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Revisiting the relationship between turgor pressure and plant cell growth

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Summary

Growth is central to plant morphogenesis. Plant cells are encased in rigid cell walls, and they must overcome physical confinement to grow to specific sizes and shapes. Cell wall tension and turgor pressure are the main mechanical components impacting plant cell growth. Cell wall mechanics has been the focus of most plant biomechanical studies, and turgor pressure was often considered as a constant and largely passive component. Nevertheless, it is increasingly accepted that turgor pressure plays a significant role in plant growth. Numerous theoretical and experimental studies suggest that turgor pressure can be both spatially inhomogeneous, and actively modulated during morphogenesis. Here, we revisit the pressure-growth relationship by reviewing recent advances in investigating the interactions between cellular/tissular pressure and growth.

Key words: turgor pressure, cell wall mechanics, mechanical signals, plant growth, tissue hydraulics, osmosis.

I. Introduction

Plant cells often have a high intracellular osmolyte content which, thanks to their rigid walls, means they can become turgid without risk of bursting. Conversely, under water stress, they lose turgidity and shrink. These observations have led some biophysicists to compare plant cells to balloons or bubbles: their surfaces are under tension and their interiors are under pressure (Fig. 1). This analogy, though simplistic, partially captures the mechanical interactions occurring during cell growth (reviewed by Beauzamy *et al.*, 2014). Wall tension

induced by turgor pressure is believed to underlie the irreversible cell expansion (reviewed by (Landrein & Ingram, 2019)). Previous biomechanical studies on plant growth mainly focused on the pressure-driven, mechanical stress-induced expansion of the primary cell wall. Wall expansion is often considered to be a passive or a protein-mediated stress relaxation, occurring through *de novo* wall material synthesis and/or wall remodeling (discussed by Cosgrove, 2022). Initial mechanical models described isotropic growth responses (Lockhart, 1965; Ortega, 1985; Wei & Lintilhac, 2003). More recently the orientation of wall materials such as cellulose microfibrils were integrated into models, conceptualizing wall stress guidance (Sampathkumar *et al.*, 2014; Zhang *et al.*, 2021; Oliveri *et al.*, 2019). Other formalisms also include pectin dynamics. It has indeed been recently proposed that some walled cells, like Arabidopsis pavement cells, may expand over short timescales when plasmolyzed due to pectin nanofilament swelling (Haas *et al.*, 2020). A similar "stored growth" phenomenon has been described in microbes (Rojas & Huang, 2017), suggesting that some walls may "grow" without turgor pressure under certain conditions.

Nonetheless, despite the key role of the wall in growth regulation, it is generally accepted that turgor pressure is the driving force of plant growth. Pressure is often treated as a passive, constant biophysical parameter, only required to initiate and maintain wall expansion. However, pressure and growth dynamics do not necessarily correlate, notably in pollen tubes (reviewed by (Chebli & Geitmann, 2007)), and the relation between turgor and growth is thus complex. Recently, several studies indicate that turgor may be biologically regulated, hinting at a more active role of turgor pressure in cell growth regulation (Cheddadi *et al.*, 2019; Long *et al.*, 2020; Dumais, 2021; Mielke *et al.*, 2021; Creff *et al.*, 2021).

In this review, we first briefly describe the basic concepts of turgor pressure and related phenomena. We then summarize recent findings regarding its relationship with plant growth on different levels. Finally, we provide our perspectives on the study of turgor pressure and plant growth regulation.

II. Turgor pressure and osmosis

"Turgor pressure" is often confused with "osmotic pressure". To clarify the difference between these two parameters, consider a cytosol with high solute concentration and the extracellular environment with low solute concentration, separated by a semipermeable plasma membrane (Fig. 1A). Water flows towards the compartment with lowest "water potential" Ψ, defined as the chemical potential of water relative to a reference state. Cellular water potential is mainly determined by osmotic and pressure potentials. Assuming the initial

cross-membrane pressure difference is 0, a solute concentration differential will create an unequilibrated chemical potential called the “osmotic potential” Ψ_Π, and cross-membrane hydraulic fluxes will spontaneously occur to equilibrate the potential gradient. Since aqueous solutions have negative osmotic potentials, the positive term “osmotic pressure” $\Pi=-\Psi_\Pi$ is often used. Water influx increases cell volume, leading to a simultaneous stretching of the walls and a compression of the protoplast. This causes the emergence of a pair of “action-reaction forces” in the form of cell wall tensile stress σ and hydrostatic pressure P, or “turgor pressure”. At equilibrium, the value of turgor pressure equals the osmotic pressure differential gradient, or “osmotic gradient” ($P=\Delta\Pi$). In unequilibrated cells, such as growing cells, the net fluid flux J is proportional to their water potential gradient $\Delta\Psi=-(\Delta\Pi-P)$.

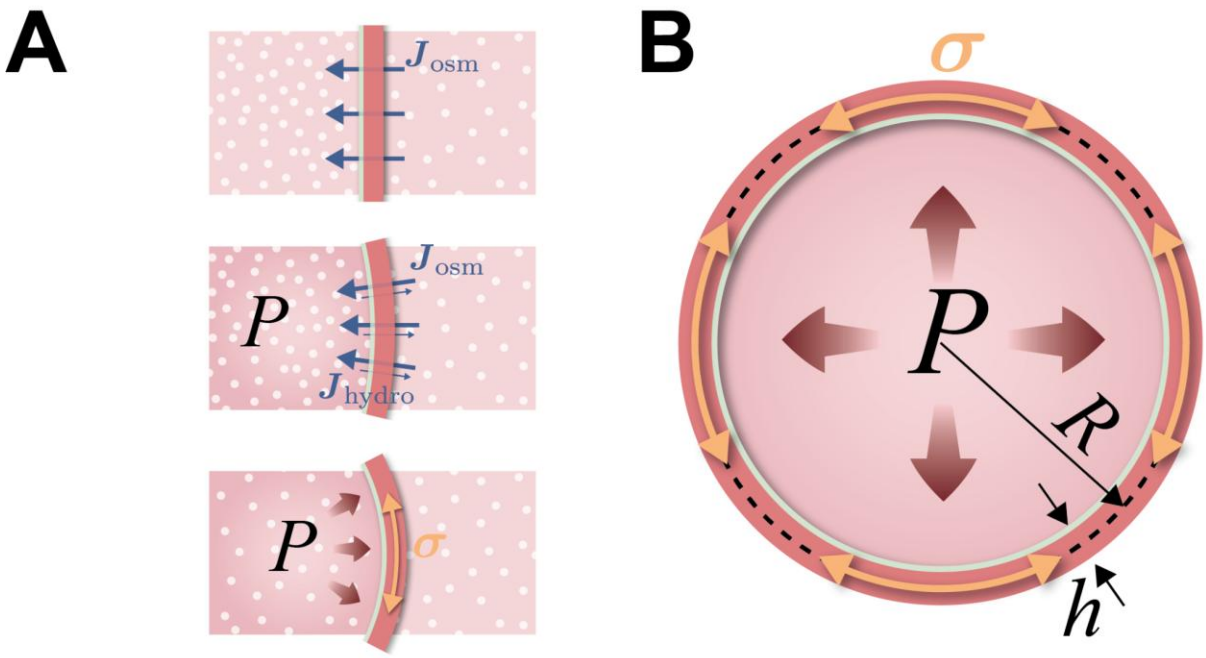


Figure 1. The hydraulics and mechanics of plant cells. (**A**) Top panel: The osmolarity differential between the cell (left hand-side) and the extracellular medium (right hand-side), separated by a semi-permeable plasma membrane (thin beige line) and a cell wall (thick pink line), induces a water flux (J_{osm}) toward the compartment with the highest osmolarity. Middle panel: This water influx increases cell hydrostatic pressure (P) which, in turn, generates a water flux toward the right hand-side one (J_{hydro}). Bottom panel: Cell wall deformation leads to the accumulation of tensile stress (σ) within the wall, which balances the hydrostatic pressure exerted on the wall. (**B**) The hydrostatic pressure (P) within an

idealized spherical plant cell embedded in a spherical wall of radius R and thickness h generates a tensile stress (σ) within the wall according to the Young-Laplace equation, see Eq.(2).

Textbox 1. Glossary

Anisotropy (dimensionless): The level of directional dependence a property, opposite to "isotropy" where this property is equal in all directions. For example, growth anisotropy describes how much an object (cell, tissue, organ) preferentially grows in one specific direction compared to other directions. A fully anisotropic growth is elongation.

Conductance ($Pa^{-1}{\cdot}s^{-1}$): Quantifies the rate at which a material allows fluid to diffuse through it. In a cylindrical cell, cell conductance ϕ_a = (A / V) L_p is a parameter that describes the efficiency of fluxes as a function of the permeability per unit membrane area L_p (unit $m{\cdot}Pa^{-1}{\cdot}s^{-1}$) and the surface-volume ratio of the cell, which is inversely proportional to the radius of the cylinder R. Note that conductance $\phi_a$and wall extensibility ϕ_w (see below) are two parameters of completely different biophysical origins.

Curvature (m^{-1}): Reciprocal of the radius of the osculating circle of a curve (a tangent circle closely adhering to a curve).

Extensibility: Quantifies the rate of deformation of a material when loaded with mechanical constraints. In plant cells, it quantifies the effect of chemical relaxation of cell wall stresses, wall material yielding, and/or the cell's ability to synthesize new wall materials upon wall stretching. In Eq.(1), the "effective cell wall extensibility" ϕ_w (unit $Pa^{-1}{\cdot}s^{-1}$) is given as a function of the apparent wall material extensibility Φ (unit also $Pa^{-1}{\cdot}s^{-1}$) and the ratio between cell radius and wall thickness R/h, depending on the exact cell geometry (Cheddadi *et al.*, 2019).

Hydrostatic pressure (Pa): The pressure that a fluid at rest experiences. In plant cells, it is also called "turgor pressure" and corresponds to the isotropic stress in the cell interior that balances external forces and constraints.

Osmotic potential (Pa): An extensive (proportional to the concentration) quantity with the same unit as pressure, given by the Van't Hoff/Morse equation: $\Psi_\Pi = -iCRT$, where i is the van't Hoff index of the compound (for example, one molecule of KCl dissolves into one

K^+ and one Cl^- ions, so i=2 for KCl), C is the concentration of the compound in mol/m^3, T is the absolute temperature in Kelvin and R the universal gas constant ~8.314 $m^3 \cdot Pa \cdot K^{-1} \cdot mol^{-1}$.

Osmotic pressure (Pa): The opposite of the osmotic potential. A reasonable assumption could be that it is constant during growth, as cells may try to maintain osmotic pressure to ensure normal physiological functions. However, constant osmotic pressure is not guaranteed, as first evaluated by Lockhart (1965), and equations describing osmolyte fluxes have been developed in e.g. pollen tube elongation (Dumais, 2021) and tumorous tissue growth (McEvoy *et al.*, 2020).

Quasi-staticity: Mechanical state very close to equilibrium where the forces experienced by a system almost totally compensate for each other. A quasi-static process happens slowly enough for the system to remain in internal physical equilibrium at each time-point considered; under this hypothesis, the pressure in an idealized cell containing only water is spatially homogeneous (but can vary in time).

Semipermeable membrane: A membrane that allows solvent molecules to pass through but retains solute molecules.

Tensile stress (Pa): Quantifies the internal force exerted on the cross section of a stretched object.

Tension (N): The pulling force in a stretched object.

Topology: The connectivity of geometric objects.

Water potential (Pa): The total energy potential of a fluid relative to pure water at rest state. It depends on many components. At the cellular scale, the major contributions are osmotic potential and pressure potential, all other potentials being negligible.

III. Turgor pressure and growth are variable

a) *The Lockhart model*

In plant cells, growth relies on osmotic water intake and cell wall expansion, often depicted as an irreversible viscoplastic yielding mechanism. This coupling implies that cell growth rate and turgor pressure both depend on the balance between

osmotic pressure and cell wall yielding threshold, Eq.(1b). Equation (1a) corresponds to the seminal Lockhart equation (Lockhart, 1965), formulated in the case of an elongating cylindrical cell. We recall some of its properties and refer readers to a recent review (Dumais, 2021) for a more thorough exploration.

$$\begin{cases} \frac{1}{l}\frac{dl}{dt} = \frac{\phi_a \phi_w}{\phi_a + \phi_w}(\Delta\Pi - Y) & (1a) \\ P = \alpha\Delta\Pi + (1-\alpha)Y & (1b) \end{cases}$$

The entanglement between hydraulics and wall mechanics is omnipresent in Eqs.(1): the proportionality coefficient in the right-hand-side of Lockhart equation, Eq.(1a), is a symmetric function of the effective cell wall extensibility, ϕ_w, and the water conductance, ϕ_a. Their respective contributions can be directly compared by the dimensionless parameter $\alpha = \frac{\phi_a}{\phi_a + \phi_w} \in [0,1]$, with $\alpha = 0.5$ corresponding to an equal contribution of the two parameters, i.e. $\phi_w = \phi_a$. One major outcome of Lockhart's model is that turgor pressure, in the general case (when $\alpha \neq 1$), is not a control variable of growth, i.e. the variable *P* is not present in the right-hand-side of Eq.(1a). Eqs.(1) highlight that both the relative expansion rate and the turgor pressure are controlled by the osmotic potential, $\Delta\Pi$, the cell wall yielding threshold, Y, and the balance between extensibility and conductance. These equations illustrate that turgor and growth result from complex interactions between fluxes and wall expansion, and that they are not unequivocally correlated. In particular, dominant mechanisms underlying growth rate and turgor pressure might not always act equivalently depending on the spatial and temporal resolution considered. Thus, turgor does not determine the rate of growth, but it is an emergent property of this system, tightly linked to growth.

After analyzing an elongating cell with extreme growth anisotropy, we will now look at an isotropically-growing spherical cell.

b) *Links between stress, turgor, and geometry*

In general, plant cells do not only elongate as described in the Lockhart model, but tend to acquire rounder shapes. Combined with their pressurized status, cells are

thus often compared to soap bubbles (Textbox 2). However, though intuitive, the bubble analogy is treacherous, because cell walls do not behave as fluid membranes. Plant cell mechanics are therefore more accurately grasped by pressurized shell models (Ali *et al.*, 2014; Bozorg *et al.*, 2014). Despite their differences, pressurized shells and bubbles share a common feature: a relationship between their shape, inner pressure (P) and the tensile stress (σ) at mechanical equilibrium. In the simple case of an isotropic, homogeneous spherical shell, this relationship is formalized by the Young-Laplace equation:

$$\sigma = \frac{PR}{2h} \quad \Leftrightarrow \quad P = \frac{2h\sigma}{R}, \qquad (2)$$

where *R* denotes cell radius, *h*, wall thickness (Fig.1B), and when the external pressure is zero. Assuming constant thickness h, this equation implies that the ratio σ/P is proportional to R, which is constant in the elongating cylinder in Lockhart's model. To examine the consequences of this difference, consider two extreme cases: first, if pressure is constant because of fast fluxes, stress increases with cell size. This could explain why plants make puzzle cells in leaves, as non-spherical shapes help decrease wall stress (Sapala et al., 2018). From a mechano-sensitive perspective, wall stress could allow cells to "measure" their shape and size, useful information in the context of morphogenesis. Second, if stress is constant, for instance because it is close to the growth threshold, then turgor decreases with size, as was observed in the growing shoot apical meristem (SAM) of *Arabidopsis thaliana* (Long et al., 2020). In general, growing tissues could be situated near either of the states described above, or somewhere in-between. As a result, the size and shape of growing cells should affect turgor, wall stress, and growth.

Having established the turgor-growth relationship in individual cells, we will next explore how multicellularity brings extra complexity to turgor-dependent tissue growth.

IV. Turgor pressure and growth are heterogenous

Turgor pressure can be different in specialized cells, such as stomatal guard cells (Nieves-Cordones *et al.*, 2022), or during invasive growth of pollen tubes (Chebli & Geitmann, 2007) or fungal hyphae (Lew, 2011). Recent studies suggest that cells within the same tissue may also have different pressures. We discuss below how this could happen, and the implications for tissue growth.

a) *Turgor pressure and growth derived from cell arrangements*

Besides physical properties, cells within a tissue can vary in size, geometry, and topology (number of neighbors). Experiments using excised *Arabidopsis* SAMs, cultured either under normal conditions, or treated with oryzalin to inhibit cell division and anisotropic growth by depolymerizing microtubules, show that cell geometry strongly correlates with turgor levels determined by atomic force microscopy (AFM). Thus smaller cells with fewer than six neighbors exhibit higher turgor than larger cells with more neighbors (Corson *et al.*, 2009; Long *et al.*, 2020). A recent model (Long *et al.*, 2020) proposed that geometry and topology are sufficient to generate these heterogeneities, even when applying the simplest assumption; homogeneous cellular properties (Fig. 2). This interpretation treats plant tissues rather like foams (see Textbox 2 for further discussion), and implies that models with more realistic cellular properties should include the emergent turgor heterogeneity as a biomechanical baseline.

b) *Pressure from tissue shape and growth heterogeneities*

In a tissue, plant cells are not mechanically isolated. A specific portion of the cell wall, the *middle lamella*, glues primary walls together. It prevents cell movement and enables propagation of mechanical stresses. Therefore, a consequence for the global quasi-staticity of the tissue is that the overall mechanical stress (σ) in any small portion (Fig. 2F) of the primary cell wall around a given cell encompasses *cell-autonomous* ($\sigma^{(CA)}$) and *non-cell-autonomous* ($\sigma^{(NCA)}$) contributions:

$$\boldsymbol{\sigma} = \boldsymbol{\sigma}^{(\mathrm{CA})} + \boldsymbol{\sigma}^{(\mathrm{NCA})} \quad (6)$$

The cell-autonomous contribution corresponds to turgor-induced stresses imposed by the considered cell on its wall (Fig.3C). The non-cell-autonomous contribution encompasses all the mechanical constraints generated by the surrounding tissues (Fig.3C). These constraints include factors such as pressure-induced contributions from deeper cells and stresses generated by growth heterogeneities. Depending on the tissue and developmental stage considered, the effects of one contribution can override those of another. In the following section we will solely focus on pressure-induced contributions, consistent with the scope of this review.

In the absence of any significant cell-autonomous contribution, *i.e.* $\left\|\sigma^{(CA)}\right\| \ll \left\|\sigma^{(NCA)}\right\|$, non-cell-autonomous mechanical stresses can trigger a mechano-sensitive response. Such

a mechanism has been suggested to play regulatory roles in various organogenic processes (Landrein & Ingram, 2019).

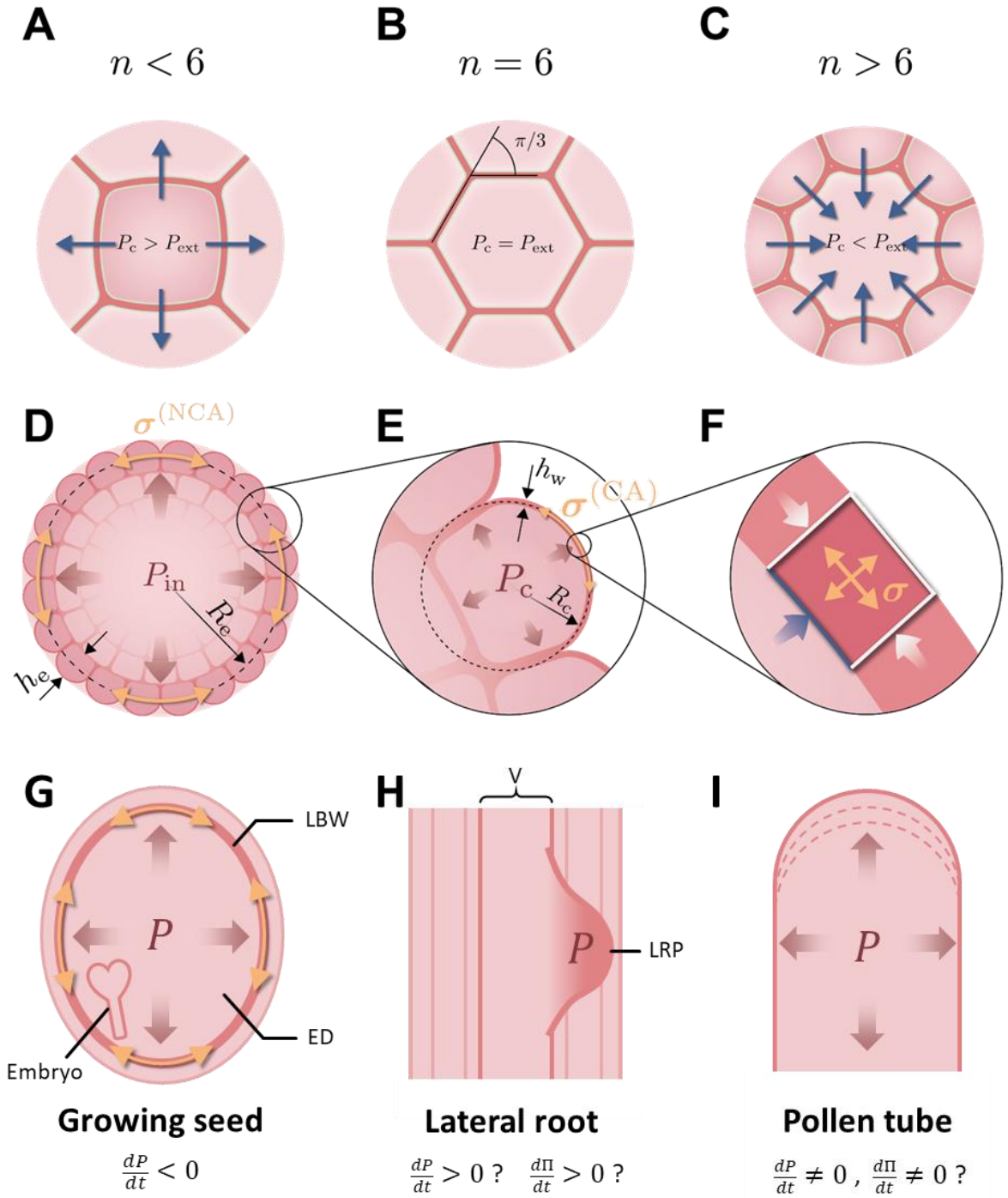


Figure. 2. A, B, C: Qualitative relation between topology (number of neighbors *n*), cell inner pressure (P_c) compared to the pressure of neighboring cells (P_{ext}), and water fluxes (blue arrows). By analogy with foam, cells with less than six neighbors feature higher pressure than their surroundings and tend to exhibit convex walls. Adapted from (Cantat *et al.*, 2013). **D, E, F: Effect of turgor pressure across scales. (D)** At the organ

level, inner tissues exert a pressure (P_{in}) on the epidermal layer (radius R_e, thickness h_e). Epidermal cells experience a non-cell-autonomous stress $\sigma^{(NCA)}$ given by the Young-Laplace equation. **(E)** At the cell level, following the same mechanism, the turgor pressure (P_c) generates a cell-autonomous stress ($\sigma^{(CA)}$) within a cell wall of radius R_c and thickness h_w. **(F)** At the subcellular level, the overall stress (σ) borne by a small piece of cell wall results from cell-autonomous (blue arrow) and non-cell-autonomous (white arrows) contributions (resp. ${f_P}^{(CA)}$ and ${f_P}^{(NCA)}$) exerted respectively by the closest cell and its surroundings on its inner and outer surfaces (resp. S_{in} and S_{ext}). **G, H, I: Examples of dynamic turgor pressure in growing plant tissues and cells. (G)** In the growing seed, the turgor pressure developed by the endosperm (ED) pushes on the load-bearing walls (LBW) of a specific layer within the testa, leading to wall expansion but also to mechanosensitive wall stiffening. Turgor pressure gradually decreases (d*P*/d*t* < 0) during seed expansion to delay wall stiffening (Creff *et al.*, 2021). **(H)** During lateral root emergence, the lateral root primordium (LRP) is proposed to rapidly build up turgor pressure by increasing aquaporin-mediated water conductance, and the overlying tissues lose turgor to facilitate rapid lateral root penetration (Péret *et al.*, 2012) V, vasculature. **(I)** The tip of the pollen tube may grow in an oscillatory manner, possibly because of osmoregulation (Dumais, 2021).

It has been established that many plant outer tissues, such as those of the SAM, seed, sepal, stem, and various animal embryonic and tumorous tissues, are pressurized from below by a fluid or another tissue (Dumais & Steele, 2000; Navis & Bagnat, 2015; Beauzamy *et al.*, 2015; Fourquin *et al.*, 2016; Ruiz-Herrero *et al.*, 2017; Robinson & Kuhlemeier, 2018; Tlili *et al.*, 2019; Zhao *et al.*, 2020; McEvoy *et al.*, 2020; Asaoka *et al.*, 2021; Verger-Serandour & Turlier, 2021). The Young-Laplace law, applicable at the cellular level, also applies to this wider, tissular scale. As Eq.(7) states, cell-autonomous and non-cell-autonomous components of the mechanical stress experienced by the primary wall within a curved tissue can be expressed similarly, but parametrized with different variables:

$$\begin{cases} ||\boldsymbol{\sigma}^{(\mathrm{CA})}|| = \frac{P_{\mathrm{c}} R_{\mathrm{c}}}{2h_{\mathrm{w}}} \\ ||\boldsymbol{\sigma}^{(\mathrm{NCA})}|| = \frac{P_{\mathrm{in}} R_{\mathrm{e}}}{2h_{\mathrm{e}}} \end{cases} \qquad (7)$$

P_c and P_{in} quantify respectively the turgor pressure within the considered cell and the pressure exerted by the compressed or expanding medium underneath the considered tissue. Similarly, the geometrical quantities R_c and h_w depict the radius of the cell and the

thickness of its primary wall, while R_e and h_e account for the curvature radius of the pressurized tissue and its thickness (Fig. 2).

Mechano-sensitive cells embedded within a pressurized tissue can therefore have access to a mechanical stimulus ($\boldsymbol{\sigma}$) directly proportional to a dimensionless geometric variable describing the shape of the tissue and quantifying its size: the ratio between its radius of curvature and its thickness: R_e/h_e. Moreover, in tissues displaying heterogeneous growth patterns, mechanical stresses arise between regions growing at different rates and/or in different directions (Shraiman, 2005), providing cells with dynamic information about their environment.

Textbox 2. Do (plant) cells act like soap bubbles?

Plant tissues have long been compared to soap foams, from cell arrangement (Lewis, 1928; Besson & Dumais, 2011) to tissue mechanics and dynamics (Corson *et al.*, 2009; Long *et al.*, 2020). However, how comparable are plant cells to bubbles? And is it safe to rely on the bubble analogy?

First, although both bubbles and plant cells have tension at their surfaces and pressure within, the mechanical origins of these factors are not the same.

Bubbles and foams are composed of two elements: a gas phase surrounded by a liquid film. Due to inner cohesive forces, the film tends to minimize its surface. Meanwhile, entropic forces in the gas tend to expand it as much as possible. Equilibrium is reached with specific shapes called “minimal surfaces”; spheres in case of isolated bubbles and polyhedral tiling with regular angles of 2π/3 (120°) in case of 2D foams (Fig. 2B). Pressure in the gas volume arises to equilibrate the surface tension in the liquid film. These two constraints, and the geometry of the minimal surface of the system are related through the Young-Laplace equation, eq.(2).

Mechanically, plant cells are more comparable to liquid-filled pressure tanks: a protoplast is embedded within a cell wall. While water influxes tend to increase the protoplast volume, the elastic properties of the surrounding walls, described as a visco-elastic shell, tend to oppose this expansion. Despite being very different in nature, an antagonistic equilibrium, similar to the one at play in soap bubbles, also emerges in plant cells. The conjunction of an expanding volume with a resisting surface generates similar constraints (i.e. pressure in volume and tensile stress on the surface).

The difference between both systems resides in the nature of the surface phases: Contrary

to a liquid film, the solid shell surrounding protoplasts can display complex structures (inhomogeneous and anisotropic) and therefore sustain complex stress and deformation patterns. As a consequence the resulting equilibrium shapes do not correspond to minimal surfaces in the general case.

However, the theory of pressurized shells states that at equilibrium, a relationship between curvature, pressure and tensile stress also emerges. Moreover, in the simple cases where the shell material is homogeneous and isotropic, this relationship is exactly the Young-Laplace equation. So, from a quasi-static perspective, where cell wall composition is excluded, similar relationships between inner pressure, curvature and surface stress can be used to describe soap bubbles/foams and plant cells/tissues.

Second, the volumetric dynamics of bubbles and plant cells are not necessarily the same. In bubbles, the liquid flows much faster in the membrane than the gas diffusion across the membrane, corresponding to α close to 0 in Eq.(1b). The Laplace pressure causes gas to gradually diffuse out of the bubbles, and in a foam, higher-pressured small bubbles tend to lose volume to their bigger neighbors and eventually disappear (von Neumann-Mullins coarsening). In plant tissues, it is likely that water fluxes across plasma membranes occur faster than the expansion of the cell walls, and the classical measurements by Boyer *et al.* (1985) and Cosgrove (1985) correspond to an α = 0.9. Direct cell-to-cell fluxes through plasmodesmata are likely relatively fast too (Rutschow *et al.*, 2011). Interestingly, thanks to the rheology of the cell wall, stress higher than the growth threshold can be constantly relaxed by wall expansion to keep wall stress relatively similar (simulated by Long *et al.*, 2020), which is not a prerequisite for foam-like dynamics (see below). Unlike bubbles that lose gas and shrink, growing cells constantly absorb water from the apoplast by osmosis and shrinkage is rarely observed. And because plant cells may likely modulate α by, for example, mechanical feedback, cell growth rate may have different trends, as discussed in Section IV. So, foam-like dynamics represent a subset of plant tissue dynamics, despite their different mechanical properties.

Lastly, the foam-like analogy is also commonly used outside the plant field. In animal tissues, cell geometry, particularly membrane curvature and cell junction angles, have been frequently used to infer membrane tension and cellular pressure in animal tissues (Roffay *et al.*, 2021). Geometrical inference without membrane curvature can also predict membrane tension and cellular pressure by energy minimization, as in foams (Ishihara *et al.*, 2013). In the plant field, energy minimization has also been classically used to predict and explain the positioning and geometry of the new cell wall formed during cell division

(particularly cell wall curvature), which can be demonstrated by soap films (Besson & Dumais, 2011). The hidden assumption in the soap film system is that the Laplace pressure in the bubbles (equivalent to turgor pressure in plant cells) is related to the membrane curvature. Soap film membranes and the new cell walls were almost always curved as demonstrated by (Besson & Dumais, 2011). Experimental data on turgor measurement or turgor removal by cell ablation also showed strong correlation between anticlinal wall convexity and turgor differences between cells on either side of the wall (Long *et al.*, 2020; Hoermayer *et al.*, 2020). Importantly, none of the above examples require tension or stress to be homogeneous.

To sum up, even though bubbles and plant cells are completely different physical entities and have very different properties, the bubble analogy, although it should be used with caution, is still useful when thinking about cells, and has been used to derive useful predictions in the shoot apical meristem (Long *et al.*, 2020), growing seeds (Creff *et al.*, 2021), and even less "foam-like" tissues such as sepal primordia (Zhao *et al.*, 2020). However, not all plant tissues can be easily compared to foams. For example, the analogy is less obvious in roots, where cuboidal cells are tightly packed into files, or in the leaf epidermis where pavement cells acquire complex, jigsaw-like shapes. How, or if, the bubble analogy may apply to these tissues, remains to be analyzed.

V. Turgor pressure and growth are actively regulated

In the previous section, we considered how nontrivial interactions between turgor and growth can emerge from uniform, constant physical parameters. Thanks to continuous cell wall modification and osmoregulation, growing cells may experience mechanical fluctuations. Here we will discuss how dynamic changes in pressure and hydraulics can contribute to diverse growth patterns.

Pollen tubes are cylindrical cells that show oscillatory growth. Whereas growth is turgor-dependent, neither the growth rate nor the oscillations were found to correlate with turgor (Benkert *et al.*, 1997; Zerzour *et al.*, 2009; Kroeger *et al.*, 2011). Recently, Dumais (2021) proposed a "Lockhart differential equation" to explain the observed growth oscillation. First, the author proposed that growing lily pollen tubes are not at osmotic equilibrium, a proposition supported by experimental measurements. Then, by testing several potential feedback mechanisms, the author predicted that a hydraulic feedback is sufficient to generate and maintain oscillatory growth. This work clearly emphasized the importance of

considering both mechanical and hydraulic regulation to explain growth dynamics (Dumais, 2021; Fig. 2I).

At the organ level, how the pressure of inner tissues affects organ growth has been studied in developing seeds, where the hydrostatic pressure of the endosperm, a zygotic compartment that fills up most of the internal space within early-stage seeds, is critical for seed growth (Beauzamy *et al.*, 2016). Endosperm pressure decreases towards the end of the growth phase and it was hypothesized that pressure drop could trigger growth arrest. However, endosperm pressure also generates tension in the testa, the developing seed coat, and it was proposed that this tension can be perceived by a specific layer of the testa, inducing wall stiffening, thus restricting growth (Creff *et al.*, 2015). Using modelling and experimental approaches, it was shown that endosperm pressure promotes seed growth initially but also restricts growth by inducing testa stiffening. Therefore, somewhat counterintuitively, the reduction of endosperm pressure during the late growth phase does not trigger growth arrest, but allows prolonged seed growth by preventing precocious testa stiffening (Creff *et al.*, 2021; Fig. 2G). This study illustrates the importance of regulating turgor during organ growth, although the associated mechanisms remain undeciphered.

The importance of regulating water fluxes during organ growth was highlighted during lateral root emergence. Lateral roots initiate deep within the root, and need to penetrate the endodermis, cortex and epidermis in order to emerge (Santos Teixeira & ten Tusscher, 2019). Lateral root initiation and emergence are controlled by auxin, which notably represses the expression of aquaporin channel proteins that mediate cross-membrane water fluxes at specific interfaces between the lateral root primordium, the overlying tissue, and the vasculature. Assuming that increasing osmotic pressure in the primordium is necessary for growth, precise patterning of aquaporins likely permits rapid build-up of high turgor in the emerging lateral root, while the surrounding tissues lose volume to accommodate lateral root penetration (Péret *et al.*, 2012) (Fig. 2H). Auxin-induced symplasmic isolation by callose deposition may also contribute to hydraulic repatterning during lateral root formation (Benitez-Alfonso *et al.*, 2013; Sager *et al.*, 2020).

VI. Perspectives

We have considered how complex relationships between turgor pressure and growth arise from tissue mechanics and hydraulics, and how physical modelling is crucial to permit understanding of the results of such complexity. We propose that turgor does not set the growth rate, but is an emergent property that drives growth. An important emerging question is to what extent this new vision of turgor pressure and tissue hydraulics adds to the existing

understanding of how plant growth is regulated? As cell wall stress and turgor pressure are interdependent, it is likely that their sensing and regulation are also biologically connected. In support of this, recent studies revealed that some wall defective mutants show turgor defects (Mielke *et al.*, 2021; Bacete *et al.*, 2021), and that wall-induced growth defects can be rescued by turgor manipulations (Verger *et al.*, 2018; Malivert *et al.*, 2021). These findings suggest that wall mechanics and turgor are biologically coordinated and may share common regulatory mechanisms. Possible candidates in the perception of these mechanical cues include receptor-like kinases (Bacete *et al.*, 2021; Malivert *et al.*, 2021) or mechanosensitive channels (Basu & Haswell, 2020), and transduction to the cytoskeleton (Colin *et al.*, 2020). Additionally, pressure is uniform in all directions, and its translation into biologically relevant signals during directional growth must rely on (bi-)directional cues such as anisotropy (Sassi *et al.*, 2014), polarity (Ali *et al.*, 2019), or gradients (Robbins & Dinneny, 2018). Finally, turgor has the potential to integrate information from biomechanics, hydraulics, physiology and metabolism, as many osmotically active compounds are energy sources, biological building blocks, and physiological regulators (Beauzamy *et al.*, 2014). Examples in cotton fiber elongation revealed involvement of all these processes (Ruan *et al.*, 2001), and it will be both informative and exciting to investigate how such information is coordinated in other growing systems.

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