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Over-expression of bacterial γ-glutamylcysteine synthetase (*GSH1*) in plastids affects photosynthesis, growth and sulphur metabolism in poplar (*Populus tremula* × *Populus alba*) dependent on the resulting γ-glutamylcysteine and glutathione levels

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ABSTRACT

We compared three transgenic poplar lines over-expressing the bacterial γ-glutamylcysteine synthetase (*GSH1*) targeted to plastids. Lines Lggs6 and Lggs12 have two copies, while line Lggs20 has three copies of the transgene. The three lines differ in their expression levels of the transgene and in the accumulation of γ-glutamylcysteine (γ-EC) and glutathione (GSH) in leaves, roots and phloem exudates. The lowest transgene expression level was observed in line Lggs6 which showed an increased growth, an enhanced rate of photosynthesis and a decreased excitation pressure (1-qP). The latter typically represents a lower reduction state of the plastoquinone pool, and thereby facilitates electron flow along the electron transport chain. Line Lggs12 showed the highest transgene expression level, highest γ-EC accumulation in leaves and highest GSH enrichment in phloem exudates and roots. This line also exhibited a reduced growth, and after a prolonged growth of 4.5 months, symptoms of leaf injury. Decreased maximum quantum yield (F_v/F_m) indicated down-regulation of photosystem II reaction centre (PSII RC), which correlates with decreased PSII RC protein D1 (PsbA) and diminished light-harvesting complex (Lhcb1). Potential effects of changes in chloroplastic and cytosolic GSH contents on photosynthesis, growth and the whole-plant sulphur nutrition are discussed for each line.

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Abbreviations: γ-EC, γ-glutamylcysteine; γ-ECS, γ-glutamylcysteine synthetase; GSH, glutathione (reduced state); *GSH1*, γ-glutamylcysteine synthetase gene; *GSH2*, glutathione synthetase gene; GSSG, glutathione (oxidized state).

INTRODUCTION

Glutathione (GSH) is an important component of the primary metabolism of plants. GSH is involved in various processes including storage and transport of reduced sulphur (Rennenberg, Schmitz & Bergmann 1979; Rennenberg 1984; Herschbach 2003), stress response to reactive oxygen (Polle & Rennenberg 1993; Foyer & Noctor 2005a,b) and heavy metals (Rauser 1995, 1999; Cobbett 2000a,b; Peuke & Rennenberg 2005a,b), as well as detoxification of xenobiotics (Rennenberg & Lamoureux 1990; Edwards & Dixon 2005). In addition, GSH is a key component to maintain the cellular redox state (Meyer & Hell 2005; Mullineaux & Rausch 2005). For example, under oxidative stress (Rouhier, Lemaire & Jacquot 2008) or during flower development (Xing, Lauri & Zachgo 2006), synthesis of various cellular proteins is under GSH-mediated redox control. The GSH-mediated redox control can occur via glutathionylation of proteins, and thus at the transcriptional and/or post-transcriptional level (Dietz 2008; Meyer 2008). GSH is synthesized in two ATP-dependent steps catalysed by consecutive action of γ-glutamylcysteine synthetase (γ-ECS), which forms γ-glutamylcysteine (γ-EC) from cysteine (Cys) and glutamate, and glutathione synthetase (GSHS), which adds glycine to the γ-EC.