

Micromechanics of Cell Walls

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Abstract In this chapter, we discuss the mechanical properties of primary and secondary cell walls with regard to cell wall structure and composition. The first sections of the chapter are devoted to defining the mechanical terms used and to giving a general introduction into the mechanical behaviour of composites. The specific structure–property relationships of primary and secondary cell walls are then discussed with a focus on unravelling the mechanical role of individual cell wall components. In these terms, the mechanical characterization of genetically modified plants is highlighted as it allows for the targeted alteration of both cell wall polymers as well as their cross-linking capacities, in turn resulting in a distinct influence on entire cell wall properties.

1 Introduction

A rigid cell wall which supplies mechanical support to the cell is a characteristic feature of the plant kingdom (see, however, Chapter 14). Its high rigidity allows trees to grow more than 100 m tall and build stems with a mass of more than 1,000 tonnes. However, to facilitate growth the cell wall cannot start off by being this rigid after initial deposition by the cell. In contrast, the cell wall during its primary phase must be flexible and plastically deformable to allow growth to a designated shape and size of the cell. This contradiction has fascinated researchers for decades, as it highlights the astonishing ability of plants to both precisely control and adapt the mechanical properties of cell walls on demand. This is all the more remarkable in view of the apoplast nature of the cell walls, meaning that the plant cells need to be able to adjust the performance of extracellular material, both during cell and organ ontogeny as well as upon external stimuli.

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With this chapter we intend to discuss the mechanics of plant cell walls, highlighting the interrelation with their structural and chemical organization. The first section defines mechanical terms and introduces the basic description of mechanical properties. Here, it is also important to briefly discuss the basic concepts in material testing and the limitations in transferring these concepts from technical materials to biological materials. The following sections deal with general composite theory and explain the analogy between technical fibre composites and the organization of the plant cell wall. The cell wall components of primary and secondary cell walls are briefly introduced along these lines. Thereafter, the structure–function relationships of primary and secondary cell wall are discussed with a focus on the interaction of cell wall components and related approaches to elucidate the underlying principles of cell wall design. Here the crucial role of cellulose fibril orientation is discussed and recent work in the field of mechanical characterization of genetically modified plants with alterations of the cell wall matrix organization is reported. In conclusion, an outlook is given reflecting scientific challenges and potential applications of plant cell wall research.

2 Mechanical Terms and Definitions

This section defines the various parameters used to describe the mechanical behaviour of a material, the experimental methods used to measure them and how this can be applied to the mechanical response of the plant cell wall. For more detailed introductory texts on the mechanics of materials see (e.g. Gere 2002; Callister 2003; Ashby and Jones 2005), for the mechanics of composite materials see (e.g. Chou 1992; Hull and Clyne 1996) and for the mechanics of biological tissues see (e.g. Wainwright et al. 1982; Vincent 1990; Niklas 1992).

The “mechanical behaviour” of a material, refers to how a piece of this material responds to applied forces or displacements, in terms of the amount and rate of deformation, the resistance (force) to deformation and the likelihood of failure. A typical mechanical test involves applying a known displacement (or force) at a given rate to a sample whilst simultaneously measuring the force (or displacement). Forces and displacements, although being the quantities actually measured in an experiment, are inappropriate to describe the material response as they are dependent on sample geometry and size. In order to compare the mechanical response of different samples, force and displacement need to be normalized, giving rise to two important mechanical quantities, stress and strain. Stress, given the symbol σ , is defined as the force acting perpendicular to a surface divided by the cross-sectional area over which this force is applied and has the units of pressure (Newtons per metre square often given in Pascals) (Fig. 1bi). Strain, with the symbol ε , is the change in length divided by a reference length and is unitless (Fig. 1biii).

Loading, of course, can be applied in different directions, meaning stress and strain are defined as tensile (resp. compressive) when the loading results in a lengthening (resp. shortening) of the sample or in a combination of both in bending.

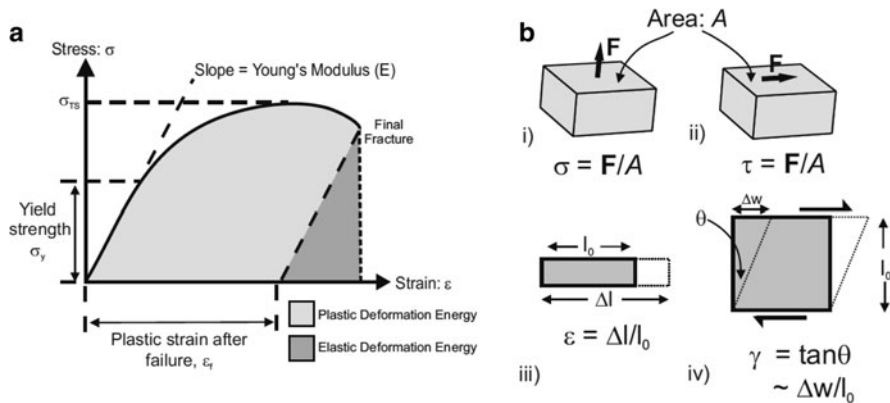


Fig. 1 (a) Example of a typical stress–strain curve for a ductile metal. The first portion of the curve is linear with a slope, E , corresponding to the Young’s modulus. Below the yield strength, σ_y , the sample deforms completely reversibly, or is linear elastic. Above this stress, the sample deforms irreversibly or plastically, until failure. The areas under the curve give the energies of deformation. The maximum stress, σ_{TS} , or the tensile strength is the maximum stress that the material can support before failure. (b) A schematic of how (i) normal and (ii) shear stresses and (iii) normal strain and (iv) shear strains are defined

Loading under shear implies the force is applied parallel to the loaded surface. Both shear and tensile loading cases are sketched in Fig. 1b. Shear stress, τ , is defined as the force acting parallel to a surface divided by the cross-sectional area over which this force is applied and has the same units as tensile stress (Fig. 1bii). Shear strain, γ , is given by the angle of shear, approximated by the sideways shear divided by a perpendicular reference length and is also unitless (Fig. 1biv). As both cross-sectional area and length change due to deformation, different definitions of stress and strain can be found in the literature. For example, engineering stress and strain (often used to present experimental data) are defined using initial area and length, whereas true stress and strain (often used in continuum mechanics modelling) are defined using the current deformed area and length which changes during deformation. The two definitions are readily converted between each other (e.g. Ashby and Jones 2005), however, in the following engineering stress and strain will be used.

The majority of mechanical testing techniques and analysis methods were originally developed by materials scientists and engineers to analyse engineering materials, and are increasingly applied to the mechanical characterization of relatively fragile biological tissues. The use of these methods on natural tissues therefore requires some caution, as many techniques are suitable only for samples with specific shapes and sizes, under the assumption of simple homogeneous microstructures. However, if these limitations are kept in mind or techniques are used in a comparative sense (i.e. two tissue types are compared using the same method), useful information about the mechanics of biological tissue can be obtained. There are several mechanical testing techniques that can be readily performed on plant tissue, such as nano-indentation, and acoustic microscopy

which gives localized mechanical information at the submicron scale, whereas micro-indentation and constant strain rate tensile testing give mechanical data at a more microscopic scale.

Micro-indentation and nano-indentation are methods in which a small sharp tip (micro- or nano-scale), with a well-defined shape, is pushed into the surface of the material being analysed while both the force and displacement are recorded (e.g. Hiller et al. 1996; Ebenstein and Pruitt 2006). From the force–displacement curve and a knowledge of the shape of the tip, mechanical parameters, such as elastic modulus and hardness, can be determined at the location of the indent (Oliver and Pharr 1992). Micro-indentation has been used in plant science to measure tissue and cell wall properties of relatively large objects such as pollen tubes (Geitmann et al. 2004; Parre and Geitmann 2005a, b), whereas nano-indentation allows for hardness and stiffness to be directly measured at the submicron level (Wimmer et al. 1997; Gindl et al. 2004). The interpretation of the results requires some care, as the theoretical models used to interpret instrumented indentation often assume that the materials are isotropic and homogeneous, a situation that is rarely true for biological tissues. Contrary, the orthotropic nature of cell walls needs to be considered when determining stiffness properties of the wall (Gindl and Schoberl 2004). Another technique operating at a similar resolution as nano-indentation is scanning acoustic microscopy (SAM). SAM uses focused acoustic waves to map acoustic impedance, a parameter combining stiffness and density, over the surface of a sample (see e.g. Clair et al. 2000; Raum et al. 2006). If the density distribution of the material being measured is known, or at least is constant over the surface, then the acoustic impedance map can be interpreted in terms of elastic modulus. Such measurements are particularly useful in highlighting areas of interest for further structural characterization within complex tissues containing regions of similar chemistry but different nanostructural architectures (e.g. Elbaum et al. 2007).

Perhaps the simplest mechanical test to perform on tissue is the constant strain rate tensile test. The advantage of tensile tests is that there are fewer constraints with respect to sample geometry than in other load cases such as compression or bending. Mechanical properties of small tissue specimens and individual fibrous cells can be determined in uniaxial microtensile tests (Page and El-Hosseiny 1983; Burgert et al. 2002, 2003; Groom et al. 2002; Köhler and Spatz 2002; Ryden et al. 2003; Abasolo et al. 2009). To calculate cell wall properties from these tests, further sample parameters such as structural/geometrical features, density or turgor pressure have to be considered.

In tensile testing, a sample is affixed by glueing or clamping to grips at both ends and is pulled at a given rate of displacement, whilst the force is measured. Such a force–displacement curve can be readily converted into the so-called stress–strain curve, characteristic for a given material. An example stress–strain curve for a ductile metal is given in Fig. 1a. Although the stress–strain curves for plant cell wall material are often more complex (see Fig. 7), the parameters used to characterize the materials response are still the same, and as such the following discussion will focus on simplified stress–strain curves.

The initial portion of a general stress–strain curve is often linear and is called the linear-elastic regime, where the deformation is completely reversible. In this regime, the path of loading corresponds exactly to that of unloading and the sample will return back to the same shape it had before deformation (Fig. 2a). In some materials, such as rubbers, however, the reversible portion of the stress–strain curve is non-linear. The slope of a linear-elastic portion of the stress–strain curve is the

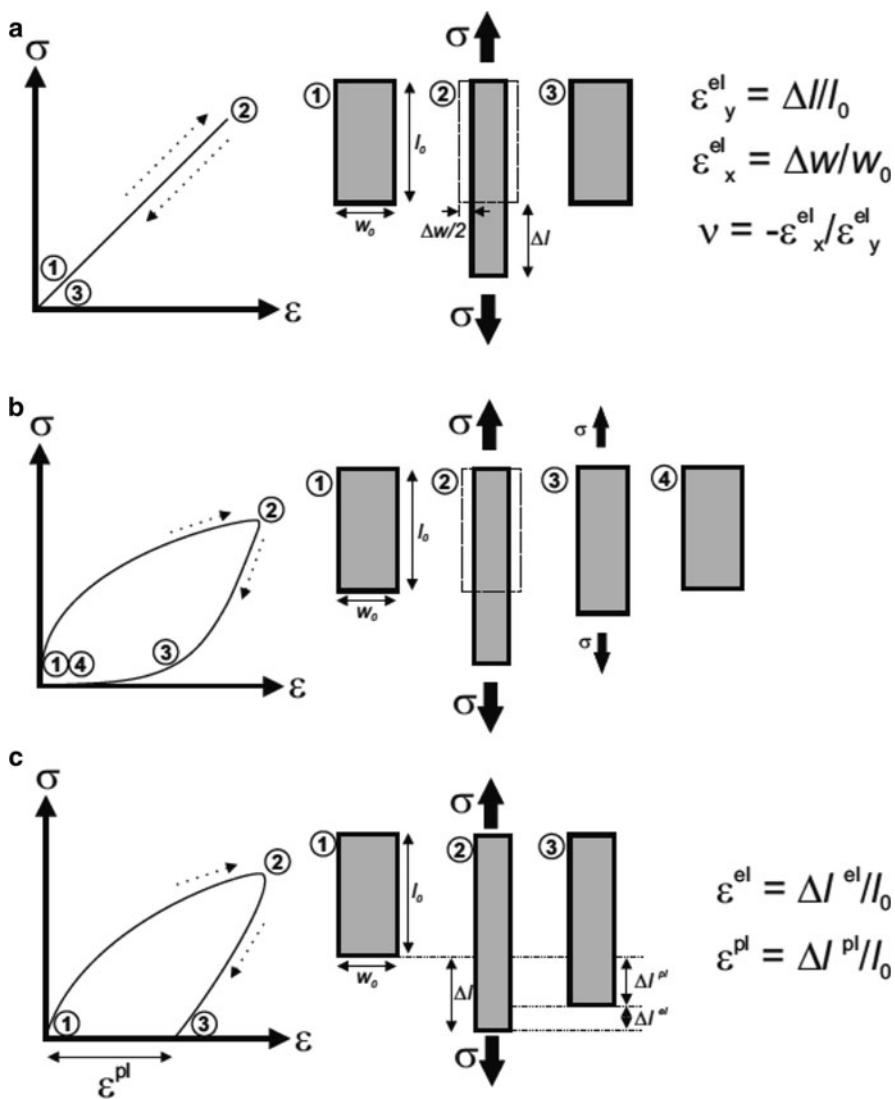


Fig. 2 Examples of stress–strain curves, for (a) linear elastic, (b) viscoelastic and (c) plastic materials with sketches of the changes in sample associated with such deformation behaviours

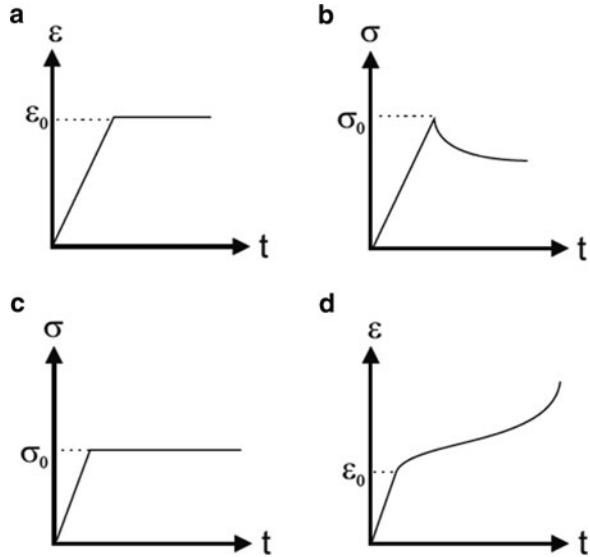
Young's or elastic modulus and is often given the symbol, E , the constant in Hooke's Law. The Young's modulus gives the stiffness of a material, in other words the resistance of a material to elastic deformation and has the same units as stress (Pascals). The higher E is, the more stress is required to deform to a given strain. Typical values of the Young's modulus range between 70 GPa for aluminium and 200 GPa for steels, normal spruce wood has an elastic modulus around 10 GPa and polymeric materials such as elastomers can have moduli in the 10–100 MPa range. An elastic modulus for shear deformation (Fig. 1bii, biv) can also be defined and is often given the symbol μ (or G). It has the same units as stress and for many materials has about 3/8 of the value of E . A third elastic modulus is the bulk modulus, K , which gives the volume change of a material as a function of pressure.

In some materials after a critical stress, or the yield strength σ_y , is reached (Fig. 1a) the deformation is no longer completely reversible upon unloading. For stresses above this yield stress, the material is said to deform plastically (Fig. 2c). From the yield point onwards the strain consists of two independent contributions, of elastic and plastic strain. By this it is meant that if the sample is unloaded or fails (Figs. 1a and 2c) then the sample will regain partially its initial length by an amount given by ε^{el} , at the point of unloading, but will have a permanent deformation given by ε^{pl} . The highest stress achieved during a tensile test is known as the ultimate tensile stress, σ_{TS} and depending on the material can also occur at the point of failure. The integrated area under the stress–strain curve has units of energy per unit volume and corresponds to the energy dissipated per unit volume of material during deformation (Fig. 1a). This area can be separated into elastic energy, which is regained upon unloading, and plastic deformation energy which is lost as heat. The deformation energy dissipated per unit volume is a useful quantity often used as a proxy for the toughness of a material. The higher toughness (area below curve) the more energy is required to fracture a given material.

Many materials display a viscoelastic response in that the elastic behaviour is time dependent with a modulus that is dependent on the rate of loading. Such a viscoelastic response is illustrated in Fig. 2b for a cyclic loading experiment. Although the loading and unloading paths are different, the stress–strain curve returns to the origin after unloading indicating fully reversible deformation. Another method to measure the viscous response of a material is to perform a relaxation test during a tensile test (Fig. 3a, b).

In a relaxation test, the sample is deformed to a given stress, σ_0 , the sample displacement is then stopped and fixed at a given strain, ε_0 , and the evolution of stress as a function of time is then followed. The rate at which the stress relaxes gives an idea of the contribution of viscous terms to the overall mechanical response. Exponential decay functions are commonly used to fit the relaxation curve, although it is often difficult to get a physical interpretation of the parameters extracted, as relaxation comes from a combination of viscoelastic and plastic deformation. A similar experiment to measure the stress–strain response is the creep test in which a fixed stress is applied to a sample and the resultant deformation observed as a function of time (Fig. 3c, d). In general, creep curves have a linear (elastic) portion corresponding to the loading, a portion of decreasing rate of creep called primary creep,

Fig. 3 Other time-dependent mechanical tests (a) and (b) Stress relaxation test in which the sample is deformed to a given strain (a) and then held at this strain whilst the stress (b) is measured with time; (c) and (d) Creep test in which a given load is applied to the sample (c) and the strain (d) measured as a function of time



followed by a linear portion of steady-state or secondary creep. During tertiary creep, the final portion of the curve displays an accelerating rate until failure.

Up until now in this introductory section, nothing has been mentioned about the fact that specimens are three-dimensional and in principle can deform in all directions. It has been assumed that both stress and strain are measured along the longitudinal tensile direction of the specimen. However, materials can deform also in directions perpendicular to the applied stress. When stretched, they often decrease in cross-section as illustrated in Fig. 2a where the longitudinal strain ϵ_y^{el} is accompanied by a decrease in sample width ϵ_x^{el} . This effect is described by the Poisson's ratio, ν , which is the negative ratio of the lateral strain to the tensile strain. Typical values of the Poisson's ratio are 0.3 for many metals, 0.5 for rubbers and 0 for materials such as cork. The Poisson's ratio, the elastic, bulk and shear moduli are in fact the four elastic constants required to describe the elastic response of an isotropic material (only three of these are independent). An isotropic elastic response is independent of the loading orientation. In general, however the behaviour of a material is direction dependent, i.e. the material is anisotropic which can, for instance, be easily seen in the directional properties in wood. This means that in order to completely describe the elastic response of anisotropic materials, elastic constants need to be defined for each independent direction in the material. In addition to the difficulty in measuring all of the constants, the fact that the materials properties are direction dependent means that a full characterization of a sample also requires a knowledge of the material orientation (the fibre orientation for example). Fibrous materials are by nature anisotropic and are typically stiffer along the fibres than perpendicular to them. Due to the symmetry of the fibre reinforcement a good approximation for their elastic response is to assume that they display an isotropic response perpendicular to the fibre. This behaviour is known as transverse isotropy and only requires five independent elastic constants in order to describe the elastic response.

3 The Cell Wall as a Composite

Composites are a class of materials made from more than one constituent phase or material. This section outlines some simple models for the mechanical response of composites, and describes how effective materials properties can be calculated as a function of the arrangement and the materials properties of the constituents. For more detailed models and references (see e.g. Chou 1992; Hull and Clyne 1996).

Consider the simplest of composites, a two-phase system under uniaxial loading, comprised of a soft material, M, and a hard material, F. Of the infinite number of possible ways to combine the two materials, there are two limiting architectures with respect to the load direction. In the first of these, the Voigt model, the two materials are oriented in layers parallel to the load, and in the second, the Reuss model, the material layers are oriented perpendicular, or in series, with respect to the load (see insets in Fig. 4a). For both these cases, it is straightforward to estimate effective Young's moduli. For the Voigt model, the effective elastic modulus of the composite, E_C , is simply the sum of the moduli of the components (E_F and E_M) weighted by the volume fractions of each phase. This gives $E_C = fE_F + (1 - f)E_M$ where f is the volume fraction of the hard material F . This model is also known in the literature as the iso-strain model, as it is derived by assuming each phase deforms the same amount. For the Reuss model, which is also known as the iso-stress model, the composite modulus is given by the weighted harmonic sum of the

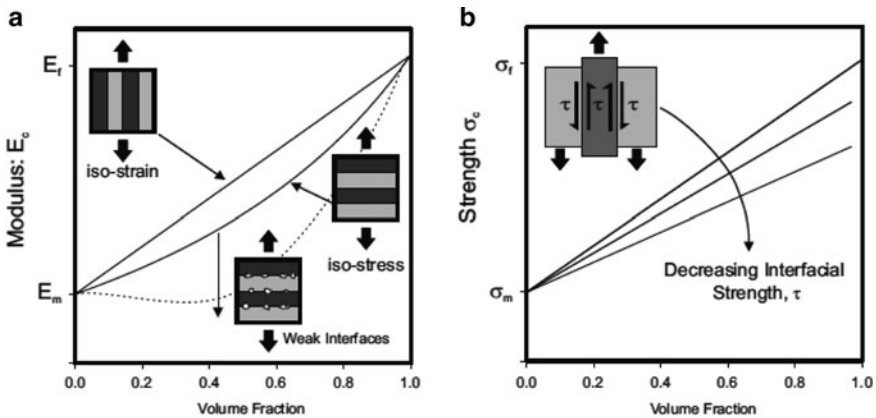


Fig. 4 (a) Schematic depiction of elastic modulus of a fibre composite, E_c , as a function of the fibre volume fraction. E_m and E_f are the matrix and fibre stiffnesses, respectively. The two solid lines indicate the upper and lower bounds of the stiffness for iso-strain and iso-stress composite models, respectively. The presence of imperfect or weak interfaces results in a reduction of the effective stiffness in the iso-stress as schematized by the dotted line. (b) Strength of a fibre composite: Strength σ_c , of a composite as a function of fibre volume fraction for an iso-strain composite with different values of interfacial shear strength. σ_m and σ_f are the respective matrix and fibre strengths

moduli, $E_c = (f/E_F + (1-f)/E_M)^{-1}$. This model, however, assumes perfect bonding at the interfaces between the two phases. A sketch of the solutions to these models is given as a function of volume fraction of the hard phase in Fig. 4a. These solutions give strict upper and lower bounds to the elastic modulus of a composite meaning the elastic moduli of real composites lie somewhere between these bounds (Hashin and Shtrikman 1963). The lower bound, however, assumes a perfect bonding between the two phases, and weaker interfaces would lead to a decrease in effective stiffness as indicated by the dotted line in Fig. 4a. Similar models can be developed to estimate other elastic constants necessary to describe the complete elastic response, for more details see (e.g. Chou 1992; Hull and Clyne 1996). The calculation of the failure strength of composite materials is more complex as it depends strongly on how easily stress is transferred via shear through the interface between the two phases. A rough sketch of these effects is made in Fig. 4b, which illustrates how a decreasing interfacial shear strength for a parallel aligned fibre composite results in a decreasing composite strength. With these simple models in mind, it can be seen that by controlling interfacial strength as well as the fibre architecture, by means of fibre's aspect ratio, a large range of material properties can be achieved.

Common technical examples include: glass or carbon fibre-reinforced epoxy resins and concrete and ceramic particle-reinforced metal matrix composites. One of the principal advantages of composite materials is that by combining different materials, a new set of physical properties can be attained that are not available in the constituents. Another important feature is that their properties can be controlled and optimized through the arrangement or architecture of the constituents (Ashby and Brechet 2003). Such an approach is used widely by nature, for example, in mineralized tissues such as bone and shell. By combining stiff and brittle ceramic particles within a soft and tough protein matrix, these mineralized tissues are made to be both tough and strong (see e.g. Fratzl and Weinkamer 2007; Dunlop and Fratzl 2010).

The analogy between technical fibre composites and plant cell walls is due to the organization of stiff cellulose microfibrils of a high aspect ratio, embedded in a matrix of various compliant polymers (Fengel and Wegener 1984; Kerstens et al. 2001; Fratzl et al. 2004; Cosgrove 2005; Fahlen and Salmen 2005). Furthermore, the high contrast in stiffness between both phases further supports this analogy. Cellulose possesses a modulus of elasticity in the axial direction under moist conditions of ~ 134 GPa, whereas matrix polymers such as hemicelluloses and lignin have much lower moduli of 40 MPa and 2 GPa, respectively (Salmén 2001). Figure 5 illustrates the fibre composite character of primary and secondary cell walls. The cellulose fibrils have a diameter of ~ 2.5 –5 nm and a length of several micrometres. While the diameter of the fibrils can be measured with relatively high precision by small angle X-ray scattering or in TEM studies (Jakob et al. 1995), the length of the fibrils is unknown because of current limitations in experimentally determining this parameter. One approach is to calculate cellulose fibril length from the degree of polymerization. This degree reflects how many β -D-glucose units are linked together by β -1,4-glycosidic

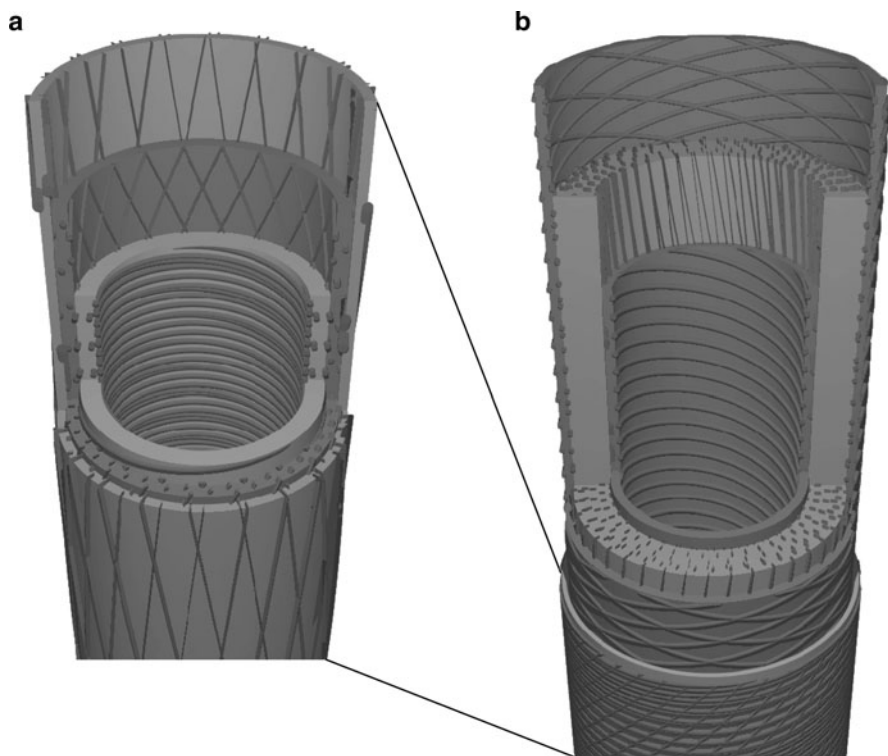


Fig. 5 View of the cell wall assembly as a composite structure (a) primary cell wall during growth with cellulose fibrils tilted toward the cell axis in the outer cell wall layers (b) secondary cell wall using the example of the wood cell structure

bonds in a linear polymer chain. Depending on the species between 7,000 and 15,000 units have been found for higher plants (Fengel and Wegener 1984). Fourteen thousand units would correspond to a cellulose fibril length of $\sim 7 \mu\text{m}$ (Somerville et al. 2004). This estimate, however, lacks precision because it requires the cell wall to be deconstructed which can lead to cellulose chain breakage. An indirect visualization in the living cell could be another principle, highlighting cellulose synthase complexes with a fluorescent marker and following their movement by spinning disc confocal microscopy (Paredes et al. 2006). However, this method can only give a lower bound to fibril length because it is only possible to view the cellulose synthase complexes in one plane of observation.

Another characteristic of the cellulose microfibrils is their para-crystalline nature which means that they consist of both crystalline as well as non-crystalline parts. Possible organization patterns of these parts could be either (1) an amorphous shell surrounding a crystalline core, (2) crystalline and amorphous segments alternating along the length axis of the fibril, or (3) a combination of both (Salmen and Bergstrom 2009). The amorphous cellulose units are quite similar to highly ordered

hemicelluloses containing either no or short side chains which probably leads to a gradual nanostructural transition between the phases.

Primary and secondary cell walls differ quite substantially in the composition of their matrices which reflects their different functions and emphasizes the crucial role of the matrix for the mechanical performance of the cell wall fibre composite. Primary walls mainly have to facilitate cell growth, which is achieved by a matrix consisting of hemicelluloses, pectin, structural proteins and aromatic substances. To make matters more complicated, the matrix assembly of primary cell walls varies substantially between grasses and other flowering plants, both by means of chemical composition and cross-linking of the macromolecules (Carpita 1996). Secondary cell walls provide mechanical stability to the plant which is supported by a more rigid matrix made up of hemicelluloses and lignin.

Hemicelluloses, of which a variety of different types can be found in all cell wall types, are heteropolymers, consisting of a large variety of neutral sugars (e.g. glucose, mannose, galactose, xylose, fucose, arabinose) and may contain uronic acids. The hemicellulose molecules typically have a backbone accompanied by side branches. Pectins are mainly found in the primary cell walls and in the middle lamella which glues the individual cells together. They are highly heterogeneous, acidic polysaccharides (Caffall and Mohnen 2009). The backbones of the side-branched molecules can be either made up of one polysaccharide unit (e.g. homogalacturonan) or of repetitive building blocks (e.g. rhamnogalacturonan) which consists of repeating disaccharides. Lignin is typically assigned to secondary cell walls, but also the primary walls become lignified after cell growth has been terminated and the cell has reached its final size and shape. Lignin polymerizes inside the cell wall from three different phenylpropane units filling voids in the basic cell wall assembly of cellulose, hemicelluloses and pectins. In secondary cell walls, lignin functions as a further reinforcing agent mainly reducing the risk of cell wall buckling under compressive loading.

In addition to the individual properties of the cell wall macromolecules, their interactions and bonding patterns are of crucial relevance for the performance of the entire cell wall. The most abundant bonding type between the macromolecules in the cell wall is hydrogen bonding. It is the exclusive bonding type found between the surface of cellulose fibrils and the surrounding matrix polymers (supposedly hemicelluloses). However, it has been reported that a fraction of xyloglucan chains can be also incorporated in cellulose fibrils (Pauly et al. 1999). A high number of hydrogen bonds is ideal to establish a tight but flexible connection between macromolecules as the bonds can be easily opened and reformed due to their low bonding strength. Hydrogen bonding is also the most abundant type between the matrix macromolecules, but also several covalent cross-links have been observed. Covalent bonds have been reported between pectin and hemicelluloses (Thompson and Fry 2000) as well as hemicelluloses and lignin (Lawoko et al. 2005; Westbye et al. 2007). Interesting features of pectins are intra-molecular ion-mediated cross-links, e.g. calcium ions in homogalacturonan and borate diesters in rhamnogalacturonan II (Carpita and Gibeaut 1993; O'Neill et al. 1996, 2004; Cosgrove 2005).

In terms of the fibre composite nature of plant cell walls, the mechanical role of cellulose is rather well understood but it is less clear for the matrix polymers. There is also a lack of knowledge on the mechanical interaction of the polymers and possible ways in which they are controlled by the plant. Despite this there are several promising approaches for a better understanding of plant cell wall mechanics which will be introduced in the following sections.

4 Elucidating the Mechanical Design of Plant Cell Walls

One possible way of determining the mechanical role of a certain mechanical component is to alter its structure or binding capacity either by genetic, enzymatic or chemical treatments and then to mechanically characterize the modified cell wall structure. Since the entire cell wall structure is retained, this method resembles a “top-down approach” which is utilized for a better understanding of both primary and secondary cell walls (Köhler and Spatz 2002; Ryden et al. 2003; Goswami et al. 2008; Abasolo et al. 2009). However, one has to be aware that all these treatments are either not highly specific or may cause compensation reactions by the plant. Therefore, further structural, (bio)chemical or genetic characterization is needed to identify clear structure–property relationships of individual cell wall components.

Another way of identifying the mechanical role of certain cell wall components is to reassemble cell wall analogues in a “bottom-up process” and then determine the mechanical performance of the entire composite structure. This approach has been mainly utilized to mimic the primary cell wall structure and study the cross-linking of the polymers (Whitney et al. 1995; Chanliaud et al. 2002). In terms of secondary cell walls, this approach is less prominent as one characteristic feature of secondary cell walls is the strictly parallel orientation of the cellulose microfibrils which can hardly be obtained in a bottom-up process based on cell wall reconstruction.

A further approach which can shed light on the load-bearing elements and the polymer interactions in plant cell walls are so-called *in situ* experiments which simultaneously combine external mechanical loading with nanostructural observations. For this purpose, mainly spectroscopic analysis (FT-IR, Raman) and X-ray analysis have been utilized (Eichhorn et al. 2000; Akerholm and Salmén 2001; Köhler and Spatz 2002; Keckes et al. 2003; Gierlinger et al. 2006; Martinschitz et al. 2008). For instance, applying an external strain to a plant segment can result in shifts of characteristic Raman bands which are indicative for the load-bearing function of this component. This has been impressively shown in plant tissue and fibre tests for the deformation of the cellulose chains (Eichhorn et al. 2000; Gierlinger et al. 2006; Peetla et al. 2006). *In situ* X-ray scattering methods can visualize the passive reorientation of cellulose fibrils toward the cell axis during tensile straining which gives insight into cellulose fibre–matrix interactions during deformation. Such a response has been shown for compression wood of conifers (Keckes et al. 2003), coir fibre bundles (Martinschitz et al. 2008) and supporting

tissue of *Aristolochia* (Köhler and Spatz 2002). One limitation of both Raman microspectroscopy and X-ray diffraction is that they are highly sensitive to changes in the molecular structure of cellulose fibrils and their orientation but changes of the matrix polymers and their interplay either cannot or can only hardly be detected. Here in situ FT-IR measurements are of advantage which have a lower resolution but are more sensitive, for instance, towards hemicelluloses (Akerholm and Salmén 2001).

5 Structure and Mechanics of Primary Cell Walls

5.1 Elastic Versus Plastic Deformation

In view of the composite analogy of cell walls as discussed in the previous sections, the primary cell wall is an exceptional mechanical structure. It provides mechanical support by being stiff and rigid, and at the same time, allows for the cell to grow and elongate. In order to achieve both conflicting properties, the cellulose–matrix interaction needs to be modulated on demand. During cell rest, the connection between cellulose fibrils and matrix polymers must remain tight to provide mechanical support; however, this connection must also be able to be loosened during cell growth.

It is well accepted that cell growth starts with a softening of the cell wall. This results in a loss of turgor pressure because the inner pressure of the cell and the stress in the cell wall counterbalance each other (Cosgrove 1993). In consequence, additional water will flow into the cell leading to a volume increase and plastic (irreversible) deformation of the cell wall (Peters et al. 2000). The cell volume increase by water uptake does not necessarily result in cell growth, but can also be utilized to pre-stress the cells (Fig. 6). In this case, the cell wall undergoes elastic rather than plastic deformation. Such systems are needed for reversible movements of living tissues in plants such as the opening and closure of stomata cells (Raschke 1975; Roelfsema and Hedrich 2002), the pulvinus cells actuating leaf movements (Toriyama and Jaffe 1972; Samejima and Sibaoka 1980; Iino et al. 2001; Moran 2007) or the leaf cells making the Venus flytrap snap (Williams and Bennett 1982; Hodick and Sievers 1989).

Hence, depending on maturity and functionality, primary walls can be either plastically or elastically deformed. This indicates that the cell to some extent must be able to control the mechanical response and deformation behaviour of its cell wall. From a mechanical perspective, it seems reasonable that this can only be achieved by adjusting and modulating the bonding pattern within and between macromolecules. A modification of the cell wall assembly takes place in order to allow the cell wall to plastically deform for cell growth (Cosgrove 2005). However, different theories exist about the biochemical agents responsible for this, e.g. expansins cleaving hydrogen bonds (McQueen-Mason et al. 1992; Cosgrove 2000; Cosgrove et al. 2002), enzymes opening and re-establishing

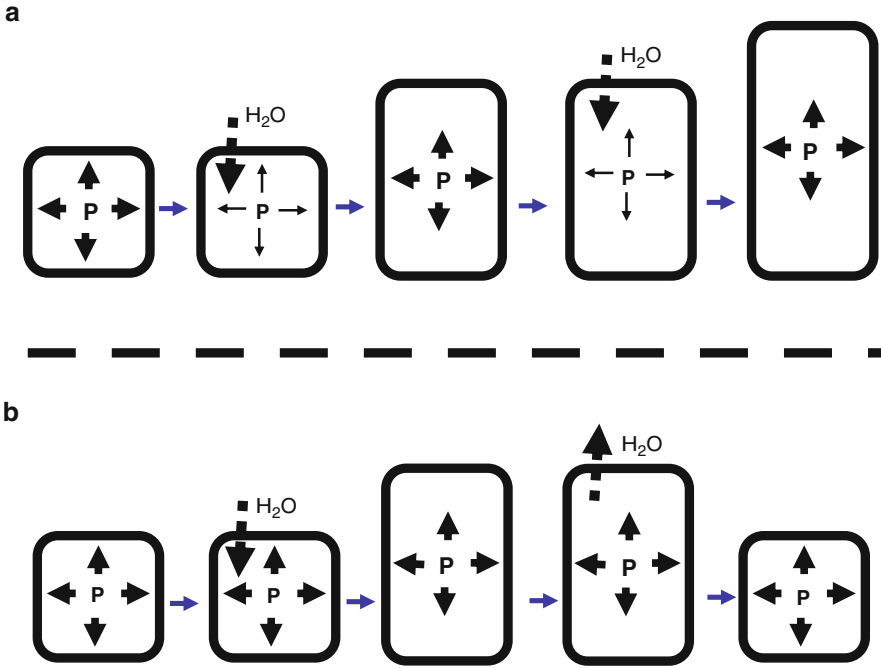


Fig. 6 (a) Plastic deformation of primary cell walls to facilitate growth (according to Peters et al. 2000) and (b) elastic deformation of primary cell walls to generate stresses. “P” in different font size illustrates the level of turgor

covalent bonds in xyloglucans (Fry et al. 1992) or hydroxyl radicals cleaving polymer linkages (Schopfer 2001; Liszkay et al. 2003). Since the mechanics of cell growth and cell wall elongation are not in the specific focus of this chapter, we refer readers to excellent review articles dealing with these questions (Cleland 1971; Taiz 1984; Cosgrove 1993, 2005; Schopfer 2006).

In terms of pure elastic deformation of cell walls for reversible organ movements, it is still unclear how relaxation and creep (see Fig. 3) of the cell wall can be avoided or limited under the pre-stressed conditions. Biological materials are normally viscoelastic, showing a time-dependent response to loading. This implies that pre-stresses are at least partly relaxed over time. This relaxation is probably due to reorientation of macromolecules and the opening and closure of labile cross-links. Therefore, in order to have a fully elastic response and retain the original self-stresses, the cell wall needs to react like a tightly bonded and fixed network.

Another important feature of either elastic or plastic deformation of cell walls is that the cell must be able to control its geometrical changes. This is achieved by controlling the cellulose fibril deposition via the microtubules which guide the movement of the cellulose synthase complexes (Paredez et al. 2006; Lindeboom et al. 2008; Wasteneys and Ambrose 2009). As a result a specific pattern of

cellulose fibril orientation in the cell wall is formed which dictates the deformation behaviour of the wall (Baskin 2005). Due to the high aspect ratio of the parallel aligned stiff fibrils, the cell wall has an anisotropic response and can be more easily deformed in a direction perpendicular than parallel to the fibril orientation. This principle is utilized to allow for polarity of growth as well as pre-stressing of primary tissues. During cell growth, the cellulose fibrils are passively reoriented and shifted from a primarily perpendicular orientation to the cell axis toward a longitudinal orientation (Preston 1982; Anderson et al. 2010) (see also Fig. 5).

5.2 Mechanical Behaviour Under Static Loading Conditions

Research on the “static” mechanical behaviour of primary cell walls is mainly driven by understanding the mechanical role of individual cell wall components and their interaction. Since most of the recent work on primary cell wall mechanics has been performed on etiolated hypocotyls of *Arabidopsis* plants, their typical cell wall characteristics will be in the focus of this chapter.

Dark grown *Arabidopsis* hypocotyls display a slender cylindrical body with a rather constant diameter along their length making them suitable for microtensile testing. In standard microtensile tests, wild type hypocotyls show a stress–strain curve with an *s*-shape pattern which is illustrated in Fig. 7a.

In the initial phase of the test, one can observe a tensile stiffening with straining followed by a phase in which stress and strain increase linearly. Beyond this phase a yielding of the sample can be observed and the increase in strain exceeds the increase in stress. From a standard tensile test, such as that illustrated in Fig. 7a, the stiffness and the ultimate stress (\sim tensile strength) of a hypocotyl can be measured.

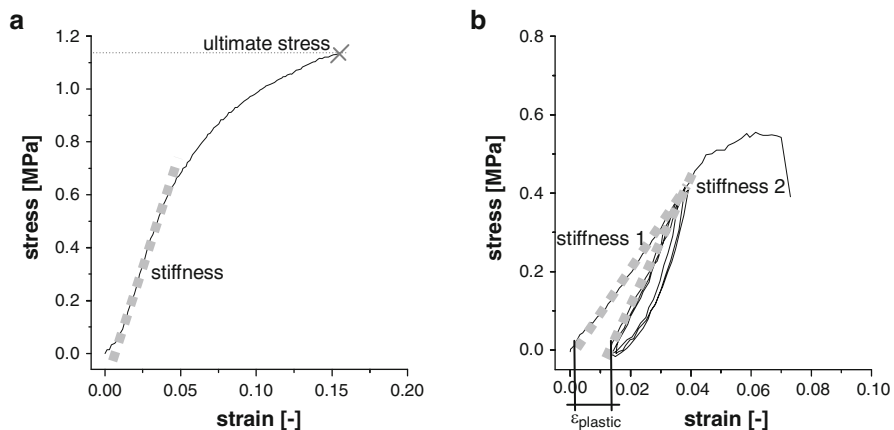


Fig. 7 Stress–strain curves of hypocotyls (a) of 6-day-old *Arabidopsis* wild type under static tensile loading; (b) of 6-day-old *Arabidopsis qua2* mutant under cyclic tensile loading

To gain further information on mechanical processes, cycling loading tests allow elastic and plastic deformations to be distinguished (Fig. 7b).

Usually the underlying objective of the hypocotyl tests is to learn about the mechanical properties of the cell walls from the mechanical performance of the hypocotyls. Here the interpretation of the initial phase of deformation in term of cell wall mechanics must be treated with care because other effects such as the hypocotyl re-alignment may play a role. But also for the other phases of the graphs one cannot conclude directly from the mechanical behaviour of the hypocotyls on cell wall mechanics. Further important parameters for the hypocotyl response which would affect the stress experienced by the cell wall are the density of the hypocotyls (e.g. how much cell wall material (mass) is agglomerated in a certain hypocotyl volume), parameters of cell geometry and the turgor pressure of the cells which stiffens the pliant primary cell wall structure. Even among *Arabidopsis* wild-type plants these parameters can vary considerably with hypocotyl age, as shown in a study on cell wall thickness (Derbyshire et al. 2007a). Therefore, structural and hydraulic parameters need to be considered in order to correlate hypocotyl properties with those of the cell wall.

5.3 Mechanical Tests of Genetically Modified Material

Various cell wall mutants have been produced to unravel crucial parameters in cell wall synthesis and for a better understanding of the mechanical role of the individual cell wall components.

Most of the mechanical tests on *Arabidopsis* hypocotyls have been focused on gaining further understanding of the interaction of the cellulose–xyloglucan network and in particular on the mechanical role of the xyloglucan side chain structure. The stiffness and strength properties of mutants with different severities of side chain modification (reduction) have been investigated. It was shown that impairing a fucosyltransferase as present in the *mur2* mutation only marginally alters the mechanical performance of the hypocotyls (Ryden et al. 2003). This observation is in good agreement with measurements by (Pena et al. 2004) and (Abasolo et al. 2009). However, a more drastic side chain modification by impairing a galactosyltransferase (*mur3*) resulted in severe and significant loss of stiffness and strength (Ryden et al. 2003). An even harsher alteration of the xyloglucan chain was achieved in the xylosyltransferase double mutant *xxt1 xxt2* (Cavalier et al. 2008). Mechanical tests revealed hypocotyl properties with reduced stiffness and strength for the *xxt1 xxt2* double mutant and even lower for the *mur3* mutant compared to the wild-type (col-0) (Fig. 8).

Another matrix component which may contribute to the primary cell wall properties is pectin. *Mur1*-mutants lack L-fucose in the shoot (Bonin et al. 1997) which leads to an alteration of xyloglucan and pectin, because the deficiency in

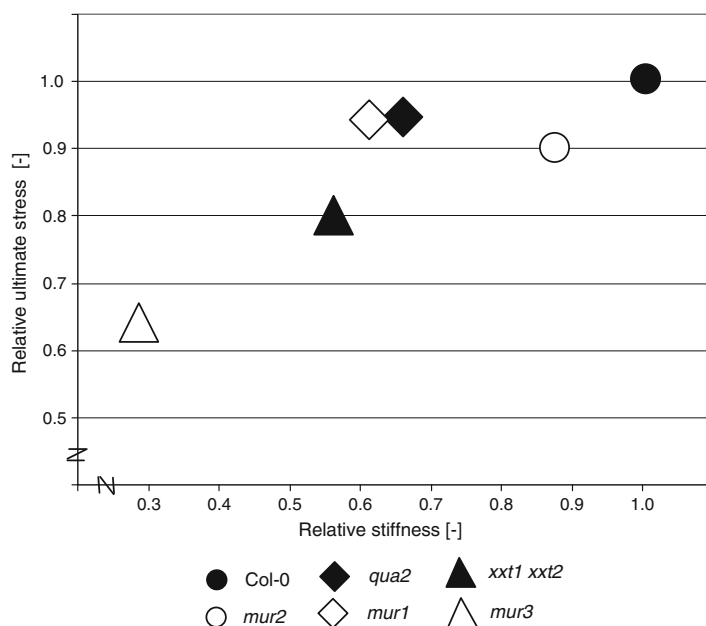


Fig. 8 Relative stiffness and ultimate stress of wild-type *Arabidopsis* hypocotyls, xyloglucan mutants and pectin mutants (4 days and 6 days old); arithmetic means are given in percentage of the wild type (col-0 = 1); detailed information on mechanical properties (arithmetic mean, SD) of col-0, *mur1*, *mur2*, *qua2* in Abasolo et al. (2009), of col-0, *mur3* in Burgert (2006) and of col-0, *xxt1 xxt2* in Cavalier et al. (2008)

fucose influences the side chain structure of the xyloglucan as well as the capability of the rhamnogalacturonan II (RGII) to form borate diesters. Interestingly, *mur1* shows different mechanical properties than the mutants which are altered exclusively on the xyloglucan side chain. The stiffness of *mur1*-hypocotyls is significantly reduced in comparison to the wild type but the hypocotyl strength is almost the same (Abasolo et al. 2009). A similar mechanical response was observed for another pectin mutant (*qua2*) which has 50% lower homogalacturonan content than the wild type (Mouille et al. 2007). Also here stiffness is reduced whereas strength is in the vicinity of the wild type (Fig. 8).

A reasonable explanation of the mechanical properties of the xyloglucan and pectin mutants may be that xyloglucan has a direct tethering function between cellulose fibrils. Pectin, however, stiffens the matrix and may prevent xyloglucan chains from unfolding in response to external tensile loading (Abasolo et al. 2009). In terms of pectins, the ion-mediated bonds may play a crucial role as they largely influence the cross-linking of pectin components and thereby the degree of matrix viscosity. This is in agreement with investigations which show that calcium ions and the degree of pectin methyl-esterification have a strong influence on pectin

properties and in consequence on cell growth processes (Willats et al. 2001; Proseus and Boyer 2006, 2007, 2008; Derbyshire et al. 2007b). Also supporting this is a study by Fleischer and co-workers showing that cell wall pore size depends on the number of borate-ester cross-links in RGII (Fleischer et al. 1999).

5.4 Mechanical Tests of Cell Wall Analogues

As discussed in Sect. 4, an alternative way to identify the mechanical role of certain cell wall components is to resemble composite structures from cell wall analogues and determine their mechanical performance. Here the stiff cell wall component is mainly bacterial cellulose which can be embedded in different matrix polymers. The addition of xyloglucan to a cellulose fibril network results in reduced stiffness and strength (Whitney et al. 1999). A similar effect was observed when pectins were added to the cellulose network (Chanliaud and Gidley 1999). Although the pure cellulose network is a reasonable reference material to use in this bottom-up approach, it is not ideal since in plant cell walls a certain amount of matrix polymer is always associated with the cellulose. The same approach was used to characterize the influence of expansins on the mechanical performance of cell wall analogues. While the deformability of cellulose/xyloglucan networks increased due to the treatment with expansins, no effect was observed for pure cellulose composites or assemblies of cellulose with glucomannan or galactomannan (Whitney et al. 2000).

6 Structure and Mechanics of Secondary Cell Walls

6.1 Structure–Property Relationships

By far more research has been conducted on the mechanics of natural secondary cell walls, mainly wood cell walls, than on primary walls (Salmen and Burgert 2009). This is mainly due to the intensive use of wood for pulp and paper and as a construction material which demands detailed knowledge on the properties of the raw material.

A secondary cell wall deposition on the primary cell wall typically happens after the cell has terminated growth and a final size and shape is reached. Several cell types die at this stage which means that the turgor pressure which formerly stabilized the system is no longer retained. Therefore, secondary cell walls have to be much thicker than primary cell walls in order to provide mechanical stability to the dead cells (see also Fig. 5). However, there are also mechanical supporting cells which stay alive with secondary cell walls, such as some hardwood fibres in tropical species or the sclerenchyma fibres in monocots. An interesting aspect of the latter fibre type is that new secondary cell wall layers can be

continuously added and therefore secondary cell wall thickness increases with ageing (Tomlinson 2006).

In the secondary xylem of dicotyledonous trees and gymnosperms, the secondary cell wall typically consists of three layers (S1–S3), with a dominance of the middle S2 layer, making up to ~80% of this sandwich structure (Fengel and Wegener 1984) (see Fig. 5b). The individual secondary cell wall layers can be distinguished because of different orientations of cellulose microfibrils. Typically, secondary cell wall layers show a strong parallel alignment of cellulose. The orientation of the fibrils toward the cell axis is called the cellulose microfibril angle. In wood, the surrounding cell wall layers (S1 and S3) show a large and rather steady angle between 50 and 80°, whereas the central S2 layer is varied between 0° and 50° depending on the mechanical function of the cell type. The ongoing consecutive deposition of secondary cell wall layers in monocots results in a multilamellar structure. Very characteristic is the alternation of thick and thin cell wall layers in bamboo, where thick cell wall layers possess a small cellulose fibril (<15°) and thin layers show a large microfibril angle (Parameswaran and Liese 1976; Liese 1987).

The cellulose microfibril angle has a strong influence on the mechanics of the cell wall; its adjustment enables the plant to control the mechanical performance of tissue according to need. The crucial role of the cellulose microfibril orientation in secondary cell walls becomes obvious when plotting the tensile stiffness of various plant tissues and fibres against the microfibril angle (Fig. 9).

For cell walls with cellulose microfibril orientation almost parallel to the cell axis in the S2 wall, high Young's moduli are measured, whereas tensile stiffness decreases with an increase of microfibril angle. This correlation also enables plants to adjust the mechanical properties of their tissues in the course of cellulose fibril deposition. This can be seen, for instance, for variations in microfibril angle between wood formed by young and mature trees (Lichtenegger et al. 1999). The juvenile wood has a high microfibril angle which makes the wood and the stem more flexible, whereas the mature wood has a low microfibril angle which results in high stiffness and a lower buckling risk.

The role of the matrix properties for the mechanical performance becomes more important with increasing microfibril angle. For instance, it has been shown for compression wood of gymnosperms with high microfibril angles (~45°) that the stiffness in the initial phase largely depends on the matrix stiffness. When the shear strength of the matrix is exceeded, then the stress–strain curve of the tissue shows a characteristic yield point and a long phase of plastic deformation typified in the typical biphasic stress–strain curves (Köhler and Spatz 2002; Keckes et al. 2003; Altaner and Jarvis 2008). A longitudinal stiffening effect by the matrix in case of high cellulose microfibril angles is also utilized in the dominating vascular bundle type of the palm tree, *Washingtonia robusta*. While the cellulose microfibril angle remains rather constant, the matrix properties are varied across the fibre cap due to changes in lignin content and lignin composition. Thereby, the plant creates a smooth transition in stiffness from the stiff inner fibre cap to its periphery, which is beneficial for the connection with the surrounding pliant parenchymatous tissue (Ruggeberg et al. 2008).

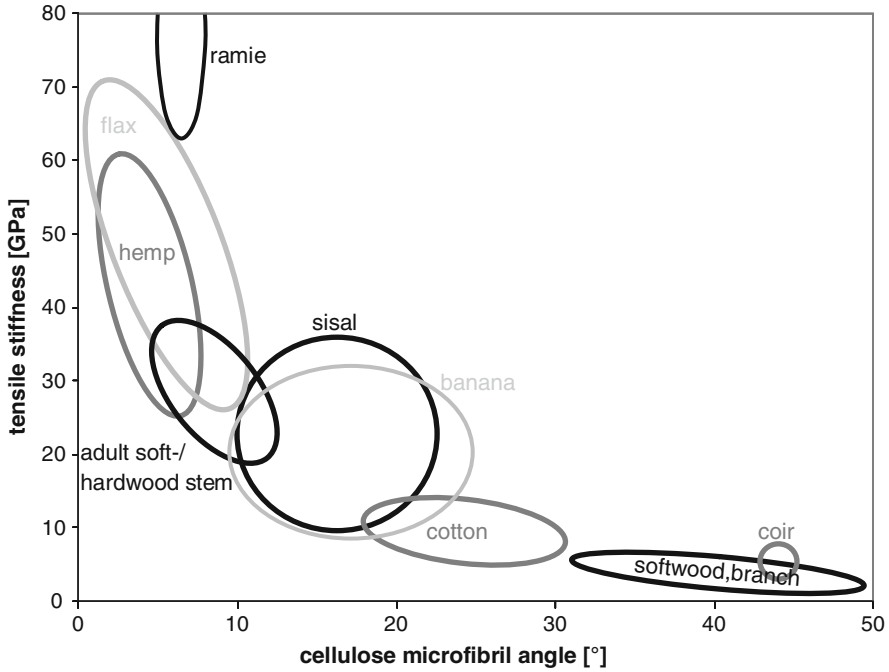


Fig. 9 Elastic modulus as a function of cellulose microfibril angle in the secondary cell wall for various species (for specific values and references see a table in Eder and Burgert (2010) giving a survey on plant fibre structure and properties)

6.2 Mechanical Tests of Genetically Modified Materials

Research on the mechanical properties of mutants with secondary cell wall alterations is still in its infancy. Most of the work has been done on tree species, mainly *Populus*. This is primarily motivated along the lines of wood modification to optimize cell wall composition for pulp and paper production. Trees with changes in lignin content and lignin composition can facilitate the chemical pulping processes (Boudet 2000; Pilate et al. 2002; Baucher et al. 2003; Boerjan et al. 2003). For instance, a downregulation of cinnamyl alcohol dehydrogenase (CAD) increased the lignin extractability (Baucher et al. 1996). In a study by Kasal and co-workers, young aspen trees with reduced lignin content and changes in lignin composition showed a lower compressive strength but no change in stiffness compared to the wild type. In contrast under tensile load, stiffness was reduced but strength was not. The bending properties were neither affected in stiffness or strength (Kasal et al. 2007). Macromechanical tests in tension, compression, bending and shear on wood from partially CAD-deficient pine trees did not reveal any significant changes compared to the wild type (Saralde et al. 2008).

7 Summary and Conclusions

The underlying structure–function relationships which result in the unique mechanical performance of plant cell walls have only been partly elucidated up to now. In this chapter, we showed current drawbacks and opportunities in terms of mechanical characterization. General mechanical terms and definitions as well composite theory have been briefly introduced to establish a solid basis for the mechanical characterization of biological materials, despite their inhomogeneous nature and complex mechanical response. For a better understanding of the mechanical role of individual components and possible adjustments of cell wall properties, the mechanical characterization of genetically modified plants appears to be a powerful approach. However, one has to bear in mind the complexity of cell walls due to the spatial organization of the polymers and the variety of weak and strong cross-links between them. Another problem is possible compensation strategies by the plant which may lead to alternative synthesis pathways as well as functional substitution of altered or diminished cell wall components.

In terms of application, the mechanical characterization of genetically modified plants will become an important step in the process of extracting optimized raw material properties for pulping as well as fuel production. A further application potential of cell walls can be foreseen in the field of biomimetics and biotemplating. The native fibre composite structure of the cell walls can inspire the improvement of technical composites by transferring design principles which are the basis for the exceptional mechanical properties (Fratzl 2006; Milwich et al. 2006). Biotemplating of plant cell walls has been utilized to create anisotropic ceramic structures (Greil 2001; Deshpande et al. 2006). Another interesting approach could be to retain the cellulose orientation but replace matrix components by nanoparticles or responsive polymers in order to build novel composites and hybrid materials as well as smart materials which have the sensor and the actuator on board.

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