

Methods S1. Supplemental experimental procedures.

Computational Model

Finite element (FE) simulations (here, ABAQUS v.6.9.1 is used) were used to interpret the cyclic nanoindentation experiments performed on the cell walls. As with all biological materials, the modeling of the load-deformation response of the plant cell wall is complex due to the various factors that affect its response. In some cases, experiments are feasible to ascertain some parameters that are needed in the model, while in other cases, reasonable assumptions must be made. For all parameters, the model can be used to study the sensitivity of the results to changes in the most critical parameters such that the results can be viewed with confidence. For the model used here, the following information is needed:

- model geometry/symmetry,
- boundary/surface constraints,
- deformation behavior,
- viscoelastic material model,
- force application procedure

Each of these components of the model is now discussed with respect to assumptions made for experiments used to determine the necessary information. Ultimately, three unknown parameters (instantaneous elastic modulus, thickness of the cell wall and turgor pressure) are determined from the depth-dependent indentations iteratively using experimental results in conjunction with finite element simulations.

Model Geometry/Symmetry: Arabidopsis cells grow in complex patterns such that a typical cell has several lobes. The purpose here is to develop a model that captures the salient features of the experimental results without adding unnecessary complexity. In order to determine the cell pressure, the simulations are repeated iteratively by adjusting specific unknown parameters until the computational results are as close as possible to the experimental results. For this reason, a two-dimensional axisymmetric model is used such that the cell is approximated as a circular disk of radius R with uniform thickness t (shown schematically in Figure S1). The radius of the cell wall is varied from 7.5 μm to 15 μm based on the range of radii observed from confocal images of the leaf surface at different developmental stages (Figure S2-S4). From numerous preliminary

simulations, it was determined that the thickness played a major role in the load-deformation response. Thus, wall thickness is chosen as one of the parameters to be determined during the iteration process. The model is discretized into a 2D axisymmetric mesh consisting of four node axisymmetric elements with reduced integration. A graded mesh is used in the model for computational efficiency. Because the stresses and stress gradients are expected to be the highest in the region of contact, a very fine mesh is used in that region, where as a coarse mesh is used elsewhere since the stress gradients are not so high. Variably sized meshes are very common in such models.

Boundary/Surface Constraints: Several assumptions related to the boundary and surface constraints are also made. First, it is assumed that only a single cell wall is affected during the indentation process. Thus, the applied loads are assumed to be small enough that there are no effects on neighboring cells. Such an interaction between groups of cells is a topic of future research. Also, the edges of the cell wall are assumed to be clamped. This assumption may lead to an overly stiff boundary, but it does reflect the extra bending stiffness expected at the cell edges due to the transition to the anticlinal wall. The lower surface of the cell wall is subject to a uniform pressure p representing the turgor pressure. The upper surface of the cell wall is assumed to be stress free, except in the region in which the indenter acts. Finally, the indenter is modeled as a 2D axisymmetric rigid surface with radius of 2.5 μm (further details regarding the simulation procedure are given below).

The simulations also ignore effects from the cell curvature that would occur if the tip engages the cell wall at an angle. Such an effect would require a much more complicated three-dimensional model to understand fully. However, our estimates of this effect are a maximum of 20 % for the curvatures expected with the greatest impact occurring at shallower depths. A study by Tsai et al (2004) on a similar topic showed the impact of tip size and indentation depth with respect to sample curvature (in this case a much harder thin film) with the impact in the range of 5-22 % of the value obtained for a flat surface.

Deformation Behavior: Assumptions regarding the deformation behavior are also needed for the model. Preliminary simulations based on small deformation behavior resulted in nonphysical, very large deformations of the cell wall due to application of turgor pressure. Therefore, it was

determined that the geometric nonlinearities present are too large to be neglected. In this case, a neo-Hookean hyperelastic material model is used for predicting the large deformation nonlinear material behavior. Such choices have been used successfully to describe the behavior of polymers and biological tissues (Soarez et al., 2010; Hollister et al., 2011). In contrast to a linear elastic material, the deformation behavior of a neo-Hookean material is nonlinear, which provides a reasonable deformations of the cell wall as can be measured from TEM images. Such a model is readily available in ABAQUS and requires two parameters to be defined: the initial shear modulus G_0 and bulk modulus K_0 . It is assumed here that the cell wall is incompressible, such that the deformation attributed towards bulk deformation is considered to be very high compared to shear deformation. Thus, K_0 is assumed to be 15 times greater than G_0 . Also, the shear modulus is related to the instantaneous modulus E_0 through the relationship $E_0 = 3G_0$.

Viscoelastic material model: To capture the time dependent behavior of the cell wall, a viscoelastic material model is assumed. For simplicity, a standard linear solid (SLS) model consisting of a Maxwell element (spring and dashpot) in parallel with an additional elastic spring is used. For the spherical indenter tip, the relationship between the applied load and displacement, based upon the Hertzian solution and reported by many authors (Cheng et al., 2005; Yuya et al., 2010), is used. This model is given by

$$P(t) = \frac{4a^3}{3R} E(t) = \frac{4a^3}{3R} \left[E_\infty + E_1 e^{-(t/\tau_1)} \right] \quad (S1)$$

where $P(t)$ is the indentation load, R is the radius of the indentation displacement, a is the contact radius, E_∞ and E_1 are the elastic moduli of the springs representing the modulus and $\tau_1 = \eta_1 / E_1$ is the relaxation time, where η_1 is the viscosity (the value of the dashpot constant). The relaxation modulus represents two different elastic regions. For early times ($t \ll \tau_1$), $E(t)$ equals the instantaneous modulus $E_0 = E_\infty + E_1$, while for late times ($t \gg \tau_1$), $E(t)$ relaxes to the long term modulus E_∞ .

In a relaxation test, the indenter tip is driven against a viscoelastic sample to a certain depth and is held at that depth for a certain period of time. During this time, the material relaxes and the load gradually falls. It is assumed that during relaxation, the contact radius a is constant. The contact radius for the half space problem is very well documented in the literature (Johnson, 1985), but is unknown for the viscoelastic spherical pressure vessel subjected to turgor pressure. The focus of relaxation test is used here to determine the ratio of modulus E_1/E_0 and the relaxation time τ_1 . Accordingly, Eq. S1 is first rewritten as

$$\frac{P(t)}{P_{\max}} = \frac{E_{\infty}}{E_0} + \frac{E_1}{E_0} e^{-(t/\tau_1)}, \quad (\text{S2})$$

where P_{\max} is the maximum force at time $t = 0$ and defined as $P_{\max} = \frac{4a^3 E_0}{3R}$. The load time plot obtained from the nanoindentation experiments can be normalized to 1 using P_{\max} . Then a least-squares fit can be used to find E_1/E_0 and τ_1 , values that are required for the computations.

Force application procedure: The steps used for finite element simulation are shown in Figure 2. For each simulation, the chosen pressure is applied first, so that the cell wall resembles a pressurized hemispherical vessel with initial strains. Because the cell wall is viscoelastic, the pressure is applied for enough time in the simulation for the wall to fully relax in order to create a model condition representing that of a plant cell *in vivo*. After the wall has relaxed, the indenter is brought into contact with the cell wall and the cyclic loading/unloading tests are performed as done in the experiments. Slope versus depth profiles are then extracted from the simulations for various depths. Results are shown in Figs. 1d and S5.