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SUPPLEMENTARY INFORMATION

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Early shaping of a leaf

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Technical comments

In trying to understand their model more fully, we encountered several issues. Firstly, λ^q determines in the model the contribution of the area constraint, related to the turgor pressure contribution, while λ^p determines the contribution of the perimeter constraint, more or less corresponding to the cell wall stiffness. The actual values of those parameters, however, are not defined in the paper or sup material. Only the ratio $\lambda_{stiff}/\lambda_{soft}$ is provided. It is the relative level of these two different λs which determines if the model is driven more strongly through a `turgor pressure'-like phenomenon, or through `cell wall growth' forces. Moreover, it is unclear if $\lambda_{stiff}/\lambda_{soft}$ defines the ratio $\lambda^q_{stiff}/\lambda^q_{soft}$, capturing cell wall elasticity, or $\lambda^p_{stiff}/\lambda^p_{soft}$ is varied as well (ie if the cells also differ in the cell size constraint).

Parameters are defined for region I, II, and III, but the paper does not state what those regions correspond to biologically. Both the simulation results and the sequence in which the data is typically presented suggest they correspond to Abaxial, Middle, and Adaxial, respectively. If this is the case, we are confused, because the squeezing force coefficients then do not seem to correspond to the FEM measurements as presented in the paper.

To clarify the relative importance of the mechanical properties attributed to the inner cell mass vs those of the epidermal cell regions, it would be critical to run the same simulations shown in Fig. 2i-l of their paper without any difference between epidermal regions (i.e., with an epidermis whose properties are homogenous). This would reveal the pure contribution of the inner tissue. Furthermore, it would be useful to run a simulation without the epidermal constraint (ie, remove the copying constraint as introduced in eq 5). This would lead to a very rapid tissue growth – as a consequence of the area for cell division being much smaller than the target area – and would hence clearly demonstrate the growth potential of the three different tissues in isolation.

It would be good to run the simulations for $\lambda_{stiff}/\lambda_{soft} = 1$ (simulations as in Sup Fig7c, but then for a ratio=1 column) while keeping all other parameters of the simulation the same as in the other cases. The marginal dependency of the final shape on the $\lambda_{stiff}/\lambda_{soft}$ as shown in Sup Fig7c suggests that the correct shape may be obtained without any difference in stiffness of the internal cells (suggesting factors other than mechanical heterogeneity are involved).

Finally, to separate the contribution of differential cell wall stiffness from differential cellular behaviour, such as cell cycle length and desired cell size, it is important to run the simulations for equal target cell size and cell division size for all tissues while only changing the parameters currently interpreted as cell wall stiffness, presumably λ^q (and λ^p?).

We look forward to seeing the software released and fully documented.