RT-PCR Analysis and Stress Response Capacity of Transgenic gshI-Poplar Clones (Populus × canescens) in Response to Paraquat Exposure

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Stress response capacity (Fv/Fm at 690 nm and F690/F735 at $F_{\rm max}$) of untransformed hybrid poplar, *Populus* × *canescens* (*P. tremula* × *P. alba*), and two transgenic lines overexpressing γ -ECS (γ -glutamylcysteine synthetase) either in the cytosol (cyt-ECS) or in the chloroplast (chl-ECS) was studied in response to the herbicide paraquat (4.0×10^{-9} to 4.0×10^{-6} M) for 21 days. Significant differences at sublethal (4.0×10^{-7} M) and bleaching (4.0×10^{-6} M) concentrations of paraquat were observed with about a two-fold and eight-fold decrease in the photosynthetic activity (Fv/Fm at 690 nm and F690/F735 at $F_{\rm max}$), respectively. None of the *gsh*I transgenic lines (cyt-ECS, chl-ECS) with elevated GSH content exhibited significant tolerance to paraquat.

Semiquantitative RT-PCR of the cyt-ECS clone was used for gene expression analysis of the nuclear encoded *rbcS* gene and the stress responsive *gst* gene. Expression of the constitutively expressed 26SrRNA ribosomal gene was probed as a control for all RT-PCR reactions. The relative intensities of gene expressions normalized to the level of 26SrRNA intensity showed a 50% decrease in the nuclear encoded *rbcS* expression and a 120% increase in the stress responsive *gst* gene expression of the paraquat treated $(4.0 \times 10^{-7} \text{ m})$ samples of the transgenic popular line (cyt-ECS).

Key words: cyt-ECS (ggs11), chl-ECS (lgl6), Paraquat Stress, Populus × canescens