

Effects of Paclobutrazol on Cadmium Accumulation of *Stellaria Media*

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Abstract: The effects of paclobutrazol (PP333) on cadmium (Cd) accumulation of *Stellaria media* were investigated through a pot experiment. The results showed that the root biomass increased with the increase of PP333 concentrations, but the shoot biomass and total biomass decreased. PP333 enhanced the photosynthetic pigment content, antioxidant enzyme activity and soluble protein content of *S. media*, and decreased the soluble sugar content in shoots of *S. media*. The low concentrations of PP333 (25 and 50 mg/L) increased the Cd content in roots of *S. media*, and high concentrations of PP333 (100 and 200 mg/L) decreased that. The Cd content in shoots increased when applied the treatments of PP333. When applied PP333 on *S. media*, the Cd accumulation amount of roots was higher than the control, and the Cd accumulation amount of shoots and whole plants were lower than the control. Therefore, PP333 could not improve the phytoremediation ability of *S. media*.

Introduction

The paclobutrazol (PP333) is a plant growth regulator, which belongs to the plant growth retardants [1]. For common plants, PP333 promotes the growth of maize seedlings, and reduces the heavy metals in plants, which improves the tolerance of maize seedlings to heavy metals [2]. PP333 also promotes the growth of sweet sorghum seedlings under copper stress, and reduces the absorption of copper [3]. For heavy metal hyperaccumulator plants, PP333 improves the chromium accumulation ability of *Houttuynia cordata* Thunb, and enhances the resistance of plant to chromium [4]. Therefore, the application of PP333 could decrease the heavy metal concentration in common plants, and increase the heavy metal concentration in hyperaccumulator plants.

Stellaria media is a widely distributed Cd-accumulator plant [5]. In this study, we used the different concentrations of PP333 to treat *S. media*, and studied the effects of PP333 on growth and cadmium (Cd) accumulation of *S. media*. The aim of the study was to screen the best PP333 concentration which could enhance the phytoremediation ability of *S. media*, and provided a reference for applying the plant hormones on other hyperaccumulators or accumulators for improving their phytoremediation ability.

Materials and Methods

Materials. The soil samples used in the experiment were inceptisol soil, which were collected from the Ya'an campus farm of Sichuan Agricultural University (29° 59' N, 102° 59' E) in August 2014. The basic properties of the soil, total Cd content and available Cd content in soil are described in the reference of Lin et al. (2014) [6]. The *S. media* seedlings with height of 10 cm were collected from the Ya'an campus farm in October 2014.

Experimental Design. The soil samples were air-dried and passed through a 5-mm sieve. Four kilograms of the air-dried soil was weighed into each polyethylene pot (18 cm high, 21 cm in diameter). Cd was added to soils as CdCl₂•2.5H₂O at 25 mg/kg in August 2014, and the soil moisture was maintained at 80% of field capacity for 2 months. Four uniform *S. media* seedlings were transplanted into each pot in October 2014, and watered every day to keep the soil moisture content

maintaining at 80% of field capacity. When *S. media* seedlings grow one month (November 2014) in Cd contaminated soil, 5 concentrations (0, 25, 50, 100 and 200 mg/L) of PP333 with 4 replicates were sprayed on the leaves of plants for each pot, respectively. The amount of each pot was 25 ml of PP333 solution. After PP333 treatment one month (December 2014), the upper mature leaves of *S. media* were collected to determine the photosynthetic pigment (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid) contents [7]. The upper young shoots (2 cm in length) were collected to determine the superoxide dismutase (SOD) activity, peroxidase (POD) activity, catalase (CAT) activity and soluble protein content [7]. Then, the whole plants were then gently removed from the soil. The treatments of plants are described in the reference of Lin et al. (2014) [6]. The Cd concentrations in roots and shoots were determined using an iCAP 6300 ICP spectrometer (Thermo Scientific, Waltham, MA, USA). The soluble sugar contents in shoots of *S. media* were determined by anthrone colorimetry with dry weight plant samples [7].

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. Root/ shoot ratio = root biomass/ shoot biomass [8], the resistance coefficient = total biomass of treatment group/ control group [9], the bioconcentration factor (BCF) = Cd content in roots (shoots)/ Cd concentration in soil [10], the translocation factor (TF) = Cd content in shoots/ Cd content in roots [11], the transfer amount factor (TAF) = (Cd content in shoots \times shoot biomass)/ (Cd content in roots \times root biomass) [12].

Results and Discussion

Biomass of *S. media*. With the increase of PP333 concentrations, the root biomass of *S. media* increased, but the shoot biomass decreased (Table 1). The whole plant biomass of *S. media* decreased with the increase of PP333 concentrations. At 25, 50, 100 and 200 mg/L PP333, the shoot biomass decreased by 31.75% ($p < 0.05$), 44.51% ($p < 0.05$), 46.57% ($p < 0.05$) and 50.70% ($p < 0.05$) respectively, compared with the control. The root/ shoot ratio increased with the increase of PP333 concentrations, and the resistance coefficient had the trend of decreasing (Table 1). So, PP333 inhibited the growth of *S. media*, but promoted the root growth of *S. media*.

Table 1 Effects of PP333 on biomass of *S. media*

PP333 concentration (mg/L)	Roots (g/plant)	Shoots (g/plant)	Whole plants (g/plant)	Root/ shoot ratio	Resistance coefficient
0	0.462 \pm 0.004e	3.779 \pm 0.029a	4.240 \pm 0.025a	0.122	1.000
25	0.480 \pm 0.004d	2.579 \pm 0.021b	3.059 \pm 0.025b	0.186	0.721
50	0.505 \pm 0.011c	2.097 \pm 0.034c	2.602 \pm 0.026c	0.241	0.614
100	0.537 \pm 0.007b	2.019 \pm 0.020d	2.555 \pm 0.023d	0.266	0.603
200	0.577 \pm 0.002a	1.863 \pm 0.013e	2.440 \pm 0.010e	0.310	0.575

Photosynthetic Pigment Content of *S. media*. PP333 increased the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid (Table 2). With the increase of PP333 concentrations, the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid increased when the dose of PP333 was not more than 100 mg/L, and decreased when the dose of PP333 was more than 100 mg/L. For the chlorophyll *a/b*, PP333 decreased the chlorophyll *a/b* of *S. Media*, and had the trend of decreasing with the increase of PP333 concentrations (Table 2).

Antioxidant Enzyme Activity of *S. media*. PP333 enhanced the antioxidant enzyme activity of *S. media* (Table 3). So, PP333 could improve the resistance of *S. media* to Cd. With the increase of PP333 concentrations, the soluble protein content of *S. media* increased when the dose of PP333 was not more than 50 mg/L, and decreased when the dose of PP333 was higher than 50 mg/L (Table 3). The soluble sugar content in shoots of *S. media* decreased by 16.01% ($p < 0.05$), 12.49% ($p < 0.05$) and 12.46% ($p < 0.05$) at 25, 50 and 100 mg/L PP333, and increased by 4.59% ($p > 0.05$) at 200 mg/L PP333.

Cd Content in *S. media*. When applied PP333 on *S. media*, the low concentrations of PP333 (25 and 50 mg/L) increased the Cd content in roots of *S. media*, and high concentrations of PP333 (100 and 200 mg/L) decreased that (Table 4). For the shoots of *S. media*, the Cd content in shoots increased when applied the treatment of PP333. The Cd content in shoots increased by 4.22% ($p < 0.05$), 8.97% ($p < 0.05$), 17.68% ($p < 0.05$) and 14.59% ($p < 0.05$) at 25, 50, 100 and 200 mg/L PP333 respectively, compared with the control. When the dose of PP333 was 100 mg/L, the Cd content in shoots got the maximum of 50.25 mg/kg. With the increase of PP333 concentrations, the BCF of roots increased when the dose of PP333 was not more than 50 mg/L, and decreased from the dose of 50 mg/L to 200 mg/L (Table 4). The BCF of shoots was higher than the control at 25, 50, 100 and 200 mg/L PP333, and the maximum was at the dose of 100 mg/L. All of the PP333 treatments improved the TF, and the maximum was at the dose of 100 mg/L (Table 4). So, PP333 could promote the Cd transport from roots to shoots.

Table 2 Effects of PP333 on photosynthetic pigment content of *S. media*

PP333 concentration (mg/L)	Chlorophyll <i>a</i> (mg/g)	Chlorophyll <i>b</i> (mg/g)	Total chlorophyll (mg/g)	Chlorophyll <i>a/b</i>	Carotenoid (mg/g)
0	1.272±0.038d	0.434±0.016c	1.706±0.012e	2.931	0.286±0.009d
25	1.413±0.009bc	0.502±0.003b	1.914±0.011c	2.815	0.303±0.005bc
50	1.434±0.022ab	0.512±0.021ab	1.946±0.013b	2.801	0.316±0.003ab
100	1.477±0.039a	0.538±0.023a	2.015±0.012a	2.745	0.323±0.013a
200	1.366±0.017c	0.486±0.008b	1.852±0.014d	2.811	0.297±0.009cd

Table 3 Effects of PP333 on antioxidant enzyme activity of *S. media*

PP333 concentration (mg/L)	SOD activity (U/g)	POD activity (U/g)	CAT activity (U/g)	Soluble protein content (mg/g)	Soluble sugar content (%)
0	123.27±1.94d	833.10±5.14c	16.17±0.35d	13.81±0.17d	8.358±0.617a
25	124.85±0.21d	1064.83±10.41a	17.11±0.38c	18.47±0.46b	7.020±0.116b
50	138.60±1.35b	1072.28±5.37a	20.62±0.01a	20.22±0.78a	7.314±0.097b
100	155.87±4.00a	989.45±3.69b	19.07±0.93b	16.61±0.51c	7.317±0.210b
200	131.65±3.78c	980.93±8.65b	18.50±0.19b	13.91±0.27d	8.742±0.370a

Table 4 Effects of PP333 on cadmium content in *S. media*

PP333 concentration (mg/L)	Roots (mg/kg)	Shoots (mg/kg)	BCF of roots	BCF of shoots	TF
0	138.79±0.03b	42.70±0.02e	5.55	1.71	0.308
25	140.38±1.60b	44.50±0.64d	5.62	1.78	0.317
50	148.48±0.72a	46.53±0.54c	5.94	1.86	0.313
100	125.84±1.25c	50.25±0.22a	5.03	2.01	0.399
200	124.83±1.20c	48.93±0.65b	4.99	1.96	0.392

Cd Accumulation of *S. media*. When applied PP333 on *S. media*, the Cd accumulation amount of roots was higher than the control (Table 5), which was benefit to the improve the phytoremediation ability of *S. media*. The Cd accumulation amount of roots increased by 5.09% ($p < 0.05$), 17.06% ($p < 0.05$), 5.40% ($p < 0.05$) and 12.35% ($p < 0.05$) at 25, 50, 100 and 200 mg/L PP333 respectively, compared with the control. However, the treatment of PP333 decreased the Cd accumulation amount of shoots, which decreased by 28.86% ($p < 0.05$), 39.53% ($p < 0.05$), 37.13% ($p < 0.05$) and 43.60% ($p < 0.05$) at 25, 50, 100 and 200 mg/L PP333 respectively, compared with the control. For the whole plants, the treatment of PP333 decreased the Cd accumulation amount of whole plants compared with the control. The treatment of PP333 also reduced the TAF (Table 5). Therefore, PP333 could not improve the phytoremediation ability of *S. media*.

Table 5 Effects of PP333 on cadmium accumulation in *S. media*

PP333 concentration (mg/L)	Roots (μg/plant)	Shoots (μg/plant)	Whole plants (μg/plant)	TAF
0	64.05±0.48d	161.34±3.40a	225.39±2.93a	2.52
25	67.31±1.26c	114.77±2.45b	182.08±4.70b	1.70
50	74.98±1.07a	97.57±2.74c	172.56±3.67c	1.30
100	67.51±1.46c	101.43±0.44c	168.94±1.90cd	1.50
200	71.96±0.56b	91.16±1.37d	163.12±1.93d	1.27

Conclusions

Applied PP333 on *S. media*, the root biomass increased with the increase of PP333 concentrations, but the shoot biomass and total biomass decreased. PP333 enhanced the photosynthetic pigment content, antioxidant enzyme activity and soluble protein content of *S. media*, and decreased the soluble sugar content in shoots of *S. media*. The low concentrations of PP333 (25 and 50 mg/L) increased the Cd content in roots of *S. media*, and high concentrations of PP333 (100 and 200 mg/L) decreased that. The Cd content in shoots increased when applied the treatment of PP333. When applied PP333 on *S. media*, the Cd accumulation amount of roots was higher than the control, and the Cd accumulation amount of shoots and whole plants were lower than the control. Therefore, PP333 could not improve the phytoremediation ability of *S. media*.

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