Assessing the hydromechanical control of plant growth

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Abstract:

Multicellular organisms grow and acquire their shapes by controlling the differential expansion and deformations of their cells. Recent research has addressed the role of the mechanical properties of cells and tissues in these processes. In the case of plants, it is generally believed that growth rate is a function of the mechanical stress exerted on the cell wall, the thin polymeric layer surrounding cells, involving an effective viscosity. Nevertheless, recent studies have questioned this view. On the one hand, it was suggested that cell wall elasticity is involved in setting growth rate. On the other hand, growth requires water uptake by the plant and it was proposed that hydraulic conductivity to water may be limiting for plant growth. To extensively assess these issues, we chose to work on the genetically identical small plants vegetatively produced by *Marchantia polymorpha* and we developed a microfluidic device to quantify growth rates, elastic properties, and hydraulic conductivity of individual plants in a controlled environment with a high-throughput. We characterized the effect of osmotic treatment and of abscisic acid on plant growth and hydro-mechanical properties. Overall, we find that the instantaneous growth rate of individuals is correlated to both their bulk elastic modulus and their hydraulic conductivity, albeit the correlation is weaker with the latter. Our results are consistent with a framework in which growth rate is determined primarily by elasticity of the wall and a chemical rate associated with remodeling of the cell wall or with changes in its composition and secondarily by hydraulic conductivity. Accordingly, the coupling between chemistry of the cell wall and hydromechanics of the cell appears as key to set growth patterns during morphogenesis.

Introduction

Plant growth and morphogenesis are complex multiscale phenomena where both (bio)chemistry and (bio)mechanics play a fundamental role. While there is little questioning that mechanical forces are involved in growth [1–5], the relevance of different hydromechanical (hydraulic and mechanical) properties in growth is still subject to diverging views, notably the role of water conductivity and elastic deformations of cells and tissues [5–7]. The high concentration inside plant cells creates a differential of water chemical potential that drives water into the cell [6], creating a high mechanical pressure called turgor that is resisted by the rigid cell wall, the polymeric thin layer around cells; the balance between turgor pressure and wall resistance sets growth rate of cells and tissues [1–5,8]. Uptake of water is dependent on the plant water conductivity, but whether growth may be limited by conductivity has been debated given the experimental data collected on specific organs or full plants [6,7]. Recently, there has been a renewed interest in this question as well as in the role of turgor pressure in growth [9]. Experimental and theoretical studies suggested that water conductivity of cells limits their growth and plays a role in the fine tuning of turgor pressure [10,11]. In this study, we further examine the relation between hydraulic conductivity and growth rate of the tissue.

The wall's ability to deform under pressure controls the growth dynamics along with the regulation of turgor pressure. This ability depends on wall elasticity or viscosity, remodeling of its architecture, and changes in its composition. Attempts to model wall expansion fall into different frameworks, most of them considering either stress (force) or strain (deformation) as the main determinant of growth rate [12]. Stress is a measure of the physical forces applied on a material, corresponding to a force per unit surface applied to the sample. The strain is a measure of said material's deformation under stress, corresponding to a relative increase of dimensions of the sample. In the case of an elastic material, the elastic modulus is the

proportionality constant between stress and strain. In this study we provide experimental evidence that elastic strain and material elasticity are key to understanding growth regulation.

In most frameworks, the strain rate, or expansion rate, of the cell wall depends on stress in the wall. This is typically the case of the Lockhart model [13] where the growth rate $\boldsymbol{G}$ is proportional to stress $\boldsymbol{\sigma}$ in excess of a threshold $\boldsymbol{Y}$ (yield stress), the proportionality factor being the extensibility $\boldsymbol{\Phi}$ which can be physically interpreted as the inverse of an effective viscosity: $\boldsymbol{G} = \boldsymbol{\Phi}(\boldsymbol{\sigma}\text{-}\boldsymbol{Y})$. This seminal description spawned a whole class of viscoplastic models of plant growth [14–17]. Some of those models include the elastic modulus in their mechanical parameters but their final formulations make the modulus dispensable to describe steady state growth, ending in description where viscosity (or extensibility) alone is relevant.
Experimental work was conducted to assess the validity of Lockhart's model [18–20] as well as to understand further the biological basis of the relevant parameters [21,22] and get an estimation of their value [19,23]. However, it is important to note that the results of the aforementioned work could also be interpreted outside of this stress derived framework, because stress is proportional to elastic strain, a measure of the reversible deformation of the sample under stress.

Notably fewer theoretical studies considered elastic strain as the relevant mechanical variable to set growth rate [24,25]. Nevertheless, mechanistic considerations on remodeling of the cell wall led to chemo-mechanical models of cell wall expansion that are compatible with such strain-based model of growth [24,26]. In this framework cell wall expansion rate is proportional to elastic strain and so is inversely proportional to elastic modulus (for a given turgor pressure). The proportionality prefactor is a time scale that would be related to the chemistry of the cell wall. Recent experimental work led to progress in the measurement of the cell wall mechanical properties and in particular of its elastic modulus [27]. Some of these studies hint at the importance of elasticity and elastic strain in the regulation of growth in the shoot apical meristem of flowering plants. Organ outgrowth was found to be dependent on cell wall remodeling (pectin methyl-esterification in this case), which was associated with a reduction in elastic modulus [28,29]. Within the shoot apex, domains growing at a slower rate were found to have

smaller elastic strain based on osmotic treatments [30] and higher elastic modulus based on atomic force microscopy [28,31,32]. In the roots of various species the fast growing regions were measured to have a lower modulus [33].

The difference in modeling between a viscous approach (based on extensibility) and an elastic approach (based on elastic modulus and a chemistry derived time constant) may seem small as they end up with the same mathematical description of growth dynamics. They however differ greatly in terms of underlying mechanisms. Overall, available experimental results do not discriminate between elastic strain and stress as the main determinant of growth rate. This is in part due to the difficulty of measuring mechanical properties and growth characteristics of the same individuals with a high throughput.

Altogether, it appears that the hydromechanical basis of growth control in plants is far from being fully understood. In order to make progress, we chose *Marchantia polymorpha* as a model organism, using a microfluidic system to grow small plants in the same conditions. While Marchantia plants provide large numbers of genetically identical progeny by vegetative propagation, microfluidic chips enable the observation of a high number of individuals over time [34–36]. We developed a microfluidic-based approach to characterize hydromechanical properties, giving us access to growth rate, elastic modulus, and hydraulic conductivity of the same individual plants with high throughput.

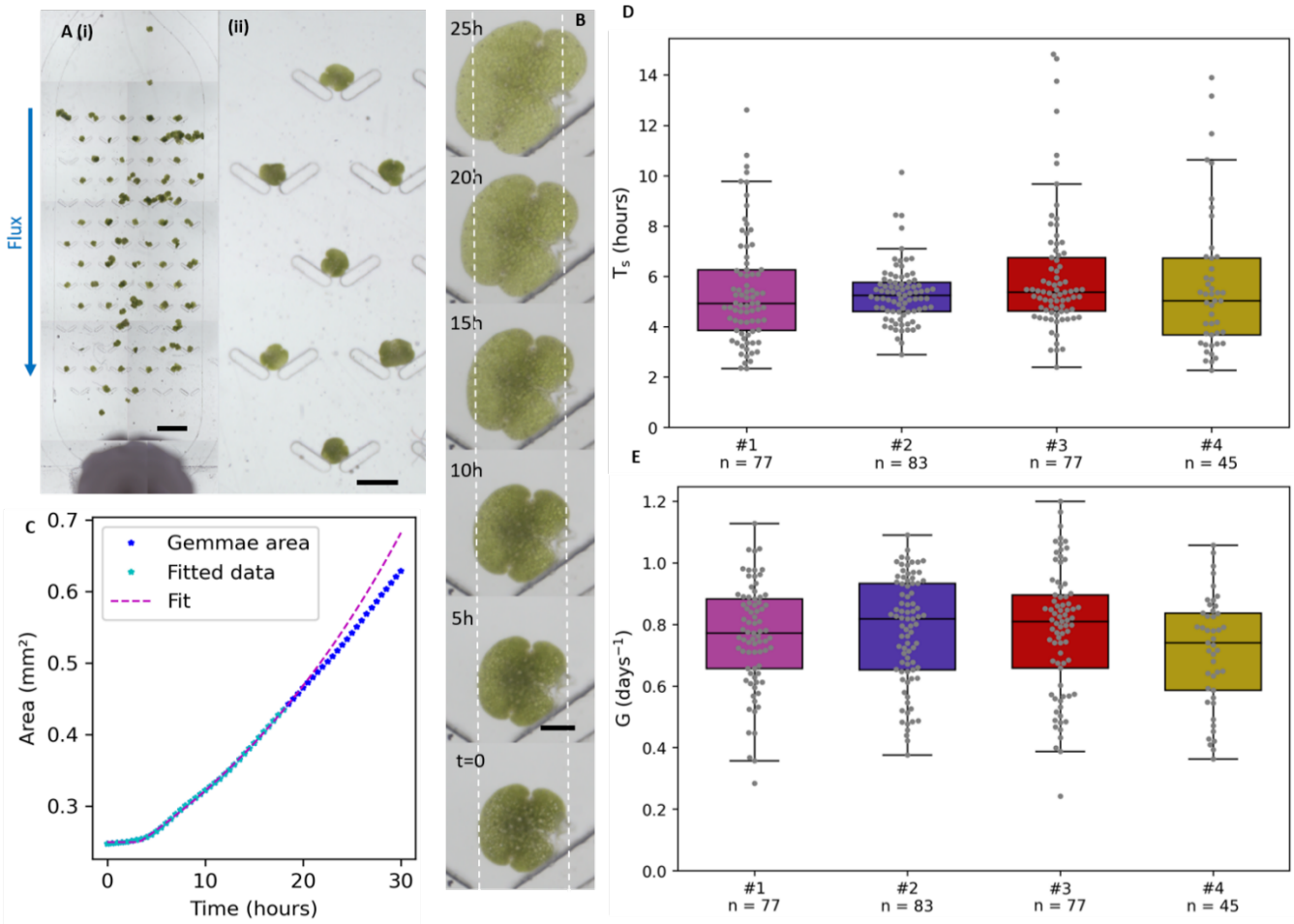
Results

High-throughput characterization of growth and hydromechanics in controlled conditions

Growth

To observe in parallel a large number of individuals in the same conditions, we designed a microfluidic chip with V-shaped traps (Fig. 1-A) to immobilize 50-100 small *Marchantia* plants (gemmae) during 30h. Gemmae were immersed in liquid growth medium (see methods for details), which was continuously renewed by slow injection in the chip during the experiments. We first optimized the collection of gemmae from the mother plant and ensured that growth of

the gemmae was robust to the choice of experimental conditions (Fig. S1). We tracked gemmae growth with a microscope (Fig. 1-B) and retrieved their area using image analysis (see methods). We observed an early phase with no visible growth, followed by a phase of approximately exponential growth (Fig. 1-C).



<u>Figure 1.</u> Growth in a microfluidic chip

A. (i) Microfluidic chip design with V-shaped traps holding several gemmae (small Marchantia plants) for parallel observation. Scale bar: 5 mm. **(ii)** Zoom on traps. Scale bar: 1mm. **B.** Growth of a gemma in time. The dashed lines serves as a marker of initial size. Scale bar: 250µm. **C.** Areal growth of the gemma represented in B (blue). The magenta dashed line is a delayed exponential fit. Fitted points (15h after growth start) are shown in light blue. **D.** Values of growth start delay for 4 independent experiments. **E.** Values of average growth rate on 15h for the same experiments as (D).

We characterized this dynamics with two parameters T_s and G, obtained by a delayed exponential fit of each growth curve up to 15h of exponential growth (see methods). T_s is the time between the immersion of plants in solution (a timing called imbibition for seeds) and the start of exponential growth, G is the increase rate of the exponential.

In total we quantified growth for n = 282 gemmae in N = 4 independent experiments (Fig. 1-EF). The median growth start time T_s over all experiments is 5.20 hours (4.93 to 5.38 hours for individual experiments medians). The variability (expressed as relative average absolute deviation, see methods for more details on statistical analysis) is of 28% over the complete distribution (from 16% to 40% in individual experiments) showing a highly variable time of growth start. The growth rate G has a median of 0.78 days^{-1} (0.74 to 0.82 days^{-1}) with a variability of 19% (17% to 20%) indicating that the growth rate of gemmae is also quite variable. For both measurements the variability in single experiments is higher than the difference across experiment medians (~10%), underlining the fact that the overall variability is representative of the variation between gemmae and not an artifact of data pooling. Such level of variability within a single sample is reminiscent of the germination time of seeds that can be largely variable even for seeds placed in identical conditions [37].

Bulk elasticity

The microfluidic system that we built also allows for rapid (~1min) change of the medium in which the gemmae are developing. We took advantage of this to design a method to measure the mechanical properties of gemmae. This is based on osmotic steps during which the control growth medium is replaced with a hyperosmotic medium (control medium supplemented with 100mM mannitol). This drives water out of plants and leads to the contraction of the gemmae by a few percent in area that can be observed and measured through image analysis (Fig.2-AB). The reverse step is applied 15 minutes later by refilling the chip with the control medium and the inflation of the gemmae is observed and quantified as well. From the curve associated with these area deformations it is possible to extract a bulk elasticity modulus from the deflation and to quantify deformation irreversibility (see methods for details). The first osmotic step appears to stop gemmae growth during the 15 minutes of treatment but for the return step and inflation we

were led to account for growth of the gemmae to get a better measurement of recovery strain (Fig. 2-B and methods for details on the fitting). We can thus quantify the elastic modulus (Fig.2-C) as well as the irreversibility of the deformation between the two steps (Fig.2-D). In this experiment (n = 63) we obtained a median bulk elastic modulus of 16.6 MPa with 14% of variability; this value of modulus is in the lower range of moduli measured for plants [1], though comparable to bulk moduli measured for other bryophytes [38]. The distribution of deformation irreversibility has a mean of 107.7% (p = 5.0e-10 in comparison to 100% that corresponds to full reversibility). This slight irreversibility could be ascribed to potential damage to the cell walls caused by the initial step and contraction, or to fast adaptation of the plant during the 15 minutes between the osmotic steps.

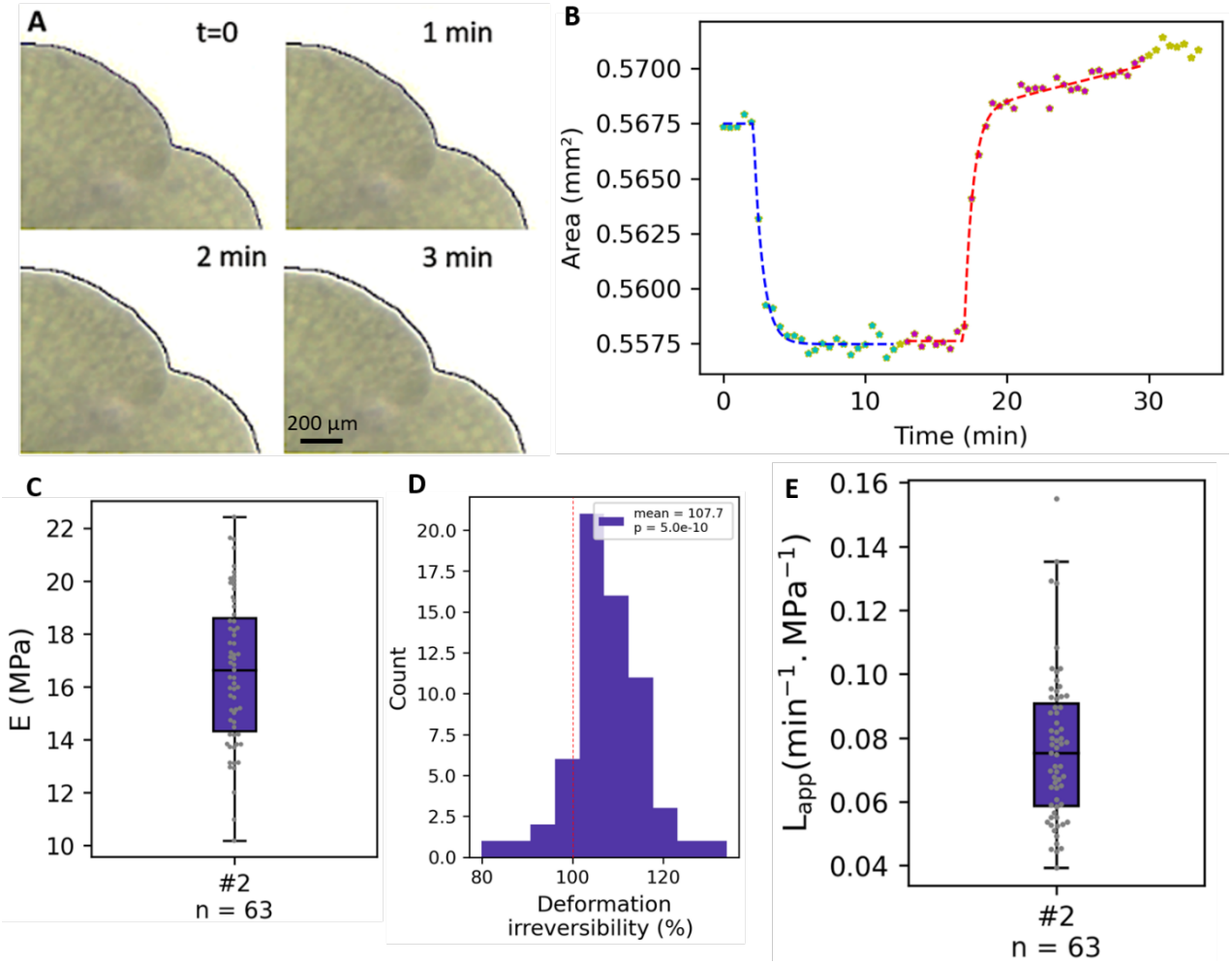
To get a better quantification of gemmae elasticity and its variability we examined this experiment (Fig.2-CD) together with the controls of the different treatments discussed hereafter (Fig.4), yielding N = 6 experiments with n = 222 individual plants. The median bulk elastic modulus is 13.95 MPa (11.9 to 16.6 MPa in individual experiments) and the variability is 16% (11% to 14% in individual experiments). Contrary to what we observed for $\boldsymbol{T_s}$ and $\boldsymbol{G}$, here the global variability comes from the difference between the experiments more than from internal variability. Regarding deformation irreversibility, individual experiments go from 101.7 to 107.7% of irreversibility, and are statistically different from 100% in 3/6 experiments. The standard error of those distributions is between 1.0 and 2.8 % of irreversibility.

Overall these measurements are very consistent and show that our method is robust. The observed low level of deformation irreversibility also supports the definition of a bulk elastic modulus for gemmae.

Hydraulic conductivity

Using the dynamics of deflation (Fig2-B), we also designed a method to extract the apparent hydraulic conductivity of the plant, based on modified Lockhart equations (see methods). Note that this is an individual-level conductivity accounting for cell membranes, cell wall, and cuticle. In the first experiment (Fig2-E, n=63) we obtain a median apparent conductivity $\boldsymbol{L_{app}}$ of 0.08 min^{-}

^{1}.MPa^{-1} with 24% of variability (n=63). When pooling all controls we get a median of 0.09 min^{-1}.MPa^{-1} (0.08 to 0.11 in individual experiment) with 22% of variability (10% to 24% in individual experiment). Like for the growth rate, the variability of individual experiments is higher than the variability between experiments median (~15%), here again indicating a high internal variability of the apparent hydraulic conductivity.



<u>Figure 2.</u> Measuring hydromechanical properties with osmotic steps

A. Illustration of the deformation of a gemmae subjected to an osmotic step (100mM mannitol). We show here a portion of the gemmae; the black line represents the contour of the gemmae at t=0 to underline the deformation. At t = 2 and 3 minutes one can observe a thin white line between the black line and the gemmae, corresponding to the contracted area. **B.** Dynamics of the area of a gemmae when subjected to an osmotic step followed by a return to control medium. The light blue dots are fitted by the blue dashed line to obtain the elastic modulus and conductivity, and the mauve dots are fitted with the red line to obtain elastic reversibility. **C.** Distributions of bulk moduli. **D.** Distribution of irreversibility levels. The red dashed line illustrates the complete reversibility to which the distribution mean is compared. **E.** Distribution of apparent conductivity.

Characterisation in perturbed conditions

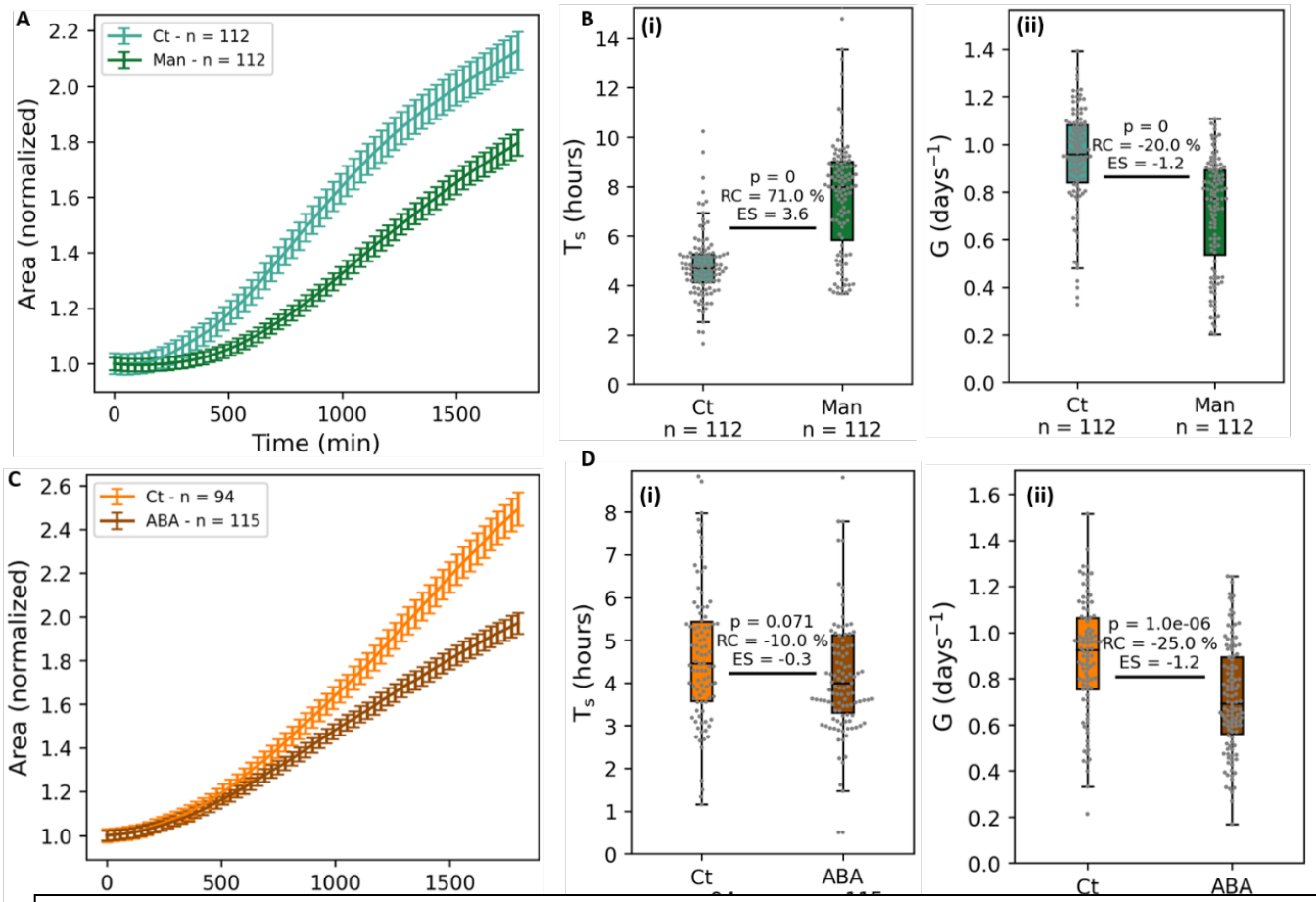
Growth

To further probe the relation between modulus and growth rate, we used our system and analysis pipeline to quantify variations due to classical treatments. We considered the change of growth dynamics caused by external physico-chemical constraints or perturbed signalling. For each condition we used two microfluidic chambers in parallel, one control and one perturbed condition, with a common gemmae pool split between them, and repeated the experiment N = 3 times. The aggregated results are presented in Fig.3 and the detailed results per experiment are available in Fig.S3.

We started with hyperosmotic treatment because this classically reduces growth. Gemmae were observed in control medium permanently supplemented with 100mM of mannitol (Man, Fig.3-A). This treatment significantly reduces the growth rate of gemmae, with a relative change RC = -20% when compared to control ($p < 1e-10$) and an effect size (see methods) ES = -1.2, as expected. Another effect of growing in a hyper osmotic environment (Fig. 3-AB) is a largely delayed growth start with a relative change RC = 71% ($p < 1e-10$) and an effect size ES = 3.6 indicating a strong change.

We then performed chemical treatment using a medium complemented with the abscisic acid (ABA) phytohormone. ABA is produced when water is lacking and enables plants to adapt to water stress [39,40]. The response to ABA was proposed to induce a delay in dormancy exit of Marchantia gemmae based on the observation of rhizoids emergence [41]. Based on these previous studies, we used a concentration of 3.5µM ABA. Unexpectedly, this treatment (Fig. 3-CD) had a small, non-significant decreasing effect on the growth start time (RC = -10%, ES = -0.3, $p = 0.071$). However it had a similar effect on growth rate reduction as the hyper-osmotic treatment did (RC = -25%, ES = -1.2, $p = 1.0e-6$), consistent with ABA-treated plants being smaller in [39].

Overall those perturbations lead to changes that are easily quantifiable with our system, in particular thanks to the observation of large numbers of individuals in identical conditions.



<u>Figure 3.</u> Growth in perturbed conditions

A. Area vs. time curves illustrating growth in hyperosmotic medium — reference medium supplemented with 100mM mannitol (shortened Man) — compared to reference medium (control, Ct). Each curve represents average and standard error for all individuals pooled from N = 3 experiments. The area curves are normalized by the initial (t=0) value for better comparison between conditions, see Fig. S3 for detail by experiment **B.** Quantification of growth start time (i) and growth rate (ii) for N=3 experiments in hyperosmotic medium. **C.** Area vs. time curves illustrating growth with abscisic acid (ABA, 3.5μM) treatment compared to reference medium (control, Ct). Each curve represents average and standard error for all individuals pooled from N = 3 experiments. The area curves are normalized by the initial (t=0) value for better comparison between conditions, see Fig. S3 for detail by experiment **D.** Quantification of growth start time (i) and growth rate (ii) for N=3 experiments with ABA.

Bulk elasticity

We then moved on to quantify the effect of those treatments on the hydromechanical properties of the gemmae (Fig.4). We performed osmotic steps on gemmae that grew 30h either in +100mM hyperosmotic medium or with 3.5µM ABA.

For the gemmae that grew in hyperosmotic medium (Fig.4-A & S4-AB, N = 3, n = 95 & 80 for control and treated sample, respectively) we observed a decrease of the bulk elastic modulus, *E*, when compared to their control (RC = -29%, ES = -2.0, p < 1e-10). In addition, we saw a small decrease of deformation irreversibility in the treated sample (RC = -4%, ES = -0.7, p = 2.0e-4) (Fig.S4-A).

Regarding the effect of ABA treatment (Fig.4-B & S4-DE, N = 2, n = 64 & 78) we can see a small, non-significant, decrease of elasticity (RC = -6%, ES = -0.2, p = 0.067). The irreversibility is unaffected by ABA treatment (Fig.S4-D).

Hydraulic conductivity

Finally, we examined the apparent hydraulic conductivity $\boldsymbol{L_{app}}$. A hyperosmotic medium (Fig.4-C & S4-C, N = 3, n = 95 & 80) slightly increased conductivity (RC = +12%, ES = 0.6, p = 0.014) in comparison to control. For ABA treated gemmae (Fig.4-D & S4-F) we observed a very large increase of $\boldsymbol{L_{app}}$ (RC = +60%, ES = 3.4, p = 5.4e-10) when compared to control.

Relation between growth rate and hydromechanical properties

We then asked if there was a relationship between the growth rate of a gemmae and its hydromechanical properties. The system we designed is well suited to answer such a question, as osmotic steps can be performed on a gemmae population after observation of its growth. It is thus possible to obtain a bulk elastic modulus and conductivity for gemmae whose growth dynamic has previously been characterized. When first looking at the correlation between growth rate $\boldsymbol{G}$ and hydromechanical properties ($\boldsymbol{E}$, and $\boldsymbol{L_{app}}$) in controlled condition, we found low

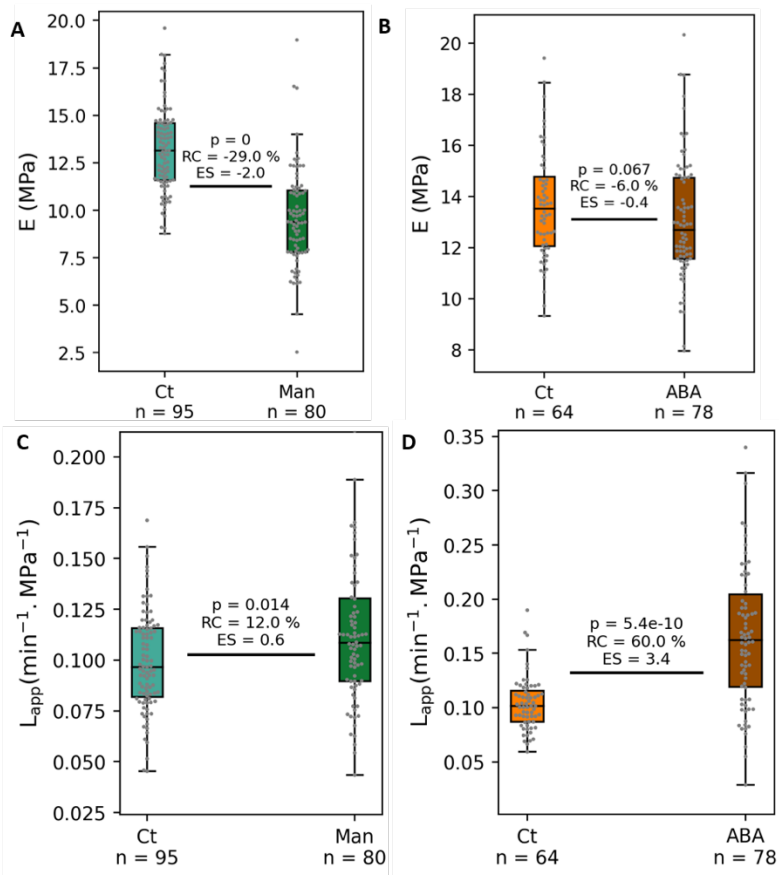


Figure 4. Hydromechanical properties in perturbed conditions

A. Elastic moduli for control (Ct) and mannitol (Man) treated gemmae. **B.** Elastic moduli for control (Ct) and abscisic acid (ABA) treated gemmae **C.** Apparent conductivity for control (Ct) and mannitol (Man) treated gemmae. **D.** Apparent conductivity for control (Ct) and abscisic acid (ABA) treated gemmae

correlations, significant for modulus $\boldsymbol{E}$ (CC = -0.197, p = 0.008) and non-significant for conductivity $\boldsymbol{L_{app}}$ (CC = 0.135, p = 0.071) (Fig.S5-B(i)C(i)). As elasticity is measured from a fast deformation (a few minutes) compared the area doubling time (several hours), we sought a more 'instantaneous' characterization of growth as close as possible in time to the measurement of elasticity. For this we measured the instantaneous growth rate $\frac{1}{A}\frac{dA}{dt}$ from the growth curve and took its average value over the last 4 time points (~2 hours, see Fig. S5-A) to define $\boldsymbol{G_{inst}}$ as the 'instantaneous' growth rate just before the step. We found a correlation coefficient of -0.503 (Spearman's rho, p < 0.0001) (Fig.5-A) between $\boldsymbol{G_{inst}}$ and the modulus $\boldsymbol{E}$ and a correlation coefficient of 0.318 (Spearman's rho, p < 0.0001) between $\boldsymbol{G_{inst}}$ and the modulus $\boldsymbol{L_{app}}$ for all controls.

We note that these correlations are based on gemmae for which both growth and elasticity/conductivity are properly quantified, reducing the number of gemmae to n = 179, lower than those observed growing (n = 289, Fig.1-DE & Fig.3) and those characterized mechanically

(n=222, Fig.2-C & Fig.4). This is due to the fact that performing osmotic steps implies a fast flow of medium that can push away some gemmae from the observation field.

In the case of elasticity we found a clear decreasing relationship between elastic modulus $\boldsymbol{E}$ and instantaneous growth rates $\boldsymbol{G_{inst}}$ (Fig.5-B). This significant correlation ($p < 0.0001$) shows that being softer goes together with faster growth, without indicating any causality of effect between those two parameters. The fact that the correlation is better when considering instantaneous growth rate suggests that the mechanical properties and instantaneous growth rate change together in time during gemmae growth. In the case of conductivity we found a positive, significant relationship between $\boldsymbol{L_{app}}$ and $\boldsymbol{G_{inst}}$ (Fig.5-C). This indicates that the hydraulic conductivity is higher in plants that grow faster.

We then further examined potential causal relations between these variables, keeping in mind that causality cannot be demonstrated only using correlations. To do so, we considered the correlation between hydromechanical properties ($\boldsymbol{E}$ and $\boldsymbol{L_{app}}$) and the growth rate after the osmotic step ($\boldsymbol{G_{post}}$, computed from the end of the re-inflation curve, see methods). We see a slightly weaker correlation between $\boldsymbol{E}$ and $\boldsymbol{G_{post}}$ than with $\boldsymbol{G_{inst}}$ and a stronger correlation between $\boldsymbol{L_{app}}$ and $\boldsymbol{G_{post}}$ than with $\boldsymbol{G_{inst}}$ (Fig.5-A). For each parameter ($\boldsymbol{E}$ and $\boldsymbol{L_{app}}$) we compared correlations with $\boldsymbol{G_{post}}$ and with $\boldsymbol{G_{inst}}$ to see if the difference between them is significant (see methods). We found that the correlations were not significantly different in the case of modulus $\boldsymbol{E}$. However, the correlation between $\boldsymbol{L_{app}}$ and $\boldsymbol{G_{post}}$ is significantly stronger than the one with $\boldsymbol{G_{inst}}$. Given that we first measure $\boldsymbol{G_{inst}}$, then $\boldsymbol{L_{app}}$, then $\boldsymbol{G_{post}}$, this suggest that the conductivity may predict the growth rate.

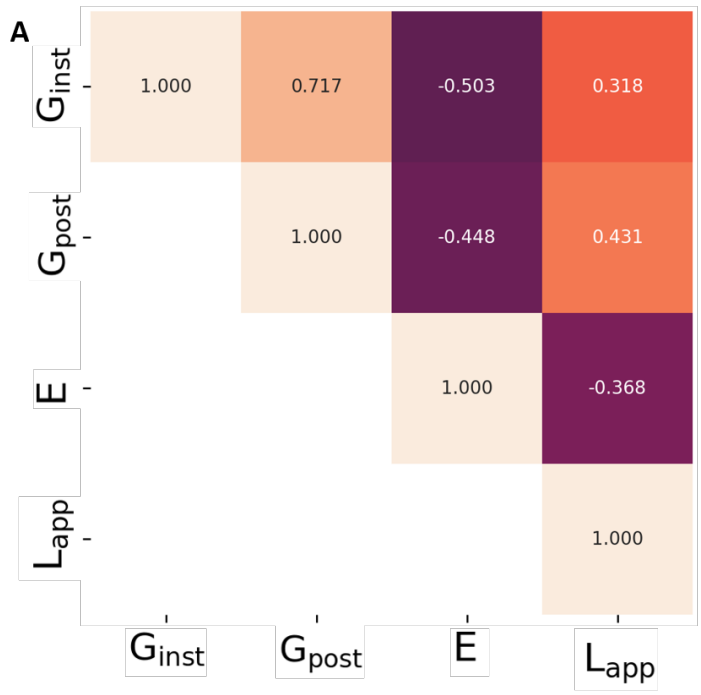
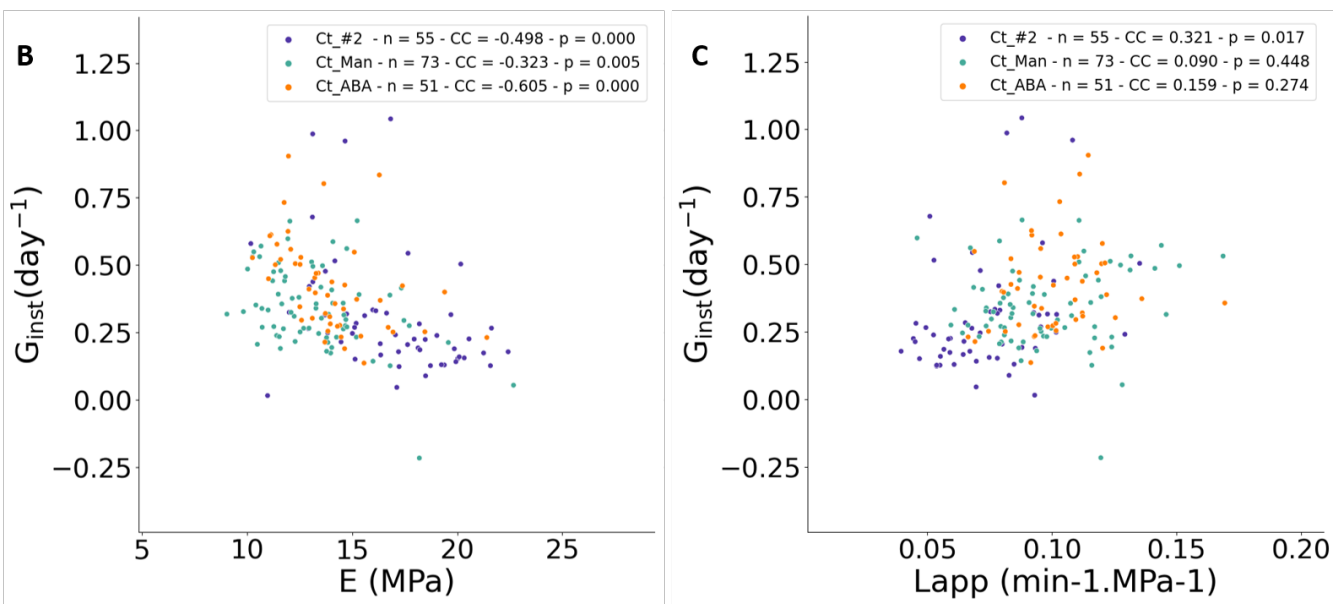


Figure 5. Link between hydromechanical properties and growth: bulk modulus E, apparent conductivity L_{app} , instantaneous growth rate G_{inst} , and growth rate avec the steps G_{post}

A. Correlation table for all control experiments pooled. All correlations have $p < 0.0001$. **B.** Correlation between G_{inst} and E for all the different controls. **C.** Correlation between G_{inst} and L_{app} for all the different controls.



Discussion

We developed a microfluidic system to study the early growth of *Marchantia* gemmae in a controlled environment and with a high throughput. The environmental control given by microfluidics allowed us to perform osmotic steps on the growing gemmae and thus directly link their mechanical properties to their growth rate. In the course of 12 independent experiments we quantified the growth of 715 individuals and the hydromechanical properties of 426 individuals. The range of values we found for the bulk elastic moduli have the same order of magnitude as those measured in a range of bryophytes [38]. For the apparent conductivity if we consider the half-thickness of the gemmae (~ 50µm) over which water has to flow to exit, and we use the median conductivity in controls (0.09 $min^{-1}.MPa^{-1}$) we get a conductivity of ~7.10^{-8} $m.s^{-1}.MPa^{-1}$ that is in the same range as the conductivity measured in roots of flowering plants [42–44].

For 179 individuals among the control experiments we had access to both hydromechanical properties and growth rate, which allowed us to show that there is a correlation between bulk elasticity of the gemmae and their instantaneous growth rate in line with previous reports of stiffer regions of the shoot apical meristem of tomato growing slower [30] and softer regions of roots growing faster [33]. A similar correlation was recently observed at cell wall level in Marchantia [45]. The weaker correlation between hydraulic conductivity and growth rate suggests that growth rate is partially limited by the plant water conductivity. However this correlation arises when considering a very large number of individuals and is not necessary significant in individual experiments where the median conductivity is larger (Fig.5-C). This suggests that the water conductivity of Marchantia in control conditions is in a critical range where it does not always limit growth. This critical range could be relevant in terms of growth control as it only takes a small relative change of conductivity to either slow down growth or to release the limitation by hydraulic conductivity. This critical range may also explain diverging results and the ensuing debate about the role of water conductivity in plant growth [6,7]. In addition, given the observed variability of growth rate in time (see Fig.S2-C for a representative curve), and the variability of hydromechanical properties across experiments, our results suggest that the regulation of instantaneous growth rate is directly linked to hydromechanical properties of the plant.

When subjecting the gemmae to water-stress by the means of a permanent hyper-osmotic environment, a delay in growth start as well as a reduced growth rate were observed, consistent with classical observations on plant growth in water-stressed conditions [46,47]. We also found gemmae to be softer after having grown in hyperosmotic medium. This was observed for the unicellular algae *Chlorella emersonii* [48] and is in line with early reports of osmotic treatment-induced extensibility variation in maize [49,50], where the increase of extensibility could be interpreted as a decrease of elastic modulus. Our results significantly strengthen those observations thanks to a tight control of experimental conditions and a large number of individuals probed. Water conductivity is slightly increased by this treatment. Even though osmotic stress generally decreases root hydraulic conductivity [42], is has been shown to increase it in some accessions [44]. Taken together the increase of conductivity and decrease of elastic moduli suggest that the reaction of the plant to osmotic stress is to adjust its hydromechanical properties to increase growth rate (Fig.5), partially compensating the growth rate reduction caused by the reduced osmotic differential.

When gemmae were treated with abscisic acid (ABA), we observed a reduction in growth rate, in line with previous observation of ABA treatment on plant growth [51]. Surprisingly ABA treatment had no visible effect on the timing of growth start. Because ABA treatment has previously been reported to delay dormancy exit in *Marchantia polymorpha*, as measured by the first appearance of rhizoids [41], this result raises the question of the appropriate criterion for dormancy exit definition. We measured a growth start around 5h while the time of rhizoids appearance is rather at the timescale of a day. We can postulate that the delay in rhizoid appearance would rather be due to a slower growth that would make them reach the gemmae periphery later, than with a delayed dormancy exit. Regarding hydromechanical properties, growth in ABA medium did not affect the bulk elasticity of gemmae, but greatly increased its water conductivity. Similar results have been found in roots in response to ABA [43]. This shows that the role ABA plays a role in water stress regulation [51] by modulating water conductivity

rather than mechanical properties. If we consider the previous observation that ABA reduces cell wall extensibility [51], this suggests that ABA also affects the dynamics of wall remodeling.

Our results on growth and mechanics taken together with previous work support a modeling framework where, in stationary conditions, the growth rate depends on $\boldsymbol{E}$ as (1): $\boldsymbol{G = \chi(P-Y)/E}$ [24], with $\boldsymbol{E}$ is the elastic modulus, $\boldsymbol{P}$ the turgor pressure and $\boldsymbol{\chi}$ is a rate corresponding to cell wall remodeling, and $\boldsymbol{Y}$ is a pressure threshold for expansion to occur. It can also be expressed as (2): $\boldsymbol{G = \chi(\varepsilon - \varepsilon_Y)}$ with $\boldsymbol{\varepsilon = P/E}$ the elastic strain and $\boldsymbol{\varepsilon_Y = Y/E}$ the deformation threshold for expansion to occur. In (1) $\boldsymbol{\chi/E}$ is the analog of the extensibility $\boldsymbol{\Phi}$ in Lockhart's model, but with a different mechanistic interpretation. Instead of viewing it as the inverse of a viscosity, as Ortega did when he derived his augmented growth equation from Maxwell linear viscoelastic model [53], we can interpret it as the capacity of the wall to remodel itself at a rate χ when under an elastic deformation $\boldsymbol{\varepsilon}$ defined by $\boldsymbol{P}$ and $\boldsymbol{E}$, as the expression in (2) suggests. This leads to a reinterpretation of plant growth modulation by osmotic and ABA treatment: osmotic treatment reduces the modulus $\boldsymbol{E}$ [49] and thus increases extensibility $\boldsymbol{\Phi}$, while ABA treatment reduces remodeling rate so $\boldsymbol{\Phi}$ decreases as well [51], assuming other parameters constant. This framework is also in better agreement with studies associating elastic strain and growth rate [30].

We may include equation (1) into the full Lockhart-Ortega equations describing the area growth of gemmae:

$$\frac{1}{A}\frac{dA}{dt} = \frac{\chi}{E}\,(P - Y) + \frac{1}{E}\frac{dP}{dt} \qquad (3)$$

$$\frac{1}{A}\frac{dA}{dt} = -L_{app}\,(\mathrm{P} - \Delta\pi) \qquad (4)$$

When osmotic pressure is constant, (3) and (4) lead to:

$$= \frac{1}{A}\frac{dA}{dt} = \frac{\chi\, L_{app}}{E\, L_{app} + \chi}\,(\Delta\pi - Y) \qquad (5)$$

The formulation (5) recapitulates our results: when conductivity, L_{app}, is very high growth rate is proportional to $\boldsymbol{\chi/E}$ and conductivity is not limiting for growth. When L_{app} is comparable to $\boldsymbol{\chi/E}$, growth rate is negatively correlated with $\boldsymbol{E}$ and positively correlated with L_{app}, as observed in our experiments.

In conclusion, we developed an experimental system to look at growth and hydromechanics in small plants with the ability to control the growth environment. We have shown that both elastic modulus and water conductivity are relevant to growth control and that altered growth conditions cause a change of these parameters. Altogether, we believe that our experimental approach and results will help overcome the challenges raised by modeling growth of cells and organisms [54].

Materials and Methods

A. Plant material

The Tak-1 wild-type accession of *M. polymorpha* was used in this study. Plants were grown on medium (0.2%, Gamborg's B5 media w/ vitamins, Duchefa) mixed with agar (1.2% plant agar, Duchefa). Plant growth takes place in a phytotron (Aralab) with 24h lighting (4600 - 5100 lux) at 22°C. The gemmae used for the experiments are taken from branch order 2 gemmae cups (see Fig. S1-A (iv)) of 3-5 weeks old plants.

B. Chip fabrication

The chip used to hold the gemmae during the experiment is made of PDMS (Sylgard 184) and molded in a brass mold. The mold was fabricated by micro-drilling (50μm drill) to allow non

uniform height of the chamber. The PDMS mix (1:10 ratio) is poured in the mold, degassed for 15 minutes in a vacuum chamber and cured at 70°C for 90 minutes. Once cut out from the mold the chip's inlet and outlet are created using a biopsy puncher. Chip thickness varies from 1mm (inlet and outlet) to 300µm (trap section) and the total chip volume is ~500µL. The gradient in thickness allows the gemmae to enter the chip in any orientation without clogging and to be gently guided to a horizontal position in the trap section for observation.

C. Experiment preparation

The PDMS chip is bonded to a 76x52x1 mm glass slide using a plasma oven (Harrick Plasma, PDC-002). The inlets are connected to glass syringes (10 & 25mL, SGE Trajan) using PTFE tubing of 1.06 mm inner diameter (Adtech) to allow for the passage of gemmae without clogging. The chip is then filled with medium using syringe pumps (Nemesys low pressure module, CETONI). Gemmae are collected in 1.5 mL tube containing medium with biocompatible surfactant (1/1000 dilution, TWEEN 20, Sigma) to avoid aggregation and sticking to the collection tube and tubing during preparation. They are then transferred to the chip manually using a 5 mL syringe (SGE Trajan). The initial medium is washed away after a constant perfusion of medium is put in place with a flow rate of 8.33µL/min (500µL/hour) and is maintained throughout the experiment. For the osmotic step, the flow rate used is 500 µL/min to ensure a fast replacement of the medium in the whole chip (~1 min).

D. Media and treatments

Control medium is 0.2% Gamborg's B5 media w/ vitamins (Duchefa) in water with pH adjusted to 5.8. Hyper-osmotic medium is composed of control medium with the addition of 100mM diluted solid D-Mannitol (Sigma). The ABA medium is composed of control medium with 3.5µM Abscisic acid. The ABA powder (Merck) is first diluted in DMSO (Sigma) at 30mM concentration before being used to make the ABA medium.

E. Image acquisition

Images are obtained at a 16X magnification using a Zeiss Axiozoom V16 and the Zen 3.3 software. Images are taken every 30 minutes for growth experiments, and every 30 seconds during osmotic steps. For growth experiments Z-stacks of 3 images, separated by 150 µm, are acquired at each position. The Z-stacks are reduced to a single Z image using the Zen post processing function 'Extended depth of focus' and the 'variance' method with default parameters. For Osmotic steps experiments there is no Z-stack acquisition to have a faster acquisition rate.

F. Image analysis

Videos (tiff image stacks) of individual gemmae are cropped from the original field of view using ImageJ. The rest of the analysis is performed using Python, the code is available on demand. The gemmae are detected in each image in several steps: First the white balance of each video is adjusted. Second a 3 channel thresholding is applied on the HSV (hue, saturation, value) representation of the original RGB image. The threshold values are adjusted manually for each experiment, but are the same for all gemmae analyzed within each experiment. Third morphological operations (binary closing with 5 µm circular elements, followed by binary opening with a 30 µm circular element) are used on the created binary mask to recover the shape of the gemmae. Finally, areas of the gemmae are measured on each image from this reconstituted binary mask.

G. Data fitting

From the image analysis we obtain experimental curves of the gemmae area in time for both the growth and osmotic step experiments (Fig. 1-C and 2-B). For the growth curve parameter extraction we fit the experimental data with a delayed exponential of the form:

$$A(t < T_{start}) = A_0$$

$$A(t > T_{start}) = A_0 e^{(t - T_{start})G}$$

It takes into account the delayed start and the exponential nature of growth. As we want to compare growth rate for a similar time period for each gemmae, we use an iterative approach. First the whole area curve is fitted to obtain a first value of $\boldsymbol{T_s}$. Then the fit is repeated on a time window stopping 15h after the $\boldsymbol{T_s}$ previously computed. This yields a new value for $\boldsymbol{T_s}$ that can be used to define a new time window ending 15h after it. This process is iterated until the value obtained for $\boldsymbol{T_s}$ converges with a precision of 0.1 % or it reaches a maximum 10 iteration in which case the fit is not validated. This ensures a robust definition of $\boldsymbol{T_s}$ and a value of $\boldsymbol{G}$ representative of the initial 15h of growth for all gemmae.

For the osmotic steps, each part of the curve (deflation and inflation) is fitted with an evolution function of the form:

$$A\left(t < T_{step}\right) = A_0$$

$$A\left(t > T_{step}\right) = (A_0 - A_{eq}).e^{\frac{T_{step}-t}{\tau}} +$$

$$A_{eq} + (t - T_{step}) * B$$

Representing an exponential transition between the two equilibrium states with the addition of a linear component to take into account the growth during the step. The $(t - T_{step})$ factor in both the exponential and linear part of the fit account for the fact that it is not experimentally possible to synchronize the imaging of all gemmae in the chip whit the exact moment where the hyperosmotic medium reach them. Thus the beginning of the deflation can happened between two time points, and leaving this time T_{step} as a fit parameters leads to more robust estimates of τ.

The elastic modulus is computed from the hyperosmotic step fit using the formula:

$$E = \frac{A_0}{|A_0 - A_{eq}|}\,\delta\pi$$

With $\delta\pi$ the change in external osmotic pressure.

The deformation irreversibility is computed as:

$$Irreversibility = \frac{|A_0 - A_{eq}|_{Second\ step}}{|A_0 - A_{eq}|_{First\ step}}$$

The hydraulic conductivity is extracted from the fit of the gemmae deflation after the hyperosmotic step. We computed it as:

$$L_{app} = \frac{1}{\tau\, E}$$

This formula comes from the resolution of a modified Lockhart model adapted to our system:

$$\frac{1}{A}\frac{dA}{dt} = \phi\,(P - Y) + \frac{1}{E}\frac{dP}{dt}$$

$$\frac{1}{A}\frac{dA}{dt} = -L_{app}\,(\mathrm{P} - \Delta\pi)$$

Where A is the area of the gemmae visible on images, L_{app} the apparent, individual-level hydraulic conductivity, accounting for cell membranes, cell wall, and cuticle. P is the turgor pressure, Y the stress threshold for growth, and E the bulk elastic modulus previously computed. We also assume $(P - Y) = 0$ as the growth stops between the two steps. We obtain the evolution of the turgor pressure as:

$$P = P0.\exp\left(-E\ L_{app}\ t\right) + \ \Delta\pi$$

Close to its final value, the area follows the same trend in time as the pressure, allowing us the extract $\tau = \frac{1}{L_{app}\ E}$ from the fit. Here again, the factor $\left(t - T_{step}\right)$ in the fit ensure that we put a low weight in the fit on the first moments of deflation and thus get a correct value for τ.
The growth rate after the step is computed from the reinflation step as:

$$G_{post} = \frac{B}{A_0}$$

For the 'instantaneous' growth rate during growth we computed

$$GR(t) = \frac{1}{A(t)}\frac{dA}{dt}(t)$$

from the experimental data using a first order discretization:

$$\frac{dA}{dt}\left(\frac{t_{n+1} + t_n}{2}\right) = \frac{A(t_{n+1}) - \ A(t_n)}{t_{n+1} - t_n}$$

and

$$\frac{1}{A\left(\frac{t_{n+1} + t_n}{2}\right)} = \frac{2}{A(t_{n+1})\ +\ A(t_n)}$$

Where the t_n are the experimental time points.

H. Statistical analysis

The variability for data in figures 1 and 2 as stated in the main text are computed as relative average absolute deviation, this means the average absolute deviation (AAD) of the sample divided by its median:

$$AAD = \frac{1}{n}\sum_{i=1}^{n}\left|x_i - \underline{x}\right|$$

$$Variability = \frac{AAD}{Median}$$

To assess the significance (p-value) of the irreversibility level when compared to 100% we used a one-sided t-test. For statistical analysis and significance (p-values) when comparing two samples the statistics used are non-parametric given the non-normal nature of data distribution in general. When 'p=0' is displayed it means that $p < 1e\text{-}10$. The paired sample comparison in figures S1, S2, 3, S3, 4, and S4 are done using Wilcoxon ranksum test. For correlations in figures S1, 2, and 4 we used the Spearman correlation coefficient as we cannot assume linear relationships between the compared quantities *a priori*.

In addition to p-values we chose to display the Relative Change (RC) and the Effect Size (ES) for each sample comparison. The RC is simply the difference between the samples median divided by the control median:

$$RC = \frac{Median_{Treated} - Median_{Ctrl}}{Median_{Ctrl}}$$

We computed a non-parametric ES as the difference between the two median divided by the average absolute deviation of the control condition:

$$ES = \frac{Median_{Treated} - Median_{Ctrl}}{AAD_{Ctrl}}$$

For example, an ES of 0.7 means that the median value of the treated sample is closer to the control median than the average point in the control sample. This gives a way to quantify the difference between two samples while taking into account the variability of the measurements. Computationally we can also understand the ES as the RC between the samples normalized by the variability of the control as defined above:

$$ES = \frac{RC}{Variability_{Ctrl}}$$

To compare the correlations between $\boldsymbol{E}$ or $\boldsymbol{L_{app}}$ and the growth rates $\boldsymbol{G_{inst}}$ or $\boldsymbol{G_{post}}$, we used http://comparingcorrelations.org/, based on an R based page including several methods to compare correlations. In every case stated in section IV all the methods gave the same result.

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Author Contributions:

Conceptualization: VL, EM, SD, AB
Methodology: VL, EM, SD
Software: VL
Validation: VL
Formal Analysis: VL, EM, AB
Investigation: VL, ND
Data Curation: VL
Writing - Original Draft Preparation: VL, AB
Writing - Review & Editing: VL, ND, EM, SD, AB
Visualization: VL
Supervision: AB
Project Administration: AB
Funding Acquisition: AB

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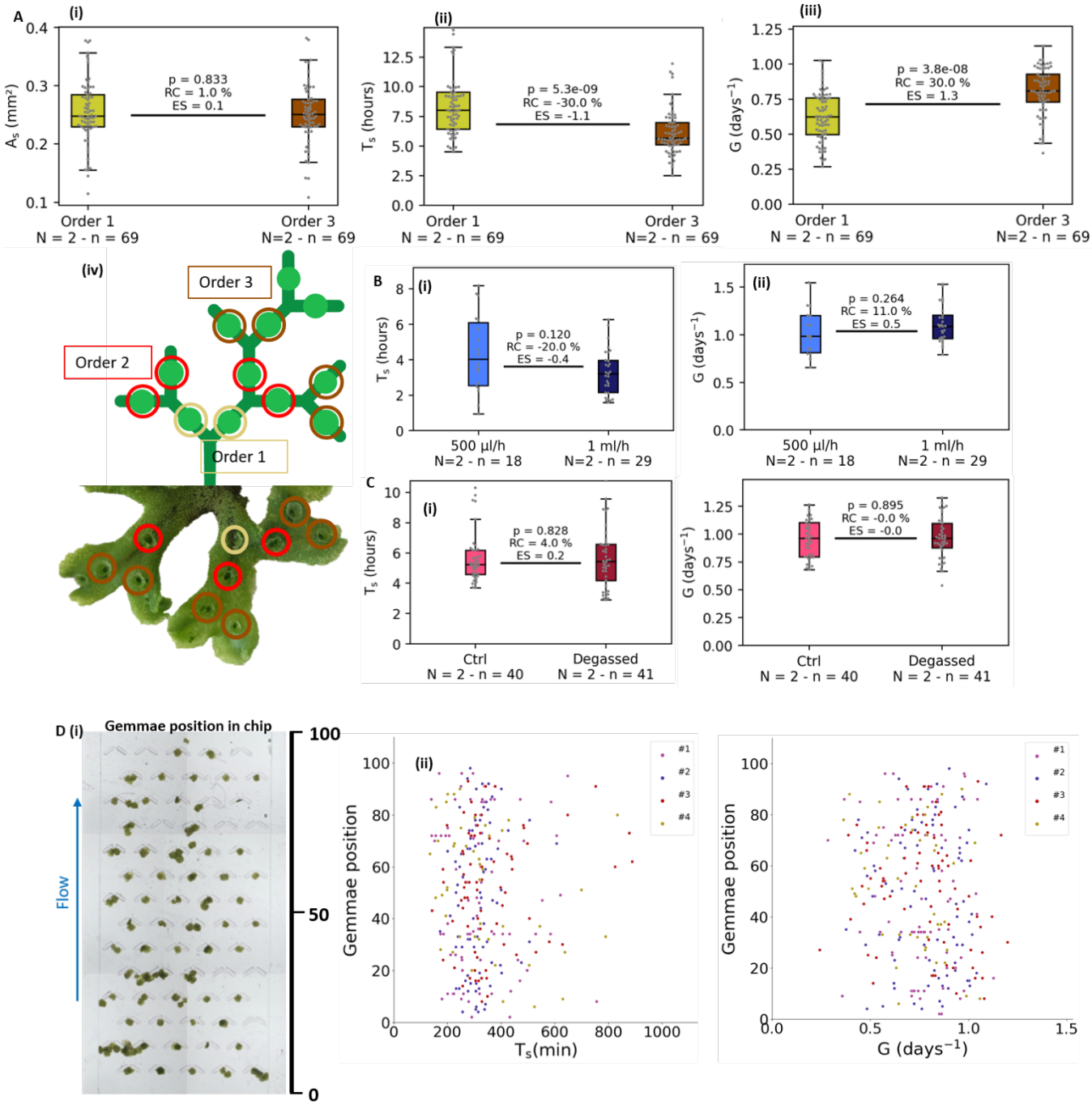
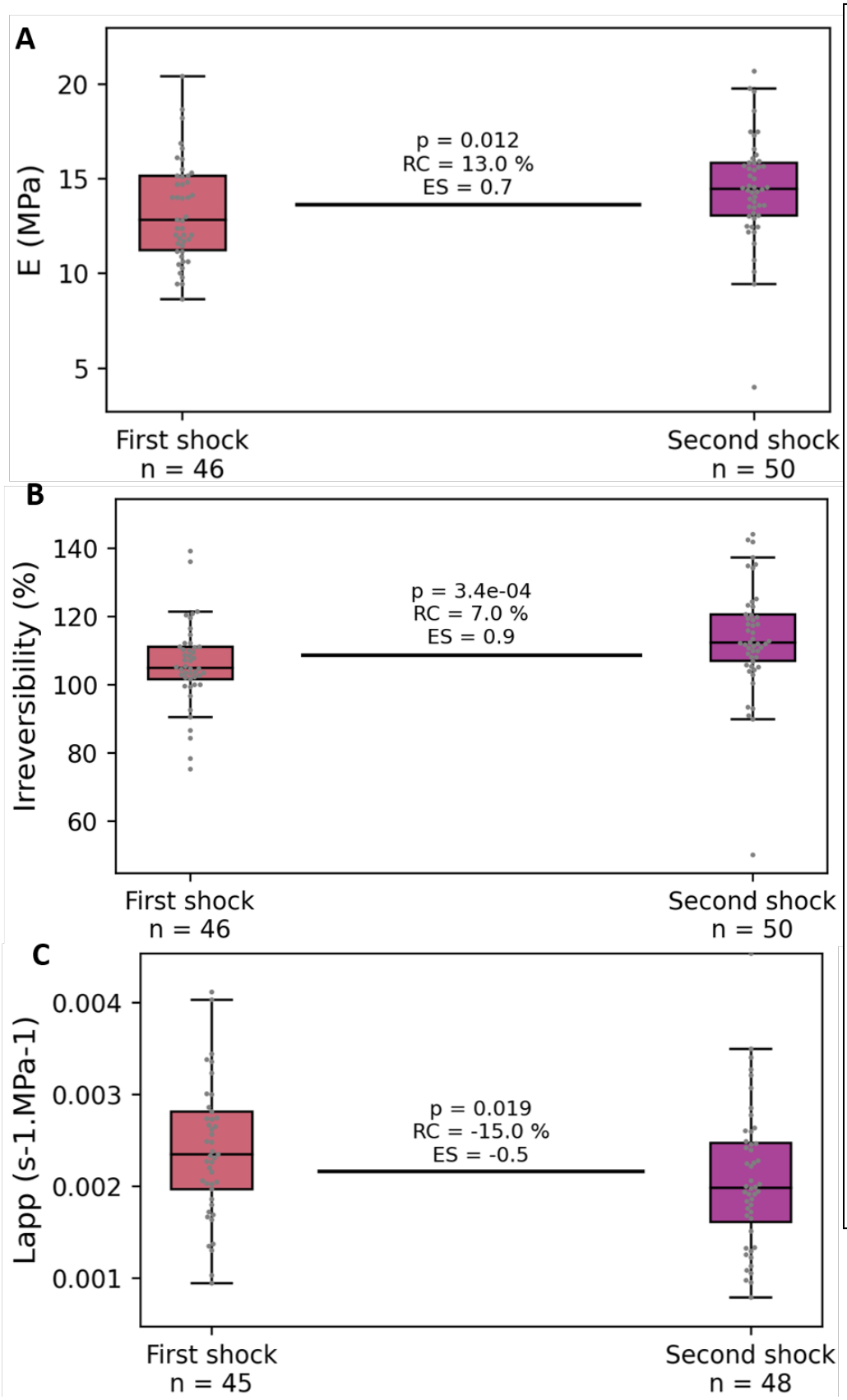


<u>Figure S1.</u> Growth in controlled conditions

A. Comparison of growth parameters for gemmae coming from old (order 1) or young (order 3) cups on the mother plant. **(i)** Areas at t=0 are similar. **(ii-iii)** Time of growth start is larger and growth rate is lower for older cups. **(iv)** Illustration of the different branch orders of cups. Light yellow are order 1 (oldest) and yellow are order 3 (young) cups. For all experiments other than the one in this figure (S1) gemmae are from order 2 (red) cups. **B.** Comparison of growth parameters for different flow rates in the chip. **(i-ii)** Both time of growth start (T_s) and growth rate (G) are similar between conditions. **C.** Comparison of growth parameters for growth in degassed medium. **(i-ii)** Both time of growth start (T_s) and growth rate (G) are similar between conditions. **D.** Illustration of gemmae position in the chip **(i)** and its correlation with T_s **(ii)** and G **(iii)** for the data in Fig.1-DE. The position of the gemmae in the chip doesn't affect the growth parameters measured (Spearman's rho < 0.15 for both).



<u>Figure S2.</u> Consecutive osmotic steps

A. Comparison of bulk moduli measured by two step cycles separated from 30 minutes. **B.** Comparison of irreversibility levels measured in the same experiment as (A). **C.** Comparison on apparent conductivity measured in the same experiment as (A).

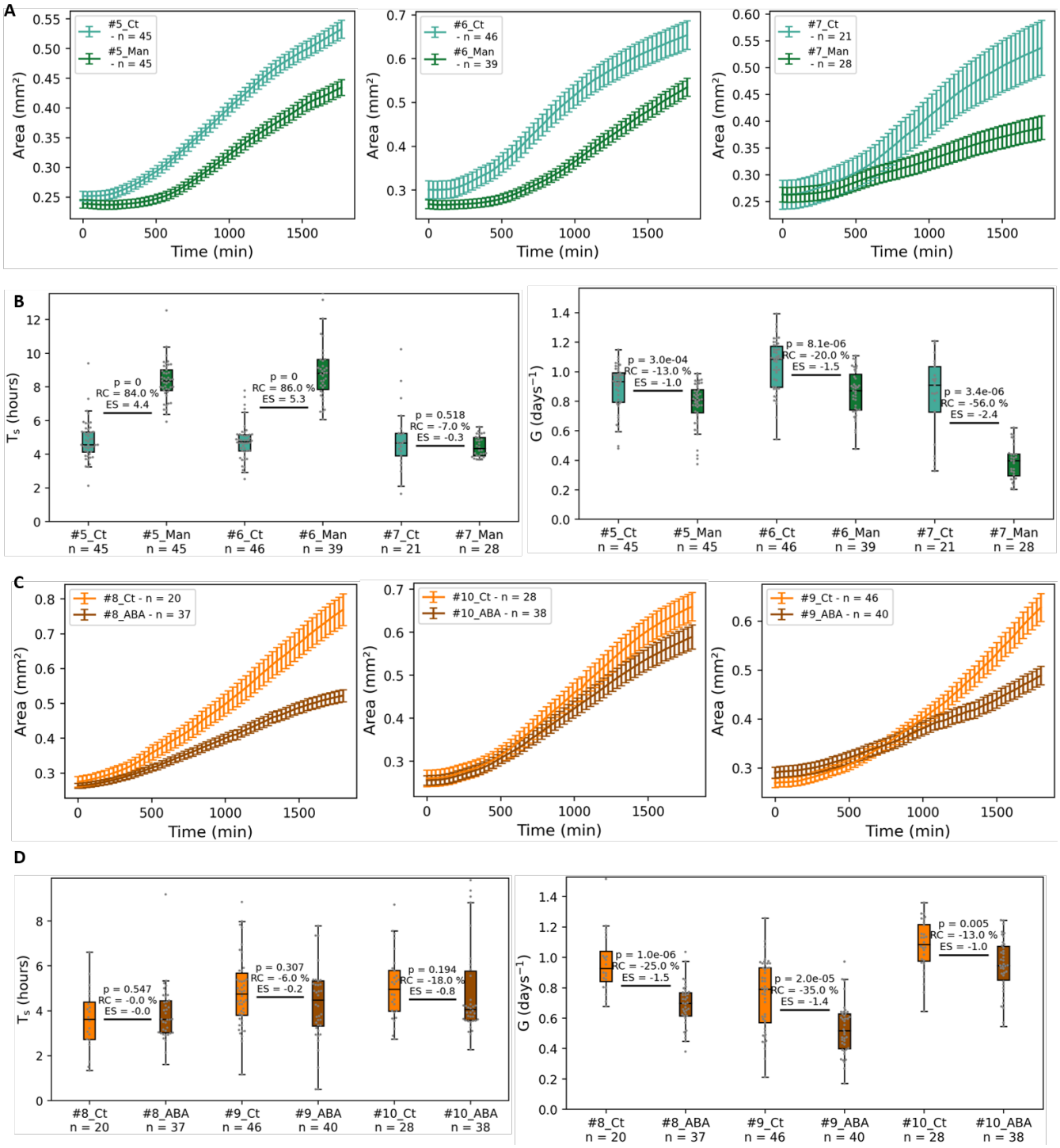
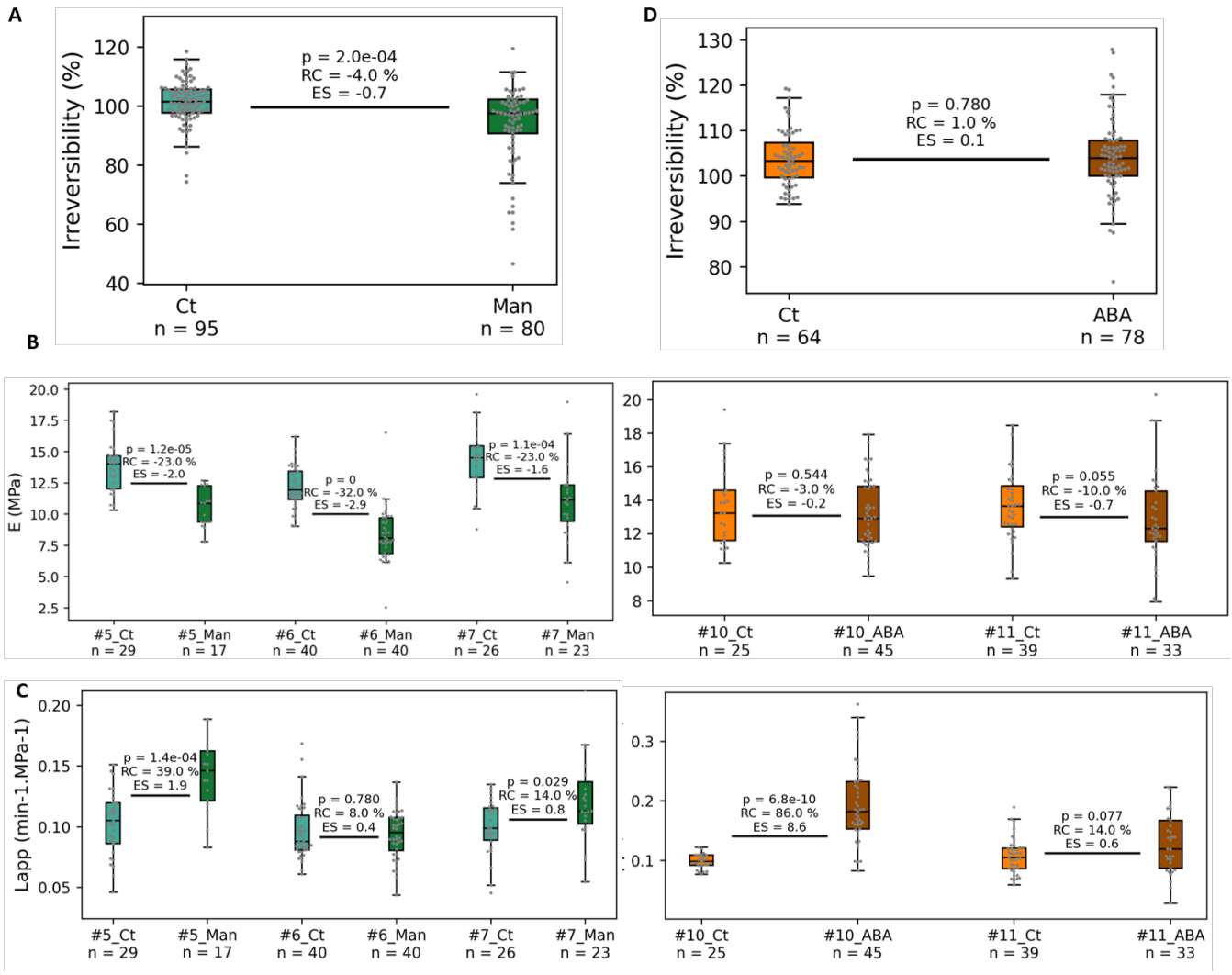


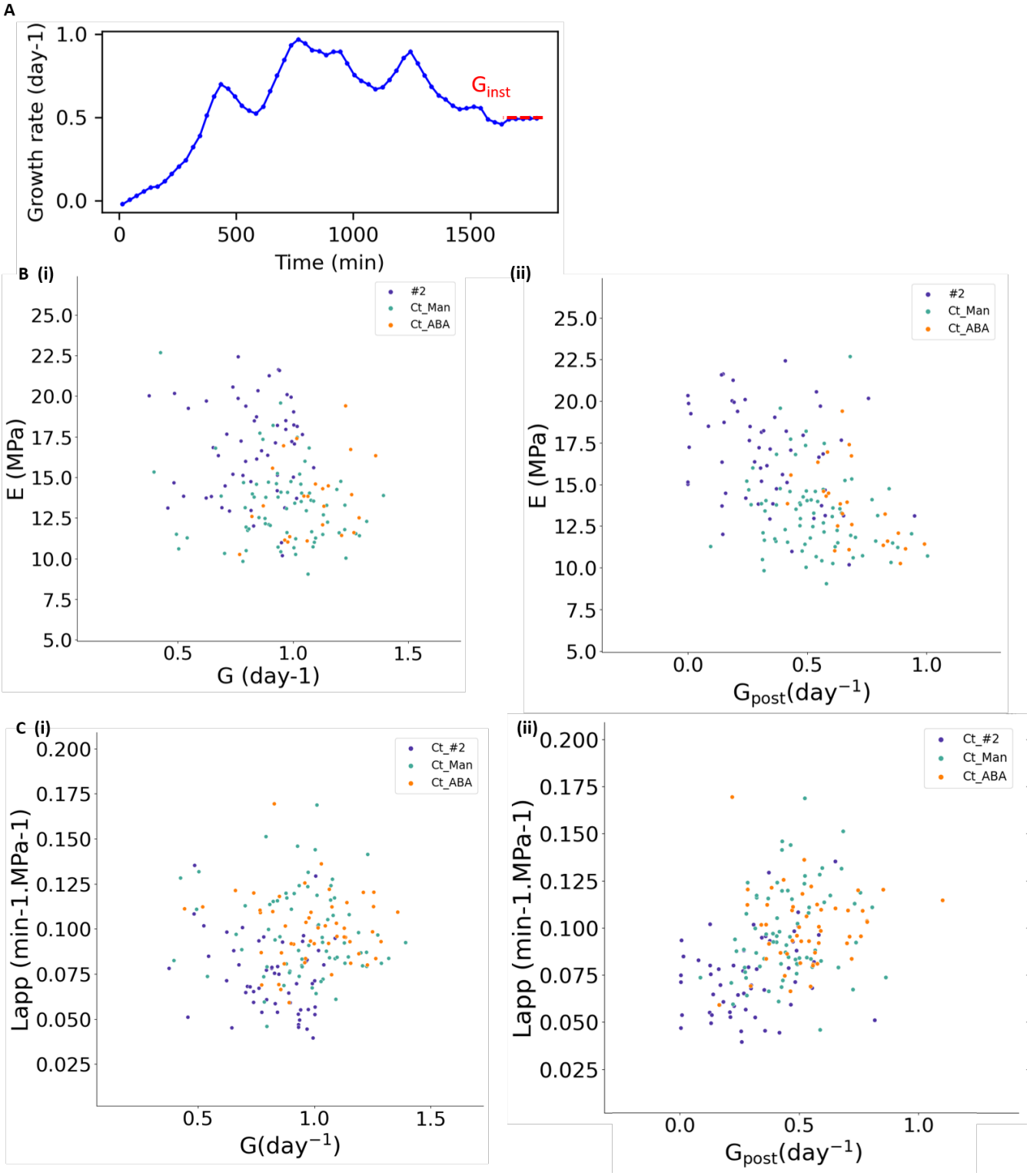
Figure S3.Growth in perturbed conditions –Detailed data

A. Growth curves per experiment for100mM mannitol treatment (Man) and control (Ct) **B.** Quantification of the growth parameters corresponding to curves in (A) **C**. Growth curves per experiment for treatment with abscisic acid (ABA, 3.5µM) and control (Ct) **D.** Quantification of the growth parameters corresponding to curves in (C)



<u>Figure S4.</u> Hydromechanics in perturbed conditions – supplementary data

A. Irreversibility levels comparison for the pooled data presented in Fig.4-A for control and mannitol treated gemmae. **B.** Detail of individual experiments pooled in Fig.4-A (Ct: control, Man: mannitol-treated). **C.** Detail of individual experiments pooled in Fig.4-C (Ct: control, Man: mannitol-treated). **D.** Irreversibility levels comparison for the pooled data presented in Fig.4-C for control and ABA treated gemmae. **E.** Detail of individual experiments pooled in Fig.4-B (Ct: control, ABA: abscisic acid-treated). **F.** Detail of individual experiments pooled in Fig.4-D (Ct: control, ABA: abscisic acid-treated)



<u>Figure S5.</u> Link between hydromechanics and growth – Additionnal data

A. Computation of instantaneous growth rate G_{inst} **B.** Correlation between the elastic modulus of gemmae and the average growth rate G (i, CC = -0.197) as well as with the growth rate after the step G_{post} (ii, CC = -0.447). **C.** Correlation between the apparent conductivity of gemmae and the average growth rate G (i, CC = 0.135) as well as with the growth rate after the step G_{post} (ii, CC = 0.431).