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# Simulating Turgor-Induced Stress Patterns in Multilayered Plant Tissues

Olivier Ali<sup>1</sup>  · Hadrien Oliveri<sup>1</sup> · Jan Traas<sup>1</sup> · Christophe Godin<sup>1</sup>

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## Abstract

The intertwining between mechanics and developmental biology is extensively studied at the shoot apical meristem of land plants. Indeed, plants morphogenesis heavily relies on mechanics; tissue deformations are fueled by turgor-induced forces, and cell mechanosensitivity plays a major regulatory role in this dynamics. Since measurements of forces in growing meristems are still out of reach, our current knowledge relies mainly on theoretical and numerical models. So far, these modeling efforts have been mostly focusing on the epidermis, where aerial organs are initiated. In many models, the epidermis is assimilated to its outermost cell walls and described as a thin continuous shell under pressure, thereby neglecting the inner walls. There is, however, growing experimental evidence suggesting a significant mechanical role of these inner walls. The aim of this work is to investigate the influence of inner walls on the mechanical homeostasis of meristematic tissues. To this end, we simulated numerically the effect of turgor-induced loading, both in realistic flower buds and in more abstract structures. These simulations were performed using *finite element* meshes with subcellular resolution. Our analysis sheds light on the mechanics of growing plants by revealing the strong influence of inner walls on the epidermis mechanical stress pattern especially in negatively curved regions. Our simulations also display some strong and unsuspected features, such as a correlation between stress intensity and cell size, as well as differential response to loading between epidermal and inner cells. Finally, we monitored the time evolution of the mechanical stresses felt by each cell and its descendants during the early steps of flower morphogenesis.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s11538-019-00622-z>) contains supplementary material, which is available to authorized users.

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24 **1 Introduction**

25 Morphogenesis of multicellular organisms relies on two distinct and complementary  
26 cellular abilities: (i) growth—the ability of cells to expand and duplicate, and (ii)  
27 *spatial awareness*—the ability of cells to locate themselves in space. The latter is  
28 required to enable spatiotemporal regulation of the former. The strong reproducibility  
29 and robustness of developmental dynamics at the scale of organisms (Vogel 2013),  
30 despite stochasticity of cellular arrangements, suggest that spatial awareness relies on  
31 some kind of supracellular *positional information field*.

32 Such a concept has been first proposed and studied by pioneers such as Turing  
33 (1952) and Wolpert (1969). These explored a *chemical spatial awareness* theory;  
34 spatiotemporal regulation of growth is mediated by chemical substances called *mor-*  
35 *phogens*, see Green and Sharpe (2015) for a general introduction to these seminal  
36 works. Ever since, abundant experimental evidence for such biochemical mechanisms  
37 has been obtained.

38 In parallel, mechanical forces have been identified as cell fate regulators (Théry et al.  
39 2006; Ladoux and Mège 2017; Vining and Mooney 2017) and it has been proposed  
40 that mechanical stress fields (the multidimensional generalization of forces) could also  
41 guide morphogenesis (Shraiman 2005; Irvine and Shraiman 2017; Keller 2012). In the  
42 context of plant tissues, this *mechanical spatial awareness* hypothesis is very appealing  
43 (Dumais 2007); epithelia are subject to pressure-induced stress field, presumably pre-  
44 scribed by tissue curvature. This echoes D’Arcy Thompson’s dictum: ‘...*the form of an*  
45 *object is a “diagram of forces”*’ (Thompson 1917). Pressure-induced stress fields could  
46 provide mechanosensitive cells with instantaneous geometrical information about the  
47 shape of the tissue they are embedded in. Moreover, from a mathematical perspec-  
48 tive, mechanical stresses are described as second-order symmetric tensors fields and  
49 therefore carry intrinsically more geometrical information (intensity, orientations and  
50 anisotropy) than scalar fields such as chemical concentrations.

51 Recently, stress sensitivity of plant cells has been studied in an increasing number of  
52 developmental systems: in the sunflower hypocotyl (Hejnowicz et al. 2000; Hejnowicz  
53 2005) as well as in *Arabidopsis thaliana* meristem (Hamant et al. 2008; Uyttewaal et al.  
54 2012) and hypocotyl (Sampathkumar et al. 2014), cell cortical cytoskeleton seems  
55 to polarize according to mechanical stress. In *Arabidopsis*, thwarting cytoskeleton  
56 dynamics (with depolymerizing drugs for instance) resulted in drastic growth-related  
57 effects: modification of cell wall deposition and cell shape (Corson et al. 2009), per-  
58 turbed growth hormone signaling (Hamant et al. 2011), as well as transcriptional  
59 activation of cell wall modifying genes (Armezzani et al. 2018). Since the cytoskeleton  
60 is a central growth regulator, its stress sensitivity is a major entry point for mechanics  
61 into the control of morphogenesis. Cell division plane alignment in the *Arabidopsis*  
62 shoot apex has also been evidenced to rely upon mechanical stress patterns (Lintilhac  
63 and Vesecky 1984; Louveaux et al. 2016). Being able to measure mechanical stresses  
64 undergone by growing plant tissues would be a major step forward. Unfortunately, in  
65 vivo quantification of forces in plants is still an unresolved experimental challenge.

To alleviate this limitation, modeling efforts have been initiated and simulating the mechanics of such systems has become an active field of research (Geitmann and Ortega 2009; Ali et al. 2014; Chickarmane et al. 2010).

Based on histological and mechanical properties of their constitutive cells, plant tissues can mechanically be compared to *visco-elasto-plastic foams* under pressure (Ortega 1985; Corson et al. 2009). Within these *foams*, since they are usually much thicker than the inner walls, the outer epidermal walls are supposed to bear most of the pressure-induced stresses, see Fig. 1b. Consequently, a common strategy in most of the existing modeling approaches is to restrict the influence of inner tissues to pressure forces applied onto the epidermis and to focus only on the mechanics of this outermost layer described as a *pressure vessel*. Such approaches either described the epidermis as a two-dimensional (2D) curved continuum (Hamant et al. 2008; Bozorg et al. 2014; Oliveri et al. 2018; Kierzkowski et al. 2012) or as a single layer of cells fixed on a flat surface (Sampathkumar et al. 2014; Sapala et al. 2018).

To go further, recent studies have attempted to grasp the complexity of fully 3D tissues through simulations based on either artificial structures (Mosca et al. 2017) or reconstructed ones from confocal images (Bassel et al. 2014; Mosca et al. 2017). However, it is not yet clear how the simple connection between tissue geometry and stress patterns, suggested by the pressure vessel model, is altered when inner walls are considered. The present work is an attempt to fill this gap. To this end, we tackle two questions: (i) to what extent the predicted stress patterns at the epidermal surface are modified when inner cells are considered as load-bearing? (ii) What are the characteristics (e.g., intensity, anisotropy, orientation) of the stresses borne by these inner cells?

To address these questions, we conducted numerical simulations of pressure-induced stress fields on 3D elastic structures at mechanical equilibrium. To this end, we used: (i) artificial structures generated from geometrical primitives such as spheres and cylinders, see Fig. 2b. (ii) Realistic ones obtained from 3D confocal stacks of real living plant tissues. More precisely, we generated meshes from segmented 3D images from a time series of the early steps of flower formation, see Fig. 2a. While studying the former enabled us to quantify the influence of inner load-bearing elements on known stress patterns, studying the latter yielded a (semi-)quantitative view of the stress patterns experienced by real tissues.

## 2 Method and Model

In this section, we detail our simulation pipeline. In the first subsection, we present our model and the method used to estimate mechanical equilibrium. In the second one, we describe how we generated triangular meshes from 3D images. In the last section, we detail the analysis procedure we applied to the simulations outputs.

## 2.1 Mechanical Modeling and Numerical Simulation of Pressurized Plant Tissues

**Static equilibrium of plant tissues under pressure** Because growth-induced deformations in plant tissues are far slower than their elastic response to loading, plant cell growth is usually depicted as a quasistatic evolution (Dumais et al. 2006; Bozorg et al. 2016); the tissues are assumed at mechanical equilibrium at any moment. This mechanical equilibrium relies on the balance between pressure forces (due to the turgidity of cytosolic compartments) and the rheological response of the cell walls, characterized by the mechanical stress field at equilibrium and noted  $\boldsymbol{\sigma}(\mathbf{x})$  hereafter. We assumed all cells to feature the same excess of pressure  $P$  relative to the outside environment. Since these walls are thin ( $\sim 0.5 \mu\text{m}$ ) compared to the characteristic length of cells ( $\sim 10 \mu\text{m}$ ), we assumed plane stress conditions, i.e., we neglected the variations of the stress field across the walls and restricted its description to a tangential 2D second-order symmetric tensor field. Equations describing this equilibrium are gathered in system 1 (Landau and Lifshitz 1959):

$$\begin{cases} \nabla \cdot \boldsymbol{\sigma}(\mathbf{x}) = \mathbf{0} & \text{in } \Omega \\ \boldsymbol{\sigma}(\mathbf{x}) \cdot \hat{\mathbf{n}}(\mathbf{x}) = P \hat{\mathbf{n}}(\mathbf{x}) & \text{on } \partial\Omega_N \\ \mathbf{u}(\mathbf{x}) = \mathbf{0} & \text{on } \partial\Omega_D \end{cases} \quad (1)$$

In system 1,  $\mathbf{u}$  stands for the displacement field and  $\Omega$  for the continuum formed by the assembly of all the walls taken together. Domains  $\partial\Omega_N$  and  $\partial\Omega_D$  represent the boundaries of  $\Omega$  where different conditions are enforced (subscripts  $N$  and  $D$ , respectively, stand for Neumann and Dirichlet). Subdomain  $\partial\Omega_N$  is the collection of walls where net pressure forces are acting, i.e., outermost walls of the tissue, while  $\partial\Omega_D$  is the collection of walls assumed to be fixed, see Fig. 2e. Depending on the structure we investigated, the latter condition was not always enforced, i.e.,  $\partial\Omega_D = \emptyset$ . When used, it was enforced by imposing a null displacement field  $\mathbf{u}(\mathbf{x})$  on  $\partial\Omega_D$ , as prescribed by the third line of system 1.

To complete the mechanical description, one needs to specify the constitutive relation between stress and strain within walls. For the sake of simplicity, we assumed this response to be linear elastic and isotropic. These assumptions yield the simplest rheological model for the cell walls: Hooke's law, depicted by Eq. 2, where  $\mathbb{H}(\mathbf{x})$  stands for the cell wall elasticity tensor (Landau and Lifshitz 1959).

$$\boldsymbol{\sigma}(\mathbf{x}) = \mathbb{H}(\mathbf{x}) : \boldsymbol{\varepsilon}(\mathbf{x}) \quad (2)$$

where “:” stands for the tensor double contraction. In the case of isotropic elasticity, the fourth-order elasticity tensor  $\mathbb{H}$  is parameterized by only two variables: The Young's modulus ( $Y$ ) and the Poisson's ratio ( $\nu$ ). The spatial dependency of the elasticity tensor accounts for the fact that in some of our simulations, we allowed different values of Young's modulus for different cell walls.

Equations 1 and 2 constitute the core of our mechanical model. They are in accordance with other state-of-the-art modeling approaches within the field of plant tissue mechanics (Hamant et al. 2008; Bozorg et al. 2014; Bassel et al. 2014; Boudon et al. 2015; Mosca et al. 2017). Numerical values of the various parameters can be found, for

143 each simulation, in the supplementary materials. Across our various simulations, we  
 144 tested pressure values from 0.1 to 10 MPa and Young's moduli between 1 and 45 MPa.  
 145 We took care to use values compatible with experimental measures (Chanliaud et al.  
 146 2002; Beauzamy et al. 2015; Milani et al. 2011; Kierzkowski et al. 2012) and existing  
 147 models (Hamant et al. 2008; Bozorg et al. 2014; Sampathkumar et al. 2014; Hervieux  
 148 et al. 2016; Mosca et al. 2017) for *realistic* structures as well as for *abstract* ones, see  
 149 section C of the supplementary materials for details.

150 **Numerical estimation of turgor-induced stress fields** The numerical framework we  
 151 used to conduct our simulations relies on two main open-source softwares: *OpenAlea*  
 152 (Pradal et al. 2008) and *Sofa* (Faure et al. 2012). The former is used to describe the  
 153 tissues and to generate the data structure, the latter to handle numerical simulations.  
 154 The general workflow of our framework is detailed in Boudon et al. (2015).

155 We chose to discretize the continuum composed by all the walls ( $\Omega$  in system 1)  
 156 using first-order finite elements (*FES*). Because bending stiffness can be neglected for  
 157 turgid cells (Weber et al. 2015; Bozorg et al. 2014), we used membrane elements.  
 158 Mechanical equilibrium is computed by solving the weak form of system 1, which  
 159 is achieved through gradient descent. For faster convergence and stability, we used a  
 160 damped implicit scheme available in the *Sofa* Module *Compliance* and based on the  
 161 methods described in Tournier et al. (2015).

## 162 2.2 Realistic Meshes Production

163 **Mesh production from segmented 3D images** In order to generate accurate sim-  
 164 ulations of realistic tissues, we needed *FE* meshes that satisfied two requirements:  
 165 on one hand, for the sake of stability and convergence, they had to be composed of  
 166 elements as regular as possible. In the present case, our triangular elements have to  
 167 be as close as possible to equilateral triangles. On the other hand, they had to account  
 168 for tissues cellularization and layering, which imposed topological constraints on the  
 169 tiling. Production of meshes managing these constraints was achieved through the use  
 170 of the *DRACO-STEM* library, described in Cerutti et al. (2017). This library provides  
 171 tools to generate regular cell-preserving triangular meshes from 3D segmented images  
 172 where cells are represented as sets of voxels labeled with the same identifier (Id). Such  
 173 segmented images can be produced from 3D confocal stacks of real tissues as well as  
 174 from artificially generated images.

175 **3D segmented images from confocal microscopy** Segmented images from the early  
 176 steps of a time series of a flower bud formation were obtained through the *Mars-Alt*  
 177 pipeline (Fernandez et al. 2010) and curated manually. Applying this segmentation  
 178 pipeline to consecutive steps of a time series enabled us to also compute the lineage of  
 179 cells within the structure. Based on this lineage information, we were able to track the  
 180 developmental history of characteristic zones defined on the last time step, see Fig. 2a.

Note that because the quality of the segmenting procedure depends heavily on the intensity signal within the initial 3D images, we were only able to reconstruct the two outermost cell layers of each time step of the flower bud time series, see Fig. 2c, d.

**3D segmented images from geometrical primitives** To complete this set of realistic meshes, we also developed algorithms to generate 3D segmented images of artificial geometrical volumes decomposed into cells. The generation of such cellularized geometrical structures involves two steps: (i) first surface meshes encompassing the desired volumes are generated from a 3D computer graphics software (in our case *Blender*). (ii) Then, the volumes defined by the meshes are populated with points, serving as seeds for a 3D centroidal Voronoi tessellation algorithm (see supplementary information).

For the present study, we generated meshes from the concatenation of simple primitives such as spheres and cylinders. We limited our analysis to three types of artificial structures: (i) a sphere, (ii) a “pill”, i.e., a cylinder closed by two hemispheres, (iii) and a “bowling pin” composed by the concatenation of two spheres of different radius, see Fig. 2b. Each structure was decomposed into five hundred cells generated from points disposed homogeneously and randomly within the considered volumes.

Structures generated from artificial geometrical volumes present some advantages compared to realistic ones. First, contrary to what happens with confocal images, there is no depth limitation with artificially generated images. From such images, we were therefore able to generate 3D structures completely filled with cells and not limited to the two outermost layers, see Fig. 2c, d. Secondly, since we restricted our analysis to axisymmetric, closed, structures; pressure forces being well balanced in such structures we did not have to fix the boundaries ( $\partial\Omega_D = \emptyset$  in (1)) in our simulations.

**Different versions of each structure to analyze the stress field** To probe the influence of inner walls on the mechanical stress pattern borne by the outermost surface, we generated for each structure several versions by varying the number of inner cell layers.

For the realistic structures, containing two cell layers, we generated three variants, see Fig. 2c, d: the first one, noted *Shell*, consists of the outermost cell walls of the epidermis, called  $L_1$ outermost periclinal walls. In the second one, noted  $L_1$ -only, all the walls surrounding the epidermal cells were taken into account, namely the  $L_1$ outermost periclinal, the  $L_1$ anticlinal and the  $L_1-L_2$  periclinal walls. Finally, in the most detailed version, noted  $L_1-L_2$ , all the walls from the two outermost cell layers were considered. This includes, on top of the previously cited, the  $L_2$ -anticlinal and the  $L_2$  periclinal walls.

With abstract structures, we were not limited to the two outermost layers and therefore added a fourth version: the *Full* one, encompassing all the walls from the two outermost layers and including also all the remaining inner walls.

## 2.3 Analysis of Simulation Outputs

**Normalized stress field** To probe the influence of inner tissues on stress patterns, we compared stress patterns between different versions of the same structure with more or



223 less inner walls. We were therefore more interested in the comparison between stress  
 224 patterns rather than by their absolute values. We, therefore, normalized every stress  
 225 field by the pressure value used to generate them:

$$226 \quad \tilde{\sigma} = \frac{\sigma}{P}. \quad (3)$$

227 This yields a dimensionless tensorial field, hereafter referred to as the *normalized*  
 228 *stress field*, which depends mostly on the geometry, the topology and the rigidity of  
 229 the underlying network of cell walls. Note that for the sake of simplicity, we will drop  
 230 the tilde notation in the rest of the text, since all stress quantities we will discuss will  
 231 be normalized.

232 **Stress field quantifiers** In the plane stress approximation, mechanical stresses within  
 233 cell walls can be expressed as 2D second-order symmetric tensors. As such, mechan-  
 234 ical stresses are fully parameterized by a set of three independent variables, we  
 235 call *quantifiers*: their *intensity*, *anisotropy* and *orientation*. Such tensors can also  
 236 be uniquely decomposed into isotropic (iso( $\cdot$ )) and deviatoric (dev( $\cdot$ )) parts:  $\sigma =$   
 237 iso( $\sigma$ ) + dev( $\sigma$ ).

238 It appeared fundamental to look at intrinsic physical quantities, which do not rely on  
 239 an arbitrary coordinate system and are directly available to cells. We therefore focus  
 240 our attention on *intensity* and *anisotropy* (respectively noted  $\sigma$  and  $\alpha_\sigma$  hereafter).  
 241 These two scalar properties can be extracted from the *isotropic/deviatoric* decompo-  
 242 sition previously mentioned. We chose to use the following definitions for these two  
 243 quantifiers:

$$244 \quad \sigma = \|\text{iso}(\sigma)\| = \frac{|\sigma_M + \sigma_m|}{2}$$

$$245 \quad \alpha_\sigma = \frac{\|\text{dev}(\sigma)\|}{\|\text{iso}(\sigma)\|} = \frac{|\sigma_M - \sigma_m|}{|\sigma_M + \sigma_m|} \quad (4)$$

246 with the 2D second-order matrix norm defined as the Frobenius norm divided by  $\sqrt{2}$ :  
 247  $\|T\| = \sqrt{\text{tr}(T^2)}/\sqrt{2}$ . The variables  $\sigma_M$  and  $\sigma_m$ , respectively, stand for the maximal and  
 248 minimal eigenvalues of the normalized stress tensor  $\sigma$  considered ( $\sigma_M \geq \sigma_m$ ).

249 The choice of the specific expressions in (4) was made such that our quantifiers  
 250 meet the usual definitions of intensity (mean value of the eigenvalues:  $T_M + T_m/2$ ) and  
 251 anisotropy (ratio between the eigenvalues difference and sum:  $T_M - T_m/T_M + T_m$ ) in the  
 252 case of tensile stresses. To avoid confusion, we checked that no significant compressions  
 253 could be observed in our simulations (see subsection C.6 in the supplementary  
 254 materials for details). Moreover, the use of absolute values enables us to define the  
 255 intensity as the natural extension of the usual vector norm to second-order symmetric  
 256 tensors.

257 With these definitions, the normalized stress field can be expressed, in the most  
 258 general way, as follows:

$$259 \quad \sigma = \pm \sigma \left( I_d + \alpha_\sigma (P_{\theta_\sigma} - P_{\theta_\sigma + \pi/2}) \right) \quad (5)$$



260 The angle  $\theta_\sigma$  quantifies the direction associated with the largest eigenvalue of  $\sigma$   
 261 and  $\mathbf{P}_\theta = \hat{\mathbf{e}}_\theta \otimes \hat{\mathbf{e}}_\theta$  is the projector on the direction given by the unit vector  
 262  $\hat{\mathbf{e}}_\theta = [\cos(\theta) \sin(\theta)]^t$ . The sign ( $\pm$ ) in front of the *rhs* of 5 corresponds to the  
 263 sign of  $\text{tr}(\sigma)$ .

264 **Cell-averaged stress field** We used subcellular resolution meshes for our simulations,  
 265 where each wall was decomposed into triangles (around 20 on average, see tables 1  
 266 and 2 in the supplementary materials). The stress tensors were computed within each  
 267 of these triangles. In order to smooth out numerical fluctuations due to mesh topology  
 268 irregularities between neighboring triangles, we applied a *closest-neighbors averaging*  
 269 *filter* on these tensors (see supplementary materials, section B) as a preprocessing step  
 270 (i.e., prior to any other analysis) on all of our raw data generated by the simulation  
 271 pipeline.

272 Since we focused the present analysis on tissue stress patterns, we ignored stress  
 273 variations at the subcellular level in most of our analyses. Therefore, we averaged our  
 274 normalized stress field quantifiers ( $\sigma, \alpha_\sigma, \hat{\mathbf{e}}_{\theta_\sigma}$ ) over each cell wall type for each cell  
 275 (the cell wall-averaged versions of the quantifiers are denoted with a bar:  $\bar{\sigma}, \bar{\alpha}_\sigma, \bar{\hat{\mathbf{e}}}_{\theta_\sigma}$ );  
 276 e.g., epidermal cells in the *L<sub>1</sub>-only*, *L<sub>1</sub>-L<sub>2</sub>* and *full* versions of a geometrical structure  
 277 feature three sets of normalized stress invariants one for the outermost periclinal walls,  
 278 one for the *L<sub>1</sub>-L<sub>2</sub>* periclinal walls and one for its anticlinal walls.

## 279 3 Results

280 We focused on three aspects in our analysis: (i) the influence of inner cell walls on  
 281 the outermost periclinal stress pattern. (ii) The characteristics of the stresses borne by  
 282 inner walls and (iii) finally, the spatiotemporal sorting/clustering of cells based on the  
 283 characteristics of the mechanical stress they experience.

### 284 3.1 Influence of the Inner Cell Walls on the Outermost Periclinal Stress Field

285 **Taking inner walls into consideration diminishes stress field heterogeneity and**  
 286 **anisotropy in the outermost walls** To estimate the mechanical influence of inner  
 287 walls, we compared the stress patterns borne by *L<sub>1</sub>* outermost periclinal walls between  
 288 various versions (*shell*, *L<sub>1</sub>-only*, *L<sub>1</sub>-L<sub>2</sub>* and *full*) of the same structure (*sphere*, *pill* and  
 289 *bowling pin*). For each version of each structure, we monitored the spatial variations  
 290 of the stress field. To do so, we looked at relative spatial fluctuations of stress intensity,  
 291 quantified by its cell-to-cell standard deviation divided by its mean value ( $\Delta\sigma/\bar{\sigma}$ ). As  
 292 Fig. 3a shows, these fluctuations are decreasing as we are considering an increasing  
 293 number of inner walls. We also monitored the anisotropy of the *L<sub>1</sub>* outermost periclin-  
 294 al stress field and observed that it was also a decreasing function of the number of  
 295 considered inner walls, see Fig. 3b. The sphere structure, represented by blue graphs  
 296 in Fig. 3a, b, serves as a reference; stresses on an exact sphere should remain homo-  
 297 geneous and isotropic for any version, the slight fluctuations and anisotropy shown on  
 298 the blue graphs of Fig. 3a, b are due to the discrepancy between discrete meshes and

299 the exact sphere. High variations observed on the two other structures (red and green  
300 graphs) appear therefore significant.

301 Put together, these two observations suggest that the mechanical influence of inner  
302 walls tends to homogenize the stress pattern borne by outermost periclinal walls and  
303 make it more isotropic.

304 **Rigidity differential between inner and outer walls increases stress field hetero-**  
305 **geneity and anisotropy in outermost walls** One key assumption of the *pressurized*  
306 *shell model* is that inner walls can be disregarded as mechanical load-bearer since  
307 they are softer than outermost ones. To test this hypothesis, we conducted simulations  
308 on the *full* versions of our geometrical structures for various values of the rigidity  
309 ratio between the outermost periclinal walls and the inner ones:  $r_R = Y_{out}/Y_{in}$ , where  
310  $Y_{out}$  and  $Y_{in}$ , respectively, stand for the Young's moduli of the  $L_1$  outermost periclinal  
311 walls and the one of all the other walls. For each value of this ratio, we computed the  
312 mean intensity value of the normalized stresses borne by each type of cell within the  
313 *full* version of a given structure and compared it to the mean intensity value of the  
314 stress field developed within the *shell* version of the same structure:  $\bar{\sigma}_{full}(cw\ type)/\bar{\sigma}_{shell}$   
315 (see Fig. 3c for a visual representation). As the rigidity ratio between outermost and  
316 inner walls increases, the intensity ratio between stress borne by outermost and inner  
317 walls grows accordingly. Conversely, when all cell walls feature the same rigidity  
318 value, stress intensity is uniformly distributed among them. As expected for springs  
319 arranged in parallel and loaded with a common force, Fig. 3c suggests that relative  
320 distribution of stress between outermost and inner load-bearing elements corresponds  
321 to the relative distribution (ratio) of rigidities.

322 In meristematic tissues, outer walls are thicker than inner ones (see supporting  
323 material of Kierzkowski et al. 2012). Structural stiffness of a plane is proportional to  
324 its thickness (Landau and Lifshitz 1959). Therefore, if composition does not differ  
325 significantly from one wall to another, one can assume that thickness ratio translates  
326 directly into stiffness ratio between outer and inner walls which should roughly lie  
327 between 3 and 10 (Kierzkowski et al. 2012 and unpublished observation made in our  
328 laboratory). Figure 3c shows that the corresponding ratios between outermost over  
329 inner stress intensities lie within the same range and that outermost stress intensity  
330 represents 60% of what it would be in the *shell* version.

331 Finally, increasing standard deviation with rigidity ratio, accounted for by increas-  
332 ing error bars in Fig. 3c, suggests that rigidity differential not only increases  
333 heterogeneity between outermost and inner stress fields, but also increase hetero-  
334 geneity within the outermost periclinal stress field itself. This is confirmed by Fig. 3d,  
335 e where outermost periclinal stress intensity fluctuations and anisotropy are, respec-  
336 tively, plotted as function of the rigidity differential.

337 **Considering inner walls drastically reduce outermost stress intensity in nega-**  
338 **tively curved regions** When surfaces grow into complex shapes, folding and crease  
339 formation are of prime importance because they define *frontiers* between organs. One  
340 output of the *pressurized shell model* is that creases, which correspond to zones of  
341 negative Gaussian curvature, feature high-intensity and highly anisotropic stresses  
342 (Bozorg et al. 2014).

When applied to a *realistic* structure displaying such negatively curved regions, our simulation pipeline produced a counterintuitive result; while creases indeed supported high-intensity and highly anisotropic stresses on the *shell* version of the structure (Fig. 4a), they supported low-intensity (but still highly anisotropic) stresses in the  $L_1$ -only (Fig. 4b) and  $L_1-L_2$  (Fig. 4c) versions. As seen in Fig. 4e, while stress relative intensity in the frontier zone is, on average, twice as big as in the central zone within the *shell* version; it decreases to almost half of the central zone mean value in the  $L_1$ -only version and to roughly 75% of it in the  $L_1-L_2$  version.

Despite this strong influence of inner walls on the relative values of stress intensity between zones, Fig. 4f shows that anisotropy remains maximal in the frontier zone, no matter if the inner walls are considered or not. Similarly, stress orientation patterns did not change drastically between the various versions, see supplementary Fig. 1.

### Stress intensity in outermost walls correlates with the area of periclinal cell faces

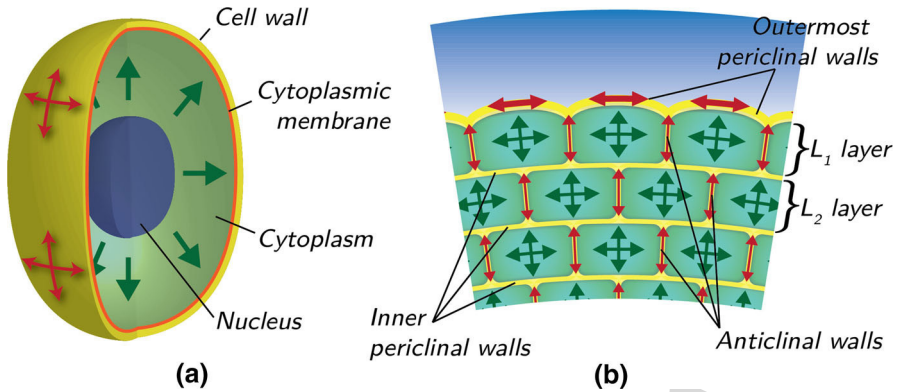
Another feature of the  $L_1$  outermost periclinal stress pattern revealed by our simulations on *realistic* structures concerns the correlation between the stress intensity borne by outermost periclinal walls and their surface area, see Fig. 5a, b: in the *surface area vs. stress intensity space*, points representing cells from the epidermis are distributed along some saturation curve. In other words, our simulations show that bigger epidermal cells experience higher stress intensities in their outermost periclinal walls. Although this correlation seems also present in the  $L_2$  layer, the saturating behavior is far less obvious, see the yellow point cloud in Fig. 5a. Stress intensity and surface area also appeared correlated in the  $L_2$  layer, but with a less obvious saturating behavior. This “stress intensity–surface area” correlation vanishes obviously in the *Shell* versions of our structures, see Fig. 5c, demonstrating that this feature emerges from the consideration of inner walls.

### Boundary conditions imposed on the innermost walls impact stress patterns at the surface

The choice of fixed boundary conditions was imposed by our experimental data. As mentioned above, when applied to 3D fluorescence intensity images, our segmentation algorithms could only reconstruct faithfully the two outermost cell layers. As Fig. 7a, b shows, the resulting structures correspond to asymmetric concave hulls. In vivo, these layers are anchored to inner tissues. To take into account the influence of these inner tissues, we fixed the innermost points of the outer two layers in our simulations. This was done using Dirichlet boundary conditions, see third line of eq. 1.

To assess the influence of such boundary conditions on the outermost periclinal stress pattern, we compared stress quantifiers (i.e., relative intensity  $\bar{\sigma}$  and anisotropy  $\bar{\alpha}_\sigma$ , averaged over the outer periclinal wall of each epithelial cell) between various versions of the same 3D structure ( $L_1$ -only,  $L_1-L_2$ , *full*), where the innermost points have been fixed, with the same quantifiers ( $\bar{\sigma}^*$ ,  $\bar{\alpha}_\sigma^*$ ) computed on the *full* version of the same structure without fixed innermost walls, see Fig. 6.

Figure 6 shows that fixed boundary conditions have a strong influence on the outermost stress pattern. As we considered more inner layers, this influence was reduced. Note that Fig. 6c, d show that depending on their curvature, various regions—of the *bowling pin* structure—are not impacted similarly by boundary conditions.



**Fig. 1** Schematic description of plant cells and tissues. **a** Sketch of a plant cell. **b** Plant cells glued together by their cell walls, forming a tissue. In both **a b**, the green and red arrows, respectively, depict turgor pressure forces and response stresses within the cell walls

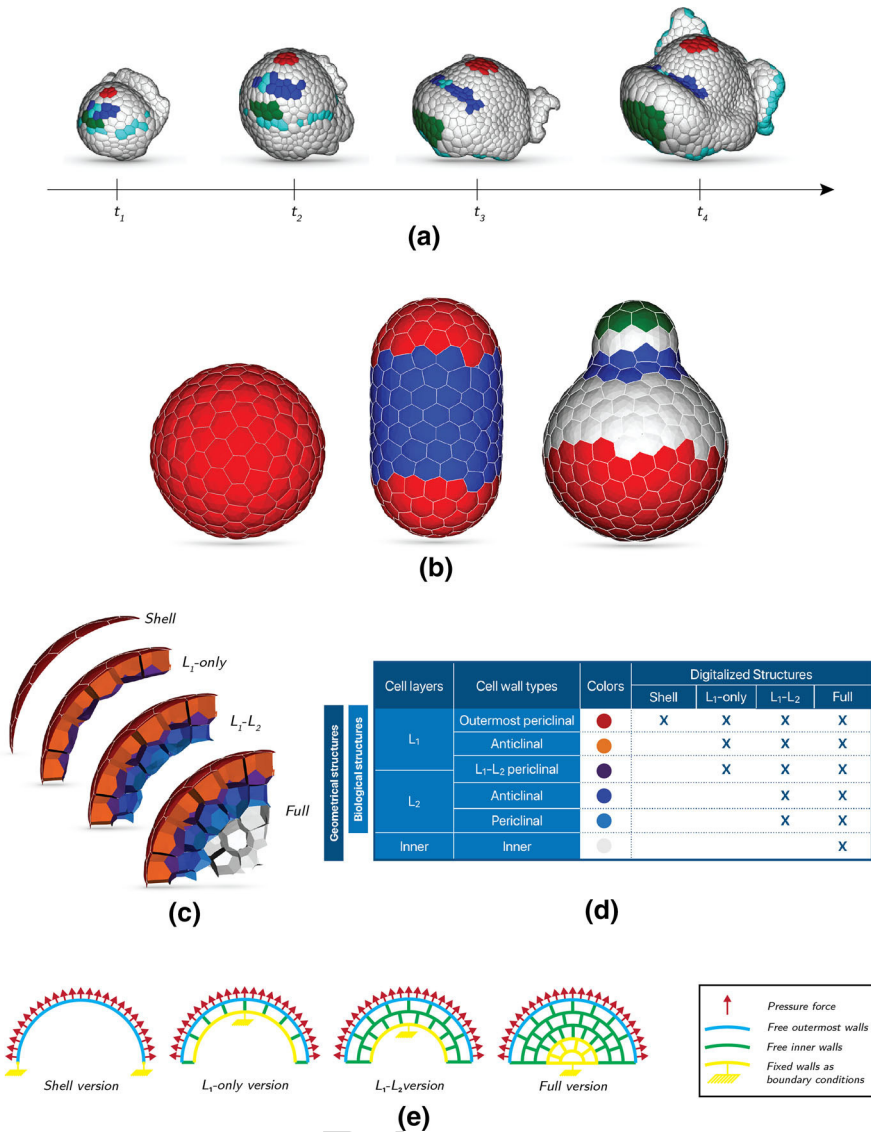
### 3.2 Characteristics of the Inner Stress Field

**Anticlinal stresses are mostly oriented in the apicobasal direction** Our simulations show that stresses borne by anticlinal walls are, when averaged within each cell, mainly aligned in the apicobasal direction (see Fig. 7a–d) and feature higher anisotropy compared to outer periclinal walls, see Fig. 7c. This suggests that stresses borne by anticlinal walls directly balance pressure forces normal to the outermost periclinal surface.

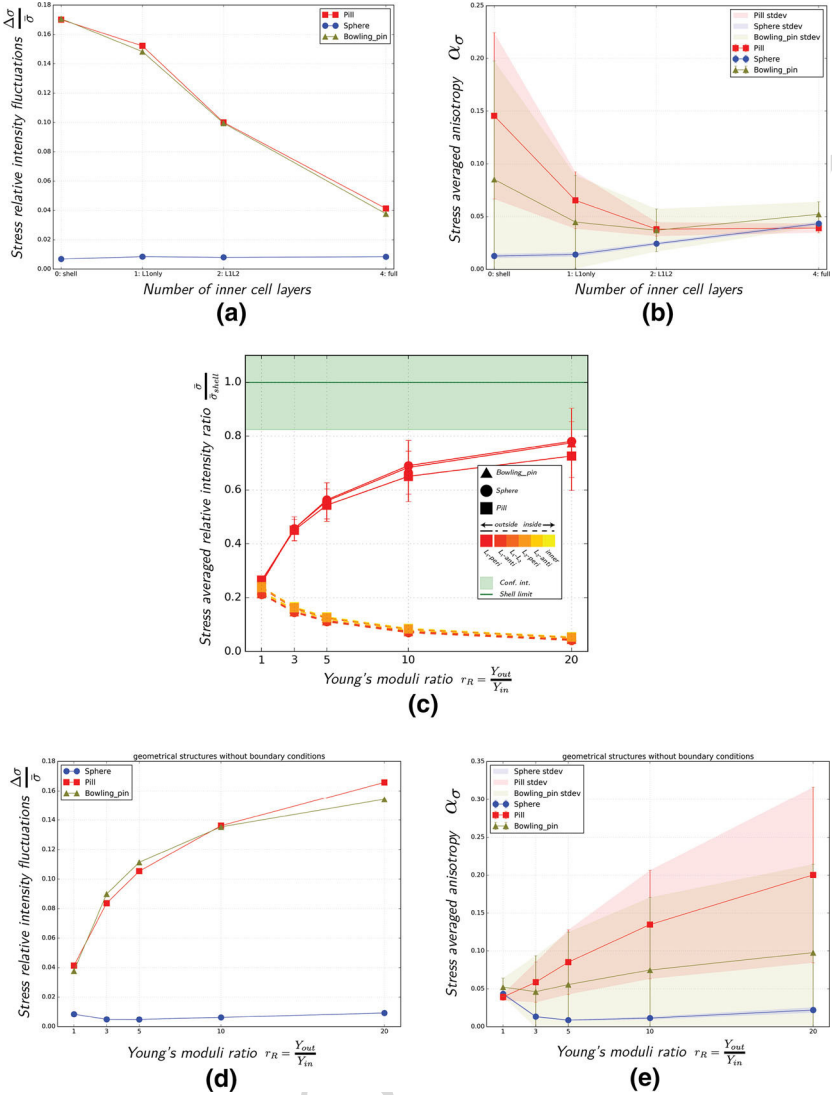
**Negatively curved regions experience low-intensity anticlinal stresses** The equilibrium of pressure forces and elastic stress highly depends on the curvature of the considered surface. As reported in the previous subsection, when inner walls were considered in our simulations, epidermal cells in negatively curved regions displayed low-intensity stresses on their outermost periclinal faces. Interestingly, the same cells also featured low-intensity stresses on their anticlinal faces, see Fig. 7e, f. Frontier zones between organs and the meristem are, therefore, mechanically characterized by low-intensity global stresses.

### 3.3 Morphologically Distinct Regions Within a Tissue Experience Specific Stress Patterns

**Mechanical stress characteristics vary significantly between morphologically different regions** For each cell of our 3D *realistic* structures, we computed a set of stress characteristics: The relative intensity and anisotropy averaged over their outermost periclinal wall and the relative stress intensity averaged over their anticlinal walls. From the previous sections, we expected these characteristics to vary from one cell to another depending on their size and localization, but Fig. 8a–c shows that in the abstract vector space composed by these characteristics (referred to as the *stress space* hereafter), points representing cells are distinctly clustering according to morpholog-



**Fig. 2** Biological and geometrical structural templates used in numerical simulations. **a** Realistic structures. Meshes generated from 3D confocal stacks. The meshes correspond to four time steps describing the early development of a flower bud. The time interval between consecutive steps is twelve hours. **b** Abstract structures generated from basic geometrical primitives (spheres and cylinders). The first structure corresponds to a cellularized sphere; the second one to a *pill* produced by the concatenation of two hemispheres with a cylinder of the same radius; the third one, the *bowling pin*, corresponds to the concatenation of two spheres of different radii. **c** Various versions of each structure. Each structure has been declined in various versions of increasing depth. The realistic structures come in three different versions: *Shell* (only the outermost cell walls are considered),  $L_1$ -only (only walls from the  $L_1$  layer have been considered),  $L_1 - L_2$  (cell walls of both  $L_1$  and  $L_2$  layers are considered). Abstract structures comes in one more version: *Full* (all walls are considered) **d** Summary of all the various versions with their cell wall compositions. **e** Fixed boundary conditions used in some simulations for the various versions of the structures (Color figure online)



**Fig. 3** Influence of the structure depth and cell wall stiffness heterogeneity on the outermost periclinal stress pattern on geometrical structures. **a** Cell-to-cell relative stress intensity fluctuations (standard deviation divided by mean value) as a function of the structures depth for each shape (sphere, pill, bowling pin). For each cell of the epidermis ( $L_1$  layer), the mean relative stress intensity is computed. **b** Averaged stress anisotropy as a function of the structures depth for each shape (sphere, pill, bowling pin). Error bars stand for standard deviation over the epidermis cells. **c** Ratio between the relative stress intensity in the full structure over the relative stress intensity in the shell structure as a function of rigidity differential between the outermost cell walls ( $L_1 - peri$ ) and the inner ones in the full structure. Each curve corresponds to a specific cell wall type. Error bars stands for standard deviation. **d** Cell-to-cell relative stress intensity fluctuations in full structures (standard deviation divided by mean value) as a function of rigidity differential between the outermost cell walls ( $L_1 - peri$ ) and the inner one. **e** Averaged stress anisotropy as a function of rigidity differential between the outermost cell walls ( $L_1 - peri$ ) and the inner one. Error bars stand for standard deviations (Color figure online)



ical identities. More precisely, Fig. 8a, b shows that on the fourth time step *realistic* structure, where morphological features—such as outgrowing initia and creases—were well established, point representing epidermal cells are clustering in distinct groups corresponding to these significant morphological features. Figure 8c shows that a clear distinction can also be made between epithelial cells and inner cells in the *stress space*.

This last sorting suggests that epithelial cells are experiencing higher stresses in their periclinal plane than along their apicobasal direction, whereas inner cells are experiencing the opposite configuration. This dichotomy originates from the rigidity differential between the inner and outer walls: stiffer outer walls bear more stress and therefore drag the corresponding point away from the first bisectrix toward the right. In simulations where the rigidity ratio  $Y_{out}/Y_{in}$  was changed, the sorting was impacted accordingly (data not shown).

### Distinct morphological fates correlate with specific trajectories within the *stress space*

In order to further assess the correlation between plant tissues morphological features and clustering of cells in the *stress space*, we looked at the point clouds corresponding to older structures (the first, second and third structures of our *realistic* time series). Since we were able to reconstruct the lineage of epidermal cells across our time series, we could follow the trajectories of each cell line (i.e., a mother cell on the first time step and its clones in the following ones) within the *stress space* as the young flower is shaping itself. In Fig. 8d, e, we see that cell lines leading to the formation of specific zones follow coherent trajectories, specific to distinct zones; In Fig. 8e, cells forming the central zone of the meristem do not move much from the center of the plane, whereas cells forming the crease and the sepal abaxial side are, respectively, moving toward the bottom right corner and the top left corner of the frame. Similarly, in Fig. 8c, trajectories of cell lines forming the crease feature a drastic and coherent shift of trajectories between the third and fourth time step, concomitant with the crease formation.

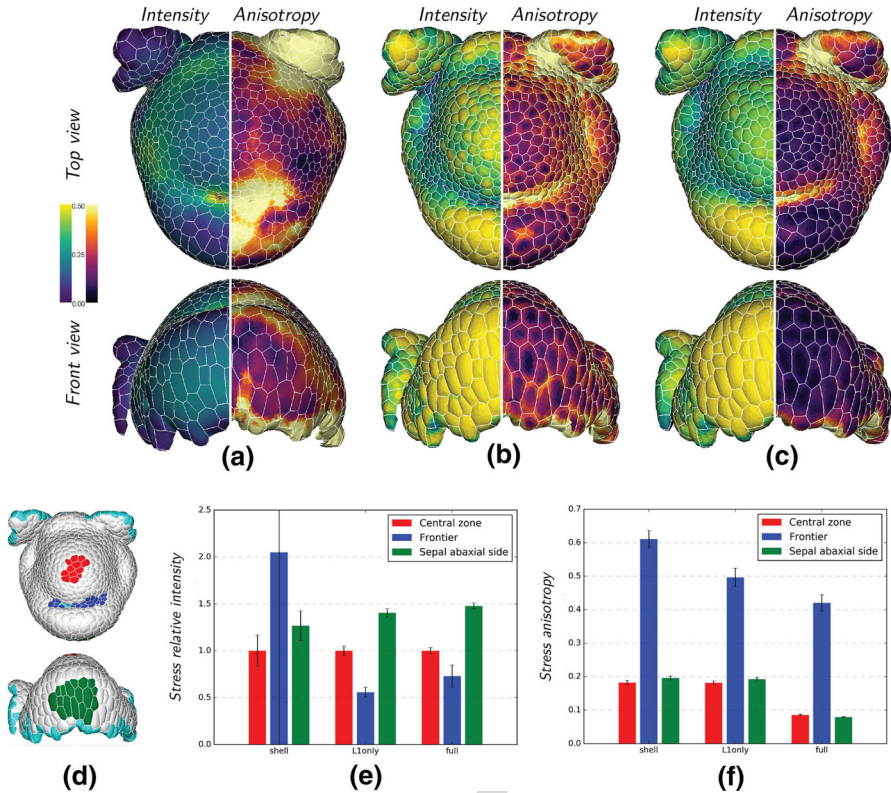
## 4 Discussion

Robust and reproducible morphogenesis relies on control mechanisms where geometry feeds back onto growth. Such regulation loops are required to translate geometry into information perceptible by cells. In plants, turgor-induced stresses emerge as such geometry-related information fields.

In order to model and simulate properly plant morphogenesis, we need to understand how this transduction from tissue geometry to mechanical stress works. In particular, how parameters such as the accuracy of tissue descriptions (number and shapes of cells, depth of the considered tissues, rheological properties) as well as the assumptions underlying simulations (e.g., boundary conditions) influence computed stress fields and therefore may modulate geometrical information available to cells.

**In order to have an informative outermost periclinal stress field, cell wall rigidity must be heterogeneous** The informativeness of a stress pattern can be quantified by its spatial directional variabilities, Fig. 3. For a given asymmetric shape (e.g., *pill*, *bowling pin*...) the variability of an informative turgor-induced stress pattern should



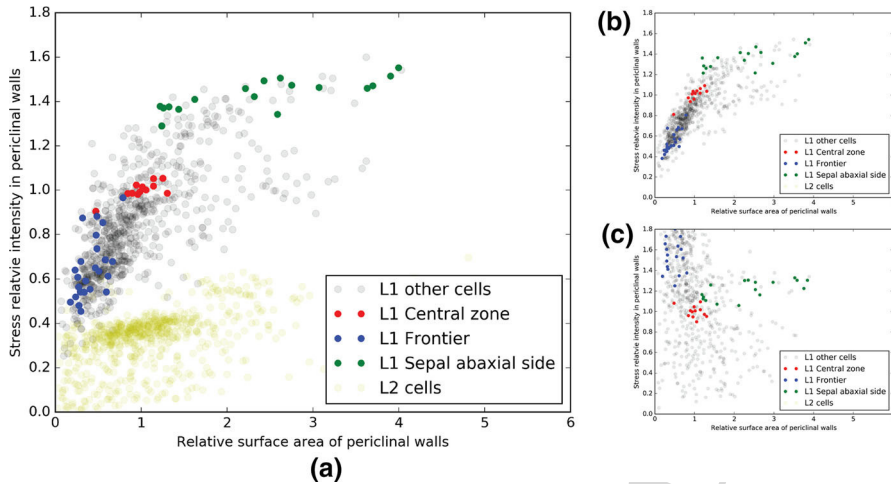


**Fig. 4** Outmost periclinal stress pattern characteristics on a realistic structure (fourth step of the time series). **a–c** Relative stress intensity (left-hand side) and anisotropy (right-hand side) heat maps computed on the three different version of the same time point: **(a)** shell, **(b)**  $L_1$  – only **(c)**  $L_1 - L_2$ . The corresponding maps of main stress directions are given as supplementary Fig. 1. **d** Visualization of the three morphological zones of interest: *Central Zone* in red, *Frontier* in blue *Sepal Abaxial Zone* in green. **e, f** Outmost periclinal stress invariants—**e** intensity (normalized by the central zone value), **f** anisotropy—averaged over zones of interest for the three versions of the same structure. Left-hand side: *Shell*, middle:  $L_1$  – only, right-hand side:  $L_1 - L_2$ . Error bars stand for standard deviations

453 correlate with that of the surface curvature field. The higher the variabilities of the  
 454 stress pattern, the more informative it is, i.e., discrimination between regions with  
 455 different geometrical features is made easier.

456 In our simulations, two main parameters influenced the stress pattern variabilities:  
 457 the number of considered inner cell layers, Fig. 3a, b, and the rigidity differential  
 458 between the outermost cell walls and the inner ones, Fig. 3d, e. While increasing  
 459 the number of considered inner cell layers decreased the outermost stress pattern  
 460 variability, rigidity differential between outer and inner walls increased it.

461 This can be qualitatively explained: by adding inner load-bearing elements to a 2D  
 462 pressurized shell one enables the stresses to distribute in 3D. This redistribution of  
 463 stresses between outer and inner walls is done in proportion to the walls rigidity (i.e.,  
 464 stiffer ones bear proportionally more stress than softer ones, see Fig. 3c). 3D struc-



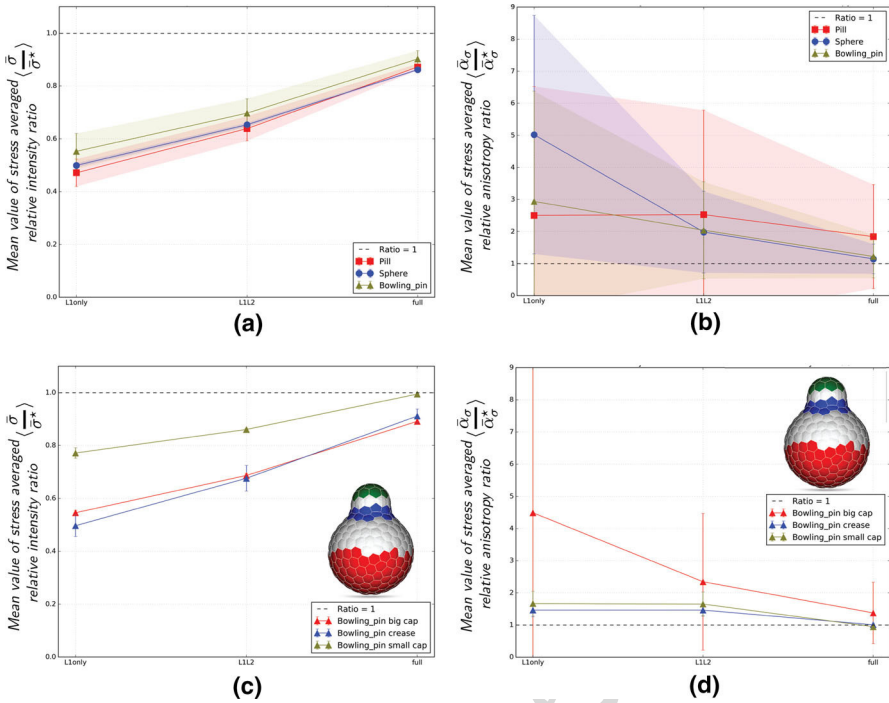
**Fig. 5** Relationship between relative stress intensity in outermost periclinal walls and epidermal cells surface areas (fourth step of the realistic time series). Relative stress intensity (normalized by its mean value in the *central zone*) as a function of cells periclinal surface area (normalized by its mean value in the *central zone*) for the three versions of the structure: **a**  $L_1-L_2$  **b**  $L_1$ -only **c** *Shell*. Each point corresponds to a single cell. The abscissa corresponds to the surface area of the cells outermost wall (*outer periclinal* for  $L_1$  cells  $L_1-L_2$  - *periclinal* for  $L_2$  ones, see table (c) ) Color code corresponds to various zones on interest in the  $L_1$  defined on c

465 tures with similar walls everywhere tend to behave mechanically as 3D homogeneous  
 466 continua, in which stress fields are known to be shape-independent and thus non-  
 467 informative. Rigidity differential between the outermost surface and the inner walls  
 468 diminishes the mechanical influence of the latter and therefore enhances contrast (and  
 469 informativeness) of the stress pattern.

470 For the sake of simplicity, we only considered isotropic cell walls here and the only  
 471 envisioned heterogeneity was between outer and inner walls. In vivo, plant cell walls  
 472 can be highly anisotropic and heterogeneous on the tissue surface (Milani et al. 2011).  
 473 A follow-up question could address the influence of these more realistic and more  
 474 complex modulations of the rigidity on the stress patterns.

475 **Negatively curved regions, such as *frontiers* between organs, experience low-**  
 476 **intensity and high-anisotropy stresses** Considering the anticlinal walls in realistic  
 477 structures helped us resolve an apparent paradox. According to the *pressurized shell*  
 478 *model* and our simulations on shells (Fig. 4a), cells lying in the creases between the  
 479 *central zone* and lateral organs experience high-intensity stresses compared to neigh-  
 480 boring zones. Since plant cells growth is fueled by turgor-induced pressure, this would  
 481 suggest fast growth of such cells, which is not observed experimentally (Burian et al.  
 482 2013; Kwiatkowska 2004).

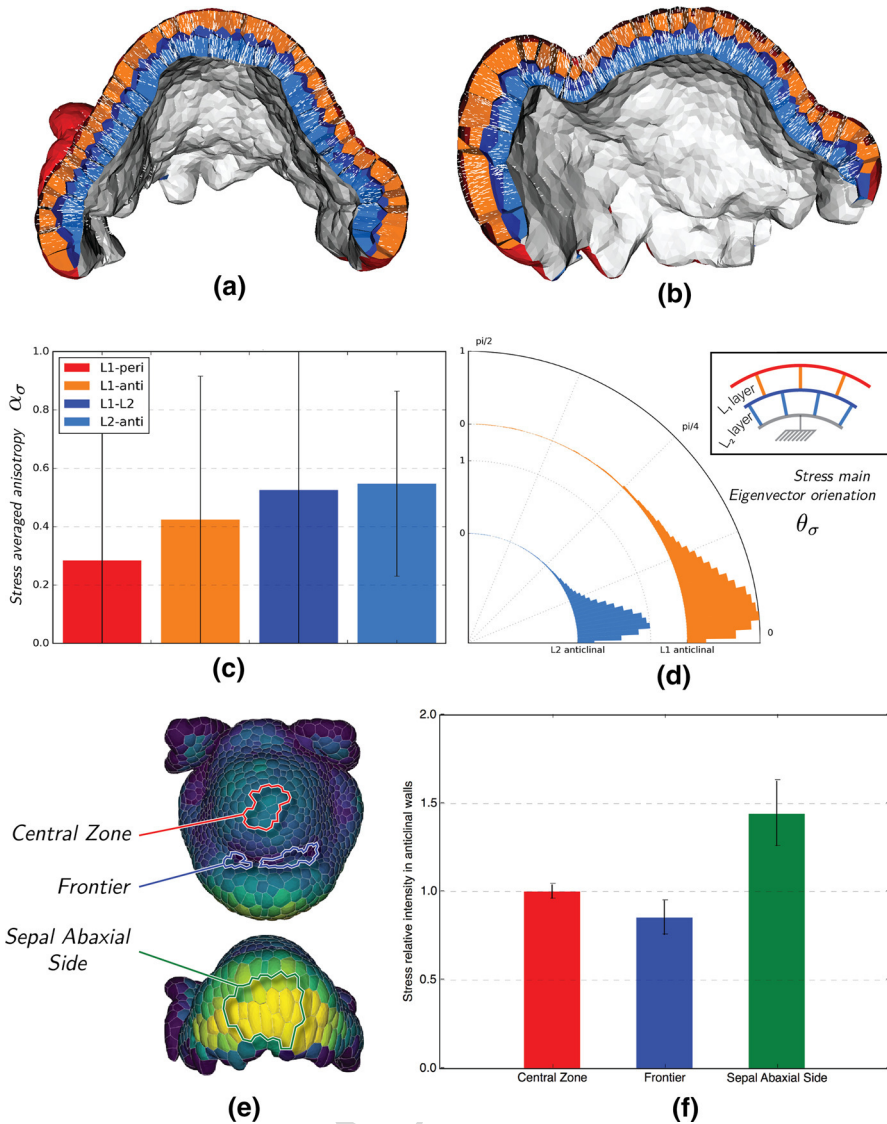
483 When anticlinal walls are taken into consideration, stress intensity in the *fron-*  
 484 *tier zone* decreases down from a relative maximum to a relative minimum, Fig. 4e.  
 485 This resolves the previously mentioned paradox: when anticlinal walls are considered,  
 486 creases between organs undergo low-intensity stresses compatible with small growth



**Fig. 6** Influence of the boundary conditions on the outermost periclinal stress pattern undergone by geometrical structures with inner walls. **a** Mean values of the outermost periclinal stress intensity ratio between structures with fixed innermost walls and a full one without fixed walls. **b** Mean values of the outermost periclinal stress anisotropy ratio between structures with fixed innermost walls and a full one without fixed walls. On both subfigures, each curve corresponds to a specific shape (red: *Pill*, blue: *Sphere*, green: *bowling pin*) and each point corresponds to a specific version of the considered shape with an increasing number of considered inner layers from left to right. Error bars stand for standard deviation over all the considered epidermal cells. **c, d** Same figures as **a, b** but only on the *bowling pin* structure. This time each graph refers to a specific zone on the structure

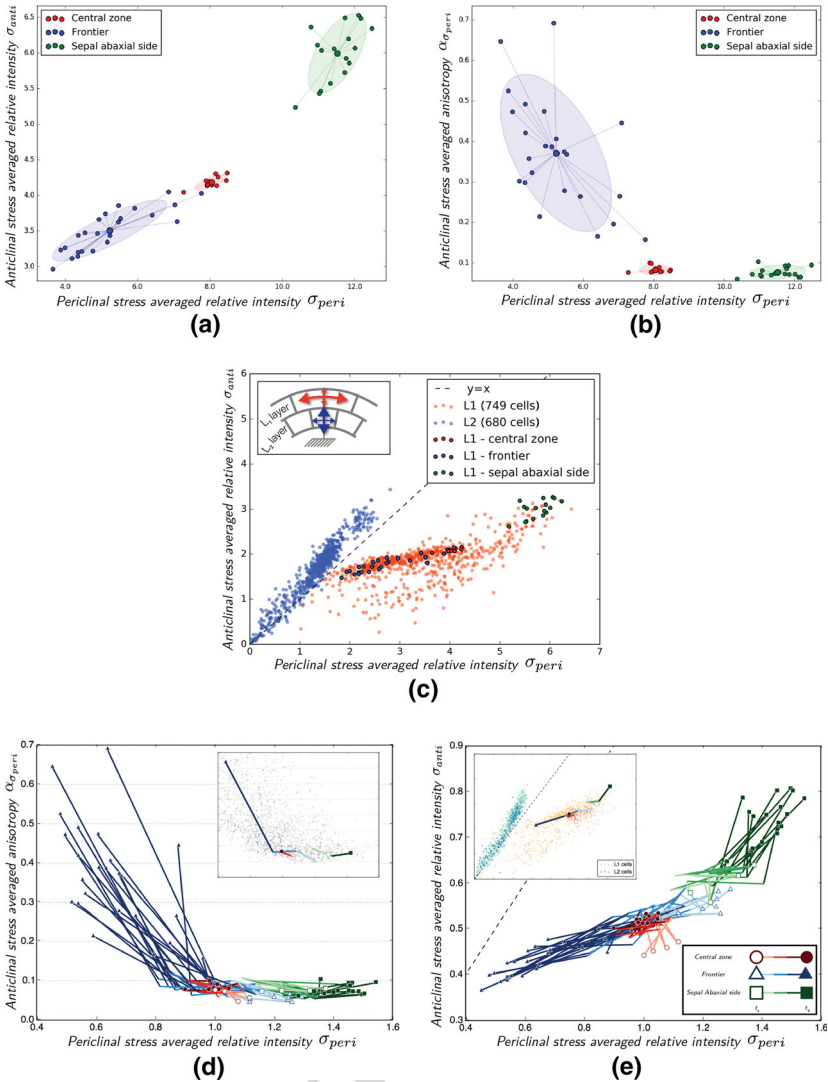
487 rates measured in such regions (Burian et al. 2013; Kwiatkowska 2004). Qualitatively,  
 488 the inversion of colors between the left-hand side panels of Fig. 4a–c suggests that all  
 489 zones with negative Gaussian curvature experience this low-intensity stresses when  
 490 inner walls are considered. Note that this is coherent with classic wounding experi-  
 491 ments performed on sunflowers (Dumais and Steele 2000): when sunflower meristems  
 492 are cut, the resulting wounds are wider in the *central zone* than in the surrounding  
 493 crease.

494 One possible explanation could be that anticlinal walls in these negatively curved  
 495 regions act as the main load-bearing elements against turgor-induced forces as the  
 496 apicobasal alignment of stresses in anticlinal walls suggests, Fig. 7a–d. However,  
 497 Fig. 7e, f shows that anticlinal stresses borne by cells in the *frontier zone* are also lower  
 498 than those borne by cells in other regions of interest. Anticlinal walls are therefore  
 499 not the main load-bearing elements in the *frontier zone*, this is confirmed by Fig. 8c:  
 500 cells of the *frontier zone*, as all epidermal cells, experience bigger stresses on their



**Fig. 7** Inner walls stress field characteristics on realistic structure (fourth step of the time series,  $L_1 - L_2$  version). **a, b** Anticlinal stress orientation (direction of the maximal stress eigenvector) in the epidermis ( $L_1$ , orange) and sub-epidermis layers ( $L_2$ , blue). **c** Histograms of inner stress anisotropy per cell wall types. Error bars stand for standard deviations. **d** Angular distributions of the anticlinal stress eigenvectors associated with the maximal eigenvector.  $\theta_\sigma = 0$  corresponds to the apicobasal direction and  $\theta_\sigma = \pi/2$  to the periclinal plane. **e** Heat map of the averaged relative anticlinal stress intensity per cells in the epidermis. **f** Histograms of the averaged relative anticlinal stress intensity per cells in the epidermis

501 outermost periclinal face than on their anticlinal ones. Note that periclinal surface  
 502 area of cells in the *frontier zone* is rather small compared to the rest of the tissue.



**Fig. 8** Characterization of cells mechanical state on realistic structure (fourth step of the time series). **a**, **b** Point clouds representing the averaged stress state of each cell of the three zones of interest defined in Fig. 4d. In **a**, the coordinates of these points correspond to the mean stress intensity in the periclinal walls (abscissa) and anticlinal walls (ordinates). In **b**, the coordinates of these points correspond to the mean stress intensity (abscissa) and anisotropy (ordinates) in the periclinal walls. The ellipses figure the covariant matrices of the various point clouds **d** 3D structures capturing the early sepal formation. Four time steps taken every 12 h. On time step  $t_4$  three topographic zones are defined on the epidermis ( $L_1$ ) layer: the central zone (CZ—red), the frontier (F—blue) and the abaxial side of the biggest sepal (AS—violet). Lineage of these zones is tracked back in older time steps and mother cells are displayed with the same color code as daughters. **f** Time evolution of cells forming the zones of interest in the peri-/anti-clinal relative stress intensities space. Each cell of each zone for of interest is depicted by a point in the plane. The lines between points represent their evolution from one time step to the next. For clarity reasons, only cells of the first ( $t_1$ ) and last ( $t_4$ ) time points are shown. Insert: global view of trajectories in the phase plane where  $L_1$  (green-blue dots) and  $L_2$  (orange) cells are superimposed



503 It remains to be seen if cell division—and the resulting increase in anticlinal walls  
504 density—account for this decrease in periclinal stress intensity.

505 **Turgor-induced stress reflects both cell-scale and tissue-scale geometries** Previ-  
506 ous numerical approaches performed on artificial structures (Mosca et al. 2017) and  
507 on realistic ones (reconstructed from confocal images of sepals Mosca et al. 2017  
508 and seedlings Bassel et al. 2014) revealed a connection between cell geometrical fea-  
509 tures and mechanical stresses. Moreover, the authors of Mosca et al. (2017), Bassel  
510 et al. (2014) suggested that such a connection could play a central regulatory role  
511 during morphogenesis. In this study, the various versions of the same realistic struc-  
512 ture suggest that this connection between cell geometry and mechanical stresses is  
513 rooted in the static geometrical and topological properties of tissues: in the 3D cases  
514 ( $L_1$ -only  $L_1-L_2$ ), we observed a correlation between outermost periclinal stress inten-  
515 sity and cell surface area, see Fig. 5a, b. Moreover, the specific shape of these point  
516 clouds suggests a saturating relation. This result gains interest when put in the context  
517 of a recent study on hypocotyls (Sapala et al. 2018). Focusing on epidermal cells,  
518 the authors obtained a similar saturation distribution profile between pavement cell  
519 periclinal surface areas and the intensity of the stress they experience, see figure 3-H  
520 of Sapala et al. (2018). Put together with our own results, this suggests that a general  
521 relationship between cells size and the intensity of the turgor-induced stress could be  
522 important for the regulation of growth in plants.

523 The fact that this correlation only appears when anticlinal walls considered (no  
524 correlation observed in Fig. 5c) reveals that the mechanical stresses experienced by  
525 cells encompass a short-range component, related to cell shape, mixed with a large-  
526 scale component, related to tissue geometry and captured by the *pressurized shell*  
527 *model*.

528 **Influence of boundary conditions** From a global standpoint, Fig. 6a, b shows that  
529 fixing inner walls results, respectively, in an underestimation of stress intensity and  
530 an overestimation of stress anisotropy on the outermost surface. This seems intuitive:  
531 fixed inner points ultimately anchor the loaded structure and end up carrying some  
532 of the pressure-induced load, explaining a drop in the global elastic stress response.  
533 Figure 4b, c shows that within each epithelial cell, the increase in anisotropy is mostly  
534 located near their anticlinal walls. When the innermost points of the structure are fixed,  
535 anticlinal walls lose some of their freedom of movement. This constrains the in-plane  
536 deformations of cells as well as the resulting elastic stresses within the outermost  
537 periclinal walls of the epidermis. The closer to the surface the fixed points, the more  
538 obvious this effect.

539 Depending on their curvature, various zones on the same structure are not similarly  
540 impacted by boundary conditions. Comparison between the small and big caps of the  
541 *bowling pin* structures, see Fig. 6c, d, suggests that as the ratio  $h/R$ —where  $h$  and  
542  $R$ , respectively, stand for the thickness of the structure (i.e., the distance between  
543 the outermost, free, surface and the innermost, fixed, one)—increases, the influence  
544 of fixed boundary conditions on the outermost stress pattern vanishes. This can be  
545 qualitatively explained: the radius of curvature is the characteristic length that most  
546 influences the outermost stress pattern (c.f. Laplace's law for spherical pressurized  
547 membrane:  $P \propto R^{-1}$ —with  $P$  the pressure differential between the two sides of the

548 membrane). When the boundary is far enough from the outermost surface (i.e., farther  
549 than this characteristic length), their influence is negligible. In realistic structures with  
550 fixed thickness, we therefore foresee that boundary conditions will impact flat zones  
551 more than highly curved ones.

552 In the previous subsection, we suggested that fixed boundary conditions generated a  
553 short-range component—reflecting cell shape—within the turgor-induced stress field.  
554 Combined with the qualitative analysis presented above, this entails that the relative  
555 importance of this component—compared to the long range one—should increase as  
556 tissues grow from small bulging primordia into flat structures as leaves and sepals.

557 Naturally, the biological relevance of fixed boundary conditions is questionable.  
558 From a mechanical point of view, fixing walls below a given depth is similar to assum-  
559 ing infinitely stiffer tissues below this depth compared to the above ones. While this  
560 has not been described in fast growing tissues, the development of vasculature in more  
561 differentiated organs could provide such a rigidity gradient. Stiffening more or less  
562 deeply cell walls could be a way to modulate the relative importance of the cell-shape  
563 and the tissue-geometry-related components within the outermost stress field.

#### 564 **Turgor-induced stress as a source of positional information field in plant tissues**

565 In conclusion, our results show that within morphologically distinct regions, cells  
566 featured specific and coherent stress characteristics. This enables an efficient clustering  
567 of cells in a low-dimensional (3D) abstract vector space, Fig. 8a–c. The pronounced  
568 segregation between clusters in this *stress space* suggests that stress-sensitive cells  
569 could plausibly discriminate between them and adapt their fate accordingly. This  
570 gives credit to the mechanical spatial awareness hypothesis.

571 Extending this analysis to the whole time series composed by our *realistic* struc-  
572 tures, we see that cell lines can be visualized as trajectories in the stress space.  
573 Figure 8d, e shows that epithelial cell lines developing toward specific morphological  
574 features correspond to coherent *streams* in the stress space. This analysis points at a  
575 number of interesting aspects. For instance, one can follow the trajectory correspond-  
576 ing to the formation of the *frontier zone* between the sepal and the meristem—blue  
577 lines in Fig. 8e. The corresponding points steadily move toward the first bisector  
578 over the course of the whole process. As mentioned earlier, the first bisector of the  
579  $\{\sigma_{\text{peri}}, \sigma_{\text{anti}}\}$ —plane discriminates strongly between epithelial cells and inner ones,  
580 see Fig. 8c. This suggests that cells forming a crease are evolving toward a mechan-  
581 ical stress environment similar to the one of inner cells. In the future, this analysis  
582 could be refined by considering more stress characteristics. The corresponding clus-  
583 ters and trajectories would consequently enable the distinction between more subtle  
584 morphological features.

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587 Das for kindly providing the time series of segmented 3D images of the growing flower bud used in this  
588 analysis. The authors also thank Gabriella Mosca for informal discussions and comments on the work.

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## References

- 591 Ali O, Mirabet V, Godin C, Traas J (2014) Physical models of plant development. *Annu Rev Cell Dev Biol* 30:59–78
- 592  
593
- 594 Armezzi A, Abad U, Ali O, Robin AA, Vachez L, Larrieu A, Mellerowicz EJ, Taconnat L, Battu V, Stanislas T, Liu M, Vernoux T, Traas J, Sassi M (2018) Transcriptional induction of cell wall remodelling genes is coupled to microtubule-driven growth isotropy at the shoot apex in *Arabidopsis*. *Development* 145(11):dev162255–11
- 595  
596  
597
- 598 Bassel GW, Stamm P, Mosca G, de Reuille PB, Gibbs DJ, Winter R, Janka A, Holdsworth MJ, Smith RS (2014) Mechanical constraints imposed by 3D cellular geometry and arrangement modulate growth patterns in the *Arabidopsis* embryo. *Proc Natl Acad Sci USA* 111(23):8685–8690
- 599  
600
- 601 Beauzamy L, Louveaux M, Hamant O, Boudaoud A (2015) Mechanically, the shoot apical meristem of *Arabidopsis* behaves like a shell inflated by a pressure of about 1 MPa. *Front Plant Sci* 6:1038
- 602  
603
- 604 Boudon F, Chopard J, Ali O, Gilles B, Hamant O, Boudaoud A, Traas J, Godin C (2015) A computational framework for 3D mechanical modeling of plant morphogenesis with cellular resolution. *PLoS Comput Biol* 11(1):e1003950
- 605  
606
- 607 Bozorg B, Krupinski P, Jönsson H (2014) Stress and strain provide positional and directional cues in development. *PLoS Comput Biol* 10(1):e1003410
- 608  
609
- 610 Bozorg B, Krupinski P, Jönsson H (2016) A continuous growth model for plant tissue. *Phys Biol* 13(6):1–14
- 611  
612
- 613 Burian A, Ludynia M, Uyttewaal M, Traas J, Boudaoud A, Hamant O, Kwiatkowska D (2013) A correlative microscopy approach relates microtubule behaviour, local organ geometry, and cell growth at the *Arabidopsis* shoot apical meristem. *J Exp Bot* 64(18):5753–5767
- 614  
615
- 616 Cerutti G, Ali O, Godin C (2017) DRACO-STEM: an automatic tool to generate high-quality 3D meshes of shoot apical meristem tissue at cell resolution. *Front Plant Sci* 8:13–15
- 617  
618
- 619 Chanliaud E, Burrows K, Jeronimidis G, Gidley M (2002) Mechanical properties of primary plant cell wall analogues. *Planta* 215(6):989–996
- 620  
621
- 622 Chickarmane V, Roeder AHK, Tarr PT, Cunha A, Tobin C, Meyerowitz EM (2010) Computational morphodynamics: a modeling framework to understand plant growth. *Annu Rev Plant Biol* 61(1):65–87
- 623  
624
- 625 Corson F, Hamant O, Bohn S, Traas J, Boudaoud A, Couder Y (2009) Turning a plant tissue into a living cell froth through isotropic growth. *Proc Natl Acad Sci* 106(21):8453
- 626  
627
- 628 Dumais J (2007) Can mechanics control pattern formation in plants? *Curr Opin Plant Biol* 10(1):58–62
- 629  
630
- 631 Dumais J, Steele CR (2000) New evidence for the role of mechanical forces in the shoot apical meristem. *J Plant Growth Regul* 19(1):7–18
- 632  
633
- 634 Dumais J, Shaw SL, Steele CR, Long SR, Ray PM (2006) An anisotropic-viscoplastic model of plant cell morphogenesis by tip growth. *Int J Dev Biol* 50(2–3):209–222
- 635  
636
- 637 Faure F, Duriez C, Delingette H, Allard J et al (2012) Sofa: a multi-model framework for interactive physical simulation. Springer, Berlin
- 638  
639
- 640 Fernandez R, Das P, Mirabet V, Moscardi E, Traas J, Verdeil J-L, Malandain G, Godin C (2010) imaging plant growth in 4d: robust tissue reconstruction and lineaging at cell resolution. *Nat Methods* 7(7):547–553
- 641  
642
- 643 Geitmann A, Ortega JKE (2009) Mechanics and modeling of plant cell growth. *Trends Plant Sci* 14(9):467–478
- 644  
645
- 646 Green JBA, Sharpe J (2015) Positional information and reaction–diffusion: two big ideas in developmental biology combine. *Development* 142(7):1203–1211
- 647  
648
- 649 Hamant O, Heisler MG, Jonsson H, Krupinski P, Uyttewaal M, Bokov P, Corson F, Sahlin P, Boudaoud A, Meyerowitz EM, Couder Y, Traas J (2008) Developmental patterning by mechanical signals in *Arabidopsis*. *Science* 322(5908):1650–1655
- 650  
651
- 652 Hamant O, Meyerowitz EM, Traas J (2011) Is cell polarity under mechanical control in plants? *Plant Signal Behav* 6(1):137–139
- 653  
654
- 655 Hejnowicz Z (2005) Autonomous changes in the orientation of cortical microtubules underlying the helical cell wall of the sunflower hypocotyl epidermis: spatial variation translated into temporal changes. *Protoplasma* 225(3–4):243–256
- 656  
657
- 658 Hejnowicz Z, Rusin A, Rusin T (2000) Tensile tissue stress affects the orientation of cortical microtubules in the epidermis of sunflower hypocotyl. *J Plant Growth Regul* 19(1):31–44
- 659  
660
- 661 Hervieux N, Dumond M, Sapala A, Routier-Kierzkowska A-L, Kierzkowski D, Roeder AHK, Smith RS, Boudaoud A, Hamant O (2016) A mechanical feedback restricts sepal growth and shape in *Arabidopsis*. *Curr Biol* 26(8):1019–1028

- 646 Irvine KD, Shraiman Boris I (2017) Mechanical control of growth: ideas, facts and challenges. *Development*  
647 144:4238–4248
- 648 Keller R (2012) Physical biology returns to morphogenesis. *Science* 338(6104):201–203
- 649 Kwiatkowska D (2004) Surface growth at the reproductive shoot apex of *Arabidopsis thaliana* pin-formed  
650 1 and wild type. *Journal of experimental botany* 55(399):1021–1032
- 651 Kierzkowski D, Nakayama N, Routier-Kierzkowska A-L, Weber A, Bayer E, Schorderet M, Reinhardt D,  
652 Kuhlemeier C, Smith RS (2012) Elastic domains regulate growth and organogenesis in the plant shoot  
653 apical meristem. *Science* 335(6):1096
- 654 Ladoux B, Mège RM (2017) Mechanobiology of collective cell behaviours. *Nat Rev Mol Cell Biol*  
655 18(12):743–757
- 656 Landau LD, Lifshitz EM (1959) *Course of theoretical physics vol 7: theory and elasticity*
- 657 Lintilhac PM, Vesecky TB (1984) Stress-induced alignment of division plane in plant-tissues grown-in-vitro.  
658 *Nature* 307(5949):363–364
- 659 Louveaux M, Julien J-D, Mirabet V, Boudaoud A, Hamant O (2016) Cell division plane orientation based  
660 on tensile stress in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 113(30):E4294–303
- 661 Milani P, Gholamirad M, Traas J, Arnéodo A, Boudaoud A, Argoul F, Hamant O (2011) In vivo analysis of  
662 local wall stiffness at the shoot apical meristem in *Arabidopsis* using atomic force microscopy. *Plant*  
663 *J* 67(6):1116–1123
- 664 Mosca G, Sapala A, Strauss S, Routier-Kierzkowska A-L, Smith RS (2017) On the micro-indentation of  
665 plant cells in a tissue context. *Phys Biol* 14(1):015003–015012
- 666 Oliveri H, Traas J, Godin C, Ali O (2018) Regulation of plant cell wall stiffness by mechanical stress: a  
667 mesoscale physical model. *J Math Biol* 78:625–653
- 668 Ortega JK (1985) Augmented growth equation for cell wall expansion. *Plant Physiol* 79(1):318–320
- 669 Pradal C, Dufour-Kowalski S, Boudon F, Fournier C, Godin C (2008) OpenAlea: a visual programming  
670 and component-based software platform for plant modelling. *Funct Plant Biol* 35(9–10):751–760
- 671 Sampathkumar A, Krupinski P, Wightman R, Milani P, Berquand A, Boudaoud A, Hamant O, Jönsson  
672 H, Meyerowitz EM (2014) Subcellular and supracellular mechanical stress prescribes cytoskeleton  
673 behavior in *Arabidopsis* cotyledon pavement cells. *eLife* 3:584–20
- 674 Sapala A, Runions A, Routier-Kierzkowska A-L, Das Gupta M, Hong L, Hofhuis H, Verger S, Mosca G,  
675 Li C-B, Hay A, Hamant O, Roeder AH, Tsiantis M, Prusinkiewicz P, Smith RS (2018) Why plants  
676 make puzzle cells, and how their shape emerges. *eLife* 7:2061
- 677 Shraiman BI (2005) Mechanical feedback as a possible regulator of tissue growth. *Proc Natl Acad Sci*  
678 102(9):3318–3323
- 679 Théry M, Racine V, Piel M, Pépin A, Dimitrov A, Chen Y, Sibarita J-B, Bornens M (2006) Anisotropy  
680 of cell adhesive microenvironment governs cell internal organization and orientation of polarity. *Proc*  
681 *Natl Acad Sci* 103(52):19771–19776
- 682 Thompson D'Arcy W (1917) *On growth and form*. Cambridge University Press, Cambridge
- 683 Tournier M, Nesme M, Gilles B, Faure F (2015) Stable constrained dynamics. *ACM Trans Graphics (TOG)*  
684 34(4):132
- 685 Turing FRSAM (1952) The chemical basis of morphogenesis. *Phil Trans R Soc Lond B* 237(641):37–72
- 686 Uyttewaal M, Burian A, Alim K, Landrein B, Borowska-Wykręt D, Dedieu A, Peaucelle A, Ludynia M,  
687 Traas J, Boudaoud A, Kwiatkowska D, Hamant O (2012) Mechanical stress acts via katanin to amplify  
688 differences in growth rate between adjacent cells in *Arabidopsis*. *Cell* 149(2):439–451
- 689 Vining KH, Mooney DJ (2017) Mechanical forces direct stem cell behaviour in development and regener-  
690 ation. *Nat Rev Mol Cell Biol* 18(12):728–742
- 691 Vogel G (2013) How do organs know when they have reached the right size? *Science* 340(6137):1156–1157
- 692 Weber A, Braybrook S, Huflejt M, Mosca G, Routier-Kierzkowska AL, Smith RS (2015) Measuring the  
693 mechanical properties of plant cells by combining micro-indentation with osmotic treatments. *J Exp*  
694 *Bot* 66:3229–3241
- 695 Wolpert L (1969) Positional information and the spatial pattern of cellular differentiation. *J Theor Biol*  
696 25(1):1–47