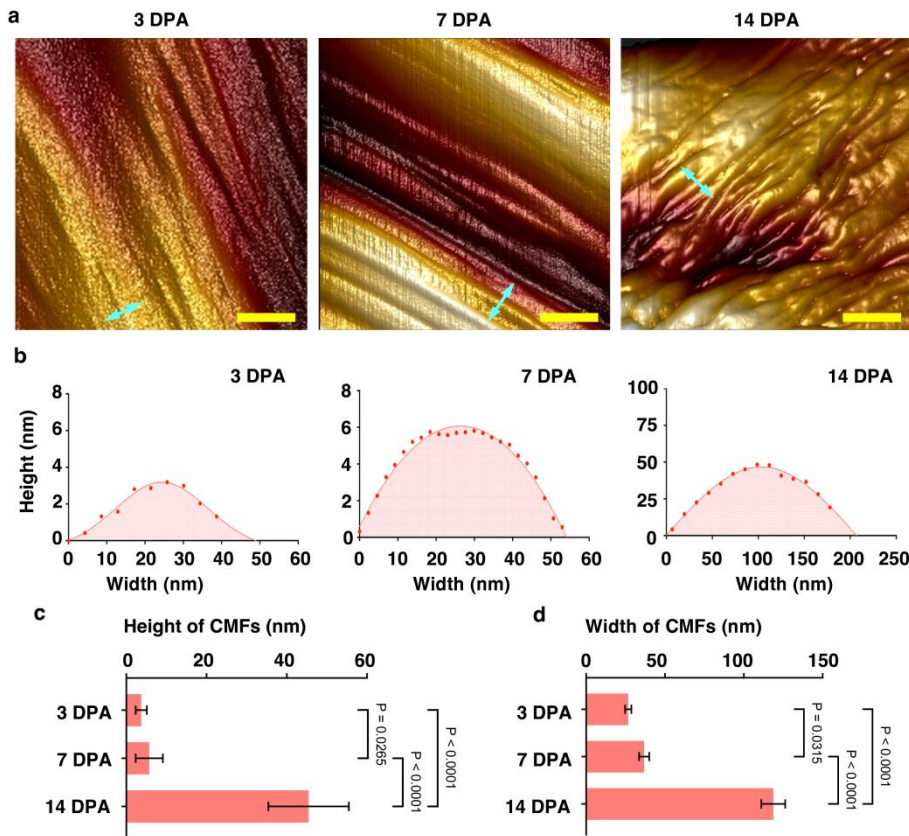


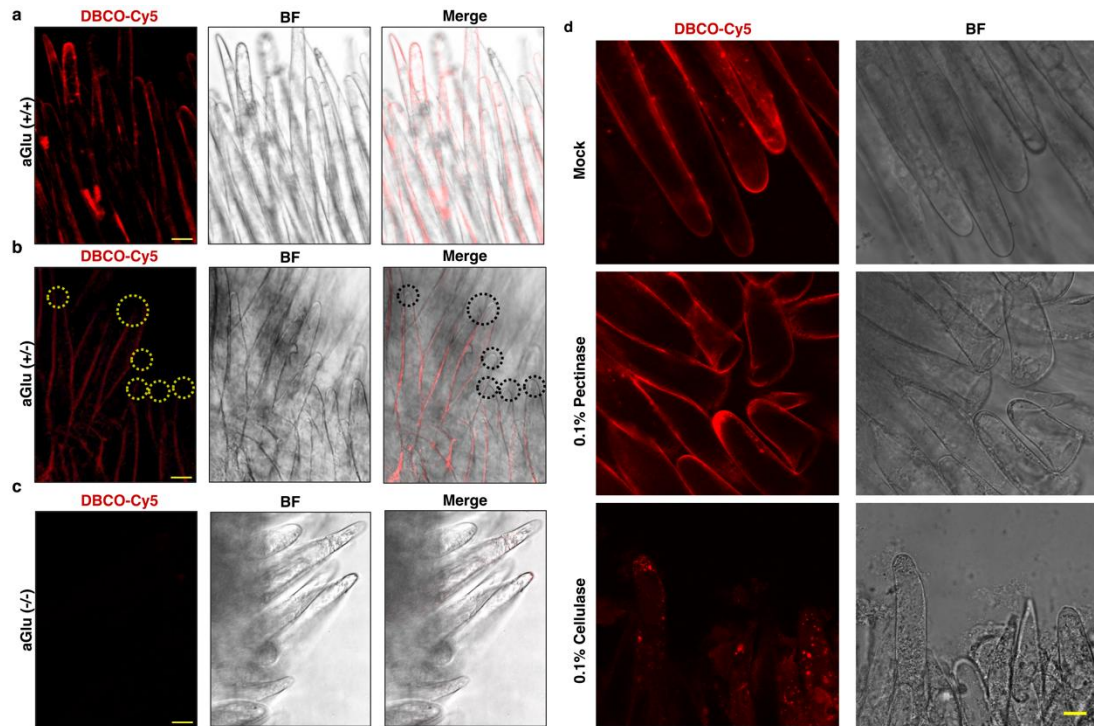
Supplementary Information

Supplementary Figures and legends



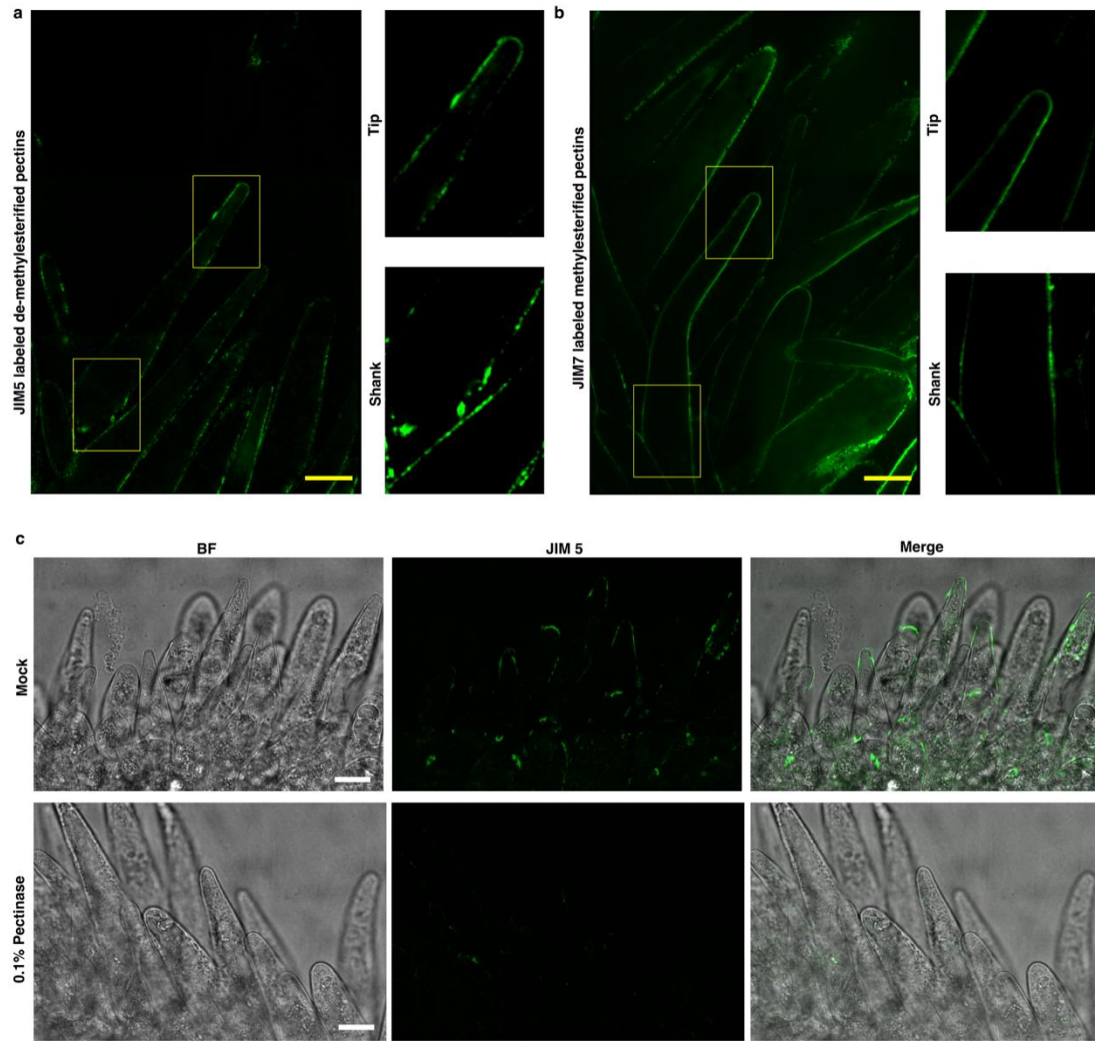
Supplementary Fig. 1 | CMFs deposited into thick bundles during cotton fiber development.

a, 3D AFM height renderings of cotton fiber primary cell walls at 3, 7 and 14 DPA. Double-headed cyan arrows indicate the positions where height profiles were extracted for CMF shape analysis. Scale bars indicate 100 nm for 3 DPA (left) and 7 DPA (middle), and 500 nm for 14 DPA (right). **b**, Representative height profiles (dotted lines) taken along the arrows in **a**. shaded curves show Gaussian fits used to estimate CMF height and width. **c** and **d**, Comparisons of the CMF bundle height (**c**) and width (**d**) based on the Gaussian fits as shown in (**b**). $n=26$ CMF bundles from nine fibers of three different ovules were considered for each group. Statistically significant differences between groups were tested using Brown-Forsythe and Welch ANOVA tests with Dunnett's test for multiple comparison. Error bars indicate S.E.M. Exact p-values were indicated in the panels.



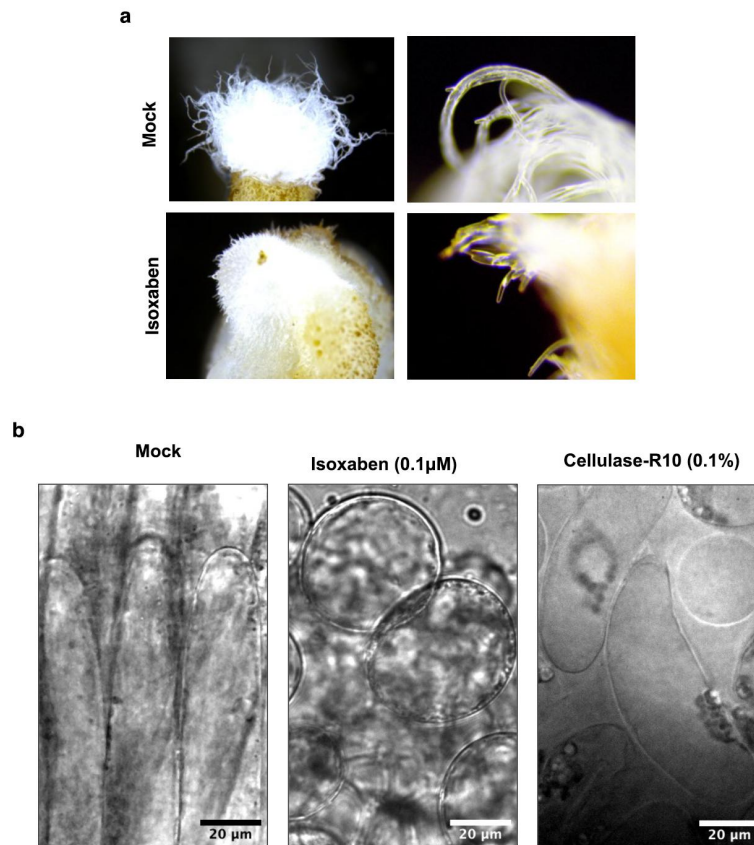
Supplementary Fig. 2 | Chase labeling of newly formed cell walls in cultured cotton fiber cells.

a, Confocal images showing the cy5-labeled cell walls in cultured cotton fibers after feeding aGlu at both periods. **b**, Confocal images showing the cy5-labeled cell walls in cultured cotton fibers after feeding aGlu at the first period and removing aGlu at the second period. Circles indicate newly formed and un-labeled cell walls of cotton fibers. **c**, Confocal images showing undetectable cy5 signals in cell walls of cultured cotton fibers without aGlu feeding. Scale bars, 10 μ m. **d**, Confocal images showing the cy5-labeled cell walls in cultured cotton fibers along with Pectinase (middle panels) or Cellulase (right panels) treatment. Scale bars, 10 μ m. 2 DPA ovules harvested from fresh flowers were used for culturing. The experiment was repeated independently three times with similar results.



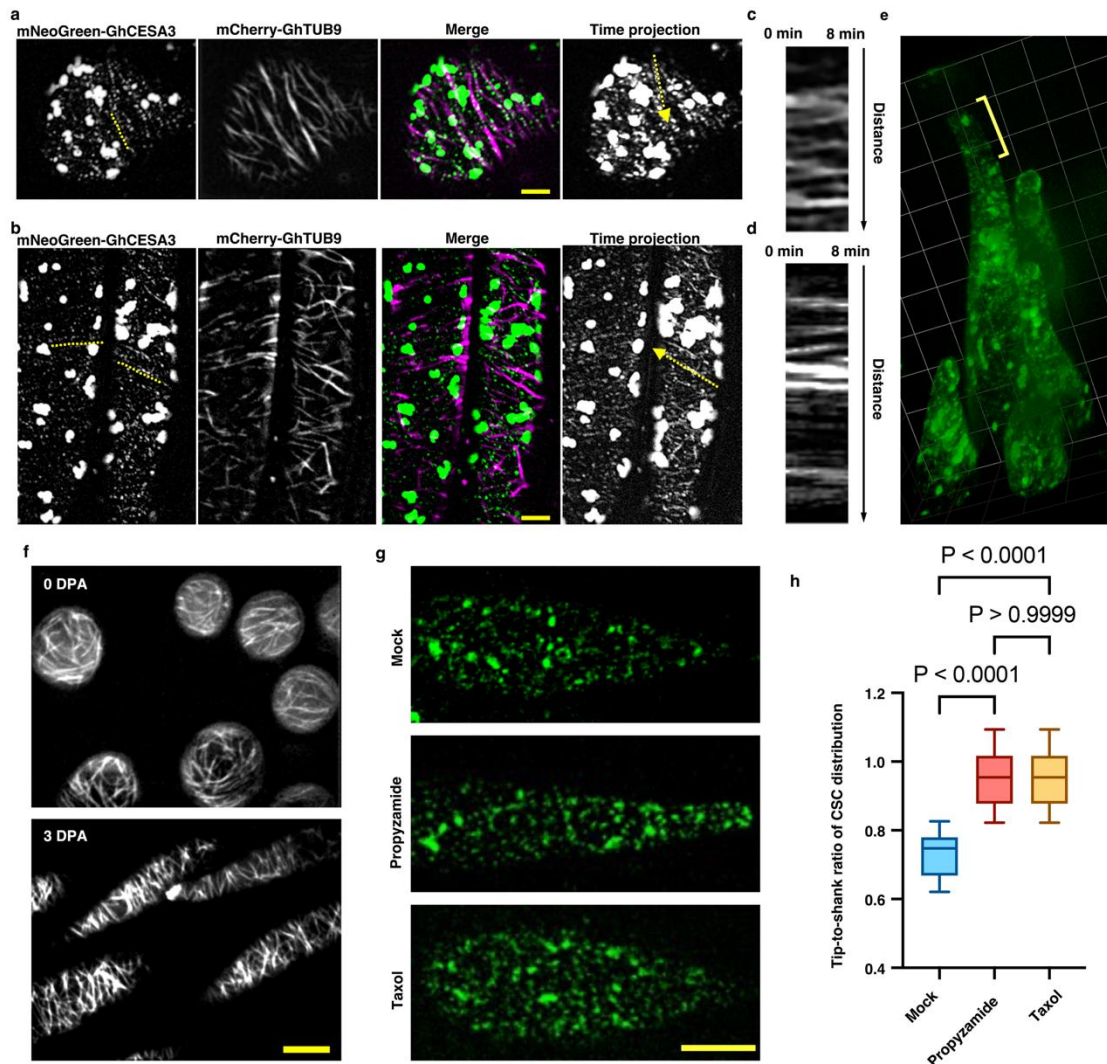
Supplementary Fig. 3 | Modification of pectins exhibit no specific spatial distribution in cotton fibers.

a, The distribution of JIM5-identified unesterified pectins in cell walls of cotton fiber tips and shanks. **b**, The distribution of JIM7-identified methylesterified pectins in cell walls of cotton fiber tips and shanks. Scale bars, 10 μm . **c**, The development of cotton fiber cells was unaffected after pectinase treatment (bottom panels). The presence of pectins in fiber cell walls was identified by JIM-5 labeling. Scale bars 20 μm . 2 DPA ovules harvested from fresh flowers were used for culturing. The experiment was repeated independently three times with similar results.



Supplementary Fig. 4 | Cellulose deposition is required for cotton fiber growth

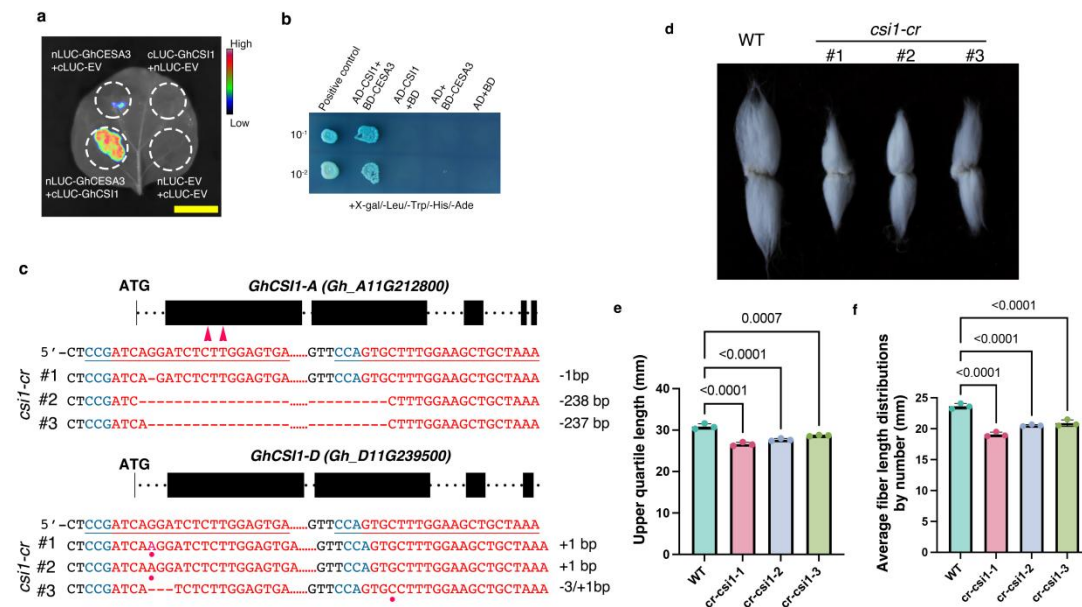
a, The development of cotton fibers was severely inhibited after 5 days of isoxaben treatment in cultured ovules (2 DPA). Left, whole-ovule views; right, higher-magnification views of fiber surfaces. **b**, Confocal images show that the anisotropic elongation of fiber cells is severely affected under either isoxabean (middle) or cellulase R-10 treatment (right). Scale bars, 20 μ m. 2 DPA ovules harvested from fresh flowers were used for culturing and treatment. The experiment was repeated independently three times with similar results.



Supplementary Fig. 5 | Dynamic behavior of CSCs and organization of microtubules in various cell types.

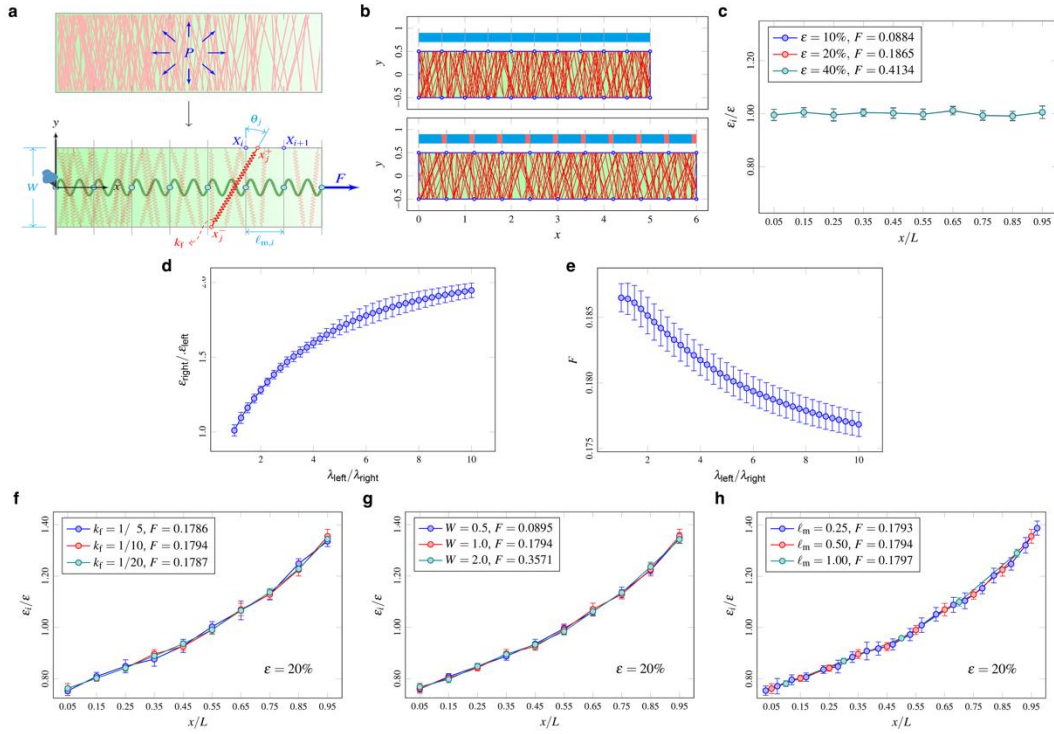
a and **b**, Live-cell imaging shows the distribution of CSCs and organization of microtubules in pavement cells (**a**) and petiole cells (**b**). The mNeoGreen-GhCESA3 foci (green) accumulate on the plasma membrane and exhibit linear trajectories distribution (yellow dotted lines) that is co-localized with microtubules (magenta). Scale bars, 5 μ m. The experiment was repeated independently over five times with similar results. **c** and **d**, Kymograph analyses show the motility of CESA3 foci along microtubules in pavement cells (**c**) and petiole cells (**d**). The areas and directions for kymograph analyses are indicated by dotted yellow arrows showing in (**a**) and (**b**), respectively. **e**, 3D reconstruction confocal images show the CSC distribution in elongating fiber cells. The yellow bracket indicates tip region of cotton fiber cell. One grid unit equals 12.28 μ m. **f**, Live-cell images show the organization of microtubules in growing fiber cells at the initiation (top) and elongation stage (bottom). Scale bars, 10 μ m. The experiment was repeated independently over five times with similar results. **g**, Representative confocal images of CSC distribution in cultured fiber cells after drug treatment. Scale bars, 10 μ m. The experiment was

repeated independently over five times with similar results. **h**, Quantification of tip-to-shank ratio of CSC density in cultured fiber cells after drug treatment. $n = 10$ fibers from at least three ovules of each group were taken into account. Statistically significant differences between groups were tested using one-way ANOVA with post-hoc Tukey test for multiple comparison. Error bars indicate SD. Exact p-values were indicated in the panels.



Supplementary Fig. 6 | Genome editing of GhCSI1 generates short cotton fibers.

a and **b** Validation of the interaction between GhCESA3 and GhCSI1 using (a), Split-luciferase complementation assay (scale bar, 2 cm) and (b), yeast two-hybrid (Y2H) assay. The experiment was repeated independently three times with similar results. **c**, Sequence confirmation of *ghcsi1* knock-out: genome editing at sgRNAs (indicated by underlines and red arrowheads) for GhCSI1-A (top panels) and GhCSI1-D (bottom panels). The nucleotide deletion (dashes) and insertion (dots) in edited lines are verified by Sanger sequencing. **d**, The genome edited lines (*csi1-cr*) show decreased fiber length. **e** and **f**, Comparison of AFIS fiber length parameters, the Upper quartile length (e) and the Average fiber length distributions by numbers (f), between the wild-type TM-1 and the genome edited *csi1-cr* lines. Both the TM-1 and *csi1-cr* lines are grown in field. At least 10 grams of raw lint fibers of each line are harvested and collected for AFIS analysis, respectively. $n = 3$ biological repeats. Statistically significant differences between groups were tested using one-way ANOVA with post-hoc Tukey test for multiple comparison. Error bars indicate SD. Exact p-values were indicated in the panels.



Supplementary Fig. 7 | Computational simulation of differentially deposited CMFs affecting cotton fiber elongation.

a, Schematic diagram of the computational simulation model. The matrix of cell walls is taken as multi-segmented springs (green), while the CMFs are considered as a mount of red springs that are vertically aligned and roughly perpendicular to the growth direction. The expansion of cotton fiber cells under turgor pressure are simulated by fixing the left end of the model and applying a constant force to the right end of the model. **b**, Representative simulation result showing strains of each region of the cell walls (red boxes) along the model with uniformly deposited CMFs. For the sake of visual presentation, only 1/50 of the CMF-associated springs in amounts are drawn in the figure. **c**, Quantification of deformation strains along the cell walls. **d** and **e**, Simulation results of right-to-left strain ratio (**d**) and changes in applied force (**e**) under different shank-to-apex CMF density distributions ratio (x/L). **f** to **h**, Testing for model robustness and reliability. The spring constant k_f for CMFs (**f**), the width of the model W (**g**), and the length of the matrix spring l_m (**h**) have no significant affection on the simulations. Ten rounds of simulations are applied for each condition. Error bars indicate S.D.

Differential Cellulose Distribution Drives Polarized Growth of Cotton Fibers – Description of the model

1 Model

This study raises a question: Do differences in the density of cellulose microfibrils (CMFs) between the tip and the shanks of a cotton fiber cell lead to variations in growth rates? Based on experimental observations, we hypothesize that the sparse distribution of CMFs in the cotton fiber cell tip and their dense distribution in the shanks might enable the tip to deform more easily under turgor pressure compared to the shanks, thereby creating spatial differences in growth rates. To test this hypothesis, we have constructed a mechanical model to calculate the deformation differences of cell walls with varying densities of CMFs under specified turgor pressures.

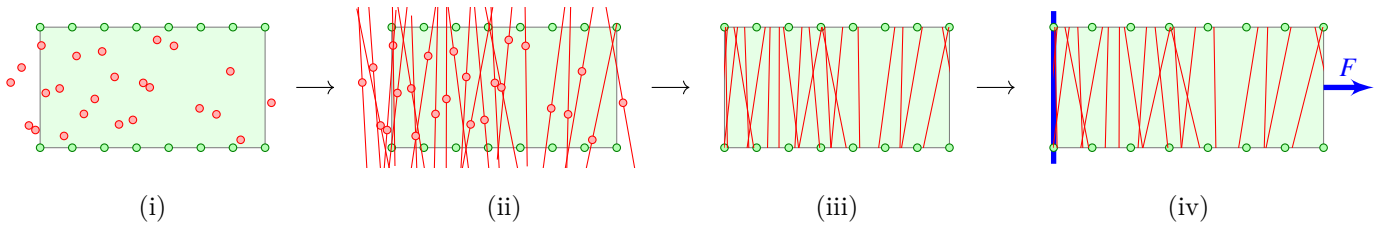


Figure 1: Model building process. (i) Within a rectangular region representing the cell wall, randomly generate a specific number of points based on a predefined density distribution; (ii) At each point, randomly generate a line segment at a random angle to the vertical axis; (iii) Retain only those line segments that lie within the rectangle, and use these as CMF springs; (iv) Fix the left side of the rectangle and stretch the right side to achieve a certain horizontal strain.

The principles of the model are illustrated in Figure S8A. CMFs are mainly oriented perpendicular to the direction of cell growth, and the growth of cotton fiber cells primarily occurs along their longitudinal axis. The well-aligned transverse arrangement of CMFs implies that under turgor pressure, the cell undergoes minimal significant deformation along the transverse axis. Therefore, the model focuses only on the stretching of the cell wall in the longitudinal axis. Imagine a rectangular cell wall (Figure S8A), with the midpoint of the left side as the origin, the growth direction defined as the x axis, and the left edge perpendicular to the growth direction as the y axis. The mechanical effects of the cell wall matrix are simplified to longitudinally arranged spring segments (green, referred to as matrix springs), and the mechanical effects of embedded CMFs are simplified to numerous transversely arranged springs (red, referred to as CMF springs). The action of turgor pressure is simplified to a force F acting along the x axis. When the cell wall is strained under the influence of F , we will calculate the strain on each matrix spring in equilibrium to test how different spatial distributions of CMF springs affect the ease of deformation of different regions of the cell wall under turgor pressure. The model construction and execution primarily involve the following four steps (Figure 1):

- (i) Within a rectangular area representing the cell wall, randomly generate several points p_j ($j = 1, 2, \dots, n$) based on a preset density distribution. The probability density of these points is uniform along

the y axis, while along the x axis, the density may be uniformly distributed or linearly decreasing. To mitigate boundary effects, points are generated not only within the rectangle but also in an extended horizontal area beyond it. This is because lines originating from points outside the rectangle may still intersect the rectangular area, as explained in step (ii).

- (ii) Construct a straight line at each randomly generated point p_j from step (i), forming a random angle θ_j with the y axis. The angle θ_j is uniformly distributed within the interval $(-\Theta, \Theta)$, where Θ is a small angle to simulate the deviation of CMF from the direction of y axis.
- (iii) Treat the line segments $\ell_{f,j}$, created by the intersection of the lines from step (ii) with the rectangle, as CMF springs. The intersection points x_j^- and x_j^+ of each CMF spring with the rectangle boundaries serve as the connection points with the matrix springs. For example, if x_j^+ is located between X_i and X_{i+1} , the force exerted by CMF spring $\ell_{f,j}$ at x_j^+ is distributed proportionally to the endpoints of matrix spring $\ell_{m,i}$ based on the distances from x_j^+ to X_i and X_{i+1} .
- (iv) Fix the left boundary of the rectangle and stretch the right boundary to apply a moderate strain horizontally. Apply the principle of energy minimization to determine the equilibrium positions of all matrix spring endpoints X_i , thereby calculating the strain on all matrix springs.

Through these steps, the computational model of this paper is established and executed. Next, we detail the energy minimization process used at mechanical equilibrium. In the x direction, the matrix springs are segmented into several parts $\ell_{m,i} = X_{i+1} - X_i$ ($i = 1, 2, \dots, m$). After applying a certain displacement to the right boundary of the cell wall X_m , the system's Hamiltonian (energy) is given by:

$$H(\{X_i\}) = \frac{1}{2}k_m \sum_{i=1}^m L_{m,i} \left(\frac{\ell_{m,i}}{L_{m,i}} - 1 \right)^2 + \frac{1}{2}k_f \sum_{j=1}^n L_{f,j} \left(\frac{\ell_{f,j}}{L_{f,j}} - 1 \right)^2 \quad (1)$$

where k_m is the spring constant of the matrix springs, assuming equal constants for the m segments and that k_m is proportional to the width of the cell wall W . $\ell_{m,i}$ and $L_{m,i}$ are the actual and free lengths of the i -th matrix spring, respectively. k_f is the spring constant of the CMF springs, assuming a uniform constant for all springs. The product $n \cdot k_f$ is proportional to the length of the rectangular cell wall $\sum_{i=1}^n L_{m,i}$. $\ell_{f,j}$ and $L_{f,j}$ are the actual and free lengths of the j -th CMF spring, respectively. It's important to note that in this model, the endpoints of the CMF springs are ultimately defined by X_i . For instance, the endpoint x_j^+ of CMF spring $\ell_{f,j}$ in Figure S8A can be expressed as a weighted average of the endpoints X_{i+1} and X_i of the matrix spring $\ell_{m,i}$ (see Appendix A for details). Equation (1) shows that the total energy of the system is a function of the coordinates of all matrix spring endpoints X_i . Therefore, the force on any matrix spring endpoint X_k can be calculated as follows:

$$f_k = -\frac{\partial H(\{X_i\})}{\partial X_k} \quad (2)$$

Note that since we are only concerned with the forces on the endpoints in the x direction, f_k represents only the horizontal force on X_k . The specific expression and derivation process for this force are detailed in Appendix A. The system's total energy is minimized to determine the coordinates of the matrix spring endpoints during mechanical equilibrium:

$$\min_{\{X_i\}} H(\{X_i\}) \quad (3)$$

2 Parameters

Prior to executing computations with our model, it is crucial to determine its parameters. Key parameters include the length L and width W of the rectangular cell wall, the spring constant k_m for the matrix

springs, the linear density of CMF springs λ_f (the number of CMF springs per unit length along the x -axis of the cell wall), the spring constant k_f for the CMF springs, and the maximum deviation angle Θ of the CMF springs. The spring constant k_m is proportional to W , reflecting the matrix's modulus of elasticity, while λ_f and k_f indicate the density and elastic modulus of the CMFs. These parameters should be set based on the macroscopic mechanical properties of the cell wall. Assume the average mass fraction of cellulose is $M_f = 35\%$, and the matrix is $M_m = 65\%$. The matrix's elastic modulus is $Y_m = 100$ MPa, density $\rho_m = 1000$ kg/m³, CMFs' longitudinal elastic modulus is $Y_f = 70$ GPa, density $\rho_f = 1650$ kg/m³ [1]. The volume fractions of cellulose and the matrix can be derived from their mass fractions and densities:

$$V_f = \frac{M_f/\rho_f}{M_f/\rho_f + M_m/\rho_m} = 24.6\%, \quad V_m = 1 - V_f = 75.4\% \quad (4)$$

Assuming the cell wall's thickness is D , then:

$$\frac{k_m}{W} = Y_m \cdot D \cdot V_m, \quad \lambda_f \cdot k_f = Y_f \cdot D \cdot V_f \quad (5)$$

In practical simulations, it is only necessary to ensure that the ratio of $\lambda_f \cdot k_f$ to k_m/W remains constant:

$$\frac{\lambda_f \cdot k_f}{k_m/W} = \frac{Y_f \cdot D \cdot V_f}{Y_m \cdot D \cdot V_m} = \frac{V_f}{V_m} \frac{Y_f}{Y_m} = \frac{24.6\%}{75.4\%} \times \frac{70 \text{ GPa}}{100 \text{ MPa}} = \frac{246}{754} \times 700 = 228.4 \quad (6)$$

To ensure a high density of CMF springs, the value of λ_f should be sufficiently large, ensuring ample CMF springs are present on each matrix spring segment.

For simplicity, in this study, we set the cell wall's length $L = 5$, width $W = 1$, and divide the matrix springs uniformly into $m = 10$ segments, each segment being 0.5 in long. The spring constants for the matrix and CMF springs are set to $k_m = 1$ and $k_f = 0.1$, respectively. Using equation (6), the average linear density of CMF springs is determined to be $\lambda_f = 2284$. If we consider a linear decrease in the density of CMF springs along the x direction, while keeping the overall average density unchanged, the density at the far left of the cell wall is set to five times that at the far right. Since pectin is uniformly distributed within the cell wall, the mass fractions of cellulose on both ends are calculated as:

$$\frac{\frac{5}{3} \times 35\%}{\frac{5}{3} \times 35\% + 65\%} = 47.3\%, \quad \frac{\frac{1}{3} \times 35\%}{\frac{1}{3} \times 35\% + 65\%} = 15.2\% \quad (7)$$

This implies that a spatial variation in cellulose mass fraction from 15.2% to 47.3% can result in a fivefold difference in cellulose content. Additionally, considering the typical orientation of CMFs in the primary walls of cotton fiber cells at angles of 70°-90° [2] to the cell's longitudinal axis, we set the maximum deviation angle Θ for CMF springs from the y direction at 20°.

We aim to investigate whether different spatial distributions of cellulose density can cause significant mechanical differences in the growth direction of the cell wall and consequently affect growth rate variations. For this purpose, we will analyze the relative strain ratios $\varepsilon_i/\varepsilon$ of each matrix spring segment to the overall average strain of the rectangular cell wall after stretching its right boundary. The strains of individual matrix springs and the overall average strain of the entire cell wall are defined as follows:

$$\varepsilon_i = \frac{\ell_{m,i}}{L_{m,i}} - 1, \quad \varepsilon = \frac{\sum_{i=1}^m \ell_{m,i}}{\sum_{i=1}^m L_{m,i}} - 1 = \frac{\sum_{i=1}^m \ell_{m,i}}{L} - 1 \quad (8)$$

Using the relative strain $\varepsilon_i/\varepsilon$ primarily aims to eliminate differences in absolute strains between segments under varying total strain conditions.

3 Implementation

The model was developed in Python 3.10. The minimization of energy was performed using the ‘minimize’ function from the ‘scipy.optimize’ library. Detailed information about this function is available at <https://docs.scipy.org/doc/scipy/reference/generated/scipy.optimize.minimize.html>. The code used in this study is available at <https://github.com/zhou-biomech/DiffCMFCottonFibers>.

References

- [1] Makato Yanagisawa, Sedighe Keynia, Samuel Belteton, Joseph A Turner, and Daniel B Szymanski. A conserved cellular mechanism for cotton fibre diameter and length control. *in silico Plants*, 4(1):diac004, 2022.
- [2] Candace H Haigler, Bir Singh, Guirong Wang, and Deshui Zhang. Genomics of cotton fiber secondary wall deposition and cellulose biogenesis. *Genetics and genomics of cotton*, pages 385–417, 2009.

Appendices

A Vertex Forces

In the model, the mechanical description of the rectangular cell wall is represented by the Hamiltonian H as shown in equation (1), where the forces on the endpoints (vertex) of the matrix springs can be expressed through the partial derivatives of H with respect to the endpoint coordinates:

$$\begin{aligned} f_k &= -\frac{\partial H(\{X_i\})}{\partial X_k} = \textcolor{green}{f_{m,k}} + \textcolor{red}{f_{f,k}} \\ &= -\frac{\partial}{\partial X_k} \left(\frac{1}{2} k_m \sum_{i=1}^m L_{m,i} \left(\frac{\ell_{m,i}}{L_{m,i}} - 1 \right)^2 \right) - \frac{\partial}{\partial X_k} \left(\frac{1}{2} k_f \sum_{j=1}^n L_{f,j} \left(\frac{\ell_{f,j}}{L_{f,j}} - 1 \right)^2 \right) \quad (9) \\ &= -k_m \sum_{i=1}^m \left(\frac{\ell_{m,i}}{L_{m,i}} - 1 \right) \frac{\partial \ell_{m,i}}{\partial X_k} - k_f \sum_{j=1}^n L_{f,j} \left(\frac{\ell_{f,j}}{L_{f,j}} - 1 \right) \frac{\partial \ell_{f,j}}{\partial X_k} \end{aligned}$$

This equation shows that the force at the endpoint X_k of the matrix spring is composed of two parts: one from matrix springs, and the other from CMF springs. Next, we will detail the derivation of the expressions for these two components of force separately.

A.1 Matrix Springs

Considering that $\ell_{m,i} = |X_{i+1} - X_i|$, the contribution of the matrix spring to the force exerted on endpoint X_k in equation (9) can be expressed as:

$$f_{m,k} = -k_m \sum_{i=1}^m \left(\frac{\ell_{m,i}}{L_{m,i}} - 1 \right) \frac{\partial \ell_{m,i}}{\partial X_k} = -k_m \sum_{i=1}^m \left(\frac{\ell_{m,i}}{L_{m,i}} - 1 \right) \frac{\partial |X_{i+1} - X_i|}{\partial X_k} \quad (10)$$

Since the derivative of $|X_{i+1} - X_i|$ with respect to X_k is nonzero only when $i = k - 1$ or $i = k$, the terms in the sum are nonzero only in these cases. In other words, the force on endpoint X_k from the matrix springs arises only from the two segments of matrix spring adjacent to that endpoint. Therefore, the expression for this force is

$$f_{m,k} = -k_m \left(\frac{\ell_{m,k-1}}{L_{m,k-1}} - 1 \right) \frac{X_k - X_{k-1}}{\ell_{m,k-1}} - k_m \left(\frac{\ell_{m,k}}{L_{m,k}} - 1 \right) \frac{X_k - X_{k+1}}{\ell_{m,k}} \quad (11)$$

Specifically, when $k = 0$ or $k = m$ (i.e., the endpoints of the cell wall), the first or second term on the right side of the formula should be omitted respectively, because the endpoint is connected only to one segment of matrix spring on the inside.

A.2 CMF Springs

Considering in Figure S8B that the length of a CMF spring is $\ell_{f,j} = \sqrt{(x_j^+ - x_j^-)^2 + w^2}$, the contribution of the CMF spring to the force exerted on endpoint X_k in equation (9) is

$$f_{f,k} = -k_f \sum_{j=1}^n L_{f,j} \left(\frac{\ell_{f,j}}{L_{f,j}} - 1 \right) \frac{\partial \ell_{f,j}}{\partial X_k} = -k_f \sum_{j=1}^n \frac{L_{f,j}}{\ell_{f,j}} \left(\frac{\ell_{f,j}}{L_{f,j}} - 1 \right) \frac{\partial (x_j^+ - x_j^-)}{\partial X_k} \quad (12)$$

In this equation, the endpoints x_j^+ and x_j^- of the CMF springs are defined by X_i . Taking the endpoint x_j^+ of CMF spring $\ell_{f,j}$ in Figure S8B, the proportions of the distance from x_j^+ to X_i and X_{i+1} to the length $|X_{i+1} - X_i|$ are

$$\alpha_{i,j}^+ = \frac{|x_j^+ - X_i|}{|X_{i+1} - X_i|}, \quad \alpha_{i+1,j}^+ = 1 - \alpha_{i,j}^+ \quad (13)$$

Assuming these proportions remain constant before and after deformation of the rectangular cell wall, the endpoint x_j^+ is represented by the following weighted formula:

$$x_j^+ = (1 - \alpha_{i,j}^+)X_i + \alpha_{i,j}^+X_{i+1} \quad (14)$$

Similarly, the other endpoint x_j^- of CMF spring $\ell_{f,j}$ can also be defined by X_i . Replacing x_j^+ and x_j^- in equation (12) with the expressions given by X_i provides the formula for $f_{f,k}$.