Cellulose factories: advancing bioenergy production from forest trees

Eshchar Mizrachi[1], Shawn D. Mansfield[2] and Alexander A. Myburg[1]

1- Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

2 - Department of Wood Science, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Author for correspondence:

Prof. A. A. Myburg

Department of Genetics

University of Pretoria

Pretoria, South Africa

Tel: +27 (0)12 4204945

Fax: +27 (0)12 3625327

Email: zander.myburg@fabi.up.ac.za

Word count

Summary: 200

Body: 3580

Conclusion: 245

Tables: none

Figures: 4 (Figure 1 and Figure 2 in colour)

Summary

Fast-growing, short-rotation forest trees such as *Populus* and *Eucalyptus* produce large amounts of cellulose-rich biomass that could be utilized for bioenergy and biopolymer production. Major obstacles need to be overcome prior to deploying these genera as energy crops, including the effective removal of lignin and subsequent liberation of carbohydrate constituents from wood cell walls. However, significant opportunities exist to both select for and engineer the structure and interaction of cell wall biopolymers, which could afford a means to improve processing and product development. The molecular underpinnings and regulation of cell wall carbohydrate biosynthesis are rapidly being elucidated, and are providing tools to strategically develop and guide the targeted modification required to adapt forest trees for the emerging bioeconomy. Much insight has already been gained from the perturbation of individual genes and pathways, but it is not known to what extent natural variation in the sequence and expression of these same genes underlie the inherent variation in wood properties of field-grown trees. Integration of data from next-generation genomic technologies applied in natural and experimental populations will enable a systems genetics approach to study cell wall carbohydrate production in trees, and should advance the development of future woody bioenergy and biopolymer crops.

Keywords: cellulose, hemicellulose, sucrose, bioenergy, *Eucalyptus*, *Populus*, systems genetics

Introduction

With the growing need for alternative sources of energy and raw materials, fast-growing plantation tree species such as *Populus* and *Eucalyptus* are important candidates for renewable sources of lignocellulosic biomass (for recent reviews on the feasability of bioenergy production from wood biomass refer to Carroll & Somerville, 2009; Hinchee *et al.*, 2009; Mansfield, 2009; Richard, 2010; Somerville *et al.*, 2010; Séguin, 2011). These two genera, broadly representing the Northern and Southern hemisphere, respectively, produce large amounts of woody biomass (>50 m^3/ha/year for eucalypts in highly productive areas such as Brazil) in relatively short rotation times, and in general, do not infringe on land dedicated to food crop production. In addition, contrary to agriculture-derived biomass, tree-derived lignocellulosics can be harvested year-round to ensure a stable, predictable and constant supply of raw material for bioenergy or biofuel production. Establishment costs and carbon footprints of multiyear forest plantations are also lower than that of annually planted crops, especially for coppicing eucalypt species, which can be grown on marginal lands (Hinchee *et al.*, 2009). Well-established industrial breeding programmes already exploit the substantial inherent genetic variation available in these genera, which can be (and has been) expanded with interspecific hybridization, and ultimately captured in clonal plantations (Grattapaglia *et al.*, 2009). The processing of wood fibre, and especially cellulose, from woody biomass has been improved and optimized for decades providing a technology base from which to develop processing plants for biofuels and biomaterials. One major consideration that is often overlooked when forecasting bioenergy feedstocks is that this bioenergy end-use will have to compete with the high value products derived from chemical cellulose and its derivatives (Figure 1), and the desired traits for many bioenergy applications are common to those desired for chemical cellulose production. Thus, the

objective of improving feedstock characteristics in trees is complementary to current tree breeding programs directed at traditional forest-reliant industries.

Cellulose-rich biomass derived from fast-growing tree species offers many advantages over agricultural feedstocks for bioenergy production, but the removal of lignin to facilitate the effective and efficient extraction of cell wall carbohydrates remains one of the primary hurdles (Studer *et al.*, 2011). To efficiently deconstruct lignocellulosic biomass, a detailed understanding of how wood cell walls are synthesized, deposited and modified *in planta* is required (Mansfield, 2009). Recent research has mainly focused on the modification of lignin, the most abundant natural biopolymer after cellulose (Vanholme *et al.*, 2008), but much remains to be learned about the possibilities for modifying and regulating the synthesis of cellulose, ultimately impacting the overall chemistry and ultrastructure of wood cell walls. While major advances have been made in understanding the biosynthesis of cellulose itself (Joshi & Mansfield, 2007), the underlying cellular and biochemical processes that influence cellulose properties in wood cell walls have not yet been fully dissected.

Most of our current knowledge of cellulose biosynthesis stems from studies in model herbaceous plants such as *Arabidopsis thaliana* and to some extent, extension of this knowledge to woody plant genera such as *Populus* (Joshi *et al.*, 2011). The poplar genome sequence (Tuskan *et al.*, 2006) has been available for five years, and as of 2011, the genome sequence of *Eucalyptus grandis* (Myburg *et al.*, in preparation) is also publically available (http://www.phytozome.net). These two landmark achievements open up new avenues for exploiting genetic variation in forest trees, and strategically improving the physicochemical properties of woody biomass. The availability of a genome sequence is particularly important for *Eucalyptus*, the most widely grown hardwood crop in the world (~ 20 million ha). With advances in next generation sequencing technologies, comparative genomics can now be applied to rapidly adopt the information learned from herbaceous models and other woody

plants such as poplars to accelerate *Eucalyptus* improvement. However, with so many candidate genes known to influence xylogenesis, how does one prioritize targets when considering forest trees as bioenergy crops? How can one expand the fundamental understanding of the biology and biosynthesis of cellulose and its interaction with other wood cell wall polymers?

Here, we provide a current summary of the general understanding of the molecular biology of cellulose production in plants and discuss how the integration of emerging functional genomics technologies with the wealth of fundamental information on wood properties in tree breeding programmes, could be used to accelerate the improvement of cellulose and bioenergy potential in trees.

An integrated view of proteins involved in cellulose biosynthesis and deposition

Historically, the biosynthesis of cellulose has focussed on the plasma membrane-located cellulose synthase (CESA) proteins that constitute the active synthesising complex (CSC; cellulose synthase complex), which is ultimately responsible for producing the polymeric glucan chains that coalesce to form cellulose microfibrils in primary and secondary cell walls of plants (Delmer, 1999; Doblin *et al.*, 2002; Saxena & Brown Jr, 2005; Somerville, 2006; Bessueille & Bulone, 2008; Taylor, 2008; Guerriero *et al.*, 2010). Building on these solid foundations, our current understanding requires an integrated view that incorporates a diverse set of proteins and regulatory mechanisms to fully understand this intricate biological process. Such a view should take into consideration the variety of cellular processes and metabolic fluxes that could, and do, influence the synthesis, deposition and physical properties of cellulose in the two distinctly different cell walls. This holistic view should also

include the inherent and tightly regulated interactions of cellulose with other cell wall biopolymers, such as lignin and hemicellulose. For example, the biosynthesis and deposition of xylan, a major constituent of the dicot secondary cell wall (Scheller & Ulvskov, 2010) is closely coordinated with the deposition of cellulose (Hertzberg *et al.*, 2001; Schrader *et al.*, 2004). Thus, to advance our fundamental understanding, and further the biotechnological objectives of improving cellulose-rich resources, research areas to be explored should focus on the transcriptional regulation of xylem forming genes; as well as post-translational modification, protein folding and protein-complex assembly; substrate (metabolite) production, transport and availability; the transport of proteins and/or polysaccharides between organelles and to the plasma membrane; and, signalling and feedback between the extracellular environment and the cytoplasm, organelles and nucleus.

Using Arabidopsis as the primary model, the current architecture of proteins and cellular processes thought to be involved in, or influence the biosynthesis and deposition of cellulose and xylan is illustrated in Figure 2. At the level of transcriptional regulation, several transcription factors have been shown to directly regulate secondary cell wall *CesA* genes in Arabidopsis (Zhong et al., 2008; Yamaguchi et al., 2010; Xie et al., 2011). Three of these - SND2, SND3 and MYB103 - appear to specifically regulate secondary cell wall *CesA* genes but not xylan or lignin genes (Zhong *et al.*, 2008). These transcription factors are part of a complex transcriptional network regulating various aspects of xylogenesis, the extent of which is still being resolved in Arabidopsis (Kubo *et al.*, 2005; Zhong *et al.*, 2006; Demura & Fukuda, 2007; Zhong *et al.*, 2007; Zhong *et al.*, 2008), as well as more recently in *Populus* (McCarthy *et al.*, 2010; Zhong *et al.*, 2010; Zhong & Ye, 2010; Zhong *et al.*, 2011).

CESA proteins are synthesized and assembled into complexes in the ER (Rudolph, 1987) and, with the help of chaperones, packaged and delivered to the Golgi (Haigler & Brown Jr, 1986). The Golgi (Figure 2) is also the site for xylan biosynthesis (Bolwell & Northcote,

1983), which can be divided, simplistically, into primer synthesis (PARVUS), chain elongation (IRX9, 10 and 14) and side chain modifications by IRX7, IRX8, PGSIP1, DUF579- and/or DUF231-containing proteins (Brown *et al.*, 2007; Lee *et al.*, 2007; York & O'Neill, 2008; Brown *et al.*, 2009; Wu *et al.*, 2009; Wu *et al.*, 2010; Brown *et al.*, 2011; Jensen *et al.*, 2011). Once the cellulose synthase complexes (CSCs) are assembled, they are transported from the Golgi to the plasma membrane via the trans-Golgi network in specialized microtubule-associated compartments (MASCs; Crowell *et al.*, 2009) that interact with actin through MYOSIN (Wightman & Turner, 2008; Szymanski, 2009). At the plasma membrane, MASCs interact with cortical microtubules, possibly but not conclusively via KINESIN, and bud vesicles containing CSCs that fuse with and become embedded in the plasma membrane (Giddings Jr *et al.*, 1980; Szymanski, 2009; Crowell *et al.*, 2010).

On the cytoplasmic face (Figure 2), the CSCs associate with cortical microtubules, putatively through kinesin-like proteins such as FRA1 (Zhong *et al.*, 2002), CSI1 (Gu *et al.*, 2010), and other microtubule associated proteins (MAPs). It is therefore apparent that cortical microtubule organization is extremely important in regulating and depositing cellulose, and the structure and orientation of said cortical microtubules is influenced by a variety of factors. From the assembly of α- and β-TUB at microtubule assembly sites containing γ-TUB and Gamma-complex proteins (Pastuglia & Bouchez, 2007; Cai, 2010), growth and modification of the microtubules is influenced by strong association with actin via KCH and MAP190 (Cai, 2010), association with other microtubules via MAP65-1, MAP 200, TBMP 200 and/or MOR1 (Cai, 2010), and association with the plasma membrane via proteins such as EB1 (Morrison, 2007), P-161 (Cai *et al.*, 2005), ATK5 (Ambrose & Cyr, 2007; Pastuglia & Bouchez, 2007), SPR1 (Nakajima *et al.*, 2004; Sedbrook *et al.*, 2004; Nakajima *et al.*, 2006), CLIPs and CLASPs (Galjart, 2005; Ambrose & Wasteneys, 2008), and PHOSPHOLIPASE-D (Cai, 2010). Microtubule length and organization is also modified by KATANIN (McNally

& Vale, 1993; Burk *et al.*, 2001; Stoppin-Mellet *et al.*, 2006; Sharma *et al.*, 2007), and therefore can impact the quality and quantity of cellulose. Transamination, tyrosylation or acetylation of microtubules can influence the binding of KINESIN proteins, while glutamination or glycylation of microtubules has been shown to influence KATANIN activity (Cai, 2010). These, and other as yet unidentified proteins, could all potentially have direct or indirect effects on cellulose deposition, via their influence on cortical microtubule dynamics.

Movement of the CSC along the membrane is believed to be driven by the force of cellulose microfibril synthesis itself against the cell wall matrix (Diotallevi & Mulder, 2007), and is guided by the cortical microtubules (Paredez *et al.*, 2006), with membrane-associated sucrose synthase (SUSY) providing UDP-glucose as substrate for the CSC (Figure 2). Towards the cell wall side, KORRIGAN (KOR - Lane *et al.*, 2001) and possibly other glycosyl hydrolases edit elongating cellulose chains as they are synthesized, while COBRA/COBL and possibly other GPI-anchored proteins, as well as the fasciclin-like arabinogalactan (FLA) proteins and/or other arabinogalactan proteins (AGPs) are thought to interact with cellulose as it is deposited, and concurrently relay signals back to the cytoplasm to regulate its synthesis (Zhang *et al.*, 2003; Seifert & Roberts, 2007; MacMillan *et al.*, 2010).

The mediation of cell wall feedback signalling is carried out by a number of pathways, and recently the Rop/Rac GTPases (Figure 2), which are regulated by RIC and Rop-GEF, have been highlighted as playing an important role in cell wall signalling, along with IQD and CTL proteins, and wall-associated kinases (WAKs) such as LRR-receptor kinases (Oikawa *et al.*, 2010). The LRR-receptor kinases include, amongst others, THESEUS (Hématy *et al.*, 2007) and KOBITO/ELD1 (Pagant *et al.*, 2002; Lertpiriyapong & Sung, 2003) both of which have been shown to impact cell wall properties. In the secondary cell wall, laccases (LAC) and other peroxidases oxidize monolignols, leading to the random coupling of lignin monomers and resulting in the synthesis of the macromolecule lignin polymer (Boerjan *et al.*,

2003; Ralph *et al.*, 2004; Mattinen *et al.*, 2008), while other as yet unidentified glycosyl hydrolases (GH) and carbohydrate binding module (CBM)-containing proteins appear to be involved in mediating cellulose-cellulose, cellulose-xylan, xylan-xylan or xylan-lignin interactions as the different biopolymers are synthesized, deposited and arranged.

In addition to the cellular processes and specific proteins involved in cellulose deposition itself, it is important to consider the metabolic flux and channelling to the various biochemical pathways that lead to the synthesis of cellulose and xylan. For example, a key metabolite is uridine diphosphate- (UDP)-glucose, which is the immediate pre-cursor for cellulose biosynthesis by CESA proteins. In addition, UDP-glucose can be readily converted to UDP-xylose for xylan biosynthesis (Figure 3). UDP-glucose is produced directly via the hydrolysis of sucrose by sucrose SUSY or indirectly by invertase (Barratt *et al.*, 2009; Kleczkowski *et al.*, 2010), which cleaves sucrose to monomeric glucose and fructose. Monomeric glucose is then converted to UDP-glucose via phosphorylation of the 6' position (HEXOKINASE/GLUCOKINASE), followed by the substitution of the phosphate to the 1' position (PHOSPHOGLUCOMUTASE) and the subsequent substitution of the phosphate group with UDP by UTP-glucose-1-phosphate uridylyltransferase (UGP). UDP-glucose can be directly employed by CESA proteins for cellulose biosynthesis, or converted to UDP-xylose via conversion to UDP-D-glucuronate by UDP-glucose 6-dehydrogenase (UGHD), followed by the removal of CO_2 by uridine-diphosphoglucuronate decarboxylase (UXS). UDP-xylose is then utilized as the backbone for xylan biosynthesis, with the addition of glucuronic acid (GlcA) and acetyl groups to the backbone or side chains to form heteroxylan.

Studies have shown that alteration in the metabolic flux of UDP-glucose can indeed affect the relative abundance and structure of cell wall polysaccharides. For example, upregulation of *SUSY* in poplar trees resulted in an increase in cell wall thickness of fibres, and production of more cellulose that displayed enhanced crystallinity (Coleman *et al.*, 2009). The combination

of *SUSY* and *UGP* overexpression in tobacco also resulted in a synergistic increase in plant height and biomass (Coleman *et al.*, 2006). It should be noted that the overall phenotypic effect of increased SUSY or UGP levels would be dependent on source and sink sugars and other metabolites (Haigler *et al.*, 2001; Coleman *et al.*, 2009; Meng *et al.*, 2009), which will vary in different plant species, and under an array of physiological conditions. These studies demonstrate that changes in metabolite levels, through intra and inter-cellular transport or enzymatic activity, could greatly influence the resulting abundance and/or structure of cell wall polysaccharides.

Towards systems genetics of cellulose production in trees

The scale of cellulose biosynthesis and biomass production in fast-growing plantation trees is vastly different from that in herbaceous models. There is an emphasis on large-scale cambial cell differentiation, cell elongation, secondary cell wall deposition and programmed cell death. The tremendous strength of the sink tissue means that the tree as a system must prioritize channelling carbon flow towards the synthesis of xylem biopolymers. Therefore, information cannot always be directly extended from herbaceous models to trees - good examples of this are the different outcomes that resulted from overexpressing *SUSY* in tobacco plants (Coleman *et al.*, 2006) as opposed to poplar (Coleman *et al.*, 2009), or the fact that for Arabidopsis, INVERTASE is necessary and sufficient for normal growth whereas direct UDP-glucose production through SUSY is not (Barratt et al., 2009). Recent findings also suggest that the transcriptional network regulating cell wall biopolymer synthesis in woody plants may be more complex and comprise novel transcription factors not previously linked to secondary cell wall formation in Arabidopsis (Zhong *et al.*, 2011). This implies the need to independently study the functions of secondary cell wall related genes in trees. Some practical considerations are that very few commercial species and clonal genotypes have

optimized transformation protocols; mature wood properties take several years to acquire; and wood properties are complex traits affected by large numbers of genes. Rigorous greenhouse studies and field trials are required for each candidate, and these carry significant economical, ecological and regulatory burdens (for recent reviews on this issue see Ahuja, 2011; Harfouche *et al.*, 2011; Strauss *et al.*, 2009). What is required is an approach that would prioritize genes or pathways that underlie variation in wood properties in mature, field grown trees.

At our disposal is a rich history of tree breeding, resulting in large, structured populations, and large amounts of genetic diversity in these populations (Sederoff *et al.*, 2009; Neale & Kremer, 2011). These resources have been exploited through the application of molecular marker technologies and forward genetics approaches in multiple forest tree pedigrees where high linkage disequilibrium (LD) has allowed the efficient identification of quantitative trait loci (QTL -Grattapaglia & Kirst, 2008), as well as in large association populations where low LD has allowed the association of single genes with wood properties (Groover, 2007; Neale & Ingvarsson, 2008). Single gene associations detected in *Eucalyptus* and *Populus* (Thumma *et al.*, 2005; Thumma *et al.*, 2009; Wegrzyn *et al.*, 2010) have not always been intuitive - for example the association between a lignin gene (*cinnamoyl CoA reductase*, *CCR*) and a physical cellulose property (microfibril angle) in *Eucalyptus* (Thumma *et al.*, 2005). This illustrates that our understanding of the causal relationship of genes and complex traits is still incomplete.

Phenotypic variation in tree breeding populations is influenced by a variety of intrinsic (and measurable) biological processes, mainly those of transcriptional and translational regulation of various biochemical pathways (Du & Groover, 2010), as well as the flux of metabolic intermediates in these pathways (Mansfield, 2009). In addition, these biological processes are strongly impacted by environmental cues and seasonal variation over the lifetime of these

long-lived organisms (Groover, 2007). A more holistic research approach encompassing genetic, biochemical and environmental variation must therefore be adopted to understand and improve wood property traits in trees.

Systems genetics (Figure 4) connects the intermediate components of a complex phenotype (e.g. transcript, protein and metabolite levels) in related individuals to measurable phenotypic traits such as wood properties or bioenergy potential, in the context of the underlying genetic variation in populations (MacKay *et al.*, 2009; Nadeau & Dudley, 2011). An extension of genetical genomics (Jansen & Nap, 2001), systems genetics is a network approach that explores the interconnectedness of the component levels of biological variation. It has been successfully applied in model organisms such as *Drosophila* (Ayroles *et al.*, 2009; Morozova *et al.*, 2009; Jumbo-Lucioni *et al.*, 2010) and mouse (Farber *et al.*, 2011). It has also been applied in humans (Plaisier *et al.*, 2009; Romanoski *et al.*, 2010), and importantly in animal breeding (Kadarmideen *et al.*, 2006; Kadarmideen & Janss, 2007; Kadarmideen & Janss, 2009), which has many similarities to plant breeding. The power of systems genetics is that it reveals emergent properties of the system, providing insight into novel gene-gene, gene-trait and trait-trait relationships that would not be detected at the level of the individual. This often allows reconstruction of complex directional gene regulatory networks and metabolic pathways (Kadarmideen *et al.*, 2006; Keurentjes *et al.*, 2007), adding insight to previously identified single gene associations and the molecular basis of QTLs. Systems genetics could also explain the biology underlying complex phenomena such as G × E interactions, epigenetic control, biotic and abiotic interactions and hybrid vigour (heterosis), which are key themes to be addressed in tree improvement in the near future.

Tree breeding programmes already make use of structured pedigrees and populations replicated across environments, and therefore present an ideal starting place for systems

genetics. Variation in transcriptomes have already been studied at the population level in *Eucalyptus* (Kirst *et al.*, 2005; Grattapaglia & Kirst, 2008) and *Populus* (Drost *et al.*, 2010). Transcriptome, proteome and metabolome profiling at the population level will allow integrated modelling of biomass production in trees. Systems genetics is complementary to fundamental biological investigations performed in model organisms and will also complement association genetics approaches and genomic selection strategies that are being implemented in forest tree breeding programs (Grattapaglia & Resende, 2011). Moreover, systems genetics will allow the identification and prioritization of candidate genes for functional genetic testing in greenhouse and field trials of forest trees.

Conclusion

Understanding how cellulose is deposited during xylogenesis in wood fibre cells has important implications for our ability to manipulate and select for bioenergy traits in trees. We also need to understand the complex genetic relationships and biochemical interactions that underlie wood property variation in tree populations. Application of next-generation DNA and RNA sequencing (Mizrachi et al., 2010), and the adoption of high throughput proteomics and metabolomics technologies in trees (Abril *et al.*, 2011; Dauwe *et al.*, 2011; Robinson and Mansfield, 2011) will allow integrated approaches to study complex relationships of genes, metabolites and wood (bio)chemistry traits at the population level. A systems genetics approach, which also includes the measurement of bioenergy potential, is a viable and increasingly cost effective method to dissect complex phenotypes in trees and will complement genomic selection efforts. It will also permit one to address the fundamental question whether the same genes linked to cell wall biosynthesis by functional genetic studies in individual genotypes are also influencing cell wall properties in natural or experimental populations. Additionally, the diversity of applications of next-generation DNA sequencing

will enable investigation of other types of regulation such as allele-specific expression, splice site variation, gene regulation by endogenous small RNAs, or epigenetic modification that may impact the bioenergy potential of forest trees. Finally, the completion of additional tree genome sequences will permit comparative genomics approaches to dissect vital biosynthetic pathways important to industrial trait development, which should form the foundations of the emerging bio-based economy.

References

Abril N, Gion JM, Kerner R, Müller-Starck G, Cerrillo RMN, Plomion C, Renaut J, Valledor L, Jorrin-Novo JV. 2011. Proteomics research on forest trees, the most recalcitrant and orphan plant species. *Phytochemistry* 72(10): 1219-1242.

Ahuja MR. 2011. Fate of transgenes in the forest tree genome. *Tree Genetics and Genomes* 7(2): 221-230.

Ambrose CJ, Wasteneys GO. 2008. CLASP modulates microtubule-cortex interaction during self-organization of acentrosomal microtubules. *Molecular Biology of the Cell* 19(11): 4730-4737.

Ambrose JC, Cyr R. 2007. The kinesin ATK5 functions in early spindle assembly in *Arabidopsis*. *Plant Cell* 19(1): 226-236.

Ayroles JF, Carbone MA, Stone EA, Jordan KW, Lyman RF, Magwire MM, Rollmann SM, Duncan LH, Lawrence F, Anholt RRH, Mackay TFC. 2009. Systems genetics of complex traits in *Drosophila melanogaster*. *Nature Genetics* 41(3): 299-307.

Barratt DHP, Derbyshire P, Findlay K, Pike M, Wellner N, Lunn J, Feil R, Simpson C, Maule AJ, Smith AM. 2009. Normal growth of *Arabidopsis* requires cytosolic invertase but not sucrose synthase. *Proceedings of the National Academy of Sciences of the United States of America* 106(31): 13124-13129.

Bessueille L, Bulone V. 2008. A survey of cellulose biosynthesis in higher plants. *Plant Biotechnology* 25(3): 315-322.

Boerjan W, Ralph J, Baucher M 2003. Lignin Biosynthesis. In. *Annual Review of Plant Biology*. 519-546.

Bolwell GP, Northcote DH. 1983. Arabinan synthase and xylan synthase activities of *Phaseolus vulgaris*. Subcellular localization and possible mechanism of action. *Biochemical Journal* 210(2): 497-507.

Brown D, Wightman R, Zhang Z, Gomez LD, Atanassov I, Bukowski JP, Tryfona T, McQueen-Mason SJ, Dupree P, Turner S. 2011. *Arabidopsis* genes *IRREGULAR XYLEM* (*IRX15*) and *IRX15L* encode DUF579-containing proteins that are essential for normal xylan deposition in the secondary cell wall. *Plant Journal* 66(3): 401-413.

Brown DM, Goubet F, Wong VW, Goodacre R, Stephens E, Dupree P, Turner SR. 2007. Comparison of five xylan synthesis mutants reveals new insight into the mechanisms of xylan synthesis. *Plant Journal* 52(6): 1154-1168.

Brown DM, Zhang Z, Stephens E, Dupree P, Turner SR. 2009. Characterization of IRX10 and IRX10-like reveals an essential role in glucuronoxylan biosynthesis in *Arabidopsis*. *Plant Journal* 57(4): 732-746.

Burk DH, Liu B, Zhong R, Morrison WH, Ye ZH. 2001. A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *Plant Cell* 13(4): 807-827.

Cai G. 2010. Assembly and disassembly of plant microtubules: Tubulin modifications and binding to MAPs. *Journal of Experimental Botany* 61(3): 623-626.

Cai G, Ovidi E, Romagnoli S, Vantard M, Cresti M, Tiezzi A. 2005. Identification and characterization of plasma membrane proteins that bind to microtubules in pollen tubes and generative cells of tobacco. *Plant and Cell Physiology* 46(4): 563-578.

Carroll A, Somerville C. 2009. Cellulosic biofuels. *Annual Review of Plant Biology* 60: 165-182.

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(1): 87-101.

Coleman HD, Yan J, Mansfield SD. 2009. Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. *Proceedings of the National Academy of Sciences of the United States of America* 106(31): 13118-13123.

Crowell EF, Bischoff V, Desprez T, Rolland A, Stierhof YD, Schumacher K, Gonneau M, Höfte H, Vernhettes S. 2009. Pausing of golgi bodies on microtubules regulates secretion of cellulose synthase complexes in *Arabidopsis*. *Plant Cell* 21(4): 1141-1154.

Crowell EF, Gonneau M, Stierhof YD, Höfte H, Vernhettes S. 2010. Regulated trafficking of cellulose synthases. *Current Opinion in Plant Biology* 13(6): 700-705.

Dauwe R, Robinson A, Mansfield SD. 2011. Recent advances in proteomics and metabolomics in gymnosperms. In C. Plomion, J. Bousquet and K. Chittaranjan (Eds.). Genetics, Genomics and Breeding of Conifers. CRC Press, Science Publishers, Inc., New Hampshire; Edenbridge Ltd., British Isles. Pp. 358-388.

Delmer DP. 1999. CELLULOSE BIOSYNTHESIS: Exciting times for a difficult field of study. *Annu Rev Plant Physiol Plant Mol Biol* 50: 245-276.

Demura T, Fukuda H. 2007. Transcriptional regulation in wood formation. *Trends in Plant Science* 12(2): 64-70.

Diotallevi F, Mulder B. 2007. The cellulose synthase complex: A polymerization driven supramolecular motor. *Biophysical Journal* 92(8): 2666-2673.

Doblin MS, Kurek I, Jacob-Wilk D, Delmer DP. 2002. Cellulose biosynthesis in plants: from genes to rosettes. *Plant Cell Physiol* 43(12): 1407-1420.

Drost DR, Benedict CI, Berg A, Novaes E, Novaes CRDB, Yu Q, Dervinis C, Maia JM, Yap J, Miles B, Kirst M. 2010. Diversification in the genetic architecture of gene expression and transcriptional networks in organ differentiation of *Populus*. *Proceedings of the National Academy of Sciences of the United States of America* 107(18): 8492-8497.

Du J, Groover A. 2010. Transcriptional regulation of secondary growth and wood formation. *Journal of Integrative Plant Biology* 52(1): 17-27.

Farber CR, Bennett BJ, Orozco L, Zou W, Lira A, Kostem E, Kang HM, Furlotte N, Berberyan A, Ghazalpour A, Suwanwela J, Drake TA, Eskin E, Wang QT, Teitelbaum SL, Lusis AJ. 2011. Mouse genome-wide association and systems genetics identify Asxl2 as a regulator of bone mineral density and osteoclastogenesis. *PLoS Genetics* 7(4).

Galjart N. 2005. CLIPs and CLASPs and cellular dynamics. *Nature Reviews Molecular Cell Biology* 6(6): 487-498.

Giddings Jr TH, Brower DL, Staehelin LA. 1980. Visualization of particle complexes in the plasma membrane of *Micrasterias denticulata* associated with the formation of cellulose fibrils in primary and secondary cell walls. *Journal of Cell Biology* 84(2): 327-339.

Grattapaglia D, Kirst M. 2008. *Eucalyptus* applied genomics: From gene sequences to breeding tools. *New Phytologist* 179(4): 911-929.

Grattapaglia D, Plomion C, Kirst M, Sederoff RR. 2009. Genomics of growth traits in forest trees. *Current Opinion in Plant Biology* 12(2): 148-156.

Grattapaglia D, Resende MDV. 2011. Genomic selection in forest tree breeding. *Tree Genetics and Genomes* 7(2): 241-255.

Groover AT. 2007. Will genomics guide a greener forest biotech? *Trends in Plant Science* 12(6): 234-238.

Gu Y, Kaplinsky N, Bringmann M, Cobb A, Carroll A, Sampathkumar A, Baskin TI, Persson S, Somerville CR. 2010. Identification of a cellulose synthase-associated protein required for

cellulose biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* 107(29): 12866-12871.
Guerriero G, Fugelstad J, Bulone V. 2010. What do we really know about cellulose biosynthesis in higher plants? *Journal of Integrative Plant Biology* 52(2): 161-175.
Haigler CH, Brown Jr RM. 1986. Transport of rosettes from the golgi apparatus to the plasma membrane in isolated mesophyll cells of *Zinnia elegans* during differentiation to tracheary elements in suspension culture. *Protoplasma* 134(2-3): 111-120.
Haigler CH, Ivanova-Datcheva M, Hogan PS, Salnikov VV, Hwang S, Martin K, Delmer DP. 2001. Carbon partitioning to cellulose synthesis. *Plant Molecular Biology* 47(1-2): 29-51.
Harfouche A, Meilan R, Altmane A. 2011. Tree genetic engineering and applications to sustainable forestry and biomass production. *Trends in Biotechnology* 29(1): 9-17.
Hématy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou JP, Höfte H. 2007. A receptor-like kinase mediates the response of *Arabidopsis* cells to the inhibition of cellulose synthesis. *Current Biology* 17(11): 922-931.
Hertzberg M, Aspeborg H, Schrader J, Andersson A, Erlandsson R, Blomqvist K, Bhalerao R, Uhlén M, Teeri TT, Lundeberg J, Sundberg B, Nilsson P, Sandberg G. 2001. A transcriptional roadmap to wood formation. *Proceedings of the National Academy of Sciences of the United States of America* 98(25): 14732-14737.
Hinchee M, Rottmann W, Mullinax L, Zhang C, Chang S, Cunningham M, Pearson L, Nehra N. 2009. Short-rotation woody crops for bioenergy and biofuels applications. *In Vitro Cellular and Developmental Biology - Plant* 45(6): 619-629.
Jansen RC, Nap JP. 2001. Genetical genomics: The added value from segregation. *Trends in Genetics* 17(7): 388-391.
Jensen JK, Kim H, Cocuron JC, Orler R, Ralph J, Wilkerson CG. 2011. The DUF579 domain containing proteins IRX15 and IRX15-L affect xylan synthesis in Arabidopsis. *Plant Journal* 66(3): 387-400.
Joshi CP, Mansfield SD. 2007. The cellulose paradox - simple molecule, complex biosynthesis. *Current Opinion in Plant Biology* 10(3): 220-226.
Joshi CP, Thammannagowda S, Fujino T, Gou JQ, Avci U, Haigler CH, McDonnell LM, Mansfield SD, Mengesha B, Carpita NC, Harris D, Debolt S, Peter GF. 2011. Perturbation of wood cellulose synthesis causes pleiotropic effects in transgenic aspen. *Molecular Plant* 4(2): 331-345.
Jumbo-Lucioni P, Ayroles JF, Chambers MM, Jordan KW, Leips J, Mackay TFC, De Luca M. 2010. Systems genetics analysis of body weight and energy metabolism traits in *Drosophila melanogaster*. *BMC Genomics* 11(1).
Kadarmideen HN, Janss LLG. 2007. Population and systems genetics analyses of cortisol in pigs divergently selected for stress. *Physiological Genomics* 29(1): 57-65.
Kadarmideen HN, Janss LLG. 2009. Population and systems genetics analyses of cortisol in pigs divergently selected for stress. *Physiological Genomics* 37(3): 303.
Kadarmideen HN, Von Rohr P, Janss LLG. 2006. From genetical genomics to systems genetics: Potential applications in quantitative genomics and animal breeding. *Mammalian Genome* 17(6): 548-564.
Keurentjes JJB, Fu J, Terpstra IR, Garcia JM, Van Den Ackerveken G, Snoek LB, Peeters AJM, Vreugdenhil D, Koornneef M, Jansen RC. 2007. Regulatory network construction in Arabidopsis by using genome-wide gene expression quantitative trait loci. *Proceedings of the National Academy of Sciences of the United States of America* 104(5): 1708-1713.
Kirst M, Basten CJ, Myburg AA, Zeng ZB, Sederoff RR. 2005. Genetic architecture of transcript-level variation in differentiating xylem of a *Eucalyptus* hybrid. *Genetics* 169(4): 2295-2303.
Kleczkowski LA, Kunz S, Wilczynska M. 2010. Mechanisms of UDP-glucose synthesis in plants. *Critical Reviews in Plant Sciences* 29(4): 191-203.
Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, Ito J, Mimura T, Fukuda H, Demura T. 2005. Transcription switches for protoxylem and metaxylem vessel formation. *Genes and Development* 19(16): 1855-1860.
Lane DR, Wiedemeier A, Peng L, Höfte H, Vernhettes S, Desprez T, Hocart CH, Birch RJ, Baskin TI, Burn JE, Arioli T, Betzner AS, Williamson RE. 2001. Temperature-sensitive alleles of *rsw2*

link the KORRIGAN endo-1,4-β-glucanase to cellulose synthesis and cytokinesis in *Arabidopsis*. *Plant Physiology* 126(1): 278-288.

Lee C, Zhong R, Richardson EA, Himmelsbach DS, McPhail BT, Ye ZH. 2007. The *PARVUS* gene is expressed in cells undergoing secondary wall thickening and is essential for glucuronoxylan biosynthesis. *Plant and Cell Physiology* 48(12): 1659-1672.

Lertpiriyapong K, Sung ZR. 2003. The elongation defective1 mutant of *Arabidopsis* is impaired in the gene encoding a serine-rich secreted protein. *Plant Molecular Biology* 53(4): 581-595.

MacKay TFC, Stone EA, Ayroles JF. 2009. The genetics of quantitative traits: Challenges and prospects. *Nature Reviews Genetics* 10(8): 565-577.

MacMillan CP, Mansfield SD, Stachurski ZH, Evans R, Southerton SG. 2010. Fasciclin-like arabinogalactan proteins: Specialization for stem biomechanics and cell wall architecture in Arabidopsis and *Eucalyptus*. *Plant Journal* 62(4): 689-703.

Mansfield SD. 2009. Solutions for dissolution-engineering cell walls for deconstruction. *Current Opinion in Biotechnology* 20(3): 286-294.

Mattinen ML, Suortti T, Gosselink R, Argyropoulos DS, Evtuguin D, Suurnäkki A, De Jong E, Tamminen T. 2008. Polymerization of different lignins by laccase. *BioResources* 3(2): 549-565.

McCarthy RL, Zhong R, Fowler S, Lyskowski D, Piyasena H, Carleton K, Spicer C, Ye ZH. 2010. The poplar MYB transcription factors, PtrMYB3 and PtrMYB20, are involved in the regulation of secondary wall biosynthesis. *Plant and Cell Physiology* 51(6): 1084-1090.

McNally FJ, Vale RD. 1993. Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* 75(3): 419-429.

Meng M, Geisler M, Johansson H, Harholt J, Scheller HV, Mellerowicz EJ, Kleczkowski LA. 2009. UDP-glucose pyrophosphorylase is not rate limiting, but is essential in *Arabidopsis*. *Plant and Cell Physiology* 50(5): 998-1011.

Mizrachi E, Hefer CA, Ranik M, Joubert F, Myburg AA. 2010. De novo assembled expressed gene catalog of a fast-growing Eucalyptus tree produced by Illumina mRNA-Seq. *BMC Genomics* 11(1).

Morozova TV, Ayroles JF, Jordan KW, Duncan LH, Carbone MA, Lyman RF, Stone EA, Govindaraju DR, Ellison RC, Mackay TFC, Anholt RRH. 2009. Alcohol sensitivity in *Drosophila*: Translational potential of systems genetics. *Genetics* 183(2): 733-745.

Morrison EE. 2007. Action and interactions at microtubule ends. *Cellular and Molecular Life Sciences* 64(3): 307-317.

Nadeau JH, Dudley AM. 2011. Systems genetics. *Science* 331(6020): 1015-1016.

Nakajima K, Furutani I, Tachimoto H, Matsubara H, Hashimoto T. 2004. *Spiral1* encodes a plant-specific microtubule-localized protein required for directional control of rapidly expanding arabidopsis cells. *Plant Cell* 16(5): 1178-1190.

Nakajima K, Kawamura T, Hashimoto T. 2006. Role of the *SPIRAL1* gene family in anisotropic growth of *Arabidopsis thaliana*. *Plant and Cell Physiology* 47(4): 513-522.

Neale DB, Ingvarsson PK. 2008. Population, quantitative and comparative genomics of adaptation in forest trees. *Current Opinion in Plant Biology* 11(2): 149-155.

Neale DB, Kremer A. 2011. Forest tree genomics: Growing resources and applications. *Nature Reviews Genetics* 12(2): 111-122.

Oikawa A, Joshi HJ, Rennie EA, Ebert B, Manisseri C, Heazlewood JL, Scheller HV. 2010. An integrative approach to the identification of *Arabidopsis* and rice genes involved in xylan and secondary wall development. *PLoS ONE* 5(11).

Pagant S, Bichet A, Sugimoto K, Lerouxel O, Desprez T, McCann M, Lerouge P, Vernhettes S, Höfte H. 2002. *Kobito1* encodes a novel plasma membrane protein necessary for normal synthesis of cellulose during cell expansion in *Arabidopsis*. *Plant Cell* 14(9): 2001-2013.

Paredez AR, Somerville CR, Ehrhardt DW. 2006. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* 312(5779): 1491-1495.

Pastuglia M, Bouchez D. 2007. Molecular encounters at microtubule ends in the plant cell cortex. *Current Opinion in Plant Biology* 10(6): 557-563.

Plaisier CL, Horvath S, Huertas-Vazquez A, Cruz-Bautista I, Herrera MF, Tusie-Luna T, Aguilar-Salinas C, Pajukanta P. 2009. A systems genetics approach implicates *USF1*, *FADS3*, and other causal candidate genes for familial combined hyperlipidemia. *PLoS Genetics* 5(9).

Ralph J, Lundquist K, Brunow G, Lu F, Kim H, Schatz PF, Marita JM, Hatfield RD, Ralph SA, Christensen JH, Boerjan W. 2004. Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenyl- propanoids. *Phytochemistry Reviews* 3(1-2): 29-60.

Richard TL. 2010. Challenges in scaling up biofuels infrastructure. *Science* 329(5993): 793-796.

Robinson AR, Mansfield SD. 2011. Metabolomics in Poplar. In C. P. Joshi, S. Difazio and K. Chittaranjan (Eds). Genetics, Genomics and Breeding of Poplar CRC Press, Science Publishers, Inc., New Hampshire; Edenbridge Ltd., British Isles. Pp. 166-192.

Romanoski CE, Lee S, Kim MJ, Ingram-Drake L, Plaisier CL, Yordanova R, Tilford C, Guan B, He A, Gargalovic PS, Kirchgessner TG, Berliner JA, Lusis AJ. 2010. Systems genetics analysis of gene-by-environment interactions in human cells. *American Journal of Human Genetics* 86(3): 399-410.

Rudolph U. 1987. Occurrence of rosettes in the ER membrane of young *Funaria hygrometrica* protonemata. *Naturwissenschaften* 74(9): 439.

Saxena IM, Brown Jr RM. 2005. Cellulose biosynthesis: Current views and evolving concepts. *Annals of Botany* 96(1): 9-21.

Scheller HV, Ulvskov P 2010. Hemicelluloses. In. *Annual Review of Plant Biology*. 263-289.

Schrader J, Nilsson J, Mellerowicz E, Berglund A, Nilsson P, Hertzberg M, Sandberg G. 2004. A high-resolution transcript profile across the wood-forming meristem of poplar identifies potential regulators of cambial stem cell identity. *Plant Cell* 16(9): 2278-2292.

Sedbrook JC, Ehrhardt DW, Fisher SE, Scheible WR, Somerville CR. 2004. The *Arabidopsis SKU6/SPIRAL1* gene encodes a plus end-localized microtubule-interacting protein involved in directional cell expansion. *Plant Cell* 16(6): 1506-1520.

Sederoff R, Myburg A, Kirst M 2009. Genomics, domestication, and evolution of forest trees.In. 303-317.

Séguin A. 2011. How could forest trees play an important role as feedstock for bioenergy production? *Current Opinion in Environmental Sustainability* 3(1-2): 90-94.

Seifert GJ, Roberts K 2007. The biology of arabinogalactan proteins.In. *Annual Review of Plant Biology*. 137-161.

Sharma N, Bryant J, Wloga D, Donaldson R, Davis RC, Jerka-Dziadosz M, Gaertig J. 2007. Katanin regulates dynamics of microtubules and biogenesis of motile cilia. *Journal of Cell Biology* 178(6): 1065-1079.

Somerville C. 2006. Cellulose synthesis in higher plants. *Annu Rev Cell Dev Biol* 22: 53-78.

Somerville C, Youngs H, Taylor C, Davis SC, Long SP. 2010. Feedstocks for lignocellulosic biofuels. *Science* 329(5993): 790-792.

Stoppin-Mellet V, Gaillard J, Vantard M. 2006. Katanin's severing activity favors bundling of cortical microtubules in plants. *Plant Journal* 46(6): 1009-1017.

Strauss SH, Tan H, Boerjan W, Sedjo R. 2009. Strangled at birth? Forest biotech and the Convention on Biological Diversity. *Nature Biotechnology* 27(6): 519-527.

Studer MH, De Martini JD, Davis MF, Sykes RW, Davison B, Keller M, Tuskan GA, Wyman CE. 2011. Lignin content in natural *Populus* variants affects sugar release. *Proceedings of the National Academy of Sciences* 108: 6300-6305.

Szymanski DB. 2009. Plant cells taking shape: new insights into cytoplasmic control. *Current Opinion in Plant Biology* 12(6): 735-744.

Taylor NG. 2008. Cellulose biosynthesis and deposition in higher plants. *New Phytologist* 178(2): 239-252.

Thumma BR, Matheson BA, Zhang D, Meeske C, Meder R, Downes GM, Southerton SG. 2009. Identification of a cis-acting regulatory polymorphism in a eucalypt COBRA-like gene affecting cellulose content. *Genetics* 183(3): 1153-1164.

Thumma BR, Nolan MF, Evans R, Moran GF. 2005a. Polymorphisms in cinnamoyl CoA reductase (CCR) are associated with variation in microfibril angle in Eucalyptus spp. *Genetics* 171(3): 1257-1265.

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam M, Ralph S, Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhalerao RR, Bhalerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V, Campbell M, Carlson J, Chalot M, Chapman J, Chen GL, Cooper D, Coutinho PM, Couturier J, Covert S, Cronk Q, Cunningham R, Davis J, Degroeve S, Dŕjardin A, DePamphilis C, Detter J, Dirks B, Dubchak I, Duplessis S, Ehlting J, Ellis B, Gendler K, Goodstein D, Gribskov M, Grimwood J, Groover A, Gunter L, Hamberger B, Heinze B, Helariutta Y, Henrissat B, Holligan D, Holt R, Huang W, Islam-Faridi N, Jones S, Jones-Rhoades M, Jorgensen R, Joshi C, Kangasjärvi J, Karlsson J, Kelleher C, Kirkpatrick R, Kirst M, Kohler A, Kalluri U, Larimer F, Leebens-Mack J, Leplé JC, Locascio P, Lou Y, Lucas S, Martin F, Montanini B, Napoli C, Nelson DR, Nelson C, Nieminen K, Nilsson O, Pereda V, Peter G, Philippe R, Pilate G, Poliakov A, Razumovskaya J, Richardson P, Rinaldi C, Ritland K, Rouzé P, Ryaboy D, Schmutz J, Schrader J, Segerman B, Shin H, Siddiqui A, Sterky F, Terry A, Tsai CJ, Uberbacher E, Unneberg P, Vahala J, Wall K, Wessler S, Yang G, Yin T, Douglas C, Marra M, Sandberg G, Van De Peer Y, Rokhsar D. 2006. The genome of black cottonwood, *Populus* trichocarpa (Torr. & Gray). *Science* 313(5793): 1596-1604.

Vanholme R, Morreel K, Ralph J, Boerjan W. 2008. Lignin engineering. *Current Opinion in Plant Biology* 11(3): 278-285.

Wegrzyn JL, Eckert AJ, Choi M, Lee JM, Stanton BJ, Sykes R, Davis MF, Tsai CJ, Neale DB. 2010. Association genetics of traits controlling lignin and cellulose biosynthesis in black cottonwood (Populus trichocarpa, Salicaceae) secondary xylem. *New Phytologist* 188(2): 515-532.

Wightman R, Turner SR. 2008. The roles of the cytoskeleton during cellulose deposition at the secondary cell wall. *Plant Journal* 54(5): 794-805.

Wu AM, Hörnblad E, Voxeur A, Gerber L, Rihouey C, Lerouge P, Marchant A. 2010. Analysis of the *Arabidopsis IRX9/IRX9-L* and *IRX14/IRX14-L* pairs of glycosyltransferase genes reveals critical contributions to biosynthesis of the hemicellulose glucuronoxylan. *Plant Physiology* 153(2): 542-554.

Wu AM, Rihouey C, Seveno M, Hörnblad E, Singh SK, Matsunaga T, Ishii T, Lerouge P, Marchant A. 2009. The *Arabidopsis* IRX10 and IRX10-LIKE glycosyltransferases are critical for glucuronoxylan biosynthesis during secondary cell wall formation. *Plant Journal* 57(4): 718-731.

Xie L, Yang C, Wang X. 2011. Brassinosteroids can regulate cellulose biosynthesis by controlling the expression of CESA genes in Arabidopsis. *Journal of Experimental Botany* 62(13): 4495-4506.

Yamaguchi M, Goué N, Igarashi H, Ohtani M, Nakano Y, Mortimer JC, Nishikubo N, Kubo M, Katayama Y, Kakegawa K, Dupree P, Demura T. 2010. VASCULAR-RELATED NAC-DOMAIN6 and VASCULAR-RELATED NAC-DOMAIN7 effectively induce transdifferentiation into xylem vessel elements under control of an induction system. *Plant Physiology* 153(3): 906-914.

York WS, O'Neill MA. 2008. Biochemical control of xylan biosynthesis - which end is up? *Current Opinion in Plant Biology* 11(3): 258-265.

Zhang Y, Brown G, Whetten R, Loopstra CA, Neale D, Kieliszewski MJ, Sederoff RR. 2003. An arabinogalactan protein associated with secondary cell wall formation in differentiating xylem of loblolly pine. *Plant Molecular Biology* 52(1): 91-102.

Zhong R, Burk DH, Morrison Iii WH, Ye ZH. 2002. A kinesin-like protein is essential for oriented deposition of cellulose microfibrils and cell wall strength. *Plant Cell* 14(12): 3101-3117.

Zhong R, Demura T, Ye ZH. 2006. SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of *Arabidopsis*. *Plant Cell* 18(11): 3158-3170.

Zhong R, Lee C, Ye ZH. 2010. Functional characterization of poplar wood-associated NAC domain transcription factors. *Plant Physiology* 152(2): 1044-1055.

Zhong R, Lee C, Zhou J, McCarthy RL, Ye ZH. 2008. A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in *Arabidopsis*. *Plant Cell* 20(10): 2763-2782.

Zhong R, Richardson EA, Ye ZH. 2007. Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of *Arabidopsis*. *Planta* 225(6): 1603-1611.
Zhong R, Ye ZH. 2010. The poplar PtrWNDs are transcriptional activators of secondary cell wall biosynthesis. *Plant Signaling and Behavior* 5(4): 469-472.
Zhong R, McCarthy RL, Lee C, Ye Z-H. 2011. Dissection of the Transcriptional Program Regulating Secondary Wall Biosynthesis during Wood Formation in Poplar. *Plant Physiology* doi:10.1104/pp.111.181354

Figure legends

Figure 1. Examples of the diversity of currently produced, high-value derivatives of wood-derived cellulose. The structure of the repeating unit of cellulose - cellobiose - is shown in the middle, with a "head-to-tail" arrangement of two glucose molecules bound via beta 1—4 linkage. The side-chain substitution of the hydroxyl groups from C_2, C_3 and/or C_6 (highlighted in red) result in the production of a variety of unique physicochemical derivatives, all of which comprise diverse industrial and commercial products (top). Pure crystalline cellulose can also be broken up into microcrystalline cellulose (bottom) by chemical disruption of the non-crystalline regions, or alternatively the entire polymer can be separated into nanocellulose crystals.

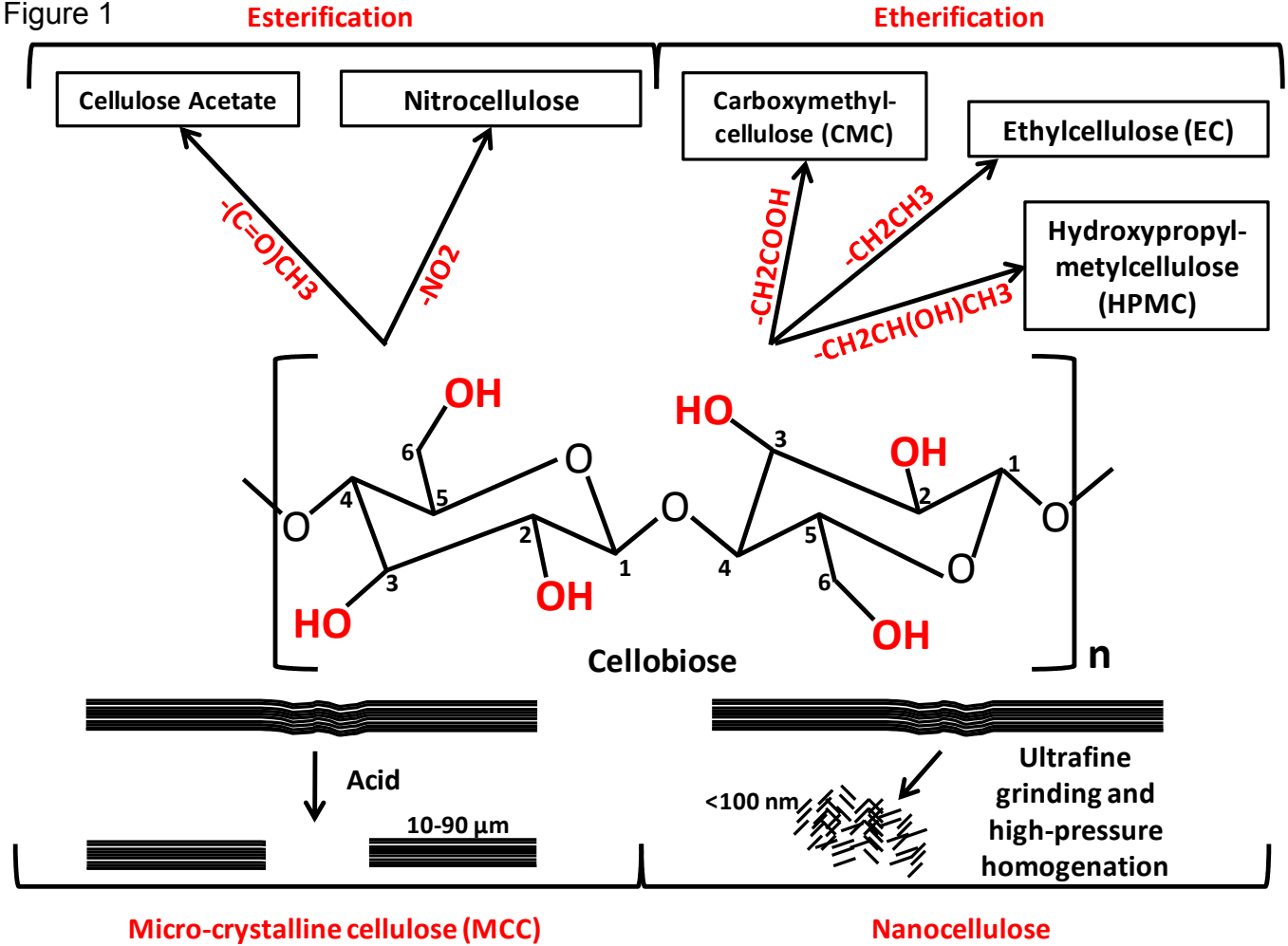
Figure 2. An integrated view of currently known proteins and some cellular processes involved in cellulose and xylan biosynthesis. Proteins are indicated as coloured circles in the cell areas they are associated with, and classes of proteins are coloured as indicated by the legend on the bottom left. Note that proximity of proteins in the figure does not imply interaction. Actin (blue beads) and microtubules (red and orange tubes) are also shown. References for the inclusion of specific proteins and full protein names can be found in the text.

Figure 3. Metabolic pathways and processes leading to cellulose and xylan biosynthesis, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG,

http://www.genome.jp/kegg/), as well as recent literature revealing putative biosynthetic enzymes involved in xylan biosynthesis (Brown *et al.*, 2007; York & O'Neill, 2008; Brown *et al.*, 2009; Oikawa *et al.*, 2010). Metabolites are represented as circles, and enzymatic processes or known enzymes of interest as boxes. BGL - beta-glucosidase; CESA - cellulose synthase; SPS - sucrose phosphate synthase; SPP - sucrose phosphate phosphatase.

Figure 4. A systems genetics approach to understanding the molecular basis of complex phenotypic traits in forest trees. Left: Systems genetics allows the molecular dissection of polygenic traits by relating phenotypic and genetic variation in experimental populations to measurable component traits (in developing cells, tissues and organs of trees) segregating in the same populations. Right: Conceptual network resulting from integrating the covariation of complex and component traits, revealing novel correlations among genes, expression modules, metabolites and complex wood phenotypes that would not be observed at the level of the individual.

Figure 1


Esterification
Etherification
Cellulose Acetate
Nitrocellulose
-(C=O)CH3
-NO2
Carboxymethyl-cellulose (CMC)
Ethylcellulose (EC)
Hydroxypropyl-metylcellulose (HPMC)
-CH2COOH
-CH2CH3
-CH2CH(OH)CH3
OH
HO
O
Cellobiose
n
Acid
10-90 µm
<100 nm
Ultrafine grinding and high-pressure homogenation
Micro-crystalline cellulose (MCC)
Nanocellulose

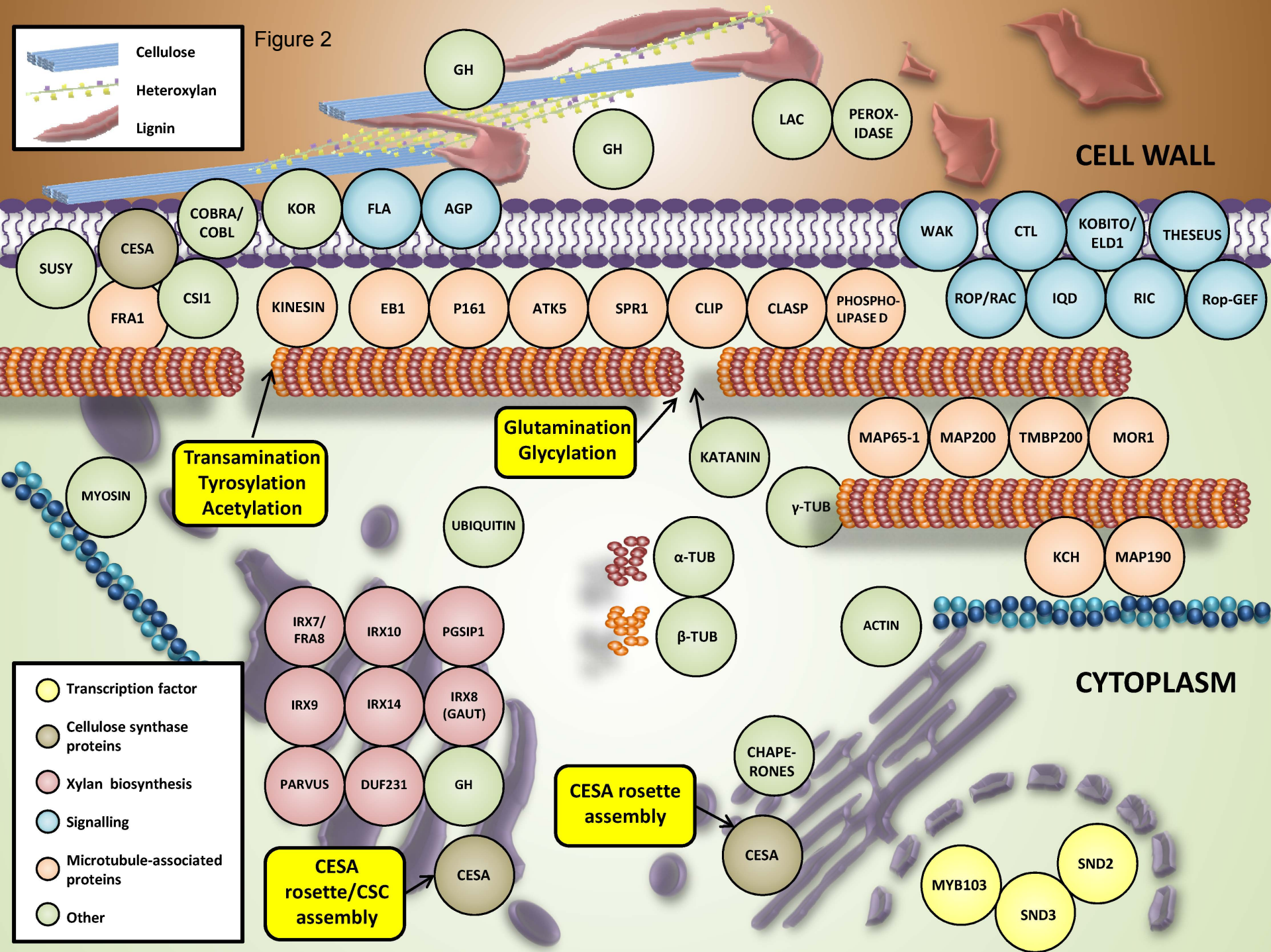


Figure 2
Cellulose
Heteroxylan
Lignin
GH
GH
GH
LAC
PEROX-IDASE
CELL WALL
COBRA/ COBL
KOR
FLA
AGP
SUSY
CESA
CSI1
FRA1
KINESIN
EB1
P161
ATK5
SPR1
CLIP
CLASP
PHOSPHO-LIPASE D
WAK
CTL
KOBITO/ ELD1
THESEUS
ROP/RAC
IQD
RIC
Rop-GEF
MYOSIN
Transamination Tyrosylation Acetylation
Glutamination Glycylation
KATANIN
MAP65-1
MAP200
TMBP200
MOR1
γ-TUB
UBIQUITIN
α-TUB
β-TUB
KCH
MAP190
ACTIN
CYTOPLASM
IRX7/ FRA8
IRX10
PGSIP1
IRX9
IRX14
IRX8 (GAUT)
PARVUS
DUF231
GH
CHAPE-RONES
CESA rosette assembly
CESA
CESA rosette/CSC assembly
CESA
MYB103
SND2
SND3
Transcription factor
Cellulose synthase proteins
Xylan biosynthesis
Signalling
Microtubule-associated proteins
Other

Figure 3

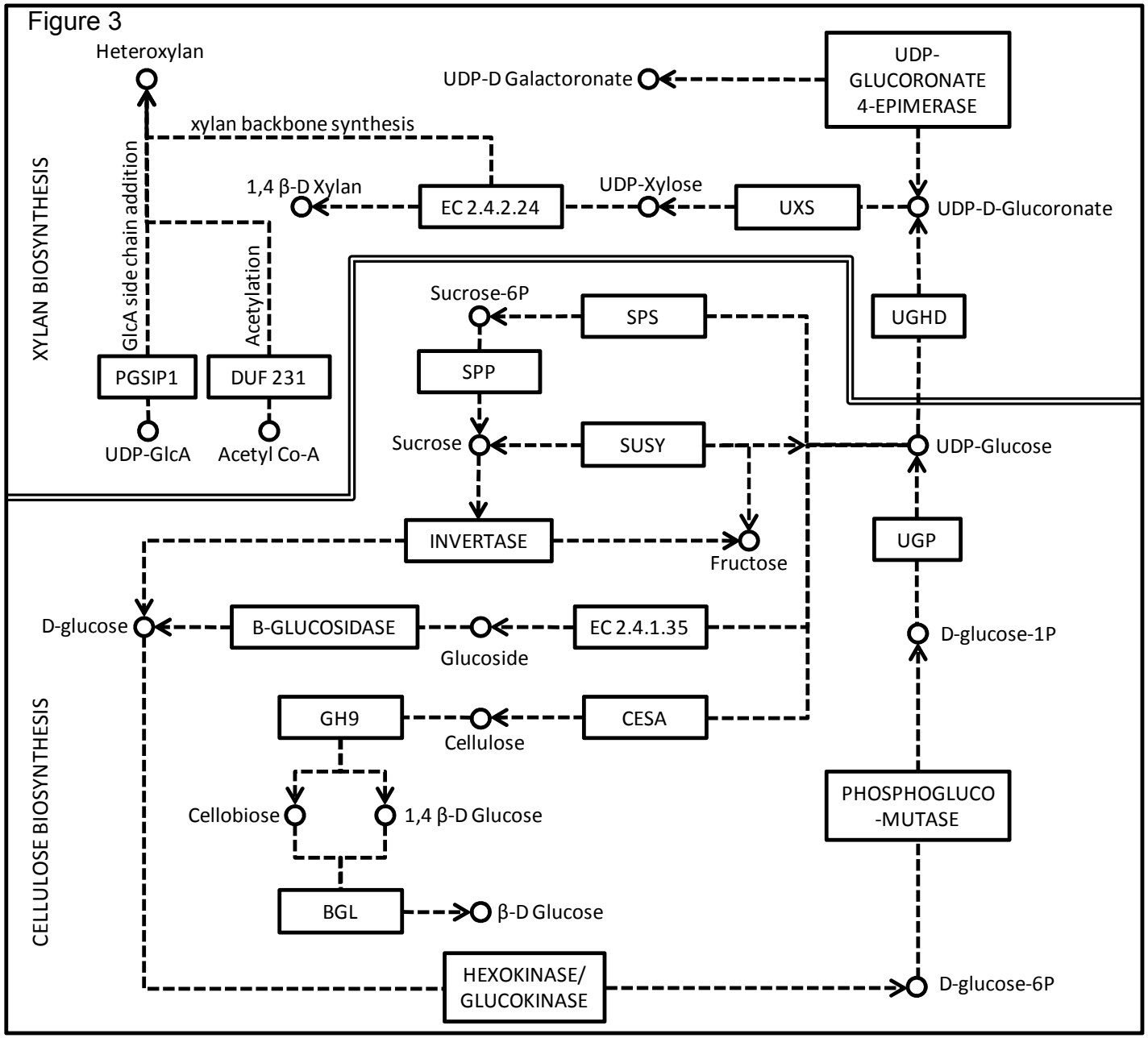
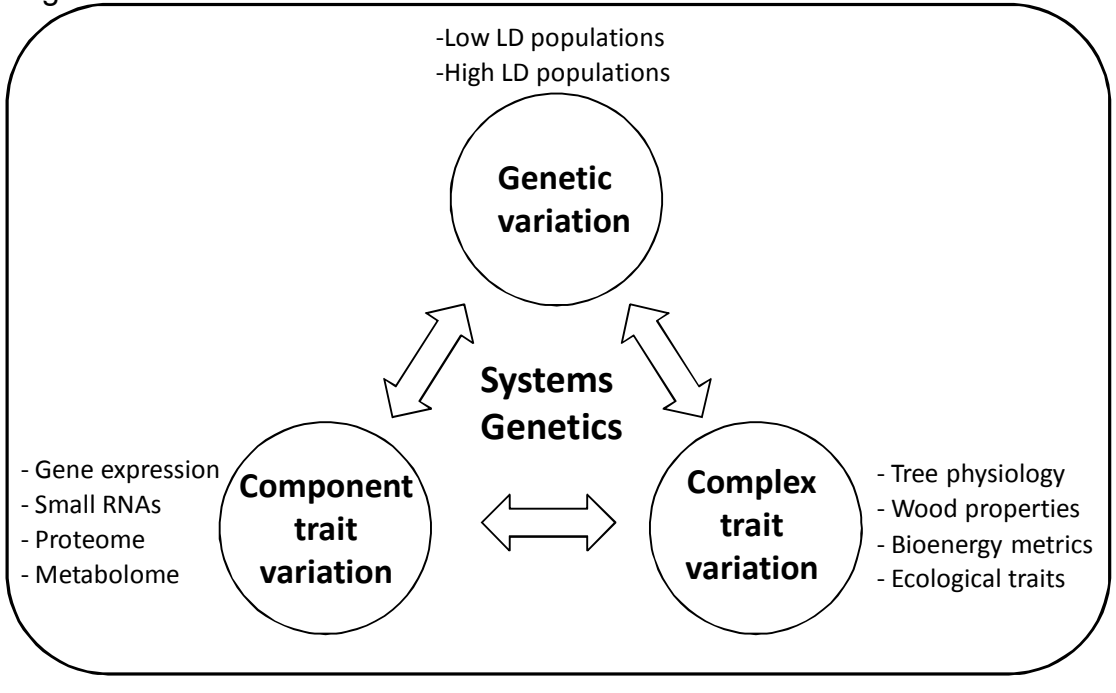
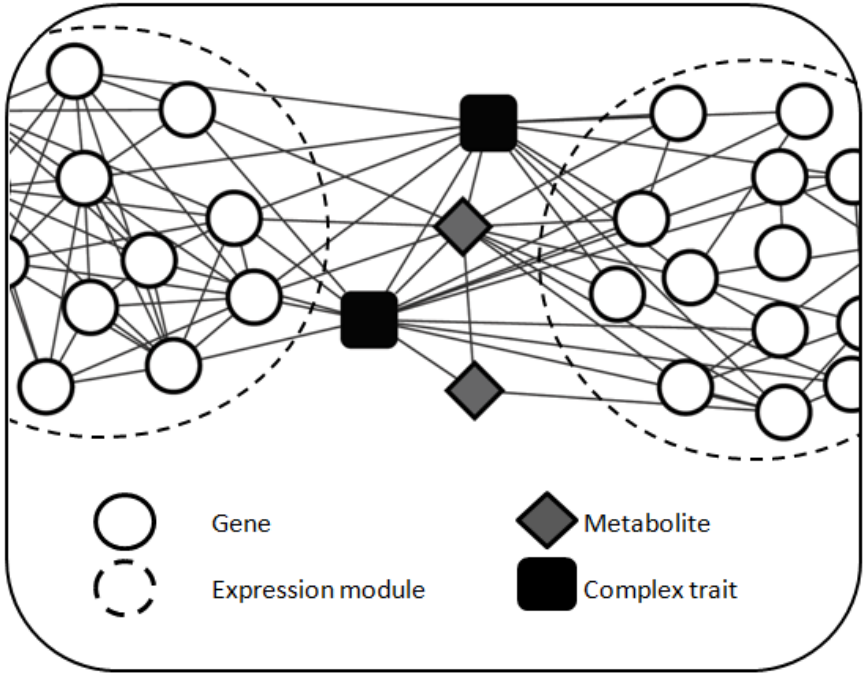

Heteroxylan
UDP-D Galactoronate
UDP-GLUCORONATE 4-EPIMERASE
xylan backbone synthesis
XYLAN BIOSYNTHESIS
GlcA side chain addition
Acetylation
1,4 β-D Xylan
EC 2.4.2.24
UDP-Xylose
UXS
UDP-D-Glucoronate
Sucrose-6P
SPS
UGHD
SPP
PGSIP1
DUF 231
UDP-GlcA
Acetyl Co-A
Sucrose
SUSY
UDP-Glucose
INVERTASE
Fructose
UGP
D-glucose
B-GLUCOSIDASE
EC 2.4.1.35
Glucoside
D-glucose-1P
GH9
CESA
Cellulose
CELLULOSE BIOSYNTHESIS
PHOSPHOGLUCO -MUTASE
Cellobiose
1,4 β-D Glucose
BGL
β-D Glucose
HEXOKINASE/ GLUCOKINASE
D-glucose-6P

Figure 4


-Low LD populations
-High LD populations
Genetic variation
Systems Genetics
Component trait variation
Complex trait variation
- Gene expression
- Small RNAs
- Proteome
- Metabolome
- Tree physiology
- Wood properties
- Bioenergy metrics
- Ecological traits


Gene
Metabolite
Expression module
Complex trait