Chapter 4

Cellulosic Fibers: Role of Matrix Polysaccharides in Structure and Function

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1. Introduction

Cellulose, being the major cell wall component and the most abundant organic matter, produced by living organisms, is not uniformly distributed within plant tissues. There are numerous cells, like the parenchyma ones, which even at maturity have thin cell wall. Thick cell walls are characteristic for the tissues with mechanical function. Among those, there are cell walls, which contain several major components, and those, the predominant component of which is cellulose. The most pure natural cellulose is considered to be present in cotton seed hairs (sometimes erroneously called "cotton fibers") – over 90% of cell wall [1]. Very close to this value is a special group of plant fibers – cellulosic or gelatinous fibers, the proportion of cellulose in which amounts for 85-90% [2,3]. The cell wall thickness in such fibers may reach 15 μm, as compared to 0.2 μm in cells with thin cell wall. So, the very significant portion of total plant cellulose may be concentrated within the gelatinous fibers, making them the important source for production of biofuels and bio-based products. An additional attractiveness of cellulosic fibers for such applications comes from the fact that gelatinous cell wall layers are devoid of lignin – the major hurdle in using plant biomass [1].

Cell wall of cellulosic fiber is of very special design, which provides unusual properties. Such fibers serve as a kind of plant "muscles" [4,5]. The revealing of mechanisms of the formation and function of cellulosic fibers is important for understanding the determinants of general plant architecture and can be useful in construction of new bio-based materials.

2. Definition of plant cellulosic fibers

In terms of plant biology, a fiber is an individual cell of mechanical tissue (sclerenchyma), characterized by the extreme cell length and well developed secondary cell wall, architecture of which is the major determinant of fiber properties. Plant fibers are one of the most economically important raw material, used both for traditional and innovative technologies [6,7]. Due to the massive cell wall, fibers comprise a significant proportion of plant biomass, thus being the valuable source of bio-based products and biofuels. For plant itself fibers are very important for the general architecture and mechanical properties of certain organs.

The functional roles of fibers within the plant and their numerous commercial applications are largely based on the characteristics of their well-developed cell wall of considerable thickness. Fibers of different origin are not uniform in their structure and cell wall composition. The thick cell walls of fully differentiated fibers can be categorized into two broad types – the xylan and the gelatinous ones [2] (Figure 1).

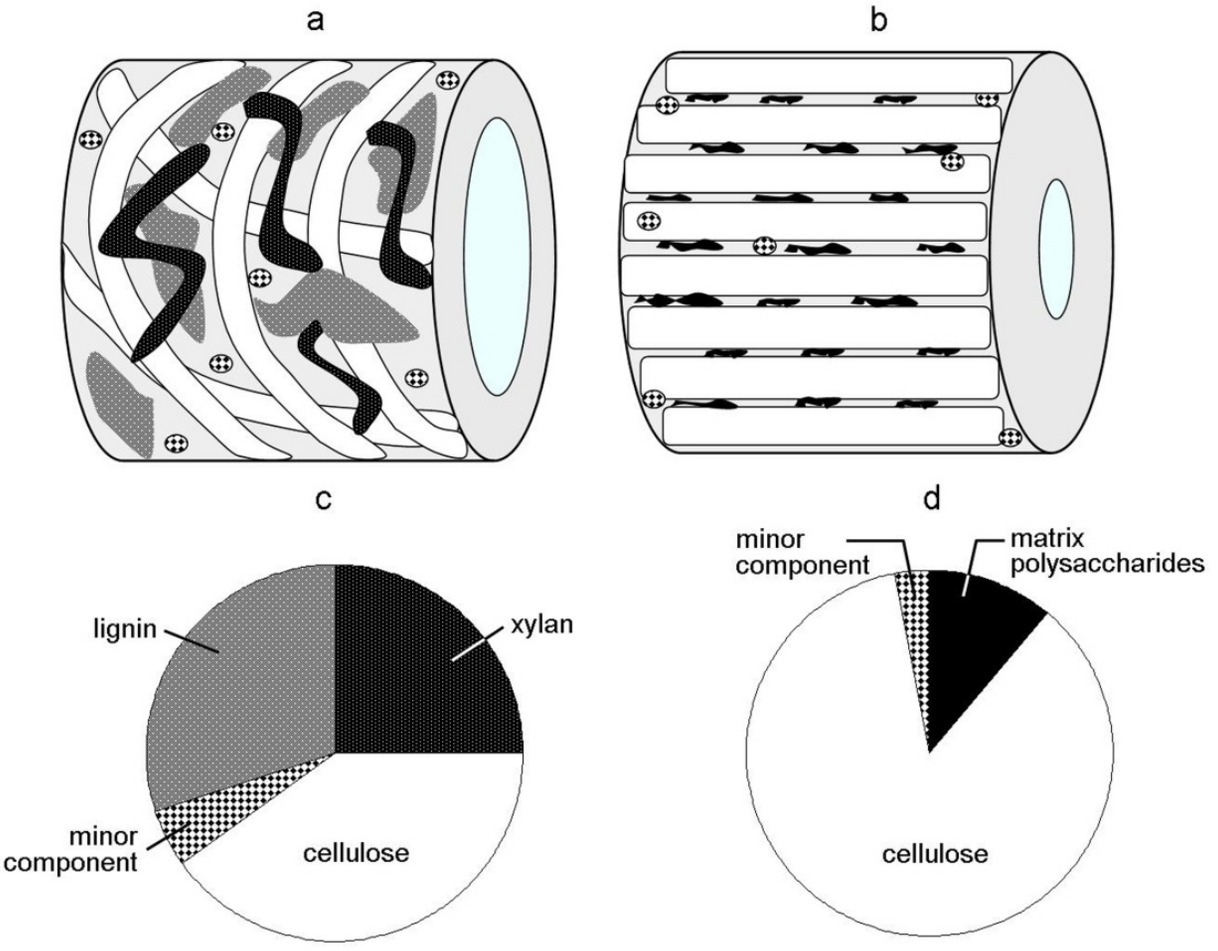

Figure 1. A scheme of structure (a, b) and content of the main components (c, d) in two types of the secondary cell walls: a, c – xylan type of cell wall, b, d – gelatinous type of cell wall.

A secondary cell wall of the xylan type is the most common one in various types of cells with secondary cell walls in land plants. The xylan type secondary cell walls are characterized by helical orientation of cellulose microfibrils, predominance of xylan in non-cellulose matrix, and high degree of lignification. The orientation of cellulose microfibrils may be significantly changed several times through the development of the xylan type secondary cell wall, leading to the formation of distinct layers, designated as S1, S2, and S3 layers (S from "secondary") in the order of deposition. Total thickness of the xylan type secondary cell walls is between 1 and 4 μm. They comprise the bulk of secondary cell walls in various types of wood cells, including vessels and wood parenchyma, and are also present in the bast fibers of some species, for example, jute and kenaf.

The second (gelatinous) type of thick cell wall is present only in fibers. It was firstly described by Th. Hartig at the end of the XIX century as a peculiar layer (G-layer) produced in the reaction wood of dicotyledonous plants (cited after [8]). The reason for such name came from the artefactual swelling of this layer in the cross-section due to the presence of certain components (e.g. alkali) of the solutions used to prepare the sample for microscopy. Fibers, which have developed G-layer of cell wall, get the name "gelatinous fibers". With modern techniques of sample preparation for microscopy, this layer doesn't look like a jelly. Moreover, G-layer was described as having exclusively high content of cellulose (up to 90%) with high degree of crystallinity [9], giving the reason for the alternative name – "cellulosic fibers". However, the justification for a term "gelatinous" was recently provided by description of the gel-like performance of G-layer upon drying (large shrinkage [8,10-12] and high rigidification [13]), and hydrogel type of structure, which has special characteristics of mesoporosity. G-layer has high content of mesopores (pore size between 2 nm and 50 nm); in tension wood fibers the pore surface areas may be more than 30 times higher than that in normal wood as was revealed by nitrogen adsorption technique [8, 14]. Mesoporosity was suggested as a new parameter for G-layer characterization [14].

Gelatinous cell-wall layer is deposited inward to the xylan type secondary wall layers; the degree of S-layer development in fibers with G-layer differs from well pronounced, like in tension wood [15], to barely detectable, as in flax [2]. Though not appropriately recognized, this type of fibers is widespread and is present in various organs of plants from many taxa [2,3]. Among others, phloem fibers of flax, hemp, and ramie, gelatinous fibers of tension wood, some fibers of bamboo and *Equisetum* belong to this group. Arabidopsis was shown to have the potential for gelatinous fiber formation [16], same as some other plant species where this type of fibers was not well known, like alfalfa [17]. Fibers able to form the gelatinous cell wall layer can originate from both primary and secondary meristems and be located within phloem or xylem [18].

Specific characteristics of the gelatinous layer of cell wall include: a) the overwhelming content of cellulose (80-90%); b) high crystallinity of cellulose; c) very low angle of cellulose

microfibrils, which are laid almost parallel to the fiber's longitudinal axis throughout the whole layer; d) considerable thickness, which in some species can reach more than 15 μm; e) the absence of xylan, f) the absence of lignin, g) special composition of matrix polysaccharides, presented mostly by galactose-containing pectins with rhamnogalacturonan backbone; h) high water content, as compared to S-layers, i) mesoporosity, j) exclusive presence in fibers, and, as discussed below, k) contractile properties.

The very peculiar characteristic of fibers with the gelatinous cell wall is their contractile properties. Such fibers may serve to move the plant parts in space. For instance, the tension wood fibres contract longitudinally during differentiation and generate longitudinal tensile stresses of up to about 70 MPa [19], providing rightning force in the tilted tree. A high-tensile growth stress generated on the surface of the xylem in the tension wood region often becomes ten times as large as that in the normal wood region [20]. The ability of a plant organ to contract is proportional to the degree of the development of fibers with G-layers [21,22].

Plants do not possess animal-type muscles, which contract due to protein-protein interactions. However, they have a different mechanism, which has the ability to move even very heavy plant parts in space. This mechanism is specifically developed in cellulosic fibers. Their contractile properties are based on tension developing within the specially designed thick cell walls. The efficacy of such fibers is remarkable. Thus, the gelatinous fibers may to a certain extent be named as "cell-wall-based plant muscles", though they do not have the ability to relax and the time-scale of their contraction is very different from that of animal muscles.

The examples of contractile action of cellulosic fibers (Figure 2) include the restoration of young tree vertical position, if disturbed [15,23], deepening of geophyte shoot in the soil by contraction of roots in the course of adaptive reaction [24], shortening of aerial roots when they reach the soil to form the effective support to heavy branches [21]; correction of lateral branch angle and, in case of apical meristem death, upward bending of lateral branch in order to transform into dominant [25-27]. The cellulosic gelatinous fibers are especially developed in the plants exposed to high mechanical stresses due to high ratio between stem height and stem diameter (most of fiber crops, like flax, hemp, ramie, nettle) [2] – this gives the properties of spring to the plant stem, helping to restore vertical position when disturbed, for example by wind. Such plants, similar to plants with developed tension wood [28,29], also exhibit the pronounced negative gravitropism, being able to turn back to vertical position a long stem, if bent, far away from the growing apical stem region. Cellulosic fibers seem to be present in the spines of cacti [30], and are developed in peduncles helping to support heavy fruits, like in the sausage tree, fruits of which may weight up to 10 kg [31]. The gelatinous fibers were demonstrated to be widely involved in the twining of vines and the coiling of tendrils [32,33].

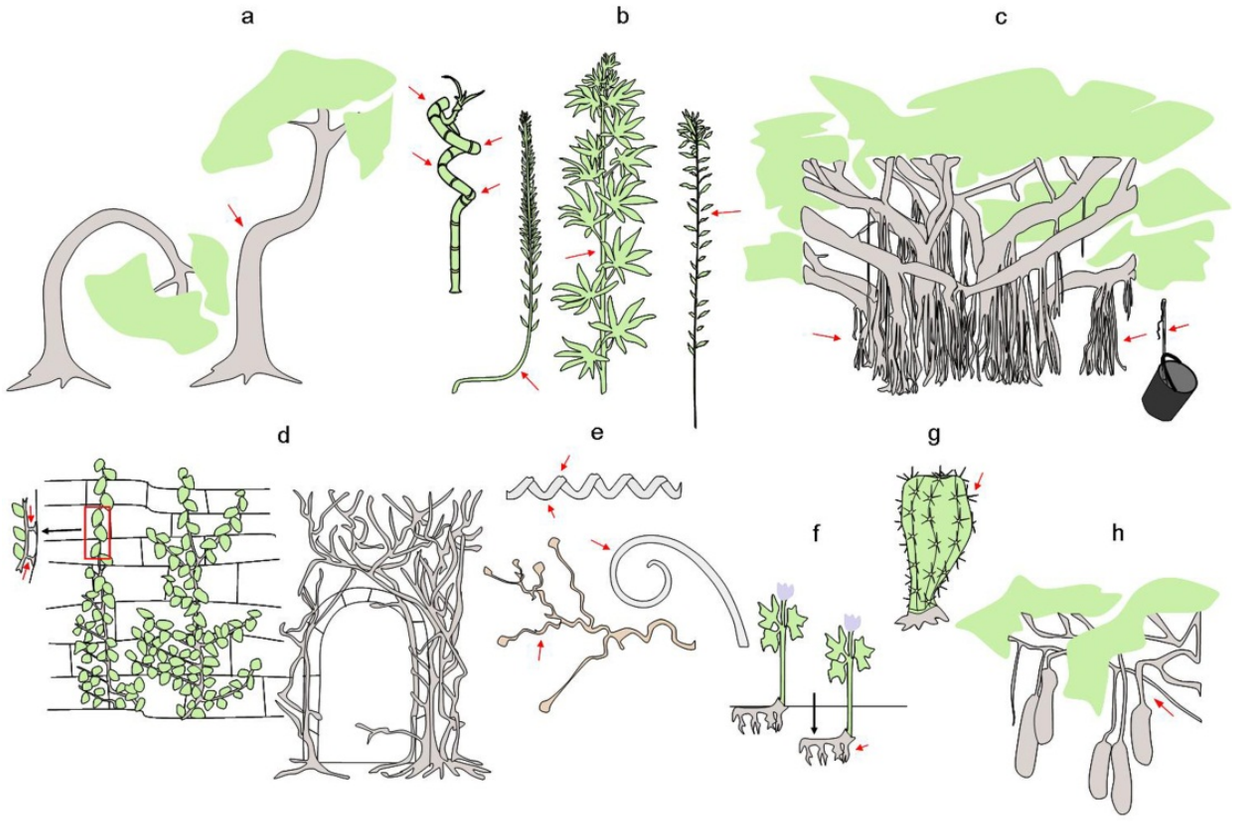

Figure 2. The examples of contractile action of cellulosic fibers: a – formation of tension wood during restoration of young tree vertical position, if disturbed, b – development of gelatinous fibers in the plants with high ratio between stem height and stem diameter, c – shortening of aerial roots after they reach the soil to form the effective support to the heavy branches (when rooted in pot, aerial roots may rise it up), d – development of cellulosic fibers in parts of stem due to which plant attaches to substrate, e – involvement of gelatinous fibers in the twining of vines, the coiling of tendrils and the expansion of climbing plants, f – deepening of geophyte shoot into the soil by contraction of roots in the course of adaptive reaction, g – presence of cellulosic fibers in the spines of cacti, h – development of gelatinous fibers in peduncles to help support heavy fruits.

3. Cellulose microfibrils in gelatinous cell wall

The very specific characteristic of cellulose microfibrils in the gelatinous cell wall is their axial orientation, which is not observed in any other cell wall type of any other but fiber cell type. This is especially remarkable, if one remembers the total thickness of G-layers. The axial orientation of cellulose microfibrils throughout the gelatinous cell wall layer was known long ago [9,34] and was confirmed by several techniques, including microbeam X-ray diffraction [35], wide-angle X-ray scattering [36], and scanning Raman microscopy [24,37]. In accordance to that, cortical microtubules, which are considered to rule the microfibril orientation, are axially oriented during deposition of the gelatinous cell wall layers [38].

In the gelatinous fibers cellulose microfibrils are characterized by a higher degree of crystallinity and a larger size of crystalline regions (crystallites) as compared with most

other plant tissues [39, 40]. The diameter of cellulose crystallites was measured in various species by several authors and though the absolute values might differ, the general conclusion was that in G-layers it was larger than in the S-layers [12,35,41-44]. The roentgen structural analysis showed that the diameter of cellulose crystal transverse sections in tension wood G-layer (6.5 nm) is markedly larger than in the neighboring S-layer of the xylan cell wall (about 3 nm), i.e., its section area is approximately fourfold higher [35]. Thus, cellulose microfibrils within the gelatinous layer exist in the form of aggregates. This allowed a supposition that individual cellulose microfibrils, each of which is formed by individual cellulose-synthesizing complex, so-called "rosette", in the gelatinous layer interact laterally [4]. Such lateral interaction is stimulated due to similar (axial) orientation of all microfibrils, the absence of lignin and of considerable amount of matrix polysaccharides, which separate microfibrils, like in S-layer. Despite the high degree of crystallinity of the cellulose, G-layer has a remarkable hygroscopicity and high water content [37,45].

Cellulose microfibrils in the gelatinous layer are under tension. This was proved by the increase in cellulose lattice spacing revealed by synchrotron radiation microdiffraction [46]. The visual demonstration of tension comes from shrinkage of G-layer along the cell longitudinal axis upon the release of tension, as observed by scanning microscopy of tension wood cross-sections [10].

For a long time, the gelatinous cell wall was believed to be composed of cellulose only [9]. Correspondingly, the ideas on tension origin were based on cellulose microfibril properties. Cellulose microfibrils themselves are virtually incontractible. So, the problem was how to get contraction, having incontractible basis of cellulose microfibrils. One of the possible solutions suggests that the contraction of the fibre is not caused by the G-layer directly, but by interaction of the G-layer with the surrounding S-layer. It was proposed that the origin of tension in cell wall of cellulosic fibers lays in the differential parameters of swelling of the S- and G-layers due to different orientation of cellulose microfibrils [36].

The cell walls with helicoidal orientation of microfibrils increase in length upon swelling, while the ones with axial orientation shrink [47]. The stress–strain-curves of cell walls show the influence of the cellulose microfibril orientation on the deformation behavior of plant tissues [48]. Within plant organism, such differences are indeed exploited in some mechanisms, for instance in opening of pine cones [49]. Fibers and sclereids located at the opposite sides of a cone scale have different angle between the long axis of the cell and the direction of cellulose microfibrils (MFA): it is high in sclerids and low in fibers. Correspondingly, the coefficient of hygroscopic expansion of fibers is significantly lower than that of sclerids. Due to that, the increase in relative humidity leads to the increase of the angle between the scale and the frame, leading to cone opening. Similar is the mechanism of wheat awn opening aimed to seed dispersal [48,50].

Similarly, the differences in swelling of S- and G-layers were suggested to explain the formation of tension in fibers of reaction wood [36]. The idea is based on the established fact that the enzymatic removal of the G-layer lead to the longitudinal extension and tangential shrinkage of tissues within the tension wood slice. It was proposed that in the living plant, a

lateral swelling of the G-layer forced the surrounding S-layers to shrink in the axial direction.

Such forces may indeed be the part of the tension creation mechanisms in gelatinous fibers. But: 1) such system would be highly dependent on humidity, same as the openings of pine cones and wheat awns; 2) in some species, like flax or ramie, S-layer in the gelatinous fibers is poorly developed [2] and hardly may serve as a mechanical counterpart of very thick G-layer; 3) mesoporosity of the G-layer is not explained; 4) tension is argumented to be developed within the G-layer itself [10,51]. Finally, specific matrix polysaccharides, which were not considered in the above hypothesis, appear at the onset of G-layer formation.

4. Matrix polysaccharides in cellulosic fibers

The presence of a polymer within a certain cell wall layer is not easy to prove. The biochemical analysis in the majority of studies is usually performed without separation into different cell wall layers, which is rather hard to achieve. In most of the experiments on the analysis of the gelatinous fiber composition, primary cell wall was not detached from the secondary one, and the xylan layers were not separated from the gelatinous ones. Moreover, such analysis is often done on the samples, like wood, which contain complex mixture of various cell types (e.g. parenchyma, vessels, fibers) at different stages of the development. A significant amount of data concerning the composition and structure of the gelatinous type cell walls was obtained by the analysis of phloem fiber bundles, which extreme strength permited their mechanical or enzymatic separation from surrounding tissues. Although gelatinous layers predominate in such fiber cell walls, the primary cell wall and S-layer of the secondary wall are also present. That's why, for instance, polygalacturonic acid (PGA) or rhamnogalacturonan I (RG I), described in numerous papers on plant fibers (e.g. [52-55] can not be attributed to a certain cell wall layer. The presence of polymers just in the G-layers must be additionally proved.

To do so, several approaches can be used or, better, combined: a) isolation of the G-layers and the biochemical analysis of constituents; b) cytochemistry, including immunocytochemistry; c) the analysis of deposition dynamics: search for the marker monomer, sugar linkage type or other specific characteristic of a certain polysaccharide, the formation of which goes in parallel to the G-layer deposition; pulse-chase experiments with labeled precursors can be especially effective since they permit to exclude the background of previously synthesized polymers; d) tracing the transcription of the identified genes, involved in the metabolism of certain cell wall polysaccharide, in the course of the G-layer formation; e) detection within G-layer of the enzyme or enzymatic activity, involved in modification of matrix polysaccharide, by various types of staining. The best way to analyze the components of the gelatinous cell walls is isolation of the G-layers, like it was done for poplar tension wood [28,56]. To this end, thin tissue sections (20 μm) are prepared and treated with ultrasound; however, this procedure permits obtaining only small amount of the material.

Among neutral monosaccharides present in polymers of the isolated G-layers, rhamnose, arabinose, galactose, and xylose were found along with glucose [56]. The cell wall matrix polysaccharides, which were identified or suggested to be present in the G-layer include xyloglucan [56], arabinogalactan proteins [57], pectic galactan [58], and, probably some arabinans [30]. Usually these polymers are considered to be the components of the primary cell walls. The obtained data on their presence in the G-layer were recently summarized in the review [5], so here we consider them only briefly.

Arabinogalactan proteins (AGPs) are highly water soluble polymers, which consist of protein backbone and carbohydrate side chains of variable structure, which can comprise over 90% of the molecule. Glycan component of AGPs has various length chains of β-(1→4)-galactan and β-(1→6)-galactan units, often decorated by terminal arabinose residues and connected to each other by (1→3,1→6)-linked branch points, which are indicative of AGPs. Protein backbone may also vary in structure and is encoded by a large gene family, the several representatives of which are always detected among the most up-regulated upon the G-layer induction genes, both in tension wood [59-62] and in fiber crops [63-65]. Carbohydrate constituents of AGPs were detected within the G-layer by immunocytochemical [57,60], cytochemical (by staining with Yariv reagent) [58,66] and biochemical [56] approaches. AGPs, different both in carbohydrate and protein part of the molecules, are present in many, if not all, plant tissues, but their exact function is still unknown.

There is not much information about another possible constituents of the gelatinous fibers – the arabinans. They were reported to be the major cell wall matrix component of cellulosic fibers in cactus spines [30]. It is not clear if the arabinans are attached to the RG I backbones.

The most substantial evidence for the matrix cell wall polysaccharides of the G-layers was collected on xyloglucan. This cross-linking glycan is composed of a backbone, which is built similar to cellulose molecule as β-(1→4)-glucan. The side chain of xylose, which is sometimes additionally substituted by galactose and further – by fucose, are attached to the backbone. Xyloglucan is the major noncellulosic polysaccharide in the isolated G-layers of poplar tension wood; its content was assessed to be 10–15% of the cell wall mass [28,56,67]. The presence of xyloglucan was detected by several methods, including the biochemical analysis of the types of bonds between monosaccharides and immunocytochemistry. Moreover, the presence of xyloglucan endotransglycosylase, an enzyme providing for connection between the regions of two different xyloglucan molecules, was demonstrated in the G-layers of the secondary cell wall. Two main functions were suggested for xyloglucan in the secondary cell walls of tension wood fibers [67]. The first one is binding of the G-layer to the neighboring xylan layer because xyloglucan and xyloglucan endotransglycosylase are localized just at the boundary between these two layers, as was shown immunocytochemically. The second supposed function is the creation of tension – it will be considered in the next chapter.

One more component of the G-layers is pectic galactan, built as a very complex rhamnogalacturonan I with a high degree of branching and a varying structure of side

chains, which are mainly composed of β-(1→4)-galactose [52,58,68]. The predominant monosaccharide in the polymer is galactose, which determines the polymer name as a galactan [69]. The side chains may include only one or two galactose residues; long chains of several tens galactose residues, likely branched side chains, which are not cleaved by galactanase; side chains, decorated with a single pentose, most likely arabinose or a galactose residue connected by other than β-(1→4) linkage [68,70]. This type of pectic galactan is fiber- and stage-specific, being present only in fibers, while forming G-layer [2,71].

Pectic galactan may be of the specific three-dimensional organization, the signs of which are revealed upon the treatment with specific glycanases. The hydrolysis of considerable part of galactose side chains of galactan as well as the partial degradation of its backbone do not change the total hydrodynamic volume, which determines the efficiency of elution from gel-filtration column, and the polymer elutes in the same part of profile, as before enzymatic treatments [68,72]. The unusual property of pectic galactan from the gelatinous fibers is the ability to form water-soluble associates, so that the charged backbone is located at the periphery of it, while the neutral side chains form the core zone (Mikshina, Gorshkova, in preparation).

The presence of the galactan within the gelatinous layer was confirmed by the analysis of the dynamics of its formation, which coincides with the G-layer deposition [71], by immunolocalization of the galactan side chains [71,73-75], by presence of tissue- and stage specific β-(1→4)-galactosidase, the substrate of which is the described galactan [75-77]. The gene of this galactosidase is highly upregulated at the onset of the G-layer formation [63, 78] and the activity is detected within fibers, forming gelatinous cell wall [64,75].

The complex galactans built mainly from β-(1→4)-galactose were found in tension wood fibers in 60-ties of the XXth century [79,80], though the linkage with the RG I backbone was not proved at that time. The content of galactose was even suggested as an indicator of the extent of the G-layer development [81]. However, these old data were actually put away for several decades due to the overwhelming notion that the G-layers were pure cellulosic, so that the published in 2008 paper describing the detection of rhamnogalacturonan I by cytochemical approaches in tension wood of several species was entitled "…gelatinous fibers contain more than just cellulose" [57].

5. Matrix polymers as the causative agent for cellulose tension in gelatinous cell wall

Presence of specific matrix polysaccharides within G-layer suggests their importance for function of cellulosic fibers, including tension creation to form contractile properties. Mellerowicz et al. [4] put forward an idea that matrix polysaccharides are entrapped by laterally interacting cellulose microfibrils. The presence of such entrapped polysaccharides between cellulose microfibrils limits their interaction and results in creation of tension, which underlies specific mechanical properties of cellulosic fibers (Figure 3).

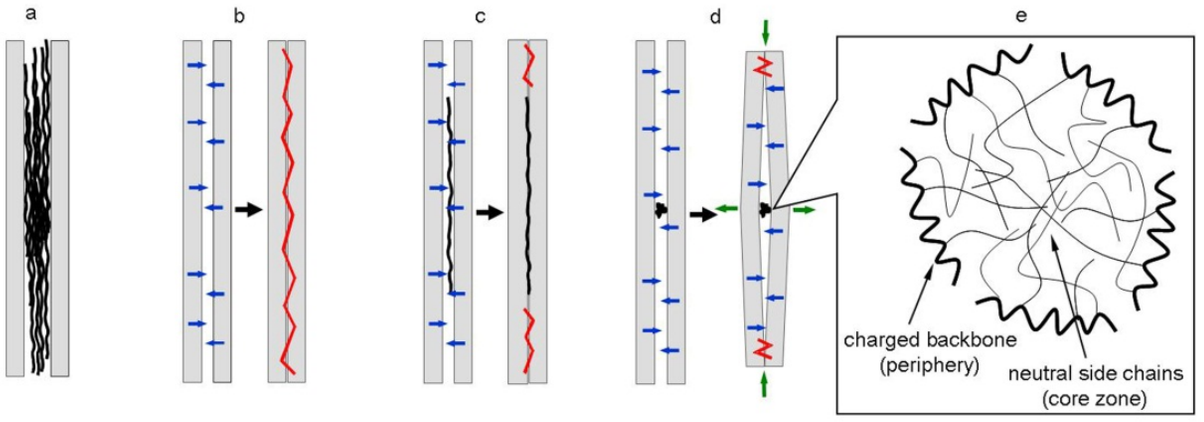

Figure 3. Possible ways of interaction between matrix polysaccharides and cellulose microfibrils in various types of cell walls: a – high content of matrix polysaccharides in xylan secondary cell wall prevents lateral interaction of cellulose microfibrils, b – microfibrils of the G-layer (gelatinous cell wall) with low content of matrix polysaccharides, cellulose microfibrils tend to lateral interactions, giving reason for higher degree of crystallinity and larger size of crystallites, c – theoretically, if matrix polysaccharides has high affinity to cellulose, being entrapped they won't cause much of tension, d – the most effective to provide longitudinal tensile stress in the cellulose microfibrils is compact polysaccharide of considerable size with low affinity to cellulose, e – a model of pectic galactan associates, in which negatively charged RG I backbone is at the periphery, and long galactose side chains form the core zone.

Originally, xyloglucan was proposed as the polymer entrapped by the laterally interacting cellulose microfibrils. Xyloglucan is, indeed, very important for the function of gelatinous fibers in tension wood of some species, like poplar. This is proved by the fact that in transgenic poplars with the expressed gene of fungal xyloglucanase, which decreased the content of xyloglucan, righting of stem basal regions in placed horizontally young plants was completely abolished, while the G-layer formation was not affected [29,82]. The expression of other endoglycanases, which decreased the levels of xylan or arabinogalactan, had no effect on the ability of transgenic plant to exhibit gravitropic reaction, restoring the stem vertical position.

However, the exact function of xyloglucan in tension wood fibers is still a matter of debate. Firstly, xyloglucan was detected in the G-layers only in limited plant species, it was never conclusively reported (though searched) to be present in thick secondary walls of cellulosic fibers in fiber crops, like flax, hemp, etc. Further evidence comes from the analysis of polysaccharides, strongly retained by cellulose microfibrils upon extraction: if entrapped between the interacting laterally cellulose microfibrils, a polymer should not be extracted by the conventional methods and should come out only after degradation of microfibrils by chemical or enzymatic means. However, due to high crystallinity of cellulose in the gelatinous layers, in natural form it is poorly degraded by specific enzymes [66] and thus, has to be first dissolved by corresponding chemicals.

To analyze the polysaccharides, which are especially strongly retained within cell wall, a special protocol was developed [83]. After removal of the extractable polysaccharides by

chelators and concentrated alkali, the residual cell wall material was dissolved in solution of lithium chloride in N,N-dimethylacetamide and afterwards cellulose was precipitated by water. Such treatment turned natural cellulose I (with parallel orientation of individual cellulose chains) into cellulose II (with antiparallel orientation of individual cellulose chains) and made it completely degradable by purified cellulase. The matrix polysaccharides, which were present in the fraction, remained in polymeric form, making possible to separate them by gel-filtration for further analysis.

We have compared the composition of matrix polysaccharides, strongly retained by cellulose microfibrils, in fibers with different proportions of the secondary cell walls of xylan and gelatinous types (Figure 4). The polymers from fibers with only xylan type secondary cell wall eluted in the region below 30 kDa. The monosaccharide analysis and antibody binding indicated that the major component of this fraction was xylan. It is known that small proportion of matrix polymers, both in the primary and the secondary cell walls get entrapped by cellulose microfibrils in the process of their crystallization [84,85]. Some polygalacturonic acid was also present, which could be originated from the primary cell wall.

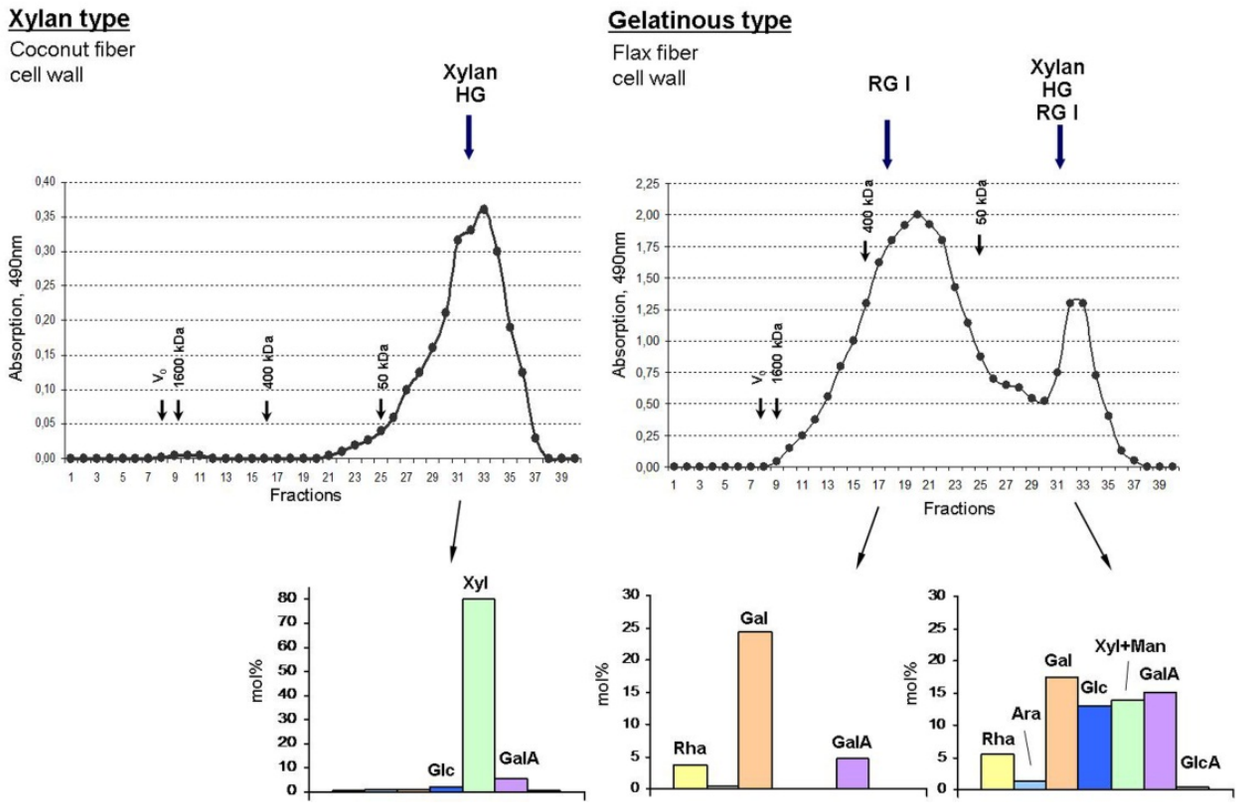

Figure 4. Elution profiles of polysaccharides strongly retained by cellulose microfibrils in the xylan and gelatinous cell walls and the relative monosaccharide content (mol%) of the main fractions of these polysaccharides.

In fibers with the G-layers, the major peak of matrix polysaccharide eluted between 100 and 400 kDa; its predominating component was pectic galactan. This galactan from flax fibers

was characterized by various techniques, including ^{1}H and ^{13}C NMR and antibody binging [83]. The ratio between high and low molecular mass peaks on the elution profile depended on the proportion of the S- and G-layers within the fiber cell wall. Antibody to xyloglucan epitopes didn't bind any fraction on the elution profile.

The proportion of pectic polymers, which were strongly retained by cellulose microfibrils, from their total content in cell wall of the gelatinous type, could be much higher than that of xylan in the S-layers. Such selectivity in entrapping of certain polymers can not be explained by their affinity to cellulose as the charged pectic molecules are far less competitive, compared to xylan. The obtained data suggest the alternative mechanism of interaction between cellulose and pectic galactan, which is specifically developed in cell walls of the gelatinous type.

The above data suggest that in cellulosic fibers it is pectic galactan that is entrapped by laterally interacting cellulose microfibrils. This polymer, due to ability to form associates, can perfectly fit the proposed function in tension creation, as illustrated in Figure 3. In the xylan type of secondary cell wall (a), high content of matrix polysaccharides prevents the lateral interaction of cellulose microfibrils. At low content of matrix polysaccharides in G-layer (b), cellulose microfibrils tend to lateral interactions, giving reason for higher degree of crystallinity and larger size of crystallites. Matrix polysaccharides with high affinity to cellulose, if entrapped (c) won't cause much of tension. Most effective would be compact polysaccharide of considerable size with low affinity to cellulose (d). Associates of pectic galactan with RG I backbone may be a good choice of Nature for such purpose. They have compact structure of considerable volume, which has poor ability (due to charged surface) to interact with cellulose (e).

Additional arguments for the important role of pectic galactans in creation of tension come from the analysis of the course of the G-layer formation and of *in muro* modifications of matrix polymers, which was in detail performed on flax cellulosic fibers.

6. Dynamics of the G-layer formation and in muro modifications of cell wall polymer

Formation of a cell wall layer is a complicated event. Partly it is based on the processes of polysaccharides' self-assembly in specific surroundings. Besides, the cell wall formation may involve modification of the interacting polysaccharides. The very illustrative example of the latter is the remodeling of the deposited G-layer in flax cellulosic fibers. In the dynamics of the G-layer formation two stages are clearly visualized at microscopic investigation of the flax fiber cell wall formation [71,86]. Under electron and/or light microscope, one can see that the inner part of the cell wall has a characteristic appearance of the loose structure where the electron dense parallel bands alternate with light regions; the outer part has much more homogenous structure (Figure 5). These two parts of the cell wall are designated as the Gn- and G-layers. During formation of the secondary cell wall, the thickness of outer layer gradually increases, while additional portions of the Gn-layer are added by the protoplast. This indicates that with time the Gn-layer is transformed into the G-layer.

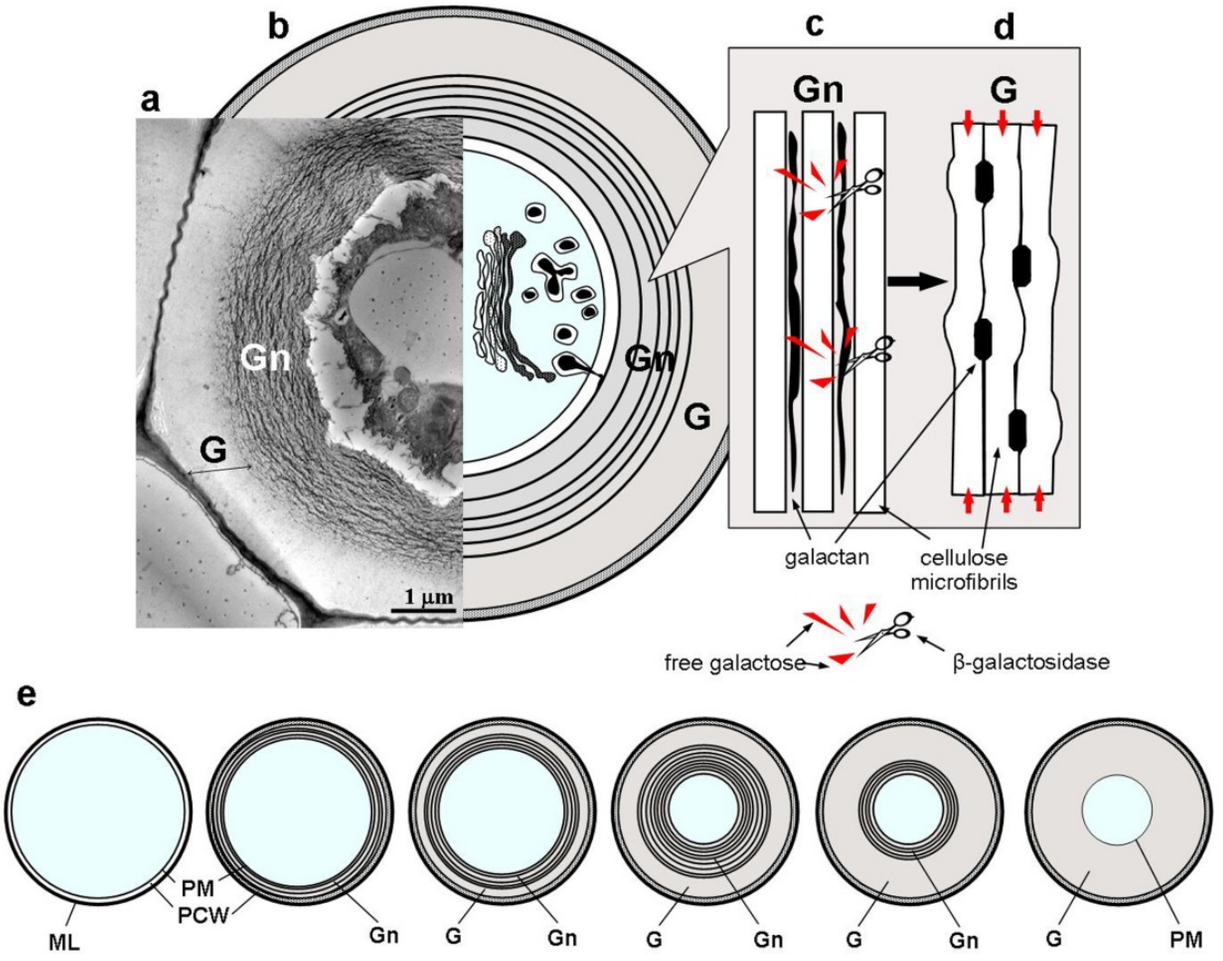

Figure 5. A model of the Gn-layer to the G-layer transformation in gelatinous fibers: a – electron microscopy of developing flax fiber cross-section; two layers (Gn and G) are obvious; b – scheme of developing flax fiber cross-section, showing a tissue-specific galactan delivered by specific Golgi vesicles to the developing Gn-layer; c – the nascent galactan is interspersed between cellulose microfibrils, preventing their association and maintaining the loosely packed morphology characteristic of the Gn-layer of secondary cell wall. During cell wall maturation, high molecular galactan partially digested by β-galactosidase, releasing free galactose; d – reducing of side chain length of galactan by galactosidase allows cellulose microfibrils to interact laterally, entrapping the galactan. Thus densely packed G-layer that is rich in crystalline cellulose is formed. The presence of entrapped galactan during lateral interactions of axially oriented microfibril causes longitudinal tensile stress in cellulose; e – dynamics of gelatinous layers deposition and remodeling in cellulosic fibers (left to right). ML – middle lamellae, PM – plasmalemma, PCW – primary cell wall, Gn – newly deposited gelatinous layer of secondary cell wall, G – mature gelatinous layer of secondary cell wall.

Transition from the Gn- to G-layer is coupled with changes in cellulose crystallinity. It is confirmed by the cytochemical analysis using the enzyme–gold complex, which showed that as distinct from G-layer, Gn-layer poorly bound cellobiohydrolase, the substrate of which is crystalline cellulose [58,87]. Besides, in pulse-chase experiments with $^{14}CO_2$ with intact flax plant, the dynamics of cellulose crystallization in fiber-enriched peels from all other analyzed samples. In roots, stem xylem, and stem apical part, which do not contain fibers with the gelatinous cell wall, the proportion of crystalline cellulose did not change during

the entire experiment, while in fibers starting at the same level as in other tissues, it increased twice through the first day of chase and only later attained the plateau, which was at much higher level than in other tissues [88] (Figure 6). This indicated that crystallization of cellulose microfibrils in the G-layer was a biphasic process: the first stage occured right after the individual cellulose chain synthesis, similar to other plant tissues, while the second stage, which gave additional increase in crystallinity, occured *in muro* – within cell wall.

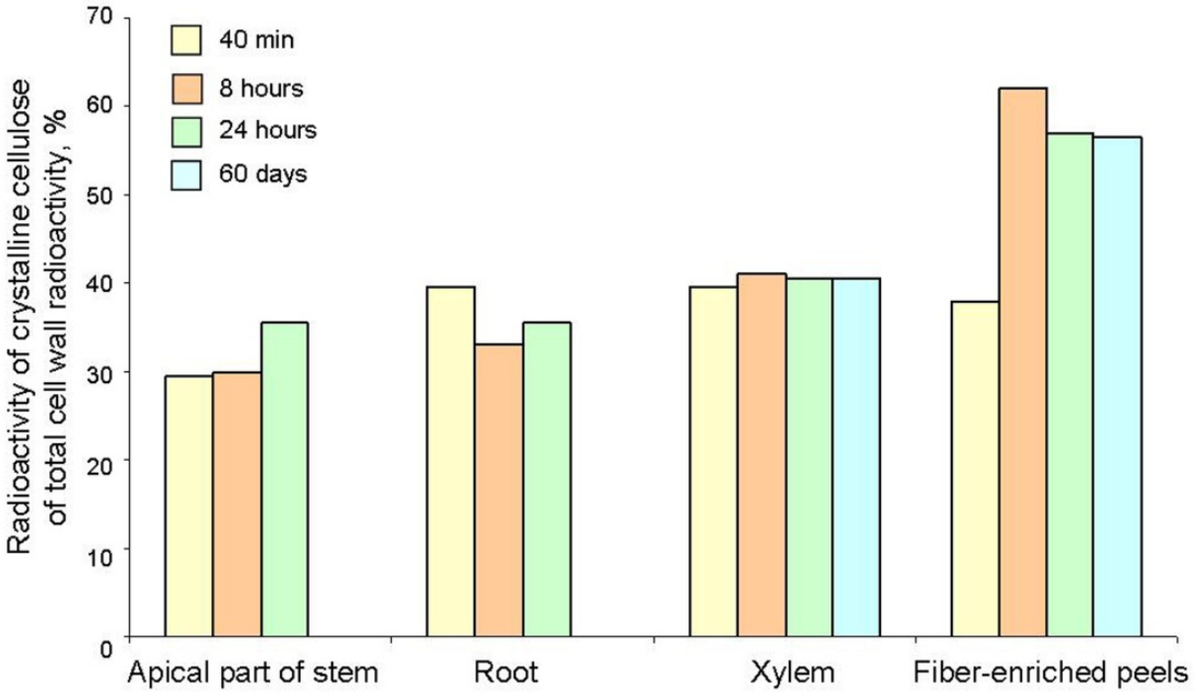

Figure 6. Radioactivity of crystalline cellulose of total cell wall radioactivity in the different part of flax plant after 40 min of photosynthesis with $^{14}CO_2$ (pulse) and during different periods of plant growth in the absence of a radioactive substrate (chase). Modified from data in [88].

The Gn- and G-layers differently bind not only cellobiohydrolase probe, but also the LM5 antibody, which is specific for β-(1→4)-galactan [89]. With LM5 the number of gold particles per area unit in the Gn-layer was fivefold higher than in the G-layer [71]. So, the reverse pattern was observed with binding the probes for cellulose crystallinity and for pectic galactan. Keeping in mind that antibody binding depends not only on the presence of the epitope but also on its availability, we consider it possible to suppose that changes in the degree of cellulose crystallization were related to *in muro* modification of tissue-specific galactan. An additional argument for such suggestion is a disappearance in the G-layer of dark bands, which are produced in the Gn-layer at galactan secretion by the Golgi apparatus and are well distinguished under electron microscope [71,74].

The pectic galactans are subjected to intensive *in muro* modifications. The investigation of galactan metabolism using the pulse-chase approach [2] confirmed that this polymer is synthesized in the Golgi apparatus, secreted outside the plasma membrane, and interacts

with cellulose microfibrils. Flax fibers, while forming the secondary cell walls, have a peculiar mechanism of polysaccharide secretion. Golgi-derived vesicles first accumulate in the cytoplasm and only later fuse with the plasma membrane to give their contents to the apoplast [74]. These Golgi derivatives as well as the layers of the secondary cell wall, especially the inner "striated" layer, bind the LM5 antibody, indicating that all these structures contain galactan. It suggests that this peculiar type of galactan secretion permitting for filling large spaces of the periplasm facilitates the contact between galactan and cellulose microfibrils when they are in the process of assembly and may be necessary for preventing lateral interaction of cellulose microfibrils right at their deposition. Such a mechanism of secretion allows to accumulate a sufficient amount of the nascent pectic galactan before it is incorporated into the cell wall. This nascent form of the pectic galactan can be collected from the tissue homogenization buffer and compared to the polymer strongly retained by cellulose microfibrils. The composition and structure of these polysaccharides together with tracing in pulse-chase experiments [68-70,83] permit to consider the entrapped by cellulose microfibrils galactan as a derivative of the nascent galactan. The comparison of these polymers revealed the following differences: the nascent polysaccharide elutes at gel-filtration as having higher molecular mass (in the 700-2000 kDa region) and has higher degree of branching and longer side chains, as compared to cell wall galactan [83].

The detected differences between the nascent and entrapped galactans suggested that they might be the result of *in muro* galactan modification by the enzyme cleaving off a part of the galactan side chains. Indeed, the histochemical staining of stems and hypocotyls with corresponding chromogenic or fluorogenic substrates shows β-galactosidase activity to be localized to developing fibers [64,76]. The gene of β-galactosidase is among the most up-regulated ones upon induction of the G-layer formation [63,78]. The substantial amounts of free galactose, which is the product of β-galactosidase action is present specifically in fibers forming gelatinous cell wall [76].

Shortening of the galactan side chains permits microfibril lateral interaction, due to which an additional portion of galactan is captured by them. The necessity of pectic galactan modification with the participation of β-galactosidase for the remodeling of cell wall supramolecular structure and transformation of the Gn-layer into mature the G-layer was demonstrated [75]. The role of fiber-specific β-galactosidase in providing the particular mechanical properties of gelatinous fibers was confirmed with transgenic flax plants (reduced galactan modifications – less mechanical strength) [75]. Antibodies raised to fiber-specific β-galactosidase of flax, revealed similar protein in the G-layers of cellulosic fibers in other plants (poplar tension wood fibers and both primary and the secondary phloem fibers of hemp), indicating that the process of the G-layer remodeling may be similar in fibers of different origin [77].

Thus, in the last several years the views on matrix polysaccharides of the gelatinous cell walls have changed dramatically: from rejecting their presence – to ascribing the major role to them in the development and function of cellulosic fibers.

7. Conclusions and perspectives for future research

Summary of our ideas on the cell wall design of cellulosic fibers and the origin of their contractile properties include the following statements, based on the considered in the current review literature data and our own results:

- Tension is caused due to lateral interaction of cellulose microfibrils and entrapment of matrix polysaccharides.
- Lateral interaction is possible because of very high cellulose content, absence of xylan and lignin.
- Similar axial orientation of all cellulose microfibrils in thick G-layer helps to cumulate tension of individual microfibrils and to develop it in the necessary direction. The effect is increased due to extreme length of fiber cells.
- The entrapped polysaccharide – complex rhamnogalacturonan I with galactan side chains of specific structure and distribution, which is able to form water-soluble associates.
- Entrapment of such associates leads to increased mesoporosity and to the development of cellulose microfibril tension.
- High hydroscopic capacity of RG I helps to keep water in the G-layer.
- Conditions for lateral interaction of cellulose microfibrils may be provided by *in muro* modification of deposited polysaccharide by fiber-specific galactosidase.
- Additional important factors may be the interaction of the G-layer with the S-layer through the action of xyloglucan-modifying enzyme, the activity of which is mainly detected at the boundary between layers, and/or different deformation behavior of the S- and the G-layers upon swelling due to different orientation of cellulose microfibrils.

Cellulosic fibers are the example of very peculiar cell wall type. Its formation includes significant reprogramming of synthesis and secretion of matrix polysaccharides, reorientation of cellulose microfibrils, active remodeling of the deposited cell wall layers, specific inter- and intra-molecular interactions between cell wall polymers. The study of these processes may give additional clues for general understanding of the plant cell wall formation, which still belongs to the most enigmatic biological processes. Of special interest is the investigation of specific three-dimensional organization of pectic galactans from cellulosic fibers in order to elucidate the largely unknown principles of supramolecular structure of complex polysaccharides. Comparison of the gelatinous cell wall formation in fibers of various organs may help to figure out the biological determinants of plant fiber yield and quality in order to improve the characteristics of plant biomass for effective conversion into biofuels and bio-based products.

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