



How the geometrical model for plant cell wall formation enables the production of a random texture

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Abstract

Cellulose synthase (CESA) molecules are the building blocks and catalytic centers of the CESA complex. The study of mutants in *Arabidopsis* has led to insight into the structure of these nanomachines. Inside the plasma membrane, the CESA molecules are arranged in complexes, which, apart from the CESA molecules proper, contain other, mostly unidentified, proteins. We developed a theory in which CESA density, together with distance between cellulose microfibrils (CMFs) being deposited and cell geometry, determines wall texture. We have shown earlier how this theory is able to explain the production of axial, helical, helicoid and crossed-polylamellate textures. In the present article we extend this theory to random wall textures.

Abbreviations: CMF – cellulose microfibril; CESA – cellulose synthase; CSAD – cellulose synthase activation domain.

Introduction

Plant cell walls form by apposition of new material from the inside of the plasma membrane. The wall matrix material of hemicellulose, pectins and glycoproteins is contained in Golgi vesicles that deliver their content into the existing cell wall after fusing of their membranes with the plasma membrane. Cellulose synthases (CESAs) are thought to be assembled in the endoplasmic reticulum and brought to the plasma membrane via the exocytotic pathway of Golgi stacks and Golgi vesicles (Haigler and Brown 1986). The CESA is only active in producing cellulose

microfibrils (CMFs) when located inside the plasma membrane.

CMFs arrange in lamellae that together form the cell wall texture. The orientation of the CMFs within a lamella is constant, but may vary from lamella to lamella. The most striking texture is the helicoidal wall, which consists of subsequent lamellae in which the orientation of the CMFs changes by a constant angle. Other wall textures are the axial, helical, crossed-polylamellate, transverse and random wall textures, and combinations of these.

CMFs are crystalline stiff rods, which can tilt and move *Acetobacter* bacteria, when they are

spun out of fixed complexes in the plasma membranes. Time-lapse studies show that the cells rotate along their longitudinal axes when they spin the cellulose ribbons, caused by the force of crystallization. When a bacterial cell, moving by cellulose synthesis, strikes the edge of a cover slip or another cell and the ribbon is anchored, the cellulose ribbon bows out, finally snapping and releasing all energy. For a few seconds, the bacterium assembles a disordered array of cellulose, before going back to its normal ribbon formation (R. Malcolm Brown Jr., personal communication). CMF stiffness, therefore, is a characteristic that one should take into account when formulating theories about their orientation mechanism. Another physical feature that must influence CMF ordering is the number of CMFs being deposited at any moment in one cell locally simultaneously, and hence the number of active CESAs making CMFs together in time.

We have formulated a geometrical model for CMF ordering during their deposition, that takes the characteristics CMF stiffness and number of active CESAs into account (Emons 1994; Emons and Kieft 1994; Emons and Mulder 1997, 1998, 2000, 2001; Mulder and Emons 2001; Emons et al. 2002; Ebskamp et al. 2004 in press). In the following paragraphs, we discuss this model and extend it to random cell wall textures.

In the model we have proposed that the default mechanism, which determines the orientation of CMFs as they are deposited in the absence of other influences, is geometrical in origin. Based on the observation that CMFs always appear approximately evenly spaced in close-packed lamellae and that their average distance apart does not depend on their orientation with respect to the cell axis, the geometrical close packing rule (Emons 1994) was formulated:

$$\sin \alpha = \frac{Nd}{2\pi R}.$$

This formula relates the CMF winding angle α to the number of CMFs being deposited (N), the distance d between them and the radius R of the cell. This explicit mathematical rule is the cornerstone of a dynamic developmental model, which rests on the assumption that new active CESAs insert into the plasma membrane through exocytosis of Golgi vesicles, or else are activated within

moving, localized regions along the cell, the CESA activation domains (CSAD, Figure 1). The rate at which new synthases become active is under cellular control and regulated, and the microtubules may well play a yet unknown role in this process, as discussed before (Emons and Mulder 1998, 2001; Ebskamp et al. 2004 in press). Once activated in the plasma membrane, the CESAs move forward propelled by the forces generated in the CMF deposition/crystallization process. In the course of time, their angle of motion with respect to the cell axis is continuously adapted to the changing number of other CESAs in their neighborhood in order to satisfy the geometrical close packing constraint. The CMFs deposited follow the tracks of the CESAs and as such constitute a 'recording' of their motion. The final ingredient of the model is that cellulose synthases have a finite active lifetime.

The elements outlined above are cast into the form of a partial differential equation describing the evolution, both in space and in time, of the

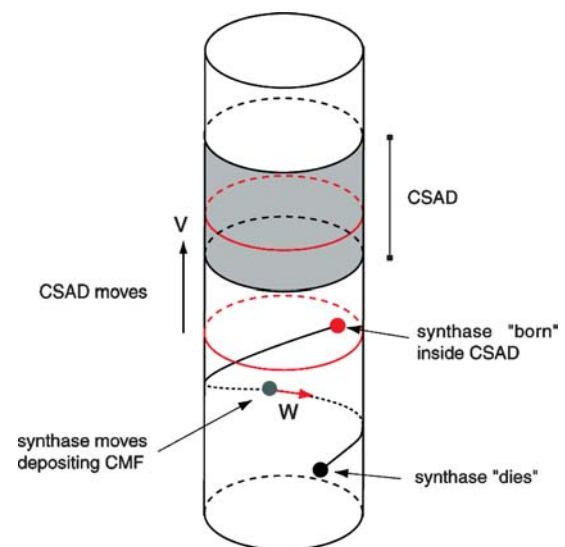


Figure 1. The cellulose synthase lifecycle. After being inserted into the plasma membrane within a CESA activation domain (CSAD: located between the red circles at the time of deposition) the synthase moves with an average speed w within the plasma membrane, leaving a CMF in its wake. The direction of motion and hence the angle the deposited CMF makes with the cell axis is determined by the local density of other synthases. The CMF synthase becomes inactive after a characteristic lifetime t^\dagger , which determines the length of the microfibrils. The CSAD itself, here shown in gray, moves with a speed v in the direction opposite to that of the CMF synthases. See color section at the end of this issue.

density of active CESAs present in the plasma membrane. This equation takes the following form on a cylindrical cell of radius R :

$$\frac{\partial N(z, t)}{\partial t} - \frac{wd}{2\pi R} N(z, t) \frac{\partial N(z, t)}{\partial z} = \varphi(N, z, t) - \varphi^\dagger(N, z, t),$$

where w is the speed with which the synthase moves and d the effective width of a CMF plus adherent matrix material, i.e. the distance between neighboring CMFs. φ is the local rate of synthase production for which we choose the following form:

$$\varphi(N, z, t) = \frac{N_*}{t_*(1-\gamma)} \left(1 - \frac{N(z, t)}{N_{\max}}\right)^\gamma$$

if $N(z, t) < N_*$ and z is located inside a CSAD

In all other cases $\varphi = 0$. The parameter γ controls the shape of the synthase production curve and lies between zero and one. Synthase production stops when the maximum density,

$$N_{\max} = \frac{2\pi R}{d},$$

is reached, which for stationary CSAD would happen after time t_* . The insertion domains are assumed to have a length l and travel at a speed v . Finally, the local rate of rosette de-activation φ^\dagger needs to be determined. This rate depends on the full evolution of the density in a time interval of length t^\dagger (= the synthase lifetime). Fortunately, the resultant equations are of a type that can be readily solved with entirely classical techniques. The solutions of these equations can be reinterpreted in terms of the tracks of the CESAs, and hence the orientations of the deposited CMFs, thus leading directly to the cell wall texture. Because of its geometrical origin, the model has only a small number (4) of relevant parameters.

We have shown that by varying these parameters several known cell wall textures can be reproduced by this fully predictive mathematical model: the axial, helical, helicoidal, and crossed

wall texture (Figure 2). In this paper we address the question of the random wall texture.

Results

Can the geometrical model describe the random wall texture?

The geometrical model for cell wall texture formation was explicitly constructed in order to explain the observed regularities of the CMF architecture in secondary cell walls, where cell wall is deposited along whole wall facets, not the banded pattern of local wall deposition in xylem cells, which is surely the atypical case. The question we address here is ‘‘Can the geometrical model also account for the so-called random wall texture which is seen in meristematic cells, at the outside of pollen tubes and root hairs and at the inside of pollen tube and root hair tips, where this texture is deposited?’’ Another term often used for this type of wall is ‘criss-cross’. At the outset we have to be a bit more precise about the notion of ‘randomness’ employed here. We have to distinguish between total randomness, in which both the locations and the orientations of the individual CMFs are without any pattern, and partial randomness, in which for instance any remaining positional and/or orientational correlations in the CMF pattern are obscured by observational limitations, leading to the impression of randomness.

Bearing this difference in possible definitions of randomness in mind, the geometrical model can in fact offer two potential explanations. Both explanations start from the observation that the areal density of CMFs in a random wall is significantly smaller than that of the more closely packed wall. This means that the average distance d between adjacent CMFs is also larger. To explain the significance of this parameter, let us recall the starting point of the geometrical model (Emons 1994). It states that the number N of fibrils in a lamella, as counted in a transverse section of the cell wall, must be related to the winding angle α through the formula

$$\sin \alpha = \frac{Nd}{2\pi R},$$

where d is the aforementioned distance between adjacent CMFs and R the radius of the cell. This

Length of the CSAD	$\lambda = \frac{l}{wt_*}$
Speed of the CSAD	$\beta = \frac{v}{w}$
Synthase lifetime	$\tau^\dagger = \frac{t^\dagger}{t}$
Synthase production curve shape	γ

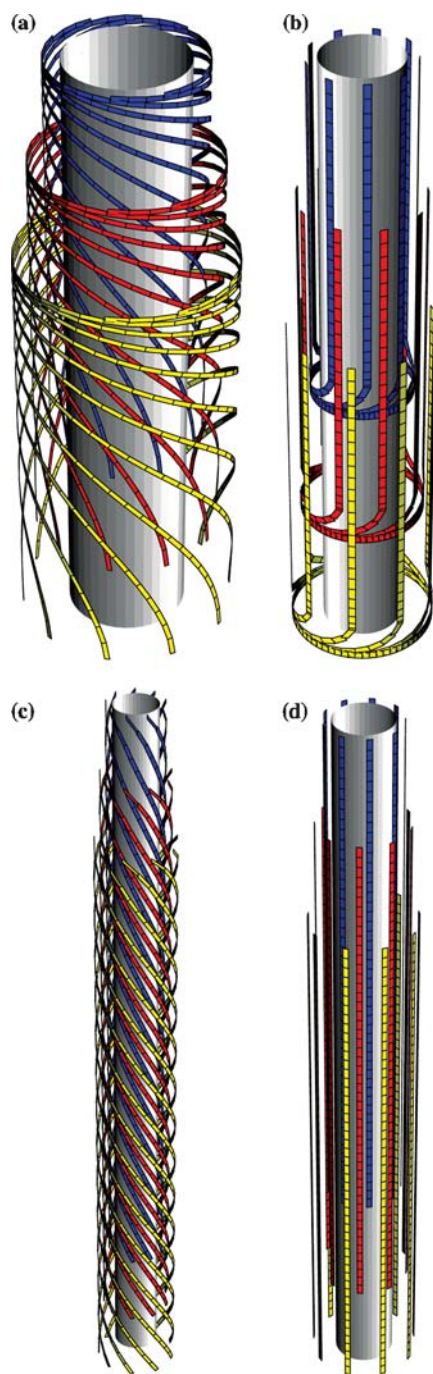


Figure 2. Different cell wall textures as predicted by the geometrical model. The ribbons shown represent the tracks CMFs obtained from the explicit solutions to the CMF evolution equation. (a) The helicoidal texture in which the angle of orientation between subsequent lamellae changes by a constant amount. (b) A crossed-polylamellate texture with alternate lamellae with transverse and axial oriented CMFs. (c) A purely axial texture. (d) A helical texture in which the CMFs have an almost constant winding angle. See color section at the end of this issue.

rule is in a sense validated *a posteriori* by the observed wall structures, which are composed of close-packed lamellae in which the mean separation between CMFs does not depend on the winding angle. However, we are as yet ignorant of the underlying physical mechanisms that cause fields of CMF synthases that are co-depositing CMFs within a single lamella to follow this geometrical 'equation of motion'. Nevertheless, it is clear that physical forces between the synthases and the CMFs that they are depositing are at work here. These forces are most likely mediated by both the plasma membrane, in which the synthases move, and the matrix polymers, that physically couple the CMFs. It is typical of such forces that they have a well-defined range of operation, beyond which they are too weak to cause a detectable influence. This is the basis of the first explanation:

1. In the primary wall the CMF synthases, and hence the CMFs they deposit, are too far apart for the physical forces between them to enforce the geometrical packing constraint. There is thus no guiding principle available, and in the absence of other factors, one expects the CMF synthases to move in random directions and consequently deposit CMFs with random orientations, i.e. a random wall.

Wolters-Arts et al. (1993) measured CMF orientation at different levels in the primary random cell wall of a number of cell types. They used meristematic, isodiametric and cylindrical parenchyma cells and cells of a suspension culture. Within the newly deposited microfibril population, various orientations were recognized on the micrographs. These measurements showed a gradual shift in CMF orientation in the different levels. Microfibrils showed predominant orientations at particular levels but microfibrils of intermediate orientation also occurred, although at a lower density. As cellulose microfibrils of intermediate orientation were not closely packed, lamellae were not formed.

On the basis of these results, we favor our second explanation for a random wall, which also follows from the geometrical model. Here we assume that, although the synthases are few in number and thus far apart, the physical forces between them are still operative over these distances. In that case, the geometrical rule would

still apply and the random wall pattern would follow one of the textures that the model so far is able to generate. Since CMFs in the random wall are seen to have all possible orientations (Figure 3), the only candidate texture is in fact the helicoidal one. This leads to the second explanation:

2. The primary cell wall is a helicoidal wall with a large spacing between the CMFs. Due to this large spacing the regularities of the pattern are not readily noticeable, leading to the 'illusion' that the texture is random.

To illustrate this second explanation, we have visualized in Figure 4 the helicoidal solution of the full geometrical model (for technical details on the necessary calculations please refer to Mulder and Emons 2001), in the case that the CMFs are spaced far apart. The inset shows a local cell wall surface section, with a few lamellae exposed. Note



Figure 3. Surface preparation of the inner cell wall at the apex of a growing root hair of *Equisetum hyemale*. The same procedure was used as described in Emons (1989). The bar represents 500 nm.

that because of the large disparity in length scale between the typical distances between CMFs (~ 10 nm) and the radius of the cell (~ 10 μm) we are unable to present these visualizations drawn to true scale. The remnants of spatial structure still visible are an artifact of this limitation. In a true surface section with a typical size of a few square μm , these regularities would not appear and the texture would be hard to differentiate from a truly random one. Nevertheless, if there is an underlying helicoidal pattern, this could show up in a more detailed statistical analysis of the observed CMF orientations, as was inferred from the above-mentioned results reported by Wolters-Arts et al. (1993). The results reported by these authors provide a foundation for the second solution of the geometrical model for a random cell wall.

Discussion

In this paper, we have shown that the geometrical model for cell wall formation suggests at least two possible explanations for the random texture. The first explanation, based on the low CMF density in the random wall, which precludes the operation of the geometrical constraints that lead to more ordered structures, is in sense a *negative* one: it places the random wall outside of the theoretical framework developed. As such it can also not be tested experimentally. The second explanation, that a random wall is in fact a very 'sparse' helicoidal wall, however, is a *positive* statement. This hypothesis can in principle be checked by accurate quantitative measurements of CMF orientations in walls traditionally presumed to be random, e.g. the inner wall at the tip of root hairs. During cell elongation, the orientation of CMFs in the outer wall may have changed. Of course the observation of a 'hidden' helicoidal structure by itself does not constitute evidence for the validity of the geometrical model, but rather allows us to analyze the random wall from the perspective the theory offers.

The geometrical model provides a conceptual framework for the alignment mechanism of CMFs, which unites examples where cortical microtubules are and are not parallel to nascent CMFs, and in which they do not directly move or channel the synthases but may be involved in their activation inside the plasma membrane. The core

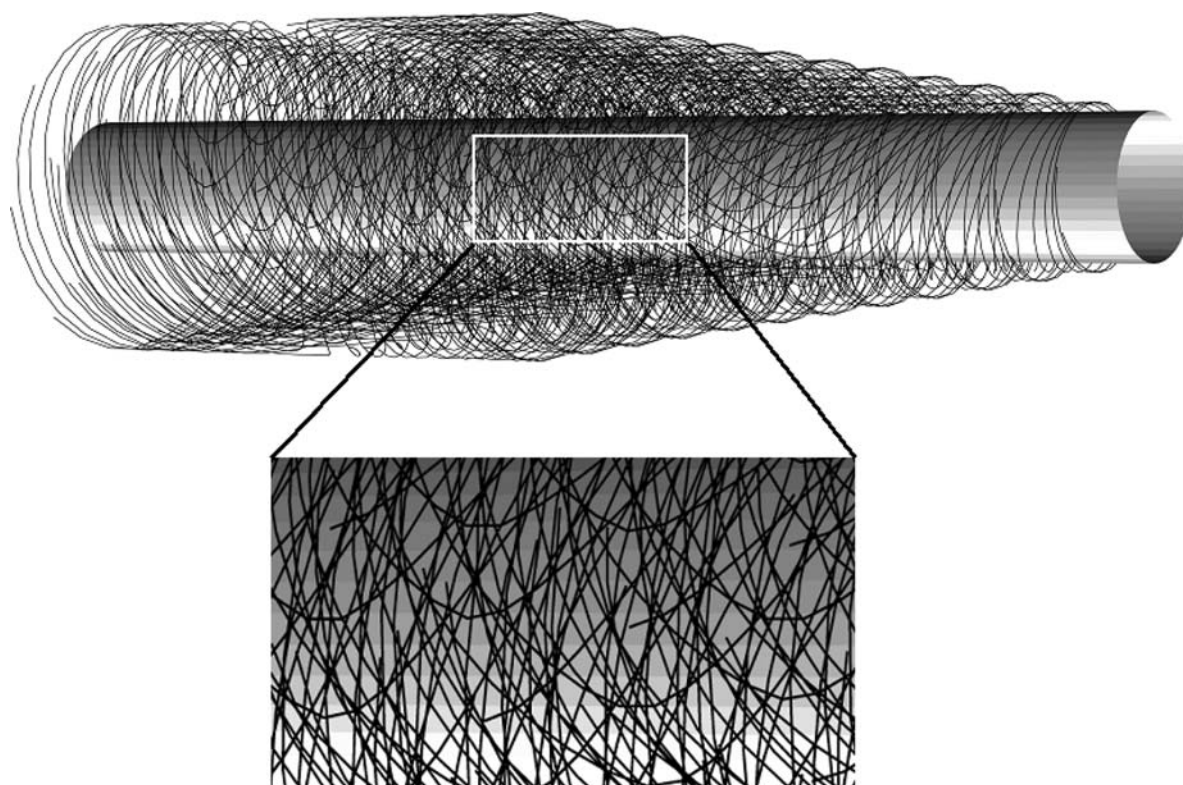


Figure 4. The random wall as a ‘sparse’ helicoidal wall: visualizations of the helicoidal solutions of the geometrical showing several generations of CMFs produced by a moving CSAD that creates a number of lamellae. The inset represents the equivalent of a small surface preparation of a section of the cell wall, showing a wide variety of CMF orientations, which, if more CMFs were drawn on true scale, would be difficult to distinguish from a random texture.

idea is as follows: by *default* CMFs go straight unless obstructed, and their alignment depends mainly on the number of CESAs simultaneously active at any position in the plasma membrane. In its current form the model is focused on secondary wall formation. Nevertheless, we believe that the example of the random cell wall presented here, shows that it is able to furnish a fresh perspective on questions concerning other aspects of cell wall formation as well.

The geometrical model does not rule out that cortical microtubules bind to the plasma membrane so tightly that synthase movement is obstructed, which could be the case in elongating cells in which both polymers are always found in line with each other and transverse to the cell elongation direction. However, recent work of the group of Wasteneys shows that even this is unlikely (Himmelspach et al. 2003; Sugimoto et al. 2003). The geometrical model surely does not rule

out, even favors, the idea that cortical microtubules are (part of) the mechanism that regulates the sites and/or amounts of CESA insertion, i.e. exocytosis or activation areas in the plasma membrane. Inferring from our knowledge of tip growing cells this would require modulation of the actin cytoskeleton (Miller et al. 1999; de Ruijter et al. 1999; Ketelaar et al. 2002, 2003), as well as of calcium ion gradients at those sites (de Ruijter et al. 1998). However, we cannot rule out that exocytosis goes on everywhere and that synthases are activated, or even assembled, locally inside the plasma membrane.

Banded secondary cell wall formation, which occurs in xylem cells, is surely a special case. The coordinated alignment of cortical microtubules and CMFs, often seen and nicely studied with modern techniques by Gardiner et al. (2003), does say something about the relationship between cortical microtubules and nascent CMFs, but does

not discriminate between a role of cortical microtubules in determining CESA insertion, like discussed above, or in directly determining CMF orientation. Although those authors are cautious about this, in a recent review Lloyd and Chan (2004), state that "...studies on secondary cell-wall formation indicate that intramembranous cellulose-synthesizing enzymes require cortical microtubules, *apparently to guide their movement*". This last part has not been proven.

The crucial factor in the geometrical model is that density of active CESAs in the plasma membrane is the default, determining factor for CMF direction control. Intuitively and scientifically, this factor is directly linked to CMF ordering since the synthases are the nanomachines that spin out the fibrils themselves. This self-ordering mechanism is tightly controlled by the cell, which controls cellulose synthase activation in the plasma membrane.

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References

- Ebskamp M., Akkerman M., Schel J.H.N., Mulder B.M. and Emons A.M.C. 2004. The cellulose synthase complex, its structure and assembly, and how its density in the plasma membrane may dictate cell wall texture to appear. To be published.
- Emons A.M.C. 1989. Helicoidal microfibril deposition in a tip-growing cell and microtubule alignment during tip morphogenesis: a dry-cleaving and freeze-substitution study. *Can. J. Bot.* 67: 2401–2408.
- Emons A.M.C. 1994. Winding threads around plant cells: a geometrical model for microfibril deposition. *Plant Cell Environ.* 17: 3–14.
- Emons A.M.C. and Kieft H. 1994. Winding threads around plant cells: applications of the geometrical model for microfibril deposition. *Protoplasma* 180: 59–69.
- Emons A.M.C. and Mulder B.M. 1997. Plant cell wall architecture. *Comments Theor. Biol.* 4: 115–131.
- Emons A.M.C. and Mulder B.M. 1998. The making of the architecture of the plant cell wall: how cells exploit geometry. *Proc. Natl. Acad. Sci. USA* 95: 7215–7219.
- Emons A.M.C. and Mulder B.M. 2000. How the deposition of cellulose microfibrils build cell wall architecture. *Trends Plant Sci.* 5: 35–40.
- Emons A.M.C. and Mulder B.M. 2001. Microfibrils build architecture: A geometrical model. In: *Molecular Breeding of Woody Plants*. Elsevier Science BV, Amsterdam, The Netherlands, pp. 111–119.
- Emons A.M.C., Schel J.H.N. and Mulder B.M. 2002. The geometrical model for microfibril deposition and the influence of the cell wall matrix. *Plant Biol.* 4: 22–26.
- Gardiner J.C., Taylor N.G. and Turner S.R. 2003. Control of cellulose synthase complex localization in developing xylem. *Plant Cell* 15: 1740–1748.
- Haigler C.H. and Brown R.M. 1986. Transport of rosettes from the Golgi apparatus to the plasma membrane in isolated mesophyll cells of *Zinnia elegans* during differentiation to tracheary elements in suspension culture. *Protoplasma* 134: 111–120.
- Himmelspach R., Williamson R.E. and Wasteneys G.O. 2003. Cellulose microfibril alignment recovers from DCB-induced disruption despite microtubule disorganization. *Plant J.* 36: 565–575.
- Ketelaar T., Faivre-Moskalenko C., Esseling J.J., de Ruijter N.C.A., Grierson C.S., Dogterom M. and Emons A.M.C. 2002. Positioning of nuclei in *Arabidopsis* root hairs: an actin-regulated process of tip growth. *Plant Cell* 14: 2941–2955.
- Ketelaar T., de Ruijter N.C.A. and Emons A.M.C. 2003. Unstable f-actin specifies the area and microtubule direction of cell expansion in *Arabidopsis* root hairs. *Plant Cell* 15: 285–292.
- Lloyd C. and Chan J. 2004. Microtubules and the shape of plants to come. *Nat. Rev. Mol. Cell Biol.* 5: 13–23.
- Miller D.D., de Ruijter N.C.A., Bisseling T. and Emons A.M.C. 1999. The role of actin in root hair morphogenesis: studies with lipochito-oligosaccharide as a growth stimulator and cytochalasin as an actin perturbing drug. *Plant J.* 17: 141–154.
- Mulder B.M. and Emons A.M.C. 2001. A dynamical model for plant cell wall architecture formation. *J. Math. Biol.* 42: 261–289.
- de Ruijter N.C.A., Rook M.B., Bisseling T. and Emons A.M.C. 1998. Lipochito-oligosaccharides re-initiate root hair tip growth in *Vicia sativa* with high calcium and spectrin-like antigen at the tip. *Plant J.* 13: 341–350.
- de Ruijter N.C.A., Bisseling T. and Emons A.M.C. 1999. *Rhizobium* Nod factors induce an increase in sub-apical fine bundles of actin filaments in *Vicia sativa* root hairs within minutes. *Mol. Plant Microbe Interact.* 12: 829–832.
- Sugimoto K., Himmelspach R., Williamson R.E. and Wasteneys G.O. 2003. Mutation or drug-dependent microtubule disruption causes radial swelling without altering parallel cellulose microfibril deposition in *Arabidopsis* root cells. *Plant Cell* 15: 1414–1429.
- Wolters-Arts A.M.C., van Amstel T. and Derksen J. 1993. Tracing cellulose microfibril orientation in inner primary cell walls. *Protoplasma* 175: 102–111.