Early shaping of a leaf

To the editor — One of the merits of computational modelling is that it can generate behaviours that would be hard to arrive at from simple intuition. However, this can raise the further problem of understanding how the model generates those behaviours. Such understanding may be critical for evaluating the model and developing further experimental tests.

In a recent issue of *Nature Plants*, Qi et al.[1] propose a model for leaf morphogenesis in which they use a combination of atomic force microscopy on the outer epidermal cell wall, and analysis of cell wall modification (pectin methyl-esterification) to infer a pattern of cell wall stiffness throughout the developing leaf primordium. They then propose a model, based on minimization of elastic energy, to see if the inferred pattern of stiffness can account for the observed morphogenesis. In their model, cell walls in the upper half of the primordium are relatively stiff, whereas those in the lower half are relatively soft (represented as pink and green, respectively, in Fig. 1). The outer epidermis has high stiffness, providing a constraint on growth. After a period of growth, a further region with soft walls (Fig. 1b) is introduced into the upper domain. The model generated a final shape that showed a reasonable match to what was observed experimentally (Fig. 1d).

From their analysis, Qi et al. conclude that mechanical heterogeneity is sufficient to produce the asymmetry in planar leaves[1]. However, it is difficult to intuit how the shape changes generated by their model arise. Plant cells grow through the expansive action of turgor pressure stretching the cell wall[2,3]. We might, therefore, expect that soft cells would grow faster than stiff cells, as they would be less able to resist the turgor. This would cause the soft green regions to bulge out relative to the stiff pink regions. In their model output, however, the upper soft region flattens and eventually becomes concave rather bulging out (arrowed in Fig. 1c,d). Also, the lower soft region tapers towards the bottom rather than bulging out. These observations suggest that the soft internal regions are growing slower, not faster, than the stiff region.

To test the idea that such an 'inverted' pattern of growth might account for the shape changes generated by the model, we used a framework developed to explore the effects of differential growth in connected tissue[4]. By establishing similar domains to Qi et al., and having the pink region grow at a higher specified growth rate than the green regions, we see a similar shape change (Fig. 1e–h). We could not recreate the domains exactly, as we were unclear from their paper how regional identities are maintained. Their simulation videos show that cells at the boundaries between pink and green regions can switch identity during growth, so the upper green region tends to 'invade' the pink (three pink cells separate the green domains in Fig. 1b; whereas one cell separates the domains in Fig. 1d). In our model, we assume that cells retain their identity and pass it on to their daughters. Although not identical to what Qi et al. describe, the shape transformation we obtain is much more similar to that obtained if pink cells have a lower specified growth rate than green (Fig. 1i–l). In the latter case, the green regions bulge out, as expected if these regions are growing faster.

The shape transformations described by Qi et al., may thus be seen to arise largely because the pink stiff region grows faster than the green soft regions. Such a situation is counter-intuitive, as we would expect stiff walls to be more resistant to turgor-induced stretching. However, Qi et al. offered the intriguing suggestion that mechanical restraint by stiff surrounding epidermal tissue may account for the morphogenetic changes observed. Stimulated by this idea, we performed simulations using differential stiffness with a constraining boundary to explore the conditions in which stiff tissues may grow faster than soft ones. Here we distinguish between specified growth (how much a region would grow if unconstrained by neighbours) and resultant growth (how much a region actually grows when constrained by boundaries or neighbours).

Consider a tissue in which one half (Fig. 2a, pink) is much more stiff than the other half (green). Next, suppose that tissue is bounded by a layer that cannot stretch (black). If both the stiff and soft tissues have the same specified growth rate, the stiff tissue grows at the expense of the soft tissue (that is, has a higher resultant growth rate) because it takes more energy to compress stiff walls (Fig. 2b). This simulation is equivalent to assuming growth is driven by insertion of material into cell walls, forcing them to become longer (growth by insertion). If we weaken the outer restraining tissue, stiff tissue can still grow faster than the soft (Fig. 2d,e). The modelling framework of Qi et al. invokes such behaviour. They state that, as the ratio in stiffness between the stiff and soft cells ($\lambda_{stiff}/\lambda_{soft}$) increases, stiff cells offer more contributions to the system's potential energy, and thus, their growth rate also increases. Without any epidermal constraint, the soft and stiff tissues grow at the same rate (Fig. 2g,h).

However, the driving force for plant growth is not believed to be through insertion of material in cell walls. Instead, growth is driven by walls yielding to turgor pressure[2,3]; with wall material being inserted secondarily to maintain wall thickness

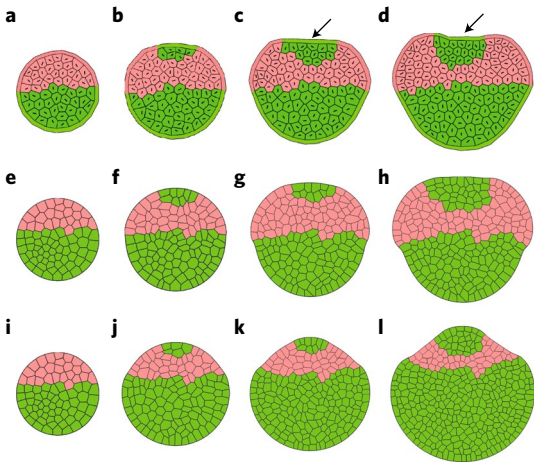

Fig. 1 | Simulations of leaf morphogenesis. a–d, Simulation results from Qi et al.[1]. Arrow points to upper soft region that becomes flattened and eventually concave, indicative of slow relative growth. Green, low stiffness; pink, high stiffness. **e–h**, Simulations based on differential specified growth rates. Green, slow; pink, fast. **i–j**, Simulations based on specified growth rates. Green, fast; pink, slow.

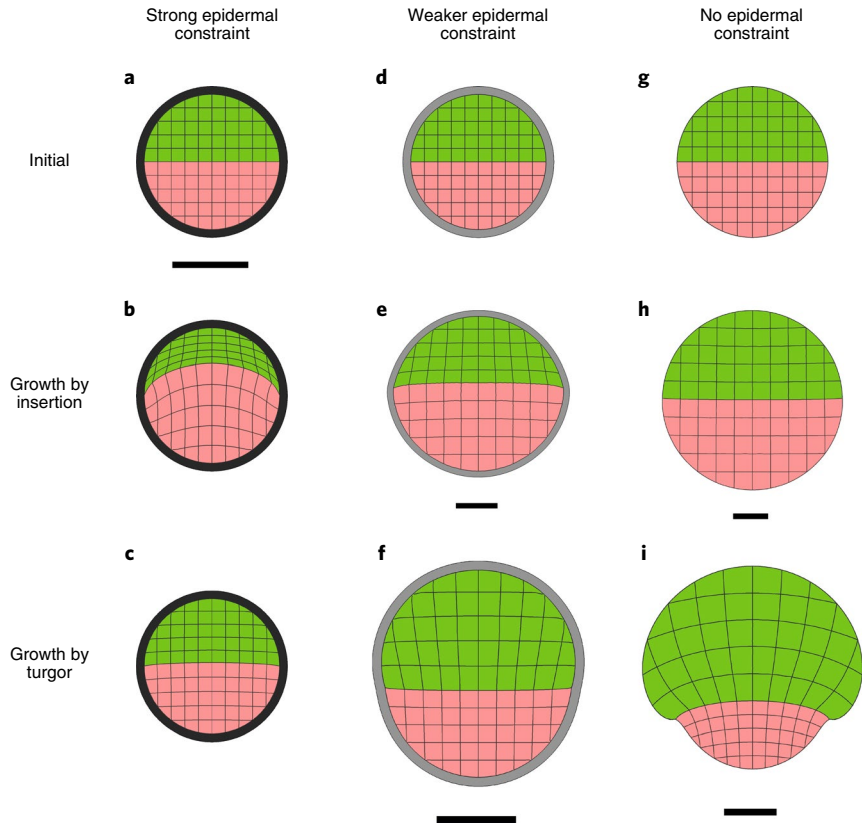

Fig. 2 | Growth of tissue under various constraints. With a strong epidermal constraint (**a**), growth by insertion allows stiff tissue to grow at the expense of soft tissue (**b**); whereas growth by turgor does not lead to differential growth (**c**). With a weaker epidermal constraint (**d**), growth by insertion still allows stiff tissue to grow more than soft (**e**), whereas growth by turgor leads to soft tissue growing more than stiff (**f**). With no epidermal constraint (**g**), growth by insertion leads to equal growth of stiff and soft tissue (**h**), whereas growth by turgor leads to soft tissue outgrowing stiff (**i**). Pink, stiff; green, soft. Unless otherwise indicated, the scale (in arbitrary units) is as in (**a**).

and strength. With such a turgor-driven mechanism, if the outer restraining tissue cannot grow, there will be no driving force for the internal cells to grow. This is because every cell in the internal tissue has the same turgor, so all internal walls have equal pressure on both sides and all tension is transmitted to the outer boundary tissue, so they bear no load. This argument applies irrespectively of the plastic or viscoelastic properties of the cell walls. We simulate this mechanism by assuming that tissue has a reduced specified growth rate in proportion to its stiffness, in which case the stiff and soft tissues remain the same size, as should be expected (Fig. 2a,c). If we weaken the bounding tissue, then growth of the internal tissue can occur but, as the soft tissue has a higher specified growth rate (yields more readily to turgor), it grows faster (Fig. 2d,f). Without any surrounding constraint, the soft tissue grows even more relative to the stiff (Fig. 2g,i). Thus, the turgor-driven mechanism for plant cell growth is not compatible with stiff cells growing faster than soft cells when put under an epidermal constraint.

Given this analysis, how can we account for the observed shape transformations of the leaf? One possibility is that turgor is not uniform and that the stiff cells have higher turgor, causing them to grow faster. This possibility is not discussed in Qi et al., and would run counter to their main conclusion that mechanical heterogeneity is sufficient to produce the asymmetry seen in planar leaves. It would also predict that the cell walls at the interface of the stiff and soft cells would bulge out (because of unequal pressure on the two sides), for which there is currently no evidence.

A second possibility is that the pattern of stiffness for internal cell walls inferred by Qi et al. is incorrect. Qi et al. only directly measure stiffness of outer epidermal walls by atomic force microscopy and infer stiffness of inner cell walls based on pectin methyl-esterification. It is possible that pectin methyl-esterification has the opposite effect on wall stiffness to the one they presume, in which case the pink domain indicates soft not stiff cells. Third, it is possible that variation in stiffness does not apply equally to all walls of a cell. The authors assume that specified growth is isotropic (equal in all orientations), but cell walls may be anisotropic such that specified growth is greater in some orientations than others. Such anisotropy would provide a range of additional options for generating the observed shape change. Further experiments and modelling should allow these hypotheses to be elaborated and tested. ❐

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Competing interests

The authors declare no competing interests.

Additional information

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