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High-resolution kinematics of gravitropic movement reveals an oscillatory dynamics. A key for efficient performance?

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Abstract

The spatial display of stem and leaves is crucial for the functioning of plants and their mutual interactions. It involves complex movements: stem change curvature and orientation to grow vertically (gravitropism) and these movements are continuously regulated by the perception of the current shape (autotropism) [1]. If a lot is known about the Physiology, very few studies have dealt with the kinematics and dynamics of these movements, and the corresponding relevant physical and biological aspects (see [2] for a recent review). In this work we focused on the reaction of a wheat coleoptiles using a novel high spatio-temporal kinematical method called "Rod PIV" combining Particle Image Velocimetry [3] and contour and median line detection of the organ: When its orientation vs the gravity was modified by tilting, the organ bent through local variation of curvature due to differential growth, and displayed a complex spatio-temporal pattern including oscillatory and propagative aspects. Moreover elongative and differential growth displayed coupled propagative oscillation. The insights of these results in terms of the modelling of the biomechanical and regulatory processes of tropic bending are discussed as well as the possible adaptive significance of these coupled oscillators for the gravitropic performance.

Introduction

Most of the plants are growing straight and vertical in all or a part of their aerial system (orthotropism). Then they display their branches in the space around in order to maximise light capture. Verticality could thus provide a selective advantage in order to reach the sunlight [4].

This orthotropism is achieved through active growth movements involving i) phototropism, driven by the perception of direction of the light gradients, and ii) gravitropism, controlled through gravity perception [5] and demonstrated readily by orthotropic movements in the dark. These active tropic movements have also been shown to be required for continuous posture control [6]. Plants are thus constantly moving and growing, although at much lower rates than animals.

Plants display two different types of growth. The primary elongation growth sits in a zone localised near the apical meristem. The secondary growth is a property of many dicots; they grow in diameter in the non-elongating basal part of the stem. Both types of growth can produce equivalent tropic motion [7], but only movements related to primary growth will be studied here.

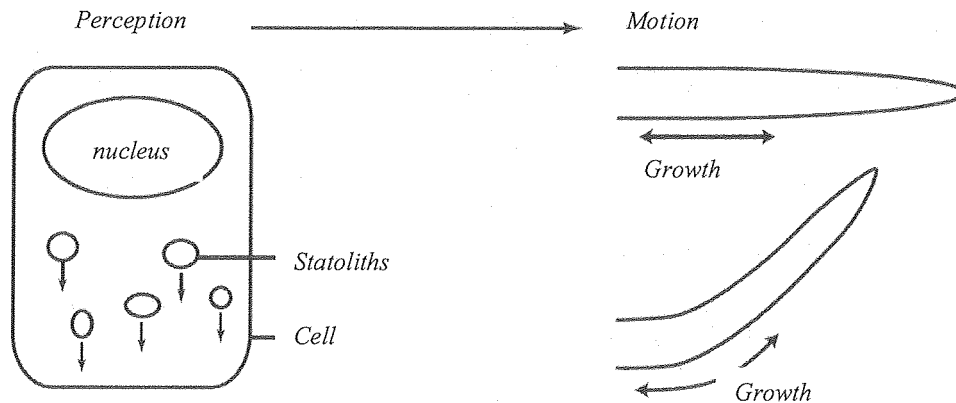


Fig 1. First cells perceive a signal. It comes from the sedimentation of the statoliths that apply a pressure on the lower wall. Many molecular mechanisms are involved in the transcription of the signal. Plants sense their local slope and respond through a transverse gradient in growth rate, called differential growth. Thereby plants can curve and reorient according to the stimuli.

Tropic movements in primary growth zone involve distributed curving powered by differential growth (see Figure 1). Many experiments have been done on such gravitropic movements since the pioneer works of Darwin, Pfeffer and Sachs (see review in [2]).

One of the classical experiments is to quantify the influence of the presentation angle on the gravitropic reaction [5, 8-10]. By looking at a tilted cylindrical organ, like coleoptiles or hypocotyls, this experiment focuses mainly on the variation in time of the apical angle. When comparing this angle with the presentation angle, a sine relation can be observed, but only for angle below 90° (see figure 2).

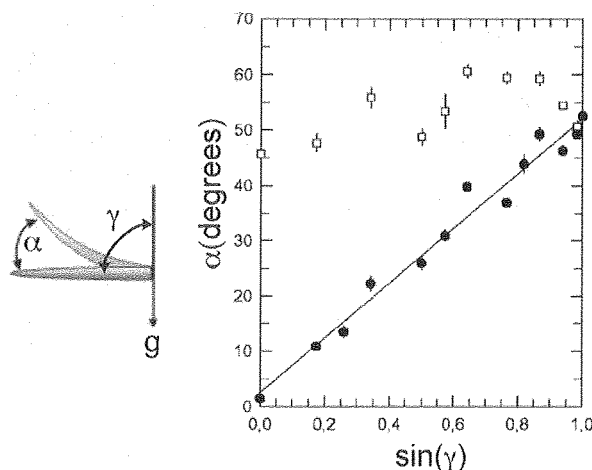


Fig. 2. From [8], Galland gives an example of sine law. *Avena* coleoptiles were placed in dark boxes and tilted from the vertical with an inclination angle γ . The orientation of the tip α were determined after 3 hours. For inclination angle below 90° the orientation of the tip is linearly related to the sine of the inclination angle (black dots). When the inclination angle is above 90°, the relation is modified (white squares).

In long time experiments, a deviation from the classical sine law occurs. As the organ is growing, and curving in an extended zone, this deviation cannot be understood by just looking at the tip angle. Indeed when the end of the stem is vertical, the basal curving still goes on. A continuous regulation of the orientation is therefore necessary to keep the apical part vertical when the basal part is curving (see figure 3). Moreover, the organ starts to straighten up its tip before it reaches the vertical avoiding overshooting. This has been called “auto tropism” because the plants need to get information on its self-shape to do so [1].

More recent works have been conducted to quantify the whole kinematics of plant growth movements. However several of them have neglected the measurement of the longitudinal growth (elemental elongation field), although growth is crucial to the mechanism (e.g. [11, 12]). Some other works have studied growth but not curvature. There are some recent studies on the whole kinematics of the two processes [13-15] but only in roots. However, the kinematics and mechanics of root gravitropism is different from that of aerial organs and in a way less complex in roots, the growth zone is really small and localised near the apex. And the root is supported by soil or nutrient solution buoyancy. There is thus no need for a postural control, and the shapes could be convoluted. In this

study we propose to analyse the whole kinematics of the straightening of wheat coleoptiles. To this aim we need to quantify at the same time the curvature and the elongation of the organ.

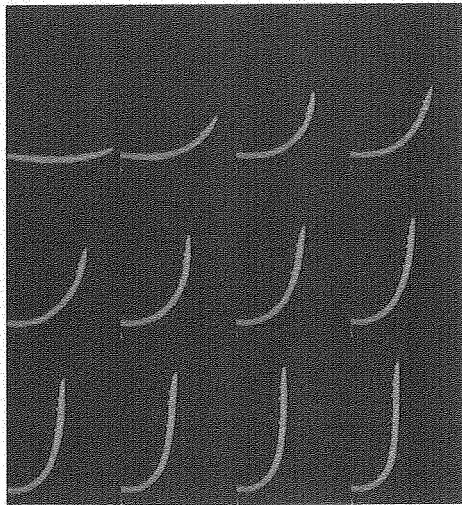


Fig 3. Straightening of a wheat coleoptile. From left to right and from top to bottom, the interval between each image is 2h. We see that the curvature is distributed over the whole length, and then once a part is vertical it remains even if the basal part continues to bend.

Material and methods

Plant material and treatments

Seeds of wheat (Recital cultivar) were soaked in water during 6 hours before being seeded. To be able to control the initial vertical of seedlings, the seeds were stuck on a tube filled with water at an angle of 30°, and the tubes placed in static holders with 1 rotational degree of freedom. The germination were set in a dark chamber at 25°C. After 3 days, when they were 10-20 mm long, they were transferred into a dark room at a 25°C, and placed in controlled initial inclination angle of 90° in the holders.

Time-lapse photography, were taken, every 10 to 15 minutes, with a camera Olympus SP-560UZ. In order to limit the photomorphogenetics effects during the photography, the flashlight of the camera was filtered to “safe” green light using a green LEE filter (139 Primary Green), (green light is far less perceived by plants than others wavelengths). Two series of experiments were performed in parallel, i) one with vertical coleoptiles, to get the growth field in the vertical position. ii) One with coleoptiles tilted 90° from the vertical. Each observation standed for 24 hours in order to let the organ to record the entire kinematics of gravitropic straightening movements.

Image analysis

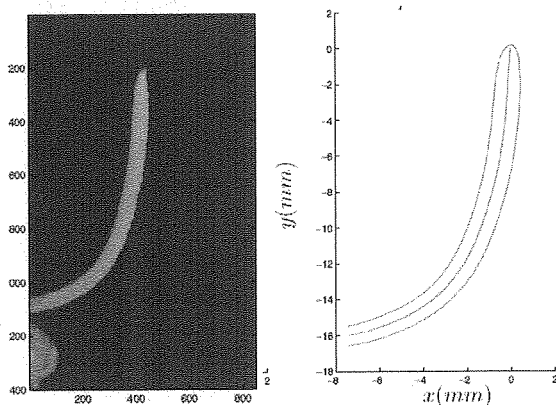


Fig. 4 The first part of the analysis process. We get the contour of the coleoptile, and then by voronoi skeletonisation we are able to find the median line.

Images were analysed with a specific Matlab program developed for this problem. As the coleoptile is a slender cylindrical organ, it can be described as a rod defined by its median line and by the radius of the rod as a function of the distance from the tip or curvilinear abscissa s . The elongation growth can then be recorded by monitoring the position of marker points $S(t)$ along the median line (see Figure 4) whereas the tropic movement can be described by changes in the local angle $a(s,t)$ and by local curvature $C(s,t)$ (i.e. the lineic rate of change in angle along the stem)

$$C(s,t) = \frac{\partial a(s,t)}{\partial s} \quad (1)$$

Where s is the curvilinear abscissa along the median line from the tip to the base and t is the time. To obtain the growth rate a fluorescent orange dye was set on the stem and used a set of markers to follow the displacement of each point. (Figure 5) In order to get the displacement $\delta(s,t)$ along the stem a modified version of the PIV (Particle Image Velocimetry) [3, 16] called Rod-PIV has been developed, in which the sub-window is moving only along the median line.

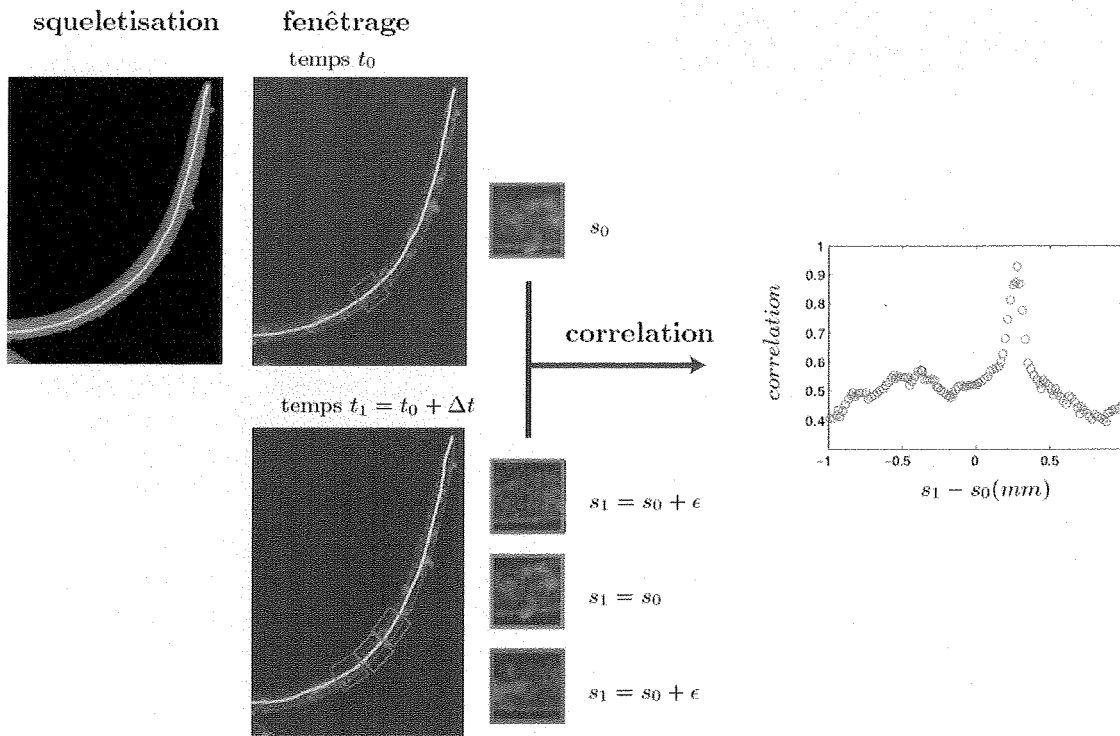


Fig. 5 Elongation along the median line. We separate Red and Green channel in the image. With the green channel we get the skeleton. Then we define sub-windows on the red channel. They are centred on the median line, as we have now directly the curvilinear abscissa s . We make the correlation of each sub-window $s = s_0$ at time t_0 with other sub-window as $t_1 = t_0 + \Delta t$, with the distance $|s_1 - s_0| < \epsilon$. Here we take $\epsilon < 1mm$. The correlation peak gives the displacement $\delta(s,t)$ on the line between two images.

By spatial derivation of the growth velocity we obtain the elemental elongation rate $E(s,t)$.

$$E(s,t) = \frac{1}{\Delta t} \frac{\partial \delta(s,t)}{\partial s} \quad (2)$$

E has been also called Relative Elemental Growth Rate or Strain rate in the literature; see [2, 17] for reviews.

As tissues elements are being stretched away by elongation, the derivative of curvature, according to position from the apex, is just a partial view of the change occurring to the organ segment (i.e. to a

group of cells). The material derivative (or derivative following growth induced points flow), has to be used, as introduced in plants studies by Silk [17])

$$\frac{D}{Dt} = \frac{\partial}{\partial t} + v(s,t) \cdot \nabla \quad (3)$$

Where v is the displacement velocity and ∇ is the gradient operator. The first term is the local variation in time on each position. The second accounts for the convection, the displacement of each point. Given the distribution of the elemental elongation rate, it is then possible to calculate the material derivative of the curvature using (3).

Results and discussion

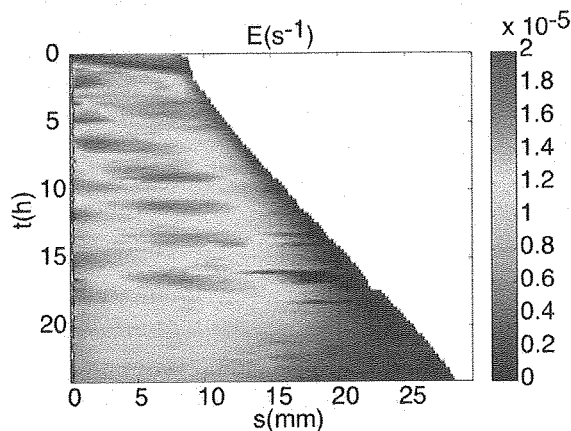


Fig 6. Measurement of the growth field according to the curvilinear abscissa in abscissa, from the apex to the base, and the time in ordinate. Oscillations appear, the period is around 2 hours.

In vertical coleoptile, the E field is not stationary (Figure 6). One can see phase of maximal elongation $E \sim 2 \cdot 10^{-5} s^{-1}$ alternating in time with a phase of minimal $E \sim 1 \cdot 10^{-5} s^{-1}$. These phases are regularly spaced in time. The period between two oscillations is approximately 2 hours. By following the position of the maximal elongation in the spatio temporal diagram figure 5, we see that the maximum is propagating from the apex to the base. There is a delay of 1 hour between the oscillation starting at the apex and reaching the base.

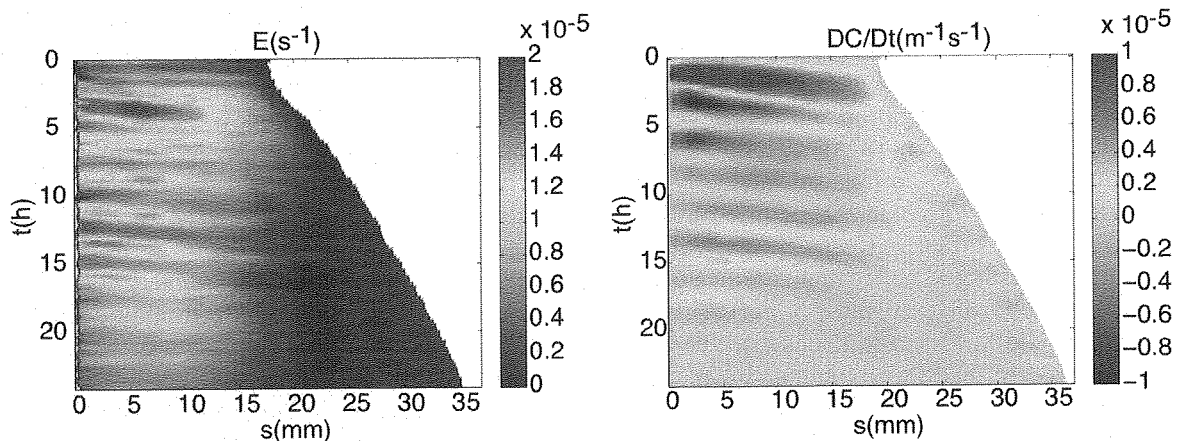


Fig 7. Left: Growth field of a tilted coleoptile. The results are comparable to previous on vertical coleoptiles. Right: Material derivative of the Curvature. After a first wave of curvature along the stem, oscillations clearly appear with the same period than the elongation pattern. The axes are the same than in the Figure 42. But the colormap accounts for the material derivative of the curvature in the right image.

The growth zone has a limited spatial extension in every coleoptile, independently of the flashlight frequency and of the temperature variations (data not shown).

In tilted coleoptiles (Figure 7), the spatio-temporal pattern of elemental elongation is unchanged. The oscillations have same frequency and amplitude. The propagation of the elongation is still present. The size of the growth zone remains the same. Therefore no redistribution or amplitude change in the elongation at the median line (i.e. the average elongation on the section) can be observed. The tropic movement is just due only to the lateral redistribution of the differential growth.

Near the base, curvature displays very little change, due to the low growth rate. Near the apex we observe oscillations of the material derivative of curvature, with a frequency similar to that of elemental elongation. Oscillations in the material derivative of curvature also displayed a spatial decay. However these oscillations were more damped. When the coleoptiles apex reached the vertical the amplitude of the motions went down.

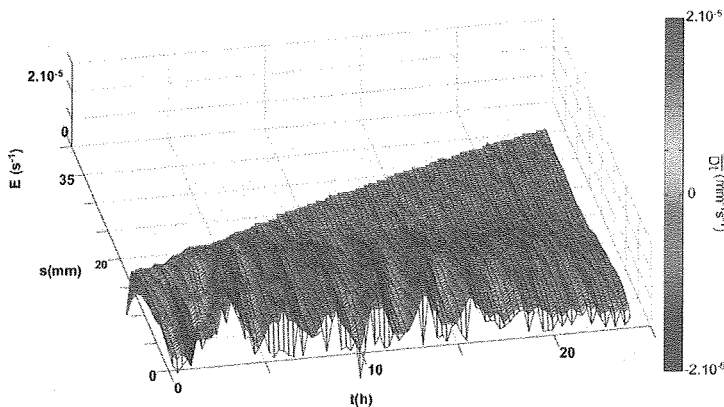


Fig 8. Surface of the growth field of a tilted coleoptile. The colour means the material derivative of curvature. The correlation between the two is really strong. When the growth is minimal the coleoptile bend otherwise it goes straight.

As a consequence the spatio-temporal field of the curvature variation is strongly correlated with the spatio-temporal field of elemental elongation (Figure 8). When the elongation is minimal, the coleoptiles curve. In the zone of maximal elongation, the coleoptile straightens up (decurve) or even bends in the opposite direction. Such coupling is not a geometric necessity and reveals a biomechanical coupling of the two processes. Despite very considerable changes in angle over time, the coleoptiles never overshoot the vertical.

Discussion

High-resolution kinematics analysis revealed that the tropic behaviour of coleoptiles is more dynamic than expected from the analysis tip angle and the sine law. The motion does not only consist on bringing the apex back to the vertical. Two different phases could be distinguished. First, the coleoptile bends on his whole length, during 3 hours, then it bends and straightens up alternatively in order to regulate its position and straighten most of its length. This last behaviour has been called auto tropism in literature [1, 18]. The straightening process could not be dependant of external stimuli, like gravity, but need to be induced internally although the inherent mechanism remains unknown. The sine law can only account for the first part of the straightening and neglect completely the auto tropic part.

It is interesting to notice that ultradian oscillatory growth pattern was observed for every coleoptile, whatever its inclination (tilted or not). This ultradian pattern was well reproducible from one plant to other and did not seem to depend on the orientation of the organ. Oscillatory growth patterns have only been described for circumnutating plants [19, 20]. In order to allow the plants to bend in different direction there is a differential growth across the section of the organ, with maxima and minima in the bending plane. In circumnutating organs the plants rotates by rotating their bending plane. So the position of the maxima and minima of elongation are moving around the cross-section of the stem. In that case the elongation on one side of the stem displays oscillations in growth between phases with maxima of elongation when the stem bends away, and minima when it bends toward the studied side. However upon gravitropic stimulation nutational movement were lost (see also [2]) the oscillations observed in this study were not due to rotations in the positions of minima and maxima around the