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BRIEF COMMUNICATION

An assessment of *Agropyron cristatum* tolerance to cadmium contaminated soil

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Abstract

A pot experiment was conducted in a greenhouse to assess the tolerance of *Agropyron cristatum* plants to cadmium contaminated soils (0, 5, 10, 25, 50, 100, 150, and 200 mg kg^{-1}) for 100 d. Results indicate that Cd in concentrations of 5 - 50 mg kg^{-1} had no significant impact on growth, relative membrane permeability (RMP), lipid peroxidation measured as malondialdehyde (MDA) content, and chlorophyll (Chl) content relative to the control. Exposure of these plants to high concentrations of Cd (100 - 200 mg kg^{-1}) caused a small reduction in growth and Chl content and a slight enhancement of RMP and MDA content compared with the control. In addition, superoxide dismutase (SOD) and peroxidase (POD) activities show an increasing trend with the increase of Cd content in soil. The Cd content in the roots was 4.7 - 6.1 times higher than that in the shoots under all Cd treatments suggesting that the plant can be classified as a Cd excluder. The translocation factor was low and similar at 25 - 200 mg kg^{-1} Cd treatments. In summary, *A. cristatum* plants tolerated Cd stress and might have potential for the phytoremediation of Cd contaminated soils.

Additional key words: antioxidant enzyme, chlorophyll, malondialdehyde, membrane permeability, phytoremediation, translocation factor.

Cd pollution of soil has become a serious environmental concern because it is associated with health risks to plants, animals, and humans. Cd-contaminated soils can be remediated by chemical and physical techniques. However, remediation of Cd-contaminated soils is very costly, destructive, and limited to small areas by using traditional physiochemical methods (Padmavathiamma and Li 2007). Therefore, we urgently need other effective methods to clean Cd contaminated soils.

There is evidence that phytoremediation is cost effective and non-invasive technology (Simon 2005). It takes advantage of the remarkable ability of plants to exclude metals from a site by accumulation in roots or by precipitation in root zones (Zhang *et al.* 2010). Some mechanisms of Cd detoxification are recognized, such as phytochelation, intracellular sequestration, active efflux from the roots, and induction of antioxidant machinery and stress proteins (Cobbett and Goldsbrough 2002, Bočová *et al.* 2012). However, different plant species and cultivars show a wide range of plasticity in Cd tolerance, from high sensitivity to the hyper-accumulation (Belkhadi *et al.* 2010, Tian *et al.* 2012). For example, legume plants are less tolerant to Cd toxicity than cereals and grasses (Metwally *et al.* 2003). Previous study shows that perennial rye-grass (*Lolium perenne*), due to accumulating heavy metals, can grow on heavy metal polluted soil and may be suitable for phytostabilisation (Wen and Fu 2008). *Agropyron cristatum,* a perennial monocotyledonous grass found in the semi-arid area of north China, has strong resistance to the drought and cold stresses. However, whether *A. cristatum* has potential for use in Cd contaminated soils remains uncertain.

To assess the potential of *A. cristatum* for phytoremediation of Cd contaminated soils, the physiological and

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Abbreviations: Chl - chlorophyll; MDA - malondialdehyde; POD - peroxidases; RMP - relative membrane permeability; SOD - superoxide dismutase; TF - translocation factor.

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biochemical changes, the antioxidant enzymes, and the characteristics of Cd accumulation and tolerance in plants subjected to different concentrations of Cd were investigated in this study.

Pot experiments were conducted in a greenhouse. The soil was collected in the Beijing Academy of Agriculture and Forestry Sciences. It had pH of 6.98, the available N of 119.3 mg kg^{-1}, P of 12.5 mg kg^{-1}, K of 200.5 mg kg^{-1}, the organic matter of 2.8 mg kg^{-1}, and total Cd of 0.83 mg kg^{-1}. After being air-dried, the soil was passed through a 5 mm sieve, and then 3 kg of the sieved soil was placed into the plastic pots (21.5 cm diameter × 18.5 cm height). Cd in the form of $CdC1_2$**.** 2.5 H_2O was added to the pots at concentrations of 0, 5, 10, 25, 50, 100, 150, and 200 mg kg^{-1}. The seeds of *Agropyron cristatum* (L.) Gaertn. were provided by *Clover Group* in China which collected them from inner Mongolia where this species is less variable than in other regions (Che *et al*. 2008). The 50 seeds of *A. cristatum* with an average germination rate over 95 % were sown into non-Cd-contaminated soil or the Cd-contaminated soil described above. The pots were arranged in completely randomized block design, each treatment consisted of 5 pots containing 40 plants each. To keep soil moisture at 70 % of field water holding capacity, all the pots were irrigated daily with tap-water to a constant mass. The experiments were conducted in the greenhouse under a 12-h photoperiod, natural irradiance, and maximum and minimum temperatures of 30 and 20 °C, respectively. After 100 d of growth, the plants were dug up, washed thoroughly with tap water followed by deionized water, the shoot height was measured, and the leaf samples were frozen in liquid nitrogen for storage at -70 °C until further use for biochemical analyses.

Lipid peroxidation in terms of MDA formation was measured by the method of Dhindsa *et al.* (1981) with slight modifications. Supernatant (1 cm^3) was mixed with 4 cm^3 of 20 % (m/v) trichloroacetic acid containing 0.5 % (m/v) thiobarbituric acid, and centrifuged at 15 000 *g* and 4 °C for 30 min. Test-tubes were heated at 95 °C for 30 min, then quickly cooled in an ice bath, and the precipitate was removed by centrifugation. The absorbances at 450, 532, and 600 nm were determined using a *UV-T6* spectrophotometer (*Persee*, Beijing, China). Leaf cell membrane damage was determined as the RMP of leaf cells using a conductivity meter (*EC215*, *Hanna*, Rome, Italy) according to the method described by Gibon *et al.* (1997) with slight modifications. Leaf tissue (100 mg) was vibrated for 30 min in deionised water followed by measurement of conductivity of bathing medium (EC_1). Then, the samples were boiled for 15 min and the final conductivity (EC_2) of the medium was measured. Finally, the RMP was calculated by using the formula: EC_1/EC_2.

Chl content was estimated using the modified method of Ma *et al.* (2012). Fresh leaf samples were crushed thoroughly with 80 % (v/v) acetone in the dark and centrifuged at 12 000 *g* and 4 °C for 10 min. The absorbances of the solution were recorded at 645 and 663 nm by the above mentioned spectrophotometer.

For analysis of antioxidant enzyme activities, 200 mg of fresh leaves was homogenized in 4 cm^3 of phosphate buffer (50 mM, pH 7.0) containing 1 % (m/v) soluble polyvinylpyrrolidone and 0.2 mM ascorbic acid. The homogenate was centrifuged at 15 000 *g* and 4 °C for 10 min and the supernatant was then used for the enzyme assays. Protein content was determined according to the method of Bradford (1976) with bovine serum albumin as a standard. The activity of SOD (EC 1.15.1.1) was determined according to the method described previously (Jiang and Huang 2001). The POD (EC 1.11.1.7) activity was measured at 470 nm as guaiacol oxidation by H_2O_2 (coefficient of absorbance 26.6 mM cm^{-1}) according to Chance and Maehly (1955).

The harvested plants were washed thoroughly with running distilled water, divided into shoots and roots, and oven dried at 80 °C for 3 d till constant mass. The dried plant tissues were weighed, ground, wet digested in H_2SO_4/HNO_3 mixture (1/5, v/v) for 24 h, and then treated with $HNO_3/HClO_4$ mixture (5/1, v/v). The Cd concentration in the digest was measured by an atomic absorption spectrophotometer (*AA6300C*, *Shimadzu*, Kyoto, Japan). The translocation factor (TF) values were estimated according to the following equation described by Shi *et al.* (2009): TF = [Cd in shoot] /[Cd in root].

All the data were subjected to one-way analysis of variance (*ANOVA*) using *SPSS 13.0* (*SPSS Inc*., Chicago, IL, USA). Duncan's multiple range tests were used to detect significant differences between means at $P < 0.05$.

Plant biomass is an important parameter for assessment of Cd accumulation in phytoremediation. The addition of Cd in the concentrations of 5 - 50 mg kg^{-1} had no impact on the shoot and root dry mass compared with the control (Table 1). Similar trends were also observed for the shoot height. The shoot and root dry masses and shoot height decreased remarkably at higher Cd concentrations (100 - 200 mg kg^{-1}), however, no significant differences were observed between these Cd treatments (Table 1).

The low Cd treatments (5 - 50 mg kg^{-1}) had no significant impact on the Chl *a* and Chl *b* content, MDA content, and RMP compared to the control (Tables 1, 2). However, the high Cd treatments (100 - 200 mg kg^{-1}) significantly reduced the Chl (*a*+*b*) content and the maximum reduction (about 37 %) was observed at 200 mg(Cd) kg^{-1}. Both MDA content and RMP showed an increasing trend at high Cd concentrations (Tables 1, 2).

The activity of SOD increased significantly with the increase of the Cd concentrations relative to the control reaching its maximum at 200 mg kg^{-1}. Similar pattern was also observed in the changes of the POD activity (Table 2).

Cd accumulations in the plants increased proportionally to the increase of Cd application (Table 2). Under Cd concentrations from 5 to 200 mg kg^{-1}, the Cd content ranged from 16.56 to 78.68 mg kg^{-1} in the shoots, and from 78.76 to 443.19 mg kg^{-1} in the roots. Thus, the Cd content in the roots was about 4.7 - 6.1 fold higher than that in the shoots. Furthermore, the TF values were less than 1 ranging from 0.21 to 0.16. Notably, the increase in Cd concentration from 25 to 200 mg kg^{-1} had no impact on the TF values (Table 2).

Table 1. Effect of different Cd concentrations in soil on some growth parameters and content of MDA and Chl in *A. cristatum* at 100 d after sowing. Values are means ± SE, $n = 5$. Different letters in individual columns indicate significant difference at $P < 0.05$.

Cd [mg kg^{-1}]	Shoot height [cm]	Shoot d.m. [g plant^{-1}]	Root d.m. [g plant^{-1}]	MDA [nmol g^{-1}(f.m.)]	Chl *a* [mg g^{-1}(f.m.)]	Chl *b* [mg g^{-1}(f.m.)]	Chl (*a*+*b*) [mg g^{-1}(f.m.)]
0	49.53±3.21a	0.37±0.021a	13.05±0.86b	21.08±1.06b	0.74±0.013a	0.38±0.011a	1.12±0.021a
5	51.24±2.83a	0.38±0.014a	12.83±1.12b	20.81±1.32b	0.72±0.014a	0.35±0.009a	1.07±0.018a
10	52.21±2.58a	0.41±0.025a	12.53±1.09b	19.83±1.19b	0.73±0.009a	0.36±0.015a	1.09±0.023a
25	53.53±3.65a	0.42±0.022a	12.68±0.66b	21.31±0.83b	0.69±0.022a	0.34±0.013a	1.03±0.031a
50	50.56±2.62a	0.39±0.018a	12.12±1.28b	20.12±0.98b	0.71±0.016a	0.37±0.012a	1.08±0.042a
100	41.67±2.18b	0.33±0.011b	16.32±1.31a	27.32±1.63a	0.52±0.012b	0.29±0.019b	0.81±0.026b
150	40.16±1.89b	0.31±0.012b	17.92±0.72a	28.81±1.02a	0.48±0.021b	0.27±0.014b	0.75±0.033b
200	38.92±2.37b	0.29±0.018b	19.16±1.23a	30.83±1.41a	0.47±0.028b	0.26±0.016b	0.73±0.039b

Table 2. Effect of different Cd concentrations in soil on RMP, the activities of SOD and POD, Cd content in shoots and roots, and TF values in *A. cristatum* at 100 d after sowing. Values are means ± SE, $n = 5$. Different letters in individual columns indicate significant difference at $P < 0.05$.

Cd [mg kg^{-1}]	RMP	SOD [U mg^{-1} protein]	POD [μmol mg^{-1}(prot.) min^{-1}]	Cd content in shoot [mg kg^{-1}(d.m.)]	Cd content in root [mg kg^{-1}(d.m.)]	TF values
0	0.24±0.011b	132.95±5.76f	3.24±0.16g	-	-	-
5	0.25±0.008b	162.72±4.81e	5.27±0.11f	16.56± 7.85f	78.76±10.85f	0.21±0.009a
10	0.27±0.012b	203.26±5.52d	6.12±0.09e	28.37± 8.39e	153.25± 9.39e	0.19±0.005b
25	0.28±0.014b	251.86±6.23c	7.15±0.07d	31.46± 9.31de	187.58± 9.31de	0.17±0.007b
50	0.26±0.013b	263.34±4.28c	7.21±0.18d	38.34±10.21d	218.92±11.21d	0.18±0.008b
100	0.47±0.023a	289.18±5.28b	7.99±0.17c	52.18±11.12c	306.28±14.12c	0.17±0.007b
150	0.48±0.018a	309.41±6.47b	8.61±0.14b	63.72± 9.89b	386.63±15.89b	0.16±0.009b
200	0.52±0.015a	332.82±4.72a	10.63±0.12a	78.68±10.06a	443.19±16.06a	0.18±0.008b

It has known that Cd does not generate ROS directly but it induces oxidative stress by interference with the antioxidant defense system (Sanita di Toppi and Gabrielli 1999). Under heavy metal stress, the ROS (O_2^-, OH^-, and H_2O_2) are formed and cause lipid peroxidation in biological membranes (Apel and Hirt 2004). Therefore, the plant cell membranes are generally considered as primary sites of metal injury. RMP and MDA content are the markers of the membrane integrity and lipid peroxidation. Cd-induced increase in RMP and MDA content were reported for some plant species (Lozano-Rodriguez *et al.* 1997, Chien *et al.* 2001). In this study, both the MDA content and RMP show an increasing trend after the Cd application but their values were similar at different Cd concentrations (Table 1). This suggests that the plants suffered only with a small Cd-induced oxidative damage due to enhancement of cell antioxidant systems which counteract oxidative stress (Sgherri *et al.* 2003, Tao *et al.* 2012). SOD is an important ROS-scavenging antioxidant enzyme (Ekmekçi *et al.* 2008) and we found stimulation of the SOD activity with the increasing concentrations of Cd (Table 2). In addition, among the H_2O_2 destroying enzymes, the Cd stress resulted in the enhancement of POD activity (Table 2). POD, which also participates in lignin biosynthesis, may build up a physical barrier against toxic heavy metals (Krantev *et al.* 2008). When plants are subjected to Cd stress, antioxidant enzymes respond in a coordinated manner to combat the Cd-induced oxidative stress (Podazza *et al.* 2012). The coordinated enhancement of the SOD and POD activities in *A. cristatum* alleviated possible damage to the structure of chloroplast, inhibition of synthesis of Chl, and finally caused a lower rate of Chl degradation.

The mechanism of metal tolerance in plants includes metal exclusion and accumulation (Hall 2002, Martin *et al.* 2012). Shi *et al.* (2009) reported that hemp plants strongly limited translocation of Cd to shoots suggesting that such Cd immobilization in root cells is an exclusion strategy. Our data shows that most Cd absorbed in the *A. cristatum* plants was mainly retained in the roots and only a small amount was transported to the shoots (Table 2). Therefore, *A. cristatum* is a Cd excluder, and Cd may be mainly bound in the roots thus contributing to regulating or minimizing its translocation to the shoots (Kim *et al.* 2007). The low Cd treatments (5 - 50 mg kg^{-1}) had no significant impact on the shoot and root biomasses and the shoot height (Table 1). Only little reduction in the root and shoot biomasses or the shoots height was observed after exposure of these plants to the high concentrations of Cd (100 - 200 mg kg^{-1}). Similar findings were reported by

Brunner *et al.* (2008) and Domínguez *et al.* (2009). Notably, *A. cristatum* plants survived and grew normally in the Cd contaminated soil without showing any toxic symptoms, especially at 200 mg kg^{-1} Cd (data not shown). Based on these results, *A. cristatum* plants had innate tolerance to the Cd stress. Mendez and Maier (2008) reported that the TF is important for estimating the potential of a plant for the phytoremediation. Plant candidates should possess an extensive root system and a large amount of biomass in the presence of high concentrations of heavy metals although keeping low TF (Alvarenga *et al*. 2008). Taken together, *A. cristatum* could be regarded as a candidate species for the phyto-remediation of Cd contaminated soil.

In conclusion, the exposure of *A. cristatum* to Cd caused little reduction of the biomass and the Chl content but an increase in the activities of SOD and POD. Most of the Cd absorbed by the *A. cristatum* plants was retained in the roots and only a small amount was transported to the shoots. These results suggest that the *A. cristatum* plants are Cd tolerant and suitable for phytoremediation of contaminated soils.

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