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RESEARCH ARTICLE

Cadmium tolerance in six poplar species

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Abstract Selection of poplar species with greater Cd tolerance and exploiting the physiological mechanisms involved in Cd tolerance are crucial for application of these species to phyto-remediation. The aim of this study is to investigate variation in Cd tolerance among the six poplar species and its underlying physiological mechanisms. Cuttings of six *Populus* species were cultivated for 10 weeks before exposure to either 0 or 200 μM $CdSO_4$ for 20 days. Gas exchange in mature leaves was determined by a portable photosynthesis system. Cd concentrations in tissues were analyzed by a flame atomic absorbance spectrometry. Subsequently, Cd amount per plant, bio-concentration factor (BCF) and translocation factor (T_f) were calculated. Nonenzymatic compounds and activities of antioxidative enzymes in tissues were analyzed spectrophotometrically. Cd exposure caused decline in photosynthesis in four poplar species including *Populus cathayana* (zhonghua 1). Among the six species, *P. cathayana* (zhonghua 1) displayed the highest Cd concentrations in tissues, the largest Cd amount in aerial parts, the highest BCF in aerial parts and T_f under Cd exposure. Under Cd stress, increases in total soluble sugars in roots but decreases in starch in roots, wood, and leaves of *P. cathayana* (zhonghua 1) were found. Induced $O_2^{\bullet-}$ and H_2O_2 production in roots and leaves, and increases in free proline, soluble phenolics, and activities of antioxidative enzymes were observed in *P. cathayana* (zhonghua 1). Based on results of this pot experiment, it is concluded that *P. cathayana* (zhonghua 1) is superior to other five species for Cd phyto-remediation, and its well-coordinated physiological changes under Cd exposure confer the great Cd tolerance of this species.

Keywords *Populus* · Cadmium · Tolerance · Accumulation · Translocation · Photosynthesis · Sugar · Phenolics

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Introduction

Cadmium (Cd) is accumulated in soils mainly due to anthropogenic activities such as mining and fertilizer application (Kramer 2010). Cd is a nonessential element for most plants except marine diatoms (Morel 2008). It can be absorbed by plants and eventually enters the human body via the food chain. Thus, Cd accumulation in the environment poses a serious threat to human health (Kaplan et al. 2011; Yaman 2006). Several technologies have been developed to reduce the Cd levels in soils. One of those technologies is phyto-

remediation; i.e., the use of plants to absorb Cd from the soil and to accumulate this toxic metal in aerial parts of plants which can be easily harvested (Kramer 2010). The success of phyto-remediation for Cd polluted soils largely depends on the selection of proper plants, which are able to grow in Cd-polluted soils and to accumulate Cd in aerial parts. In the last decade, attention has been paid to Cd hyper-accumulation plants (Kramer 2010). However, only few plant species such as *Arabidopsis halleri*, *Noccaea caerulescens* (formerly *Thlaspi caerulescens*), and *Sedum alfredii* have been identified, which are able to tolerate high of Cd concentrations (Hanikenne and Nouet 2011; Lu et al. 2008). Since these herbaceous Cd hyper-accumulators display slow growth and little biomass production, the applicability of these plants for phyto-remediation in Cd-polluted fields is limited (Meighan et al. 2011). Alternatively, the usage of *Populus* species has been proposed for phyto-remediation because these woody species are characterized by fast-growth, deep-rooting systems and can be managed in short rotation coppicing plantations (Di Lonardo et al. 2011; Fischerova et al. 2006; Laureysens et al. 2004, 2005; Marmiroli et al. 2011; Pietrini et al. 2010; Unterbrunner et al. 2007; Vangronsveld et al. 2009; Wu et al. 2010; Yadav et al. 2010; Zhao and McGrath 2009).

Previous studies have shown that poplar plants display differential accumulation of Cd in their roots, wood, bark, and leaves (Elobeid et al. 2012; He et al. 2011; Laureysens et al. 2004, 2005). In most cases, the Cd concentrations in the aerial parts of poplar plants are no more than 100 μg g^{-1}, which is the threshold value for Cd hyper-accumulators (Milner and Kochian 2008). However, large amounts of aboveground biomass can be obtained for poplars within one growing season in the temperate zone (Yadav et al. 2010). Therefore, the total Cd amounts accumulated in aerial parts of poplar plants are still by far higher than those accumulated by herbaceous hyper-accumulators (Di Lonardo et al. 2011; Pietrini et al. 2010). These results indicate that poplar plants are promising for Cd phyto-remediation. The genus *Populus* consists of five sections with about 30–40 species occurring worldwide, mainly in the Northern Hemisphere (Polle and Douglas 2010). It is demonstrated that different clones of the same poplar species may exhibit variable heavy metal tolerance due to intraspecific genetic dissimilarity (Castiglione et al. 2009). It is believed that there exists a huge variation in Cd tolerance among different poplar species. However, little information is available on this trait in different poplar species (Marmiroli et al. 2011).

The responses of poplar plants to Cd exposure have been investigated at the physiological and molecular levels (Elobeid et al. 2012; Durand et al. 2010; He et al. 2011; Kieffer et al. 2008; Marmiroli et al. 2011; Polle et al. 2012; Zacchini et al. 2011). Cd exposure leads to decline in photosynthetic rates, foliar chlorophyll concentrations, and chloroplast degradation (He et al. 2011; Kieffer et al. 2009). Biomass reduction is observed in poplar plants exposed to various levels of Cd and has been ascribed to interference with the auxin metabolism (Elobeid et al. 2012; He et al. 2011; Pietrini et al. 2010). Recently, variation in Cd tolerance has been studied in ten poplar clones belonging to six *Populus* species in hydroponics (Zacchini et al. 2009). The bio-concentration factor (BCF), which is calculated as the ratio of Cd concentrations in plants to that in the solution and the translocation factor (T_f) defined as the ratio of Cd concentrations in aerial parts of a plant to that in roots showed marked variations among these poplar clones (Zacchini et al. 2009). Soluble sugars and alcohols are important metabolites derived from photosynthesis. Inhibition of photosynthesis causes decreases in soluble carbohydrates in *Populus* × *canescens* exposed to Cd, and this may limit the transport and repair activities required to cope with excess Cd (He et al. 2011). Cd entry into the symplast inevitably leads to over-production of reactive oxygen species (ROS) such as $O_2^{\bullet-}$ and H_2O_2 in plant cells (He et al. 2011; Hermans et al. 2011). To scavenge excess ROS in plant cells, nonenzymatic antioxidants such as free proline and soluble phenolics and antioxidative enzymes play important roles (He et al. 2011). Up to date, however, limited information is available on the physiological mechanisms involved in variations in Cd accumulation and translocation in different poplar species.

Here, we used six *Populus* species varying in growth characteristics and water use efficiency (Cao et al. 2012) to investigate Cd accumulation, translocation and biochemical stress, and defense reactions as the basis of Cd tolerance. Poplar plants grown in sand filled pots were exposed to either 0 or 200 μM $CdSO_4$ for 20 days. Variation in Cd tolerance and the physiological mechanisms involved in these variations in the six poplar species were studied. The hypothesis is that variation in Cd tolerance exists among the six poplar species and the great Cd tolerance in poplar species is conferred by well-coordinated physiological changes and transport activities into different plant tissues. To examine this hypothesis, photosynthesis, photosynthetic pigments, Cd concentrations, Cd amount per plant, bio-concentration factors and translocation factors, soluble sugars and starch, ROS ($O_2^{\bullet-}$ and H_2O_2), free proline and soluble phenolics, as well as antioxidative enzyme activities were analyzed in the six poplar species. The implications for Cd tolerance and the underlying physiological mechanisms are discussed in the context of the evaluation and selection of poplar plants for phyto-remediation of Cd-polluted soils.

Materials and methods

Plant materials and Cd exposure

Cuttings (about 15 cm in length and 1 cm in diameter) of the six *Populus* species [*Populus cathayana* (clone ID, zhonghua 1), *Populus deltoides* (clone ID, N30), *Populus* × *euramericana*

(clone ID, S66), *Populus alba* × *Populus glandulosa* (clone ID, 84K), *Populus nigra* (clone ID, I35), *Populus popularis* (clone ID, H20); cuttings of each species were derived from the same clone] collected from a breeding program (Cao et al. 2012) were rooted and planted in 10-l plastic pots (10 kg sand for each pot). One cutting with a sprout was planted in each pot. Plants were cultivated for 10 weeks in a greenhouse (day/night temperature, 25/18 °C; relative air humidity, 50–60 %; light per day, natural light) before Cd exposure. Thirty milliliters nutrient solution (full strength Hoagland solution) in the morning and 30 ml distilled water in the evening were carefully irrigated to each plant to avoid runoff. For Cd treatment, 12 plants with a similar height for each species were selected and divided into two groups with six plants in each group. The apex of each plant was marked prior to the Cd treatment in order to distinguish tissues formed before and during the Cd treatment. Each plant of each group was treated with either 0 or 200 μM $CdSO_4$. Plants were grown for 20 days in absence or presence of Cd being supplied daily with the nutrient solution. In this way, plant roots may sense a Cd concentration more than 200 μM $CdSO_4$ due to some solution retention in the rhizosphere leading to increases of Cd concentration.

Gas exchange measurement and harvest

Before harvest, three mature leaves (leaf plastochron index= 7–9) of each plant were selected for gas exchange measurements. Net photosynthetic rate (A), stomatal conductance (g_s), and transpiration rate (E) were determined using a portable photosynthesis system (LiCor-6400; LiCor Inc., Lincoln, NE, USA) as described previously (He et al. 2011).

After photosynthesis measurement, the root system of each plant was carefully washed using tap water before exposure to 50 mM $CaCl_2$ for 5 min to remove the Cd^{2+} at the root surface. Subsequently, the roots and aboveground tissues of each plant were harvested. The aboveground tissues were separated into wood, bark, and leaves. Fresh weight of each sample was recorded before samples were wrapped with tinfoil and immediately frozen in liquid nitrogen. Frozen samples were ground into fine powder in liquid nitrogen with a mortar and a pestle and stored at −80 °C for further analysis. Fresh powder (about 30 mg) from each tissue per plant was dried at 60 °C to determine the fresh/dry mass ratio, which was used to calculate the dry weight (biomass) of each tissue. Further physiological and biochemical analyses were performed in different tissues of the six poplar species except that no root materials were left for *P. nigra* and *P. popularis* in some analyses as stated in the following sections.

Analysis of Cd and foliar pigments

To analyze Cd concentrations in different tissues, fine powder (about 100 mg) from roots, wood, bark, and leaves was digested in a mixture (7 ml concentrated HNO_3 and 1 ml concentrated $HClO_4$) at 170 °C. Subsequently, Cd was determined by a flame atomic absorbance spectrometry (Hitachi 180-80, Japan) as described (He et al. 2011). The total Cd amount in each plant was calculated by multiplying the Cd concentration in each tissue with the biomass of that tissue.

To determine chlorophyll and carotenoid contents in leaves, fine powder of fresh leaves (about 40 mg) was extracted for 24 h in 5 ml of 80 % acetone in darkness. The contents of chlorophyll a, chlorophyll b, and carotenoids in the extracts were determined spectrophotometrically as suggested (He et al. 2011).

Bio-concentration factor and translocation factor

BCF was defined as the ratio of metal concentration in plant roots or aerial tissues to that in the soil or solution (Shi et al. 2010). The translocation factor (T_f) indicated the ability of plants to translocate cadmium from the roots to the shoots (Shi et al. 2010). T_f was calculated as the cadmium concentration in aerial tissues of a plant divided by the cadmium concentration in roots (Shi et al. 2010).

Analysis of total soluble sugars and starch

The concentrations of total soluble sugars and starch were analyzed by the anthrone method as described (Yemm and Willis 1954). The fine powder of fresh tissues (about 100 mg) was homogenized in 3 ml of 80 % ethanol and incubated in an ultrasonic bath for 30 min at 80 °C. After centrifugation (6,000×g, 25 °C, 10 min), the supernatant was collected. The pellet was extracted again as mentioned above, and the supernatant was collected and combined with the previous one. After adding 2 ml of anthrone reagent to the supernatant, the mixture was heated in boiling water for 7 min. After the mixture was cooled to the room temperature, the absorbance of the mixture was recorded spectrophotometrically at 620 nm. The standard curve was established by using a serial of diluted solutions of glucose.

The pellet obtained after the extraction of the soluble sugars was further extracted by $HClO_4$ for the determination of starch. Subsequently, starch (expressed as glucose equivalent) in the supernatant was determined as above.

Determination of MDA, $O_2^{\bullet-}$, and H_2O_2

The malonaldehyde (MDA) concentrations in plant materials were analyzed spectrophotometrically at 450, 532, and 600 nm as described previously (Lei et al. 2007).

Concentrations of the superoxide ($O_2^{\bullet-}$) and H_2O_2 in plant materials were determined spectrophotometrically at 530 and 410 nm, respectively, as described previously (He et al. 2011).

Analysis of proline and soluble phenolics

Free proline was determined spectrophotometrically according to Tamas et al. (2008) with a minor modification (He et al. 2011). The standard curve was generated using a serial of diluted solutions of L-proline (Amresco Inc., Solon, USA).

Soluble phenolics in plant materials were determined spectrophotometrically using the Folin–Ciocalteus reagent as reported previously (Luo et al. 2008). Standard curve was prepared using a serial of diluted solutions of catechin (Sigma, St Louis, USA).

Assay of enzyme activities

Soluble proteins in plant materials were extracted and quantified as described previously (Luo et al. 2008). The soluble protein extracts were also used for the assays of enzyme activities.

The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was determined according to Polle et al. (1990). Activities of guaiacol peroxidase (GPX, EC 1.11.1.7) after Sun et al. (2009), catalase (CAT, EC 1.11.1.6) after Ma and Cheng (2003), and ascorbate oxidase (AAO, EC 1.10.3.3) after Tamas et al. (2006) were determined.

Statistical analysis

All statistical tests were performed with Statgraphics (STN, St. Louis, MO, USA). For BCF and T_f analysis, one-way ANOVA was applied with species as the only factor. For other experimental variables, two-way ANOVAs were applied with $CdSO_4$ (Cd) and species as two factors. When the interaction was significant, a posteriori comparison of means was made. To reduce the chance of type I errors, all P values of these multicomparisons were corrected by the Tukey honestly significant difference method. Data were tested for normality prior to the statistical analysis. Differences between means were considered significant when the P value of the ANOVA F test was <0.05.

Results

Photosynthesis and biomass

Photosynthetic rates (A) of mature leaves declined by about 19–48 % in four poplar species (*P. cathayana*, *P. deltoides*, *P.* × *euramericana*, and *P. alba* × *P. glandulosa*) exposed to 200 μM Cd compared to those in leaves of poplar plants grown under control conditions, but remained unaffected in leaves of Cd exposed *P. nigra* and *P. popularis* (Table 1). Under Cd exposure, the strongest effect on A was found in *P.* × *euramericana* (−48 %) and the least in *P. nigra* (−1.4 %). Marked differences in the photosynthetic rates were also among the different poplar species (Table 1). Photosynthetic rate was highest in *P. deltoides* and lowest in *P. alba* × *P. glandulosa* irrespective the Cd treatments. g_s and E showed Cd responses similar to those found for A (Table 1).

Concentrations of chlorophyll a significantly declined by about 21–32 % in three poplar species (*P. deltoides*, *P.* × *euramericana*, *P. alba* × *P. glandulosa*), but were unaffected in the other three species under 200 μM Cd conditions compared to those without Cd exposure (Table 1). Similarly, concentrations of chlorophyll b markedly decreased by about 26–35 % in *P. cathayana*, *P.* × *euramericana* and *P. alba* × *P. glandulosa*, but remained unchanged in the other three species under Cd exposure in comparison with those under the control conditions (Table 1). The most marked decrease in concentrations of chlorophyll (a+b) was found in *P.* × *euramericana* (−33 %) and the least decrease in *P. nigra* (−7 %) due to Cd exposure. Carotenoid concentrations also markedly decreased in leaves of *P. deltoides*, *P.* × *euramericana*, and *P. alba* × *P. glandulosa* exposed to Cd in comparison with those under control conditions (Table 1). Among the six species, concentrations of chlorophyll (a+b) were highest in *P. nigra* and lowest in *P. deltoides* (Table 1).

During the whole experimental period and the Cd exposure phase, biomass increments in poplar plants were monitored (Table S1). Total root biomass of *P. alba* × *P. glandulosa* and *P. nigra* was markedly lower in the plants exposed to 200 μM Cd than those grown under controls. Wood and leaf biomass formed during Cd exposure were markedly reduced in plants exposed to Cd compared to those of controls. Notably, Pn, whose photosynthetic activity was unaffected by Cd, showed a strong growth reduction of the aboveground tissues under Cd exposure (Table S1). In general, biomass increments were largest in *P.* × *euramericana* and least in *P. popularis* among the six species.

Cd concentration, BCF, and T_f

To evaluate plant's ability to accumulate Cd from the soil, Cd concentrations were analyzed in roots, wood, bark, and leaves of the different poplar species (Fig. 1, Table S2). Mean Cd concentrations were 43-, 9-, 23-, and 25-fold elevated in roots, wood, bark, and leaves, respectively, of poplar plants exposed to 200 μM Cd compared to those under control conditions. Among the six poplar species, *P. cathayana* displayed the highest Cd concentrations in the analyzed tissues of Cd-exposed plants. Cd concentrations in bark and leaves of *P. cathayana* were 101.6 and 135.0 μg g^{-1} DW, respectively, which is above the threshold value defined for Cd hyperaccumulation. The species-specific differences in Cd concentrations were significant in roots and leaves.

Based on the Cd concentrations in root, wood, bark, and leaf tissues and their corresponding biomass, the total Cd

Table 1 CO_2 assimilation rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and photosynthetic pigments (mg g^{-1} DW) in leaves of the six poplar species exposed to either 0 or 200 μM $CdSO_4$ for 20 days

Species	Cd (μM)	A	g_s	E	Chl a	Chl b	Chl (a+b)	Car
Pc	0	14.5±0.9 c	0.61±0.03 e	7.29±0.45 ef	5.76±0.59 def	1.91±0.06 de	7.70±0.85 cde	1.09±0.08 bc
	200	10.9±0.9 b	0.58±0.04 e	7.12±1.82 def	4.85±0.35 abcd	1.44±0.03 abc	6.28±0.44 abc	1.05±0.07 abc
Pd	0	19.3±0.5 e	0.49±0.02 d	6.67±1.74 cd	5.33±0.15 cde	1.52±0.01 abcd	6.86±0.19 bcd	1.14±0.02 cd
	200	15.7±1.1 cd	0.49±0.04 d	6.90±2.63 de	4.15±0.51 ab	1.16±0.05 a	5.33±0.68 a	0.92±0.10 ab
Pe	0	16.5±0.4 d	0.42±0.02 bcd	6.10±1.86 abc	5.88±0.38 def	2.00±0.05 ef	7.91±0.65 def	1.10±0.03 bc
	200	8.6±0.3 a	0.35±0.03 ab	5.62±1.78 ab	3.99±0.12 a	1.26±0.01 a	5.26±0.17 a	0.88±0.02 a
Pg	0	12.3±0.5 b	0.39±0.02 abc	6.03±2.02 ab	6.81±0.36 fg	2.46±0.01 f	9.25±0.39 f	1.43±0.09 e
	200	7.4±0.3 a	0.34±0.01 a	5.50±1.28 a	4.64±0.22 abc	1.68±0.02 bcde	6.35±0.32 abc	1.05±0.05 abc
Pn	0	14.7±0.7 c	0.48±0.02 d	7.64±1.96 f	7.04±0.52 g	1.95±0.04 ef	9.02±0.69 ef	1.44±0.10 e
	200	14.5±0.3 c	0.45±0.03 cd	7.48±1.21 ef	6.01±0.27 efg	1.73±0.03 cde	7.76±0.40 cdef	1.33±0.08 de
Pp	0	16.6±0.9 d	0.45±0.02 cd	7.07±2.07 def	5.16±0.43 bcde	1.52±0.03 abcd	6.70±0.55 abcd	1.10±0.10 bc
	200	14.8±0.3 cd	0.33±0.02 a	6.15±2.31 bc	4.86±0.29 abcd	1.33±0.01 ab	6.18±0.35 ab	1.05±0.03 abc
P values	Cd	–****	–**	–**	–****	–****	–****	–***
	Species	–****	–****	–****	–***	–***	–**	–****
	Cd×Species	ns	ns	ns	ns	ns	ns	ns

Data indicate means ± SE (n=6). *Different letters behind the values in the same column* indicate significant difference between the treatments. P values of the ANOVAs of $CdSO_4$ (Cd), species, and their interactions were also shown

Chl a chlorophyll a, *Chl b* chlorophyll b, *Chl (a+ b)* sum of chlorophyll a and b, *Car* carotenoid, *Pc P. cathayana*, *Pd P. deltoides*, *Pe P.×euramericana*, *Pg P. alba×P. glandulosa*, *Pn P. nigra*, *Pp P. popularis*, *ns* not significant

*P≤0.05; **P≤0.01; ***P≤0.001; ****P≤0.0001

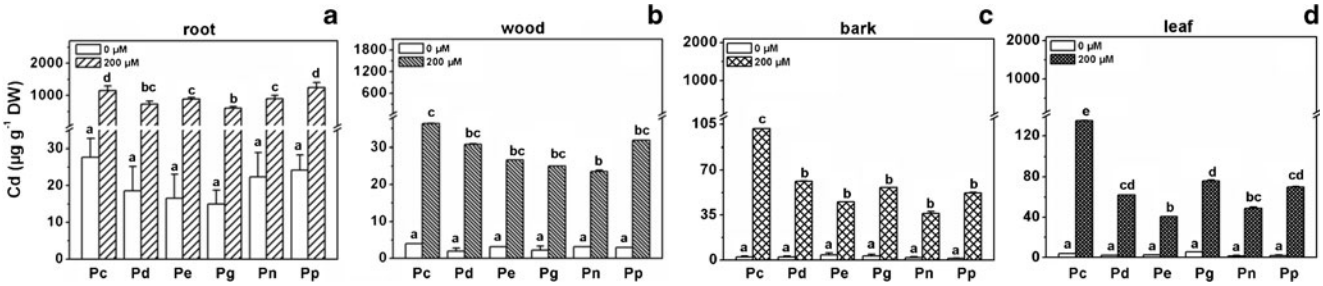

Fig. 1 Cd concentrations in roots, wood, bark, and leaves of the six poplar species exposed to either 0 or 200 μM $CdSO_4$ for 20 days. Bars indicate means ± SE (n=6). *Different letters on the bars for the same tissue* indicate significant difference between the treatments. The results of statistical analyses are shown in Table S2. Pc *P. cathayana*, Pd *P. deltoides*, Pe *P.×euramericana*, Pg *P. alba×P. glandulosa*, Pn *P. nigra*, Pp *P. popularis*

amounts accumulated in each species were calculated (Fig. 2, Table S2). Among the six poplar species, the Cd amount in aerial parts was largest in *P. cathayana* (566.4 μg), mainly due to its high Cd concentrations and its relatively higher biomass in comparison with those of other five species.

To further evaluate the ability of different poplar species to accumulate Cd, their BCF was determined (Fig. 3, Table S3). BCF in the aerial parts of the six species displayed a gradual decrease in the order *P. cathayana* > *P. alba* × *P. glandulosa* > *P. popularis* > *P. deltoides* > *P. nigra* > *P.*× *euramericana*. BCF was significantly higher in the aerial parts of *P. cathayana* than in those of the other five species. BCF in the roots varied from 84 to 175 in the six species and relatively larger BCFs were found in *P. popularis* and *P. cathayana* than in the other analyzed species. In addition to the BCF, the translocation factor (T_f) is often used to evaluate the ability of plants to translocate heavy metals from roots to the aerial tissues (Zacchini et al. 2009). Among the six poplar species, T_fs varied from 4.4 to 10.4 % (Fig. 3b, Table S3). T_fs were significantly higher in *P. cathayana* (up to 124 %) and *P. alba* × *P. glandulosa* (up to 139 %) than in the other four species.

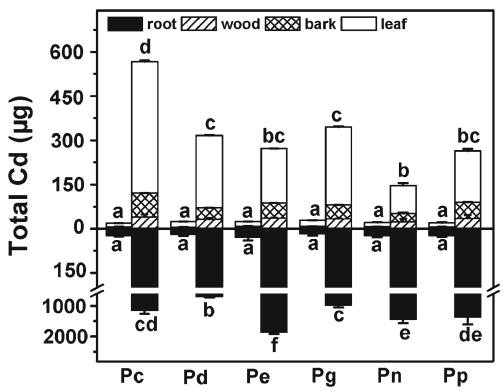

Fig. 2 Total Cd amount in roots, wood, bark, and leaves of the six poplar species exposed to either 0 or 200 μM $CdSO_4$ for 20 days. Bars indicate means ± SE (n=6). *Different letters on the bars for the same tissue* indicate significant difference between the treatments. The results of statistical analyses are shown in Table S2. Pc *P. cathayana*, Pd *P. deltoides*, Pe *P.×euramericana*, Pg *P. alba×P. glandulosa*, Pn *P. nigra*, Pp *P. popularis*

Total soluble sugars and starch

Soluble sugars and starch are important metabolites, which are derived from photosynthesis and may be involved in osmotic regulation, membrane lipid biosynthesis and oxidant detoxification under Cd exposure (He et al. 2011). Concentrations of total soluble sugars and starch in different tissues of poplar plants were examined (Fig. 4, Table S4). In general, the mean concentrations of total soluble sugars were enhanced in roots, bark, and leaves of poplar plants exposed to Cd compared to those under the control. Among the six species, the concentrations of total soluble sugars were markedly higher in wood of *P. nigra* and *P. deltoides* than in wood of other four species. Similarly, concentrations of total soluble sugars were higher by 11–43 % and 12–44 % in leaves of *P. cathayana* and *P. nigra*, respectively, compared to those in the other four species. Whereas Cd exposure led to significant decreases in concentrations of starch in roots of *P. alba* × *P. glandulosa*, it resulted in increases in starch concentrations in bark of *P. nigra* and *P. popularis* and in leaves of *P. nigra*. Starch concentrations were higher in bark and leaves of *P. cathayana* than those in other species.

MDA, $O_2^{\bullet-}$, and H_2O_2

MDA, an indicator for membrane lipid oxidation, is often used to evaluate the ability of plants to tolerate abiotic stresses. Interestingly, no effects of Cd exposure on MDA concentrations were found in these poplar plants (Table S5). However, species-specific differences in MDA concentrations were found. MDA concentrations in roots of *P. alba* × *P. glandulosa* were higher than those in *P. cathayana*, *P. deltoides*, and *P.* × *euramericana*. Similarly, MDA concentrations in bark and leaves differed among the species. The highest MDA concentrations were found in bark and leaves of *P. nigra* and the lowest in bark and leaves of *P. deltoides*.

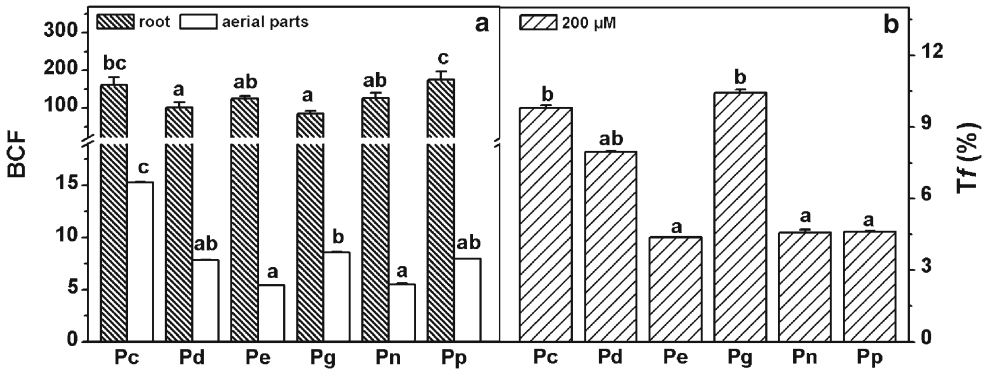

Fig. 3 The bio-concentration factor (BCF) and translocation factor (T_f) in the six poplar species exposed to 200 μM $CdSO_4$ for 20 days. *Different letters on the bars* indicate significant difference between the species. *Bars* indicate means ± SE (n=6). The results of statistical analyses were shown in Table S3. Pc *P. cathayana*, Pd *P. deltoides*, Pe *P.*×*euramericana*, Pg *P. alba*×*P. glandulosa*, Pn *P. nigra*, Pp *P. popularis*

ROS such as $O_2^{\bullet-}$ and H_2O_2 are often accumulated in plants exposed to heavy metals. $O_2^{\bullet-}$ concentrations were significantly elevated by 45 and 53 % in roots of *P. cathayana* and *P. deltoides*, respectively, under 200 μM Cd in comparison with those under control conditions, but remained unchanged in roots of *P.* × *euramericana* and *P. alba* × *P. glandulosa* (Fig. 5, Table S6). In wood, $O_2^{\bullet-}$ concentrations were markedly enhanced by 39 and 47 % in *P.* × *euramericana* and *P. nigra*, respectively, under Cd addition compared to those under the control. In leaves, $O_2^{\bullet-}$ concentrations were increased in *P.* × *euramericana*, *P. alba* × *P. glandulosa*, and *P. popularis* under 200 μM Cd compared to those without Cd addition. Among the analyzed poplar species, $O_2^{\bullet-}$ production was the highest in wood, bark, and leaves of *P. deltoides*.

In wood, H_2O_2 concentrations were significantly increased by 65 and 45 % in *P. nigra* and *P. popularis*, respectively, with Cd exposure than those without Cd addition. In leaves, H_2O_2 concentrations were elevated by 31 % in *P. cathayana* and by 41 % in *P. nigra* under Cd addition in comparison with those under the control. Among the tested poplar species, H_2O_2 concentrations were the highest in wood of *P. deltoides*, bark of *P.* × *euramericana*, and leaves of *P. popularis*.

Free proline and soluble phenolics

Free proline and soluble phenolics are important antioxidants in plants (Grace 2005; Hall 2002). Upon Cd exposure, concentrations of free proline and soluble phenolics were altered in poplar plants (Fig. 6, Table S7). In roots of *P. cathayana* and *P. deltoides*, free proline concentrations were markedly elevated by 52 and 43 %, respectively, under Cd addition condition compared to those under the control. In leaves of *P.* × *euramericana*, *P. alba* × *P.*

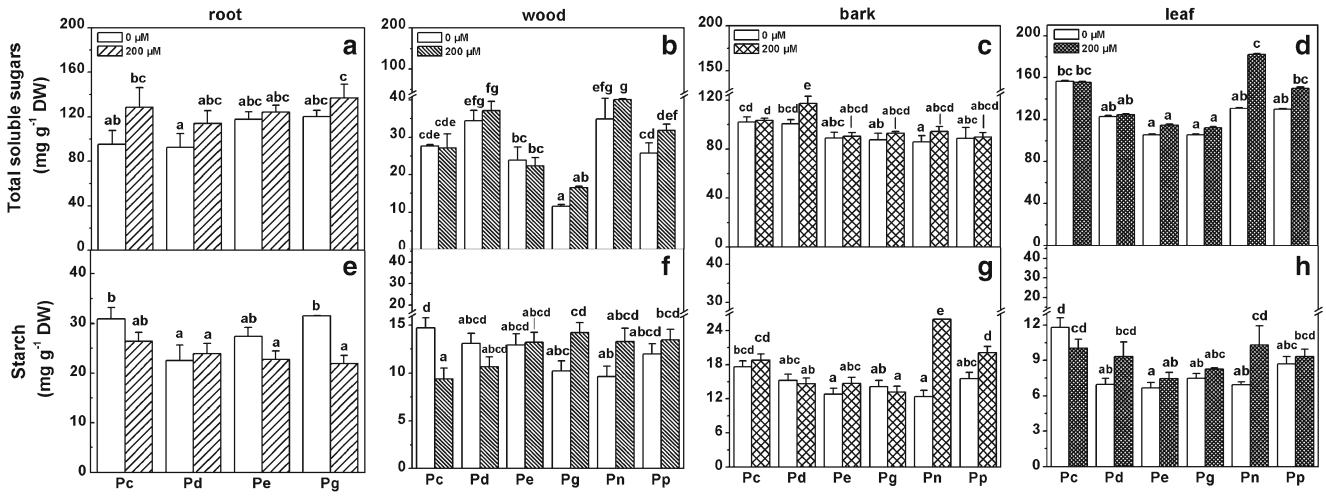

Fig. 4 Total soluble sugars and starch in roots, wood, bark, and leaves of poplar species exposed to either 0 or 200 μM $CdSO_4$ for 20 days. *Bars* indicate means ± SE (n=6). No root materials left for *P. nigra* and *P. popularis* to perform these analyses. *Different letters on the bars for the same tissue* indicate significant difference between the treatments. The results of statistical analyses are shown in Table S4. Pc *P. cathayana*, Pd *P. deltoides*, Pe *P.*×*euramericana*, Pg *P. alba*×*P. glandulosa*, Pn *P. nigra*, Pp *P. popularis*

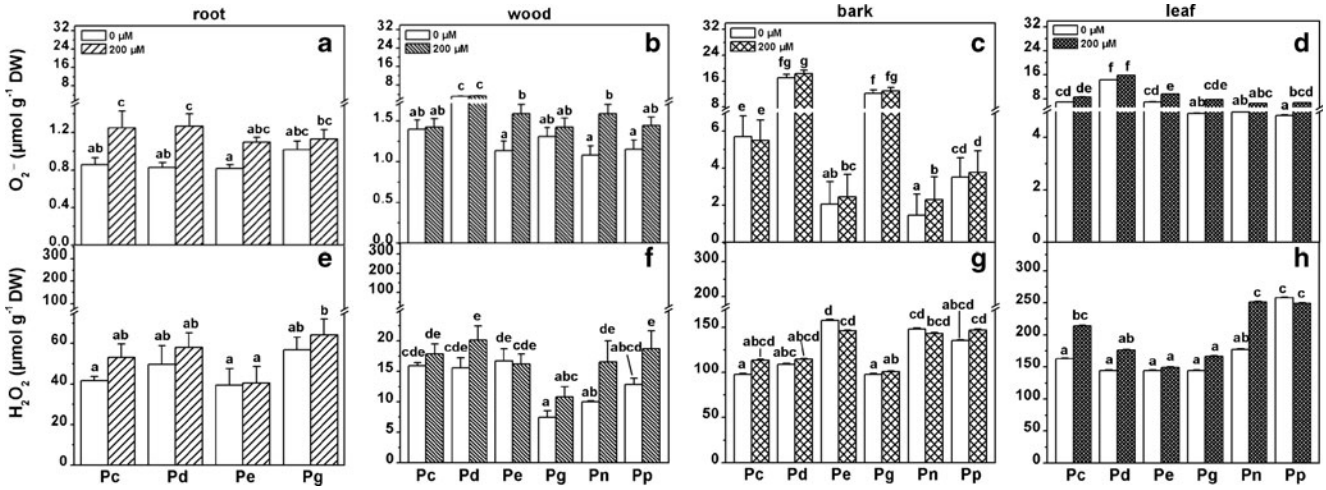

Fig. 5 $O_2^{\bullet-}$ and H_2O_2 in roots, wood, bark, and leaves of poplar species exposed to either 0 or 200 μM $CdSO_4$ for 20 days. *Bars* indicate means ± SE (n=6). No root materials left for *P. nigra* and *P. popularis* to perform these analyses. *Different letters on the bars for the same tissue* indicate significant difference between the treatments. The results of statistical analyses were shown in Table S6. Pc *P. cathayana*, Pd *P. deltoides*, Pe *P.*×*euramericana*, Pg *P. alba*×*P. glandulosa*, Pn *P. nigra*, Pp *P. popularis*

glandulosa, and *P. nigra*, free proline concentrations were enhanced by 27–29 % under Cd exposure in comparison with those under the control. Among the analyzed poplar species, concentrations of free proline were highest in wood and bark of *P.* × *euramericana* and in leaves of *P. nigra*.

The concentrations of soluble phenolics increased by 47 % in roots of *P. deltoides*, by 38–168 % in wood of *P.* × *euramericana*, *P. nigra*, and *P. popularis*, by 67 % in leaves of *P. nigra* in response to Cd compared with those found under control conditions. Among the examined poplar species, concentrations of soluble phenolics were highest in roots of *P. alba* × *P. glandulosa*, in wood of *P. nigra*, in bark of *P.* × *euramericana*, and in leaves of *P. nigra*.

Enzyme activities

Antioxidative enzymes play an essential role in detoxification of ROS in plants exposed to various abiotic and biotic stresses (Gratao et al. 2005; Pandhair and Sekhon 2006). Regulation of APX, GPX, CAT, and AAO activities were found in *P.* × *canescens* exposed to Cd (He et al. 2011). Under Cd exposure, the activity of APX was markedly enhanced by 59 % in wood of *P. deltoides* in comparison with that under control conditions. In contrast, APX was significantly inhibited by 43–49 % in leaves of four poplar species (*P.* × *euramericana*, *P. alba* × *P. glandulosa*, *P. nigra*, and *P. popularis*) compared to those under control conditions (Fig. S1, Table S8). Among the analyzed species,

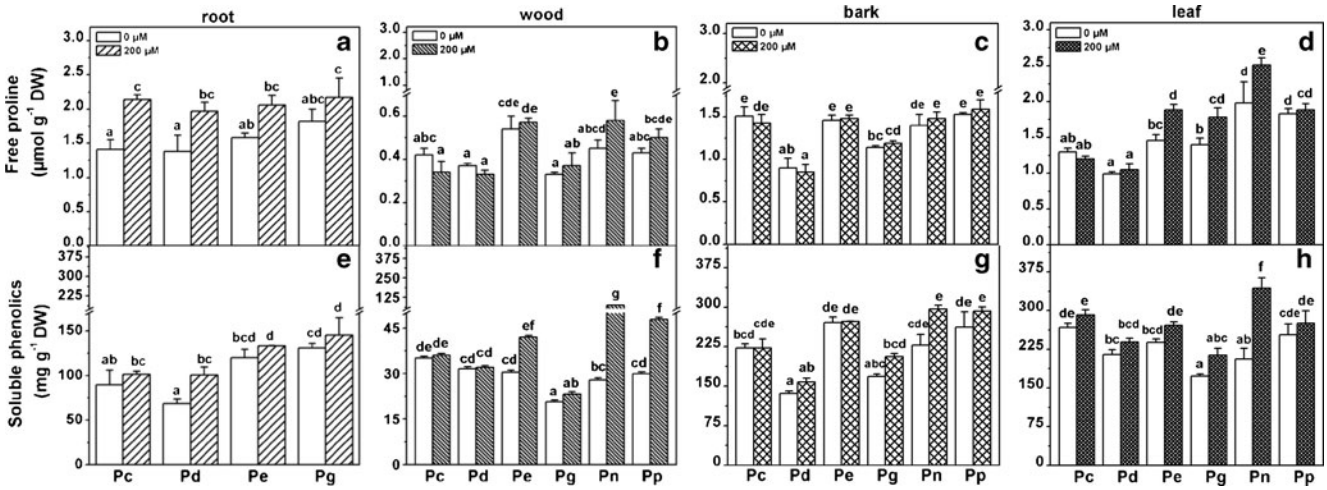

Fig. 6 Free proline and soluble phenolics in roots, wood, bark, and leaves of poplar species exposed to either 0 or 200 μM $CdSO_4$ for 20 days. *Bars* indicate means ± SE (n=6). No root materials left for *P. nigra* and *P. popularis* to perform these analyses. *Different letters on the bars for the same tissue* indicate significant difference between the treatments. The results of statistical analyses were shown in Table S7. Pc *P. cathayana*, Pd *P. deltoides*, Pe *P.*×*euramericana*, Pg *P. alba*×*P. glandulosa*, Pn *P. nigra*, Pp *P. popularis*

the activity of APX was highest in roots and leaves of *P. cathayana*, in wood of *P. alba* × *P. glandulosa*, and in bark of *P. nigra*. The activities of GPX were elevated in roots of *P. cathayana* and *P.* × *euramericana* and in wood of *P. nigra* and *P. popularis*, but were inhibited in leaves of the four species (*P. deltoides*, *P.* × *euramericana*, *P. nigra*, and *P. popularis*) exposed 200 μM Cd in comparison with those under control conditions. Among the analyzed poplar species, GPX activity was highest in roots of *P. cathayana*, in wood of *P. nigra*, in bark of *P. deltoides*, and in leaves of *P. alba* × *P. glandulosa*. The activities of CAT were higher in roots and bark, but lower in leaves of poplar plants exposed to 200 μM Cd than those under the control. CAT activities were highest in wood and leaves of *P. alba* × *P. glandulosa* and in bark of *P. cathayana* among the investigated species. In contrast to other antioxidative enzymes, the activities of AAO were inhibited in wood, bark of *P. nigra* and in leaves of *P. alba* × *P. glandulosa* in response to Cd. AAO activities were highest in roots of *P.* × *euramericana*, in wood of *P. deltoides*, in bark of *P. cathayana*, and in leaves of *P. alba* × *P. glandulosa* among the tested poplar species.

Discussion

Variation in Cd tolerance among the six poplar species

Although many studies on the Cd responses of plants have been carried out, no explicit indicators are available for evaluating Cd tolerance in plants (Di Lonardo et al. 2011; Pietrini et al. 2010; Zacchini et al. 2009). In this study, we have integrated plant photosynthesis, biomass, and Cd accumulation capacity to assess Cd tolerance in the six poplar species. Among the six poplar species, the highest Cd concentrations in roots, wood, bark, and leaves of *P. cathayana* indicate that this poplar species has the ability to accumulate relatively higher Cd in different tissues than the other five species (Fig. 1). Furthermore, the largest Cd amount in aerial parts of *P. cathayana* (Fig. 2) suggests that this poplar species is superior to the other five poplar species for Cd phyto-remediation because aboveground Cd accumulation is ideal for easy harvests (Kramer 2005). Additionally, the highest BCF in aerial parts and a relatively high T_f of *P. cathayana* (Fig. 3) also demonstrate that this poplar species has a stronger capacity to accumulate and translocate Cd to aboveground parts of plants than the other analyzed poplar species. Due to strong Cd accumulation (Figs. 2 and 3) and a marked decline in *A* and chlorophyll b concentrations (Table 1), the biomass of *P. cathayana* during Cd exposure tended to decrease, but no marked reduction was detected (Table S1). This finding indicates that dry mass accumulation may reduce by a long-term Cd exposure in *P. cathayana* although it is uninhibited by Cd exposure within 20 days under the conditions of current study. However, previous studies on poplar plants exposed to Cd have demonstrated that for a short-term Cd treatment (≤14 days) a strong negative impact happened on proteins involved in the Calvin cycle and the light-dependent reactions of photosynthesis, but for a long-term Cd exposure (up to 56 days), the impact of Cd on protein abundance from photosynthesis and Calvin cycle was less pronounced (Kieffer et al. 2008, 2009). These findings suggest that poplars may well adapt to Cd after a long-term exposure to minimize the negative effects on photosynthesis and growth (biomass production). Although the performance of *P. cathayana* (zhonghua 1) remains to be examined under a long-term Cd exposure in field conditions, current data suggest that *P. cathayana* (zhonghua 1) is superior to the other five poplar species in terms of Cd tolerance.

Beside *P. cathayana*, the Cd amounts in aerial parts of the other five poplar species decreased in the order *P. alba* × *P. glandulosa* > *P. deltoides* > *P.* × *euramericana* > *P. popularis* > *P. nigra* (Fig. 2), but Cd concentrations in roots, wood, bark, and leaves, BCF in aerial parts, and T_f of these poplar species showed different orders (Figs. 1 and 3). Notably, the Cd induced decreases in biomass production were most pronounced in *P. nigra* exposed to Cd (Table S1), although photosynthesis remained unchanged in this species and Cd accumulation was lower than in the other species. Obviously, this species is very Cd sensitive. This finding furthermore shows that the photosynthetic response is an insufficient indicator for Cd tolerance.

Since the purpose of phyto-remediation is to remove Cd from the soil using Cd tolerant plants to absorb and accumulate Cd in aboveground tissues, an ideal plant for this purpose should have not only the ability to cope with high Cd concentrations in the plant body but should also produce a large amount of harvestable biomass (Kramer 2010; Pietrini et al. 2010; Shi and Cai 2009; Wu et al. 2010). Most previous studies have emphasized Cd concentrations in plants, especially in hyper-accumulators, but the low biomass production of those plants has largely been ignored (Di Lonardo et al. 2011; Kramer 2010; Pietrini et al. 2010). Obviously, the removal Cd from the soil during the phyto-remediation process depends on the Cd concentrations in and the biomass amounts of the aerial tissues/organs. Fast growing woody plants such as the *Populus* species used in this study exhibit rapid biomass production. Although the Cd concentrations in the aerial tissues of poplars (<200 μg Cd g^{-1} dry weight) were lower than those observed in hyper-accumulators (>200 μg Cd g^{-1} dry weight) (Kramer 2010), tolerant species such as *P. alba* (Di Lonardo et al. 2011) and *P. cathayana*, which can maintain high biomass production under Cd stress, may sequester similar Cd amounts as hyper-accumulators or even more and may therefore be especially suitable for phyto-remediation of Cd polluted soils.

Cd tolerance in *P. cathayana* is associated with well-coordinated physiological changes and defense reactions

The highest Cd concentrations in different tissues of *P. cathayana* among the six poplar species (Figs. 1 and 2) and its high biomass production indicate that this poplar species may have more efficient means to detoxify Cd than the other species studied here. Among the physiological mechanisms involved in Cd tolerance, the availability of carbohydrates for defense and repair may play important roles. Our data suggest that increases in total soluble sugars in roots of *P. cathayana* may have contributed to osmotic regulation and oxidant detoxification under Cd exposure as observed for *P.* × *canescens* (He et al. 2011). Recently, it was found that *P. euphratica*, which is a highly Cd sensitive poplar species, was unable to counteract Cd induced wilting (Polle et al. 2012).

It has often been observed that ROS production is induced in plants under Cd exposure, which is mainly due to impairment of the mitochondrial and photosynthetic electron transfer chains by Cd (Bi et al. 2009; Heyno et al. 2008; Rodriguez-Serrano et al. 2009). As in previous studies with other poplar species (He et al. 2011; Schützendübel et al. 2002), Cd-exposure-induced ROS ($O_2^{\bullet-}$ and H_2O_2) production in roots and leaves of *P. cathayana* (Fig. 5). To avoid ROS induced injury, $O_2^{\bullet-}$ and H_2O_2 need to be scavenged by elevated antioxidants (e.g., free proline, soluble phenolics, and antioxidative enzymes). Free proline and soluble phenolics were important nonenzymatic antioxidative compounds activated in *P.* × *canescens* under Cd stress (He et al. 2011). In addition to nonenzyme antioxidants, antioxidative enzymes such as APX, GPX, and CAT are of great importance for the plant defense system against ROS such as $O_2^{\bullet-}$ and H_2O_2 (Najeeb et al. 2011; Schützendübel and Polle 2002). Here, we show that *P. cathayana* responded to Cd exposure with increases in concentrations of free proline in roots and with elevated activities of APX, GPX, and CAT in roots (Figs. 6 and S1). These results indicate that the antioxidant system is upgraded under Cd exposure and that well-orchestrated physiological changes to prevent injury confer greater Cd tolerance in *P. cathayana* (zhonghua 1) than in the other poplar species analyzed.

In conclusion, Cd exposure caused a decline in photosynthesis in *P. cathayana* (zhonghua 1), *P. deltoides*, *P.* × *euramericana*, and *P. alba* × *P. glandulosa*, but not in *P. nigra* and *P. popularis*. Among these six poplar species, *P. cathayana* (zhonghua 1) showed the highest Cd concentrations in roots, wood, bark, and leaves, the largest Cd amount in aerial parts, the highest BCF in aerial parts and T_f under Cd exposure, indicating that *P. cathayana* (zhonghua 1) is superior to the other five poplar species in terms of Cd tolerance under the greenhouse conditions. Under Cd stress, increases in total soluble sugars in roots but decreases in starch in root, wood, and leaves of *P. cathayana* (zhonghua 1) have been found. Induced ROS ($O_2^{\bullet-}$ and H_2O_2) production in roots and leaves of *P. cathayana* (zhonghua 1), and increases in concentrations of free proline and soluble phenolics and activities of antioxidative enzymes (APX, GPX, and CAT) have been observed in this species. These data suggest that the well-coordinated physiological changes in *P. cathayana* (zhonghua 1) exposed to Cd confer the greater Cd tolerance in this than in the other poplar species under the current experimental conditions.

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References

Bi YH, Chen WL, Zhang WN, Zhou Q, Yun LJ, Xing D (2009) Production of reactive oxygen species, impairment of photosynthetic function and dynamic changes in mitochondria are early events in cadmium-induced cell death in *Arabidopsis thaliana*. Biol Cell 101:629–643

Cao X, Jia J, Li H, Li M, Luo J, Liang Z, Liu T, Liu W, Peng C, Luo Z-B (2012) Photosynthesis, water use efficiency and stable carbon isotope composition are associated with anatomical properties of leaf and xylem in six poplar species. Plant Biol. doi:10.1111/j.1438-8677.2011.00531.x

Castiglione S, Todeschini V, Franchin C, Torrigiani P, Gastaldi D, Cicatelli A, Rinaudo C, Berta G, Biondi S, Lingua G (2009) Clonal differences in survival capacity, copper and zinc accumulation, and correlation with leaf polyamine levels in poplar: A large-scale field trial on heavily polluted soil. Environ Pollut 157:2108–2117

Di Lonardo S, Capuana M, Arnetoli M, Gabbrielli R, Gonnelli C (2011) Exploring the metal phytoremediation potential of three *Populus alba* L. clones using an in vitro screening. Environ Sci Pollut Res 18:82–90

Durand TC, Sergeant K, Planchon S, Carpin S, Label P, Morabito D, Hausman JF, Renaut J (2010) Acute metal stress in *Populus tremula* × *P. alba* (717-1B4 genotype): Leaf and cambial proteome changes induced by cadmium^{2+}. Proteomics 10:349–368

Elobeid M, Göbel C, Feussner I, Polle A (2012) Cadmium interferes with auxin physiology and lignification in poplar. J Exp Bot. doi:10.1093/jxb/err384

Fischerova Z, Tlustos P, Szakova J, Sichorova K (2006) A comparison of phytoremediation capability of selected plant species for given trace elements. Environ Pollut 144:93–100

Grace S (2005) Phenolics as antioxidants. In: Smirnoff N (ed) Antioxidants and reactive oxygens pecies. Blackwell, London, pp 141–168

Gratao PL, Polle A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol 32:481–494

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11

Hanikenne M, Nouet C (2011) Metal hyperaccumulation and hypertolerance: a model for plant evolutionary genomics. Curr Opin Plant Biol 14:252–259

He J, Qin J, Long L, Ma Y, Li H, Li K, Jiang X, Liu T, Polle A, Liang Z, Luo Z-B (2011) Net cadmium flux and accumulation reveal tissue-specific oxidative stress and detoxification in *Populus* × *canescens*. Physiol Plant 143:50–63

Hermans C, Chen JG, Coppens F, Inze D, Verbruggen N (2011) Low magnesium status in plants enhances tolerance to cadmium exposure. New Phytol 192:428–436

Heyno E, Klose C, Krieger-Liszkay A (2008) Origin of cadmium-induced reactive oxygen species production: mitochondrial electron transfer versus plasma membrane NADPH oxidase. New Phytol 179:687–699

Kaplan O, Ince M, Yaman M (2011) Sequential extraction of cadmium in different soil phases and plant parts from a former industrialized area. Environ Chem Lett 9:397–404

Kieffer P, Dommes J, Hoffmann L, Hausman JF, Renaut J (2008) Quantitative changes in protein expression of cadmium-exposed poplar plants. Proteomics 8:2514–2530

Kieffer P, Planchon S, Oufir M, Ziebel J, Dommes J, Hoffmann L, Hausman JF, Renaut J (2009) Combining proteomics and metabolite analyses to unravel cadmium stress-response in poplar leaves. J Proteome Res 8:400–417

Kramer U (2005) Phytoremediation: novel approaches to cleaning up polluted soils. Curr Opin Biotechnol 16:133–141

Kramer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517–534

Laureysens I, Blust R, De Temmerman L, Lemmens C, Ceulemans R (2004) Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture: I. Seasonal variation in leaf, wood and bark concentrations. Environ Pollut 131:485–494

Laureysens I, De Temmerman L, Hastir T, Van Gysel M, Ceulemans R (2005) Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture. II. Vertical distribution and phytoextraction potential. Environ Pollut 133:541–551

Lei YB, Korpelainen H, Li CY (2007) Physiological and biochemical responses to high Mn concentrations in two contrasting *Populus cathayana* populations. Chemosphere 68:686–694

Lu LL, Tian SK, Yang XE, Wang XC, Brown P, Li TQ, He ZL (2008) Enhanced root-to-shoot translocation of cadmium in the hyperaccumulating ecotype of *Sedum alfredii*. J Exp Bot 59:3203–3213

Luo ZB, Calfapietra C, Scarascia-Mugnozza G, Liberloo M, Polle A (2008) Carbon-based secondary metabolites and internal nitrogen pools in *Populus nigra* under free air CO_2 enrichment (FACE) and nitrogen fertilisation. Plant Soil 304:45–57

Ma FW, Cheng LL (2003) The sun-exposed peel of apple fruit has higher xanthophyll cycle-dependent thermal dissipation and antioxidants of the ascorbate-glutathione pathway than the shaded peel. Plant Sci 165:819–827

Marmiroli M, Visioli G, Maestri E, Marmiroli N (2011) Correlating SNP genotype with the phenotypic response to exposure to cadmium in *Populus* spp. Environ Sci Technol 45:4497–4505

Meighan MM, Fenus T, Karey E, MacNeil J (2011) The impact of EDTA on the rate of accumulation and root/shoot partitioning of cadmium in mature dwarf sunflowers. Chemosphere 83:1539–1545

Milner MJ, Kochian LV (2008) Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system. Ann Bot 102:3–13

Morel FMM (2008) The co-evolution of phytoplankton and trace element cycles in the oceans. Geobiology 6:318–324

Najeeb U, Jilani G, Ali S, Sarwar M, Xu L, Zhou WJ (2011) Insights into cadmium induced physiological and ultra-structural disorders in *juncus effusus* L. and its remediation through exogenous citric acid. J Hazard Mater 186:565–574

Pandhair V, Sekhon BS (2006) Reactive oxygen species and antioxidants in plants: an overview. J Plant Biochem Biot 15:71–78

Pietrini F, Zacchini M, Iori V, Pietrosanti L, Bianconi D, Massacci A (2010) Screening of poplar clones for cadmium phytoremediation using photosynthesis, biomass and cadmium content analyses. Int J Phytoremediat 12:105–120

Polle A, Douglas C (2010) The molecular physiology of poplars: paving the way for knowledge-based biomass production. Plant Biol 12:239–241

Polle A, Chakrabarti K, Schurmann W, Renneberg H (1990) Composition and properties of hydrogen peroxide decomposing systems in extracellular and total extracts from needles of Norway spruce (*Picea abies* L., Karst.). Plant Physiol 94:312–319

Polle A, Klein T, Kettner C (2012) Impact of cadmium on young plants of *Populus euphratica* and *P.* × *canescens,* two poplar species that differ in stress tolerance. New For. doi:10.1007/s11056-011-9301-9

Rodriguez-Serrano M, Romero-Puertas MC, Sparkes I, Hawes C, del Rio LA, Sandalio LM (2009) Peroxisome dynamics in *Arabidopsis* plants under oxidative stress induced by cadmium. Free Radic Biol Med 47:1632–1639

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Schützendübel A, Nikolova P, Rudolf C, Polle A (2002) Cadmium and H_2O_2-induced oxidative stress in *Populus* × *canescens* roots. Plant Physiol Bioch 40:577–584

Shi GR, Cai QS (2009) Cadmium tolerance and accumulation in eight potential energy crops. Biotechnol Adv 27:555–561

Shi GR, Liu CF, Cai QS, Liu QQ, Hou CP (2010) Cadmium accumulation and tolerance of wwo safflower cultivars in relation to photosynthesis and antioxidantive enzymes. Bull Environ Contam Toxicol 85:256–263

Sun YB, Zhou QX, Wang L, Liu WT (2009) Cadmium tolerance and accumulation characteristics of *Bidens pilosa* L. as a potential Cd-hyperaccumulator. J Hazard Mater 161:808–814

Tamas L, Bocova B, Huttova J, Mistrik I, Olle M (2006) Cadmium-induced inhibition of apoplastic ascorbate oxidase in barley roots. Plant Growth Regul 48:41–49

Tamas L, Dudikova J, Durcekova K, Halugkova L, Huttova J, Mistrik I, Olle M (2008) Alterations of the gene expression, lipid peroxidation, proline and thiol content along the barley root exposed to cadmium. J Plant Physiol 165:1193–1203

Unterbrunner R, Puschenreiter M, Sommer P, Wieshammer G, Tlustos P, Zupan M, Wenzel WW (2007) Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environ Pollut 148:107–114

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, van der Lelie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16:765–794

Wu FZ, Yang WQ, Zhang J, Zhou LQ (2010) Cadmium accumulation and growth responses of a poplar (*Populus deltoids* × *Populus nigra*) in cadmium contaminated purple soil and alluvial soil. J Hazard Mater 177:268–273

Yadav R, Arora P, Kumar S, Chaudhury A (2010) Perspectives for genetic engineering of poplars for enhanced phytoremediation abilities. Ecotoxicology 19:1574–1588

Yaman M (2006) Comprehensive comparison of trace metal concentrations in cancerous and non-cancerous human tissues. Curr Med Chem 13:2513–2525

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514

Zacchini M, Pietrini F, Scarascia-Mugnozza G, Iori V, Pietrosanti L, Massacci A (2009) Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. Water Air Soil Pollut 197:23–34

Zacchini M, Iori V, Scarascia-Mugnozza G, Pietrini F, Massacci A (2011) Cadmium accumulation and tolerance in *Populus nigra* and *Salix alba*. Biol Plant 55:383–386

Zhao FJ, McGrath SP (2009) Biofortification and phytoremediation. Curr Opin Plant Biol 12:373–380