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Wood of trees: Cellular structure, molecular formation, and genetic engineering[oo]

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ABSTRACT

Wood is an invaluable asset to human society due to its renewable nature, making it suitable for both sustainable energy production and material manufacturing. Additionally, wood derived from forest trees plays a crucial role in sequestering a significant portion of the carbon dioxide fixed during photosynthesis by terrestrial plants. Nevertheless, with the expansion of the global population and ongoing industrialization, forest coverage has been substantially decreased, resulting in significant challenges for wood production and supply. Wood production practices have changed away from natural forests toward plantation forests. Thus, understanding the underlying genetic mechanisms of wood formation is the foundation for developing high-quality, fast-growing plantation trees. Breeding ideal forest trees for wood production using genetic technologies has attracted the interest of many. Tremendous studies have been carried out in recent years on the molecular, genetic, and cell-biological mechanisms of wood formation, and considerable progress and findings have been achieved. These studies and findings indicate enormous possibilities and prospects for tree improvement. This review will outline and assess the cellular and molecular mechanisms of wood formation, as well as studies on genetically improving forest trees, and address future development prospects.

Keywords: cambium, cell wall, *Populus*, wood formation, xylem

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INTRODUCTION

Wood is an invaluable asset to human society and has been used to make construction materials and furniture, as well as paper, packaging materials, and many others. Wood is now also regarded as a feasible bioenergy source for substituting fossil fuels as well as an ideal raw material for replacing the use of plastic and chemical products. In 2020, global wood product production was 473 million cubic meters of sawn wood, 368 million cubic meters of wood-based panels, and 401 million tons of paper and paperboard (https://www.forestresearch.gov.uk/tools-and-resources/statistics/). Furthermore, forests fix approximate 82% of terrestrial biomass, or around 450 billion tons of carbon in wood (Bar-On et al., 2018), making them an important CO_2 sink for mitigating global warming. The high capacity of wood carbon storage as well as net-zero carbon emissions make wood utilization an effective strategy for reducing the global increase in CO_2 concentrations (Griscom et al., 2017; Lewis et al., 2019). However, as the world's population grows and the industrialization process continues,

the forest area keeps shrinking. The global forest area decreased by around 4.7 million hectares (0.1%) per year between 2010 and 2020 (https://www.fao.org/forestry/en/). Therefore, growing wood on limited forest land by breeding trees with high productivity appears to be a potential solution, while success is dependent on our understanding of how to make trees grow fast, and with desired wood qualities. Molecular biotechnology has shown rising potency in crop improvement since its inception in the 1980s. Similarly, knowledge concerning forest tree growth and wood qualities has advanced from traditional structural descriptions to mechanistic understanding at the cellular and molecular levels, with significant advances in the elucidation of molecular mechanisms of wood formation. By applying advanced knowledge and cutting-edge technologies, a great deal of exploration and study has been undertaken in the creation of fast-growing and high-quality forest trees. As a result, the findings indicate a high potential and promising application for improving trees. A timely assessment to analyze and comprehend these new discoveries and outcomes would provide new perspectives for future development, which would aid in advancing forest tree improvement and making renewable wood resources more effective in utilization.

UNDERSTANDING OF WOOD FORMATION

Wood cell organization and properties

Wood is formed as an accumulation of secondary xylem tissue, which consists of a variety of cell types, including tracheary elements (TEs, referred to as tracheids in gymnosperms and vessel elements in angiosperms), fibers, and xylem parenchyma cells, all of which are arranged in both axial and radial patterns (Figure 1) (Butterfield, 2003). TEs are characterized by their robust secondary cell walls (SCWs) and arranged in an axial manner, forming a lengthy pathway for the transportation of water and soluble substances. The tracheids seen in gymnosperms constitute up to 90% of the wood volume due to the absence of fibers. The vessel elements that exist in angiosperm wood typically possess bigger diameters but exhibit restricted axial expansion. Fibres serve as the main strucural cells in angiosperms, characterized by their limited radial expansion and the presence of thick and lignified SCWs. The xylem paraenchyma cells play a role in storing water, non-structural carbohydrates, and lipids, as well as facilitating cell and tissue communication through the presence of abundant plasmodesmata. Ray cells are a type of xylem paraenchyma cell that have a radial arrangment found in both angiosperms and gymnosperms.

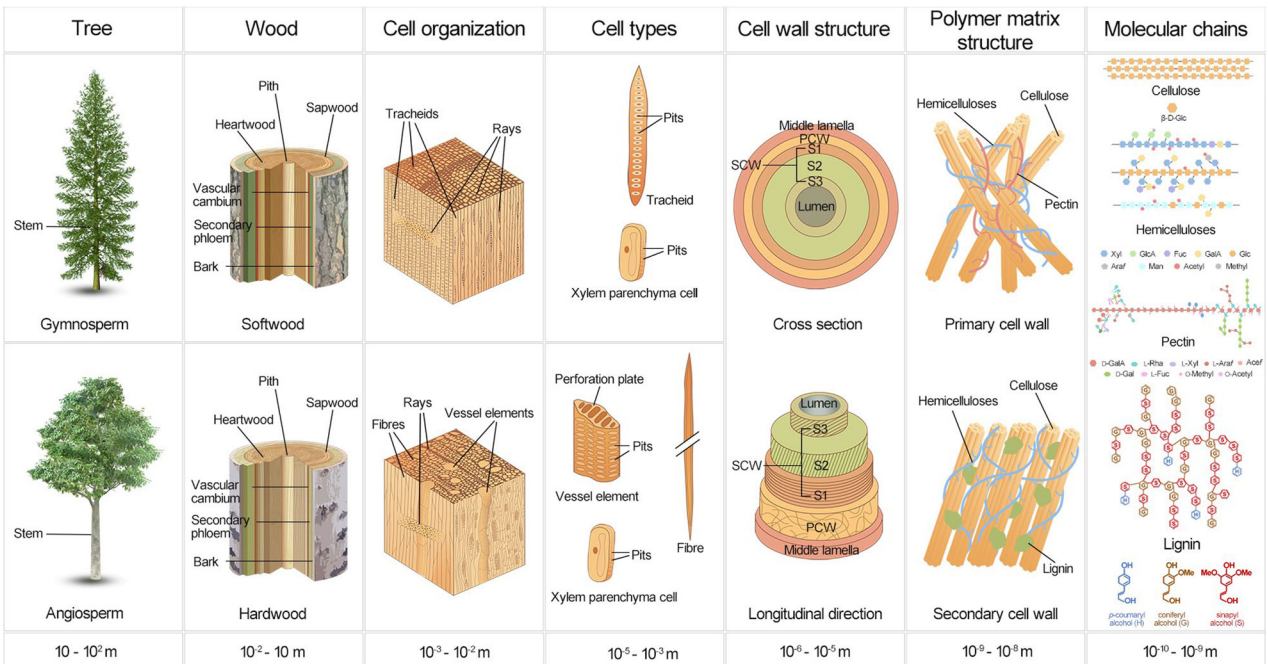

Figure 1. Schematic illustration of wood structure at hierarchical scales

Wood primarily consists of secondary xylem tissue derived from the stems of gymnosperm (softwood) and angiosperm (hardwood) trees. The stem exhibits a hierarchical structure, with the outermost part known as the bark, followed by the secondary phloem, vascular cambium, secondary xylem (comprising sapwood and heartwood), and then the central pith. Softwood mostly consists of tracheids and ray parenchyma cells, whereas hardwood is characterized by the presence of vessel elements, fiber cells, and ray parenchyma cells. Wood has a laminated cell wall structure and is made up of various biopolymers, namely cellulose, hemicelluloses, pectin, and lignin, which are stored within the cell walls. Ace, aceric acid; Ara, arabinose; Gal, galactose; GalA, galacturonic acid; Glc, glucose; GlcA, glucuronic acid; Fuc, fucose; Man, mannose; Rha, rhamnose; Xyl, xylose.

These cells facilitate communication between the xylem and phloem tissues. Moreover, ray cells exhibit the capacity to sustain their metabolic activities over long periods of time, which contributes to the dispersal of phenolic compounds in neighboring cells, resulting in the pigmentation of the heartwood (Blokhina et al., 2019). Axial parenchyma cells are often found in angiosperms and are closely arranged around vessel elements, and their proportions; however, they exhibit considerable variation across various species, ranging from 1% to 30% (Słupianek et al., 2021). The proportion of axial parenchyma cells exhibits a positive correlation with the size of vessel elements, as indicated by an extensive analysis encompassing 2,332 species of woody angiosperms (Morris et al., 2018). Other cell types can also be observed in certain tree species. For instance, in certain conifers, there are epithelial cells that are enclosed by resin canals. These cells are responsible for the production of resin, which serves as a defense mechanism against biotic threats (Baas et al., 2004).

The origin of all cell types within wood may be attributed to the vascular cambium features. The arrangement and frequency of cell division within the vascular cambium dictate the organization of cells within the xylem tissue (Fischer et al., 2019; Luo and Li, 2022). The vascular cambium is usually defined by a limited number of layers consisting of narrow and thin-walled cells (Bossinger and Spokevicius, 2018; Shi et al., 2019). The vascular cambium cell generates the axial cell system in wood through periclinal divisions occurring in the tangential plane. Additionally, the cambium undergoes anticlinal division to ensure a balanced growth in its circumference. The ultimate size of wood cells is dictated by the flexibility of the primary cell walls (PCWs) and the development of SCWs. PCWs are thin and relativly extensible, surrounding all vasuclar cells to accommodate their growth and are constructed mainly using cellulose, hemicellulose and pectin. SCWs, including S1, S2, and S3 layers, are deposited on TEs and fibers, while the thickness, chemical makeup, and cellulose microfibril angle of the S1, S2, and S3 layers vary. Wood SCW is mainly made up of cellulose, hemicelluloses, and lignin (Figure 1). Differences in the makeup, organization, pattern, size and shape of cell types, as well as the structures of SCWs and wood extractives, contribute to the considerable variation that exists in wood characteristics among different tree species. The biomacromolecules that make up wood cell walls are often regarded as a carbon-neutral resource. In order to achieve the most effective wood processing efficiency in the production of pulp and paper or fermentable sugar-based biorefineries, it is desirable to utilize wood with high cellulose and hemicellulose contents while minimizing the presence of lignin or facilitating its easy removal.

Genomic understanding of tree wood formation

For tree species, genome sequencing endeavors to shed light on tree evolution and to find genes related to wood formation: These are blooming. By the end of February 2022, the draft genomes of 357 tree species, comprising 266 arboreal, 71 shrub, and 20 vine species, have been assembled, accounting for approximately 36% of all sequenced plants (Borthakur et al., 2022), and these numbers are continuously increasing. The genomes of trees may be employed to elucidate the genetic basis underlying specific wood properties, such as wood cell types, cell size, cell wall thickness, and SCW composition. *Amborella trichopoda* is the ancestral angiosperm, with a xylan-rich SCW. According to studies of the *Amborella* genome, genes relevant to such specialized wood properties first emerged as a result of the ancient angiosperm genome duplication (Project et al., 2013). *Tetracentron sinense* is one of four early diverging eudicot lineages and is distinguished by the primary form of vessel elements, which is indicated by an atypical SCW that resembles tracheids. The genome of *Tetracentron sinense* contains two copies of *VND* genes, which are master transcription factors for vessel element differentiation and formation. Multiple *VND* copies are the result of whole-genome duplication events (Liu et al., 2020; Li et al., 2021b). *Eucalyptus grandis*, a tree species with fast growth and superior wood properties, has a comparatively small genome of 640 Mb and 34% of its genes occur in tandem duplication. Tandem duplication has resulted in the expansion of 10 families with 174 genes encoding phenylpropanoid biosynthesis (Myburg et al., 2014). The genome sequences of *Melia azedarach*, *Azadirachta indica* and *Toona sinensis* (also called Chinese mahogany) from the Meliaceae family have been compared. The results show the genes related to cellulose and hemicellulose biosynthesis, as well as their corresponding regulatory transcription factors, are significantly expanded in *Toona sinensis* (Cui et al., 2023). *Ochroma pyramidale*, also known as balsa, is a fast-growing tree the wood of which is soft and light, while *Mesua ferrea*, often called ironwood, generates wood with a high density and hardness. Comparison analysis of their genomes showed that the different regulatory constituents related to SCW biosynthesis exist (Sahu et al., 2023). Current surveys of angiosperm tree genomes have shown that expanded genes involved in wood formation via the whole genome or tandem duplications and subsequent increasing complexity of regulatory networks may contribute to varied wood properties.

Gymnosperms are significant tree components in forest ecosystems and produce wood with a different cellular structure from angiosperm wood. About 13 families and slightly more than 1,000 species of gymnosperms exist today (Wang and Ran, 2014). Conifers (*Phylum Pinophyta*) encompass 615 species, accounting for 39% of the world's forests and 45% of total wood production (Filer and Farjon, 2013). Since the publication of the first gymnosperm genome of Norway spruce in 2013 (Nystedt et al., 2013), a handful of gymnosperm genomes have been obtained (Liu et al., 2021b; Niu et al., 2022; Sun et al., 2022). The biggest challenge is assembling the huge gymnosperm genome, for example, 9.87 Gb of *Ginkgo biloba* (Liu et al., 2021b), 10.97 Gb of *Larix kaempferi* (Sun et al., 2022), and even more, 25.4 Gb of Chinese pine (*Pinus tabuliformis*) (Niu et al., 2022). The big genome of gymnosperms is mainly due to long introns and an abundance of transposable elements rather than whole-genome duplication as of angiosperms. Although no clear conclusions have been drawn thus far from the genome

about the molecular mechanisms by which gymnosperms have unique wood structures and properties, it would be expected that the sequenced genomes of high quality should be able to shed more insights into gymnosperm wood formation.

Transcript profiling at the single-cell level is being used to track the genes expressed in the highly specialized vascular cambium or differentiating xylem cells and to achieve a comprehensive understanding of the molecular process of wood formation and precise spatial regulation of interest genes for genetic manipulation of wood properties (Li et al., 2021a; Xie et al., 2022; Du et al., 2023; Liao and Wang, 2023). Meanwhile, when paired with other emerging technologies, single-cell transcriptomic profiling allows us to better understand the wood formation process. In *Populus* stems, hallmarks of transition from primary to secondary vascular tissues were characterized using a spatial transcriptome approach combined with high-resolution anatomic analysis and single-cell sequencing. The most remarkable observation is the presence of two types of cambium-like cell pools within secondary vascular tissues, which provides a new perspective on vascular cambium initiation during wood development (Du et al., 2023; Li et al., 2023). The developmental lineages of ray and fusiform cells in developing xylem were determined by single-cell and laser capture microdissection transcriptome profiling on four divergent woody angiosperms representing core and base eudicots (Tung et al., 2023). Furthermore, small conditional RNA sequencing data can be used to forecast gene redundancy during the wood-forming process (Chen et al., 2021). All of these studies are effective ways for identifying essential genes and regulatory networks that are involved in wood formation and modulating the chemical and mechanical properties of wood at the cellular or species level. The determination of how these genes or networks are implicated in the genetic regulation of wood qualities in tree species necessitates further genetic studies.

Vascular cambium establishment and its activity

Vascular cambium cells in tree stems originate beneath the shoot apical meristems, begin with procambium, and then fascicular cambium in primary vascular bundles, and are cylindrically joined by interfascicular cambium, allowing woody plants to generate a “tree ring” (Larson, 1994; Zhu et al., 2018). The development of cylindrical vascular cambium occurs quickly within the internode beneath shoot apical meristems, allowing the tree to further organize a functioning xylem network for overall plant growth. Combining single-cell RNA sequencing with spatial transcriptome analysis uncovered discrete dynamic molecular maps of cell identity and differentiation in the stem vascular cambium and shoot apical meristem (Li et al., 2023). *PopREVOLUTA*, an *HD-ZIP III* gene in *Populus*, regulates vascular cambium initiation in *Populus* stems (Robischon et al., 2011). *PtrHB4* is another member of the *Populus HD-ZIP III* gene family which controls interfascicular cambium establishment, as evidenced by the loss of *PtrHB4* activity in *Populus*, which resulted in distinct vascular bundles similar to those found in primary growth stems (Zhu et al., 2018). Multiple regulators, including hormones, peptides, and transcription factors, function together to control the formation, maintenance and proliferation of the vascular cambium through a complex of regulatory networks (Figure 2).

Auxin has long been known to play a crucial role in promoting cambial activity in woody plants. Exogenous auxin application to the apical bud increases cambial cell division and secondary xylem differentiation (Gouwentak, 1941). The maximum concentration of auxin is found in the cambial zone of gymnosperm and angiosperm species, which has an important correlation with cambium activity and gradually decreases toward differentiation of xylem and phloem cells (Tuominen et al., 1997; Uggla et al., 1998). Auxin transporters are involved in the establishment of auxin concentration gradients in the cambial zone (Schrader et al., 2003; Zheng et al., 2021). *PIN5* in *Populus*, the expression of which is controlled by two *MADS-box* genes, *VCM1* and *VCM2*, modulates the content of soluble auxin in cambium cells (Zheng et al., 2021). Other *PINs* in *Populus*, such as *PttPIN1* and *PttPIN12*, are expressed in the cambial zone (Schrader et al., 2003). Within the auxin signaling system, the *INDOLEACETIC ACID* (*IAA*) and *AUXIN RESPONSE FACTOR* (*ARF*) genes have a role in controlling cambium activity. Overexpression of a mutant version of *PttIAA3* alters auxin responsiveness and cambial cell division activity in *Populus* (Nilsson et al., 2008). The PaC3H17-PaMYB199 module is involved in regulating cambium division, which is enhanced by auxin in *Populus* (Tang et al., 2020).

Cytokinin concentration is high on the phloem side next to the cambium and gradually declines through the cambial zone and adjacent xylem (Immanen et al., 2016). The expression peak for the genes encoding cytokinin receptors, including *HK3a*, *HK3b*, *CRE1a* and *CRE1b* in *Populus* and *CRE1* (*CYTOKININ RECEPTOR 1*) in birch, is located in the dividing cambial cells (Nieminen et al., 2008). Transgenic *Populus* expressing *cytokinin oxidase/dehydrogenase 2* (*CKX2*), encoding an enzyme to degrade cytokinin, driven by the phloem-specific *CLE41b* promoter shows disruption of the cytokinin gradient pattern and restriction of the cambial activity (Fu et al., 2021), and expressing *CKX2* under the promoter of a birch *CRE1* gene in *Populus* impairs secondary growth (Nieminen et al., 2008). In another study, expression of the cytokinin biosynthesis genes *ROCK4* and *ROCK3* under the control of the cambium-specific *HB8* promoter in *Populus* led to increased radial stem growth (Riefler et al., 2022). These studies indicate that the cytokinin level in the cambium area is critical to cambium activity. However, the RNA interference-based knockdown of the *histidine kinase* (*HK*) genes encoding cytokinin receptors specifically in secondary phloem significantly compromised the division activity of cambial cells (Fu et al., 2021). These results suggest the possibility that cytokinin may act in a non-cell-autonomous manner to regulate cambial activity.

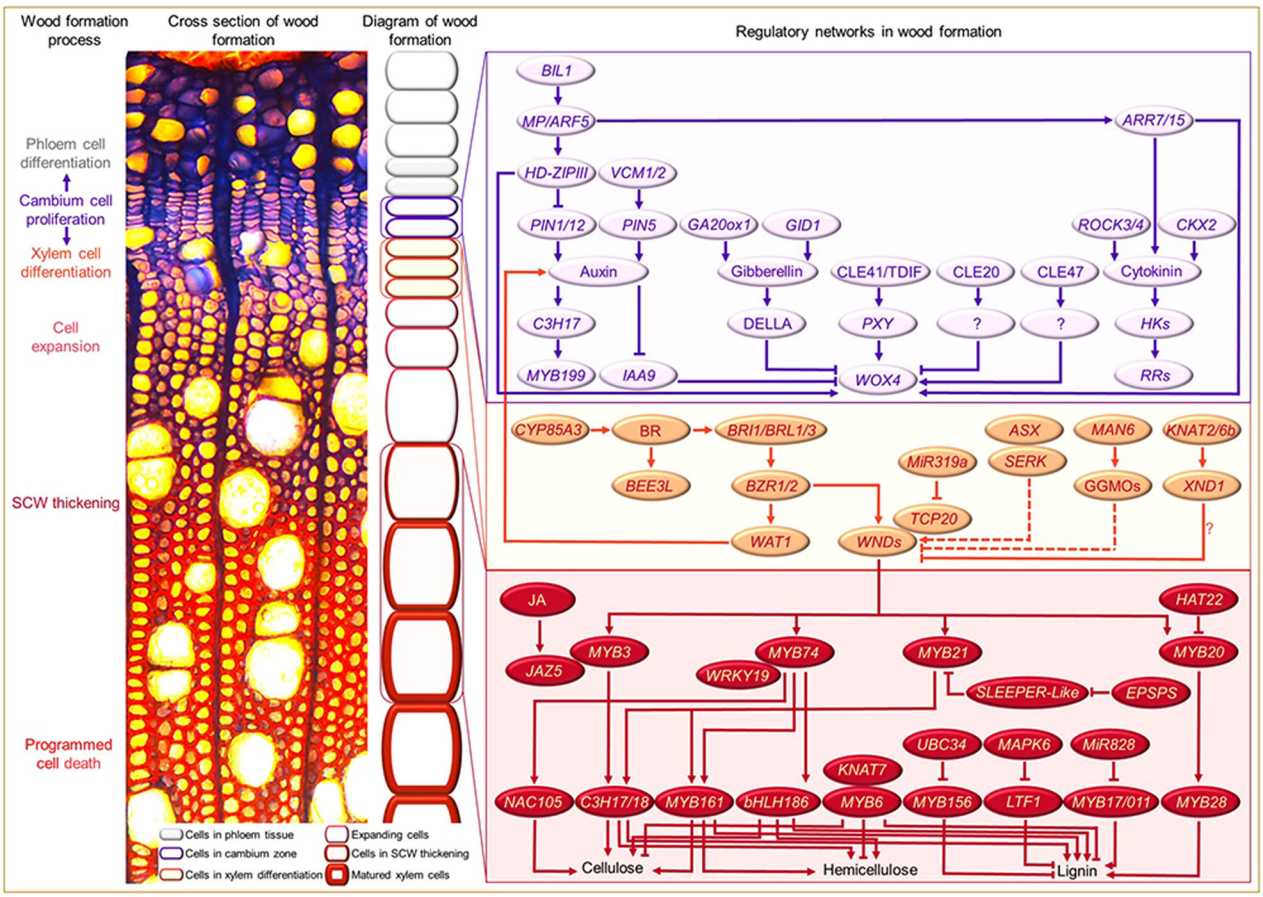

Figure 2. Regulatory networks in the process of wood formation in trees

Left: A cross-section shows the *Populus* stem secondary growth tissue stained with safranin and astra blue. Middle: A schematic representation illustrates the sequential stages involved in wood formation. Right: A schematic illustration of the regulatory networks and signaling pathways identified so far in various stages of wood formation.

WUSCHEL-RELATED HOMEOBOX 4 (WOX4) is regarded as a key regulator of cambial activity. In *Populus*, *PttWOX4* genes are specifically expressed in the cambial region during vegetative growth. In *PttWOX4a/b RNAi* trees, primary growth was not affected, whereas the width of the vascular cambium was severely reduced and secondary growth was greatly diminished (Kucukoglu et al., 2017). Small peptides, auxin, and gibberellin (GA) signaling have been reported to link to the regulation of *WOX4* expression and cambium cell division in trees. The aspen receptor kinase PttPXY and its peptide ligand PttCLE41 make up a signal module that activates the *WOX4*-directed pathway to control the rate of cambial cell division and the organization of woody tissue (Etchells et al., 2015). On the other hand, the peptide PtrCLE20 negatively regulates cambial activity in *Populus* (Zhu et al., 2020). The gene encoding PtrCLE20 is expressed in developing xylem cells, while its coded peptide, PtrCLE20, acts in the cambium to inhibit its proliferation activity. CLE41 and CLE20 may form a pair of positive-negative signals via the *WOX4*-directed pathway to regulate cambium activity (Zhu et al., 2020). A vascular cambium-specific expressed peptide PttCLE47 was also identified as being involved in cambium cell proliferation (Kucukoglu et al., 2020). The ubiquitin receptor PagDA1 can affect the WOX4 stability in *Populus*. PagDA1 interacts with PagWOX4 and causes it to be degraded via the 26S proteasome pathway, suggesting that PagDA1 is involved in affecting cambium activity (Tang et al., 2022).

Gibberellin is involved in cambium cell proliferation. The highest levels of bioactive GA concentration have been found in developing xylem (Israelsson et al., 2005). *PdGA20ox1* from *Pinus densiflora*, which encodes GA20 oxidase, a crucial enzyme that catalyzes the generation of bioactive GA forms from GA12, enhances xylem width and cell number in transgenic *Populus* (Jeon et al., 2016). Overexpression of *Arabidopsis AtGA20ox1* showed earlier flowering and taller stems in aspen (Eriksson et al., 2000). In a similar way, overexpression of the GA receptor gene *GID1* in *Populus* makes the cambial cells accumulate and enhances the secondary growth (Mauriat and Moritz, 2009). According to these findings, GA is implicated in cambium activity and differentiation. In addition, the interaction between auxin and GA has been evidenced in accompaniment in cambium activity. DELLA proteins from

the GA signaling pathway and Aux/IAA proteins from the auxin signaling pathway have been shown to interact with PtARF7 in *Populus*, controlling *PtWOX4* expression (Hu et al., 2022a).

Differentiation of secondary xylem

Following cambium division, newly proliferated cells on the interior differentiate into secondary xylem, resulting in increased stem diameter in trees (Figure 2). Secondary xylem differentiation is a time-ordered process that is governed by an array of molecular processes. Single-cell sequencing and pseudotime trajectory analysis of cell development reveal that different developmental phases have varied gene regulation (Li et al., 2023). Uncovering the molecular pathways that drive secondary xylem differentiation have been conducted and a number of regulatory networks have been elaborated for their function in the process (Figure 2).

PtrHB5 and *PtrHB7/8* are the orthologs of *Arabidopsis POPCORONA* and *AtHB8* in *Populus*, respectively. Both genes correspondingly regulate xylem differentiation during secondary growth in *Populus*, and overexpression of *PtrHB7* causes more cells to differentiate into xylem (Du et al., 2011; Zhu et al., 2013). The *Populus* PtoIAA9-PtoARF5 module has been shown to mediate auxin-triggered cell differentiation of early developing xylem in the process of wood formation (Xu et al., 2019). PtoARF5.1 can bind to the promoters of *PtoHB7* and *PtoHB8* and stimulate their expression, resulting in enhanced cell differentiation toward xylem. With auxin treatment, PtoIAA9 protein is degraded and releases PtoARF5 from the module, inducing PtoARF5-activated gene expression, in turn, activated PtoIAA9 switches off auxin signaling in a self-controlled manner during wood formation (Xu et al., 2019).

Brassinosteroids (BR) have a critical role in the regulation of wood formation by regulating cell differentiation and SCW biosynthesis (Du et al., 2020). The knockouts of the vascular-enriched BR receptors *BRI1*, *BRL1*, and *BRL3* showed discontinuous cambial ring and patterning defects in derived secondary vascular tissues (Wang et al., 2022a). *BEE3* (*brassinosteroid-enhanced expression 3*) is an important participant in BR signaling (Friedrichsen et al., 2002). Overexpression of a *BEE3-like* gene in *Populus*, *PagBEE3L*, resulted in increased secondary xylem formation (Noh et al., 2015). AtCYP85A2 is a bifunctional cytochrome P450 monooxygenase that catalyzes the conversion of castasterone to brassinolide, which is the final rate-limiting step in the BR-biosynthetic pathway in *Arabidopsis* (Shimada et al., 2001; Shimada et al., 2003; Kim et al., 2006). *PtCYP85A3* is one of three *Populus AtCYP85A2* homologous genes. *PtCYP85A3* overexpression increased xylem formation without changing cellulose and lignin composition or cell wall thickness in transgenic *Populus* (Jin et al., 2017). A number of receptor-like kinases have been found in *Populus* that are specifically expressed in developing xylem cells, but the majority of which have functions uncharacterized yet (Song et al., 2010). A recent study has revealed that one receptor-like kinase, ASX (attenuation of secondary xylem), regulates xylem differentiation but does not affect cambium division, although its ligand molecules remain unknown (Xie et al., 2023). In cell suspension culture, galactoglucomannan oligosaccharides (GGMOs) do not affect cell division but increase the differentiation of divided cells into tracheary components (Benová-Kákosová et al., 2006). This implies that GGMOs could function as signal molecules in the regulation of xylem cell development. The mannanase gene *PtrMAN6* is identified and characterized in *Populus* for the generation of GGMOs, which play an important role in the regulation of SCW thickening during xylem development (Zhao et al., 2013).

NAC transcription factors are essential regulators controlling fiber and vessel element differentiation during xylem development. Key regulators of fiber differentiation have been identified as *NAC SECONDARY WALL THICKENING PROMOTING FACTOR1* (*NST1*) and *SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN1* (*SND1*) in *Arabidopsis* (Zhong et al., 2006; Mitsuda et al., 2007; Zhong et al., 2007). Four *NST1/SND1 Populus* orthologs have been identified and shown to coordinately influence SCW production in wood and phloem fibers (Takata et al., 2019). *PtrWND1B*, an *SND1 Populus* homolog, is found to be expressed specifically in secondary xylem fiber cells, and *PtrWND1B* suppression specifically decreased fiber cell wall thickening (Zhao et al., 2014). Furthermore, *PtrWND1B* undergoes alternative splicing, which functions as a mechanism to govern the steady thickening of fiber cell walls (Zhao et al., 2014). In *Populus*, the downstream hierarchical transcription factors and protein-protein interactions of PtrSND1-B1 are proposed to have 76 direct targets and 55 interactions (Lin et al., 2013; Chen et al., 2019; Wang et al., 2020a). In the context of PtrSND1-B1 regulation, PtrMYB074 was detected to form a dimeric complex with PtrWRKY19, resulting in the sequential activation of *PtrbHLH186*, subsequently leading to the development of fiber cells characterized by shorter length and thicker SCWs in great numbers as well (Liu et al., 2022). VASCULAR-RELATED NAC-DOMAIN6 (VND6) and VND7, on the other hand, govern xylem vessel differentiation (Kubo et al., 2005; Yamaguchi et al., 2010). *PdeNAC2*, a *Pinus densiflora VND* homolog, can trigger the development of vascular element-like cells in *Arabidopsis* and *Populus* and shares 17 direct target genes with *AtVND6* (Kim et al., 2023). TCP20 interacts with WND6 in *Populus* to promote xylem differentiation during wood formation, and *TCP20* is a direct target of miR319a, the expression level of which gradually decreased from primary to secondary growth (Hou et al., 2020). Another NAC domain protein XYLEM NAC DOMAIN1 (XND1) functions as a negative regulator of xylem vessel differentiation via inhibiting expression of *VND7* during TE differentiation in *Arabidopsis* (Zhao et al., 2017). In *Populus*, *PagXND1* is directly regulated by *KNAT2/6b*, *KNOTTED-like homeobox* genes, which negatively regulate xylem differentiation (Zhao et al., 2020).

Cell expansion occurs during the xylem development process, which controls the cell size of wood cells. Different

tree species often produce wood with varied cell sizes. EXPs (expansins) are cell wall-loosening proteins that allow the PCW to be extended. The expansin gene family is divided into four subfamilies: *EXPA*, *EXPB*, *EXLA*, and *EXLB*. *Populus PttEXPA1* is abundant in wood-forming tissues. *PttEXPA1* overexpression enhanced fiber diameter and vessel element length (Gray-Mitsumune et al., 2008). Thirty-six *EXP*s have been identified in the *Populus* genome, and their functions during the formation of wood are yet unknown (Sampedro et al., 2006; Yin et al., 2023). PMEs (pectin methylesterases) alter the cell wall and the pectin-rich middle lamella to facilitate cell growth. PMEs are distributed bilaterally on each side of the cambium during the active stage of cambial daughter cell expansion (Micheli et al., 2000). PttPME1 was prevalent in wood-forming tissue and removed the methylester group from homogalacturonan (HG), altering HG methylesterification patterns and, as a result, regulating xylem cell expansion in *Populus* (Siedlecka et al., 2008; Allario et al., 2018). XETs (xyloglucan endo-transglycosylases) can change the xyloglucan-cellulose architecture of plant cell walls, consequently regulating their expansion (Stratilová et al., 2020). In developing wood, 16 *Populus XET* genes are expressed, and *PtxtXET16-34* is uniquely expressed in developing wood. *PtxtXET16-34* overexpression increased vessel element growth but not fiber expansion in *Populus* (Nishikubo et al., 2011). The xylem cell size may be regulated through potassium transport. ENLARGED VESSEL ELEMENT (EVE) is found to contribute to vessel element enlargement in *Populus*, which is engaged in potassium uptake (Ribeiro et al., 2020).

Ethylene signaling may be involved in regulating the expansion of cells and SCW deposition in xylem development. ACC (1-aminocyclopropane-1-carboxylic acid) synthase (ACS) catalyzes ethylene precursor ACC synthesis. In *Populus*, the *ACS* gene is expressed during xylem development (Sundell et al., 2017), and *ERF118*, an *Ethylene Response Factor* (*ERF*), has also been shown to be involved in xylem development based on its expression pattern and co-expression networks (Seyfferth et al., 2018). Ethylene enhanced the expression of *Populus ERF139* during xylem differentiation to regulate the radial size of vascular elements in relation to SCW formation (Wessels et al., 2019). Furthermore, *Populus ERF85* overexpression revealed a role in regulating xylem fiber width and SCW deposition (Seyfferth et al., 2021).

SCW biosynthesis in the process of wood formation

SCW biosynthesis is an essential process in the formation of wood. A number of excellent reviews have covered cell wall biosynthesis in plants in general (Meents et al., 2018; Zhong et al., 2019; Zhang et al., 2021). Here, we focus on wood SCW formation, which is a primary process for storing photosynthetic products in trees as well as a determinant of wood properties.

Cellulose is the most abundant unique biopolymer in nature, with widespread applications in bioenergy and high value bioproducts. Cellulose accounts for 40%–60% of the content of the SCW, which forms microfibrils bundled with beta-(1,4)-linked glucan chains (Figure 1). The arrangement of cellulose microfibrils is a partly crystalline, amporphous, well-oriented structure. Most wood cellulose microfibrils are found to contain multiple glucan chains and have a crystalline-ordered core of 2.2–35 nm diameter (Tai et al., 2023). The proportion of crystallites in cellulose microfibrils determines cell wall accessibility during industrial processing. Hemicelluloses are polysaccharides with beta-(1,4)-linked backbones with diverse substitutions, including xyloglucans, xylans, mannans, and glucomannans in wood (Figure 1). The amount and types of hemicellulose vary widely in different species and cell types. Hemicelluloses may contribute to strengthening the cell wall through binding with cellulose and lignin (Scheller and Ulvskov, 2010). In softwood, both glucomannan and xylan link to the cellulose microfibril surfaces, increasing the elastic modulus in compression (Salmén, 2022). In hardwood, xylan interacts with cellulose microfibrils, increasing the elongation at break under tension (Berglund et al., 2020). Lignin is a polymer synthesized mainly from the amino acid phenylalanine. Each cell type and cell wall layer have distinct lignin types to assist their specific functions, and different interactions with lignin influence cell wall stiffness and flexibility. In softwood, lignin binds to all xylan and mannan, and lignin-cellulose contacts are abundant, but lignin remains hydrated. In hardwood, lignin binds all xylans and cellulose microfibers (Kirui et al., 2022). The biosynthesis of the SCW is controlled by the specifically coordinated expression of numerous genes, and a general picture of the biosynthesis of cellulose, hemicellulose, and lignin has been drawn (Scheller and Ulvskov, 2010; Hao and Mohnen, 2014; McNamara et al., 2015; Kieber and Polko, 2019; Vanholme et al., 2019; Zhu and Li, 2021; Pedersen et al., 2023).

Cellulose synthase (CesA) is the enzyme in charge of cellulose synthesis. Multiple CesAs collaborate to form a cellulose synthase complex (CSC) on the plasma membrane, making multiple glucan chains at the same time that are bundled into microfibrils. The *Arabidopsis* genome contains 10 *CesA* genes, and the *Populus* genome contains nearly twice as many, with 18 (Suzuki et al., 2006). Two types of CSCs with different CesA components exist for PCW (CesA1, CesA3, and CesA6) and SCW (CesA4, CesA7, and CesA8), respectively, in *Arabidopsis* (Desprez et al., 2007; Persson et al., 2007). Both types of CSCs are engaged in cellulose synthesis for SCWs in *Populus* by analysis of protein abundance during cell wall thickening of secondary xylem (Song et al., 2010). The composition of CesAs in CSCs varies during wood formation among tree species. The SCW CesA stoichiometry in *Populus* developing xylem was 3:2:1 for CesA8, CesA4, and CesA7, whereas in Norway spruce it was 1:1:1, similar to that in *Arabidopsis* (Kieber and Polko, 2019). The stoichiometry of CesAs in CSCs could also alter as the wood forms. The cellulose-enriched gelatinous (G) layer is generated during tension wood formation with a CesA

stoichiometry of 8:3:1 in *Populus* (Zhang et al., 2018). In addition, two types of CSCs in *Populus* have distinct effects on SCW structure and microfibril crystallinity, as: Indicated by interference with the expression of the *CesA* genes using RNA interference (RNAi) (Xi et al., 2017). More specifically, *PtrCesA4*, *PtrCesA7*, and *PtrCesA8* knockdown in transgenic *Populus* resulted in collapsed vessels, thinner fiber cell walls, and expanded fiber lumen widths, as well as a decrease in cellulose content (Abbas et al., 2020). Genome editing knocking out *PtrCesA4*, *PtrCesA7A/B*, or *PtrCesA8A/B* resulted in the disappearance of the G layer, one-layer-walled fiber, and a 90% decrease in cellulose (Xu et al., 2021). Several CesA-interacting proteins have also been characterized during wood formation in trees. *KOR* (*KORIGAN*) is a membrane-bound endoglucanase that has been shown to correlate with cellulose deposition in the secondary xylem of *Populus* and spruce (Maloney and Mansfield, 2010; Maloney et al., 2012). RNAi *KORs* in *Populus* led to reduced SCW thickness and cellulose content, as well as crystalline cellulose (Maloney and Mansfield, 2010; Yu et al., 2014).

The most predominant hemicellulose are xylans and mannans in the SCWs. The backbone of xylans is β-1,4-linked xylose, which is often substituted with glucuronic acid in the eudicot and varying amounts of arabinose in conifer (Figure 1). Mannans have a β-1,4-linked mannose and glucose backbone and substitute with galactose, which are the most abundant hemicellulose of the SCW in conifer. Synthesis of hemicellulose requires varied enzymes and mainly depends on glycosyltransferases for backbone and side chains and methyltransferases, acetyltransferases for modification. The core xylan biosynthesis complex containing IRX9/IRX9L, IRX14/IRX14L, belonging to the glycosyltransferase (GT) 43 family, and IRX10/IRX10L of GT47 family. In *Populus*, five (*GT43A-E*) of seven *GT43* genes (*GT43A-G*) were highly expressed in the developing wood (Lee et al., 2011). *GT43A/B/E* genes correspond to the *Arabidopsis IRX9* clade and GT43C/D genes to *AtIRX14* clade, which showed higher specificity for SCW (Ratke et al., 2015). Downregulation of *GT43* via RNAi in *Populus* reduced xylose content, supporting its role in xylan backbone biosynthesis (Lee et al., 2011; Ratke et al., 2018). The reducing-end sequence at the xylan backbone is biosynthesized by *GT8* and *GT47* family genes, such as *GAUT12/IRX8*, *GATL1/PARVUS* and *FRA8/IRX7* in *Arabidopsis* (Smith et al., 2017). In *Populus*, *GAUT12-1/2* (also called *GT8D1/2*), *GATL1.1* and *GATL1.2* and *GT47C* are also responsible for biosynthesis of the reducing-end sequence of xylan (Lee et al., 2009; Li et al., 2011; Biswal et al., 2015; Biswal et al., 2018b; Derba-Maceluch et al., 2023). *SPR1*, an *atypical aspartic proteases and nucellins* gene, was also reported to involve in regulating xylan reducing-end biosynthesis, as indicated by its strong expression in developing xylem and the reduction of xylose content in its suppression in *Populus* (Derba-Maceluch et al., 2023). Hemicellulose is acetylated during biosynthesis in the Golgi using acetyl-coenzyme A (CoA) as a donor substrate, which is transported from cytoplasmic pools to the Golgi by REDUCED WALL ACETYLATION (RWA) proteins. Four RWA proteins have been found in *Arabidopsis* (RWA1-4) as well as in *Populus* (RWA-A-D) (Pawar et al., 2017b). Downregulation of *RWAs* in *Populus* reduced wood xylan and xyloglucan acetylation (Pawar et al., 2017b; Derba-Maceluch et al., 2020). In *Populus*, a protein called Domain of Unknown Function 231 (DUF231) is also involved in the acetylation of xlyan (Yang et al., 2017). Furthermore, *PtrDUF579-3* is expressed specifically in *Populus* xylem, and localized in the Golgi apparatus to act in glucuronoxylan methylation during the formation of wood (Song et al., 2016).

Lignin is the main component of wood cell walls and is polymerized by monolignols, which include *p*-coumaryl (H), coniferyl (G), and sinapyl (S) alcohols (Figure 1) synthesized in the cytosol or near the endoplasmic reticulum of monolignol-producing cells via the phenylpropanoid and monolignol biosynthesis pathways, which have been extensively reviewed (Boerjan et al., 2003; Weng and Chapple, 2010; Vanholme et al., 2019). Since the first cloning of *OMT* (*lignin-bispecific O-methyltransferase*) in monolignol synthesis in trees in 1991, all discovered genes for monolignol biosynthesis in woody trees have been functionally verified (Bugos et al., 1991; Li et al., 2006; Chanoca et al., 2019; De Meester et al., 2022b). Although there may be differences between herbaceous plants and woody trees, the metabolic processes for monolignol synthesis have been largely described (Halpin, 2019). It is proposed that at least 21 genes encode enzymes that catalyze 37 reactions on 24 intermediate metabolites, resulting in lignin monomer diversity in different tissues of *Populus* (Wang et al., 2018). Other lignin monomers, in addition to the conventional three, are found in some plant species, and these monomers may have substantial implications for cell wall function. A BAHD family acyltransferase found in *Populus* catalyzes monolignol *p*-hydroxybenzoylation, hence altering the *p*-hydroxybenzoylated lignin structures (Zhao et al., 2021). The structural relevance of lignin *p*-hydroxybenzoylation on lignin physiochemical properties is shown by a significant change in lignin solvent dissolving rate. The monolignols are transported across the plasma membrane by unresolved process and subsequently polymerized in the apoplast by peroxidases or laccases (LAC). A mitochondrial ascorbate peroxidase (PtomtAPX) was identified to catalyze lignin polymerization coupling to the programmed cell death (PCD) process during the early stages of SCW formation in *Populus* (Zhang et al., 2022). Class III peroxidases (PRXs) are plant-specific enzymes involved in lignin formation (Hoffmann et al., 2020). Ninety-three *PRXs* were identified in *Populus* genome (Ren et al., 2014), while their function in lignin biosynthesis is yet to be characterized during wood formation (Christensen et al., 1998). Several LACs in *Populus* have been functionally characterized, indicating their importance in cell wall structure and integrity (Ranocha et al., 2002; Bryan et al., 2016). *PtoLAC14* has shown to be essential for lignin polymerization by promoting G unit deposition (Qin et al., 2020). *LAC19/25/32* loss of function mutants generated via clustered regularly interspaced palindromic repeats (CRISPR) editing in *Populus* led to decreased lignin staining and vessel collapse (Guo et al., 2023).

Monolignol biosynthesis is regulated at transcriptional, post-transcriptional as well as post-translational levels (Figure 2). MYB and WND transcription factors usually directly bind to promoters of the genes of the monolignol biosynthesis pathway to regulate their expression as activators or repressors. Overexpression of *MYB55* or *MYB92* resulted in increased SCW thickness and deposition of lignin in *Populus* (Jiao et al., 2019; Sun et al., 2020). *MYB10* and *MYB120* in *Populus* both act as positive regulators to promote monolignol biosynthesis by reprogramming of phenylpropanoid metabolism from anthocyanin biosynthesis (Kim et al., 2021; Jiang et al., 2022). *PdWND3A*, a homolog of *Arabidopsis VND4* and *VND5*, promotes expression of *F5H* (*Cald5H*) (Yang et al., 2019), which encodes a key enzyme controlling the biosynthesis of sinapyl alcohol for S-monolignol in trees (Osakabe et al., 1999). In addition, JAZ5 plays a negative role by interacting with MYB3 and WND6, which are known as activators for lignin biosynthesis (Zhao et al., 2023). PagUNE12 (UNFERTILIZED EMBRYOSAC12) is recently reported as an activator in lignin biosynthesis in *Populus* (Song et al., 2023). MYB1 in both *Pinus* and *Eucalyptus* repressed lignin biosynthesis, suggesting their conserved functions during wood formation (Bomal et al., 2008; Soler et al., 2017). PtrEPSPS, a 5-enolpyruvylshikimate 3-phosphate synthase, is a key enzyme in the shikimate pathway. An isoform of EPSPS has been identified as a transcriptional repressor by binding to the promoter of a *SLEEPER*-like transcriptional regulator, which itself specifically binds to the promoter and represses the expression of *PtrMYB021* (known as *MYB46* in *Arabidopsis*), which regulates lignin biosynthesis (Xie et al., 2018). LTF1 (lignin biosynthesis-associated transcription factor) represses the expression of monolignol biosynthesis genes in *Populus* (Gui et al., 2019). Loss of function *LTF1* mutant showed increased lignin content and overexpression of *LTF1* decreased the overall lignin content and led to dwarfism. Mitogen-activated protein kinase 6 interacted with LTF1 and phosphorylated LTF1 to decrease its stability by degradation via 26S proteasome (Gui et al., 2019).

Several of the transcription factors involved in regulating lignin biosynthesis and the genes encoding enzymes in the monolignol biosynthesis pathway are regulated by microRNAs. MYB171 and MYB011, which activate *PAL1* and *CCR2* expressions, are miR828 targets in *Populus* (Wang et al., 2022b) (Figure 2). MiR6443 directly targets *F5H2* transcripts to regulate S lignin biosynthesis (Fan et al., 2020). MiR397 and miR408 target laccase genes (Lu et al., 2013; Li et al., 2019). Overexpression of miR408 delays lignification, and modestly reduces lignin content, S/G ratio and degree of lignin polymerization in *Populus* (Guo et al., 2023). Post-translational modifications such as phosphorylation and ubiquitination have been reported in several proteins of lignin biosynthesis in trees. Phenylalanine ammonia-lyase (PAL) is an enzyme that is responsible for the first reaction step in the phenylpropanoid pathway. PAL2 in *Populus* has been proposed to be phosphorylated by the calmodulin-like domain protein kinase (CDPK) family to regulate its turnover (Allwood et al., 1999). PtrAldOMT2, a 5-hydroxyconiferaldehyde O-methyltransferase enzyme, converts 5-hydroxyconiferaldehyde to sinapaldehyde for syringyl monolignal biosynthesis, and its phosphorylation turns off O-methyltransferase activity and the phosphorylation sites are conserved from 46 diverse plant species (Wang et al., 2015b). PtoMYB156 and PtoMYB221 are transcriptional repressors of monolignol biosynthesis and their repression activity can be modified by E2 ubiquitin-conjugating enzyme 34 (PtoUBC34) (Zheng et al., 2019) (Figure 2).

In addition to lignin biosynthesis, the entire biosynthesis of SCWs is finely regulated at the transcriptional level (Figure 2). Overexpression of *PtBRI1.2* promoted the accumulation of PtBZR1 (BRASSINAZOLE RESISTANT1) in the nucleus, which subsequently activated *PtWND4B/6B* to up-regulate expression of SCW biosynthesis genes in *Populus* (Jiang et al., 2021). *PtrHAT22*, a *HD-Zip II* transcription factor, is predominantly expressed in secondary developing xylem tissues. Overexpression of *PtrHAT22* showed arrested growths and decreased the contents of lignin, cellulose, and thickness of SCW. PtrHAT22 represses the expression of *PtrMYB20*, *PtrMYB28*, and *PtrCOMT2* by directly binding their promoters (Ren et al., 2022). Overexpression of *MYB6* showed reduced SCW deposition, accompanied by repressed expression of the SCW biosynthetic genes. MYB6 interacted physically with KNAT7 and formed functional complexes that acted to repress SCW development in *Populus* (Wang et al., 2019). PdMYB3/21 activated the *PdC3H17/18* promoters, of which overexpression in *Populus* increased secondary xylem width and secondary wall thickening in stems, whereas dominant repression of them had the opposite effects on these traits (Chai et al., 2014).

Programmed cell death during the formation of wood

Wood cells are dead cells that retain their cell walls. The final stage of wood formation occurs when xylem cells undergo PCD, which is a cell-autonomous, active, and organized process that involves the recruitment of certain hydrolases such as proteases, nucleases, and ribonucleases (RNase) (Daneva et al., 2016). The morphology of cells and the timing of PCD progression vary depending on the types of xylem cells. Vessel elements differentiate rapidly and die after a few days of development from the vascular cambium, although fibers and tracheids remain alive for considerably longer, estimated to be about 1 month in trembling aspen (*Populus tremula*) and Norway spruce (*Picea abies*) (Bollhöner et al., 2012). Thus, xylem vessel elements quickly autolyze, but tracheids and fibers slowly autolyze (Courtois-Moreau et al., 2009). As a result, tracheids and fibers appear to share at least a few aspects of the cell death pathway. The vessel elements, on the other hand, appear to have evolved a distinct cell death program that differs from the ancient program found in tracheids (Bollhöner et al., 2012). Ray parenchyma

cells can be the only living cells in the mature secondary xylem and can live for decades before dying, depending on species (Nakaba et al., 2006). The rupture of the tonoplast, which releases hydrolytic enzymes from the vacuole and activates cytoplasmic enzymes by acidification of the cytoplasm, is an evident feature of PCD of vessel elements, rapidly initiating post-mortem clearance, dismantling the membrane system, and degrading nuclear DNA and organelles (Bollhöner et al., 2012; Escamez and Tuominen, 2014). The distinctions of PCD in fibers, based on morphological changes during xylem cell death, are that, in addition to vacuolar disintegration, nuclear DNA integrity is compromised, and cytoplasmic contents are gradually degraded (Courtois-Moreau et al., 2009). *Metacaspase9* (*MC9*) is required for vessel cell death by degrading vessel cell contents after vacuolar rupture in *Arabidopsis* (Bollhöner et al., 2013), and *AtMC9* homologs *PttMC13* and *PttMC14* were shown to be substantially expressed during xylem maturation in *Populus* (Bollhöner et al., 2018). However, the precise roles of *PttMC13* and *PttMC14* in cell death remain unknown. Caspase-3-like activity is observed in a pattern of a developmental gradient in *Populus* secondary xylem and is associated with the 20S proteasome involved in TE PCD (Han et al., 2012). In *Populus*, two *aspartic protease* (*AP*) genes, *AP17* and *AP45*, were shown to be highly expressed in secondary xylem fibers. CRISPR/CRISPR-associated protein 9 mutations of *AP17* or *AP45* delayed secondary xylem fiber PCD, but *AP17* overexpression produced premature PCD in xylem fibers, demonstrating a positive modulation of fiber PCD. Interestingly, both the *ap17ap45* mutant and the *AP17* overexpression plants significantly increased saccharification yield, suggesting that PCD of xylem cells might be a target for wood engineering (Cao et al., 2022).

WOOD GENETIC ENGINEERING

Wood is a valuable renewable biomass resource that is utilized in the production of pulp and paper, fiber materials, chemical products, and biofuels. The chemical composition of wood influences its usage in many productions, and genetic engineering technologies can improve the efficiency of wood use by modifying its composition and structure. Furthermore, the structure of the wood cell wall controls the characteristics of the wood, and biotechnology can be utilized to make forest trees that grow fast and have superior wood properties. As summarized in Table 1, genetic engineering of trees for improved wood and fast growth has made significant progress in the last 30 years or so.

Engineering wood composition by modifying genes involved in cell wall biosynthesis

Wood components mainly contain lignin, cellulose, and hemicelluloses. The content and structure of these components have a great influence on the utilization of wood. For example, the production of pulp requires the removal of lignin, which consumes a lot of energy and produces a lot of pollutants. Cellulose content and crystalline structure affect the quality of the produced fiber and have a great influence on the conversion of cellulose to sugar for fermentation to produce biofuels. In this way, it is an effective strategy to improve the efficiency of wood utilization by genetically modifying the biosynthesis of wood components so that wood can accumulate the components and structures we desire.

The lignin biosynthetic pathway has been extensively elucidated, and lignin is the earliest genetic engineering target for wood modification. *COMT* and *CAD* were the first to be applied to modify the biosynthesis of wood lignin. Inhibiting their expression via constitutive promoters such as 35S resulted in accumulation of some intermediates from the monolignol biosynthetic pathway, producing colored wood (Van Doorsselaere et al., 1995; Baucher et al., 1996; Tsai et al., 1998). Suppression of *COMT* expression resulted in changes in monolignol composition (Van Doorsselaere et al., 1995). By inhibiting the expression of *4CL* in *Populus* under greenhouse conditions, the lignin content can be reduced by more than 40%, and growth can be promoted at the same time (Hu et al., 1999; Voelker et al., 2010). Gene regulation can change not only the lignin content but also the monomer composition of lignin. While inhibiting the expression of *4CL* and overexpressing the *Cald5H* gene simultaneously, the lignin content in *Populus* decreased and the monomer composition (S/G) ratio increased (Li et al., 2003). So far, genes encoding enzymes in the monolignol biosynthesis pathway have been applied to genetically modify wood lignin (Table 1) (Chanoca et al., 2019).The results reveal that the lignin content can be reduced and the composition of monolignols can be altered. Simultaneously, altering the genes of the monolignol biosynthetic pathway may affect the metabolic flow, and engineered wood frequently accumulates unusual lignin monomers, such as intermediates of the monolignol biosynthetic pathway, resulting in the appearance of colored wood. Recently, genome editing is also applied to lignin modification in trees. Multiplex CRISPR genome editing showed a variety of editing lignin biosynthetic genes, resulting in edited *Populus* with varied lignin levels, with lignin reduced by up to 49.1% and the carbohydrate-to-lignin ratio increased by up to 228% (Sulis et al., 2023).

Cellulose is another target to modify in wood. Cellulose is considered to be synthesized by CSCs composed of CesA subunits (Song et al., 2010; Lei et al., 2012). Suppression of *CesA* gene expression affects cellulose deposition. Suppression of *PtdCesA8* in *Populus* decreased cellulose content, resulting in collapsed xylem vessels (Joshi et al., 2011). Genome editing of *CesA4* to increase its homo/heterodimerization capacity in *Populus* showed increased cell wall thickness, 33% more cellulose content and 20% cellulose degree of polymerization (DP) (Nayeri et al., 2022). Interfering with the expression of *CesA*s from two types of CSCs in *Populus* has different effects on wood cellulose. Interfering with the expression of *PtrCesA7A* reduces the crystallinity of

Table 1. A list of genetic modifications of wood in trees

Species	Gene modified	Promoter used	Method	Growth condition	Main effects of transgenic trees	Reference (ordered by year)
Populus tremula × *P. alba*	*COMT*	35S	Antisense	Greenhouse	Lignin S/G ratio decrease	Van Doorsselaere et al., 1995
P. tremula × *P. alba*	*CAD*	35S	Antisense	Greenhouse	Little lignin change; colored xylem	Baucher et al., 1996
P. tremuloides	*COMT*	35S	Antisense	Greenhouse	Lignin S/G ratio changes; colored xylem	Tsai et al., 1998
P. tremuloides	*4CL*	35S	Antisense	Greenhouse	Lignin decrease; growth increase	Hu et al., 1999
P. tremula × *P. alba*	*CAD*; *COMT*	35S	Antisense	Greenhouse and field	Lignin S/G ratio changes; colored xylem	Lapierre et al., 1999
P. tremula × *P. alba*	*CCoAOMT*	35S	Antisense	Greenhouse	Lignin decrease and S/G ratio increase; colored xylem	Meyermans et al., 2000
P. tremula × *P. alba*	*CCoAOMT*	35S	Antisense	Greenhouse	Lignin decrease; colored wood	Zhong et al., 2000
P. tremula × *P. alba*	*COMT*	35S	Co-suppression	Greenhouse	Lignin decrease; colored xylem	Jouanin et al., 2000
P. tremula × *P. alba*	*F5H*	C4H	Upregulation	Greenhouse	Lignin S/G increase	Franke et al., 2000
P. tremula × *P. tremuloides*	*GA20ox1*	35S	Upregulation	Greenhouse	Improving growth rate and biomass; longer xylem fibers	Eriksson et al., 2000
P. tremula × *P. alba*	*CAD*; *COMT*	35S	Antisense	Field	Improving pulping efficiency in *CAD* suppression	Pilate et al., 2002
P. tremuloides	*4CL* + *CAld5H* (*F5H*)	4CL	*4CL* downregulation and *CAld5H* upregulation	Greenhouse	Lignin decrease and S/G ratio increase	Li et al., 2003
P. tremula × *P. alba*	*F5H*	C4H	Upregulation	Greenhouse	Improving pulping efficiency	Huntley et al., 2003
P. tomentosa	*4CL*	35S	Antisense	Greenhouse	Lignin decrease; colored wood	Jia et al., 2004
P. tomentosa	*CCoAOMT*	35S	Antisense	Greenhouse	Lignin decrease; colored wood	Lu et al., 2004
Leucaena leucocephala	*COMT*	35S	Antisense	Greenhouse	Lignin decrease; cellulose increase	Rastogi and Dwivedi, 2006
P. tremula × *P. alba*	*CCR*	35S	Antisense	Greenhouse and field	Lignin decrease; cellulose increase; colored xylem; improving pulping efficiency; growth affected	Leplé et al., 2007
P. alba × *P. grandidentata*	*C3'H*	35S	RNAi	Greenhouse	Lignin decrease	Coleman et al., 2008
P. tremula × *P. alba*	*CCoAOMT*	35S	Antisense	Field	Lignin decrease; S/G ratio increase; improving pulp quality; pulp yield increase	Wei et al., 2008
Picea abies	*CCR*	35S	Antisense	Greenhouse	Lignin and H unit decrease; smaller stem width	Wadenbäck et al., 2008
P. alba × *P. tremula*	*GT47C*	35S	RNAi	Greenhouse	Glucuronoxylan decrease; SCW thickness reduced	Lee et al., 2009
Pinus radiata	*4CL*	35S	RNAi	Greenhouse	Lignin and G unit decrease; collapse tracheids; dwarfed	Wagner et al., 2009
P. tremula × *P. alba*	*F5H*	C4H	Upregulation	Greenhouse	S-unit increase	Stewart et al., 2009
P. alba × *P. grandidentata*	*SuSy*	35S; 4CL	Heterologous overexpression	Greenhouse	Cellulose increase	Coleman et al., 2009
P. tremula × *P. alba*	*4CL*	35S	Antisense	Field	Lignin and S/G ratio decrease; colored wood	Voelker et al., 2010
P. tremula × *P. tremuloides*	*C4H*	35S	Antisense	Greenhouse	Lignin decrease; wood density decrease	Bjurhager et al., 2010

Continued

Table 1. Continued

Species	Gene modified	Promoter used	Method	Growth condition	Main effects of transgenic trees	Reference (ordered by year)
P. tremuloides	*CESA8a*	35S	Co-suppression	Greenhouse	Cellulose decrease; lignin and hemicellulose increase	Joshi et al., 2011
P. trichocarpa	*GT8D1/2*	35S	RNAi	Greenhouse	Xylan decrease; lignin increase; brittle stem	Li et al., 2011
P. alba × *P.tremula*	*GT43B*; *GT8D*	35S	RNAi	Greenhouse	Xylan decrease; SCW thickness reduced	Lee et al., 2011
P. trichocarpa	*4CL*	35S	Downregulation	Greenhouse	Lignin decrease	Min et al., 2012
P. tomentosa	*4CL*; *CCoAOMT*	35S	Antisense	Field	Lignin decrease	Wang et al., 2012
P. alba × *P. grandidentata*	*C3'H*	35S	RNAi	Greenhouse	Lignin decrease; H unit increase	Ralph et al., 2012
P. tomentosa	*4CL*	35S	Sense and antisense	Field	Lignin decrease; enhanced growth	Tian et al., 2013
P. deltoides × *P. euramericana*	*KOR1/2*	35S	RNAi	Greenhouse	Cellulose decrease	Yu et al., 2014
P. trichocarpa	*4CL*	35S	Antisense	Field	Lignin decrease	Stout et al., 2014
P. tremula × *P. alba*	*CCR*	35S	Antisense and co-suppression	Greenhouse and field	Lignin decrease; colored xylem	Van Acker et al., 2014
P. tremula × *P. alba*	*FMT*	35S or CesA8	Heterologous overexpression	Greenhouse	Saccharification yield increase	Wilkerson et al., 2014
P. tremula × *P. tremuloides*	*SUS1/2*	35S	RNAi	Greenhouse	Wood density decrease	Gerber et al., 2014
P. davidiana × *P. bolleana*	*FLA6*	35S	Antisense	Greenhouse	Cellulose and lignin decrease;	Wang et al., 2015a
P. deltoides	*GAUT12*	35S	RNAi	Greenhouse	Xylan decrease; growth increase	Biswal et al., 2015
P. tremula × *P. alba*	*4CL1*		CRISPR-Cas9	Greenhouse	Lignin and S/G ratio decrease; colored wood	Zhou et al., 2015
Eucalyptus urophylla × *E. grandis*	*C3'H*; *C4H*	35S	Antisense	Greenhouse	Lignin decrease	Sykes et al., 2015
P. alba × *P. tremular* var. *glandulosa*	*GA20ox1*	35S	Heterologous overexpression	Greenhouse	Enhanced growth; xylose and glucose increase	Park et al., 2015
P. tremula × *P. tremuloides*	*PXY*; *CLE41*	ANT; PP2	Tissue-specific upregulation	Greenhouse	Biomass increase	Etchells et al., 2015
P. deltoides	*KOR1-5*	35S	RNAi	Greenhouse	Cellulose and lignin S/G ratio decrease; reduced growth	Kalluri et al., 2016
P. alba × *P. grandidentata*	*MOMT4*	PAL2	Upregulation	Greenhouse	Lignin decrease; cellulose increase	Cai et al., 2016
P. tremula × *P. tremuloides*	*IPT7*	LMX5	Heterologous overexpression	Greenhouse	Biomass increase	Immanen et al., 2016
P. tremula × *P. tremuloides*	*SWN1*	NST3	Heterologous overexpression	Greenhouse	Cell wall content and wood density increase	Sakamoto et al., 2016
P. tremula × *P. tremuloides*	*AXE1*	35S	Heterologous overexpression	Greenhouse	Reduced xylan acetylation; lignin S/G ratio decrease	Pawar et al., 2017a
P. tremula × *P. tremuloides*	*RWA*	35S or GT43B	RNAi	Greenhouse	Reduced hemicellulose acetylation	Pawar et al., 2017b
P. deltoides	*DUF231*	Ubq3	Upregulation	Greenhouse	Cellulose increase; acetylated xylan increase	Yang et al., 2017
Pinus taeda	*4CL*	Xylem-specific	RNAi	Greenhouse	Lignin decrease	Edmunds et al., 2017
P. nigra L. × *P. maximowiczii*	*4CL*	35S	Antisense	Field	Lignin decrease	Xiang et al., 2017
P. tremula × *P. alba*	*CAD1*	35S	RNAi	Greenhouse	Lignin decrease	Van Acker et al., 2017
P. tremula × *P. alba*	*CSE*	35S	RNAi	Greenhouse	Lignin decrease; cellulose increase	Saleme et al., 2017

Continued

Table 1. Continued

Species	Gene modified	Promoter used	Method	Growth condition	Main effects of transgenic trees	Reference (ordered by year)
P. tremula × *P. alba*	*FMT*	35S	Heterologous overexpression	Greenhouse	Saccharification yield increase	Kim et al., 2017
P. deltoides × *P. euramericana*	*CesA7A*	35S	RNAi	Greenhouse	Crystalline cellulose decrease	Xi et al., 2017
P. deltoides	*GAUT4*	35S	RNAi	Greenhouse	Improving biomass yields and sugar release	Biswal et al., 2018a
P. deltoides	*GAUT12*	Ubq3	Upregulation	Greenhouse and field	Hemicellulose increase; growth enhanced	Biswal et al., 2018b
P. tremula × *P. tremuloides*	*GT43BC*	35S or GT43B	RNAi	Greenhouse	Xylan decrease; growth enhanced	Ratke et al., 2018
P. deltoides	*EPSPS*	Ubq3	Upregulation	Greenhouse	Lignin increase	Xie et al., 2018
P. alba × *P. glandulosa*	*HCT*; *C3'H*	35S	Downregulation	Greenhouse	Lignin decrease	Zhou et al., 2018
P. deltoides	*IQD10*	35S	RNAi	Greenhouse	Cellulose increase	Badmi et al., 2018
P. tremula × *P. tremuloides*	*SWN1*	NST1/ SND1	Heterologous overexpression	Greenhouse	Wood density increase	Nuoendagula et al., 2018
P. tomentosa	*DWF4*	35S	Upregulation	Greenhouse	Improved growth	Shen et al., 2018
P. trichocarpa	*CAD1*; *CCR2*	35S	RNAi	Greenhouse	Lignin decrease	Yan et al., 2019
P. deltoides	*WND3A*	Ubq3	Upregulation	Greenhouse	Lignin and S/G ratio increase	Yang et al., 2019
P. alba × *P. tremular var. glandulosa*	*GA20ox1* + *MYB221*	DX15	Upregulation	Greenhouse and field	Biomass increase; lignin decrease; holocellulose increase	Cho et al., 2019
P. trichocarpa	*CESA4*; *CESA7a/b*; *CESA8a/b*	35S	RNAi	Greenhouse	Cellulose decrease; lignin and hemicellulose increase	Abbas et al., 2020
P. tremula × *P. tremuloides*	*AnAXE1*	35S or WP	Heterologous overexpression	Field	Cell wall acetylation reduction; growth inhibition	Derba-Maceluch et al., 2020
P. tremula × *P. tremuloides*	*HjAXE*	GT43B	Heterologous overexpression	Greenhouse	Xylan acetylation reduction	Wang et al., 2020b
P. deltoides × *P. euramericana*	*4CL1*	WND1B or XCP1	Suppression	Greenhouse and field	Lignin decrease	Cao et al., 2020
P. tremula × *P. alba*	*4CL1*		CRISPR-Cas9	Greenhouse	Lignin S-unit decrease	Tsai et al., 2020
P. trichocarpa	*C3H3*; *C4H1*; *C4H1/ C4H2/C3H3*	35S	RNAi	Greenhouse	Lignin decrease	Kim et al., 2020
P. tremula × *P. alba*	*CCR2*		CRISPR-Cas9	Greenhouse	Lignin decrease	De Meester et al., 2020
P. tomentosa	*LAC14*		CRISPR-Cas9	Greenhouse	Lignin S/G ratio increase	Qin et al., 2020
P. deltoides × *P. euramericana*	*LTF1*	WND1B or XCP1	Suppression	Greenhouse and field	Lignin decrease	Gui et al., 2020
P. tomentosa	*miR6443*	35S	Suppression via STTM	Greenhouse	Lignin S-unit increase	Fan et al., 2020
P. tremula × *P. tremuloides*	*ERF123*	35S	Upregulation	Greenhouse	Improved saccharification efficiency; cellulose increase; lignin and xylan decrease	Hori et al., 2020
P. trichocarpa	*CESA4*; *CESA7a/b*; *CESA8a/b*		CRISPR-Cas9	Greenhouse	Cellulose decrease	Xu et al., 2021
P. tremula × *P. tremuloides*	*AnAXE1*; *HjAXE*	35S	Heterologous overexpression	Greenhouse and field	Cell wall acetylation reduction	Pramod et al., 2021
P. tremula × *P. alba*	*PMT1*	AtC4H	Heterologous overexpression	Greenhouse	Improved saccharification yield	Lapierre et al., 2021
P. tremula × *P. alba*	*CCR2*	SNBE	CRISPR-Cas9 + overexpression of *AtCCR1*	Greenhouse	Lignin decrease	De Meester et al., 2021
P. alba × *P. glandulosa*	*CSE1*; *CSE2*		CRISPR-Cas9	Greenhouse	Lignin decrease	Jang et al., 2021

Continued

Table 1. Continued

Species	Gene modified	Promoter used	Method	Growth condition	Main effects of transgenic trees	Reference (ordered by year)
P. tremula × *P. alba*	*CSE1/CSE2*		CRISPR-Cas9	Greenhouse	Lignin decrease	de Vries et al., 2021
P. alba × *P. glandulosa*	*HCT*	35S	RNAi	Greenhouse	Lignin and S/G ratio decrease; cellulose increase	Su et al., 2021
P. tremula × *P. alba*	*PHBMT1*		CRISPR-Cas9	Greenhouse	Depletion of lignin p-hydroxybenzoylation	Zhao et al., 2021
P. trichocarpa	*HSFB3-1*; *MYB092*		CRISPR-Cas9	Greenhouse	Lignin decrease; cellulose increase	Liu et al., 2021a
P. alba × *P. glandulosa*	*miR393*	35S	Suppression via STTM	Greenhouse	Lignin increase; growth enhanced	Chu et al., 2021
P. alba × *P. glandulosa*	*MYB120*	35S	Dominant suppression via SRDX	Greenhouse	Lignin decrease	Kim et al., 2021
P. alba × *P. glandulosa*	*GA20ox1* + *MYB3*	DX15	Upregulation	Greenhouse and field	Enhanced growth; cellulose increase	Cho et al., 2021
P. davidiana × *P. bolleana*	*BRI1*	35S	Upregulation	Greenhouse and field	Enhanced growth	Jiang et al., 2021
P. tremula × *P. alba*	*KNAT7*	DX15	Antisense	Greenhouse	Lignin decrease; S/G ratio increase; improved saccharification efficiency	Ahlawat et al., 2021
P. alba	*CESA4*		CRISPR-Cas9	Greenhouse	Cellulose decrease; hemicellulose increase; stunt growth	Nayeri et al., 2022
P. trichocarpa	*FLA40/45*		CRISPR-Cas9	Greenhouse	Lignin increase; growth enhanced	Zhen et al., 2022
P. alba × *P. grandidentata*	*PHBMT1*	35S or C4H	Upregulation	Greenhouse	Lignin *p*-hydroxybenzoylation increase	de Vries et al., 2022
P. tremula × *P. alba*	*DCS/CURS2*	CesA8	Heterologous overexpression	Greenhouse	Lignin increase; cellulose decrease	De Meester et al., 2022a
P. tremula × *P. tremuloides*	*HpSK*	C4H	Heterologous overexpression	Greenhouse	Lignin H unit increase and S, G units decrease	Hu et al., 2022b
P. alba × *P. grandidentata*	*MdCHS3*	C4H	Heterologous overexpression	Greenhouse	Lignin decrease	Mahon et al., 2022
P. tomentosa	*miR828*	35S	Suppression via STTM	Greenhouse	Lignin increase	Wang et al., 2022b
P. alba × *P. grandidentata*	*QsuB*	C4H	Heterologous overexpression	Greenhouse	Lignin decrease	Unda et al., 2022
P. tremula × *P. tremuloides*	*MYB46*	CesA18	Upregulation	Greenhouse	Lignin S/G ratio and acetylated hemicellulose decreased	Nakano et al., 2022
P. tomentosa	*MYB115*	35S	Upregulation	Greenhouse	Lignin decrease; S/G ratio increase; reduced cellulose DP; enhanced saccharification	Fan et al., 2022
P. tremula × *P. tremuloides*	*ASPR1*	35S	RNAi	Field	Growth decrease	Derba-Maceluch et al., 2023
P. deltoides	*RWA-C*	Ubq3	Upregulation	Greenhouse	Xylan acetylation increase; lignin and S/G ratio increase	Zhang et al., 2023
P. trichocarpa	*PAL2/4/5*; *C3H3*; *CAD1*; *AldOMT2*; *C4H1*; *CCoAOMT1/2*		CRISPR-Cas9	Greenhouse	Lignin decrease	Sulis et al., 2023
P. alba × *P. grandidentata*	*CPL*	CesA8	Heterologous overexpression	Greenhouse	Lignin decrease; growth decrease	Mottiar et al., 2023
P. alba × *P. glandulosa*	*miR408*; *LAC19/25/32*	35S	Upregulation; CRISPR-Cas9	Greenhouse and field	Lignin and S/G ratio decrease	Guo et al., 2023

CRISPR-Cas9, clustered regularly interspaced palindromic repeats – CRISPR-associated protein 9; H, *p*-coumaryl; P., Populus; RNAi, RNA interference; SCW, secondary cell wall; S/G, sinapyl: coniferyl alcohol ratio; STTM, short tandem target mimic.

cellulose content, while interfering with the *PtrCesA3D* of other types of CSC increases the crystallinity of cellulose (Xi et al., 2017). Cellulase KORRIGAN is thought to be linked to CSC (Vain et al., 2014). Suppression of *KORRIGAN* orthologs in white spruce and *Populus* led to reduced cellulose content (Maloney and Mansfield, 2010; Maloney et al., 2012; Yu et al., 2014). Modifying the metabolic pathways that lead to the generation of uridine diphosphate (UDP)-glucose, the substrate for cellulose synthesis, can be employed as another strategy for modifying cellulose in trees. Sucrose synthase (SuSy) catalyzes the conversion of sucrose into UDP-glucose and fructose. Overexpression of a cotton *SuSy* in *Populus* led to enhanced wood density and thicker, more crystalline cell walls (Coleman et al., 2009). However, suppression of two endogenous *SuSy* genes in *Populus* resulted in no significant changes in the relative composition of cellulose, lignin, and hemicellulose, but significantly decreased wood density (Gerber et al., 2014).

Several studies have reported that wood hemicelluloses and pectin can be modified by regulating their biosynthesis genes. Suppression of *PdGAUT12.1* via RNAi resulted in reduction in pectin and xylose, while yielding 4% to 8% more glucose release upon enzymatic saccharification and also displayed enhanced growth up to 50% (Biswal et al., 2015). Downregulation of *GAUT4*, which encodes an α-1,4-galacturonosyltransferase that synthesizes HG pectin, increased growth and improved saccharification of cell wall biomass in *Populus*, which probably is due to the reduction of pectin which enhances sugar release from cell walls (Biswal et al., 2018a). In addition, in *Populus* overexpression of *PL1-27*, encoding a pectate lyase degrading HG, resulted in increased solubility of pectin as well as hemicelluloses, giving higher yields of sugars during saccharification (Biswal et al., 2014). The high acetylation of hemicellulose hinders the conversion of wood biomass to sugar during saccharification. In *Populus*, downregulation of *RWA*s, which are responsible for xylan acetylation, reduced 25% of wood acetylation and increased glucose production from cell wall biomass saccharification (Pawar et al., 2017b). Since wood xylan acetylation is a promising target to improve wood saccharification, acetyl xylem esterase (AXE) from fungus was induced into *Populus* for deacetylation of xylan. The transgenics showed reduced xylan acetylation, with 25% more glucose yield in saccharification (Pawar et al., 2017a). Heterologous overexpression of *HjAXE* from *Hypocrea jecorina* under control of the *PtGT43B* promoter in *Populus* also led to 27% higher glucose yield after saccharification (Wang et al., 2020b).

Modification of regulatory networks during wood formation

A network of regulatory genes that affect the development of wood in trees has been well documented (Du and Groover, 2010; Ye and Zhong, 2015; Luo and Li, 2022). Wood cell wall composition as well as cell wall structure can often be modified by manipulating the regulatory genes. Heterologous overexpression of *SWN1* from rice under the control of the *NST3* promoter resulted in a 57% increase in the physical strength of the stem of transgenic *Populus* (Sakamoto et al., 2016). Overexpression of *Arabidopsis SND1* in *Populus* resulted in enhanced SCW thickening (Nakano et al., 2022). The upregulation of the alternative-spliced *PtrWND1B* transcript in *Populus* resulted in a considerable increase in xylem fiber cell wall thickness (Zhao et al., 2014). Several MYB transcription factors that regulate SCW formation have been studied in order to modify the composition of wood SCWs. Overexpression of *AtMYB46*, which is directly downstream of *SND1* in the regulatory network for SCW biosynthesis, resulted in a lower lignin S/G ratio and acetylated hemicellulose in *Populus* (Nakano et al., 2022). *MYB115* overexpression decreased lignin quantity and increased the S/G ratio, which improved saccharification and bioethanol yield (Fan et al., 2022). Similarly, *KNAT7* overexpression reduced lignin amounts while increasing the S/G ratio and saccharification efficiency (Ahlawat et al., 2021). *ERF123* overexpression in *Populus* improved saccharification efficiency by increasing cellulose content and decreasing xylan and lignin (Hori et al., 2020). In addition to TFs, microRNAs that directly regulate monolignol biosynthetic genes are modified to reduce lignin content and hence improve saccharification efficiency. MiR6443 directly targets *F5H2*, which is responsible for S-unit lignin synthesis. Overexpression of miR6443 in *Populus* resulted in a decrease in the *F5H2* transcript, a reduction of S lignin, and an increase of 13.2%–14.6% hexoses yield (Fan et al., 2020). MiR408 targets the *laccase* genes, which are required for lignin polymerization, and overexpression of *miR408* in *Populus* reduced lignin content, S/G ratio, and degree of lignin polymerization, resulting in enhanced saccharification efficiency of 85%–92% (Guo et al., 2023).

Engineering trees for fast growth and desired wood quality

Promoting tree growth is another strategy to obtain high wood production with desired wood quality. Genes involved in GA synthesis have repeatedly been reported in tree engineering. Overexpression of *Arabidopsis AtGA20ox1* in *Populus* showed increased growth rate, longer xylem fibers and more wood biomass (Eriksson et al., 2000). Similarly, overexpression of pine *PdGA20ox1* in *Populus* resulted in nearly three-fold increase in woody biomass, fiber length and cellulose/xylan content (Park et al., 2015). By using a xylem-specific promoter (*DX15*) to drive *PdGA20ox1* expression, the transgenics had increases of wood biomass up to 300% similar to that constitutive promotor (Jeon et al., 2016). When *PtrMYB221* and *GA20ox1* were expressed in *Populus* under the control of a xylem-specific promoter, the transgenics had an increase in biomass and a 16% reduction in lignin concentration, as well as enhanced saccharification efficiency (Cho et al., 2019). Transgenic *Populus* transformed with cytokinin biosynthetic gene *IPT7* driven by promotor *PttLMX5* showed nearly two-fold increases in wood growth (Immanen et al., 2016). Modifying BR biosynthesis and signaling

pathway genes also enhanced tree growth. Overexpression of *CYP85A3* or *DWF4* in *Populus* increased plant height and stem diameter, resulting in a biomass increase (Jin et al., 2017; Shen et al., 2018). Overexpression of *PtBRI1.2* resulted in faster growth, an increase of 15%–22% in plant height, and 44%–61% in stem diameter in transgenic plants (Jiang et al., 2021). Co-expression of *PttPXY* driven by a cambium-specific promotor (*ANTEGUMENTA*) and *PttCLE41* driven by phloem-specific promotor (*PHLOEM PROTEIN2*) increased the rate of wood formation (Etchells et al., 2015). Overexpression of *SWN1*, a transcription factor regulating SCW formation from *Oryza sativa*, under control of the *SND1* promoter led to an increased cell wall thickness and wood density in *Populus* (Nuoendagula et al., 2018). These studies suggest a possibility of engineering fast growth tree and wood properties through manipulating biosynthesis of hormones or their signaling.

Modification of wood in a cell-specific manner

A large number of laboratory studies have shown that genetic engineering of trees can significantly improve the chemical composition of wood, cell wall structure, and conversion and utilization efficiency of wood, but at the same time, some field experiments have shown that the growth of transgenic trees is affected (De Meester et al., 2022b). For instance, studies have been conducted on the suppression of genes involved in the monolignol biosynthesis pathway, including *4CL*, *CAD*, and *CCoAOMT*, in both greenhouse environments and field conditions. The maintenance of reduced lignin content was achieved in the field, while a growth penalty was detected (Pilate et al., 2002; Wei et al., 2008; Voelker et al., 2010; Wang et al., 2012; Tian et al., 2013; Stout et al., 2014; Xiang et al., 2017). To overcome the growth penalty, engineering wood formation through cell-type specific manner has been attempted in recent years. Modification of lignin biosynthesis in xylem fibers has been shown to result in lignin reduction with little growth penalty in *Populus* (Cao et al., 2020; Gui et al., 2020). In another study, knockout *CCR2* in *Populus* had significant lignin reduction and was extremely dwarfed. Reintroduction of the *CCR2* expression in a vessel-specific manner led to normal growth with 18% less lignin (De Meester et al., 2021). The results of these studies indicate that the influence of lignin on plant growth displays variability depending on the specific type of xylem cell. The influence of modifications in lignin within fiber cells on growth is relatively negligible; nevertheless, preserving the integrity of lignin within vessels plays a pivotal role in facilitating growth. These observations could possibly be related to the physiological activity of these cells. The cells of the vessels are crucial in permitting the long-distance transportation of water and minerals. The preservation of the structural integrity of their cell wall's lignin is crucial for the optimal performance of their function. Fiber cells primarily function as a means for providing mechanical reinforcement, with their lignin modification having limited impact on growth. Without a doubt, these findings present potential avenues for the study of innovative genetic engineering strategies with the objective of improving favorable wood characteristics while simultaneously maintaining consistent rapid tree growth.

As delineated in Table 1, endeavors have been undertaken to genetically manipulate the process of wood formation in trees utilizing diverse methodologies. The majority of the aforementioned studies have primarily been undertaken within controlled greenhouse environments. However, a limited number of studies have explored the potential implications of these findings in the context of field cultivation, specifically in relation to paper and biofuel production (Cho et al., 2019; Gui et al., 2020; Pramod et al., 2021; Derba-Maceluch et al., 2023). The utilization of genetic technology in the cultivation of plantation forest trees necessitates the transition from proof-of-concept studies to field demonstrations, which represents a crucial stage on the advancement of tree biotechnology.

PERSPECTIVES

Over the past three decades or more, there has been a substantial accumulation of molecular and genomic research pertaining to wood formation. Consequently, our comprehension of the processes involved in wood formation has seen notable advancements. Concurrently, endeavors aimed at genetically modifying trees and formulating novel approaches for cultivating rapidly growing trees possessing diverse favorable wood characteristics have exhibited encouraging results in laboratory environments. Nevertheless, the process of wood formation is a multifaceted molecular and cellular developmental process. The current understanding of wood formation in trees remains insufficient. The complete elucidation of wood formation remains elusive. The primary focus of what is currently being investigated is the formation and regulation of wood cell wall components, including lignin, cellulose, and hemicellulose, as well as the activity of cambium division. In order to comprehensively understand wood formation, it is important to investigate various facets at multiple levels, such as tissue structure, cell differentiation, cell wall synthesis, distinct genomic components, signal transduction, regulatory elements and networks, and others. There remain many unknowns yet to be unveiled. The genetic regulation of wood properties is evident in the great variation observed among tree species in terms of cell size, cell length, cell wall thickness, and the proportion of different cell types present in wood. What is the fundamental mechanism underpinning their genetic regulation? The identification of the genetic underpinnings responsible for these wood characteristics will offer a more robust foundation for manipulating timber to possess desirable wood features.

An additional facet of future research pertains to the transfer of successful laboratory research findings to practical applications in the field, such as in the context of cultivating fast-growing forest trees with desirable wood characteristics on a large-scale plantation. Currently, this

subject presents a multitude of obstacles that necessitate resolution. These challenges encompass both research-related concerns as well as policy-oriented matters that require attention. In the realm of scientific research, studies conducted in controlled laboratory settings predominantly focus on *Populus* species, with a significant proportion of these studies being carried out in greenhouse environments. In order to ensure the reliability and accuracy of laboratory findings, it is imperative to validate the results through systematic field trials. Additionally, it is crucial to assess the adaptability of genetically engineered trees to their surrounding environment. Furthermore, the effectiveness of the ultimate harvest features of genetically engineered trees must be proven. However, it is imperative to enhance public awareness and understanding of genetically modified trees through the dissemination of scientific knowledge to the general population, with the provision of policy backing.

However, the pursuit of carbon neutrality has emerged as a key objective in addressing worldwide environmental challenges, and harnessing the carbon sequestration capabilities of trees represents a crucial stride in this endeavor. The acquisition of wood from forest trees has the potential to not only store and fix carbon dioxide but also serve as a viable substitute for a range of chemicals and energy commodities derived from fossil fuel sources. The significance of forest trees in addressing global environmental issues is increasing. In this particular context, there is a strong anticipation for the comprehensive elucidation of the mechanism via which forest trees undergo the process of converting solar energy and carbon dioxide into wood biomass, as it has great potential for the development of trees that possess efficient carbon sequestration capabilities. Such trees could serve as viable alternatives to fossil energy and materials. The potential for study in this area is vast, and resulting outputs are expected to provide novel solutions to global environmental challenges.

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CONFLICTS OF INTEREST

The authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

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