

# Plant Cell and Molecular Biology

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jihomoravský kraj

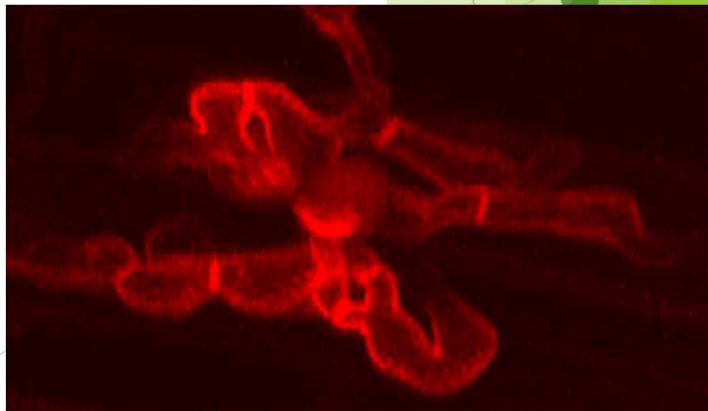


Oddělení  
experimentální  
biologie rostlin

# OUTLINE of the talk

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

*Arabidopsis thaliana*



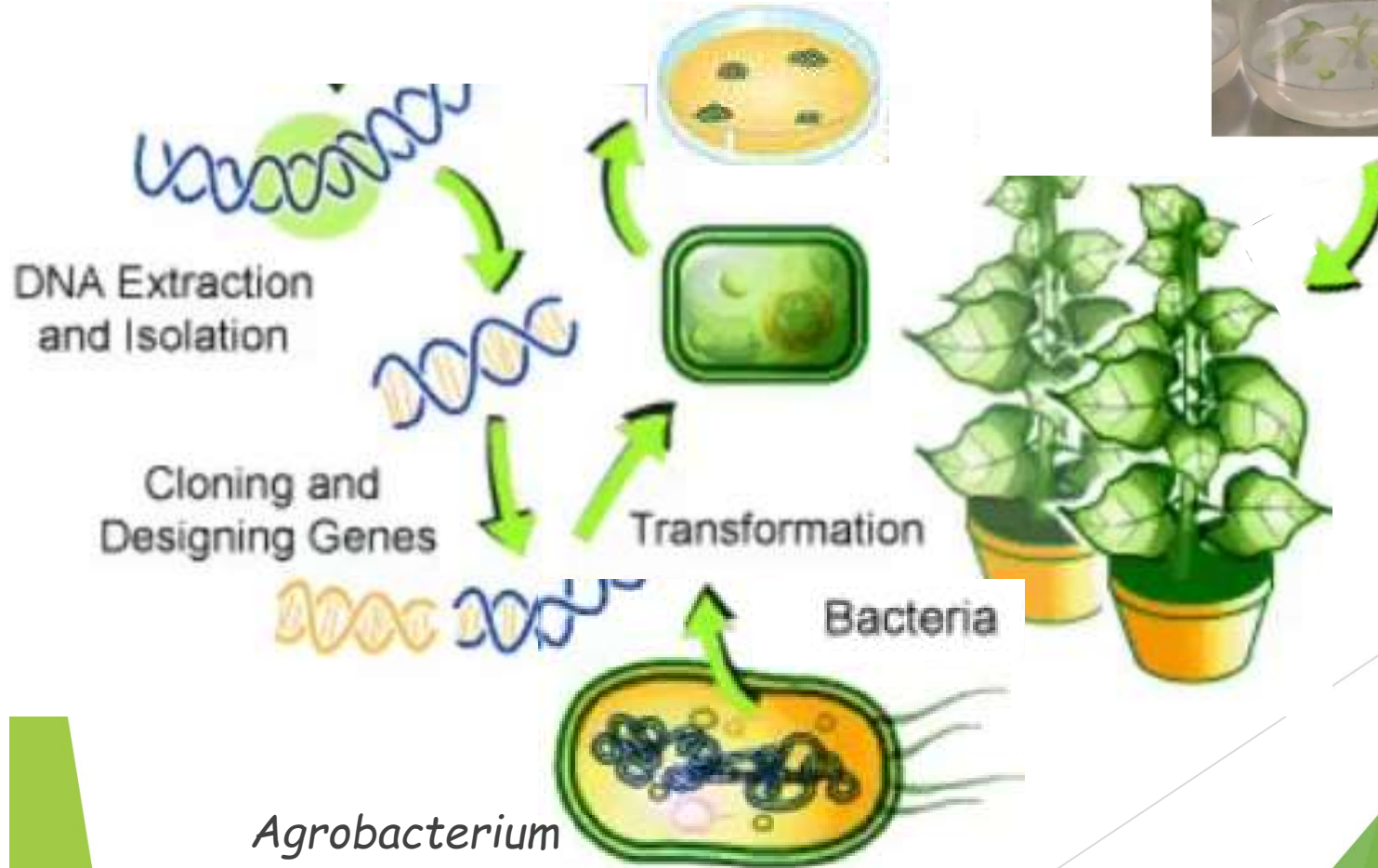
# How to make a genetically modified or genome-edited plant?



# Transformation

## ► Tissue cultures

- tobacco
- rice



*Arabidopsis thaliana*

## ► Floral dip



*Arabidopsis*



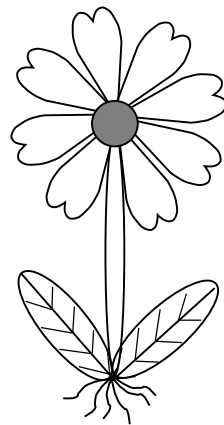
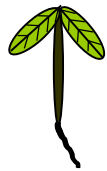
How to regulate (trans)gene  
expression?



# Chemically inducible gene expression systems in plants

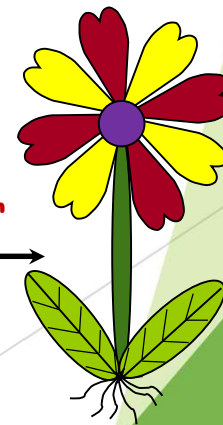
- ▶ regulate (trans)gene expression at a particular developmental stage and for a specific duration using chemical inducers.
- ▶ Expression can be **SWITCHED ON** or **OFF** using chemical inducers.
  - ▶ Gene overexpression, knock-down expression by amiRNAs, knock-out gene by combining the system with CRISPR/Cas9 (Gehrke et al., 2023)
- ▶ Essential for expression of gene products that interfere with regeneration, growth or reproduction...

Meristem  
defect



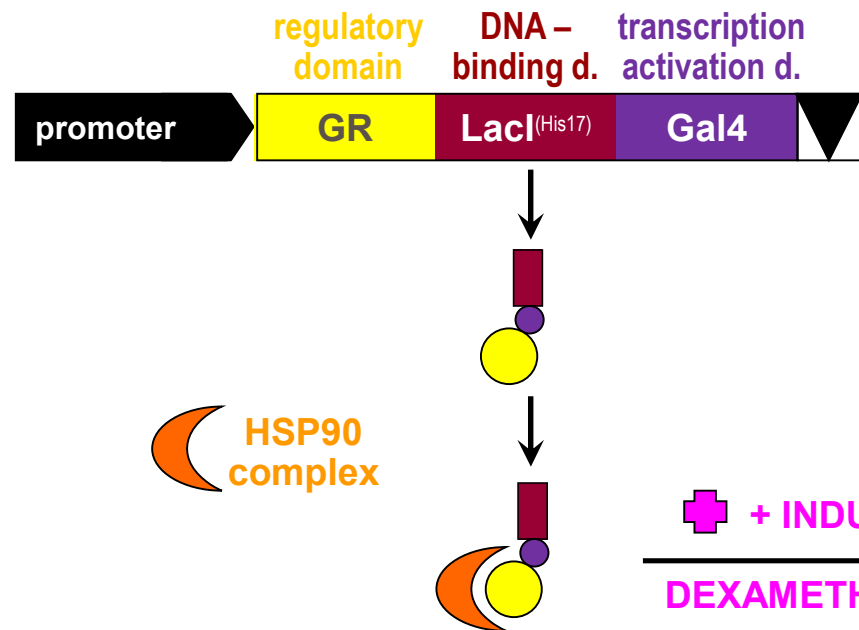
+ Inducible  
gene

+ inducer

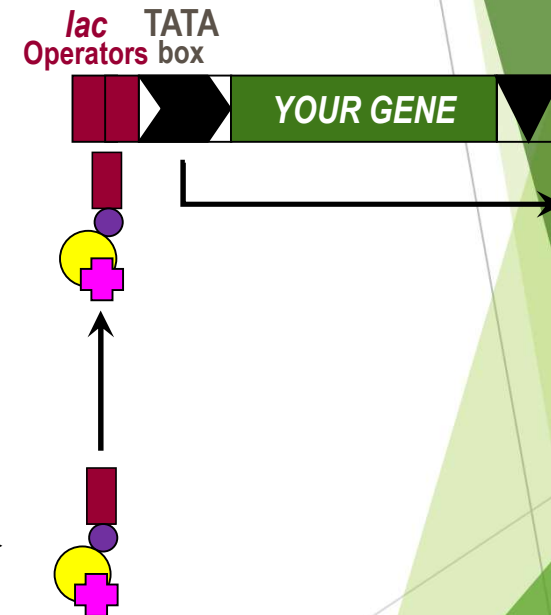


# The chemically inducible transcription activation system pOp/LhGR

## LhGR ACTIVATOR



## pOp REPORTER



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

## An ideal inducible system

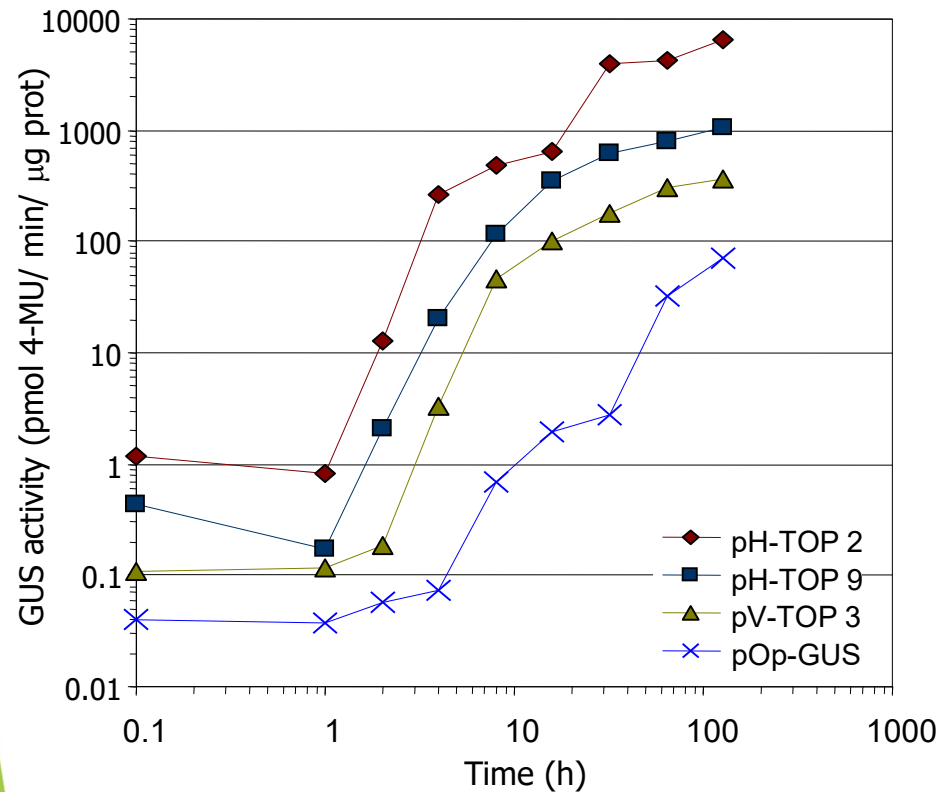
- ▶ High induced expression (e.g. 1000x or more).
- ▶ No uninduced expression (not leaky).
- ▶ Rapid uptake and wide distribution of inducer.
- ▶ No toxicity, no physiological effects in plants.
- ▶ Convenient application by a number of methods.
- ▶ Functional in several plant species.

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



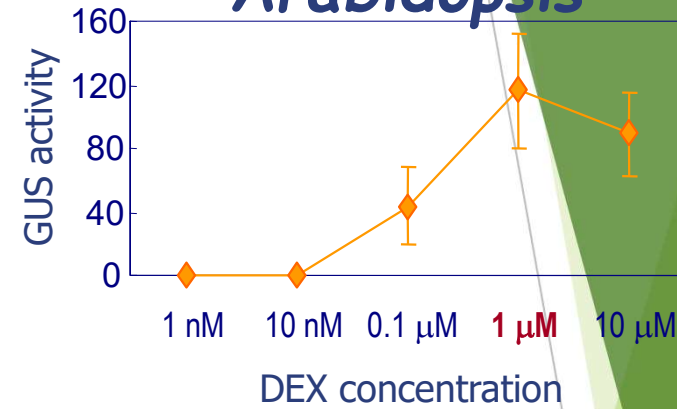
# The pOp6/LhGR is highly inducible, fast & v. sensitive

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

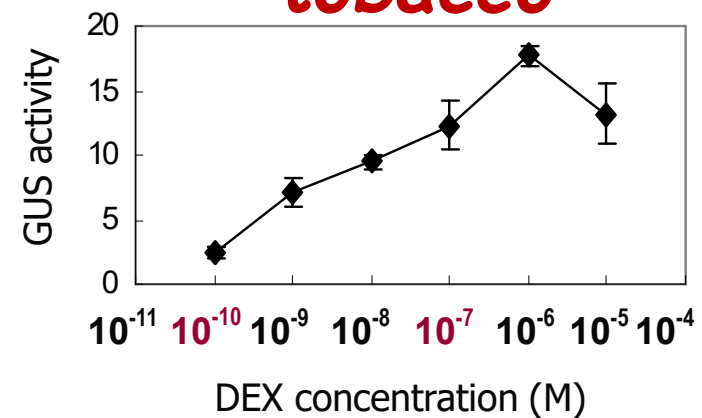


- ▶ Increase of GUS activity in 2h!

## Arabidopsis



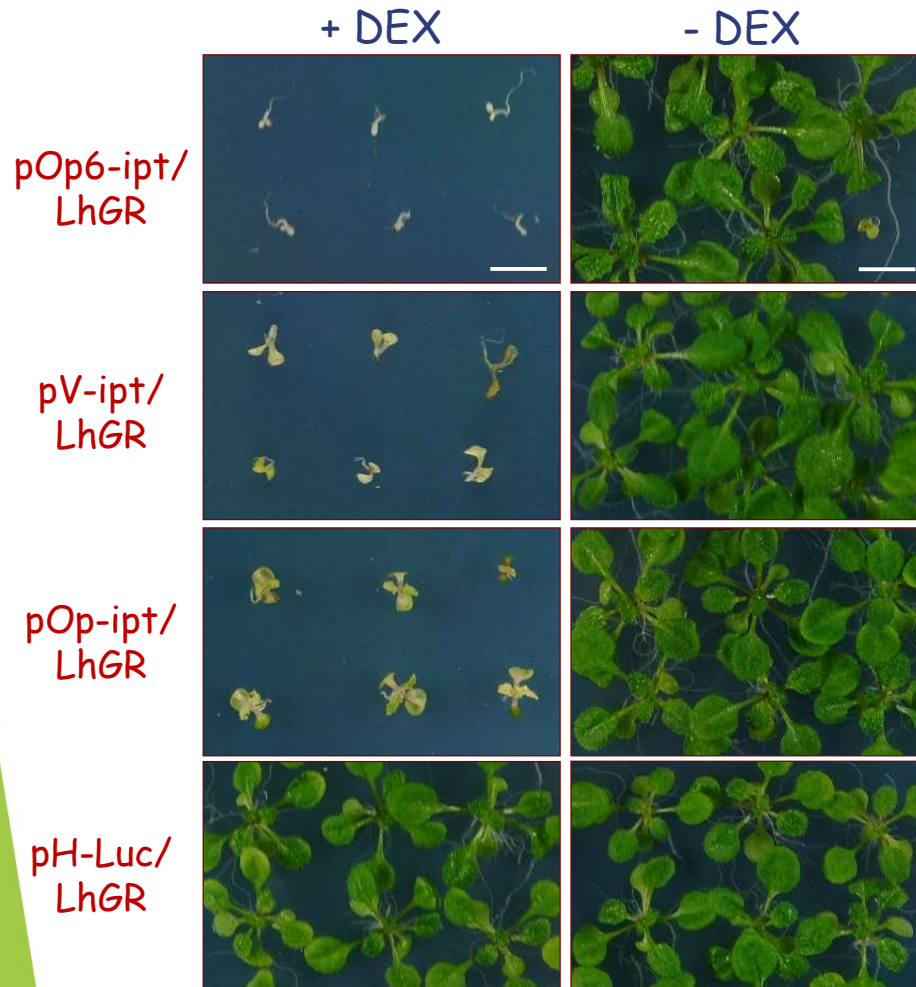
## tobacco



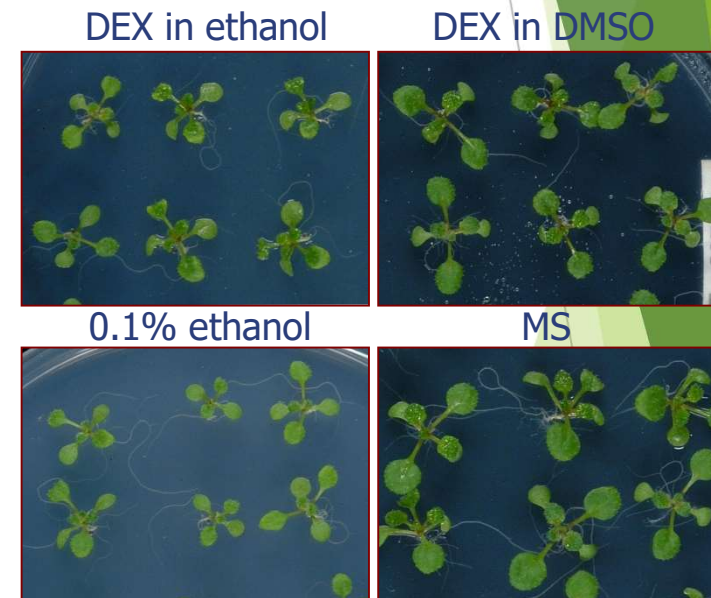
- ▶ The most sensitive system for tobacco!

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

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

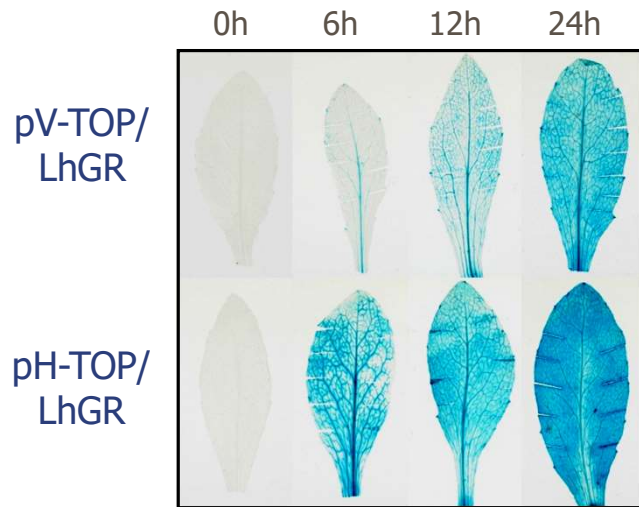


- ▶ neither DEX nor LhGR affects endogenous processes in plants ... **though ethanol does!**

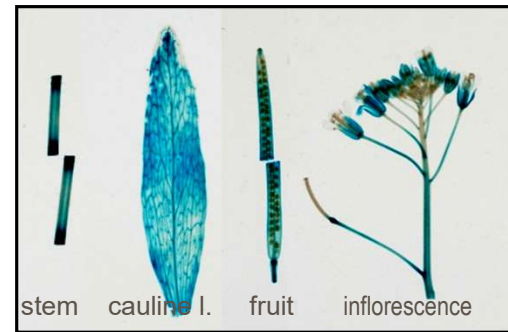


Arabidopsis seedlings were grown on plates in the presence or absence of 10  $\mu$ M DEX.

# The pOp6/LhGR system is inducible by various methods

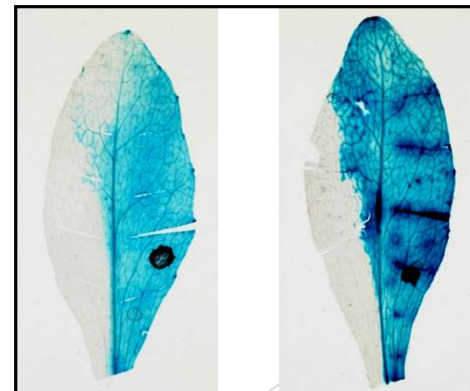
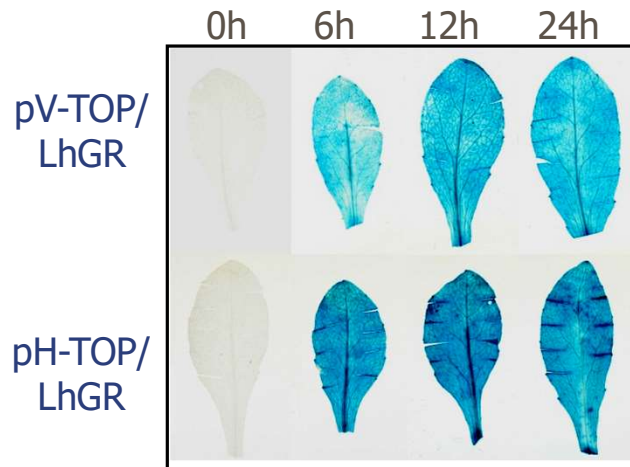


## Watering plants with DEX



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

## Painting plants with DEX



*A leaf half painted with DEX*

# The pOp6/LhGR system is functional in several species

**Arabidopsis**

(Craft, Samalova et al., 2005)

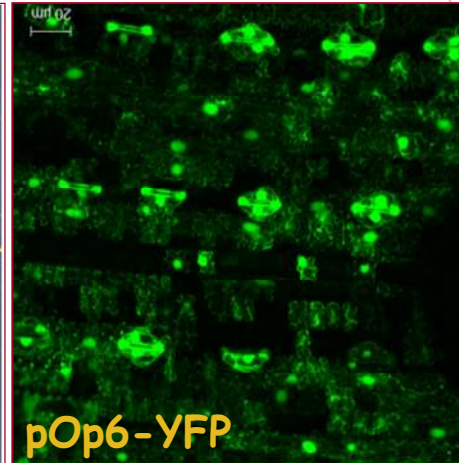
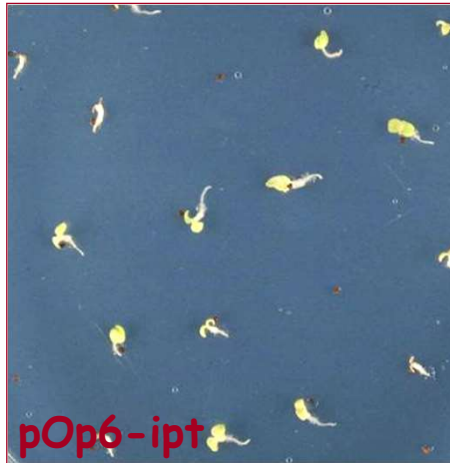
**Tobacco**

(Samalova et al., 2005)

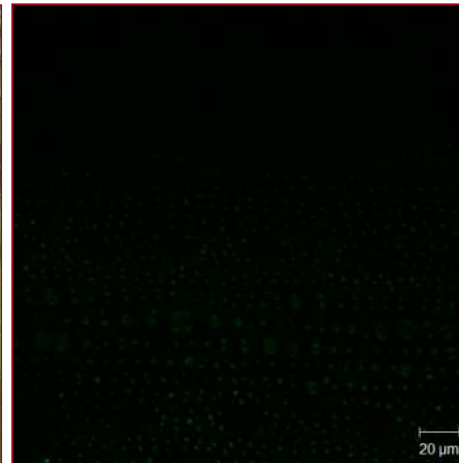
**Rice**

(Samalova & Moore, 2021)

+ DEX



- DEX

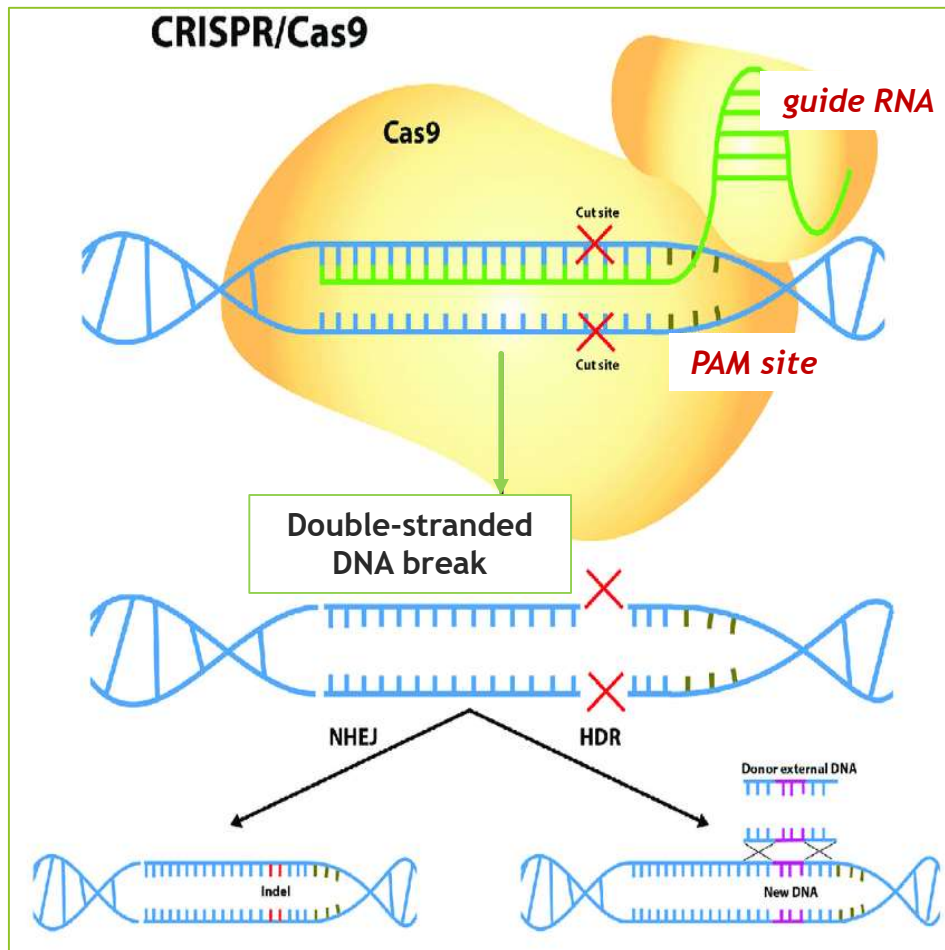


- ▶ *Maize, potato, tomato, Cardamine hirsuta, citrus...*
- ▶ Detailed step-by-step protocols in Samalova et al., 2019



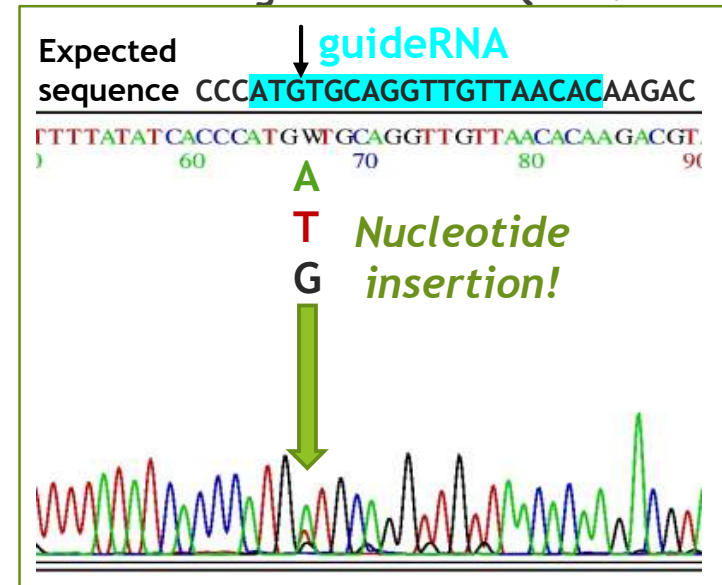
# CRISPR/Cas9 bacterial system adapted to edit the genome of various species ~ "genetic scissors"

- ▶ The ability of Cas9 (nuclease) to target a specific site of genomic DNA using gRNA
  - ▶ 2020 Nobel Prize in chemistry awarded to E. Charpentier a J. Doudna



## ▶ Genome-edited organism

- ▶ Changes in the open reading frame (ORF) generate a stop codon!  
Creating "knock-out" (KO mutant)



**CRISPR:** Clustered Regularly Interspaced Short Palindromic Repeats  
**PAM:** Protospacer Adjacent Motifs

[https://www.youtube.com/watch?v=4YKFw2KZA5o&ab\\_channel=naturevideo](https://www.youtube.com/watch?v=4YKFw2KZA5o&ab_channel=naturevideo)

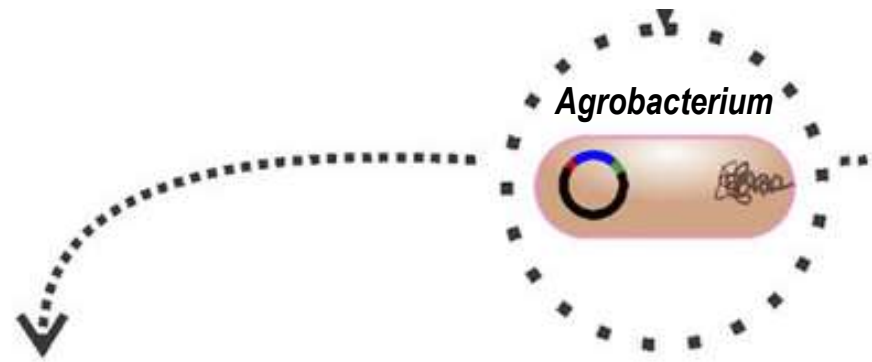
The background features abstract, overlapping green geometric shapes. On the left, a solid green triangle points downwards. On the right, a complex arrangement of semi-transparent green polygons in various shades (from light lime to dark forest green) creates a layered, architectural effect. A thin, light gray line extends from the bottom right towards the center of the page.

# Transient gene expression and fluorescent proteins



# Transient gene expression assay

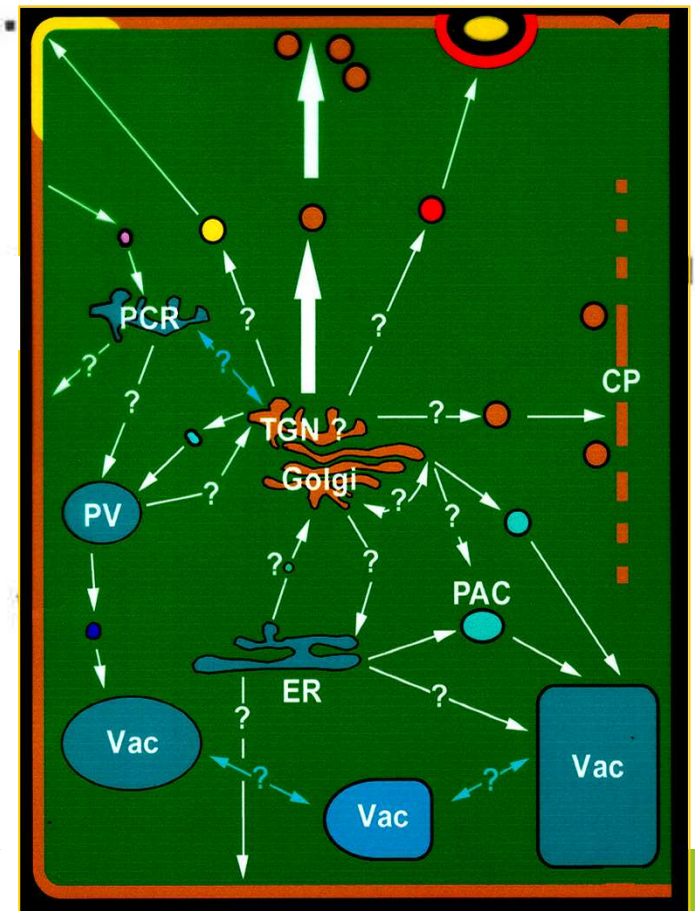
- ▶ **AGROINFILTRATION** method
- ▶ *Agrobacterium* infiltrated into tobacco plants



## 3. TRANSIENT GENE EXPRESSION ASSAY



- ▶ e.g. to study plant endomembrane trafficking



# Use of fluorescent proteins (FP) in cell biology

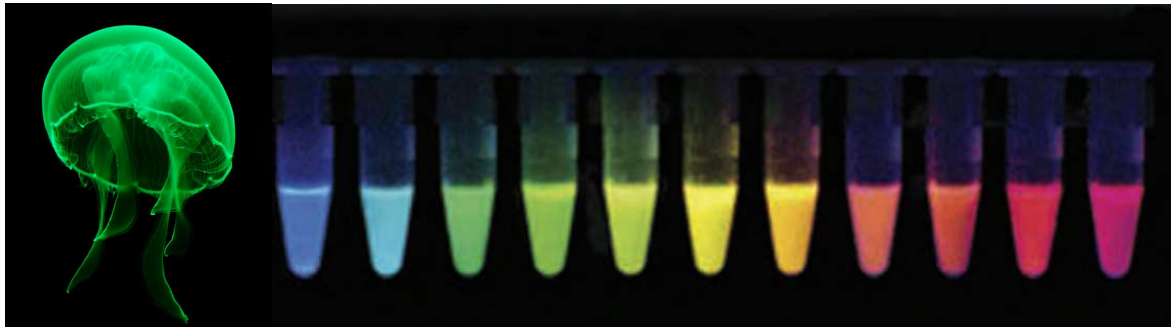
- ▶ Protein localization, protein-protein interactions...

GFP ~ green FP from jellyfish *Aequorea victoria*

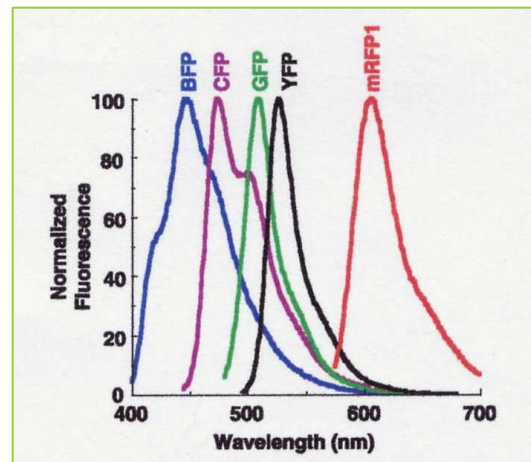
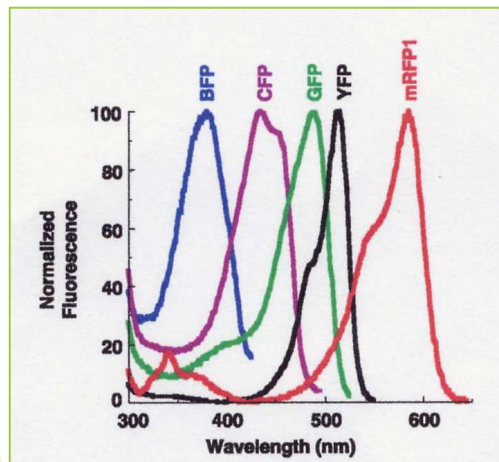
YFP - yellow FP mutant variant of GFP

mRFP1 - monomeric red FP from *Discosoma coral*

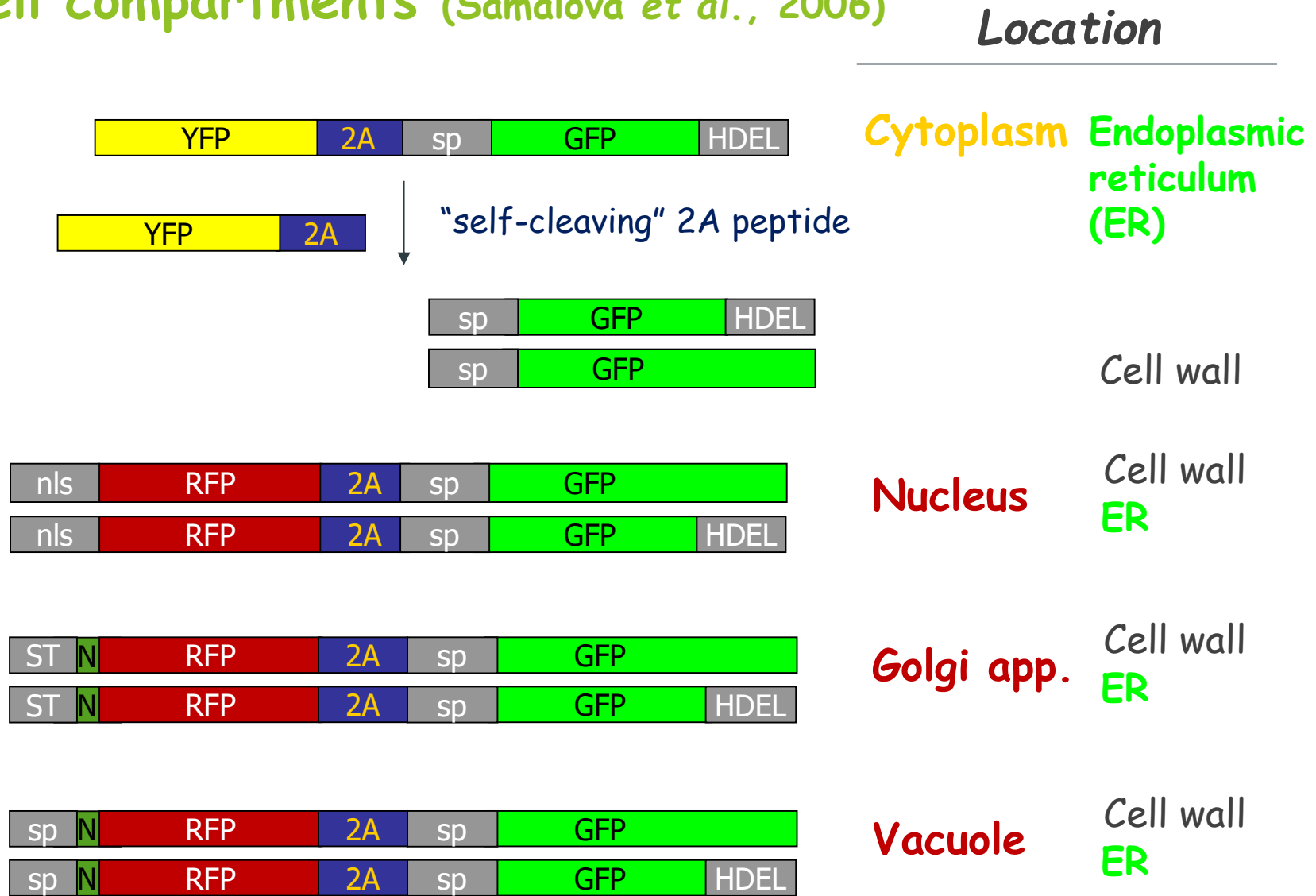
- ▶ CLSM ~ confocal laser scanning microscope
- ▶ Generates optical slices through live specimens.



- ▶ Excitation spectra
- ▶ Emission spectra

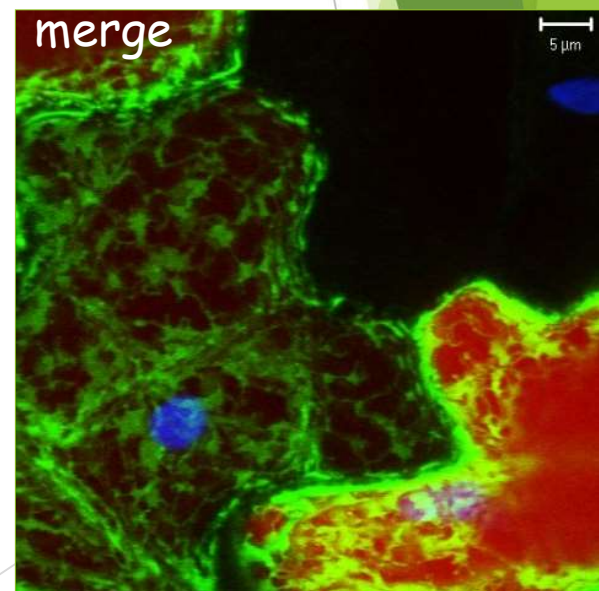
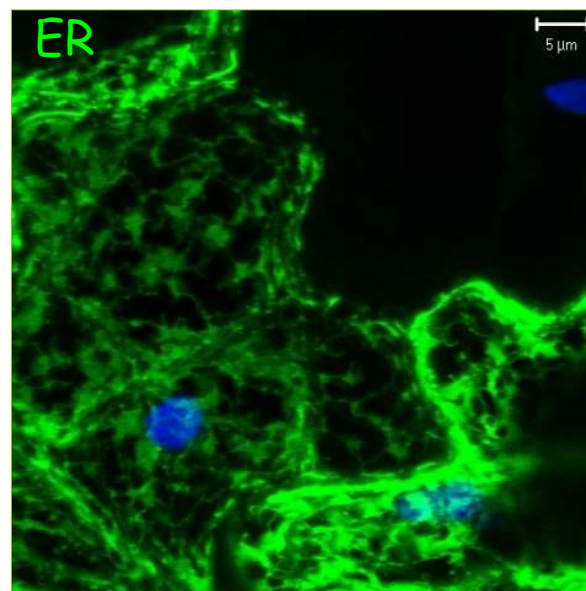
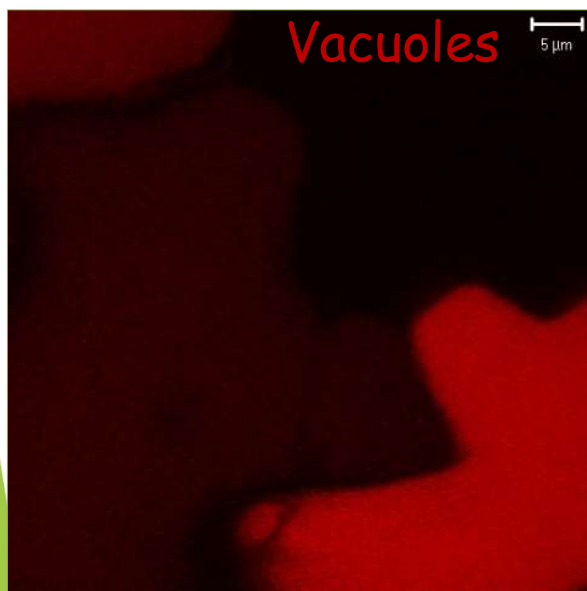
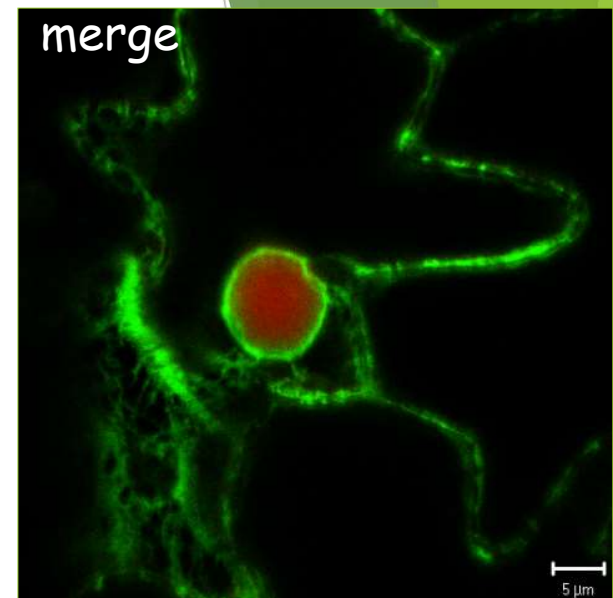
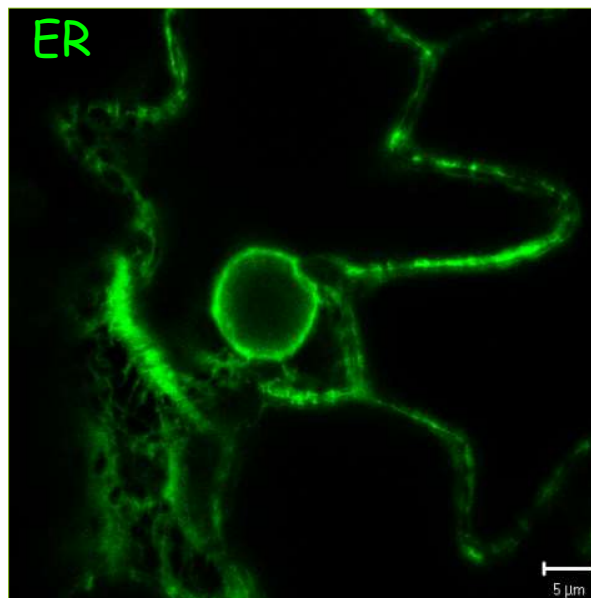
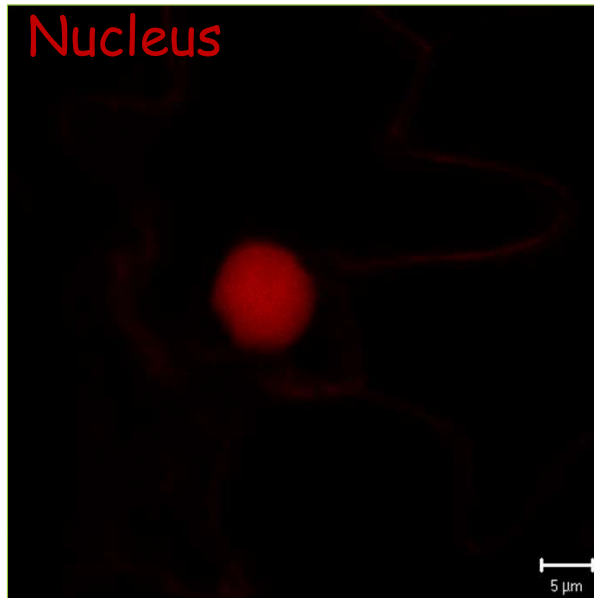


# Targeting fluorescent fusion proteins into different cell compartments (Samalova et al., 2006)

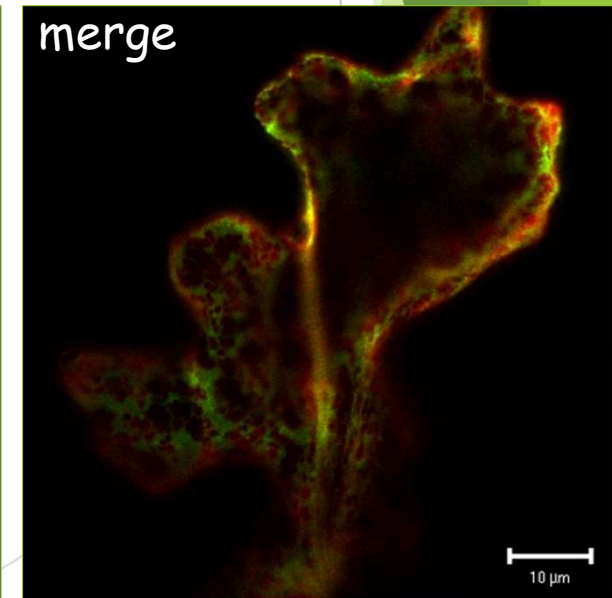
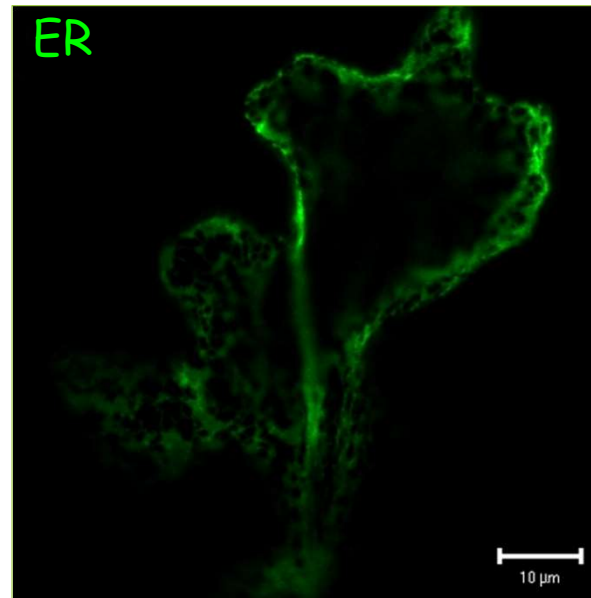
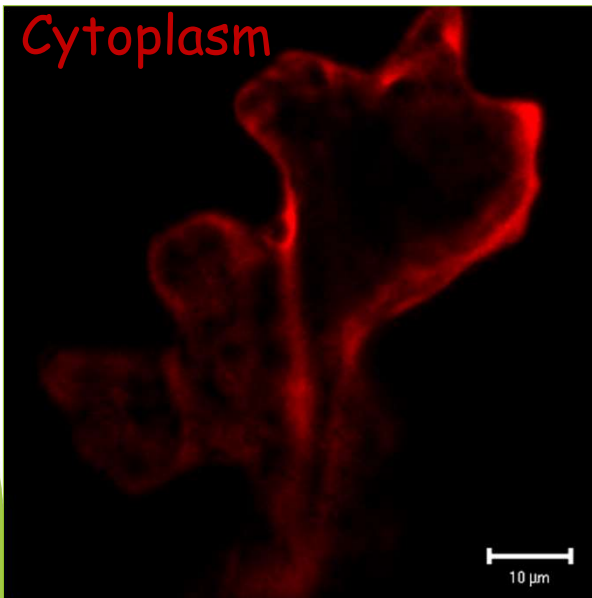
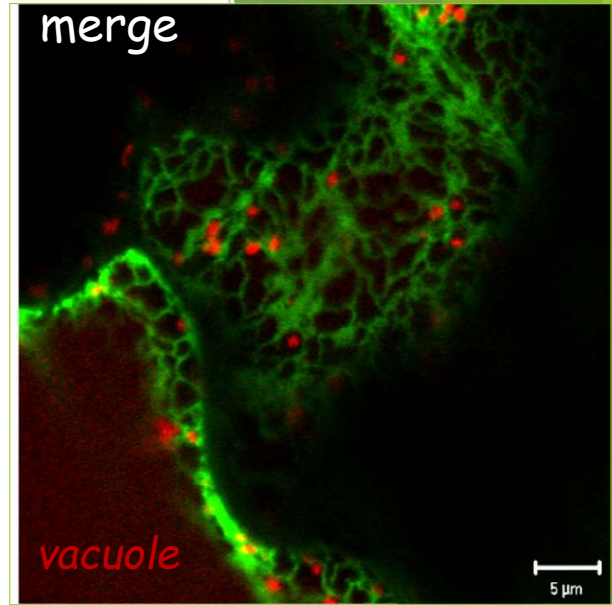
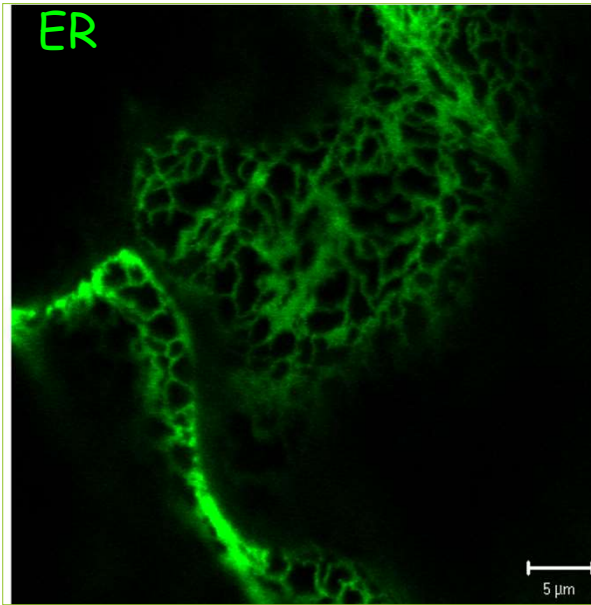
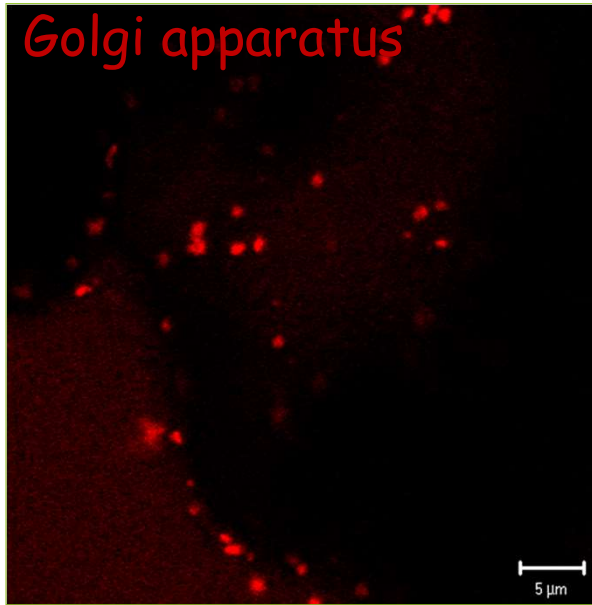




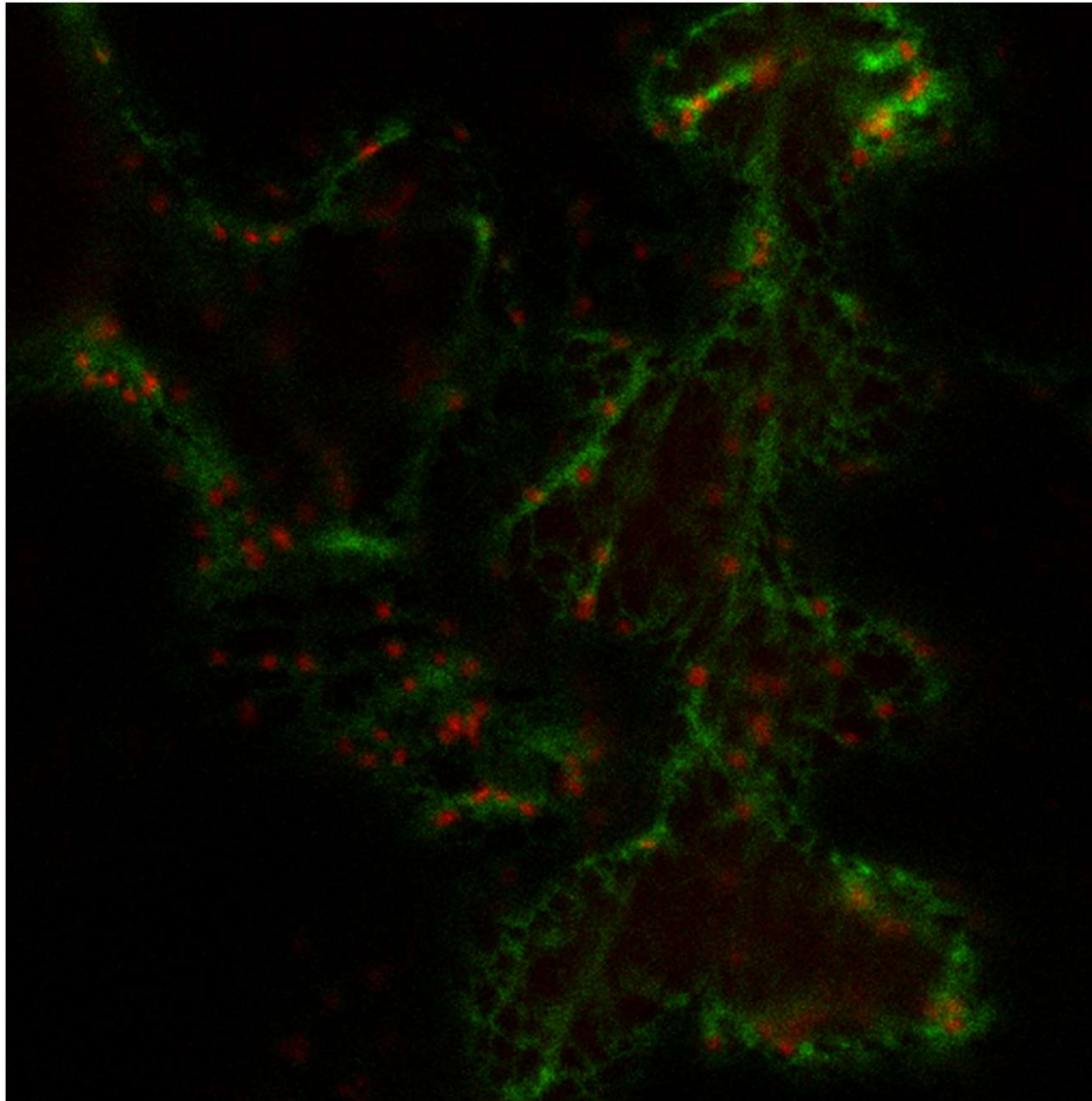
nls RFP 2A sp GFP HDEL



sp N RFP 2A sp GFP HDEL



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



PLANTS  
ARE  
MOVING!

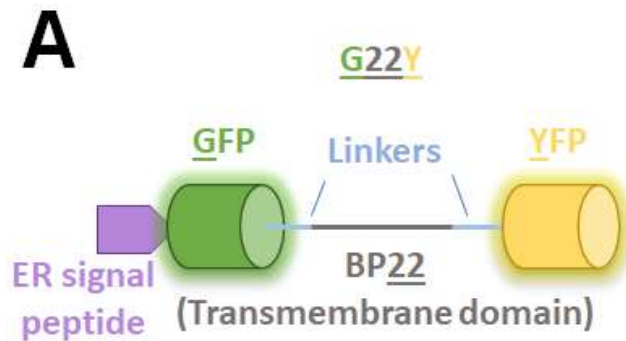


The background features abstract, overlapping green geometric shapes in various shades, including light lime green, medium green, and dark forest green. These shapes are primarily located on the left and right sides of the slide, framing the central text. The overall aesthetic is clean and modern.

**Create your own compartment :)**

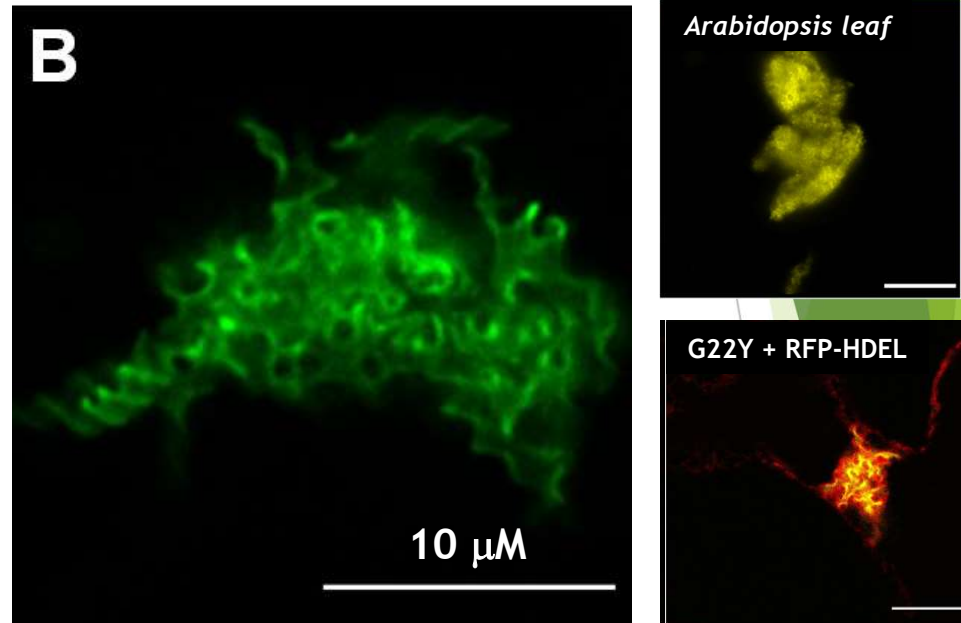
# A tool for plant synthetic biology

- substantial expansion of the endomembrane system in each cell of the plant (Sandor, Samalova *et al.*, 2024)



**OSER**

*Samalosome*



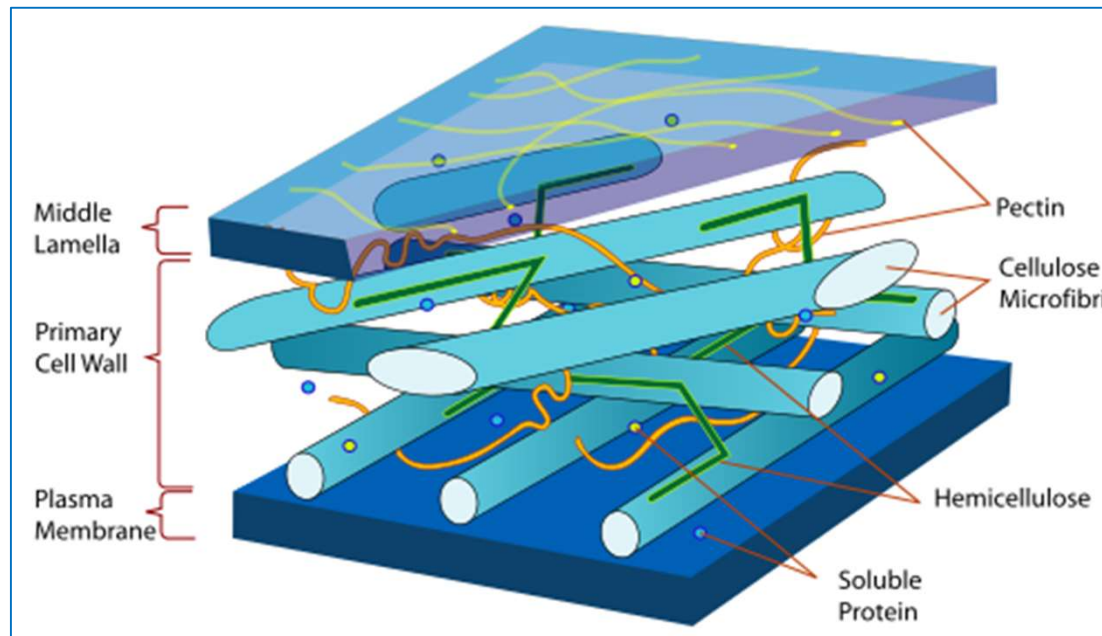
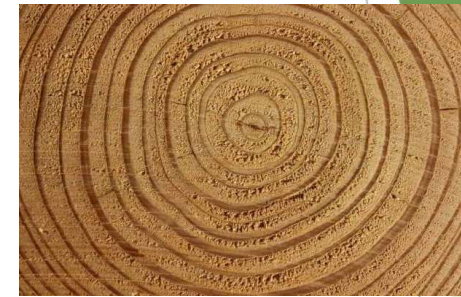
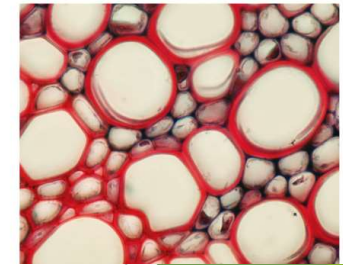
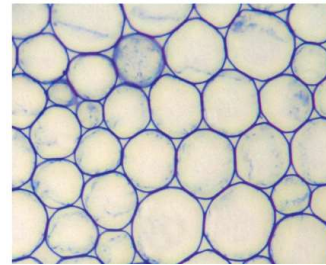
- *Organised Smooth Endoplasmic Reticulum*
- Potential applications of the synthetic compartment for the metabolic engineering of plants, e.g. recombinant or toxic proteins.
- No detrimental effects in plants!



Plant cell wall (*CW*)

# CW is crucial for plant growth & development

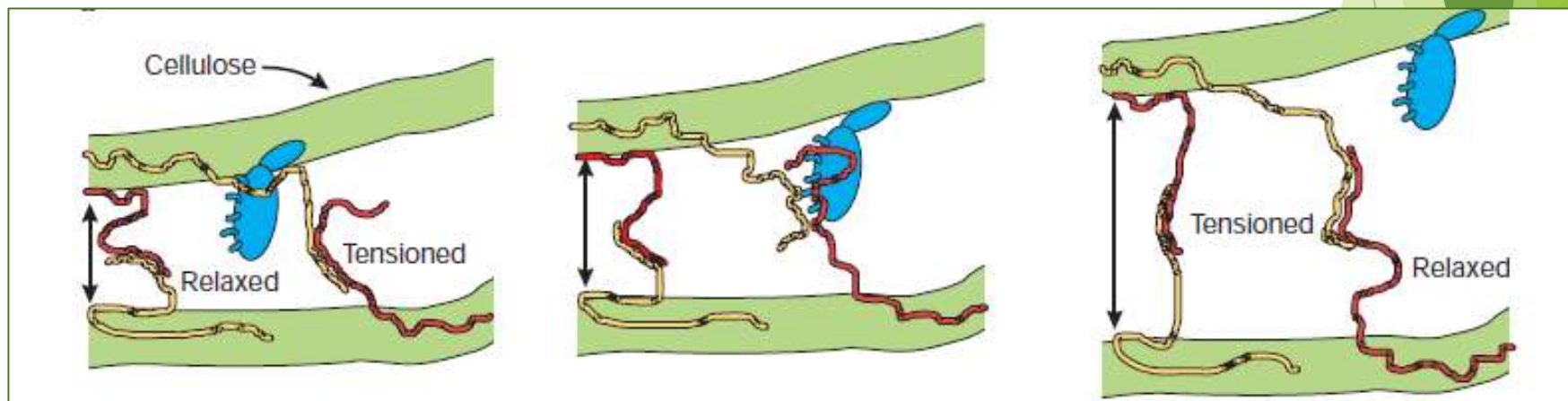
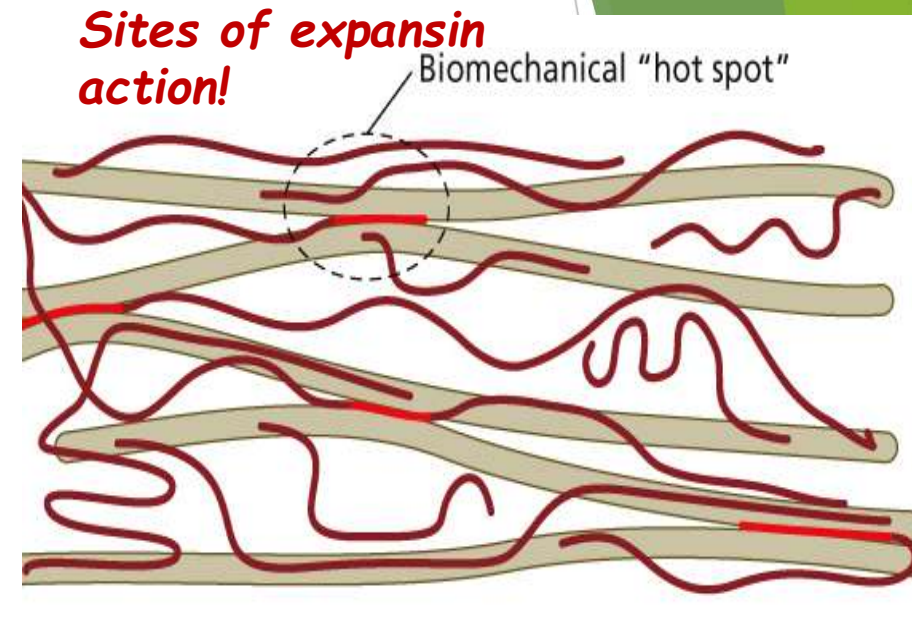
- ▶ shapes the plant body
- ▶ movement of solutes and nutrients
- ▶ protects plants from the environment
- ▶ intercellular communication (Wolf *et al.*, 2012)
- ▶ **Cellulose is the most abundant biopolymer on Earth!**



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

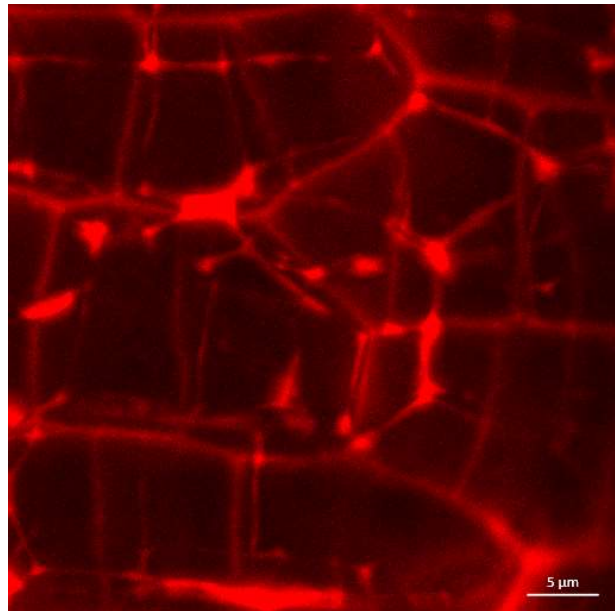
# How do plant cells grow?

- ▶ Plant CW combine strength with extensibility ...
- ▶ Wall extensibility may be controlled at limited regions, '**biomechanical hotspots**' (Cosgrove, 2014; 2018).
- ▶ **EXPANSINS** are small proteins that disrupt the non-covalent bonds between CW polysaccharides, thus relaxing wall stresses and allowing turgor-driven **cell expansion** (Cosgrove, 2000).

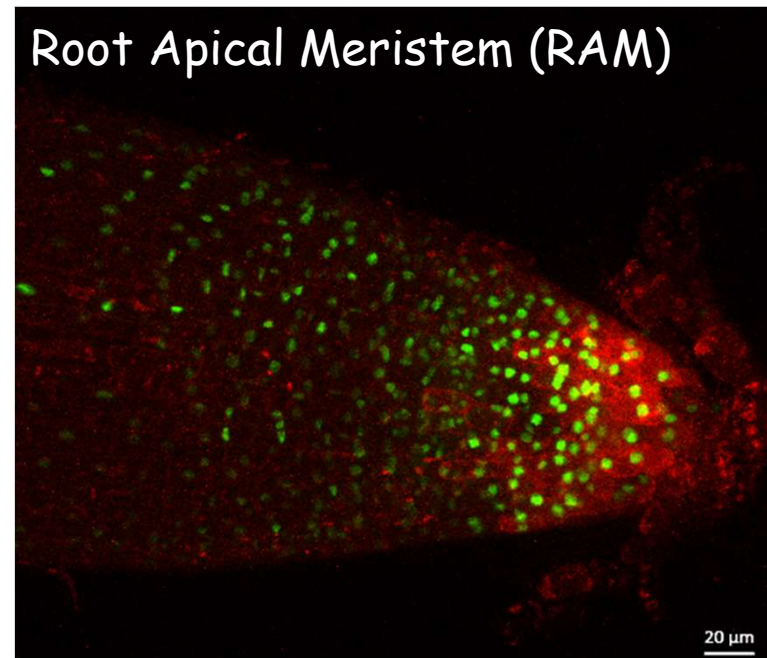
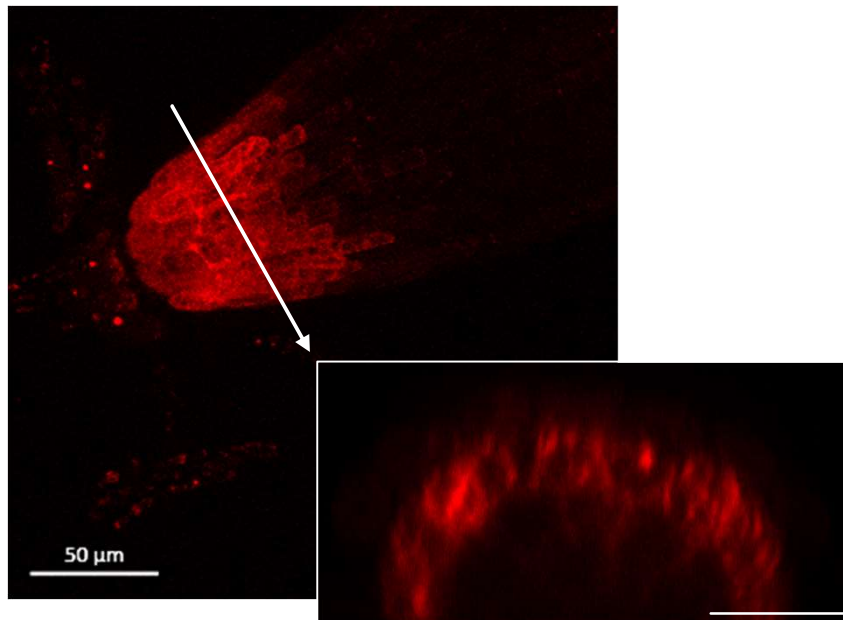




# EXPANSINS are localized in the cell wall

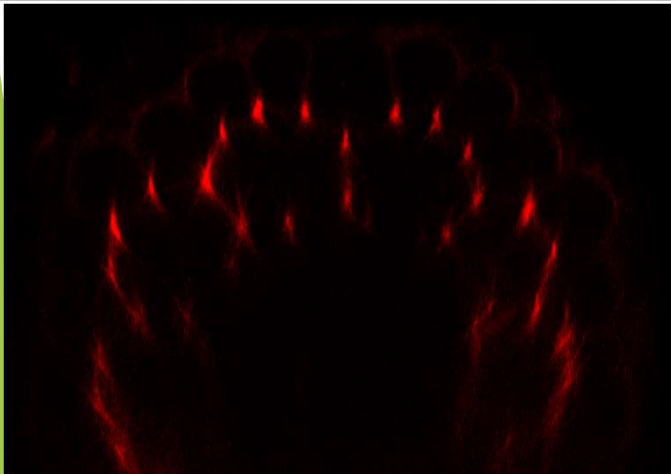
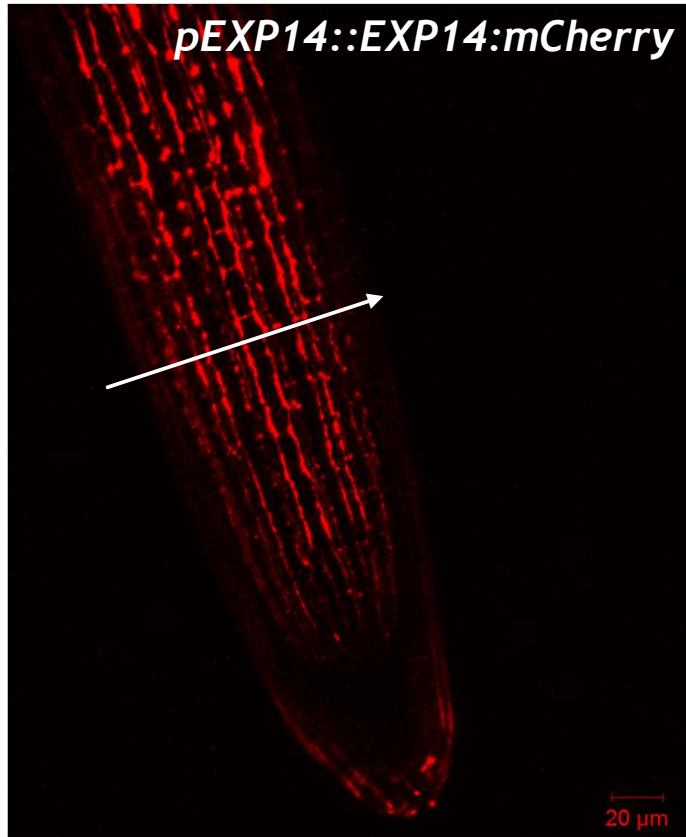


- EXPANSINS localised to the CW *in vivo* for the first time! (Samalova *et al.*, 2024)
  - Use of mCherry (RFP) instead of pH sensitive GFP

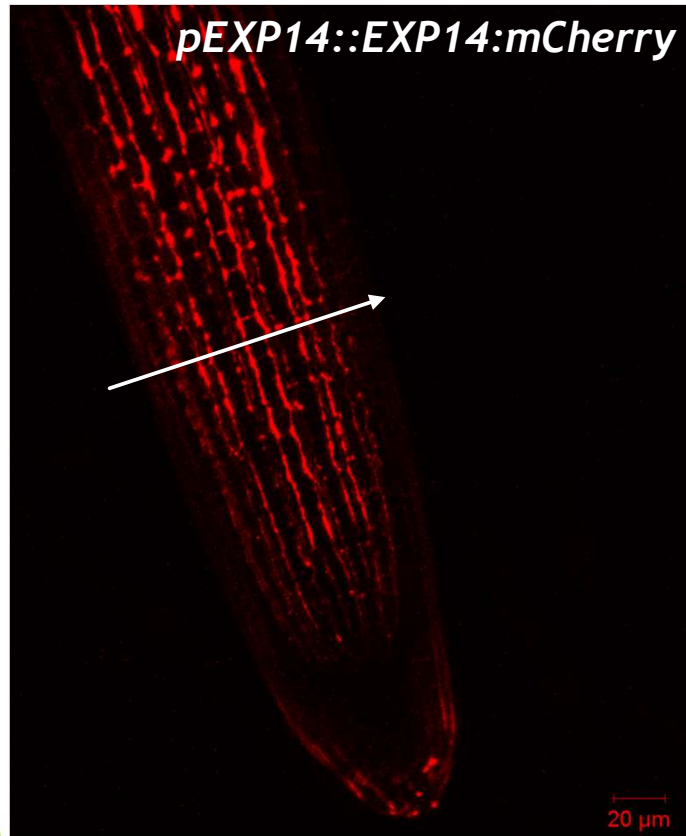




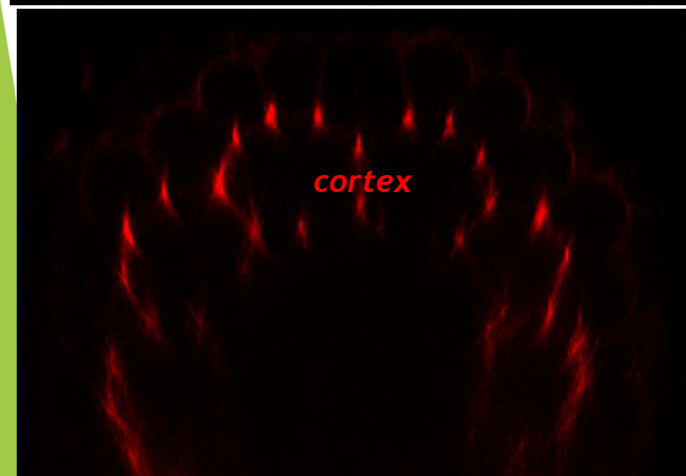
# EXPANSINS are localized into various root tissues



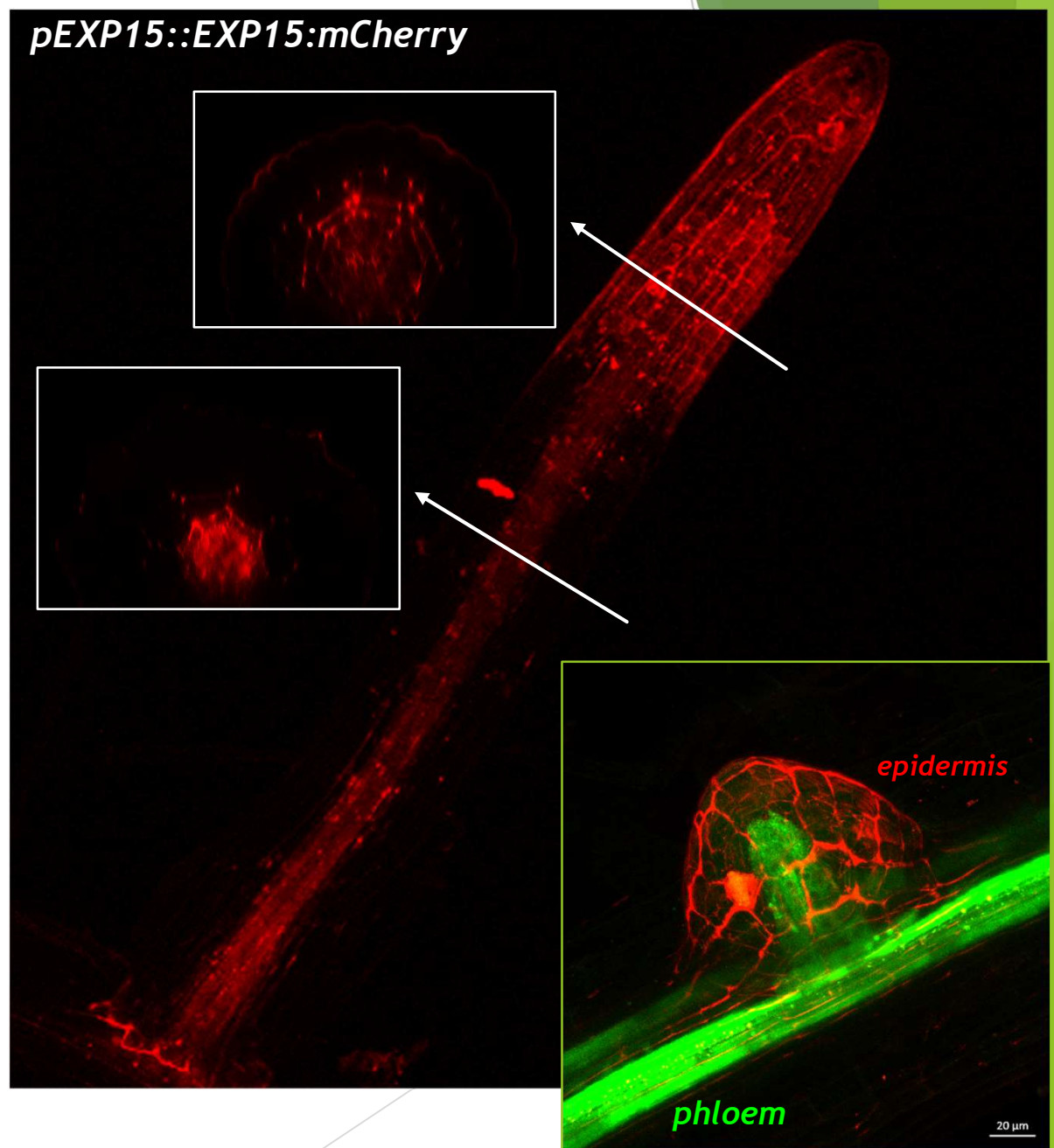
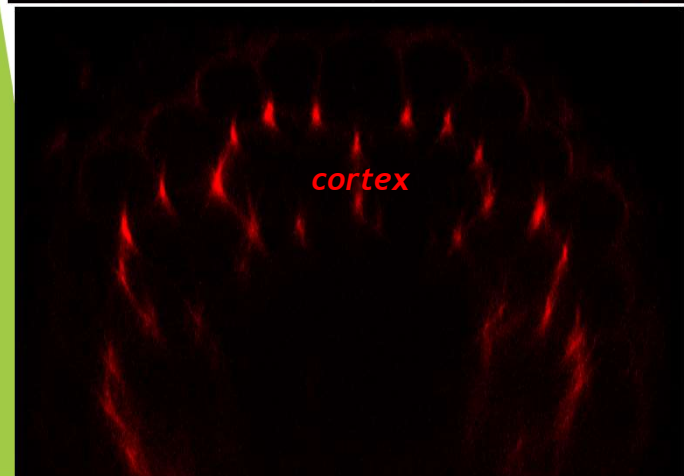
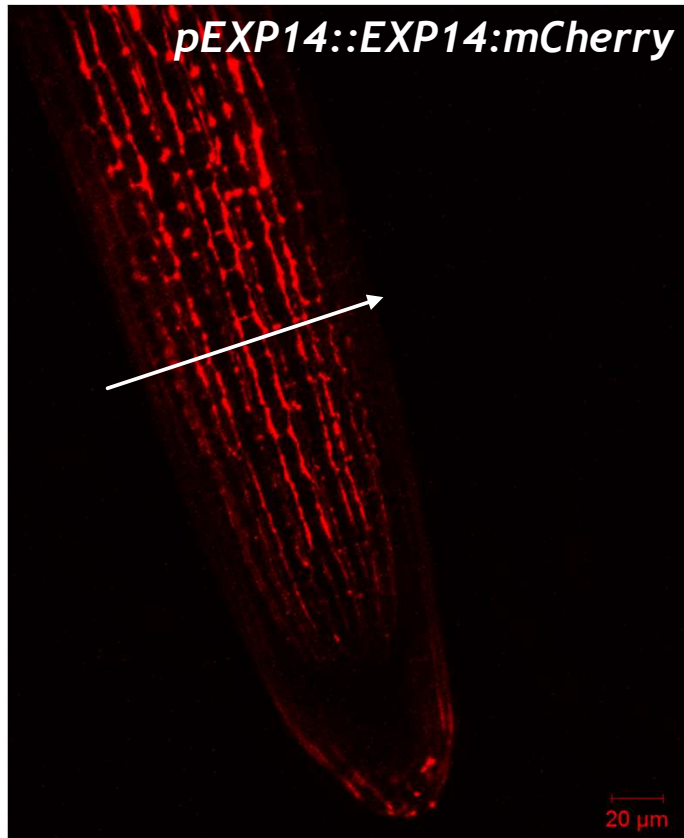
# EXPANSINS are localized into various root tissues



- ▶ 3D projection of Z-stack (combined optical slices) taken by a confocal microscope.



# EXPANSINS are localized into various root tissues

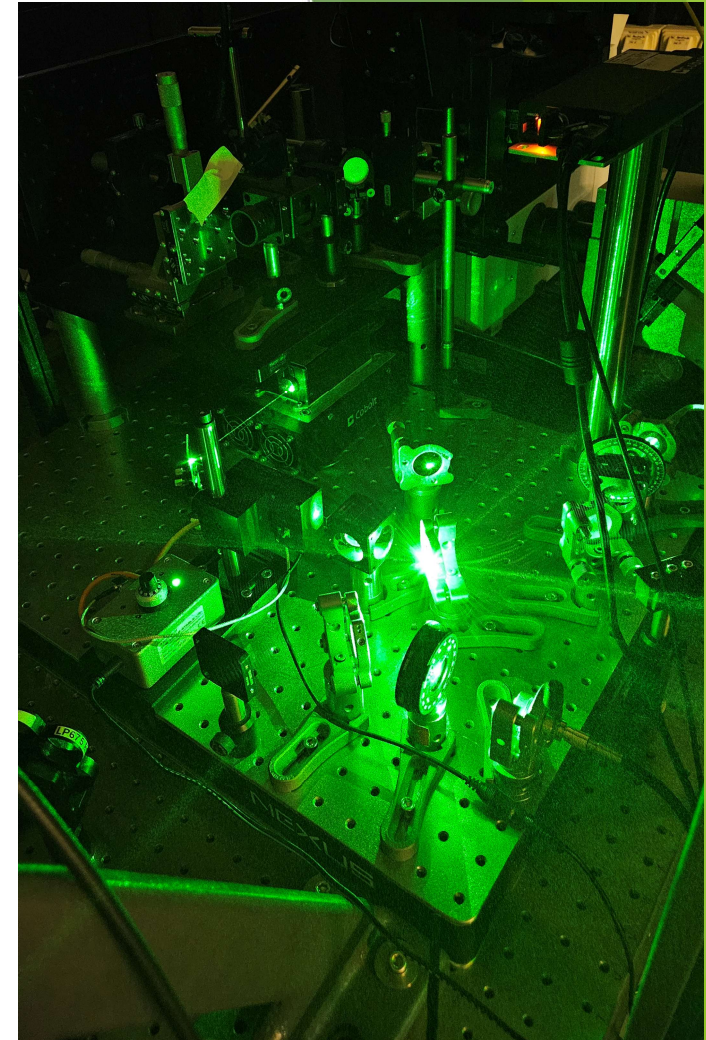
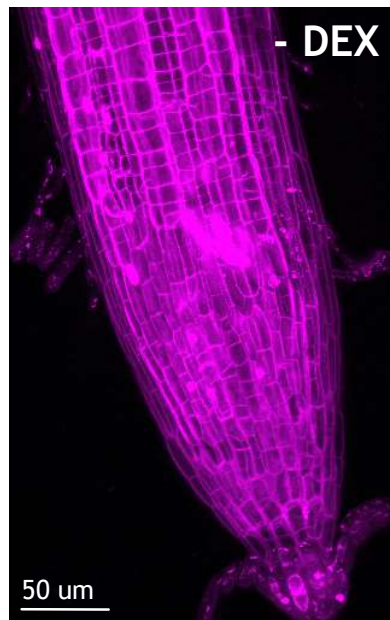
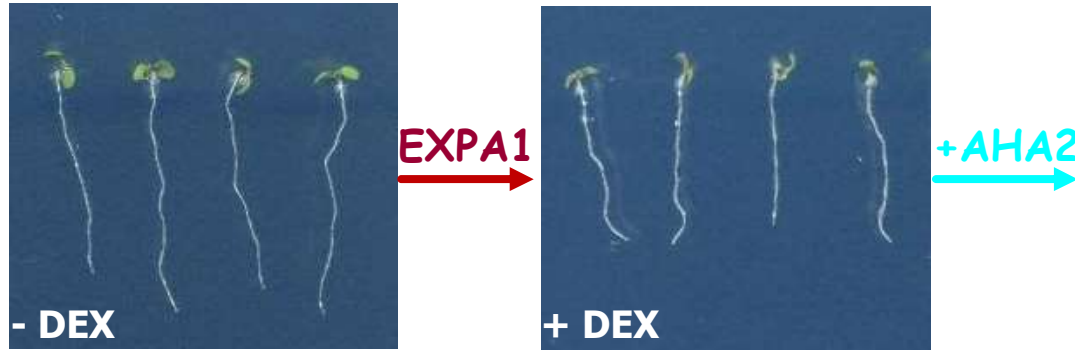




# Overexpression of *EXPA1* makes the plants smaller by “stiffening” cell walls

- ▶ Changes in biomechanical properties of CWs.

*pRPS5A>>EXPA1*



nature photonics

Keshmiri, Cikes, Samalova *et al.* 2024

**Brillouin light scattering anisotropy microscopy for imaging the viscoelastic anisotropy in living cells**



# Overexpression of *EXPA1* leads to smaller, compact plants that are more resistant to (a)biotic stresses

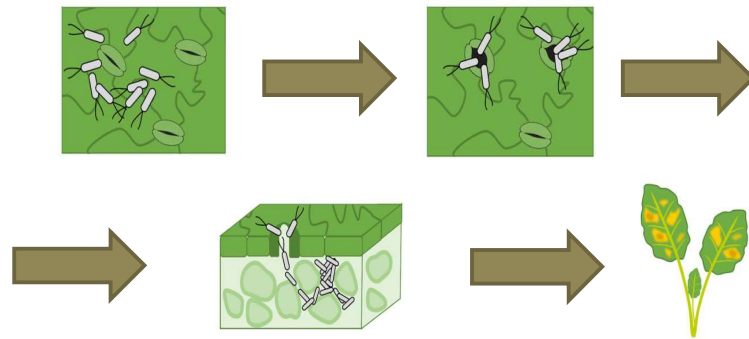


➤ Exploring a role of *EXPANSINS* under stress:

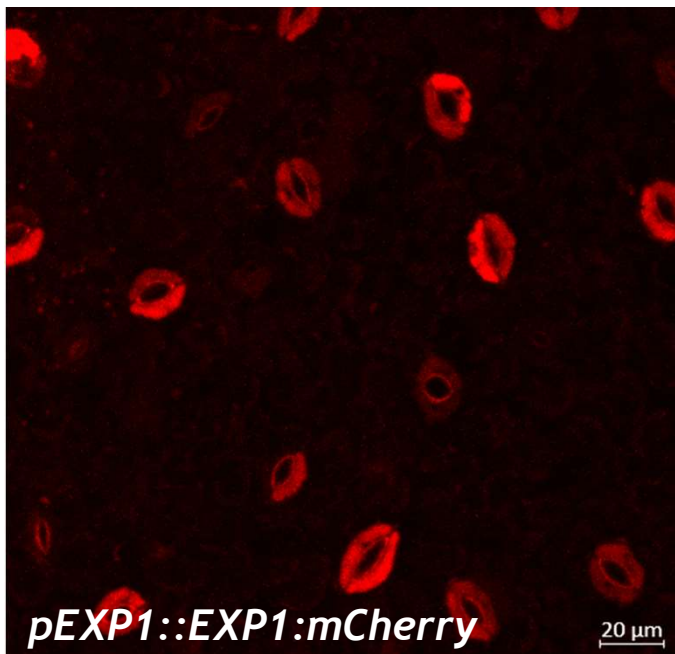


# Plants overexpressing *EXPA1* are more resistant to bacteria *Pseudomonas syringae*

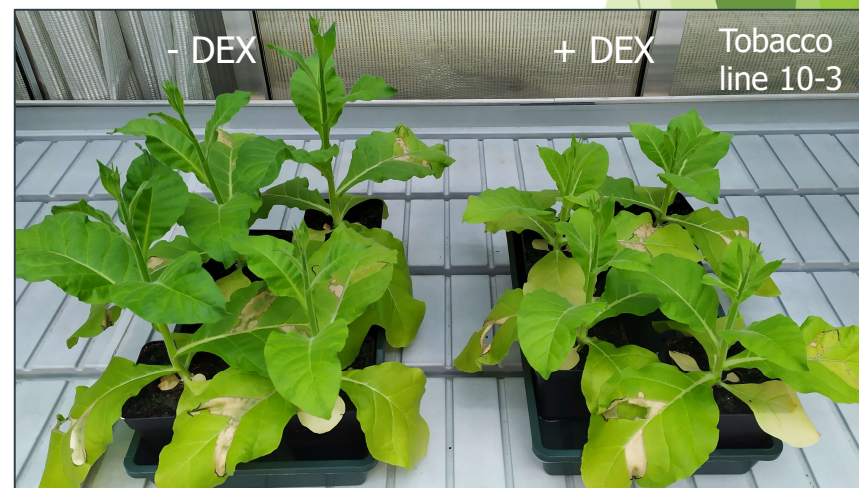
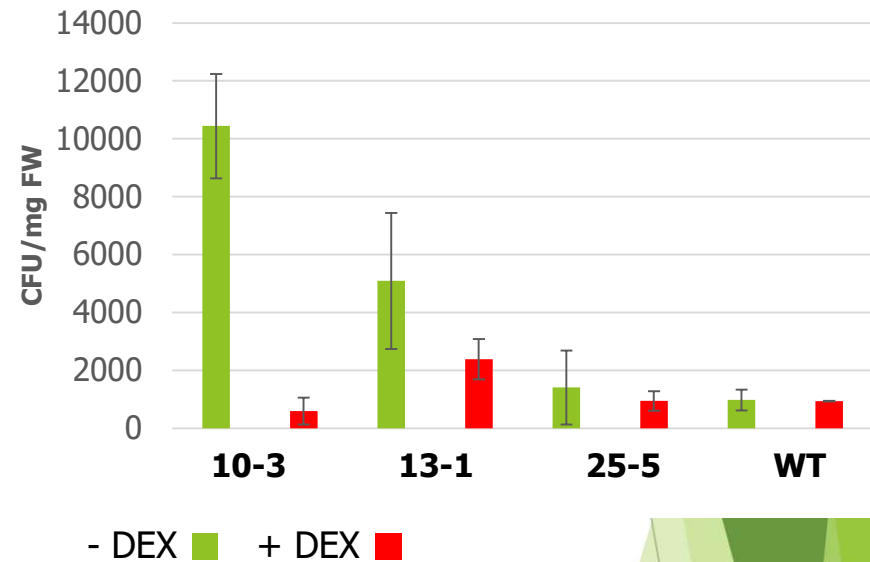
- *P. s.* is an aggressive bacterial pathogen.
- Enters plants through stomata!



***EXPA1* localizes in stomata!**



## Bacterial plant infection



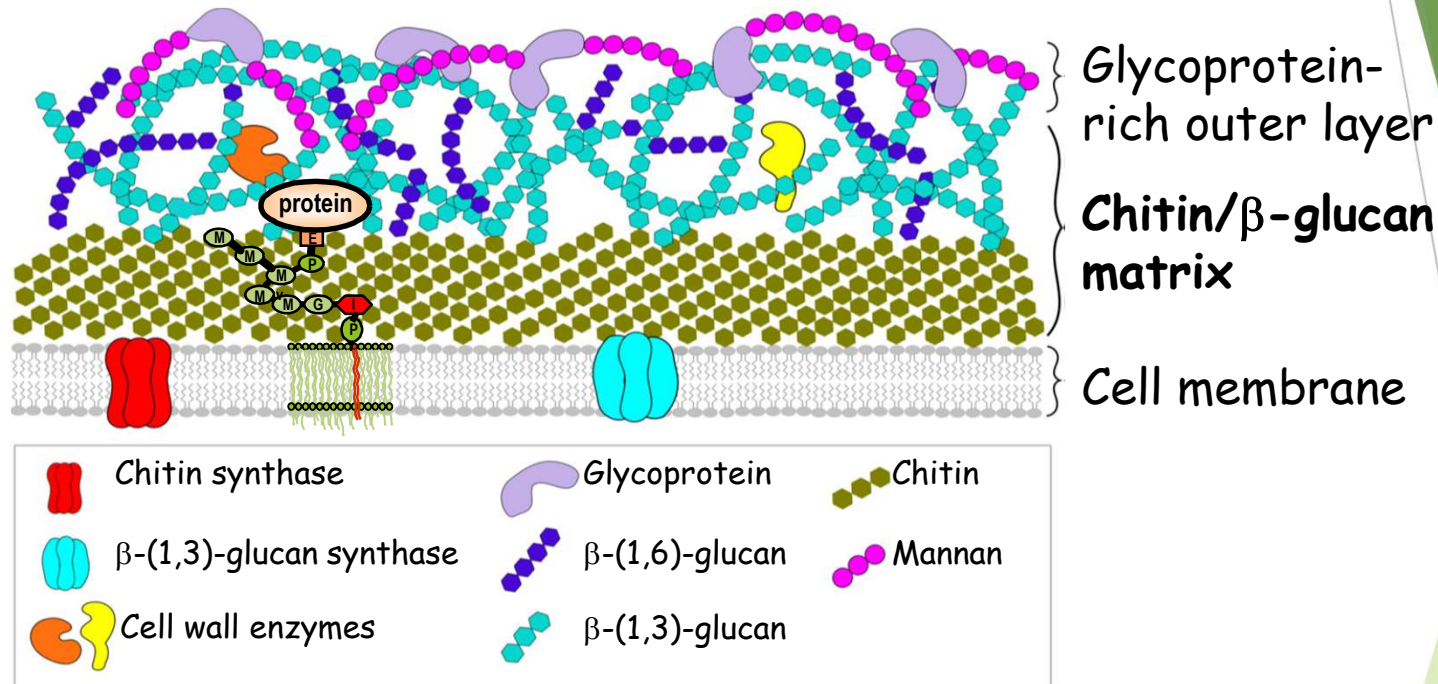


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Not only plant cells have the *CW*...

# Unique composition of the fungal cell wall

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



- ▶ **GPI** (GlycosylPhosphatidylInositol) Anchored Proteins = **GAP**
  - ▶ Cell wall modifying enzymes
  - ▶ e.g. Glucan Elongation (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.

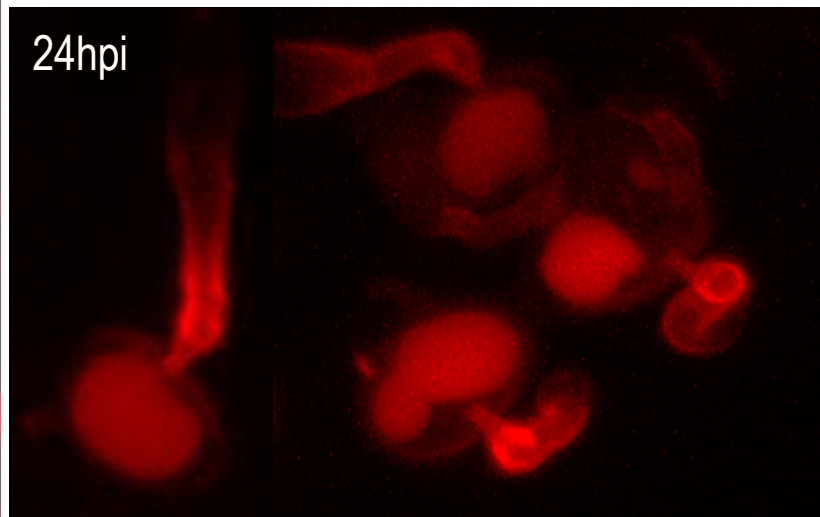
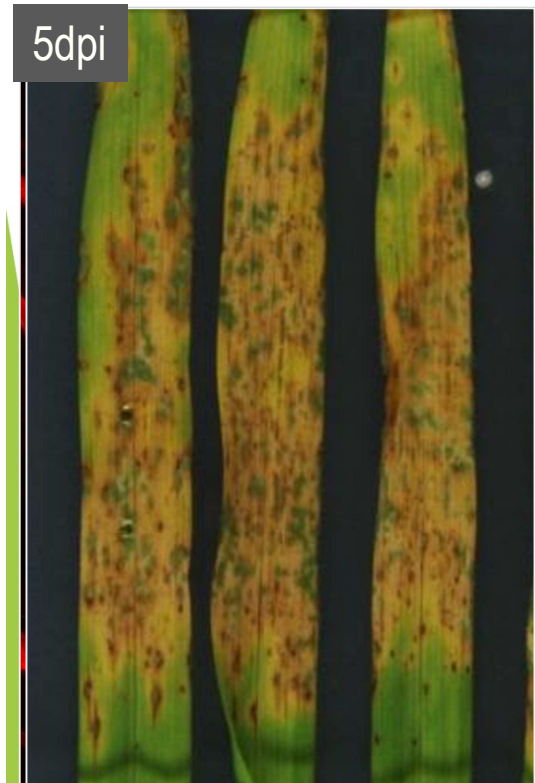
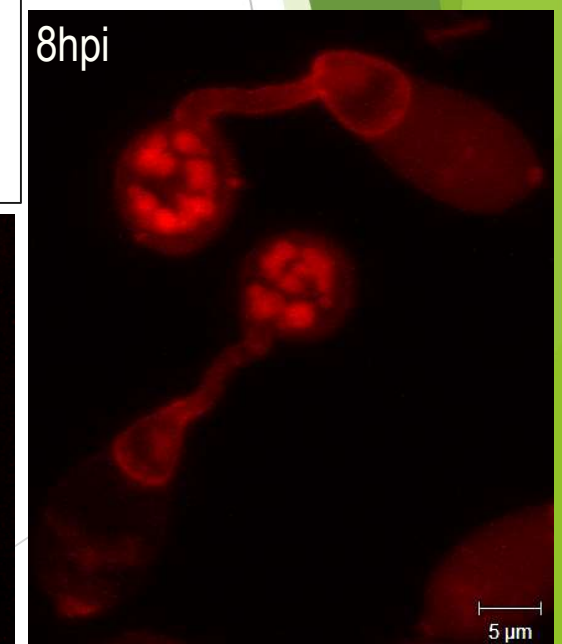
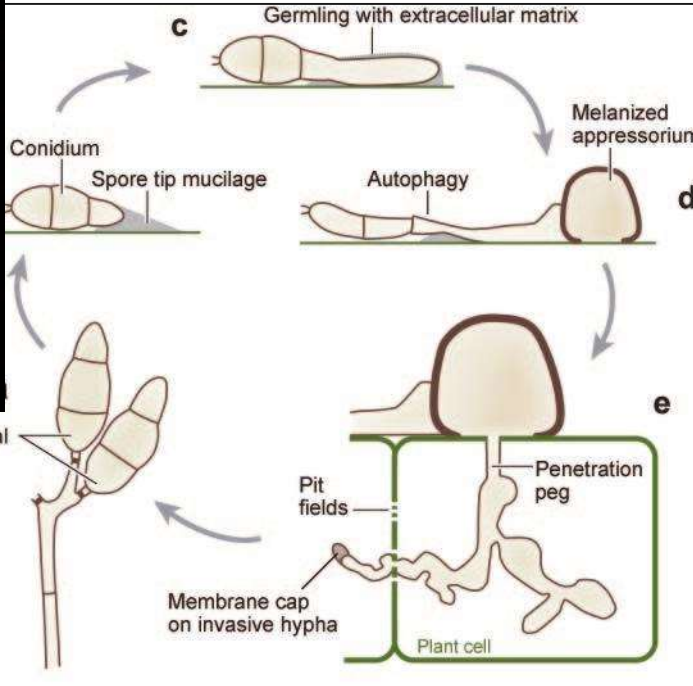
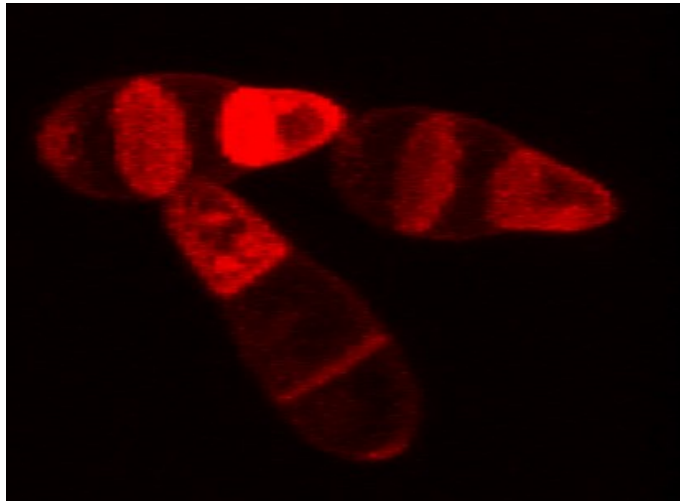
### ➤ **Food security & climate change**





# Magnaporthe oryzae asexual life-cycle

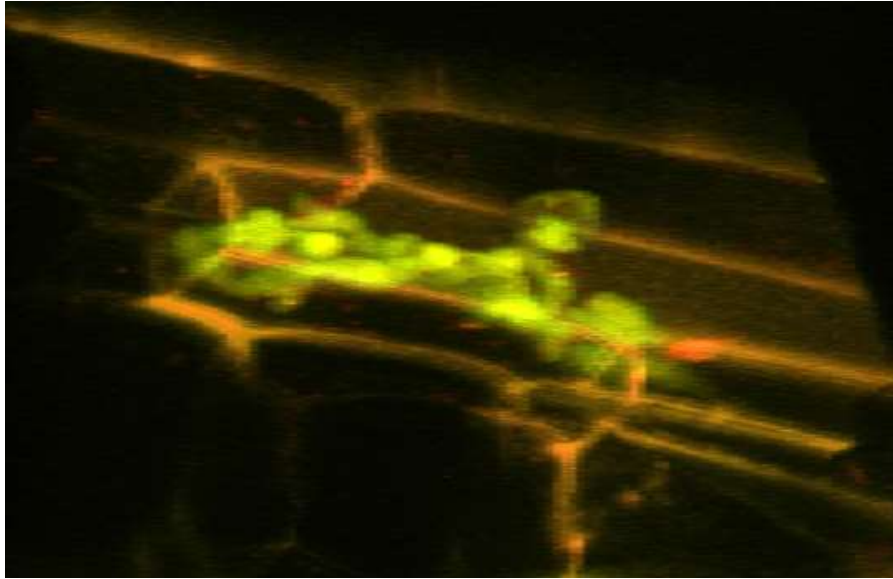
*pGEL3::mCherry:GEL3*



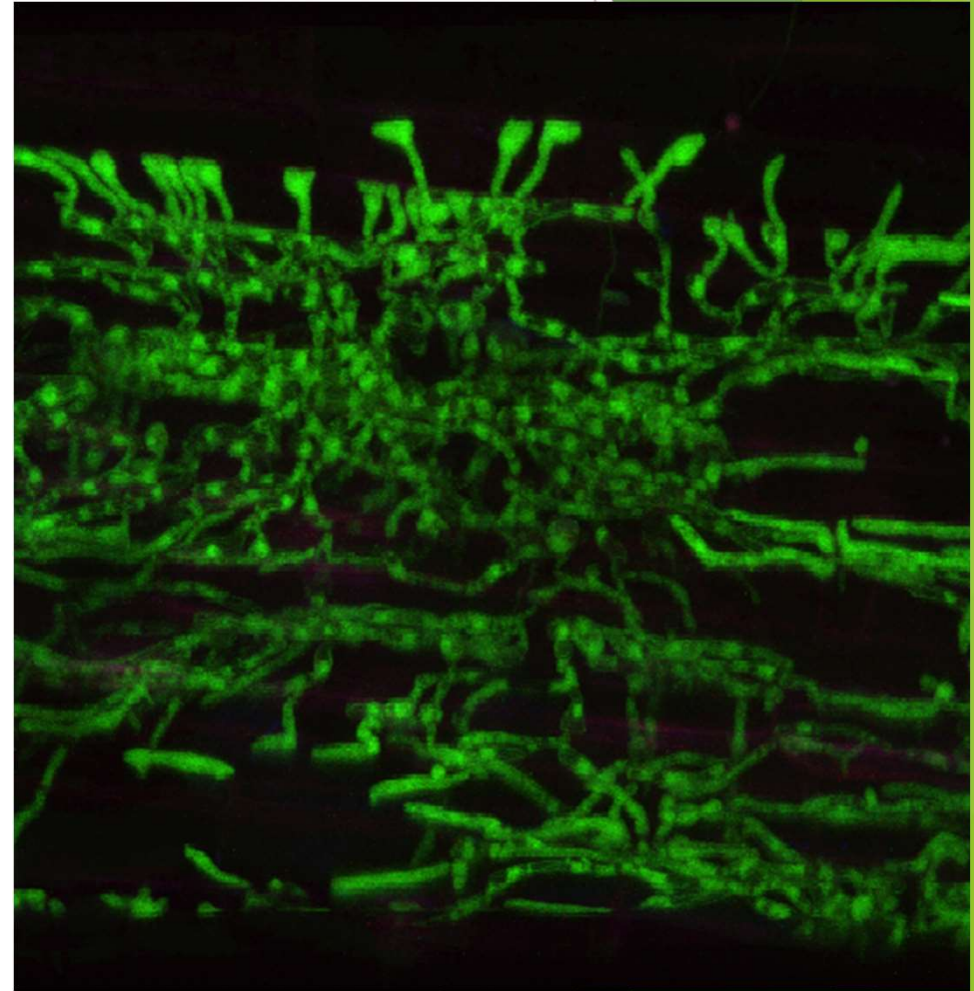
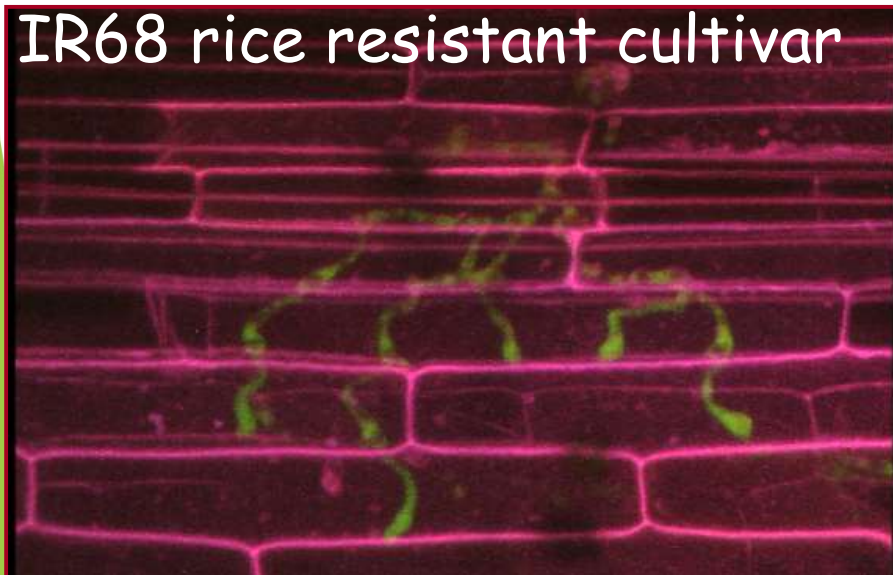
Samalova et al., 2017



# ROS toxicity alone is NOT sufficient to kill *Magnaporthe oryzae* in resistant rice!

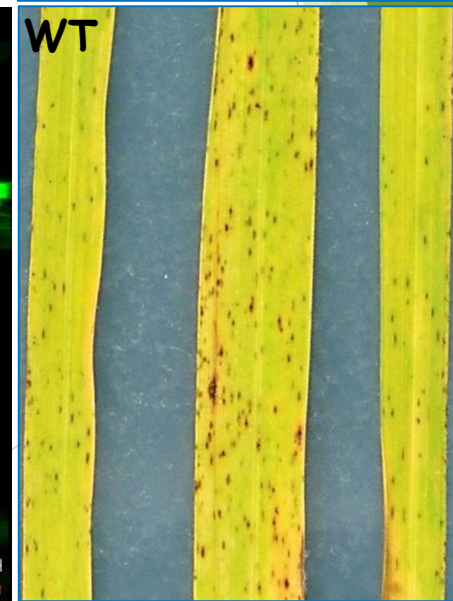
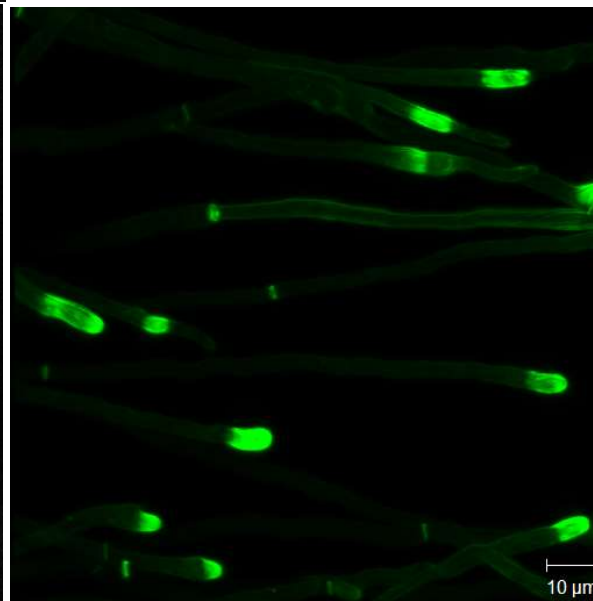
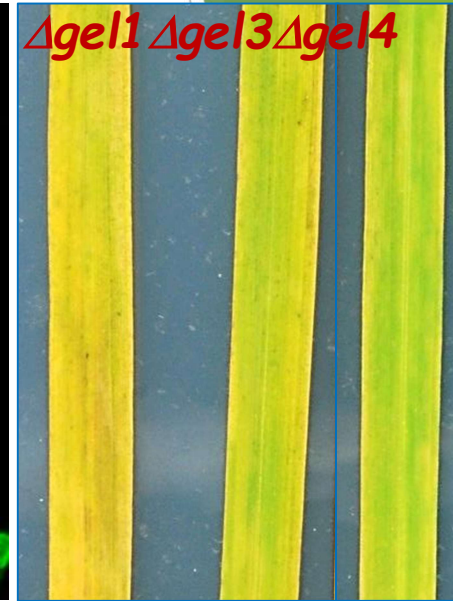
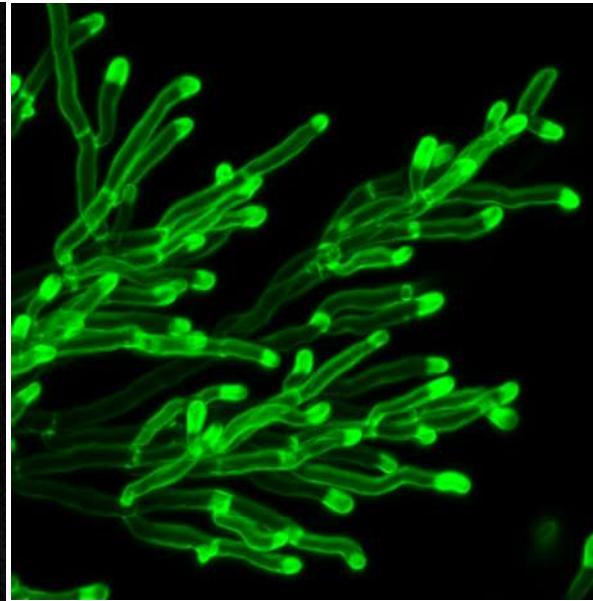


IR68 rice resistant cultivar



- ▶ Exploring redox state in susceptible & resistant (Samalova *et al.*, 2013; 2014)

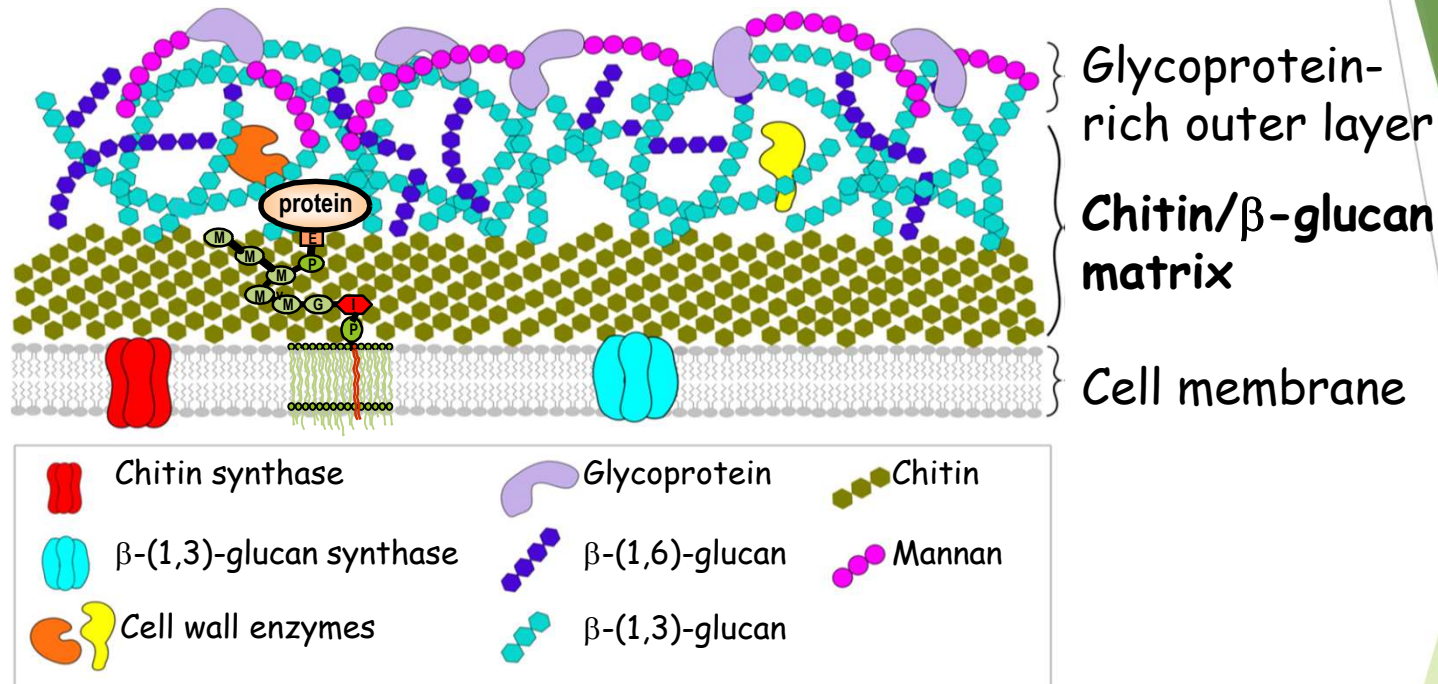
*Triple  $\Delta gel1 \Delta gel3 \Delta gel4$  KO has reduced mycelial growth, hyper branching phenotype and is non-pathogenic!!!*





# Unique composition of the fungal cell wall

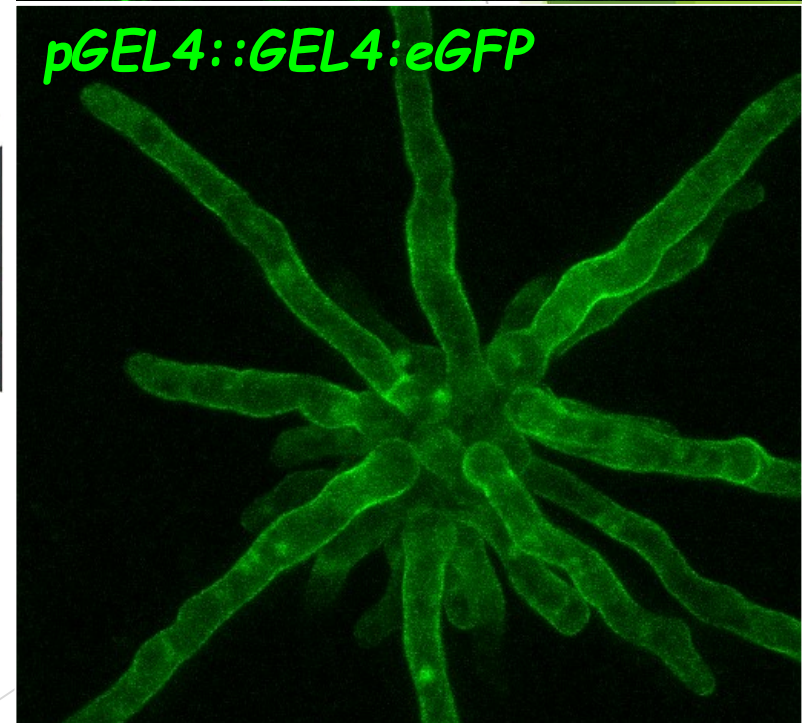
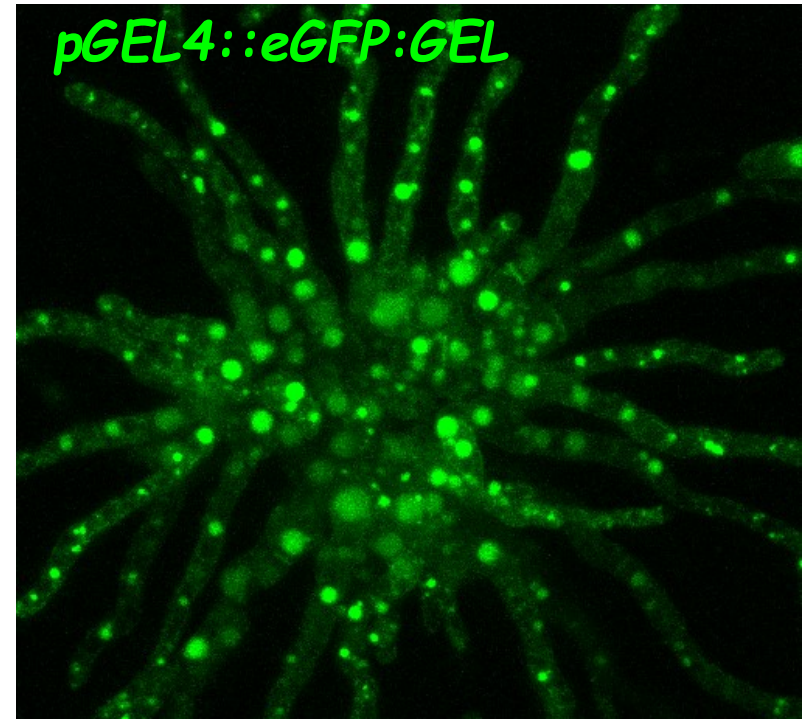
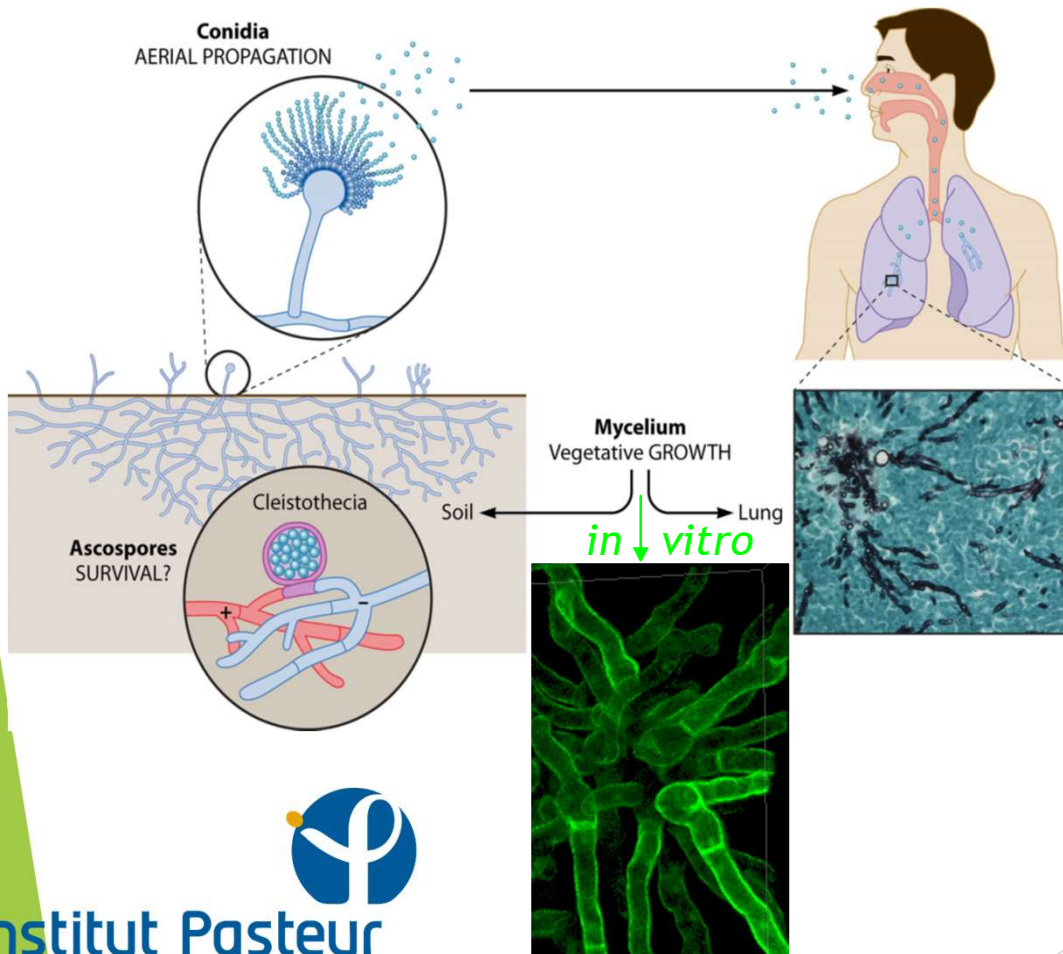
- ▶ makes it an ideal target for the development of *fungicides!*



- ▶ **GPI** (GlycosylPhosphatidylInositol) Anchored Proteins = **GAP**
  - ▶ Cell wall modifying enzymes
  - ▶ e.g. Glucan Elongation (Ge) proteins elongating β-1,3-glucan chains

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

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

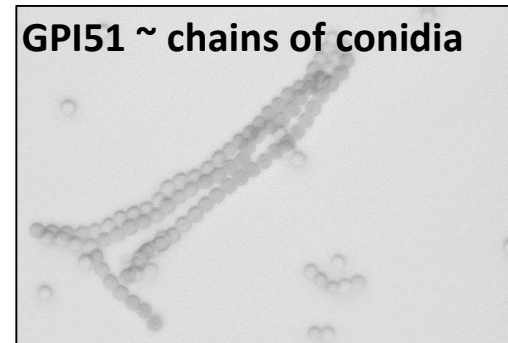
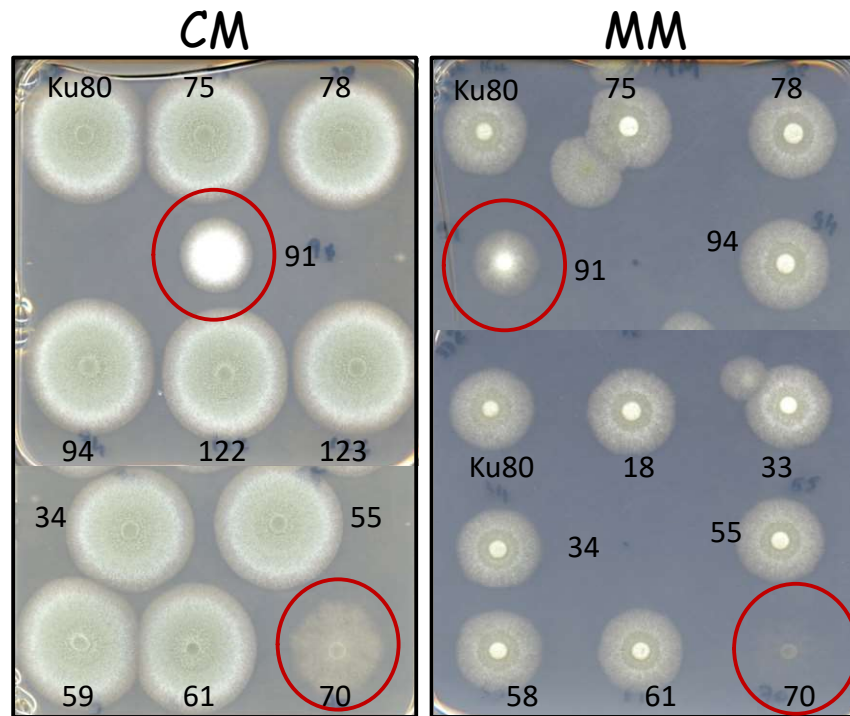




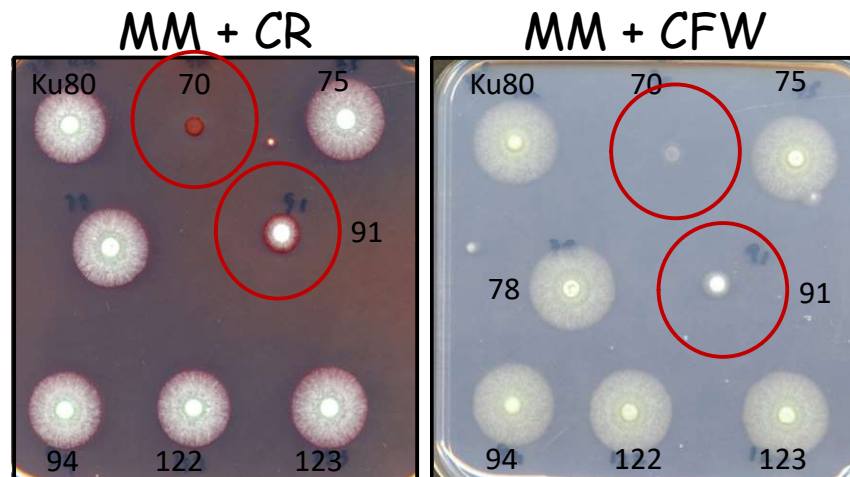
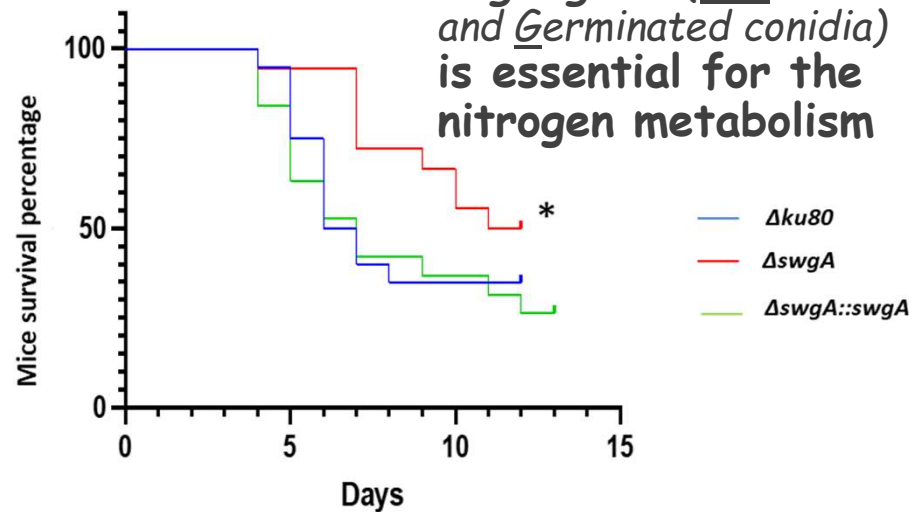
# How to knock-out 132 genes in one summer . . .

► Single KOs of **all GAP** proteins!!!

- Growth defects /phenotype
- Spore phenotype



► *swgA* gene (SWollen and Germinated conidia) is essential for the nitrogen metabolism



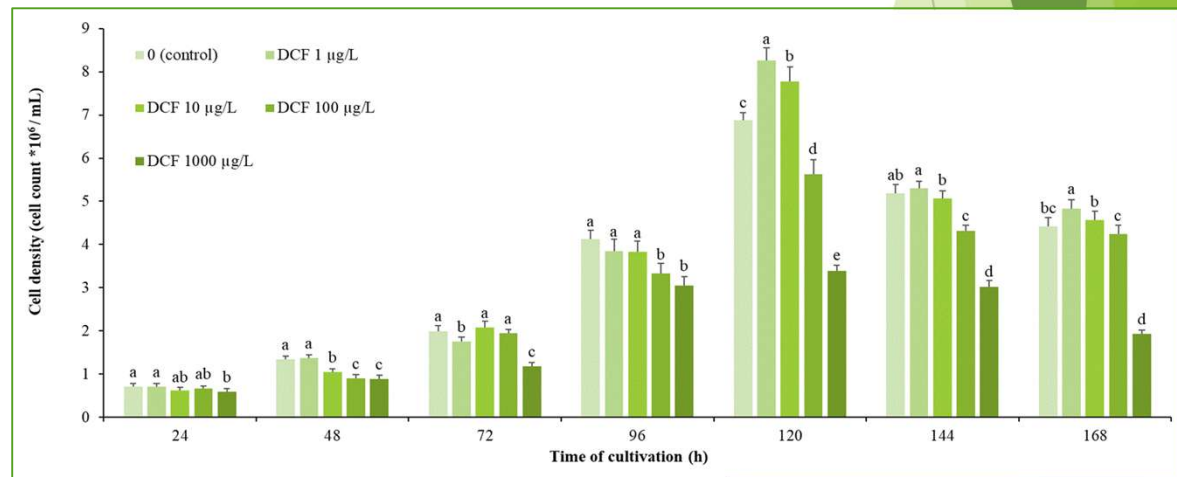
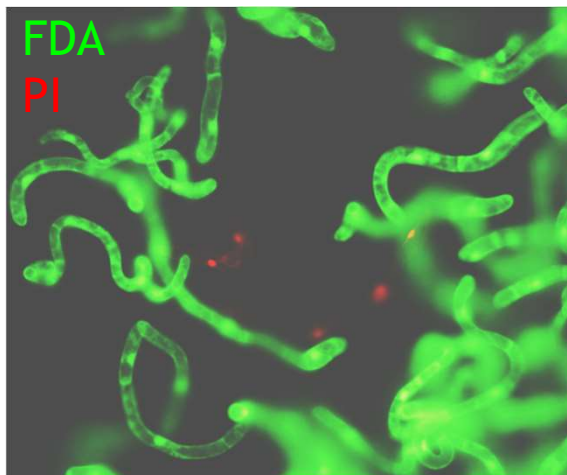
► Samalova et al., 2020, 2023

# Vliv léčiv na rostliny - model rostlinná buněčná suspenze

- ▶ **Odd. EBR - Laboratoř organických polutantů**  
**doc. M. Kummerová, Dr. Š. Zezulka**
- ▶ Léčiva jako součást znečištění životního prostředí
- ▶ *Nicotiana tabacum*, cv. Bright-Yellow 2 (BY-2)
- ▶ Využití při studiu:
  - ▶ programovaná buněčná smrt
  - ▶ produkce sekundárních metabolitů
  - ▶ signalizace v rostlinných buňkách
  - ▶ regulace buněčného cyklu
  - ▶ **stresové reakce** (toxické kovy, organické polutanty)

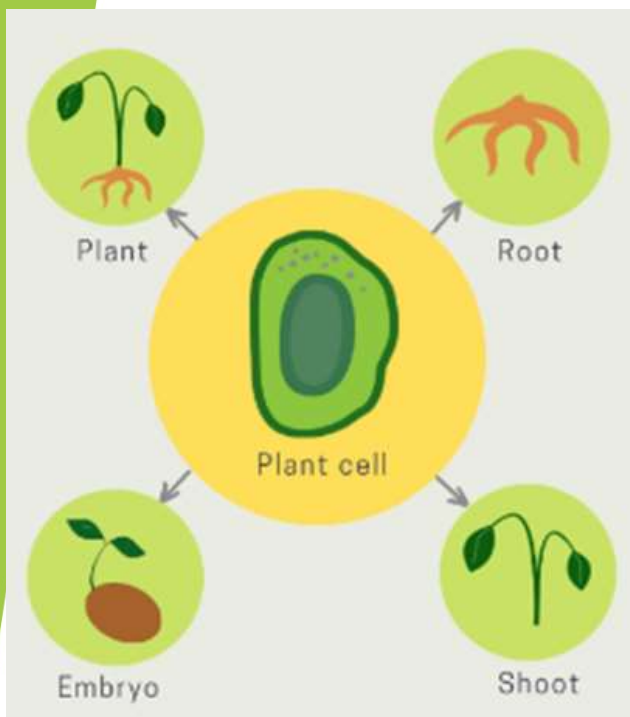


*Test viability a růstová křivka hustoty suspenze v čase (Svobodníková et al., 2019, Ecotox. Environ)*



# Využití rostlinných tkáňových kultur

- Odd. EBR - Laboratoř rostlinných tkáňových kultur
- Dr. Hana Cempírková



Využití totipotence rostlinných buněk

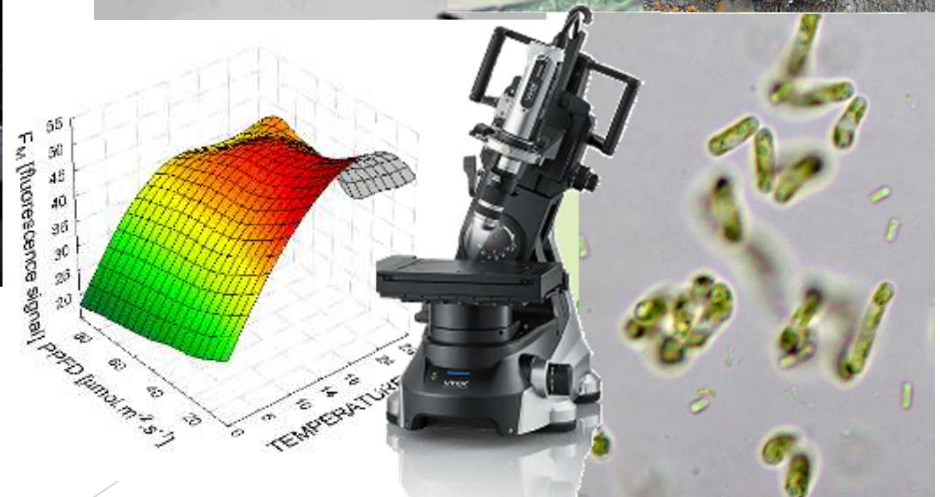
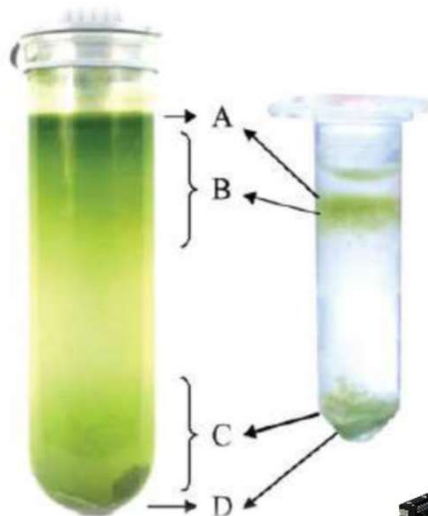
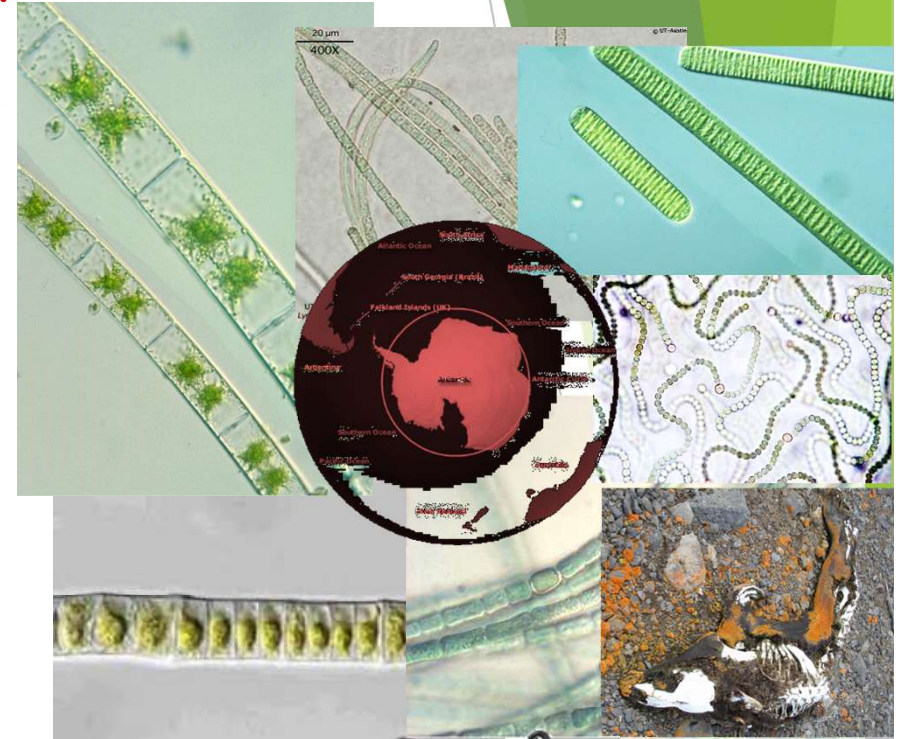
- Sterilní prostředí, živiny v tekutém/ztuženém médiu
- Možnost ovlivnění růstu kultur přidavkem rostlinných fytohormonů/růstových regulátorů
- Příklady využití:
  - Množení rostlin (= mikropropagace)
  - Molekulární biologie (transgenóza)
  - Záchrana ohrožených druhů
  - Šlechtění
  - Uchování genetických zdrojů





# Studium extremofilních organismů z Antarktidy

- ▶ **Odd. EBR - Laboratoř fotosyntetických procesů**  
**Prof.M.Barták, doc.J.Hájek, Dr.P.Váczi**
- ▶ Vzorky z polárních oblastí - experimentální algologie
- ▶ Suspenzní kultury řas a cyanobakterií,
  - ▶ axenické kultury, izolované chloroplasty, thylakoidy
- ▶ automatizované systémy při studiu fotosyntézy - **fotobioreaktory**
  - ▶ vliv extrémního prostředí (UV, t, vysoký svit)
  - ▶ studium fotochemických reakcí fotosyntézy
  - ▶ antioxidační systémy
  - ▶ sekundární metabolity





# Laboratoř minerální výživy a vodního provozu



doc. RNDr. Vít Gloser, Ph.D.

VitGloser@sci.muni.cz

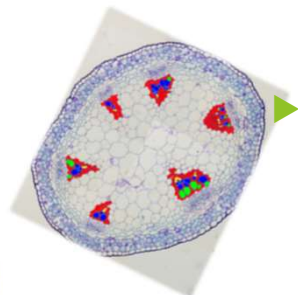
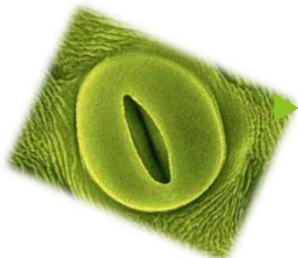
## Jaká témata řešíme?

### Rychlé reakce rostlin na podmínky prostředí

- ▶ Mechanismy signalizace dostupnosti vody
- ▶ Signalizace dostupnosti živin v médiu
- ▶ Mechanismy regulace rychlosti dálkového transportu

### Přizpůsobení rostlin změnám v prostředí

- ▶ Změny struktury orgánů v reakci na podmínky prostředí
- ▶ Efektivita využití vody a živin
- ▶ Regulace vývoje vodivých pletiv faktory prostředí





**Thank you for your attention!**