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Review

Structure, Modification Pattern, and Conformation of Hemicellulose in Plant Biomass

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Abstract: Different forms of plant biomass have been utilised for various applications in daily life and have gained increasing attention as replacements for fossil fuel-based products in the pursuit of a sustainable society. Plant cell walls, the primary carbon sink of plant biomass, have a high-order polysaccharide architecture consisting of cellulose, hemicelluloses, pectins, lignin and some proteins. Hemicelluloses are a group of polysaccharides that interact with cellulose, which is fundamental to the different properties and functionality of the plant cell walls. However, for industrial applications, the complex polysaccharide architecture poses a barrier to their efficient use. Understanding the molecular basis of plant cell walls – especially cellulose-hemicellulose interactions – is therefore critical to improving the utilisation of plant biomass. Recent research has revealed that the detailed structures, modification patterns, and conformation of hemicelluloses play an influential role in their interaction with cellulose. In this review, we discuss the latest insights into hemicelluloses across different forms of plant biomass and how their structures affect cell wall assembly. Additionally, we explore recent findings on how alterations in hemicellulose structure and modification patterns affect the usability of plant biomass, including the extractability of polysaccharides and the digestibility of biomass by glycoside hydrolases for biofuel production. Furthermore, we address unsolved questions in the field and propose future strategies to maximize the potential of plant biomass.

Key words: biomass, plant cell wall, hemicellulose, mannan, xylan, xyloglucan

INTRODUCTION

Plants make up 80 % of all biomass on Earth [1], most of which is stored in plant cell walls, a complex matrix of polysaccharides surrounding plant cells. In human history, we have been utilising such natural plant materials for many applications, such as construction, textile, paper, foods, forage, and feedstocks for biofuels. These renewable sources have recently gained more attention as a means to address global challenges, including climate change, and to promote a sustainable society.

The plant cell walls are a complex network of polysaccharides, consisting of cellulose, hemicelluloses, pectins, lignin, and glycoproteins. The cell walls in developing tissues are called primary cell walls, and are thin yet flexible and extensible, allowing cell expansion and elongation [2, 3]. In mature tissues, such as woody parts of plants, on the other hand, thick layers of cell walls called secondary cell walls are deposited inside the primary cell walls, which provide mechanical support to cells [4]. These completely different properties are attributed to the composition of polysaccharides and their interactions in the cell wall architecture. Cellulose is a flat ribbon-like composite structure made of β-1,4-glucan chains that assemble into a fibril and interact with the other cell wall components. Hemicellulose is a term for a group of non-cellulosic polysaccharides that share a β-1,4-linked backbone with a variety of backbone composition and side chain structures [5]. Hemicelluloses are known to interact with cellulose microfibrils and are thought to play important roles in determining cell wall properties possibly by arranging the cellulose microfibril network. Over the past decade, it has been reported that certain periodic substitutions of hemicellulose play an important role in its interaction with cellulose. Understanding the precise structures and how they affect cell wall architecture helps design optimal processes for industrial applications and the production of novel plant-based materials. In this review, we discuss current views on hemicellulose fine structures, patterning, conformations, and interactions with cellulose found in

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Abbreviations: AcGGM, acetylated galactoglucomannan; Ara*f*, L-arabinofuranose; Ara*p*, L-arabinopyranose; AXe, arabinoxylan with an even distribution of Ara*f* substitutions; β-GGM, β-galactoglucomannan; *esk1*, *eskimo1*; Fuc, L-fucose; Gal, galactose; GAXc, glucuronoarabinoxylan with clustered GlcA substitutions; Glc, glucose; GlcA, glucuronic acid; hsGAX, highly substituted glucuronoarabinoxylan; MD, molecular dynamics; MeGlcA, 4-*O*-methyl-glucuronic acid; PDSD, proton-driven spin diffusion; *RAW*, *REDUCED WALL ACETYLATION*, ssNMR, solid-state nuclear magnetic resonance; *tbl29*, *trichome birefringence-like 29*; *xax1a*, *xylosyl arabinosyl substitution of xylan 1a*; Xyl, xylose.

different forms of plant biomass as well as how these structures and interactions affect biomass recalcitrance. We present perspectives of future directions for research on hemicellulose, for a better use of plant biomass.

HEMICELLULOSES IN PLANT BIOMASS

Hemicelluloses are in general tightly bound to the cell wall matrix (with some exceptions) as demonstrated by the requirement of alkali treatment for their extraction [5]. Although most of them fall into similar structures, the precise structures vary depending on the plant species and tissue. Here, we focus on the major hemicelluloses in biomass – xylan, β-mannans, and xyloglucan.

Xylan consists of a β-1,4-linked xylosyl (Xyl) backbone with modifications, such as glucuronic acid (GlcA), L-arabinofuranose (Ara*f*), and acetyl group.

β-Mannans are composed of β-1,4-linked mannosyl (Man) and varying amounts of glucosyl (Glc) residues. The Man may carry α-1,6-linked galactosyl (Gal) residues and acetyl groups at *O*-2/3 of Man. Recently, a new structure has been discovered [6, 7], which is distinct from the conventional β-mannans mentioned above. It has a backbone of strictly repeating Glc-Man with β-1,2-linked Gal substitutions on the α-1,6-linked Gal. This structure was named β-galactoglucomannan (β-GGM) in comparison to the conventional acetylated galactoglucomannan (AcGGM).

Xyloglucan has a backbone made of β-1,4-linked Glc, with up to three-quarters (depending on plant species) of which are substituted with Xyl. The Xyl sidechains are further substituted with varying mono- or disaccharides, including predominantly Gal, L-fucose (Fuc), and Ara*f*, while other less abundant modifications have also been reported [8, 9]. More detailed structures in different biomass will be discussed in the following sections.

These hemicellulose structures have long been studied; therefore, their basic structures are well known. In the past, the structural analysis of polysaccharides relied on acid or enzymatic hydrolysis of polysaccharides into small mono- and oligosaccharides, therefore, losing structural information such as patterning and distribution of decorations. In addition, the structural analysis of hemicelluloses requires strong alkali extraction, which also results in the loss of some information such as acetyl esterification, and interactions between hemicelluloses and cellulose. Over the last decade, however, those limitations have been overcome by the use of a variety of glycoside hydrolases with different substrate specificities, enabling us to analyse overlooked pattern structures on hemicelluloses. Furthermore, the cutting-edge techniques of solid-state ^{13}C nuclear magnetic resonance (ssNMR) allow the analysis of intact cell wall architecture without disturbing the original interactions between hemicelluloses and cellulose [10−18]. Generally, the sensitivity of a ^{13}C spectrum is low due to the natural abundance of ^{13}C, which is only about 1 %. To enhance the sensitivity, plants can be grown in the presence of ^{13}C-labelled Glc or $^{13}CO_2$, enriching the ^{13}C content in plant biomass. With ^{13}C-enriched biomass, two-dimensional experiments, such as INADEQUATE and proton-driven spin diffusion (PDSD) can be performed. These experiments allow observation of covalently bound ^{13}C-^{13}C correlation peaks and through-space proximity of ^{13}C, respectively, leading to high-resolution peaks that distinguish chemically similar cellulose and hemicelluloses. Furthermore, in ssNMR, detected chemical shifts are sensitive to the conformational changes and dynamic behaviour of atoms, bringing us closer to understanding the molecular mechanism of cell wall architecture formation and how to improve the use of natural resources.

Hemicelluloses in woods.

Woods are a great source of secondary cell walls. The cell wall chemistry of different woods has been intensively characterised in the 1950s−1980s [19, 20]. The woods are categorised based on taxonomical differences – hardwood from deciduous trees and softwood from coniferous trees. The variation regarding hemicellulose structures between plant species is less compared to that of the primary cell walls; however, there are clear distinctions in hemicellulose compositions and structures between hardwood and softwood [21].

In hardwood, xylan is the predominant hemicellulose (15−30 %) [5, 19], which carries α-1,2-linked 4-*O*-methyl-glucuronic acid (MeGlcA) and acetyl groups on C2- and C2- and/or C3-hydroxyl of xylosyl residues, respectively. It has recently been reported that these modifications on xylan show periodic patterns in Arabidopsis (*Arabidopsis thaliana*, a model eudicot plant with xylan structure similar to hardwood) [14, 20, 22, 23]. With regards to [Me]GlcA modifications on xylan, two domains have been described (Fig. 1A): a major domain with evenly spaced [Me]GlcA modifications (predominantly every 6, 8, 10 xylose residues) and a minor domain with [Me]GlcA spaced every 5, 6 or 7 Xyl residues [14, 22], by which overall glucuronidation per Xyl falls into 10−12 % [20, 22]. The even pattern on xylan decorations as well as a trivial amount of consecutive [Me]GlcA decoration have also been found in hybrid aspen [24]. Acetylation was also found to be evenly spaced [23, 24]. Although xylan in solution adopts a flexible threefold helical screw conformation [25], it changes the conformation in cell walls. Molecular dynamics (MD) simulations predict that xylan with the even pattern of decorations could adopt a conformation compatible for interaction with cellulose [22, 23]. Indeed, ssNMR revealed that xylan is induced to a flat, twofold helical screw conformation when it binds to cellulose [11]. This means that all substitutions orient on one side of the xylan chain, permitting xylan to dock onto cellulose by hydrogen bonding or stacking interactions presumably with the hydrophilic surfaces of cellulose. On the other hand, xylan with the uneven pattern of decorations disrupts the interaction with cellulose due to steric hindrance of the randomly oriented decorations, leaving the unbound xylan with a flexible threefold helical screw conformation [11, 14, 23, 26]. There is a small amount of AcGGM (2−5 %) in hardwoods with a ratio of Man:Glc = 1−2:1 (Fig. 1A) [19]; however, no structural patterning has been reported. Little is known about conformation of AcGGM and its interaction with cellulose in hardwood.

Unlike hardwoods, the major hemicellulose of softwood is AcGGM (15−20 %) [5, 19, 20]. Although the structure of softwood AcGGM is similar to that in hardwood, there are two structurally distinct populations – one with the ratio of Man:Glc:Gal = 4:1:0.1−0.3 and another with the ratio of Man:Glc:Gal = 3:1:1−1.2 (Fig. 1B) [19, 27]. Those two

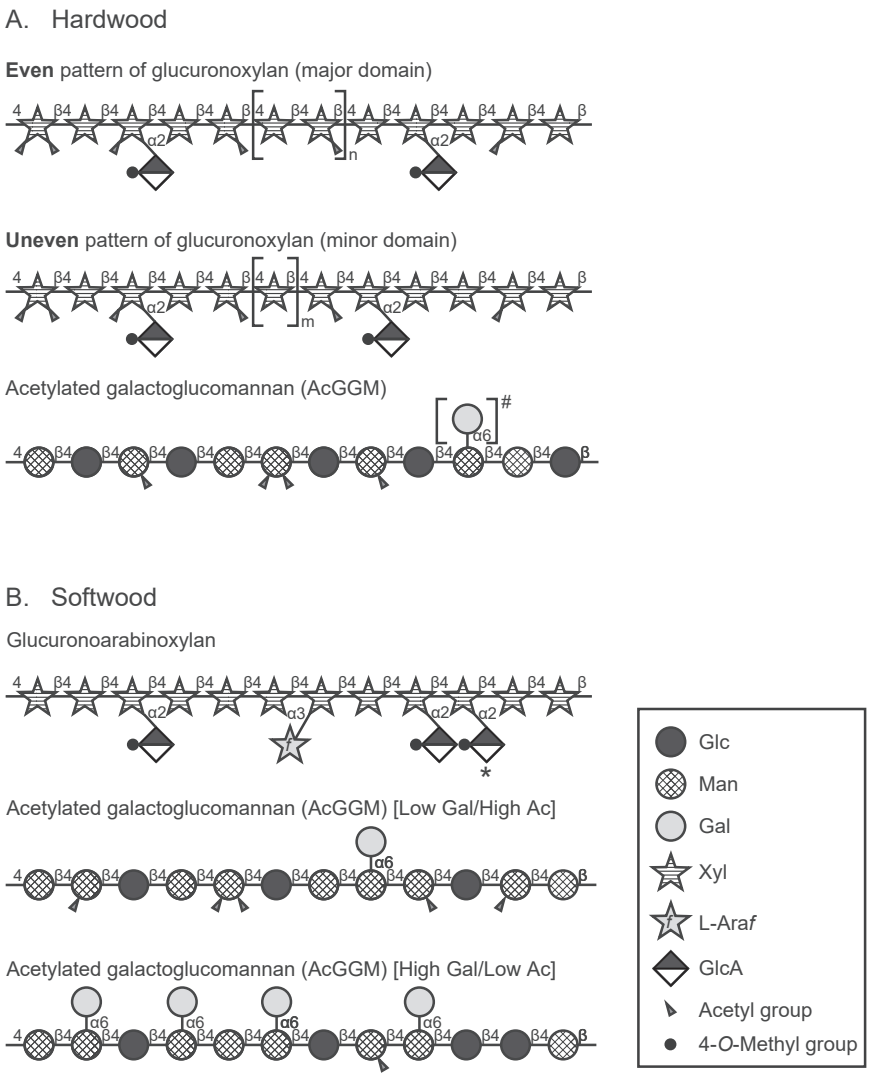

Fig. 1. Hemicellulose structure and patterns in wood.

(A) Major hemicelluloses in hardwoods. Wood xylan consists of domains with even spacing of MeGlcA (spaced predominantly every 6 Xyl residues; n = 0, 1, 2, 3…) and domains with uneven pattern of MeGlcA distribution (m = 0, 1, 2, 3). Note that domains with even and uneven MeGlcA spacing are found on the same xylan molecule [22]. Acetylation is also evenly distributed on the xylan backbone [23, 24]. There is no structural pattern on hardwood acetylated galactoglucomannan (AcGGM) so the arrangement of Glc and Man is random. Number (#) indicates that hardwood AcGGM is occasionally branched by a Gal side chain but the frequency is very low. AcGGM has acetylation at *O*-2 and/or *O*-3 of Man. (B) Major hemicelluloses in softwoods. Softwood glucuronoarabinoxylan lacks acetylation but is modified with Ara*f* and MeGlcA residues. MeGlcA on softwood xylan is precisely spaced every 6 Xyl residues and Ara*f* is found in two Xyl residues apart from MeGlcA [29]. Asterisk (*) indicates consecutive glucuronidation, which is a minor component [32]. Softwood AcGGM has no structural pattern. There are two different fractions of AcGGM in softwood: one with low Gal and high acetylation and another with high Gal and low acetylation [19, 27].

AcGGM fractions have different degrees of acetylation, 0.4 and 0.1, respectively [19, 27]. The ssNMR on spruce (*Picea abies*) exhibited that AcGGM is in close proximity with cellulose, implying the interaction of glucomannan with cellulose in the cell walls [13]. Furthermore, the ssNMR on pine wood (*Pinus radiata*) revealed that a fraction of AcGGM irreversibly changes its conformation by drying and rehydration and no longer interacts with cellulose [12]. It is unclear whether a certain fraction of AcGGM is sensitive to the process; however, it suggests that the structural difference of AcGGM may affect their hydrodynamic behaviour and interaction with cellulose in the cell wall architecture.

Softwoods have less xylan (7−10 %) and their structure is slightly different compared to hardwoods (Fig. 1B); however, the even spacing of substitutions is also conserved in softwoods. Softwood xylan lacks acetylation but is modified with Ara*f* and MeGlcA residues. MeGlcA on softwood xylan is precisely spaced every 6 Xyl residues and Ara*f* is found two Xyl residues apart from MeGlcA [20, 22, 28, 29]. Minor portions of consecutive glucuronidation have also been reported in softwoods [28, 30−32]. MD simulation suggests that the even spacing of decorations allows softwood xylan to form the twofold screw conformation, which is important for binding to cellulose [26, 29]. *In vivo* interaction of softwood xylan with cellulose was experimentally evident by ssNMR [12, 13].

Despite the detailed information on hemicellulose structures and decoration patterns, understanding how cell wall architecture is formed and how individual hemicelluloses contribute to the material properties needs further investigation.

Hemicelluloses in grass cell walls.

Grasses, such as rice and wheat, are not only important crops for human nutrition and animal forage but also an important feedstock for biofuel production – especially the remaining biomass after harvest (i.e., field residues and process residues). Xylan is the main component of grass cell walls comprising up to 50 % of the dry weight of grasses and cereal grains [21]. Similar to dicots, grass xylan is modified with [Me]GlcA at *O*-2 and acetyl groups at *O*-2 and/or *O*-3 positions of Xyl residues. However, grass xylan is also modified with Ara*f* residues [33, 34]. This Ara*f* can be further substituted with β-1,2-Xyl or esterified at *O*-5 to hydroxycinnamic acid groups (e.g., ferulic acid or *p*-coumaric acid) [35−39]. The type and frequency of grass xylan side chains depend on tissues reflecting the different functionalities of xylan molecules. Cereal endosperm arabinoxylan is highly decorated with Ara*f* with less acetylation, which gives arabinoxylan water retention properties to maintain the hydration of the seed (Fig. 2A). On the other hand, xylan in vegetative grass tissues has decorations of [Me]GlcA, Ara*f* and acetyl groups, and cross-links cellulose fibres or lignin, providing high mechanical support and waterproofing properties to these tissues (Fig. 2B). Grass xylan ferulation mediates xylan-xylan cross-links via diferulate bridge formation or cross-links xylan to lignin [40, 41]. The [Me]GlcA substitutions of xylan may also link to lignin through esters [39−41].

It was recently proposed that grass cell wall xylan is structurally diverse, consisting of at least three different xylan domains (Fig. 2B): an arabinoxylan with even distribution of Ara*f* substitutions (AXe); a highly substituted glucuronoarabinoxylan (hsGAX) and a glucuronoarabinoxylan with clustered [Me]GlcA substitutions (GAXc) [42]. GAXc was shown to comprise two subdomains: a domain with clustered [Me]GlcA modifications spaced every 5 or 7 Xyl residues and a domain with evenly distributed [Me]GlcA, predominantly every 6 Xyl residues [39]. It has been proposed that those xylan populations are important to form a high-ordered cell wall architecture through xylan-cellulose interaction,

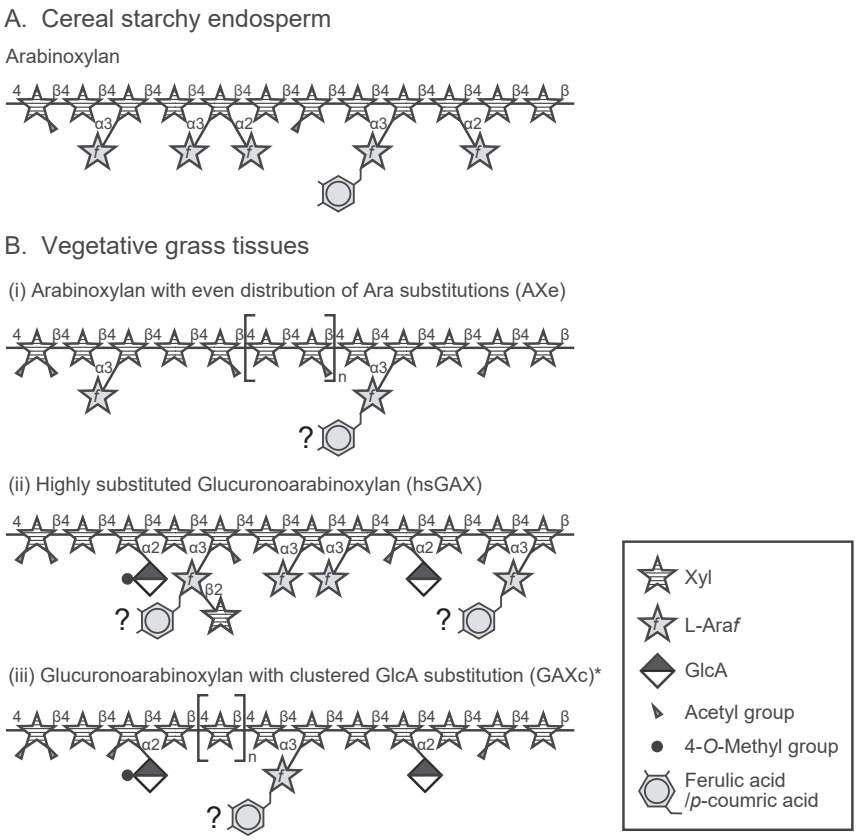

Fig. 2. Xylan structures in grass cell walls.

(A) In the starchy endosperm of cereal grains, xylan lacks [Me]GlcA modifications and Xyl residues can be doubly modified with Ara*f* side chains. (B) The cell walls of vegetative grass tissues contain both Ara*f* and [Me]GlcA modifications [34]. At least three different types of xylan are present in grass cell walls [42]: (i) an arabinoxylan with even distribution Ara*f* substitutions (AXe); (ii) a highly substituted glucuronoarabinoxylan (hsGAX); (iii) a glucuronoarabinoxylan with clustered GlcA modifications (GAXc). GAXc consists of two subdomains: a domain with predominantly even distribution of [Me]GlcA residues and a subdomain with clustered [Me]GlcA residues spaced every 5 or 7 Xyl residues [39]. Some Ara*f* residues on these xylans could be feruloylated (indicated by question marks). The arrangement of acetyl and feruloyl substitutions on different xylans and the position relative to other xylan side chains is unknown [42]. Note that both GlcA modified with a 4-*O*-methyl group and its non-methylated counterpart are present on grass xylan.

xylan-xylan, and xylan-lignin cross-links [39, 42]. Although questions regarding which xylan type(s) interact with cellulose/lignin remain open, the twofold screw conformation of xylan that interacts with highly ordered cellulose was evident by ssNMR of never dried Brachypodium (*Brachypodium distachyon,* a model grass system) tissues [16].

Hemicelluloses in fruits.

Fruit and their processed residues are potential sources of bioenergy and production of valuable polysaccharides for applications. The cell walls in fruit are mainly primary cell walls. Unlike the other biomass, fruit primary cell walls are rich in pectic polysaccharides, consisting of 25 % of total mass [43]. Xyloglucan is the major hemicellulose in the primary cell walls [5, 8, 43]. There are two types of xyloglucans with distinct repeating units – XXXG- and XXGG-types (Fig. 3) [5, 8]. The former is a common structure for most eudicots where the xyloglucan oligosaccharide unit has xylosylation on three out of four Glc residues from the non-reducing end. On the other hand, the latter unit possesses two Xyl modifications occurring on cellotetraose and is specifically found in Solanaceae plants, such as tomato, potato and tobacco [5, 8]. The Xyl residues are further substituted; for example, of the XXXG unit in Arabidopsis, β-1,2-Gal residues can decorate at the second and third position from the non-reducing end of the unit and an α-1,2-Fuc can be further added on Gal at the third position. For the XXGG-type in tomato, on the other hand, the Gal substitutions are often replaced by α-1,2-Ara*f* and the third Glc on the backbone can be acetylated. These diverse side chains depend on plant species, and 24 types of side chains have been identified across the plant kingdom [8]. The MD simulation suggests a trisaccharide side chain {α-Fuc-(1→2)-β-Gal-(1→2)-α-Xyl-} on xyloglucan induces a flat conformation of the backbone, which helps xyloglucan bind to cellulose [44, 45]. However, how different side chains affect the formation of cell wall architecture is largely unknown. A number of *in vitro* studies have shown that xyloglucan

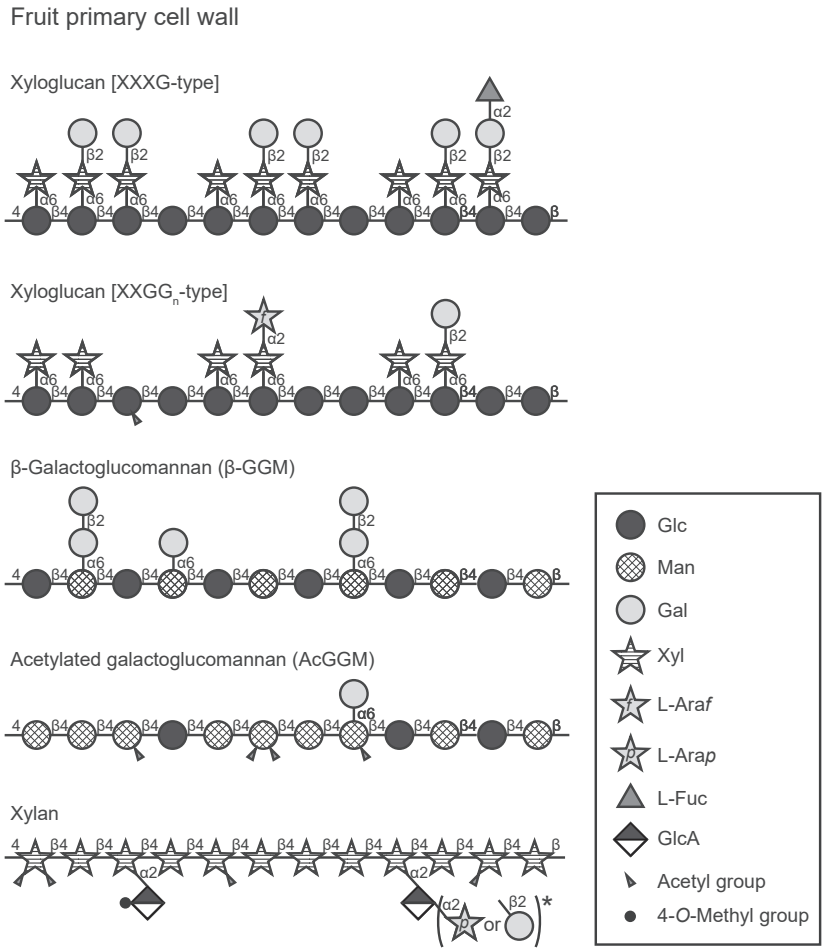

Fig. 3. Hemicelluloses in fruit primary cell walls.

Xyloglucan is made of XXXG-unit and Xyl side chains at the second and third positions are further decorated by varying mono- or di-saccharides (e.g., Gal and Fuc). The XXGG-type xyloglucan is mainly found in Solanaceae family plants [5, 8]. Various side chains and acetyl-esterification have been reported in different plants and tissues. β-Galactoglucomannan (β-GGM) has been found exclusively in eudicot primary cell walls [6, 7, 55]. β-GGM has a backbone made of Glc-Man repeats so that all decorations of Gal or di-Gal side chains on Man are evenly spaced. Acetylated galactoglucomannan is also present in primary cell walls but no pattern has been reported. Xylan in primary cell walls has [Me]GlcA decorations at every 6 Xyl residues [52, 53]. The [Me]GlcA side chains can be further decorated by α-1,2-linked Ara*p* [52, 53]. Asterisk (*) indicates β-1,2-linked Gal found in primary cell walls of the Myrtaceae family together with α-1,2-linked Ara*p* [53]. GlcA can be modified with a 4-*O*-methyl group.

interacts with cellulose likely via stacking interactions [46−50]; nonetheless, the interaction *in vivo* has not been investigated in detail. The ssNMR on Arabidopsis seedlings has shown unexpectedly little xyloglucan interacting with cellulose [51]; however, it could be due to drying and treatment of materials prior to the analysis.

Although xyloglucan is the main hemicellulose in the primary cell walls, primary cell wall-specific xylans and mannans have also been reported [6, 52, 53]. β-GGM is the primary cell wall-specific hemicellulose exclusively found in eudicots including tomato, apple, and kiwifruit (Fig. 3) [6, 7, 54]. AcGGM does exist in the primary cell walls with a slight structural variation (Fig. 3) [6]. However, it is unclear whether two structures, β-GGM and AcGGM, coexist in the same cell walls or they are localised in different cell types, due to lack of their localisation information. Since an exhaustive analysis of the mannan structure did not find hints of β-GGM in basal angiosperms and monocots, β-GGM is thought to have evolved after eudicots had emerged [55]. The unique β-GGM structure could be beneficial for its interaction with cellulose. MD simulations on the interaction between mannooligosaccharides cellulose showed that β-Glc-(1→4)-β-Man repeating structure has an adsorption energy equal to cellooligosaccharides [56, 57]. In addition, due to the alternating structure of the backbone, the decoration patterns are evenly spaced, making the side chains oriented on one side of the chain if the backbone forms a twofold screw conformation. The MD simulations suggest that β-GGM indeed interacts with cellulose [58]; however, the structure used for the simulation carries only the α-Gal side chain. Thus, the effects of the β-Gal-(1→2)-α-Gal disaccharide side chains on the interaction with cellulose are still unknown. A high field ssNMR analysis of Arabidopsis callus revealed that β-GGM is a rigid molecule, suggesting that it is likely to interact with cellulose in the cell wall [6]. Given the significant amount of β-GGM in the primary cell walls, it is likely that β-GGM is one of the major components of human intake. It would be interesting to investigate whether β-GGM has beneficial effects on the human gut microbiome similar to the well-studied mannans, such as konjac glucomannan and legume galactomannans [59−61].

Xylan structures in fruit have been less studied but xylan in primary cell walls, in general, is more complex and diverse compared to secondary cell wall xylan. In Arabidopsis primary cell wall, a xylan with an α-1,2-L-arabinopyranose (Ara*p*) onto the α-1,2-GlcA side chains on the xylan backbone has been reported (Fig. 3) [52, 53]. The spacing between [Me]GlcA residues was precisely every six Xyl [52]. In *Eucalyptus grandis,* primary cell wall xylan [Me]GlcA decorations are also modified with β-1,2-Gal [62], and this has now been confirmed in multiple Myrtaceae family species including, *Psidium guajava* (guava), *Myrcianthes pungens* (guabiyu), and *Plinia cauliflora* (jaboticaba) [53]. The edible fruits of these plant species are commercially available worldwide (guava) and in some countries in South America (guabiyu and jaboticaba), yielding substantial amounts of food waste and processed residues [63]. Although the presence of these xylan structures in the fruit needs to be investigated, they are the potential source of unusual xylan structures as a functional molecule in the food and health industries [64].

Hemicellulose structural patterns govern wall extractability and recalcitrance.

The degree, type, and distribution of substitutions on hemicelluloses are major factors affecting cell wall recalcitrance and hemicellulose extractability by forming crosslinks with the other wall polysaccharides and lignin [14, 38, 39, 65], by influencing interactions with cellulose and by impairing the action of hydrolytic enzymes [66, 67]. These features of plant cell walls are relevant to the industrial use of biomass that shapes our lives. For example, hemicellulose extractability is an important factor influencing papermaking processes and quality or polysaccharide valorisation, and cell wall recalcitrance is critical for biofuel production processes.

Although no decoration pattern has been reported for AcGGM, acetylation and Gal side chains seem to be influential to its interaction with cellulose, which thus could be key factors to regulate their extractability. As mentioned above, two populations of softwood AcGGMs are comprised of different amounts of Gal and acetyl groups, where the one with more Gal (or less acetylation) exhibited less extractability [19, 27]. In the case of leguminous galactomannan (no Glc in the backbone), it has been shown that the increased amounts of Gal decreased the incorporation into bacterial cellulose composites [68], suggesting that the Gal decoration had a negative effect on the interaction with cellulose. These two studies appeared to indicate the opposite effects of Gal modification on the interaction with cellulose; however, it may be due to the experimental systems using different mannans and celluloses (AcGGM and cellulose or galactomannan with bacterial cellulose). Further systemic investigation will be required in order to understand the relationship between the decorations of AcGGM and cellulose interaction. Polysaccharide engineering will be a powerful tool to fine-tune the level of decoration for such experiments.

Acetyl groups on xylan may affect xylan extractability. Sequential subcritical water extraction was performed on birch wood and showed that tightly bound xylan possesses higher glucuronidation and less acetylation [69]. *REDUCED WALL ACETYLATION* (*RAW*) downregulation in hybrid aspen (*Populus tremula* × *tremuloides*) exhibited a reduction of xylan acetylation, leading to lower xylan extractability in an alkali fraction [70]. Furthermore, it has been shown in Arabidopsis that a drastic reduction of xylan acetylation (by a mutation of xylan acetyltransferase *eskimo1* (*esk1*) (also known as *trichome birefringence-like 29* (*tbl29*)) led to an alteration in [Me]GlcA even distribution on xylan, resulting in a xylan incompatible for cellulose binding [14]. Interestingly, the ssNMR demonstrated that the reduction of the xylan acetylation in *esk1/tbl29* resulted in more dynamic, unbound xylan [14]. Together, these data suggest that not only the degree of acetylation but also the distribution of xylan modifications, influence xylan-cellulose interactions, determining xylan extractability.

Since hemicelluloses in plant biomass interact with cellulose and crosslink with lignin, modified hemicellulose structures alter the cell wall architecture, affecting susceptibility to enzymatic digestion. For example, in Brachypodium, sugarcane (*Saccharum* spp.) and Arabidopsis, improved saccharification of plant biomass has been demonstrated by removing xylan

GlcA decorations [39, 71, 72]. This finding was interpreted as improved accessibility of hydrolytic enzymes to cellulose due to a looser tethering of cell wall components as a consequence of reduced xylan-lignin crosslinking. Numerous studies have shown indirect evidence of the crosslinking via [Me]GlcA in woods, such as Japanese beech (*Fagus crenata*), Japanese red pine (*Pinus densiflora*) and spruce, through selective oxidation or the use of a glucuronoyl esterase [73−77]. In grass biomass, tight polysaccharide tethering can also be maintained via feruloyl-Ara*f* [40, 41]. Indeed, Brachypodium *xylosyl arabinosyl substitution of xylan 1a* (*xax1a*) mutants with a significant reduction in feruloyl-Ara*f* side chains exhibited reduced cell wall recalcitrance [39]. Although xylan structures differ between eudicots and grasses, modifying the xylan structures to disrupt xylan-lignin crosslinks can be a common strategy for improving biomass digestibility.

FINAL REMARKS

More than 340,000 vascular plant species cover our planet [78], providing us with abundant resources of a variety of polysaccharides that crosslink and interact with each other, forming complex and tightly knit cell walls. The architecture of wall components causes the biomass to be resistant to degradation, which diminishes the nutritional value of biomass for animals and the cost of biofuel production, but these properties could be beneficial for human gut health by influencing dietary fibre properties. For the best use of valuable biomass, it is important to understand the detailed structures of individual polysaccharides and their interactions in the cell wall architecture. Recent researchers' attention has focused on the patterning of hemicellulose decorations since it has a pivotal role in determining compatibility for the interaction with cellulose. Molecular mechanisms of the interaction between hemicelluloses and cellulose and the contribution of a variety of modifications to the interaction will be the next important questions to address. For this, the enigma of the nature of plant cellulose, such as the number of cellulose chains and the ways of assembly into a microfibril, needs to be solved, by which we would get more clues for molecular mechanisms of cellulose-hemicellulose interactions and thus the assembly of the plant cell wall. In addition, knowledge of biosynthesis and polysaccharide engineering by molecular biology approaches would have a great contribution to answering the questions. Indeed, given the recent advanced technology of genetic modification of plants, polysaccharide engineering for desired properties of biomass has become feasible. Finally, we would like to note that the discovery and analysis of structural patterns of hemicellulose were enabled by a range of glycoside hydrolases with a variety of substrate specificities, therefore the exploration of new carbohydrate-degrading enzymes is equally important.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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