Ability of transgenic poplars with elevated glutathione content to tolerate zinc(2+) stress

András Bittsánszkya, Tamás Kömivesb,*, Gábor Gullnerb, Gábor Gyulaia, József Kissa, László Heszkya, László Radimszkyc, Heinz Rennenbergd

*Department of Genetics and Plant Breeding, HAS-SIU, Szent István University, 2103 Gödöllő, Páter K. u. 1, Hungary
bPlant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest, P.O.B. 102, Hungary
cResearch Institute for Soil Science and Agricultural Chemistry, Hungarian Academy of Sciences, 1525 Budapest, P.O.B. 35, Hungary
dAlbert-Ludwigs-Universität, Institut für Forstbotanik und Baumphysiologie, 79085 Freiburg i. Br., Georges-Köhler-Allee 53/54, Germany

Abstract

Phytoremediation potentials of four poplar lines, *Populus nigra* (N-SL clone), *Populus canescens*, and two transgenic *P. canescens* clones were investigated using in vitro leaf discs cultures. The transgenic poplars overexpressed a bacterial gene encoding γ-glutamylcysteine synthetase in the cytosol (11ggs) or in the chloroplasts (6LgI), and therefore, they contained an elevated level of glutathione. Leaf discs of poplar clones were exposed to different concentrations of ZnSO₄ for 21 days. Zinc(2+) was phytotoxic only at high concentrations (10⁻² to 10⁻¹ M) at all *P. canescens* lines, but *P. nigra* was more sensitive. Transgenic poplars showed elevated heavy metal uptake as compared to the nontransformed clones. Treatments with zinc(2+) strongly induced the activity of glutathione S-transferase enzyme in untransformed poplar lines but to a lesser extent in the transgenic clones. These results suggest that transgenic poplars are more suitable for phytoremediation of soils contaminated with zinc(2+) than wild-type plants.

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1. Introduction

Poplar plants have been shown to be excellent candidates for phytoremediation purposes. They can be cultivated at high rates of growth and produce a large biomass. Poplar trees have an extensive root systems ensuring efficient uptake of water containing the pollutants from the soil. Poplars allow several cycles of decontamination, their leaves can be easily collected and the contaminated biomass substantially reduced by incineration. The wood harvested may be used as valuable raw material for paper industry and the production of matches (Dietz and Schnoor, 2001; Kömives and Gullner, 2000; Kömives et al., 2003).

Although poplars are known to take up several inorganic pollutants including heavy metals, such as cadmium (Koprivova et al., 2002), mercury (Rugh et al., 1998), and zinc (Di Baccio et al., 2003), their heavy metal tolerance is limited (Dietz and Schnoor, 2001).

The remediative capacity of poplars can be significantly increased by genetic manipulations. Recently, poplar plants were transformed to overexpress the bacterial gene encoding γ-glutamylcysteine synthetase (γ-ECS, EC 3.2.3.3), which is the rate-limiting regulatory enzyme in the biosynthesis of the ubiquitous tripeptide thiol compound glutathione (GSH, γ-L-glutamyl-L-cysteinyl-glycine; Noctor and Foyer, 1998). The transformed poplars contained higher levels of GSH and its precursor γ-L-glutamyl-L-cysteine (γ-EC) than the wild-type (Noctor et al., 1998). The increased production of GSH contributes to the antioxidative protection of plant cells against oxidative stress caused by various environmental factors (Noctor and Foyer, 1998). The glutathione
S-transferase isoenzymes (GSTs, E.C. 2.5.1.18.), which catalyze conjugation reactions between GSH and a number of xenobiotics, play crucial roles in the degradation of toxic substances (Cummins et al., 2003; Gullner et al., 2001; Jablonkai, 2003; Jablonkai and Hatzios, 1991; Kömives and Gullner, 2000). GSH, as the metabolic precursor of the heavy metal chelating phytochelatins, plays an important role also in the heavy metal detoxification in plants (Cobbett, 2000). Poplars overexpressing γ-ECS exposed to cadmium also contained higher amounts of phytochelatins and accumulated higher amounts of cadmium than wild-type plants. Although the overexpression of γ-ECS allows greater tissue cadmium accumulation, it has only a marginal effect on cadmium tolerance (Rennenberg and Will, 2000; Koprivova et al., 2002).

Little information is available about the physiological response and the base of tolerance of poplar plants to toxic concentrations of zinc (Di Baccio et al., 2003). In the present study, the stress reactions of two wild-type poplar hybrids, Populus nigra (N-SL) and Populus canescens (P. tremula×P. alba), as well as two transgenic poplar lines overexpressing γ-ECS in the cytosol (11ggs, cyt-ECS) or in the chloroplasts (6LgI, chl-ECS) were investigated following Zn exposure. The uptake of zinc and some other heavy metals by poplar leaf discs was also examined.

2. Materials and methods

2.1. Plant material

Two untransformed poplars, P. nigra and P. canescens (Populus tremula×Populus alba), as well as two genetically transformed P. canescens poplar lines overexpressing the *Escherichia coli* gshI gene encoding γ-ECS in the cytosol (11ggs) or in the chloroplasts (6LgI) were used for the experiments. Vector construction, transformation, identification, and characterization of transformants were published earlier (Noctor et al., 1998). The *P. nigra* N-SL clone was selected for general stress resistance at the Forestry Research Institute, Sárvár, Hungary (Kiss et al., 2001).

2.2. Leaf disc culture and biochemical analyses

Discs (8 mm in diameter) from leaves of micropropagated poplar cut clones were cut and placed onto woody plant media (WPM) supplemented with a concentration series of ZnSO₄ (10⁻⁵ to 10⁻¹ M), as described earlier (Gyulai et al., 1995). After a 21-day long exposure to ZnSO₄, heavy metal contents of discs (Cd, Cr, Cu, and Zn) were determined by inductively coupled plasma emission spectrometry (Zarcinas et al., 1987). GST activities were determined spectrophotometrically by measuring the formation of the conjugate reaction product at 340 nm using 1-chloro-2,4-dinitrobenzene as substrate (Habig et al., 1974).

2.3. Statistics

At least three independent parallel experiments were carried out in each case. The significant differences between mean values were evaluated by Student’s t-test. Differences were considered to be significant at P=0.05.

3. Results and discussion

3.1. Phytotoxic effects of Zn

A general obstacle in phytoremediation is finding appropriate plant species that can tolerate greater concentrations of pollutants in soils. Zn and other heavy metals are multisite inhibitors of several metabolic pathways and therefore phytotoxic at high concentrations (Cobbett, 2000; Schützendübel et al., 2002). High Zn concentrations brought about significant reductions in foliage and total dry mass in *Populus×euramericana* I-214 clones (Di Baccio et al., 2003). In our experiments, Zn applied at 10⁻¹ M concentration was found to cause severe phytotoxic, necrotic symptoms at all poplar clones studied. At 10⁻² M concentration, ZnSO₄ led to bleaching of leaf discs, nevertheless, with retained growing activity. High sensitivity of chloroplasts to ZnSO₄ was also observed in the transgenic clones. No toxic effects were observed at lower Zn concentrations (10⁻³ to 10⁻⁵ M) on discs of transgenic or wild-type clones of *P. canescens*. The phytotoxicity of ZnSO₄ on leaf discs of *P. nigra* showed somewhat different patterns as compared to *P. canescens* clones. Zn treatments (10⁻² to 10⁻⁵ M) caused brownish leaf disc color with retained growing activity. These results show an unexpectedly higher sensitivity of *P. nigra* towards ZnSO₄ as compared to either transgenic or control clones of *P. canescens*.

3.2. Heavy metal uptake

Heavy metal contents (Cd, Cr, Cu, and Zn) in leaf discs of the four poplar clones are shown in Fig. 1. The endogenous Zn content of discs increased gradually with the exogenous Zn supply. The Zn uptake of the transgenic and untransformed clones did not differ. The Cd, Cr, and Cu contents showed a linear increase with the applied ZnSO₄ concentration in all clones. The heavy metal contents measured in the leaf discs of the transgenic 6LgI clone were slightly but not significantly higher than in the untransformed clones. The highest heavy metal concentrations were detected in the transgenic 11ggs clone, which accumulated significantly more Cd, Cr, and particularly Cu than the other clones. Enhanced heavy metal uptake in 11ggs poplar clones found earlier (Rennenberg and Will, 2000; Koprivova et al., 2002) and in this study are related to the higher GSH and γ-EC concentrations in this clone. It is interesting to speculate if higher GSH levels result in
enhanced phytochelatin production as observed in other heavy metal treated plants (Cobbett, 2000). The marked accumulation of Cd, Cr, and Cu in the transgenic 11ggs clone indicates the suitability of this poplar plant to in situ phytoremediation.

3.3. GST activity

It was shown earlier that the foliar GST activities did not differ significantly between the transgenic 11ggs and 6LgI and the wild-type *P. canescens* hybrids in the absence of stress effects (Gullner et al., 2001). In the present study, markedly induced GST activities were measured in the leaf discs of untransformed poplar clones and of the transgenic 6LgI clone, while the activity did not change significantly in the 11ggs clone (Fig. 2). The induction of enzyme activity was observed at lower zinc(2+) concentrations (10⁻⁵ to 10⁻² M), but at the very phytotoxic 10⁻¹ M concentration, the GST activities were very low. The lower inducibility of the stress indicator GST activity in the transgenic clones as compared to the untransformed poplars showed that the Zn exposure caused a stronger stress effect in the untransformed clones. This effect was most evident at 10⁻² M Zn(2+) concentration. The low inducibility of GST in the transgenic clones indicates the marked stress tolerance of these clones to Zn exposure.

Phytoremediation efficiency is strongly influenced by the ability of the plant to escape deleterious concentrations of the toxic form of the pollutant and the active oxygen species that might be generated in the treated tissue. GST enzymes are known to possess GSH peroxidase activity, and thereby, they contribute to the detoxification of active oxygen.

![Fig. 1. Heavy metal (Zn, Cu, Cr, and Cd) contents in leaf discs of the untransformed poplar clones *P. nigra* (N-SL, -D-), *P. canescens* (-O-), and of the transgenic *P. canescens* 11ggs (-●-), and 6LgI (-○-) clones after 21 days of exposure to different concentrations of ZnSO₄ by in vitro leaf disc culture. Mean values ± S.D. are shown (n=3).](image1)

![Fig. 2. The induction of glutathione S-transferase activity in leaf discs of the untransformed poplar clones *P. nigra* (N-SL, -D-), *P. canescens* (-O-), and of the transgenic *P. canescens* 11ggs (-●-) and 6LgI (-○-) clones after 21 days of exposure to different concentrations of ZnSO₄. Mean values ± S.D. are shown (n=3).](image2)
species (Kömives et al., 2003). Recently, a functional theta class GST in Aspergillus nidulans was found to be involved in resistance to the heavy metals cadmium and nickel (Rai et al., 2003). This finding suggests that increased levels of GST contribute to elevated detoxification capacity in poplar leaf discs treated with Zn(2+) as well. The present results show that transgenic poplars are more suitable for the phytoextraction of soils contaminated with Zn(2+) than wild-type plants although by a mechanism still unknown.

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References

Rai R, Tate JJ, Cooper TG. Ure2, a prion precursor with homology to glutathione S-transferase, protects Saccharomyces cerevisiae cells from heavy metal ion and oxidant toxicity. J Biol Chem 2003;278:12826–33.