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Effects of living hyperaccumulator plants and their straws on the growth and cadmium accumulation of *Cyphomandra betacea* seedlings

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A B S T R A C T

To determine whether the living hyperaccumulator plants and their straws have the same effects on the growth and heavy metal accumulation of common plants, two pot experiments (intercropping experiment and straw mulch experiment) were conducted to study the effects of living hyperaccumulator plants (*Solanum photeinocarpum*, *Tagetes erecta*, *Galinsoga parviflora* and *Bidens pilosa*) and their straws on the growth and cadmium (Cd) accumulation of common plant *Cyphomandra betacea* seedlings. Intercropping with *T. erecta* or *B. pilosa* promoted the growth of *C. betacea* seedlings compared with the monoculture, while intercropping with *S. photeinocarpum* or *G. parviflora* inhibited that. Intercropping with *S. photeinocarpum* decreased the Cd contents in the roots and shoots of *C. betacea* seedlings compared with the monoculture, but intercropping with the other plants did not. In the straw mulch experiment, the straw of *S. photeinocarpum* or *T. erecta* promoted the growth of *C. betacea* seedlings compared with the control, while the straw of *G. parviflora* or *B. pilosa* did not. The straw of *S. photeinocarpum* or *T. erecta* decreased the Cd contents in the shoots of *C. betacea* seedlings, and the straw of *G. parviflora* or *B. pilosa* increased the shoot Cd contents. Thus, intercropping with *S. photeinocarpum* and applying *S. photeinocarpum* or *T. erecta* straw can reduce the Cd uptake of *C. betacea*.

1. Introduction

The intercropping usually promotes or inhibits the growth of plants when the two plants' roots contact each other (Li et al., 2008). The root exudates of plants can change the soil heavy metal forms to reduce or enhance their bioavailability (Tatár et al., 1998; Yang et al., 2000). Intercropping also enhances the heavy metal uptake of plants without enhancing the bioavailability of soil heavy metal (Lin et al., 2014d). There are three results for the heavy metal uptake of intercropping plants: the uptake of both plant species is enhanced (Lin et al., 2014e; Tang et al., 2016), the uptake of one plant species is enhanced while the other is reduced (Whiting et al., 2001; Liu et al., 2005), and no obvious changes in uptake occur in the two plant species (Yang et al., 2003). Thus, intercropping must not promote or inhibit the heavy metal uptake in plants, and only suitable combinations of plants could enhance or reduce the heavy metal uptake. Intercropping with heavy metal hyperaccumulator plants may remedy heavy metal-contaminated soil by reducing the heavy metal contents in crops for safe production (Whiting et al., 2001; Liu et al., 2005).

Returning a crop's straw to the field is the usual procedure in agricultural production (Wang and Zhou, 2013). This can improve the physical structure of the soil, increase the organic matter and mineral element contents in the soil, and improve the soil enzyme activities, resulting in increased crop yield and quality (Huang et al., 2012; Zhu et al., 2010; Song et al., 2016). Under heavy metal-contaminated soil condition, applications of hyperaccumulator straw, accumulator straw or tolerant plant straw have different effects on the heavy metal uptake of different plant species. When applying the straw of the Cd-hyperaccumulator plant *Youngia erythrocarpa* to Cd-contaminated soil, the Cd-extraction capabilities of the hyperaccumulator plant *Galinsoga parviflora* and accumulator plant *Capsella bursa-pastoris* are significantly enhanced, and applying the straws of other hyperaccumulator plant species (*Bidens pilosa*, *Solanum photeinocarpum* or *Sigesbeckia orientalis*) reduces the Cd-extraction capabilities of these two plant species or has no obvious effects (Wang et al., 2016b; Hu et al., 2015). The straws of the Cd-accumulator plant species *Cardamine hirsuta* and *Conyza*

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canadensis significantly increase the Cd-extraction capability of *G. parviflora*, but significantly reduce the Cd-extraction capability of *C. bursa-pastoris* (Tang et al., 2015; Lin et al., 2015). The Cd-tolerant plant *Mazus japonicus*' straw improves the Cd extraction capability of *G. parviflora* (Lin et al., 2014c), but reduces the Cd-extraction capability of *C. bursa-pastoris* (Wang et al., 2016a). Thus, like the intercropping, only a suitable plant straw could enhance or reduce the heavy metal uptake of other plants.

Cyphomandra betacea is a Solanaceae fruit tree with agricultural and ornamental values, and it is rich in protein and mineral elements (Mey et al., 1969). However, with the development of industry and agriculture, farmland soil has become seriously contaminated by heavy metals, and the safety, quality and yield of fruits in some orchards have been influenced by heavy metals, especially Cd (Li et al., 2006; Tang et al., 2011). The main cultivated areas of *C. betacea* in China are Yunnan and Guizhou provinces, where have been contaminated by Cd (Yuan et al., 2012). In previous studies, the applications of straws from the Cd-accumulator plant species *C. canadensis* and the Cd-tolerant plant *Plantago asiatica* decrease the Cd contents in *C. betacea* seedlings, but the straws of other Cd-accumulator plant species (*Trifolium repens*, *Eclipta prostrata* and *Stellaria media*) and Cd-tolerant plants (*Ranunculus sieboldii*, *Digitaria sanguinalis* and *Clinopodium confine*) increase the Cd contents (He et al., 2016, 2017). In this study, the seedlings and the straws of Cd-hyperaccumulator plant species *S. photeinocarpum* (Zhang et al., 2011), *Tagetes erecta* (Rungruang et al., 2011), *G. parviflora* (Lin et al., 2014a) and *B. pilosa* (Sun et al., 2009) were used to study the effects of living hyperaccumulator plants and their straws on the growth and Cd accumulation of *C. betacea* seedlings under Cd-contaminated soil conditions. The aim of the study was to determine whether both hyperaccumulator living plants and their straws could affect the growth and the Cd accumulation of *C. betacea* seedlings, and which hyperaccumulator plant could reduce the amount of Cd accumulated in *C. betacea* seedlings to provide a reference for the phytoremediation of soils contaminated with heavy metals.

2. Materials and methods

2.1. Materials

The inceptisol soil (Purple soil in the Genetic Soil Classification of China) samples used in the experiment were collected from the Ya'an campus farm of Sichuan Agricultural University (29°59′ N, 102°59′ E) in May 2014. The basic properties of the soil and its Cd content are described in Lin et al. (2014b): pH 7.02, organic matter 41.38 g kg^{-1}, total nitrogen (N) 3.05 g kg^{-1}, total phosphorus (P) .31 g kg^{-1}, total potassium (K) 15.22 g kg^{-1}, alkali soluble N 165.30 mg kg^{-1}, available P 5.87 mg kg^{-1}, and available K 187.03 mg kg^{-1}. The total Cd content was 0.101 mg kg^{-1} and the available Cd content was 0.021 mg kg^{-1}. The soil samples were air-dried and passed through a 5-mm sieve. Then, 3.0 kg of air-dried soil was weighed and placed into each polyethylene pot (15 cm high, 18 cm diameter). According to other crops intercropping under Cd stress, such as hyperaccumulator plants intercropping with cherry seedlings (Lin et al., 2014b) and different varieties of radish mutual intercropping (Lin et al., 2014d); the straw mulching under Cd-contaminated soil conditions, such as using tolerant plant straw and accumulator straw to affect Cd accumulation of *Cyphomandra betacea* seedlings (He et al., 2016, 2017), Cd was added to the soil samples as $CdCl_2 \cdot 2.5H_2O$ at a dose of 10 mg kg^{-1}. Soil moisture was maintained at 80% of field capacity for one month.

The seeds of *C. betacea* were collected from a three-year-old tree in October 2013, and stored at a constant temperature of 4 °C before the experiment. The seeds of *C. betacea* were directly sown in the Ya'an campus farm (uncontaminated soil) in May 2014, and the seedlings of *C. betacea* were transplanted into pots when they grew four euphyllas.

The seedlings of Cd-hyperaccumulator species *S. photeinocarpum*, *T. erecta*, *G. parviflora* and *B. pilosa* with four euphyllas were collected from the Ya'an campus farm (uncontaminated soil) in June 2014.

The shoots of Cd-hyperaccumulator species *S. photeinocarpum*, *T. erecta*, *G. parviflora* and *B. pilosa* were also collected from the Ya'an campus farm (uncontaminated soil) in October 2013, dried at 75 °C to a constant weight, and then cut into small pieces of ~ 1 cm.

2.2. Intercropping experiment

The intercropping experiment was conducted at the Ya'an campus farm from June to August in 2014. In June 2014, five treatments were applied: the monoculture of *C. betacea*, *C. betacea* intercropped with *S. photeinocarpum*, *C. betacea* intercropped with *T. erecta*, *C. betacea* intercropped with *G. parviflora* and *C. betacea* intercropped with *B. pilosa*. Three uniform *C. betacea* seedlings were transplanted into each pot of the monoculture, and the intercropping treatments consisted of two uniform *C. betacea* seedlings and one hyperaccumulator plant seedling. Three plants of each pot evenly distributed in three corners, and the spacing between any two plants was 15 cm. Each treatment was repeated six times. The soil moisture was maintained at 80% of field capacity during the plant seedlings' growth process. At 40 days after planting (August 2014), *C. betacea* and hyperaccumulator plants were harvested.

2.3. Straw mulch experiment

The straw mulch experiment was conducted at the Ya'an campus farm from July to September in 2014. In July 2014, three uniform *C. betacea* seedlings were transplanted into each pot, and the straws of four hyperaccumulator plant species were independently mulched on the soil surface at 2 g kg^{-1} (6 g pot^{-1}) (Lin et al., 2014c). Three plants of each pot evenly distributed in three corners, and the spacing between any two plants was 15 cm. The five treatments applied in the experiment were the control (no straw applied) and the straw of each of the four hyperaccumulator plant species (*S. photeinocarpum*, *T. erecta*, *G. parviflora* and *B. pilosa*). Each treatment was repeated six times. The soil moisture was maintained at 80% of field capacity during the *C. betacea* seedlings' growth process. At 40 days after planting (September 2014), all of the *C. betacea* were harvested.

2.4. Sample analysis

The third or fourth mature leaves from the top of plants were collected to determine the activities of SOD, POD and CAT (Hao et al., 2004), and the photosynthetic pigment (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid) contents were measured (Hao et al., 2004). The plants were then harvested, and the soils in the centre of each pot were collected (repeated six times). The roots, stems and leaves were washed with tap water, further washed with deionized water three times, and then dried at 80 °C to a constant weight for dry weight determination. The plant samples were finely ground for digestion, and the Cd concentrations were determined using an iCAP 6300 ICP spectrometer (Thermo Scientific, Waltham, MA, USA) (Bao, 2000). The pot soils were air-dried and ground into powder (granule diameter, 1 mm) to determine the soil pH levels and the available Cd concentrations. Soil pH was measured in a 1:2.5 (w/v) suspension of soil and deionized water, and the available Cd in the soil was extracted with DTPA-TEA and analysed with an iCAP 6300 ICP spectrometer (Bao, 2000).

2.5. Statistical analyses

Statistical analyses were conducted using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). The results were statistically analysed by one-way ANOVA with Duncan's multiple range test at the $p = 0.05$ confidence level. The translocation factor (TF) = Cd content in shoots/ Cd content in roots (Rastmanesh et al., 2010).