TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2013) 37: 950-955

doi:10.3906/bot-1110-9

Cadmium detoxification in *Populus* × *canescens*

Hui-Ping DAI[1], Chan-Juan SHAN[2], Genliang JIA[3], Chao LU[4], Tu-Xi YANG[5], An-Zhi WEI[5,*]

[1]College of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, Shaanxi, P.R. China
[2]Henan Institute of Science and Technology, Xinxiang, P.R. China
[3]College of Science, Northwest Agriculture and Forestry University, Yangling, Shaanxi, P.R. China
[4]College of Agronomy, Northwest Agriculture and Forestry University, Yangling, Shaanxi, P.R. China
[5]College of Forestry, Northwest Agriculture and Forestry University, Yangling, Shaanxi, P.R. China

Received: 12.10.2011 • **Accepted:** 29.04.2013 • **Published Online:** 06.09.2013 • **Printed:** 30.09.2013

Abstract: Cadmium is one of the most toxic heavy metals and affects all viable cells, even at low concentrations. It is introduced to agricultural soils mainly by phosphate fertilisers and causes many toxic symptoms in cells. Phytochelatins (PCs) are heavy metal-binding peptides that play an important role in sequestration and detoxification of heavy metals in plants. In this study, after a 12-week cultivation, the plants were treated with 5 CdSO4·7 H_2O concentrations of 0 (control), 10, 30, 50, and 70 μM for 28 days. The effects of Cd on growth and on glutathione (GSH) and PC contents were investigated in the roots, bark, wood, and leaves of *Populus* × *canescens*. The accumulation of Cd increased with external Cd concentrations. The accumulation of Cd in roots was higher than that in bark, wood, and leaves. Dry mass production of different tissues decreased under Cd treatment, especially for leaves. In roots, bark, wood, and leaves, exposure to Cd caused an appreciable decline in GSH contents and an increase in PC synthesis proportional to Cd concentrations in the growth medium. At the same Cd concentration, PC production was higher in roots than in bark, wood, and leaves. Taken together, the majority of Cd was accumulated by *Populus* × *canescens* roots in the form of a Cd-PC complex.

Key words: Cadmium accumulation, cadmium tolerance, glutathione, phytochelatins

1. Introduction

Cadmium is one of the most hazardous and ubiquitous trace elements present in the soil and is toxic for all biological systems (Prashant & Girjesh, 2010; Tran & Popova 2013). Its levels have increased in the environment due to anthropogenic activities, including the expansion of industry and agricultural practices such as waste water irrigation, application of sewage sludge, and excessive use of fertilisers and pesticides. Cadmium can be easily taken up by plant roots and transported to above-ground tissue organs (Shamsi et al., 2008; White & Brown, 2010; Dai et al., 2011, 2012a, 2012b, 2012c, 2013). High concentrations of Cd are toxic to plants because they can lead to the inhibition of germination and root elongation, leaf chlorosis and withering, and reduction of plant biomass (Wang et al., 2008). Cadmium can also disturb plant metabolic processes and lead to root growth retardation, suberisation, damage to internal and external root structures, decrease in root hydraulic water conductivity, interference with nutrient absorption, and translocation. Thus, Cd can lead to nutrient imbalance, decrease chlorophyll content, interfere with enzymatic activities related to photosynthesis, and decrease stomatal openings and conductance (Vandecasteele et al., 2003; Benavides et al., 2005).

Phytochelatins (PCs) are well known as the principal heavy metal-detoxifying peptides in plants, fungi, and microalgae (Grill et al., 1985). Phytochelatins form a family of peptides with a structure based on repetitions (n = 2–11) of the γ-Glu-Cys dipeptide followed by C-terminal glycine. PCs are structurally related to glutathione (GSH), which is a substrate for their synthesis (Cobbett, 2000a; Clemens, 2006; Dai et al., 2011; Wójcik & Tukiendorf, 2011). PCs are enzymatically synthesised from the substrate GSH in response to heavy metal exposure (Grill, 1985). Furthermore, PCs are synthesised enzymatically from reduced GSH. The reaction, catalysed by a c-glutamylcysteine dipeptidyl transpeptidase (EC 2.3.2.15, commonly referred to as phytochelatin synthase), is activated by metal ions, especially Cd^{2+} (Grill et al., 1989). Thus, plants can withstand Cd toxicity by maintaining high levels of phytochelatin or its precursor, GSH, which functions as a heavy metal ligand (Cánovas et al., 2004). Upon heavy metal exposure, GSH concentrations drop as a consequence of PC biosynthesis initiation.

* Correspondence: weianzhi@126.com

Previous studies indicated that GSH may contribute in several other ways to heavy metal tolerance besides its involvement in PC biosynthesis. It may sequester toxic metal ions in the cytosol, and such complexes may activate PC synthase, transfer metal ions to newly synthesised PCs, or transport them to the vacuole (May et al., 1998; Xiang et al., 2001). Interestingly, recent findings of Wojas et al. (2008) suggested that the high ability of PC synthesis in transgenic tobacco plants over-expressing an *Arabidopsis thaliana* (L.) Heynh. PC synthase is insufficient to cope with the metal load if the antioxidant system is simultaneously hampered.

In the present study, Cd tolerance and accumulation in different tissues of grey poplar was investigated. The aim of this study was to validate whether a PC-based transport mechanism was involved in Cd^{2+} translocation to the roots, bark, wood, and leaves in grey poplar.

2. Materials and methods

2.1. Cultivation of plants and Cd exposure

The experiments were performed in the orchard of the Northwest Agriculture and Forestry University, Yangling (34°20′N, 108°24′E), P.R. China. Plantlets of *Populus* × *canescens* (*P. tremula* × *P. alba*) were produced by micropropagation (Leplé et al., 1992) and cultivated in a climate chamber (day/night temperature, 25/18 °C; relative air humidity, 50%–60%; light per day, 16 h; photosynthetic photon flux, 150 μmol m^{-2} s^{-1}). The nutrient solution included 5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 5 mM KNO_3, 2 mM $MgSO_4 \cdot 4H_2O$, 1 mM KH_2PO_4, 0.1 mM EDTA-Fe, 461 mM H_3BO_3, 9.11 mM $MnCl_2 \cdot 4H_2O$, 0.321 mM $CnSO_4 \cdot 5H_2O$, 0.761 mM $ZnSO_4 \cdot 7H_2O$, and 0.51 mM $H_2MoO_4 \cdot H_2O$ (Leplé et al., 1992). After 4 weeks, the rooted plantlets were transferred to an aerated Hoagland nutrient solution in a growth room with the same environmental conditions as the climate chamber. The nutrient solution was exchanged every 3 days. After a 12-week cultivation, the plants were treated with 5 $CdSO_4 \cdot 7\ H_2O$ concentrations of 0 (control), 10, 30, 50, and 70 μM $CdSO_4$ by adding $CdSO_4$ to the nutrient solution.

2.2. Analysis of Cd content

To analyse Cd in different tissues, fine powder (ca. 100 mg) from roots, stems, and leaves was digested in a mixture (7 mL of concentrated HNO_3 and 1 mL of concentrated $HClO_4$) at 170 °C according to the method of Schützendübel et al. (2001). Subsequently, Cd contents in extracts were determined by flame atomic absorbance spectrometry (Hitachi 180-80, Japan). A standard curve was prepared using a series of diluted solutions of a commercially available standard (National Criterion Solutions, National Analysis Centre, Beijing, China).

2.3. Determination of glutathione

Extraction and measurement of GSH was carried out according to the method of Griffith (1980). The different tissue materials were homogenised in 5% (m/v) sulphosalicylic acid, and the homogenate was centrifuged at 10,000 × *g* for 10 min. A volume of 1 mL of supernatant was neutralised using 0.5 mL of potassium phosphate buffer (pH 7.5). Change in absorbance at 412 nm was measured (E = 4.2 mM^{-1} cm^{-1}).

2.4. Determination of PC content

Plant material frozen in liquid nitrogen was extracted in 5% sulphosalicylic acid and 6 mM diethylenetriamine-pentaacetic acid by the method of Zhang (2005). The homogenate was centrifuged at 10,000 × *g* and 4 °C for 10 min to remove cellular debris and precipitated proteins. Total nonprotein thiols in the supernatant were then quantitated spectrophotometrically with Ellman's reagent at 412 nm by the method of Zhang et al. (2005).

2.5. Statistical analysis

The experiment had a completely randomised design with 6 replicates per treatment for each time point. Data were subjected to analysis of variance (ANOVA) to examine the effect of time, treatment, and clone. Statistical analysis was conducted using CoStat 6.2 (CoHort Software, CA, USA). Separation of means was performed by LSD test at $\alpha = 0.05$ significance level.

3. Results

3.1. Cadmium accumulation

In the roots, bark, wood, and leaves, the amount of Cd increased with the increase in Cd concentrations in the growing medium and time of exposure (Table 1). For almost all Cd treatments the Cd concentration per gram of plant dry weight was higher in roots (5- to 19-fold) compared to the control. Taking into account the leaf, bark, wood, and root biomass, the amount of Cd was higher in leaves (1- to 5-fold), bark (3- to 10-fold), and wood (3- to 5-fold), compared to the control. Taken together, the exposure of *Populus* × *canescens* to 10, 30, 50, and 70 μM $CdSO_4$ led to significant increases in Cd concentrations as follows: roots > wood > bark > leaves.

3.2. Biomass accumulation

To analyse the toxic effects of Cd on plant growth, the root, bark, wood, and leaf biomass was recorded (Table 2). Plant growth decreased with increases in Cd concentration after 28 days of treatment. The leaves showed the highest biomass, followed by roots, wood, and bark.

3.3. Effect on PC synthesis and accumulation

The concentrations of PCs and GSH in roots, bark, wood, and leaves of plants exposed to different Cd treatments are shown in Table 3. Increasing intracellular Cd concentrations induced the accumulation of PCs.

Table 1. Cd content of *Populus* × *canescens* grown with 0 (control), 10, 30, 50, and 70 μM Cd for 28 days. Values are means of 6 replicates ± standard deviation. ** and * represent significant differences at α = 0.01 and 0.05, respectively.

Organ	Cadmium concentration (μM L^{-1})				
	0	10	30	50	70
Leaves	4.8 ± 0.9**	7.8 ± 1.2**	10.8 ± 1.4**	14.7 ± 1.6**	26.7 ± 1.7**
Bark	6.9 ± 0.8**	22.1 ± 1.3**	55.1 ± 1.6**	65.3 ± 1.5**	74.5 ± 1.8**
Stem	1.8 ± 0.3**	6.1 ± 0.5**	6.8 ± 0.6**	7.6 ± 0.7*	9.2 ± 0.6**
Roots	225.6 ± 8.4*	1136.2 ± 52.1*	1461.6 ± 61.2*	2060.1 ± 46.6**	4461.8 ± 58.3**

Table 2. Leaf dry weight (LDW), bark dry weight (BDW), wood dry weight (WDW), and root dry weight (RDW) of *Populus* × *canescens* grown with 0 (control), 10, 30, 50, and 70 μM $CdSO_4$ for 28 days. Values are means ± standard error (n = 6). The g unit represents dry mass. ANOVA α values for the primary effects and interactions of metal × origin (M × O) are provided.

Cd treatment	LDW (mg g^{-1})	BDW (mg g^{-1})	WDW (mg g^{-1})	RDW (mg g^{-1})
0 μM	2.33 ± 0.18*	0.67 ± 0.06*	1.3 ± 0.06*	1.55 ± 0.08*
10 μM	2.17 ± 0.58*	0.60 ± 0.17*	1.23 ± 0.31*	1.47 ± 0.34*
30 μM	2.05 ± 0.43*	0.55 ± 0.12*	1.19 ± 0.25*	1.37 ± 0.29*
50 μM	1.82 ± 0.34*	0.51 ± 0.09*	0.94 ± 0.17*	1.21 ± 0.17*
70 μM	1.49 ± 0.18*	0.43 ± 0.06*	0.87 ± 0.06*	1.16 ± 0.08*

Biomass	Primary effects		Interactions
	Metal	Origin	M × O
ANOVA α values			
Leaves	0.000	0.000	0.009
Bark	0.000	0.000	0.002
Wood	0.000	0.000	0.000
Roots	0.000	0.000	0.003
Total	0.000	0.000	0.000

PCs were detected after 28 days of Cd exposure and increased with Cd concentrations and exposure time (Table 3). The increasing concentrations of Cd caused a continuous decrease in GSH contents, while PC levels remained high. Furthermore, the roots showed the highest PC concentrations, followed by bark, wood, and leaves. In roots, Cd hypertolerance was due to a reduction in the uptake of metals and promotion of chelation and sequestration.

4. Discussion

It has been reported that *Populus* × *canescens* root is able to accumulate Cd in a time- and dose-dependent manner (Dai et al., 2012a). Restricted transport of Cd from root to shoot (bark and wood) is often accompanied by a higher Cd tolerance. Conversely, it seems that enhanced translocation may be the reason for increasing sensitivity. In the present study, we also found that the highest growth inhibition and the most distinct Cd toxicity symptoms

Table 3. Changes in GSH and PC concentrations in *Populus* × *canescens* after 28 days at 0 (control), 10, 30, 50 and 70 μM $CdSO_4$ exposure. Each point represents the mean of 6 biological replicates ± standard error. ANOVA α values for the primary effects and interactions of metal × origin (M × O) are provided.

	Organ	Cd treatment (μM)				
		0	10	30	50	70
GSH nmol g^{-1} FW	Leaves	48.8 ± 3.3**	43.1 ± 2.6	41.6 ± 2.2	34.5 ± 2.1	33.3 ± 1.3
	Bark	36.6 ± 2.3*	33.6 ± 2.5	32.2 ± 1.8	31.0 ± 1.7	28.3 ± 1.5
	Stem	30.6 ± 2.8*	29.0 ± 2.1	26.7 ± 1.6	25.2 ± 1.4	22.9 ± 1.2
	Roots	28.8 ± 2.6*	27.3 ± 2.5	26.1 ± 2.1	25.1 ± 1.1	23.0 ± 1.3
PCs μmol g^{-1} FW	Leaves	16.7 ± 11.7	44.4 ± 12.2	48.1 ± 10.8	54.6 ± 7.5	76.9 ± 14.9
	Bark	180.7 ± 3.7	198.8 ± 4.4	214.9 ± 6.9	227.5 ± 4.1	321.2 ± 5.6
	Stem	15.3 ± 2.9	26.3 ± 2.7	40.1 ± 3.9	48.8 ± 4.0	56.6 ± 4.9
	Roots	23.8 ± 2.3	53.2 ± 3.6	68.1 ± 4.1	72.8 ± 4.3	98.5 ± 4.6

	Primary effects		Interactions
	Metal	Organ	M × O
ANOVA α values			
Leaves	0.000	0.001	0.001
Bark	0.000	0.001	0.002
Wood	0.000	0.000	0.001
Roots	0.000	0.005	0.003

were apparent when the Cd concentration was higher in the root compared to the shoot (bark and wood) (Table 1). Similar results were also obtained by Cd-tolerant and Cd-sensitive ecotypes of *Arabidopsis thaliana* (Wójcik & Tukiendorf, 2004). The maximal accumulation of Cd was reached after 28 days of 70 μM Cd treatment, irrespective of the concentration applied. Most of the Cd accumulated in different tissues was bound to a thiol-rich fraction with a molecular weight close to that of the PCs. This result suggested that PCs may constitute the principal mechanism in heavy metal sequestration, as in *Arabidopsis thaliana* (Cobbett, 2000b). Plant tolerance of heavy metals is often related to transport processes, which permit compartmentalisation of heavy metals and prevent their accumulation in the cytoplasm (Brune et al., 1995; Pal & Ral, 2010; Wójcik & Tukiendorf, 2011). The role of GSH and PCs in Cd detoxification and tolerance was well characterised in *Populus* × *canescens* with an altered level of GSH or PC synthesis. The GSH level alters in response to different types of biotic and abiotic stresses. It has been suggested that GSH may act both as a stress sensor and as a part of a very compact signal transduction system (Xiang et al., 2001; Maughan & Foyer, 2006; Szalai et al., 2009).

In conclusion, our results confirmed our earlier finding that *Populus* × *canescens* roots were more resistant to Cd due to an efficient Cd detoxification system. Furthermore, our results suggested that PCs can be used for long-term Cd toxicity. Our studies reveal a tolerating mechanism in poplar to Cd and will provide a solid foundation for poplar breeding in Cd-contaminated soil.

Acknowledgements
We would like to acknowledge the financial support of the 948 Project of the State Forestry Administration of China (2008-4-33 and 2013JK0724).

References

Benavides MP, Gallego SM & Tomaro ML (2005). Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology* 17: 21–34.

Brune A, Urbach W & Dietz KJ (1995). Differential toxicity of heavy metals is partly related to a loss of preferential extraplasmic compartmentation: a comparison of Cd, Mo, Ni, and Zn-stress. *New Phytologist* 129: 403–409.

Cánovas D, Vooijs R, Schat H & de Lorenzo V (2004). The role of thiol species in the hypertolerance of *Aspergillus* sp. P37 to arsenic. *The Journal of Biological Chemistry* 279: 51234–51240.

Clemens S (2006). Evolution and function of phytochelatin synthase. *Journal of Plant Physiology* 163: 319–332.

Cobbett CS (2000a). Phytochelatin biosynthesis and function in heavy metal detoxification. *Current Opinion in Plant Biology* 3: 211–216.

Cobbett CS (2000b). Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology* 123: 825–832.

Dai HP, Jia GL, Feng SJ, Wei AZ, Song H, Yang TX & Wang CF (2011). Phytoremediation with transgenic poplar. *Journal of Food, Agriculture & Environment* 9: 710–713.

Dai HP, Jia GL, Gao PX, Chen JW, Wei Y, Yang TX, Wang CF, Wang Y, Sa WQ & Wei AZ (2012a). Influence of photosynthesis and chlorophyll synthesis on Cd accumulation in *Populus* × *canescens*. *Journal of Food, Agriculture & Environment* 10: 1020–1023.

Dai HP, Shan CJ, Jia GL, Yang TX, Wei AZ, Zhao H, Wu SQ, Huo KK, Chen WQ & Cao XY (2013). Responses to cadmium tolerance, accumulation and translocation in *Populus* × *canescens*. *Water, Air & Soil Pollution* 224–1504.

Dai HP, Shan CJ, Lu C, Jia GL, Wei AZ, Sa WQ & Yang TX (2012b). Response of antioxidant enzymes in *Populus* × *canescens* under cadmium stress. *Pakistan Journal of Botany* 44: 1943–1949.

Dai HP, Wei Y, Yang TX, Sa WQ & Wei AZ (2012c). Influence of cadmium stress on chlorophyll fluorescence characteristics in *Populus* × *canescens*. *Journal of Food, Agriculture & Environment* 10: 1281–1283.

Griffith OW (1980). Determination of glutathione and glutathione disulphide using glutathione reductase and 2-vinyl pyridine. *Annals of Clinical Biochemistry* 106: 207–211.

Grill E, Löffler S, Winnacker EL & Zenk MH (1989). Phytochelatins, the heavy-metal binding peptides of plants, are synthesized from glutathione by a specific glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). *Proceedings of the National Academy of Sciences of the United States of America* 86: 6838–6842.

Grill E, Winnacker EL & Zenk MH (1985). Phytochelatins: the principal heavy-metal complexing peptides of higher plants. *Science* 230: 647–676.

Leplé JC, Brasileiro ACM, Michel MF, Delmotte F & Jouanin L (1992). Transgenic poplars expression of chimeric genes using four different constructs. *Plant Cell Reports* 11: 137–141.

Maughan S & Foyer CH (2006). Engineering and genetic approaches to modulating the glutathione network in plants. *Plant Physiology* 126: 382–397.

May MJ, Vernoux T, Leaver C, Montagu MV & Inzé D (1998). Glutathione homeostasis in plants: implications for environmental sensing and plant development. *Journal of Experimental Botany* 49: 649–667.

Namdjoyan S, Namdjoyan S & Kermanian H (2012). Induction of phytochelatin and responses of antioxidants under cadmium stress in safflower (*Carthamus tinctorius*) seedlings. *Turkish Journal of Botany* 36: 495–502.

Pal R & Rai JPN (2010). Phytochelatins: peptides involved in heavy metal detoxification. *Applied Biochemistry and Biotechnology* 160: 945–963.

Prashant KR & Girjesh K (2010). The genotoxic potential of two heavy metals in inbred lines of maize (*Zea mays* L.). *Turkish Journal of Botany* 34: 39–46.

Schützendübel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Heyser R, Godbold DL & Polle A (2001). Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in Scots pine roots. *Plant Physiology* 127: 887–898.

Shamsi I, Jilani G, Zhang G & Kang W (2008). Interactive effects of cadmium and aluminum on growth and antioxidative enzymes in soybean. *Biologia Plantarum* 52: 165–169.

Szalai G, Kellős T, Galiba G & Kocsy G (2009). Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. *Journal of Plant Growth Regulation* 28: 66–80.

Tran TA & Popova LP (2013). Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turkish Journal of Botany* 37: 1–13.

Vandecasteele B, Lauriks R, Vos BD & Tack FMG (2003). Cd and Zn concentration in hybrid poplar foliage and leaf beetles grown on polluted sediment-derived soils. *Environmental Monitoring and Assessment* 89: 263–283.

Wang KS, Huang LC, Lee HS, Chen PY & Chang SH (2008). Phytoextraction of cadmium by *Ipomoea aquatica* (water spinach) in hydroponic solution: effects of cadmium speciation. *Chemosphere* 72: 666–672.

White P & Brown P (2010). Plant nutrition for sustainable development and global health. *Annals of Botany* 105: 1073–1080.

Wójcik M & Tukiendorf A (2004). Phytochelatin synthesis and cadmium localization in wild type of *Arabidopsis thaliana*. *Plant Growth Regulation* 44: 71–80.

Wójcik M & Tukiendorf A (2011). Glutathione in adaptation of *Arabidopsis thaliana* to cadmium stress. *Biologia plantarum* 55: 125–132.

Wojas S, Clemens S, Hennig J, Skłodowska A, Kopera E, Schat H, Bal W & Antosiewicz DM (2008). Overexpression of phytochelatin synthase in tobacco: distinctive effects of *AtPCS1* and *CePCS* genes on plant response to cadmium. *Journal of Experimental Botany* 59: 2205–2219.

Xiang C, Werner BL, Christensen EM & Oliver DJ (2001). The biological functions of glutathione revisited in *Arabidopsis* transgenic plants with altered glutathione levels. *Plant Physiology* 126: 564–574.

Zhang HY, Xu WZ, Guo JB, He ZY & Ma M (2005). Coordinated responses of phytochelatins and metallothioneins to heavy metals in garlic seedlings. *Plant Science* 169: 1059–1065.