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The Plant Cell Walls:

Complex Polysaccharide Nano-Composites

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http://www.glycopedia.eu/e-chapters/the-plant-cell-walls/article/introduction

INTRODUCTION

Observing a section of cork with a microscope that he had himself devised, Robert Hooke, discovered that the tissue had a honeycomb-like structure made of small spaces contained by walls, that he coined cells (from the latin word cellula, small compartment). He recorded his observations in his famous book Micrographia (1665). This conception of fundamental units was the basis of the studies by Marcello Malpighi (1628–1694) and Nehemiah Grew (1641–1712) of the microstructure of plant anatomy. In spite of being both eukaryotic, plant and animal cells differ fundamentally by the fact that the plant cell is encased in a rigid thick and strong wall around the plasma membrane, when the animal cell is surrounded by a thin and soft extracellular membrane. The presence of a thick wall around the cell membrane is an extracellular matrix shared by the prokaryotic cell of bacteria.

The wall serves several functions at the levels of the cell and of the whole plant:

(i) a **morphological role**: it determines the shape of the cells and their association and organization into tissues, and ultimately the plant morphology;

(ii) a **physical barrier** bringing support, strength, and rigidity to the plant. The mechanical strength of the cell wall allows the cell to sustain the internal turgor pressure;

(iii) a **biological barrier** against pathogens;

(iv) **a metabolic role**: it takes part in cell communication and transport and secretion system; it has a role in cell differentiation and plant growth; it can be source of oligosaccharide fragments that have hormone-like action, especially in defense mechanism against pathogen infection, inducing reaction against the attack.

The cell wall-associated kinases (WAKs) of angiosperms link the plasma membrane to the carbohydrate matrix and can directly regulate plant cell wall functions (Anderson, 2001); (iv) a natural composite material with multiple unique properties, with economic value for wood and fiber products (lumber and paper industries, and construction) and source of energy. Cell walls from crop plants are also important for food and feed.

There are three distinct anatomical regions in most plant cell walls: the ***primary wall***, a thin layer which is the first laid down against the plasma membrane and develops during cell expansion; the thick ***secondary wall***, deposited against the primary wall and made of additional sub-layers; the ***middle lamella***, which joins adjacent cells together. Not all plant cells have a secondary wall, but all have a primary wall and middle lamella. Each region of the cell walls has its own chemical composition and arrangement of its constituting polymers.

The plant cell wall is a dynamic structure which undergoes continuous rearrangements accompanying the phases of extension and anisotropic expansion (Baskin, 2005).

PLURALITY OF CELL WALL TYPES

The constituents of the plant cell walls vary depending on the stage of development and the type of tissue. In fact, when analyzing the structure-function relationship, one should always consider the plurality of plant cell types (~35 different types in plants, (Cosgrove, 2005)) with characteristic walls since their composition and macromolecular organization are adapted to a particular mechanical or physiological role. The multiplicity of cell wall types in the plant kingdom not only is due to the diversity of tissues types but also to the botanical origin. However, the fundamental macromolecular constitution of the cell walls relies on a common basis of polysaccharides, polyphenols and proteins, and it is the variability of the primary structure of these macromolecules, their relative proportion and their mutual arrangement that creates such a multiplicity.

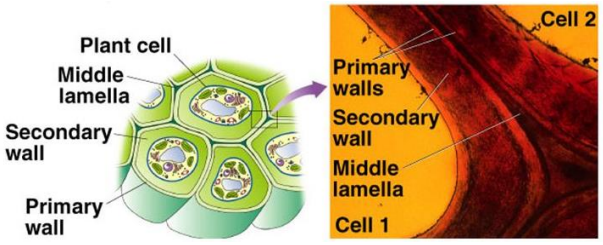

Figure 1. The plant cells and their walls

Each region of the wall harbors a specific network of polymers adapted to its particular role in the plant tissue.

Middle lamella – this is the outermost layer, constituting the glue that binds adjacent cells. It is composed primarily of pectic polysaccharides and lignin.

Primary wall –this is the first wall deposited by cells before and during active growth. It is the outer wall and elongates and expands over the life of the plant cell. The primary wall of dicot cells is comprised of pectic polysaccharides (ca. 30%), cross-linking glycans (hemicellulose; ca 25%), cellulose (15-30%) and protein (ca. 20%) (Darvill *et al.*, 1980). The actual content of the wall components varies with species and age. All plant cells have a middle lamella and primary wall.

Secondary Wall - some cells deposit additional layers inside the primary wall. This occurs after growth stops or when the cells begin to differentiate (specialize). The secondary wall is mainly for support and is comprised primarily of cellulose and lignin. Often distinct layers, S1, S2 and S3 can be distinguished - which differ in the orientation, or direction, of the cellulose microfibrils.

Reaction wood layers – due to various stresses, plants, and specially trees, differentiate secondary walls with modified or additional layers such as the gelatinous layer (G-layer) of tension wood. [for a review see *The biology of reaction wood*, B. Gardiner et al. Eds, Springer, 2014]

In most models of plant cell wall, and in numerous reviews entitled "The plant cell wall …", the presentation of the sole primary wall is often described as representative (see for instance Somerville, 2004; Cafall, 2009). Considering that the primary wall is only a few percent of the mature walls, this leads to a somewhat reductive and misleading view of the actual plant cell wall as a whole, given the importance of the lignocellulosic biomass. This situation arises from the biological importance of primary wall during the biosynthetic elaboration of a plant, together with from the uncertain knowledge of the complex interconnections occurring between the polymers in the secondary wall due to the presence of lignin.

POLYSACCHARIDE DIVERSITY

During photosynthesis plants fix the carbon from carbon dioxide into sugars which are incorporated into complex polysaccharides. This process makes plant cell walls a terminal carbon sink and constitutes the dominant carbon sequestration system on Earth. All plant cell walls share in common the characteristic of having a complex composite structure organized on a cellulose framework. They are essentially made of polysaccharides, more than 90% in the primary walls and between 65 and 70% in the secondary walls. Their composition is based on three different categories of polysaccharides, cellulose, and the so-called matrix polysaccharides: pectic polysaccharides and hemicelluloses. Such a diversity of complex carbohydrate polymers is due to the great diversity of monosaccharide constituents allied to the diversity of inter-glycosidic linkages. This results in a great heterogeneity in the structure and function of the plant cell walls (Burton *et al.*, 2010).

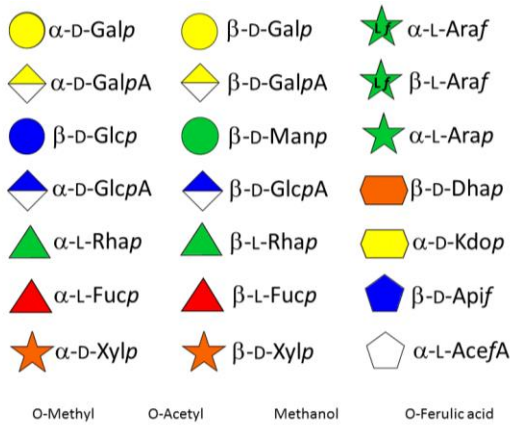

Figure 2. Major monosaccharide constituents of plant polysaccharides. Figure 1 displays a schematic representation of monosaccharides units considered in the present study, using the Symbol Nomenclature for Graphical Representations of Glycans (Varki et al., 2015)

More than fifteen different monosaccharides of the D and L series engaged in various modes of glycosidic linkages with α and β configurations are found in the cell wall polymers. It is interesting that most of these monosaccharides originate from the activated form of glucose, UDP-α-D-glucose, via common nucleotide sugar interconversion pathways (Feingold & Avigad, 1980; Reiter &Vanzin, 2001) catalyzed by a series of oxidation, reduction, epimerization, and/or decarboxylation reactions leading to activated sugars that can be used directly by glycosyltransferases. As many as 30 different nucleotide sugars have been identified in plants. In some cases, the free form of certain sugars can be incorporated into polysaccharides. (Bar-Bedel & O'Neill 2011)

Glycosyl transferases are needed to assemble the numerous monosaccharides in the various polysaccharides.

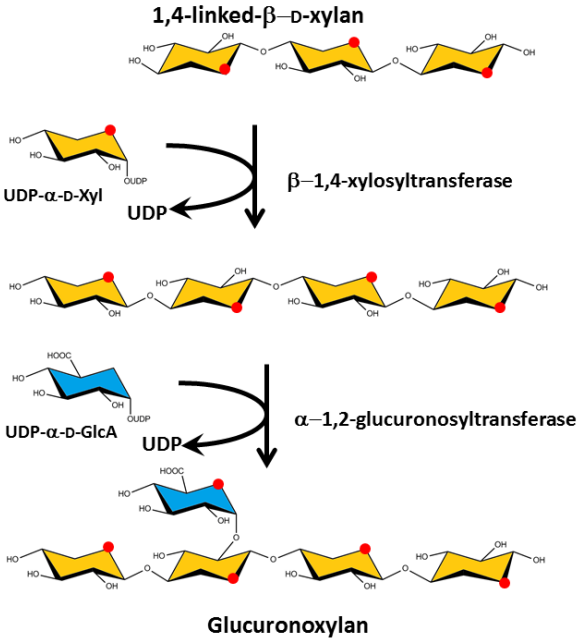

Figure 3. The biosynthetic steps leading to glucuronoxylan.

The compositional and structural complexity of plant cell wall poysaccharides is defined by an estimate of 120 different glycosyl transferases which contribute to specify the sugar series (D or L), the position of the glycosidic linkages and their anomeric configuration, as well as the eventual branching pattern on the main chain and substitution by non-carbohydrate groups, such as methyl and acetyl groups

The sites and synthesis pathways of cellulose and the matrix polysaccharides are different. Cellulose synthesis is engineered in large enzymatic membrane complexes, called the rosettes, situated in the plasma membrane, which extrude already organized microfibrils into the extracellular space (Saxena & Brown, 2005). By contrast, the backbones of the matrix polysaccharides are formed in the Golgi apparatus and exported through the plasma membrane to be secreted into the wall where they form a network held by hydrogen bonding together with chemical cross-linking (Proseus &Boyer, 2005; Cosgrove, 2005).

The precise primary structure of the non-cellulosic matrix polysaccharides varies according to various factors, including botanic origin, growth, and influence of external factors, such as stresses. Interestingly, the principal polysaccharides in the developing primary walls, the pectic polymers, essentially consist of (1→4)-linked D-glycopyranosidic main chains in which the glycosidic bonds have the α configuration, contrary to the most typical structural polysaccharides, cellulose and hemicelluloses, which also consist of (1→4)-linked D-glycopyranosidic chains, but have glycosidic bonds in the β configuration with an equatorial configuration at C1 and C4. This stereochemical difference underscores the structural importance of the glycosidic configuration on the final conformational characteristics of the polysaccharide backbones that governs their biochemical and physical properties, such as their susceptibility to chemical and enzymatic hydrolysis, and their capacity of interaction and association, leading to their determining impact on the mechanical behavior.

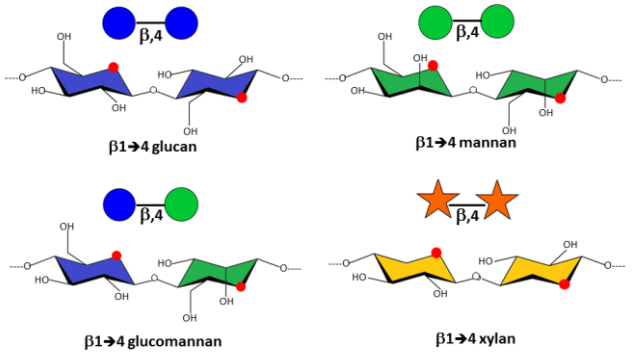

Figure 4. Importance of the glycosidic bond configuration of polysaccharide chains. Examples of the equatorial-equatorial configuration found in plant polysaccharides.

CELLULOSE, PECTINS,……

Cellulose. Cellulose is the most abundant natural polymer on earth. Cellulose accounts for 15–30 % dry weight of the primary cell wall and 20-35 of the secondary walls.

Its apparently simple primary structure is that of a homopolymer consisting of long linear chains of β-(1→4)-linked D-glucopyranose residues associated into the repeating disaccharide unit of cellobiose. The degree of polymerization (DP) of cellulose extends between 2000 - 14000 residues. It is an insoluble polysaccharide which possesses a tension resistance comparable to steel. The β configuration of the (1→4)-linked D-glucopyranosidic residues with the two equatorial positions of the aldehydic bond confers stiffness to the molecule and is responsible for its particular resistance to acid hydrolysis and for its remarkable mechanical strength. It has an extended conformation which gives rise to fibrous structures.

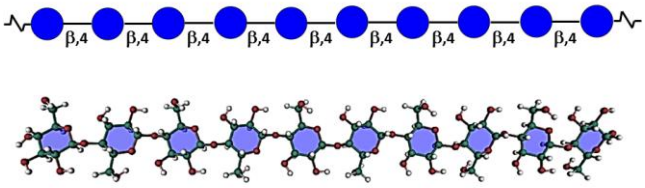

Figure 5. Schematic and 3D representations of the structure of one chain of cellulose

The regular repetitive structure of native cellulose chains results in a particular arrangement organized into microfibrils in which crystalline and amorphous (para-crystalline) domains coexist. Aggregates of 30 to 40 β-(1→4)-linked-D-glucan chains are hydrogen bonded to one another into the fundamental microfibril (MF), the dimensions of which vary slightly between primary and secondary walls, with a width in the range of 3-5 nm depending on the origin and developmental stage, and a length of a few µm (Nishiyama, 2010).

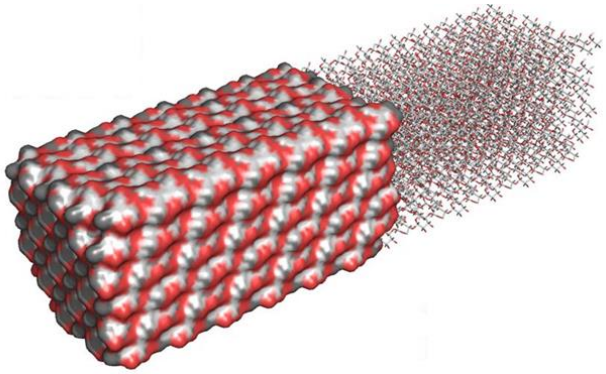

Figure 6. 3D structure of an "idealized" crystalline arrangement of Cellulose made up of 36 chains. (*Perez & Samain, 2010*)

According to the formation of intra- and inter-molecular hydrogen bonds, several allomorphs of crystalline cellulose have been defined. The most represented of these allomorphs are found in the plant cell walls and consist of cellulose I. This latter consists of a mixture of the crystalline forms Iα and Iβ, the former corresponding to a triclinic one chain unit, whereas the latter adopts a monoclinic two-chain unit cell (*Sugiyama et al.*, 1991). The fine structure and hydrogen bonding system in cellulose Iα and Iβ was resolved from synchrotron X-ray and neutron fiber diffraction (Nishiyama *et al.*, 2002, 2003).

The long debate as to whether the chains were packed in a parallel or antiparallel manner was cleared by a combination of

reducing end labeling and electron crystallography concluding to the parallel-up arrangement (Koyama *et al.*, 1997).

The deposition of cellulose fibrils perpendicular to the axis of elongation, determined by the orientation of cortical microtubules, influences the direction in which the cell elongates by restricting lateral swelling and allowing longitudinal expansion (Thomas *et al.*, 2013).

(For a review of the structure of cellulose, see S. Perez, Glycopedia, and Structure and Engineering of Celluloses, S. Perez & D. Samain (2010) Advances in Carbohydrate Chemistry and Biochemistry, 64, 25-116)

Pectic polysaccharides. Pectic polysaccharides typically consist of a group of polymers in which galacturonic acid (Gal*p*A) predominates and gives these polysaccharides anionic properties. Due to their negatively charged nature pectic substances are involved in various physiological and mechanical processes during cell growth and differentiation. Aside from the acidic polymers, pectins also comprise a few neutral polysaccharides such as arabinans and arabinogalactans often interconnecting the anionic moieties. Because of their particular acidic structure, the anionic pectic polysaccharides will be treated here separately from the neutral ones.

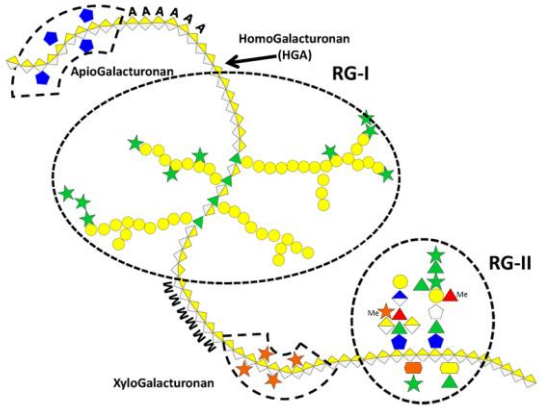

Figure 7. General scheme of the main constituents found in pectins (Perez et al., 2000)

Homogalacturonan (HG). This is the most represented form of pectic polymers, essentially localized in the primary walls, and consists of a unsubstituted backbone of α-1,4-linked D-GalpA residues. It is often referred to as polygalacturonic acid or linear galacturonan.

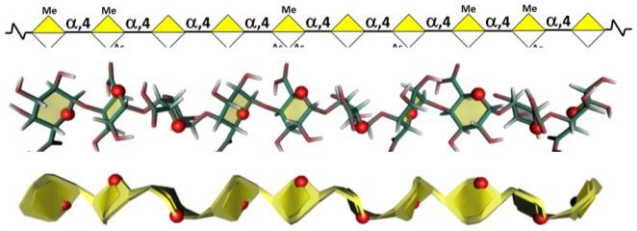

Figure 8. Schematic and 3D representations of homogalacturonan.

A number of Gal*p*A residues within the backbone may have their carboxyl group at C-6 methyl esterified, and may be acetyl esterified at O-2 and/or O-3 positions, depending on the plant origin. HGs with more than 50% methyl esterified residues are known as high methyl-esterified HGs. Blocks of galacturonate residues allow HGs to form gels by association of chains through divalent ions, particularly calcium ions (Braccini *et al.*, 1999). Both methyl and acetyl esters influence the gelling characteristics of pectins.

About 20 consecutive blocks of unmethylated Gal*p*A residues are required for Ca2+-induced cross-linking. Such sequences may be modified in muro by wall-bound pectin methylesterases which catalyse the demethylesterification of HG, involved in the primary wall remodeling accompanying the developmental processes of stem elongation and cellular adhesion (Micheli, 2001). In this way HGs structure contributes to the mechanical properties of the cell wall.

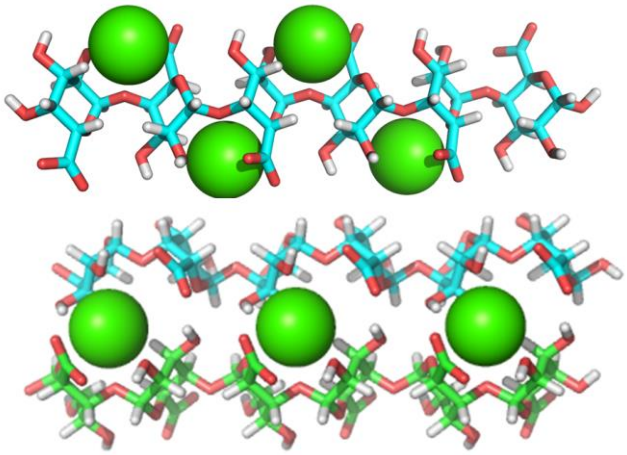

Figure 9. Three-dimensional representations of the interactions occurring between Ca2+ and one homogalacturonan chain (having two-fold conformation) and two-chains of homogalacturonan inducing the so-called "egg-box" structure (Braccini & Perez, 2000).

Rhamnogalacturonan-I (RG I). This highly represented pectic polysaccharide has a backbone composed of the alternating disaccharide repeat unit [→ 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 →]n.

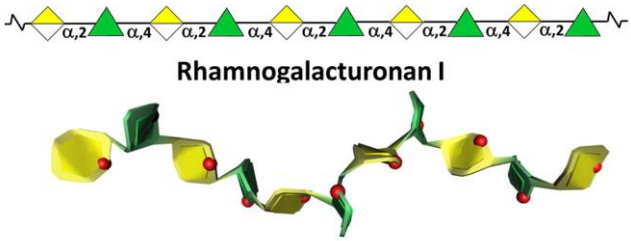

Figure 10. Schematic and 3D representation of the structure of Rhmanogalacturonan backbone.

In most cases, RGI does not exist as the simple linear chain, but is highly ramified with single terminal β-D-galactosyl and/or α-L-arabinosyl residues at positions O-4 or O-3 of the rhamnosyl residues whereas the α-D-Gal*p*A residues are often O-acetyl esterified at O-2 and/or O-3. A proportion of the latter may as well be substituted at O-4 with polymeric side chains of (1→5)-α-L-arabinans, (1→4)- α-D-galactans, arabinogalactans-I (AG-I)

or arabinogalactans-II (AG-II) (Yapo, 2001), which are, therefore, integral part of RGI.

Rhamnogalacturonans-II (RG II). RG II is a relatively minor component of the primary wall, accounting for 0.5% to 8 % of the primary walls. This low molecular weight (5–10kDa) macromolecular component of the primary walls is considered the most structurally complex cell wall polysaccharide with about 12 different monosaccharide residues interconnected by more than 20 glycosidic types of linkages. It consists of a homogalacturonan backbone comprising seven to nine (→ 4)-α-D-GalpA-(1 →) residues, some of them being methyl-esterified, carrying up to five kinds of oligosaccharide side chains, the number of which varies with the plant origin (Pabst *et al.*, 2013). Rare sugars are found in these side chains such as D-apiose, L-aceric acid (3-C-carboxy-5-deoxy-L-xylose), 2-O-methyl L-fucose, 2-O-methyl D-xylose, L-galactose, 2-keto-3-deoxy-D-lyxo-heptulosaric acid, and 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo). Such a diversity of unusual sugar residues renders RG II rather insensitive to degradation by wall-bound enzymes, and accounts for its high stability.

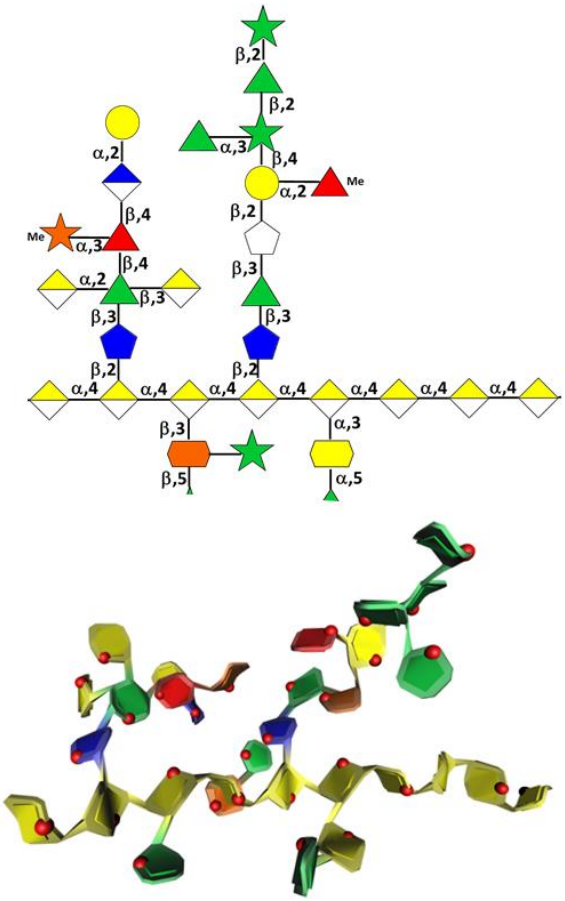

Figure 11. Schematic and 3D representatin of the structure of Rhamnogalacturonan II. (ref)

Thus, treating plant primary walls with an endo α-1,4-polygalacturonase releases the low molecular weight RG II. RG II's temporal stability becomes even further strengthened when one monomeric structure interacts with a second one and self-assembles through boron esterification to form a 1,2-borate dimer.

The ester cross-links the apiofuranosyl residue of the 2-O-methyl-D-xylose-containing side chains in each of the subunits of the dimer (Ishii *et al.*, 1999). It is noteworthy that RG II is a highly conserved structure in the plant kingdom.

Type	Composition	Non-Carbohydr. Group	Main Chain Glycosidic Linkage	Side Chain Glycosidic Linkage
Homogalacturonans	D-GalpA	Me ester (C_6) OAc (C_2,C_3) Ca^{2+}	α (1→4) D-GalpA	
Rhamnogalacturonans I	D-GalpA L-Rhap	OAc (C_2,C_3)	4)-α-D-GalpA-(1,2)-α-L-Rhap-(1,	α(1→5)Arabinan β(1→4)Galactan
Rhamnogalacturonans II	D-GalpA + 12 different sugars	Borate diester	α (1→4) D-GalpA	>20 different linkages

Pectic Galacturonans

Hemicelluloses. They have originally been defined as plant cell wall polysaccharides that are not solubilized by water but are solubilized by aqueous alkali (e.g. 1 and 4M KOH).This rather broad category of wall polysaccharides, made of neutral sugar backbones branched with monosaccharides and/or side chains, is present, albeit in different proportions and with different structures, in primary and secondary walls.

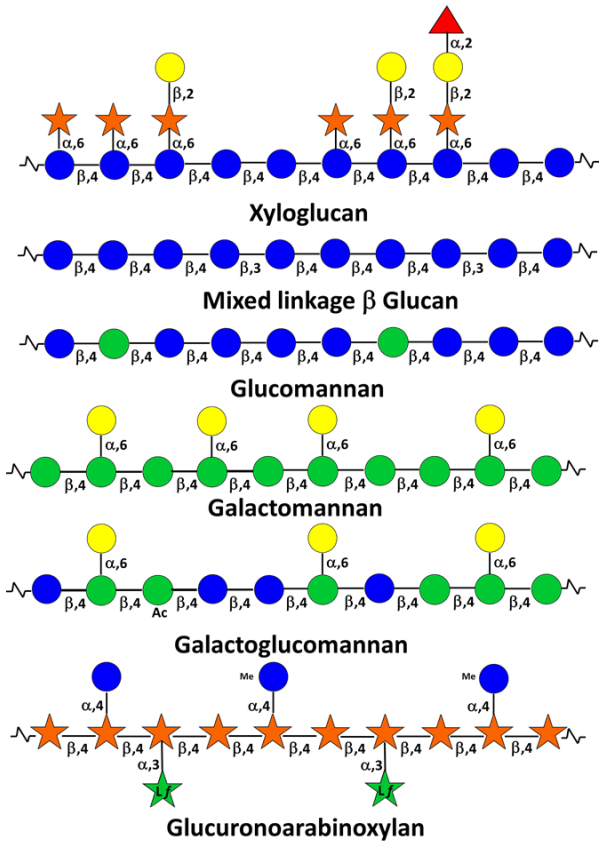

Figure 12. Primary structures of main plant cell wall polysaccharides

They also present a certain degree of variability according to botanical origin and whether they belong to one or the other wall compartment.

A remarkable aspect of hemicelluloses is to have a backbone of 1,4-linked β-D-pyranosyl residues (e.g. Glc*p*, Man*p*, and Xyl*p*). This results in a conformational homology between hemicelluloses and cellulose inducing complementarity in their non-covalent associations within microfibrils.

Typically the hemicelluloses from primary walls comprise xyloglucans, xylans and galactoglucomannans. In addition, mixed-linked β-(1→3,1→4) glucans are found in some monocotyledons of the *Poaceae* group (Scheller & Ulvskov, 2010). Those from secondary walls are essentially xylans, glucomannans and galactoglucomannans.

PRIMARY WALL HEMICELLULOSES

Xyloglucan (XG). They consist of a β-(1→4)-linked D-glucopyranosyl backbone carrying various side chains of D-xylopyranosyl-α-1→,D-galactopyranosyl-β-(1→2)-D-xylopyranosyl-α-1→,L-arabinofuranosyl-(1 -→2)-D-xylopyranosyl-α-1→, and (except in grasses) L-fucopyranosyl-α-(1→2)-D-galactopyranosyl-β-(1→2)-D-xylopyranosyl-α-1→ (Fry, 1989). The cellulose-like backbone is responsible for the strong and particular interaction between XGs and cellulose microfibrils. An interesting feature of this typical primary wall hemicellulose is that a certain regularity of distribution of the side chains constitutes repetitive subunits in its primary structure, the structure of which is denoted with a one-letter code (Fry *et al.*, 1993; O'Neill & York 2003). XGs 'structures vary between plant species.

Type	Composition	Non-Carbohydr. Group	Main Chain Glycosidic Linkage	Side Chain Glycosidic Linkage	DPn	Origin
Cellulose	D-Glcp	-	β (1→4)		10^4 - 10^5	
Xyloglucan	D-Glcp	OAc (C_6 Gal)	β (1→4)	Xylp-α(1→6) Galp-β(1→2) Fucp-α(1→2)		Gymnosperms Dicots Monocots
Mannan	D-Manp	OAc (C_3)	β (1→4)			Seeds;
(Galacto)glucomannan	D-Manp D-Glcp D-Galp	OAc (C_6,C_3)	β (1→4)	Galp-α(1→6)	100-400	Softwood
Arabinoxylan	D-Xylp	OAc (C_2,C_3)	β (1→4)	Araf-α(1→2) Araf-α(1→3)		Seeds; Monocots
Glucuronoarabinoxylan (GAX)	D-Xylp D-GlcpA	OAc (C_2,C_3) OMe (4-GlcpA)	β (1→4)	GlcpA-α(1→2) Araf-α(1→2)		Dicots; Monocots; Seeds
Arabinogalactan (Type I)	D-Galp	Protein	β (1→4)	α Araf- (1→5 β Galp	≤40	Monocots Dicots
Arabinogalactan (Type II) AGP	D-Galp	Protein	β (1→3)	βGalp(1→6)$_n$ α Araf-	≤40	Dicots
Arabinans	L-Araf		α(1→5)	Araf-α(1→2) Araf-α(1→3)	≤50	Seeds; Dicots

Primary Wall Cellulose and Main Hemicellusosic Polysaccharides

Whereas terminal α-L-fucopyranosyl residues are present in the primary walls of a wide range of dicots, non-graminaceous monocots, and gymnosperms, in some other plant species, including solanaceae, fucose is rarely present and short arabinofuranosyl side chains are found. In spite of structural variability, XGs seem to be conserved in all plant species (Caffall & Mohnen, 2009). The general role of XGs as the main load-bearing component between cellulose microfibrils has been recently questioned but they were confirmed to be implicated in primary wall mechanics (Park & Cosgrove 2012). In addition to their structural function in binding tightly to cellulose microfibrils via hydrogen bonds in a way that tethers them (Fry, 1989, Hayashi 1989), XGs are the source of oligosaccharides mediated by the action of wall-bound xyloglucan endotransglycosylase (XET).These fragments have shown regulatory activities during plant cell growth and elongation (McDougall & Fry 1989), suggesting that xyloglucan metabolism plays a role in the control of the elongation of plant cells (Takeda *et al.*, 2002).

Glucuronoarabinoxylan (GAX). This is the main type of the xylan family of hemicelluloses found in some primary walls. In grasses, and more generally the commelinid monocots, the glucuronoarabinoxylans (GAX) constitute the major hemicellulose (Vogel 2008), representing about 20% of the primary walls, and exceed the proportion of XG. GAX has also been found in the primary walls of dicots (*Arabidopsis*, Darvill *et al.*, 1980). As members of the xylan group, primary wall GAXs possess the 1,4-linked β-D-xylopyranosyl backbone, which is branched by single α-D-glucopyranosyl uronic acid and α-L-arabinofuranosyl residues attached at position 2 and 3, respectively, to the β-(1→4)-D-xylopyranose backbone, which might also be acetylated at position 2 and/or 3. A particular feature of grass GAX is the esterification of arabinofuranosyl residues at O-5 by ferulic acid (Wende & Fry, 1997). As a result, GAX in grass primary walls are covalently cross-linked via 5,5'-diferulate esters, and interact between cellulose microfibrils (Saulnier *et al.*, 1999).

Mannan. They consist of a linear β-(1→4)-linked D-mannopyranosyl backbone. Mostly abundant in the endosperm of some seeds, these true mannans (main chain comprising only mannose) are only minor components of dicot plant primary walls, as shown in primary walls from *Arabidopsis thaliana* by the method of polysaccharide analysis using carbohydrate gel electrophoresis (PACE) (Handford *et al.*, 2003).

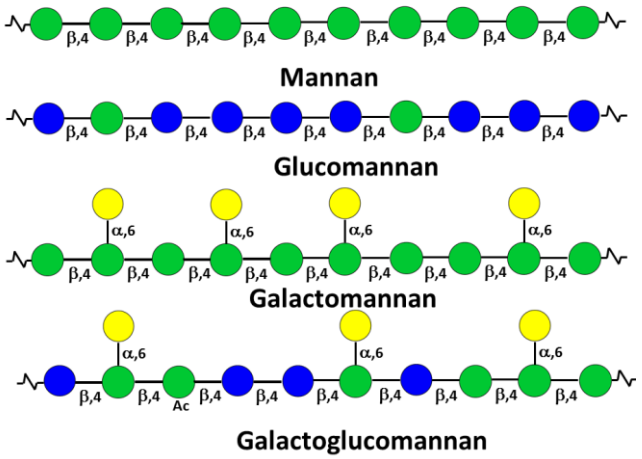

Figure 13. Primary structures of some of the main mannan containing plant cell wall polysaccharides.

Glucomannan (GM) and (Galacto)glucomannan (GGM). These types of mannose-containing hemicelluloses are often,

and abusively, referred to as "mannan" in spite of the fact that their main chain consists of a backbone in which β-(1→4)-linked D-mannopyranosyl and D-glucopyranosyl residues are alternating, making them by definition true glucomannans. Mannose is generally predominating. GM is a minor polymer in the primary walls of dicots and grasses.
In GGMs, the mannose-glucose main chain is substituted by single-unit side chains of α-D-galactopyranose bonded to the main chain by (1→6)-bonds. Mainly the mannose units, but also some glucose units, may be substituted with galactose units. To the difference of the GGM found in the secondary walls of gymnosperms, where the ratio of galactose, glucose, and mannose are generally about 1:1:3, respectively, this ratio in dicot primary walls is closer to 1:1 between glucose and mannose with a variable lower proportion of galactose (Cartier *et al.*, 1988).

Mixed-Linkage Glucans (MLG). They have a linear backbone of glucose consisting of cellotriosyl and cellotetraosyl units, (i.e. β-(1→4)-linked D-glucopyranosyl residues) linked together by β-(1→3) linkages. The length of the β-(1→4)-linked segments may be more than 3 or 4 glucosyl residues. Absent from the dicots cell walls, the MLGs are a key distinguishing feature of the grasses in which they are distributed almost exclusively within the *Poaceae* (Fry *et al.*, 2008; Fincher, 2009). In grasses the MGLs and GAXs replace the XGs and pectic polymers from dicots cell walls. They accumulate extensively in primary walls of coleoptiles during the elongation phase of growth, and undergo a turnover by a hydrolysis mechanism upon cessation of growth. However, they have been recently found in the secondary walls of mature stems of rice, suggesting that they may have a structural and mechanical role (Vega-Sanchez *et al.*, 2013).

SECONDARY WALL HEMICELLULOSES

The thick secondary walls are laid down only after the cambial cells have stopped enlarging. They have a layered structure in which the layers are conventionally denoted S1, S2, etc., in the order in which they are laid down (Harris, 2006). Some cell types, such as parenchyma cells of the cotyledons and the endosperms of seeds have a non-lignified secondary wall. However, most of the secondary walls of higher plants are lignified. These are essential for the supportive and conductive function of tissues. Secondary walls are mainly found in tracheary elements (tracheids in seedless vascular plants and gymnosperms, and vessels in angiosperms) and fibers in the primary xylem and the secondary xylem (wood).

To the difference of primary walls, secondary walls do not contain pectic polysaccharides. The predominant non-cellulosic polysaccharides belong to three families of hemicelluloses, namely, heteroxylans, glucomannans and galactoglucomannans. Xyloglucan has been detected in dicots and grasses secondary walls, but only in very minor proportion.

Xyloglucan (XG). Fucosylated xyloglucan was demonstrated, via the activity of the xyloglucan-specific enzyme xyloglucan endotransglycosidase (XET) and XG visualization immunolabeling, in poplar (Bourquin *et al.*, 2002) suggesting that XG may be considered to play a role in fully differentiated secondary walls and in developing wood.

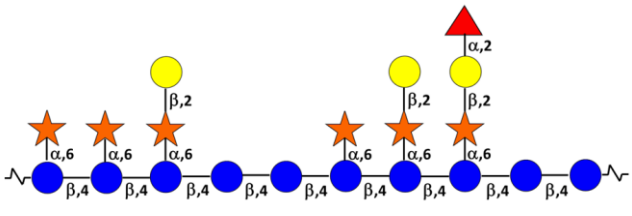

Figure 14. Structural motifs and sequences found in xyloglucan

Glucuronoxylan (GX). GXs are one of the major hemicelluloses in the secondary walls of dicots in which they account for 15-30%. Single α-(1,2)-linked D-glucuronic acid and 4-O-methyl- D-glucuronic acid residues are attached on average every 10 xylosyl residue to the β-(1→4)-D-xylopyranose backbone. In the native state GXs are often acetylated at C-2 or C-3, containing between 3-13% acetyl group.

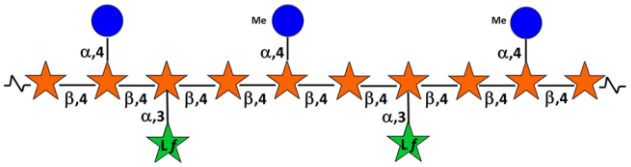

Figure 15. Structural motifs and sequences found in xyloglucan

(Arabino)glucuronoxylan (AGX) and glucurono)arabinoxylan (GAX). They are two variants of xylans differing in the relative proportions of the single substituents of 4-O-methyl-α- D-glucuronic acid and α-L-arabinofuranose residues attached at position 2 and 3, respectively, to the β-(1→4)-D-xylopyranose backbone. AGX are found in dicots secondary wall but are typically present in the cell walls of lignified supporting tissues of gymnosperms in which they constitute a significant proportion of the hemicelluloses together with the galactoglucomannans. They are highly substituted by 4-O-Me GlcA , with one glucuronic acid every 5-6 xylose residues. The GAX found in secondary cell walls of monocotyledons has fewer side-chains than the GAX of primary cell walls. This is thought to result in a stronger GAX-cellulose interaction (Vogel, 2008).

Glucomannan (GM) and Galactoglucomannan (GGM). Both these hemicelluloses are built on a backbone alternating short sequences (3-5) of β-(1→4)-linked D-mannopyranosyl interspaced by single β-(1→4)-D-glucopyranosyl residues. GMs are more typical of dicots secondary walls where they account for only a few percent. They are also present in coniferous tracheids, possibly with a minor amount of α-(1→6)-D-galactopyranose substituents. On the other hand, GGMs are characteristic components of gymnosperm secondary walls in which they are often the major matrix polysaccharide (≥ 30%) beside GAXs. Glucose, galactose and mannose occur typically in coniferous species in the average ratio of 1:1:3, respectively, with some variations having higher proportions of mannose (Dutton & Joseleau 1977; Lundqvist *et al.*, 2002). Here again, the mannosyl residues may be acetylated.

	Xyloglucan (XG)	Glucuronoxylan (GX)	Glucurono Arabinoxylan (GAX)	Glucomannan (GM)	Galactogluco Mannan (GGM)	β-1,3-1,4 Glucan
Primary Wall						
Dicot	20 - 35	-	5	3 - 5	m*	-
Grass	2 - 5	-	20 - 40	2	-	2 - 15
Conifer	10	-	2	-	m	-
Secondary Wall						
Dicot	m	20 -30	-	2 - 5	0 - 3	-
Grass	m	-	40 -50	0 - 5	-	m
Conifer	-	-	5 - 15	m	10 - 30	-

Main hemicelluloses occurring in the primary and secondary walls of plants

Type	Composition	Non-Carbohydrate Group	Main Chain Glycosidic Linkage	Side Chain Glycosidic Linkage	DPn	Origin
Cellulose	D-Glcp	_	β – (1→4)	_	10^4 - 10^5	
Mannan	D-Manp	O-Ac (C_3)	β – (1→4)			Seeds; Seaweeds
Glucomannan	D-Manp D-Glcp	O-Ac (C_2,C_3)	β – (1→4)		60 - 100	Hardwood
Galactoglucomannan	D-Manp D-Glcp D-Galp	O-Ac (C_2,C_3)	β – (1→4)	α – (1→6)	100 - 400	Softwood;
Glucuronoxylan	D-Xylp D-GlcpA	OAc (C_2,C_3) OMe (4-GlcA)	β – (1→4)	GlcpA-α(1→2)	100-400	Dicots; Monocots
Glucuronoarabinoxylan (GAX)	D-Xylp D-GlcpA	OAc (C_2,C_3) OMe (4-GlcA)	β – (1→4)	GlcpA-α(1→2) Araf-α(1→3)	100	Softwood; Dicots; Monocots
Galactan	D-Galp		β – (1→4)	Galp-β(1→6)	40 - 300	Normal Wood Compression Wood
Arabinogalactan (Type II)	D-Galp L-Araf		β – (1→3)	Galp-β(1→6) Araf-α(1→6) Arap-β(1→3		Softwood

Secondary Wall Main Polysaccharides

THE PLANT CELL WALLS ARE COMPLEX NANOCOMPOSITES OF POLYSACCHARIDES

Whether primary or secondary, the plant cell walls are built on the cellulose network organized around the cellulose microfibril unit (MF).

Depending on the developmental stage, the tissue type, and the cell wall layer, cellulose microfibrils (MFs) are differentially embedded in pectic polysaccharides, hemicelluloses and lignin. Many fundamental biological, physical, mechanical and chemical properties of plants depend on the fine organization of these structural polymer constituents at the ultrastructural and nanoscale levels. It is well accepted that the major load-bearing components in cell walls are MFs.

Many fundamental biological, physical, mechanical and chemical properties of plants depend on the fine organization of these structural polymer constituents at the ultrastructural and nanoscale levels. It is well accepted that the major load-bearing components in cell walls are MFs.

Primary walls

The Xyloglucan-Cellulose interaction. In the growing primary walls, the main MFs are oriented transverse to the growth direction (Szymanski & Cosgrove, 2009). The strength of the cell wall is due to the interaction between cellulose MFs and the matrix polysaccharides. In the resulting scaffold, xyloglucan and arabinoxylan bind to cellulose surface and tie together the MFs into a strong network. It has been shown for a long time that XG interacts with cellulose forming non-covalent association mediated by hydrogen bonds (Valent & Albersheim, 1974). This privileged association involving the β-1,4-D-glucopyranosyl chain of cellulose and the highly substituted β-1,4-D-glucopyranosyl chains of XGs results in the extensive topochemical distribution of XGs coating cellulose microfibrils within the supramolecular cell wall edifice (McCann *et al.*,1990; Ruel & Joseleau 1993; Fujino *et al.*, 2000). This partly explains the load-bearing function of XGs (Cosgrove 1997; Chanliaud et al. 2002). Several studies showed that the Xyl/Glc ratio affect the binding of XGs to cellulose, the less substituted XGs, the highest binding yields. Moreover, the terminal fucosyl residues of XGs differentially affect the binding, depending on the crystallinity of cellulose, suggesting that the surface status of native cellulose microfibrils affects the XG/Cellulose interaction (Abeces *et al.*, 1993; Chambat *et al.*, 2005). The influence of XG side chains in the binding in vitro to cellulose was earlier rationalized by conformational dynamics simulations (Levy *et al.*, 1997). The XG backbone adopts a helical conformation in solution, which, together with the arrangements of side chains, prevents self-association in solution while at the same time favoring adoption of a flat conformation upon interacting with cellulose (Levy *et al.*, 1991). Recent computer simulation studies showed that every cellulose Iβ surface was capable of binding xyloglucan oligomers (Hanus & Mazeau 2006).

The XG/Cellulose association may be modulated during anisotropic cell expansion by hydrolysis of XG through the action of endogenous XET in a process of selective primary wall loosening (Fry *et al.*, 1992) accompanied by mechanical constraints and changes in the orientation of cellulose microfibrils (Burgert & Frazl 2009). However, the key tethering role of XG to cellulose microfibrils during growth has been recently questioned on the basis of two- and three-dimensional magic-angle-spinning (MAS) solid-state NMR and uniformly 13C-labeled nuclear magnetic resonance studies indicating that the interaction was actually weakly pronounced (Dick-Perez et al., 2011), and prompted Park & Cosgrove (2012) to revise the role of XG in the dynamics of the growing primary wall.

The pectic network, In the classical models of primary walls, the Xyloglucan/Cellulose network is the load bearing component. On the other hand, the pectic polymers form another network (Carpita & Gibeaut 1993). Several studies, including molecular modeling have shown that the arabinan and/or galactan side chains of pectins adsorb to cellulose microfibrils (Zykwinska *et al.*, 2008). However, the cation-based cross-linking of acidic pectic polymers (essentially Ca2+) greatly influences the capacity of extension of the primary walls as well as their porosity. An important factor in this process is the methyl- de-esterification *in muro* of homogalacturonans by wall-bound methylesterases that modulates the extent of their binding capacity. As a result, the gel-forming pectins act as hydrophilic plasticizers between the microfibrils, keeping the growing cell wall both pliant and strong (Szymanski & Cosgrove, 2009).

Although the arrangement *in vivo* of the various pectic polysaccharides is not known, it is suggested that RG-I and RG-II form a continuous covalent cross-linking with the HG backbone (Vincken *et al.,* 2003). In complexing with boron to form a borate diol ester, two molecules of RG-II can establish cross-links via their apiofuranosyl residues (Ishii *et al.,* 1999). A study of T1 relaxation time in two- and three-dimensional magic-angle-spinning (MAS) indicates that the interaction of pectin is restricted to the surface of microfibrils (Dick-Peremay *et al.,* 2011). The most significant structural feature displayed by RG-II is certainly its "disk-like" shape that results form the most complex arrangements of its 31 constituent monosaccharides. Boron complexation accompanied by Ca2+ pairing provide stabilization of the two disks.

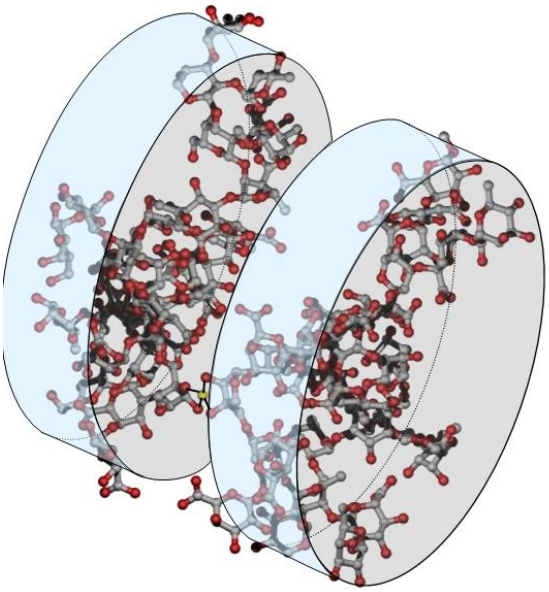

Figure 15. Schematic representation of the formation throughout Boron-boron interaction of the functiobal dimeric organisation of Rhamnogalacturonan II. (Perez et al., 2003)

This results in an architectural motif that has never been depicted for any plant cell components. This motif may be considered as a "clef de voute" that provides stable links in key regions within the pectic network. Once biosynthesised and located in the cell wall, RG-II, with its insensibility towards all cell wall enzymes, offers a structure (mRG-I) that will remain stable for the entire lifetime of the cell wall. When this monomeric structure (presumably linked to the HGA pectic backbone) interacts with another same structure throughout Boron and Ca2+ binding, a very stable spatial anchor is formed that will resist any temporal changes This is of course in contrast to the dynamical interactions that are undergone by the HGA moiety, where parallel and antiparallel chain pairings may occur to stabilise the gel constituent of an expanding medium in a highly time dependent fashion (Perez *et al.,* 2003).

It is suggested that arabinans are anchoring pectins in the wall and that galactans, in filling the gaps in the wall networks, may control the pore size (Voragen *et al.,* 2003). Structural glycoproteins are found in most primary walls (1-5%). The most studied are the hydroxyproline-rich glycoproteins (HRGP), the arabinogalactan proteins (AGP), the glycine-rich proteins (GRPs), and the proline-rich proteins (PRPs). Their role in the cell development has been suggested to be their involvement in recognition and signaling (Showalter 1993; Ellis *et al.,* 2010). The case of these glycoproteins is not treated in the present review.

Secondary walls. The arrangement of cellulose MFs, determines the mechanical and physical properties of the tissue. In the S1, S2, and S3 layers constituting generally the fiber walls, the MFs may be aligned at a particular angle to the cell axis. The MF angle increases, with regard to the cell axis, resulting in a highly anisotropic structure of the fiber wall.

The Hemicellulose-Cellulose interaction. The role of hemicelluloses in secondary walls, illustrated here with wood cell walls, is mostly a mechanical function of support in fibers and tracheids, and therefore differs greatly from the role of hemicelluloses and pectins in the expending primary walls. A common standpoint is that cellulose fibrils are coated with hemicelluloses and that the complex is embedded in a lignin-hemicellulose matrix, as visualized by the technique of immunolabeling in transmission electron microscopy (Ruel & Joseleau 2005). In the coating, the D-pyranosyl backbone of the hemicellulose forms a strong non-covalent hydrogen-bond association with cellulose microfibrils. Additionally, the charged carboxyl groups of glucuronoxylans are arranged face-to-face in such a way that repelling forces prevent aggregation of the cellulose microfibrils and favor their parallel arrangement (Dammström *et al.,* 2009). The O-acetylation degree of xylans may modulate the binding to cellulose. However, the hemicelluloses show a much lower degree of orientation than that of cellulose (Salmén *et al.,* 2012).

A modeling study by molecular dynamics simulations of the interaction of xylan fragments having 5 skeletal β-(1→4) xylosyl residues (X5) onto the (110) surface of cellulose microfibrils illustrated the affinity of the selected xylan fragments for crystalline cellulose (Mazeau & Charlier 2012). The calculations confirmed that xylan in solution is readily adsorbed on cellulose microfibrils and that the xylan fragments have a tendency to get aligned with respect to the molecular axis of cellulose in the microfibrils. Curiously, the study concluded that substitution of the X5 backbone by either Glc*p*A and/or Ara*f* side chains had no major influence on either the conformation or the efficiency of the interaction. In an attempt to quantify the strength of the interaction at the interface between cellulose and hemicellulose, another molecular dynamics (MD) simulation study (Zhang *et al.,* 2015) emphasized the contact area as well as hydrogen bonds, together with the covalent bonds in backbone of hemicellulose chain as the various controlling parameters at the interface.

The other main hemicelluloses of the woody plants secondary walls are glucomannans and galactoglucomannans. As well as the close affinity between mannans and cellulose (Chanzy *et al.,* 1982), and due to the configuration of their sequences of β-(1→4)-linked D-mannopyranosyl interspaced by single β-(1→4)-D-glucopyranosyl residue, GM and GGM closely associate to cellulose. There is a hierachical organization in the secondary wall that is influenced by the degree of acetylation of the hemicelluloses which results in a parallel arrangement of xylans relative to cellulose and glucomannan, and in wich xylans may more interact with the accessible glucomannan than the cellulose itself (Åkerholm & Salmén, 2001; Salmén, 2015).

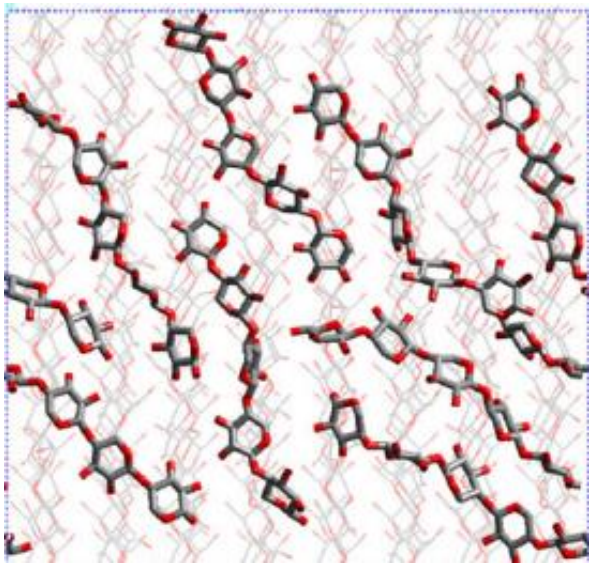

Figure 15. A schematic representation of one of the possible interaction of xylan onto the 110 crystalline surface of cellulose (Mazeau & Charlier 2012).

The close interaction of hemicelluloses with the surface of cellulose microfibrils was evidenced by spectroscopic techniques such as dynamic infrared (Dammström *et al.,* 2009)and microscopic techniques such as atomic force microscopy (Fahlén and Salmén 2004) and immuno-transmission electron microscopy using antibodies directed against xylans and glucomannans (Joseleau, 2007; Maeda *et al.,* 2013).

The reticulation of cell wall polysaccharides through phenolic substances, and particularly lignin (Ruel *et al.,* 2002), is an important factor of terrestrial plant mechanical resistance. This aspect will not be dealt with in this review, and will the included in another review to be published in glycopedia by K Ruel & J-P Joseleau).

MODELS OF PLANT CELL WALL ARCHITECTURE

The plant cell walls can be viewed as a supramolecular complex of polymers intertwined in an organized manner. How the various and complex polysaccharides, proteins, phenolic compounds and inorganic ions are organized and interact to form the structural entity of the plant cell wall remains in great part unknown. Several models have proposed tentative structures of primary and secondary walls. All of them have a basis of cellulose-hemicellulose interconnection dominated by hydrogen bonding. This is the cellulose-xyloglucan which features the basic framework of primary walls, whereas cellulose-other hemicelluloses relationships predominate in the secondary walls, in addition to the lignin-hemicellulose matrix.

Building an integrated model of the primary wall structure(s) is difficult because it is a dynamic structure that changes during plant growth and undergoes remodeling and rearrangements. Because the lignified secondary walls provide mechanical strength to the plant, modeling the structural organization of the constituting polymers must take into account the orientation of their respective deposition. The anisotropic properties of hemicelluloses, and to some extent that of lignin, are important features when modeling secondary walls (Åkerholm & Salmén, 2003; Salmén *et al.,* 2012).

The architectural complexity and diversity of plant cell walls is such that imaging the archetype of the plant cell wall is a difficult enterprise. That requires a systemic approach. The progress of analytical, spectroscopic, genomic, and live imaging techniques, will help elaborating models relating structure and specific function.

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