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Research article

Populus × *canescens* grown on Cr-rich tannery waste: Comparison of leaf and root biochemical and proteomic responses

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ABSTRACT

Treatment of tannery effluents generates large amounts of sediments containing concentrated doses of metals (mainly chromium). Such waste is most commonly disposed of by landfilling, which is hazardous to the ecosystem due to Cr leaching. Afforestation of disposal sites with fast growing trees could stabilize contaminants in the soil and prevent them from spreading. The aim of this study was to examine the adaptation of *Populus* × *canescens* Sm. to tannery waste using biochemical and proteomic methods. We analyzed changes in the leaves and fine roots of poplar planted in soil or tannery waste. We found no obvious symptoms of metal stress, such as: elevated hydrogen peroxide levels or lipid peroxidation, but we observed activation of many elements of antioxidative system. Comparison of 2-DE protein profiles of leaves and fine roots from poplar grown on soil or tannery waste revealed increased expression of glycolytic enzymes and proteins involved in the synthesis of cell wall components, changes in the levels of proteins associated with photosynthesis, stress-related proteins, proteasome subunits and methionine biosynthesis enzymes. This experiment demonstrated that proteomic analysis has the potential to link the effects of Cr-rich tannery waste with biological consequences.

1. Introduction

The tanning industry is one of the main sources of chromium pollution, with landfilling being the most common way of solid tannery waste management. As tannery wastes are toxic to most of living organisms, such areas become, to some extent, excluded from the surrounding ecosystem. Research concerning the phytoremediation of tannery waste has focused mainly on evaluating the potential of selected plant species for use in the treatment of wastewater and contaminated soil (Calheiros et al., 2008) or on the effects of the waste on growth and yield of crop plants (Lopez-Luna et al., 2012). Knowledge about Cr-containing tannery waste toxicity to plants is based mostly on the observed impact of specified chromium salt doses (Oliveira, 2012). The effect of tannery waste on plant physiology is similar to the effects of applying chromium ions (Sinha et al., 2007), although it cannot be explained by the influence of Cr alone. Waste toxicity can be mitigated or even completely removed by high levels of organic matter, a suitable pH or high concentrations of macro and micronutrients, which can promote the growth of plants that are able to utilize them (Shukla et al., 2011).

The application of small doses of solid tannery waste increases plant biomass, root and shoot lengths, the number of leaves and their surface area (Giachetti and Sebastiani, 2006; Sinha et al., 2007). Shukla et al. (2011) observed an increase in canopy area, diameter at breast height and total height in five woody plant species selected for the phytoremediation of tannery sludge dumps. Growth on soils containing tannery waste can also promote flowering (Singh et al., 2011).

However, tannery waste negatively affects some cellular processes. For example, mitotic index reduction, induction in micronuclei and chromosomal and mitotic aberrations in the root cells

Abbreviations: ACN, acetonitrile; ADK, adenosine kinase; APOX, ascorbate peroxidase; AsA, ascorbate; CAT, catalase; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate; 2-DE, two-dimensional gel electrophoresis; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; DTNB, 5,5'-dithiobis-2-nitrobenzoic acid; DTT, dithiothreitol; DW, dry weight; EDTA, ethylenediaminetetraacetic acid; FW, fresh weight; GS, glutamine synthetase; GPOX, guaiacol peroxidase; GSH, glutathione; GR, glutathione reductase; GSSG, glutathione disulfide; H_2O_2, hydrogen peroxide; IPG, immobilized pH gradient; MDA, malondialdehyde; MDHAR, monodehydroascorbate reductase; 2-ME, 2-mercaptoethanol; SDS, sodium dodecyl sulphate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SOD, superoxide dismutase; PMSF, phenylmethylsulphonyl; PVP, polyvinylpyrrolidone; PVPP, polyvinylpolypyrrolidone; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TFA, trifluoroacetic acid.

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have been observed (Gupta et al., 2012). Photosynthetic activity can either be stimulated or decreased in plants growing on tannery waste. An increase in chlorophyll and carotenoid contents has been observed, but only for a short period of time. The contents decrease over the longer term (Sinha et al., 2007; Gupta and Sinha, 2009; Chandra et al., 2009). The impacts on mineral nutrition are strongly dependent on the chemical composition of the waste and the plant species present. Usually there is an increase in macronutrient (N, P, Ca, Mg, and S) concentrations in plant organs (Giachetti and Sebastiani, 2006). Metal accumulation in roots (Fe, Mn and Cr), leaves (Cd and Cu) or seeds (Zn) has also been seen (Sinha et al., 2007; Chandra et al., 2009). Common consequences of such disorders are morphological changes, such as reductions in root and shoot lengths (Nagajyothi et al., 2009) and changes in leaf structure (Gupta and Sinha, 2009).

Tannery waste exposure leads to increased production of free radicals in leaves and roots of plant, which can cause oxidative stress (Sinha et al., 2007). The scale of this effect is dose and duration dependent. For example, an increase in lipid peroxidation may occur, but is not always observed (Sinha et al., 2007; Gupta and Sinha, 2009). Sinha et al. (2007) found an initial increase in cysteine content, followed by decreases at larger doses of tannery sludge, possibly due to reductions in the activity of enzymes involved in the sulfate reducing pathway (ATP sulfurylase and adenosine 5-phosphosulfate sulfotransferase) under metal stress. Growth on tannery waste can also lead to fluctuations in the levels of other non-protein thiols and changes in the concentration of low molecular antioxidants, such as ascorbic acid (Chandra et al., 2009; Gupta and Sinha, 2009). The protein content in tissues also rose, possibly due to the accumulation of proline or the induction of stress protein biosynthesis (Chandra et al., 2009).

Previous studies have mostly concentrated on the morphology of the plants, analysis of their mineral nutrition and oxidative stress generation (lipid peroxidation). However, there is a lack of detailed research on the molecular effects of growth on tannery waste. The objective of this study was to depict changes in the metabolism of poplars grown on tannery waste and to identify mechanisms allowing plant growth in such stressful and harsh conditions. Our paper is the first to describe the protein profile and the antioxidative system status of plants cultivated on solid tannery waste.

A hybrid poplar (*Populus* × *canescens* Sm.) was chosen for the study, as due to its tolerance to various abiotic stress conditions it can be used both as an energy crop and for phytoremediation (Abril et al., 2011). Our earlier results suggested that *P.* × *canescens* was able to grow well on tannery waste and showed no statistically significant decrease ($p > 0.05$) in biomass (Zemleduch and Lorenc-Plucińska, 2011). It accumulated Cr (759 mg kg^{-1} DW) in the roots above the critical level (30 mg kg^{-1} DW) and compared to the saplings growing on the control soil contained higher concentrations (mg kg^{-1} DW) of N (8690 vs 18,300), S (1320 vs 2050), Ca (4683 vs 6810), Na (712 vs 4668) and Fe (1605 vs 4753) while P (2389 vs 1584) and Zn (221 vs 91) concentrations was lower (Zemleduch and Lorenc-Plucińska, 2011), possibly due to the antagonistic effect of Cr (Giachetti and Sebastiani, 2006; Sinha et al., 2007).

2. Materials and methods

2.1. Plant material and experimental conditions

One year old hybrid poplar *P.* × *canescens* Sm. (*Populus tremula* L. × *Populus alba* L.) root suckers were dug up from the old poplar plantation at the Institute of Dendrology, Polish Academy of Sciences (ID PAS), and planted on solid tannery waste from an active landfilling site run by one of the biggest Polish tanneries or on unpolluted soil taken from the ID PAS poplar plantation. Details about the landfill site, the waste and the soil characteristics have been described previously in Zemleduch and Lorenc-Plucińska (2011). The tannery waste comprised pressed sediment obtained from wastewater treated with appropriate coagulants, such as Pix 113 (iron (III) sulphate), Pax 15 (polyaluminium chloride) and lime, and mixed with chrome trimmings, fleshings and shavings as well as unfinished leather splits. It contained phytotoxic levels of chromium (III) (23,026 mg kg^{-1} DW of total and 156 mg kg^{-1} DW of bioavailable forms of Cr). Essential differences in soil nutrition levels were also noted between the control soil and tannery waste in the concentrations (mg kg^{-1} DW) of total N (900 vs 18,100), S (2000 vs 39,300), P (374 vs 5418), K (1577 vs 455), Mg (840 vs 3583), Ca (1269 vs 126,801), Cu (4.97 vs 27.17) Fe (6242 vs 17,165), Zn (23 vs 173), Ni (2 vs 25), Pb (14 vs 45) and organic matter (15,100 vs 407,000), as well as some physical parameters, such as pH (5.21 vs 7.34), conductivity (mS cm^{-1}) (21.6 vs 2480), cation exchange capacity (CEC, in cmol kg^{-1}) (1.69 vs 67.76) and clay content (in %) (2 vs 6). Bioavailable forms of all elements were also higher in the waste than in the control soil.

Saplings were grown for 22 weeks in a shaded polytunnel, in 2 L pots filled with soil or tannery waste. Saplings were hand-watered daily or as necessary with tap water to maintain relative soil humidity of 60–80%. At the end of the experiment, fine roots (diameter < 2 mm) and healthy leaves from 6 plants per growth variant were collected, mixed, frozen in liquid nitrogen and stored at −80 °C for biochemical and molecular analyses. Mycorrhiza associated with the roots were regarded as part of the roots.

All the spectrophotometric measurements were done using Beckman Coulter DU-640 UV–VIS (Beckman Instruments, Inc., Fullerton CA, USA).

2.2. Concentration of malondialdehyde (MDA)

MDA was taken as an indicator of lipid peroxidation levels and was measured using the thiobarbituric acid (TBA) reaction (Heath and Packer, 1968). Leaf or fine root extracts (0.15 g FW 1.5 ml^{-1}) in 10% (w/v) trichloroacetic acid (TCA) with 0.25% (w/v) TBA were boiled for 30 min, cooled rapidly and centrifuged at 12,000 *g* for 10 min at 4 °C. The absorbance of the supernatant at 450, 532 and 600 nm was determined using a spectrometer. The MDA concentration was estimated using the formula: C (μmol l^{-1}) = $6.45(A_{532} - A_{600}) - 0.56A_{450}$ (Yang et al., 2009) and expressed as μmol g^{-1} FW.

2.3. Measurement of hydrogen peroxide

Hydrogen peroxide concentration was determined according to the method described by Patterson et al. (1984). A 0.5 g sample was homogenized with 0.2 g of activated carbon and 2 ml of 5% (w/v) TCA, filtered through Miracloth and then centrifuged at 25,000 g for 10 min at 20 °C. Exactly 100 μl of supernatant was made up to 2 ml with 50 mM K-phosphate buffer (pH 8.4) and incubated with 1 ml of colorimetric reagent in the dark for 15 min. The reagent was made by mixing 1:1 (v/v) 0.6 mM 4-(2-pyridylazo)resorcinol (disodium salt) and 0.6 mM potassium titanium oxide oxalate. The absorbance at 508 nm was measured against the K-phosphate buffer. The blank was 5% TCA instead of the supernatant. The H_2O_2 concentration was calculated using a standard curve and expressed as μmol g^{-1} FW.

2.4. Measurement of ascorbate and glutathione

Reduced (AsA) and oxidized ascorbate (DHA) contents were determined according to Law et al. (1983). Leaf or fine roots extracts