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## **Aquaporin TIP1 expression and its regulation in the growing root apex under two levels of osmotic stress**

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► **To cite this version:**

Marie-Béatrice Bogeat-Triboulot, Rémy Merret, Bruno B. Moulia, David Cohen, Agnès A. Guilliot, et al.. Aquaporin TIP1 expression and its regulation in the growing root apex under two levels of osmotic stress. Réunion du groupe “ Xylème-Eau-Croissance ”, Apr 2011, Nancy, France. 19 p. hal-02805346

**HAL Id: hal-02805346**

**<https://hal.inrae.fr/hal-02805346>**

Submitted on 6 Jun 2020

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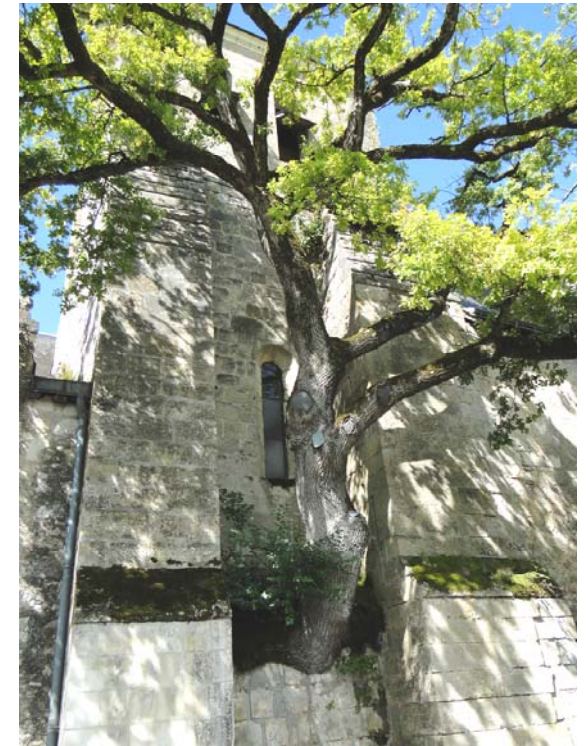


**Aquaporin *TIP1s* expression and their regulation  
in the growing root apex  
under two levels of osmotic stress**



## Roots

- anchor plants
- uptake water and nutrients



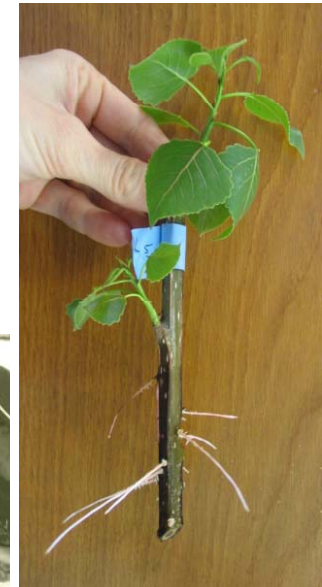
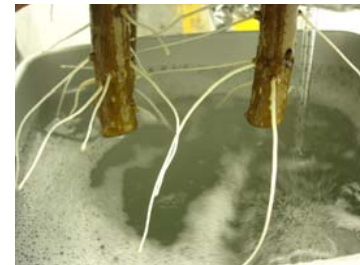
## Under drought, growth of roots

- allows the prospection of distant and wetter soil horizons
- contributes to plant acclimation and tolerance to water deficit

## ↪ Control of root growth under water deficit ?



*Populus deltoïdes x nigra* cv Soligo  
Cuttings grown in hydroponics



## Root growth under osmotic stress

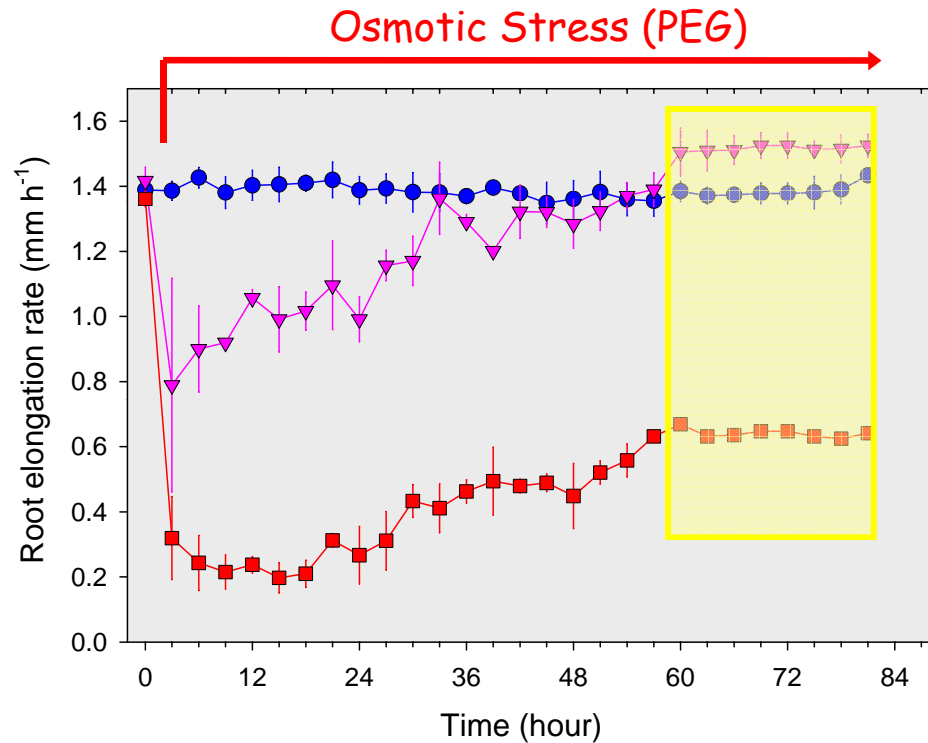
21°C, continuous dark

- Control

2 levels of continuous and stable osmotic stress (PEG 3350 g mol<sup>-1</sup>):

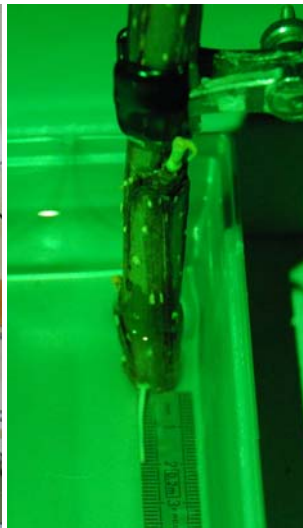
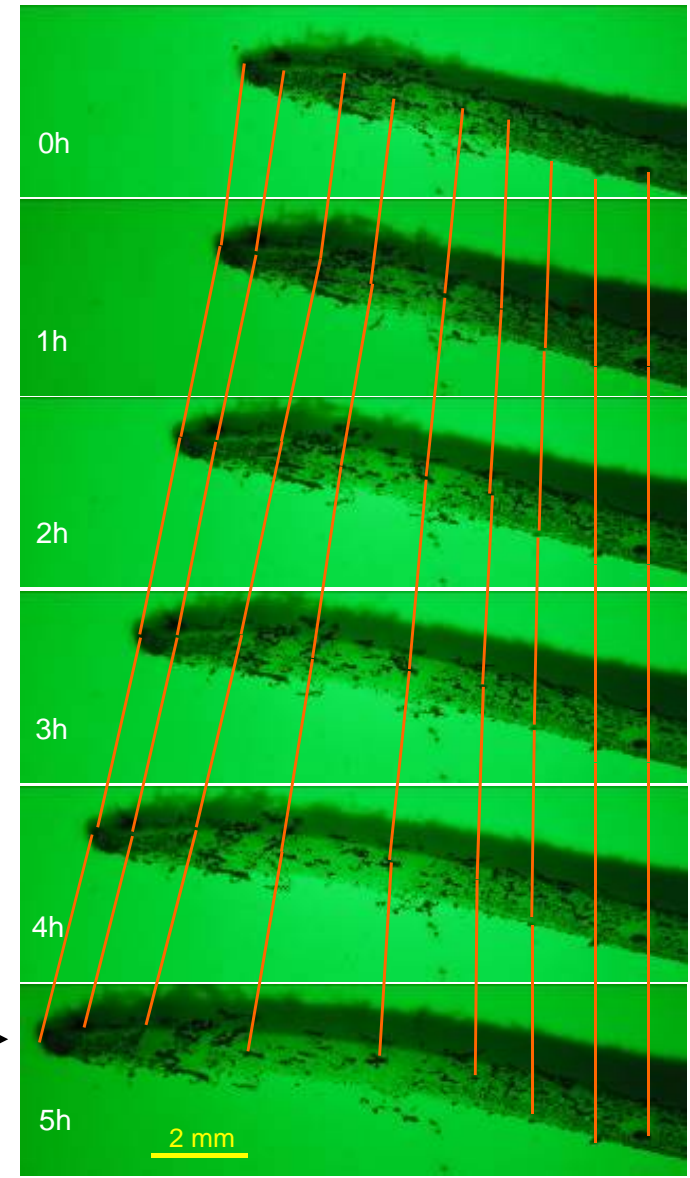
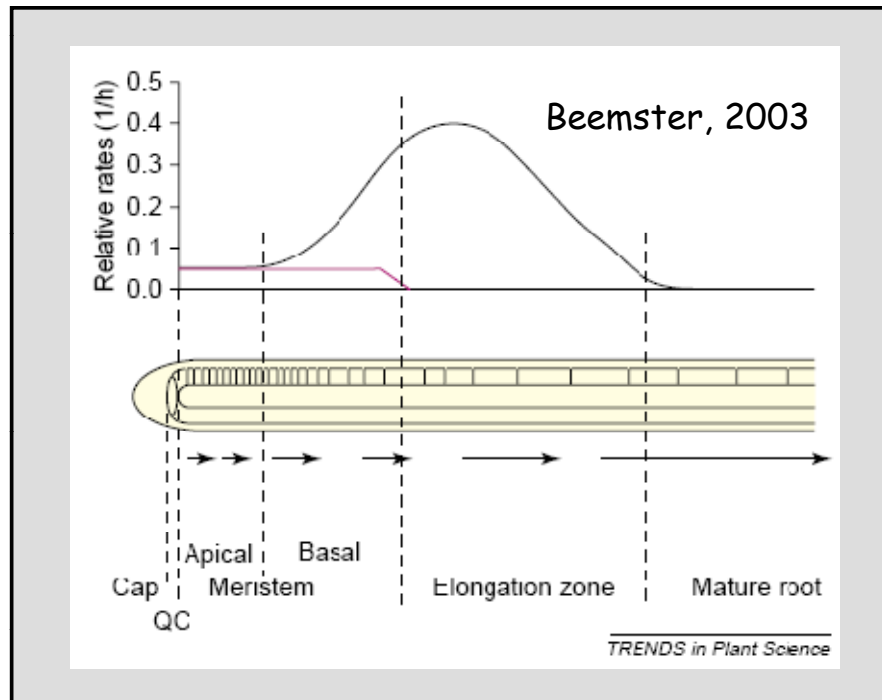
- Moderate stress :  
(90 mosmol kg<sup>-1</sup>, 100g L<sup>-1</sup> PEG)

- High stress :  
(260 mosmol kg<sup>-1</sup>, 200g L<sup>-1</sup> PEG)



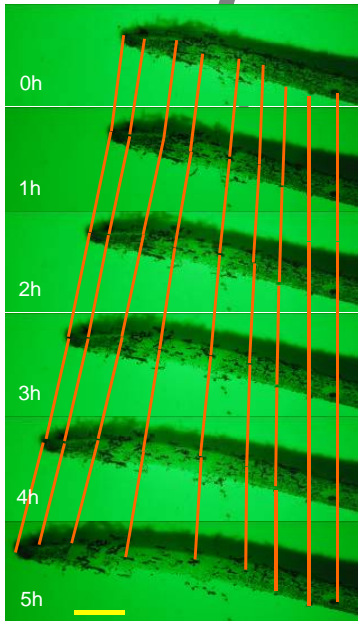
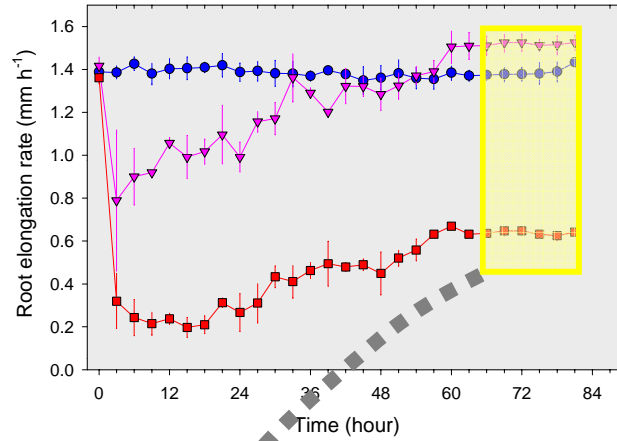
- steady state
- 3 contrasted elongation rates
  - control
  - moderate stress : slightly higher than control
  - high stress : lower than control

# Root elongation results from cell division and cell expansion



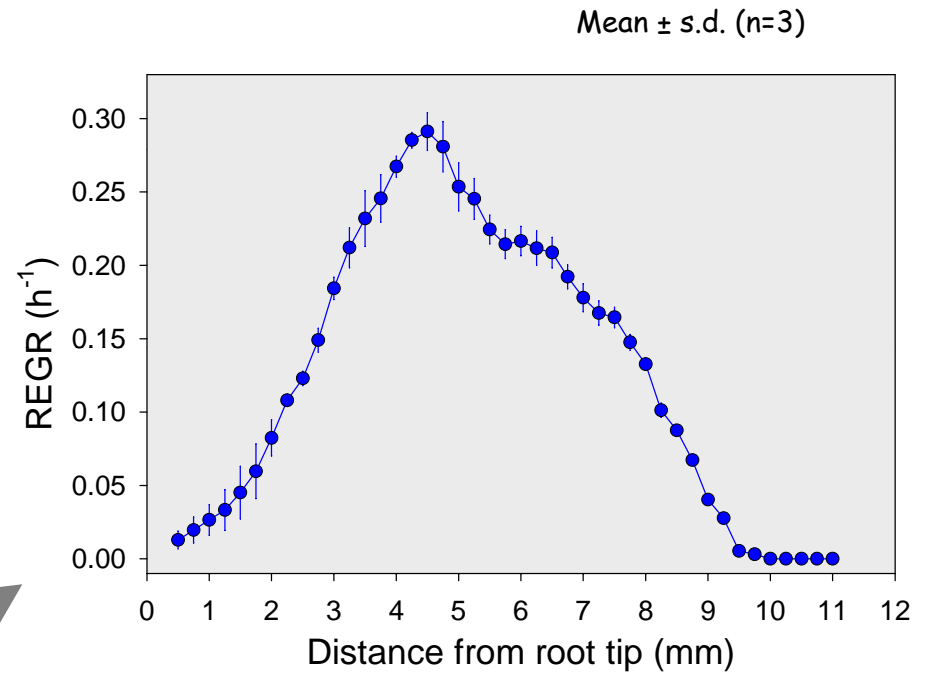
Carbon particles deposition & *in vivo* time laps photography

# Relative Elemental Growth Rate

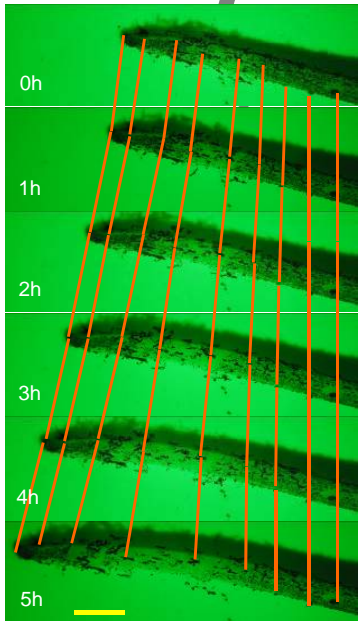
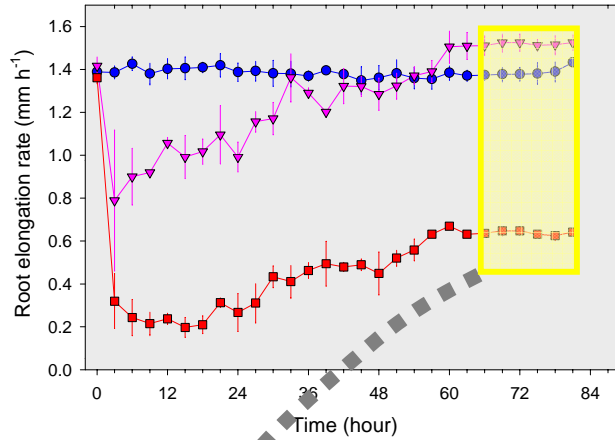


kinematic analysis

Kineroot  
(Basu et al, 2007)

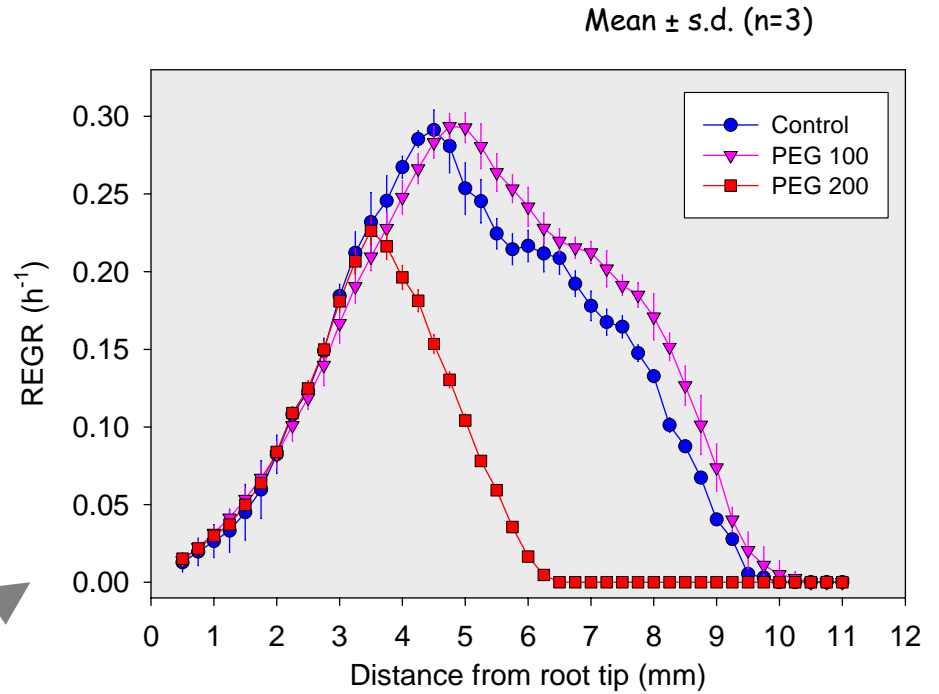


# Relative Elemental Growth Rate



kinematic analysis

Kineroot  
(Basu et al, 2007)

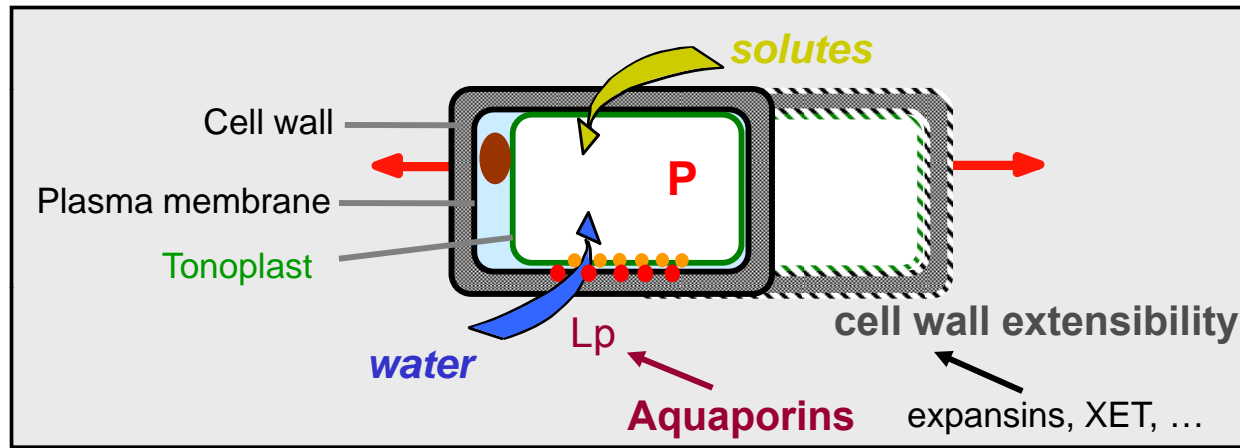


- REGR differentially affected along the growth zone
- Cell expansion rate differentially affected

# Are growth alterations due to variations of hydraulic conductivity ?

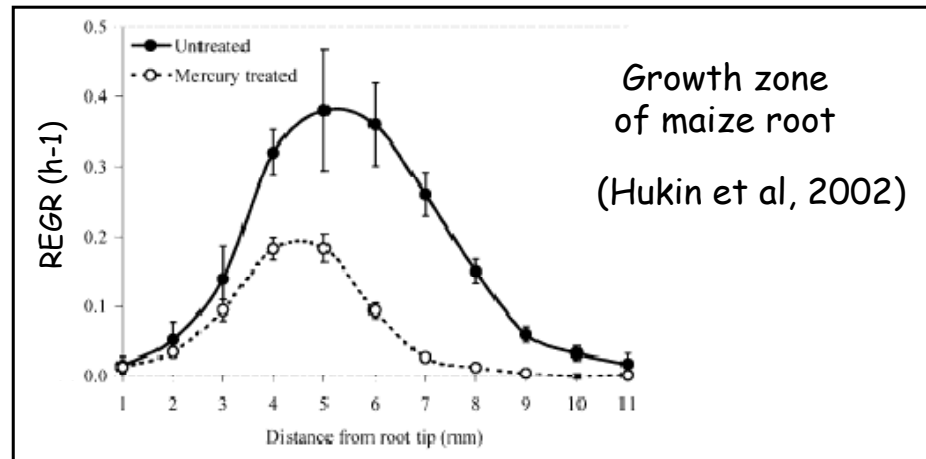
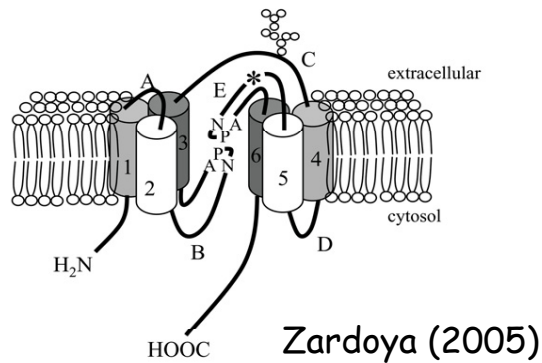
Biophysical model of cell expansion (Lockhart, 1965):

$$\frac{1}{V} \cdot \frac{dV}{dt} = \frac{L \phi}{L + \phi} \cdot (P - Y)$$



Hydraulic limitation of cell expansion (Cosgrove, 1993; Boyer & Silk, 2004)

↳ Involvement of aquaporins ?



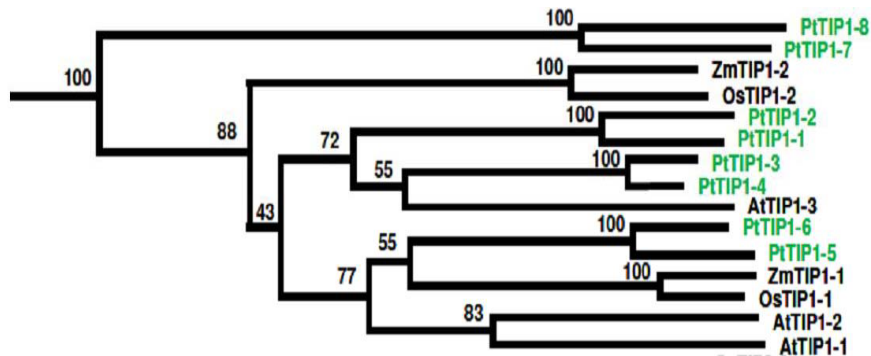


## Aquaporin family : several classes

- PIP : Plasma membrane intrinsic protein

-TIP : Tonoplast intrinsic protein

& NIP, SIP, XIP ...



Gupta and Sankaramakrishnan, 2009

Pt : *Populus trichocarpa*

Os : *Oryza sativa*

At : *Arabidopsis thaliana*

Zm : *Zea mays*

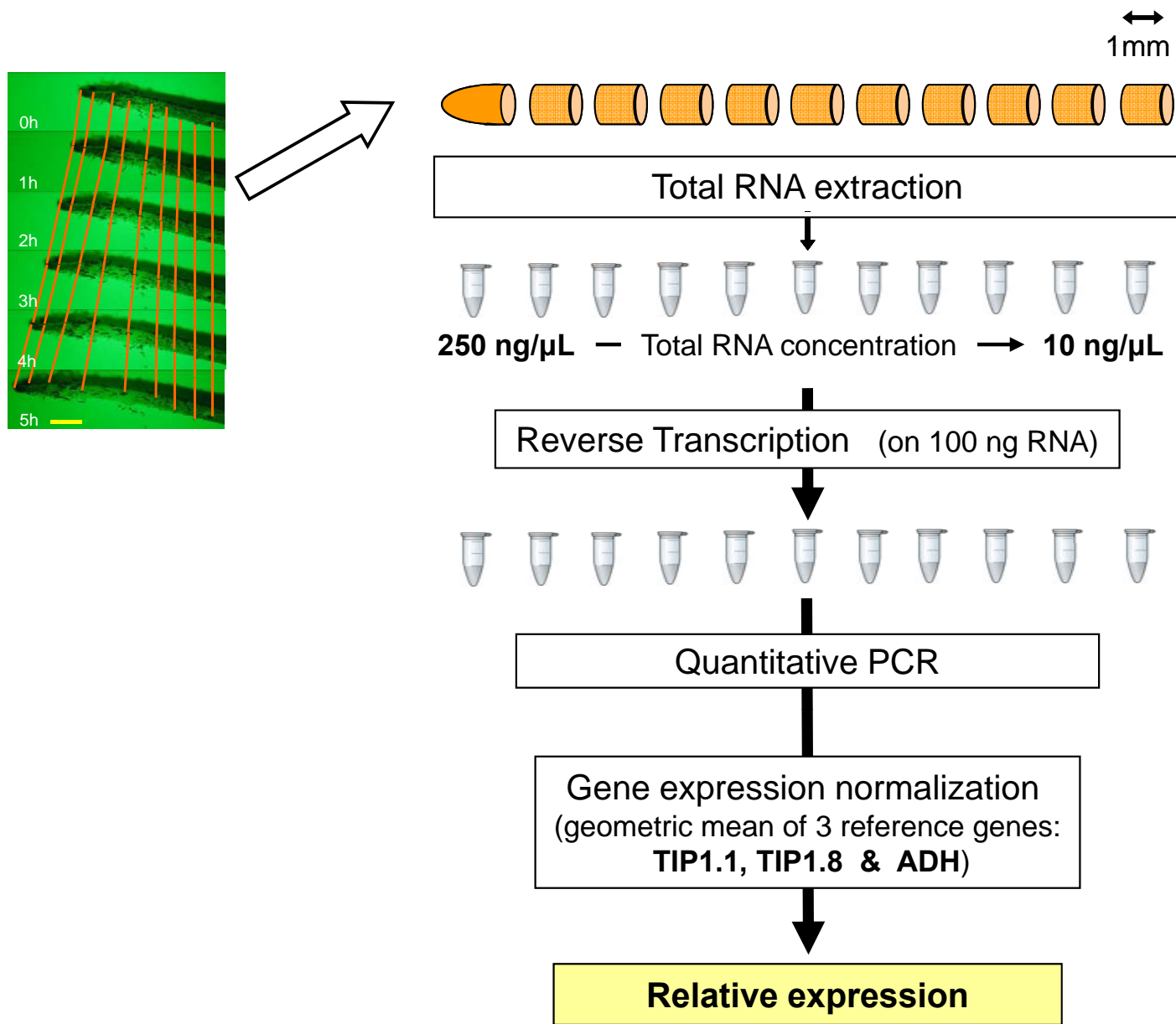


**8 *TIP1s* in *Populus trichocarpa* genome  
4 paralog pairs (gene duplication)**

## *Our questions*

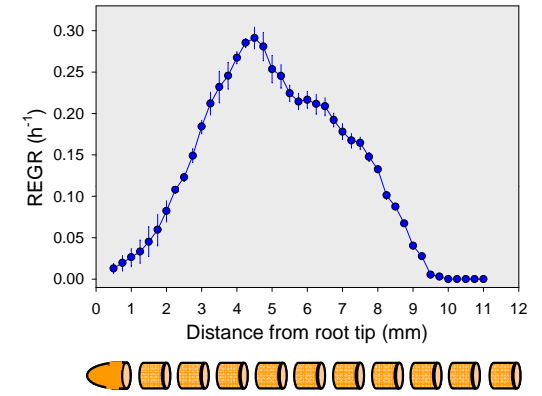
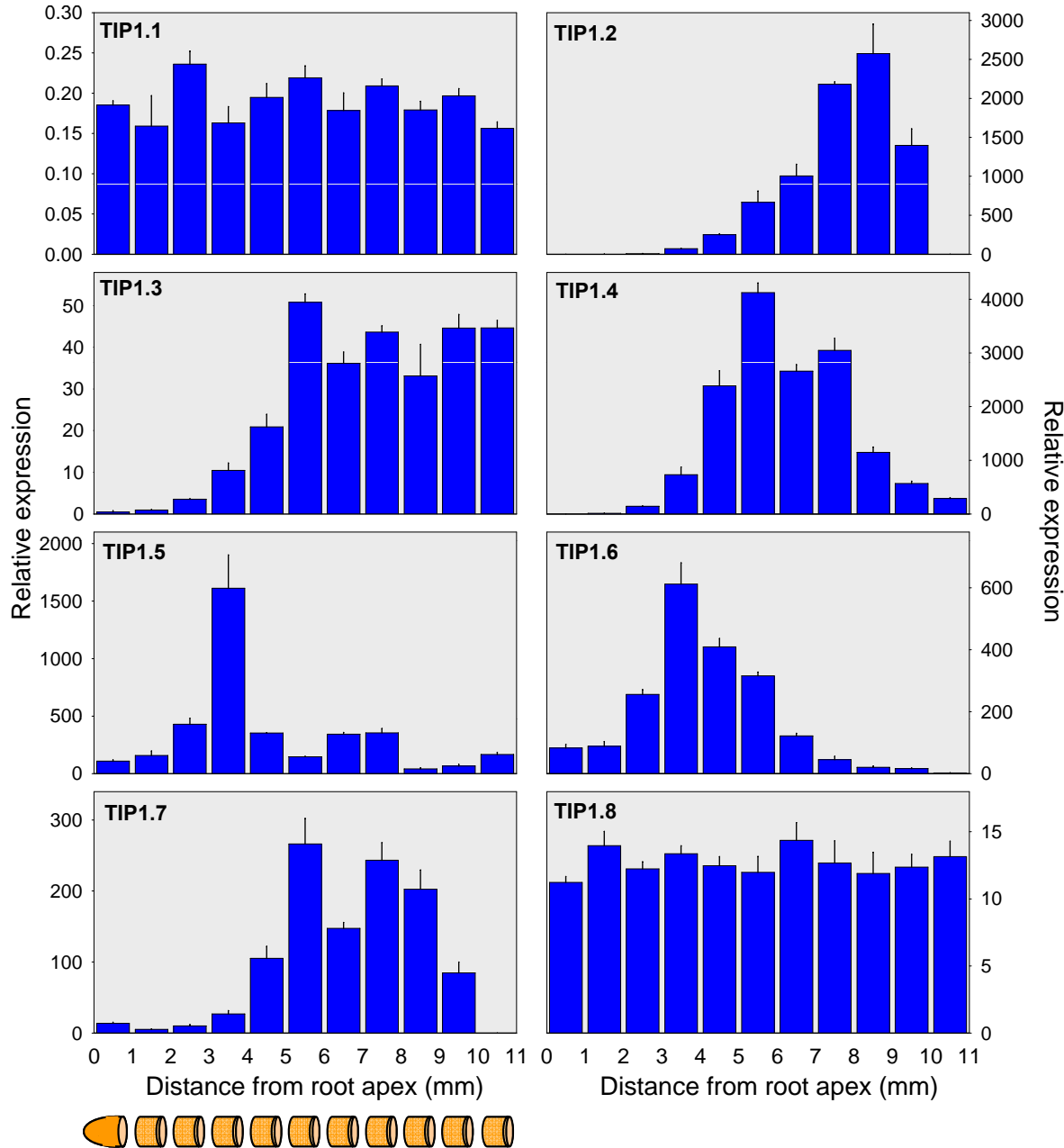
- ↪ 1- Expression of *TIP1s* in the growing zone of the poplar root ?
- ↪ 2- How is the expression level of *TIP1s* affected by osmotic stress ?
- ↪ 3- Can we access to the regulation of *TIP1s* expression ?

# 1- Relative expression of TIP1s in the growing zone of the root



# 1- Relative expression of TIP1s in the growing zone of the root

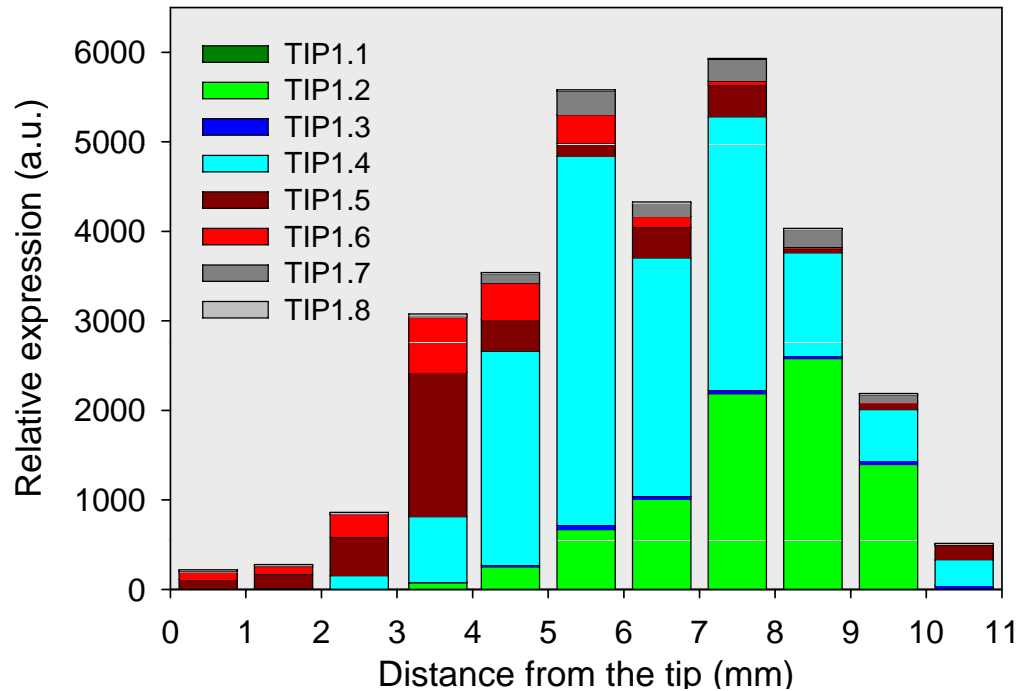
Mean  $\pm$  s.d. (n=3)



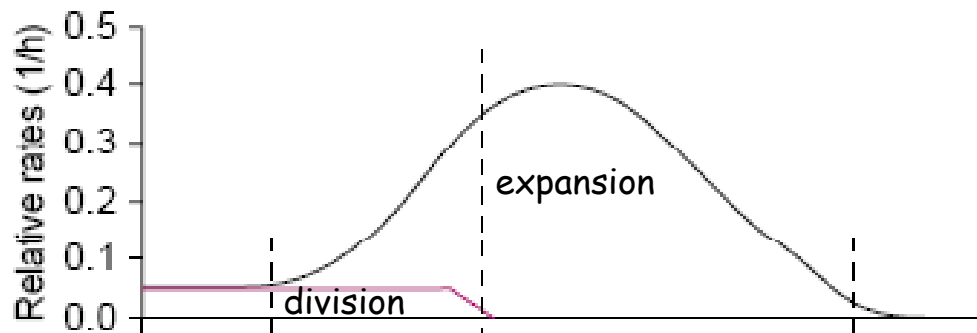
Relative expression along the root growth zone:

- stable for *TIP1.1* et *TIP1.8*, as expected
- highly contrasted pattern for the 6 other *TIP1s*
- differences between paralog pairs
- different levels of expression

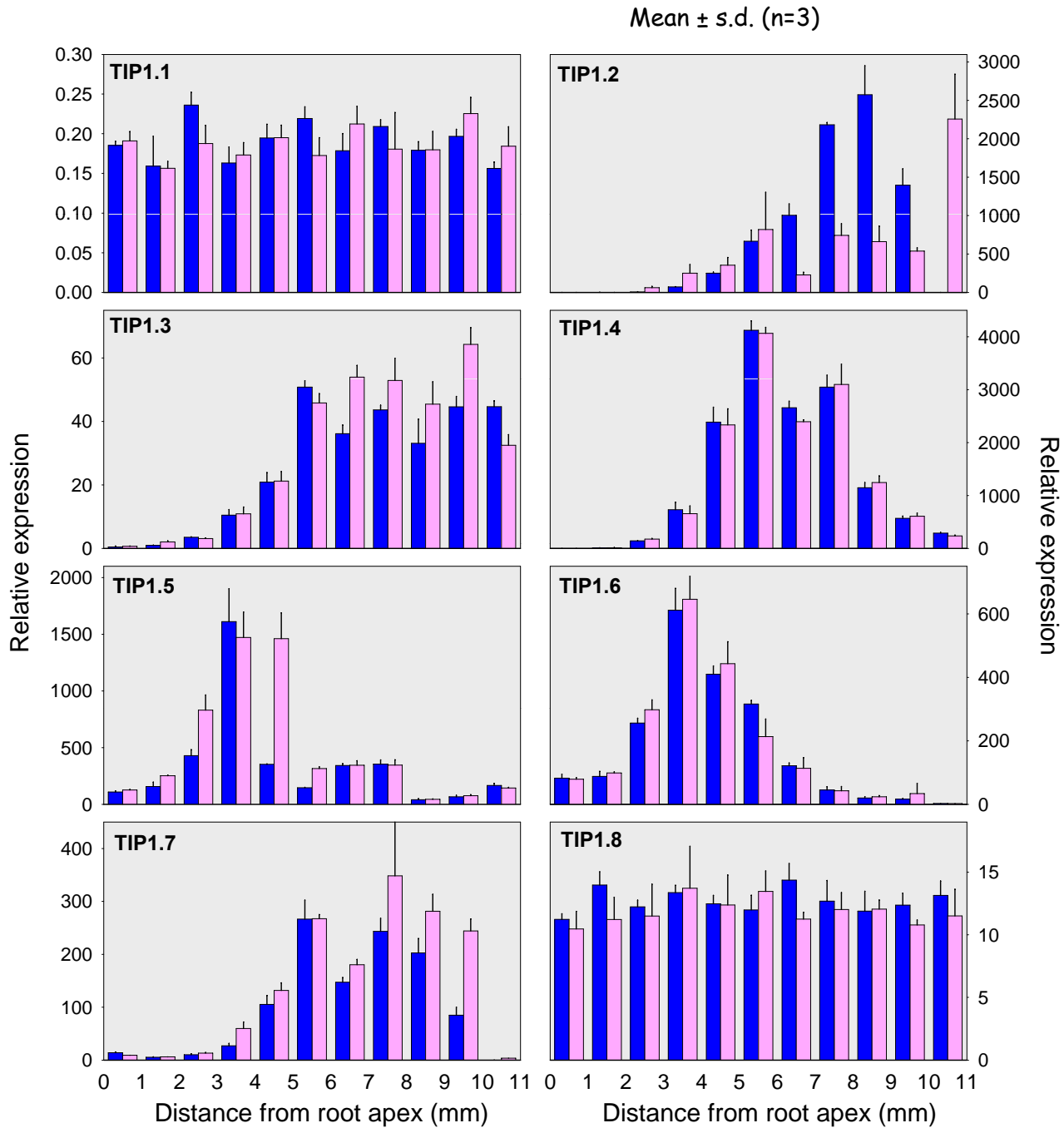
# 1- Relative expression of TIP1s in the growing zone of the root



- Level of expression differs between paralogs
- High expression level of *TIP1s* in the growth zone
- Meristem : *TIP1.5* & *TIP1.6*
- Cell expansion : *TIP1.4*
- Distal part : *TIP1.2*

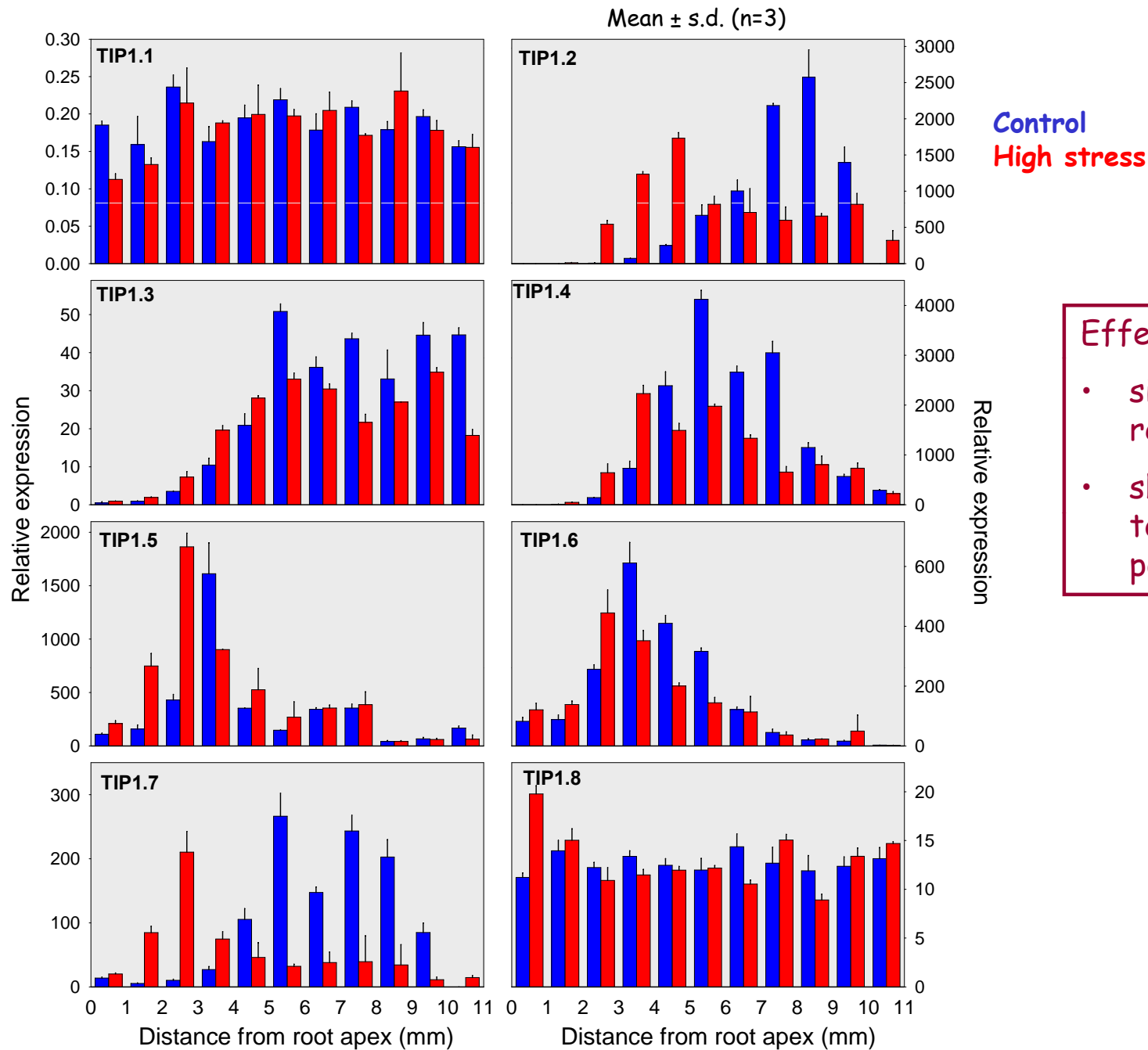


## 2- Relative expression of TIP1s under moderate osmotic stress



Low impact of moderate stress

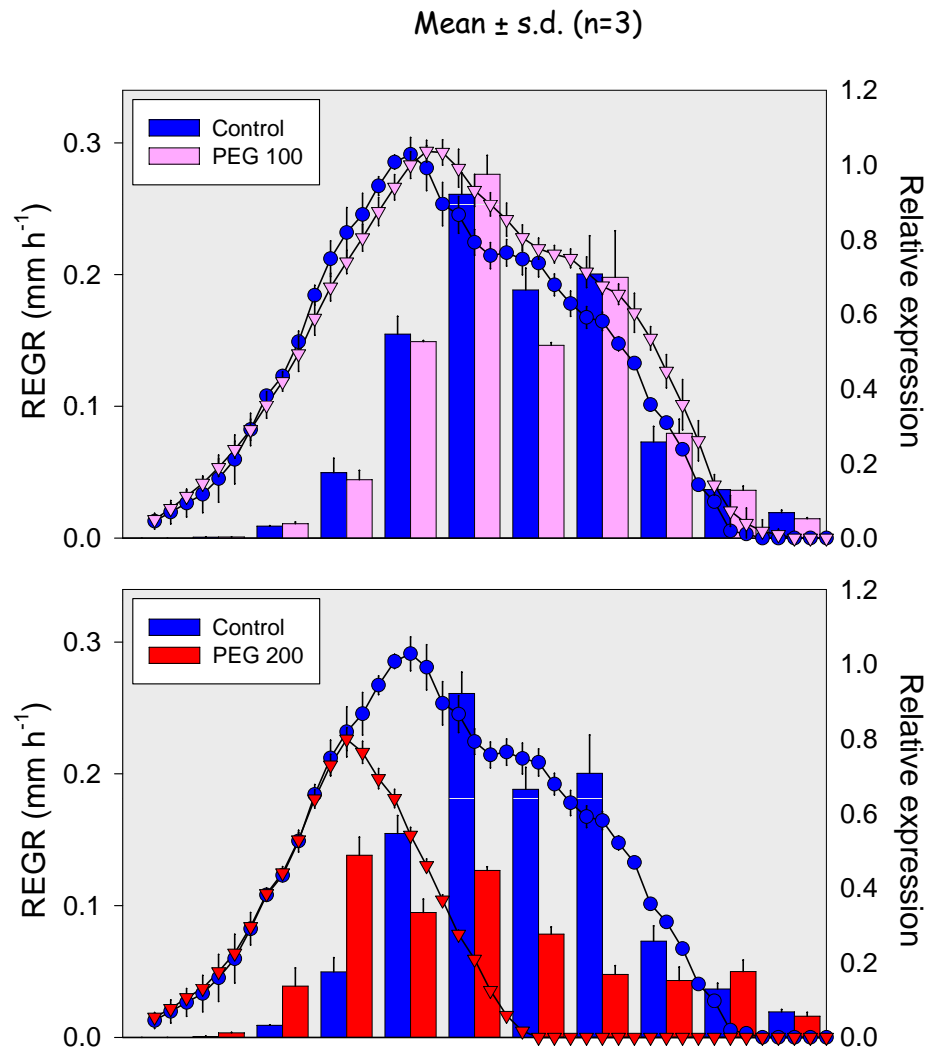
## 2- Relative expression of TIP1s under high osmotic stress



### Effect of high stress:

- similar or lower relative expression
- shift of the maxima towards a more apical position

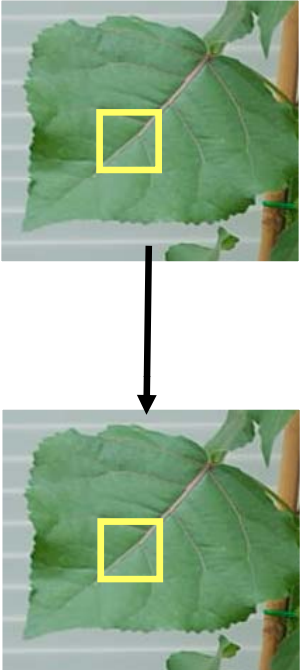
## 2- Relative expression of TIP1.4 and cell expansion



Control  
Moderate Stress  
High stress

For the 3 growth states :  
overlapping of REGR pattern and  
*TIP1.4* expression pattern  
-> putative link between *TIP1.4*  
and cell expansion

### 3- Can we access to the regulation of TIP1s expression ?

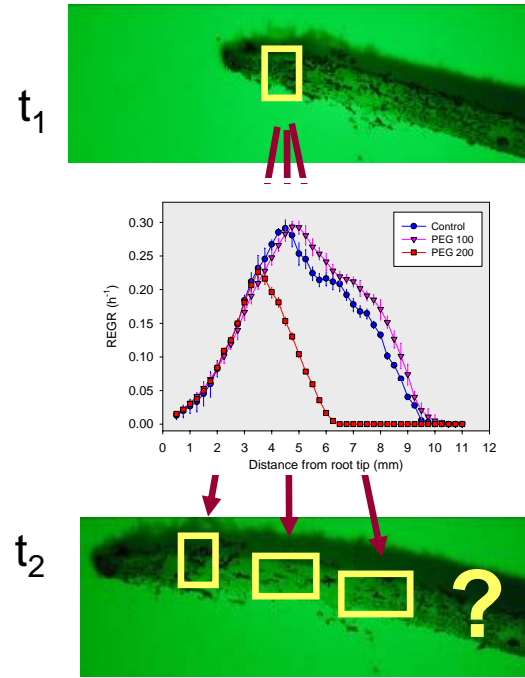


$t_1$

$t_2$

**Mature organ :**

Regulation of gene expression = temporal variation of transcript density

$$\delta\rho / \delta t$$


**Growing root**

In a **growing organ**, it is necessary to take into account cells movement and their expansion.

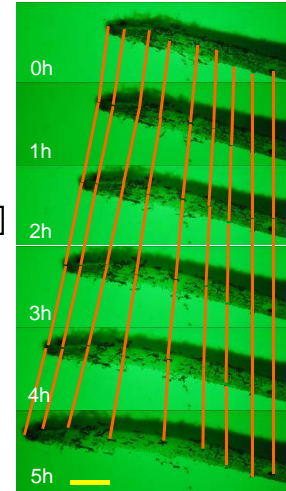
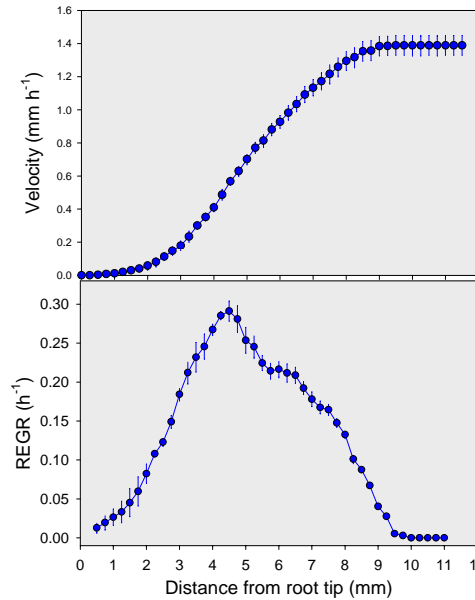
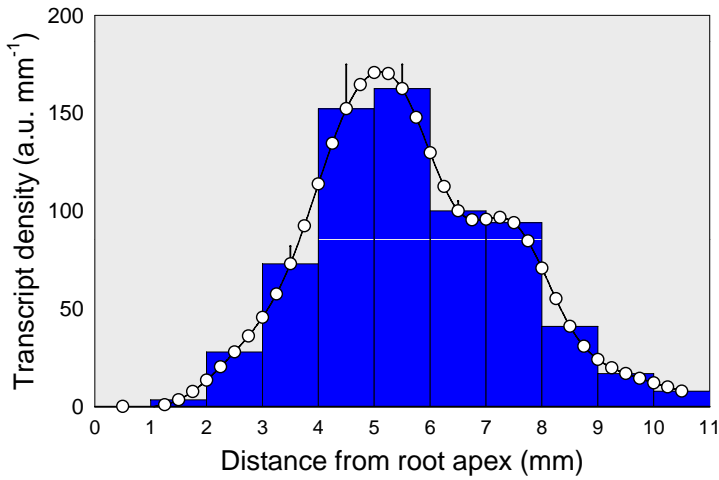
$$D_{(x)} = \underbrace{\frac{\partial \rho}{\partial t}}_{\text{Temporal variation}} + \underbrace{v \frac{\partial \rho}{\partial x}}_{\text{Convection}} + \underbrace{\rho \frac{\partial v}{\partial x}}_{\text{Dilution}}$$

**Net accumulation rate**

The **continuity equation** (issued from a fluid mechanics formalism) gives access to the **net accumulation rate of transcript**, i.e., the **regulation of gene expression**

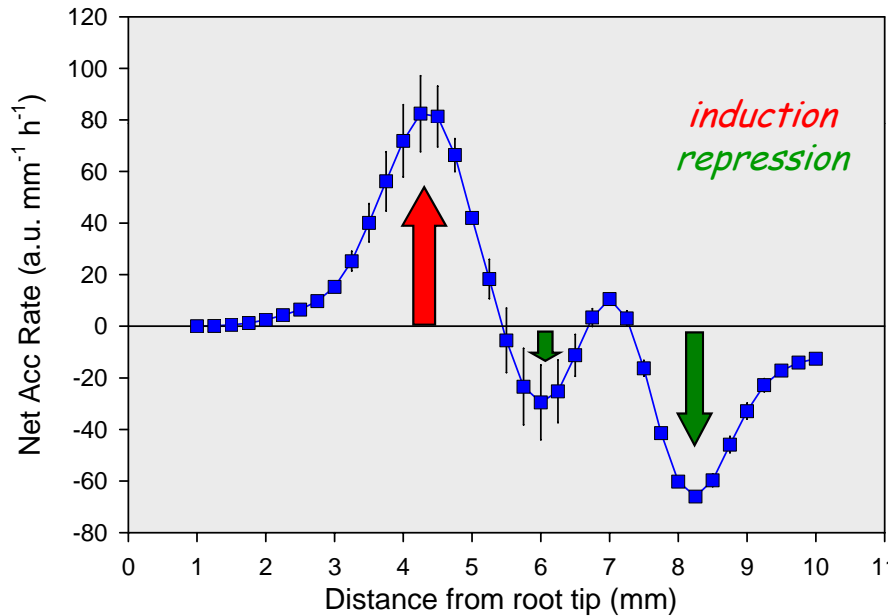


### 3- Spatial pattern of local net transcript accumulation rate



**Continuity equation**

$$D_{(x)} = \frac{\partial \rho}{\partial t_{(x)}} + v \frac{\partial \rho}{\partial x_{(x)}} + \rho \frac{\partial v}{\partial x_{(x)}}$$

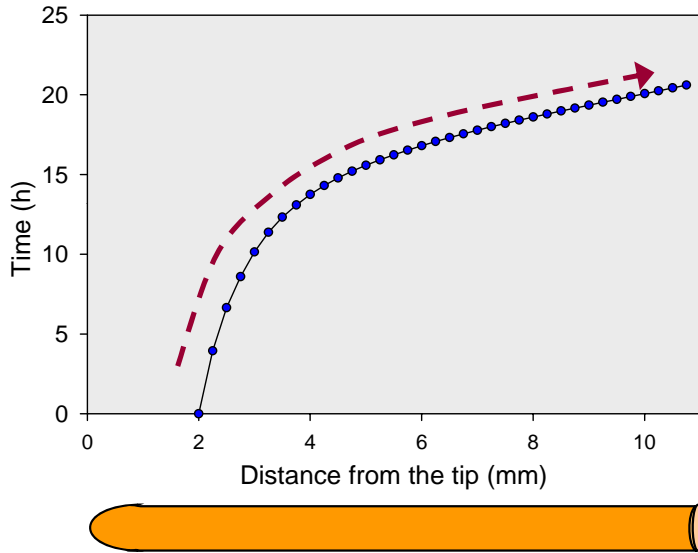


*Merret et al. (2010)*

### 3- Spatio-temporal pattern of regulation in a 'moving particule'

If steady state :

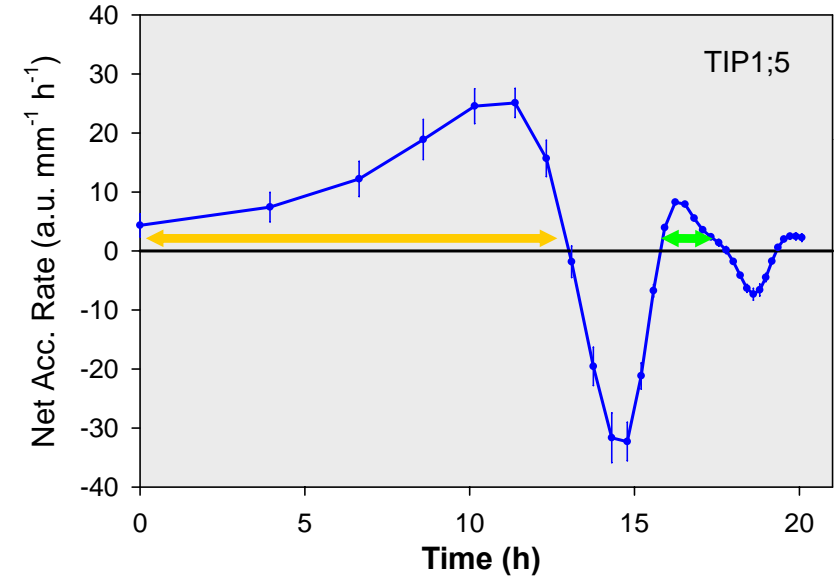
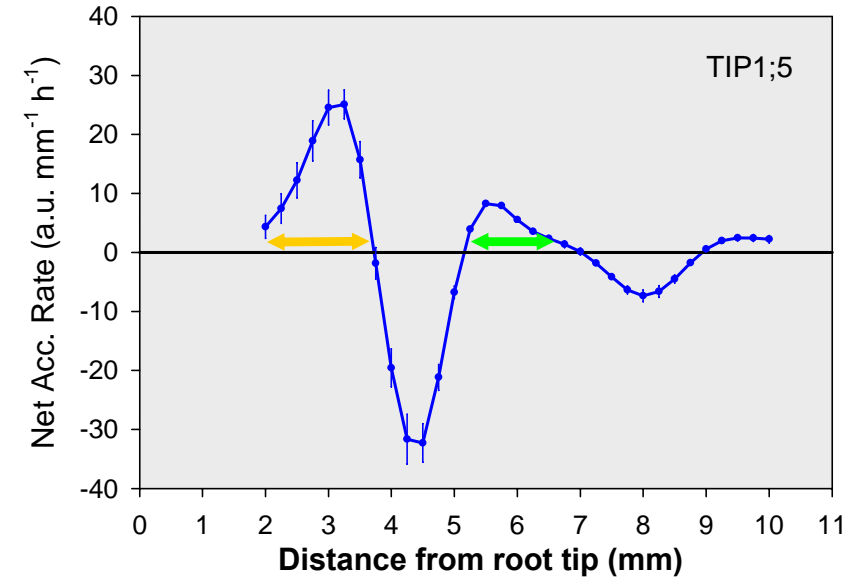
Particule trajectory  
time = f (position)



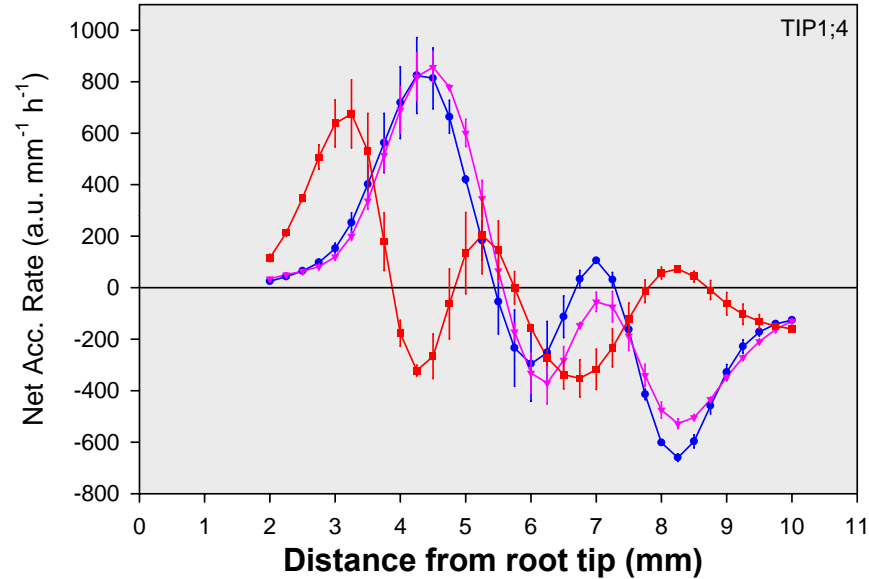
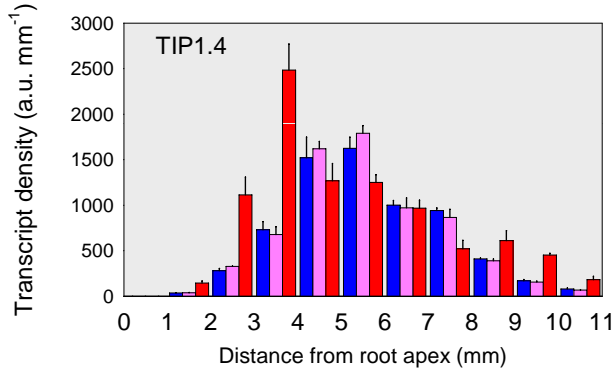
spatial coordinates



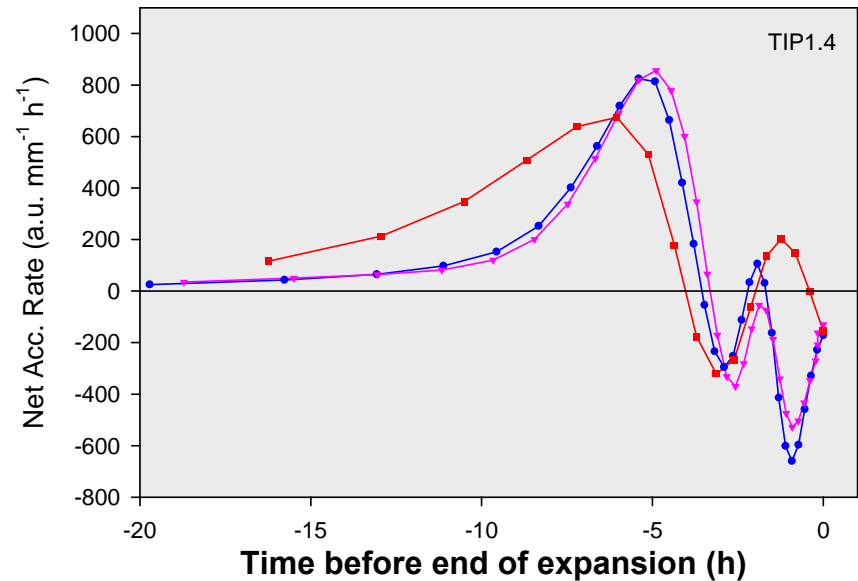
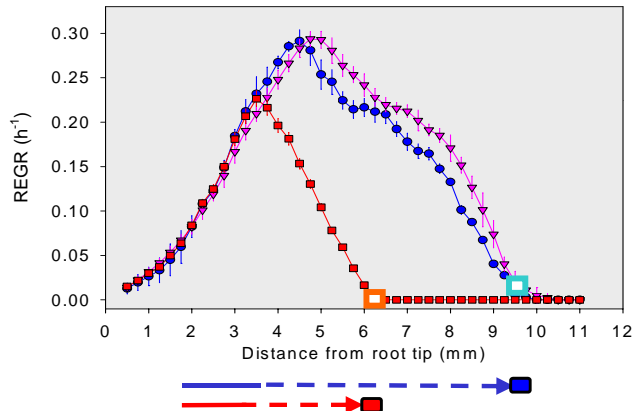
temporal coordinates



### 3- Spatio-temporal pattern of *TIP1.4* regulation during cell expansion



Spatial regulation of *TIP1.4* expression during cell expansion differs between treatments



Temporal regulation of *TIP1.4* expression during cell expansion very similar in all treatments

-> tight temporal regulation of *TIP1.4* expression



Project 12000158



Project GPLA0628G - 2006/2010

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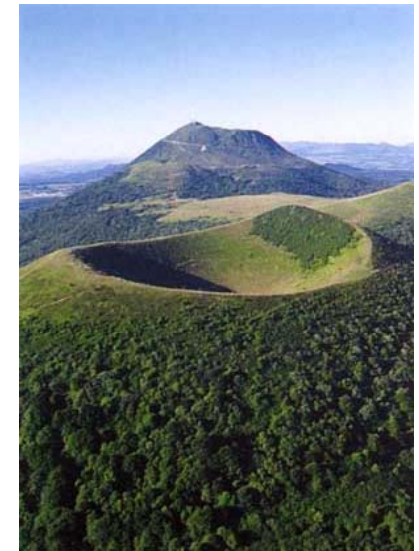
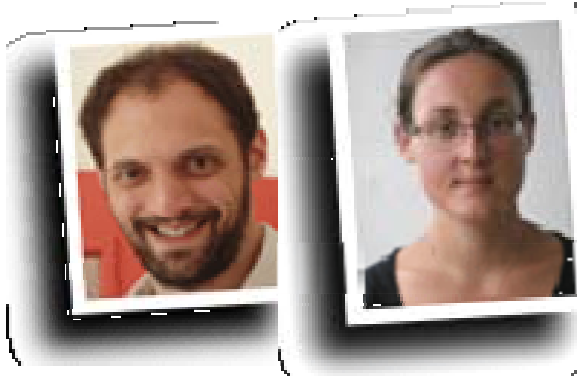
**Rémy Merret <sup>1</sup>**

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**Thanks for your attention**