under Cd stress, for the carotenoid content in $C. \text{betacea}$, the order from large to small was ranked: intercropping with $V. \text{umbellata}$ > intercropping with $V. \text{radiate}$ > Three intercropping > monoculture. (Figure 3). Compared to the monoculture, intercropping significantly increased the carotenoid content in $C. \text{betacea}$ and the content of carotenoid of intercropping with $V. \text{radiate}$, $V. \text{umbellata}$ and three intercropping were 18.29% ($p < 0.05$), 27.24% ($p < 0.05$) and 15.31% ($p < 0.05$), respectively, higher than the monoculture.

Figure 3. Carotenoid content. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$).
3.4. Total chlorophyll b content in C. betacea

Under Cd stress, all intercropping patterns can increase the total chlorophyll content in C. betacea than the monoculture and the differences can do reach significant levels (Figure 4). The content of total chlorophyll of intercropping with V. radiate, V. umbellata and three intercropping were 22.85% ($p < 0.05$), 32.16% ($p < 0.05$), 19.02% ($p < 0.05$), respectively, higher than the monoculture.

3.5. Total chlorophyll b content in C. betacea

Under Cd stress, all intercropping patterns reduced the chlorophyll a/b of C. betacea compared to the monoculture (Figure 5).

4. Conclusions

According to the experiment, under Cd stress, all intercropping treatments increased the chlorophyll a, chlorophyll b, carotenoid and total chlorophyll contents in C. betacea, and intercropping with V. umbellata was the highest in chlorophyll a, chlorophyll b, carotenoid and total chlorophyll contents. On the contrary, all intercropping patterns decreased the chlorophyll a/b compared to the monoculture and the content of intercropping with V. umbellata was lowest. Therefore, intercropping with V. umbellata had strong resistance so that it had a better effect on the photosynthetic pigment than on other treatments.

Acknowledgements

This work was financially supported by the 2016 Innovation Training Program of University Student (201610626032) and the National Natural Science Foundation of China (31560072).

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