

# Plant Experimental Biology

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CEITEC

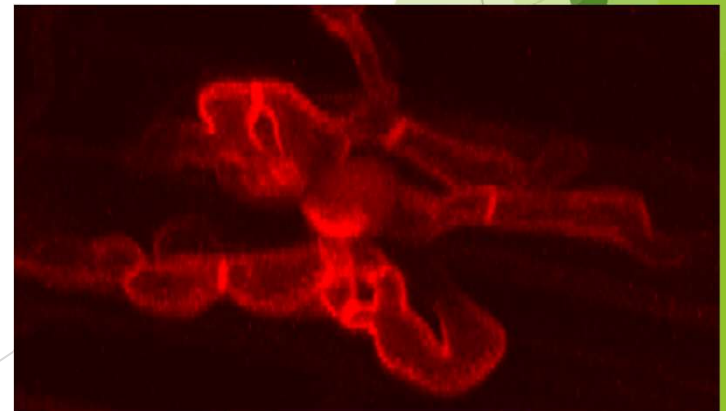


Oddělení  
experimentální  
biologie rostlin

## SUMMARY

- ▶ How to make a transgenic plant?
  - ▶ Tobacco, rice
  - ▶ *Arabidopsis thaliana*
- ▶ How to regulate (trans)gene expression?
  - ▶ The pOp6/LhGR system
  - ▶ CRISPR/Cas9
- ▶ Transient gene expression
- ▶ Fluorescent proteins and protein localization
- ▶ Plant endomembrane system
- ▶ Plant cell wall
  - ▶ Expansins & abiotic stresses
- ▶ Fungal cell wall
  - ▶ *Magnaporthe oryzae* - a model organism
  - ▶ *Aspergillus fumigatus*

*Arabidopsis thaliana*

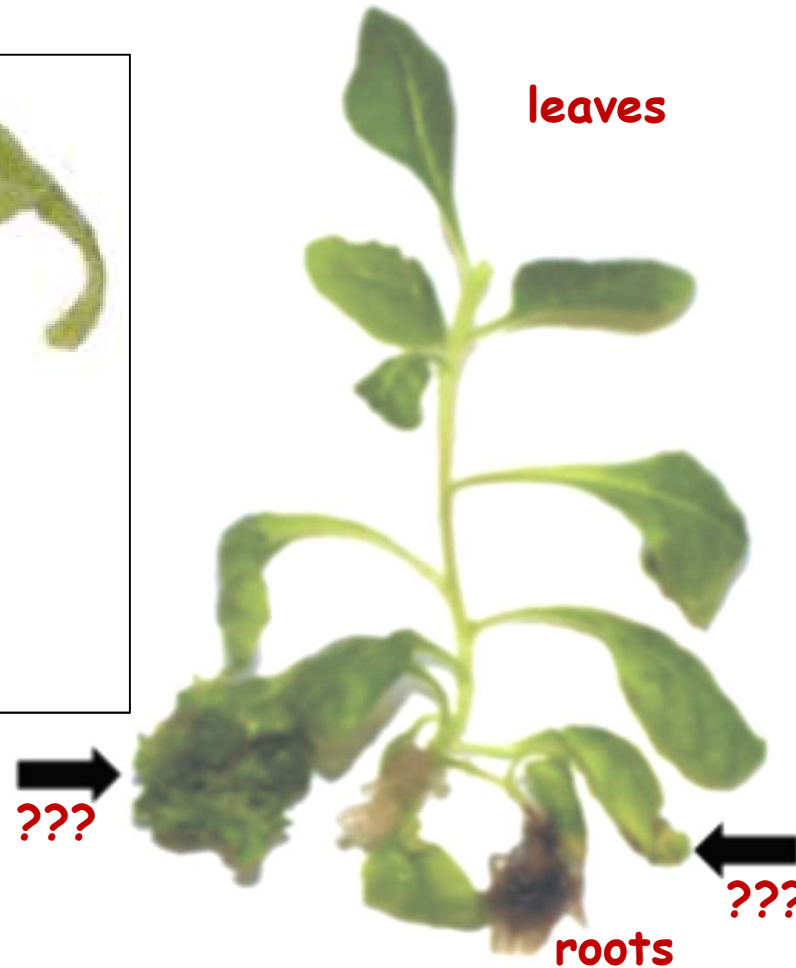


# My 1<sup>st</sup> transgenic tobacco plant that I made during my PhD (in the last century...:)

- ▶ Re-generated *in vitro* using tissue cultures



- ▶ Ectopic meristems developing into shoot-like structures...
- ▶ Introduced *IPT* gene encoding isopentenyl transferase which catalysis the first step in hormone cytokinin biosynthesis...



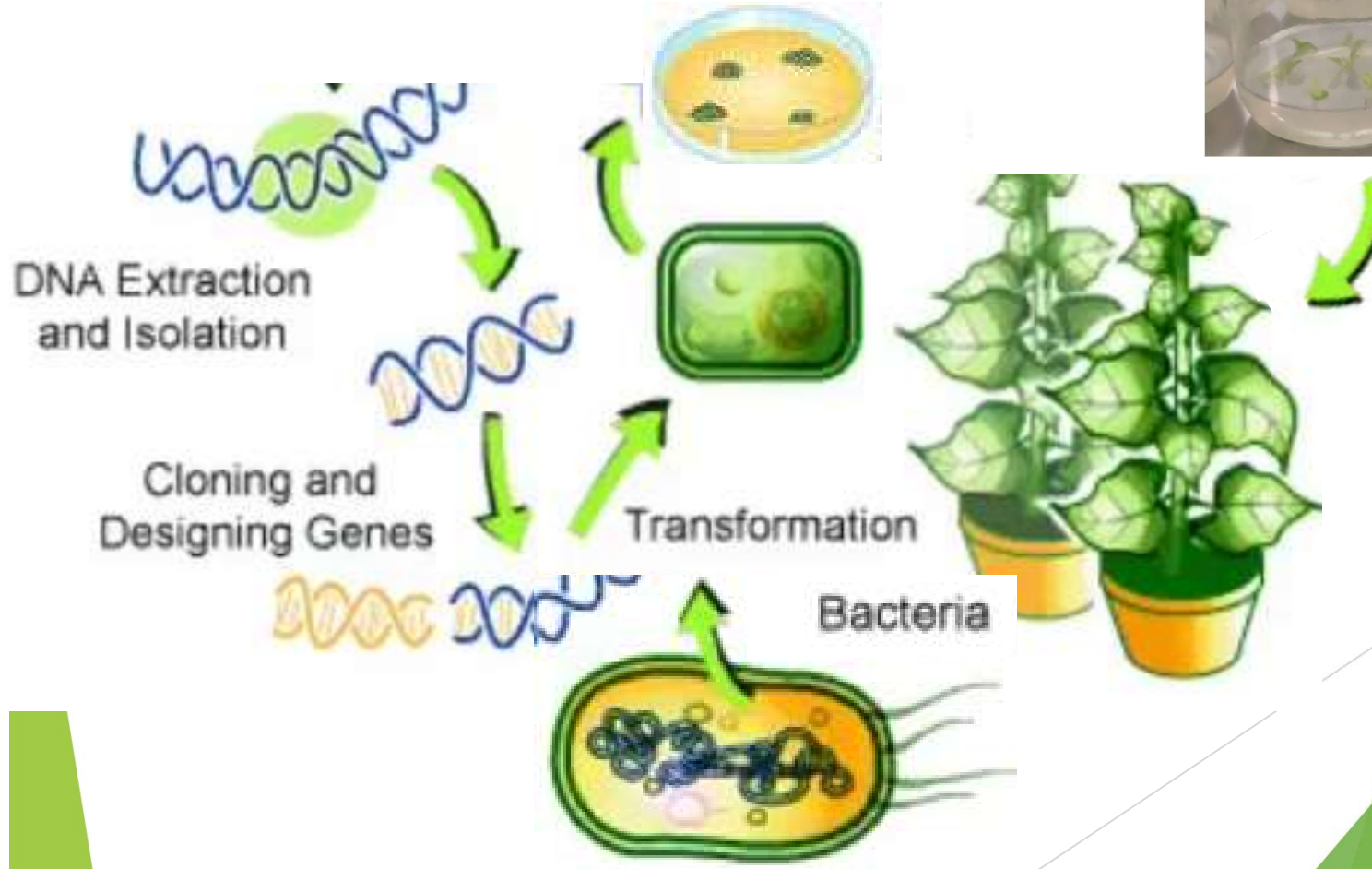
The background features abstract, overlapping green geometric shapes, primarily triangles and polygons, in various shades of green, creating a modern and scientific aesthetic. The shapes are layered, with some appearing more prominent than others, and they are set against a plain white background.

How to make a transgenic plant?

# Transformation

## ► Tissue cultures

- tobacco
- rice



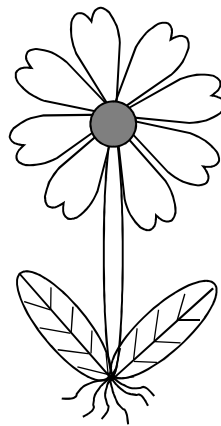
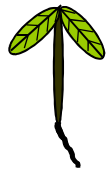


How to regulate (trans)gene  
expression?

## Chemically inducible gene expression systems in plants

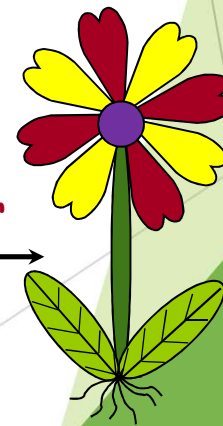
- ▶ used to regulate (trans)gene expression at a particular developmental stage and for a specific duration using chemical inducers.
  - ▶ Allow precise temporal and spatial control of a (trans)gene.
- ▶ Expression can be **SWITCHED ON** or **OFF** using chemical inducers.
  - ▶ Gene overexpression or knock-down expression by amiRNAs.
- ▶ Essential for expression of gene products that interfere with regeneration, growth or reproduction...

Meristem  
defect



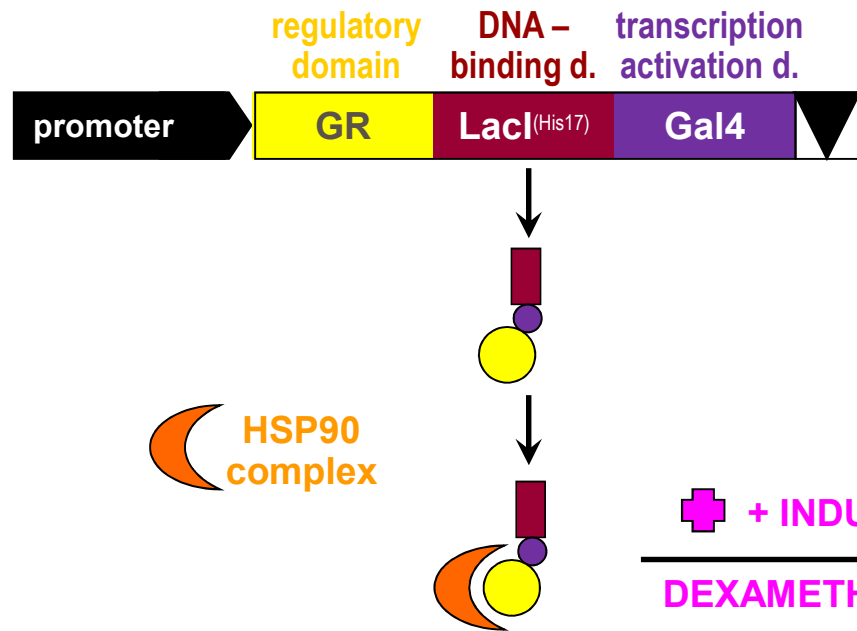
+ Inducible  
gene

+ inducer

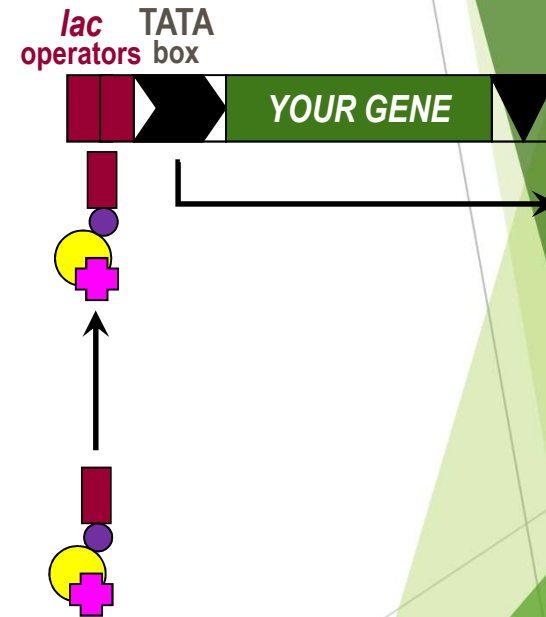


# The chemically inducible transcription activation system pOp/LhGR

## LhGR ACTIVATOR



## pOp REPORTER



- Developed in the laboratory of Dr Ian MOORE
- Use world-wide today...
- An "ideal" inducible system?



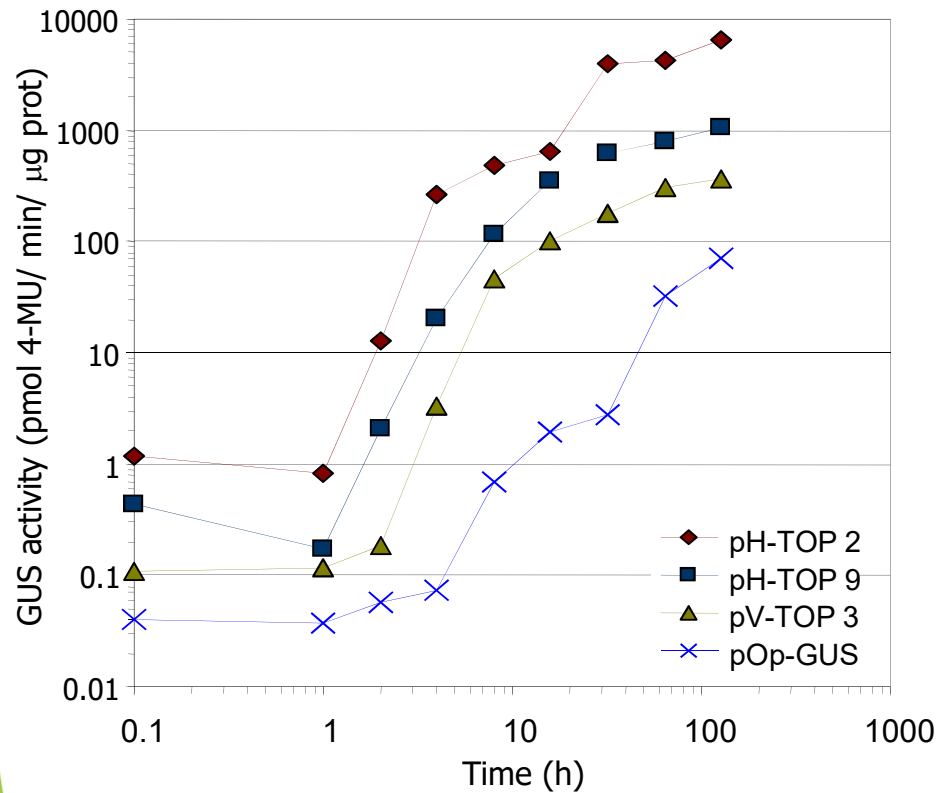
## An ideal inducible system

- ▶ High induced expression (e.g. 1000x or more).
- ▶ No uninduced expression (not leaky).
- ▶ Rapid uptake and wide distribution of inducer.
- ▶ No toxicity, no physiological effects in plants.
- ▶ Convenient application by a number of methods.
- ▶ Functional in several plant species.

Depend on the type of application, the gene being expressed and the plant species!

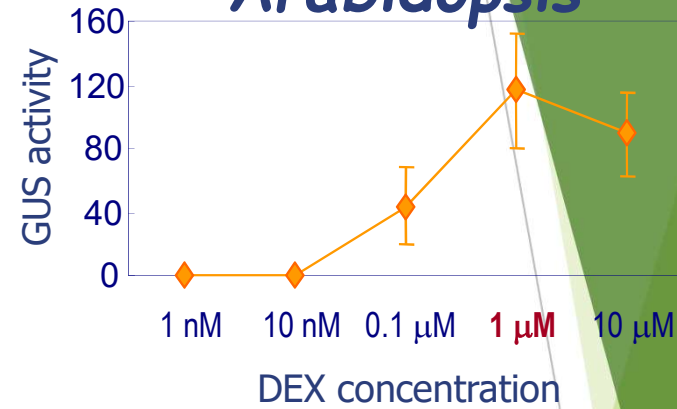
# The pOp6/LhGR is highly inducible, fast & v. sensitive

- ▶ 10,000-fold induction of GUS activity (log scale !)

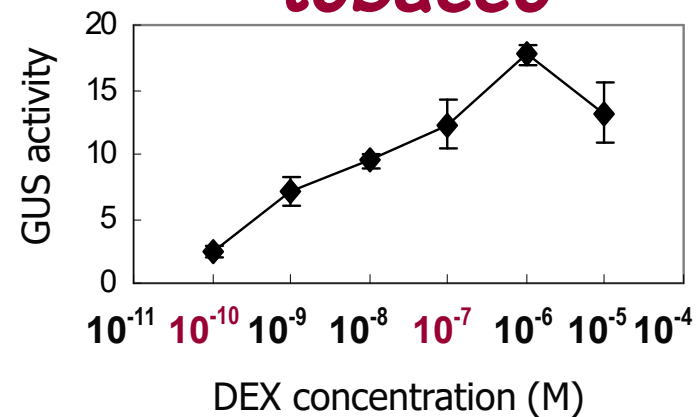


- ▶ Increase of GUS activity in 2h!

## Arabidopsis



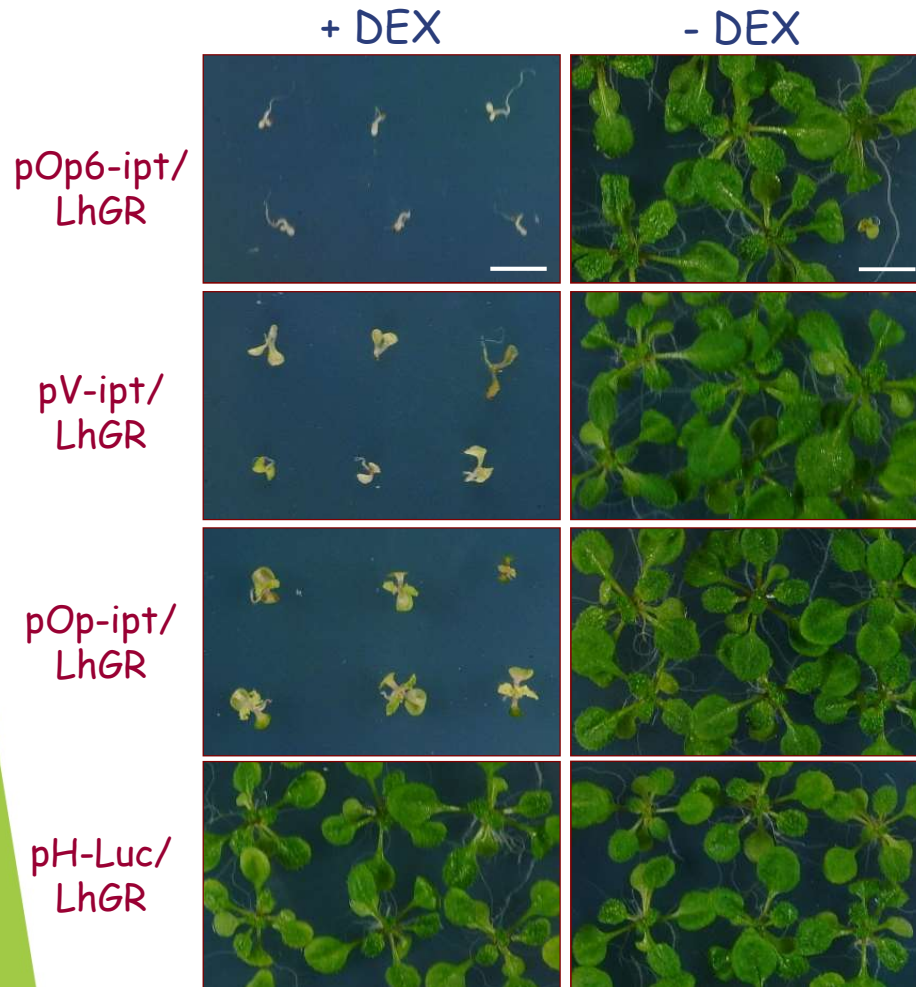
## tobacco



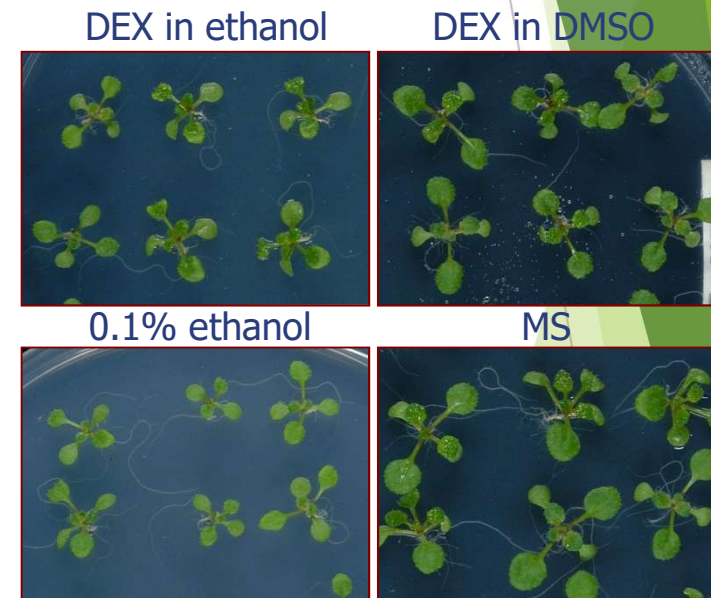
- ▶ The most sensitive system for tobacco!

# The pOp6/LhGR system is tightly regulated & not toxic!

- ▶ Basal expression levels tested with *ipt* gene
  - ▶ from *Agrobacterium* (cytokinin biosynthesis)
  - ▶ physiologically strong transgene

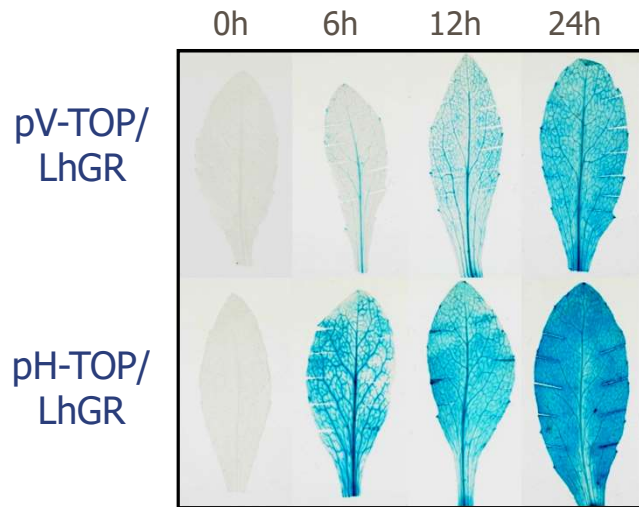


- ▶ neither DEX nor LhGR affects endogenous processes in plants ... **though ethanol does!**

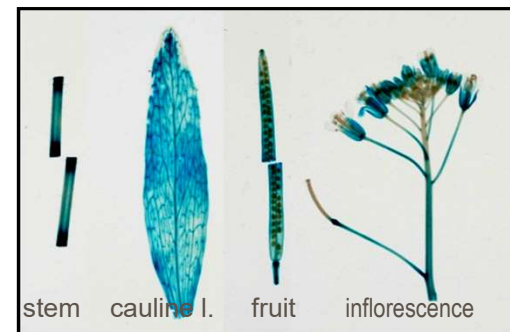


Arabidopsis seedlings were grown on plates in the presence or absence of 10  $\mu$ M DEX.

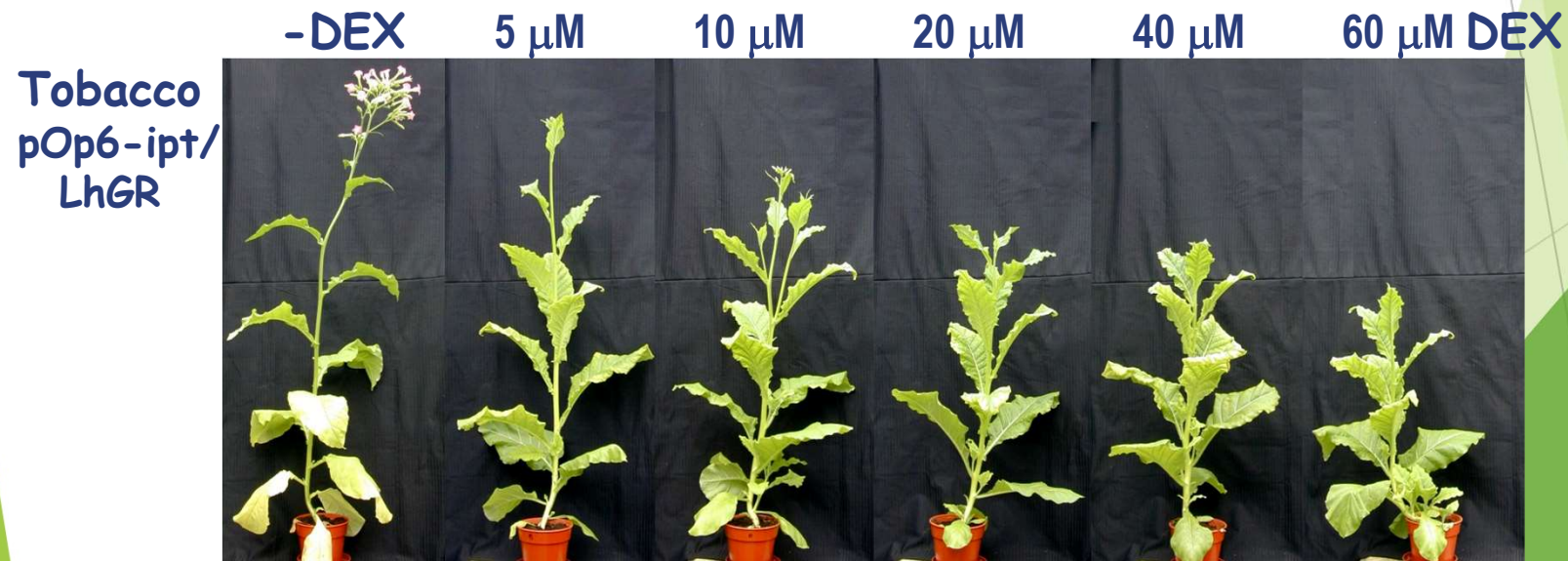
# The pOp6/LhGR system is inducible by various methods



## Watering plants with DEX

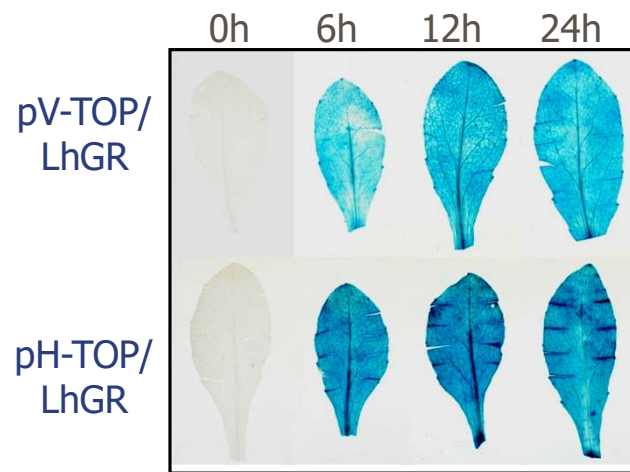


*DEX distribution through tissues (24h after watering).*

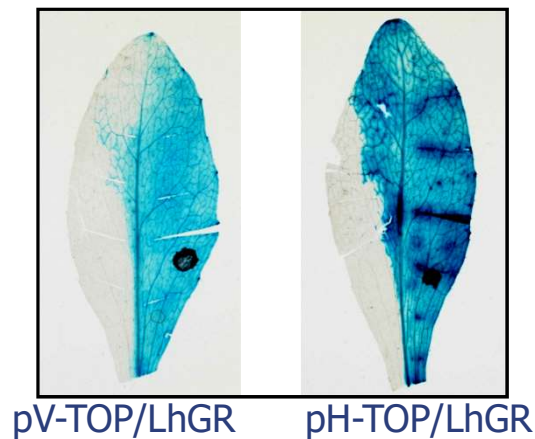


# The pOp6/LhGR system is inducible by various methods

## Painting plants with DEX

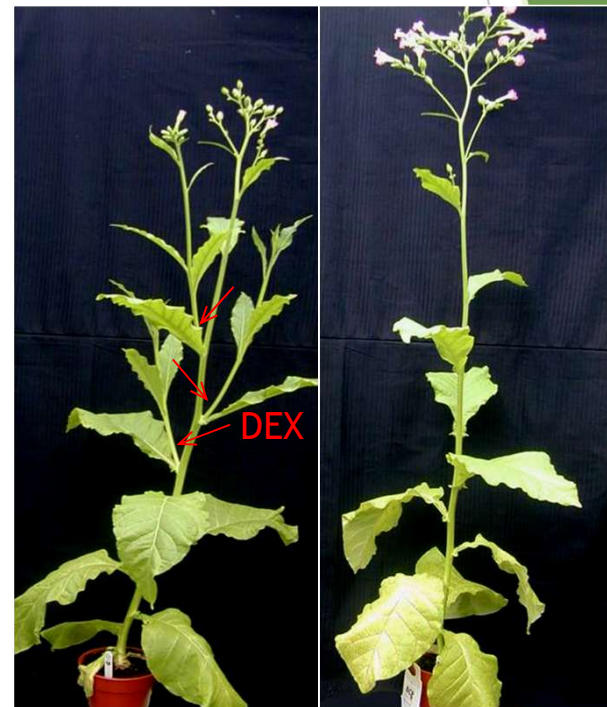


## A leaf half painted with DEX



application  
on axillary  
buds

Control



Tobacco  
pOp6-ipt/LhGR

# The pOp6/LhGR system is functional in several species

**Arabidopsis**

(Craft, Samalova et al., 2005)

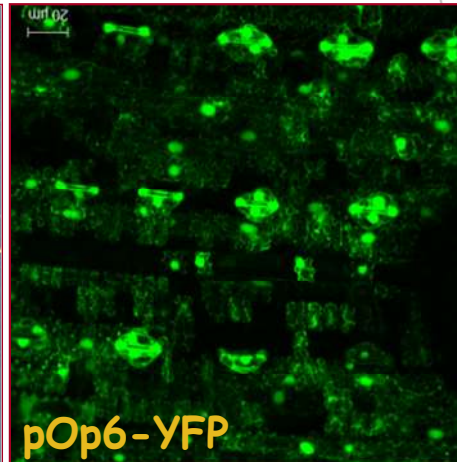
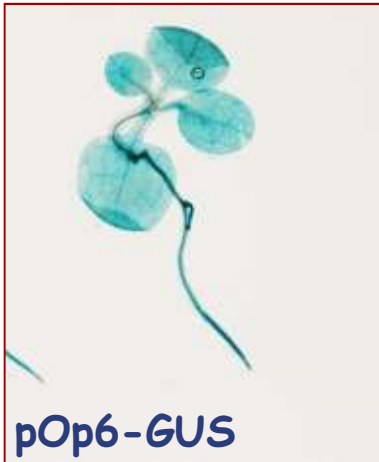
**Tobacco**

(Samalova et al., 2005)

**Rice**

(Samalova & Moore, 2021)

+ DEX



- DEX



- ▶ *Maize, potato, tomato, Cardamine hirsuta, citrus...*
- ▶ Detailed step-by-step protocols in Samalova et al., 2019

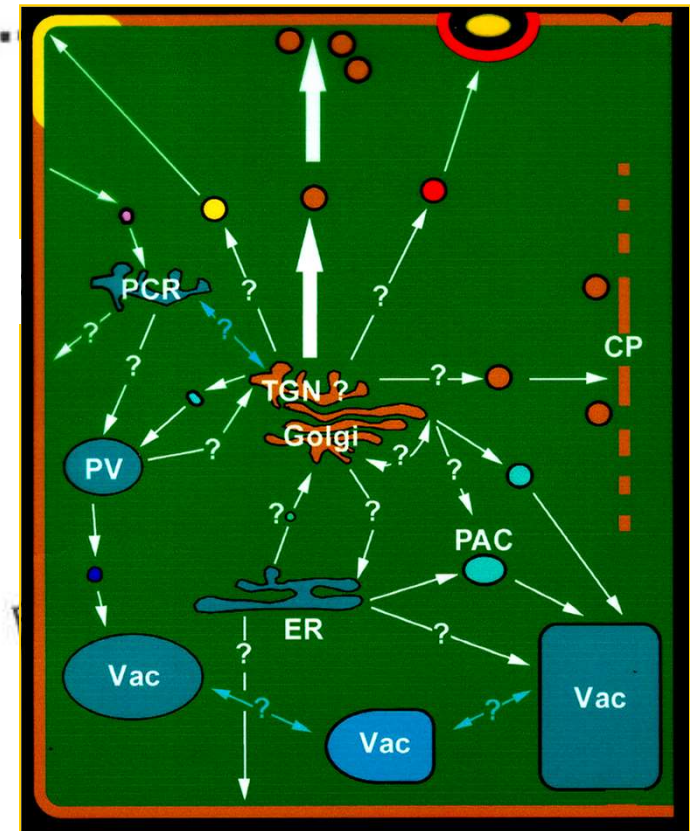
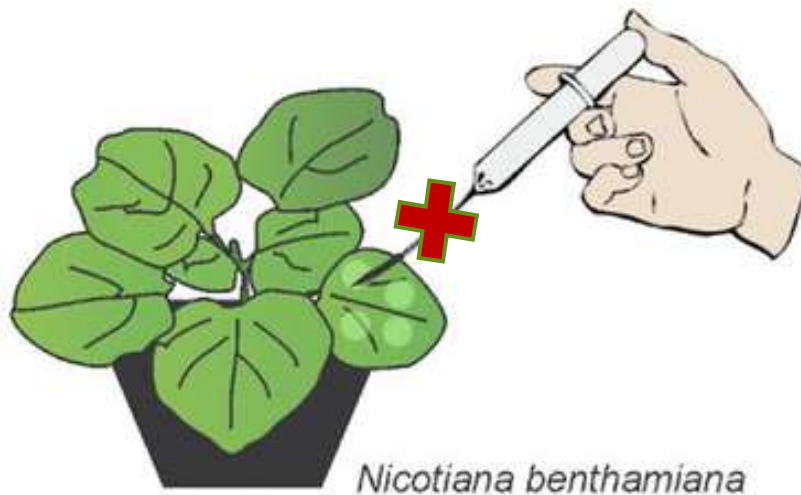
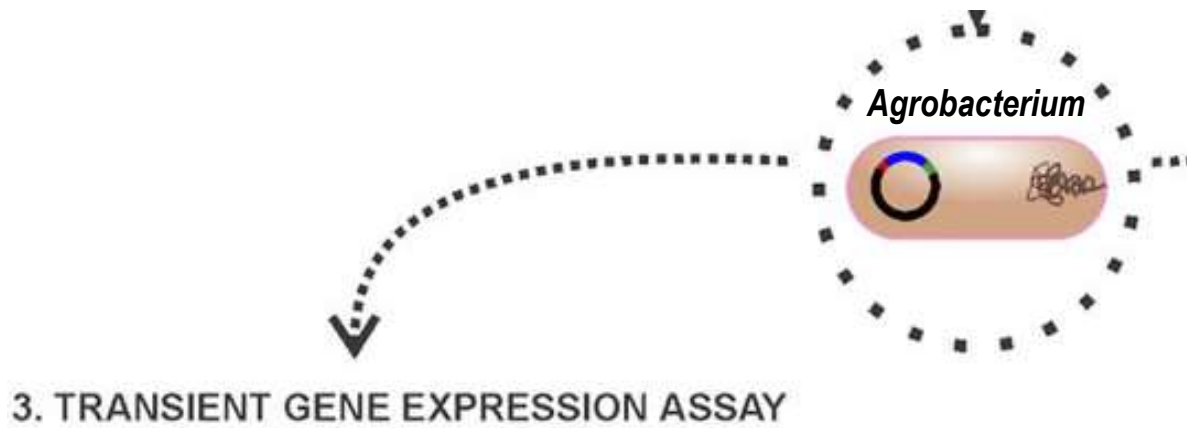
The background features abstract, overlapping green geometric shapes in various shades, including light lime green, medium green, and dark forest green. These shapes are primarily located on the left and right sides of the slide, framing the central text. The shapes are layered and semi-transparent, creating a modern, layered effect.

# Transient gene expression and fluorescent proteins

# Transient (gene) expression assay

- ▶ **AGROINFILTRATION** method
- ▶ *Agrobacterium* infiltrated into tobacco plants

- ▶ e.g. to study plant endomembrane trafficking





# Use of fluorescent proteins (FP) in cell biology

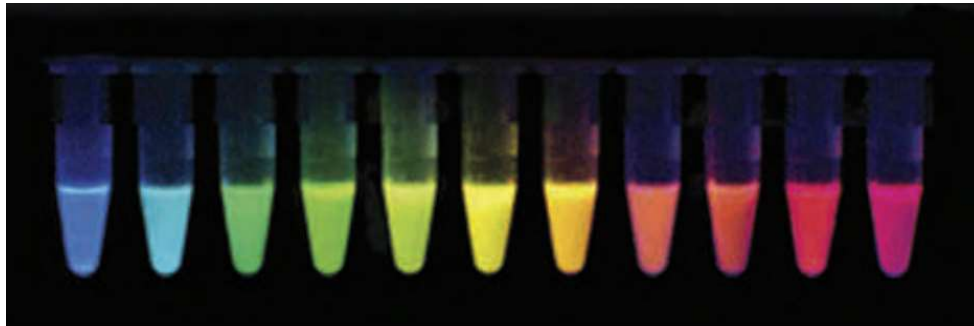
- ▶ Protein localization, protein-protein interactions...

GFP ~ green FP from jellyfish *Aequorea victoria*

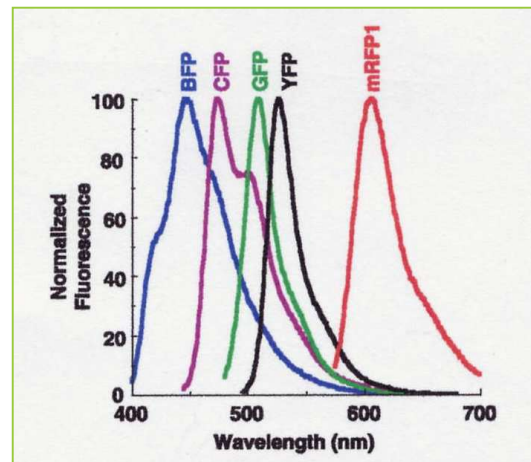
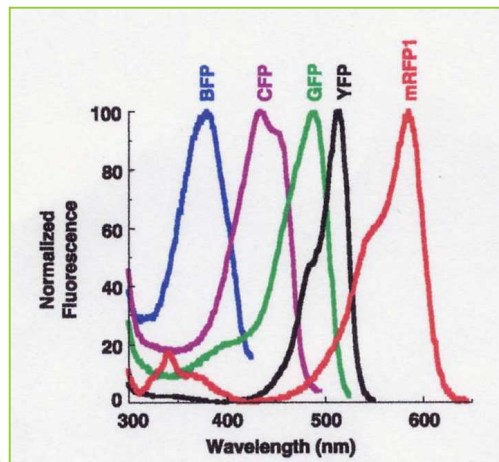
YFP - yellow FP mutant variant of GFP

mRFP1 - monomeric red FP from *Discosoma coral*

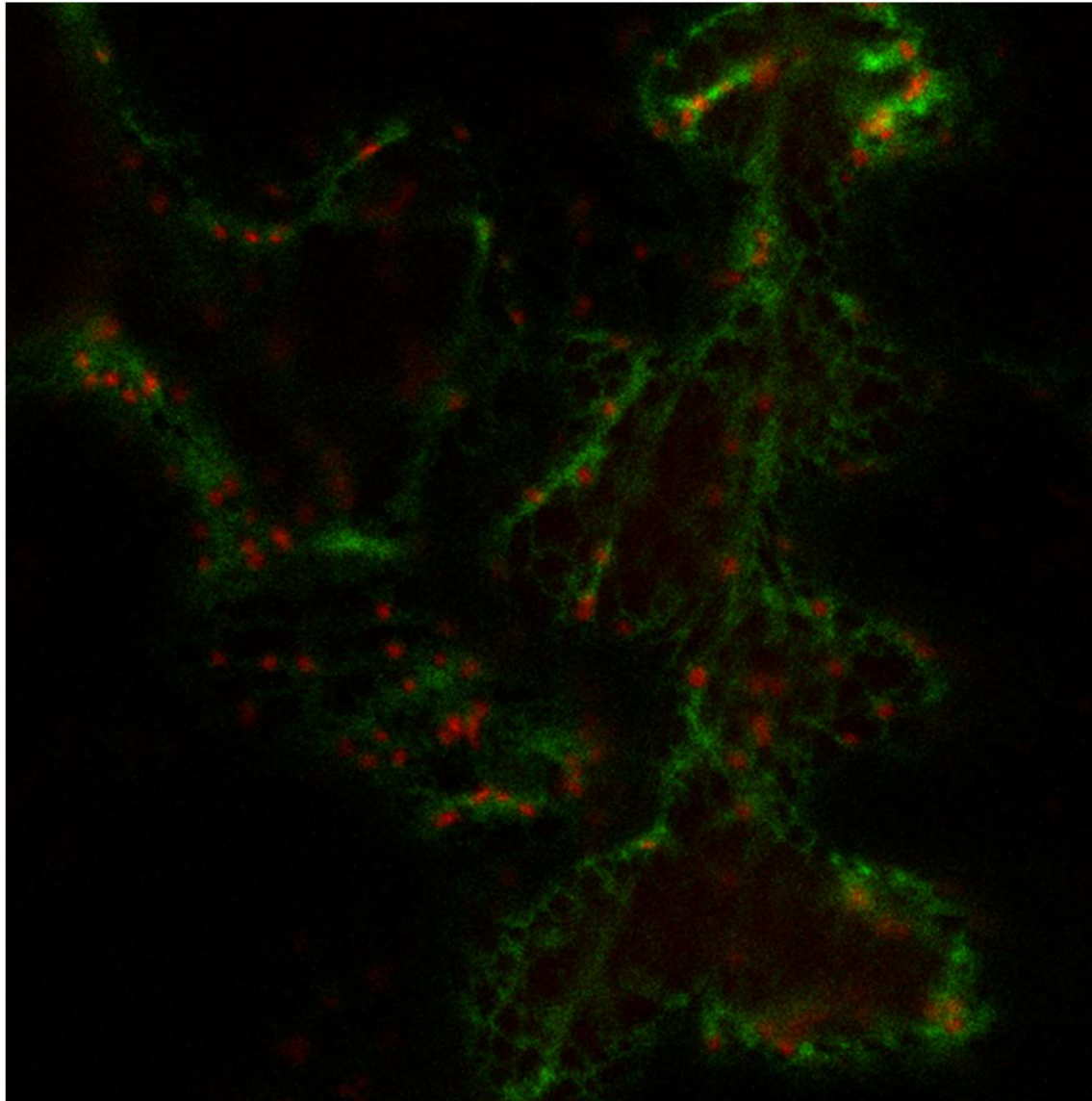
- ▶ CLSM ~ confocal laser scanning microscope
- ▶ Generates optical slices through live specimens



- ▶ Excitation spectra
- ▶ Emission spectra



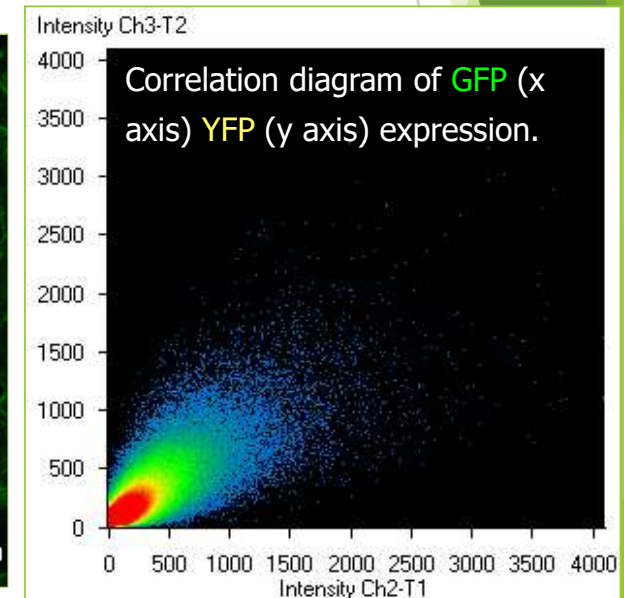
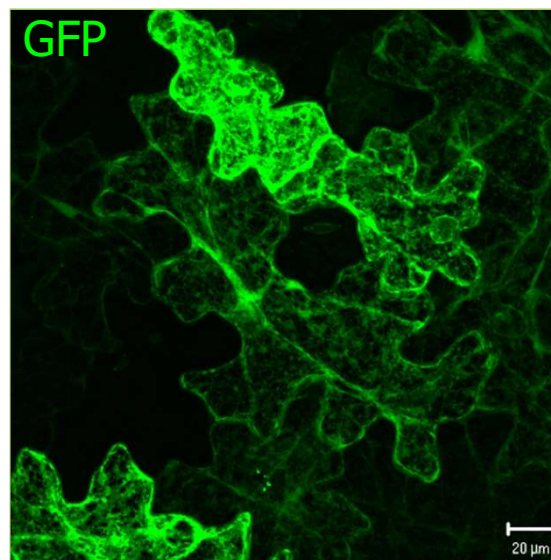
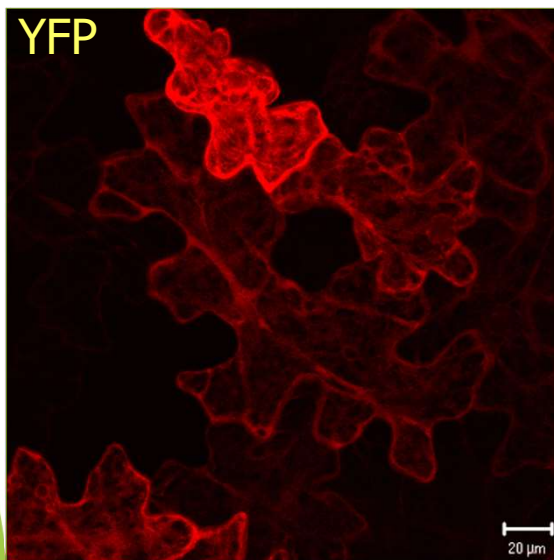
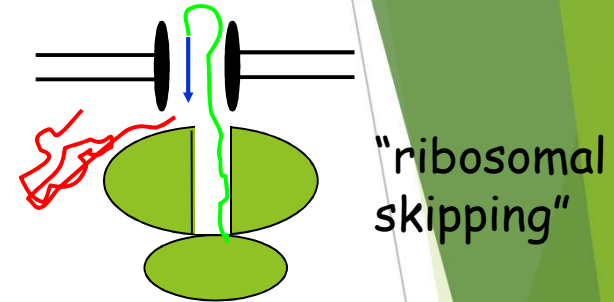
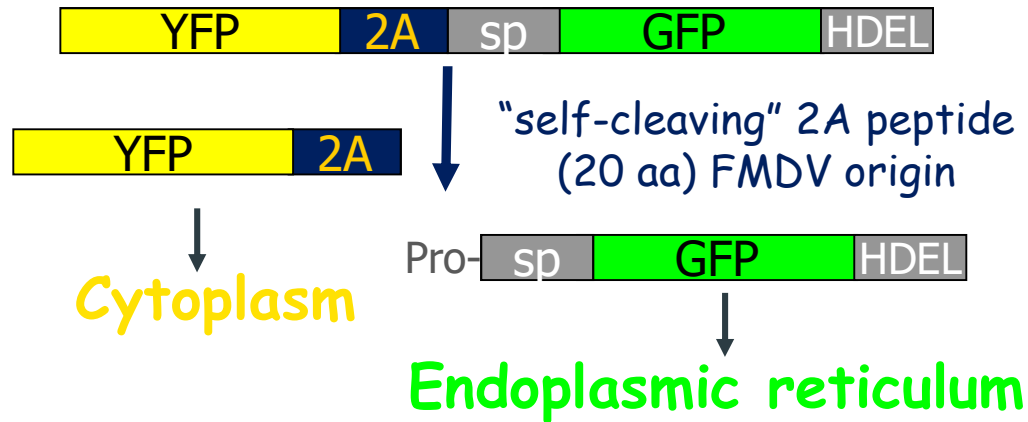
The **Golgi apparatus** moving along the **ER network** in living tobacco cells....



**PLANTS  
ARE  
MOVING :)**

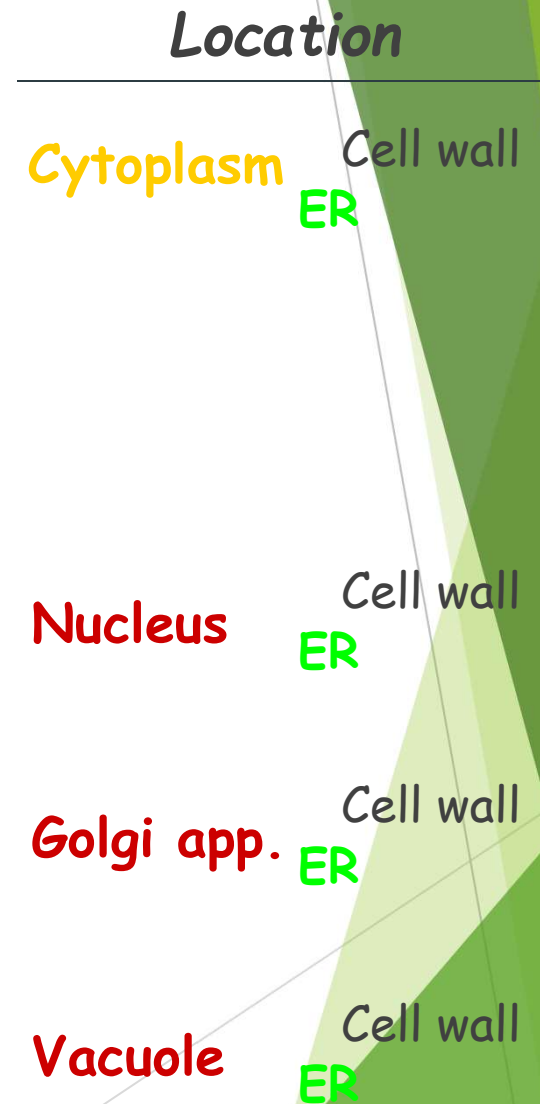
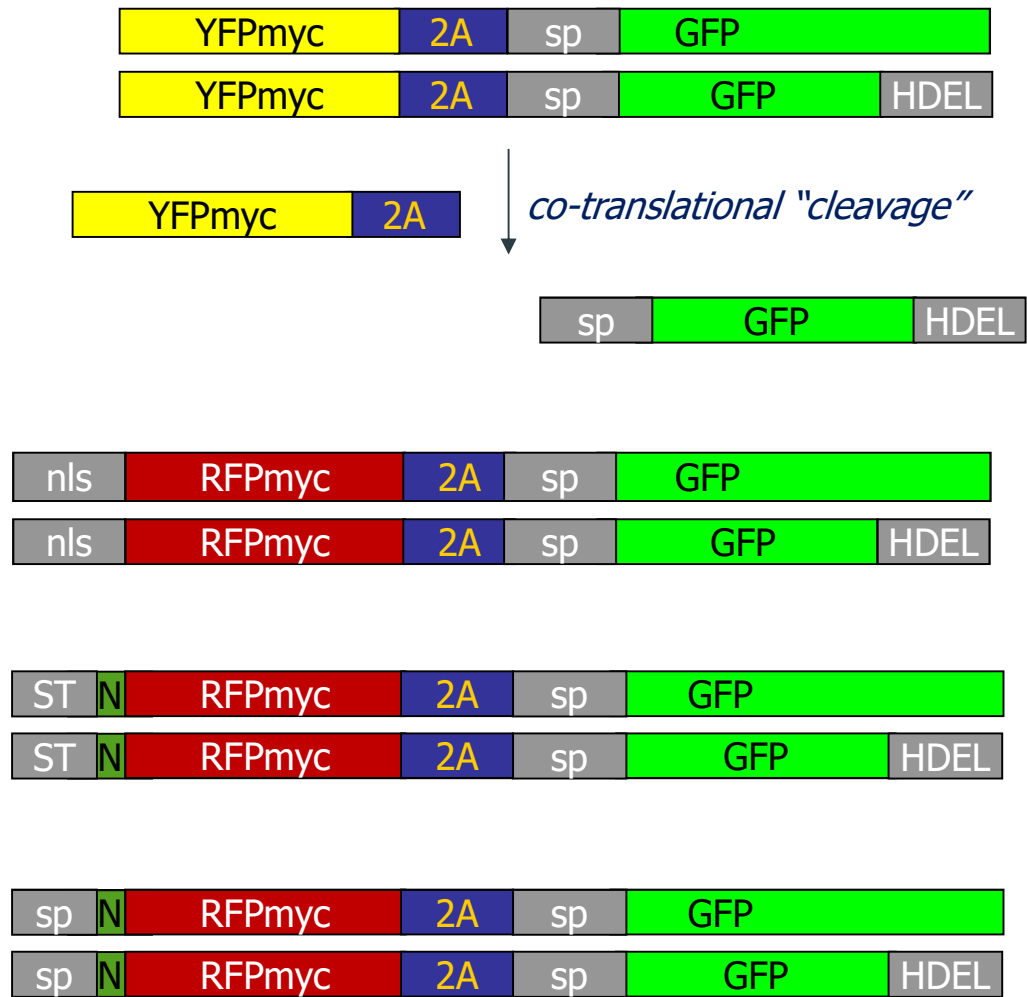
## Co-ordinated expression of two FPs

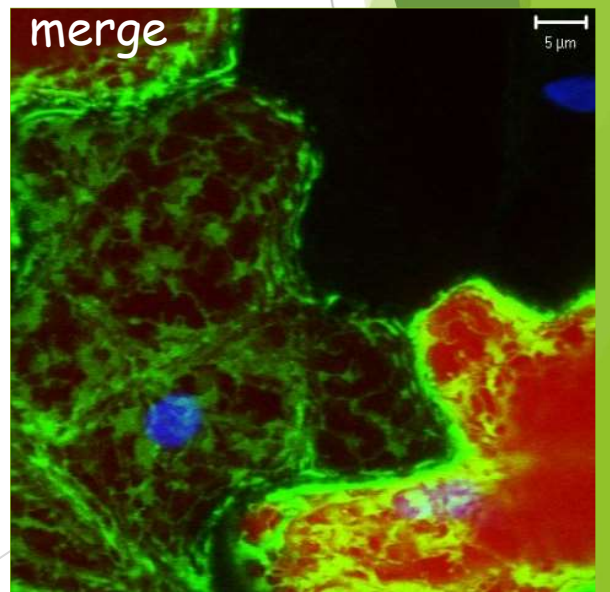
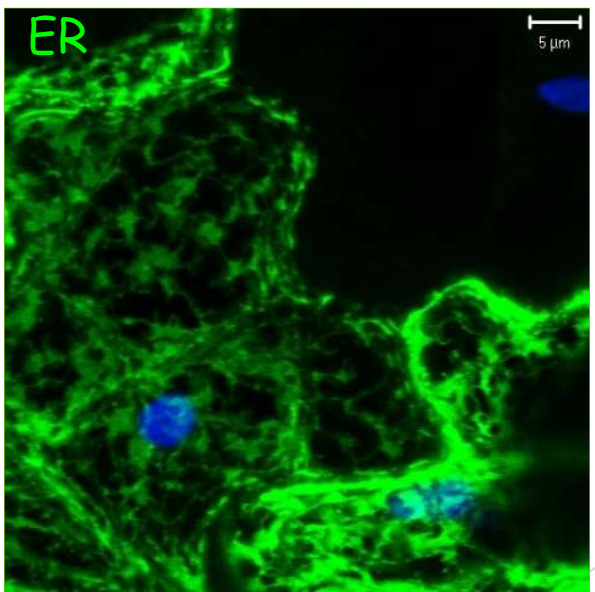
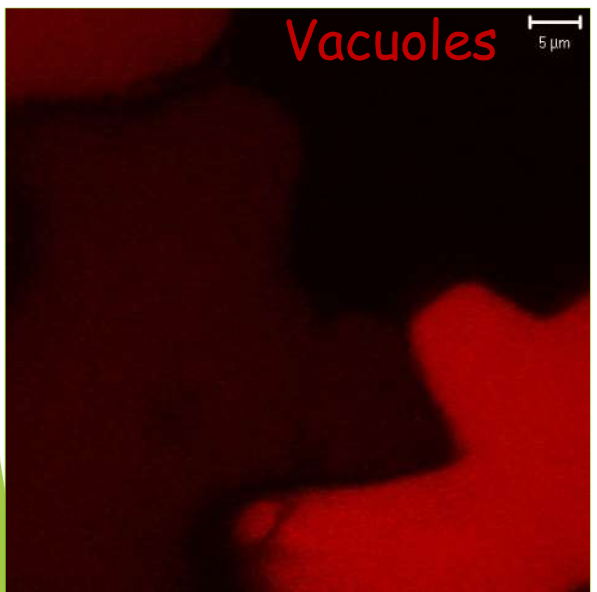
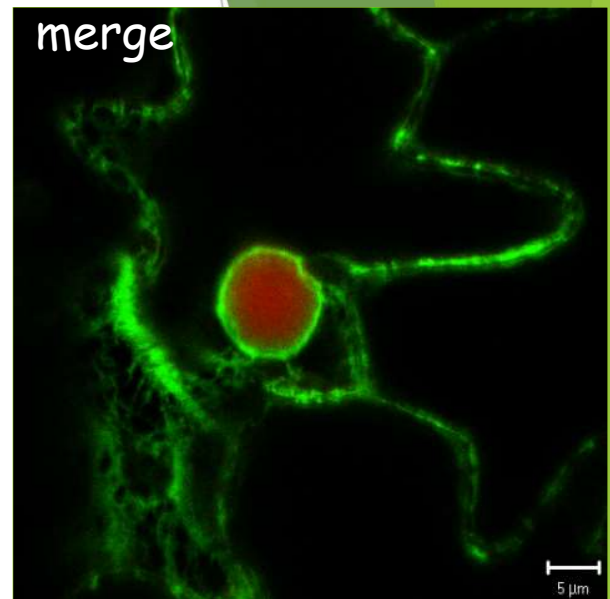
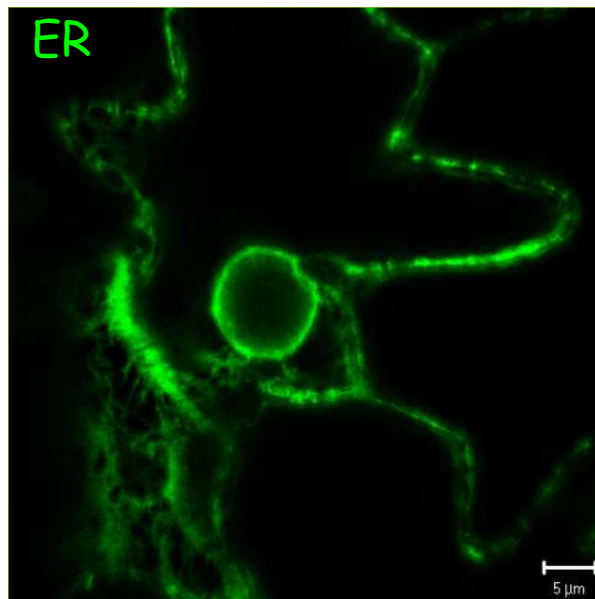
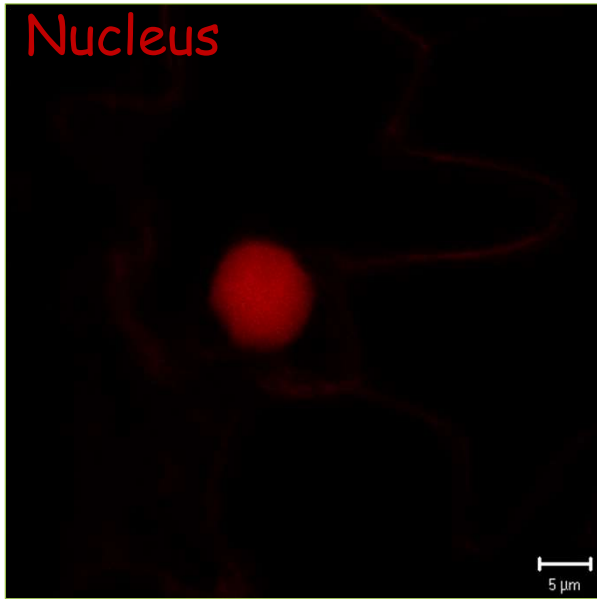
- ▶ to develop ratio-metric fluorescence-imaging assays (Samalova et al., 2006)

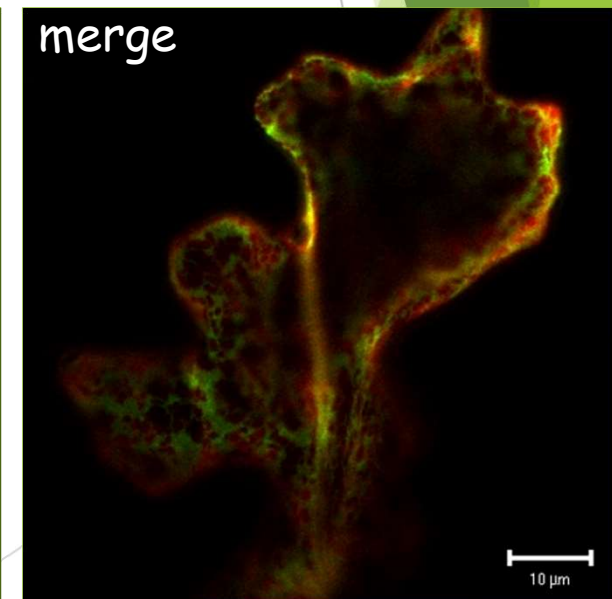
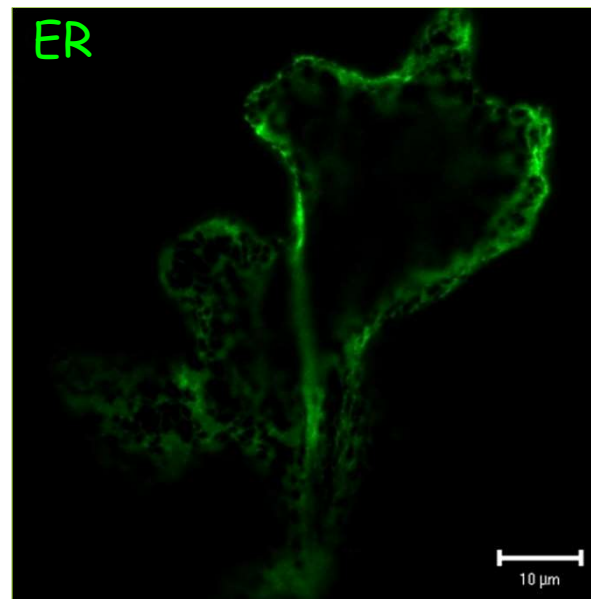
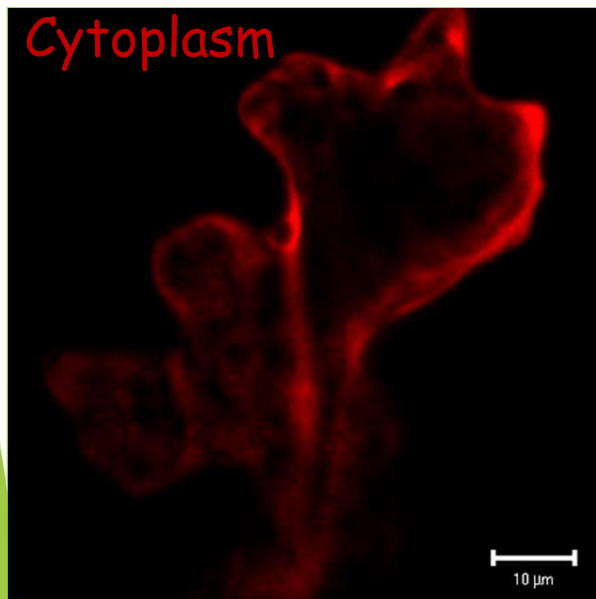
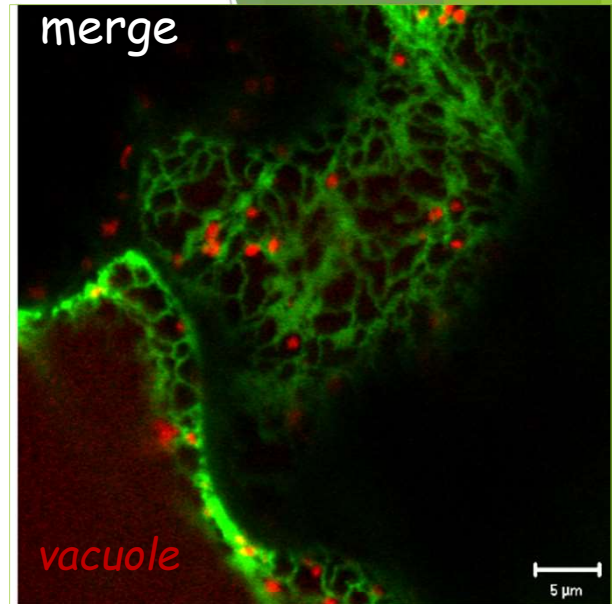
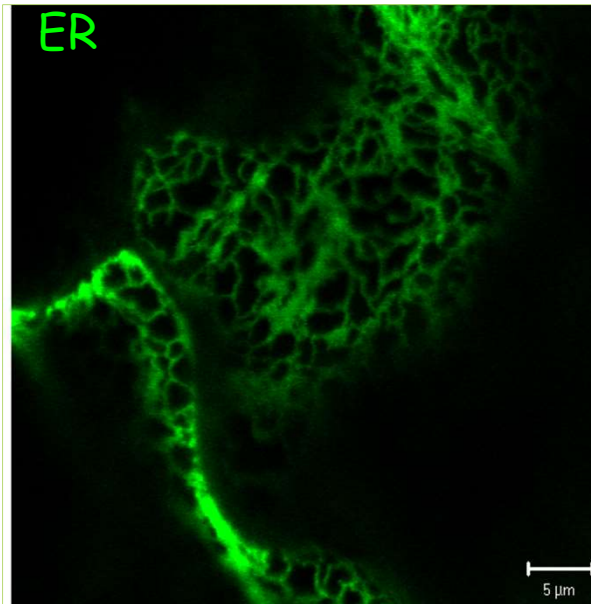
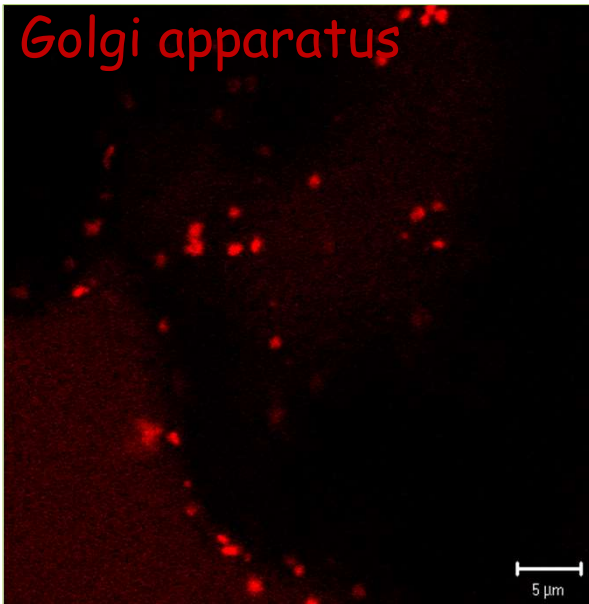


- ▶ YFP predicts GFP accumulation, at a similar ratio.

# Targeting of 2A fusions into different cell compartments





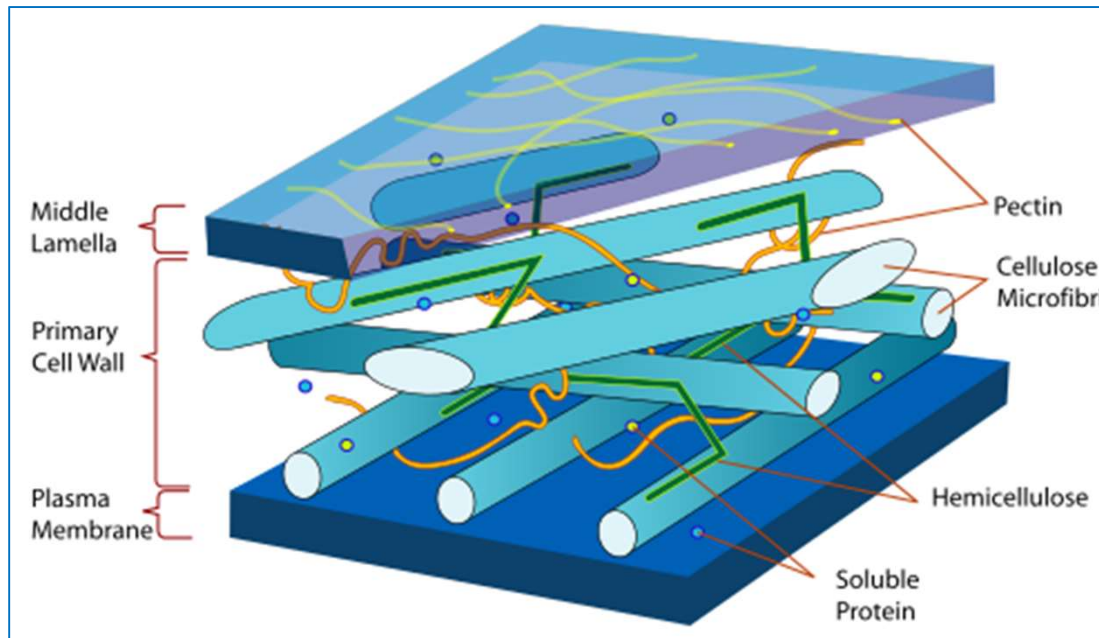




Plant cell wall (CW)

## CW is crucial for plant growth and development

- ▶ shapes the plant body
- ▶ movement of solutes and nutrients
- ▶ protects plants from the environment
- ▶ intercellular communication (Wolf *et al.*, 2012)
  - ▶ Cellulose is the most abundant biopolymer on Earth!

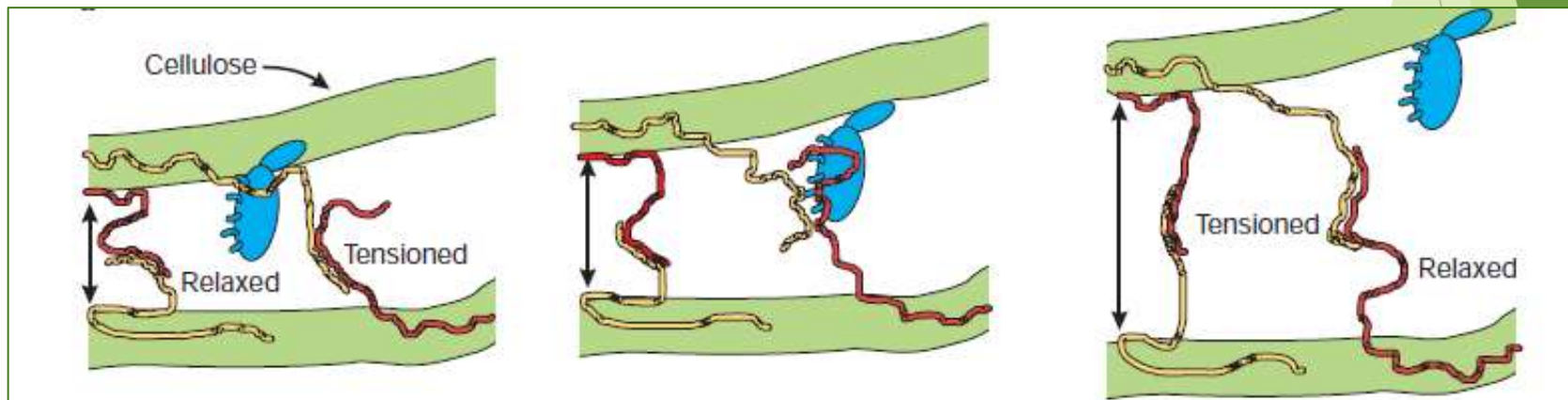
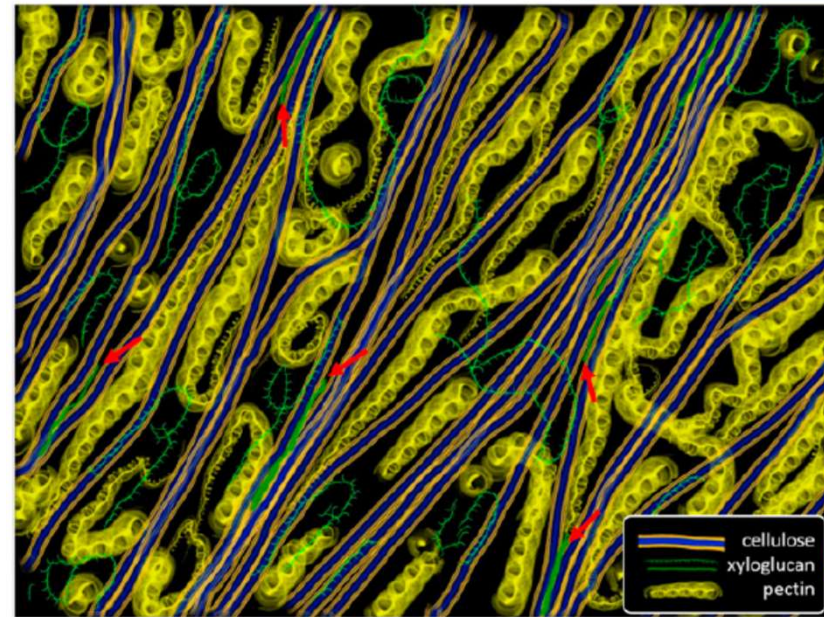


- ▶ Load-bearing *cellulose microfibrils*
- ▶ embedded into viscoelastic matrix of *hemicellulose and pectins*.

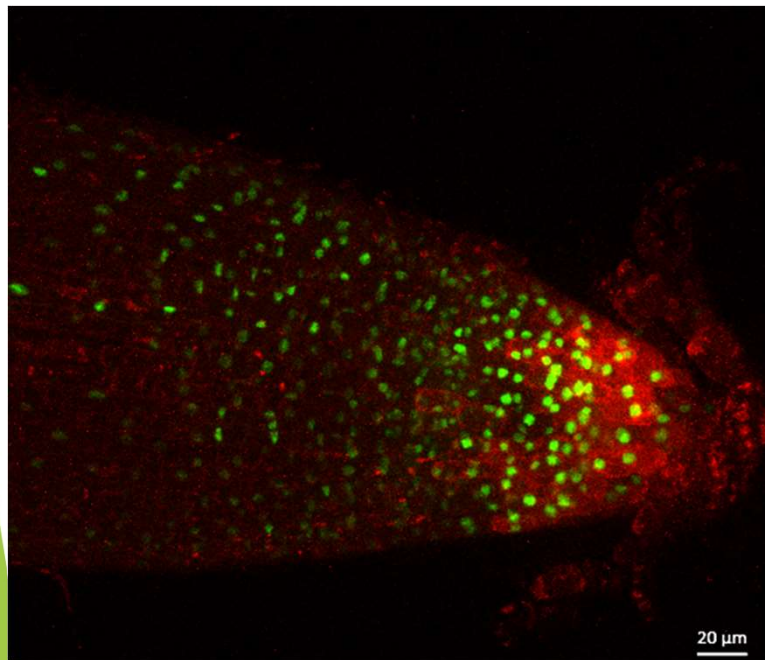
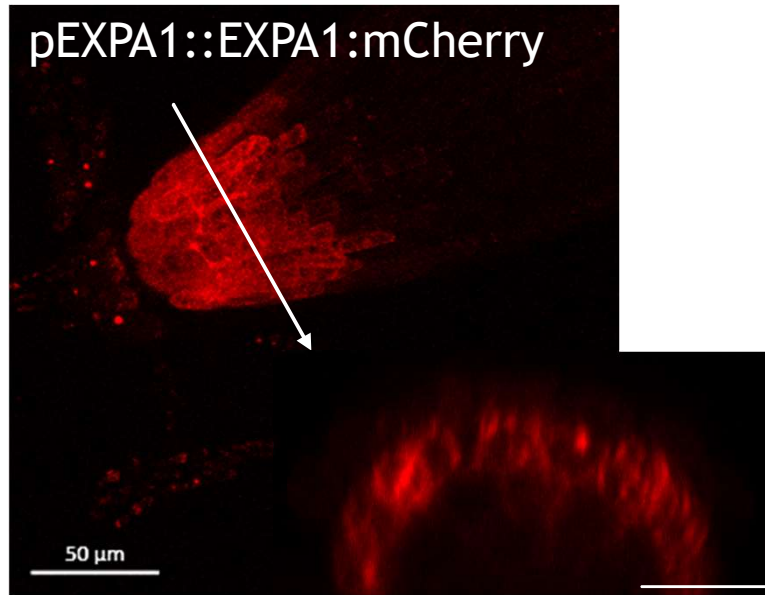


# Plant CWs combine strength with extensibility

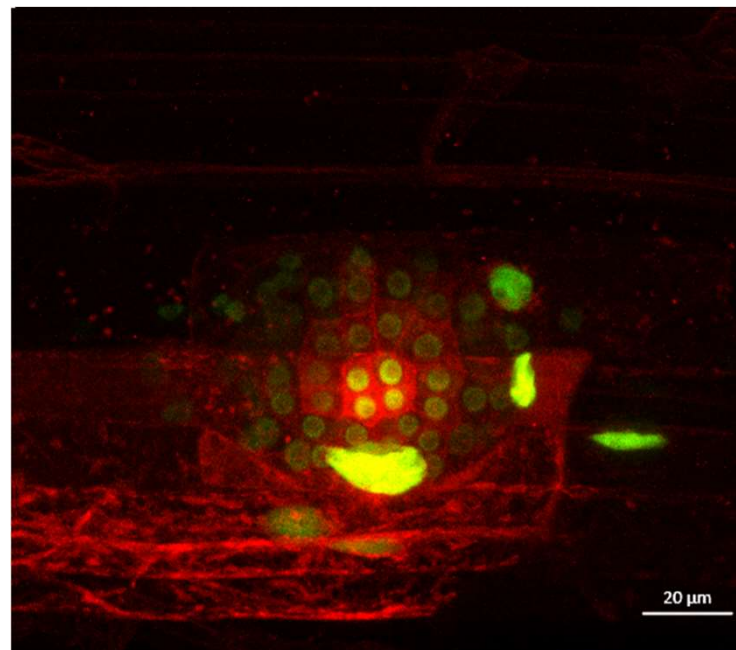
- ▶ Wall extensibility may be controlled at limited regions, '**biomechanical hotspots**' (Cosgrove, 2014; 2018).
- ▶ **EXPANSINS** discovered as the most pH-responsive substance in the CW (McQueen-Mason *et al.*, 1992).
- ▶ do not have a hydrolytic activity
- ▶ but disrupt the non-covalent bonds between CW polysaccharides, thus relaxing wall stresses and allowing turgor-driven cell expansion (Cosgrove, 2000).



# EXPANSINS are localized in the cell wall

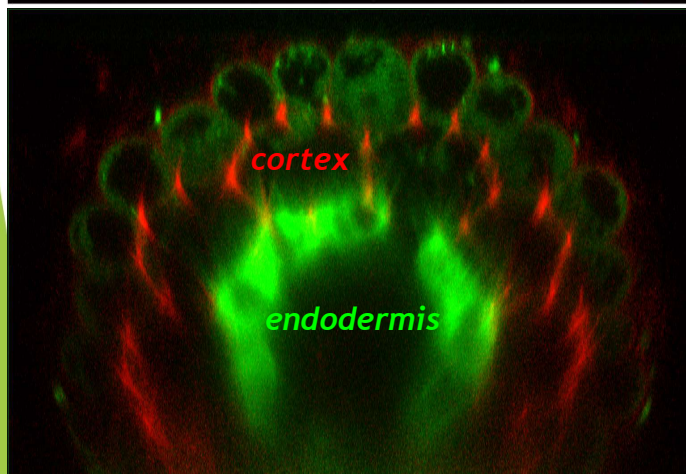
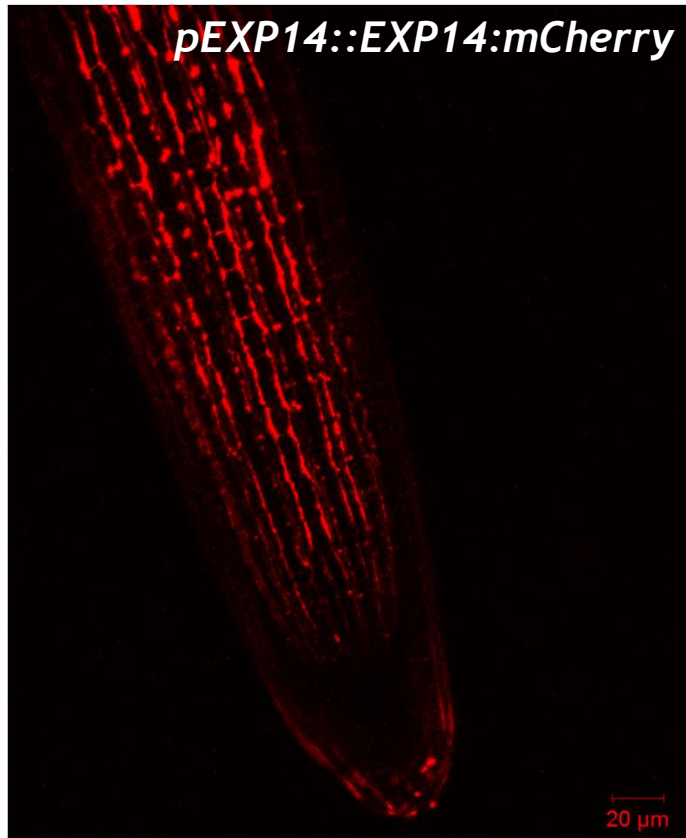


- EXPANSINS localised to the CW *in vivo* for the first time!
- Use of mCherry (RFP) instead of pH sensitive GFP

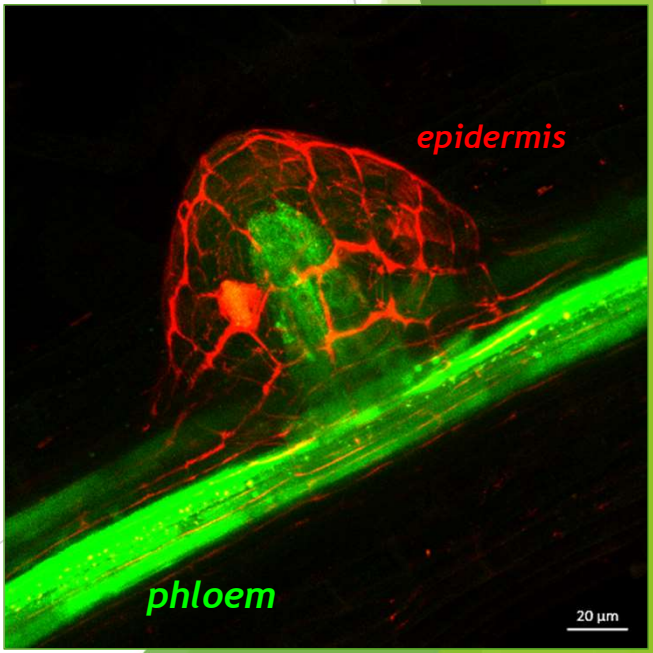
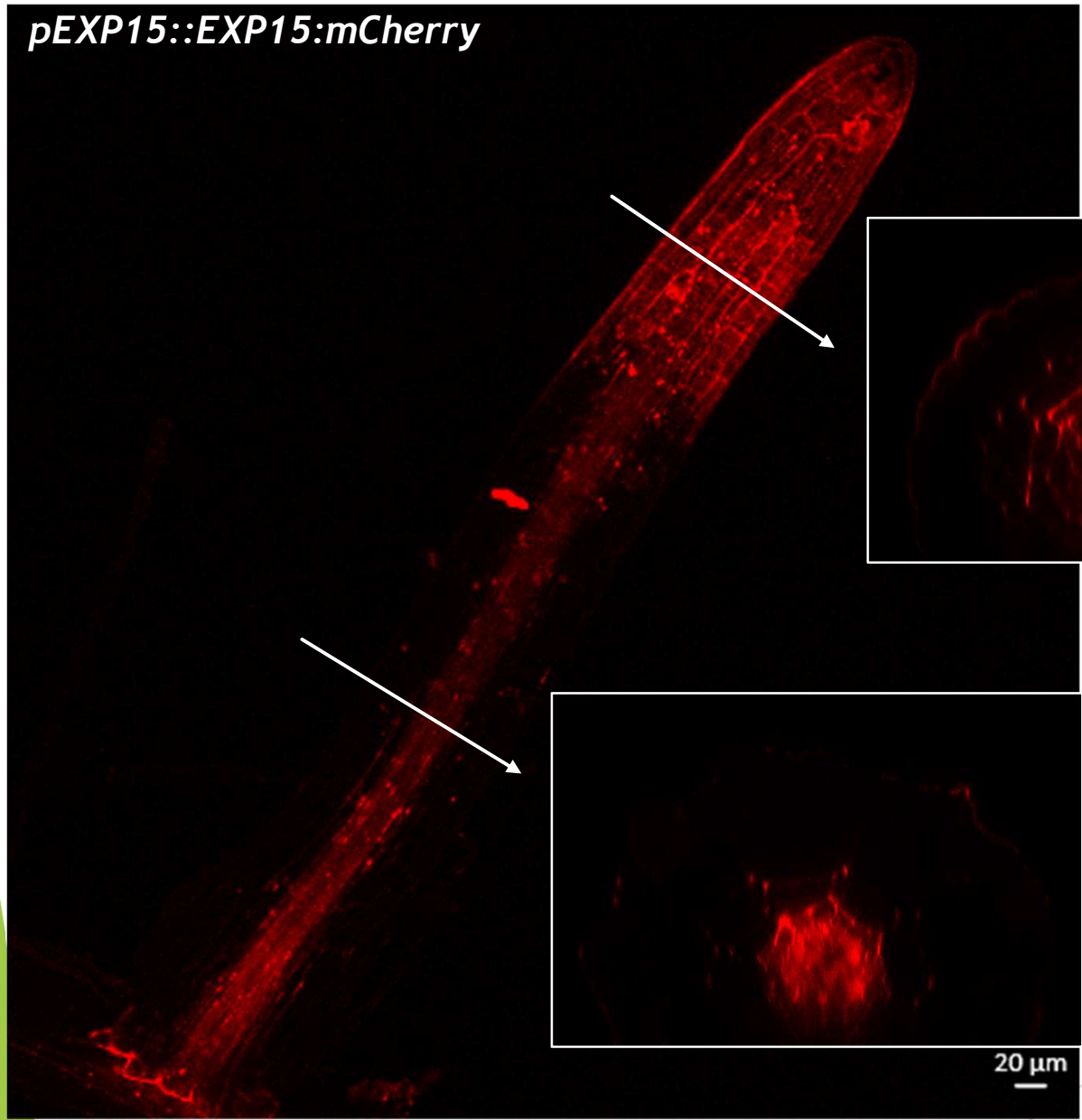


# EXPANSINS are localized into various root tissues.

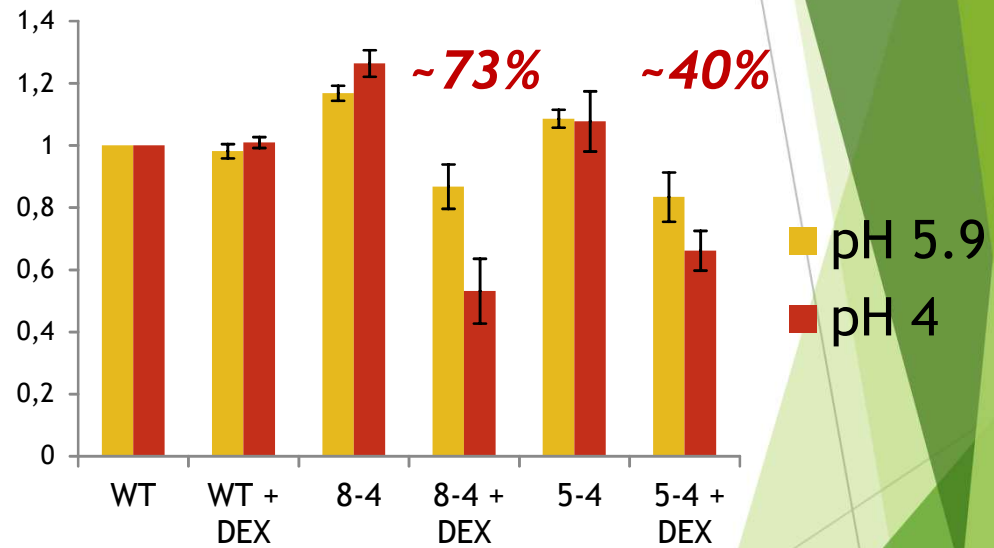
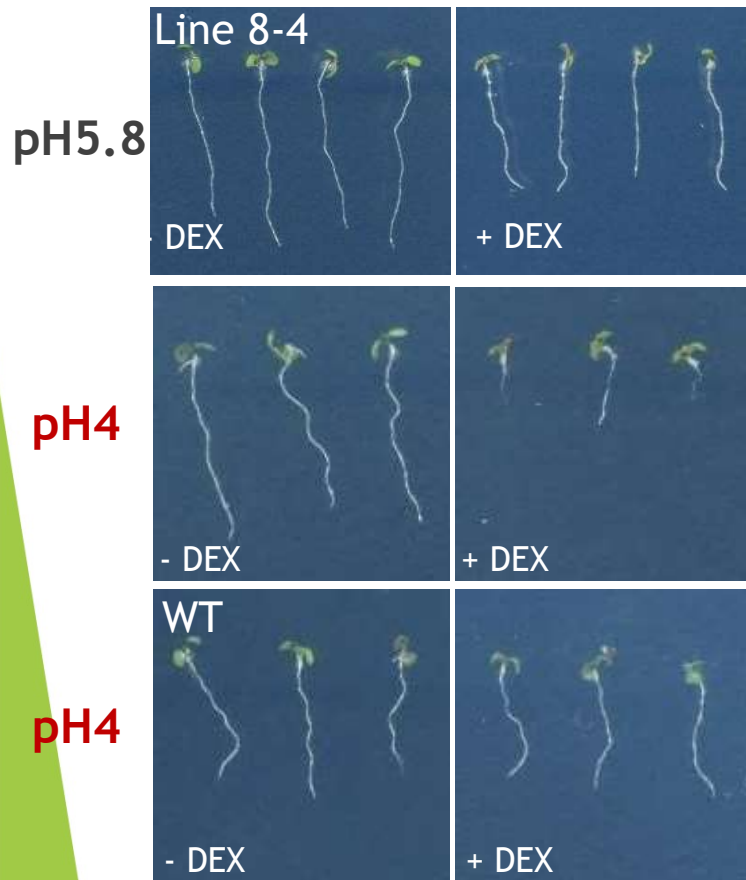
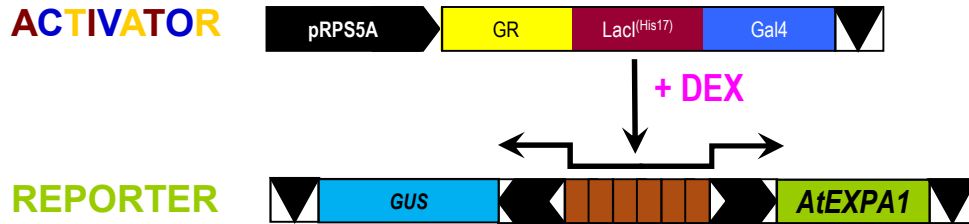
- ▶ 3D projection of Z-stack (combined optical slices) taken by CLSM with airyscan detector



# EXPANSINS are localized into various root tissues.

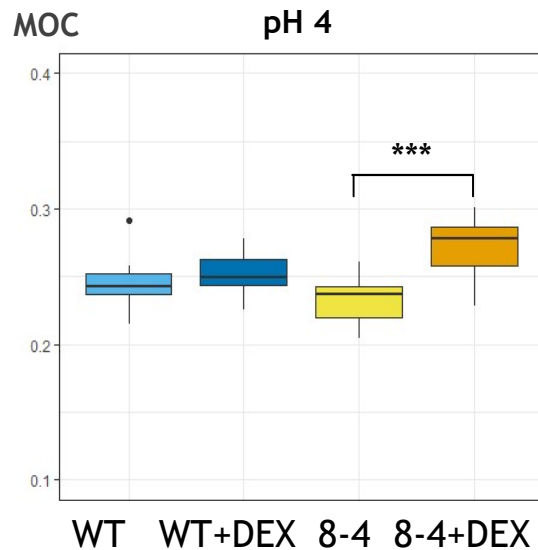
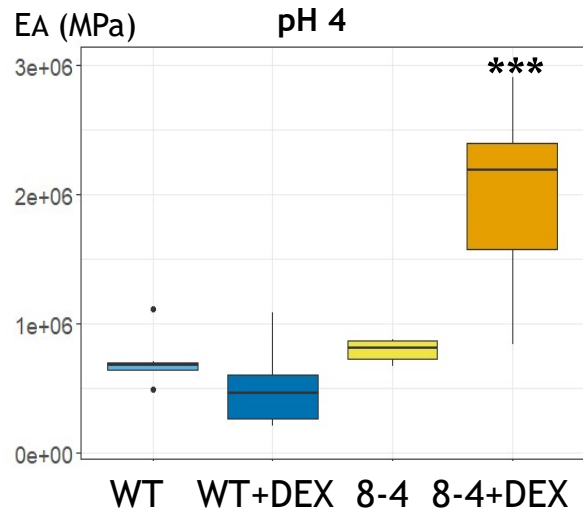


# Overexpression of *EXPA1* reduces the root size



► Significant **reduction of root size** of *EXPA1* overexpressing plants (7day-old *Arabidopsis* seedlings).

# Overexpression of *EXPA1* changes biomechanical properties "stiffens" CW

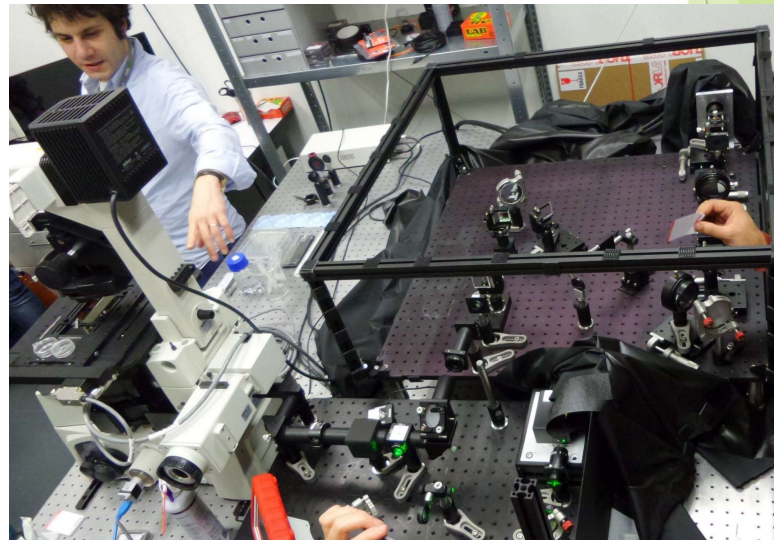


▶ *Samalova et al., 2020 BioRxiv*

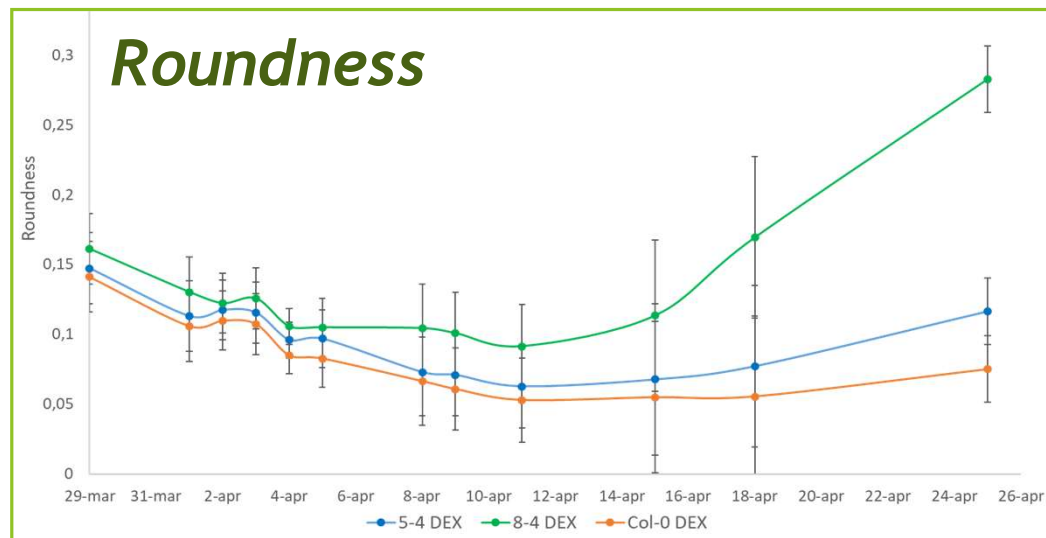
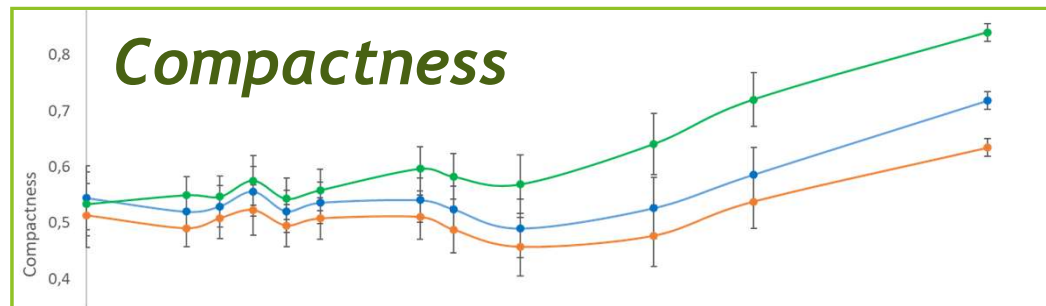
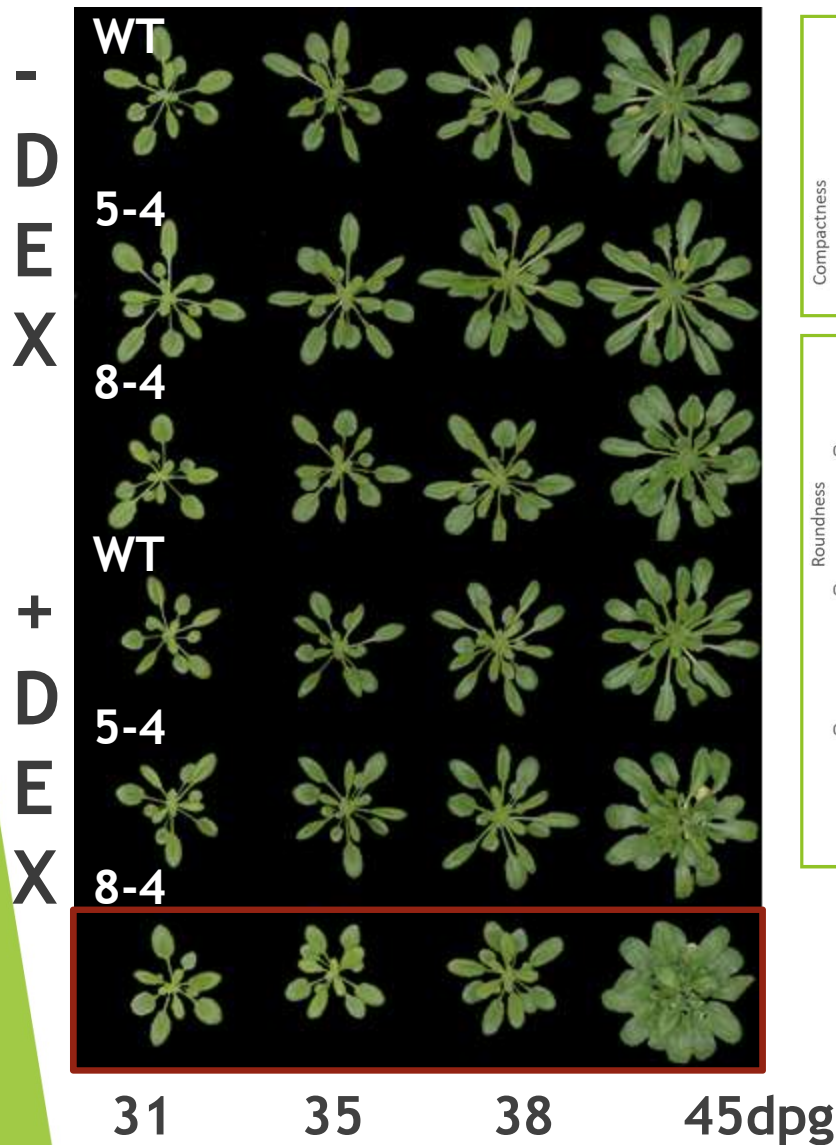
▶ **Atomic Force Microscopy (AFM)**  
(Dr Alexis Peaucelle, INRAE, Versailles)



▶ **Brillouin Light Scattering (BLS)**  
(Dr Kareem Elsayad, Biocentre, Vienna)



# Overexpression of *EXPA1* leads to smaller, compact plants



- 8-4 DEX
- 5-4 DEX
- Col-0 DEX



# EXPANSINs can improve stress tolerance of plants



➤ Explore the role of **EXPANSINs** under stress:

➤ **ABIOTIC**

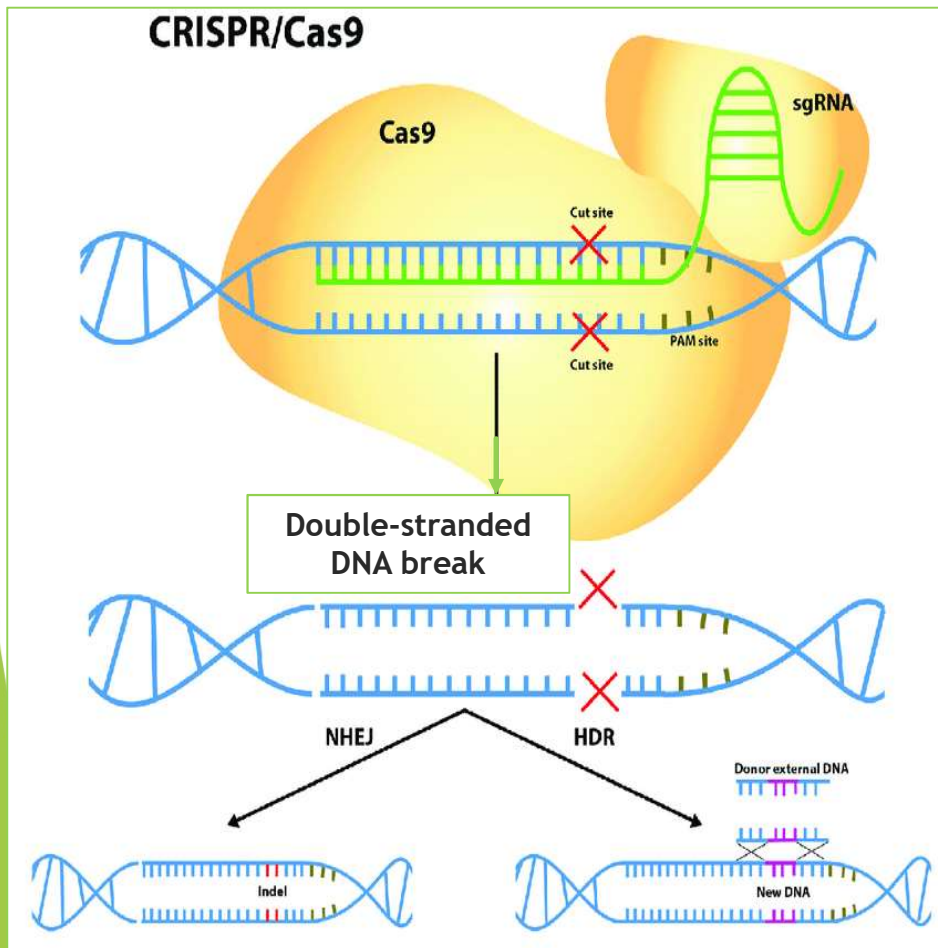
- drought
- salt
- heat
- cold
- $H_2O_2$
- Cd ...

➤ **BIOTIC** - viruses, bacteria, fungi, brown planthopper



# Knocking-out multiple *EXPANSINs* genes

- ▶ CRISPR/Cas9 - multicomplex mutagenesis (Richter *et al.*, 2018)
- ▶ To create *expa1 expa10 expa14 expa15* multiple knock-out



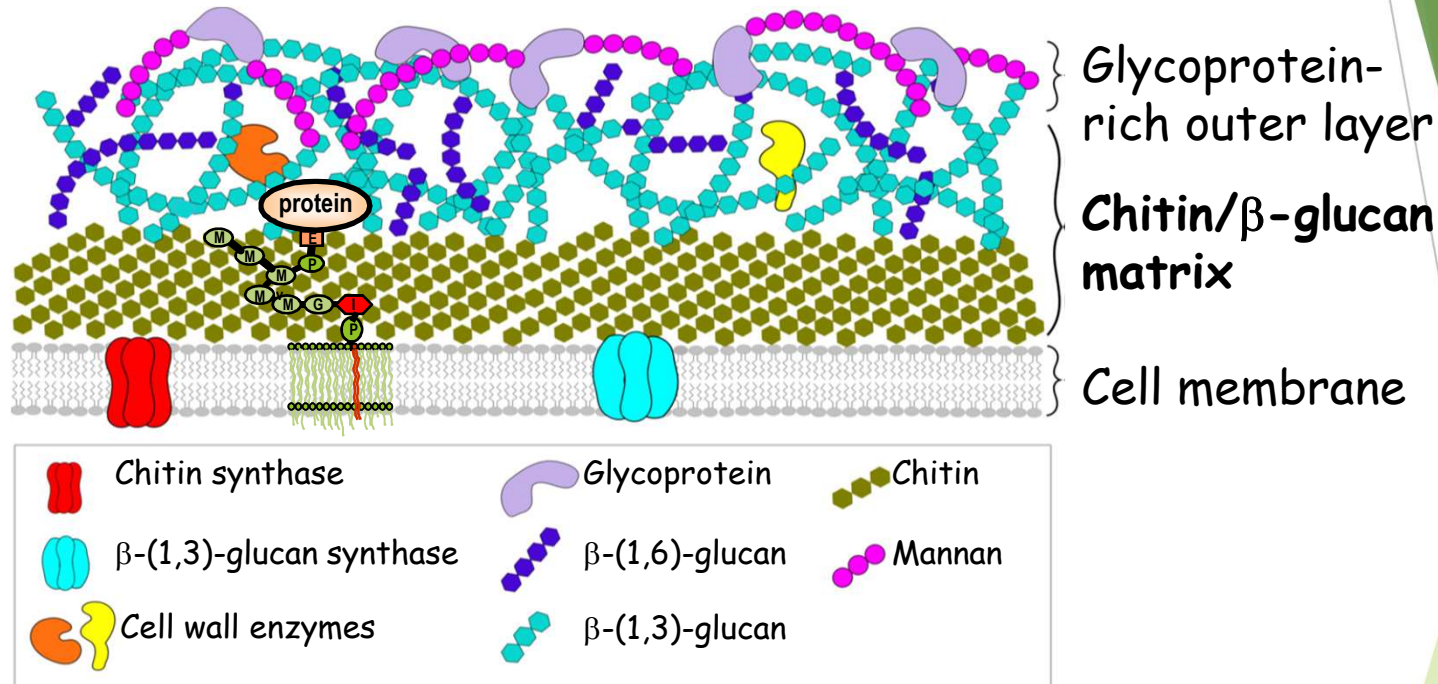
- Insertion changes the reading frame!
- Creates a stop codon after 47 aa (in the first exon of *EXPA10*).

The background features abstract, overlapping green geometric shapes in various shades, including light lime green, medium green, and dark forest green. These shapes are primarily located on the left and right sides of the slide, framing the central text. The overall aesthetic is clean and modern.

Not only plant cells have the *CW*...

# Unique composition of the fungal cell wall

- ▶ makes it an ideal target for the development of *fungicides!*



- ▶ **GPI** (**G**lycosyl**P**hosphatidyl**I**nositol) **A**nchored Proteins = **GAP**
  - ▶ CW modifying enzymes
  - ▶ E.g. **G**lucan **E**longation (**Ge**) proteins elongating β-1,3-glucan chains

## *Magnaporthe oryzae* the most devastating pathogen of rice!

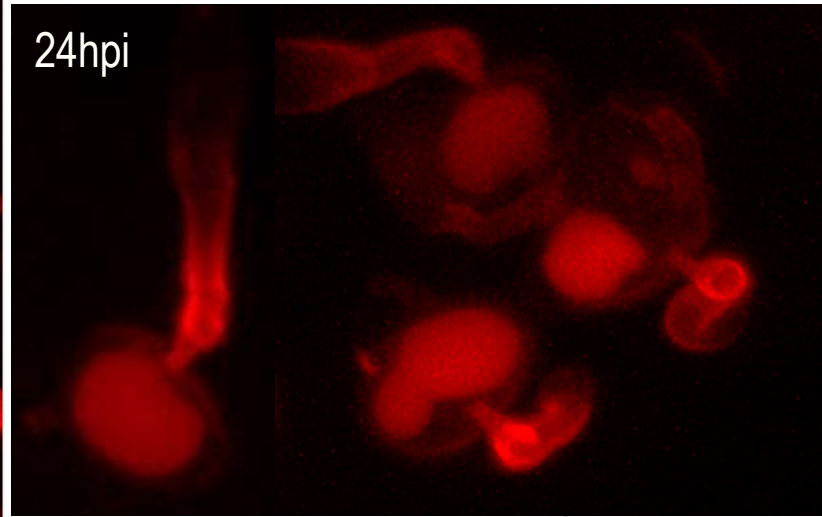
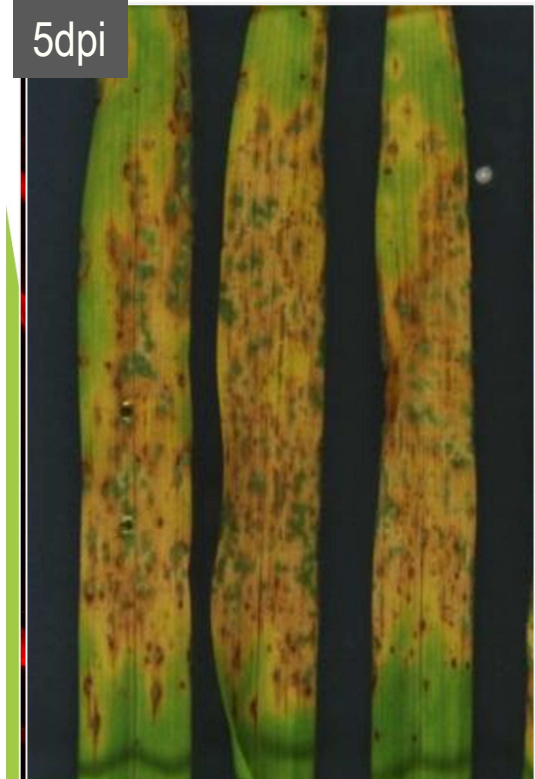
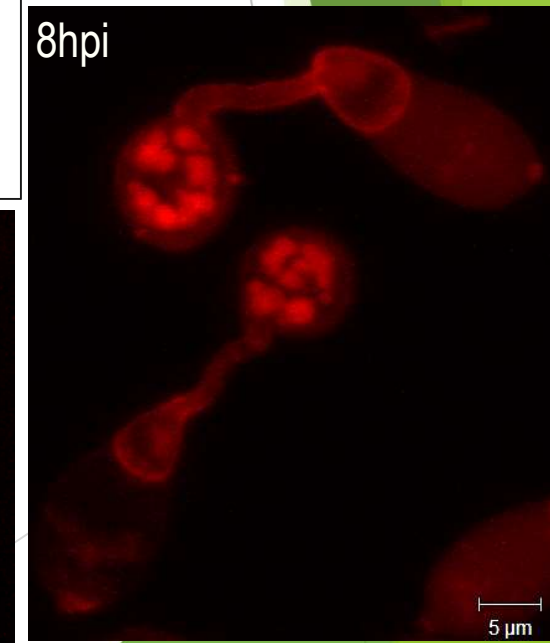
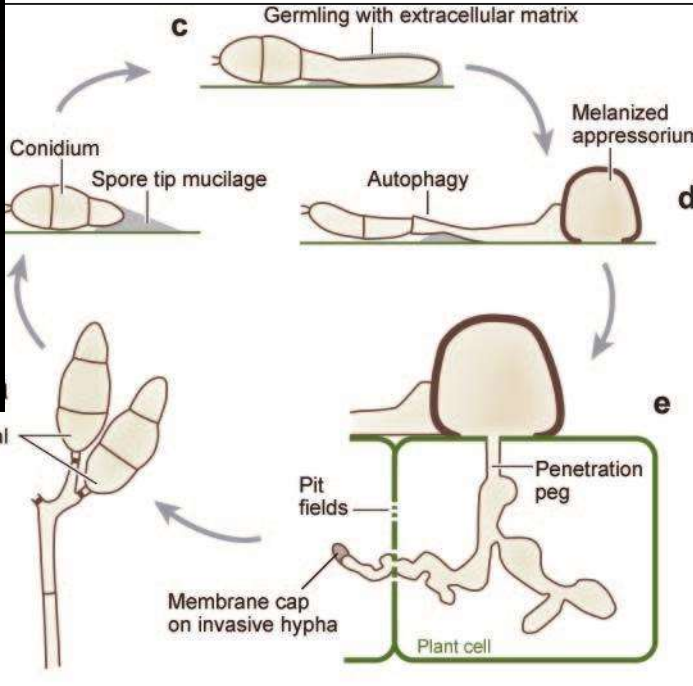
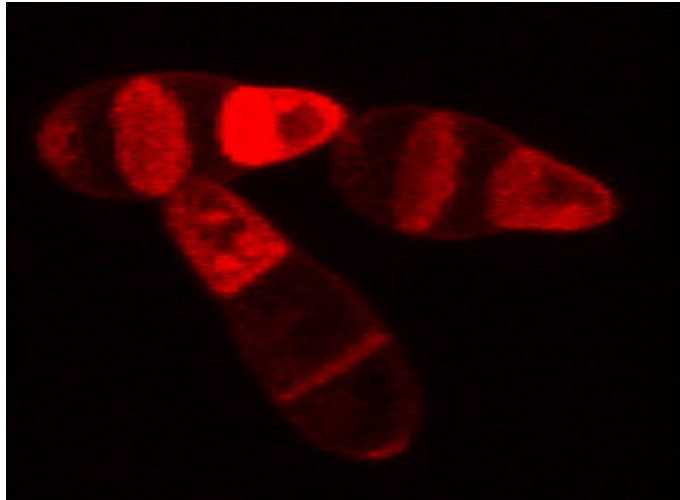
- ▶ **Model organism for plant pathogens: 1<sup>st</sup> sequenced** (Dean *et al.*, 2005)
- ▶ Hemibiotrophic filamentous *Ascomycete* fungus causing **rice blast!**
- ▶ Haploid, short (asexual) life cycle, gene deletions by homologous recombination.

### ➤ Food security & climate change



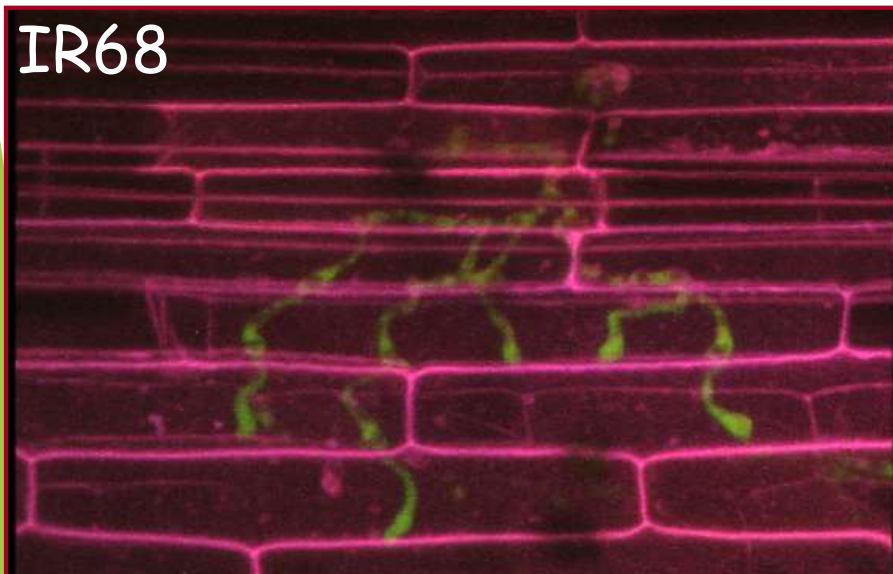
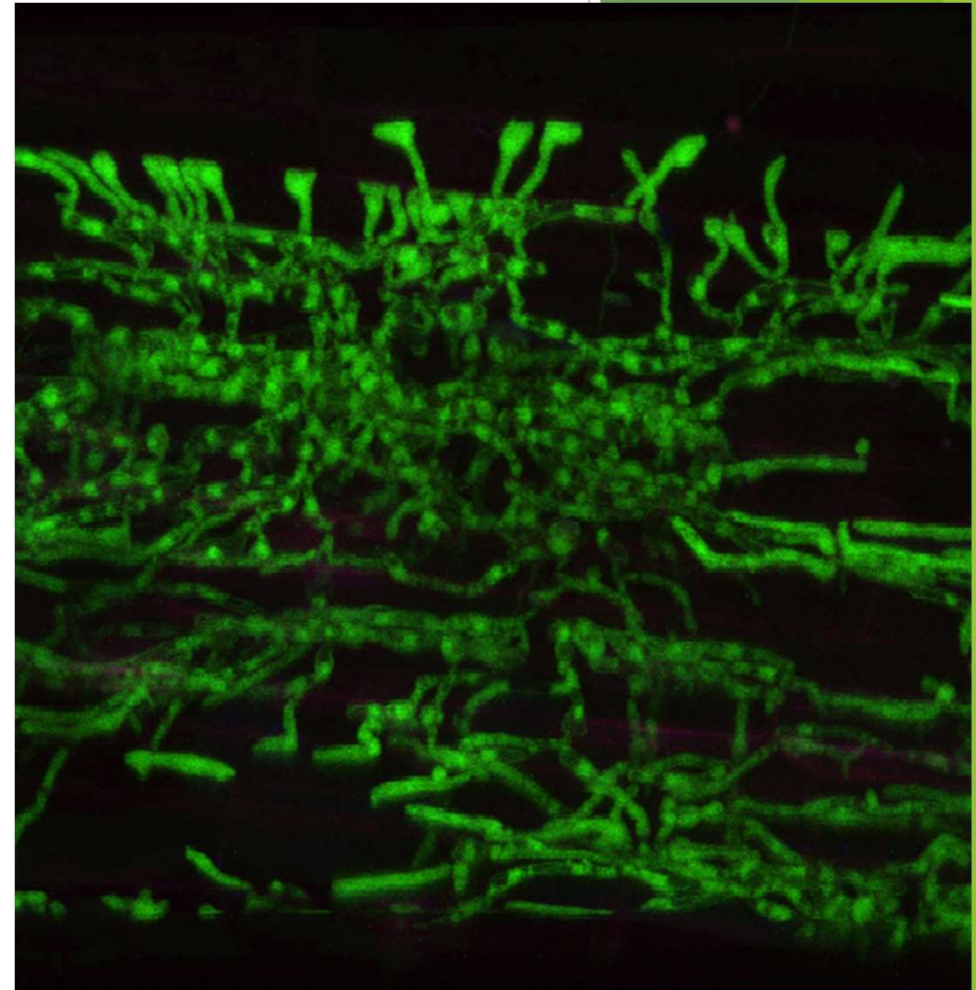
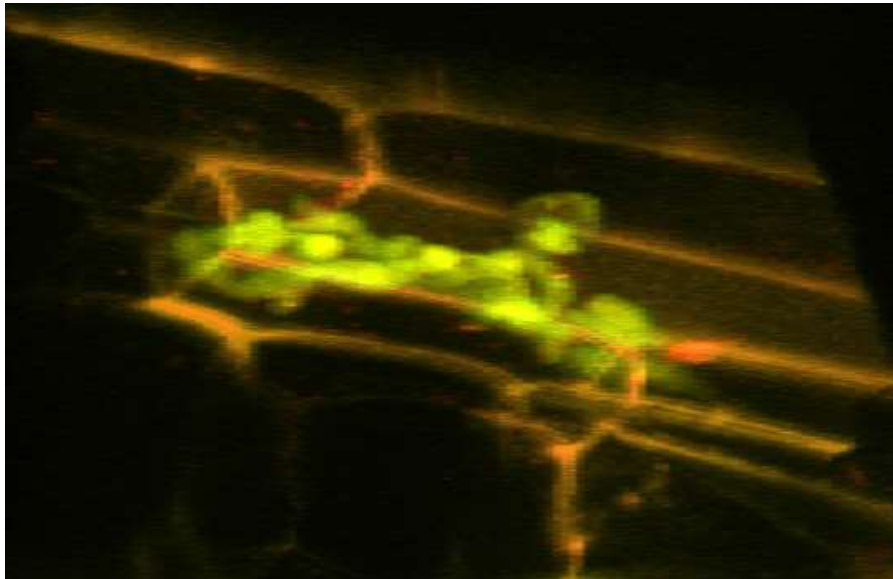
# Magnaporthe oryzae asexual life-cycle

*pGEL3::GEL3:mCherry*



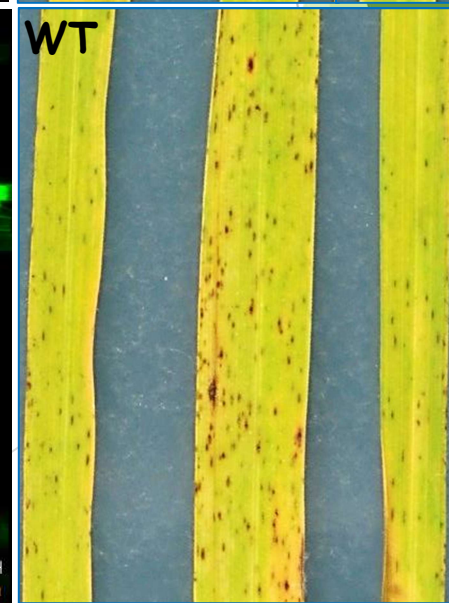
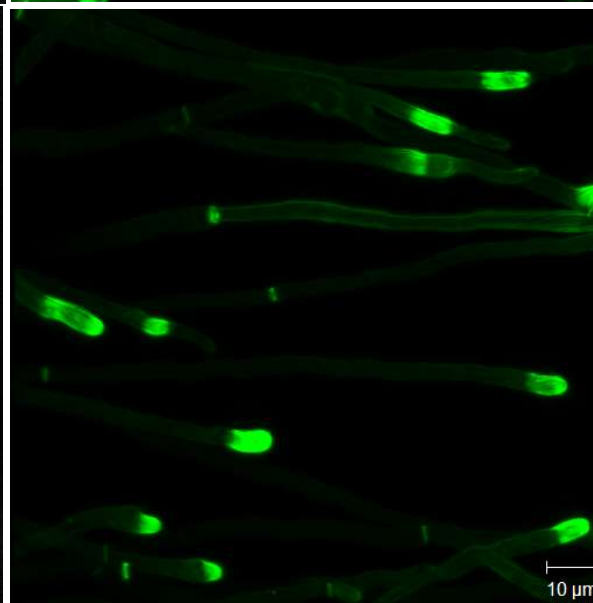
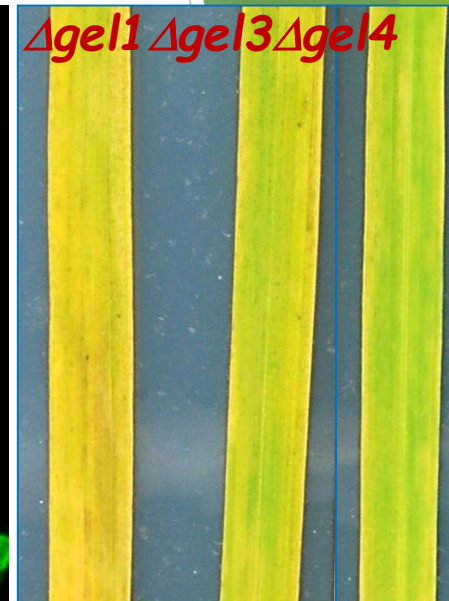
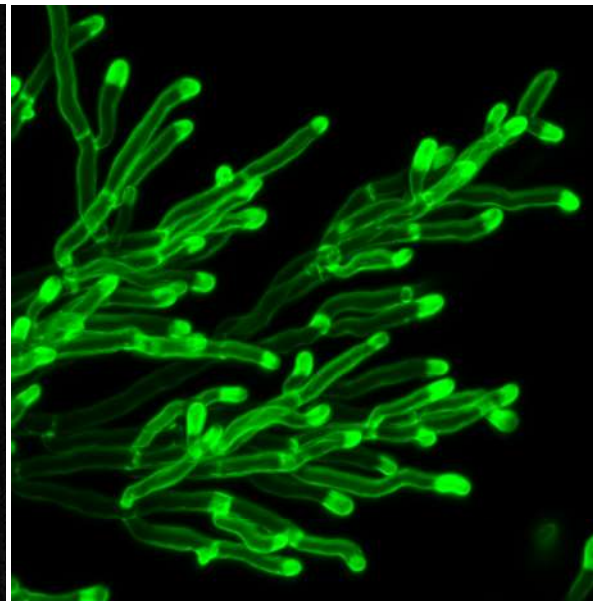
Samalaova et al., 2017

## Exploring redox state in susceptible & resistant rice



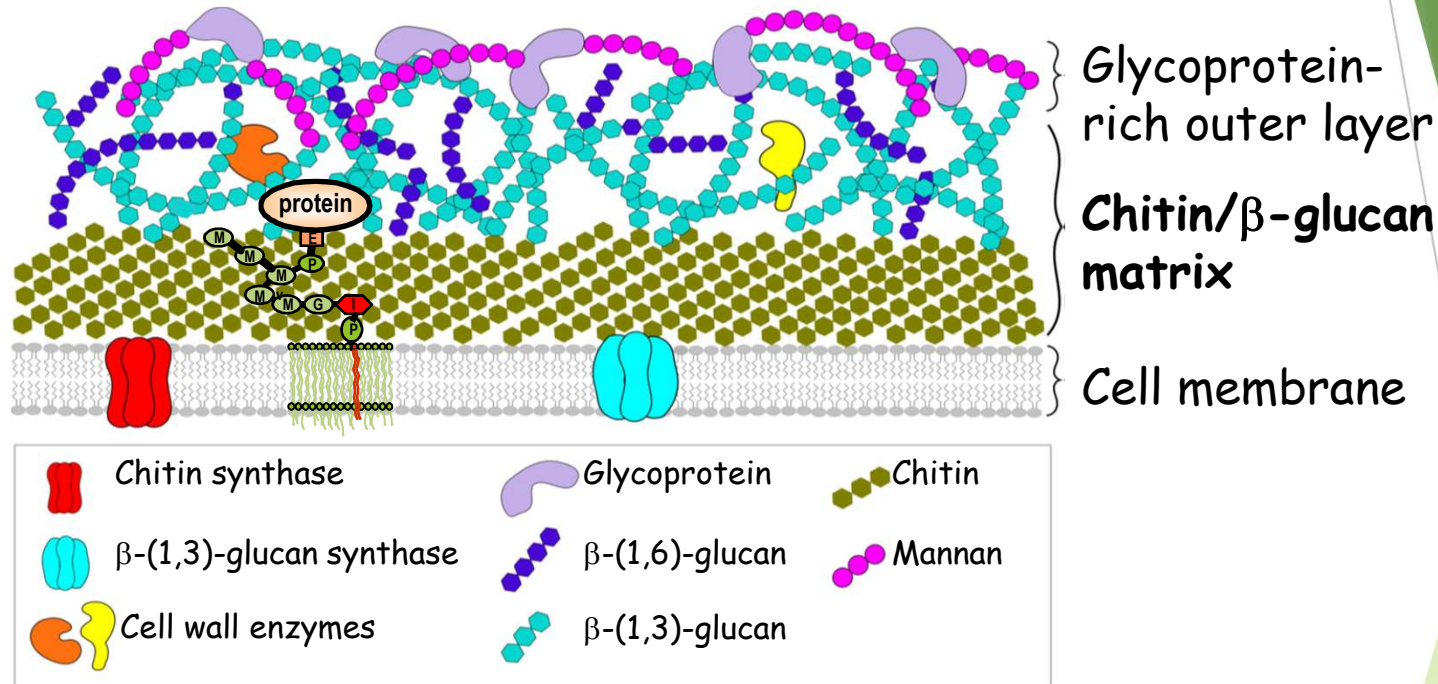
- ▶ ROS toxicity alone is NOT sufficient to kill *Magnaporthe oryzae* in resistant rice! (Samalao *et al.*, 2013; 2014)

*Triple  $\Delta gel1 \Delta gel3 \Delta gel4$  KO has reduced mycelial growth, hyper branching phenotype and is non-pathogenic!!!*



# Unique composition of the fungal cell wall

- ▶ makes it an ideal target for the development of *fungicides!*

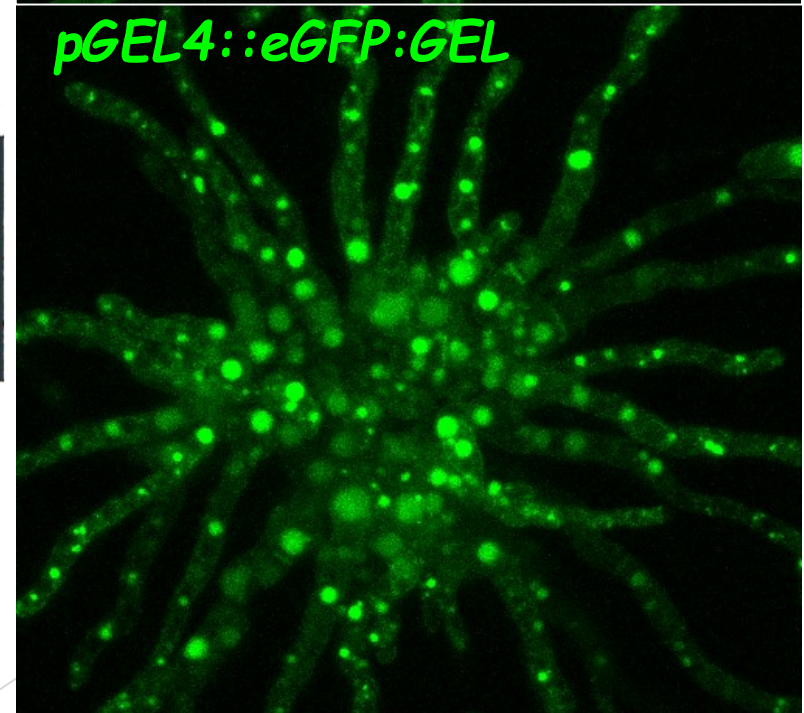
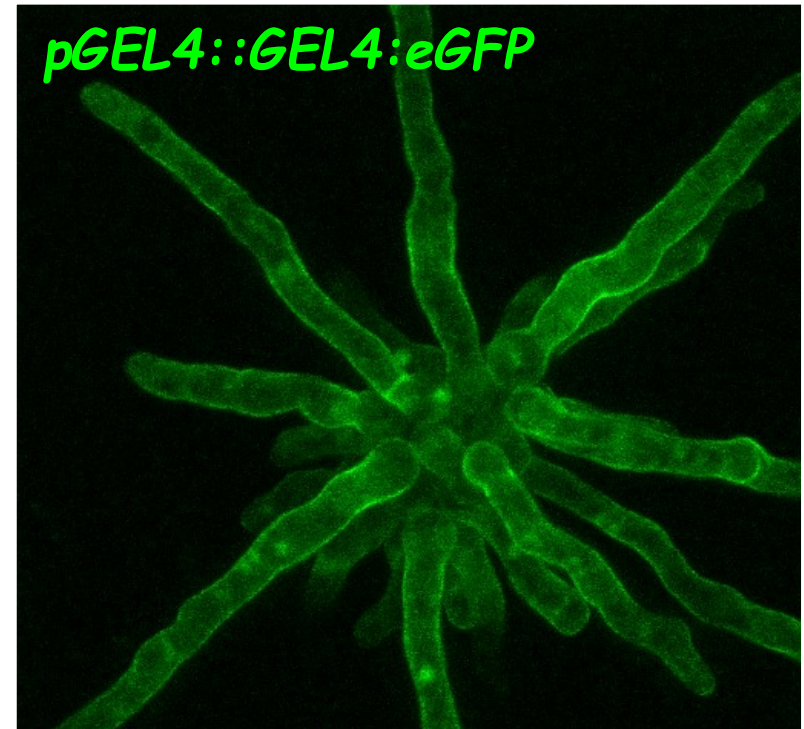
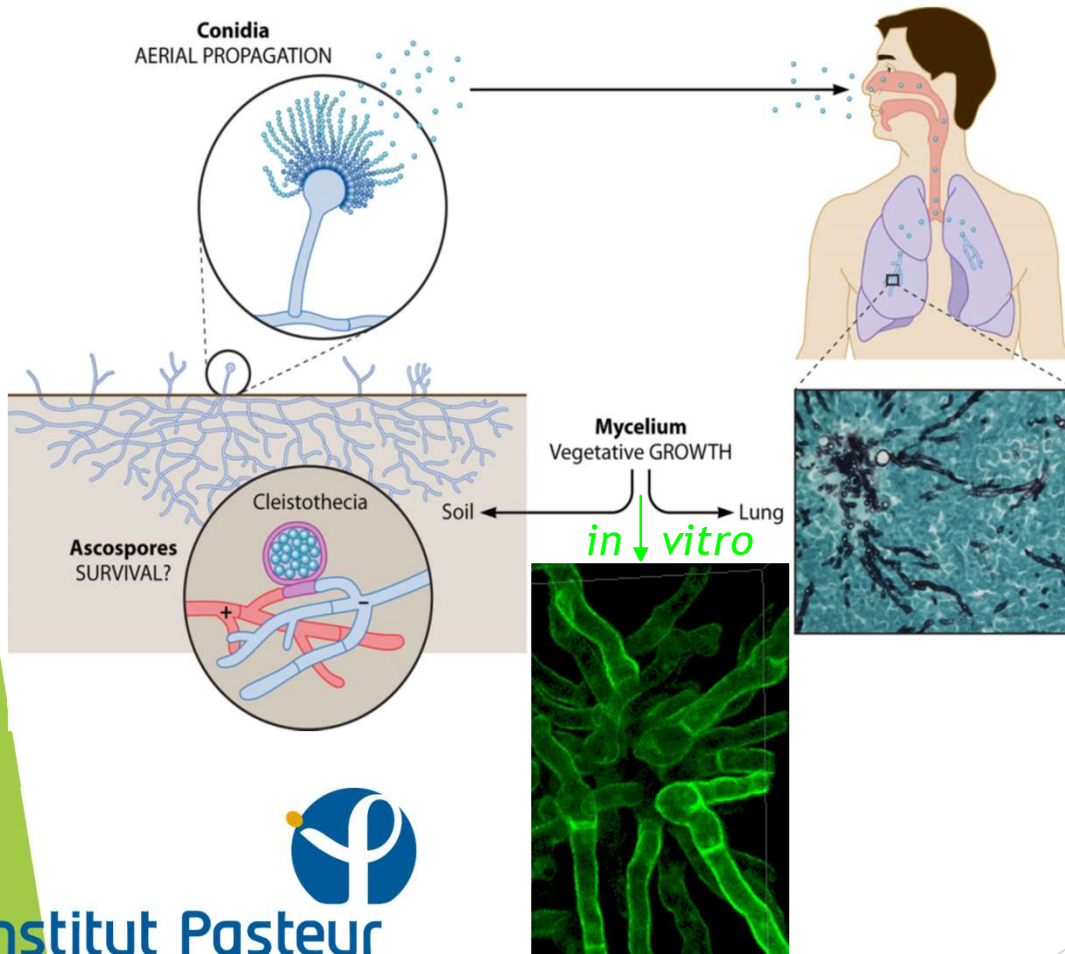


- ▶ **GPI** (**G**lycosyl**P**hosphatidyl**I**nositol) **A**nchored Proteins = **GAP**
  - ▶ CW modifying enzymes
  - ▶ E.g. **G**lucan **E**longation (**Ge**) proteins elongating β-1,3-glucan chains

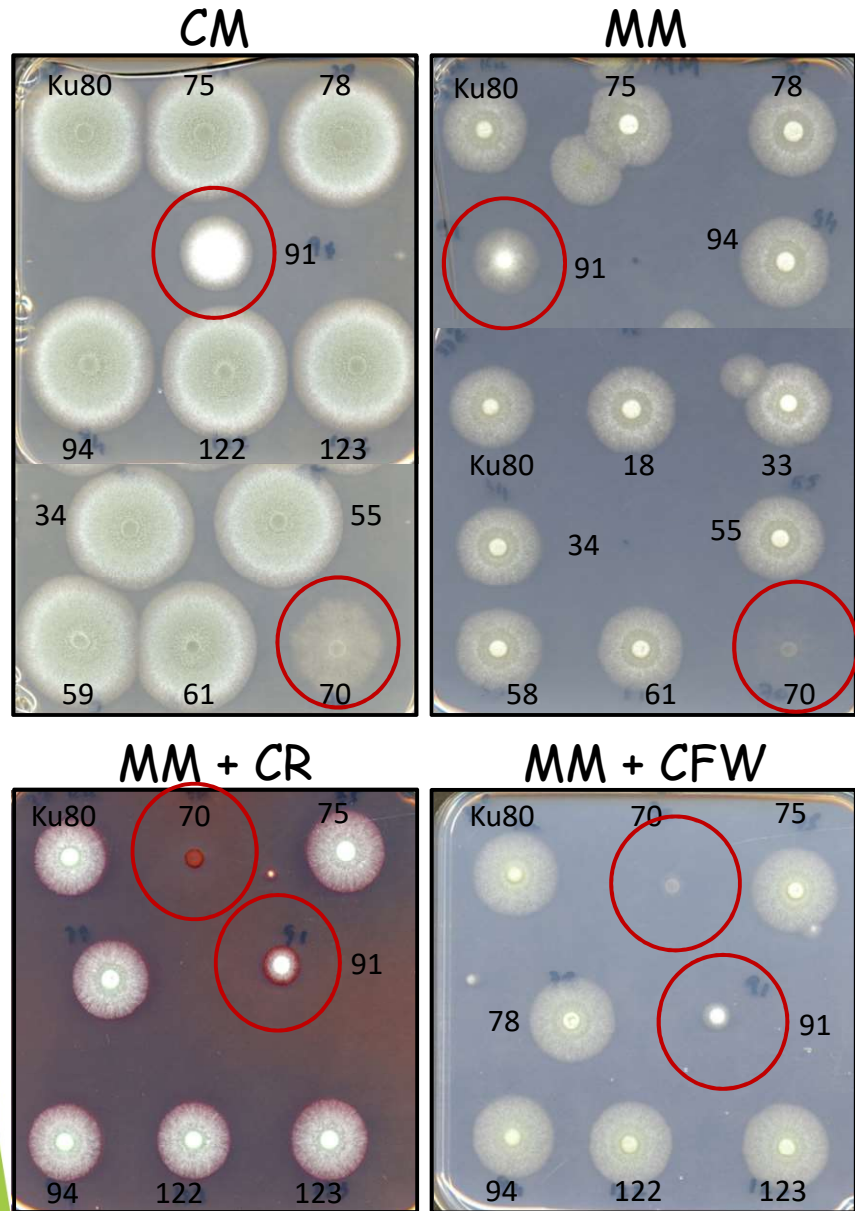


# *Aspergillus fumigatus* is a fungal saprotroph BUT opportunistic human pathogen!

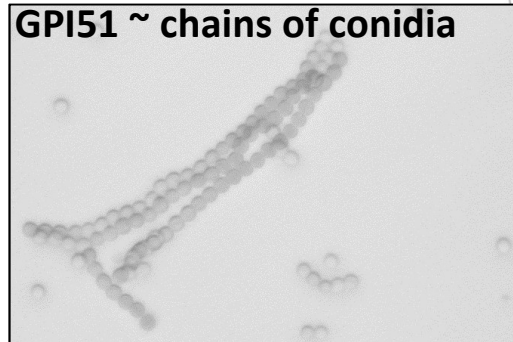
- Causes **aspergillosis** in immunocompromised patients... deadly



# How to knock-out 132 genes in one summer . . . .



- ▶ Single KO of **all GAP** proteins!!!
- ▶ Growth defects /phenotype on different type of media:
  - ▶ Complete & minimal medium
  - ▶ MM+CR or CFW ~ **CW stress**
  - ▶ MM+ SDS ~ **PM stress**
  - ▶ MM+H<sub>2</sub>O<sub>2</sub> ~ **oxidative stress**
- ▶ Spore phenotype



▶ A candidate gene is in a medical trial to test for reduced immune response....(Samalova *et al.*, 2020)

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