

**Tight control of division plane orientation is necessary to optimize the growth capacity of tissues and organs in *Arabidopsis thaliana***

Analysis of replum development in the *trm1234* mutant



Magalie Uyttewaal

The SPACE team (Spatial Control of Cell Division)  
Institut Jean-Pierre Bourgin, INRAE Versailles

# Diversity of fruit shapes among Angiosperms and among families



Snyders Frans 1660, Musée du Louvre



Volume 32  
Number 11  
June 6, 2022

CellPress

Boualem et al., 2022, Current Biology

In numerous species fruit shape is correlated to ovary shape



WT



*rf1*



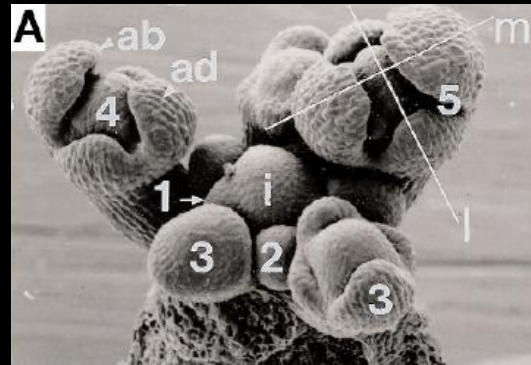
WT ♀



*rf1* ♀

Simonini and Østergaard 2019  
, Monforte et al., 2014, Boualem et al., 2022

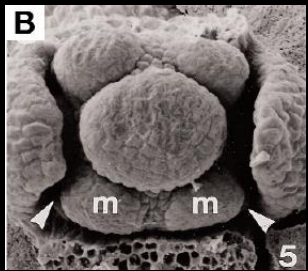
# Arabidopsis female reproductive organ development: from the SAM to the mature pistil



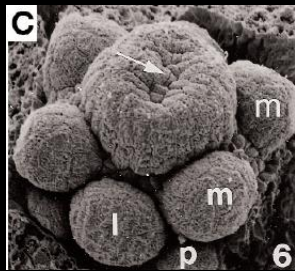
Stage 1-4

Organ Specification

Arabidopsis gynoecium is composed of 2 fused carpels that arise from a single primordium



Stage 5



Stage 6



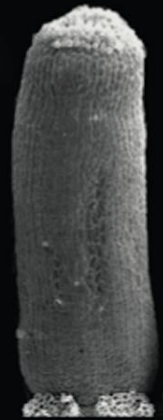
Stage 7



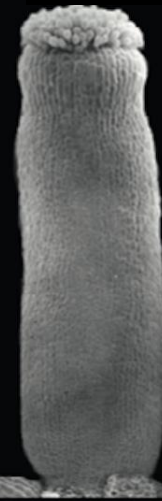
Stage 8



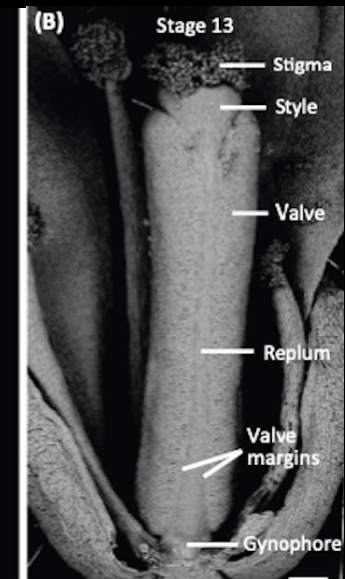
Stage 9



Stage 11



Stage 12

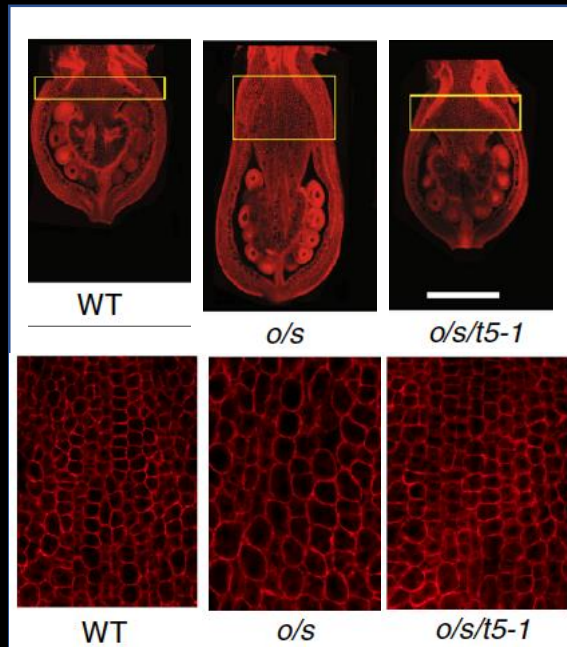


Stage 13

Promotion of organ growth through cell division and elongation

Fertilization

# Determinants of gynoecium and fruit size and shape are related to growth and division effectors and TRM genes



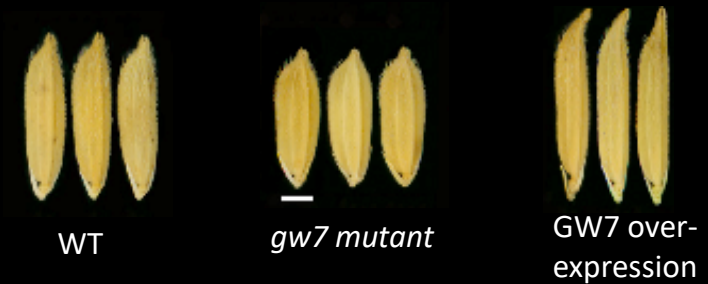
Wu et al., 2018

Differential cell patterning in round versus elongated ovary (Pan et al 2020, Wu et al 2018).

The TRM-OVATE module controls fruit shape in several species (Wu et al., 2018, Boualem et al., 2022)



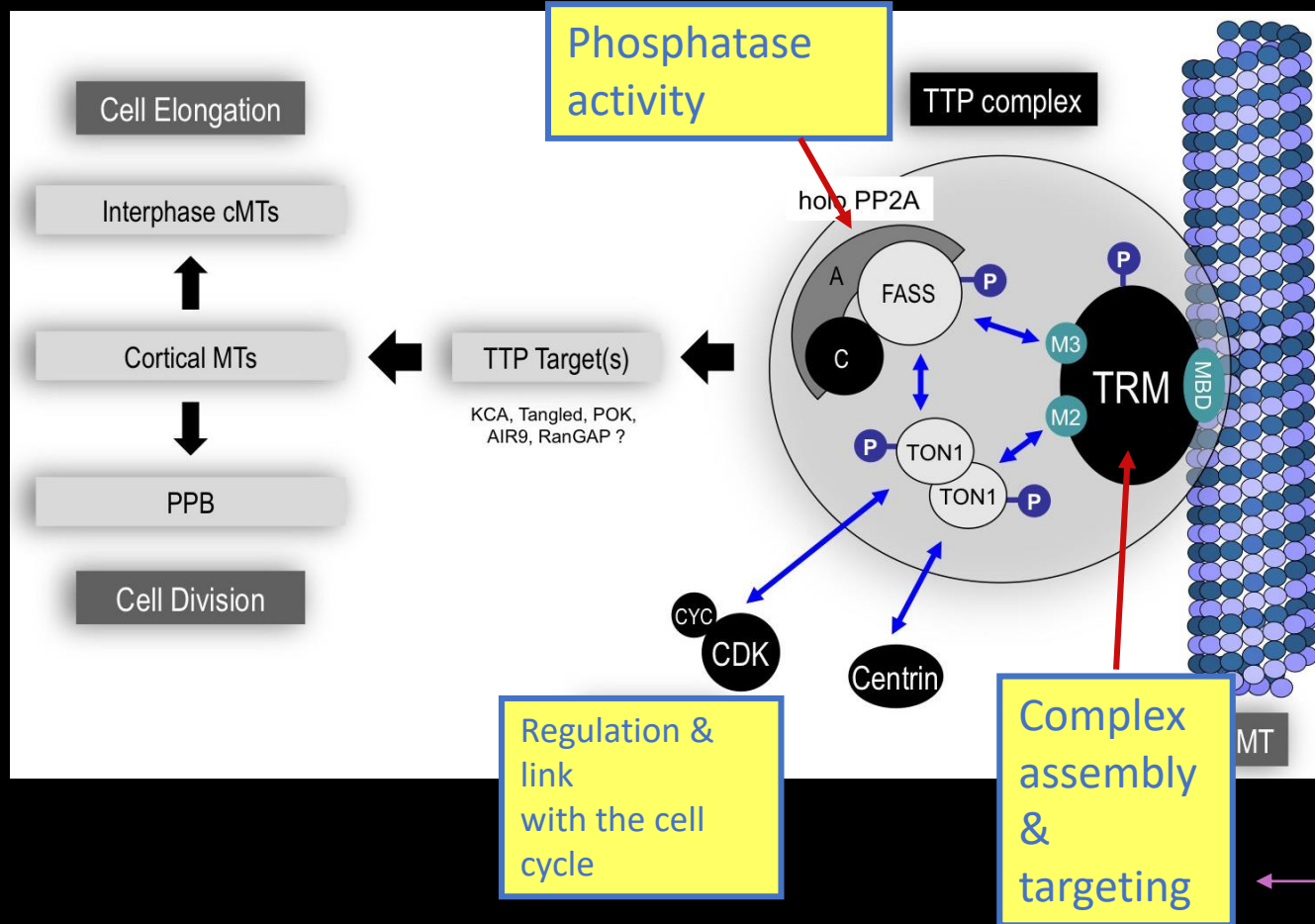
Grain shape in rice (Wang et al., 2015)



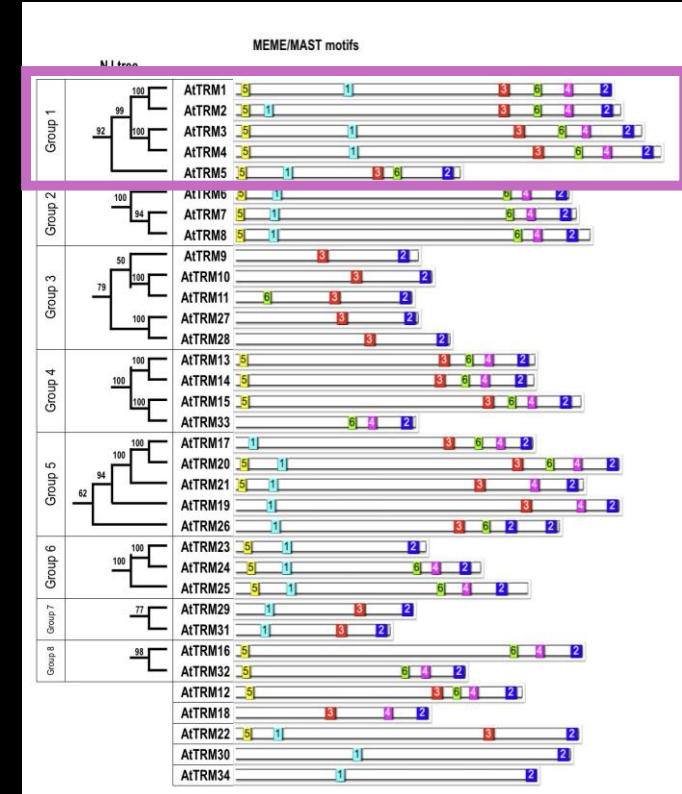
Species	Gene	Trait	Reference
Maize	ZmGW7-GRMZM2G061562	kernel size	Li et al., 2022
Melon	TRM	fruit shape	Boualem et al., 2022
Maize	GRMZM2G403003	root hair length	Liu et al., 2021
Cotton	OsGL7 Homolog	seed size	Liu et al., 2020
Tomato	TRM	fruit shape	Wu et al., 2018
Cucumber	TRM	fruit shape	Wu et al., 2018
Wheat	TaGW7	fruit shape and weight	Wang et al., 2019
Rice	OsGL7/OsGW7	grain size	Wang et al., 2015
Rice	OsGW7	grain shape, yield, quality	Wang et al., 2015
Arabidopsis	TRMs	Silique size and shape	Unpublished data, SPACE group

# The TTP protein complex regulates cortical microtubule arrays

## The TTP complex



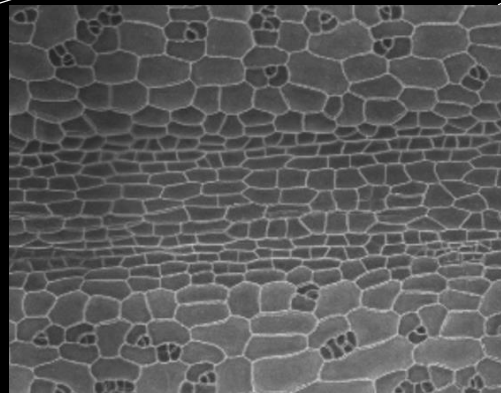
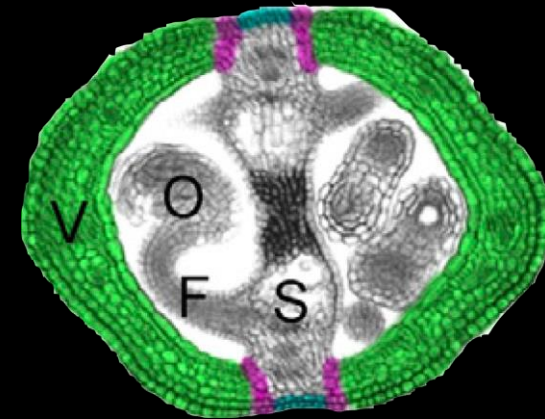
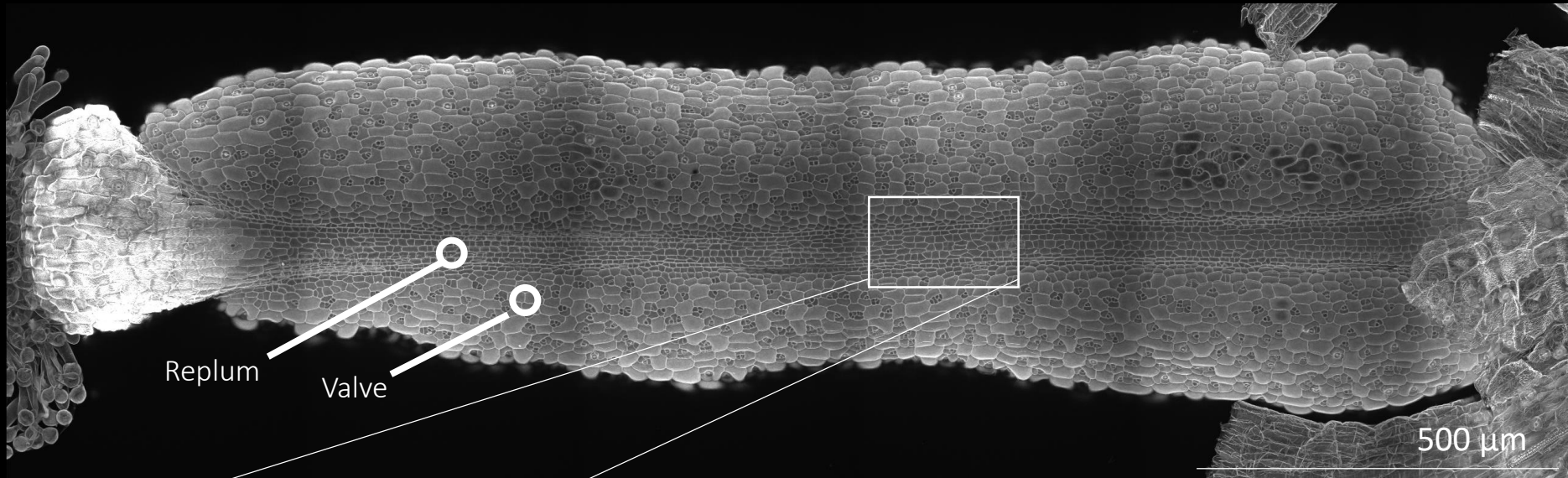
## The Arabidopsis TRM family



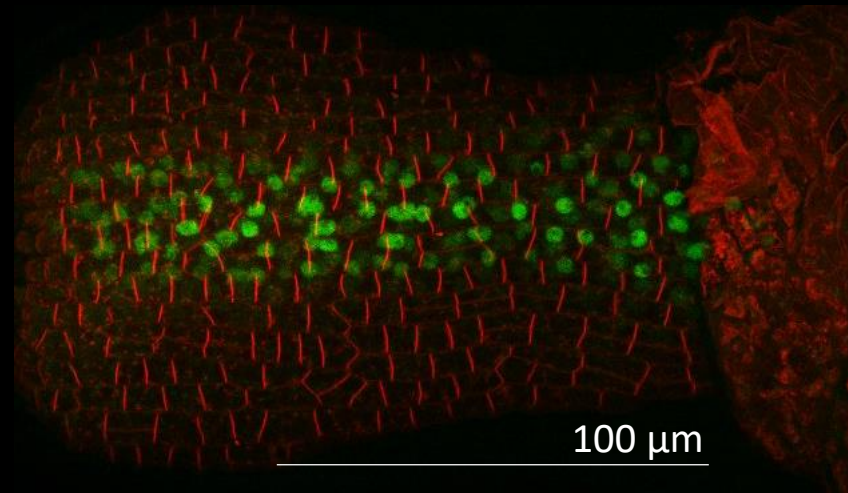
Sub-group 1

Drevensek et al., 2012  
 Spinner et al., 2013  
 Schaefer et al., 2017

# The Arabidopsis Gynoecium: a complex organ with parallel cell files in the replum

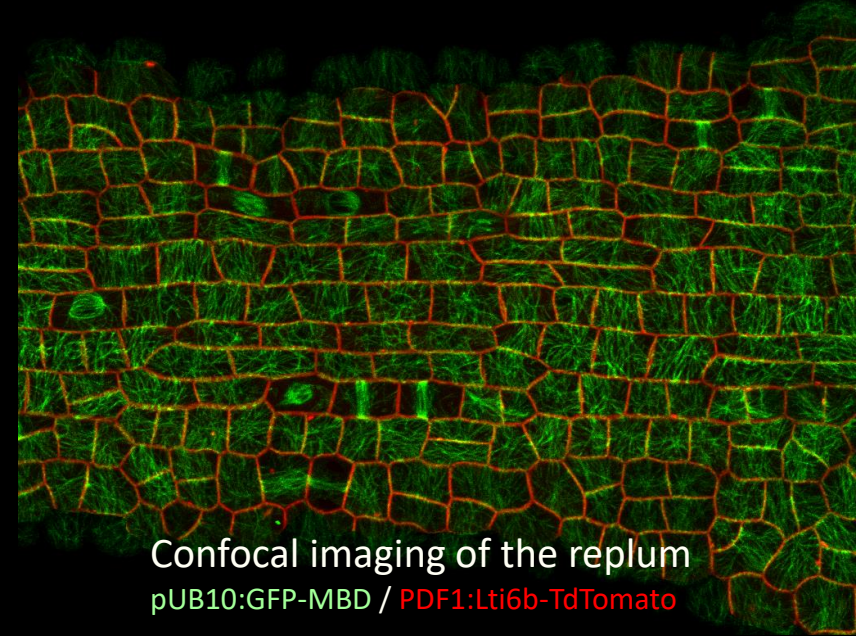
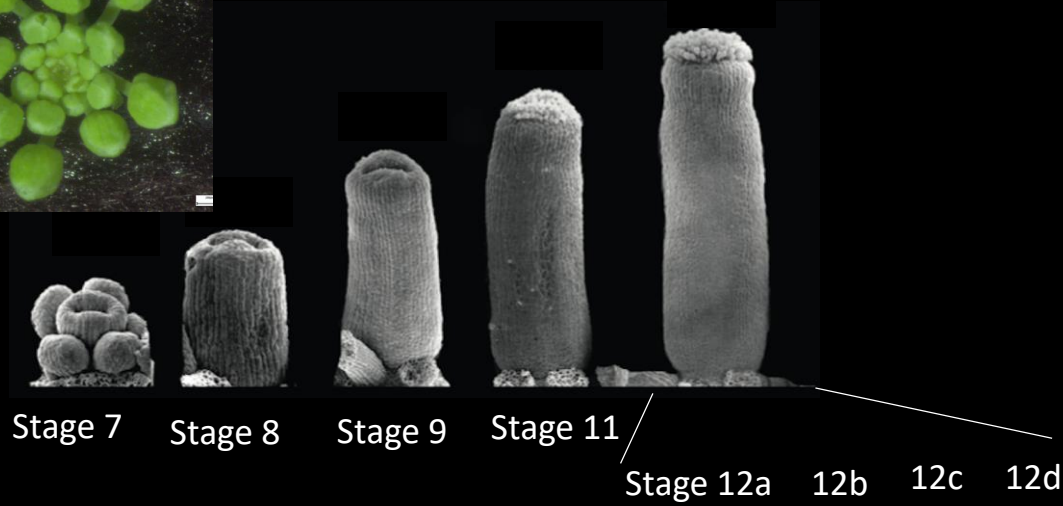


The replum epidermis is made of parallel cell files

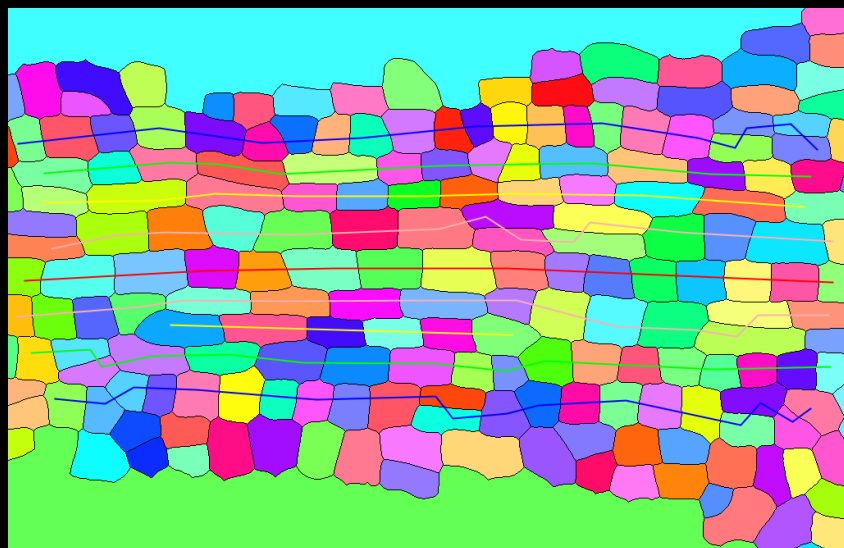


Cell files identifiable from early stages of development (FM4-64, RPL1-GFP)

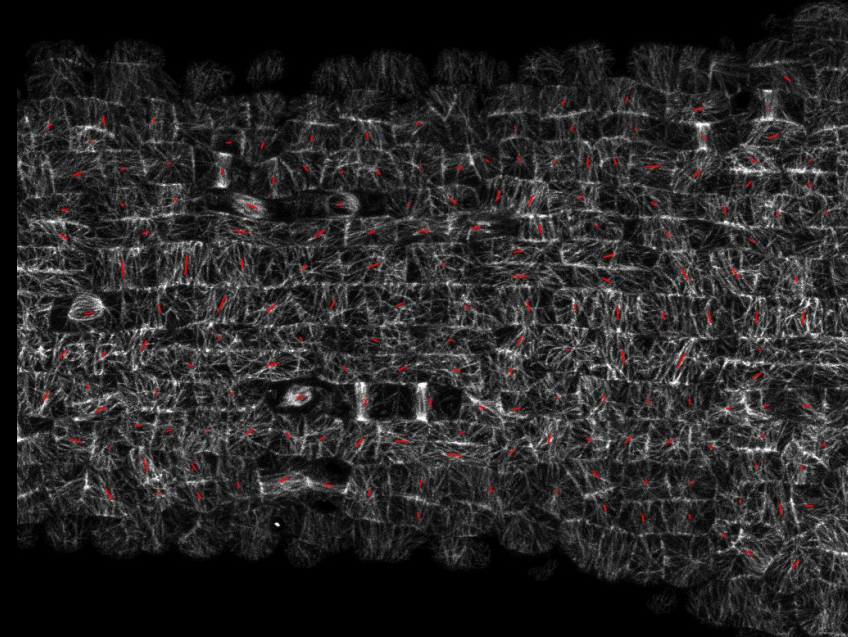
# Quantitative analysis of cellular and sub-cellular parameters of the quadruple *trm1234* mutant



Confocal imaging of the replum  
pUB10:GFP-MBD / PDF1:Lti6b-TdTomato

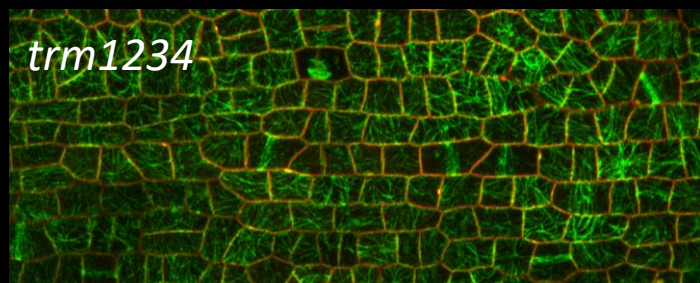
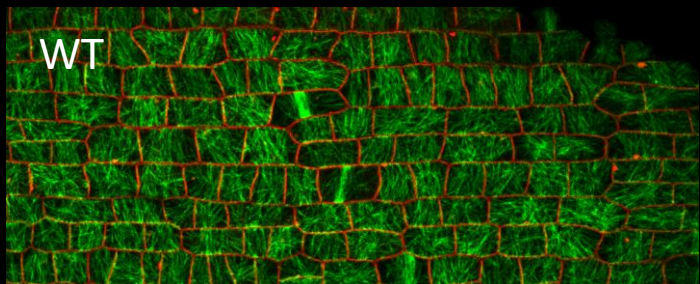


- 2D segmentation
- Cell morphology
- Cell file identification
- Transverse angles
- Skeletonization and vertex analysis

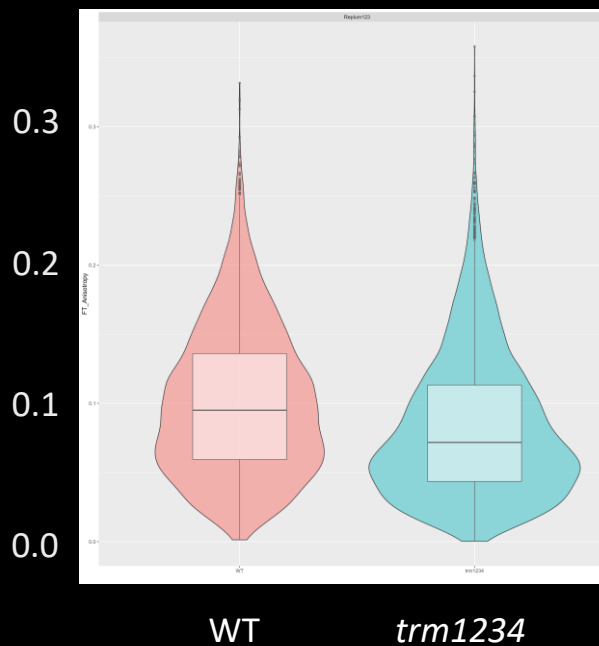


- MT array anisotropy and orientation (FibrilTool)
- Mitotic index

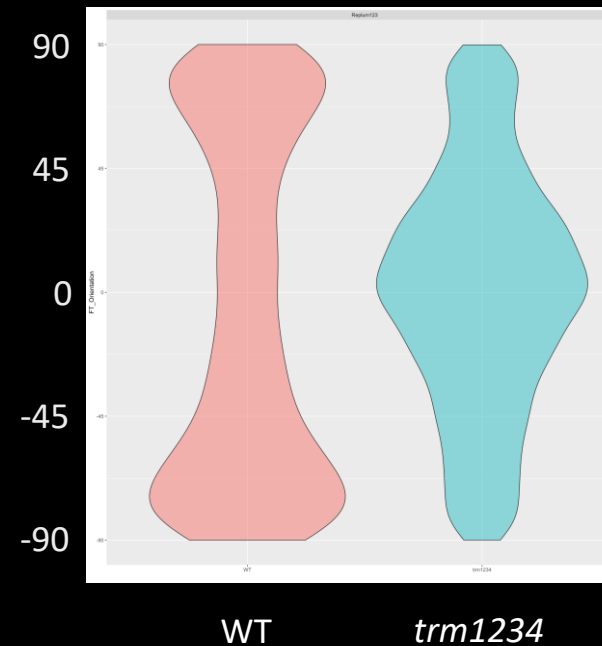
# TRM1234 control interphasic cortical microtubule array organization



### Anisotropy of cMT arrays



### Orientation of cMT arrays

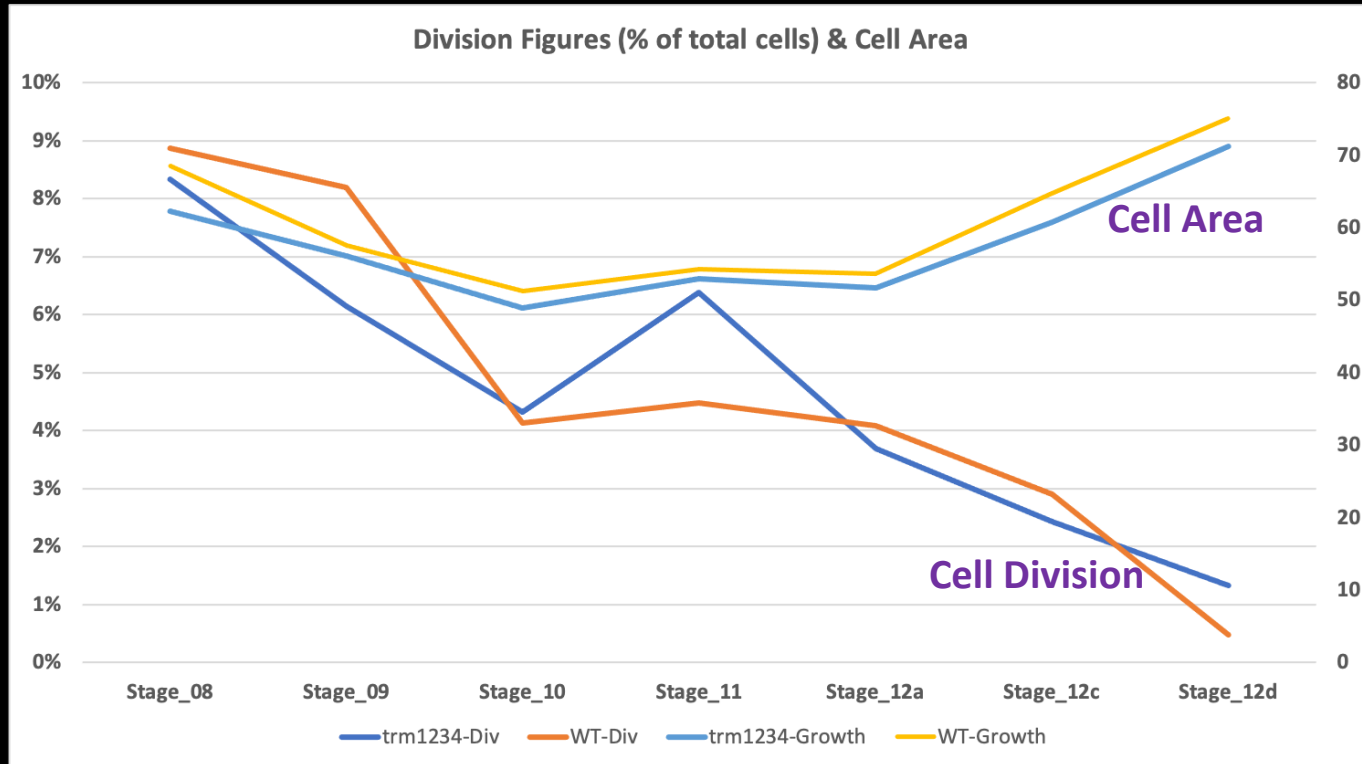


Repleum apico-basal axis

(FibrilTool analysis)



# Cell growth and cell division during replum development



WT

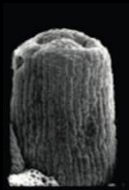
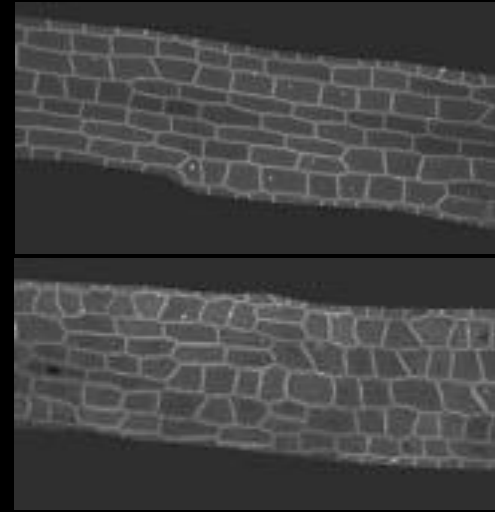
*trm1234*

WT

*trm1234*

WT

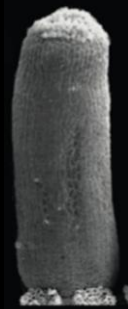
*trm1234*



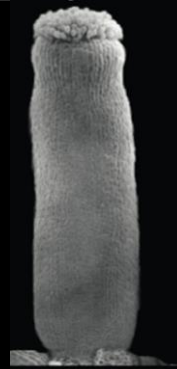
Stage 8



Stage 9



Stage 11

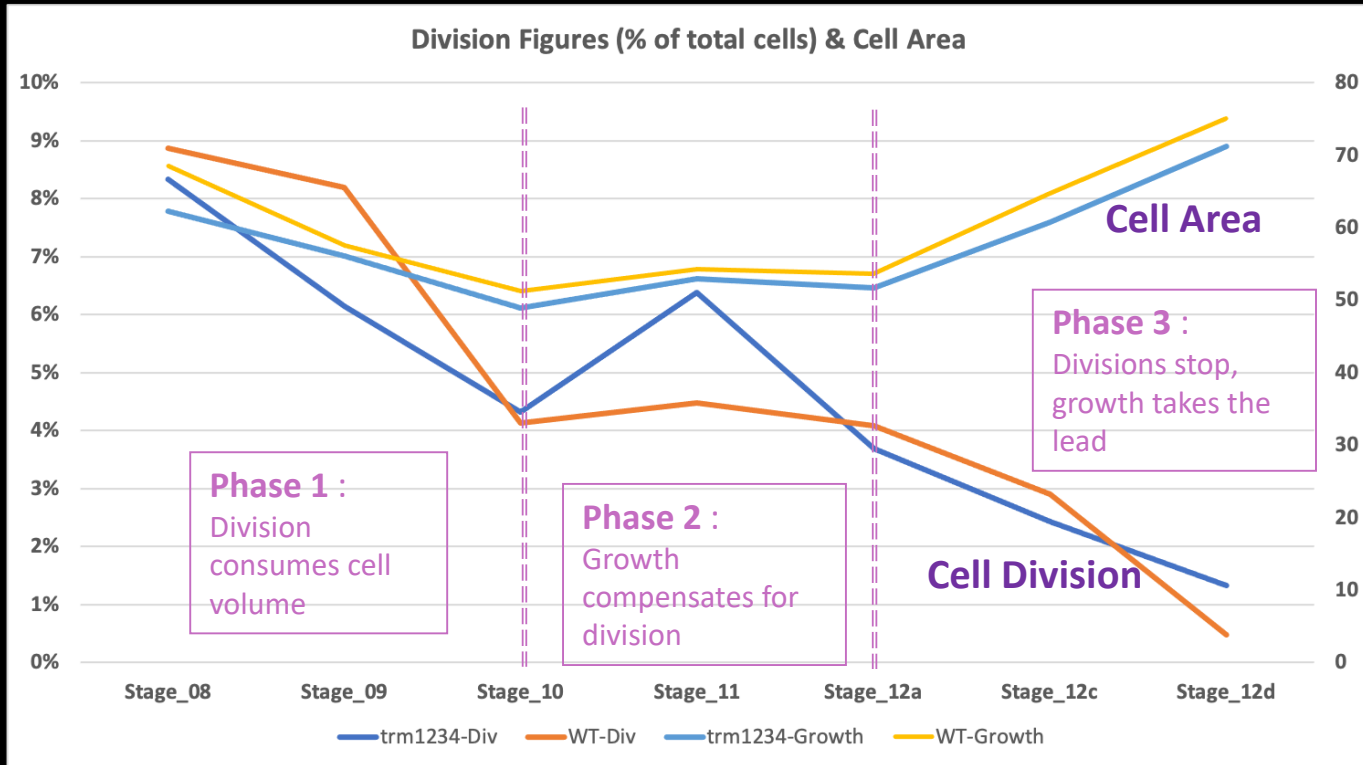


Stage 12c

Stage 12a

Stage 12d

# Cell growth and cell division during replum development



WT

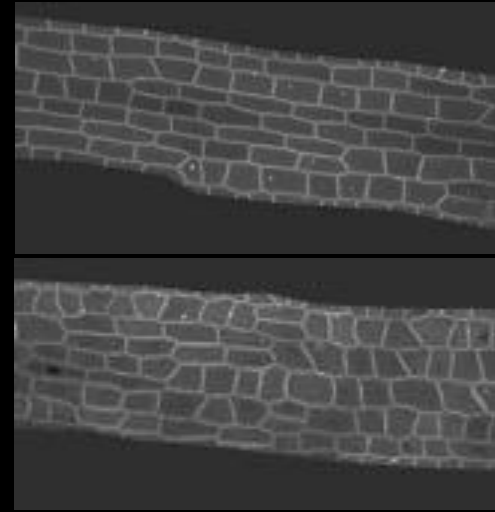
trm1234

WT

trm1234

WT

trm1234



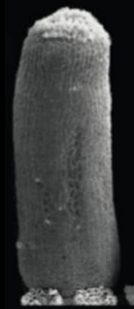
Cell growth is not strongly affected in *trm1234*,...



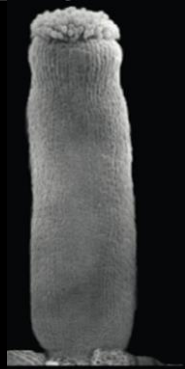
Stage 8



Stage 9



Stage 11

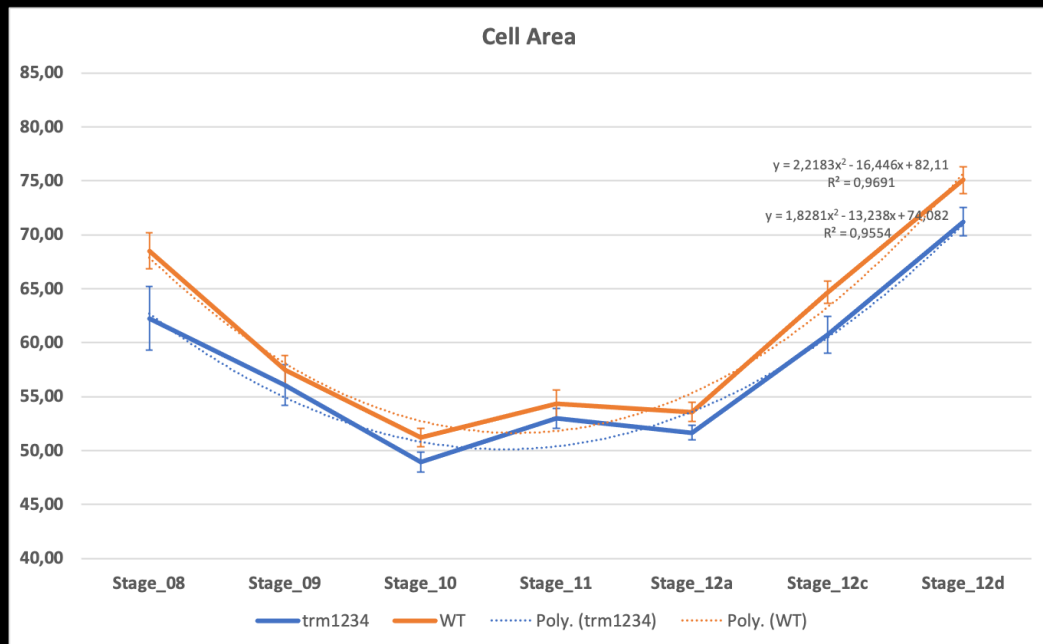


Stage 12c

Stage 12a

Stage 12d

# ... Cell elongation is reduced in *trm1234*

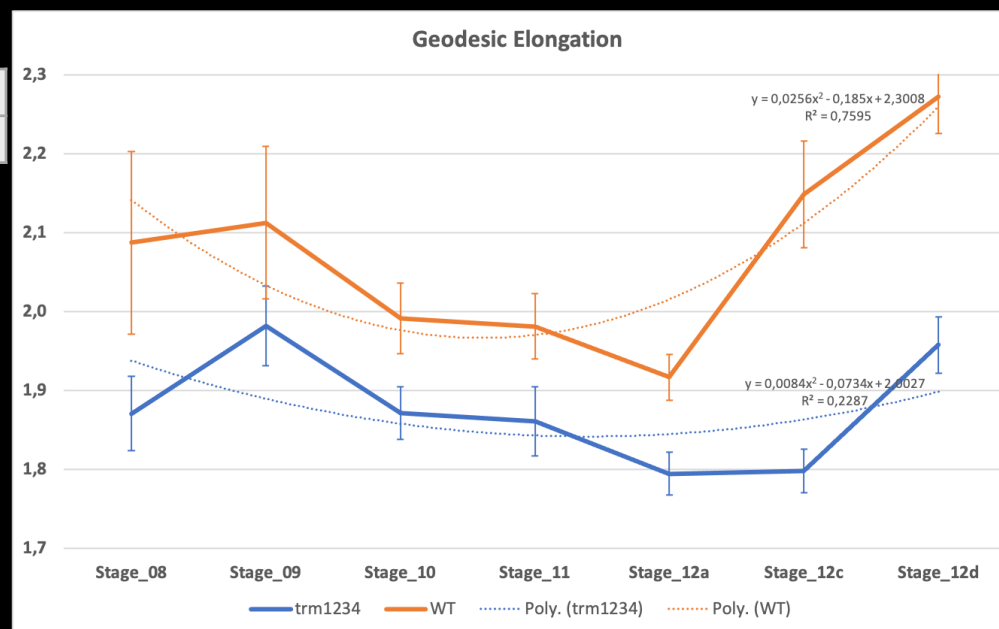
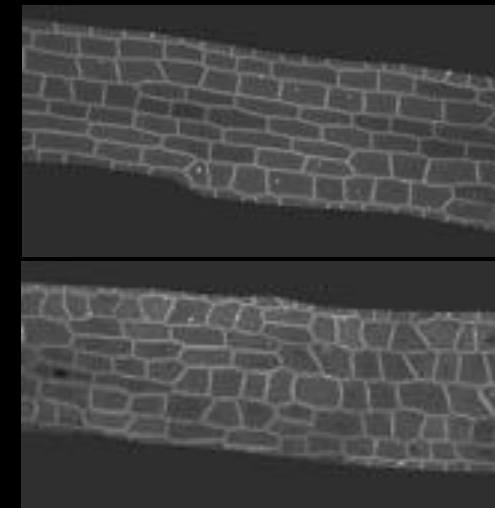


Stage_08	Stage_09	Stage_10	Stage_11	Stage_12a	Stage_12c	Stage_12d
+ 10,0 %	+ 2.5 %	+ 4.6 %	+ 2.5 %	+ 3.7 %	+ 6.5 %	+ 5.4 %

WT  
trm1234

WT

trm1234

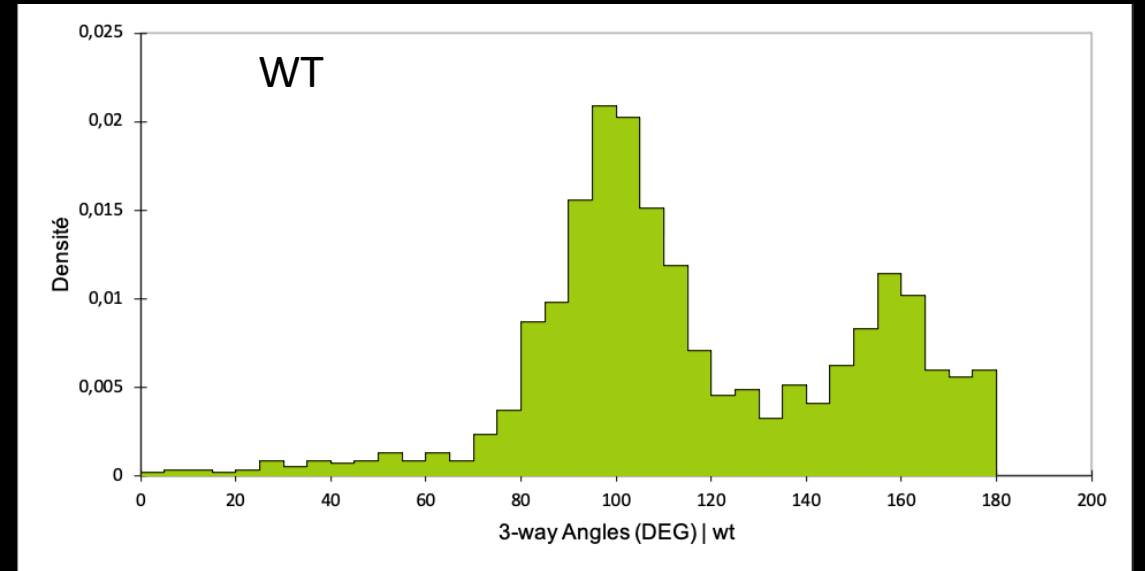
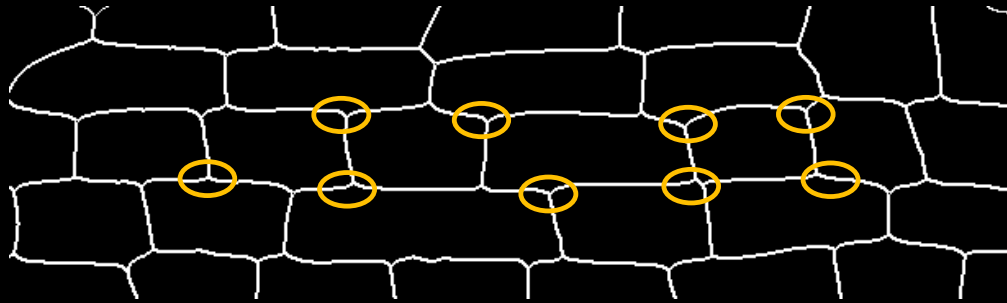


WT

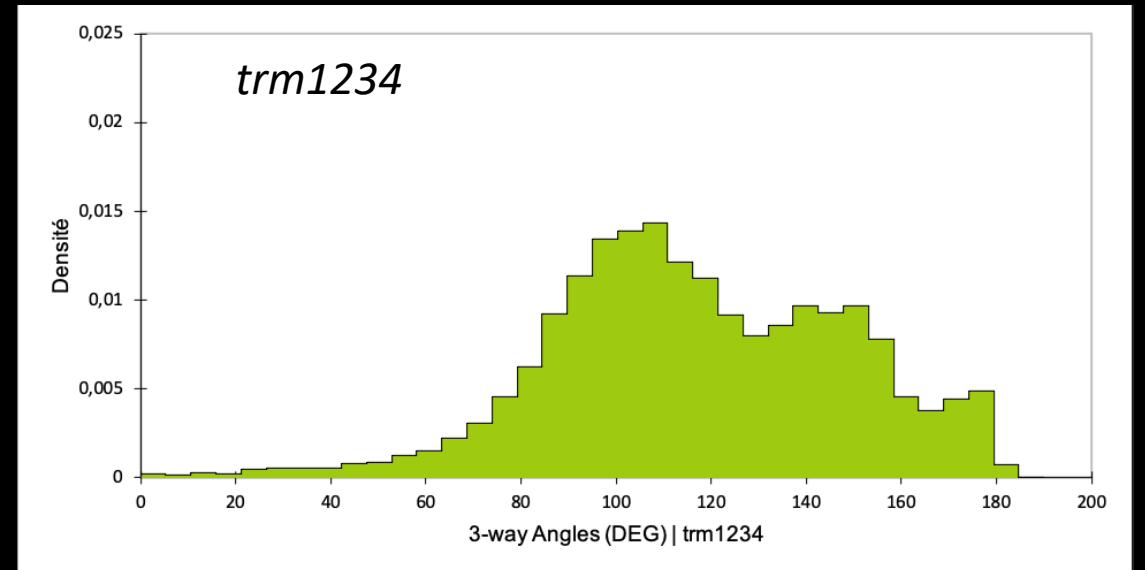
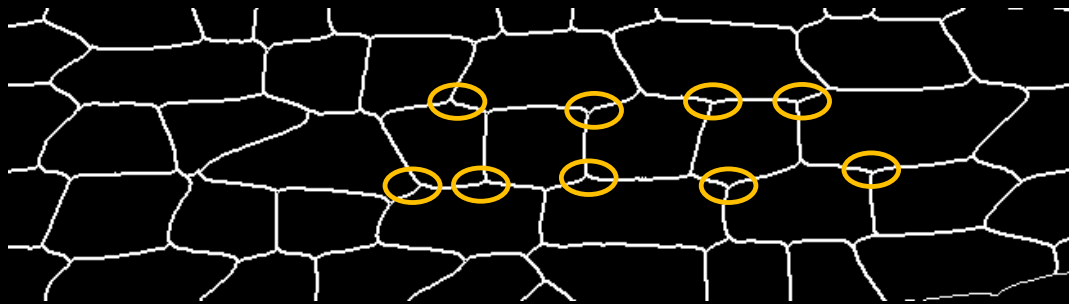
trm1234

# The cellular topology of the replum is altered in *trm1234*

WT

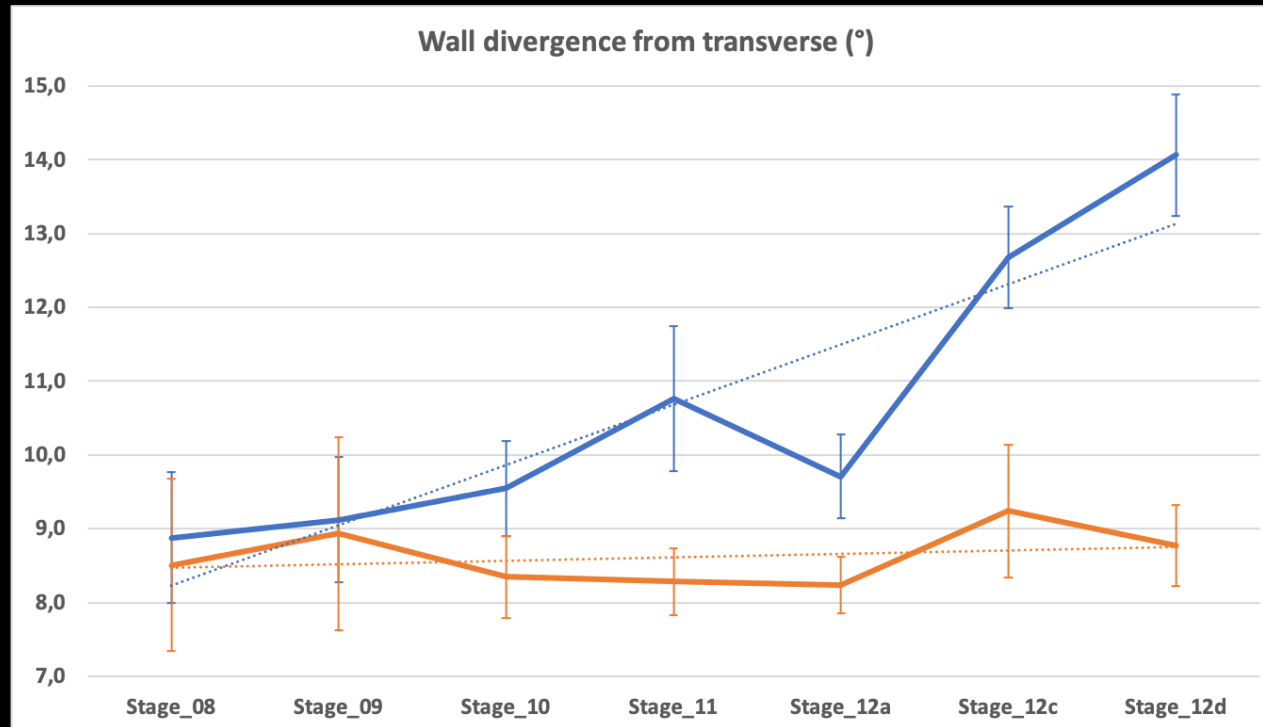


*trm1234*

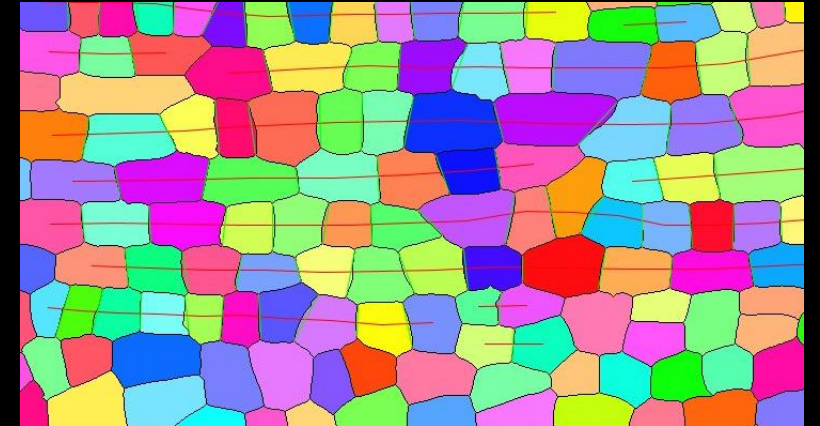


BoneJ skeleton Analysis

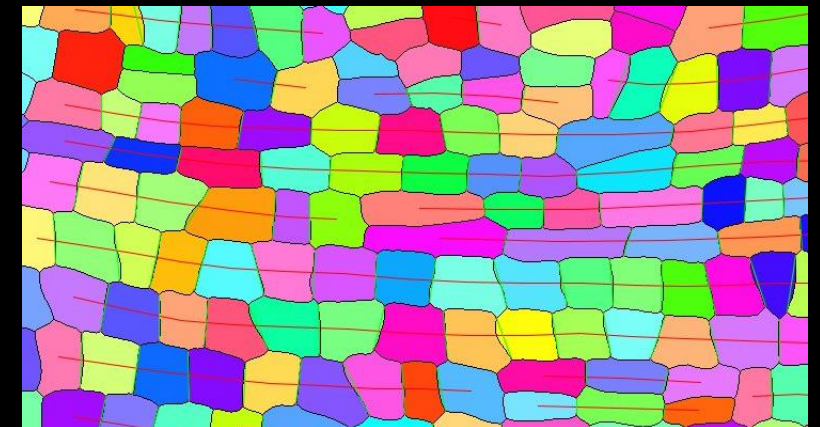
# Walls diverge from transverse in the *trm1234* mutant



*trm1234*

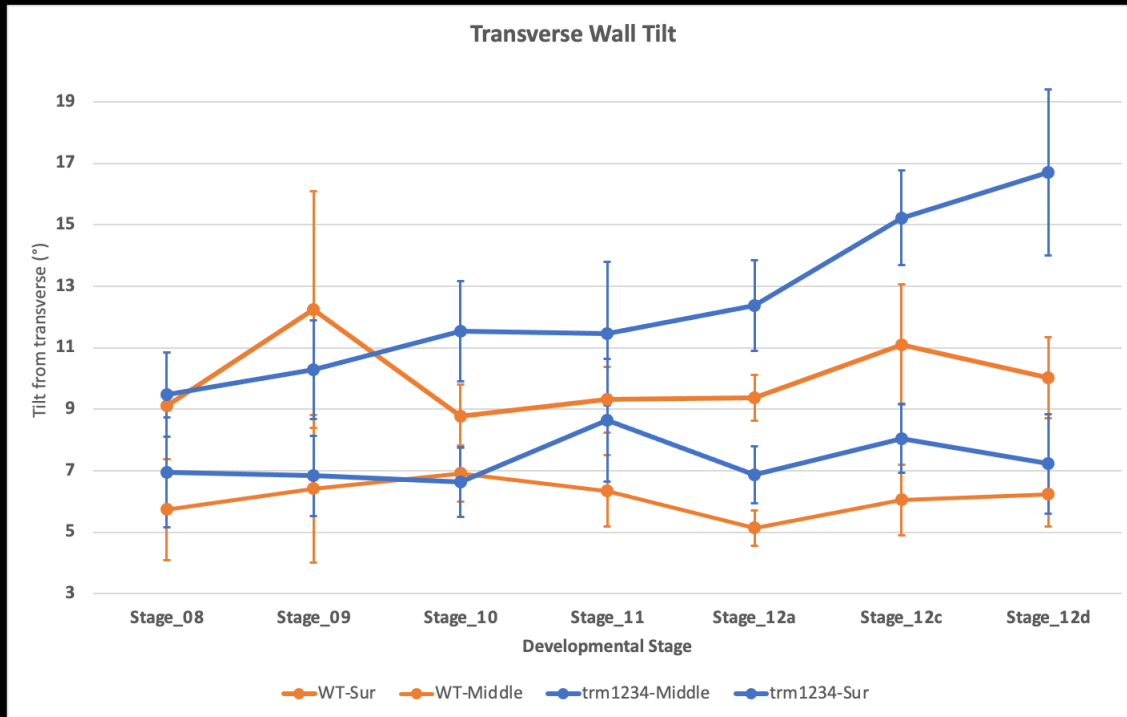


WT



Cell File Angle Tool (IJPB-plugin in Fiji, Schaefer et al., 2017)

# Recent cell walls are only slightly tilted the *trm1234* mutant

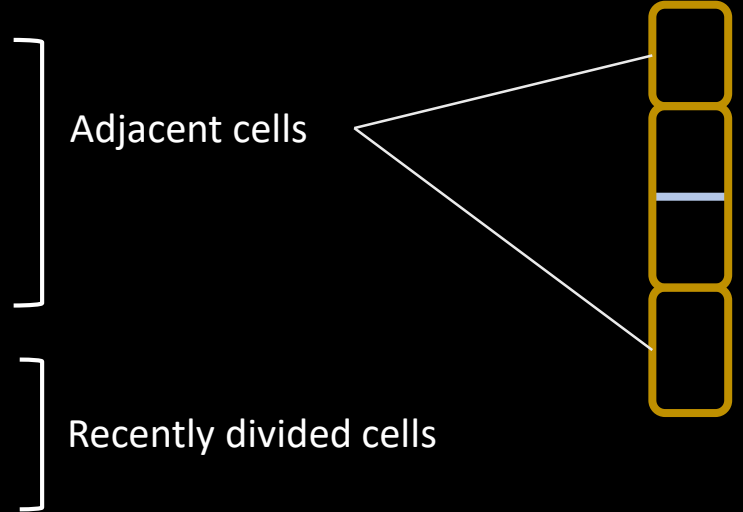


*trm1234*

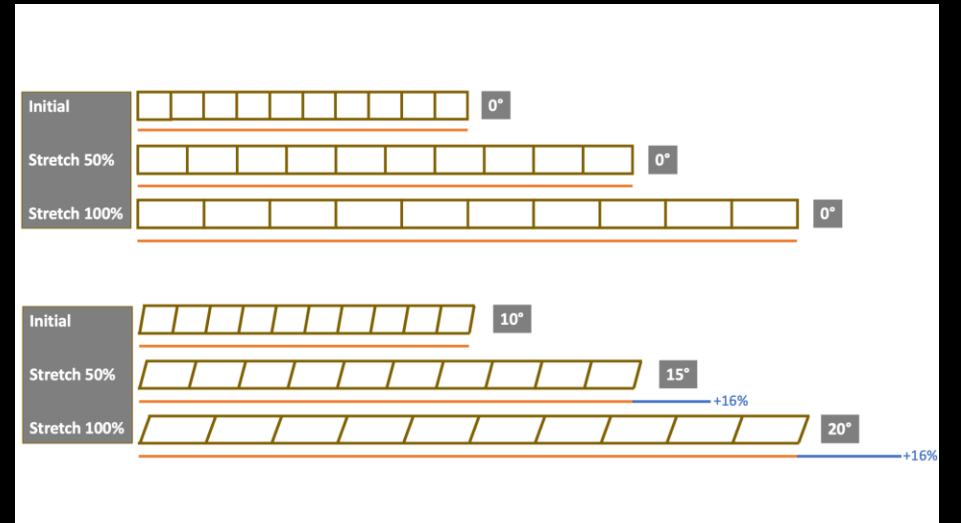
WT

*trm1234*

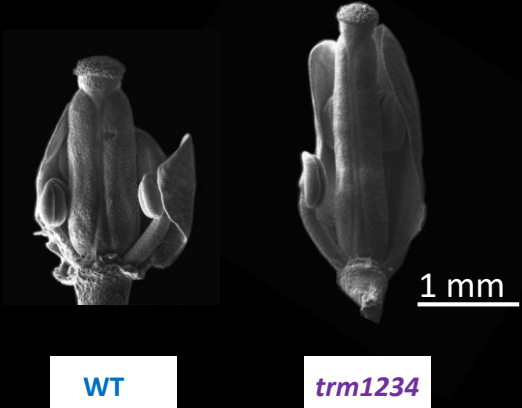
WT



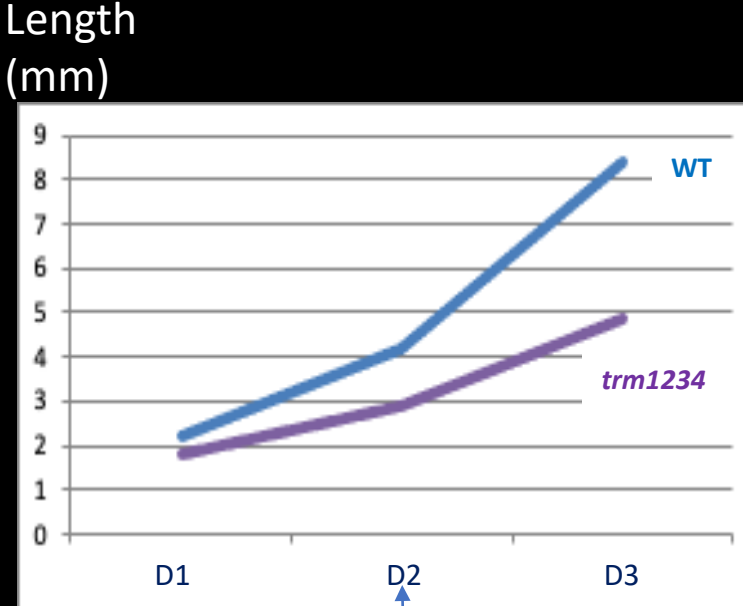
Non-transverse angles can be amplified by elastic stretching



As a consequence, pistil growth, fruit size and seed number are reduced in the *trm1234* mutant



Stage 12

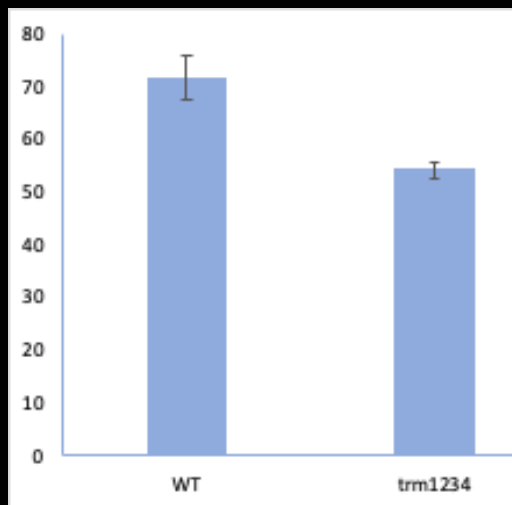


Pistil growth after petal emergence

Pollinization

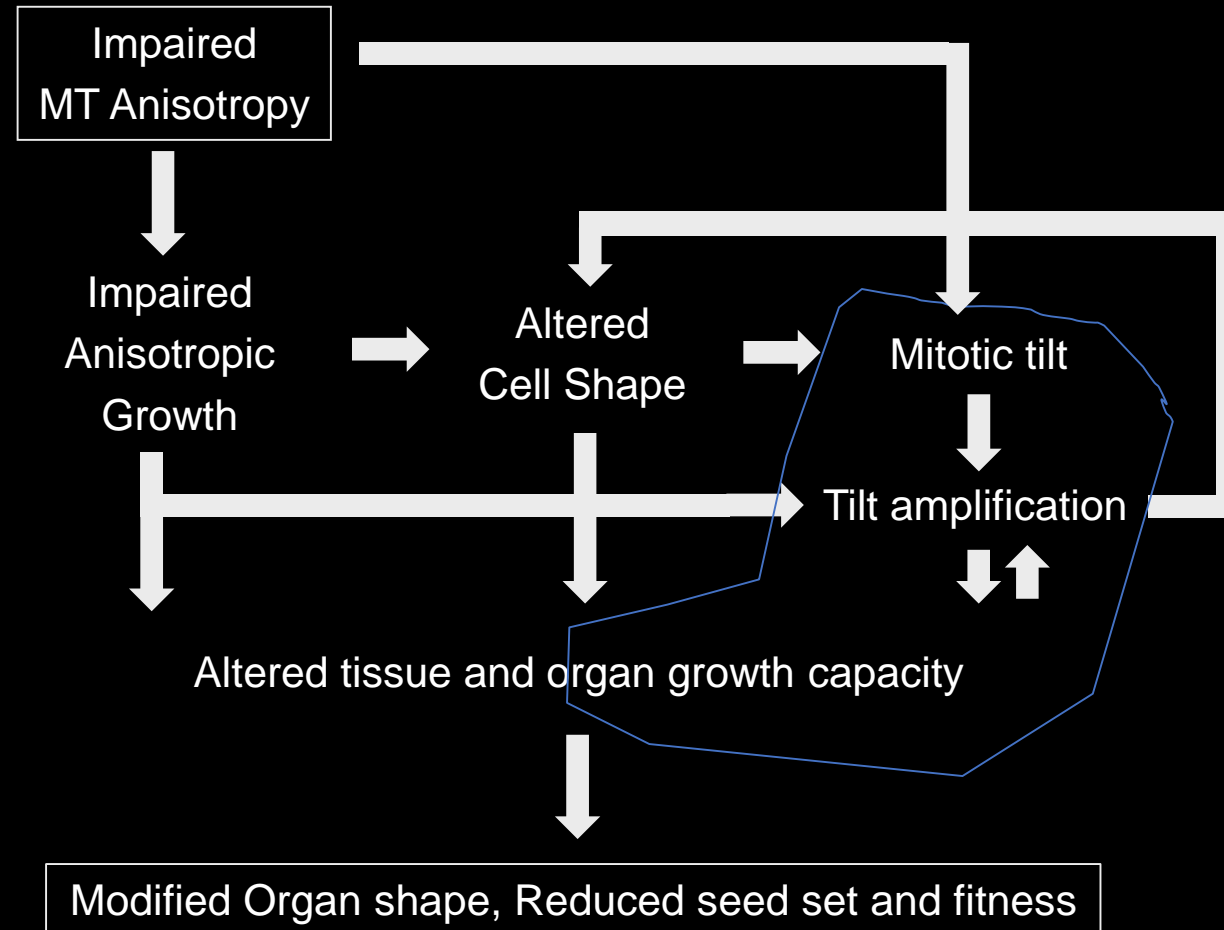


Mature siliques



Seed number per silique

# Cellular function of TRM 1-2-3-4 in fruit development, from sub-cellular events to organ shape





# Thanks to

## SPACE team:

**David Bouchez**

**Martine Pastuglia**

**Katia Belcram**

**Bérengère Dalmais**

**Aloise Ducamp**

**Zoé Bomsel**

**Past members**

**Marie-Ludivine Moreau-de Tauzia**

**Chie Koderu**

**Coralie Goncalves**

## The plant observatory:

OV – Cytologie / Imagerie, Gladys Cloarec

OV – Plant facilities

## Collaborations:

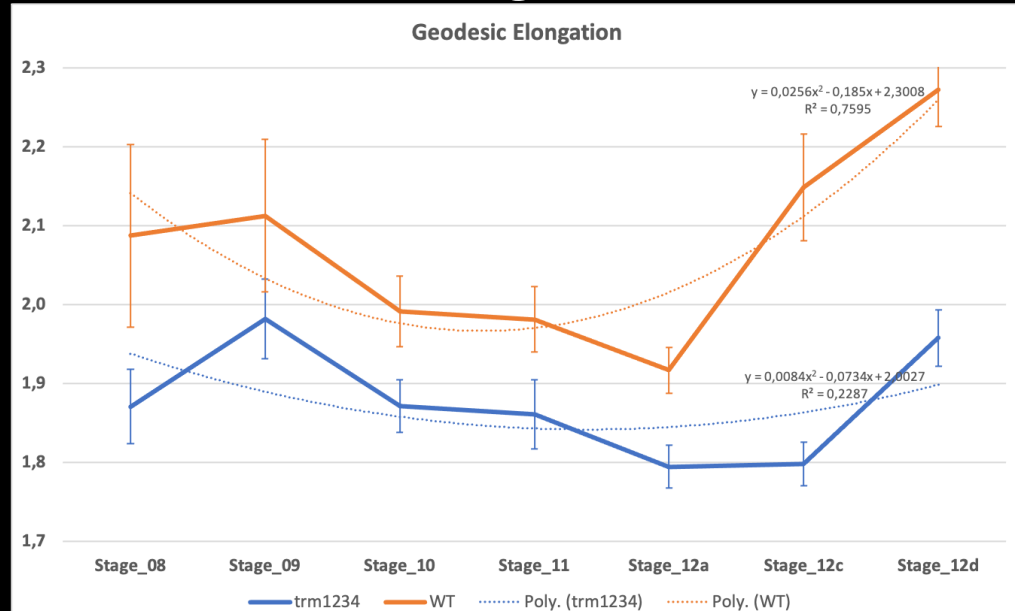
Sarah Robinson, Léo Serra (Sainsbury Laboratory, Cambridge)

Philippe Andrey, Eric Biot, Sandrine Lefranc (MIN group, IJPB-INRAE de versailles)



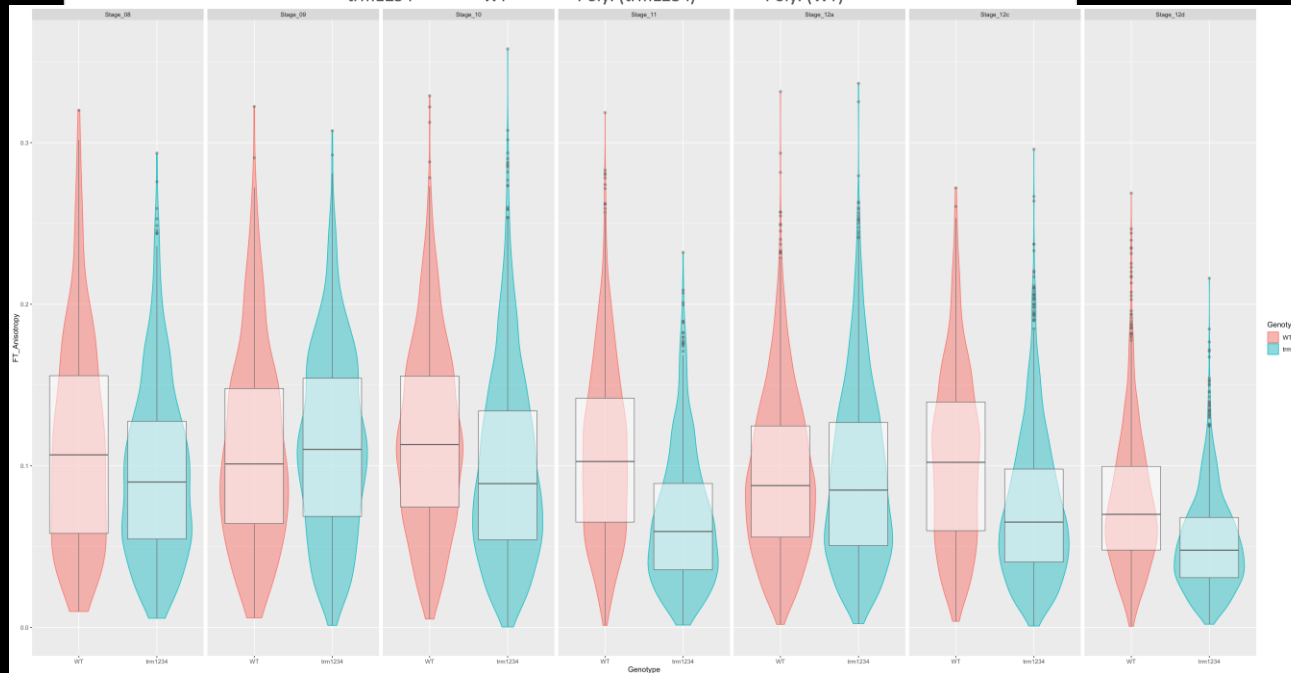


# ... Cell elongation is reduced in trm1234



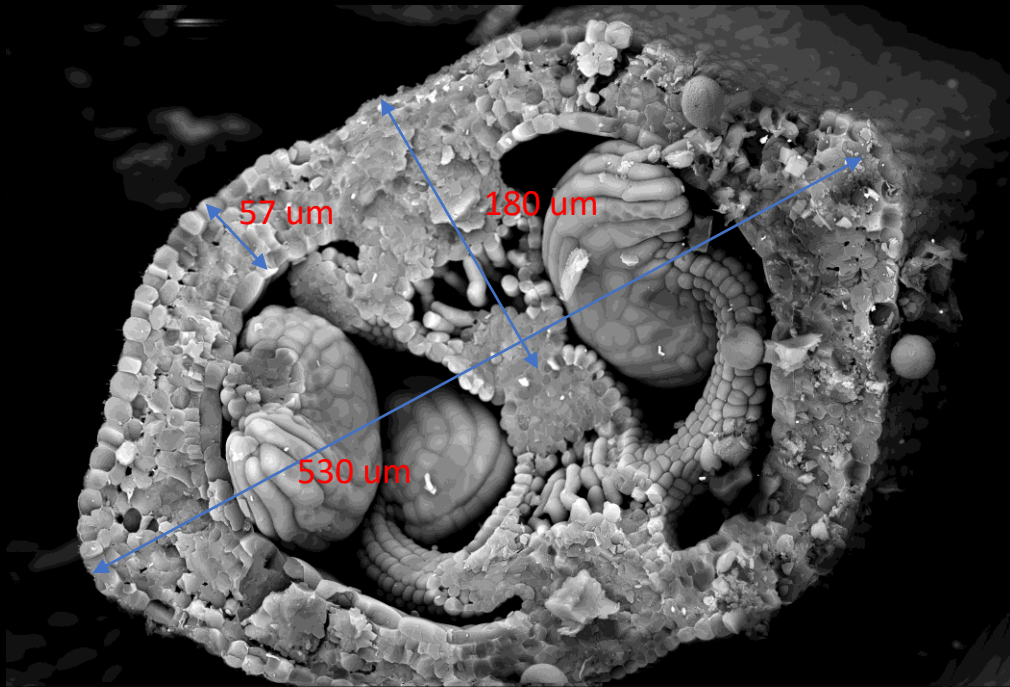
WT

trm1234



(anisotropic growth requires an highly ordered interphasic cortical MT array)

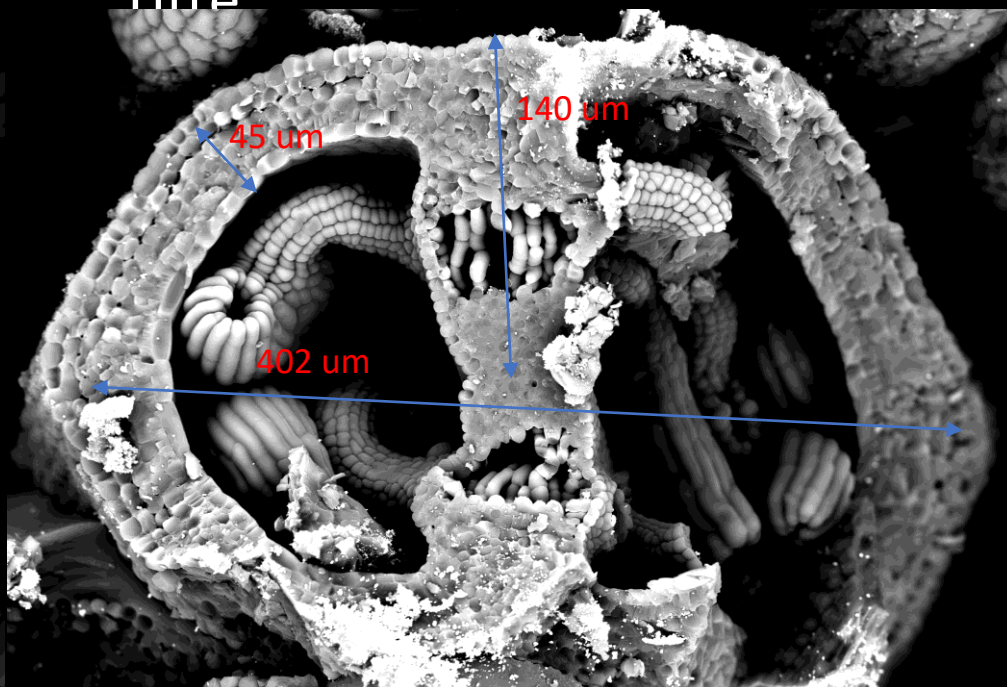
WT



20 μm\* EHT = 25.00 kV Signal A = NTS BSD WD = 5.5 mm Date : 19 Nov 2021  
Mag = 650 X e : 11:53:37  
Name = RAY

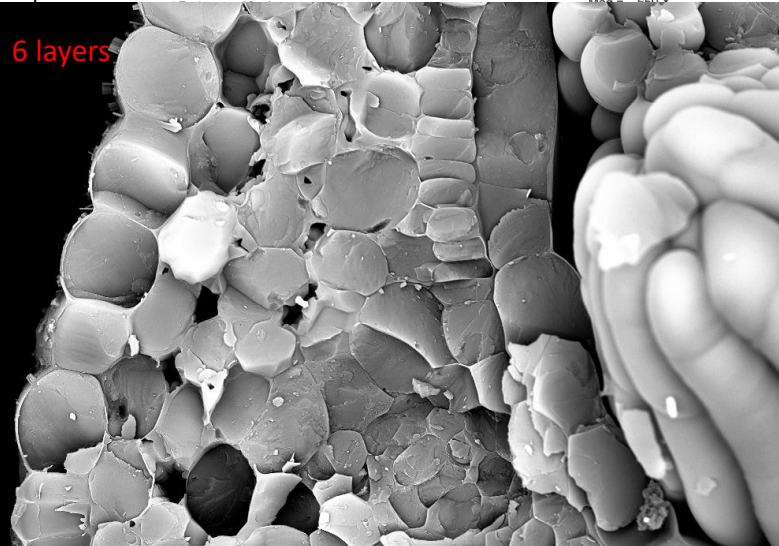
Titre

*trm1234*



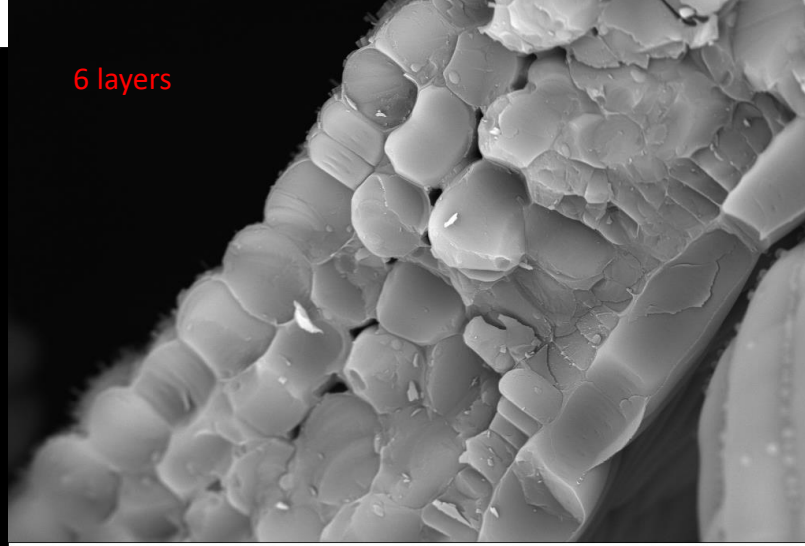
20 μm\* EHT = 25.00 kV Signal A = NTS BSD WD = 5.5 mm Date : 19 Nov 2021  
Mag = 650 X e : 11:53:37  
Name = RAY

6 layers



10 μm\* EHT = 25.00 kV Signal A = NTS BSD WD = 5.5 mm Date : 19 Nov 2021  
I Probe = 16 pA Mag = 3.00 K X Time : 12:00:15  
User Name = RAY

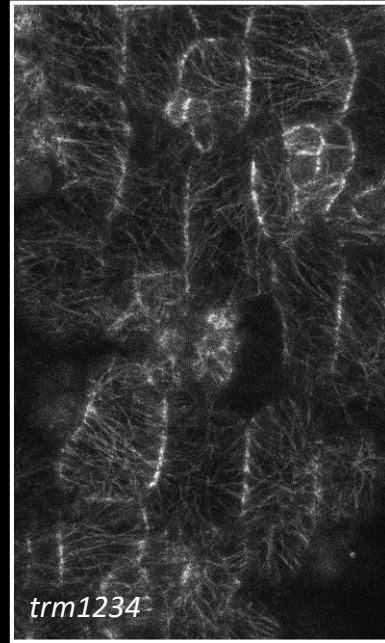
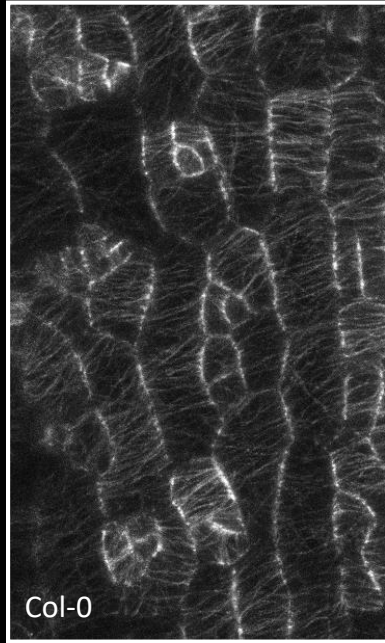
6 layers



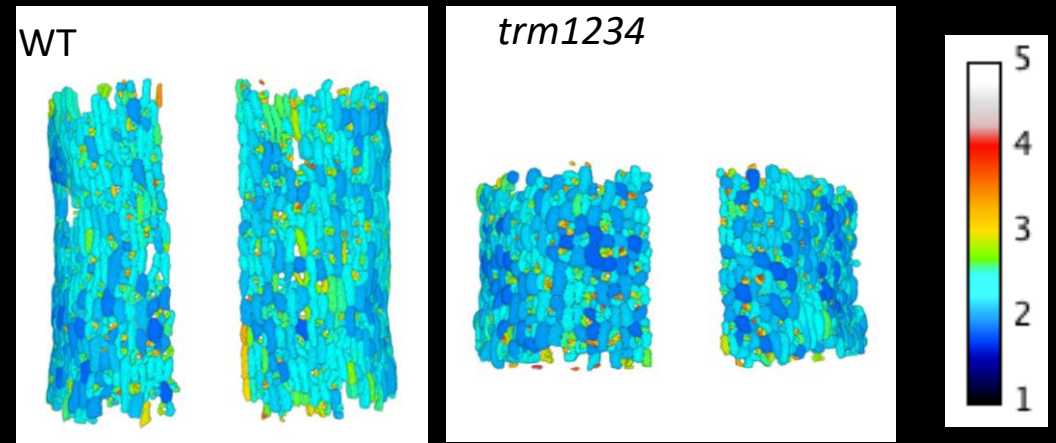
10 μm\* EHT = 25.00 kV Signal A = NTS BSD WD = 5.5 mm Date : 19 Nov 2021  
I Probe = 16 pA Mag = 3.00 K X Time : 16:16:52  
User Name = RAY

# 3D morphological cell fruit parameters

(mCherry-TUBULIN6, outer epidermal cell face)

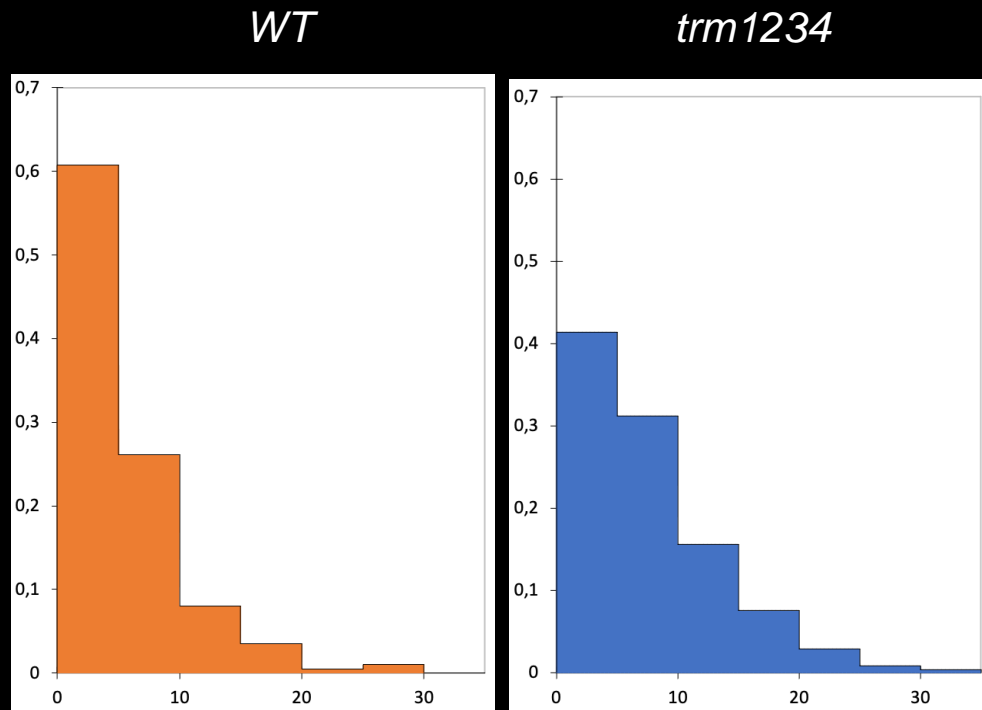


*shortest/longest perimeters*



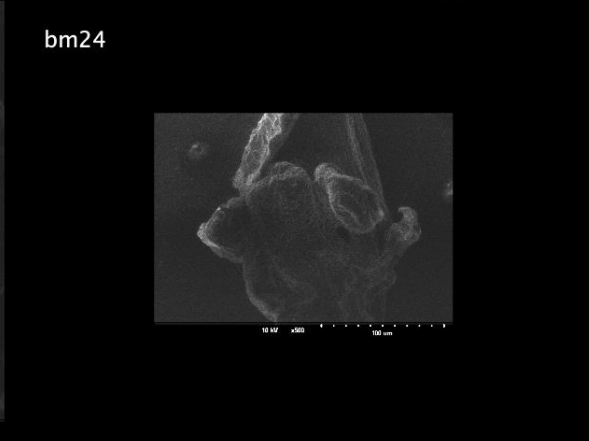
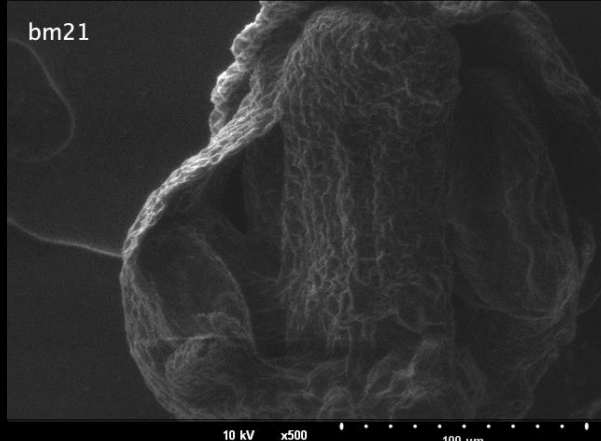
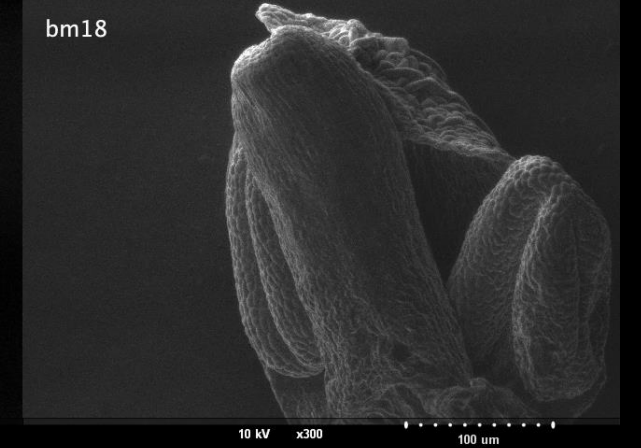
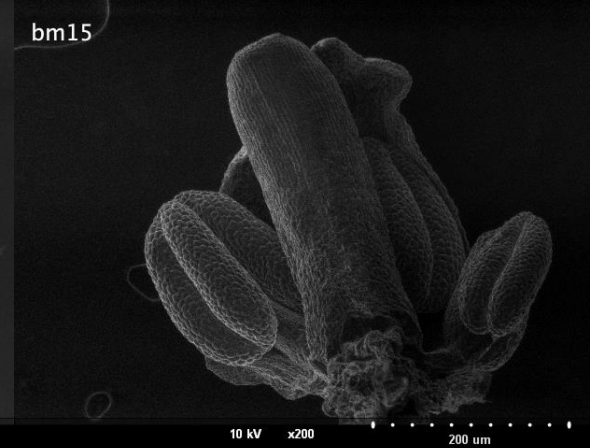
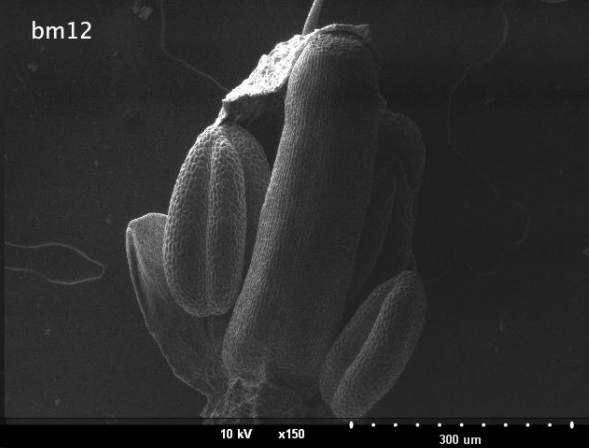
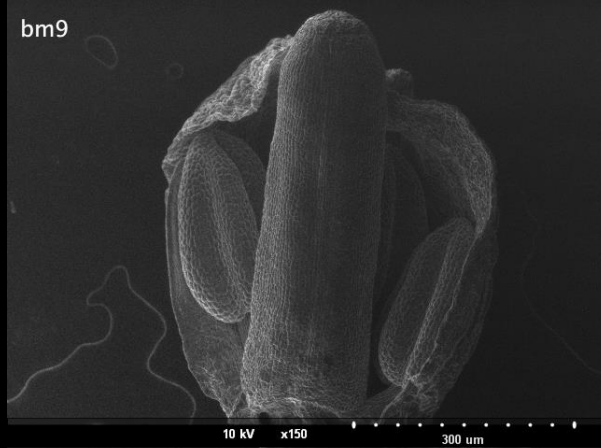
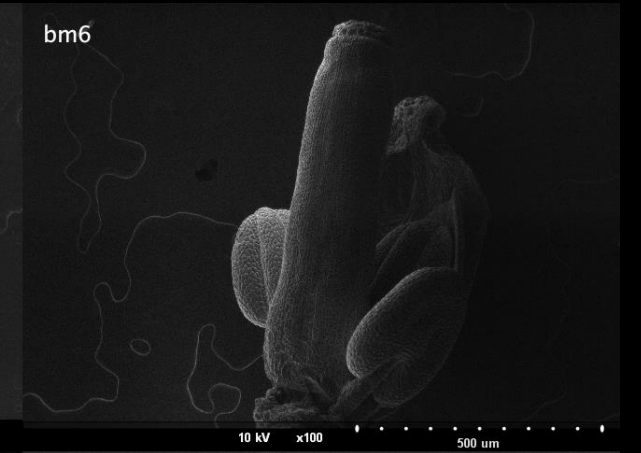
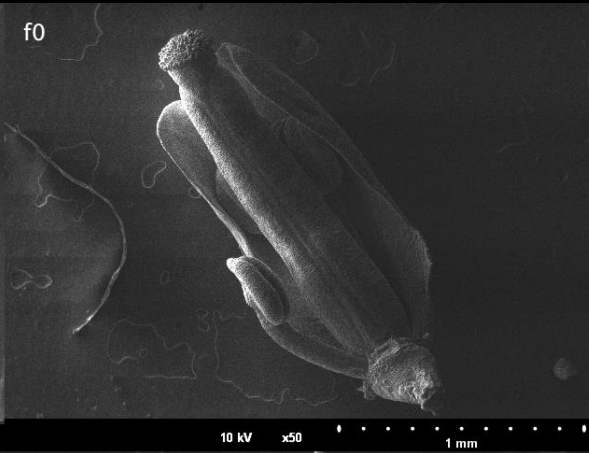
Mitotic figures are slightly more tilted in the *trm1234* mutant

Orientation of mitotic MT arrays (PPB & phragmoplast)



WT :  $5.09 \pm 4.63$  (n = 199)  
*trm1234* :  $7.54 \pm 5.94$  (n = 237)

# Morphology of the WT gynoecium



# Morphology of the *trm1234* mutant gynoecium

