

Effects of hyperaccumulator plant straw on antioxidant enzyme activities and osmotic substances and malondialdehyde content of lettuce under cadmium stress

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Abstract. Lettuce (*Lactuca sativa*) is one of the most common green leafy vegetables, which is widely cultivated all over the world, but extremely sensitive to cadmium (Cd) contaminated in soil. The effects of three hyperaccumulator plants straw (*Solanum nigrum*, *Bidens pilosa* and *Galinsoga parviflora*) on the antioxidant enzyme activities and osmotic regulators and malondialdehyde (MDA) content under Cd stress were studied by pot culture. Compared with no straw, three kinds of hyperaccumulator straw of *S. nigrum* straw, *B. pilosa* straw and *G. parviflora* straw increased the content of soluble protein and proline in lettuce, the activity of peroxidase (POD) and catalase (CAT) in lettuce was also increased, and the content of MDA was decreased. The stress resistance of lettuce was improved and the toxicity of Cd to lettuce was alleviated by using three kinds of hyperaccumulator plant straw.

1 Introduction

Straw returning is considered to be one of the most promising eco-agricultural measures in China's cropland and has net mitigation potential, economic benefits, and air pollutant reduction [1]. However, plant straw may release some allelochemical to inhibit plant growth, such as reduce biomass and the content of photosynthetic pigments, while improving soil environment [2]. In farmland, heavy metals content may be increased by diffuse deposition, flowing water, application wastewater, pesticides, and fertilizer [3]. Cadmium (Cd) is a major agricultural problem in soil, and it is not essential element for crop growth but easily ingested and accumulated in plants, causing potential damage to plants [4]. There are many ways to alleviate Cd toxicity. Returning the hyperaccumulator plant straw to the field in this experiment is one of green environmental protection methods. Lettuce (*Lactuca sativa*) is one of the most common green leafy vegetables and widely planted all over the world. It is very sensitive to Cd and is easy to accumulate in edible parts. Moreover, its toxic symptoms are not obvious, which greatly increases the risk of Cd entering human body [5]. *Solanum nigrum*, *Bidens pilosa* and *Galinsoga parviflora* are common hyperaccumulator plants in farmland, which have the characteristics of convenient sampling, large number and wide distribution. This experiment explored the effects of hyperaccumulator plant straw on antioxidant enzyme activities and osmotic substances and MDA content of lettuce under Cd pollution; it provides a new idea for leaf vegetables to improve their resistance to heavy metals.

2 Materials and Methods

2.1. Materials

In June 2015, three kinds of Cd hyperaccumulator plants were collected from the surrounding farmland (not contaminated by heavy metals) in the Sichuan Agriculture University: *S. nigrum*, *B. pilosa* and *G. parviflora*, after removing the seeds, they are washed with distilled water. After 15 minutes of killing fresh at 105 °C, they are dried to constant weight at 75 °C. Cutting the dried straw into about 1 cm segments and set aside.

Lettuce is a common glass lettuce in Sichuan, and it has high purity, good quality, good tolerance and resistance, and can be planted all year round and sustainable harvest for more than 50 days.

The tested soil was paddy soil, which was taken from farmland not polluted by heavy metals around Chengdu campus of Sichuan Agricultural University. Its basic physical and chemical properties are as follows: pH 6.36, organic matter 18.10 g/kg, total nitrogen 1.04 g/kg, total phosphorus 1.36 g/kg, total potassium 20.78 g/kg, hydrolyzable nitrogen 43.90 mg/kg, available phosphorus 92.71 mg/kg, available potassium 134.25 mg/kg, available Cd not detected. CdCl₂·2.5H₂O analytical pure solution was added to the tested soil for heavy metal Cd.

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2.2. Experimental design

In June 2015, the air-dried and crushed soil was sifted through 300 meshes and loaded into barrels, each containing 20 kg. The Cd concentration was 10 mg/kg by adding analytical pure CdCl₂·2.5 H₂O solution [7], after 30 days of moist storage, all contaminated soils are fully blended and packed in a pot of 3 kg per pot.

Full lettuce seeds were select for disinfection, evenly spread them on wet gauze, put them into 20 °C light incubator for germination, and then sow them in a pot dish with nutritive soil to raise seedlings. After one week of lettuce seedling raising, and three kinds of hyperaccumulator plant straw were applied to the prepared Cd contaminated soil, 6 g per pot, and mixed evenly, with no straw as the control. The experiment was divided into four treatments: no straw, *S. nigrum* straw, *B. pilosa* straw and *G. parviflora* straw. After a week of balancing and watering, selected healthy lettuce seedlings and transplant them into Cd-contaminated soil prepared with straw. Four plants were transplanted in each pot and nine pots were treated in each pot. After transplanting, the seedlings were placed in plastic greenhouse, and the soil moisture was maintained at about 80%. After the lettuce grew 50 d, antioxidant enzyme activities and osmotic substances and MDA content of lettuce were determined.

2.3. Index determination

Superoxide dismutase (SOD) activity was determined by NBT method; the activity of peroxidase (POD) was determined by guaiacol method; catalase (CAT) activity was determined by ultraviolet absorption method [7]. According to Bradford's method, the soluble protein

content was determined by Coomassie brilliant blue method. The content of proline was determined by sulfosalicylic acid method, the content of soluble sugar was determined by anthrone colorimetry, determination of MDA by thiobarbituric acid [7].

2.4. Statistical analyses

All data are collated with Excel 2010 software; SPSS 20.0 was used for variance analysis and Duncan's new complex range method was used for multiple comparisons.

3 Result

3.1. Effects of soil application of hyperaccumulator plant straw on antioxidant enzyme activities in lettuce leaf

The activities of antioxidant enzymes in lettuce leaves increased after the application of hyperaccumulator plant straw (Table 1). The activity of SOD in lettuce leaves treated with *B. pilosa* straw increased significantly by 20.04% compared with the control ($P < 0.05$). As far as POD activity of lettuce leaves was concerned, the application of *S. nigrum* straw, *B. pilosa* straw and *G. parviflora* straw increased 63.13% ($P < 0.05$), 20.00% ($P < 0.05$) and 13.06% ($P < 0.05$) respectively compared with the control. The CAT activity of lettuce leaves from high to low was as follows: *B. pilosa* straw > *G. parviflora* straw > *S. nigrum* straw > no straw. The CAT activity of lettuce leaves was significantly increased by applying *B. pilosa* straw, which was 20.88% higher than that of the control ($P < 0.05$).

Table 1. Effects of hyperaccumulate plant straw on antioxidant enzyme activity of lettuce

Plant straw	SOD activity (U/g)	POD activity (U/g)	CAT activity (U/g)
No straw	104.37±4.99c	370.36±25.45d	24.62±2.08d
<i>S. nigrum</i> straw	100.08±10.33d	604.18±28.28a	27.96±1.27c
<i>B. pilosa</i> straw	125.29±5.35a	444.45±28.28b	29.76±2.12a
<i>G. parviflora</i> straw	105.63±3.21b	418.72±31.11c	28.53±1.27b

Different lowercase letters in the same column indicate significant difference ($P < 0.05$).

3.2 Effects of soil application of hyperaccumulator plant straw on osmotic substances and malondialdehyde (MDA) content in lettuce

Application of hyperaccumulator plant straw reduced the MDA content of lettuce leaves, and increased the soluble protein and proline content of lettuce (Table 2). As far as MDA content was concerned, the treatments with *S. nigrum* straw, *B. pilosa* straw and *G. parviflora* straw were significantly lower than those of the control, which decreased by 2.12% ($P < 0.05$), 30.02% ($P < 0.05$) and

15.06% ($P < 0.05$), respectively. The application of hyperaccumulator plant straw increased the soluble protein content of lettuce, the soluble protein content of lettuce with *B. pilosa* straw was the highest, 47.63% higher than that of the control ($P < 0.05$), followed by the treatment with *G. parviflora* straw, 29.64% higher than that of the control ($P < 0.05$). The order of soluble sugar content in lettuce from high to low was: *S. nigrum* straw > no straw > *B. pilosa* straw > *G. parviflora* straw. As far as proline content was concerned, the treatment with *G. parviflora* straw had the highest proline content, which increased by 186.59% compared with the control ($P < 0.05$), followed by *B. pilosa* straw and *S. nigrum* straw.

Table 2. Effects of hyperaccumulate plant straw on the Contents of osmotic substances and MDA of lettuce

Plant straw	MDA content (nmol/g)	Soluble protein content ($\mu\text{g/g}$)	Soluble sugar content (%)	Proline content ($\mu\text{g/g}$)
No straw	10.36 \pm 0.63a	88.66 \pm 6.68d	0.47 \pm 0.01b	11.63 \pm 0.41d
<i>S. nigrum</i> straw	10.14 \pm 0.12b	102.91 \pm 7.87bc	0.59 \pm 0.01a	19.15 \pm 0.41c
<i>B. pilosa</i> straw	7.25 \pm 0.65d	130.89 \pm 12.34a	0.45 \pm 0.01b	28.41 \pm 1.23b
<i>G. parviflora</i> straw	8.80 \pm 0.24c	114.94 \pm 10.80b	0.38 \pm 0.03c	33.33 \pm 0.82a

Different lowercase letters in the same column indicate significant difference ($P < 0.05$).

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4 Conclusion

Under Cd stress, the toxicity of Cd to lettuce was alleviated by applying hyperaccumulator plants straw (*S. nigrum*, *B. pilosa* and *G. parviflora*) in soil. The activity of antioxidant enzymes and the content of osmotic regulators were increased, and the content of MDA was decreased. Under the condition of Cd pollution, the application of three kinds of hyperaccumulate plant straw can alleviate the toxicity of heavy metals to vegetables, and the reduction of Cd content in soil by application hyperaccumulator plants and the safe production of vegetables need to be further studied.

References

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