January 2007

Over-expression of UDP-glucose pyrophosphorylase in hybrid poplar affects carbon allocation

Heather D. Coleman
University of British Columbia

Thomas Canam
Eastern Illinois University, tcanam@eiu.edu

Kyu-Young Kang
University of British Columbia

David D. Ellis
National Center for Genetic Resources Preservation

Shawn D. Mansfield
University of British Columbia

Journal of Experimental Botany, Vol. 58, No. 15/16, pp. 4257–4268, 2007
doi:10.1093/jxb/erm287
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Over-expression of UDP-glucose pyrophosphorylase in hybrid poplar affects carbon allocation

Heather D. Coleman[1], Thomas Canam[1], Kyu-Young Kang[1], David D. Ellis[2] and Shawn D. Mansfield[1,*]

[1] *Department of Wood Science, University of British Columbia, Vancouver, BC, V6T 1Z4 Canada*

[2] *National Center for Genetic Resources Preservation, Fort Collins, CO 80521, USA*

Received 13 September 2007; Revised 15 October 2007; Accepted 16 October 2007

Abstract

The effects of the over-expression of the *Acetobacter xylinum* UDP-glucose pyrophosphorylase (UGPase) under the control of the tandem repeat Cauliflower Mosaic Virus promoter (2×35S) on plant metabolism and growth were investigated in hybrid poplar (*Populus alba*×*grandidentata*). Transcript levels, enzyme activity, growth parameters, leaf morphology, structural and soluble carbohydrates, and soluble metabolite levels were quantified in both transgenic and wild-type trees. Transgenic 2×35S*::*UGPase poplar showed impaired growth rates, displaying reduced height growth and stem diameter. Morphologically, 2×35S*::*UGPase trees had elongated axial shoots, and leaves that were substantially smaller in size when compared with wild-type trees at equivalent developmental stages. Biochemical analysis revealed significant increases in soluble sugar, starch, and cellulose contents, and concurrent decreases in lignin content. Lignin monomer composition was altered in favour of syringyl moieties. Detailed soluble metabolite analysis revealed that 2×35S*::*UGPase trees had as much as a 270-fold increase in the salicylic acid 2-*O*-β-D-glucoside (SAG), a compound typically associated with the stress response. These data suggest that while it is possible to alter the allocation of carbon in favour of cellulose biosynthesis, whole plant changes result in unexpected decreases in growth and an increase in defence metabolites.

Key words: Carbon allocation, cellulose, hybrid poplar, sucrose metabolism, UDP-glucose, UDP-glucose pyrophosphorylase.

Introduction

To maximize resource acquisition and to minimize exposure to deleterious phenomena, trees constantly monitor endogenous and environmental cues, and use this information to regulate resource allocation. Ultimately, these responses are controlled by gene expression patterns, resulting in the synthesis of a variety of metabolites, including carbohydrates, which can accumulate, participate in metabolite channelling and/or be polymerized into more complex macromolecules. Studies have shown that altered carbon partitioning can manifest changes in the chemical composition of plants by the altered regulation of genes involved in the synthesis of lignin or cellulose (Li *et al*., 2003; Canam *et al*., 2006). Despite these findings, how carbon partitioning occurs and the factors that regulate plants to produce more or less cellulose still remain largely unanswered. It may be possible to alter and/or regulate carbohydrate utilization by increasing the availability of precursor substrates such as UDP-glucose in the production of cellulose, or ADP-glucose in starch synthesis. The availability of soluble carbohydrates would therefore be a key component; to altering and/or regulating carbohydrate utilization, thus by increasing available sugar metabolites in the sink cells (i.e. UDP-glucose), there may be the potential to augment cellulose production and content.

Two pathways have been identified that lead to the direct production of UDP-glucose. The first is based on the cleavage of sucrose by sucrose synthase (SuSy; EC 2.4.1.13) liberating fructose and UDP-glucose, while the second relies on the hexose phosphate pool and the active phosphorylation of glucose 1-phosphate by UDP-glucose pyrophosphorylase (UGPase, EC 2.7.7.9). UGPase is considered to be a key enzyme in carbohydrate biosynthesis and plays an important role in sucrose

* To whom correspondence should be addressed: shawnman@interchange.ubc.ca

metabolism (Coleman *et al*., 2006). In sink tissues, UGPase works in co-ordination with the sucrolytic enzymes (i.e. SuSy, fructokinase, and SPS/SPP) in the metabolism of sucrose and hexose phosphates, while in source tissues, UGPase works closely with SPS in the synthesis of sucrose (Kleczkowski, 1994). As such, the dual functionality of UGPase makes it an interesting target for altering cellulose production; it has the potential to increase the amount of sucrose in source tissues, and concurrently decrease sucrose in sink tissues, thereby modulating the solute potential gradient and facilitating sucrose channelling to sink tissues.

Previous investigations have focused primarily on down-regulating UGPase enzyme activity, as evidence suggests that UGPase is present in ample supply in plants (Appeldoorn *et al*., 1997; Magel *et al*., 2001). However, the results of such studies are mixed, ranging from no observed phenotypic effect in potato tubers despite a decrease in UGPase by 96% (Zrenner *et al*., 1993) to substantial decreases in soluble sugar concentrations in potato tubers with a 30–50% reduction in activity (Spychalla *et al*., 1994; Borokov *et al*., 1996). In *Arabidopsis*, similar reductions in soluble sugars were observed when UGPase activity was reduced by ~50% (Johansson, 2003). In contrast, when UGPase activity was up-regulated in tobacco, increased plant biomass and changes in carbohydrate metabolism were observed, albeit without altering partitioning to cellulose (Coleman *et al*., 2006). Studies evaluating native UGPase gene expression patterns in poplar clearly demonstrate the onset of UGPase up-regulation during late cell expansion and secondary cell wall formation, which is consistent with the theory that UGPase contributes in providing the immediate substrate for cellulose synthesis, and is co-ordinately up-regulated with the cellulose synthase complex (Hertzberg *et al*., 2001).

The current study attempts to further elucidate the role of UGPase in secondary cell wall biosynthesis in hybrid poplar, and its effects on carbon partitioning. Poplar trees were transformed with UGPase from *Acetobacter xylinum* under the control of the constitutive (2×35S) promoter and were used to evaluate changes in tree growth characteristics, biochemistry, and cell wall chemistry. The *Acetobacter*-derived UGPase gene was chosen for these studies since it shows a high specificity for UDP-glucose (Brede *et al*., 1991), in contrast with the broader substrate specificity of plant-derived non-specific UDP-sugar pyrophosphorylases (Kotake *et al*., 2004).

Materials and methods

Cloning of UGPase and plasmid construction

UGPase (M76548) was cloned from *Acetobacter xylinum* ATCC #23768 and inserted into the pBIN cloning vector under the control of the enhanced tandem CaMV 35S (2×35S) constitutive promoter (Datla *et al*., 1993; Kay *et al*., 1987). Sequence analysis was used to confirm the proper insertion of the promoter and gene into the binary vector.

Plant transformation and maintenance

Hybrid poplar (*Populus alba*×*grandidentata*) was transformed using *Agrobacterium tumefaciens* EHA105 (Hood *et al*., 1993) using a standard leaf disc inoculation. Binary plasmids were inserted into EHA105 using the freeze–thaw technique, and incubated overnight in liquid Woody Plant Media (WPM: McCown and Lloyd, 1981) with 100 μM acetosyringone. Leaf discs were cut and co-cultured with EHA105 for 1 h at room temperature, blotted dry, and plated abaxially onto WPM supplemented with 0.1 μM each α-naphthalene acetic acid (NAA), 6-benzylaminopurine (BA), and thiadiazuron (TDZ) and solidified with 3% (w/v) agar and 1.1% (w/v) phytagel (WPM 0.1/0.1/0.1). After three days the discs were transferred to WPM 0.1/0.1/0.1 supplemented with carbenicillin disodium (500 mg l^{-1}) and cefotaxime sodium salt (250 mg l^{-1}). Following three additional days, the discs were transferred to WPM 0.1/0.1/0.1 containing carbenicillin, cefotaxime, and kanamycin (25 mg l^{-1}). After 5 weeks, shoots and callus material were transferred to WPM with agar and phytagel, 0.01 μM BA, carbenicillin, cefotaxime, and kanamycin. Once individual shoots were visible, plantlets were transferred to solidified WPM with 0.01 μM NAA and carbenicillin, cefotaxime, and kanamycin to induce rooting. After two consecutive 5-week periods on this media, shoot tips were isolated to solidified antibiotic-free WPM with 0.01 μM NAA.

Plants were confirmed as transgenic by PCR screening of genomic DNA employing gene-specific oligonucleotides: specifically, UGP-F (5′-ATCGAGGAATTCTGCCTCGT-3′) and UGP-R (5′-TCGCAAGACCGGCAACAGGATT-3′).

All shoot cultures, including transgenic and non-transformed control lines, were maintained on solid WPM with 0.01 μM NAA in GA-7 vessels at 22 °C under a 16 h photoperiod with an average photon flux of 40 μmol m^{-2} s^{-1}.

Plant growth and biomass

Tissue culture plantlets were transferred into 7.5 l pots containing a 50% peat, 25% fine bark, and 25% pumice soil mixture in the greenhouse, and covered with 16 oz clear plastic cups for 1 week to aid in acclimation. Each poplar line, transgenic and wild type, was represented by 12 clonally-propagated trees. The greenhouse trees were harvested after 4 months growth, at which time tree height, from base to tip, and stem diameter (10 cm above root collar) were measured.

Developmental stages of tissues were standardized by employing a plastochron index, where leaf plastochron index PI=0 was defined as the first leaf greater than 3 cm in length, and where PI=1 was the leaf immediately below PI=0. Portions of the stem from each plant spanning PI=5 to PI=15, and from PI=15 to PI=25 were excised and dried at 105 °C for 48 h for dry weight determination, and retained for further analysis. Leaves were also collected in ten node groups (PI 6–15, PI 16–25) and analysed using an Area Meter (Li-Cor Environmental, Lincoln NE), and then dried at 105 °C for 48 h for dry weight determination. Developing xylem was scraped and flash frozen in liquid nitrogen for future analysis of enzyme activity, RNA transcript abundance, and soluble sugar analysis.

Transcription levels

Real-time PCR was used to determine transcript abundance of each transgene. Leaf and developing xylem samples weighing approximately 1 g (FW) were ground in liquid nitrogen, and RNA was extracted according to the method of Kolosova *et al*. (2004). One μg of RNA was used for the synthesis of cDNA using Superscript II

Reverse Transcriptase (Invitrogen, Carlsbad, CA) and dT_{16} primers according to the manufacturer's instructions. Samples were run in triplicate with Brilliant SYBR Green QPCR Master Mix (Stratagene, La Jolla, CA) on an Mx3000P Real-Time PCR System (Stratagene). The primers for the RT-PCR analysis of the *Acetobacter xylinum* UGPase were AU-RTF (5′-TGGAAGCAACCCGCGTCATC-3′) and AU-RTR (5′-GCCAAGGCCCAGCGGTTCC-3′). Conditions for the RT-PCR reactions were as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 62 °C for 1 min, and 72 °C for 30 s. Transcript levels were based on standard curves derived from known concentrations of plasmid DNA run under the same conditions. Transcript abundance of the two native poplar UGPase genes, as per Meng *et al.* (2007) was concurrently evaluated by RT-PCR using the following primers: PtUGP1F (5′-GGCTTCTTCAGATTTGCTTCTG-3′), PtUGP1R (5′-CCAGTTTCACACCAGATTTCAC-3′) and PtUGP2F (5′-GCAACTTCAGATCTGCTTCTTG-3′), PtUGP2R (5′-TCCAATTTCACACCAGATTTTG-3′).

In addition, the transcription abundance of key genes involved in lignin and cellulose production in the developing xylem were also quantified, including: phenylalanine ammonia-lyase, PAL-RT-FW (5′-AAAGGTGCCGAAATTGCCATGG-3′), PAL-RT-RV (5′-TGCAGAAATCAAGCCCAAGGAG-3′); cinnamate 4-hydroxylase, C4H-RT-FW (5′-GTGGGGAATTGCTGAGCTTGT-3′), C4H-RT-RV (5′-CGCAACTTCTTCTGGATTTCA-3′); coumarate 3-hydroxylase, C3H-RT-FW (5′-ATGGCTTCGTTGGATGTTTC-3′), C3H-RT-RV (5′-ATCCATAATAGCTCTAGTGA-3′); caffeoyl CoA 3-*O*-methyltransferase, CCOMT-RT-FW (5′-TTTGCATGCTTCCTGTTGGTGA-3′), CCOMT-RT-RV (5′-AATGCAGCCCCTCACTTGATCC-3′); cinnamoyl CoA reductase, CCR-RT-FW (5′-ATGGTTTACTCTATGTGCTTCTCT-3′), CCR-RT-RV (5′-GCTCCTCCTTCACAAACCTTAA-3′); cinnamyl alcohol dehdrogenase, CAD-RT-FW (5′-ATGAAGTGGTTGGTGAGGTTGT-3′), CAD-RT-RV (5′-ACACCGACAACATCTCCAACTT-3′); ferulate 5-hydroxylase, F5H-RT-FW (5′-AGCTCGCAGACGTGGTGGGTTTAG-3′), F5H-RT-RV (5′-GAAATAACCAGCAACCTCAGCATCT-3′); caffeic acid 3-*O*-methyltransferase, COMT-RT-FW (5′-GCCAGTGCTTCAGTTCTACCAA-3′); COMT-RT-RV (5′-GGTCGAGTTCAATGGCTGTTTT-3′); 4CL-RT-FW (5′-GCACCTAAAACTCACCATCTCTCC-3′), 4CL-RT-RV (5′-AAGGTTTTTCGGGATGTAGATGTC-3′); sucrose synthase, SuSyPt-RT-FW (5′-CCATGGATTGCTCTTGCTCTGC-3′), SuSyPt-RT-RV (5′-GCAACACGCAAATCCTCAACAA-3′); and cellulose synthase, CESA-RT-FW (5′-AGAGCTGTGATCATTATGCGACTG-3′), CESA-RT-RV (5′-ACCCAAGAAAATGCAAACCAGATC-3′). The genes selected and employed for transcript analysis of the lignin branch of the phenylpropanoid pathway and cellulose biosynthesis were based on previously reported high levels (highest of each isoform) during EST expression profiling of the cambial zone and tension wood formation in poplar (Sterky *et al*., 2004). Critical threshold (ct) values for all genes were quantified in triplicate and normalized to β-actin transcript levels.

Enzyme activity

Leaf and developing xylem samples (approximately 1 g FW) were ground in liquid nitrogen with 1 mg of insoluble PVPP and 4 vols of extraction buffer (50 mM HEPES–KOH pH 7.5, 10 mM $MgCl_s$, 1 mM EDTA, 2 mM DTT, 1 mM PMSF, 5 mM ε-amino-*n*-caproic acid, 0.1% v/v Triton X-100, and 10% v/v glycerol). The samples were centrifuged at 14 000 rpm for 20 min at 4 °C. The extract was passed through a desalting column (DG 10: Bio-Rad) that was pre-equilibrated with ice-cold extraction buffer without Triton X-100 and PVPP. Extracts were collected into prechilled vials and used immediately. UGPase activity was determined spectrophotometrically at 340 nm as per Appeldoorn *et al.* (1997) using 100 µl of plant extract and a molar extinction coefficient of 6.22 mM cm^{-1}. Total protein content of the extracts was determined using a Bio-Rad Protein Assay (Bio-Rad, Hercules, CA).

Soluble carbohydrate and starch analysis

Soluble carbohydrates (glucose, fructose, and sucrose) were extracted overnight at –20 °C from ground freeze-dried plant material using methanol:chloroform:water (12:5:3 by vol.). The samples were centrifuged, the supernatants removed, and the remaining pellet washed twice with fresh methanol:chloroform:water (12:5:3 by vol.) and all fractions pooled. Five ml of water was added to the combined pooled supernatant and centrifuged to facilitate phase separation. The aqueous fraction was removed to a round-bottom flask and rotary evaporated to dryness. The samples were resuspended in 3 ml of distilled water and analysed using anion exchange HPLC (Dionex, Sunnyvale, CA) on a DX-600 equipped with a Carbopac PA1 column and an electrochemical detector, as per Coleman *et al.* (2006).

The residual pellets were hydrolysed using 4% sulphuric acid at 121 °C for 4 min. The liberation of glucose represented starch content, and was directly quantified by HPLC using similar conditions.

Cell wall compositional analysis

Greenhouse-grown plant stem material was ground using a Wiley mill to pass through a 40-mesh screen, and then Soxhlet extracted with acetone for 24 h. The extractive free material was used for all further analyses. Lignin content was determined using a modified Klason, where extracted ground stem tissue (0.2 g) was treated with 3 ml of 72% H_2SO_4 as per Coleman *et al.* (2006). Carbohydrate concentrations in the hydrolysate were determined using high-performance liquid chromatography (HPLC) (Dionex DX-500, Dionex, CA) equipped with an ion exchange PA1 (Dionex) column, a pulsed amperometric detector with a gold electrode, and a Spectra AS 3500 auto-injector (Spectra-Physics, Los Angeles, CA). 20 µl of hydrolysate was loaded on the column equilibrated with 250 mM NaOH and eluted with deionized water at a flow rate of 1.0 ml min^{-1}, followed by a post column addition of 200 mM NaOH at a flow rate of 0.5 ml min^{-1}. Each experiment was run in duplicate.

Determination of α-cellulose content

α-cellulose was determined from the extract-free wood using a modified microanalytical method developed by Yokoyama *et al.* (2002). In short, 200 mg of ground extract-free wood was weighed into a 25 ml round bottom flask and placed in a 90 °C oil bath. The reaction was initiated by the addition of 1 ml of sodium chlorite solution (400 mg 80% sodium chlorite, 4 ml distilled water, 0.4 ml acetic acid). An additional 1 ml of sodium chlorite solution was added every half hour and the samples removed to a cold waterbath after 2 h. Samples were then filtered through a coarse crucible, dried overnight, and holocellulose composition determined gravimetrically. Fifty mg of this dried holocellulose sample was weighed into a reaction flask and allowed to equilibrate for 30 min. Four ml of 17.5% sodium hydroxide was added and allowed to react for 30 min, after which 4 ml of distilled water was added. The sample was macerated for 1 min, allowed to react for an additional 29 min and then filtered through a coarse filter. Following a 5 min soak in 1.0 M acetic acid, the sample was washed with 90 ml of distilled water and dried overnight. The α-cellulose content was then determined gravimetrically.

Monolignol analysis

Thioacidolysis (Rolando *et al*., 1992) was used to determine the lignin monomer ratios (syringyl:guaiacyl; S:G), using 10 mg of

oven-dried extractive-free wood and tetracosane as an internal standard (2 ml of 25 mg ml^{-1} in dichloromethane). The silylation reaction proceeded for a minimum of 2 h, and gas chromatography was carried out on a ThermoFinnigan Trace GC-PolarisQ ion trap system with an AS2000 autosampler and a split/splitless injector. The GC was equipped with a 30 m, 0.25 mm internal diameter J&W DB-5 column. The GC conditions were as follows: initial injector temperature of 250 °C, detector temperature of 270 °C, and initial oven temperature of 130 °C. Following a 2 µl injection, the oven remained at 130 °C for 3 min and then ramped at a rate of 3 °C min^{-1} to 260 °C and held for 5 min.

Soluble metabolite analysis

Liquid nitrogen-frozen developing xylem tissue was ground using a dental amalgam mixer, and extracted using a two-phase methanol:chloroform extraction as per Robinson *et al*. (2005). In short, 600 µl of methanol was added to 20 mg of frozen tissue to stop biological activity. The samples were vortexed and 40 µl of water, 10 µl of internal standard (10 mg ml^{-1} ribitol in water) and 10 µl of lipophilic internal standard (10 mg ml^{-1} nonadecanoic acid methyl ester in methanol) were added. The samples were then incubated for 15 min at 70 °C with constant agitation, and then centrifuged for 5 min at 13 000 rpm. The supernatants were removed, and 800 µl of methanol was added to the pellets, which was vortexed to resuspend the pellets, and then incubated at 35 °C for 5 min with constant agitation. The samples were centrifuged for 5 min at 13 000 rpm and then the supernatants were removed and combined with the previous methanol supernatant. 600 µl of water was added to the pooled methanol extracts, vortexed and centrifuged at 4000 rpm for 15 min. A 900 µl sample was removed from the upper potion (water/methanol) and dried at 40 °C using a Speedvac.

The pellet was resuspended in 50 µl of methoxyamine hydrochloride solution (20 mg ml^{-1} in chloroform) and incubated for 2 h at 60 °C with constant agitation. Following the addition of 200 µl of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), the samples were incubated at 60 °C for 30 min with constant agitation. Samples were left at room temperature overnight to allow the reaction to progress to completion, and then filtered through tissue paper to remove particulates. GC/MS analysis was carried out using a 30 m, 0.25 mm internal diameter Restek Rtx-5MS column using the following conditions: inlet temperature 250 °C, injector split ratio 10:1, resting oven temperature 70 °C, and GC–MS transfer line temperature of 300 °C. Following a 1 µl injection, the oven remained at 70 °C for 2 min and then ramped at a rate of 8 °C min^{-1} to 325 °C and held for 6 min.

Results

Transformed hybrid poplar trees regenerated from the UGPase-*Agrobacterium*-treated leaf explants were propagated from single shoots from individual unique explants, and represented independent transformed lines which were confirmed by PCR screening of genomic DNA to amplify a diagnostic fragment specific to the UGPase gene. From the independent transgenic lines, six 2×35S::UGPase transformants (based on high real-time qPCR expression levels) and corresponding control (wild-type) shoots were propagated *in vitro* as shoot cultures, and a minimum of 12 individual trees of each line were transferred into the greenhouse and grown for four months under 16 h days supplemented with overhead lighting with a radiant flux density of 300 W m^{-2}. At harvest, all of the transgenic lines showed substantially altered growth characteristics, demonstrating significantly impaired height and diameter growth (Fig. 1A, B). Furthermore, the trees displayed significantly reduced internodal length (Fig. 1D), as well as decreased stem dry weight (data not shown).

Morphologically, the 2×35S::UGPase transgenic lines also displayed a greater abundance of significantly smaller leaves per stem, and consistently had elongated axial shoots at each leaf node (Fig. 2). A quantitative evaluation of leaf characteristics confirmed that the 2×35S::UGPase leaves were 75% smaller, had reduced total leaf area per stem and reduced total leaf dry weight (Fig. 1C). In addition, the trichomes appeared to be more highly concentrated in the 2×35S::UGPase leaves when compared with the wild-type poplar (data not shown).

Transcription levels

Real-time quantitative PCR was used to evaluate transcript abundance of the exogenous UGPase gene in the transgenic lines (Table 1). All the 2×35S::UGPase transgenic lines had significantly higher transcript levels in the leaf material compared with the stem (developing xylem). Transcript abundance ranged from 68–172 copies μg^{-1} total RNA in leaf tissue and 2.1–27.9 copies μg^{-1} total RNA in the developing xylem, among the transgenic poplar trees. The expression of the two native poplar UGPase genes was also shown to be affected in most lines (see Supplementary Table 1 at *JXB* online), with smaller increases in PtUGP1 when compared with the observed increases in PtUGP2.

Transcript abundance of genes involved directly in cellulose and lignin biosynthesis in the developing xylem were also compared using real-time quantitative PCR. PAL, 4CL, CST, CQT, and COMT showed no change in the transgenic lines relative to wild-type levels, while all other genes in the lignin biosynthetic pathway were up-regulated (Fig. 3). SAD, CAD, C3H, and F5H were increased between 2–3-fold relative to wild-type levels, and C4H was increased to 4-fold the wild-type level. CCR and CCoAMT showed the highest relative increases at over six times the abundance seen in wild-type trees (see Supplementary Table 2 at *JXB* online). Similarly, the CesA gene family was also shown to be up-regulated approximately 4-fold over the corresponding wild-type CesA transcript levels, while SuSy expression levels remained comparable with the wild-type trees.

Enzyme activity

UGPase activity was determined using an indirect assay measuring the production of the reduced form of nicotinamideadenine dinucleotide (NADH). All of the 2×35S::UGPase transgenic trees showed consistent increases in UGPase activity in the leaf tissue compared

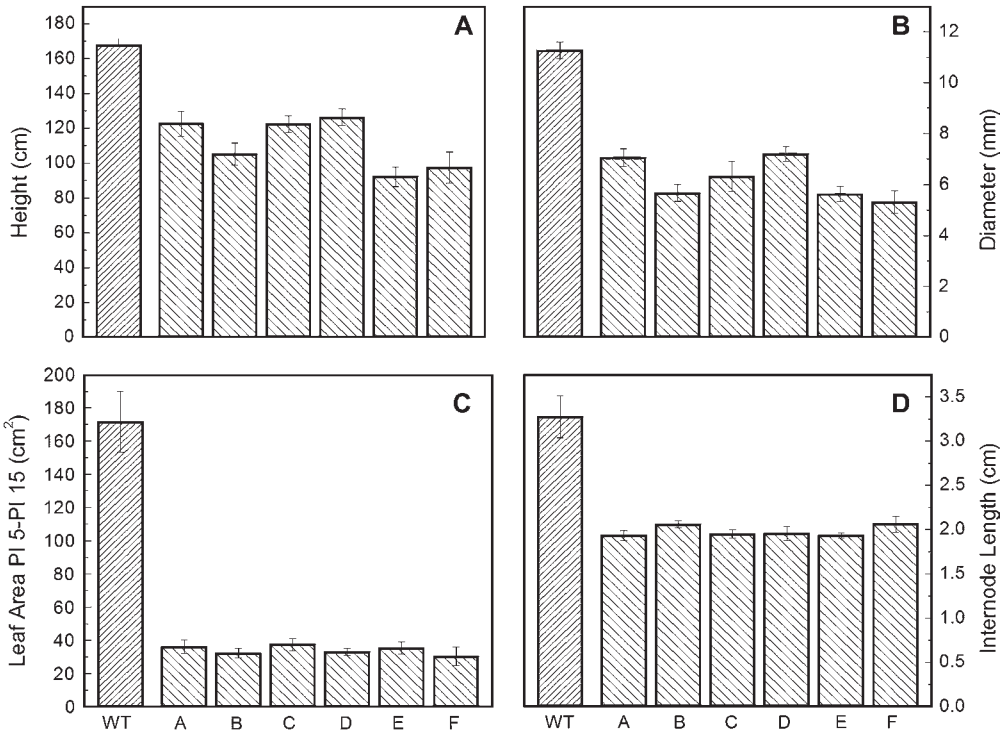

Fig. 1. Plant height (A), diameter (B), leaf area (C), and internode length (D); for transgenic and wild-type trees. Mean (±SE) were calculated from 10 plants per line. All transgenic lines are statistically significant different from the control trees at α=0.05.

to the wild-type trees (Table 1). However, by contrast, only one line (2×35S::UGPase line C) demonstrated a statistically significant increase in enzyme activity in the developing xylem, despite the slight general upward trend in activity in the other transgenic lines.

Soluble carbohydrates

In general, all transgenic lines showed elevated levels of total soluble sugars in the leaf tissue, which was a result of a commensurate increase in all three soluble sugars quantified; sucrose, fructose, and glucose (Table 2). The total soluble carbohydrate concentrations, as well as concentrations of each individual sugar, were significantly increased in the leaves of five out of the six 2×35S:: UGPase transgenic lines evaluated relative to the controls. The exception was Line F, which despite having higher glucose and fructose levels did not have statistically significant higher levels of sucrose, and thus total carbohydrates. By contrast, significant increases in total soluble carbohydrates, as well as significant increases in glucose and sucrose, were only found in two 2×35S:: UGPase lines (B, C) in the developing xylem.

Starch content

Three of the six 2×35S::UGPase transgenic lines (A, B, F) had significant increases in starch content in the leaves compared with starch levels in control leaves (Table 3). As well, five of the six 2×35S::UGPase transgenic lines (A, B, C, D, F) had significantly elevated starch in the developing xylem relative to control trees, with two lines (C, F) displaying three times the concentration of wild-type trees. And, although not statistically significant the sixth transgenic line (line E) did have higher levels of starch accumulation in the developing xylem, similar to the other transgenics.

Cell wall chemistry

The total cell wall carbohydrate content, as a measure of percent total dry weight, of the 2×35S::UGPase lines increased dramatically compared with the wild-type trees, where all six lines showed statistically significant changes in polymeric cell wall moieties. Wild-type hybrid poplar trees were shown to be composed of ~64.4% total carbohydrates, while the 2×35S::UGPase transgenic trees ranged from 69.2% to 73.1%. Although all cell wall carbohydrates (arabinose, galactose, glucose, xylose, mannose, and rhamnose) were shown to be elevated in the 2×35S::UGPase transgenic lines, the most significant changes were observed in glucose content (Table 4). The observed increases in glucose content were reflective of the overall increase in cellulose composition in all transgenic lines compared with the corresponding wild-type trees (Fig. 4), as determined by α-cellulose quantification.

The total cell wall lignin content of the 2×35S::UGPase transgenic lines was concurrently shown to be

Fig. 2. Image depicts axial shoot elongation and leaf size in a representative 2×35S::UGPase (right) and wild-type (left) poplar (inset is a close up of elongated axial shoots of transgenic poplar).

significantly decreased. As measured by percentage weight of total dry mass, the total lignin was reduced from 23.7% of the dry weight in wild-type trees to a range of 18.7–20.1% in the 2×35S::UGPase transgenic lines. This represents a decrease of 12–21% across the lines. Furthermore, the drop in total lignin content appears to be directly related to a reduction in the acid-insoluble lignin moieties (Table 4).

Significant increases in the syringyl:guaicyl (S:G) ratio in the lignin was also observed in the stems of these 2×35S::UGPase transgenic lines (Table 5), with the % mol S concentration increasing from an average of 69.2% in wild-type poplar, to between 76.6–78.2% in the six 2×35S::UGPase lines with decreased lignin content. The increase in syringyl-based lignin monomers is proportionately related to a decrease in the guaiacyl monomers. Although significant, the change in the S:G ratio had a minor effect on the level of the *p*-hydroxyphenyl monomer (H-lignin) composition. However, H-lignin is only minor component in poplar trees relative to total lignin content.

Soluble metabolite analysis

Total soluble metabolites, extracted independently from leaf tissue and developing xylem, were evaluated by metabolite profiling with GC/MS analysis. Table 6 lists 18 (of ~280 compounds investigated) whose levels are changed 2-fold or greater in 2×35S::UGPase trees compared with the wild type. In general, many of the identified compounds are associated with plant/tree defence or stress, including myo-inositol, galactinol, galactitol, and pinitol. In addition, compounds related to carbon allocation, such as maltose and other carbohydrates were identified. However, a single compound was shown to dominate the pooling metabolites in the 2×35S::UGPase transgenic lines; salicylic acid 2-*O*-β-D-glucoside was dramatically increased in all transgenic lines with increases relative to wild-type poplar ranging from 175–270-fold (Table 6; see Supplementary Table 3 at *JXB* online).

Discussion

This study investigated the effects of over-expressing the *Acetobacter xylinum* UGPase gene in hybrid poplar under the control of a constitutive (2×35S) promoter on tree growth and cell wall biochemistry. Transcript quantification revealed a much higher level of transcript abundance in the leaves of the 2×35S::UGPase lines relative to the stem (developing xylem). Consistent with the observed elevated levels of transcription, the total UGPase enzyme activity followed a similar pattern in the leaves. The effect of the introduction of the exogenous UGPase transgene from *Acetobacter xylinum* on the transcript levels of the two native poplar UGPase genes was also examined, and an increase in both PtUGP1 and PtUGP2 was shown, in both leaf and developing xylem (see Supplementary Table 1 at *JXB* online). However, it was apparent that altered levels of expression of the two genes responded differently, with increases ranging from ~2–4-fold in developing xylem while a maximum 2-fold change was apparent in the leaf tissue. Meng *et al*. (2007), however, have shown tight post-transcriptional/ translational control for these two genes, and therefore, increases in transcript abundance, while expected with an increase in sucrose content, may not necessarily correlate to an increase in protein/enzyme activity.

The 2×35S::UGPase transgenic lines were shown to be substantially smaller than wild-type trees, with decreased height growth, diameter, and stem dry weight (biomass). Morphologically, the 2×35S::UGPase trees, despite having a significantly greater number of leaves, had a reduced total leaf area and biomass compared with the corresponding wild-type trees. These same lines exhibited elongation of the axial shoots, which is consistent with weakening or loss of apical dominance resulting in changes in the auxin/cytokinin ratios. For example, in pea plants it has been shown that the removal of the apex resulted in the elongation of lateral shoots, with the exogenous

Table 1. *Transcript level and enzyme activity in leaf tissue and developing xylem for transgenic and wild-type trees*

Mean (±SE) were calculated from three plants per line. Bold denotes significant difference from control values at α=0.10.

Transgenic line	UGPase transcript level (copy number μg^{-1} total RNA)		UGPase enzyme activity (μmol NADH min^{-1} mg^{-1} total protein)	
	Leaf	Developing xylem	Leaf	Developing xylem
Control			0.18±0.05	0.71±0.05
2×35S::UGPase A	99.47±14.94	13.13±12.10	**0.50±0.10**	0.75±0.01
2×35S::UGPase B	172.99±15.17	20.79±11.65	**0.34±0.05**	0.80±0.05
2×35S::UGPase C	89.81±44.64	2.07±1.23	**0.54±0.12**	**1.69±0.40**
2×35S::UGPase D	89.61±48.89	19.24±17.07	**0.47±0.02**	0.68±0.06
2×35S::UGPase E	67.51±25.22	12.66±11.13	**0.39±0.08**	0.86±0.18
2×35S::UGPase F	76.60±42.27	27.92±25.99	**0.84±0.16**	0.82±0.05

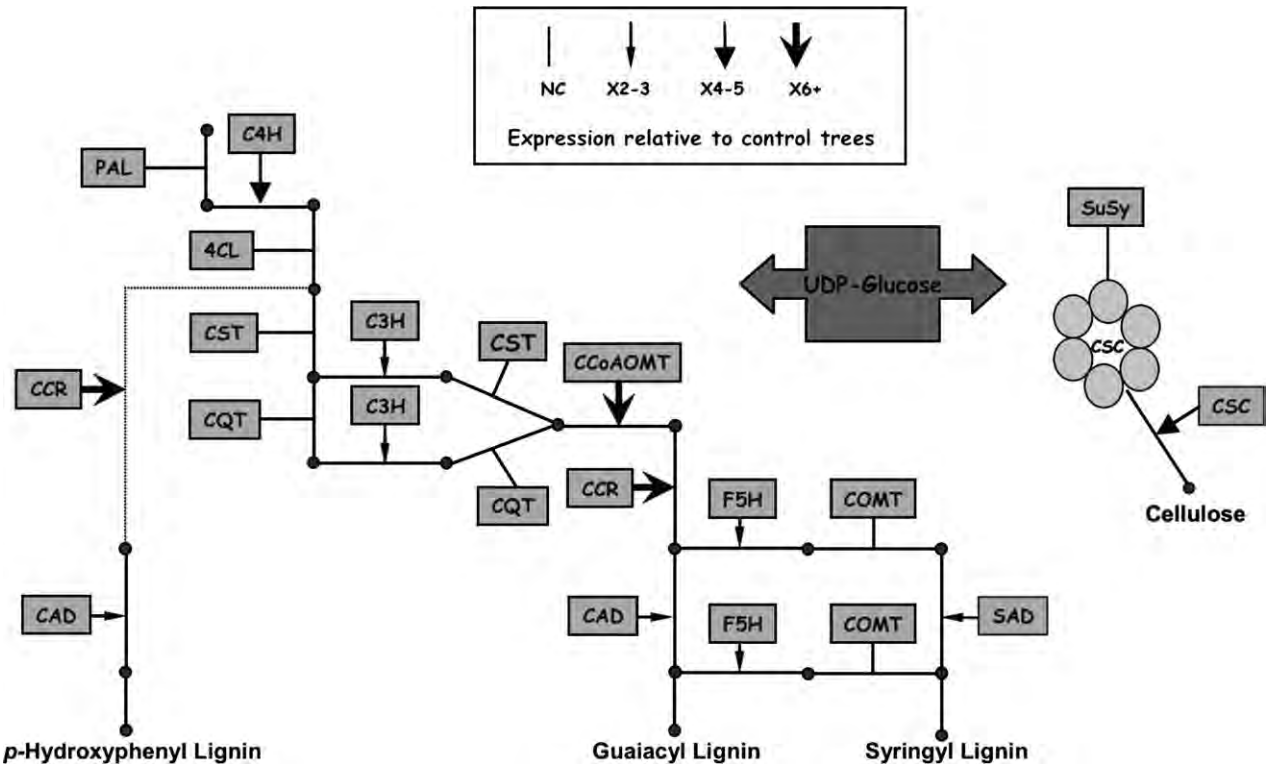

Fig. 3. Schematic representation of the effect of over-expression of 2×35S::UGPase on the transcript abundance of the lignin and cellulose biosynthetic genes in the developing xylem. All mRNA levels were calculated by qPCR from threshold cycle values and are relative to controls and normalized with respect to β-actin transcript abundance.

application of NAA resulting in the retention of unaltered phenotype (Li *et al*., 1995). The same result was seen when the apex was removed from chick pea plants, or when exogenous cytokinins were applied (Turnbull *et al*., 2004).

To date, most of the work elucidating the function of UGPase is mixed and inconclusive, and has evolved from a limited number of studies down-regulating UGPase activity. For example, reductions from 30% to 96% in UGPase activity by antisense suppression showed no change in plant biomass in either *Arabidopsis* or potato, respectively (Johansson, 2003; Zrenner *et al*., 1993). In the case of *Arabidopsis*, Johansson (2003) speculated that the 30% reduction in *Arabidopsis* UGPase expression did not confer a phenotype as multiple UGPase isoforms exist permitting a compensatory control of pathway regulation (Johansson, 2003). These studies imply that UGPase activity is abundant in the plant, and mis-regulation will likely have little or no effect on plant phenotype.

Contrary to this, the over-expression of transgenic UGPase in tobacco resulted in significantly improved growth rates, both under ubiquitous and tissue-specific promoters (Coleman *et al*., 2006). The improved growth characteristics were attributed to the potential role of

Table 2. *Total soluble carbohydrates (mg g^{-1}) in leaves and developing xylem of transgenic and wild-type trees*

Mean (±SE) were calculated from three plants per line. Bold denotes significant difference from control values at α=0.10. Tissue (collected between plastichron index 3–5) was collected from greenhouse plants between 10.00 h and 12.00 h.

Leaf	Glucose	Fructose	Sucrose	Total
Control	7.49±2.07	2.61±0.58	49.90±3.19	60.00±2.57
2×35S:UGPase A	**18.79±1.10**	**6.00±0.75**	**68.96±4.25**	**93.75±5.60**
2×35S:UGPase B	**14.57±6.29**	**4.93±1.22**	**61.93±3.95**	**81.42±8.22**
2×35S:UGPase C	**17.54±3.76**	**5.90±1.61**	**66.33±4.76**	**89.77±6.79**
2×35S:UGPase D	**13.60±3.11**	**6.59±1.68**	**65.60±6.63**	**85.79±11.08**
2×35S:UGPase E	**24.35±3.35**	**6.06±1.83**	**64.45±3.74**	**94.86±2.56**
2×35S:UGPase F	**19.71±4.45**	**6.89±0.56**	51.30±4.67	77.91±9.12
Developing xylem	Glucose	Fructose	Sucrose	Total
Control	3.61±0.87	0.00±0.00	23.71±5.47	27.31±6.12
2×35S:UGPase A	**8.65±3.12**	**8.65±3.12**	35.05±7.90	43.71±10.64
2×35S:UGPase B	**5.82±0.97**	0.00±0.00	**43.33±1.93**	**49.15±1.05**
2×35S:UGPase C	**13.19±4.76**	0.00±0.00	**61.95±4.51**	**75.14 ±9.15**
2×35S:UGPase D	3.37±0.51	0.00±0.00	27.83±6.70	31.20±6.55
2×35S:UGPase E	4.38±1.41	0.00±0.00	40.52±10.72	50.06±15.77
2×35S:UGPase F	3.38±0.53	0.00±0.00	35.06±6.36	38.44±6.71

Table 3. *Starch content (mg g^{-1}) in leaves and developing xylem of transgenic and wild-type trees*

Mean (±SE) were calculated from three plants per line. Bold denotes significant difference from control values at α=0.05. Tissue (collected between plastichron index 3–5) was collected from greenhouse plants between 10:00 h and 12:00 h.

	Leaf	Developing xylem
Control	2.6±0.1	8.3±0.7
2×35S::UGPase A	**4.3±0.5**	**18.3±4.2**
2×35S::UGPase B	**4.2±0.3**	**16.0±4.0**
2×35S::UGPase C	1.3±0.0	**27.6±6.3**
2×35S::UGPase D	2.9±0.4	**11.2±0.6**
2×35S::UGPase E	2.4±0.0	14.0±6.4
2×35S::UGPase F	**3.7±0.1**	**31.2±7.9**

UGPase in altering sink strength as it participates in the catabolism of sucrose into hexose phosphates. Although similar results were not observed in the current study investigating hybrid poplar, the differences may be attributed to the innate differences in mechanisms of carbon translocation, with tobacco employing an active mechanism (Burkle *et al*., 1998) and poplar using a passive system moving assimilate along a sucrose concentration gradient (Turgeon and Medville, 1998). UGPase in source tissues tends to act in the formation of sucrose, providing the substrate-to-sucrose transporters for active loading from the leaves (Kleczkowski, 1994). In poplar, photoassimilate accumulating in sink cells must be lower than the solute concentration in source cells, and this occurs primarily by temporary storage in starch, or permanent storage in structural carbohydrates such as cellulose. UGPase in sink tissue tends to function in the breakdown of sucrose, while in source cells it is thought to act in the synthesis of sucrose (Kleczkowski *et al*., 2004), thus creating the concentration gradient necessary for the passive flow of carbon skeletons required for active metabolism.

Altered UGPase activity in other plant species has also been shown to manifest changes in carbohydrate biochemistry, however, these changes are inconsistent and conflicting. In potato, Zrenner *et al*. (1993) showed no change in carbohydrate metabolism with a 96% reduction in UGPase enzyme activity, while other studies with less extreme reductions in UGPase activity showed significant decreases in stored tuber carbohydrate concentrations (Spychalla *et al*., 1994; Borokov *et al*., 1996). Similarly, a 30% reduction in UGPase activity resulted in a decrease in soluble sugar and starch content in *Arabidopsis* (Kleczkowski *et al*., 2004). Consistent with these findings, tobacco plants over-expressing UGPase showed increases in stem glucose and fructose levels compared with non-transgenic control plants (Coleman *et al*., 2006). In the current study, significant increases in all soluble carbohydrates were observed in the leaf tissue of 2×35S::UGPase hybrid poplar. In the developing xylem, the 2×35S:: UGPase transgenic lines showed significant, yet smaller, increases primarily in glucose and sucrose only.

These findings support the theory that UGPase activity can contribute to photoassimilate generation in source tissue and, in the case of symplastic loading plants like hybrid poplar, that this elevated activity in sink tissue can augment carbon allocation. In all of the 2×35S::UGPase transgenic lines, there was a significant increase in transcript abundance enzyme activity, and, total soluble carbohydrates. In addition, many of the transgenic lines showed increased levels of starch accumulation pointing to the accumulation of storage carbohydrates resulting from the pooling of available soluble sugars. It appears that the expression of the exogenous UGPase in the leaf tissue is resulting in the biosynthesis of carbohydrates in source tissue and is probably working co-ordinately with SPS in the synthesis of sucrose, as has been proposed

Table 4. *Chemical composition of stem material of transgenic and wild-type trees*

Mean (±SE) were calculated from three plants per line. Bold denotes significant difference from control values at α=0.10.

	% Carbohydrates						% Lignin	
	Arabinose	Galactose	Glucose	Xylose	Mannose	Rhamnose	Acid soluble	Acid insoluble
Control	0.4±0.0	1.0±0.1	43.2±0.2	17.7±0.7	1.5±0.1	0.6±0.1	3.0±0.0	20.7±0.1
2×35S::UGPase A	**0.6±0.0**	**1.4±0.1**	**49.0±1.4**	18.8±0.7	**2.0±0.1**	0.6±0.0	**3.3±0.1**	**15.4±0.5**
2×35S::UGPase B	0.5±0.0	**1.7±0.2**	**50.6±0.9**	18.0±0.3	1.8±0.1	0.5±0.0	3.4±0.1	**15.9±0.5**
2×35S::UGPase C	**0.6±0.0**	1.3±0.3	47.7±2.9	18.9±1.2	1.8±0.1	0.6±0.0	**3.5±0.2**	**16.4±0.9**
2×35S::UGPase D	**0.6±0.0**	1.3±0.2	46.6±2.1	19.6±0.9	1.8±0.2	0.7±0.0	3.4±0.1	**17.5±0.7**
2×35S::UGPase E	**0.5±0.0**	1.1±0.1	**46.3±0.8**	18.8±0.6	1.9±0.2	0.6±0.0	3.2±0.1	**17.6±0.3**
2×35S::UGPase F	**0.5±0.0**	1.2±0.1	**48.8±0.7**	17.4±0.4	1.8±0.1	0.5±0.1	**3.2±0.1**	**16.9±0.7**

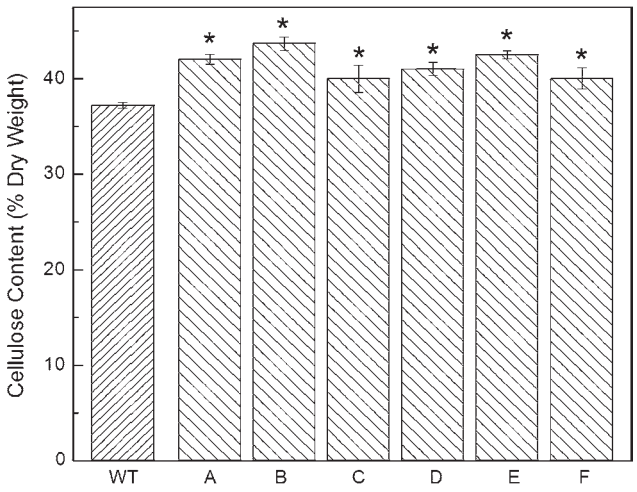

Fig. 4. α-cellulose content of stem material from transgenic and wild-type trees. Mean (±SE) were calculated from three plants per line. Symbol denotes significant difference from control values at α=0.10.

Table 5. *Syringyl, guaiacyl and* p*-hydroxyphenyl monomer contents (moles) of transgenic and wild-type trees as determined by thioacidolysis*

Mean (±SE) were calculated from three plants per line. Bold denotes significant difference from control values at α=0.05.

	p-Hydroxyphenyl	Guaiacyl	Syringyl
	Mean	Mean	Mean
Control	0.02±0.01	30.78±0.25	69.20±0.26
2×35S:UGPase A	0.06±0.02	**21.68±1.25**	**78.26±1.27**
2×35S:UGPase B	0.05±0.01	**21.87±0.65**	**78.08±0.66**
2×35S:UGPase C	0.07±0.02	**23.20±0.23**	**76.73±0.20**
2×35S:UGPase D	**0.08±0.00**	**23.29±0.39**	**76.63±0.39**
2×35S:UGPase E	**0.11±0.00**	**22.63±1.08**	**77.26±1.08**
2×35S:UGPase F	0.11±0.01	**23.28±1.08**	**76.61±1.07**

by Kleczkowski (1994). Interestingly, the introduction of the exogenous UDP-glucose pyrophosphorylase from *Acetobacter* appears to have manifested an increase in transcript abundance of both endogenous poplar UGPase genes, and therefore it is difficult to ascertain if the observed effects are independent of the native gene/protein.

Despite not having measured metabolic flux, it appears that the increased biosynthesis of sucrose in the source tissue has resulted in an increase in the transport of photoassimilate to the sink tissue, as in all transgenic lines an accumulation of both storage and structural carbohydrates was apparent. Although changes in hemicellulose-derived carbohydrates were observed, the most dramatic increase in cell wall carbohydrate chemistry was observed in glucose concentrations, which is derived from cellulose. In these lines, increases in cellulose content ranged from ~2.8% to 6.5% (Fig. 4). These findings are consistent with the theory that an increase in cellulose content would coincide with an increased supply of precursor to the cellulose synthase complex (CSC) from elevated levels of soluble metabolites synthesized and probably transported to the sink (wall developing tissue). Furthermore, this is supported by the 4-fold increase in the secondary wall-specific CSC gene expression levels in the transgenic lines. Confirmation of this theory and our results are also consistent with the microarray findings of Hertzberg *et al*. (2001) who showed that UGPase is up-regulated during the period of late expansion and secondary cell wall formation. Similar changes in structural carbohydrates were not observed in tobacco plants transformed with the same constructs (Coleman *et al*., 2006), which can be attributed to a lack of any measurable change in stem sucrose content compared with the stem of the poplar.

In addition to the observed increases in carbohydrate composition in the 2×35S::UGPase transgenic trees, there was also an associated decrease in lignin content. This is not thought to be the result of a decrease in the rate of lignin deposition, but rather the result of a change in the ratio of cellulose to lignin. This was confirmed using real-time PCR analysis of the lignin-branch of the phenylpropanoid biosynthetic genes toolbox, which showed that there was no decrease in the transcripts of any of the key lignin biosynthetic gene isoforms surveyed, rather in many of the genes there was an increase (Fig. 3; see Supplementary Table 2 at *JXB* online).

Correlated to the change in the relative amount of lignin, was a change in lignin monomer composition in

Table 6. *Metabolites identified in the developing xylem of 2×35S::UGPase hybrid poplar relative to levels in wild-type trees*

Fold change relative to wild-type poplar	Compound
232.6	Salicylic acid 2-*O*-β-D-glucoside
11.4	Maltose
5.8	Unknown
5.3	Myo-Inositol
5.1	Maltotriose methoxyamine
4.4	Galactinol
4.1	D-pinitol
3.0	Unknown
2.7	Galactitol
2.5	Unknown
2.5	Glucose
2.5	Galactose
2.3	Sucrose
2.2	Unknown
2.2	Sorbose
2.1	4-amino butyric acid
2.0	Fructose
2.0	Picein

favour of syringyl units. These biochemical findings were supported by real-time PCR evaluations, which demonstrated significant augmentation of ferulate 5-hydroxylase (F5H) consistent with the change in monomer composition of the cell wall lignin. These findings suggest that the elevated transcript abundance of lignin biosynthetic genes may be related to changes in sugar concentrations rather than a change in cellulose deposition. Sucrose has been shown to serve as a signal molecule regulating gene expression (Wiese *et al*., 2004), and consequently influences associated metabolic pathways and morphological development (Lunn and McRae, 2003; Gibson, 2005). A compelling body of evidence indicates that carbohydrates, particularly the hexose glucose, are essential sources of carbon skeletons and function as important signalling molecules (reviewed in Smeekens, 2000; Rolland *et al*., 2002; Gibson, 2005).

In *Arabidopsis*, a relationship between carbon availability and lignin accumulation has been established (Rogers *et al*., 2005), clearly demonstrating that metabolizable carbohydrates positively influence the abundance of lignin. Further, concurrent transcriptome analysis lends support to the hypothesis that carbohydrates are not merely a source of carbon skeletons for lignification, but also function as a signal to enhance the capacity to synthesize these key cell wall macromolecules. In addition, diurnal fluxes in lignin biosynthetic capacity were suggested to be modulated at the transcriptional level by at least three different stimuli: light, the circadian clock, and available hexose carbohydrates. The absolute abundance of these transcripts is shaped by the amount of available carbohydrates. The link between sugar signalling and lignification is particularly interesting, as carbohydrate-mediated changes in vegetative development have been well documented in dark-grown seedlings (Roldan *et al*., 1999; Baier *et al*., 2004).

Metabolic profiling was used to investigate changes in 'global' cell wall metabolism as a result of this single gene mis-regulation. In addition to the altered levels of soluble carbohydrates identified by HPLC, the 2×35S::UGPase transgenic poplar trees differentially accumulated several metabolites, many of which have been identified as products of sugar metabolism, such as galactose, maltose, and sorbose, or as metabolites commonly associated with abiotic or biotic stress (Table 5). Aside from being an integral component of cell wall hemicellulose, the monosaccharide galactose is also known to be a major carbohydrate participating in the formation of the raffinose family of oligosaccharides known to be elevated in response to stress, particularly in *Populus*. Maltose, on the other hand, is the immediate by-product of starch degradation, while sorbose is a precursor ketose monosaccharide involved in the biosynthesis of ascorbic acid, which has been shown to influence leaf development, growth, and size (Chen and Gallie, 2006), and may be responsible directly or indirectly for the alteration of leaf morphology in the current study. In addition, other stress-related compounds were identified including myo-inositol, galactinol, and pinitol. Myo-inositol and galactinol are the immediate precursors to the formation of raffinose family of oligosaccharides, while pinitol is a methylated cyclitol, derived from myo-inositol, thought to be an important osmolyte in plants responding to drought stress.

Most interesting was the extremely high quantities of a single compound, the glycoside of salicylic acid (salicylic acid 2-*O*-β-D-glucoside [SAG]), which was found at significantly elevated levels (up to a 270-fold increase compared with the wild-type trees) in the developing xylem of all 2×35S::UGPase transgenics trees (Table 6; see Supplementary Table 3 at *JXB* online). Salicylic acid 2-*O*-β-D-glucoside was also identified in the leaf tissue of all transgenic lines, where it was absent in the wild-type trees. The formation of SAG is has been shown in tobacco to be catalysed by a UDP-glucose:SA glucosyltransferase, which employs UDP-glucose as the sole glucose donor (Lee and Raskin, 1999). Furthermore, SAG has been shown to be induced by the presence of salicylic acid, which has been shown to accumulate in tobacco leaves following TMV infections, resulting in an accumulation of SAG as a major product and glucosyl salicylate (GS) as a minor, less stable metabolite (Lee and Raskin, 1998). These results suggest that the accelerated generation of UDP-glucose, manifested by the over-expression of 2×35S::UGPase in hybrid poplar, resulted in the substantial accumulation of SAG. It is tempting to speculate that the allocation of UDP-glucose to salicylic

acid may be a direct response of the inability of the cellulose synthase complex effectively to utilize the intracellular UDP-glucose channelled to the formation of cellulose, which was increased by ~6%. However, the accumulation of SAG, among other compounds, has also recently been observed in transgenic aspen over-expressing sucrose phosphate synthase (Hjältén *et al*., 2006), which should have provided another sink for UDP-glucose. Salicylic acid has been shown to act as a signalling molecule in local defence reactions and also in the induction of systemic resistance (Durner *et al*., 1997), and as such the increases in salicylic acid, and hence SAG and/or GS, may be the catalyst for the increases in other plant defence metabolites and may improve herbivore-plant interactions, as shown by Hjältén *et al*. (2006).

In summary, the over-expression of UGPase in hybrid poplar resulted in significant increases in soluble sugars, more so in the leaves than the developing xylem. These increases in sugar appear to provide increased substrate to both cellulose and starch synthesis, resulting in changes in the chemical composition of the stem, with as much as a 6.6% increase in cell wall cellulose content being observed. Contrary to the results observed in tobacco, in hybrid poplar results suggest that the over-expression of UGPase under the control of a ubiquitous promoter can alter carbon partitioning to starch and cellulose. However, the alterations in sucrose metabolism, specifically in the symplastically loading plant *Populus*, appears to cause other downstream repercussions within the plant, particularly with respect to carbohydrate signalling and sensing, which can augment cell wall biosynthesis, as is evident in the altered lignin biosynthesis. In addition to changes in wood chemistry, the trees also produced more defence-related metabolites, which may explain the decreased energy directed to growth.

Supplementary data

The data reported in this manuscript are supported by supplementary data which are available at *JXB* online. Included in the supplemental data is transcript abundance of the native poplar UDP-glucose pyrophosphorylase genes in leaf and developing xylem tissue (Supplementary Table 1), as well as transcript abundance of the cell wall biosynthetic genes involved in lignin and cellulose deposition for transgenic and wild-type trees (Supplementary Table 2). In addition, quantification of the fold change in salicylic acid 2-*O*-β-D-glucoside in the developing xylem of all transgenic 2×35S::UGPase hybrid poplar relative to levels in wild-type trees is available (Supplementary Table 3).

Acknowledgements

The authors would like to thank Lisa McDonnell, Ji-Young Park, Ahn Nguyen, Andrew Robinson, and Victoria Maloney for their technical assistance. Funding for this project to SDM is acknowledged from the National Science and Engineering Research Council of Canada, CellFor Inc., and the Canadian Forest Service.

References

Appeldoorn NJG, de Bruijn SM, Koot-Gronsveld EAM, Visser RGF, Vreugdenhil D, van der Plas LHW. 1997. Developmental changes of enzymes involved in conversion of sucrose to hexose-phosphate during early tuberisation of potato. *Planta* **202,** 220–226.

Baier M, Hemmann G, Holman R, Corke F, Card R, Smith C, Rook F, Bevan MW. 2004. Characterization of mutants in Arabidopsis showing increased sugar-specific gene expression, growth, and developmental responses. *Plant Physiology* **134,** 81–91.

Borovkov AY, McClean PE, Sowokinos JR, Ruud SH, Secor GA. 1996. Effect of Expression of UDP-glucose pyrophosphorylase ribozyme and antisense RNAs on the enzyme activity and carbohydrate composition of field-grown transgenic potato plants. *Journal of Plant Physiology* **147,** 644–652.

Brede G, Fjaervik E, Valla S. 1991. Nucleotide sequence and expression analysis of the *Acetobacter xylinum* uridine diphosphoglucose pyrophosphorylase gene. *Journal of Bacteriology* **173,** 7042–7045.

Burkle L, Hibberd JM, Quick WP, Kuhn C, Hirner B, Frommer WB. 1998. The H^+-sucrose cotransporter NtSUT1 is essential for sugar export from tobacco leaves. *Plant Physiology* **118,** 59–68.

Canam T, Park J-Y, Yu KY, Campbell MM, Ellis DD, Mansfield SD. 2006. Varied growth, biomass and cellulose content in tobacco expressing yeast-derived invertases. *Planta* **224,** 1315–1327.

Chen Z, Gallie DR. 2006. Dehydroascorbate reductase affects leaf growth, development, and function. *Plant Physiology* **142,** 775–787.

Coleman HD, Ellis DD, Gilbert M, Mansfield SD. 2006. Up-regulation of sucrose synthase and UDP-glucose pyrophosphorylase impacts plant growth and metabolism. *Plant Biotechnology Journal* **4,** 87–101.

Datla RSS, Bekkaoui F, Hammerlindl JK, Pilate G, Dunstan DI, Crosby WL. 1993. Improved high-level constitutive foreign gene translation using an AMV RNA4 untranslated leader sequence. *Plant Science* **94,** 139–149.

Durner J, Shah J, Klessig DF. 1997. Salicylic acid and disease resistance in plants. *Trends in Plant Science* **2,** 266–274.

Gibson SI. 2005. Control of plant development and gene expression by sugar signalling. *Current Opinion in Plant Biology* **8,** 93–102.

Hertzberg M, Aspeborg H, Schrader J, *et al*. 2001. A transcriptional roadmap to wood formation. *Proceedings of the National Academy of Sciences, USA* **98,** 14732–14737.

Hjältén J, Lindau A, Wennström A, Blomberg P, Witzell J, Hurry V, Ericson L. 2006. Unintentional changes of defense traits in GM trees can influence plant-herbivore interactions. *Basic and Applied Ecology* **5,** 434–443.

Hood EE, Gelvin SB, Melcher LS, Hoekema A. 1993. New *Agrobacterium* helper plasmids for gene transfer to plants. *Transgenic Research* **2,** 208–218.

Johansson H. 2003. Gene regulation of UDP-glucose synthesis and metabolism in plants. PhD Thesis, Umea University, Sweden.

Kay R, Chan A, Daly M, McPherson J. 1987. Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. *Science* **236,** 1299–1302.

Kleczkowski LA. 1994. Glucose activation and metabolism through UDP-glucose pyrophosphorylase in plants. *Phytochemistry* **37,** 1507–1515.

Kleczkowski LA, Geisler M, Ciereszko I, Johansson H. 2004. UDP-glucose pyrophosphorylase. An old protein with new tricks. *Plant Physiology* **134,** 912–918.

Kolosova N, Miller B, Ralph S, Ellis BE, Douglas C, Ritland K, Bohlmann J. 2004. Isolation of high-quality RNA from gymnosperm and angiosperm trees. *BioTechniques* **36,** 821–824.

Kotake T, Yamaguchi D, Ohzono H, Hojo S, Kaneko S, Ishida H-K, Tsumuraya Y. 2004. UDP-sugar pyrophosphorylase with broad substrate specificity toward various monosaccharide 1-phosphates from pea sprouts. *Journal of Biological Chemistry* **279,** 45728–45736.

Lee H, Raskin I. 1998. Glucosylation of salicylic acid in *Nicotiana tabacum* cv. Xanthi-nc. *Phytopathology* **88,** 692–697.

Lee H, Raskin I. 1999. Purification, cloning, and expression of a pathogen inducible UDP-glucose: salicylic acid glucosyltransferase from tobacco. *Journal of Biological Chemistry* **274,** 36637–36642.

Li C-J, Guevara E, Herrera J, Bangerth F. 1995. Effect of apex excision and replacement by 1-naphthylacetic acid on cytokinin concentration and apical dominance in pea plants. *Physiologia Plantarum* **94,** 465–469.

Li L, Zhou Y, Cheng X, Sun J, Marita JM, Ralph J, Chiang VL. 2003. Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. *Proceedings of the National Academy of Sciences, USA* **100,** 4939–4944.

Lunn JE, MacRae EA. 2003. New complexities in the synthesis of sucrose. *Current Opinion in Plant Biology* **6,** 208–214.

Magel E, Abdel-Latif A, Hampp R. 2001. Non-structural carbohydrates and catalytic activities of sucrose metabolizing enzymes in trunks of two *Juglans* species and their role in heartwood formation. *Holzforshung* **55,** 135–145.

Meng M, Geisler M, Johansson H, Mellerowicz EJ, Karpinski S, Kleczkowski LA. 2007. Differential tissue/organ-dependent expression of two sucrose- and cold-responsive genes for UDP-glucose pyrophosphorylase in *Populus*. *Gene* **389,** 186–195.

McCown BH, Lloyd G. 1981. Woody Plant Medium (WPM): a mineral nutrient formulation for microculture for woody plant species. *Horticultural Science* **16,** 453.

Robinson AR, Gheneim R, Kozak RA, Ellis DD, Mansfield SD. 2005. The potential of metabolite profiling as a selection tool for genotype discrimination in *Populus*. *Journal of Experimental Botany* **56,** 2807–2819.

Rogers LA, Dubos C, Cullis IF, Surman C, Poole M, Willment J, Mansfield SD, Campbell MM. 2005. Light, the circadian clock, and sugar perception in the control of lignin biosynthesis. *Journal of Experimental Botany* **56,** 1651–1663.

Rolando C, Monties B, Lapierre C. 1992. Thioacidolysis. In: Lin S, Dence C, eds. *Methods in lignin chemistry*. Berlin, Germany: Springer-Verlag, 334–349.

Roldan M, Gomez-Mena C, Ruiz-Garcia L, Salinas J, Martinez-Zapater JM. 1999. Sucrose availability on the aerial part of the plant promotes morphogenesis and flowering of *Arabidopsis* in the dark. *The Plant Journal* **20,** 581–590.

Rolland F, Moore B, Sheen J. 2002. Sugar sensing and signaling in plants. *The Plant Cell* **14,** S185–S205.

Smeekens S. 2000. Sugar-induced signal transduction in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **51,** 49–81.

Spychalla JP, Scheffler BE, Sowokinos JR, Bevan MW. 1994. Cloning, antisense RNA inhibition, and the coordinated expression of UDP-glucose pyrophosphorylase with starch biosynthetic genes in potato tubers. *Journal of Plant Physiology* **144,** 444–453.

Sterky F, Bhalerao RR, Unneberg P, *et al.* 2004. A *Populus* EST resource for plant functional genomics. *Proceedings of the National Academy of Sciences, USA* **101,** 13951–13956.

Turgeon R, Medville R. 1998. The absence of phloem loading in willow leaves. *Proceedings of the National Academy of Sciences, USA* **95,** 12055–12060.

Turnbull CGN, Raymond MAA, Dodd IC, Morris SE. 2004. Rapid increases in cytokinin concentration in lateral buds of chickpea (*Cicer arietinum* L.) during release of apical dominance. *Planta* **202,** 271–276.

Yokoyama T, Kadla JF, Chang HM. 2002. Micoranalytical method for the charnacterization of fiber components and morphology of woody plants. *Journal of Agriculture and Food Chemistry* **50,** 1040–1044.

Wiese A, Elzinga N, Wobbes B, Smeekens S. 2004. A conserved upstream open reading frame mediates sucrose-induced repression of translation. *The Plant Cell* **16,** 1717–1729.

Zrenner R, Willmitzer L, Sonnewald U. 1993. Analysis of the expression of potato uredinediphosphate-glucose pyrophosphorylase and its inhibition by antisense RNA. *Planta* **190,** 247–252.