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RESEARCH PAPER

Over-expression of *gsh1* in the cytosol affects the photosynthetic apparatus and improves the performance of transgenic poplars on heavy metal-contaminated soil

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Keywords

Cell number; cell size; chloroplast; γ-glutamylcysteine synthetase; glutathione; mesophyll structure.

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ABSTRACT

Recent studies of transgenic poplars over-expressing the genes *gsh1* and *gsh2* encoding γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase, respectively, provided detailed information on regulation of GSH synthesis, enzymes activities and mRNA expression. In this experiment, we studied quantitative parameters of leaves, assimilating tissues, cells and chloroplasts, mesophyll resistance for CO_2 diffusion, chlorophyll and carbohydrate content in wild-type poplar and transgenic plants over-expressing *gsh1* in the cytosol after 3 years of growth in relatively clean (control) or heavy metal-contaminated soil in the field. Over-expression of *gsh1* in the cytosol led to a twofold increase of intrafoliar GSH concentration and influenced the photosynthetic apparatus at different levels of organisation, *i.e.*, leaves, photosynthetic cells and chloroplasts. At the control site, transgenic poplars had a twofold smaller total leaf area per plant and a 1.6-fold leaf area per leaf compared to wild-type controls. Annual aboveground biomass gain was reduced by 50% in the transgenic plants. The reduction of leaf area of the transformants was accompanied by a significant decline in total cell number per leaf, indicating suppression of cell division. Over-expression of γ-ECS in the cytosol also caused changes in mesophyll structure, *i.e.*, a 20% decrease in cell and chloroplast number per leaf area, but also an enhanced volume share of chloroplasts and intercellular airspaces in the leaves. Transgenic and wild poplars did not exhibit differences in chlorophyll and carotenoid content of leaves, but transformants had 1.3-fold fewer soluble carbohydrates. Cultivation on contaminated soil caused a reduction of palisade cell volume and chloroplast number, both per cell and leaf area, in wild-type plants but not in transformants. Biomass accumulation of wild-type poplars decreased in contaminated soil by more than 30-fold, whereas transformants showed a twofold decrease compared to the control site. Thus, poplars over-expressing γ-ECS in the cytosol were more tolerant to heavy metal stress under field conditions than wild-type plants according to the parameters analysed. Correlation analysis revealed strong dependence of cell number per leaf area unit, chloroplast parameters and mesophyll resistance with the GSH level in poplar leaves.

INTRODUCTION

The sulphur-containing tripeptide glutathione (GSH) is the major low molecular mass peptide in plants and is present at millimolar concentrations within cells. It is involved in many cellular processes through its influence on intracellular redox state (Noctor & Foyer 1998), its function as a transport form and reservoir of reduced sulphur (Rennenberg 2001) and its significance in the cross-talk between sulphur, nitrogen and carbon metabolism (Kopriva & Rennenberg 2004). In addition, GSH plays an important role in the defence of plant cells against reactive oxygen species (Noctor & Foyer 1998), xenobiotics (Edwards & Dixon 2005) and heavy metals (Cobbett & Goldsbrough 2002).

Lines of the poplar hybrid, *Populus tremula* × *P. alba*, over-expressing the bacterial genes *gsh1* or *gsh2* encoding γ-glutamylcysteine synthetase (γ-ECS) or glutathione synthetase, respectively, are widely known as good models for studies of the effects of enhanced glutathione (GSH) biosynthesis in plants (Noctor *et al.* 1998; Kopriva & Rennenberg 2004; Rennenberg & Peuke 2005). Therefore, these poplar transformants are among the best-characterised transgenic plants (Noctor *et al.* 1998). In laboratory experiments, transgenic poplars over-expressing γ-ECS in the cytosol did not differ from wild-type lines morphologically during initial growth and development (Noctor *et al.* 1996; Gullner *et al.* 2001) but had distinct functional features. Transformants were more tolerant towards chloroacetanilide herbicides than