Chinese Science Bulletin 2005 Vol. 50 No. 1 33-38

# A newly-discovered Cd-hyperaccumulator *Solanum nigrum* L.

### WEI Shuhe, ZHOU Qixing, WANG Xin, ZHANG Kaisong, GUO Guanlin & MA Lena Qiying

Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China Correspondence should be addressed to Zhou Qixing (e-mail: zhouqixing2003@yahoo.com, shuhewei@tom.com)

Abstract A systematic investigation was conducted to screen for cadmium-hyperaccumulator from 54 species in 20 weed families using outdoor pot-culture experiment and small-scale field experiment. The results from the outdoor pot-culture experiment showed that Cd concentrations in the stems and leaves of Solanum nigrum L. growing in a soil spiked with 25 mg/kg Cd were up to 103.8 and 124.6 mg/kg (DW), respectively, which was greater than 100 mg/kg, minimum Cd concentration for a Cd-hyperaccumulator. The Cd enrichment factor (EF, concentration ratio in plant to soil) in shoots was as high as 2.68. Moreover, Cd accumulation in shoots was greater than that in roots (TF, concentration ratio in shoots to roots) and the plant biomass growth was not inhibited at the Cd concentrations tested compared with the control. The results of the small-scale field experiment also showed that the characteristics of Cd accumulation in S. nigrum were all consistent with the characteristics of Cd-hyperaccumulators. Thus S. nigrum can be classified as a Cd-hyperaccumulator. This work is important for further research in the areas of hyperaccumulators screening, and plant-tolerance physiology and evolution. It provides a patentable new plant species for phytoremediation of Cd-contaminated soils.

Keywords: hyperaccumulator, cadmium, *Solanum nigrum* L., remediation of contaminated soils.

### DOI: 10.1360/982004-292

Phytoremediation emerged in early the 1980s is an important technology for remedying contaminated sites. One of the most promising phytoremediation technologies is phytoextraction using hyperaccumulators to remove heavy metals from contaminated soils. The term hyperaccumulator was first used by Brooks et al.<sup>[11]</sup> to refer to plants that can accumulate more than 1000 mg/kg Ni dry weight (DW) in their shoots. Although more than 400 hyperaccumulators have been reported<sup>[2]</sup>, so far traction technology has not been applied to remediate contaminated soils. This is partially due to the low efficiency of these known hyperaccumulators in absorbing pollutants from soils. Thus, identification of more effective hyperaccumulators is still a key step for successful phytoreme-

diation.

In recent years, much researches on hyperaccumulators has been conducted in China<sup>[3]</sup>. However, hyperaccumulators with application patent owned by the People's Republic of China for phytoremediation are still scarce. In addition, the area of farmland contaminated by heavy metals is as large as  $2.5 \times 10^7$  hm<sup>2</sup> and agricultural products polluted by heavy metals account for approximately  $1.2 \times 10^7$  t in a year in China. Traditional technologies such as physical and chemical remediation are difficult to apply to such a large area of contaminated soils, especially considering their economical and technological acceptance. Thus, remediation of contaminated soils in China has been  $slow^{[3]}$ . With advantages such as simplicity in operation and applicability to large scale with economical and technological feasibility, phytoremediation has emerged as an irreplaceable technology for remedying contaminated sites containing low concentration of pollutants, making its commercial application feasible in China<sup>[4]</sup>. Undoubtedly, the study on identification of hyperaccumulators in China is still of significance in both theory and practice. Thus, a systematic screening investigation of hyperaccumulator from weed species in agricultural fields in Shenvang area was carried out using outdoor pot-culture method and small-scale field experiment.

#### 1 Materials and methods

() Screening of weed species for Cd-hyperaccumulator. All experiments were carried out in Shenyang Station of Experimental Ecology, the Chinese Academy of Sciences (123°41′N and 41°31′E) and tested plants were collected from agricultural fields in the station and its surrounding area. The station is located at a temperate zone with 3100—3400 of the annual accumulative temperature higher than 10 , and the frostless duration of 127—164 d in a year. Burozem distributed in the station is a relatively clean compared with the National Soil-Environmental Quality Standard of China (NSEQSC, GB 15618, 1995)<sup>[5]</sup>. Soil samples were collected from the surface (0—20 cm) from fields with concentrations of heavy metals below the background concentrations.

The single Cd treatment (T<sub>1</sub>) and the mixed Cd, Pb, Cu and Zn treatment (T<sub>2</sub>) were designed according to the current situation of soil pollution in China and the NSEQSC. In treatment T<sub>1</sub>, concentration of Cd was added at 10 mg/kg, whereas in treatment T<sub>2</sub>, concentrations of Cd, Pb, Cu and Zn were added at 10, 1000, 400, and 1000 mg/kg, respectively. The concentrations of added Cd, Pb, Cu and Zn are 10, 2, 1 and 2 times of the values of the third level standards of the NSEQSC, respectively, which correspond to the current levels of heavy metals in contaminated soils in northeast China<sup>[6,7]</sup>. The added heavy metals were CdCl<sub>2</sub> · 2.5H<sub>2</sub>O, Pb(CH<sub>3</sub>COO)<sub>2</sub> · 3H<sub>2</sub>O, CuSO<sub>4</sub> · 5H<sub>2</sub>O, and ZnSO<sub>4</sub> · 7H<sub>2</sub>O, respectively. Mean-

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while, the treatment with no heavy metal addition is regarded as the control  $(CK_1)$ .

During spring of 2002, the soil samples were sieved through a 4 mm sieve, then mixed with heavy metal according to the above-mentioned concentrations and filled plastic pots ( $\zeta = 20$ cm, H = 15cm), and equilibrated for 2 weeks. Weed seedlings of similar age were transplanted into the pots with treatments  $CK_1$ ,  $T_1$ , and  $T_2$ . There were 2-6 seedlings (4 seedlings for Solanum nigrum L.) in each pot based on plant size. The number of seedlings for each treatment was kept the same. The pots were put outdoors, so the plants grew under open fields. Loss of water were made up using tap water (no Cd, Pb, Cu and Zn detected) to sustain 80% of soil water-holding capacity. No fertilizer was added. All treatments were replicated three times. The plants were harvested after they reached physical maturity or when the frost started. A total of 54 weed species in 20 families were examined. In particular, there were 13 investigated species in the composite family.

() Identification of Cd-hyperaccumulator using concentration gradient experiment. Based on the outdoor pot-culture experiment, S. nigrum exhibited characteristics of Cd-hyperaccumulator<sup>[8]</sup>. However, the Cd concentration added to the soil was too low for S. nigrum to accumulate over 100 mg/kg of Cd in the shoots, the minimum concentration for a Cd-hyperaccumulator. To further examine the potential of S. nigrum as a Cd-hyperaccumulator, a concentration gradient experiment was carried out. There were 6 treatments including the control (CK<sub>2</sub>) without external Cd addition, and treatments  $R_1$ — $R_5$ , with Cd added as CdCl<sub>2</sub>·2.5H<sub>2</sub>O at 10, 25, 50, 100 and 200 mg/kg Cd, respectively. Two seedlings of S. nigrum of 3.5 cm in height were transplanted into the pots during spring of 2003. The plants were harvested after 88 d when they reached their physiological maturity. The treatment of both soil and plant were the same as the previous experiment.

( ) Confirmation of *S. nigrum* as a Cd-accumulator using small-scale field method. To validate *S. nigrum* as a Cd-hyperaccumulator under field conditions, a small-scale field experiment was carried out concurrent with the concentration gradient experiment. The area consisted of 8 m<sup>2</sup> (Length = 4 m, Width = 2 m). The basic properties of the soil were the same as those of the first experiment. Cd was added to the soil at 50 mg/kg as CdCl<sub>2</sub> · 2.5H<sub>2</sub>O.

An area of 8 m<sup>2</sup> soil was excavated to 50 cm in June, 2003, which is sufficient for the root system of *S. nigrum* to grow in soil spiked with Cd. The air-dried soil was divided into two equal parts after being sieved through a 4 mm sieve. One half was used as the control (CK<sub>3</sub>) when no external Cd was added. The other half was treated with Cd. The soils included the control were placed back in the field with one half as CK<sub>3</sub> and the other half as soil spiked

with Cd. A piece of plastic sheet was used to separate the two treatments. Twenty seedlings of *S. nigrum* 4 cm in height were transplanted into the two plots (each with 10 seedlings) after the treated soils equilibrated for two weeks. A plastic canopy with 80 cm of inner height was used to cover the field to ensure sufficient plant growth before frost in late September. When the plants reached the maturity after growing for 91 d, *S. nigrum* and corresponding soil samples were collected.

() Metal analysis and data processing. Harvested plant samples were divided into roots, stems, leaves and inflorescences and carefully washed with deionized water before rinsing with tap water. The samples were then dried at 105 for 5 min, then at 70 in an oven until completely dried. The dried plant samples were ground to powder. Soil samples were air-dried and ground using a mortar and pestle and passed through a 0.149 mm sieve. The plant and soil samples were digested with a solution containing 87% of HNO<sub>3</sub> and 13% of HClO<sub>4</sub> The concentrations of heavy metals were determined using a Hitachi atomic absorption spectrophotometer (AAS)<sup>[8]</sup>. Soil organic matter was determined using the method of Lu<sup>[9]</sup>. The value of pH was determined using a pH meter (PHS-3B) at soil to water ratio of 1: 2.5. The average and standard deviation (SD) of three replicates for each treatment were calculated using Microsoft Excel software and analysis of variance was performed<sup>[10]</sup>. The results showed that the background concentrations of heavy metals Cd, Pb, Cu and Zn in the tested soil were 0.2, 14.2, 12.4, and 39.9 mg/kg dry weight, respectively, with pH value of 6.5 and organic matter of 1.52 %.

### 2 Results

() Characteristics of *S. nigrum* accumulating Cd. Usually, when the concentration of a heavy metal in soils is not higher than the critical value that affects the growth of a plant, the aboveground biomass of a plant will not decrease significantly even though the concentration of the heavy metal in soils is very high. Once when it exceeds the critical value, would the growth of a plant be adversely inhibited, followed by occurrence of abnormal leaf color, reduction in plant height and decrease in aboveground biomass<sup>[11-13]</sup>.

The results of the outdoor pot-culture experiment showed that the leaf color and plant height of *S. nigrum* growing under the condition of treatment  $T_1$  (10 mg/kg Cd added) and  $T_2$  (10, 1000, 400 and 1000 mg/kg Cd, Pb, Cu and Zn added) did not differ significantly (*P*<0.05) from that of the control (CK<sub>1</sub>). The average plant height of *S. nigrum* in CK<sub>1</sub> was 23.5 cm but the average plant height in  $T_1$  and  $T_2$  was 23.8 and 22.5 cm, respectively. Compared with the control, the aboveground biomass of *S. nigrum* growing in treatment  $T_1$  and  $T_2$  did not decrease significantly (*P*<0.05), suggesting its tolerance to Cd (Fig. 1).



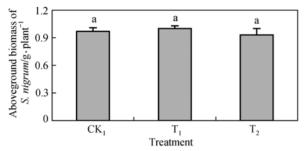


Fig. 1. Aboveground dry biomass of *S. nigrum* in the field pot-culture experiment.

Our data suggested that *S. nigrum* had high tolerance to Cd, typical of a hyperaccumulator<sup>[14]</sup>.

The concentrations of Cd, Pb, Cu and Zn in *S. ni*grum are listed in Table 1. Growing in a soil spiked with 10 mg/kg Cd (T<sub>1</sub>), *S. nigrum* accumulated up to 31.8 mg/kg Cd (DW) in its shoots, which was higher than that in its roots (27.8 mg/kg), i.e. translocation factor (TF, concentration ratio of shoots to roots) was greater than one. The Cd enrichment factor (EF, concentration ratio of plant to soil) in its shoots was up to 3.17. Growing in a soil spiked with Cd-Pb-Cu-Zn (T<sub>2</sub>), *S. nigrum* roughly accumulated same amount of Cd as that in T<sub>1</sub>. In other words, Cd accumulation by *S. nigrum* under T<sub>2</sub> is a feature of a Cd-hyperaccumulator, with Cd EF and TF both greater than one.

Based on its high tolerance to Cd and its ability to accumulate Cd, *S. nigrum* can be considered as a Cd-hyperaccumulator<sup>[8]</sup>.

() The potential of *S. nigrum* endurancing and accumulating Cd. In the outdoor pot-culture experiment with a concentration gradient, the leaf color of S. nigrum did not change significantly (P<0.05) with Cd concentration compared with the control  $(CK_2)$ . The average height of S. nigrum under treatments R3-R5 (Cd addition concentrations = 50, 100 and 200 mg/kg, respectively) was reduced significantly (P < 0.05) to 19.3, 18.0 and 16.5 cm, respectively. The shoots biomass of S. nigrum was also decreased significantly (P<0.05). However, neither the average height nor shoot dry mass of S. nigrum was reduced significantly in treatments R<sub>1</sub> and R<sub>2</sub> (Cd addition concentrations = 10 and 25 mg/kg, respectively) compared with the height of 29.2 cm for CK<sub>2</sub> (Fig. 2), indicating high tolerance of S. nigrum to Cd when growing in a soil containing Cd of 10–25 mg/kg<sup>[3]</sup>. The reduction in its shoots biomass growing in a soil spiked with Cd > 50mg/kg may not necessarily indicate that the plant is not a Cd-hyperaccumulator, implying that 50 mg/kg can be considered as the critical concentration of the plant in improving contaminated soil by Cd. In fact, the key role of S. nigrum in extracting Cd should be attributed to its capability to accumulate Cd even at a reduced biomass.

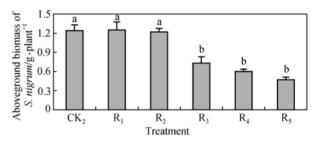


Fig. 2. Aboveground dry biomass of *S. nigrum* growing under the conditions of the concentration gradient experiment.

Treatment	Plant tissue	Cd		Dh (tatal)	C (t-t-1)	7(+++-1)
		Total	EF	Pb (total)	Cu (total)	Zn (total)
CK1	Root	0.1±0.01		0.8±0.09	31.1±0.57	103.2±2.06
	Stem	$0.4{\pm}0.02$		1.3±0.08	6.1±0.36	84.9±1.42
	Leave	$0.5 \pm 0.04$		1.1±0.02	7.2±0.55	33.3±1.46
	Inflorescence	0.3±0.05		$0.2 \pm 0.07$	5.4±0.54	25.8±1.18
	Shoot	$0.3 \pm 0.09$		1.2±0.13	6.8±0.53	47.2±1.89
Tı	Root	27.8±1.14d	2.76±0.11			
	Stem	61.8±1.91b	6.16±0.19			
	Leave	74.4±1.73a	7.41±0.17			
	Inflorescence	10.8±1.59e	$1.07 \pm 0.16$			
	Shoot	31.8±1.07c	3.17±0.11			
T <sub>2</sub>	Root	27.5±1.34d	2.74±0.13	213.7±7.03a	79.8±5.11a	380.4±8.10a
	Stem	59.5±1.49b	5.93±0.15	54.5±3.76c	8.4±0.75b	184.8±7.88b
	Leave	75.1±1.80a	7.48±0.18	75.1±4.32b	12.6±1.87b	101.5±9.95c
	Inflorescence	11.6±0.57e	1.15±0.06	11.6±2.23d	10.7±1.19b	31.4±3.42d
	Shoot	31.6±1.82c	3.15±0.18	51.7±1.13c	11.4±0.55b	73.76±2.37

Table 1 Accumulation of heavy metals by S. nigrum under pot-culture experiment/mg · kg<sup>-la</sup>)

a) EF is enrichment factor, and data are expressed as mean  $\pm$  standard deviation (SD).

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Cadmium concentration in the stems and leaves of *S. nigrum* were 103.8 and 124.6 mg/kg in treatment R<sub>2</sub> (Table 2), which was greater than 100 mg/kg, the criteria for a Cd-hyperaccumulator. The Cd enrichment factor in its shoots was also greater than 1. Furthermore, the Cd concentration in shoots of the plant was higher than that in its roots, i.e. TF>1. Thus, *S. nigrum* growing under a soil spiked with 25 mg/kg Cd has the basic properties of a Cd-hyperaccumulator<sup>[8,15,16]</sup>. With the increase in Cd concentration added to a soil, Cd accumulation by *S. nigrum* also increased, and Cd concentration in its stems and leaves was mostly greater than 100 mg/kg.

The Cd concentration in stems and leaves of *S. ni-grum* reached the criteria of a Cd-hyperaccumulator. In addition, Cd concentration in its shoots was higher than that in its root, and the plant had a strong tolerance to Cd at 25 mg/kg. Based on our data, thus, *S. nigrum* can be considered as a Cd-hyperaccumulating plant.

() Cadmium accumulation in *S. nigrum* under small-scale field experiment. The results of the smallscale field experiment showed that the average concentration of Cd accumulated in roots, stems and leaves was up to 96.3, 129.4 and 194.7 mg/kg, respectively (Table 3) when growing for 91 d in a soil spiked with 50 mg/kg Cd. The Cd enrichment factor in shoots was as high as 2.01. Cadmium accumulation in its shoots was higher than that in its roots. Data of *S. nigrum* obtained in the small-zone field experiment was consistent with the basic properties of a Cd-hyperaccumulator<sup>[2,14,15]</sup>. Furthermore, the results were similar to those under treatment  $R_3$  where Cd was spiked to the soil at 50 mg/kg in the concentration gradient experiment. Cadmium concentrations in roots, stems and leaves of treatment  $R_3$  were 97.4, 135.5, and 194.3 mg/kg with EF of 2.02 (Table 2). Our data obtained using the pot-culture method was consistent with those obtained using the field experiment.

The average aboveground biomass of 10 *S. nigrum* plants under the small-scale field experiment is listed in Table 3. The *t*-test showed that the variance (t = 5.690>2.101, v = 18) in the aboveground biomass of *S. nigrum* surviving in the small-scale field experiment with Cd-addition of 50 mg/kg was similar to that in the concentration gradient experiment. In other words, data obtained using the pot-culture method was consistent with those from the small-scale field experiment.

In summary, *S. nigrum* can be further validated as a Cd-hyperaccumulator by the small-scale field experiment based on established criteria for Cd-hyperaccumulator<sup>[3,16]</sup>.

### 3 Discussion

As for judging standards of hyperaccumulators, the original and the foremost one was the critical concentration of a plant accumulating heavy metals, in other words, the metal contents in stems or leaves of a hyperaccumula-

Treatment	Root	Stem	Leave	Florescence	Shoot	EF <sup>a)</sup>
R <sub>1</sub>	28.8±1.55d	61.5±2.25b	75.5±2.49a	11.2±0.60e	36.6±1.61c	3.63±0.1
$R_2$	59.9±1.11d	103.8±3.50b	124.6±1.63a	23.7±1.43e	67.3±1.43c	2.68±0.0
R <sub>3</sub>	97.4±2.43c	135.5±3.40b	194.3±4.71a	33.3±0.63d	101.1±1.99c	2.02±0.0
$R_4$	113.1±0.65d	203.6±3.57b	264.8±1.11a	40.2±0.91e	131.3±4.91c	1.31±0.0
R <sub>5</sub>	157.4±5.63d	252.4±2.02b	291.4±1.05a	45.3±0.15e	167.2±5.63c	0.83±0.0

Table 2 Cd-accumulating characteristics of S. nigrum under the concentration gradient experiment/mg  $\cdot$  kg<sup>-1</sup>

a) EF is the Cd enrichment factor in the shoots.

Table 3 Cd-accumulation and aboveground biomass of S. nigrum in the small-scale field experiment

Plant –	Cd accumulation/mg $\cdot$ kg <sup>-1</sup>					– EF	Aboveground biomass /g · plant <sup>-1</sup>	
	Root	Stem	Leave	Inflorescence	Shoot	- Er	Treatment	CK <sub>3</sub>
1	96.3	128.6	203.0	38.0	97.0	1.94	4.41	6.54
2	82.7	136.4	189.9	38.3	84.5	1.69	3.19	5.06
3	102.7	132.5	194.2	43.5	104.6	2.13	2.94	5.87
4	92.5	127.3	203.0	47.5	93.7	1.89	3.29	5.67
5	98.9	132.3	193.1	33.2	100.0	2.01	2.97	4.23
6	95.9	123.7	195.0	35.4	98.0	1.97	4.10	6.75
7	93.7	129.9	196.9	34.9	96.1	1.93	3.78	6.15
8	99.7	127.2	191.0	39.4	108.7	2.21	3.03	4.46
9	98.2	126.1	188.2	42.0	105.4	2.14	2.90	4.53
10	102.3	130.1	193.2	34.8	105.1	2.14	4.01	4.62
Average	96.3	129.4	194.7	38.7	99.3	2.01	3.45	5.39
SD	5.81	3.67	5.01	4.52	7.11	0.16	0.56	0.93
CV <sup>a)</sup>	6.03	2.84	2.57	11.68	7.15	7.78	16.08	17.18

a) CV is coefficient of variance, and EF is the Cd enrichment factor in the shoots.

tor are the most important parameter showing its ability to accumulate heavy metals. The minimum Ni concentration of a Ni-hyperaccumulator should be at least 1000 mg/kg, which was first suggested by Brooks et al.<sup>[1]</sup>, and later the standard was lowered to 500 mg/kg<sup>[17]</sup>. Molaisse et al.<sup>[18]</sup> recommended 1000 mg/kg for a Cu-hyperaccumulator. while Reeves<sup>[19]</sup> suggested 10000 mg/kg for a Zn-hyperaccumulator based on the fact that Zn concentration in plants can easily exceed 1000 mg/kg. After extensive researches, Chaney et al.<sup>[14]</sup> pointed out that, for a Cd-hyperaccumulator, the criterion should be 100 mg/kg. In addition, hyperaccumulating plants should have higher translocation ability and hyper-tolerance of heavy metals. However, Wenzel et al.<sup>[20]</sup> suggested that 50 mg/kg can be used for a Cd-hyperaccumulator due to the lack of Cd-hyperaccumulating plants based on 100 mg/kg. At present, the standards for hyperaccumulators have essentially become unanimous. Hyperaccumulators should share two basic properties: the critical concentration property and the translocation property. The critical contents of heavy metals accumulated in stems or leaves of hyperaccumulators are extensively used as reference values suggested by Baker and Brooks with 10000 mg/kg for Zn and Mn, 1000 mg/kg for Pb, Cu, Ni, Co and As, 1 mg/kg for Au and 100 mg/kg for  $Cd^{[15]}$ . The translocation property is described that the contents of heavy metals accumulated in shoots (including stems and leaves) of a plant should be higher than those in its roots, i.e. TF>1. On the basis of our work, endurance property and enrichment factor property should be considered as judging standards of hyperaccumulators too<sup>[3,8,21]</sup>. The enduring characteristic means that hyperaccumulators should have strong endurance to heavy metals contamination. For plants tested under experimental conditions, the aboveground biomass (the sum of dry stems, leaves and inflorescence) of hyperaccumulators should not significantly decreased compared with the control when they are growing in soils contaminated by heavy metals seriously. At least the aboveground biomasses of plants should not be significantly reduced when the polluted levels in soils are high enough to make the contents of heavy metals absorbed by plants reaching the critical concentration standards what hyperaccumulators should accumulate. Enrichment factor characteristic is identified as the concentration ratio in plant shoots to soils should be higher than 1, and at least the EF should be higher than 1 when the content of a heavy metal in soil is roughly equal to the minimum of a hyperaccumulator. According to these standards, S. nigrum can be validated as a Cd-hyperaccumulator, because the plant displayed strong tolerance to and translocation ability of Cd. Specifically, enrichment factor of Cd in shoots was greater than 1 and the accumulation of Cd in stems and leaves of the plant exceeded 100 mg/kg when the concentration of Cd added to soil was 25 mg/kg.

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Thlaspi caerulescens has been studied extensively as the only Cd hyperaccumulator until now. According to Baker et al.<sup>[22]</sup>, the average Cd concentration accumulated by T. caerulescens growing in a metal mine area was 164 mg/kg. Brown et al.<sup>[23]</sup> reported that Cd concentration in leaves of T. caerulescens was 1800 mg/kg when Cd content of in soil was 1020 mg/kg. In particular, Cd accumulation by T. caerulescens growing in south France was up to 2800 mg/kg<sup>[24]</sup>. Zhao et al.<sup>[25]</sup> pointed out that the significant differences in metal-accumulation among species of the same plant can be attributed to different ecotypes of a plant. After having regarded Arabidopsis thaliana as a model plant of gene, some researchers are attempting to consider T. caerulescens as a model plant of hyperaccumulator to further explore hyperaccumulating phytophysiology, biochemistry and molecular biology in view of its simultaneous hyperaccumulation of Zn and Ni besides Cd<sup>[26]</sup>. Using an agar-based medium, Gardea-Torresdey et al.<sup>[27]</sup> demonstrated that *Convolvulus arvensis* has a strong ability to accumulate Cd. The Cd concentrations in stems and leaves of C. arvensis were all 750 mg/kg, and that in its roots was 3000 mg/kg when Cd concentration in the growing medium was 20 mg/L. Rosa et al.<sup>[28]</sup> also examined Cd accumulation of Salsola kali using an agar-based medium. When Cd concentration in the culturing medium was 20 mg/L, Cd accumulation in stems, leaves and roots of S. kali was 2075, 2016 and 2696 mg/kg, respectively. However, these plants do not meet the basic properties of hyperaccumulators although their Cd accumulation was very high. If the biomass is very huge, this kind of plant mentioned above would also show effective remediation role to contaminated soil for their higher concentrations of heavy metals. Thus, Assuncao et al. [29] thought that Cannabis sativa has a potential to be used for remediation of Cd-contaminated soils. When Cd contents in soils were 26.6 and 82.0 mg/kg, Cd accumulation in stems, leaves and roots of C. sativa was 18.1, 9.4, 109.2 and 58.8, 73.0, 1368.2 mg/kg, respectively. The plant biomass was 10.0  $t/hm^2$  and root systems stretched to a depth of 0.5 m. In order to enhance the efficiency of phytoremediation, some researchers also attempt to apply amendments to promote the uptake of a hyperaccumulator to heavy metals. Ethylene diamine tetracetic acid (EDTA) was used by Turgut et al.<sup>[30]</sup> to strengthen the ability of two cultivars of *Helian*thus annuus remedying Cd. They reported that the concentration of Cd accumulated by the two plants was increased significantly after 0.1 g/kg of EDTA was added to the medium. Although many researches have involved in research on Cd-hyperaccumulators and some plants which can extract Cd from contaminated soils effectively, there are seldom practical examples in application of hyperaccumulating plants to remediate contaminated soils by Cd. Obviously, the road from the identification of hyperaccumulators to successful phytoremediation practice is very

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far. We think that the adoption of modern agricultural technology combined with phytoremediation would be a short cut for commercial application of phytoremediation<sup>[31]</sup>. Although *S. nigrum* has been identified as a new Cd-hyperaccumulating plant in this study, research on the matching technology related to the Cd-hyperaccumulator is necessary in the future. However, the plant — *S. nigrum* surviving in unpolluted ecosystem showed hyperaccumulative characteristics only after a short-term induced by heavy metal, which may have some significances in screening hyperaccumulators even in the studies of plant tolerant physiology, inheritance and evolution.

**Acknowledgements** This work was supported by the National Natural Science Foundation of China as the outstanding young scientist grant (Grant No. 20225722) and as the overseas young scientist grant (Grant No. 20428707).

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(Received September 7, 2004; accepted December 6, 2004)