

Cellulosic Microfibril and Its Embedding Matrix within Plant Cell Wall

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ABSTRACT: Cellulose is the homopolymer of (1→4)-β-D-glucopyranose. Glucan chains are aggregated to form microfibrils with cross dimensions ranged from 2 to 20 nm. The variation in each of microfibrillar dimension, hydrogen-bonding network and molecular orientation depends on their parent nature. Cellulosic microfibrils isolated from bacteria and certain algae (10–25 nm in diameter) were found to be thicker than those extracted from the primary cell walls in higher plants (3–10 nm in diameter). Three biopolymers, namely hemicellulose, lignin and/or pectin contribute to the embedding matrix enveloping the cellulosic microfibrils. The secondary cell wall was found to be differentiated into three consecutive layers (S₁, S₂, S₃). Within each layer, cellulose microfibrils are found parallel and of high order with a different microfibrillar angle. The bundling process of cellulose microfibrils in both primary and secondary cell walls may involve the aggregation of the closely arranged cellulosic microfibrils. Some models for the microfibril construction have been proposed. Along the longitudinal microfibril's axis, crystalline regions are alternated with amorphous ones. The most important methods of estimating the crystallinity of cellulose microfibrils such as X-ray diffraction (XRD), ¹³C solid-state NMR spectroscopy (¹³C-NMR), small-angle neutron scattering (SANS) and sum frequency generation (SFG) spectroscopy were concluded.

KEYWORDS: Biopolymers, Microfibril, Hydrogen-bonding, Crystallinity of cellulose.

I. INTRODUCTION

The cell wall of higher plants (Fig.1a and 1c) is constituted of a mixture of polysaccharides (Cosgrove, 2005) and can be differentiated into primary wall and secondary wall (Li *et al.*, 2014). The differences between primary and secondary cell walls in chemical composition and structure are responsible for the biodiversity in the plant kingdom (Popper, 2008). Beside cellulose and hemicellulose, the primary cell wall contains pectin while the secondary cell wall contains lignin (Figure 1b) and the later wall is usually thicker and more rigid than the former (Roberts *et al.*, 2000).

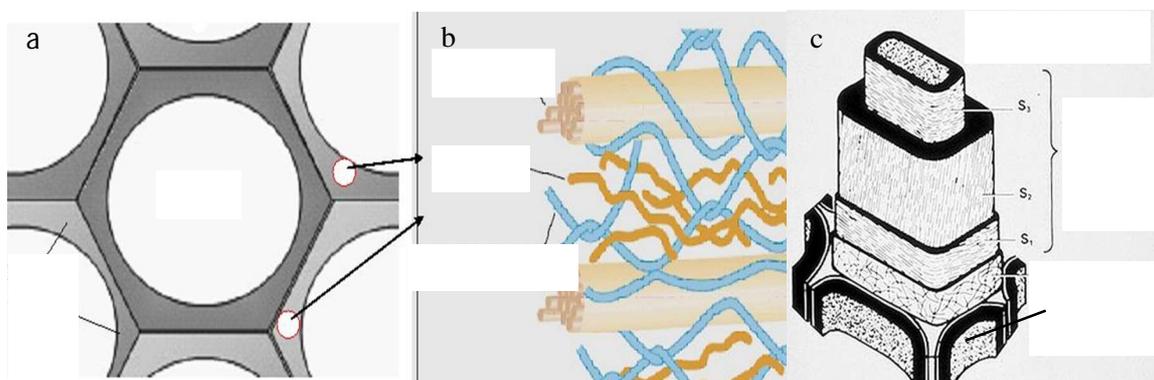


Fig.1. Representative schematic for the plant cell wal: a) cross section, b) cellulose microfibrils embedded in biomatrix and c) ultrastructure of perennial cell wall.

II. CHEMICAL STRUCTURE OF WOOD POLYMERS

2.1. Cellulose

Cellulose is the homopolymer of (1→4)-β-D-glucopyranose (Matthews *et al.*, 2010; Brett, 2000; Somerville, 2006). Although its chemical composition is simple, understanding its conformation and the packing procedure in microfibrils is still a mystery to some extent (Matthews *et al.*, 2010). A cellulosic chain is consisted of repeated cellobiose units (Fig.2a) that generates diverse architectures, reactivity and functions increasing its importance (Klemmet *et al.*, 2005). A Single cellulosic chain may be composed of about 10.000 glucopyranose subunits (Zabel and Morrel, 1992) or more. The adjacent chains are strengthened together by hydrogen bonding and van der Waals forces (Fig.2b).

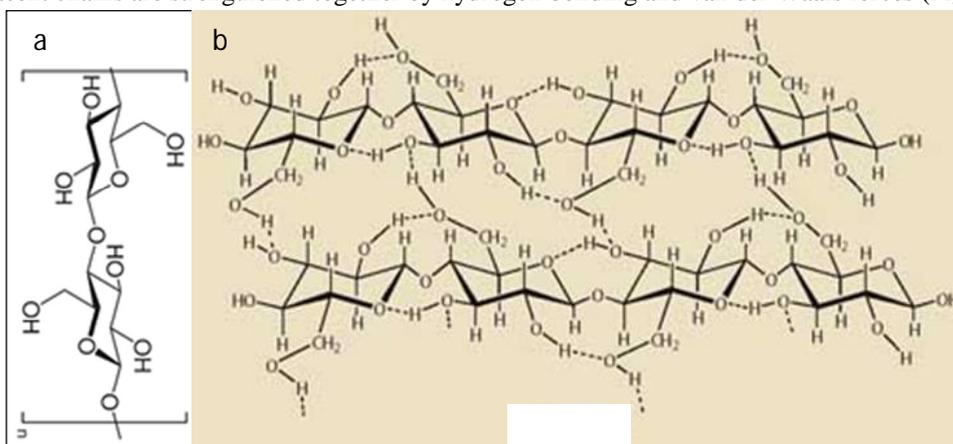


Fig.2. Chemical structure of cellulose: a) the cellobiose unit and b) two glucan chains conjugated by hydrogen bonding network.

2.2. Hemicelluloses

Hemicelluloses are the second division of polysaccharides in plant cell walls beside cellulose. It is constructed from simple sugar monomers, namely glucose, galactose, mannose, xylose, arabinose and glucuronic acid (Fig. 3). It has β-(1→4)-linked backbones in an equatorial configuration. Hemicelluloses are found as different compounds such as xylans (Fig. 4a), glucomannans (Fig. 4b), xyloglucans, mannans and beta-(1→3,1→4)-glucans. These compounds are present in all plants, except for beta-(1→3,1→4)-glucans, that are confined to Poales and some other groups. The hemicelluloses structure varies widely in nature due to species and cell types. The most important biological effect of hemicelluloses is their roll in strengthening the cell wall by interaction with the other biopolymers. Hemicelluloses are synthesized by glycosyltransferases present in the Golgi membranes (Scheller and Ulvskov, 2010). Xyloglucan is the most dominant hemicellulose in primary walls of dicotyledons and it plays a great role in forming the cross-links between cellulosic microfibrils (Keegstra *et al.*, 1973). Comparing to cellulose which is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with weak strength and is easily hydrolyzed by dilute reagent (acid, base or myriad hemicellulase enzymes).

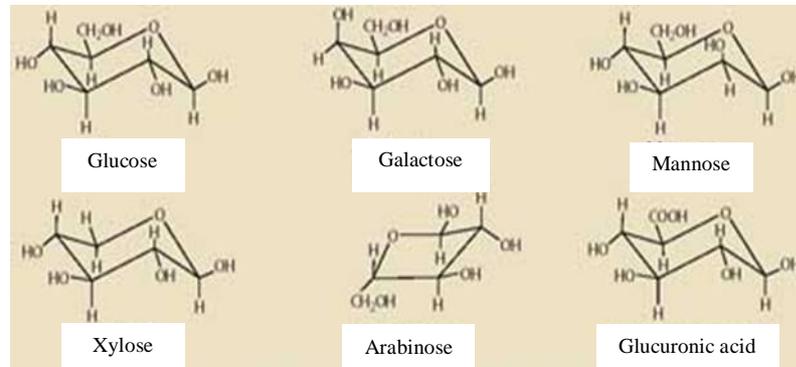


Fig.3. Simple sugar monomers constituting the hemicelluloses.

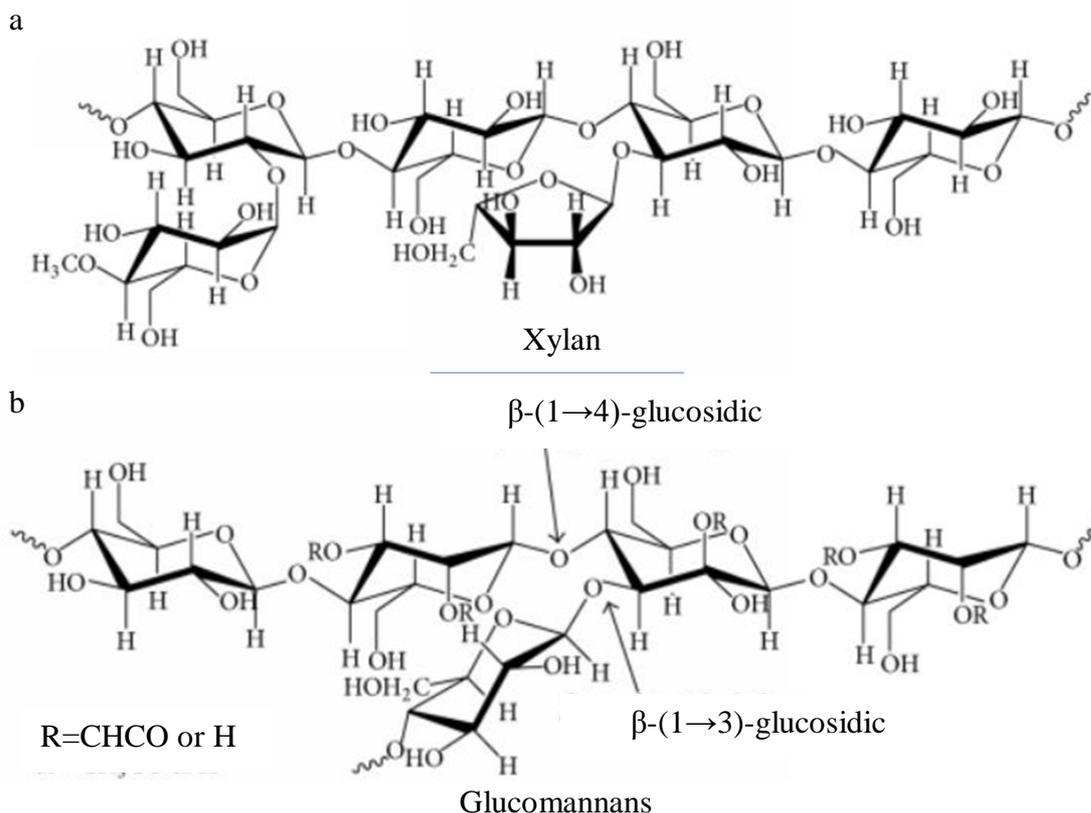


Fig.4. Chemical structure of hemicelluloses: a) xylans and b) glucomannans.

2.3. Lignin

Lignin is a biopolymer consisted of phenylpropane monomers. The complexity of lignin is arisen from the cross-linking occurred between its monomers (Fig. 5) by different chemical bonds (Fig. 6). Its resistant to biochemical effects has been proved, although some organisms such as white-rot fungi and actinomycetes have the ability to secrete necessary enzymes to break lignin apart. The initial reactions are mediated by extracellular lignin and manganese peroxidases (Crawford, 1986; Kirk and Farrell, 1987; Basaglia *et al.*, 1992). Since lignin is the most recalcitrant component of the plant cell wall, increasing its content will reduce the surface area available to enzymatic penetration and activity (Haug, 1993).

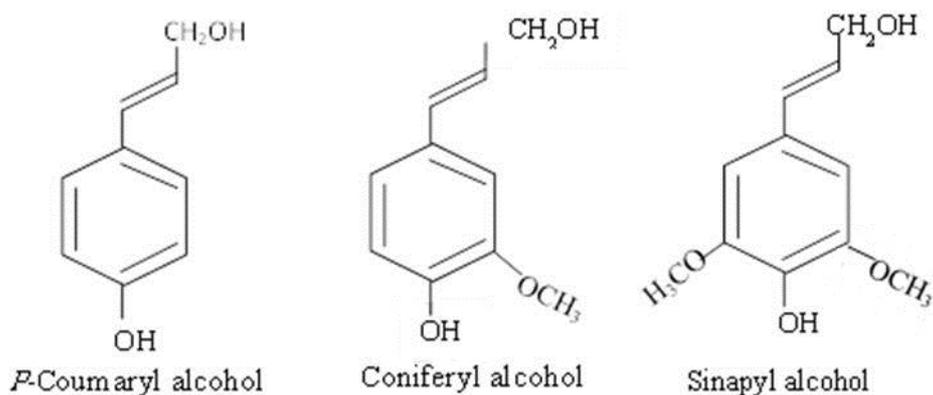


Figure 5. The three common monolignols: paracoumaryl alcohol (1), coniferyl alcohol (2) and sinapyl alcohol

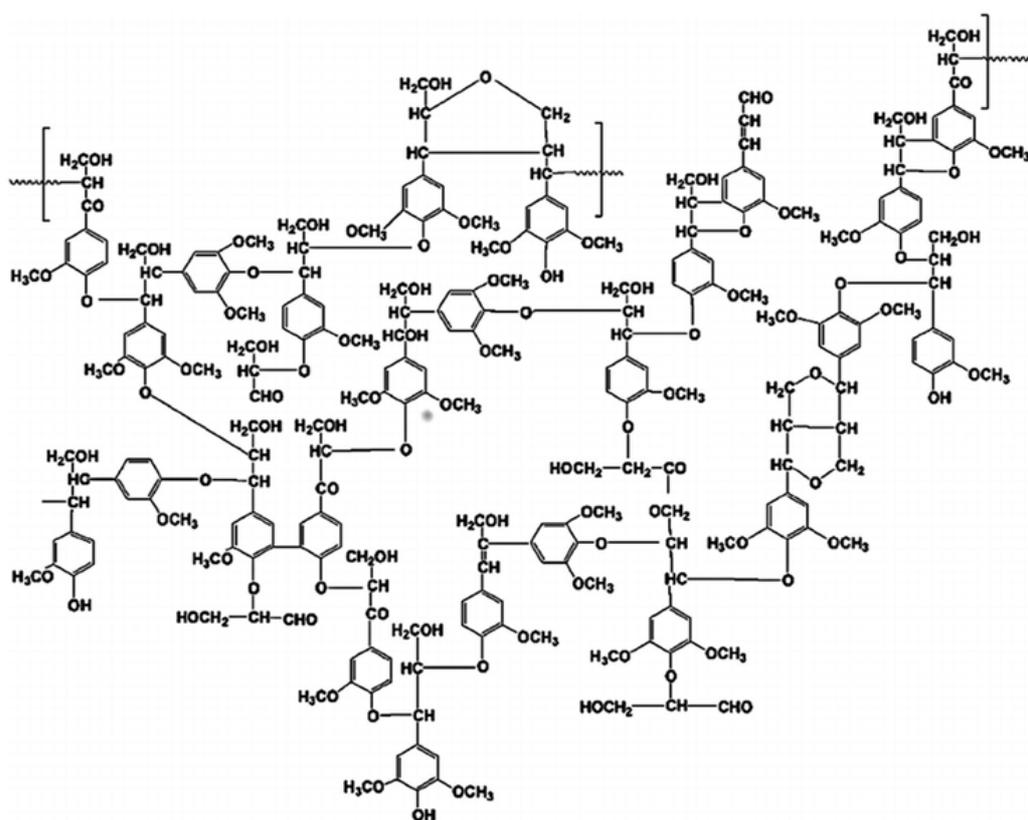


Fig.6. Lignin matrix in hardwoods.

2.4. Pectin

Pectins are a group of heterogeneous polysaccharides containing 1→4-linked α-D-galactosyluronic acid. Three types of pectin were isolated from primary plant cell walls, namely homogalacturonan (HG), rhamnogalacturonan-I and substituted galacturonans. The HG is a linear polymer of 1→4-linked α-D-galactosyluronic residues, in which some of the carboxyl groups are methyl esterified (Fig. 7). They may also be O-acetylated at the C₂ and C₃ positions. Pectin has rhamnopyranosyl residues inserted in the galactosyluronic backbone at 1 to 4% substitution. The

rhamnogalacturonan-I (RG-I) chains are large substituted side chains. Between 20 and 80% of the rhamnopyranosyl residues are substituted at C-4 with neutral and acidic oligosaccharide side chains. The predominant side chains contain large linear and branched α -L-arabinofuranosyl and/or β -D-galactopyranosyl residues (Sharma *et al.*, 2006).

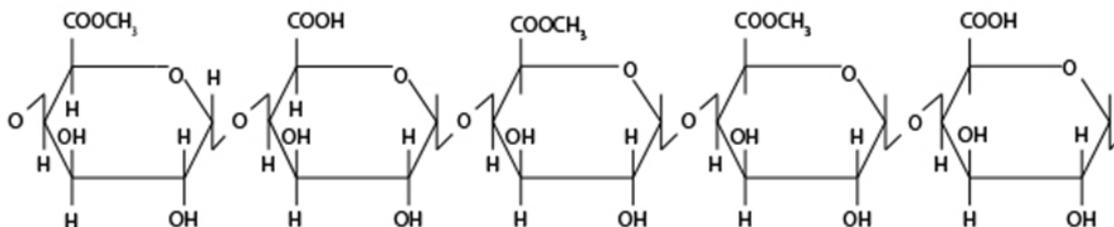


Fig. 7. Chemical structure of pectin.

Cellulose microfibrils are found to be coated with xylogalacturonan, a pectin polysaccharide, during the synthesis of cellulose microfibrils in quince seed mucilage. The xylogalacturonan coating prevents the coalescence of cellulose microfibrils into large bundles (Ha *et al.*, 1998). In primary cell walls of Arabidopsis, pectin directly interacts with cellulose microfibrils. It has been estimated that 25–50% of the glucan chains are contacted with pectin polysaccharides (Wang *et al.*, 2012).

3. Microfibril Organization

Glucan chains are aggregated just after they are biosynthesized to form microfibril aggregates with different cross dimensions (Fig. 8). The variation in each of microfibrils' dimensions, hydrogen-bonding network and molecular orientation depends on their parent's nature (French *et al.*, 2004; Atalla, 1984; Habibiet *al.*, 2010).

Upon wood formation, cellulosic microfibrils in the secondary cell wall are precipitated as three consecutive layers (S₁, S₂, S₃). Within each layer the, cellulose microfibrils are highly ordered and parallel, however, the microfibril angle is oriented differently from one layer to another (Plomionet *al.*, 2001; Barnett *et al.*, 2004). Along with embedded hemicelluloses and lignin, the multi-layered cellulose microfibril organization makes the wood cell wall an ideal structure owing to strength and rigidity it provides (Chaffey, 1999; Plomionet *al.*, 2001; Li *et al.*, 2014).

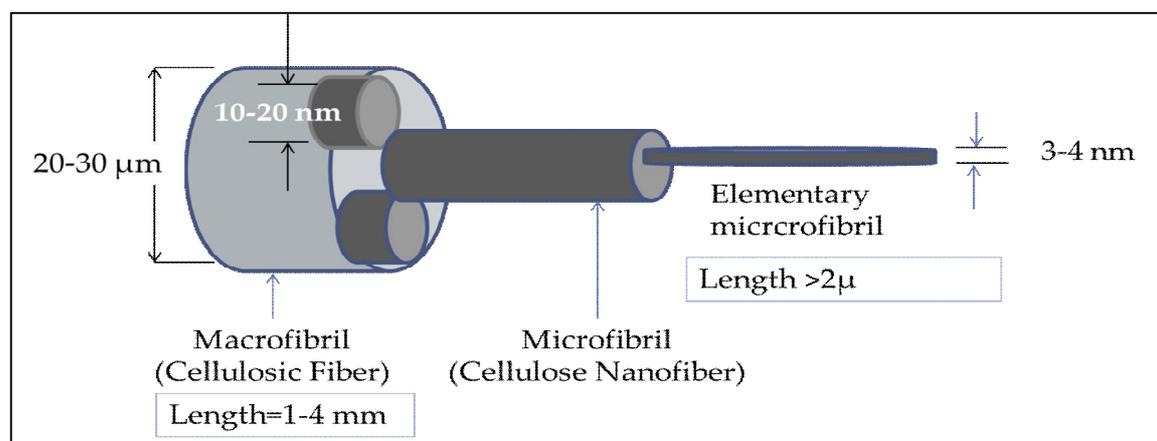


Fig.8. Dimensions of the different aggregates of cellulosic microfibrils.

In both primary and secondary cell walls, cellulose microfibrils often exist as bundles (Anderson *et al.*, 2010; Fernandes *et al.*, 2011; Thomas *et al.*, 2013; Zhang *et al.*, 2013). The bundling process may involve the aggregation of closely arranged cellulosic microfibrils; Moreover, although its bundling process itself is not completely understood, the other polymers of wood (hemicellulose, lignin and pectin) may also affect the bundling of cellulosic microfibrils (Keegstra *et al.*, 1973; Cosgrove, 1997; Keegstra, 2010 and Scheller and Ulvskov, 2010).

4. Unit Cell of Cellulose

Some models for the microfibril construction have been proposed to describe their supramolecular configuration (Fig.9). These models differ essentially in the describing the organization manner and distribution of the amorphous regions within the microfibril (Rowland and Roberts, 1972), along the longitudinal microfibril's axe, crystalline regions are alternated with amorphous ones.

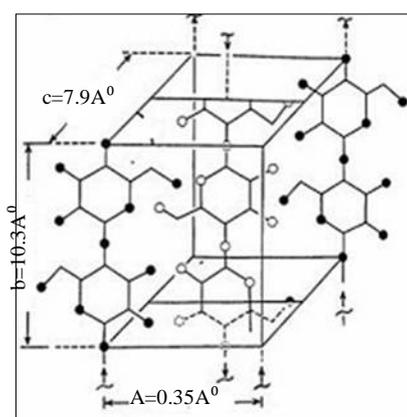


Figure 9. The unit cell of cellulose.

5. Crystallinity of Cellulose

Different methods have been developed to estimate the crystallinity of cellulose microfibrils such as X-ray diffraction (XRD), ^{13}C solid-state NMR spectroscopy (^{13}C -NMR), small-angle neutron scattering (SANS) and sum frequency generation (SFG) spectroscopy. The XRD can roughly provide relative crystallinity index, which is based upon the proportions of crystalline and amorphous materials in the cell wall. The XRD may be influenced by amorphous fraction or non-cellulosic polysaccharides (Segal *et al.*, 1959; Harris *et al.*, 2010; Park *et al.*, 2010; Harris *et al.*, 2012).

Cellulose crystallinity assessed by ^{13}C -NMR spectroscopy is dependent on comparing the relative intensities of peaks that correspond to C_4 atoms in the interior of the cellulose versus C_4 atoms that are on the surface of the cellulose microfibrils, which can be used to estimate crystallite size (Boottenet *et al.*, 2011; Dick-Perez *et al.*, 2011). However, this method has trouble differentiating between amorphous cellulose regions and cellulose chains at the surface of crystalline microfibrils. The ^{13}C -NMR is the best crystallinity analysis method for thick microfibrils (10–25 nm in diameter) isolated from bacteria and certain algae (Brett, 2000). Nonetheless, cellulose microfibrils from the primary cell walls in higher plants are relatively thin, ranging from 3–10 nm in diameter (Thomas *et al.*, 2013; Zhang *et al.*, 2013).

A model in which regions of crystalline cellulose are interconnected by amorphous cellulose regions has gained popularity in recent years. These amorphous regions can be detected by SANS method and are highly accessible through acid hydrolysis (Fernandes *et al.*, 2011).

The SFG is a nonlinear laser spectroscopy technique used to analyze surfaces of materials and their interfaces with surrounding atmosphere. It is useful to deduce their composition, orientation distributions, and some structural information of molecules at gas–solid, gas–liquid and liquid–solid interfaces. SFG can investigate a monolayer surface sensitively else for solids or fluids (Shen, 1989). The SFG spectroscopy was recently used to detect the asymmetric distribution of C_6H_2 and $\text{O}_3\text{H}-\text{O}_5$ group in crystalline cellulose microfibrils (Hieu *et al.*, 2011; Barnette *et al.*, 2012). The SFG is also a desirable method to estimate the content of crystalline cellulose due to the absence of spectral interference from other cell wall polymers such as hemicellulose, lignin and pectin (Barnette *et al.*, 2012). In addition, SFG can also detect subtle changes in cellulose ordering and packing in secondary cell wall (Park *et al.*, 2013).

6. Intra- and inter bonding network

In the crystalline regions of cellulosic microfibrils, the chains are tightly contacted together side-by-side by a network of intra- and intermolecular hydrogen-bonds as well as van der Waals forces (Atalla, 1984; Brett, 2000; French *et al.*, 2004; Somerville, 2006; Habibi *et al.*, 2010). The hydrogen-bonding network and molecular orientation within

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cellulosic microfibril vary widely depending on the parent cellulosic resource and the used maceration method (French *et al.*, 2004; Atalla, 1984). Hydrogen bonds appear between hydroxyl groups and oxygen atoms both within a single glucan (glucopyranose) chain and between neighboring chains, the cellulosic chains are crystallized by the stress effect of intra- and inter-chain hydrogen bonding to form cellulose microfibrils with high axial stiffness (Gillis *et al.*, 1969).

In higher plants, the small elementary microfibrils (3 nm in width) can aggregate into larger ones (microfibrils). The dimensions of microfibrils vary according to their location in the cell wall (5–10 nm in primary cell walls and 30–50 nm in secondary cell walls (Davies *et al.*, 2003; Zhang *et al.*, 2014) as shown in Figure 1. The length of cellulose microfibrils termed as the degree of polymerization (DP) varies significantly among its parent. The DP of cellulose microfibrils is estimated to range from hundreds to thousands of glucose units in primary walls, and up to 15,000 glucose units in secondary walls (Brett, 2000; Somerville, 2006).

The crystallinity, molecular structure and the hydrogen-bonding system in cellulose I_β has been determined using synchrotron and neutron diffraction data recorded from oriented fibrous samples prepared by aligning cellulose microcrystals from tunicin. In addition, the X-ray data are used to determine the C and O atom positions. The resulting structure consisted of two parallel chains with slightly different conformations and organized in a "parallel-up" fashion, with all hydroxyl-methyl groups adopting the TG conformation. The hydrogen atoms positions in hydrogen-bonding network can be determined from a Fourier-difference analysis using neutron diffraction data collected from hydrogenated and deuterated samples. The hydrogen atoms involved in the intramolecular O₃...O₅ hydrogen bonds have accurate-defined positions. Inversely, intramolecular hydrogen bond formed between O₂ and O₆ covered a wider volume, indicative of multiple geometry with partial occupation. Using a recent infrared analysis indicates that crystals of cellulose I_β have an inherent disorganization of the intermolecular hydrogen bonding network that maintains the cellulose chains in sheets despite their high crystallinity (Nishiyama *et al.*, 2002^a).

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