Review

The *Arabidopsis* wood model – The case for the inflorescence stem

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**A B S T R A C T**

*Arabidopsis thaliana* has successfully served as a model to discover genes and proteins that have roles in a wide range of plant traits, including wood-related traits, such as lignin, cellulose and hemicellulose biosynthesis, secondary growth regulation, and secondary cell wall synthesis. Both the radially thickened hypocotyl and the inflorescence stem have been studied. In this review, we address lingering doubts regarding the utility of *Arabidopsis* as a model for wood development by highlighting studies that provide new biochemical and biophysical evidence that extend support for the *Arabidopsis* inflorescence stem as a model for wood development beyond what is currently thought.

We describe different aspects of *Arabidopsis* that make it a highly versatile tool for the study of wood development. One would likely utilise the radially thickened hypocotyl because of its more fully developed vascular cambium for traits related specifically to secondary (i.e., cambial) growth. It is more productive to utilise the inflorescence stem for wood-like biophysical traits. Accession variation has been underexploited as a powerful method to discover genes governing wood-like traits. We discuss recent findings that survey the accession variation in *Arabidopsis* for biochemical and biophysical properties of various wood traits, such as microfibril angle, tensile strength and cellulose/hemicellulose content. Furthermore we discuss how larger-scale studies of this nature using plants grown in long days (as opposed to the current short-day paradigm) could accelerate gene discovery and our understanding of cell wall and wood development. We highlight some relatively unexplored areas of research relating to the secondary cell wall composition, architecture and biophysical properties of the inflorescence stem, and how these traits are relevant to wood formation. The *Arabidopsis* inflorescence stem has other characteristics, expressed genes and traits held in common with woody species that have not been widely characterised or discussed to date. We discuss how this conservation may indicate the more general potential for “true” woodiness in herbaceous species, in the context of so-called secondary woodiness.

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1. Does the concept of the Arabidopsis “wood model” have merit?

In our admittedly anecdotal experience, the use of Arabidopsis thaliana as a model for wood development has been questioned, at times quite strongly over the years, despite its extensive use for this purpose by laboratories all over the world. Arabidopsis is a small rosette plant, perhaps as far from a tree in stature and architecture as an angiosperm plant can be. Nonetheless, it is clear that wild-type accessions (ecotypes) of this species are capable of making some form of wood [1,2, see photo therein].

Arabidopsis gained favour as a model for wood development with the demonstration of a classical vascular cambium in radially thickening hypocotyl/rosette compressed stems grown in short-day environmental conditions [3]. The Arabidopsis in vitro tracheary element system has proven to be an excellent tool for elucidating the molecular mechanisms involved in secondary cell wall synthesis and the role(s) of microtubules in vascular patterning [4]. The Arabidopsis root has also played a pivotal role in our understanding of vascular development, particularly with regard to the role of cytokinin in this process [5,6]. Such contributions of the root model continue, with the finding that the CLAVATA3/ENDOSPERM SURROUNDING REGION-45 (CLE45) peptide [7] appears to be the ligand for the BARELY ANY MERISTEM-3 (BAM3) receptor, with a role in primary protophloem differentiation [8]. Comparative genomic data suggest that the Arabidopsis, Eucalyptus and Populus xylem-related gene sets appear to be about equally similar to one another [9], with significant similarity to pine [9,10], suggesting that xylem transcriptional networks are conserved across these species. Furthermore, it is clear that genes that regulate wood and wood-like characteristics appear to be functionally conserved, at least between woody angiosperm species and Arabidopsis [11-14], with concomitant microsynteny in at least one case [13]. There are also expected developmental differences in Arabidopsis relative to woody species, such as asynchronous cell division among adjacent files in the vascular cambium, atypical of woody species [15]. However, the discovery of wood formation in the Arabidopsis soc/ful double mutant [16] revealed the innate potential for wood formation in this species via allelic variation. Additionally, lignin, which makes up about 30% of typical secondary cell walls of wood, is present in Arabidopsis and its synthesis and deposition has been comprehensively studied using Arabidopsis as a model with excellent inroads made into identifying key genes and transporters. These insights bear close relevance to lignin in trees [17-19]. Thus, we think that the answer to the question: “Does the concept of the Arabidopsis wood model have merit?” is “yes”.

The purpose of this review is to examine strengths and weaknesses of the more recent (and more or less ad hoc) extension of the radially thickened hypocotyl model to the use of the inflorescence stem. We discuss findings that provide a stronger biophysical/biophysical justification for its use as well as the potential for more rapid progress via the power of short rotation growth in studies of accession variation.

2. The Arabidopsis radially thickened hypocotyl and inflorescence stem have different utilities for the study of wood-like traits

2.1. The radially thickened hypocotyl model and its use for analysing secondary growth

Arabidopsis has served successfully as a model to discover genes and proteins that have roles in a wide range of plant traits. These include wood-related traits, such as cellulose and hemicellulose biosynthesis, secondary growth regulation and secondary cell wall synthesis. The hypocotyl is perhaps the Arabidopsis organ of choice as a secondary growth/wood trait model due to its established capacity for secondary growth that initiates shortly after germination under the right growth conditions, particularly in short-day (i.e., 8 h light) photoperiods [3,20]. Many genes, particularly transcription factors, have been identified as having roles in secondary growth and the development of other aspects of wood-like characteristics using this model (see [4,20,21] for reviews).

2.2. The secondary growth and cell wall thickening of the inflorescence stem

A critical biophysical shortcoming of the radially thickened hypocotyl as a wood model system is the fact that, unlike the inflorescence stem, the hypocotyl is by and large not load-bearing. In contrast, like a tree trunk, the inflorescence stem is a load-bearing organ. Regardless, a perceived limitation of the Arabidopsis inflorescence stem as a ‘true’ model for wood formation is that it undergoes only limited secondary growth in the classical sense of a tree-like vascular cambium (Fig. 1). This perception has spurred several lines of research to create more ‘wood-like’ phenotypes/models in Arabidopsis (discussed in [22]). Ironically, non-woody Arabidopsis lines may have been inadvertently bred through an unconscious selection over the decades away from wild-type secondary growth [23]. Nevertheless, several microarray studies have used the Arabidopsis inflorescence stem to identify genes and networks that are involved in activation of secondary cell wall development and cellulose synthesis [24-26]. Despite the lack of a well-defined vascular cambium in the inflorescence stem, parallels continue to arise between the inflorescence stem vascular cambium and those of woody plants. These are evinced by similar expression patterns of and cross-complementation by the SHORT ROOT (SHR) transcription factor gene in Arabidopsis and poplar [27].
The inflorescence stem makes thickened, lignified secondary cell walls in abundance (Fig. 1) [28], presumably because of its role in supporting the inflorescence and later, the developing fruits. Such traits have lent themselves to detailed experiments using the inflorescence stem to reveal that the MYB103 transcription factor has a key role in syringyl lignin biosynthesis in Arabidopsis [29]. A measurable cellulose microfibril angle is also observable in these organs [30–32]. The cellulose microfibril angle, a key trait of wood, is the overall angle of the microfibrils relative to the longitudinal axis of the wood cell. Moreover, the inflorescence stem can be exploited for other wood-related biophysical traits such as tensile strength and stiffness, as well as bending strength [30,31,33].

3. The microfibrils of wood and the Arabidopsis inflorescence stem

Wood cells in trees have thick secondary cell walls that contain cellulose microfibrils that are composed of arrays of individual cellulose polysaccharides that cohere to each other laterally as sheets via hydrogen bonds. Sheets cohere to each other via van der Waals forces between them. Plant microfibrils are generally 3 nm in diameter, and may be arranged into microfibrils, which can vary widely in diameter, dependent on species and physiological state [34]. The arrangement of microfibrils in woody cell walls has long been of particular significance to the construction timber industries because of its correlation with wood stiffness. In general terms, smaller microfibril angles correlate with stiffer wood [35,36]. Consequently, much effort is devoted to the pheno-
typic analysis of tree breeding lines for higher stiffness and lower microfibril angle, particularly in softwood species, which have far more variable microfibril angle than hardwoods [37]. Such data are used as part of the selection process for breeding elite timber of improved quality and stiffness.

A few recent studies of Arabidopsis cell walls indicate that, like woody cells of trees, Arabidopsis cell walls have a measurable microfibril angle and that this phenomenon can be exploited for understanding the underlying molecules and processes contributing to the architecture of the microfibrils in the cell wall.
likely viable alternative to FESEM [30,31], particularly if automation of the scanning process can be implemented. The extent and functional significance of microfibril aggregation into microfibrils and how this might relate to the biomechanics of woody cells are additional unanswered questions that could be addressed using the Arabidopsis inflorescence stem model.

4. Accession variation as a gene identification tool for wood-like traits

Accession variation analysis is a powerful tool for plant gene discovery that has proven useful for studying diverse traits such as flowering time, climate adaptation, development and defence responses, which are all beyond the scope of this review. It is clearer with each published study that higher numbers of accessions lend increasing statistical power to accession variation and genome-wide association studies for the discovery of small-effect loci. See Ref. [31], for a brief overview of recent publications. Accession variation studies for wood traits have lagged behind this rapid progress, perhaps due in part to a lack of a clear foundation (and also likely some scepticism) about the use of Arabidopsis for traits of direct interest to and use by forestry researchers, but this situation is beginning to change. As already discussed briefly above in the context of microfibril angle, encouraging evidence supporting the potential utility of large-scale accession variation studies in a new battery of biochemical and biophysical wood-related traits has been revealed [31]. Twelve different wild-type natural accessions, chosen to represent several common laboratory strains as well as a wide range of geographic origins, were grown under long-day and short-day conditions. Inflorescence stems were harvested from these plants and dried. The inflorescence stem bases were assayed for tensile strength, tensile stiffness, neutral carbohydrate content and lignin content. Correlation analyses (including flowering time, leaf number, rosette diameter and inflorescence stem diameter to assess potential effects due to differences in resource allocation) were undertaken. Carbohydrate analyses revealed strong correlations consistent with the biophysical effects of known large-effect (i.e., Mendelian) mutants in hemicellulose biosynthesis [31]. Many significant correlations (and some trends just below the significance threshold) consistent with woody traits, such as microfibril angle and stiffness, were observed. Negative correlations between lignin content and microfibril angle, as well as all tensile resistance characteristics, were reported. It was suggested that this might mean that lignin might have more of a role in resisting compressive forces [31]. Trivial correlations with flowering time (except for stem diameter) were ruled out and most importantly, the general correlations were seen to be independent of daylength [31]. The significance of the last finding lies in the fact that useful data on wood-like traits in Arabidopsis can be gleaned from plants grown in long-day conditions, which is often thought not to be useful due to perceived limitations to secondary growth and wood-like characteristics under such conditions. Growth under long-day conditions would save considerable amounts of time and space, making truly large-scale (i.e., >1000 accessions) accession variation and genome-wide analyses for wood-like traits feasible.

Also supporting the use natural variants to study the underlying genetics of Arabidopsis wood-like phenotypes, a survey of 32 Arabidopsis accessions has shown variation in the amount of ‘hypocotyl secondary growth’ with particular emphasis on variation in xylem:phloem ratio in this tissue [40]. Specifically, ‘hypocotyl secondary growth’ in two recombinant inbred line populations was linked to a locus for xylem:phloem ratio that was coincident with a flowering time QTL, and their analysis, including that of various accessions, suggested that expression a major flowering time gene, FLC, is correlated with the amount of ‘hypocotyl secondary growth’ xylem expansion and fibre differentiation (vernation was a not a factor included in these experiments) [40]. It is not known whether variation in flowering time loci (such as FLC expression) correlates with natural variation of wood-like properties of inflorescence stems, however no correlation was found between flowering time and inflorescence stem biomechanical or cell wall properties, or diameter, in the study of [31]. These two reports raise the question of correlation between flowering time loci and other linked genes, with specific inflorescence and/or rosette short stem secondary growth properties such as biomechanics, cell wall properties, diameter, xylem:phloem ratio, etc. It is important to consider this in the context of using large-scale genome-wide association studies of inflorescence stem wood properties. If genome-wide association studies indeed find flowering time loci that are linked with inflorescence stem wood properties, then a question arises: are there perhaps flowering time loci that have undiscovered biological mechanisms – for example by affecting cell differentiation, cell wall expansion and development, in addition to modulating responses to flowering time cues (such as bioactive gibberellins, vernalisation, long days, and autonomous cues)?

Despite the growing number of accessions used in large-scale genome-wide association studies, it should be noted that low levels of polymorphism in some critical genes might result in a lack of phenotypic variation and thus a potential limitation the statistical power necessary for associations to be supported. In such cases, reverse genetics approaches can also provide insight into gene function, including knock-out and knock-down/overexpression approaches. The use of high-density reverse genetics and transformation in trees is limited and this adds weight to the case for using Arabidopsis as a model for wood formation.

We believe that there is significant scope for exploitation of the Arabidopsis inflorescence stem and accession variation analysis approaches for elucidating the underlying genes and proteins that contribute to specific woody traits such as cellulose microfibril angle and crystallinity, tensile strength, stiffness and other biomechanical traits, as well as hemicellulosic polysaccharides and lignin. Further expansion of such approaches to non-neutral carbohydrates (e.g. uronic acids) is currently unexplored and this will surely provide additional insight to genes contributing to woody traits.

5. Cell wall proteins and biomechanical traits

Many research papers, reviews and textbooks describe the secondary cell wall as a complex matrix composed of cellulose, lignin (when present), and hemicelluloses. Perhaps because of this complexity, cell wall proteins are often not taken into consideration, or are considered ancillary at best. However, proteins in plant cell walls, including woody cell walls, likely have integral roles in cell wall structure and function. For example, an extensin-like protein has been found in secondary cell walls in developing xylem and also in dried wood. This protein persists in wood for many years and may affect its physical properties [41]. The proteome of the plant secondary cell wall matrix is an almost entirely unexplored realm which may hold many key insights into the structure and ultimately the functions of various plant cell walls, including those of wood. The Arabidopsis inflorescence stem is proving to be a tractable experimental system for exploring this realm.

5.1. Fasciclin-like arabinogalactan proteins

Fasciclin-like arabinogalactan proteins (FLAs) are plant-specific arabinogalactan proteins that contain one or two copies of the evolutionarily ancient fascinl (FAS) intercellular adhesion protein domain. The FAS domain is conserved across many phyla [30]
and is itself a member of the "β-grasp fold" protein superfam-
ily that is suggested to be ubiquitously evolutionarily conserved
with a lineage dating back to the last common ancestor of all life
on earth [42]. The specific functions of FLAs in plants have been
evasive until recently. Experiments on Arabidopsis inflorescence
stems and on trees indicate a role for FLAs in plant secondary
cell wall biomechanics [30]. A sub-group of FLAs, known as the
Group A FLAs [30] that have a single FAS domain flanked on either
side by arabinogalactan glycosylation sites are predicted to be
embedded in the extracellular matrix [43]. This group of FLAs is
conserved in angiosperms including tree species and they have
roles in tensile strength and stiffness in Arabidopsis inflorescence
stems [30]. Specifically, T-DNA insertion double knockout mutant
Atf11/12 inflorescence stems had a 30% reduction in uniaxial
tensile strength in both fresh and dried inflorescence stems and a
15% and 26% reduction in tensile stiffness in fresh and dried inflo-
rescence stems, respectively. Conversely, 3-point bending tests
(flexural tests) revealed little difference between Atf11/12 and
wild-type stems. A key distinction between uniaxial tensile tests
and 3-point bending tests is that the latter involves compressive,
tensile and shear forces, all of which can contribute to failure within
the sample (Fig. 2; see Box 1). The difference between the uniaxial

**Box 1**

**Strength and stiffness testing in wood and Arabidopsis**

Wood biomechanics can be measured in different ways, and
is often done to quantify strength and stiffness properties. The
biomechanical properties of any plant, whether Arabidopsis
or a tree, ultimately derive from the forces it must withstand
in its environment. Plants thus experience tensile, compres-
sive, and shear forces. Superior resistance to these forces is
what is ultimately desired in wood. Thus, biomechanical tests
designed to measure these properties are frequently used in
timber grading (particularly structural timber) for commercial
sale as well as in tree breeding programs to select and breed
superior trees with increased stiffness and strength. Such tests
are also used with Arabidopsis to identify candidate genes
with effects on strength and stiffness in mutant analysis and/or
large-scale accession variation studies. 'Strength' is measured
by the amount of force required to ultimately rupture and break
the material, and 'stiffness' is measured by the force required
to displace (stretch) the material over a unit length. Wood and
Arabidopsis inflorescence stem strength and stiffness can be
measured using a 3-point bending test (Fig. 2). In this test, com-
pressive, tensile and shear forces occur within the sample until
it finally ruptures. Failure can occur due to loss of tension, com-
pression, or shear resistance, or any combination of the three.
Tensile tests (uniaxial tests; Fig. 2) involve pulling the sample
apart to measure tensile strength and stiffness. This type of
test is not frequently used for wood due to the high likelihood
of experimental artefacts. However, tensile tests are used for
Arabidopsis inflorescence stems. In contrast to the 3-point test,
sample failure in a tensile test is solely due to loss of resistance
to the tensile force, so the data are very specific. Tensile and
bending tests each have their merits. If a material is weaker or
less stiff in resisting tensile forces, this would be detected in a
tensile test, but not necessarily in a 3-point bending test. If a
material is weaker or less stiff in resisting compressive forces,
then this could be detected in a 3-point bending test. 4-point
bending tests are frequently used for wood because there are
no shear forces between the central supports, but these tests
are not as frequently used for Arabidopsis stems.
wall proteins such as FLAs with structural and biomechanical roles in plants.

The conservation of the FAS domain across phylogenetic kingdoms and phyla underscores conserved roles for FAS domain proteins not only in cell adhesion, but also in biomechanical functions in the animal and plant kingdoms. For example, FAS-protein (i.e. perisitin) knockout mice have been shown to have reduced heart-tissue and skin stiffness [46,47], which reinforces the significance of this class of proteins to biomechanics. Detailed structural protein analysis of a bacterial fascin 1 protein from Rhodobacter sphaeroides has led to a model for the function of eukaryotic FAS proteins that proposes a newly identified site within the C-terminal FAS domain is the ligand binding site, although its binding partner is currently unknown, and that N-terminal FAS I partners may prevent ligand binding [48]. This model could start to explain the significant role that FLAs play [30] in plant cell wall biomechanics. The fact that some FAS domain-containing proteins are evolutionarily adapted to function in the plant cell wall with arabinogalactan side chains attests to the importance and malleability of the FAS domain in biomechanical roles in contrasting cellular contexts. In plants, it could be that arabinogalactan moieties are ligands that bind to the FAS domain. This type of interaction could contribute to the tensile properties of the plant cell wall.

5.2. Arabidopsis cell wall proteomics

Little is currently published about the proteome, including the glycoproteome, of the Arabidopsis secondary cell wall. Although this is likely because such studies are difficult due to the complex nature of the cell wall, the work that has been published is revealing. The N-glycosylated sub-proteome of mature (late stages of flowering) Arabidopsis inflorescence stems was examined. These experiments involved enrichment for soluble cell wall N-linked glycoproteins using lectin-based Concanavalin A sepharose affinity chromatography. Many glycosyl hydrolases were reported among the various glycoproteins in the extracellular secretory pathway resulting from the enrichment, along with aspartyl and serine proteases, oxidoreductases, lectin-like proteins, and Group-A and Group-C FLAs FLAs [49]. Concanavalin A affinity chromatography was also used successfully to isolate and identify glycoproteins from Arabidopsis seedlings and barley grains [50]. What other proteins, such as proteoglycans, arabinogalactan proteins and FLAs, are embedded in the secondary cell wall? We suggest that more extensive proteome and glycoproteome (or even proteoglycome) examination and analysis of the Arabidopsis secondary cell wall will shed light on new proteins in the complex architecture of plant cell walls including potentially unknown contributors to biophysical properties. Such proteomic analyses of secondary cell walls are also under-explored areas of research in industrially important forest trees and textile crops.

Care should be taken in secondary cell wall proteomic analyses to avoid discarding highly glycosylated proteins (e.g. proteoglycans and glycoproteins) during the fractionation from cell wall polysaccharides such as pectins and hemicelluloses. In a proteomic study of cell wall preparations of Arabidopsis suspension-cultured cells that identified carbohydrate active enzymes, peroxidases and proteases using multidimensional protein identification technology, many classes of arabinogalactan proteins were not captured because of the wall preparation and clean-up procedures in which highly glycosylated proteins were probably discarded [51]. However, glycoprotein and proteoglycan purification could prove to be even more daunting than was previously thought. Proteoglycans can be covalently cross-linked to primary cell wall hemicelluloses such as pectin and arabinoxylan [52], rendering them extremely difficult to isolate. It is thus conceivable to extrapolate that cross-links also occur between proteoglycans and/or glycoproteins and the hemicelluloses within secondary walls. There is a range of emerging methods for analysis of plant proteins that are post-translationally modified [53,54] and such methods could be valuable in studies of proteins of plant cell secondary walls.

6. Cellulose structure and biomechanical traits

Cellulose synthesis in higher plants is complex and incompletely understood. Several models describe the processes involved. A rigorous review [55] on this topic has examined some of the accepted models of cellulose biosynthesis, taking care to separate what is experimentally validated versus what remains hypothetical (yet often dogmatically accepted as fact). The Arabidopsis inflorescence stem has been used as a tool in recent publications to test these hypotheses. Here we discuss two aspects of cellulose structure, degree of polymerisation and crystallinity, in the context of the Arabidopsis wood model.

6.1. Degree of polymerisation

The degree of cellulose polymerisation is another area of wood- and cellulose-related research that has been relatively unexplored in Arabidopsis. It holds potential for insights into the complex processes that lead to different forms of cellulose and materials found in different plant cell walls. In plants, the degree of cellulose polymerisation is estimated to range from 500 to 15,000 glucan subunits [56].

The degree of polymerisation in commercial cellulose-based materials appears to be important for their biomechanical properties. For example, cotton seed fibres are used worldwide in textiles because of the high-tensile properties of the cellulose, and the high degree of polymerisation of cotton seed fibre cellulose (~14,000–15,000 glucan subunits) [56] has been suggested to be an important contributing factor to the high tensile strength of cotton. Conversely, primary cell wall cellulose has a much lower degree of polymerisation, sometimes varying to as low as 500 and as high as 2000–4000 within an individual plant [56]. The degree of polymerisation of wood cellulose can vary with species and age. For example in one study, measurement of the degree of polymerisation of aspen wood cellulose was 4600 whereas Nalita wood (Tremat orientalis) had an average degree of polymerisation of 3400 that increased with age in this fast-growing species from about 3100 to 3600 in 12- to 30-year old trees [57]. In a study of extracted Eucalyptus grandis wood pulp, the degree of polymerisation ranged from 5000 to 7000. Interestingly, this was positively correlated with the tensile index and pulp stiffness indicating there may be a relationship between the degree of cellulose polymerisation and material properties in paper-making [58], with a very low degree of polymerisation (<1000) leading to poor paper quality. Alkaline methods of pulping to remove lignin can significantly reduce the degree of polymerisation, as these methods can degrade the glycosidic bonds present in polysaccharides, and therefore result in reduced pulp quality. Shorter cellulose polymers in synthetic and bacterially produced cellulose films have reduced strength [59]. Care must be taken in the use of methods used to isolate cellulose for the measurement of the degree of polymerisation as various cell wall extraction and solubilisation procedures can depolymerise the cellulose chains. Nitration is a commonly used technique to prevent depolymerisation [60].

Relative cellulose index is a type of measurement that combines the degree of cellulose polymerisation as well as cellulose structure. Relative cellulose index measurements of ball-milled Arabidopsis tissue reveal significant differences among tissue types, indicating that cellulose degree of polymerisation and structure are different in different cell types [61]. Given the range and functional
properties attributed to the degree of cellulose polymerisation in other species, clearly there is scope to survey accession variation in the degree of polymerisation in Arabidopsis inflorescence stems and secondary cell walls and to explore the relationship between the degree of polymerisation and the functional properties of secondary cell walls.

6.2. Crystallinity

Cellulose molecules can be arranged in highly crystalline arrays, particularly in the secondary cell walls of wood. However, it can also be found in less crystalline (more amorphous) forms, particularly in primary cell walls. Crystalline cellulose usually takes the forms of 1-α or 1-β cellulose which differ in how the cellulose chains are longitudinally staggered in the lattice, with the 1-β form being more stable. The 1-α form can be converted to the 1-β form by mechanical bending [62]. Cotton contains larger cellulose crystals and these are more of the 1-β crystal form (a comparison of cotton with linen, another strong cellulosic textile fibre, would be of great interest). Similarly, in gymnosperm wood the 1-β form predominates, with primary walls containing more of the 1-α form [62]. The level, magnitude, and type of cellulose crystallinity can contribute to the properties of the cell wall. For example, the more crystalline the cellulose in the cell wall, the greater the tensile strength of the cell wall will be (largely due to hydrogen bonding), and the more resistant the wall will be to expansion. This could then also affect the ultimate shape of the cell.

Insights into Arabidopsis cellulose crystallinity have begun to emerge. Cortical microtubules, particularly microtubule polymer mass, can affect the extent of cellulose crystallinity during rapid growth in mcr-1 temperature sensitive mutants. Mutants at the permissive temperature were identical to wild type, with long parallel cortical microtubules. At the restrictive temperature (29°C), the mcr-1 microtubules were much shorter and more disordered in their orientations. These changes in microtubule length and orientation were correlated with a higher degree of crystallinity in the mutant plants at the restrictive temperature. This crystallinity in turn resulted in isotropic cell expansion affecting the ultimate cell shape and plant stature [63], highlighting the role that cellulose crystallinity plays in physicochemical properties of cell walls.

Two cellulose synthase A (cesA) mutants, ageus and irc1-2 (a cesA1 and a cesA3 mutant, respectively) that have reduced cellulose crystallinity have been reported [64]. These mutant proteins are both functional subunits of the cellulose synthase complex, but they result in the production of cellulose microfibrils with decreased crystallinity [64]. Similarly, both mutants encode cesA subunits that have amino acid substitutions in their amino-terminal transmembrane-spanning domains, well-removed from the active sites of the enzyme subunits. Saccharification assays revealed that these mutants had substantially lower cellulose hydrolytic efficiencies relative to wild-type, particularly when they were combined in a double mutant [64]. These authors suggested that directed approaches to introducing analogous mutations to the Arabidopsis cesA1 ageus and cesA3 irc1-2 mutants in woody species could result in trees with superior pulping and saccharification characteristics for biofuel production [64].

Arabidopsis KORRIGAN1 and its poplar orthologue PtcCel9A1 are membrane-bound endo-β-(1 → 4)-glucanases that have been implicated in affecting cellulose crystallinity based on observations of decreased cellulose crystallinity in Arabidopsis KORRIGAN1 overexpression lines and increased crystallinity in KORRIGAN1 reduced-expression lines in regions of secondary growth [14]. The specific role of KORRIGAN1 is currently unclear and several hypotheses as to its mechanism of action, such as release of a primer molecule or control of cellulose chain length via release of the microfibril from cellulose synthase or potentially trimming “imperfections” in the microfibrils have been put forward, as reviewed in Ref. [55]. It will be interesting to follow the progress and application of the research in Arabidopsis cellulose crystallinity in secondary cell walls, and how this relates to that of various tree species.

7. Non-cell-autonomous signal molecules, woodiness and secondary woodiness

The vascular cambium, the cylinder of meristematic cells found in the stems of all species that have secondary radial growth, is responsible for the production of wood. The economic importance of wood has given rise to a multitude of studies directed towards understanding the molecular basis of vascular cambium function. From these, a complex regulatory network is beginning to emerge (see [4,20,21] for excellent reviews), which continues to grow in complexity as we discuss at various points in this review. Several non-cell autonomous signalling molecules participate in the regulation of the vascular cambium and/or xylem/phloem differentiation. The small-molecule fundamental plant growth regulators auxin and cytokinin [51] have not surprisingly been identified with roles in the regulation of the vascular cambium and/or radial growth (Fig. 3). Our understanding of auxin’s role in secondary growth is deepening, with several interesting reports. Basipetally transported auxin stimulates vascular cambium cell initiation and division, demonstrated using a cut inflorescence stem system to which exogenous auxin was applied [65]. Furthermore, this study demonstrated that this process is dependent on two genes encoding cell-autonomous receptor-like kinase family receptors, MORE LATERAL GROWTH-1 (MOL1) and REDUCED LATERAL GROWTH-1 (RUL1), which are named for their mutant phenotypes. Thus, MOL1 is an inhibitor of vascular cambium cell initiation and RUL1 stimulates vascular cambium cell initiation [65]. These data are consistent with the radial auxin gradient decreasing away from the vascular cambium (Fig. 3) and the stimulatory role of auxin in maintaining homeostasis of the meristematic state of the vascular cambium [66]. In this state, peak level auxin stimulates cell division, but inhibition of differentiation into vascular cells in tree cambium (Fig. 3). Under lower auxin concentrations, differentiation and maturation of the vascular cambium daughter cells occurs, likely in a concentration-dependent manner in conjunction with other factors [5,66] (Fig. 3). Consistent with this picture, the walls are thin (wat) mutation affects fibre cell wall, but not vessel wall thickness in inflorescences [67], which has obvious implications for our future understanding of wood formation in hardwood species. The molecular function of the tonoplast-localised WAT1 protein is not well understood, but auxin levels are sharply decreased in the wat1 mutant and inflorescence stems are significantly shortened [67]. The revoluta (rev) mutant also has low/disrupted auxin transport and fibre cells in this mutant are substantially lacking in lignin [68]. Several other signal molecules participate in vascular cambium maintenance and/or the regulation of xylem differentiation (Fig. 3), as we discuss below.

7.1. Gibberellin and secondary growth

The apparent stimulation of secondary growth in hypocotyls and roots of plants during bolting is an interesting phenomenon that has been described in Arabidopsis [40,69]. The so-called “Phase I/Phase II” growth stage changes, in which cambial meristem cells that previously differentiated into parenchymal primary cells undergo a change of developmental fate to secondary, thickened fibre cells in the hypocotyl [3] is brought about by floral induction and/or growth of the inflorescence stem [40]. This secondary growth stimulation is brought about in Arabidopsis and rosette....
plants generally by basipetally transported gibberellin from the inflorescence stem to the hypocotyl (Fig. 3) [69]. Gibberellins have been implicated in regulating secondary growth in poplar, particularly via transgenic approaches [5,70,71]. Xylogenesis and fibre cell elongation are affected by GA20 oxidase activity (a key step in producing precursor gibberellins) and these effects are governed by different GA receptors [71]. Thus, parallels between Arabidopsis and woody species are again seen.

7.2. Jasmonic acid

Ninety-two genes were identified that were preferentially expressed in the basal sections of inflorescence stems. These genes were profiled in comparison to apical section as putative secondary growth genes. Numerous jasmonic acid–regulated genes were identified within this set [72]. T-DNA insertion mutants in the jasmonic acid signalling repressor jasmonate zim-domain 10 (jaz10) gene had significantly enhanced radial expansion of vascular cambial regions in the stem. Conversely, the jasmonic acid signal transduction mutants corona-tine-insensitive1-1 (coi1-1) and the basic/helix-loop-helix transcription factor mxy3 had reduced thickness in their vascular cambium regions [72]. Mechanostimulation and jasmonic acid were shown to induce JAZ10 expression. The mechanostimulation-induced ethylene signalling transcription factor mutant ethylene response factor104 (erf104) also had reduced-thickness vascular cambium regions, thus connecting jasmonic acid and ethylene signalling. The mechanostimulated induction of the vascular cambium by jasmonic acid and ethylene signal transduction pathways likely results from physical stresses encountered by the stem base during stem elongation as a signal for increased xylogenesis, secondary thickening and wood formation to support the growing inflorescence.

7.3. Xylogen

Xylogen, an arabinogalactan protein, positively regulates in vitro xylem cell differentiation in the Zinnia tracheary element differentiation system (Fig. 3) [73]. In Zinnia, xylogen is secreted from xylem cells and it stimulates additional xylem development. Xylogen is a member of an 11–gene family in Arabidopsis with a wide variety of expression patterns [74]. Phylogenetic analysis reveals two potential orthologues of this gene in Arabidopsis as well as poplar [74], so it is possible that one or more xylogen orthologues have roles in wood formation in poplar.

7.4. CLE41/44-TDF and ethylene

In contrast to xylogen, the CLAVATA3/EMBRYO-SPECIFIC REGION 41/44-TRAJECTORY ELEMENT DIFFERENTIATION FACTOR (CLE41/44-TDF) genes encode a highly evolutionarily conserved polypeptide ligand [75,76], that inhibits xylogenesis (Fig. 3). Overexpression of CLE41 using a Cauliflower Mosaic Virus 35S promoter or the xylem-specific IRREGULAR XYLEM-3 promoter results in dramatic disruption of both xylem and phloem (and presumably vascular cambium) development [76]. CLE41/44-TDF signalling is mediated by the receptor PHLOEM INTERCALATED WITH XYLEM/TDF RECEPTOR (PXY/TDR) [76,77]. Null mutants of pxy/tdr also have disrupted vascular development (reviewed in Ref. [21]), but this phenotype is mild, suggesting redundancy or compensatory signalling pathways in xylogenesis and vascular cambium homeostasis. Analysis of microarray data from inflorescence stems of pxy/tdr null mutants revealed strong increases in transcript abundance of several APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) transcription factor genes relative to wild-type controls, suggesting a role for ethylene in vascular development [78]. Double erf109-erf018 mutant inflorescence stems had reduced average numbers of cells per vascular bundle relative to wild-type. Triple pxy/tdr-erf109-erf018 mutants had the greatest reduction in vascular bundle cell number. Ethylene-overproducing mutants had enhanced vascular cell division and the pxy/tdr mutant phenotype was increased in ethylene insensitive mutants [78]. Taken together, these experiments reveal that an ethylene-mediated signalling pathway compensates for loss-of-function of the PXY/TDR receptor. This ethylene-mediated pathway is normally suppressed in the presence of a functional PXY/TDR receptor (Fig. 3) [78]. Interestingly, the stimulation of cambial cell initiation and cell division by auxin was demonstrated to occur in a PXY-dependent manner [65], so ethylene apparently does not compensate for this...
auxin transport-stimulated process, at least not directly. It is possible that the receptors MOL1 and RUL1 that regulate this process downstream of PXY [65], also have CLE peptides as ligands. These findings are likely to be highly relevant for wood development in both hardwood and softwood tree species, as ethylene has long been implicated in xylegenesis and reaction wood formation in tree species [79]. Other peptide ligands have been implicated in vascular development, if not wood formation per se (reviewed in [75]).

Most of the research on the molecular basis of vascular cambium homeostasis regulation and/or vascular cell differentiation has been carried out in angiosperm species, including Arabidopsis. Conifer species also form and maintain a vascular cambium and make wood. Understanding the similarities and differences in the regulation of wood development in hardwood (angiosperm) and softwood (gymnosperms-conifers) species will strengthen our understanding of this fundamental process. Inroads are starting to be made in bridging this gap using data acquired first in the Arabidopsis wood model. The perfect evolutionary conservation of the CLE41/44 peptide ligand sequence extends to conifers (Strabala et al., submitted for publication). Furthermore, the genes encoding these peptides are expressed in phloem and not in xylem (Strabala et al., submitted for publication; Fig. 3), as they are in Arabidopsis [76,77]. One of these genes is expressed at approximately half the level of the other in Pinus radiata phloem and roots (Strabala et al. submitted), intriguingly paralleling AtCLE41 and AtCLE44 expression in inflorescence stems and roots [7]. This observation raises the question as to why the relative differential expression levels of genes presumably fulfilling identical functions in such widely divergent species [80] would be conserved over approximately 300 million years of evolution. This possibly reflects a conserved aspect of regulation of vascular differentiation that is not fully understood. The redundancy of CLE genes/peptides currently appears to be unique among the known CLE-based meristematic regulation systems. Shoot apical meristem regulation appears to only utilise CLV3 [81], and the root apical meristem, only CLE40 [82], based on mutant data. In contrast, neither cle41 mutants, nor cle41/42 double mutants had discernable phenotypes [76], strongly suggesting functional redundancy of the CLE41 and CLE44 (and perhaps CLE42, although its expression levels are substantially lower than either CLE41 or CLE44 [7]) genes.

The CLE42 peptide/gene, despite its clear ability to create phenotypes indistinguishable from CLE41/44-TDIF when exogenously applied or overexpressed [7,83], is only very weakly expressed relative to CLE41/44 [7]. GUS expression analysis of the CLE42 promoter reveals that the gene is only weakly expressed in the vascular cambium or developing vasculature, with preferential expression in the shoot apical meristem and axillary buds [84]. Exogenous CLE41 or CLE42 application had almost no effect in pxy/tdr mutants, so CLE42 may have a PXY/TDR-mediated role in axillary bud formation [84]. Such a role would certainly be consistent with the “shrub-like” overexpression phenotypes of these genes [7].

7.5. Is the presence of a vascular cambium indicative of the potential for woodiness?

The vascular cambium is a feature held in common among conifer and dicot angiosperm species, despite ancient evolutionary divergence [80]. The vascular cambium is not observed in monocot angiosperm species (which do not make wood in the classical sense, although some species have wood-like stems as discussed later in this section) irrespective of their more recent evolutionary divergence from dicots [80]. This distinction raises an interesting and important question: although the vascular cambium is required to make wood, does the presence of a vascular cambium indicate/predict the ability to make wood? An increasing amount of evidence suggests that this may be the case.

As described in the opening section of this review, the xylem transcriptomes of woody and non-woody angiosperms are remarkably similar to one another as well as to pine. The capacity for secondary woodiness (the evolution of woody traits from herbaceous ancestors) and/or insular woodiness (secondary woodiness resulting from geographic isolation and the lack of woody competitor species) is well-established in a wide range of dicotyledonous species [16,85]. The conservation of genes that have roles in both the shoot apical meristem and the vascular cambium may place a strong selective pressure on the retention of wood-forming potential [22, reviewed in 86]. However, we think that there is strong selective pressure on genes that are specific to the vascular cambium as well. No known woody plant species lacks putative CLE41/44-TDIF gene orthologues (Table 1). We suggest that the presence of these genes in herbaceous plants is indicative of the capacity for secondary woodiness.

The results of a TBLASTN search of the NCBI/DBJ/EMBL whole-genome sequence, general nucleotide and EST databases against “green plants” (TAXID 33090) are shown in Table 1. Perfect matches and hits with single amino acid differences from CLE41/44 were included in the table. Virtually all the perfectly conserved hits come from herbaceous and/or woody/shrubby/perennial dicots. All monocots (except one) lack a perfectly conserved orthologue. The one exception to this is Phoenix dactylifera (date palm). A closer examination of the P. dactylifera genome sequence is consistent with this sequence being derived from a bona fide CLE gene (T. Strabala, unpublished observations). Perhaps as the exception that proves the rule, stem thickening in many palm species appears to come from diffuse secondary growth or so-called secondary thickening meristems, because palms, like all monocots, lack a vascular cambium [87, and references therein]. It is possible that other monocot species that display secondary growth characteristics, such as from the genera Cordyline [88] and Dracena, may also retain a gene or genes encoding a CLE41/44-TDIF peptide, which might suggest a more generic role for CLE41/44-TDIF in secondary growth, either with or without a vascular cambium. It could be argued that the single amino acid changes seen in the other monocot CLE41/44-like putative peptide sequences could still function as CLE41/44-TDIF peptides. However, the apparent non-vascular cambium role played by CLE42 with its single amino acid difference relative to CLE41/44-TDIF [84], tends to mitigate against such a hypothesis in vivo. Moreover, different CLE peptide sequences have different affinities for CLE receptors [89]. Even single amino acid changes can affect the binding affinity of a peptide ligand, to the point of creating an antimorphic phenotype [90,91], so such differences would likely be significant. Understanding where in monocot plants these CLE41/44-TDIF-like genes are expressed, with particular attention paid to the vasculature, is a logical first step in understanding their role(s) in monocot development. Examination of the phenotypes of transgenic monocots that overexpress CLE41/44-TDIF would additionally be of great interest in understanding the differences in vascular growth among these taxa.

Although Arabidopsis is capable of wood development, even the most extreme woody phenotype displayed by this species is severely pleiotropic [16] and cross sections of these plants reveal that they lack many characteristics of woody species, e.g. the retention of a substantial region of parenchymal pit tissue [16,22]. It is unknown what the orthogonal suppressor of overexpression of constans/fruittul (soc/ful) double mutations would look like in a truly woody species. Generation of SOC/FUL knock-downs in transgenic poplar would likely be very enlightening as to the exact roles played by these genes in wood formation. On the other hand, the soc/ful double mutant bears strong resemblance to secondarily woody members of the Brassicaceae, which have paedomorphic features (e.g. a lack of rays) [22]. It is possible that the transition to “true” woodiness requires mutations in many more genes. It is also
Table 1
CLE41/44-TDIF and related peptide sequences in land plant species.

<table>
<thead>
<tr>
<th>CLE41/44-TDIF</th>
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<td>GVPSPNPISN (CLE42)</td>
<td>Arabidopsis thaliana</td>
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<tr>
<td><strong>Species:</strong></td>
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<tr>
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<td>Zinnia elegans (TDIF)</td>
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<td>Phoenix dactylifera</td>
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Results of a TBLASTN search of GenBank/EMBL/DDBJ entries using the CLE41/44-TDIF peptide sequence as the query. The BLAST search was performed on EST entries from "green plants" (TAXID 33090), using the PAM30 matrix (best for short sequences), low complexity filter off, and an E value threshold of 10,000 using only the conserved CLE41/44-TDIF peptide sequence as a query. Perfect matches and putative peptide sequences with a single mismatch are shown here. Key to the colour code: black lettered binomials: herbaceous dicots; orange-lettered binomials: woody/shrubby dicots; blue lettered binomials: monocots. On the amino acid sequences, the black highlighted white letters are the tandemly conserved characteristic amino acids of the CLE41/44-TDIF family [7]. Turquoise highlighted letters represent changes in the sequence of any of these three conserved amino acids. Grey background letters represent other single amino acid differences from the CLE41/44-TDIF consensus.

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possible that true woodiness cannot be achieved in species that are capable of secondary woodiness. However, the comparison of the Arabidopsis model system to woody species is likely to continue to yield additional clues towards settling such issues.

8. Evidence for other wood-like features in the inflorescence stem

The focus of the Arabidopsis wood model so far has largely been on the vascular cambium and the xylem vessel and fibre cells, with secondarily thickened cell walls. However, a few publications highlight other features of Arabidopsis growth with potential relevance to wood and wood modelling as discussed below.

8.1. Phloem fibres

Phloem fibres (often referred to as bast fibres) have very thick secondary cell walls and are the main cells used in the commercially important textile fibres of flax, jute, ramie and hemp. Their cell walls frequently, but not always, contain lignin (flax fibres contain very low levels of lignin [92]). Phloem fibres can be found in trees on the inner surface of bark. Arabidopsis has long been known to have phloem fibres, but these have not received extensive attention and analysis. Often times, they are absent and their presence is not necessarily readily reproducible. Nonetheless, phloem fibres have been reported in the Arabidopsis C24 and Columbia accessions [93], both in the radially thickened hypocotyl and in the inflorescence stem, and are located either as single cells or in clusters in the inner cortex of these tissues. More phloem fibres are often apparent in the radially thickened hypocotyl than the inflorescence stem [93]. Phloem fibres have also been visualised in a range of other Arabidopsis accessions [31]. As in Columbia and C24, they can be seen in the basal regions of the inflorescence stem as single cells or clusters of cells, with cell walls histologically similar to those of interfascicular fibres (Fig. 1).

The molecular pathways that control secondary cell wall formation in xylem vessels and fibres of Arabidopsis may also be involved in controlling secondary cell wall formation of phloem fibres. NO APICAL MERISTEM/ATAF/CUP-SHAPED COTYLEDON (NAC) transcription factors participate in the control of this process in Arabidopsis xylem vessels and fibres [94]. NAC orthologues are known to control secondary cell wall development in poplar [95]. Overexpression of poplar secondary cell wall NAC orthologues in Arabidopsis resulted in the ectopic formation of phloem fibre cells in the inflorescence stem cortex (among other phenotypes) [95]. This apparent overlap in the molecular control of secondary cell wall formation in xylem and phloem fibres suggests that Arabidopsis phloem fibre development has relevance to wood in addition to xylem vessels and fibres. Examination of Arabidopsis phloem fibres in mutants, recombinant inbred lines and accession variants should thus provide additional insight into secondary cell wall development.

8.2. Rays

Rays are features of wood in trees and stems of woody plants like cotton as well as herbaceous species such as tobacco. Rays are made up of living parenchymal cells that originate from the vascular cambium. In trees, they often extend across wood growth rings. Ray cells serve to transport nutrients and assimilates radially across the woody stem, as well as to provide storage of carbohydrates. Rays have been reported in Arabidopsis inflorescence stems grown under particular conditions, which included adding weights to the stem shoot apices [96]. The “rays” were single files of two to seven cells.

This report suggests further scope for Arabidopsis wood modelling by extending it to other cell types found in wood.

8.3. Storied interfascicular cambium

In addition to the discovery that ray-like cells can be produced in Arabidopsis inflorescence stems, storied cambial cells appear under the same conditions that stimulate ray development [96]. These researchers go on to suggest that they are similar to storied cambial cells of trees [96]. This suggestion becomes more compelling with the awareness that storied cambium is found in some woody genera of the family Brassicaceae, of which Arabidopsis is a member [97]. The implication of this work is that, from the perspective of the vascular cambium, Arabidopsis could be a closer model to wood than has been traditionally thought, particularly when inflorescence stems are used. Researchers are encouraged to pay particular attention to the interfascicular region of the inflorescence stem for observation of the cambial cells that develop into xylem fibres as they may have been overlooked in the past.

8.4. “Tension wood” G layers

As alluded to briefly in Section 2.2, Arabidopsis inflorescence stems appear to respond to repeated bidirectional mechanical bending by the development of shorter, more flexible, apparently thinner-walled inflorescence stems as compared to un-bent stems [33]. It is unclear from this study whether a typical woody angiosperm response to bending or other thigmomorphogenic stimuli, i.e., tension wood, is present. The stains used for the microscopic examination of the stems in these experiments do not readily detect the gelatinous cellulose G-layer present in tension wood. Evidence for a G-layer in other herbaceous species (e.g. Nicotiana) has been noted, but no evidence was found for tension wood in radially thickened hypocotyls [98]. A more recent study utilising gravistimulated, decapitated Arabidopsis inflorescence stems reported the presence of a cellulose-rich G layer [99]. The G-layer was only seen in plants cultivated under high light combined with short-day conditions. The plants had also had their inflorescence stems repeatedly decapitated before allowing the experimental inflorescence stems to elongate. This interesting result appears to be rather preliminary and further experimentation is necessary to provide more support and detail to the findings. It would also be of interest to determine if Arabidopsis inflorescence stems might be stimulated to form tension wood using a third approach, i.e., unidirectional bending of long-day-grown inflorescence stems, possibly by using a fan, to simulate a prevailing wind. If successful, such a technique could bring the study of tension wood into the realm of accession variation analysis. The G-layer might also be more readily detected using confocal microscopy [98] or the use of specific stains.

9. Future prospects and concluding remarks

The developmental biological underpinnings of the Arabidopsis radially thickened hypocotyl justifying its use as a wood model were established some time ago [3]. The Arabidopsis inflorescence stem has also been used extensively as a wood model, arguably with less experimental justification than exists for the radially thickened hypocotyl. However, studies in the last few years have explicitly examined the inflorescence stem for wood-like characteristics such as cellulose microfibril angle, biomechanical characteristics and cell wall carbohydrate content and have compared their correlations with such characteristics in wood [30,31,33]. This work has given the use of the inflorescence stem a firmer biochemical/biophysical footing as a model for wood development.

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The inflorescence stems of long-day-grown plants appear to be ideal for many experiments regarding wood-like traits. The radially thickened hypocotyl model [3] generally requires several months of growth under short-day conditions, with concomitant increases in space considerations for experiments. In contrast, inflorescence stems grown in long days can be produced in a few weeks and they have more wood-like biomechanical properties when dried than short-day-grown inflorescence stems, owing to a lower proportion of primary parenchymal tissue relative to secondary thickened cells [31]. Growth in long-day conditions appears not to be a barrier for further studies of secondary growth in hypocotyls as well, because flowering stimulates the expansion of xylem in the hypocotyl [40,69].

The ability to grow more plants faster with inflorescence stems that are still useful for the examination of wood-like traits readily lends itself to the application of large-scale accession variation studies. Current limiting factors that might slow progress are the labour-intensive and/or expensive techniques that currently must be applied to examine traits such as microfibril angle and neutral carbohydrate content. The use of synchrotron light sources (which are becoming more generally available and can be automated) should significantly speed up the analysis of microfibril angle. Other analytical techniques that could be brought to bear on secondary cell wall studies include near-infrared spectroscopy and Fourier-transform infrared spectroscopy. Near-infrared spectroscopy has been used to predict wood properties in various tree species, e.g. [100,101]. It is potentially valuable to use similar techniques on Arabidopsis inflorescence stems. However, careful calibration is required [100]. Fourier-transform infrared spectroscopy has been used to examine cell wall composition including cellulose, lignin, and hemicelluloses in primary cell walls of Arabidopsis natural accessions [102]. Such a technique could presumably be used in secondary cell wall analysis as well. In any case, more development of higher throughput methods of detailed cell wall component analysis is required before the use of thousands of accessions can be considered feasible for such studies, but this is well within reach.

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References


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