Effects of intercropping with reciprocal hybridization F1 generation of Solanum photoinocarpum on growth and physiology of Cyphomandra betacea seedlings

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Abstract. To study the effects of positive and negative hybridization F1 generation of two ecotypes (mining and farmland) of hyperaccumulator plants on the growth and the fruit quality safety of Cyphomandra betacea under cadmium stress. In this experiment, C. betacea seedlings were used as research materials, intercropping S. photoinocarpum of parents and positive and negative hybridization F1 generation with C. betacea seedlings, study the effects intercropping with S. photoinocarpum on growth and physiology of C. betacea seedlings. The biomass, photosynthetic pigments content, soluble protein content and antioxidant enzymes activity of intercropping treatments were all lower than monoculture, the above shows that in the physical growth and resistance of C. betacea seedlings intercropping with S. photeinocarpum were all showed the intercropping disadvantage, especially the worst growth C. betacea seedlings was intercropping with S. photoinocarpum positive hybridization F1 generation.

1. Introduction
Ecotype refers to different groups in the same species that have certain structural or functional differences due to adaptation to different habitats [1]. Different ecotypes represent different genotypes, so even if they are transplanted into the same habitat, they can still maintain their stable differences, and different ecotypes can be freely crossed [2]. Different ecotype plants not only show different absorption or tolerance to heavy metals, but also often have significant differences in response characteristics to environmental factors. For example, mine ecotype hyperaccumulator plants grow in mining areas environments with high levels of heavy metals, such as around factories that emit a large amount of heavy metals, the enrichment capacity is usually strong, but the growth is slow and the biomass is small, while the hyperaccumulator plants of the same type of farmland ecology grow without heavy metal pollution or heavy metal content has the opposite effects.

Cross breeding can be used to cross-breed the genes of parents to recombine, create mutations and select new breeds [3]. According to the complementarily model, it is possible to breed an inbred line containing all favorable alleles from crossbreeding under sufficient recombination and selection pressure. Therefore, the correct selection of parents and the reasonable combination are the keys to the success or failure of cross breeding [4]. Hybrid breeding with hyperaccumulator plants of different ecotypes, and screening of their offspring, may result in improved varieties that integrate the dominant traits of super-enriched plants of different ecotypes. Therefore, it is practical to use hybrid plants of different ecotypes to improve the varieties feasible methods [5-6]. At present, in terms of heavy metal...
pollution, hybrid breeding is mainly used in the breeding of rice varieties with low metal enrichment or low absorption [7]. However, no studies have been reported on the use of different ecotypes of hyperaccumulator plants for hybridization to further increase the heavy metal accumulation capacity of hyperaccumulator plants. Hybrid breeding between hyperaccumulator plants of different ecological types can open a new path for the remediation of heavy metal pollution. In this experiment, the effects of intercropping with reciprocal hybridization F1 generation of potential cadmium hyperaccumulator Solanum photeinocarpum on growth and physiology of C. betacea seedlings were studied.

2. Materials and methods

2.1 Materials
The mine ecotype S. photeinocarpum collected from Tangjiashan lead zinc mine, Hanyuan Country, Sichuan Province, China, and the farmland ecotype S. photeinocarpum collected from the farm of Ya’an campus of Sichuan Agricultural University, Yucheng Country, Sichuan Province, China. Positive hybridization: Farmland ecotype is the female parent, and mine ecotype is the male parent. Negative hybridization: Mine ecotype is the female parent, and farmland ecotype is the male parent [8].

2.2 Experimental design
The soil from the farm of Sichuan Agricultural University was dried by air. The 21 cm × 20 cm (diameter × height) plastic basin was used to load 3.0 kg of air dried soil screened by 6.72 mm (3 mesh) and 10 mg/kg cadmium (added into the soil in the form of CdCl₂·2.5H₂O analysis pure form) was added [9]. The soil was kept moist and placed for 30 days. The soil was turned over and mixed irregularly to make the soil fully and evenly mixed. In the same month, C. betacea seeds were placed in the climate box for breeding. In August 2015, the seeds of parents and F1 hybrids of S. photeinocarpum were placed in the climate box for breeding.

The parents of S. photeinocarpum and F1 generation seedlings (about 3 cm high, 2 true leaves spread) were mixed with C. betacea seedlings (about 10 cm high, 3 true leaves spread) in a pot. There were 1 plant in each pot for mixed planting of S. photeinocarpum and 3 plants in each pot for single planting of C. betacea, 2 plants in each pot for mixed planting, each treatment repeated 6 times. In the process of cultivation, water in time to keep the field water capacity of the soil at about 80%, and pay attention to weed removal and pest control.

Two months after the growth of the plants, determination of the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) by acetone-ethanol mixed (1: 1) extraction method [10]; determination of superoxide dismutase (SOD) activity by nitrogen blue tetrazole method, the peroxidase (POD) activity by guaiacol method, and the catalase (CAT) activity by potassium permanganate titration [10]. The soluble protein content was determined by the Coomassie Brilliant Blue G250 method and expressed as the number of milligrams (mg/g) of soluble protein per gram of fresh weight sample [10]. Then, the whole plant and C. betacea were sampled respectively, and the soil was stored separately. Plant samples were rinsed with tap water, and then rinsed repeatedly with deionized water. They were killed at 105 °C for 15 min, then dried to constant weight at 70 °C and weighed.

3. Results and discussion

3.1 Biomass of C. betacea seedlings
Intercropping with S. photeinocarpum decreased the root, stem, leaf and shoot biomasses of C. betacea seedlings, compared with the monoculture of C. betacea seedlings (Table 1). Compared with the monoculture of C. betacea seedlings, intercropping with farmland ecotype, mine ecotype, positive and negative of S. photeinocarpum decreased the root biomass of C. betacea seedlings by 8.95% (p > 0.05), 6.33% (p > 0.05), 13.76% (p <0.05), and 11.57% (p <0.05), respectively, and decreased the
shoot biomass of *C. betacea* seedlings by 16.78% (*p* < 0.05), 15.67% (*p* < 0.05), 22.39% (*p* < 0.05), and 20.75% (*p* < 0.05), respectively.

### 3.2 Photosynthetic pigment content in *C. betacea* seedlings

Intercropping with *S. photeinocarpum* decreased the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents in leaves of *C. betacea* seedlings, compared with the monoculture of *C. betacea* seedlings (Table 2). Compared with the monoculture of *C. betacea* seedlings, intercropping with farmland ecotype, mine ecotype, positive and negative of *S. photeinocarpum* decreased the total chlorophyll content in *C. betacea* seedlings by 21.27% (*p* < 0.05), 17.45% (*p* < 0.05), 23.86% (*p* < 0.05), and 22.42% (*p* < 0.05), respectively, and decreased the carotenoid content in *C. betacea* seedlings by 12.03% (*p* < 0.05), 7.80% (*p* > 0.05), 17.82% (*p* < 0.05), and 16.93% (*p* < 0.05), respectively.

| C. betacea | Roots (g/plant) | Stems (g/plant) | Leaves (g/plant) | Shoots (g/plant) |
|------------|----------------|----------------|-----------------|-----------------|
| Monoculture | 0.458±0.011a | 0.456±0.009a | 0.903±0.024a | 1.359±0.033a |
| Int. farmland | 0.417±0.013ab | 0.446±0.014a | 0.685±0.020b | 1.131±0.034b |
| Int. mine | 0.429±0.014ab | 0.450±0.013a | 0.696±0.023b | 1.146±0.036b |
| Int. positive | 0.395±0.017b | 0.397±0.016b | 0.659±0.028b | 1.056±0.044b |
| Int. negative | 0.405±0.021b | 0.404±0.018b | 0.673±0.031b | 1.077±0.049b |

Values are mean ± SD (*n* = 6). Different lowercase letters indicated significant differences among treatments at 0.05 levels.

### 3.3 Antioxidant enzyme activity of *C. betacea* seedlings

Intercropping with *S. photeinocarpum* decreased the soluble protein content and antioxidant enzyme activity of *C. betacea* seedlings, compared with the monoculture of *C. betacea* seedlings (Table 3). Compared with the monoculture of *C. betacea* seedlings, intercropping with farmland ecotype, mine ecotype, positive and negative of *S. photeinocarpum* decreased the soluble protein content in *C. betacea* seedlings by 12.42% (*p* < 0.05), 3.13% (*p* > 0.05), 18.89% (*p* < 0.05), and 18.36% (*p* < 0.05), respectively, reduced the SOD activity of *C. betacea* seedlings by 17.37% (*p* < 0.05), 8.07% (*p* < 0.05), 26.85% (*p* < 0.05), and 22.81% (*p* < 0.05), respectively, reduced the POD activity of *C. betacea* seedlings by 5.11% (*p* > 0.05), 50.0% (*p* > 0.05), 13.77% (*p* < 0.05), and 13.12% (*p* < 0.05), respectively, and reduced the CAT activity of *C. betacea* seedlings by 21.58% (*p* < 0.05), 4.85% (*p* > 0.05), 26.27% (*p* < 0.05), and 23.79% (*p* < 0.05), respectively.

### 4. Conclusions

The effects of intercropping with *S. photeinocarpum* on growth and physiology of *C. betacea* seedlings were studied in this experiment. The biomass, photosynthetic pigments content, soluble protein content and antioxidant enzymes activity of intercropping treatments were all lower than
monoculture, the above shows that in the physical growth and resistance of C. betacea seedlings intercropping with S. photeinocarpum were all showed the intercropping disadvantage, especially the worst growth C. betacea seedlings was intercropping with S. photeinocarpum positive hybridization F1 generation.

Table 3. Antioxidant enzyme activity of C. betacea seedlings.

| C. betacea       | Soluble protein content (mg/g) | SOD activity (U/g) | POD activity (U/g/min) | CAT activity (mg/g/min) |
|------------------|-------------------------------|-------------------|------------------------|------------------------|
| Monoculture      | 3.447±0.116a                  | 99.04±3.69a       | 3813.51±130.43a        | 50.70±1.39a            |
| Inter. farmland  | 3.019±0.108b                  | 81.84±2.87c       | 3618.78±84.84a         | 39.76±0.93b            |
| Inter. mine      | 3.339±0.127a                  | 91.05±2.39b       | 3794.46±102.30a        | 48.24±1.57a            |
| Inter. positive  | 2.796±0.79b                   | 72.45±1.48d       | 3288.43±92.69b         | 37.38±1.05b            |
| Inter. negative  | 2.814±0.91b                   | 76.45±1.91cd      | 3313.14±123.63b        | 38.64±1.15b            |

Values are mean ± SD (n = 6). Different lowercase letters indicated significant differences among treatments at 0.05 levels.

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