

INNOVATIVE INSIGHT ON THE MECHANICAL PRINCIPLES OF THE CELL WALL BASED ON CRYO-ELECTRON TOMOGRAPHY OF THE SUPRAMOLECULAR 3D NANO-ARCHITECTURE OF ARABIDOPSIS PLANT

MEJORA INNOVATIVA EN LOS PRINCIPIOS MECÁNICOS DE LA PARED CELULAR EN BASE A TOMOGRAFÍA CRIOELECTRÓNICA DE LA ARQUITECTURA SUPRAMOLECULAR 3D DE PLANTA DE ARABIDIOPSIS

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Identification code: T3-27

Abstract

A cutting-edge cryo-electron tomography technique developed at the Lawrence Berkeley National Lab revealed the architecture of the cell-wall of the Arabidopsis plant with a resolution never seen before. This kind of plant, the Arabidopsis, is typically used in biology due to large size of cells and ease of manipulation. This new finding may be extremely important for wood technology, because it places us in a better position to improve our understanding of the nano-mechanics, which is one of the key aspects to better understand the wood properties and eventually manipulate the timber at the structural scale. Given the geometrical configuration of the cell wall, the challenge of this research was conducting a detailed mechanical analysis in order to get an insight in two fundamental questions: (i) why has the cell wall such mechanical properties? and (ii) which is the reason behind such a cell wall architecture? To this end, an extensive series of computational models that included masking, homogenization, nonlinear buckling and viscoelasticity were elaborated. The results of the analysis were mostly unexpected, and suggested that, despite the loss of stiffness, the capacity to store and dissipate energy, the stability, the load transfer and the ductility are the vital “design criteria” for plants to decide how the cell needs to be organized.

Keywords: cell wall; timber properties; homogenization; viscoelasticity; buckling; nanoscale

Resumen

Una técnica puntera de tomografía crioeléctrica desarrollada en el Lawrence Berkeley National Lab reveló la arquitectura de la pared celular de planta de Arabidopsis con una resolución jamás vista anteriormente. Este tipo de planta, la Arabidopsis, se emplea habitualmente en biología debido al gran tamaño de sus células y la facilidad de manipulación. Este nuevo hallazgo podría ser extremadamente importante para la tecnología de la madera al situarnos en mejor posición para mejorar nuestro entendimiento del funcionamiento mecánico en la nanoescala, lo cual es uno de los aspectos clave para comprender las propiedades mecánicas de la madera y eventualmente poder modificarlas en la escala estructural. Dada la configuración geométrica de la pared celular, el reto de esta investigación consistió en llevar a cabo un análisis mecánico detallado con el fin de responder dos preguntas fundamentales: (i) ¿a qué se deben las propiedades mecánicas de la pared celular?; (ii) ¿cuál es la razón de esa arquitectura celular? Con este fin se desarrollaron una gran cantidad de modelos numéricos que incluyeron segmentación, homogenización, pandeo no lineal y viscoelasticidad. Los resultados del análisis fueron sorprendidos y sugieren que, a pesar de la pérdida de rigidez, la capacidad de almacenar y disipar energía, la estabilidad, la transmisión de carga y la ductilidad son los “criterios de diseño” fundamentales de las plantas para decidir la organización de las paredes celulares.

Palabras clave: pared celular; propiedades de la madera; homogenización; viscoelasticidad; pandeo; nanoescala

1. INTRODUCTION

The origin of timber structural properties is mainly attributed to the nanoscale, also termed as ultramicroscale [1]. This scale normally ranges from several to thousands of nanometers and thus comprise the study of microfibrils, cross links and other polymeric structures that are organized casting cell-wall fragments. The organization of those fragments, and the organization of the entire cells, are the main actors dictating the timber engineering properties. In fact, it is possible to predict structural properties of wood in a hierarchic fashion so that the properties of each scale are obtained from the result of the corresponding inferior structure using the so called multiscale-models [2].

Nevertheless, those multi-scale models mostly lack in the nanostructure (NS) or are built using major assumptions of it. The investigations at the NS are indeed rather scarce not only for the woods, but also for most plants mainly because of the limitations in the resolution of the measuring methods. Better understanding of the NS is thus a paramount aspect to better understand the mechanics of woods and plants. In addition, the NS is also a crucial aspect for developmental biology, because the mechanical properties of the cell wall control the growth and morphogenesis, i.e. in addition to the synthesis of new polymers, the cells are constantly subjected to membrane (turgor) pressure and the deformation against that pressure dictates the diameter increase of lumens.

Several computational models of the NS have been reported in the past, but they have been based on major assumptions because the NS was not entirely known. Specifically, the models were based on pseudorandom fiber networks [3-5]. Randomness is likely to be an inadequate analysis approach for pattern-organized networks due to high fiber orientation dependency. Another remarkable assumption was considering fibers as perfectly straight and slender (high length to diameter ratio). This let modelling fibers as linear elements as against of considering their entire volume, which is much less computationally expensive. However, as presented in subsequent sections, those assumptions are unrealistic.

In this context, a cutting-edge cryo-electron tomography technique developed recently at the Lawrence Berkeley National Lab revealed the architecture of the cell-wall of Arabidopsis plants with a resolution never seen before. This Arabidopsis plants are commonly used in biology due to large size of cells and ease of manipulation. This new detailed data allowed for the first time the recreation of a detailed computational model at the NS that can reveal important insights on two fundamental questions: (i) why has the cell wall such mechanical properties? and (ii) which is the reason behind such cell wall architecture? This paper is devoted to present the main characteristics of such a mechanical model and the main conclusions regarding the above questions.

2. STARTING POINT

The typical measured NS of the secondary cell wall (SCW) and primary cell wall (PCW) are shown in Figure 1.

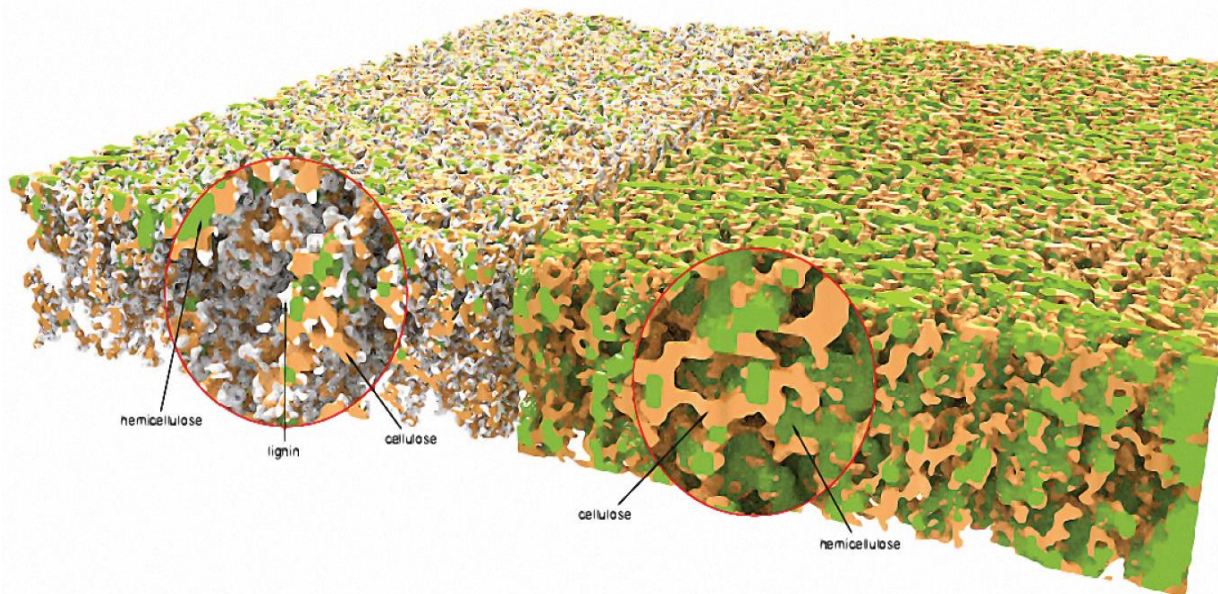


Figure 1: Typical nanostructure of the secondary cell wall (left) and primary cell wall (right). The cellulose is represented in brown, hemicellulose in green and lignin in white. The dimensions of both cell wall fragments are $250 \times 400 \times 50$ nm in the T, R and L directions, respectively. Note the cells are mostly cylindrical so that an analogy with the wooden L, R and T axes can be established.

As it can be seen in the Figure 1, both SCW and PCW are very complex structures composed mainly by cellulose, hemicellulose and lignin. The cellulose is mainly constituting microfibrils, which are relatively aligned with the L axis. The hemicellulose is mostly shaping the cross links, being smaller links that bond different microfibrils together. The orientation of these links is less fixed in comparison to microfibrils, but it is mostly orientated along T and R axes of the cell. Also, it is thought that part of the hemicellulose is glued together at the external surface of the cellulose microfibrils creating a small external layer at each microfibril. The exact shape of the lignin is unknown, but it is thought to conform small globules. As it will be presented later, such lignin globules were not actually measured because we used cells in youth (unlignified) cells, however we considered the presence of lignin in subsequent analyses to investigate the potential impact. All these compounds, cellulose, hemicellulose and lignin, are commonly assumed isotropic with Young moduli close to 30 GPa, 10 GPa and 6 GPa, respectively.

3. MASKING AND HOMOGENIZATION

Due to the complexity of the measured data, the possibilities of an exhaustive mechanical analysis of the NS were apparently very limited, as it seems rather impossible to vary the geometry and see what implications this may have. However, as it is presented in the next subsection, the apparently chaotic NS actually follows very well organized patterns.

In addition to the complexity of the geometry, there was no mean to know the exact composition of each individual fiber out of the tomographs - one can only know the average ratio of hemicellulose to cellulose of the entire structure, but it is not possible to ascertain whether a certain fibril is a microfibril or a cross-link as well as its initial and final locations.

In order to overcome this difficulty, we applied a masking procedure to differentiate the hemicellulose from the cellulose by taking advantage of the smaller size of cross links in comparison to microfibrils, the known hemicellulose ratio, and its 'logical' position. The masking procedure consisted of progressively removing the peripheral volume of each fiber until only the 50% of the initial volume of the SCW and PCW remained. As the hemicellulose is thought to mainly be located

at cross links and periphery of fibrils, and the volume of microfibrils (both length and diameter) is significantly larger than that of the cross-links, we attributed the cellulose to this 50% remaining volume. Although not exact, this filtering should provide us a reasonable split of cellulose and hemicellulose. This way we could differentiate the cellulose from hemicellulose as shown in Figure 1.

Once the initial structural composition was known, the first mechanical analysis consisted of determining the ‘effective’ elastic properties for both SCW and PCW. This was accomplished by homogenization based on strain equivalence principle. The homogenization is a standard procedure applied in composites and porous materials to estimate the overall physical or mechanical properties (effective properties) of a complex structure by only analyzing a small fraction that adequately represents the entire material. Such small fraction is termed the representative volume element (RVE) and, for mechanical problems, the estimation typically comprises loading the RVE in different directions to subsequently apply an equivalence principle. Displacements, strain and stresses are customarily calculated via finite element method. By this equivalence principle one can determine whether there exists an ideally homogeneous material so that its effective properties (stiffness) adequately represent the average strain or energy stored in the complex RVE fraction. If the homogenization succeeds, i.e. if there exists such a homogenous material, the output could be very valuable to account for the NS of the cell wall at larger scales. The typical displacement results of the SCW during one homogenization test are shown in the Figure 2.

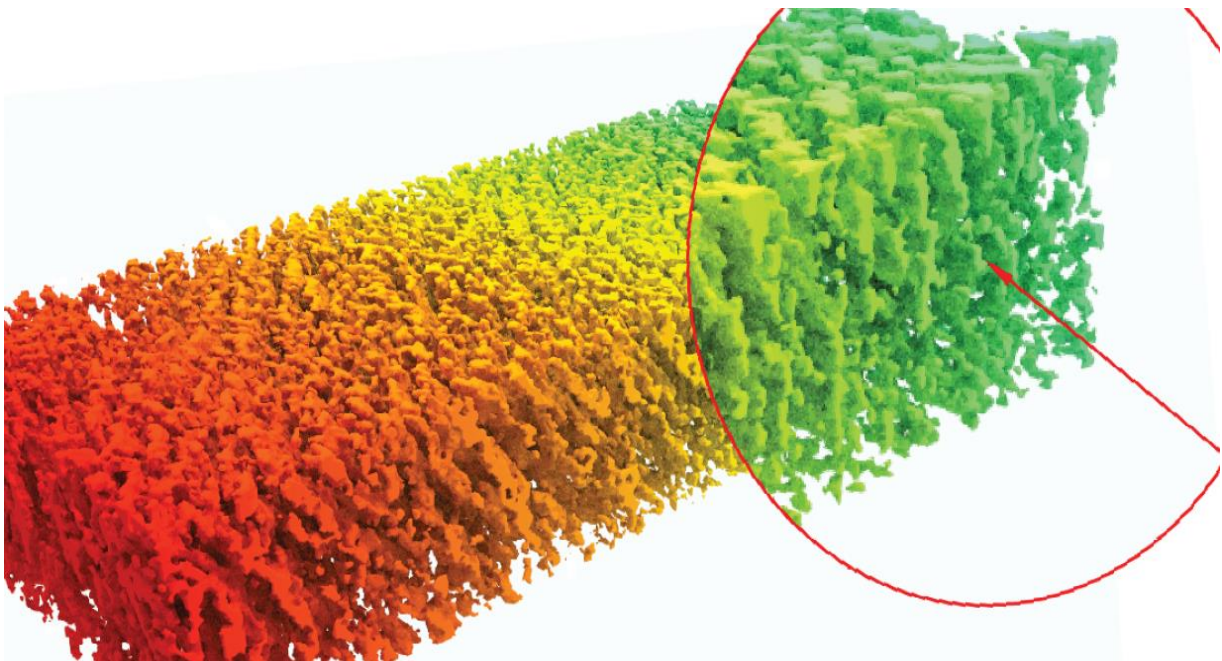


Figure 2: Typical calculated displacements of the SCW during a homogenization experiment. For a sake of simplicity, hot colors indicate large displacement.

Homogenization of the geometries shown in Figure 1 resulted in the following Young moduli for SCW: $E_L=59$ MPa, $E_R=5.6$ MPa and $E_T=25$ MPa while the moduli of the PCW were $E_L=521$ MPa, $E_R=63$ MPa and $E_T=161$ MPa. Also we computed the strain equivalence principle under different potential elastic constitutive hypotheses and verified that both SCW and PCW can be modelled as an orthotropic material with an error of about 6%, but it is not possible to isotropic, transverse isotropic or cubic material assumptions because the errors would be unacceptable. In addition, we discovered that the principal material axes of the SCW were slightly deviated respect to the L, T and R geometrical axes of the cell, however for the PCW the mechanical axes were almost coincident with

the geometrical ones. This indicates that fibers remain primarily vertical and cross links are reasonably well aligned with R and T axes at the PCW but not so at the SCW, suggesting thus a more elaborated structure. Computing different RVE sizes we also verified that relatively small fractions of the cell wall of about $250 \times 400 \times 50$ nm were large enough for homogenization, as the results were consistent with larger RVE.

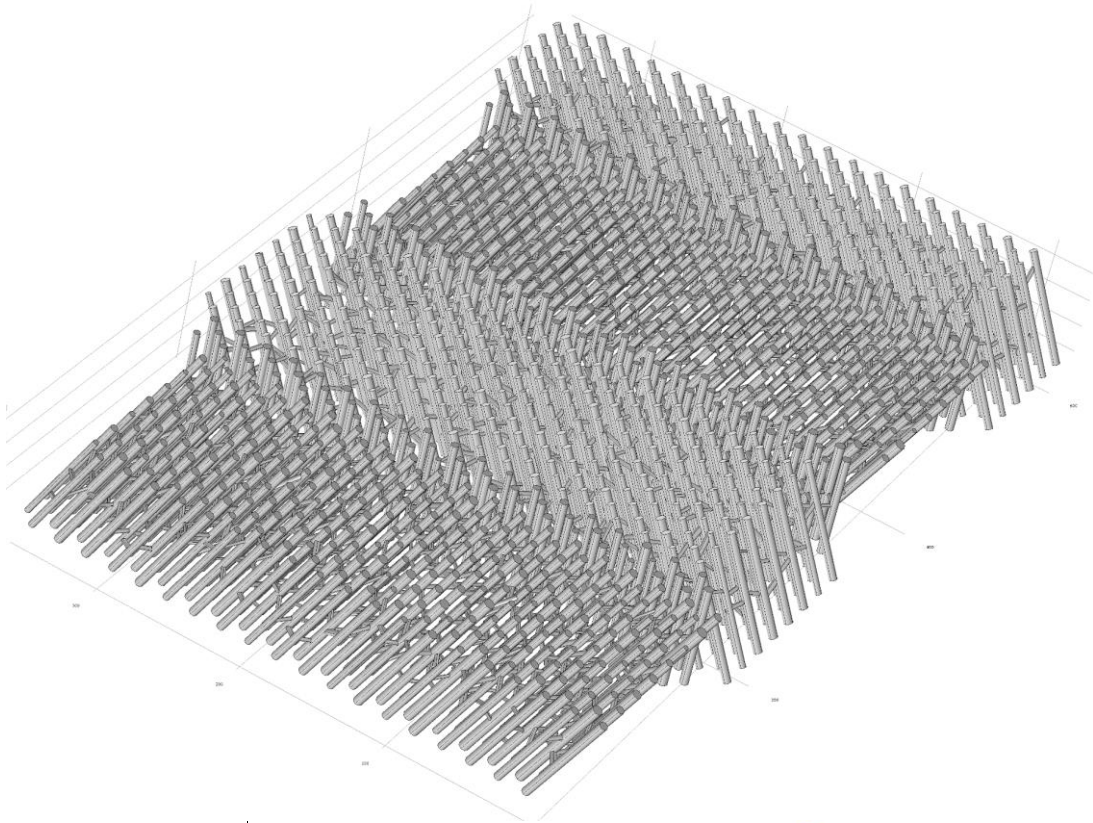
Unexpectedly, the PCW showed denser (smaller void fraction) and stiffer NS than the SCW. This superior stiffness of PCW can be however explained because the measured cell wall was at an early growth stage and thus the SCW was not yet lignified. Also, with respect to the longitudinal direction, one could argue that at the first stages, when only the PCW exists, the plants have primary support necessities so that it is safer to produce a rigid PCW to bear the self-weight. On the other hand, the increased stiffness of PCW on the transverse direction might have very interesting additional implications. Because the cells are always subjected to membrane (turgor or hydric) pressure, and if the PCW is stiffer than SCW, then the SCW would constantly be subjected to considerable compressions in transverse direction as long as it is not lignified. This transverse pressure is surprisingly similar to the well-known confining pressure of reinforced concrete that significantly increases the compressive strength and stability of columns when subjected to compressive forces. So, it is possible that the increased stiffness of the PCW respect to the SCW is a strategy of the plant to increase strength as long as SCW is not lignified.

4. PATTERN RECOGNITION AND IDEAL MODELS

Although the measured structure is apparently intractable, under the magnifying glass a rather organized pattern of microfibrils fairly oriented into $-15/3/+15/3/-15/3$ layer configuration connected by short and sturdy cross-links was revealed at the SCW. Also the PCW showed a rather organized structure similar to SCW except that the network was denser and microfibrils were more vertically aligned along the L axis, which reinforced our homogenization conclusions. In addition, such exhaustive analysis of the tomographic data let us to establish the average lengths, diameters, connectivity and angles of the different cell-wall constituents. With all these recognized patters we could elaborate an ideal model of the SCW, which is presented in the Figure 3a.

Because the real tomography models were too complex, the geometry could not be easily modified and thus hampered any detailed study of the NS. However, for the discovered ideal (artificial) model it was possible to intentionally vary the geometry of the SCW. Hence, we used the ideal model to investigate the implications of the NS architecture. But before doing so, a series of finite element models were constructed to first verify the best modelling approach, see an illustration of the four tested approaches in the Figure 3b.

(a)



(b)

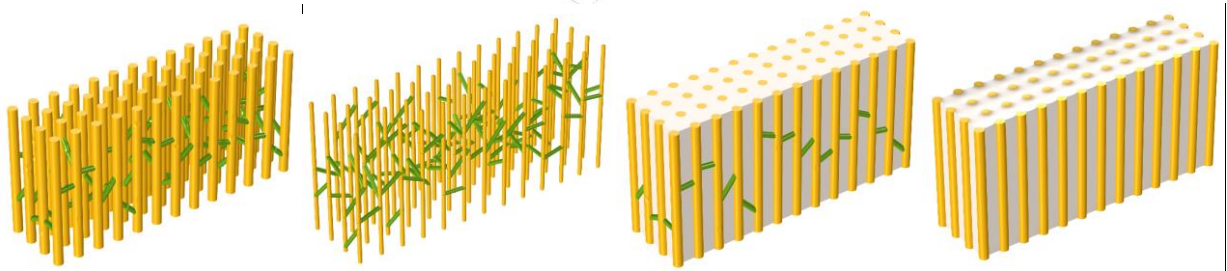


Figure 3: (a) Idealized model of the SCW with -15/3/+15/3/-15/3 layers, and (b) corresponding modelling approaches from left to right: NET, BEAM, NETMAT, MAT

The four modeling approach consisted of: first, modelling the entire volume of microfibrils and cross links (termed as NET); second, modelling all constituents as beam elements (BEAM); third, including into the NET model a potential matrix of inter-fibrillar material (NETMAT) and; fourth, suppressing the cross-links from the NETMAT, see an illustration in the Figure 3b. The comparison of all models showed that the simplification of NET into BEAM is non-consistent because of the reduced slenderness of cross links, also the NETMAT and MAT models were discarded because the addition of any inter-fibrillar material, even when it was of very low stiffness, produced an enormous impact on the mechanical properties rendering the cross-links to meaningless. Therefore, we decided to use the NET model, i.e. modelling the entire volume of fibers without inter-fibrillar material, in order to perform our analysis of the NS.

But before that step, we first computed the stiffness of the ideal geometry of the SCW using the NET model and compared it with the real geometry. Results showed that the stiffness of the NET ($E_L=1520$ MPa $E_R=4$ MPa, $E_T=9$ MPa) was in surprising agreement with the measured geometry, except in the longitudinal (axial) direction, which was about 25 times higher. As presented in next section, we attribute such divergence in the longitudinal direction due to the tortuosity (twist) of the

microfibrils. If tortuosity is considered in the NET model, the response of NET is reasonably similar to measured model in all directions. This reinforces the geometrical pattern idealization and the NET approach, as a suitable method to model the SCW. An illustration of the computation of the ideal model with the NET approach is presented in Figure 4, showing the elastic energy density of the SCW when subjected to turgor pressure.

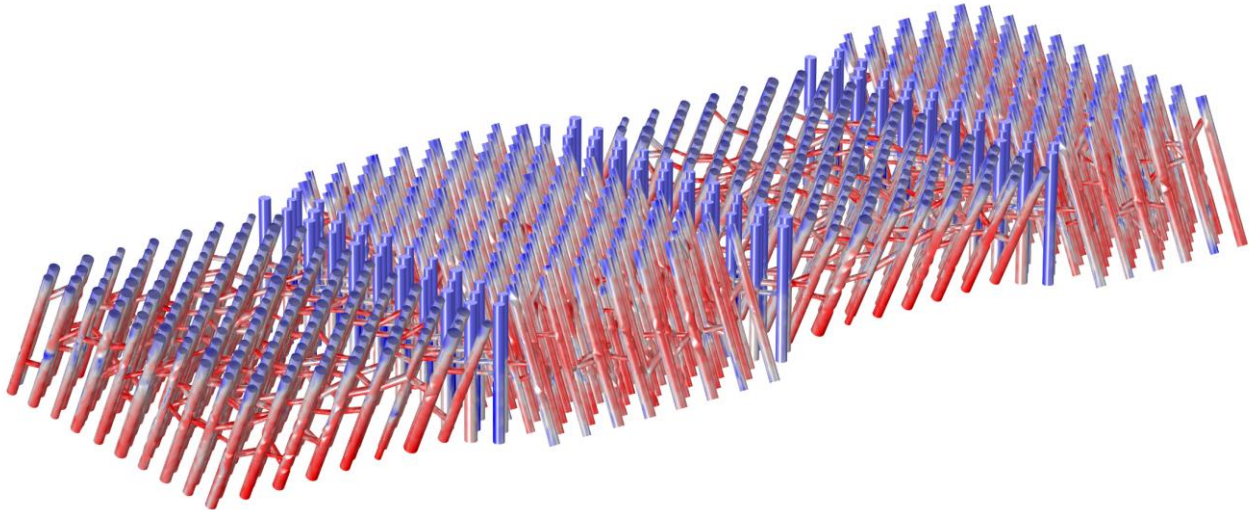


Figure 4: Elastic strain energy of the SCW when loaded by turgor pressure. For a sake of simplicity, hot colors indicate high elastic energy density.

5. DETAILED MECHANICAL ANALYSIS

Once the real and ideal models of the SCW were constructed we started a detailed analysis of the cell wall. The analysis comprised an extensive program of hundreds of simulations so that only the main results and conclusion are in the following outlined:

- 1) When introducing the tortuosity (twist) in the NET, the response is in surprising agreement with the real model, as the tortuosity decreases the stiffness about 90% in the longitudinal direction, but only about 5-10% in the transverse directions. Furthermore, the tortuosity seems to be an extremely important aspect for the cell wall because, although it decreases stiffness and the buckling load alike, it also increases 9 times the ductility and 40% the elastic and viscoelastic energies. The ductility (capacity to undergo large deformations after buckling) could be crucial for plants to prevent breakage against extreme loading. The high elastic energy storage could be vital for plants to recover its original position after high deformation or energy dissipation events, acting like springs. And finally, the high viscoelastic energy could provide plants with an outstanding capacity to dissipate part of the loads loading in the form of heat, especially under cyclic forces such as the wind, which also could be crucial to prevent breakage. An illustration of nonlinear buckling, elastic and viscoelastic analyses of ideal straight and tortous microfibrils is presented in Figure 5a, 5b and 5c, respectively.

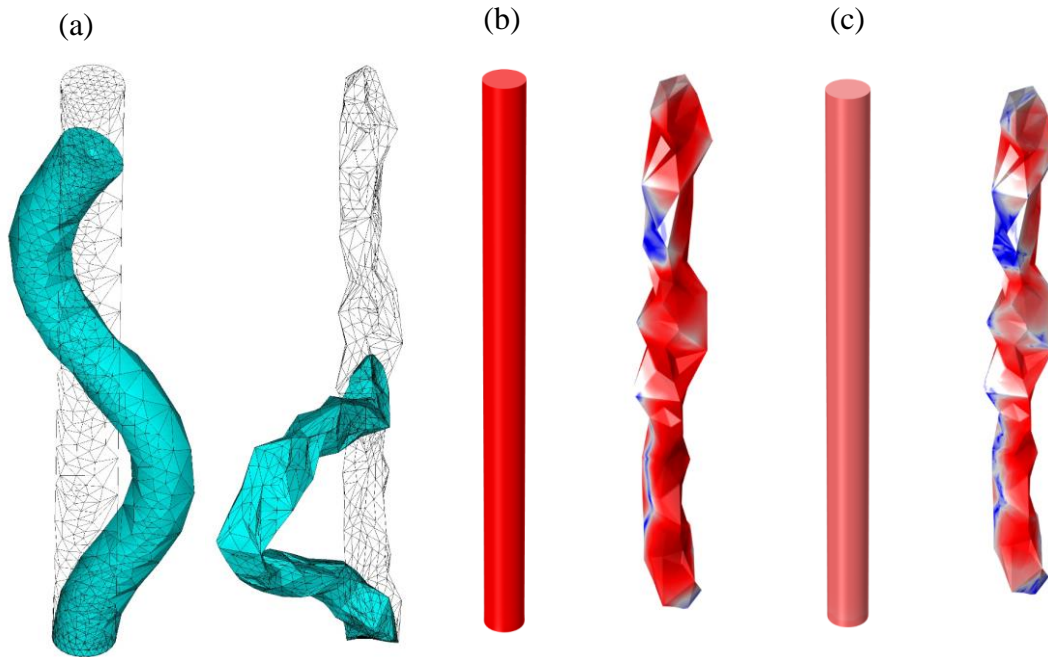


Figure 5: Effect of the tortuosity on the microfibrils: (a) increased ductility after buckling of a real fiber (right) respect to an ideally straight fiber (left), (b) increased elastic energy storage capacity of a real fibril and (c) increased viscoelastic energy dissipation capacity.

- 2) If adding lignin globules into the real geometry as shown in Figure 1, the stiffness of the SCW increases quadratically according to the following equations with a fitness above $R^2=0.98$:

$$\Delta E_L = 0.1005 \cdot X_{lig}^2 + 15.218 \cdot X_{lig} \quad (1)$$

$$\Delta E_R = 9.9094 \cdot X_{lig}^2 - 26.548 \cdot X_{lig} \quad (2)$$

$$\Delta E_T = 4.0008 \cdot X_{lig}^2 + 6.8503 \cdot X_{lig} \quad (3)$$

where ΔE is the percentage stiffness increase in the corresponding directions, and X_{lig} is the volume fraction of lignin in the SCW. These results indicate that the effect of lignin is significantly larger in the transverse directions, especially in radial (turgor) direction. This means that rather than reinforcing against axial loading, the lignin should play a very important role in regulating cell wall growth and casting the perpendicular-to-the-grain properties.

- 3) A reticular (rectangular) versus diamond (cross) cross-link arrangement did not significantly affected for axial and shear loadings, although a cross-arrangement would reduce stiffness in transverse directions at the price of increasing elastic energy storage and promoting more evenly distributed load.
- 4) Small number of sturdy cross-bridges vs. large thin cross-bridges showed negligible influence on the axial direction but significant effect on transverse direction as stiffness is reduced with sturdy cross-links. However, the measured sturdy cross-links tend to better distribute the load leading to more energy storage in microfibrils.

- 5) Cross link inclination showed reduced influence only in shear stiffness.
- 6) Finally, we studied distinct orientations in the layers of the SCW, see an illustration in Figure 6. As expected, the inclination of microfibrils has a paramount impact on the longitudinal elastic modulus as it drops rapidly when fibrils are inclined, but it also leads to increased elastic energy storage as well as increased stiffness in transverse direction.

But apart from stiffness and elastic energy, there are a number of further aspects to note as the supramolecular organization of the cell wall shares several interesting features with some well-known problems of structural and mechanical engineering. Firstly, such inclined arrangement would indeed cast a helical (spiral) configuration when looked at larger scales, thus shaping a spring. This kind of configuration is not only optimal to store elastic energy, but also it is surprisingly similar to that used on cylindrical spiral reinforced concrete columns. The helical reinforcement in those structures is well-known for its optimal confining pressure generation that significantly increases the strength of columns under compression. Also these types of columns are known for resisting famous earthquakes because of their superior ductility. Similar helical structures have also been used as energy absorbers for civil and automotive engineering. Recent research on natural fiber reinforced composites has proved that this kind of structure has outstanding energy absorption capacity of impact loads, mainly due to multiple failure mechanisms as for instance micro-fiber breakage or interlaminar separation. Thus, aside from the high elastic energy storage that could be used for shape recovering, this spiral layered configuration is presumed to optimally improve compression strength and ductility of the cell wall at the larger greater (micro) scale, as well as providing high impact specific impact absorption energy capacity.

On the other hand, as each of the cell-wall layers is thin and most microfibrils are oriented along an specific angle, the cell wall architecture may be regarded as a characteristics conventional laminates composites as those used in civil, mechanical and aerospace engineering. These laminates are commonly designed using the classical laminate theory so that the plies or laminates (layers) are oriented in several directions to provide high stiffness and strength not only in one (longitudinal) but in several directions. Furthermore, laminates are commonly designed to satisfy two guidelines: symmetry and balancing. A symmetric laminate is that showing reflection symmetry on the ply orientation respect to the mid surface. Symmetry is normally desired in composites because it prevents the coupling of the bending and membrane (in-plane) responses, i.e. a bending force in a symmetric laminate would not generate membrane deformation, and a bending moment would not cause membrane strain. This uncoupling is essential for composites to prevent the wrapping and twisting when shrinking or swelling during temperature changes. The measured cell wall sample was not fully symmetric, but it would approach the symmetry if neglecting the narrow transition ply (3°) and dividing the mid-layer by two. This feature may be essential for cells to prevent wrapping and twisting during grow as they are subjected to turgor (membrane) pressure.

A balanced laminate is that in where any positive angle ply has its negative counterpart. This opposite alignment uncouples the axial and shear membrane response, i.e. when subjected to a membrane force, the laminate does not show shear strain and vice versa. This is important for composites to prevent skewing deformation during temperature changes. Also for balanced composites, the bending is relatively tends to be rather uncouple from twisting, i.e. a bending moment only may cause small twisting. The cell wall is neither a balanced composite, but again, it would approach the balance if neglecting the transition layers (3°) and dividing the central layer. This could prevent cell wall from shear deformations during growing (turgor pressure), and twisting deformation when bending.

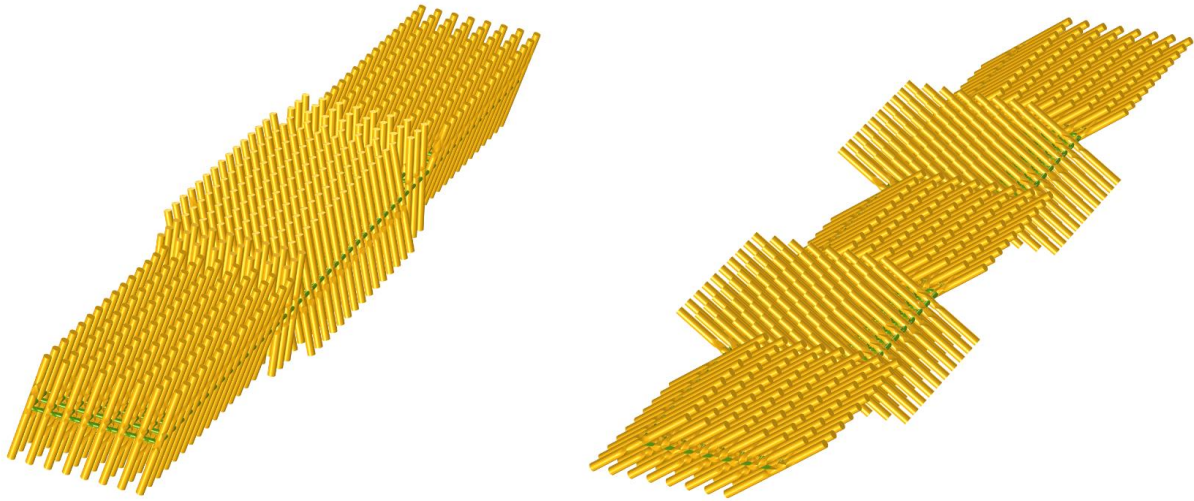


Figure 6: Distinct layered configurations of the SCW, at the left the measured configuration for Arabidopsis (-15/3/+15/3/-15/3), at the right an alternative (more inclined) configuration.

6. OVERALL CONCLUSION

The detailed segmented tomography of Arabidopsis vitreous sections let us to carry out a detailed numerical analysis of the cell wall at the nanoscale. The found structure was apparently intractable, but under the magnifying glass, a rather organized pattern of microfibrils fairly oriented into -15/3/+15/3/-15/3 configuration connected by short and sturdy cross-links was revealed at the SCW, while a similar but denser and more vertically aligned architecture showed at PCW. A suite of simulations that combined masking, homogenization and finite element methods, let us to calculate the mechanical stiffness and the fitness of different elastic laws to emulate the mechanical response of the wall, as well as study the potential effect of lignification. Results indicated that an orthotropic material reasonably can model both PCW and SCW, and these homogenized properties can be extracted out of relatively small cell-wall fractions. The addition of lignin led to a quadratic increase of the SCW stiffness, being especially critical in the transverse directions and thus indicating a primordial influence on controlling cell growth and perpendicular-to-the-grain properties. Unexpectedly, the primary cell wall showed a denser and more perpendicularly oriented nanoarchitecture at this stage of growth that yield to larger stiffness than secondary cell wall. The increased stiffness may be justified due to the primary support necessities of plants at early growth stages as long as the cell is not lignified. It is interesting that as long as cell is not lignified, the SCW is expected to bear significant transverse compression loading, and this may have a major contribution in improving the strength and stability at early developmental stages.

The recognized patterns and the homogenization also let us to select an ideal model out of several possible modelling approaches as a suitable method to emulate the response of the complex data and thus investigate the implications of modified geometrical configurations. Far from the stiff, and most probably brittle, polymeric constituents, the cell wall rather casts at the very nanoscale an admirably flexible and ductile structure with superior load transfer capabilities as well as elastic and viscoelastic energetic capacities. It is interesting that these versatile characteristics are obtained after scarifying stiffness in various ways, as with the inclination and tortuosity of microfibrils, short and sturdy cross-links and even the link of cross-links to potentially surrounding hemicellulose. It is also remarkable

that other potential polymeric compounds occupying the void space in the form of globules, such as lignin, would have very high impact increasing the cell -wall stiffness, even at low concentrations.

When looking at larger scale, the layered secondary cell wall configuration is presumed to cast a structure close to a helical laminate composite that is balanced and symmetric. This recalls to several well-known structures of civil, mechanical and aerospace engineering such as spiral reinforced concrete columns, impact energy absorbers, or classical laminate composites used in spaceships. It is surprising, that when summing up the principles these structures are made for, one can find similar benefits as those found at the very cell-wall nanoscale, namely superior flexibility, load transfer, ductility and high energetic capacity using the less possible amount of mass, even when that means a drop in stiffness. These characteristics should benefit plants with a magnificent plastic response to external stimulus, as it enables for undergoing large deformations, redistributing the load, and storing and dissipating high amounts of energy, while recovering original shape. Also when breaking, high energy dissipation is expected. In addition, the symmetrical and balanced configuration should critically contribute in stabilizing the cell, by uncoupling different loads, preventing wrapping, skewing and twisting during growth (turgor pressure) or axial compressions.

Because one of the greatest unknowns in the mechanics of woods and plants is still the nanostructure and its mechanical properties, this investigation should provide a significant contribution to improve our understanding of the mechanics of natural materials.

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