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Effects of Colchicines on Cadmium Accumulation of Post Generation of *Bidens pilosa*

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Abstract: The pot experiment was conducted to study the effects of colchicines on cadmium (Cd) accumulation of post generation of *Bidens pilosa*. The 0.3 % of colchicines treating with 48 h did not change the number of chromosomes in two ecotypes (farmland ecotype and mining ecotype) of *B. pilosa*. The colchicines treatments increased the biomass, photosynthetic pigment content, Cd contents and Cd accumulation of post generation of *B. pilosa* compared with their respective control. For farmland ecotype of post generation of *B. pilosa*, the Cd accumulation amount in roots and shoots increased by 16.30% and 29.82%, respectively compared with the control; for mining ecotype of post generation of *B. pilosa*, the Cd accumulation amount in roots and shoots increased by 2.67% and 25.01%, respectively compared with the control. Therefore, the colchicines could promote the growth and Cd accumulation of post generation of *B. pilosa*.

Introduction

Cadmium (Cd) is one of the most toxic heavy metals, and is widely present in the soil [1]. The industrial waste, excessive mining and irrational agricultural measures have led to the increase of Cd concentration in soil [2]. As a non-essential element, the excessive accumulation of Cd in the crop will lead to a decline in crop yield and quality, and which will enter into the human body through the food chain [3]. So, reducing the uptake and accumulation of Cd in crops and ensuring the safety of agricultural production is imminent.

Bidens pilosa is a Cd-hyperaccumulator [4] with strong resistance and fecundity, and a broad distribution worldwide [5]. Compared with other hyperaccumulator or accumulator species such as *Solanum nigrum* [6] and *Siegesbeckia orientalis* [7], the remediation ability of *B. pilosa* is low and requires improvement. To enhance the potential of *B. pilosa* to remediate Cd-contaminated soil, two ecotypes (farmland ecotype and mining ecotype) of *B. pilosa* were treated with 0.3% of colchicines. The aim of the study was to determine whether the colchicines could enhance the phytoremediation ability of post generation of *B. pilosa*, and to provide a reference for improving the phytoremediation ability of other hyperaccumulator or accumulator species.

Materials and Methods

Materials. Seeds of two ecotypes (mining and farmland) of *B. pilosa* were collected from the Tangjiashan lead-zinc mine (29°24′N, 102°38′E, with typical dry-hot valley climate) and farmland of the Ya'an campus farm of the Sichuan Agricultural University (29°59′N, 102°59′E, with humid subtropical monsoon climate), respectively, in August 2013. The seeds were air dried and stored at 4 °C.

Colchicines Treatment. The seeds of two ecotypes of *B. pilosa* were sown in farmland of the Ya'an campus farm in March 2014. When the first pairs of plant euphyllas expanded, 0.3% of colchicines treated the top of stems, and used the plastic film covering to keep moisture for 48 h. When the seeds of *B. pilosa* mature, they were collected, air dried and stored at 4 °C. After microscopic examining the chromosome of post generation of *B. pilosa*, the 0.3 % of colchicines treating with 48 h did not change the number of chromosomes in two ecotypes of *B. pilosa*.

Experimental Design. The experiment was conducted in the greenhouse of the Ya'an campus farm from February to June 2015. The soil samples were air dried and passed through a 5-mm sieve. Three kilograms of the air-dried soil was weighed into each polyethylene pot (18 cm high and 21 cm in diameter). Cd was added to soils as $CdCl_2{\cdot}2.5H_2O$ at 10 mg/kg [8] in February 2015. The soil moisture was maintained at 80 % of field capacity for 2 months. The seeds of two ecotypes of post generation of *B. pilosa* were sown in farmland of the Ya'an campus farm in March 2015. Four uniform seedlings of *B. pilosa* (two pairs of euphyllas expanded) were transplanted into each pot in April 2015, and the soil moisture content was maintained at 80 % of field capacity from the time the plants were transplanted into the pots until the time the plants were harvested. Treatments were replicated four times. 2 months later (June 2015), the upper mature leaves of *B. pilosa* were collected to determine the photosynthetic pigment (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid) contents [9]. The plants were then gently removed from the soil, and the roots, stems, and leaves were washed with deionized water and dried at 80 °C to constant weight for dry weight and Cd content determination [10].

Statistical analyses. Statistical analyses were conducted using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way analysis of variance with least significant difference (LSD) at the p = 0.05 confidence level. The following calculated were used: translocation factor (TF) = Cd content in shoots/ Cd content in roots [11]; translocation accumulation factor (TAF) = (Cd content in shoots×biomass in shoots) / (Cd contents in roots × biomass in roots) [12].

Results and Discussion

Biomass. The biomass of mining ecotype of *B. pilosa* was higher than the farmland ecotype of *B. pilosa* (Table 1). The colchicines treatments increased the root, stem, leaf and shoot biomasses of two ecotypes of *B. pilosa* compared with their respective control. For the farmland ecotype of *B. pilosa*, the colchicines treatments increased the root and shoot biomasses by 12.58% ($p < 0.05$) and 10.29% ($p < 0.05$) respectively compared with the control. For mining ecotype of *B. pilosa*, the colchicines treatments increased the root and shoot biomasses by 1.66% ($p > 0.05$) and 11.39% ($p < 0.05$) respectively compared with control. The root/ shoot ratio of mining ecotype of *B. pilosa* was higher than the farmland ecotype of *B. pilosa* (Table 1). The colchicines treatments improved the root/ shoot ratio of farmland ecotype of *B. Pilosa*, and reduced the root/ shoot ratio of mining ecotype of *B. pilosa.*

Table 1 Biomass of post generation of *Bidens pilosa*

Treatments	Roots (g/plant)	Stems (g/plant)	leaves (g/plant)	Shoots (g/plant)	Root/ shoot ratio
Farmland control	0.453±0.0071c	0.913±0.0071d	1.118±0.011c	2.031±0.018c	0.223
Mining control	0.541±0.014ab	0.971±0.0071c	1.215±0.021bc	2.186±0.028b	0.248
Farmland treatment	0.510±0.014b	1.001±0.013b	1.239±0.027ab	2.240±0.040b	0.228
Mining treatment	0.550±0.013a	1.140±0.014a	1.295±0.021a	2.435±0.035a	0.226

Photosynthetic Pigment Content. The chlorophyll *a* content in mining ecotype of *B. pilosa* was higher than the farmland ecotype of *B. pilosa*, but there were no significant differences of the chlorophyll *b*, total chlorophyll and carotenoid contents between two ecotypes of *B. pilosa* (Table 2). The colchicines treatments increased the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid in two ecotypes of *B. pilosa*. The colchicines treatments increased total chlorophyll contents in farmland ecotype of *B. pilosa* by 39.25% ($p < 0.05$) compared with the control, and by 28.02 ($p < 0.05$) for that in mining ecotype of *B. pilosa* compared with the control. The chlorophyll a/b of mining ecotype of *B. pilosa* was also higher than the farmland ecotype of *B. pilosa* (Table 2). The colchicines treatments reduced the chlorophyll a/b of two ecotypes of *B. pilosa* compared with their respective control.

Table 2 Photosynthetic pigment content in post generation of *Bidens pilosa*

Treatments	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Chlorophyll a/b	Carotenoid (mg/g)
Farmland control	1.233±0.025c	0.357±0.012b	1.590±0.037b	3.454	0.439±0.009b
Mining control	1.357±0.003b	0.374±0.050b	1.731±0.080b	3.628	0.468±0.001b
Farmland treatment	1.636±0.048a	0.578±0.032a	2.214±0.080a	2.834	0.603±0.023a
Mining treatment	1.656±0.011a	0.560±0.036a	2.216±0.047a	2.957	0.596±0.008a

Cadmium Content. The Cd contents in roots, stems, leaves and shoots of mining ecotype of *B. pilosa* were higher than the farmland ecotype of *B. pilosa* (Table 3). The colchicines treatments increased the Cd contents in roots, stems, leaves and shoots of two ecotypes of *B. pilosa* compared with their respective control. For the farmland ecotype of *B. pilosa*, the colchicines treatments increased the Cd contents in roots and shoots by 3.30% ($p < 0.05$) and 17.92% ($p > 0.05$) respectively compared with the control. For mining ecotype of *B. pilosa*, the colchicines treatments increased the Cd contents in roots and shoots by 0.99% ($p < 0.05$) and 12.23% ($p < 0.05$) respectively compared with control. The TF of mining ecotype of *B. pilosa* was higher than the farmland ecotype of *B. pilosa* (Table 3). The colchicines treatments enhanced the TF of two ecotypes of *B. pilosa* compared with their respective control.

Table 3 Cadmium content in post generation of *Bidens pilosa*

Treatments	Roots (mg/kg)	Stems (mg/kg)	Leaves (mg/kg)	Shoots (mg/kg)	TF
Farmland control	187.15 ±4.04a	75.01 ±1.73b	112.41±3.66c	95.60±2.77c	0.511
Mining control	195.32 ±6.00a	77.86 ±0.20ab	131.23 ±1.85b	107.52± 0.98b	0.550
Farmland treatment	193.32 ±4.71a	80.75 ±3.48ab	138.56 ±3.80b	112.73± 3.53ab	0.583
Mining treatment	197.26 ±3.89a	84.84 ±3.05a	152.21± 4.45a	120.67±3.73a	0.612

Cadmium Accumulation. Compared with the farmland ecotype of *B. pilosa*, the Cd accumulation amounts in roots, stems, leaves and shoots of mining ecotype of *B. pilosa* were higher (Table 4). The colchicines treatments increased the Cd accumulation amounts in roots, stems, leaves and shoots of two ecotypes of *B. pilosa* compared with their respective control. For the farmland ecotype of *B. pilosa*, the colchicines treatments increased the Cd accumulation amounts in roots and shoots by 16.30% ($p < 0.05$) and 29.82% ($p > 0.05$) respectively compared with the control. For mining ecotype of *B. pilosa*, the colchicines treatments increased the Cd accumulation amounts in roots and shoots by 2.67% ($p < 0.05$) and 25.01% ($p < 0.05$) respectively compared with control. The TAF of mining ecotype of *B. pilosa* was lower than the farmland ecotype of *B. pilosa* (Table 3). The colchicines treatments enhanced the TAF of two ecotypes of *B. pilosa* compared with their respective control.

Conclusions

The colchicines treatments did not change the number of chromosomes in two ecotypes (farmland ecotype and mining ecotype) of *B. pilosa*. The colchicines treatments increased the biomass, photosynthetic pigment content, Cd contents and Cd accumulation of post generation of *B. pilosa* compared with their respective control. For farmland ecotype of post generation of *B. pilosa*, the Cd accumulation in roots and shoots increased by 16.3% and 29.8%, respectively compared with the control; for mining ecotype of post generation of *B. pilosa*, the Cd accumulation in roots and shoots increased by 2.7% and 25.0%, respectively compared with the control. Therefore, the colchicines could promote the growth and Cd accumulation of post generation of *B. pilosa*, which could use to enhance the phytoremediation ability of *B. pilosa* for Cd-contaminated soil.

Table 4 Cadmium accumulation amount in post generation of *Bidens pilosa*

Treatments	Roots (μg/plant)	Stems (μg/plant)	Leaves (μg/plant)	Shoots (μg/plant)	TAF
Farmland control	84.78±0.51d	68.48±1.04d	125.67±2.82d	194.51±3.87d	2.29
Mining control	105.67±0.48b	75.60±0.36c	159.44±0.53c	235.04±0.89c	2.22
Farmland treatment	98.59±0.33c	80.83±2.45b	171.68±0.99b	252.51±3.44b	2.56
Mining treatment	108.49±0.37a	96.72±2.28a	197.11±2.54a	293.83±4.82a	2.71

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