Growth dynamics of the shoot apical meristem: global, cellular and sub-cellular approach

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The shoot apical meristem (SAM) is responsible for the whole shoot morphogenesis (stem and lateral organ formation). Different SAM zones contribute to organ initiation and its self-perpetuation. They exhibit various cytohistological traits, as well as growth rate and anisotropy, which dynamically evolve during SAM ontogeny. These zones are believed to be linked with the gene expression patterns through differential mechanical properties of the meristem tissue (Green, 1999), among which the outmost cell layer seems to play a crucial role.

The recent years have seen a growing interest in the modeling of SAM morphogenesis, in link with the genetic regulation and mechanical aspects. In order to make realistic hypothesis for these models and test them, there is a need for empirical data at different levels: (1) at the whole SAM level; (2) at the individual cell level; and (3) at the cell wall level. Here, we present a method, which has proven to be useful in obtaining information on the growth at the whole SAM level and which with further improvements can provide us with data on the cellular and cell wall levels. The analysis using this method is performed for shoot apices of *Arabidopsis thaliana* and *Anagallis arvensis*, two small dicot herbs. Computations are performed with the aid of original codes written in Matlab (The Mathworks, Natick, MA, USA).

The sequential replica method and existing protocols for growth and geometry assessment

The sequential replica method is a non invasive method allowing one to follow the shape and growth of an individual SAM surface for several days. Briefly, replicas (molds) are taken from the surface of an individual shoot apex at 12-24 h intervals for up to several days (Williams and Green, 1988).The molds are then filled with epoxy resin in order to prepare casts, which are observed in a scanning electron microscope, providing an indirect observation of the meristem surface at different instants. To reconstruct the 3-D shape of the surface, two images (a stereopair) are taken for every cast, one from a top view and the other tilted by 10º in the microscope chamber. Vertices, i.e. contacts of three anticlinal walls of neighboring cells, are used as marker points to reconstruct the 3-D shape of the apex surface. Their position is digitized on the first image and then semi-automatically recognized on the second. Differences in relative vertex positions on the two images are used to compute the third coordinate. Based on the comparison of successive replicas the same cells and vertices can be recognized on the individual apex surface at consecutive instants. This enables computation of growth parameters for each vertex based on the deformation of the triangle formed by its three nearest neighbors. Next, the parameters are computed for each cell as the average for its vertices (Goodall and Green, 1986; Dumais and Kwiatkowska, 2002). Growth is described by the principal directions of growth, growth rates along these directions, growth anisotropy and areal cell growth. For each 3-D reconstruction, shape of the SAM surface can be also quantified by means of curvature directions and Gaussian curvature.

The method has been successfully used to describe zones of different growth on the wild type *Arabidopsis* and *Anagallis* apices, showing that different growth patterns occur in inflorescence and vegetative

meristems (e.g. Kwiatkowska, 2006) and zones of different growth evolve in time, rather than being a steady field of growth. The existing computation method is thus sufficient to analyze growth and geometry on the whole SAM level. However, the stereoscopic reconstruction technique had several drawbacks which lead to imprecision in the reconstruction, and the growth computation protocols are based on averaging for SAM portions composed of more than a single cell. Therefore, we need to improve the computational part of the protocols in order to use the replica method at smaller scale (cell, cell wall).

Improvement of the stereoscopic reconstruction technique

Imprecision in the existing reconstruction technique is due to errors in the placement of each vertex on the two images of the stereopair, perspective deformation of scanning electron microscope images, and lens deformation of the microscope. The first step of the improved reconstruction technique involves an automatic robust matching of the features of the two images in order to recover the epipolar geometry. This is followed by an auto-calibration stage to correct the perspective deformation through recovering focal length for both images (Zhang 1998), dense matching of the paired images (Zitnick and Kanade, 2000) and, finally, triangulation leading to a dense high resolution reconstruction of the SAM surface (Hartley and Zisserman, 2000) (fig.1). This dense reconstruction can be afterwards approximated by a continuous surface, allowing a much more precise quantification of the SAM geometry, good enough for studies at the cell wall level.

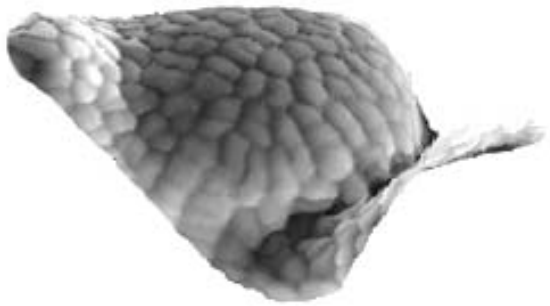

fig. 1. A dense reconstruction of the meristem surface

New questions to be answered and new empirical data to come from the improved protocols

The whole SAM level:

Previous studies show that morphogenesis at the SAM involves rather dramatic changes in both geometry and growth. We therefore speculate that some regions at the SAM surface are especially affected by tensile, compressive and shear stress. In order to identify such SAM regions we have to combine the local growth and shape analysis with an analysis of the vertex displacement field with respect to an arbitrarily chosen fixed region of the meristem. This is achieved through the superposition of landmarks (vertices) on the consecutive reconstructions with the aid of the procrustes algorithm (Eggert et al., 1997).With a good choice of the reference region and a careful analysis, non radial displacements can be interpreted, for example, as a consequence of shear occurring on the meristem surface.

The cellular level:

New models of meristem growth preferentially incorporate a realistic cell behavior, where cell shapes are preserved during the growth through a good choice of parameters of cell growth and divisions (e.g. Smith et al. 2006, Nakielski and Barlow 1995). Several rules have been proposed to link cell growth, shape and division planes, however, few experimental observations had been provided for their falsification.

The first rules have been proposed long ago by the plant biologists Hofmeister (1863), Sachs (1878) and Errera (1888). Hofmeister's rule states that if an organ grows in different directions, cell division planes are perpendicular to the direction of the fastest growth. According to the Errera's rule, new walls follow the shortest path that will halve the parental cell. The Sachs' rule states that the new cell wall meets parental walls at a right angle. A more general rule formulated by Hejnowicz and Romberger (1984) states that the cell division plane is always perpendicular to one of the cell principal growth directions. Using the improved protocols to compute growth for individual cells we aim to perform a statistical analysis of the presumable link between principal growth directions, cell shape and the orientation of division plane. We will also check whether the cell behavior is the same in the different zones of the SAM.

A preliminary analysis of the relation between maximal cell growth and the new cell wall orientation was already made. A Monte-Carlo method was used in order to exclude from the analysis the cells for which the direction of maximal growth cannot be robustly determined (due to cell growth being nearly isotropic, too small growth rates in comparison to the noise, or special cell shape). Then we computed the angle between the direction of maximal growth and the normal to the new cell wall.

One difficulty in our data interpretation comes from the fact that the growth rates we compute are averaged value for the time lapse between two replicas. A cell division wall visible on a replica (at time T2) could appear just before the replica was made, or just after the preceding replica (made at T1). As a result the growth computed between T1 and T2 is the sum of growth before *and* after cell division, in unknown proportions. We thus need to compute also the direction of maximal growth between times T0 and T1, and to compare its orientation with that of the new cell wall.

Preliminary results show that, for *Anagallis* cells, the direction of maximal growth computed for the timelapse T1-T2 is clearly correlated with the new cell wall orientation, PDG max being most often in the direction normal to the new cell wall (fig 2). On the contrary there is no clear relation between the direction of maximal growth between T0 and T1 and orientation of the new cell wall in T2. However, a comparison between growth in two successive time intervals suggests that some cells switch their growth orientation upon cell division. More data are needed to draw clear conclusions, and other factors (cell shape, curvature, position on the meristem) have to be taken into account in the analysis.

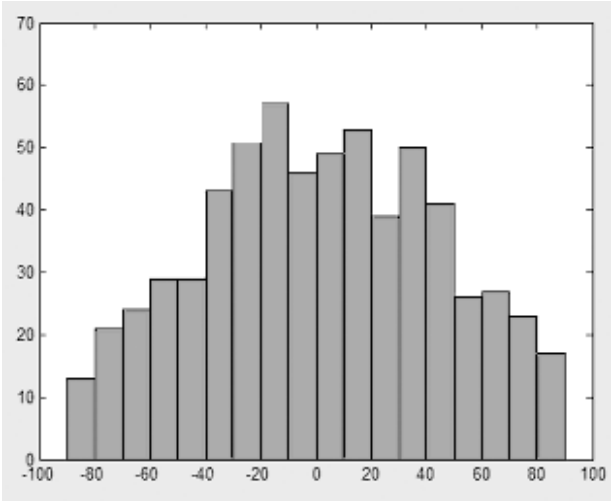

fig. 2. Angle (in degrees) between normal to the new cell wall and direction of maximal growth

The cell wall level:

Just after division, the daughter cells do not have the typical shape of older cells, which are most often pentagons or hexagons with the cell walls meeting at approximately 120°. Since the new cell walls, as stated by Sachs' rule, are supposed to appear always at right angles with respect to the old ones, the angle between walls has to change in time. This change of cell shape is possible only if there is a difference in growth rates between the different walls of the same cell. Two antagonistic models have been proposed. D'Arcy Thompson (1942) speculates that the new formed cell wall gradually stiffens so that its tension progressively approaches the older walls tension, while Korn (1980) supposes that the young cell wall is not able to grow for a few cell cycles. Both these models can explain theoretically the changes in angles between cell walls (Lloyd 1991). We aim to test them by following actual changes in cell wall growth and angles between walls, thus providing both important data for SAM modeling and a clue on the development of cell wall properties after its formation.

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References

D'Arcy Thompson W, 1942. On growth and form. Cambridge University Press, Cambridge.

Dumais J, Kwiatkowska D. 2002. Analysis of surface growth in shoot apices. Plant Journal 31, 229-241.

Eggert DW, Lorusso A, Fisher RB. Estimating 3-D rigid body transformations: a comparison of four major algorithms. Machine Vision and Applications 9, 272-290.

Goodall CR, Green PB. 1986. Quantitative analysis of surface growth. Botanical Gazette 147, 1-15.

Green PB. 1999. Expression of pattern in plants: combining molecular and calculus-based biophysical paradigms. American Journal of Botany 86(8): 1059-1076.

Hartley R, Zisserman A, 2000. Multiple view geometry in computer vision. Cambridge University Press.

Hejnowicz Z, Romberger JA. 1984. Growth tensor of plant organs. Journal of Theoretical Biology 110, 93-114.

Korn RW, 1980. The changing shape of plant cell: transformations during cell proliferation. Annals of Botany 46, 649-666.

Kwiatkowska D, 2006. Flower primordium formation at the Arabidopsis shoot apex: quantitative analysis of surface geometry and growth. Journal of Experimental Botany 57(3), 571-580.

Lloyd CW, 1991. How does the cytoskeleton reads the laws of geometry in aligning the division plane of plant cells? Development Supplement 1, 55-56.

Nakielski J, Barlow PW, 1995. Principal directions of growth and the generation of cell patterns in wild-type and gib-1 mutant roots of tomato (Lycopersicon esculentum Mill.) grown in vitro. Planta 196(1), 30-39.

Smith RS, Guyomarc'h S, Mandel T, Reinhardt D, Kuhlemeier C, Prusinkiewicz P. 2006. A plausible model of phyllotaxis. PNAS 103(5) , 1301-1306.

Williams MH, Green PB. 1988. Sequential scanning electron microscopy of a growing plant meristem. Protoplasma 147, 77-79.

Z. Zhang, 1998. Determining the epipolar geometry and its uncertainty : a review. IJCV 27(2), 161-198.

L. Zitnick, T. Kanade, 2000. A cooperative algorithm for stereo matching and occlusion detection. IEEE Transactions on Pattern Analysis and Machine Intelligence 22 (7), 675-684.