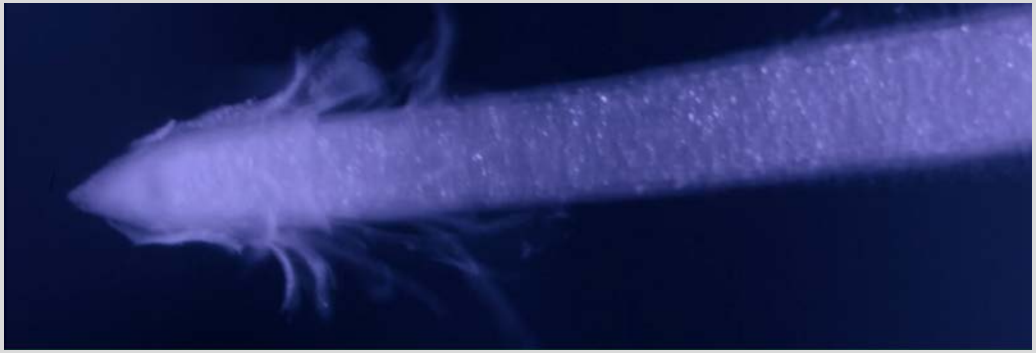
Root growth, cell processes and responses to environment

Marie-Béatrice Bogeat-Triboulot



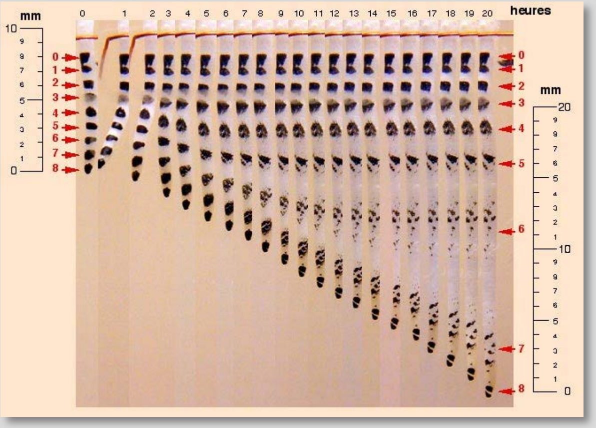
Root growth, cell processes and responses to environment

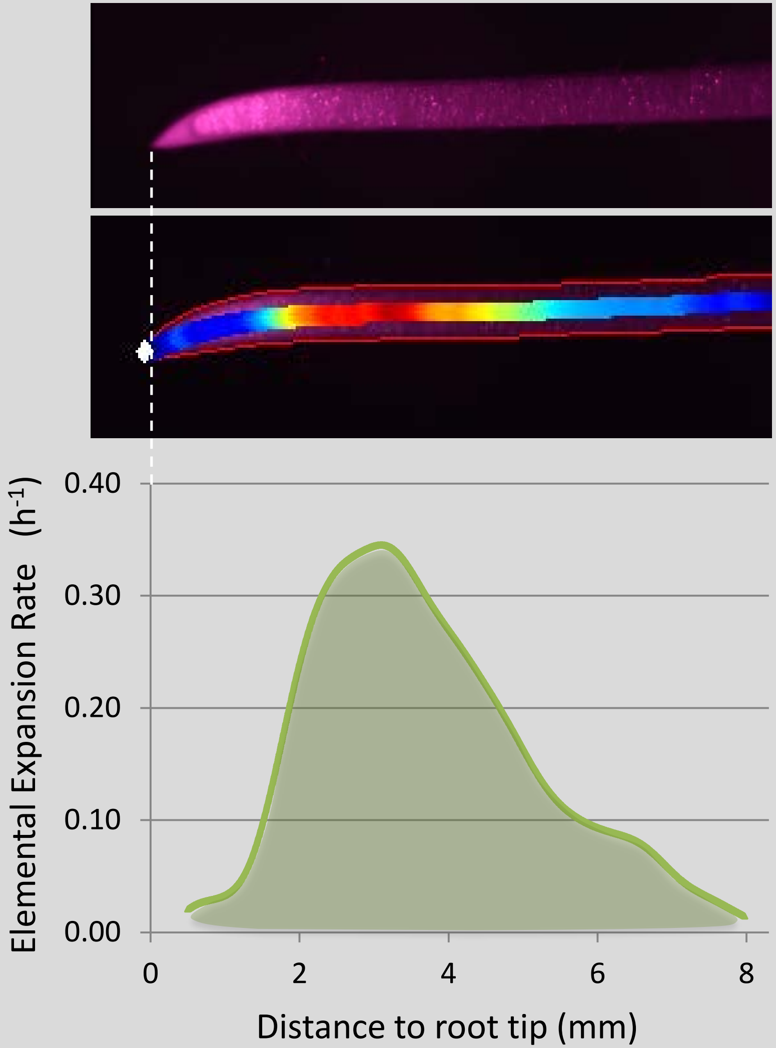
Marie-Béatrice BOGEAT-TRIBOULOT

UMR SILVA

INRA - Nancy - France

Root growth = expansion of material





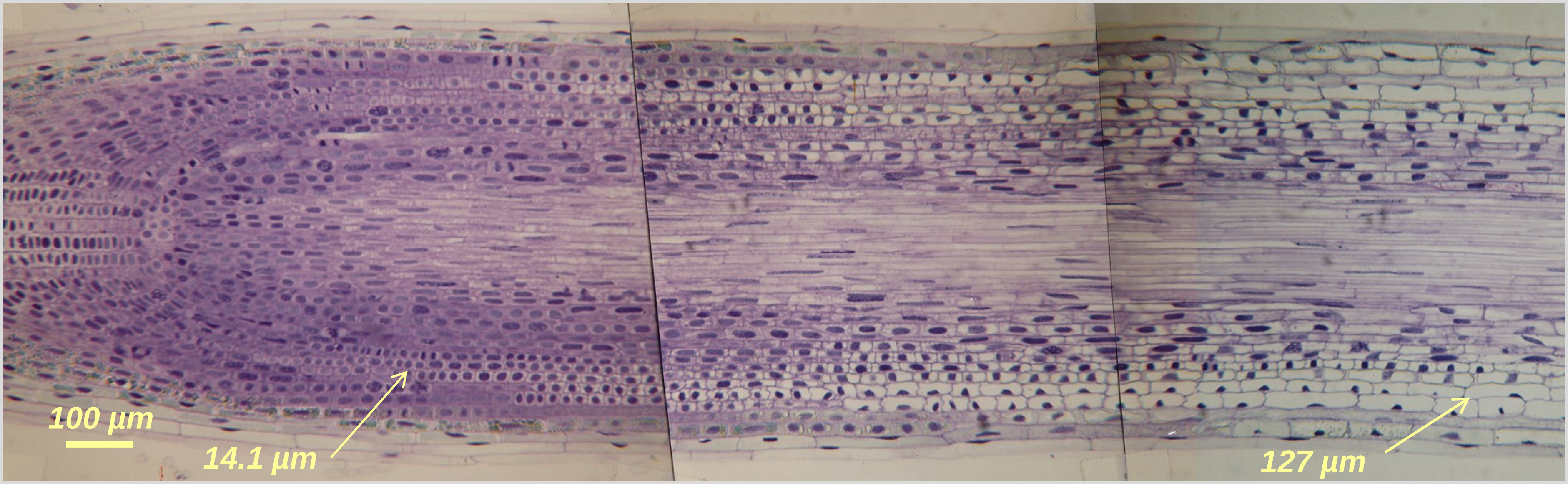
- **quantified** by the **Elemental Expansion Rate (EER)** or **strain rate**

  mm mm^{-1} h^{-1}

surface under the curve : proportional to the root growth rate

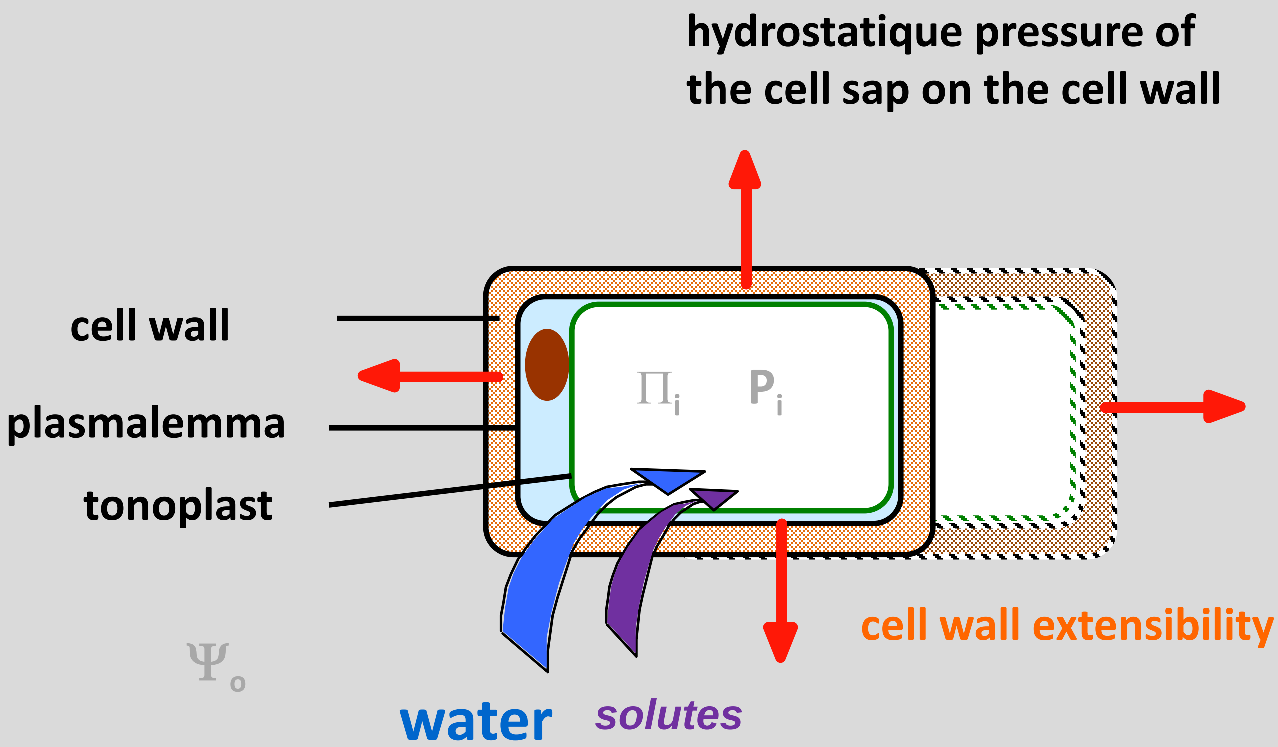
Cell elongation

Pinus pinaster root apex



Cell length : 15 µm ⇨ 320 µm : x 20!

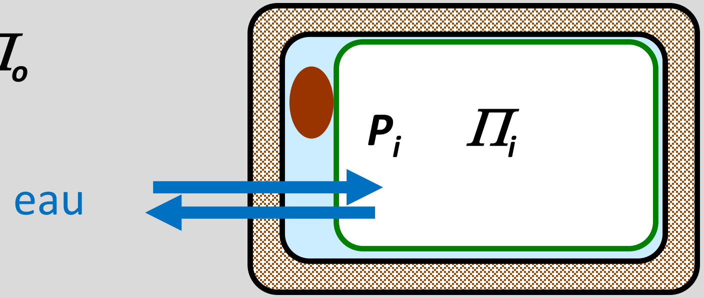
The expanding cell



- Cell wall deformation and synthesis
- Water and solutes inflow

Water flow into cell

$\Psi_o = P_o - \Pi_o \approx - \Pi_o$



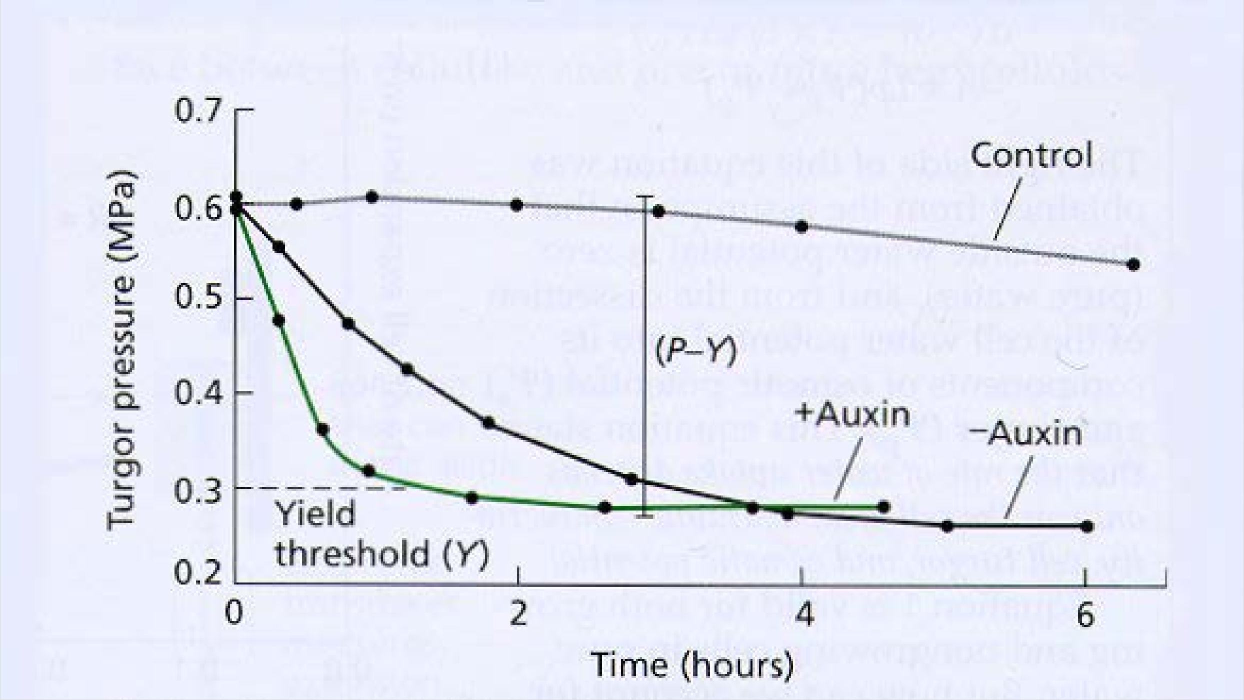
- Le potentiel hydrique de la cellule : $\Psi_i = P_i - \Pi_i$
  - P = pression de turgescence : force hydrostatique
  - Π = pression osmotique (due aux solutés dissous)

- A l'équilibre
  - $(\Delta P - \sigma \Delta \Pi) = 0 \Rightarrow J_v = 0$ flux in = flux out
  - $P_o \approx 0, \; P_i = \sigma \Delta \Pi$
  - origine de la turgescence : paroi rigide et milieu hypotonique

σ = coefficient de réflectivité
Si la membrane plasmique est semi-perméable :
$\sigma \approx 1$

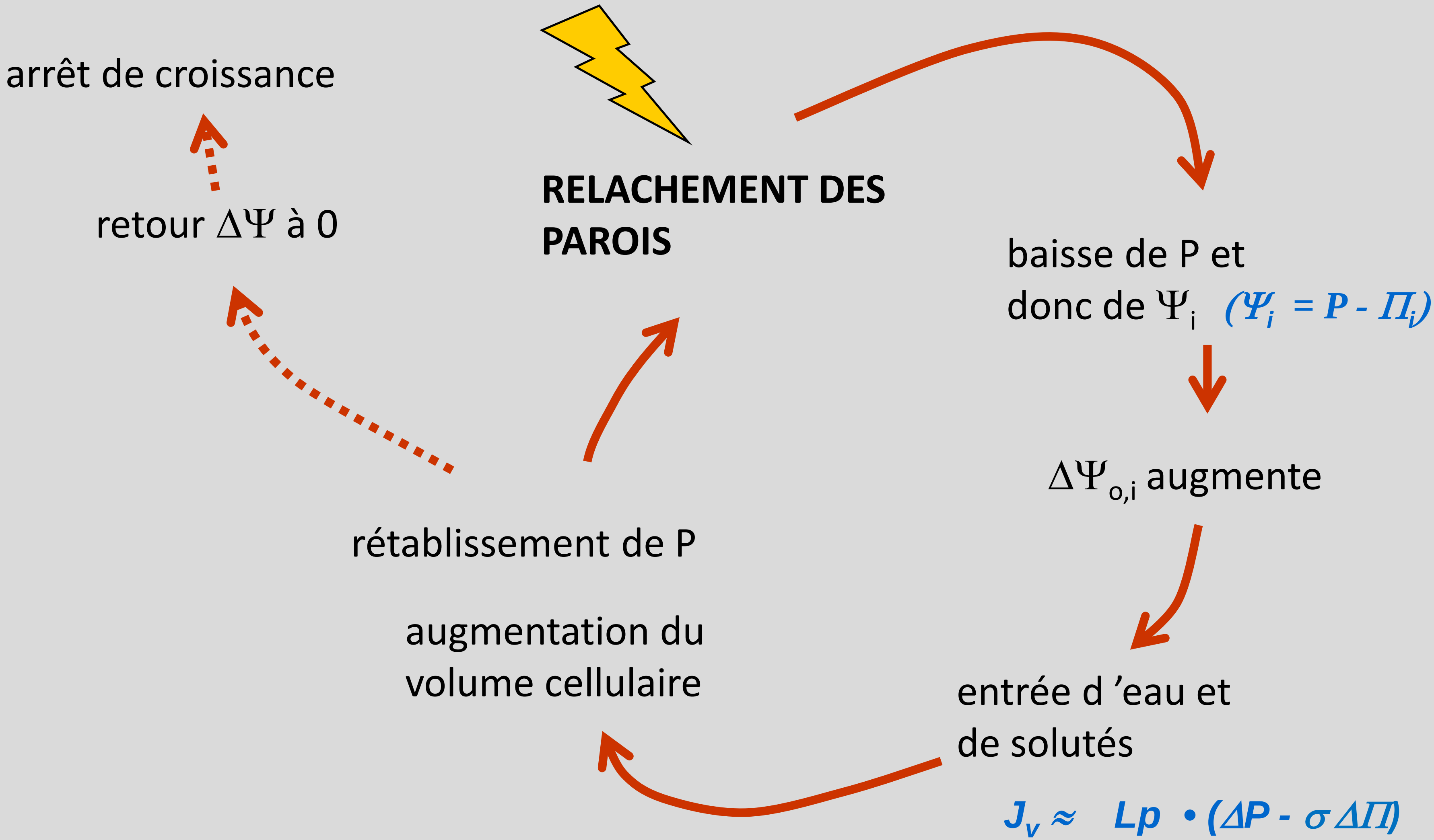
- Si déséquilibre de statut hydrique : flux directionnel
  - $J_v = Lp \bullet (\Delta P - \sigma \Delta \Pi) = Lp \bullet (P_i - \sigma \Delta \Pi)$
  - Lp = conductivité hydraulique membranaire

Cell wall relaxation reduces turgor pressure



- Excised pea growing stem segment
  - connected to a water source
  - not connected to a water source, without auxin
  - not connected to a water source, with auxin (increases cell wall relaxation)

Cell wall relaxation : the basis of cell expansion



Growth rate depends linearly on turgor pressure

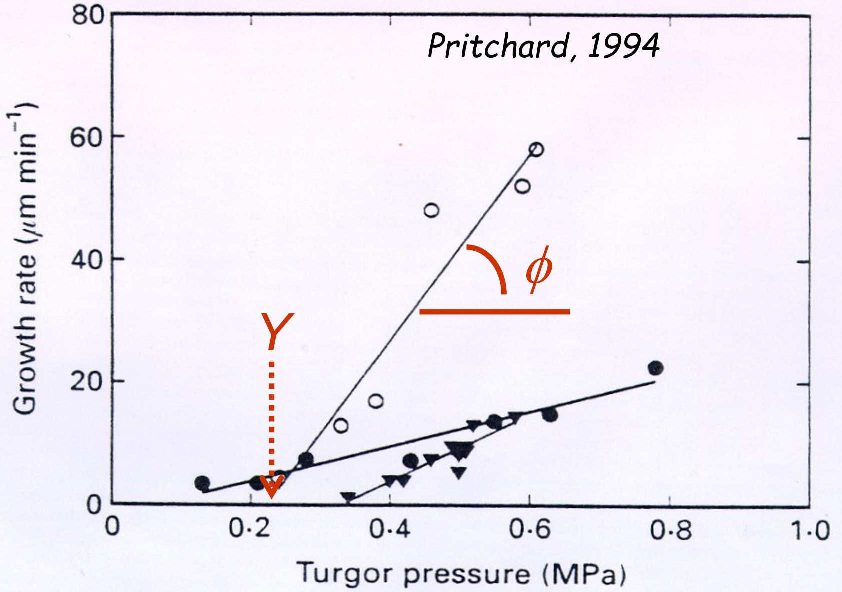


Figure 3. Root growth rate as a function of the turgor pressure; maize (○) and wheat (●) roots were bathed in a series of mannitol solutions (Pritchard *et al.*, 1990*b*, 1991). Pea roots (▼) were grown in soils of different density (Greacen & Oh, 1972).

Growth Rate = ϕ *(P - Y)*

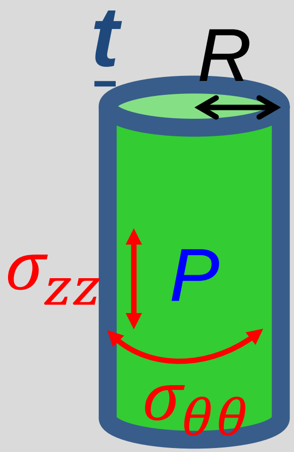
La croissance est linéairement proportionnelle à la pression de turgescence (le moteur de la croissance), au delà d'un seuil minimum

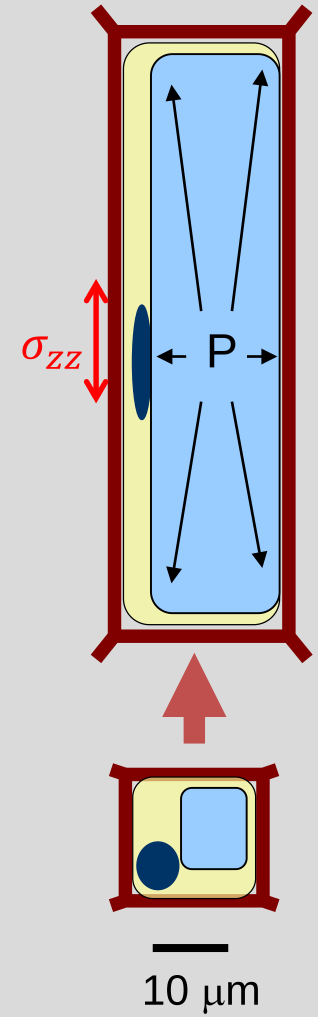
Wall tension and anisotropic growth

→ Turgor pressure **P**

→ Wall tension σ

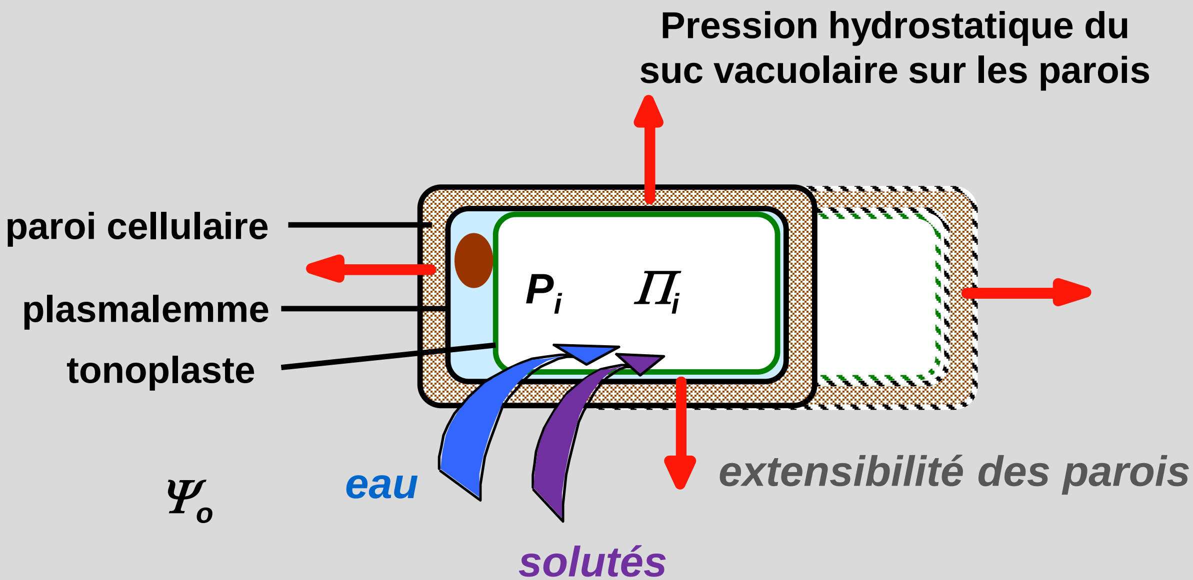
$$\sigma_{zz} = \frac{R}{2t} P \qquad \sigma_{\theta\theta} = \frac{R}{t} P = 2\sigma_{zz}$$





- Cell wall rheogical properties are different longitudinally and axially
- role of cellulose microfibrils orientation

Cell expansion parameters



$\Psi_i = P_i - \Pi_i$

- **Force motrice** du grandissement cellulaire :
  - Pression de turgescence
- **Contrôle** du grandissement cellulaire :
  - extensibilité des parois (ø , Y)
  - conductivité hydraulique volumique cellulaire (-> Lp)
  - Disponibilité en solutés / en eau

A biophysical model of cell expansion : Lockhart (1965)

Hydraulics

$1/V\ (dV/dt) = L\ [\sigma\ (\Pi_i - \Pi_o) - P\ ]$

Rheology of cell walls

$1/V\ (dV/dt) = \phi\ (P - Y)$

$$\frac{1}{V} \cdot \frac{dV}{dt} = \frac{L\,\phi}{L+\phi} \cdot (\sigma \Delta \Pi_{i,o} - Y)$$

If L >> ϕ : Lϕ/(L+ϕ) $\approx$ ϕ

Cell expansion is limited by the cell wall mechanical properties

If L << ϕ : Lϕ/(L+ϕ) $\approx$ L

Cell expansion is limited by the membrane hydraulic conductance

Lockhart (1965)

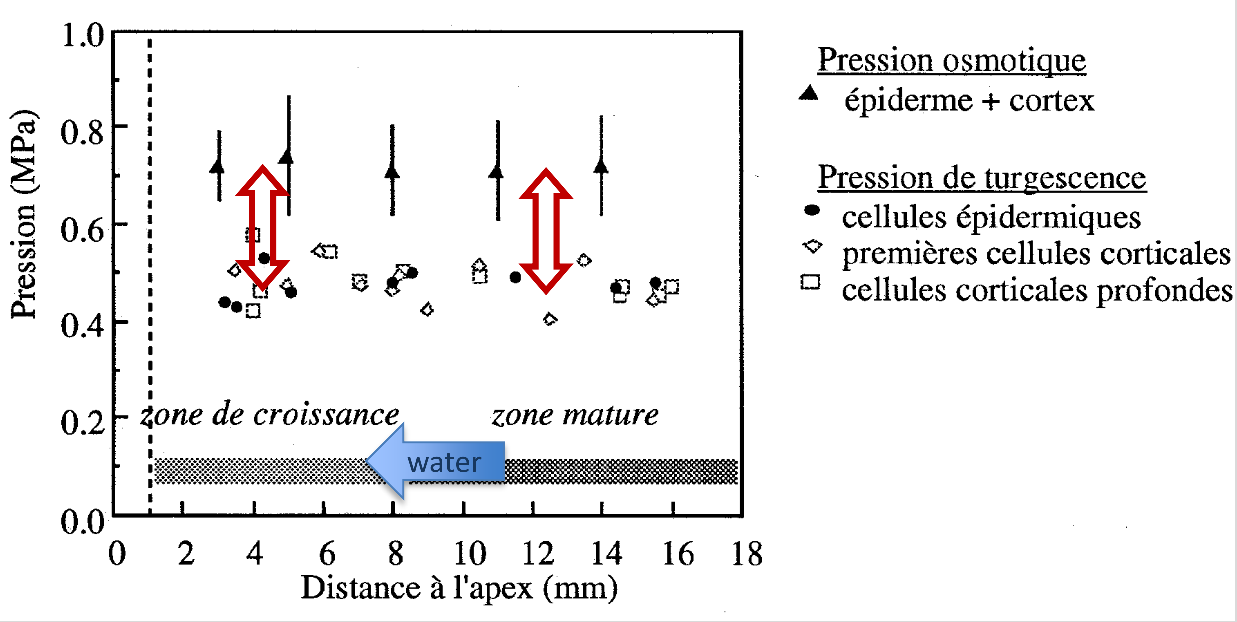
$$\frac{1}{V}\cdot\frac{dV}{dt} = \frac{L\,\phi}{L+\phi}\cdot(\sigma\Delta\Pi_{i,o} - Y)$$

- établi pour <u>une</u> cellule et pour un <u>régime stationnaire</u>

- conceptuel mais ne reflète pas tout, ne prend pas en compte:
  - régime transitoire
  - échelle spatiale : cellule / tissu (approximation pour un tissu)

Intensité du 'gradient de potentiel induit par la croissance'

Triboulot et al, 1995

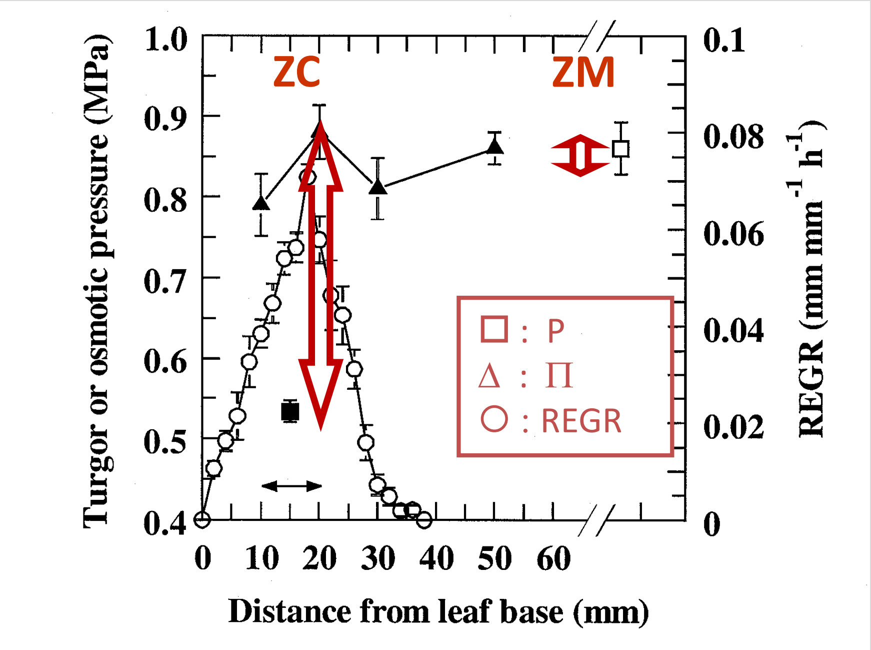


- P et Π $\pm$ constant le long de la zone de croissance et au delà
- $\Psi_i \sim \Psi_o$

  ↳ GIWP-gradient très faible :

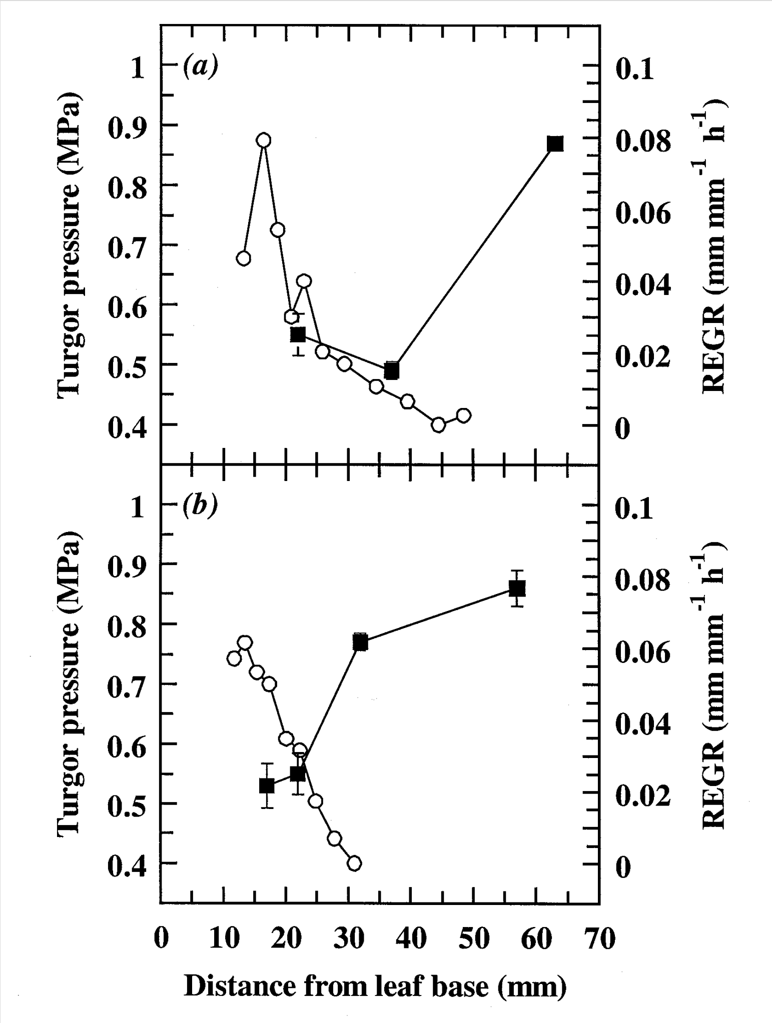
  - Lp non limitante
  - croissance contrôlée par propriétés mécaniques

Intensité du 'gradient de potentiel induit par la croissance'



ZC d'une feuille de fétuque

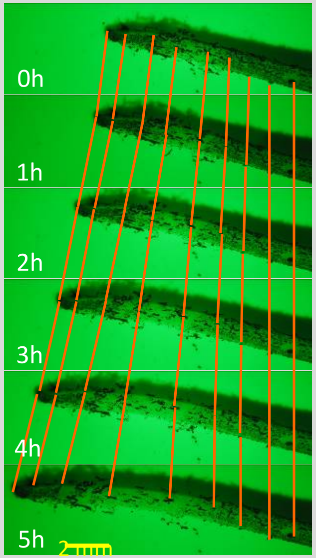
- ✓ Π ~ constant dans ZC et ZM
- ✓ P passe de ~ 5 bars dans ZC à ~ 9 bars dans la zone mature



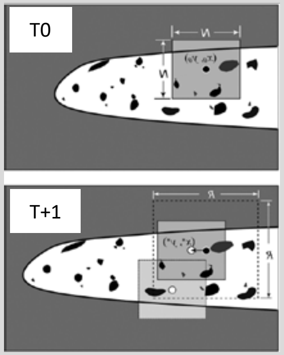
↳ GIWP-gradient voisin de 0.3 MPa : Lp joue un rôle dans la limitation de la croissance

Martre et al, 1999

Spatial characterisation of growth : time lapse photography and kinematics

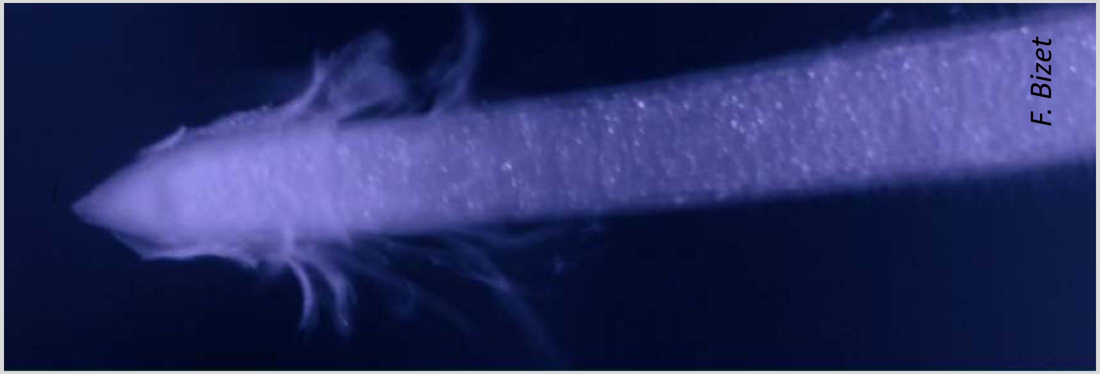


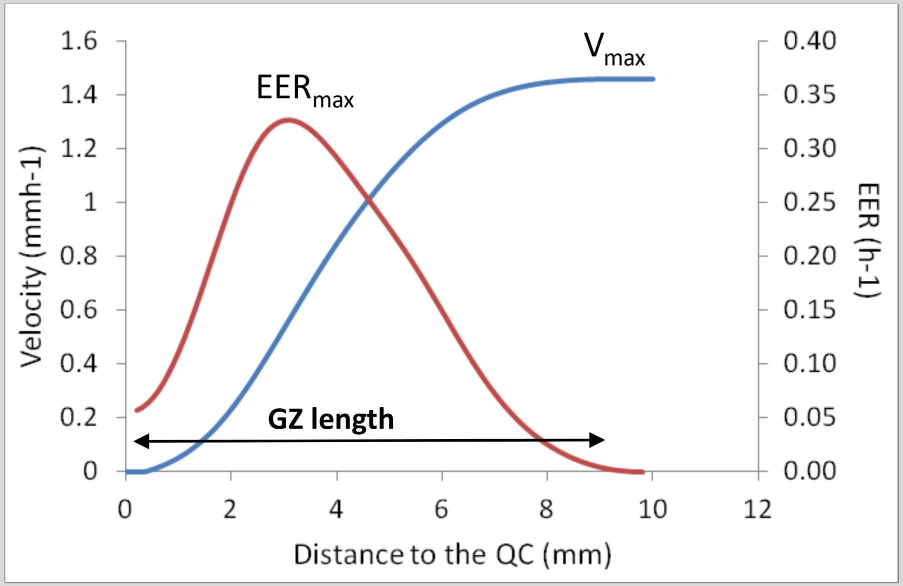
particule image velocimetry



Kineroot - Basu *et al.*, 2007
Kymorod - Bastien et al, 2016

Infra-red illumination : monitoring of natural marks
- no thigmomorphogenesis response
- renewing of marks -> long-time monitoring

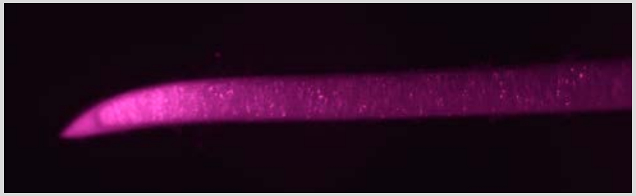


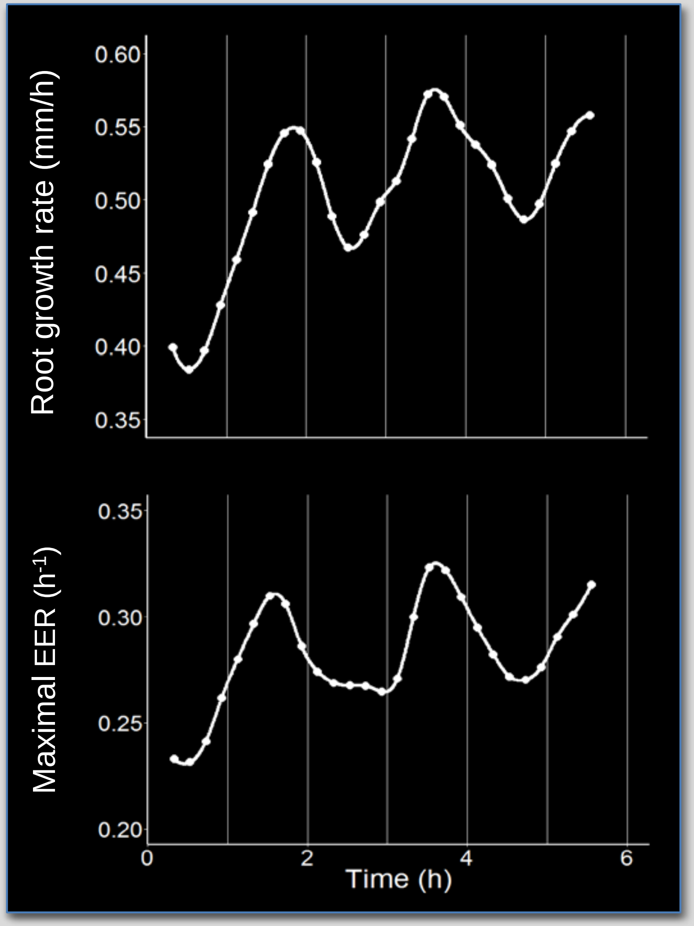


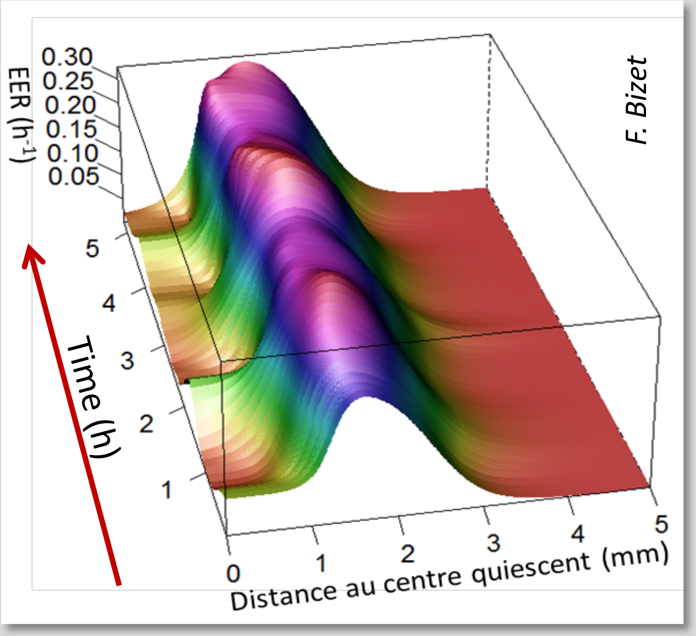
Long term monitoring highlights temporal oscillations

Infra-red illumination

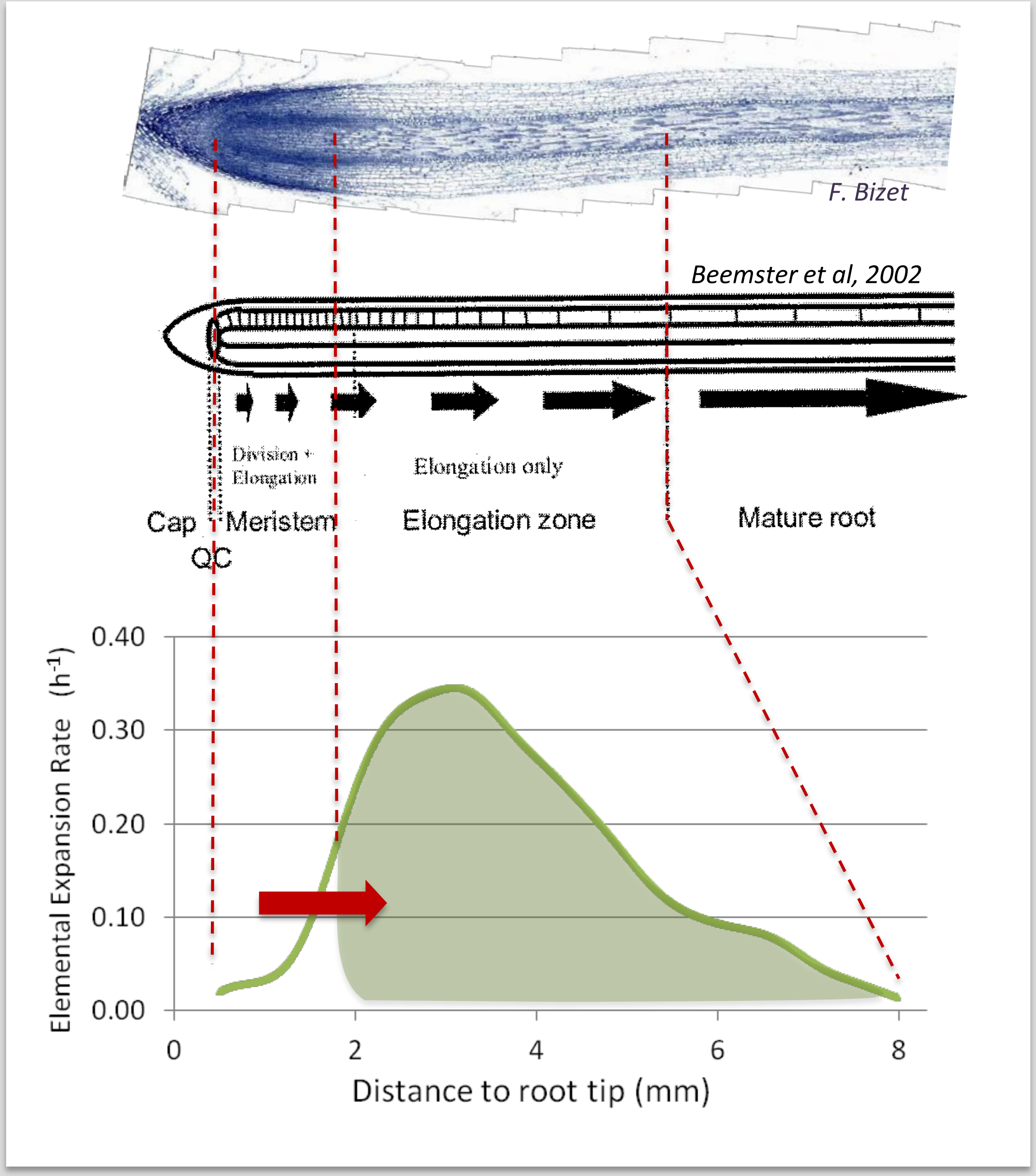
- natural marks
- renewing of marks -> long-time monitoring







Root growth = cell production + cell elongation

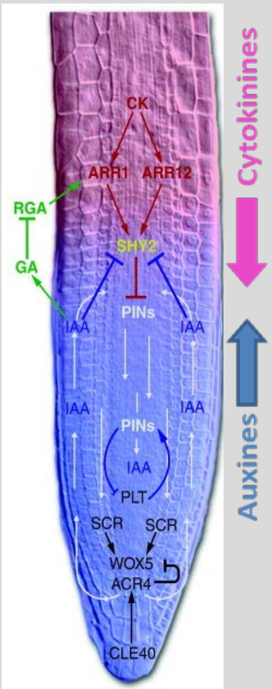


- quantitatively , most of growth is due to cell elongation

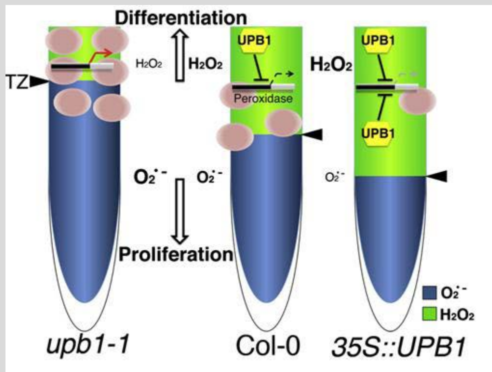
- meristem provides cells to the elongation zone

➔ **cell proliferation rate affects root growth rate**

Controls of transition from cell proliferation to cell expansion



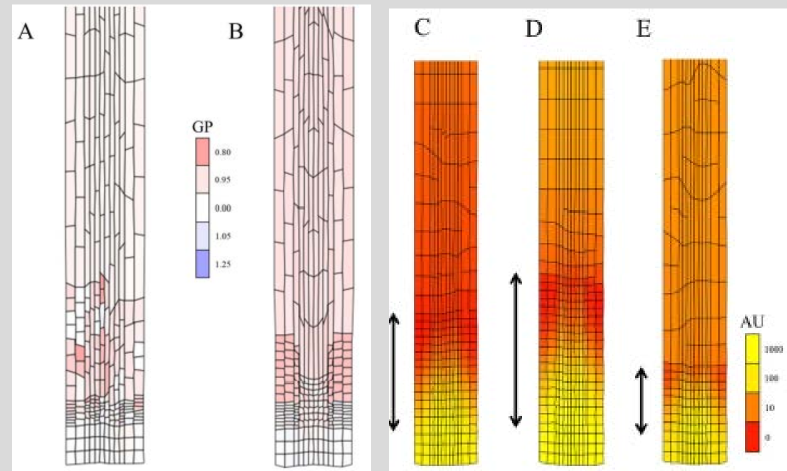
Perilli et al. (2012)



Tsukagochi et al. (2012)

timer/counter

spatial cue

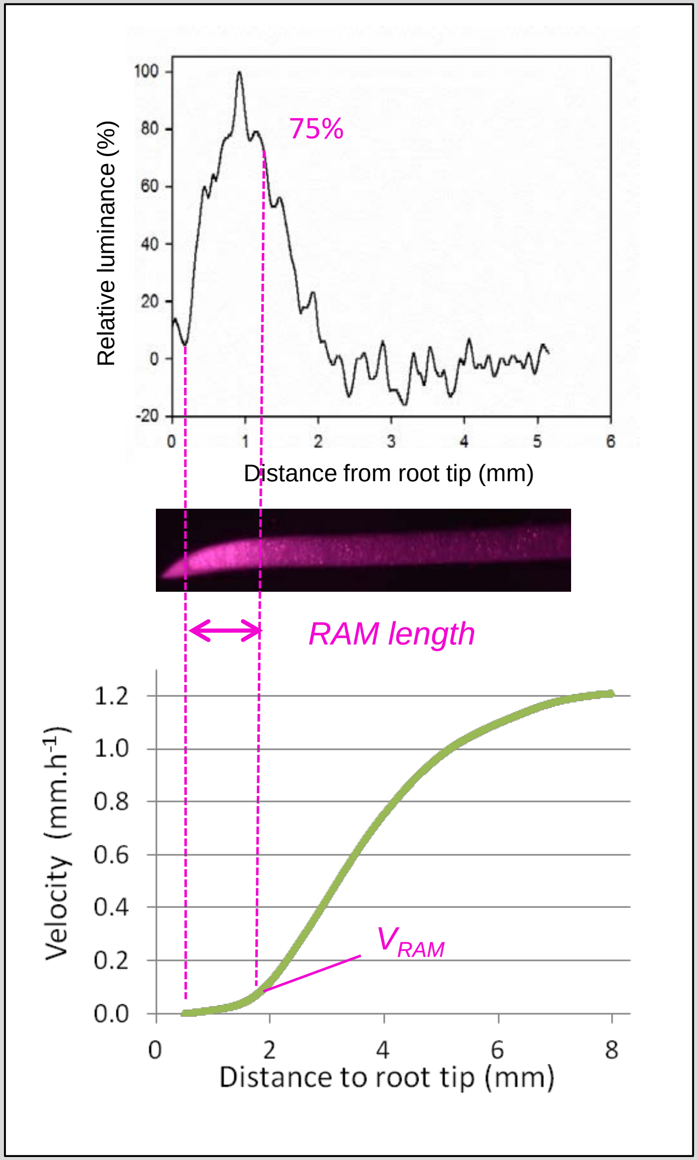


De Vos et al. (2014)

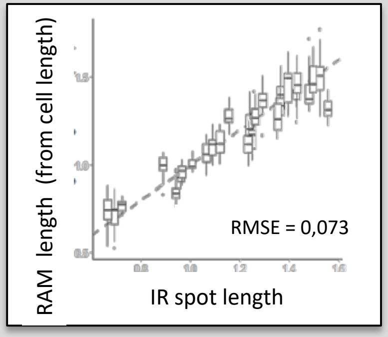
- cell-autonomous regulatory rules are insufficient to simulate smoothed developmental zones
- spatial cues solve the problem

Regulation of cell status (dividing/ expanding) ⇨ meristem size ⇨ cell proliferation rate ⇨ root growth rate

Root apical meristem characterisation

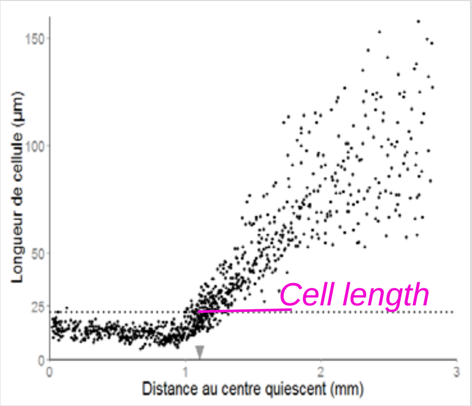


- **RAM length** determined from luminance profile (Bizet et al, 2015)



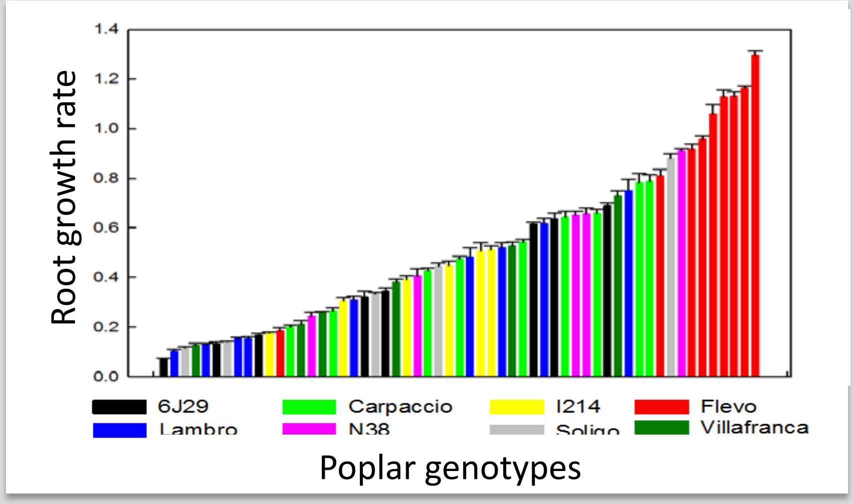
- **Cell production rate** =

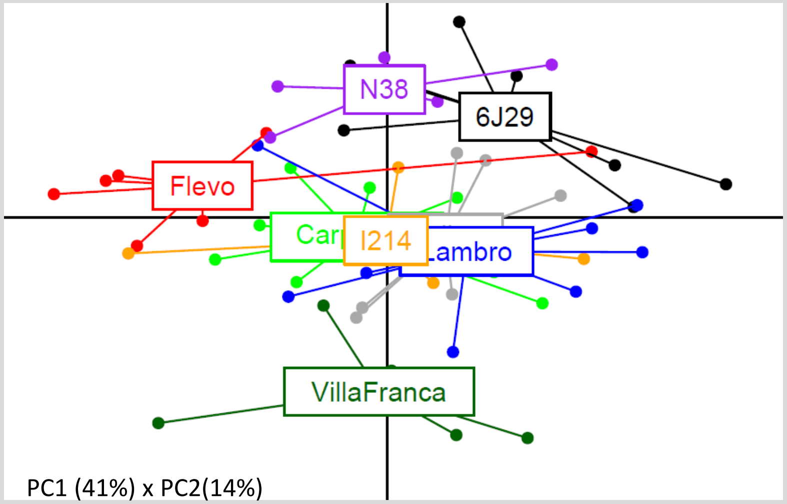
$$\frac{\text{Velocity}_{RAM}}{\text{Cell length}_{RAM}}$$



Variability of root growth components in poplar

- Inter-species variability of root growth rate is mostly explained by cell production rate (Gazquez and Beemster, 2017)
- Growth rate highly variable among roots within a root system, among species.
- origin?

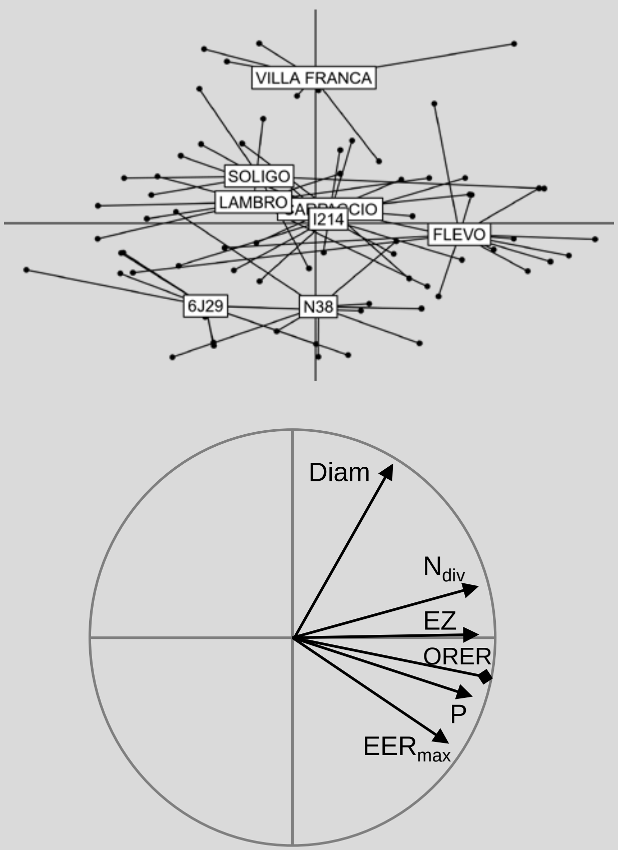




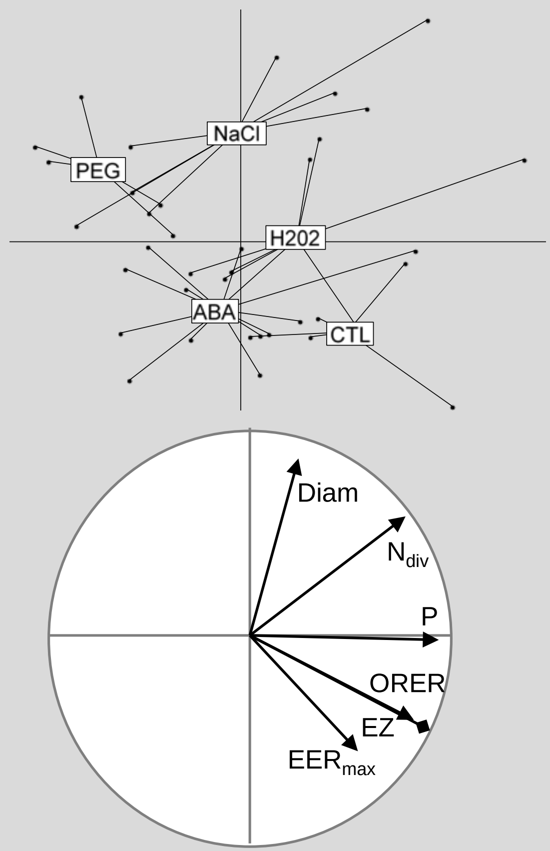
Youssef et al, 2018
J Exp Bot

Variability of root growth components in poplar

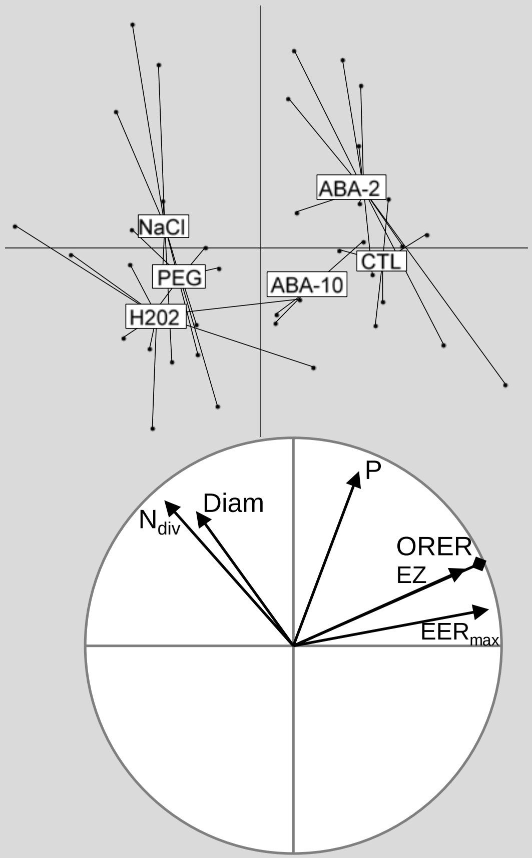
Species and cultivars



24h of chemical treatments



1h of chemical treatments



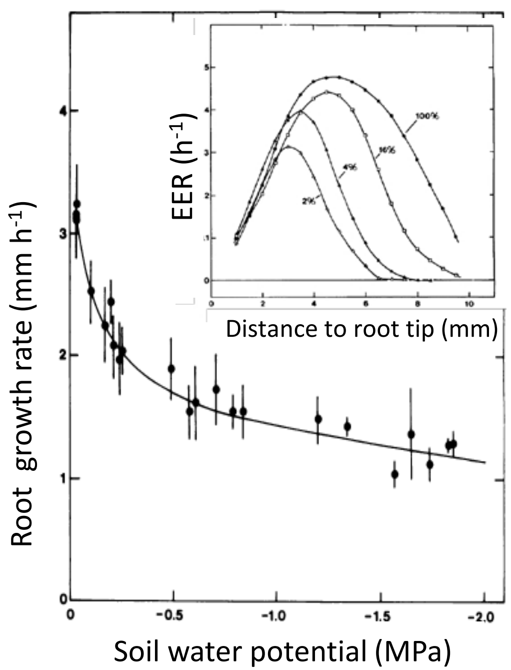
- All parameters correlated
- Steady state growth rate : more driven by P than EER max
- Short term response : mainly due to the high responsiveness of EER

Youssef et al, 2018
J Exp Bot

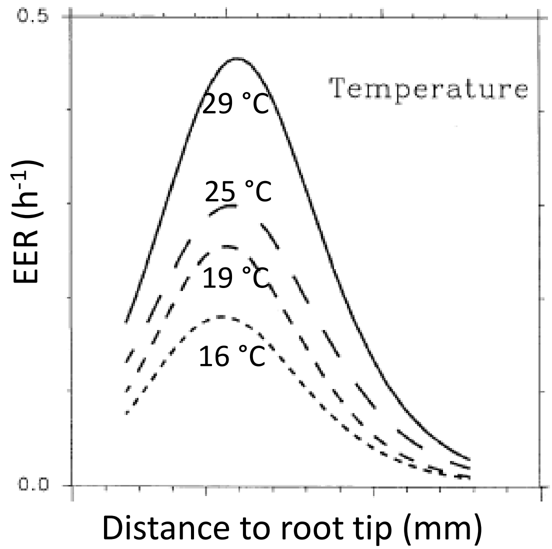
Root growth is highly responsive to environment

- Sensitive to water availability, nutrient availability, temperature, soil mechanical impedance, …

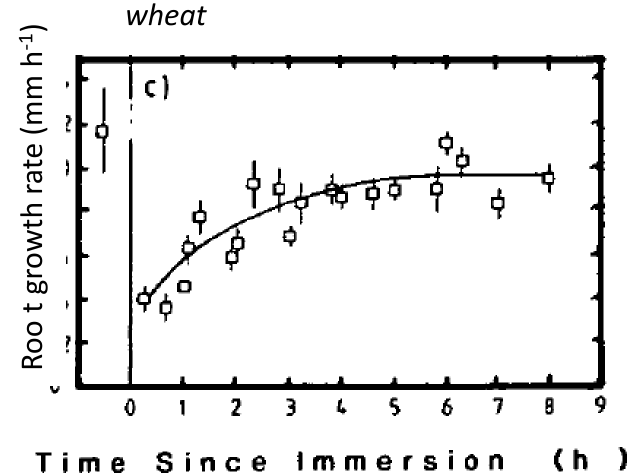
Sharp et al. (1988) *maize*

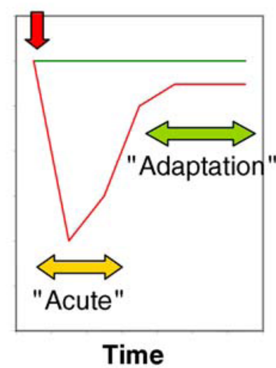


Silk (1992)
maize



Pritchard et al. (1991)
wheat





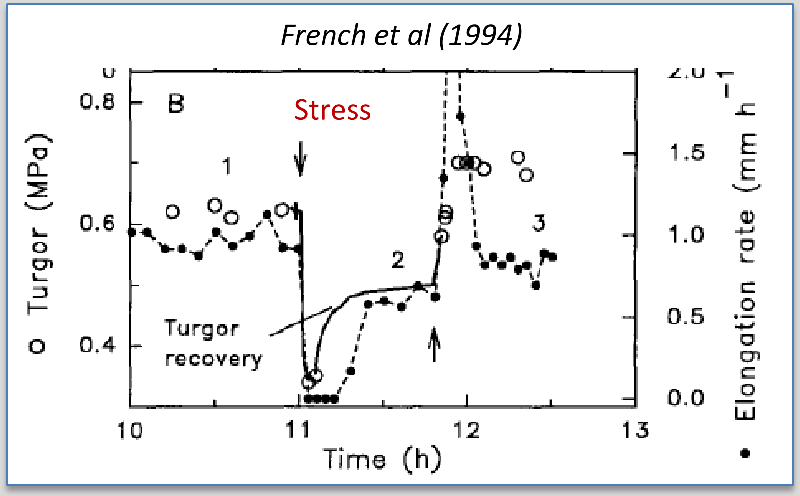
Skirycz & Inze (2010)

steady state *versus* transition phase

Dynamics of root growth in response to osmotic stress

- Root growth is sensitive to water deficit

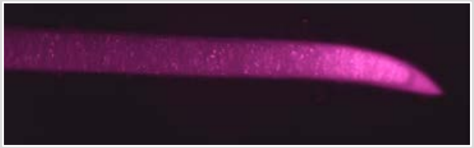
- time course of cell turgor recovery (motor of cell expansion) differs from that of growth : there are biological responses in addition to mechanical limitation



What's happen during the transition phase ?

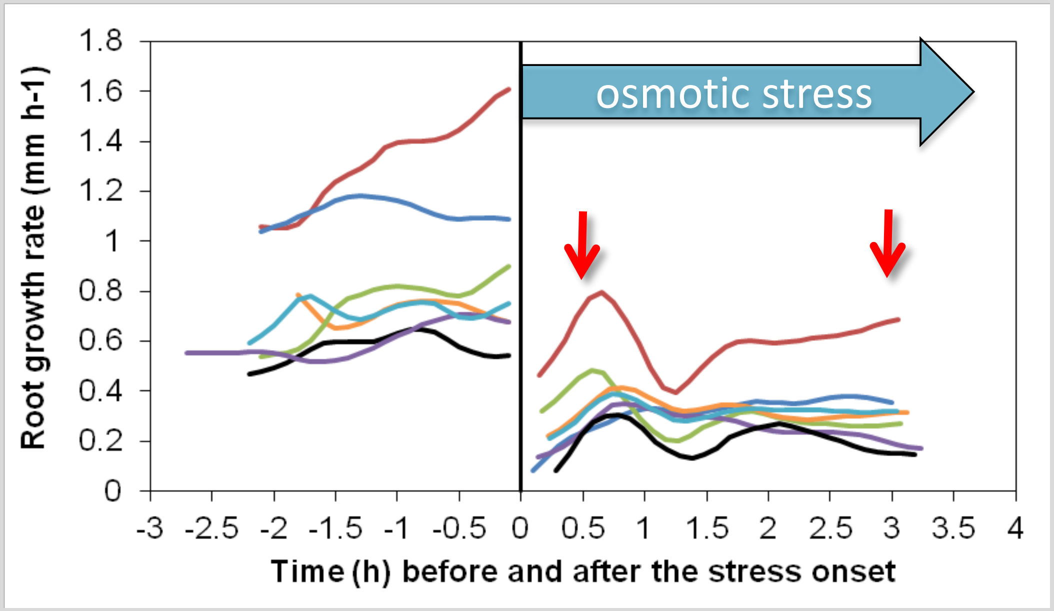
- cell proliferation rate
- cell expansion rate
- transcriptome rewiring in the division zone and the elongation zone

Poplar as a model species



- Clonal propagation
- Easy cultivation in hydroponics
- Fast adventitious rooting
- Plagiotropic roots
- Fast growing root
- Large growth zone
  -> easy manipulation

Dynamics of root growth in response to osmotic stress



Populus nigra

PEG 4000 g/mol (Ψ = -0.35 MPa)

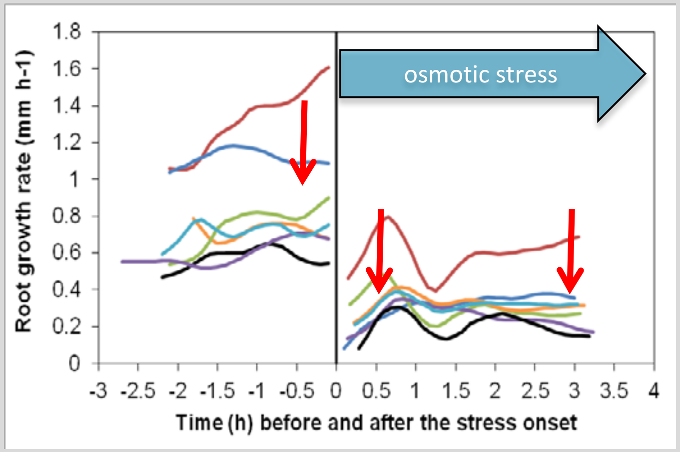
- ➢ strong growth reduction
- ➢ return to stable growth rate after 2 hours

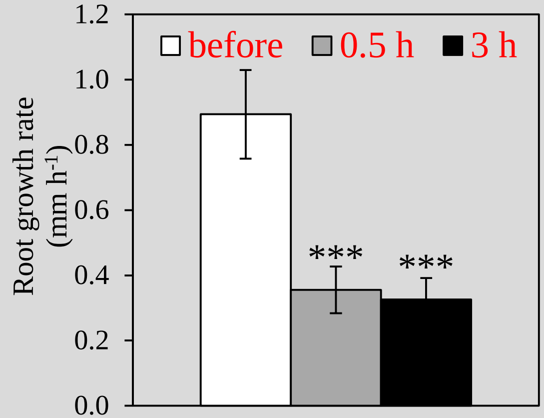
-> 2 snapshots during the transition phase, after 0.5h and 3h of stress

- kinematics -> growth components
- transcriptome in the division zone and the elongation zone

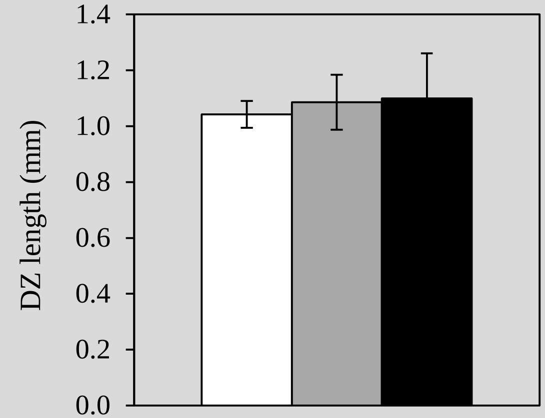
Royer et al, 2016
J Exp Bot

Rapid impairment of cell division & cell expansion

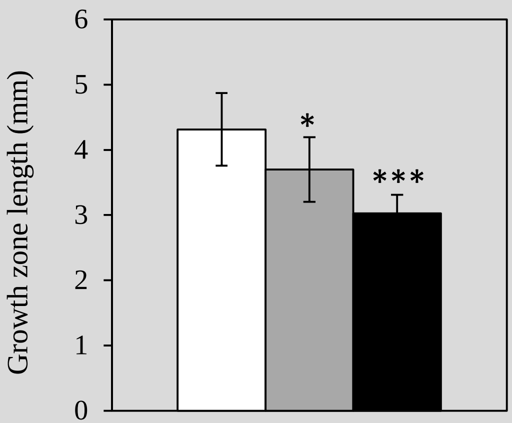




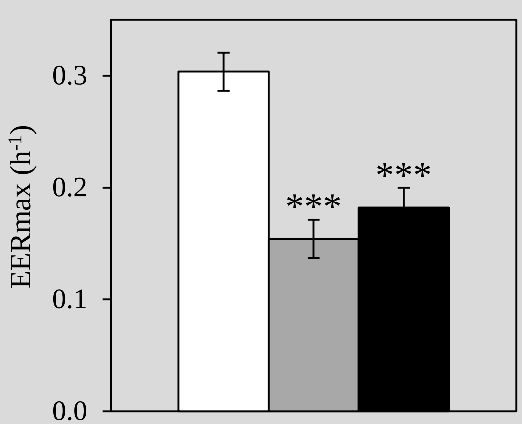
↘ root growth rate



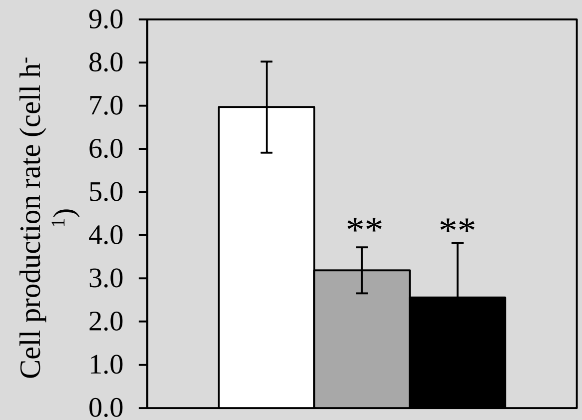
→ division zone length



↘ growth zone length

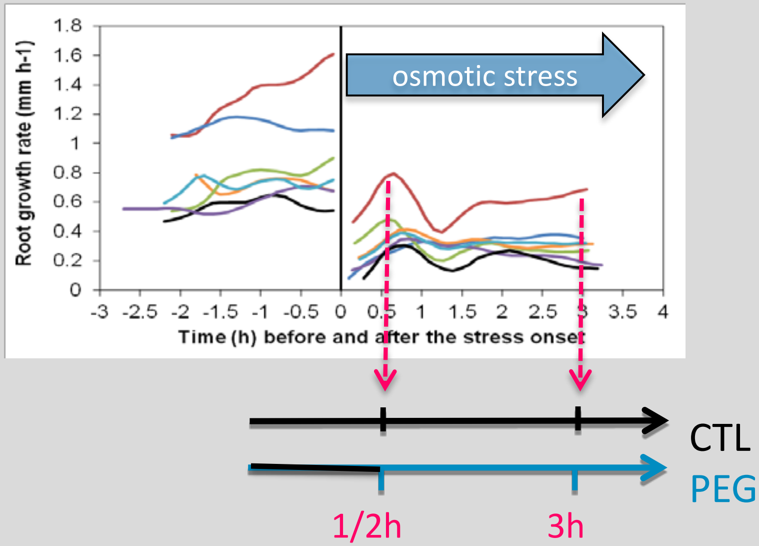


↘ EERmax



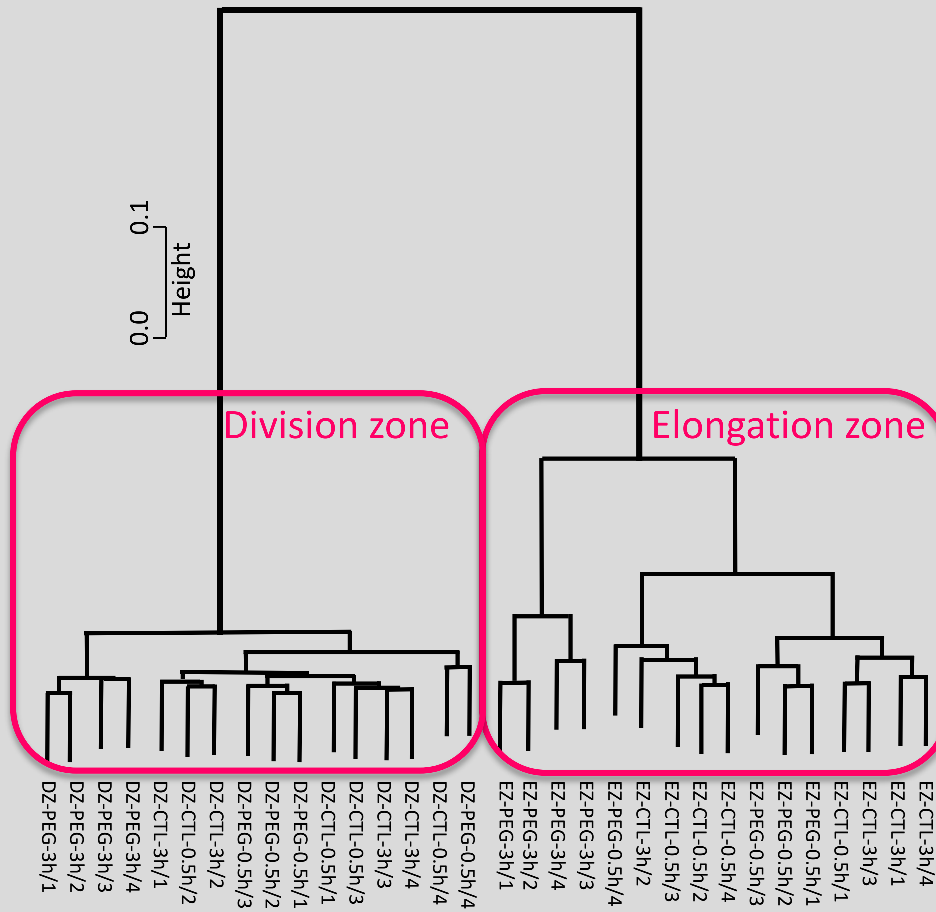
↘ cell production rate
(↘ cell division rate)

Dynamics of molecular controls



32 Librairies & RNA sequencing

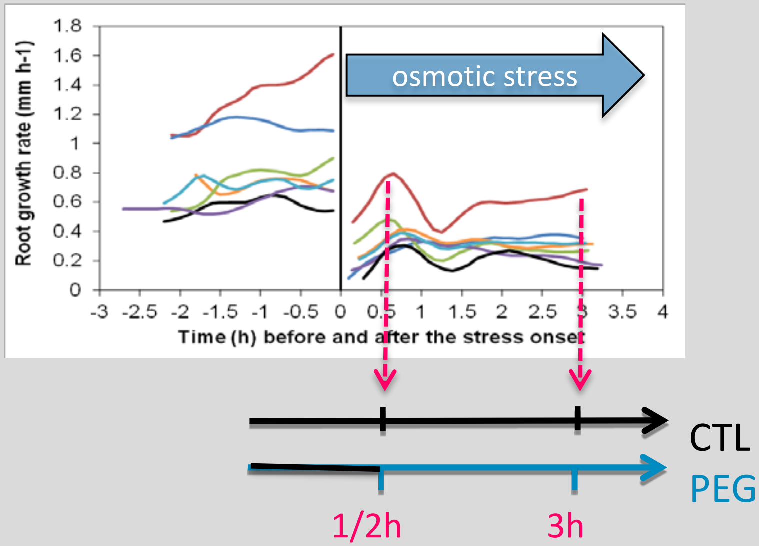
30 million paired-end reads 100 bp, HiSeq 2500

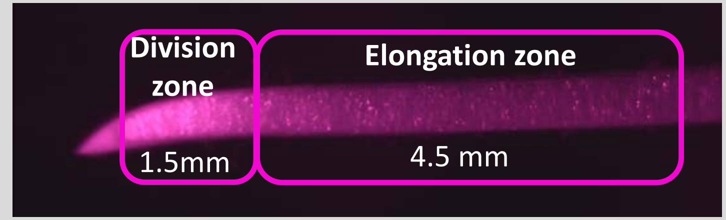


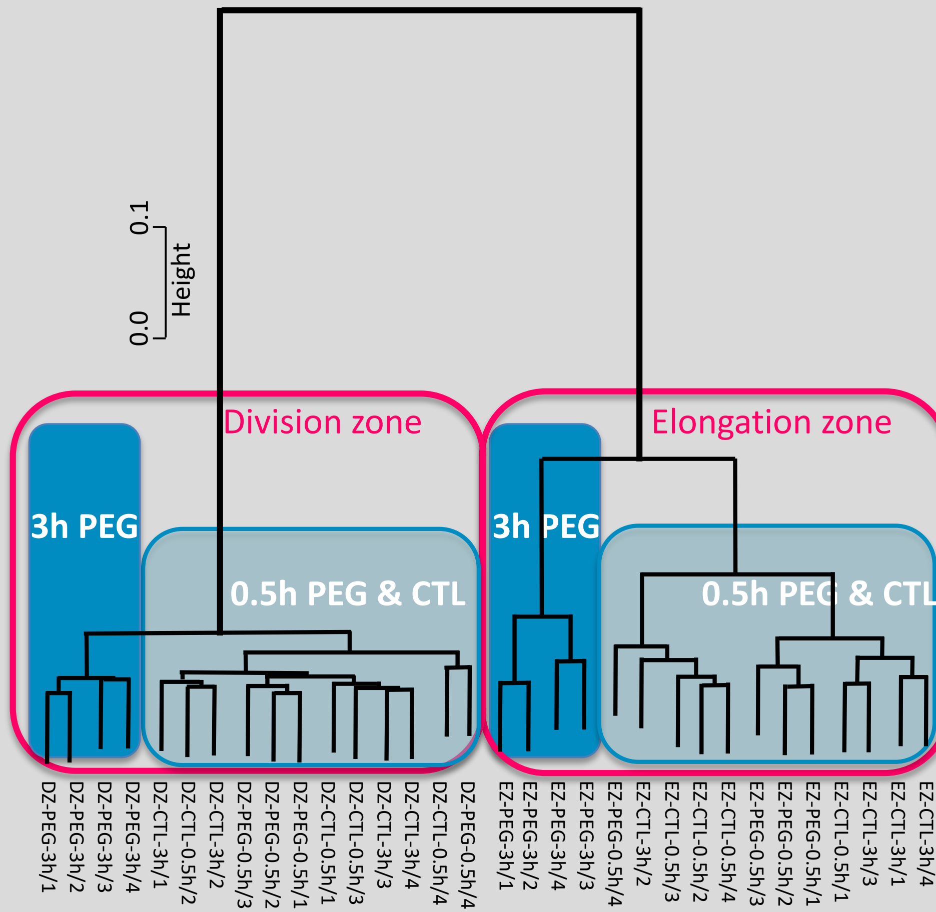
- **Expression patterns are spatially structured**

  distinct cell process ⇔ distinct genes & GO

Dynamics of molecular controls







- **Minor changes after 0.5h**
- **Strong remodelling after 3h**

-> molecular response took time to fully deploy

Interferences between growth effectors and hormonal pathways

TRANSCRIPTOME remodelling & GO enrichments

- **Activation of regulatory processes** with a strong emphasis on genes related to **hormone pathways** **(up to 20 % of DEGs)**

Abscissic Acid (+)
Ethylene (+)
Auxin (+)
Cytokinins (+/-)
Salicylic acid (+)
Gibberellins (-)
Jasmonates (+)
Brassinosteroids (-)

Modification of biosynthesis, catabolism, signaling cascade & transport

Control of hormonal status & local hormone maxima

osmotic stress

GROWTH

↘ EERmax
↘ growth zone length

↘ cell production rate
↘ cell division rate

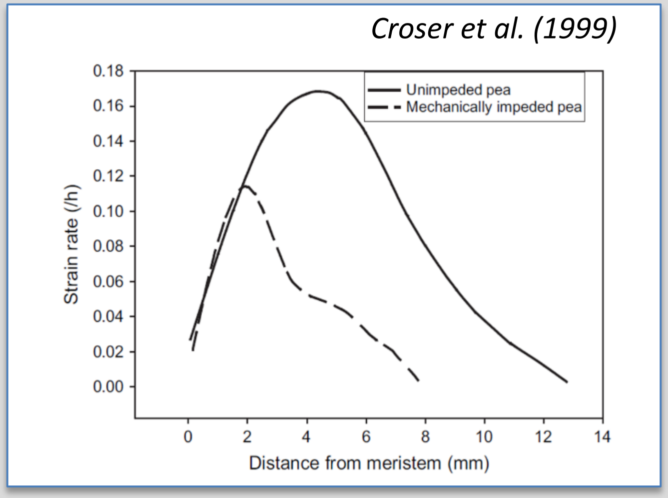
retroaction ?

- **Promotion of growth effectors** suggesting the facilitation of cell expansion
  - cell wall-strengthening ***XTHs*** **(-)**
  - cell wall-loosening ***EXPs*** (*EXPA1* and *EXPA8)* **(+)**
  - cell wall-loosening ***PECTINE LYASEs*** **(+)**
  - Membrane hydraulic conductivity : ***aquaporins*** **(+)**

- **No regulation of core cell cycle genes**
  - low regulation level?
  - impact of expansive growth in DZ ?
  - post-transcriptional regulation of cell cycle components ? (Skirycz *et al.*, 2011)

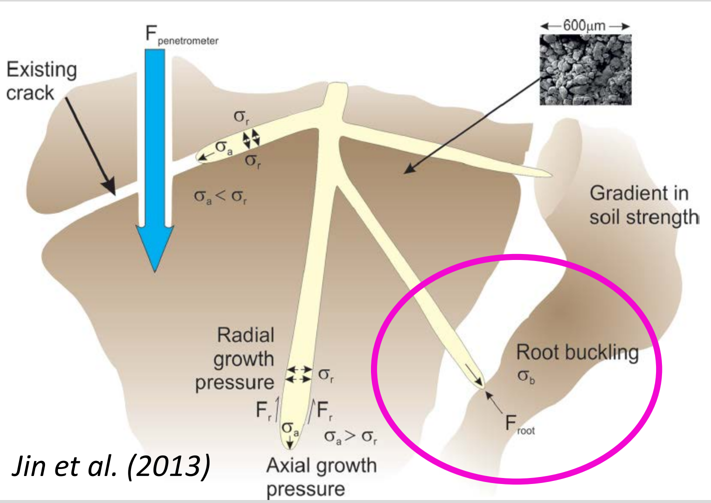
Dynamics of root growth in response to mechanical resistance

- Root growth is sensitive to soil impedance
- Reduction of cell proliferation and cell expansion rates

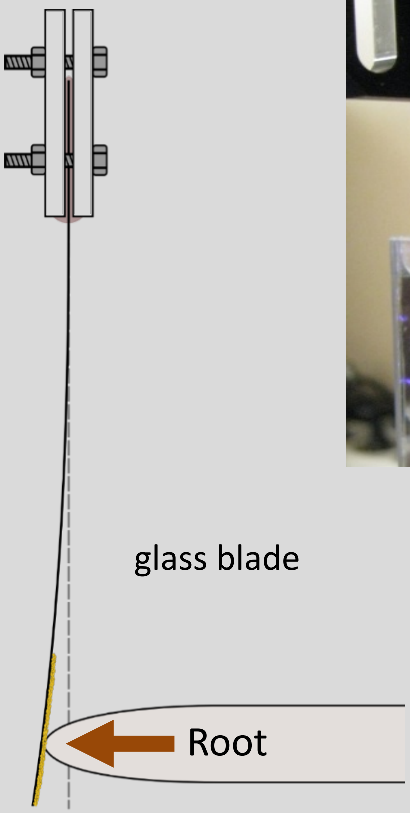


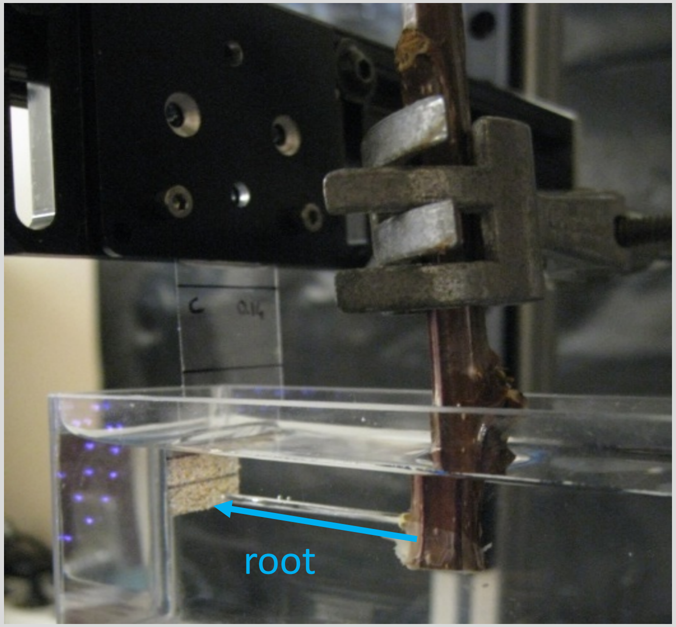
What is the growth response of a root encoutering an obstacle?

- force applied by the root
- growth rate
- curvature



3D kinematics



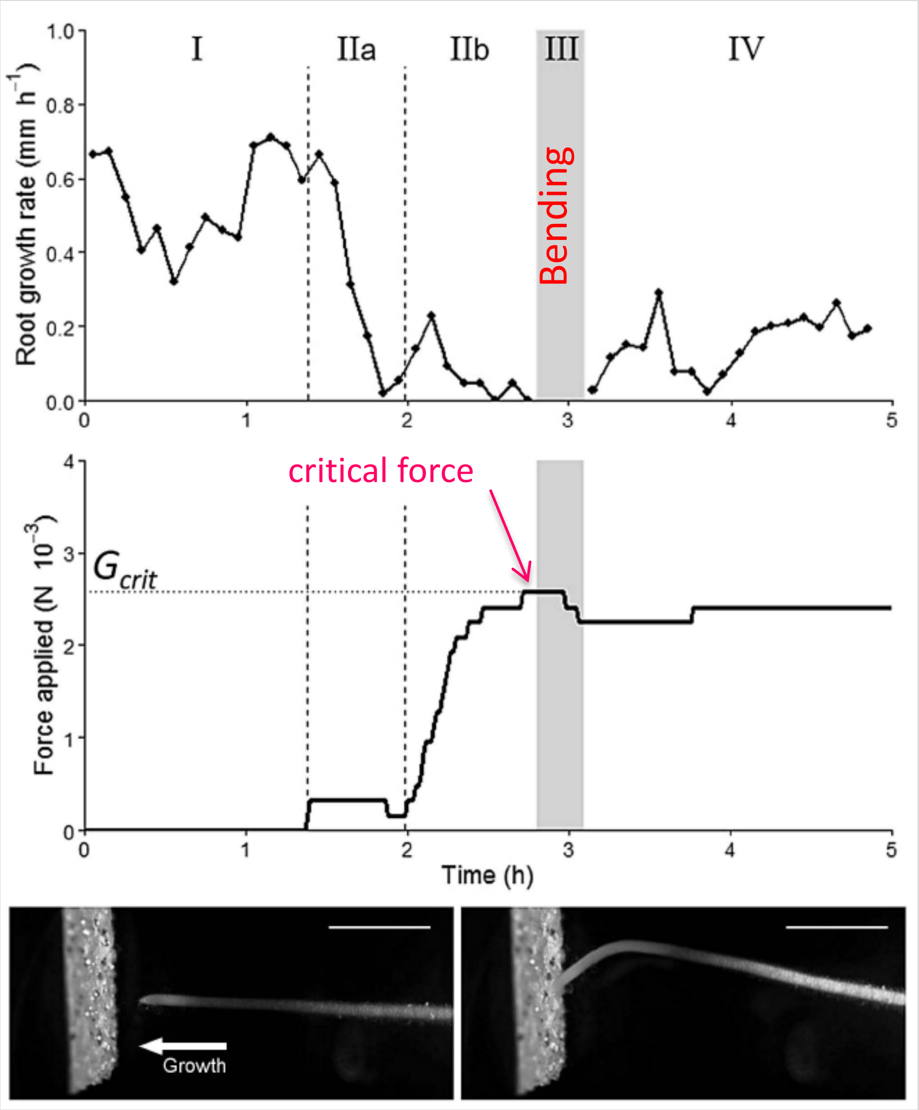


- Adventitious root (*P. euramericana* cv Soligo)
- Hydroponic culture
- Obstacle = home-made submersible sensor of force

  *force = k * deformation*
- Time-lapse photography from two orthogonal directions -> 3D kinematics

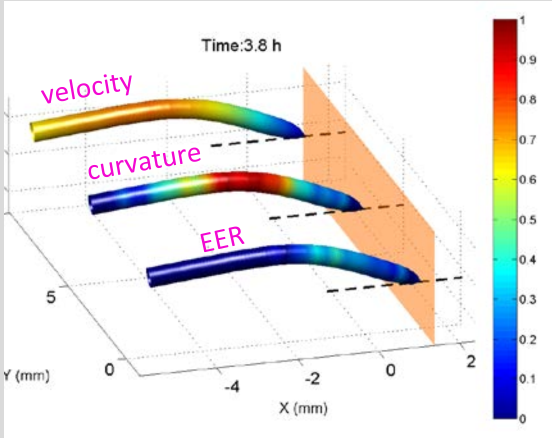
Bizet et al, 2016
J Exp Bot

Dynamics of the response

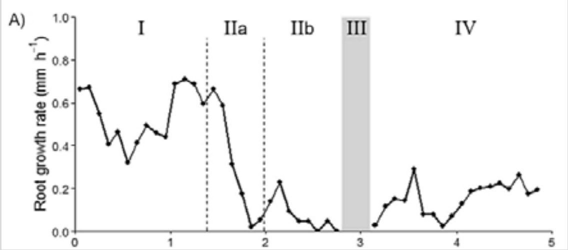


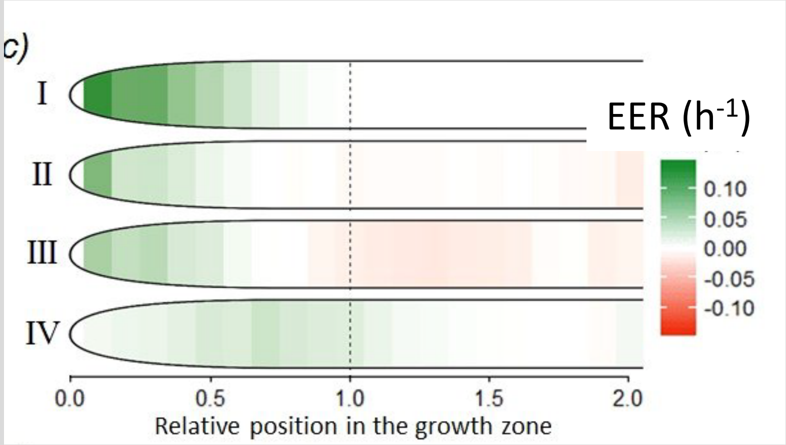
- Several consecutive phases
  - « touch » response
  - growth recovery and force build-up
  - bending of the root & the critical force is reached
  - after bending : the root continues to grow with a slower rate

Dynamics of cell expansion



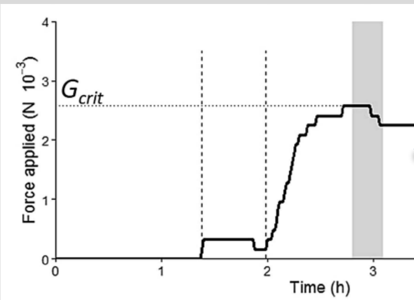
3D reconstruction is necessary because buckling does not occur in a 2D plan

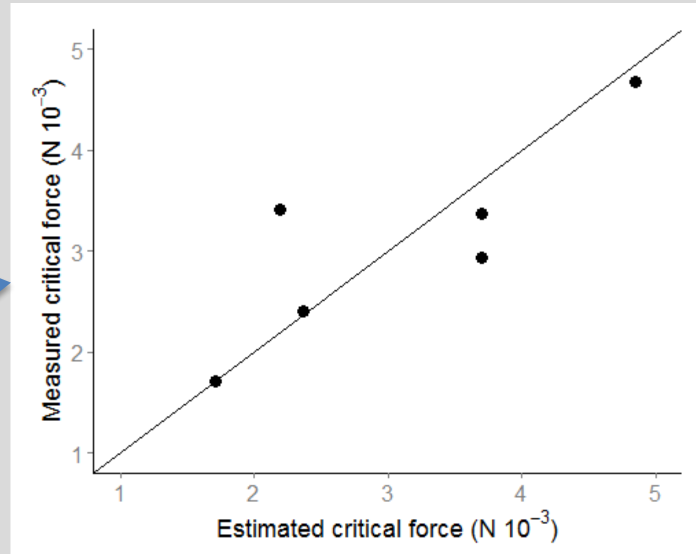


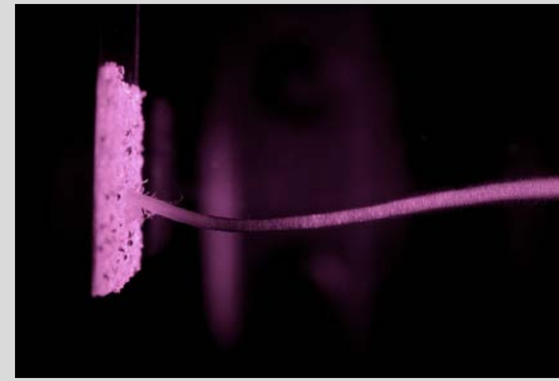


- shortening of the growth zone
- tissue contraction at the junction between elongation zone and mature zone, where curvature was maximal
  = zone of mechanical weakness
- after buckling : lower cell expansion rate but longer elongation zone

Is root bending a purely mechanical phenomena?







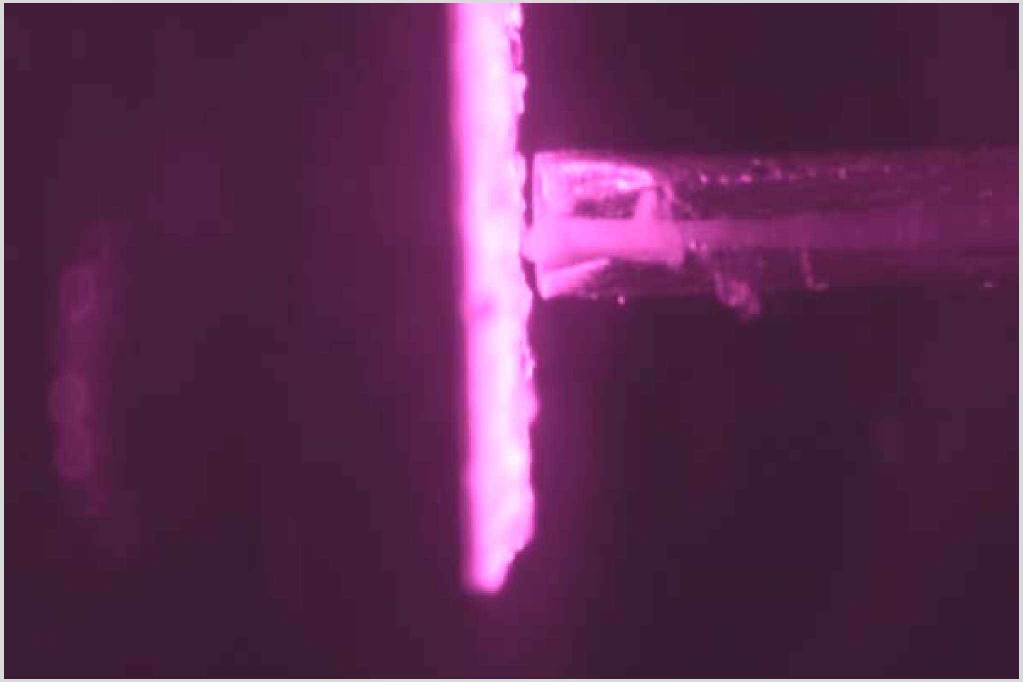
$$F = \frac{E \cdot \pi^2 \cdot (\pi \cdot d^4 / 64)}{(0.7 \cdot L)^2}$$

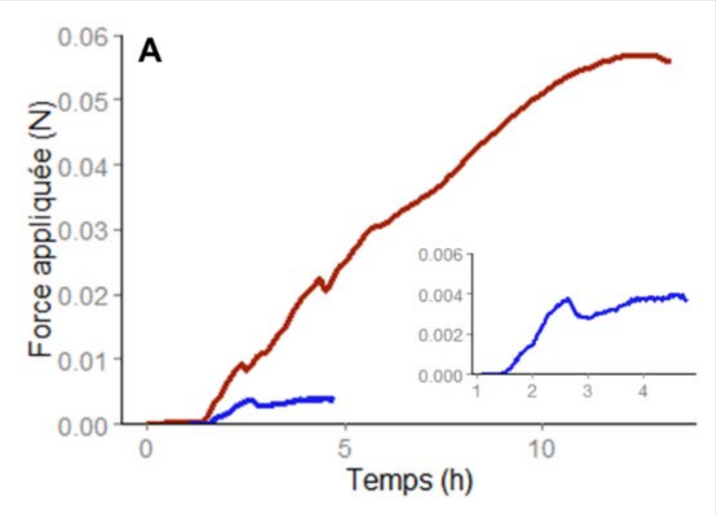
beam theory

- root dimensions
- Young's elastic modulus (32 ± 5 MPa)

- Bending was predicted from root mechanical properties
- No biologically mediated accommodation to mechanical forces influenced bending during this short period of time. Bending was purely mechanical = **BUCKLING**

Bracing root increases the applied force





- Lateral bracing of the root increased the force applied by the growing root by preventing buckling

Thank you for your attention

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