A biomechanical model for the study of plant morphogenesis: Coleocheate orbicularis, a 2D study species.

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Abstract

Pattern formation in the morphogenesis in plants is a phenomenon strongly determined by the genetic programs of particular species. However, because organs are groups comprised of specialized cells that are adherent and not capable of migration, physical processes (cell wall expansion, diffusion) are though to be major aspect influencing the plant development. In the present study, *Coleocheate orbicularis*, a probable ancestor of land plant species has been used as a model species to study the mechanics of cell expansion in multicellular colonies. Image analysis methods have been developed in order to analyze the kinematics of cell expansion and division, and a simple analytical model were used to estimate both viscosity and cell wall division properties. Numerical computation using the finite element method has been used to simulate the growth of entire colonies using parameters derived from live imaging. Comparison with live experiments showed strong similarities between measured and simulated colonies. Also, parameter analysis showed that not only wall mechanical properties, but also division rate and orientation are factors strongly influencing pattern formation.

Introduction

In growing plant meristems, new organs emerge at precise location, time and through well defined mechanisms. The determination of models for such behaviours is, however, particularly complex due to the large number of factors, either physical, chemical or biological, taking part in the regulation of cell expansion through space and time. Recent advances in molecular biology have greatly contributed to the understanding of the regulation of gene expression in various plant organs, for example floral, apical and root meristems, and the construction of realistic predicting models of genetic regulatory networks is now possible [1]. However, the emergence of most complex shapes cannot be fully understood without considering: 1) the spatial structure of the problem which determines the nature of cell to cell signalling network and 2) the physical driving forces that impose expansion and deformations to that structure with time.

In this context, simulation tools and methods are essential to explore and improve our understanding of the mechanisms of development in plants. We have been developing dynamic computational methods of plant morphogenesis at cellular level. The segmentation of live microscopy data was used to estimate parameters of a simple viscous model. Morphogenetic, transport and biomechanical model of cell wall expansion have been developed in order to analyses coupled mechanisms in morphogenesis. Most importantly, a software platform was developed to bring a flexible environment for the development and the

coupling of models (genetic regulation, spatio-mechanical factors, and signal transduction) at different levels of interests of the plant structure (e.g Plant - Cell - wall).

Live imaging and segmentation of growing *Coleocheate*

Coleochaete: model system

Coleocheate is a small group of fresh water microscopic algae of about ten species. The development of *Coleocheate* species shows interesting morphological diversity, from the generation of irregular branched segmented filaments in *C. divergens* or *C. nitellarum* to symmetric dichotomous branching thalli in *C. soluta* or almost perfect symmetric centrifugal expansion in *C. orbicularis* and *C. scutata*. (*Fig. 1*). Among all *Coleocheate* species, *C. orbicularis* appeared to be the most simple of all, as its expansion remains circular and is well adapted to a first generation of models.

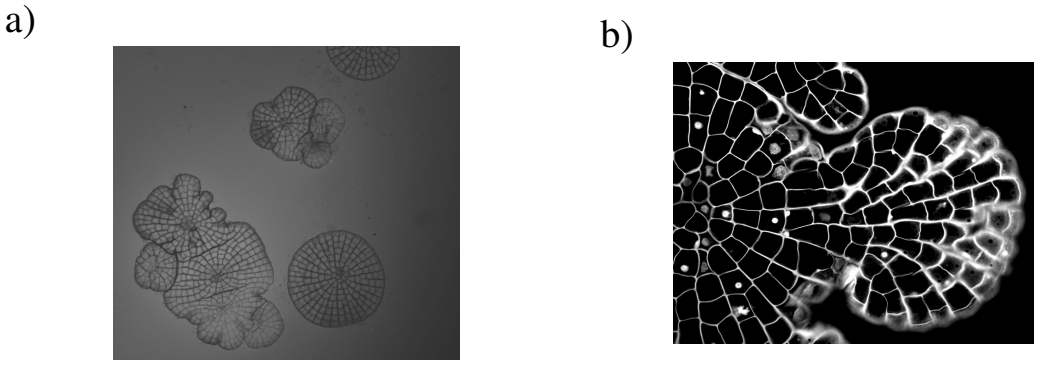

Fig. 1: a) the development of Coleocheate orbicularis, when unconstrained generate 2D circular tissues. Other morphologies may appear when contact between several colonies occurs or in the case of injuries b) (e.g. cell ablation).

Image segmentation

A cellular architecture can be described as a strongly hierarchical graph object constituted of different types of sub units e.g. a cell wall is constituted of two vertices (point at the crossing of three or more cells in a 2D system), a cell is constituted of walls, a tissue is constituted of cells etc… Each of this sub-units bear specific local information e.g. vertices are associated with spatial coordinates, walls are associated with mechanical properties and cell objects are associated with genetic information (*Fig. 2*). Image segmentation is used to extract information about this cellular architecture of live plant tissues as well as their kinematics and can be used as template for numerical simulations.

The segmentation of images is based on a marked watershed algorithm [2]. In marked watershed computation, the grey-scale image is seen as a topographic surface. The algorithm is initiated by a finite set of points (called seeds) on the images. This topography is then flooded from below by letting each seed be a source of water, which creates progressively larger basins of pixels having identical pixel label. A pixel in contact with a particular basin with intensity lowers than the basin is then associated to it. When the simulation reaches the maximum pixel intensity, each pixel of the image is associated to at least one initial seed label. A further processing is needed to reconstitute the whole architecture of cells. The complete architecture of the cellular structure (*Fig. 2*) is then reconstructed based only on pixels in contact with at least three basins, in a scale increasing order: a wall is defined by two

vertices sharing the same two basins. A cell is constituted of all the walls having the same basin in common.

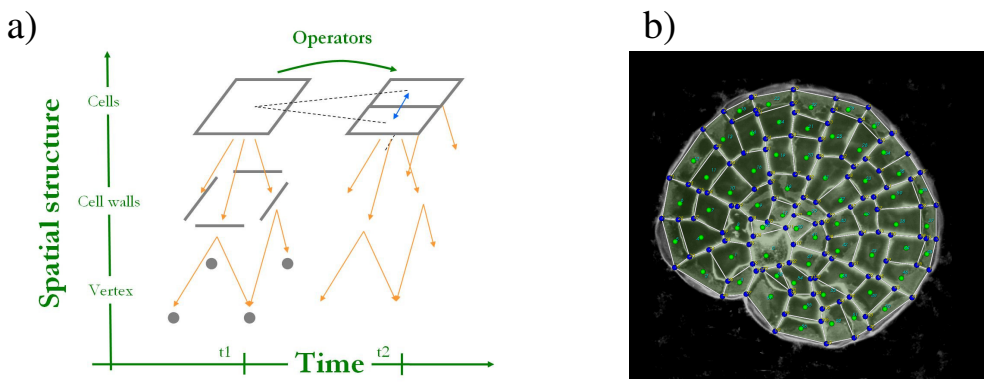

Fig. 2: a) Plant cellular architecture has a strongly hierarchical structure which entities are defined at specific spatial scales (vertex-wall-cell-tissue-organ …). b) The cellular structure of Coleocheate using watershed segmentation and reconstructed with the algorithms we have developed.

Mechanics of cell wall expansion

From a mechanical point of view, the cell can be seen as a closed thin walled structure, maintained in tension by turgor pressure (*Fig. 3*). The mechanism of cell expansion is then generally believed to result from a driving force, turgor, acting on a yielding cell wall material, where both of these factors are ultimately controlled by the plant metabolic activity [3]. More recent papers have proposed various other yielding behaviour ([4], [5] , [6]), but in the absence of strong experimental evidence, we chose to assume the simple viscous hypothesis to derive an analytical formulation of cell expansion (*Fig. 3*).

Kinematics: as it is observed experimentally that radial expansion and division concern exclusively the first layer of cells at the periphery of the colony [7]. Cell size (radial l_r and tangential l_t) in this layer is assumed remain constant. Finally, cell expansion in assumed to be linear in the layer of cell at the edge, and null for interior cell. Therefore, velocity field can easily be expressed as a function of the rate of radial expansion $\partial R/dt$ and the radial position r

$$\begin{cases} \mathbf{v} = \dfrac{r - R + l_r}{l_r}\mathbf{e_r}; r \in [R - l_r, R] \\ \mathbf{v} = 0; r < R - l_r \end{cases} \qquad (1)$$

The strain rates on $[R-l_r, R]$ in the cylindrical coordinate are $\dot{\varepsilon}_r = \dot{R}/R$ and $\dot{\varepsilon}_t = \dot{R}/l_r$.

Tensile forces in cell walls: Strain in cell walls in therefore constant and depend only on orientation. Therefore, assuming a perfect viscous material property, it is possible to relate expansion rate of the colony with tensile forces in cell walls:

$$\begin{cases} t_r = \mu.S_t.\dot{R}/R \\ t_t = \mu.S_r.\dot{R}/l_r \end{cases} \qquad (2)$$

where t_t is the tensile force in wall, μ is the viscosity coefficient ($Pa.s$) of the wall, S_t and S_r are cross sections of cell wall in tangential and radial direction. Wall cross sections were

assumed to remain constant throughout deformation i.e. no explicit cell wall synthesis is considered in the model.

Expansion of the colony: From the equilibrium of forces acting on cell wall, it is possible to derive an expression of radial growth of the system:

$$\dot{R} = \frac{(P - P_0)hRl_r l_t}{(S_t.l_r l_t / R + S_r R)\mu} \qquad (3)$$

When the radius becomes large relative to the dimensions of the cell, the expression of μ reduces to $(P - P_0)h.l_r l_t /(S_r \dot{R})$ and can easily be used for the determination of the viscosity coefficient from experimental data.

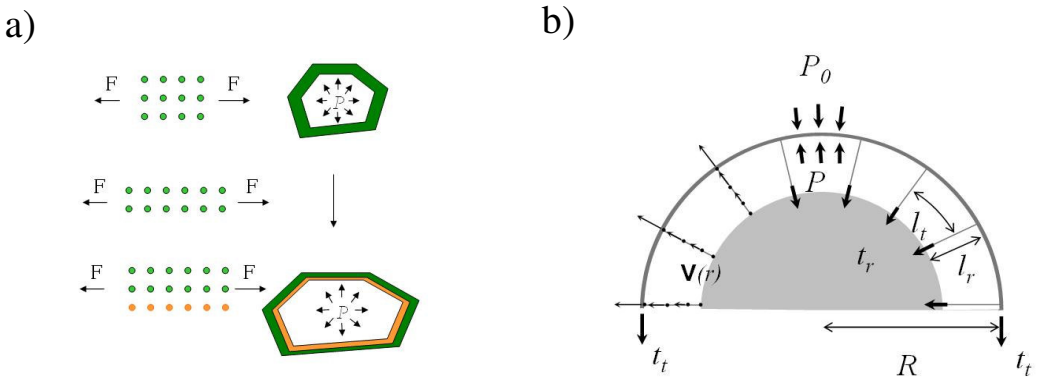

Fig. 3: a) Biomechanical model for cell expansion in morphogenesis: Cell wall response to turgor pressure through a viscous yielding of cell wall. b) the analytical model consist of a perfectly circular colony of radius R, with a constant radial l_r and tangential l_t cell length. The velocity field v *is centrifuge and linear for boundary cells. Forces in the structure consists of pressure inside (P) and outside (P_0) the cell, tensile forces in walls in the radial (t_r), and tangential direction (t_t).*

Multicellular computations

Software for the simulation of plant cell proliferation has been developed on the basis of previous work. [8]. Cells consist of individual automatons, characterized by a genetic activation state, whose behaviour is determined as a function of the signals it receives from its neighbourhood.

Computation of cellular wall expansion was performed using the finite element method. Because the structures being analysed are two dimensional, Euler Bernoulli beam hypothesis has been adopted. Time integration was carried according to a forward-Euler finite difference scheme and the scaled gradient conjugate method is used for solving the system of linear equations. The finite element mesh used for each wall consists of the two vertices defining its boundary and a mid point between them. Interior cells were considered to be rigid. The external force at the origin of expansion is turgor pressure, applied at each node of the finite element mesh.

The division of a cell occurs along one of two principal directions, parallel and perpendicular to the edge of the colony. A cell divides when its volume reaches a threshold, in the plane of minimal distance between existing walls. Threshold values for cell division where based on fixed cell size dimensions. During the simulation, the volume thresholds are drawn randomly using a normal distribution determined from experimental cell size distribution.

Results and discussion

The evolution of the size of the colonies, as measured from live imaging experiments, showed a linear increase of the radius with time (e.g number of cell has a quadratic growth). As expansion takes place at the periphery of the colony, the increase in radius of the colony is determined by the individual cells in the radial direction. The predicted evolution of radius with time from the analytical models and the finite elements simulations was compared to experimental data (*Fig. 4*) and reproduced similar linear radial expansion rate.
Investigations into the key mechanical behaviour occurring in morphogenesis have been carried out by simulating some strongly contrasting scenarios (*Fig. 4*), emphasizing potential mechanisms triggered by genetic activation of cells. Bending deformations were shown to be major mechanism influencing the way structures are established, as it has been emphasized by [9]. When moment of Inertia is decreased, the symmetry of expansion disappears because forces in radial walls tended to be homogenized and their expansion to vary with cell size. For the lowest values, however, bending of outside walls became the main factor driving the establishment of the structure. Each outside wall gives birth to an outgrowth, starting a new direction of expansion.

In the simple analytical model derived in the previous section, turgor pressure, cell size and viscosity coefficient were found to influence expansion rate identically. However, when such parameters are modified locally as it is found in real organisms, different types of mechanisms could be observed: locally increased turgor pressure was most efficient to generate outgrowth and initiated a second colony. The reason is that only an increase in turgor pressure can generate lateral forces at the early stage of the simulation. Lateral forces induce higher anticlinal division rate. An increased number of cells had higher turgor pressure which then accelerated the formation of the outgrowth. In all other cases, vertical forces on both sides of the wall are in equilibrium at the beginning of the simulation, until the outside walls are deformed enough to generate lateral forces. For the same reason, cell ablation generates secondary circular outgrouth as it is found on live injured tissues: The ablation of cell induced a decrease of turgor pressure in the neighbourhood of cells being isolated. It resulted in local isotropic expansion as opposed to dominant radial expansion in mature *Coleocheate*.

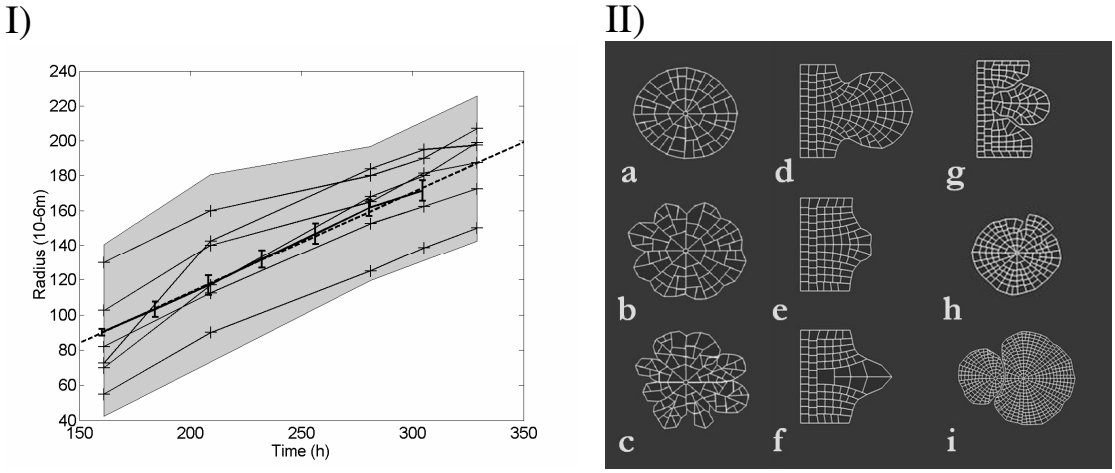

Fig. 4: I) Growth curve of the radius of the colonies as observed experimentally (+), as predicted by the analytical model (--) and simulated (—) and the 95% confidence interval in shaded grey. II) Cell proliferation patterns in various conditions: a-c) circular growth with various wall bending properties (moment of inertia of 2.10^{-21} m^4 - $5.10^{-22}m^4$ - 2.10^{-22} m^4). d-f) outgrowth resulting from local variation in turgor pressure (d), viscosity (e) and cell size (f). g-h) outgrowths resulting from the forces released from ablated cells. i) simulations of colonies getting in contact

Conclusion

In this study, we have been developing a mechanical computational framework for the simulation of morphogenesis in plant at the cellular level. A first set of simulations showed a range of possible forces and corresponding mechanisms can drive a biological system to establish its structure. Using models and computer simulations, it will be possible in the future to complement experimental investigations by isolating certain factors or visualising hypotheses to exclude incompatible biological assumptions. Integrating self-organised models for the regulation cell proliferation is now essential to go further in the understanding of the mechanisms, and particularly how the genetic programs interact with the key physical variables responsible for emergence of geometrical patterns.

Coleocheate orbicularis was used in this study as a first experimental system. The colonies adhere to a plastic or a glass substrate and grow as a cell monolayer. This simplistic structure and kinematics of growth makes *C. orbicularis* a unique and compelling new system for the study of the biomechanics of morphogenesis: High throughput time lapse microscopy can be implemented using computer controlled microscope with a robotic stage, automatic segmentation techniques can be used to extract and analyse kinematics of growth, simple analytical models can be developed and used to compared with numerical simulations.

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