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A new scenario for gravity detection in plants: the position sensor hypothesis

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Abstract. The detection of gravity plays a fundamental role during the growth and evolution of plants. Although progress has been made in our understanding of the molecular, cellular and physical mechanisms involved in the gravity detection, a coherent scenario consistent with all the observations is still lacking. In this perspective paper we discuss recent experiments showing that the response to inclination of shoots is independent of the gravity intensity, meaning that the gravity sensor detects an inclination and not a force. This result questions some of the commonly accepted hypotheses and leads to propose a new “position sensor hypothesis”. The implications of this new scenario are discussed in the light of different observations available in the literature.

1. Introduction

Gravity perception by plants plays a key role in their development and acclimation to their environment, from the direction of seed germination to the control of the posture of adult plants. This can be demonstrated by the ability for the shoot to recover a vertical posture independently from light clues when inclined to different angles. This ability is broadly observed among the plant world, from small wheat coleoptiles to trees [1] (Fig 1a and b). Roots also are sensitive to gravity and can adjust gravitropically their direction to grow deeper in the soil (Fig. 1c). This phenomenon has been named gravitropism (from *gravi*, gravity and the Greek *tropein*, to turn). The observation and the study of gravitropism go back to the 19th century with the pioneering work of Julius von Sachs (1868) and of the Darwins (1880) (reviewed in [2–4]). More than a century later, our understanding has significantly improved, but many questions remain open [4–6].

Among the key issues is the sensing mechanism. A candidate model for the sensing mechanism should meet two requirements: 1) It should explain how cells sense their change in orientation at the cellular level. It should explain in particular what organelles are involved, what variable is sensed and leads to the primary physiological reaction, what molecular players are involved, and what is the timing of the different phases. 2) It should be consistent with the characteristics of the macroscopic response of the plant, such as the characteristic times to start the response and to converge to the vertical, the influence of the angle of tilt and of the duration of the tilting stimulus [7, 8]. The research tactic to achieve the identification of the gravisensing mechanisms has thus involved studies at both

the cellular level and the macroscopic level of the organ. Currently the prevailing scenario is the following.

1.1. The prevailing scenario

The perception of gravity starts in specific cells that act as statocytes (from *stato*, static position and *cyte*, cell). Indeed the suppression of these specific cells strongly inhibits gravitropism [9, 10]. Statocytes are located in different organs. In coleoptiles they are found in a thin layer near conducting tissues; in young stems, within a thin layer near the endodermis; in roots, within few columns of cells located in the central root cap, called the columella; and statocytes have recently been localized in the secondary phloem of mature woody stems [11]. These cells contain specialized organelles called statoliths. Being denser than the surrounding intra-cellular fluid, the statoliths move in the direction of the gravity Fig. 1d and exert a force, presumably on the plasma membrane, that provides the information about the direction of gravity (the “statolith hypothesis”) [5]. When the orientation of the organ with respect to the gravity vector changes, the statoliths change position, exert force on a new part of the cell, which in turn induces the relocalisation of membrane transporters called PIN proteins. The PIN proteins then redirect the flow of auxin, the major plant hormone, leading to a differential growth between the two faces of the organ and ultimately to the organ bending back to the desired orientation with respect to gravity. (Chodlony-Went hypothesis) [2].

Although some consensus has been reached about the main scenario described previously, many questions remain open, from the early steps of gravity sensing to the auxin

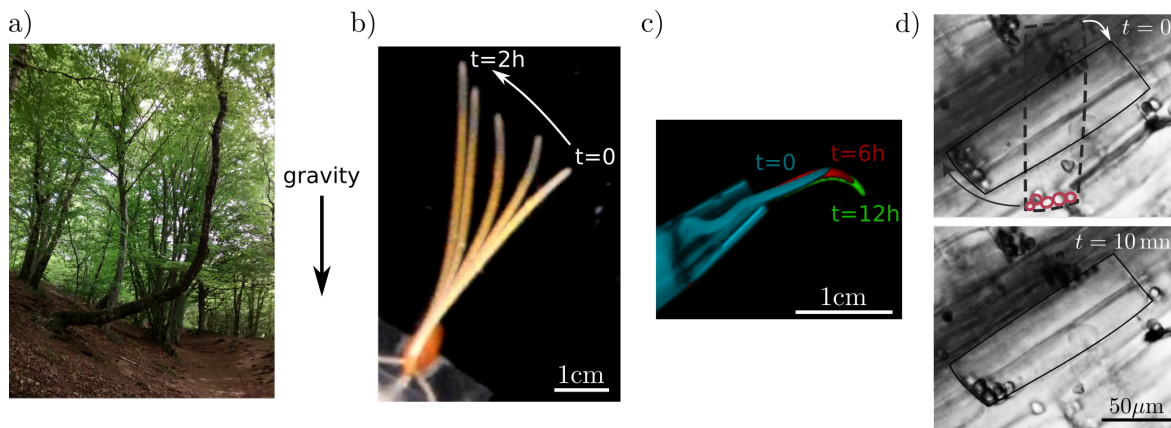


Figure 1. Illustration of gravitropism: a) on a tree, b) on a wheat coleoptile, c) on a lentil root; d) pictures of a statocyte (delineated in black) in wheat coleoptile showing the position of the statoliths just after inclination and 10 min later. Their previous positions are delineated with dotted red lines.

pathway leading to differential growth. In this perspective paper, we focus on the gravity detection mechanism, reviewing results obtained both at the cellular and macroscopic plant scale (for a focus on the more downstream events involving auxin transport and growth response, see [12]).

1.2. Open questions and hypotheses at the cellular and macroscopic scales

At the cellular scale, the exact nature and roles of the statoliths, and whether or not they are necessary for a graviperception is still a matter of debate. It is generally accepted that the amyloplasts (i.e. organelles filled with starch grains) are the main statoliths in the statocytes (the “starch-statolith hypothesis” [5, 13, 14]). Indeed in experiments using mutants deprived of starch and displaying little if any cellular sedimentation of amyloplasts, the macroscopic response to a gravi-stimulation is dramatically diminished compared to the wild type [15–18]. However, a response still exists suggesting that amyloplasts may not be the sole statoliths or that they act in gravity detection as “enhancers” without being necessary. On the other hand, experiments carried out by

artificially moving the statoliths using strong magnetic field gradients [19,20] unambiguously show that the plant bends in the direction of the displacement. Another study implicated statoliths by using mutants having a rigid vacuole [21]. The rigidity of the vacuole prevented the statoliths from moving and as a result, the response of the mutant dramatically decreased. In conclusion, there is no doubt that amyloplasts convey information about the gravity field, but other actors may also play this role. Mitochondria and Golgi apparatus have been shown to sediment (although much more slowly than statoliths) in oat coleoptiles and have been proposed as secondary statoliths [22], although this point is still disputed. Other authors called for an alternative mechanism resulting from the sensing of the pressure due to the weight of the cytoplasm acting on the membrane or cell wall [17] (the “protoplast pressure hypothesis”). The two pathways (the statolith-enhanced pathway and the protoplast pressure pathway) may even co-exist [23].

The nature of the signal detected by the statocytes is another fundamental question which remains largely open. And, here

also, different hypotheses have been developed. Some authors argue that the statocytes are sensitive to the pressure exerted by the statoliths on the membrane or on the endoplasmic reticulum [24, 25]. This could be achieved as the statoliths promote the opening of mechano-sensitive ion channels, either directly or through interaction with the actin cytoskeleton [26–28]. A second hypothesis relies on the role of protein complexes (called TOC) located in the envelope membrane of plastids which could contribute by functioning as a gravity signal transducer [29]. In this case, the proximity between the TOC and interactors in the plasmic membrane is the only requirement ; no pressure is needed. But only the first hypothesis has been fully tested in the framework of macroscopic reactions (sine law, $g \times t$ transients... as detailed later) [30].

During the sedimentation of statoliths, certain physiological reactions have been documented in hypocotyls and in roots: a change in apoplast pH [31], an increase in intracellular reactive oxygen species concentration [32] and changes in cytosolic calcium concentration [33]. Even if the involvement of these actors in gravity signaling pathway is sustained, the relationship between them is also not completely deciphered [34]. At the end of the signaling pathway, the differential cell elongation leading to bending is triggered by redirected auxin flux to the lower side of the plant organ [35]. Numerous studies have showed that the directionality of auxin flow within tissues is determined by a polar cellular localization of auxin export carriers, in particular PIN-FORMED (PIN) proteins [36, 37]. After an inclination of roots or hypocotyls, the localization of the PIN proteins, mainly PIN3, becomes repolarized, [38–40], leading to the redirected auxin flux. However the mechanisms describing how

the sedimentation of statoliths triggers a relocalisation of PIN have not yet been elucidated.

Surprisingly, questions are also open at the organ scale. Even though a precise knowledge of the response at the organ level might help discriminate between proposed explanations, only few studies have quantitatively looked at the bending kinematics and at its sensitivity. They fall into three very different approaches. In the first type, the focus is on the response of the organ to different inclinations from the vertical. The response varies linearly with the sine of the inclination angle, a relation known as the “sine law” [41]. However, despite its naming as a “law” and its popularity in textbooks, few reliable measurements and assessments of the “sine law” can be found in the literature, and concern a very limited number of organs and species ([7, 42, 43], see [4] for a review). In the second type, the focus is on the response to transient exposure to gravity. In this case the gravity is “switched on and off” either using microgravity experiments in which transient gravity is mimicked through centrifugation, or using clinostat experiments on Earth in which a transient compensation of the gravity sensing is provided by changing the plant inclination continuously before significant statolith downward motion can occur [6, 44]. Measurements suggest that the response to transient gravity is proportional to the dose, namely the product of the gravity intensity with the time of exposure (the “reciprocity rule”). Finally, the third type relies on varying the mass of the amyloplasts in the statocytes either through drastic hormonal treatments [15], or through a range of starch-less and starch-excess mutants [30] and studying their response to tilting (on Earth). It was found that the macroscopic responses increases with the mass of the statoliths.

1.3. The standard synthesis: the “gravity-force sensing hypothesis” and the “starch-statolith weight model”... and its call into question

Combining such a wide range of phenomena and hypotheses for gravity sensing at the cellular and macroscopic scales into a single framework has remained a challenge. However a standard synthesis has been widely accepted (more or less implicitly) to explain the cellular and macroscopic responses related to processes upstream of the development of a gradient in auxin concentration. It is based on a “gravity-force sensing hypothesis” [23]. Its most accepted version is the “starch-statolith weight model”, which stipulates that the statoliths sediment after tilting, and that the detected signal is the force exerted by the sedimented or sedimenting statoliths on the lateral side of the cell, or on the side of internal structures like the cytoskeleton, or the endoplasmic reticulum. In this hypothesis, gravisensing should then depend on the mass of the statoliths and on the gravity intensity.

In this perspective paper, we discuss recent results we have obtained on shoot gravitropism, showing that the response of a shoot is insensitive to the gravity intensity and, hence, insensitive to the weight of the statoliths or protoplast, but solely depends on the inclination between the shoot and the gravity vector [45]. The gravity sensor in plants thus works as an inclination sensor and not as a force sensor. This finding dismisses the “gravity force sensing” paradigm, requiring an alternative “position sensor model” to unify the cellular and macroscopic results.

At the cellular level, we point out that the collective motion of the statoliths during tilting experiments on Earth is actually not the sedimentation of a suspension, but the

dynamics of a grain-pile. We discuss the strong implications of these two results and how they help us to revisit the gravisensing pathway. We then propose an alternative theory, the “statolith position hypothesis”. Our hypothesis is that the relevant parameter sensed by the statocytes is the position of the statolith pile. In a vertical organ, the statoliths settle down and form a pile at the bottom of each cell. When inclined, they avalanche on the lower side of the cell, and their new position provides the information about the direction of gravity. We will show that this “position sensor model” can provide an explanation for the sine law and also gives an interpretation for the response to transient stimuli, the reciprocity rule, and may also account for the phenotype of the starch mutants. We finally speculate about a possible mechanism to connect this statolith-pile position with the distribution of the auxin transporters (PINs), and the onset of a lateral gradient in auxin concentration.

2. Response of a shoot to permanent stimuli: influence of gravity intensity

A shoot initially inclined bends up actively and goes back to its original orientation with respect to gravity (often but not always vertical). Having a proper quantitative knowledge of the response is crucial to phenotype mutants and to compare this phenotype with microscopic modeling of statolith motion and sensing. However, relatively few studies address the issue and they are often difficult to compare. One difficulty comes from the choice of a quantitative measurement of “the gravitropic response” [4]. Some authors have chosen to measure the angle of the tip of the stem after a given time [42, 46], while others measure the differential growth between the top and the

bottom face of the inclined stem [47], or use the temporal variation of the angle [1]. Another difficulty to properly measure the gravitropic response comes from the fact that the movement is not controlled solely by gravity perception. A cross-talk with a second concurrent sensing comes into play: the sensing by the organ of its own local curvature independently of gravity, called proprioception [48]. A minimal unifying model for the combined control through gravisensing and proprioception has been developed and validated on many species [1, 49]. This approach opened new perspectives and suggested that the response to inclination should be revisited and that care should be taken when analyzing the macroscopic response. Measurements should be done when gravisensing is dominant compared to proprioception, which means when the curvature is negligible, i.e. at the beginning of the bending. It also means that measure should be done on organs sufficiently stiff so that they do not bend under their weight when inclined.

To go beyond the specificity of each species and find general tendencies, it is useful to work with a defined dimensionless number. With these precautions in mind, it is possible to define a relevant dimensionless measure for the gravitropic response of shoots, as proposed by Bastien et al. [1, 49]. The dimensionless gravitropic response number introduced by Bastien et al. relies on the comparison of the bending velocity to the growth velocity. In the following, we explain how this definition can be derived from kinematic arguments, before presenting the response of shoots to permanent stimulus at various inclinations and gravity intensities and discussing the so called ‘‘sine law’’.

2.1. A valid measure of the gravitropic response

To define a valid macroscopic quantification of response of an organ (shoot or root) to a change in gravity condition, we treat the growth mechanism at the origin of bending. Let us consider an initially straight stem, for which there is no proprioception. Once the shoot is inclined, the change in gravity direction induces an asymmetric flux of auxin and differential growth. A relevant response would then correspond to the measure of the relative asymmetry in auxin concentration, namely the difference in auxin between the top and the bottom sides compare to average concentration of auxin. This dimensionless response $\tilde{\Delta}$ can then be written as:

$$\tilde{\Delta} = \frac{\phi_{\text{low}} - \phi_{\text{up}}}{\phi_{\text{low}} + \phi_{\text{up}}} \quad (1)$$

where ϕ_{low} is the auxin concentration in the bottom half, and ϕ_{up} the auxin concentration in the top half of the stem.

It is known that the growth rate in shoots is an increasing function of the auxin concentration until an optimum concentration is reached [50, 51]. Therefore, in a finite range of auxin concentration, it is possible to linearize the law and to assume that the elongation rates $\dot{\epsilon}_{\text{low}}$ and $\dot{\epsilon}_{\text{up}}$ (i.e. the relative change in length per unit of time on each side) are proportional to the concentration of auxin:

$$\dot{\epsilon}_{\text{low}} = k\phi_{\text{low}} \quad \text{and} \quad \dot{\epsilon}_{\text{up}} = k\phi_{\text{up}}. \quad (2)$$

The proportionality factor k may depend on genetic factors or environmental factors such as temperature. The difference in elongation rate between the two sides induces a bending as shown in Fig. 2. An initially straight piece of shoot of length L and diameter $2R$ ends up after a time dt in a curved cylinder characterized by a length at the bottom equal

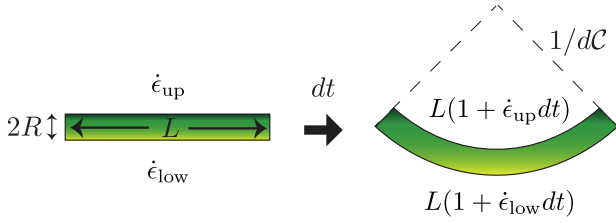


Figure 2. Bending resulting from the relative growth between the two sides of a beam.

to $L(1 + \dot{\epsilon}_{low}dt)$, and a length at the top equal to $L(1 + \dot{\epsilon}_{up}dt)$. One can then easily show that the difference in lengths induces a curvature dC given by

$$2RdC = dt(\dot{\epsilon}_{low} - \dot{\epsilon}_{up}). \quad (3)$$

Using equations 1, 2 and 3, the response $\tilde{\Delta}$ can then be expressed as a function of the mean elongation rate $\dot{\epsilon}_{mean} = (\dot{\epsilon}_{low} + \dot{\epsilon}_{up})/2$:

$$\tilde{\Delta} = \frac{RdC/dt}{\dot{\epsilon}_{mean}}. \quad (4)$$

The measurement of $\tilde{\Delta}$ requires the measurement of the rate of change in curvature along the stem dC/dt and of the relative elemental growth rate $\dot{\epsilon}_{mean}$ at each position along the reacting organ [4, 52]. Curvature and elongation changes can be measured through image analysis [1, 49] and recently an automatic and user-friendly tool has been released to do so [53]. However, as it involves double spatial derivative and a time derivative, these measurements are extremely sensitive to noise, and require cumbersome experiments. This limit can be overcome by considering the averaged curvature and averaged growth rate in the growth zone of length ℓ . The curvature can be approximated by $C \approx (\theta_{tip} - \theta_{base})/\ell$, where θ_{tip} and θ_{base} are the angles at the tip and at the base of the stem, and the growth rate can be approximated by $\dot{\epsilon}_{mean} \approx (dL/dt)/\ell$ where L is the length of the stem.

The gravitropic response is then given by:

$$\tilde{\Delta} = R \frac{d\theta_{tip}/dt}{dL/dt} \quad (5)$$

because θ_{base} is independent of the time.

Therefore, the relevant gravitropic response is made dimensionless by comparing the speed of the bending to the speed of growth. Measuring the velocity at which the shoot comes back to vertical, i.e. $d\theta_{tip}/dt$, as done in several studies [42], is thus a good estimate of the gravitropic response, but only if the growth rate remains constant. A shoot growing two times faster because of environmental or genetic changes will come back to the vertical two times faster, but it does not mean that the plant is twice more sensitive to gravity, as noticed by some authors [15]. This trivial effect linked to the growth velocity is properly taken into account by using the dimensionless response $\tilde{\Delta}$. The relevance of this formulation has been tested by Chauvet et al. [45] where it was shown that changing the temperature of the growth chamber modifies the growth velocity and the bending velocity but not the ratio of the two, meaning that the gravitropic response is the same. A last important remark is that the above analysis only holds at the first instants of the bending, when the curvature is sufficiently small so that the proprioception can be neglected.

2.2. Response to a permanent stimulus: the sine law.

Having defined a valid measure of the gravitropic response, one can now analyse how the response depends on the stimulus applied to the shoot. The simplest experiment consists in studying how a shoot goes back to the vertical when initially inclined at an angle θ_{init} from the vertical. In this configuration and using the dimensionless response $\tilde{\Delta}$, the “sine

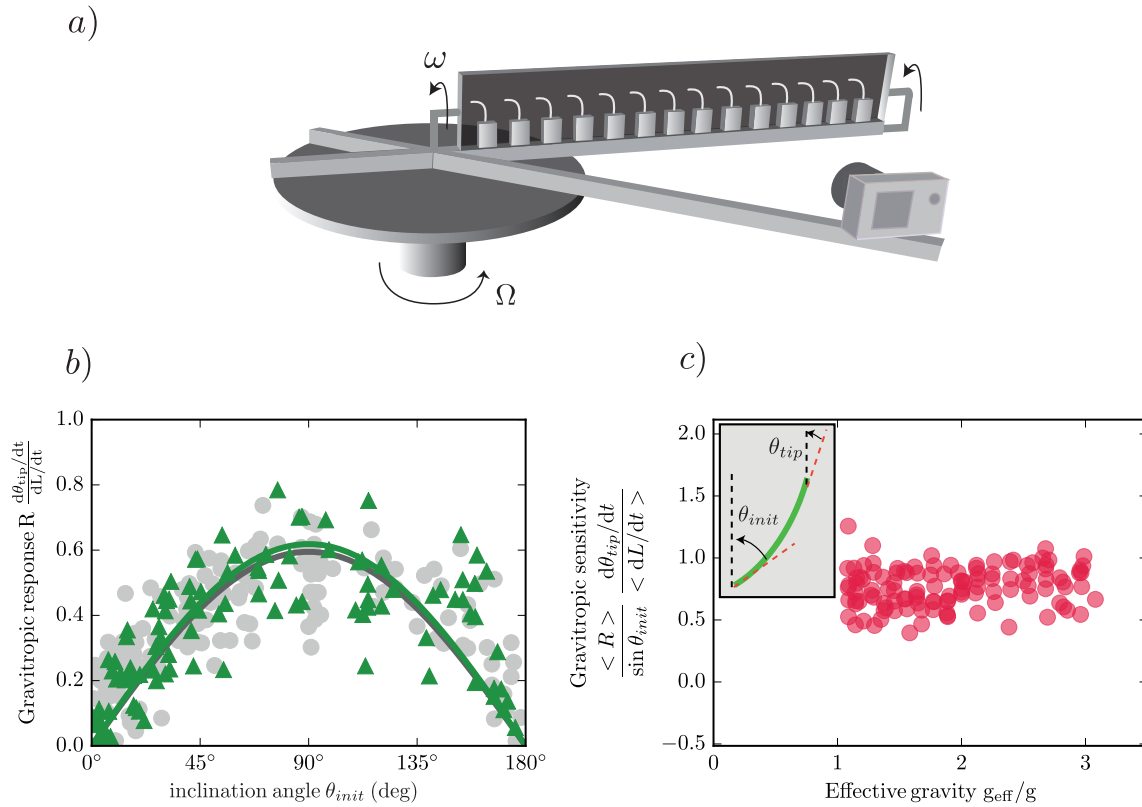


Figure 3. a) Sketch of the experimental setup: a clinostat rotating at a slow rotation velocity (ω) is fixed on a table rotating at a fast rotation speed (Ω). The slow rotation disrupts the response to Earth’s gravity and the fast rotation creates an effective gravity g_{eff} which increased with the distance from the center of the rotating table and its rotation speed. Plants are grown in individual small boxes loaded into the clinostat and their kinematics induced by the local g_{eff} is recorded using a camera synchronised with the clinostat rotation (ω). The sine law for wheat coleoptiles; b) gravitropic response as a function of the inclination angle θ for gravity intensities equal to 1 (gray symbols) and 2.5 (green symbols) the earth gravity (data from [45]), c) gravitropic response normalized by the sine of the inclination angle as a function of the effective gravity (data from [45]).

law” has been observed on a set of species sampling the major clades of angiosperms (flowering plants) namely rosids, asterids and commelinids [45]. The law stipulates that the response of the shoot inclined at an angle from vertical varies linearly with the sine of the inclination angle [42].

The results are presented in Fig. 3b for wheat coleoptiles (grey symbols). When increasing the initial inclination angle, the gravitropic response increases roughly linearly, reaches a maximum around 90 degrees when

the plant is put in a horizontal position, and decreases when the shoot is further inclined upside down. Data are well fitted by a sine law. The novelty of the study was to investigate the influence of the gravity intensity g on the response, independently of the inclination angle θ_{init} . This was achieved by developing a growth chamber on a rotating table (Fig. 3a) able to induce an additional centrifuge acceleration and to mimic hyper gravity conditions under an effective gravity g_{eff} . Data in green in Fig. 3b have been

obtained for an effective gravity $g_{\text{eff}} = 2.5g_{\text{earth}}$ and follow quantitatively the same “sine law” as for $g_{\text{eff}} = g_{\text{earth}}$. This independence holds over a whole range of gravity intensity as shown in Fig. 3c where the response normalized by $\sin \theta_{\text{init}}$ is plotted versus g_{eff} .

The independence of the gravitropic response with gravity intensity has also been observed for different organs from species broadly representative of land angiosperms and in hypo-gravity condition down to $0.1g_{\text{earth}}$ using a specific clinostat mounted on a rotating table (Fig. 3a). Counterintuitively, this study thus concludes that gravisensing in plants appears to be independent of the amplitude of gravity. It implies that gravity sensing in plants works as a clinometer, sensing inclination angles, and not as an accelerometer or a force sensor. In this sense, the gravity sensor in plants contrasts with the inner ear system of vertebrates, which is based on the deflection of ciliar cells induced by the force exerted by otoliths (small grains attached to the cells). Whereas vertebrates do not detect the difference between transient accelerations and inclinations, plants do, which may be a good strategy to be less sensitive to vibrations induced for example by wind. In the next section we discuss how this observation has implication for the understanding of the gravity sensor at the cellular level.

3. The Position Sensor Hypothesis

The observations reported in [45], and discussed in the previous section, strongly constrains the various hypotheses of gravity detection at the cellular level discussed in the introduction, as they show that the response is insensitive to the gravity intensity and only varies with the inclination angle. More precisely, a gravitropic response independent of

g_{eff} over a wide range dismisses the scenarios based on the measure of the statoliths weight, such as the assumption that the signal is triggered by the pressure exerted by the statolith on the endoplasmic reticulum or on the membrane, or the assumption that the weight of the statoliths induces a deformation of the actin network and activates the signal. If the weight of the statoliths was the measured signal, the gravitropic response should depend on the gravity intensity. By the same token, it discards the “protoplast pressure hypothesis” which has been proposed as an alternative candidate mechanism to explain the behavior of starchless mutants.

The result presented in section 2 thus strongly suggests that the relevant stimulus is the position of the statoliths. When the shoot is inclined, the final position of the grains within the cell is independent of the gravity intensity but only depends on the inclination. As long as the gravity is not zero, the statoliths will move to the lower corner of the cell (Fig. 1d). The averaged position of the statolith pile is thus a good candidate for the relevant stimulus detected by the statocytes. This idea is also supported by other experiments, showing that no gravitropic response is observed if the displacement of statoliths is impeded by a rigid vacuole [21], or showing in roots that the asymmetry in auxin fluxes is correlated to the motion of the statoliths [35]. Therefore, we propose a “position sensor hypothesis” stipulating that the signal controlling the gravisensing is the position of the statolith assembly within the cells.

This hypothesis raises several questions and needs to be confronted with our current knowledge of the response of plants to different stimuli. In the following we discuss the implications of the “Position Sensor

Hypothesis” for our understanding of the gravisensing chain. A first question concerns the angular sensitivity of plants, which are able to respond to small inclination angles ($\leq 10^\circ$). How a position sensor can be so sensitive is a non trivial question. A second question concerns the origin of the “sine law”, and how it can be explained within this hypothesis. The third point concerns the response to transient stimuli and how the position sensor hypothesis may reconcile the “sine law” and the “reciprocity rule”. The last question concerns the response of starch-less or starch-excess mutants, and whether or not the observation of their response might be compatible with the “Position Sensor Hypothesis”.

4. Implications of the Position Sensor Hypothesis

4.1. Sensitivity to small inclinations: a liquid behavior of the statoliths?

Assuming that the position of the statoliths is the relevant parameter implies that the statoliths have to move and change position to induce a signal. From a physical point of view this is not as trivial as it sounds. Statoliths are not isolated elements free to move in a clear fluid. First, they are embedded in a complex and highly heterogeneous medium (the cytoskeleton and the vacuole) whose mechanical properties are not well understood. Second, when the plant is upright, they form a dense assembly of particles at the bottom of the cell in which the motion of one grain can be strongly affected by the surrounding grains. Their motion is thus collective and may be compared to a submarine granular avalanche [25, 55, 56]. Everyday experience tells us that a packing of grains resting at the bottom of

a container full of liquid will not move when the container is inclined, unless the inclination becomes higher than a critical angle, typically around 25 degrees for spherical grains. In the physics of granular media, this is called the pile angle, and reflects the difficulty for the grains to flow due to the friction between them and to the geometrical entanglement in the packing [54]. If statoliths were behaving like simple passive grains (like sand grains), they would not move when the plant is inclined at an angle less than 25 degrees and the “position sensor hypothesis” would then predict no gravitropic response. A plant inclined at small angle would grow inclined without ever reaching the vertical, meaning that an angle threshold would exist in the detection. This is not what is observed, as evidenced by the sine law in Fig. 3b showing that even at small angles a response exists and that the plant indeed comes back to the vertical.

However, statoliths are not sand grains and may not behave like passive grains. When observing the dynamics of the statoliths in the statocytes, statoliths seem to follow erratic trajectories and look as though they were constantly agitated [25, 56, 58, 59]. This constant agitation arguably helps the grains to rearrange and to move with respect to their neighbors, even at small inclinations. The pile of agitated statoliths could then flow like a liquid to the lower corner of the cell and may not behave like a classical granular medium. The remarkable sensitivity of plants at low inclination angles could thus be explained within the “Position Sensor Hypothesis”, thanks to the agitation of the statoliths.

In this scenario, the origin of the agitation becomes an important question that should be studied further. Some studies [25, 56–58] suggest that statoliths experience two kinds

of fluctuating motions: large saltation motion together with small vibration-like dynamics. The fluctuations have been analyzed recently using tools from statistical physics of thermal colloidal systems, assuming that the agitation was of Brownian origin [59]. However, the size of the statoliths being relatively large (between 3 to 8 μm), it is not obvious that thermal fluctuations are sufficient to explain the erratic motion observed in the statocytes.

The cell activity, and more precisely the dynamics of the actin cytoskeleton [60] is a good candidate as a source of agitation for the statoliths. Indeed, experiments using actin inhibitory compounds show that the cytoskeleton actually plays a role although the reports are contradictory [61]. The response of stems [58, 62, 63] or roots [64] when inclined to the horizontal seems faster and stronger using drugs that depolymerize the actin network, whereas drugs that stabilise polymerization or prevent actin depolymerisation decrease the response [64]. This observation could be rationalized within the “Position Sensor Hypothesis”. At high inclination, the cytoskeleton and its permanent activity actually hinders the avalanche of the grains and may slow down their displacement and resuspend the statoliths [65, 66], playing the role of an inhibitor for gravity perception. At small inclination by contrast, the activity facilitates the motion of the grains that would be jammed otherwise. The scenario that the gravity sensor is based on the avalanches of an active granular medium needs to be confirmed by more detailed studies of the motion of the statoliths, of their agitation and their interaction with the cytoskeleton.

4.2. The sine law

One of the most robust results on the gravitropic response is the “sine law” presented in section 2. The text-book explanation for the variation of the response as a function of the inclination angle relies on a force argument: the statoliths exert a lateral force on the side wall of the cell, which is proportional to $M_b g \sin \theta$, where M_b is the buoyancy-corrected mass of the statoliths. However, this explanation does not fit with the recent observation that the gravitropic response is independent of the gravity intensity. Thus, it is legitimate to wonder how the “sine law” may be explained within a scenario where the relevant stimulus is the position of the statoliths.

A plausible mechanism is described in Fig. 4. Let us speculate that the proximity or the contact of the grains with the membrane induces a flux of auxin [39, 67]. One can think of a mechanism involving a key-lock system or steric interactions which may locally perturb most membrane trafficking. When the plant is in a vertical position, the statoliths have sedimented at the bottom of the cell, and the local auxin fluxes at each contact have a symmetric distribution between the two lateral sides of the cell as drawn in Fig. 4a. The resulting total flux is thus vertical, aligned with the longitudinal axis of the cell, which induces no differential growth between the two sides. When the cell is inclined at an angle θ , the statoliths flow in the lower corner and form a pile with a free surface which is horizontal thanks to the liquid-like behavior previously discussed. In this configuration, there are more contacts between statoliths and the membrane on the lower side of the cell than on the top side, and thus more local fluxes of auxin. If one assumes that the

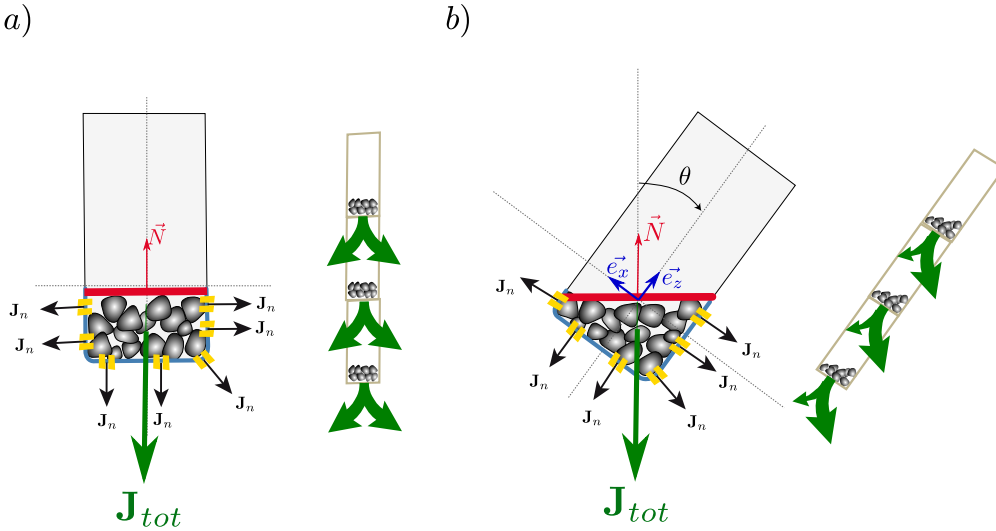


Figure 4. Sketch illustrating the scenario proposed for the “position sensor hypothesis”: the proximity or the contact between statoliths and the membrane induces local auxin fluxes (black arrows). The resultant total flux (green arrows) is thus aligned with the cell axis when the cell is vertical (a), but present an asymmetry when the cell is inclined (b). The red line represents the free surface of the statolith pile.

elementary flux per unit of contact area is uniform, the integral over the total contact area gives by geometrical argument a total flux which is aligned with the normal \vec{N} to the free surface. The free surface being horizontal, the total flux is then again aligned with the vertical. The total flux \mathbf{J}_{tot} is thus no longer aligned with the axis of the cell but makes an angle θ with the cell longitudinal axis. The lateral component of the flux along the \mathbf{e}_x direction is then proportional to the sine of the inclination angle θ . This lateral flux could then give rise to a differential growth and to the gravitropic response at the plant scale. In this cartoon where the proximity of the statoliths to the membrane is the stimulus, the “sine law” results from the geometrical asymmetry of the position of the statoliths in the cell since the lateral flux evolves proportionally to $\sin(\theta)$. A way to further investigate the relevance of this scenario would be to look for a “sine law” in the transduction signal itself, for example in the auxin distribution

or in the PIN relocalisation [38]. In any case, the position sensor hypothesis provides a new interpretation of the “sine law”, that is consistent with the insensitivity of gravisensing to the intensity of the gravity vector.

4.3. The reciprocity rule

We now discuss the second type of experiment focused on the response of plants to a transient stimulus. These studies have been mostly performed by the microgravity community, the goal being to determine the gravity detection threshold [68–70]. The procedure is the following. A plant is exposed to an effective gravity g_{eff} perpendicular to its axis during a time lapse t_0 , and then put back in a zero gravity condition, either in space or in a clinostat. The plant then actively bends up in response to this transient stimulus. The amplitude of the response appears to increase when increasing the gravity intensity g_{eff} or when increasing the time of exposure t_0 . Data

suggest that the response is proportional to the dose, i.e. to the product of the gravity intensity by the time of exposure $g_{\text{eff}} \times t_0$, a result known as the “reciprocity rule”. Because the goal was the determination of the minimal dose to get a response, most of the experiments have been performed at a low level of gravity ($g_{\text{eff}} < g_{\text{earth}}$).

The “reciprocity rule” explicitly involves the gravity intensity and appears a priori incompatible with the “sine law” presented in section 2 showing that the response to a permanent stimulus is independent of g_{eff} . This incompatibility may seriously question the position sensor hypothesis. However, the sine law and the reciprocity rule originate from two very different types of experiments. The first one corresponds to a permanent stimulus (gravity is continuously present and the base of the plant remains inclined during the whole experiment), the second one corresponds to transient stimulus during a finite time. Here we show that the two types of procedure may be reconciled when thinking in terms of statolith avalanches as illustrated in Fig. 5. When a plant is inclined, the final and steady position of the statoliths in the lower corner of the cell is independent of the gravity intensity, providing an explanation for the sine law as discussed above. Only the time necessary for the statoliths to flow and to reach their final position varies (linearly) with g_{eff} as sketched in Fig. 5a and 5b. As long as this avalanche time in achieving the final position is negligible compared to the time lag for the onset of the next steps in the signal transduction pathway, it has negligible effect on the observed gravitropic response.

During a transient stimulus, when the plant is inclined during a time t_0 , the statoliths may not reach their final position if t_0 is shorter than the time of avalanche. In this transient

regime, the maximum excursion of the grains is expected to be proportional to their velocity V times the time t_0 . The velocity V results from the balance between the gravity $M_b g_{\text{eff}}$ and the viscous drag $\eta V D$ (η being the viscosity of the cytosol and D the typical size of the pile) and is thus proportional to the gravity $V = M_b g_{\text{eff}} / \eta D$. As a result, the maximum excursion should scale as $M_b g_{\text{eff}} t_0 / \eta D$, and the maximum deflection angle of the free surface would be proportional to $g_{\text{eff}} \times t_0$. This is illustrated in Fig. 5c, d and e. When comparing two plants inclined during the same time t_0 but under two different gravity $g_{\text{eff}} = g$ (Fig. 5c) and $g_{\text{eff}} = 0.5g$ (Fig. 5d), the excursion of the statoliths is two times shorter for the lower gravity (the excursion being measured by θ_s^{max} , i.e. the deflection of the free surface of the pile compared to the lower side of the cell). However, if the time of excursion is also multiplied by two (Fig. 5e) to get the same dose $g_{\text{eff}} \times t_0$, one recovers approximately the same excursion θ_s^{max} . If the position is the relevant stimulus as stipulated by the position sensor hypothesis one thus recovers the reciprocity rule: the response is proportional to θ_s^{max} and thus to $g_{\text{eff}} t_0$. In experiments where plants are put in zero gravity after the exposure time instead of coming back to the vertical, one can imagine that the statoliths will leave the membrane when g_{eff} is zero (as shown in lentil root statocytes [71]), leading to the same phenomenology.

This interpretation of the “reciprocity rule” based on the avalanche dynamics only holds if the time for the detection of the statolith positions and for the signaling is sufficiently short compared to the avalanche time. Indeed, the response at the plant scale is controlled by the slower process at the microscopic scale. Thus, the response

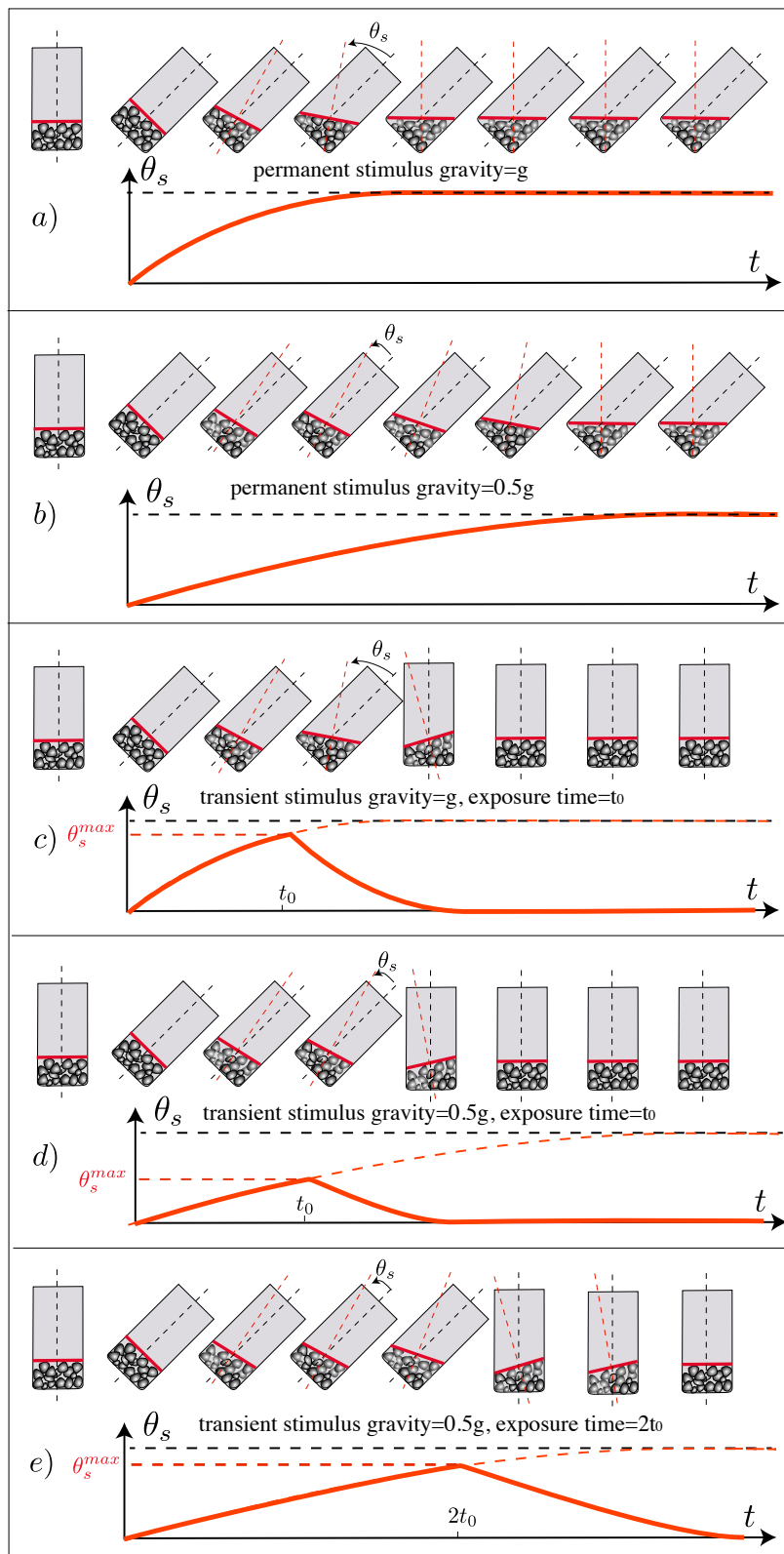


Figure 5. Sketch of the statolith avalanche dynamics in different experiments. The angle θ_s made by the free surface of the statoliths pile with the base of the cell is plotted as a function of time; (a) and (b) permanent inclination at two different gravity intensities g . (c), (d) and (e) transient inclination at two different gravity intensities g and two exposition times

to transient stimuli reflects the statolith avalanche dynamics only if detection and signaling processes are faster. This is expected to be true in the low gravity conditions corresponding to the microgravity experiments exhibiting the reciprocity rule, but it will perhaps no longer be true for Earth gravity level, at least when dense statoliths are involved.

In the “Position Sensor Hypothesis”, the reciprocity rule would then be the signature of the physical dynamics of statolith displacement and not an intrinsic gravisensitive response of the plant. These considerations show that there is a need to perform detailed experiments under transient stimuli, looking both at the response at the plant scale under various levels of gravity intensity, and at the cell scale investigating the avalanche dynamics of the statoliths.

4.4. The starch-less and starch-excess mutants

The last facts usually presented in favour of the “gravity-force sensing hypothesis” are the effect of changing the overall mass of the amyloplasts acting as statoliths in the statocytes. This can be achieved either through changing the growth conditions (e.g. light vs dark) or through the use of starch-depleted or starch-excess mutants. Kiss and coworkers used these two methods in a series of quantitative studies of the cellular and macroscopic responses (reviewed in [72]). They considered five genotypes of *Arabidopsis thaliana*: the wild type (WT), a starch-excess mutant (*sex1*), two reduced starch mutants (ACG 20 and ACG 27), and a starch-less mutant (ACG 21). From their observations, they concluded that a correlation exists between the mass or volume of the

statoliths and the gravitropic response. We have re-analyzed their data to check that their conclusion still holds with the dimensionless gravitropic response $\tilde{\Delta}$ (equation 5) introduced in section 2, since mutation or treatment conditions may affect the growth rate. We have also extracted an estimate of the volume of the statoliths by measuring their apparent area in the published pictures. Our analysis has been limited to the dark-grown (dg) hypocotyls of the starch-deficient mutants [30] and to the light-grown (lg) hypocotyls of the starch-excess mutants [72] for which sufficient data were published. Data are reported in table 1. We find that the plant response $\tilde{\Delta}$ seems to increase with the volume of the amyloplasts inside statocytes.

One difficulty in interpreting these experiments is that changing the growth conditions or the genotype not only affects the size of the statoliths but also their density and number. Nevertheless, the global trend supports the idea that varying the overall mass and weight of the starch-statolith changes the gravitropic sensing, in agreement with the “gravity-force model”. However, these results are not a priori incompatible with the “position-sensor hypothesis” we propose. Fig. 6 shows that increasing the amount of statoliths at a given inclination increases the contact area between the pile and the lateral side of the cell. If one assumes that the relevant signal triggering the gravitropic response is the asymmetry of the contact area as proposed in Fig. 4, one may expect an influence of the volume of the statolith pile on the response. The “position-sensor hypothesis” could then be compatible with the macroscopic responses that have been observed by Kiss and coworkers. At this stage, it is nevertheless difficult to discriminate between the different scenarios. Further studies on mutants analysing both the plant kinematic

Table 1. Quantitative estimates of the amyloplasts and macroscopic response of the starch mutants in arabidopsis hypocotyl. For dark-grown material, the sizes of amyloplasts (in terms of apparent area) range from 6.5 (for WT_{dg}) to 1.5 μm^2 (for ACG27_{dg}) and the starch content of each amyloplast compared to the WT_{dg} was slightly reduced in the ACG20_{dg}, reduced in the ACG27_{dg}, and the ACG21_{dg} amyloplasts were completely deprived of starch. For light-grown material, the size was larger than the dark-grown ones: the WT_{lg} amyloplasts are about two times bigger than the WT_{dg} and the *sex1*_{lg} are two times bigger than the WT_{lg}. But the light also largely reduces the amount of amyloplasts for the WT_{lg}. Taken together these genotypes can be ranked on the volume and mass of their amyloplasts as follows: WT_{dg} > ACG20_{dg} > ACG27_{dg} \gg ACG21_{dg} and WT_{lg} \gg *sex1*_{lg} \gg WT_{lg}. The plant response $\tilde{\Delta}$ seems to increase with the volume of the amyloplasts inside statocytes. The Δ of the light-grown WT_{lg} was 6 times lower than the one of dark-grown WT_{dg}, and the $\tilde{\Delta}$ of the light-grown *sex1*_{lg} was more than 4 times higher than WT_{lg} restoring the level of the $\tilde{\Delta}$ found for the dark-grown WT_{dg}. It may also be noted that the value for $\tilde{\Delta}$ for the WT_{dg} is similar to the one reported for inflorescences of the same genotype by [45].

genotype	growth conditions	Plastid size (μm^2) and starch content	Relative plastid size	θ_{init} (rad)	$d\theta/dt^{**}$ (rad/h)	dL/dt (mm/h)	R^* (mm)	$\tilde{\Delta}$	$\tilde{\Delta}/\tilde{\Delta}_{WT}$	ref
WT (WS)	Dark-grown	6.5*, normal	1	$\pi/2$	0.57	0.33	0.4	0.68	1	[30]
ACG20 (WS)	Dark-grown	4.5*, slightly-reduced	0.70	$\pi/2$	0.42	0.28	0.4	0.60	0.88	[30]
ACG27 (WS)	Dark-grown	1.5*, reduced and smaller	0.23	$\pi/2$	0.37	0.28	0.4	0.53	0.78	[30]
ACG21 (WS)	Dark-grown	1.5*, no starch	0.24	$\pi/2$	0.03	0.25	0.4	0.05	0.08	[30]
WT (Col0)	Light-grown	14.4 \pm 0.8, very few	1	$\pi/2$	0.03	0.05	0.22	0.12	1	[72]
<i>sex1</i> (Col0)	Light-grown	30.9 \pm 0.9	2.14	$\pi/2$	0.06	0.03	0.28	0.52	4.4	[72]

* measured from plates using Fiji, ** measured from the kinetics graphs.

response and the statolith properties and dynamics would provide precious information.

5. Conclusion

Gravitropism, more than a century after Darwin, remains an active subject. In this perspective paper we have discussed the results

of recent experiments on the response of plant shoots to inclination at different gravity intensities. The observation that the response is independent of the gravity intensity strongly suggests that the gravity sensor in plants behaves like a clinometer rather than an accelerometer. We propose a new scenario in which the gravity sensors, the statocytes,

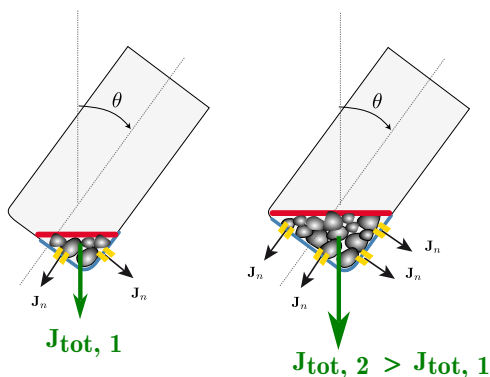


Figure 6. Sketch illustrating the effect of the statoliths volume variation on the resultant total flux of auxin for the “position sensor hypothesis”.

behave like position sensors, being sensitive to the position of the statoliths within the cell.

In this “position sensor hypothesis” the motion of the statoliths is facilitated by the activity of the cytoskeleton, which may explain the high sensitivity of plants even at small inclination angle. This scenario also provides a coherent framework to interpret and reconcile the response to permanent stimuli (“the sine law”), as well as the response to transient stimuli (“the reciprocity rule”), or to mutations in the starch content of statoliths. The “position sensor hypothesis” has also important implications on the different molecular hypotheses of gravitropic perception. On one hand, it discards mechanisms based on the measure of forces, such as the detection of the statolith weight by the actin network or the endoplasmic reticulum, and the “protoplast pressure hypothesis”. On the other hand it is compatible with the mechanisms involving the proximity of the statoliths to subcellular elements (endoplasmic reticulum [29], actin cytoskeleton [21]) or a change of the intracellular trafficking due to statolith asymmetric distribution within the cell.

At this stage, all the conjectures discussed in the paper remain to be confirmed or

contradicted, which should motivate more detailed analysis coupling experiments at different scales: at the plant scale to analyze the kinematics, at the cell scale to progress in our understanding of the motion of the statoliths, and at the molecular scale to disentangle the signaling pathway.

A last crucial remark is that a better understanding of the gravisensing is not sufficient to apprehend and describe the gravitropic movement and more generally the control of the posture of plants. Proprioception, i.e. the ability of plants to feel their own curvature leading to the tendency of plants to unbend independently of gravitational stimulus, also plays an important role. The development of a recent model combining gravisensing and proprioception to predict the gravitropic macroscopic motion opens new perspectives and could serve as a base to develop more elaborate models taken into account the details of the gravisensing chain discussed in this paper [48].

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References

- [1] Bastien R, Bohr T, Moulia B and Douady S 2013 *Proc. Natl. Acad. Sci. U.S.A.* **110** 755–760

- [2] Firm R D and Digby J 1980 *Annu. Rev. Plant Physiol.* **31** 131–148
- [3] Whippo C W and Hangarter R P 2009 *Am. J. Bot.* **96** 2115–2127
- [4] Moulia B and Fournier M 2009 *J. Exp. Bot.* **60** 461–486
- [5] Morita M T 2010 *Annu. Rev. Plant Biol.* **61** 705–720
- [6] Gilroy S 2008 *Curr. Biol.* **18** R275–R277
- [7] Perbal G and Perbal P 1976 *Physiol. plant.* **37** 42–48
- [8] Perbal G, Jeune B, Lefranc A, Carnero-Diaz E and Driss-Ecole D 2002 *Physiol. Plant.* **114** 336–342
- [9] Fukaki H, Wysocka-Diller J, Kato T, Fujisawa H, Benfey P N and Tasaka M 1998 *The Plant J.* **14** 425–430
- [10] Blancaflor E B, Fasano J M and Gilroy S 1998 *Am. J. Plant Physiol.* **116** 213–222
- [11] Gerttula S, Zinkgraf, M, Muday G, Lewis D, and Ibatullin F M, Brumer H, Hart F, Mansfiel S D, Filkov V and Groover A 2015 *The Plant Cell* **27** 2800–2813
- [12] Vanneste S and Friml J 2009 *Cell* **136** 1005–1016
- [13] Nèmec B 1900 *Deutsch. Bot. Ges., XVIII, S* **241–245**
- [14] Haberlandt G 1900 *Ber. Dtsch. Bot. Ges.* **18** 261–272
- [15] Pickard B G and Thimann K V 1966 *The J. Gen. Physiol.* **49** 1065–1086
- [16] Kiss J Z, Hertel R and Sack F D 1989 *Planta* **177** 198–206
- [17] Weise S E and Kiss J Z 1999 *Int. J. Plant Sci.* **160** 521–527
- [18] Fitzelle K J and Kiss J Z 2001 *J. Exp. Bot.* **52** 265–275
- [19] Kuznetsov O A and Hasenstein K H 1997 *J. Exp. Bot.* **48** 1951–1957
- [20] Hasenstein K H and Kuznetsov O A 1999 *Planta* **208** 59–65
- [21] Toyota M, Ikeda N, Sawai-Toyota S, Kato T, Gilroy S, Tasaka M and Morita M T 2013 *The Plant J.* **76** 648–660
- [22] Shen-Miller J 1970 *Planta* **92** 152–163
- [23] Barlow P W 1995 *Pant Cell Environ.* **18** 951–962
- [24] Darwin F 1903 *Nature* **67** 571–572
- [25] Leitz G, Kang B H, Schoenwaelder M E and Staehelin L A 2009 *The Plant Cell* **21** 843–860
- [26] Hasenstein K H 2009 *Gravitational and Space Res.* **22** 2133
- [27] Yoder T L, Zheng H q, Todd P and Staehelin L A 2001 *Am. J. Plant Physiol.* **125** 1045–1060
- [28] Perbal G and Driss-Ecole D 2003 *Trends Plant Sci.* **8** 498–504
- [29] Strohm A K, Barrett-Wilt G A and Masson P H 2014 *Front. Plant Sci.* **5** 148
- [30] John Z K, Mary M G, Allison J M and Kathi S S 1997 *Plant Cell Physiol.* **38** 518–525
- [31] Fasano J M 2001 *The Plant Cell* **13** 907–922
- [32] Joo J H, Bae Y S and Lee J S 2001 *Am. J. Plant Physiol.* **126** 1055–1060
- [33] Toyota M, Furuichi T, Tatsumi H and Sokabe M 2008 *Am. J. Plant Physiol.* **146** 505–514
- [34] Kolesnikov Y S, Kretynin S V, Volotovskiy I D, Kordyum E L, Ruelland E and Kravets V S 2015 *Protoplasma* **253** 987–1004
- [35] Band L R, Wells D M, Larrieu A, Sun J, Middleton A M, French A P, Brunoud G, Sato E M, Wilson M H, Peret B, Oliva M, Swarup R, Sairanen I, Parry G, Ljung K, Beeckman T, Garibaldi J M, Estelle M, Owen M R, Vissenberg K, Hodgman T C, Pridmore T P, King J R, Vernoux T and Bennett M J 2012 *Proc. Natl. Acad. Sci. U.S.A.* **109** 4668–4673
- [36] Petrášek J, Mravec J, Bouchard R, Blakeslee J J, Abas M, Seifertová D, Wiśniewska J, Tadele Z, Kubeš M, Čovanová M, Dhonukshe P, Skůpa P, Benková E, Perry L, Křeček P, Lee O R, Fink G R, Geisler M, Murphy A S, Luschnig C, Zažímalová E and Friml J 2006 *Science* **312** 914–918
- [37] Wiśniewska J, Xu J, Seifertová D, Brewer P B, Ružička K, Blilou I, Rouquié D, Benková E, Scheres B and Friml J 2006 *Science* **312** 883–883
- [38] Friml J, Wiśniewska J, Benková E, Mendgen K and Palme K 2002 *Nature* **415** 806–809
- [39] Kleine-Vehn J, Ding Z, Jones A R, Tasaka M, Morita M T and Friml J 2010 *Proc. Natl. Acad. Sci. U.S.A.* **107** 22344–22349
- [40] Rakusová H, Gallego-Bartolomé J, Vanstraelen M, Robert H S, Alabadí D, Blázquez M A, Benková E and Friml J 2011 *The Plant J.* **67** 817–826
- [41] Dumais J 2013 *Proc. Natl. Acad. Sci. U.S.A.* **110** 391–392
- [42] Iino M, Tarui Y and Uematsu C 1996 *Plant, Cell and Environment* **19** 1160–1168
- [43] Galland P 2002 *Planta* **215** 779–784
- [44] Brown A H, Chapman D K, Johnsson A and Heathcote D 1995 *Physiol. Plant.* **95** 27–33
- [45] Chauvet H, Pouliquen O, Forterre Y, Legué V and Moulia B 2016 *Sci. Rep.* **6** 35431

- [46] Tanimoto M, Tremblay R and Colasanti J 2008 *Plant Mol. Biol.* **67** 57–69
- [47] Myers A B, Glyn G H, Digby J and Firn R D 1995 *Ann. Bot.* **75** 277–280
- [48] Hamant O and Moullia B 2016 *New Phytol.* **212** 333–337
- [49] Bastien R, Douady S and Moullia B 2014 *Front. Plant Sci.* **5** 136
- [50] Galston A W and Hand M E 1949 *Am. J. Bot.* **36** 85–94
- [51] Hopkins W G and Hüner N P A 2009 *Introduction to plant physiology, 4th edition* (Wiley)
- [52] Silk W K 1984 *Ann. Rev. Plant Physiol.* **35** 479–518
- [53] Bastien R, Legland D, Martin M, Fregosi L, Peaucelle A, Douady S, Moullia B and Höfte H 2016 *The Plant J.* **88** 468–475
- [54] Andreotti B, Forterre Y and Pouliquen O 2013 *Granular media: between fluid and solid* (Cambridge University Press)
- [55] Sack F D, Suyemoto M M and Leopold A C 1985 *Planta.* **165** 295–300
- [56] Sack F D, Suyemoto M M and Leopold A C 1986 *Am. J. Bot.* **73** 1692–1698
- [57] Saito C, Morita M T, Kato T and Tasaka M 2005 *The Plant Cell* **17** 548–558
- [58] Nakamura M, Toyota M, Tasaka M and Morita M T 2011 *The Plant Cell* **23** 1830–1848
- [59] Zheng Z, Zou J, Li H, Xue S, Wang Y and Le J 2015 *Mol. plant* **8** 660–663
- [60] Guo M, Ehrlicher A J, Jensen M H, Renz M, Moore J R, Goldman R D, Lippincott-Schwartz J, Mackintosh F C and Weitz D A 2014 *Cell* **158** 822–832
- [61] Blancaflor E B 2013 *Am. J. Bot.* **100** 143–152
- [62] Yamamoto K and Kiss J Z 2002 *Am. J. Plant Physiol.* **128** 669–681
- [63] Hou G, Kramer V L, Wang Y S, Chen R, Perbal G, Gilroy S and Blancaflor E B 2004 *The Plant J.* **39** 113–125
- [64] Mancuso S, Barlow P W, Volkmann D and Baluka F 2006 *Plant Signaling Behav.* **1** 52–58
- [65] Friedman H, Vos J W, Hepler P K, Meir S, Halevy A H and Philosoph-Hadas S 2003 *Planta* **216** 1034–1042
- [66] Volkmann D, Baluška F, Lichtscheidl I, Driss-Ecole D and Perbal G 1999 *The FASEB J.* **13** 143–147
- [67] Dharmasiri S, Swarup R, Mockaitis K, Dharmasiri N, Singh S, Kowalchuk M, Marchant A, Mills S, Sandberg G, Bennett M *et al.* 2006 *Science* **312** 1218–1220
- [68] Volkmann D and Tewinkel M 1998 *Adv. Space Res.* **21** 1209–1217
- [69] Laurinavicius R, Svegzdiene D, Buchen B and Sievers A 1998 *Adv. Space Res.* **21** 1203–1207
- [70] Driss-Ecole D, Legué V, Carnero-Diaz E and Perbal G 2008 *Physiol. Plant.* **134** 191–201
- [71] Driss-Ecole D, Jeune B, Prouteau M, Julianus P and Perbal G 2000 *Planta* **211** 396–405
- [72] Vitha S, Yang M, Sack F D and Kiss J Z 2007 *Am. J. Bot.* **94** 590–598