Lignin and Biomass: A Negative Correlation for Wood Formation and Lignin Content in Trees[1]

Evandro Novaes, Matias Kirst, Vincent Chiang, Heike Winter-Sederoff, and Ronald Sederoff*

School of Forest Resources and Conservation (E.N., M.K.) and University of Florida Genetics Institute (M.K.), University of Florida, Gainesville, Florida 32611; and Forest Biotechnology Group, Department of Forestry and Environmental Resources (V.C., R.S.), and Department of Plant Biology (H.W.-S.), North Carolina State University, Raleigh, North Carolina 27695

Studies in populations of forest tree hybrids have shown a negative correlation of biomass growth (usually measured as wood volume) and lignin content (Kirst et al., 2004; Novaes et al., 2009). The control of growth and lignin appears to be highly regulated, implying that selection for improved growth rate could effect a reduction in lignin content. Trees with increased biomass and reduced lignin would provide a yield advantage for pulp and paper production, as well as for production of biofuels. The purpose of this article is to briefly review the evidence for the correlation between lignin content and biomass growth and to discuss this correlation in a metabolic context. We review evidence that the regulation of the balance between lignin biosynthesis and biomass growth is mediated, at least in part, at the level of transcription, and expand previous results to show specific regulation within gene families of monolignol biosynthesis. Finally, we suggest a model of physiological control for the regulation of the relationship of wood formation and lignin content in trees.

Wood, used by human societies for millennia, remains one of the world's most abundant raw materials for industrial products and renewable energy. Wood is the secondary xylem of vascular plants, a tissue formed from the terminal differentiation of the inner side of the cambial meristem for vertical and horizontal transport of water, nutrients, and extractives. The secondary cell wall structure and composition of wood are the primary determinants of its physical and chemical properties, and of its energy content. Wood is typically composed of about 25% lignin, and 70% cellulosic carbohydrates, with roughly 45% cellulose and 25% hemicelluloses (Sjostrom, 1993).

THE PROPERTIES OF LIGNIN IN WOOD

Lignin is a complex phenolic polymer that provides an embedding material for the cellulosic polymers of the secondary cell walls. It is also the major polymer in the middle lamellae between adjacent cell walls (Plomion et al., 2001; Boerjan et al., 2003). Lignin provides the hydrophobic surface that allows plants to transport water to heights greater than 100 m (Carder, 1995; Koch et al., 2004) and contributes to the mechanical strength that can support trees weighing more than 2,000 metric tons (Fry and White, 1938). Lignin's physical and chemical properties also serve as a barrier against the invasion of pests and pathogens (Vance et al., 1980; Bhuiyan et al., 2009). For the forest products industries, lignin is the major barrier to efficient extraction of cellulose fibers for pulp and paper production. For the bioenergy industries, lignin is a barrier to saccharification for production of liquid biofuel (Li et al., 2003; Chen and Dixon, 2007).

LIGNIN VARIATION

While lignin is fundamental to growth and adaptation of herbaceous and woody plants (Sarkanen and Ludwig, 1971; Boerjan et al., 2003; Ralph et al., 2007; Heitner et al., 2010; Vanholme et al., 2010), it is not clear how much lignin is needed or how much its composition may vary. Within a plant, lignin content can vary greatly in different tissues; for example, lignin is very low in young shoots and high in wood. In different tree species the lignin content of wood can vary from 15% to 40% (Sarkanen and Ludwig, 1971). Within a species, average lignin content of wood is much less variable, often ranging only a few percent (Einspahr et al., 1964; van Buijtenen et al., 1968). Lignin content is also increased or decreased in wood formed under gravitropic stimulation or mechanical stress (known as reaction wood; Timell, 1969, 1986; Andersson-Gunneras et al., 2006). In softwoods (gymnosperms), compression wood may be up to 40% lignin, and in hardwoods (angiosperms), tension wood fibers have a specialized gelatinous cell layer that is almost devoid of lignin (Timell, 1969).

Lignin composition is also highly variable. There are two main types of subunits in lignin. One derived

[1] This work was supported by the Consortium for Plant Biotechnology Research (grant no. GO12026–225); by the Department of Energy, Office of Science, Office of Biological and Environmental Research (grant no. DE–FG02–05ER64114); and by the National Science Foundation, Genes and Genomes System Cluster in the Division of Molecular and Cellular Biosciences (grant no. 0817900).

* Corresponding author; e-mail ron_sederoff@ncsu.edu.

www.plantphysiol.org/cgi/doi/10.1104/pp.110.161281

from coniferyl alcohol monomers forms guaiacyl (G) units, resulting in a lignin typical of softwoods. A second monomer sinapyl alcohol forms syringyl (S) units after polymerization. A mixed type of lignin with both G and S units is typical of hardwoods, and is characterized by the ratio (S/G ratio) of subunits. The S/G ratio varies greatly among hardwoods (Sarkanen and Ludwig, 1971).

In herbaceous and field crops, significant reductions in lignin have been achieved by traditional breeding, spontaneous mutation, and by transgenesis. In general, reduced lignin is associated with depressed yields (Pedersen et al., 2005). However, in many cases, lignin can be reduced without reducing yield or fitness. Genetic background and diverse environments have a significant role.

LIGNIN EVOLUTION

Because of its roles in water transport, mechanical support, and biodefense, lignin is intimately associated with the evolution of vascular plants (Robinson, 1990). Early large plants may have had higher lignin contents than we find today. Ferns and lycopods, the predominant plants of the middle to late Paleozoic (350–250 million years ago [Mya]), are likely to have had lignin contents of 30% to 50% or more. During the Mesozoic (250–65 Mya), when gymnosperms were dominant, lignin contents of wood were 30% to 35%. In the late Cretaceous (80 Mya) and following, angiosperms and pinaceous gymnosperms with lignin contents of 30% or less, replaced earlier flora. Although the lignin content of angiosperms varies greatly, with grasses having 5% to 10% (van Soest, 1982; Schaefer et al., 1985) and some tropical hardwoods were reported to have more than 40% (Fengel and Wegener, 1983), an average lignin content of 20% has been estimated for modern land plants (Robinson, 1990).

LIGNIN IS AN ENERGY-RICH CARBON SINK

While lignin clearly confers an adaptive advantage, it is also a substantial metabolic sink for reduced carbon (C). The strength of the C sink for lignin versus other cell wall components may have implications that extend beyond the simple balance of C partitioning. Lignin has a higher energy content than cellulose or hemicelluloses. One gram of lignin has on average 2.27 KJ, 30% more than the energy of cellulosic carbohydrate (Shafizadeh and Chin, 1977; White, 1987). The energy content of lignin is similar to that of coal (McLaughlin et al., 1996). Because lignin is more reduced than the cellulosics, the energetics of plant growth suggests that more wood could be formed if the content of lignin were reduced. Analysis of metabolic stoichiometry indicates that it takes the energy equivalent of 2.7 to 3.0 g of Glc to produce 1 g of lignin (Amthor, 2003).

EARLY INDICATIONS

An early indication of a negative correlation between biomass growth and the lignin content of wood was proposed for loblolly pine (*Pinus taeda*) carrying a natural mutation in the gene encoding *CINNAMYL ALCOHOL DEHYDROGENASE* (*CAD*), the last enzyme of the monolignol biosynthetic pathway. A null allele *cad-n1* was associated with a significant increase in second year shoot elongation and fourth year wood volume (Wu et al., 1999). This effect was significant but variable, depending on genetic background, in subsequently tested populations of 15-year-old pines (Yu

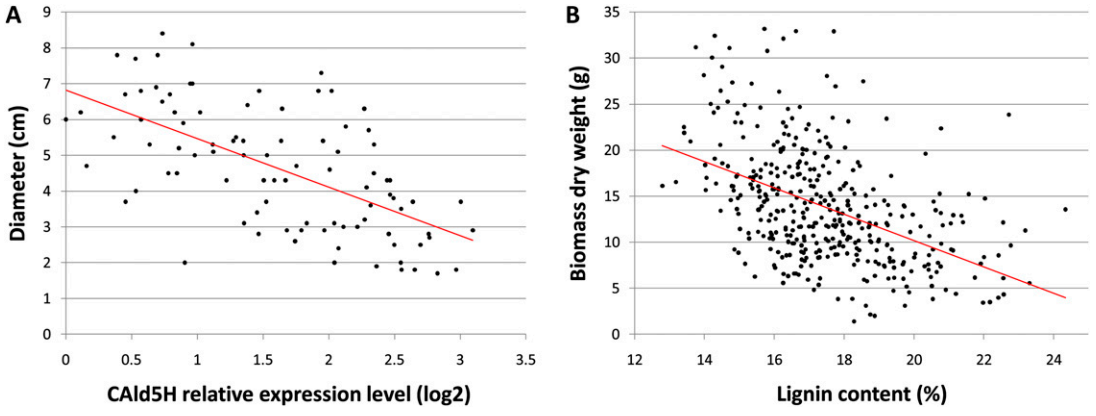

Figure 1. A, Diameter growth and transcript level for CAld5H (for coniferaldehyde 5-hydroxylase; also known as F5H, for ferulate 5-hydroxylase) are negatively correlated ($r = -0.62$; P value < 0.0001) in a hybrid backcross population of *Eucalyptus* (Kirst et al., 2004). CAld5H was the gene with the highest correlation to diameter growth. Similar results from *Populus* are shown in Figure 2. B, The negative correlation ($r = -0.48$; P value < 0.0001) between growth and lignin is based on measurements made in 396 genotypes of *Populus* family 52-124 (Novaes et al., 2009).

et al., 2006). In another survey using loblolly pine clones (age 6), the clone means ranged from 30.0% to 32.6% in lignin content. A negative correlation of growth and lignin content was small with an r^2 of 0.13 (B. Li, unpublished data). A negative correlation between lignin and biomass growth in woody angiosperms was observed in a transgenic aspen (*Populus tremuloides*) down-regulating *4-COUMARATE-CoA LIGASE* (*4CL*) in the monolignol biosynthesis pathway (Hu et al., 1999). *4CL* transgenic plants exhibited a 45% reduction in lignin levels that was compensated by a 15% increase in cellulose and 17% to 57% increase in hemicelluloses. This reduction in the lignin to cell wall carbohydrate ratio was associated with significantly enhanced growth in all vegetative organs (Hu et al., 1999).

NEGATIVE CORRELATION OF LIGNIN WITH BIOMASS

Analyses of lignin and growth in interspecific mapping populations of *Eucalyptus* and *Populus* indicate a clear association between cell wall composition and plant growth. Microarray analysis of transcript abundance in a segregating population of *Eucalyptus grandis* × *globulus* hybrids found that transcripts from most of the genes encoding enzymes of the monolignol

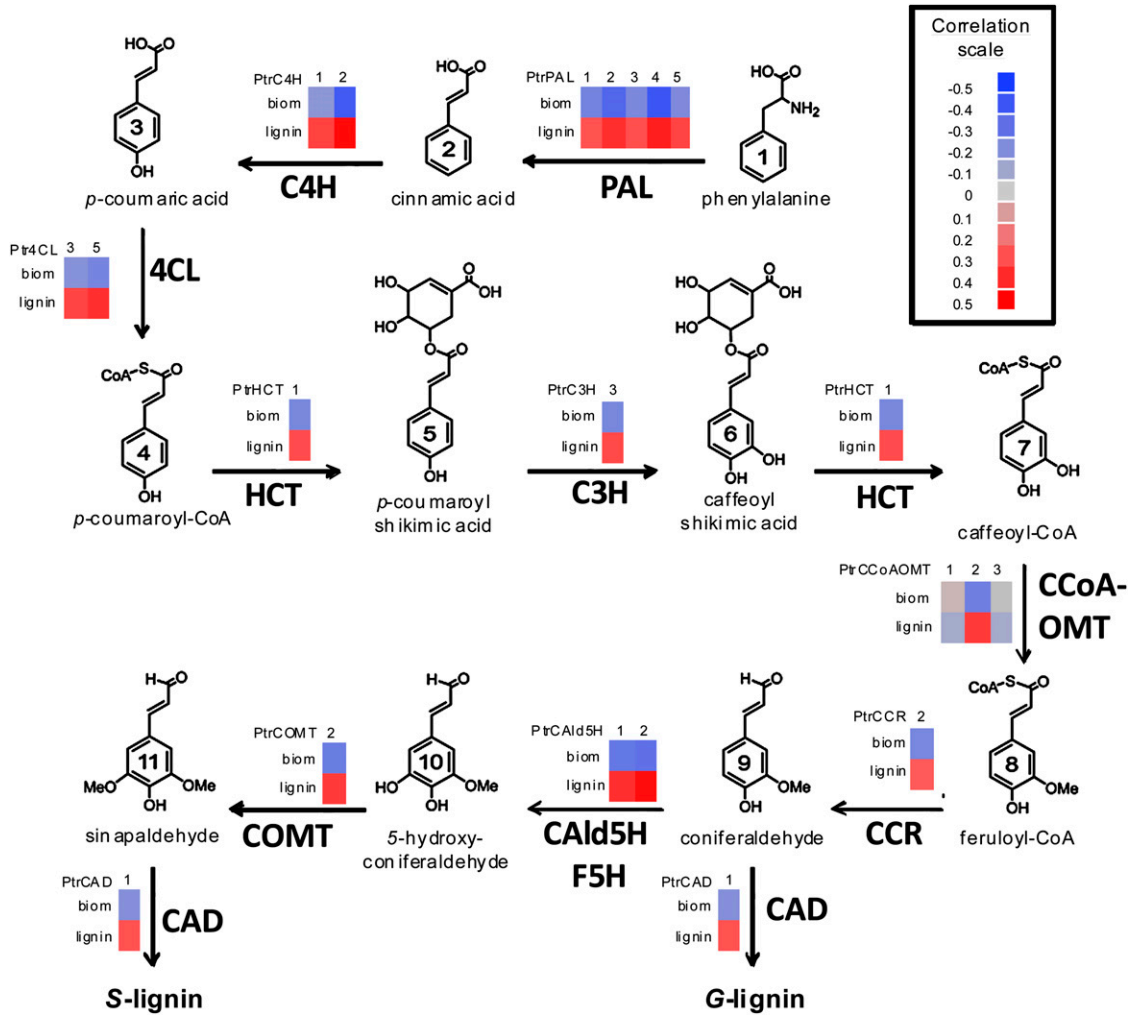

Figure 2. Correlations (Spearman) of biomass growth (biom) and lignin levels with transcript abundance of specific monolignol biosynthetic genes in a interspecific pseudo-backcross pedigree of *Populus* (family 52-124). Analysis of lignin and microarrays estimates of relative abundance are from Novaes et al. (2009) and Drost et al. (2010). Each step of the pathway is shown with the specific genes known to be highly expressed in differentiating xylem in *P. trichocarpa* (Shi et al., 2010). Abbreviations of the monolignol biosynthetic enzymes are as follows: PAL, Phe ammonia-lyase; C4H, cinnamate 4-hydroxylase; HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase; C3H, *p*-coumarate 3-hydroxylase; CCoAOMT, caffeoyl-CoA *O*-methyltransferase; CCR, cinnamoyl-CoA reductase; CAld5H, coniferaldehyde 5-hydroxylase; COMT, caffeate 3-*O*-methyltransferase.

biosynthesis pathway were coordinately down-regulated in fast-growing individuals (Fig. 1A; Kirst et al., 2004). Chemical analysis of wood from individual trees demonstrated a 10% reduction in lignin among the fastest-growing trees relative to slow growers and an increase in the S/G ratio in slow-growing trees (Kirst et al., 2004).

More recently, an interspecific pedigree of *Populus* (*Populus trichocarpa* × *deltoides*) with 396 clonally replicated genotypes was characterized for growth and wood composition under two nitrogen levels (Novaes et al., 2009). Highly significant negative genetic correlations ($r = -0.48$, P value < 0.001) were observed between plant growth and lignin content (Fig. 1B). The above-ground biomass was also negatively correlated with S/G ratio (−0.59). Cellulose was positively correlated with growth ($r = 0.58\%$, P value < 0.001).

While the primary points of regulation of C partitioned to lignin or cellulose biosynthesis, are not known, it is clear that there is coordinated transcriptional control of genes involved in monolignol biosynthesis. Similar to the results previously observed in *Eucalyptus* (Kirst et al., 2004), expression of lignin biosynthesis genes was coordinated with above-ground growth in the segregating family of *Populus* analyzed by Novaes et al. (2009). Analysis of this population with whole-transcriptome microarrays developed to discriminate among members of gene families (Fig. 2; Drost et al., 2009, 2010) detected a negative correlation between biomass growth and transcript levels for most of the genes recently inferred to be important in lignin biosynthesis in *P. trichocarpa* (Shi et al., 2010). The correlation with biomass is not observed in most gene family members not significantly expressed in xylem tissue.

COORDINATE CONTROL

The coordinated regulation of lignin biosynthesis genes is associated not only with developmental variation (e.g. growth) but also occurs in response to environmental cues, such as nitrogen, Suc, and Phe availability (Anterola et al., 2002; Kirst et al., 2004; Scheible et al., 2004). This coordinated transcription extends beyond the phenylpropanoid pathway, including genes in the biosynthesis of aromatic amino acid precursors for Phe and *S*-adenosylmethionine (Anterola et al., 2002; Kirst et al., 2004). Quantitative trait locus (QTL) mapping of phenotypes and expression QTL mapping of transcript level variation provides locations of genomic regions containing candidate genes responsible for coordinate control. Quantitative trait mapping in the *P. trichocarpa* × *deltoides* population identified the location of many potential pleiotropic regulators. A major QTL, identified on LGXIII, is responsible for an estimated 56% of the heritable variation in the cellulose to lignin ratio, and at least 20% of the heritable variation of several growth traits, including stem diameter and biomass accumulation in root and shoot (Novaes et al., 2009). Transcription factors may underlie many QTLs and more generally, the negative association of lignin and biomass.

TRANSCRIPTION FACTORS AND LIGNIN BIOSYNTHESIS

Several transcription factors have been implicated in the control of lignin biosynthesis during formation of secondary xylem (Raes et al., 2003; Zhou et al., 2009; Zhong et al., 2010). NAC domain transcription factors have been implicated in both Arabidopsis (*Arabidopsis thaliana*) and *Populus*, including wood-associated NAC domain factors that are functional orthologs of SND1 (for secondary wall-associated NAC domain protein 1; Zhou et al., 2009; Zhong et al., 2010). One LIM factor, up to eight MYBs, and a KNOX transcription factor are direct positive and negative regulators of genes in the monolignol biosynthetic pathway, affecting transcript abundance, enzyme activity, and lignin content or composition (S/G ratio; Tamagnone et al., 1998; Kawaoka et al., 2000; Dixon et al., 2002; Mele et al.,

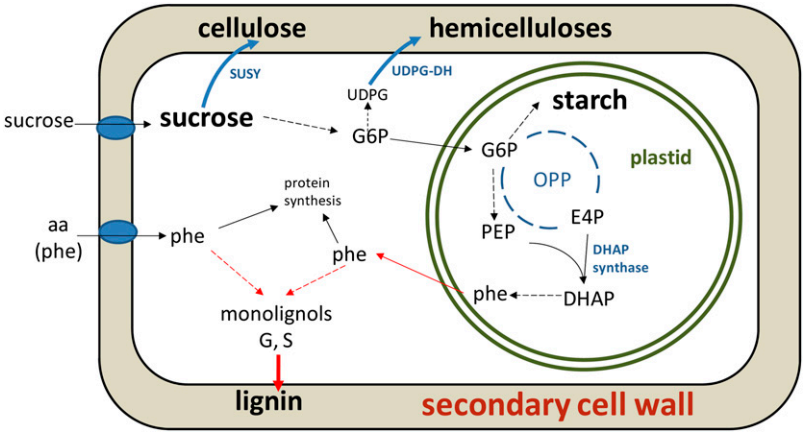

Figure 3. Model for C partitioning during wood formation. Suc is the substrate for cellulose biosynthesis via Suc synthase (SUSY) in wood-forming cells. Glycolytic breakdown of Suc generates the precursors for hemicelluloses and starch accumulation. Phe may be transported from phloem or be synthesized in the cells. Precursors for hemicelluloses are derived from UDP-Glc (UDPG) through oxidation by UDP-Glc dehydrogenase (UDPG-DH; Tenhaken and Thulke, 1996). aa, Amino acids; DHAP, 3-deoxy-D-arabino-heptulosonate 7-phosphate; E4P, erythrose 4-P; G6P, Glc-6-P; OPP, oxidative pentose phosphate; PEP, phospho*enol*pyruvate.

2003; Goicoechea et al., 2005; Bomal et al., 2008; Zhou et al., 2009; Zhong et al., 2010). No microRNAs have yet been implicated in control of lignin biosynthesis.

Suc May Be a Key Regulator of Growth and Lignin

The negative correlation between growth and lignin content may be thought of as a competition for C allocation to lignin versus C allocation to cellulosics (cellulose and hemicelluloses) because these two classes of molecules are the major C sinks in the formation of the wood cell wall (Sjostrom, 1993; Higuchi, 1997). Lignin biosynthesis requires the formation of monolignol precursors from Phe with methylation from *S*-adenosylmethionine, and the cellulosics are derived from Suc (for cellulose) and hexoses (for hemicelluloses; Fig. 3). Differentiating xylem itself, is a sink tissue and its cells are entirely dependent on the import of reduced C from the photosynthetically active leaves via the phloem. Suc and amino acids, including Phe, are transported in the phloem and unloaded into wood-forming cells through specific transporters (Couturier et al., 2010; Merchant et al., 2010). Suc imported from the phloem can either be directly converted to cellulose or other cell wall carbohydrates through the activity of Suc synthase (Konishi et al., 2004). Suc can also be stored in the vacuole or be cleaved into Glc and Fru by invertases, principally for glycolysis (Trethewey et al., 1998). The relative activities of Suc synthase and invertase in sink tissues are the key determinants of C partitioning between cell wall synthesis, storage, or biosynthesis of other cell components required for growth (Winter and Huber, 2000; Koch, 2004; Coleman et al., 2009). Suc also functions as a signal that regulates metabolic pathways through changes in transcription and enzyme activities mediated through Suc-non-fermenting Related protein Kinases (Halford and Paul, 2003; McKibbin et al., 2006). In addition, Suc stimulates cell division through activation of cyclins (Riou-Khamlichi et al., 2000; Beemster et al., 2002). Although growth may be controlled at least in part by Suc, there are many ways the composition of the walls might be altered, and components negatively correlated.

METABOLIC REGULATION OF LIGNIN BIOSYNTHESIS

Wood-forming cells are heterotrophic and have to import all of their energy and many other components for biosynthesis. Phe as the precursor for lignin biosynthesis is either imported from the phloem or synthesized de novo by the plastid-localized shikimate pathway. Glc-6-P and phospho*enol*pyruvate can be transported into plastids (Kammerer et al., 1998). Glc-6-P can be metabolized in the plastid to synthesize starch or erythrose 4-P through the oxidative pentose phosphate pathway. A positive correlation between lignin biosynthesis, starch accumulation, and reduced growth rates in poplar (*Populus fremontii* × *angustifolia*) has been observed (Harding et al., 2009). Erythrose 4-P and phospho*enol*pyruvate are the substrates for 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, the first critical commitment of the shikimate pathway and subsequent synthesis of aromatic compounds, including the phenylpropanoids. The relative contributions of Phe synthesized de novo through the shikimate pathway versus its import through the phloem is unclear and likely to vary with environmental conditions such as drought or nitrogen availability (Fritz et al., 2006). Specific reduction in lignin, without reduction in other secondary metabolites would have to be regulated downstream in the phenylpropanoid pathway. Phenolic conjugates (Dauwe et al., 2007) may limit the accumulation of phenolic acids and serve a storage function to limit feedback inhibition of the secondary C pathway, thus potentiating the continued flux of C to this pathway at the expense of growth.

Many other potential points of regulation could exist based upon signaling by phenolics, carbohydrates, or compounds of nitrogen metabolism. All major classes of phytohormones may have important roles in xylogenesis (Aloni et al., 1990; Uggla et al., 1998; Andersson-Gunneras et al., 2006), and therefore could affect both growth and lignin. An integrated genetic, transcriptional, and metabolic approach could provide a systematic path to determine the mechanism of control.

Received June 15, 2010; accepted July 12, 2010; published October 6, 2010.

LITERATURE CITED

Aloni R, Tollier MT, Monties B (1990) The role of auxin and gibberellin in controlling lignin formation in primary phloem fibers and in xylem of *Coleus blumei* stems. Plant Physiol **94:** 1743–1747

Amthor JS (2003) Efficiency of lignin biosynthesis: a quantitative analysis. Ann Bot (Lond) **91:** 673–695

Andersson-Gunneras S, Mellerowicz EJ, Love J, Segerman B, Ohmiya Y, Coutinho PM, Nilsson P, Henrissat B, Moritz T, Sundberg B (2006) Biosynthesis of cellulose-enriched tension wood in Populus: global analysis of transcripts and metabolites identifies biochemical and developmental regulators in secondary wall biosynthesis. Plant J **45:** 144–165

Anterola AM, Jeon JH, Davin LB, Lewis NG (2002) Transcriptional control of monolignol biosynthesis in Pinus taeda: factors affecting monolignol ratios and carbon allocation in phenylpropanoid metabolism. J Biol Chem **277:** 18272–18280

Beemster GT, De Vusser K, De Tavernier E, De Bock K, Inze D (2002) Variation in growth rate between Arabidopsis ecotypes is correlated with cell division and A-type cyclin-dependent kinase activity. Plant Physiol **129:** 854–864

Bhuiyan NH, Selvaraj G, Wei Y, King J (2009) Role of lignification in plant defense. Plant Signal Behav **4:** 158–159

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. Annu Rev Plant Biol **54:** 519–546

Bomal C, Bedon F, Caron S, Mansfield SD, Levasseur C, Cooke JE, Blais S, Tremblay L, Morency MJ, Pavy N, et al (2008) Involvement of Pinus taeda MYB1 and MYB8 in phenylpropanoid metabolism and secondary cell wall biogenesis: a comparative in planta analysis. J Exp Bot **59:** 3925–3939

Carder A (1995) Forest Giants of the World: Past and Present. Fitzhenry and Whiteside, Markham, Canada

Chen F, Dixon RA (2007) Lignin modification improves fermentable sugar yields for biofuel production. Nat Biotechnol **25:** 759–761

Coleman HD, Yan J, Mansfield SD (2009) Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. Proc Natl Acad Sci USA **106:** 13118–13123

Couturier J, de Fay E, Fitz M, Wipf D, Blaudez D, Chalot M (2010) PtAAP11, a high affinity amino acid transporter specifically expressed in differentiating xylem cells of poplar. J Exp Bot **61:** 1671–1682

Dauwe R, Morreel K, Goeminne G, Gielen B, Rohde A, Van Beeumen J, Ralph J, Boudet AM, Kopka J, Rochange SF, et al (2007) Molecular phenotyping of lignin-modified tobacco reveals associated changes in cell-wall metabolism, primary metabolism, stress metabolism and photorespiration. Plant J **52:** 263–285

Dixon RA, Achnine L, Kota P, Liu C, Reedy MSS, Wang L (2002) The phenylpropanoid pathway and plant defence-a genomics perspective. Mol Plant Pathol **3:** 371–390

Drost DR, Benedict CI, Berg A, Novaes E, Novaes CR, Yu Q, Dervinis C, Maia JM, Yap J, Miles B, et al (2010) Diversification in the genetic architecture of gene expression and transcriptional networks in organ differentiation of Populus. Proc Natl Acad Sci USA **107:** 8492–8497

Drost DR, Novaes E, Boaventura-Novaes C, Benedict CI, Brown RS, Yin T, Tuskan GA, Kirst M (2009) A microarray-based genotyping and genetic mapping approach for highly heterozygous outcrossing species enables localization of a large fraction of the unassembled Populus trichocarpa genome sequence. Plant J **58:** 1054–1067

Einspahr DW, Goddard RE, Gardner HS (1964) Slash pine wood and fiber property heritability study. Silvae Genet **13:** 103–109

Fengel D, Wegener G (1983) Wood—Chemistry, Ultrastructure, Reactions. Walter de Gruyter, Berlin, pp 133–181

Fritz C, Palacios-Rojas N, Feil R, Stitt M (2006) Regulation of secondary metabolism by the carbon-nitrogen status in tobacco: nitrate inhibits large sectors of phenylpropanoid metabolism. Plant J **46:** 533–548

Fry W, White JB (1938) Big Trees. Stanford University Press, Stanford, CA

Goicoechea M, Lacombe E, Legay S, Mihaljevic S, Rech P, Jauneau A, Lapierre C, Pollet B, Verhaegen D, Chaubet-Gigot N, et al (2005) EgMYB2, a new transcriptional activator from eucalyptus xylem, regulates secondary cell wall formation and lignin biosynthesis. Plant J **43:** 553–567

Halford NG, Paul MJ (2003) Carbon metabolite sensing and signalling. Plant Biotechnol J **1:** 381–398

Harding SA, Jarvie MM, Lindroth RL, Tsai CJ (2009) A comparative analysis of phenylpropanoid metabolism, N utilization, and carbon partitioning in fast- and slow-growing Populus hybrid clones. J Exp Bot **60:** 3443–3452

Heitner C, Dimmel D, Schmidt J (2010) Lignins and Lignans: Advances in Chemistry. Taylor & Francis Ltd., Boca Raton, FL

Higuchi T (1997) Biochemistry and Molecular Biology of Wood. Springer-Verlag, Heidelberg

Hu WJ, Harding SA, Lung J, Popko JL, Ralph J, Stokke DD, Tsai CJ, Chiang VL (1999) Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. Nat Biotechnol **17:** 808–812

Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, Weber A, Flugge UI (1998) Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: the glucose 6-phosphate/phosphate antiporter. Plant Cell **10:** 105–117

Kawaoka A, Kaothien P, Yoshida K, Endo S, Yamada K, Ebinuma H (2000) Functional analysis of tobacco LIM protein Ntlim1 involved in lignin biosynthesis. Plant J **22:** 289–301

Kirst M, Myburg AA, De Leon JP, Kirst ME, Scott J, Sederoff R (2004) Coordinated genetic regulation of growth and lignin revealed by quantitative trait locus analysis of cDNA microarray data in an interspecific backcross of eucalyptus. Plant Physiol **135:** 2368–2378

Koch GW, Sillett SC, Jennings GM, Davis SD (2004) The limits to tree height. Nat Biotechnol **428:** 851–854

Koch K (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Curr Opin Plant Biol **7:** 235–246

Konishi T, Ohmiya Y, Hayashi T (2004) Evidence that sucrose loaded into the phloem of a poplar leaf is used directly by sucrose synthase associated with various beta-glucan synthases in the stem. Plant Physiol **134:** 1146–1152

Li L, Zhou Y, Cheng X, Sun J, Marita J, Ralph J, Chiang V (2003) Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. Proc Natl Acad Sci USA **100:** 4939–4944

McKibbin RS, Muttucumaru N, Paul MJ, Powers SJ, Burrell MM, Coates S, Purcell PC, Tiessen A, Geigenberger P, Halford NG (2006) Production of high-starch, low-glucose potatoes through over-expression of the metabolic regulator SnRK1. Plant Biotechnol J **4:** 409–418

McLaughlin SB, Samson R, Bransby D, Wiselogel A (1996) Evaluating physical, chemical, and energetic properties of perennial grasses as biofuels. *In* Bioenergy '96: Partnerships to Develop and Apply Biomass Technologies. Proceedings of the Seventh National Bioenergy Conference, Nashville, TN. Department of Energy, Oak Ridge National Laboratory, Oak Ridge, TN, pp 1–8

Mele G, Ori N, Sato Y, Hake S (2003) The knotted1-like homeobox gene BREVIPEDICELLUS regulates cell differentiation by modulating metabolic pathways. Genes Dev **17:** 2088–2093

Merchant A, Peuke AD, Keitel C, Macfarlane C, Warren CR, Adams MA (2010) Phloem sap and leaf delta13C, carbohydrates, and amino acid concentrations in Eucalyptus globulus change systematically according to flooding and water deficit treatment. J Exp Bot **61:** 1785–1793

Novaes E, Osorio L, Drost DR, Miles BL, Boaventura-Novaes CR, Benedict C, Dervinis C, Yu Q, Sykes R, Davis M, et al (2009) Quantitative genetic analysis of biomass and wood chemistry of Populus under different nitrogen levels. New Phytol **182:** 878–890

Pedersen JF, Vogel KP, Funnell DL (2005) Impact of reduced lignin on plant fitness. Crop Sci **45:** 812–819

Plomion C, Leprovost G, Stokes A (2001) Wood formation in trees. Plant Physiol **127:** 1513–1523

Raes J, Rohde A, Christensen JH, Van de Peer Y, Boerjan W (2003) Genome-wide characterization of the lignification toolbox in Arabidopsis. Plant Physiol **133:** 1051–1071

Ralph J, Brunow G, Boerjan W (2007) Lignins. *In* F Rose, K Osborne, eds, Encyclopedia of Life Sciences. Wiley & Sons, Chichester, UK, pp 1–10

Riou-Khamlichi C, Menges M, Healy JM, Murray JA (2000) Sugar control of the plant cell cycle: differential regulation of Arabidopsis D-type cyclin gene expression. Mol Cell Biol **20:** 4513–4521

Robinson JM (1990) Lignin, land plants, and fungi—biological evolution affecting phanerozoic oxygen balance. Geology **18:** 607–610

Sarkanen KV, Ludwig CH (1971) Lignins: Occurrence, Formation, Structure and Reactions. John Wiley and Sons, New York

Schaefer D, Steinberger Y, Whitford WG (1985) The failure of nitrogen and lignin control of decomposition in a North American desert. Oecologia **65:** 382–386

Scheible WR, Morcuende R, Czechowski T, Fritz C, Osuna D, Palacios-Rojas N, Schindelasch D, Thimm O, Udvardi MK, Stitt M (2004) Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen. Plant Physiol **136:** 2483–2499

Shafizadeh F, Chin PPS (1977) Thermal degradation of wood. *In* IS Goldstein, ed, Wood Technology: Chemical Aspects. American Chemical Society Symposium Series, Washington, DC, pp 57–81

Shi R, Sun YH, Li Q, Heber S, Sederoff R, Chiang VL (2010) Towards a systems approach for lignin biosynthesis in Populus trichocarpa: transcript abundance and specificity of the monolignol biosynthetic genes. Plant Cell Physiol **51:** 144–163

Sjostrom E (1993) Wood Chemistry. Academic Press, San Diego

Tamagnone L, Merida A, Parr A, Mackay S, Culianez-Macia FA, Roberts K, Martin C (1998) The AmMYB308 and AmMYB330 transcription factors from antirrhinum regulate phenylpropanoid and lignin biosynthesis in transgenic tobacco. Plant Cell **10:** 135–154

Tenhaken R, Thulke O (1996) Cloning of an enzyme that synthesizes a key nucleotide-sugar precursor of hemicellulose biosynthesis from soybean: UDP-glucose dehydrogenase. Plant Physiol **112:** 1127–1134

Timell TE (1969) The chemical composition of tension wood. Svensk Papperstidning **72:** 173–181

Timell TE (1986) Compression Wood in Gymnosperms, Vol 3. Springer-Verlag, Berlin

Trethewey RN, Geigenberger P, Riedel K, Hajirezaei MR, Sonnewald U, Stitt M, Riesmeier JW, Willmitzer L (1998) Combined expression of glucokinase and invertase in potato tubers leads to a dramatic reduction in starch accumulation and a stimulation of glycolysis. Plant J **15:** 109–118

Uggla C, Mellerowicz EJ, Sundberg B (1998) Indole-3-acetic acid controls cambial growth in scots pine by positional signaling. Plant Physiol **117:** 113–121

van Buijtenen JP, Einspahr DW, Peckham JR (1968) Micropulping loblolly pine grafts selected for extreme wood specific gravity. Silvae Genet **17:** 15–19

van Soest PJ (1982) Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, NY

Vance CP, Kirk TK, Sherwood RT (1980) Lignification as a mechanism of disease resistance. Annu Rev Phytopathol **18:** 259–288

Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W (2010) Lignin biosynthesis and structure. Plant Physiol **153:** 895–905

White RH (1987) Effect of lignin content and extractives on the higher heating value of wood. Wood and Fiber Science **19:** 446–452

Winter H, Huber SC (2000) Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes. Crit Rev Biochem Mol Biol **35:** 253–289

Wu RL, Remington DL, MacKay JJ, McKeand SE, O'Malley DM (1999) Average effect of a mutation in lignin biosynthesis in loblolly pine. Theor Appl Genet **99:** 705–710

Yu Q, Li B, Nelson CD, McKeand SE, Batista VB, Mullin TJ (2006) Association of the cad-n1 allele with increased stem growth and wood density in full-sib families of loblolly pine. Tree Genet Genomes **2:** 98–108

Zhong R, Lee C, Ye Z-H (2010) Functional characterization of poplar wood associated NAC domain transcription factors. Plant Physiol **152:** 1044–1055

Zhou J, Lee C, Zhong R, Ye ZH (2009) MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in *Arabidopsis*. Plant Cell **21:** 248–266