



# 4PMI: Plant Phenotyping Platform for Plant and Microorganisms Interactions Phenotyping innovations, opportunities and challenges

Christophe Salon, Céline Bernard, Mickaël Lamboeuf, Christian Jeudy

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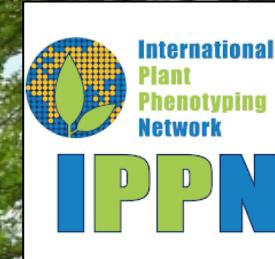
HAL Id: hal-02733820

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Submitted on 2 Jun 2020

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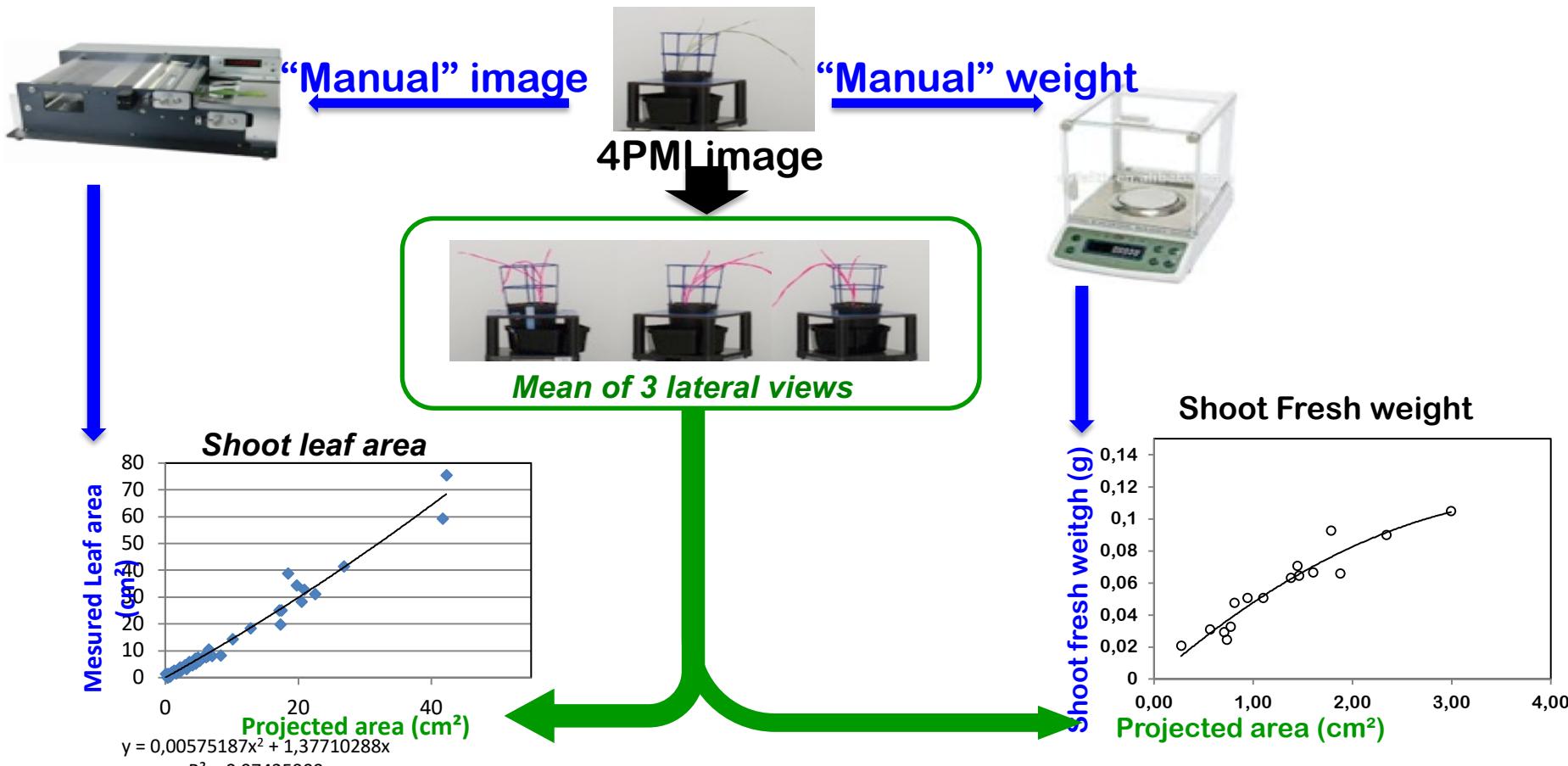


**4PMI: Plant Phenotyping Platform for Plant and  
Microorganisms Interactions**

**Phenotyping innovations, opportunities and challenges**  
**(Christophe Salon, Céline Bernard, Mickael Lamboeuf, Christian Jeudy,  
UMR Agroécologie, INRA, Dijon, France)**



# Shoot phenotyping: basics



# Shoot phenotyping: 1st example

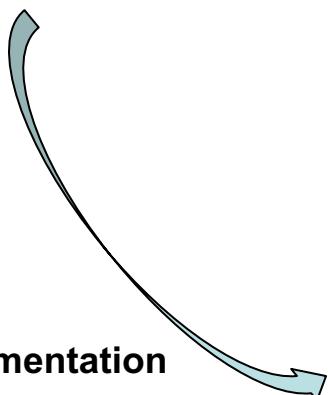
*Micro tom phenotyping, coll. C Rothan (BFP Bordeaux, France)*



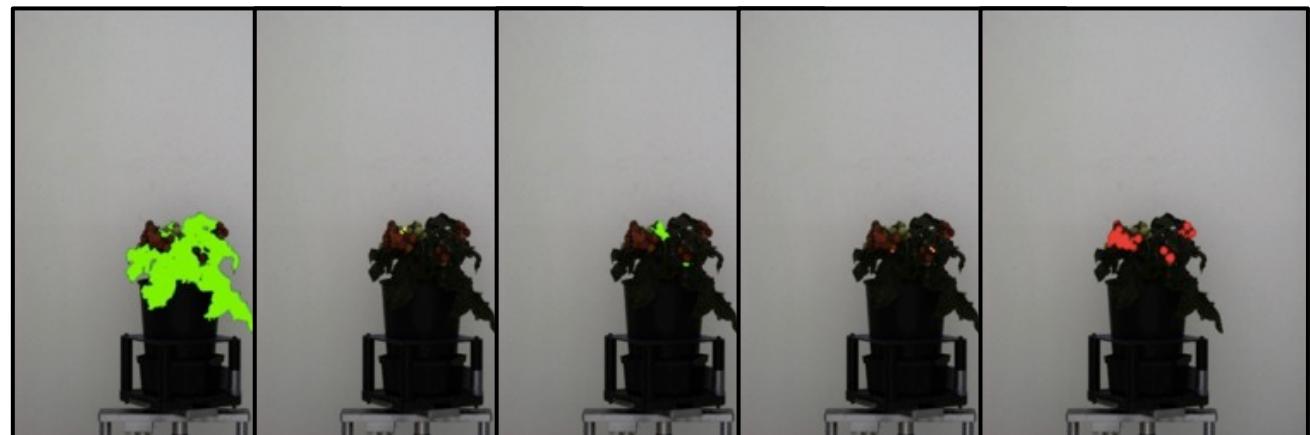
Tomato in pots



Original image

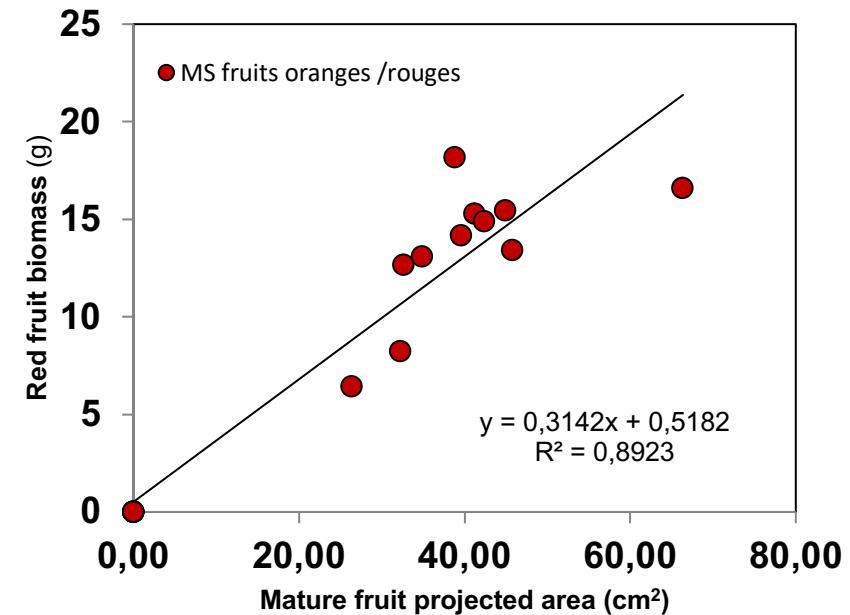
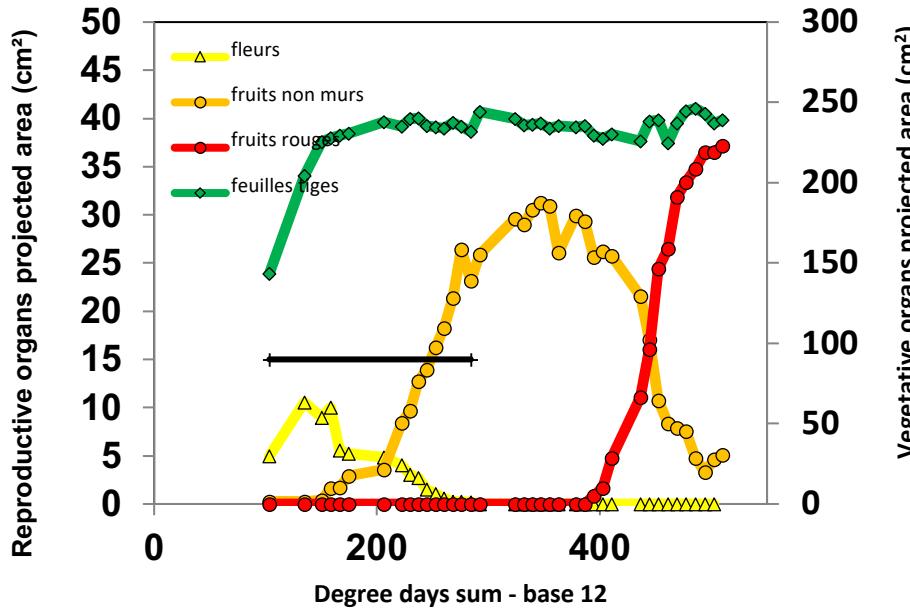


Segmentation



# Shoot phenotyping: 2nd example

*Micro tom phenotyping, coll. C Rothan (BFP Bordeaux, France)*



- Phenology and fruit maturing followed non destructively from image analysis.
- Fruit detection realized = f(image analysis).



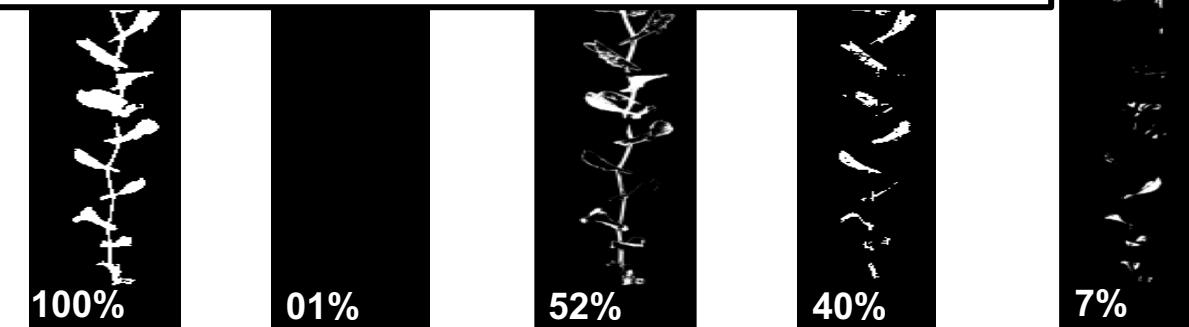
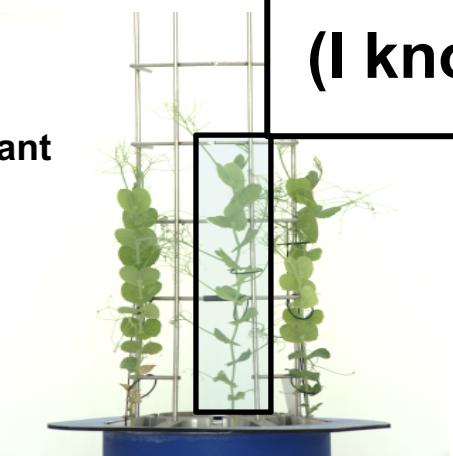
## PEA plants

Chlorosis  
Sensible



**1800 plants capacity !**

Tolerant



Algorithms to identify symptoms on 300 genotypes

# Root phenotyping: why go into trouble ?

- **Crop breeding programmes:** root traits rarely used as selection criteria, a focus on adaptation to high-input systems,
  - Improve crop resource-use efficiency through:
    - (i) physiological utilization of acquired resources,
    - (ii) resource acquisition
- **Technical difficulties:**
  - Access to roots ,
  - Root diversity,
  - Plasticity of RSA (abiotic and biotic factors including plant and microorganisms interactions) in order to enhance its efficiency.

## We wish:

- To visualize (harvest) roots, at high resolution, dynamically and non destructively, for a large number of biological units, various species.
- To estimate structural (and functional?) traits, avoid shading roots, oxygen shortage and pH, nutrient unregulated conditions
- To study plant-plant and plant-microorganism interactions

## ...and access various descriptors of RSA:

### First priority

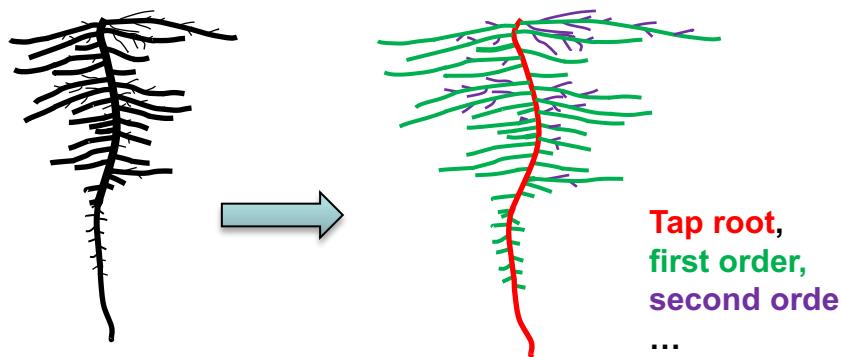
Root projected area  
 Nodule projected area  
 Nodule number  
 Total root length  
 Root depth, prospection

### then

Main and lateral root length  
 Number of lateral roots, of secondary roots on lateral roots  
 Number and position of nodules on each root  
 Apical diameter of roots

### Notes:

Number: total, by segment-segment length  
 Projected area: individual, by class  
 Position: individual, by class  
 Nodule efficiency: individual, by class  
 Estimated biovolume: a root ≠ cylinder  
 Biomass estimation: calibration



Also structures arising from plants and microorganisms : nodules, mycorrhiza



# Many opportunities

Soil



Agar plates, petri dishes

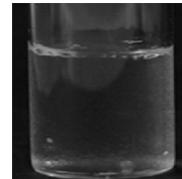
Hargreaves  
Plant a.  
297.

X Ray tomography



Mooney et al. 2012. Plant Soil, 352:1-22  
Moradi et al. Plant Soil (2009) 318:2

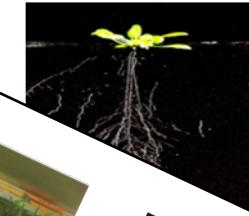
Artificial



Downie et al. 2012

DOI: 10.1371/journal.pone.0044276

Soil and Rhiz-



Tube  
0:1096-1108

MRI

LEGATO

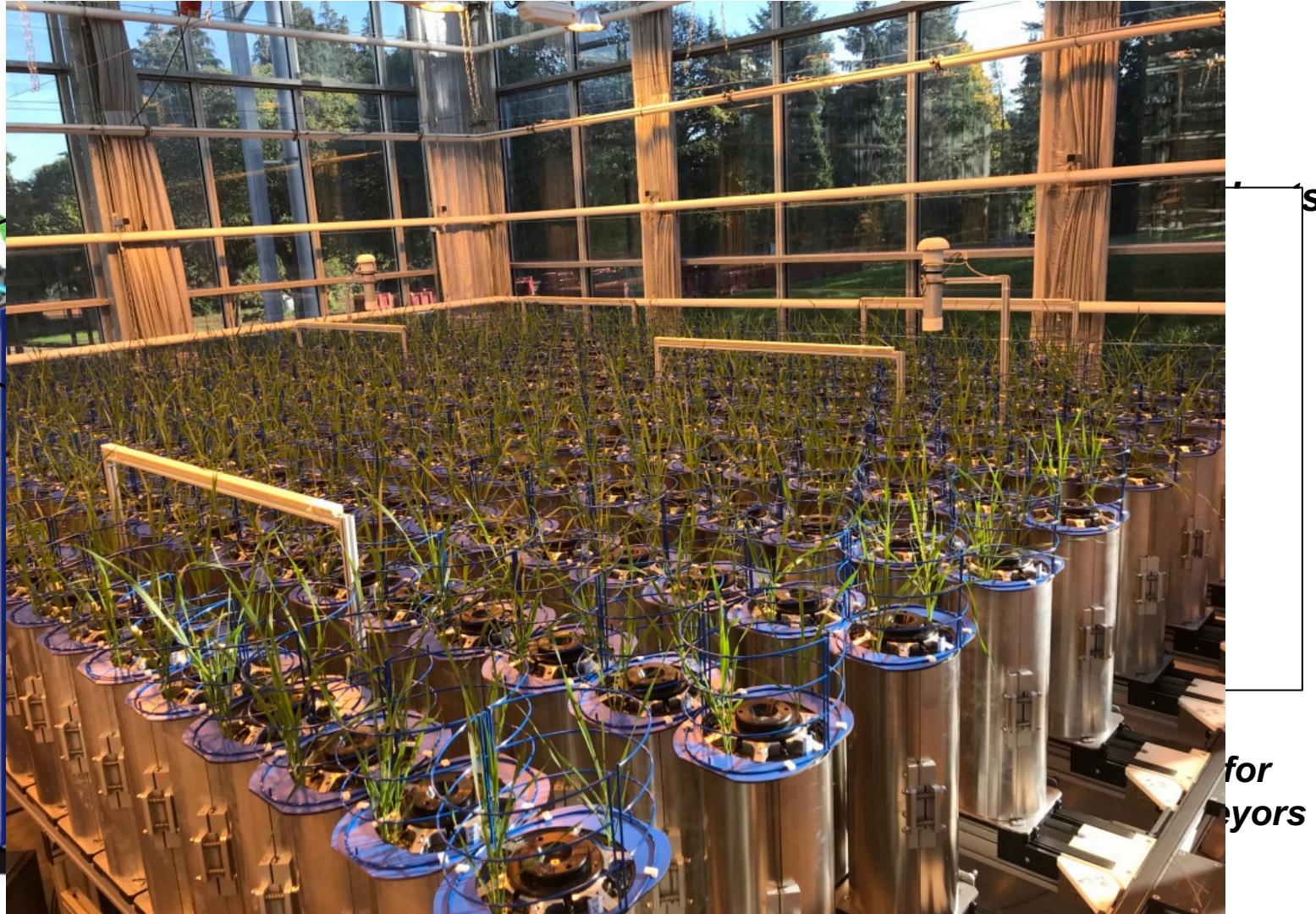


Clark et al. 2011. PNAS, 108(15): 5156-5161

is. 156:455-465.

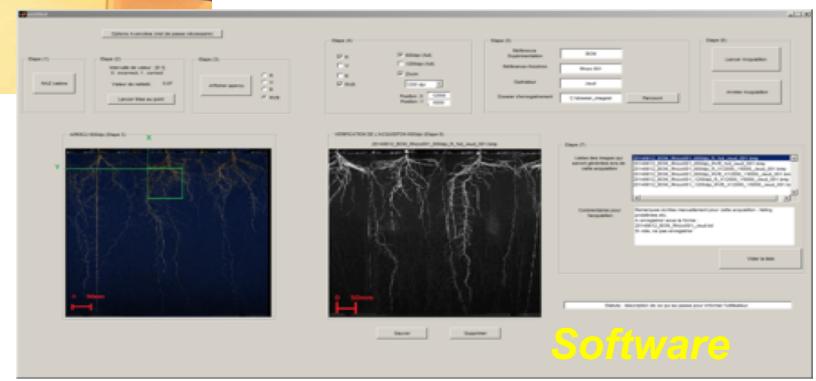
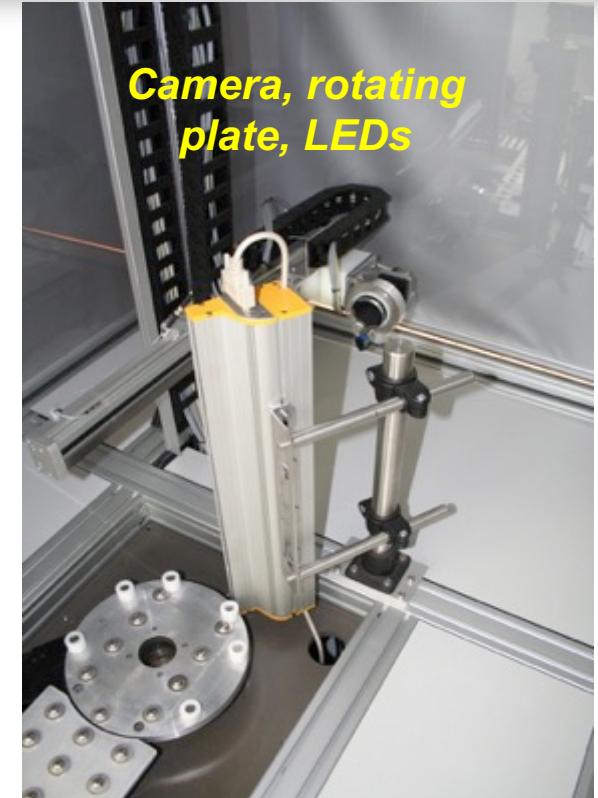
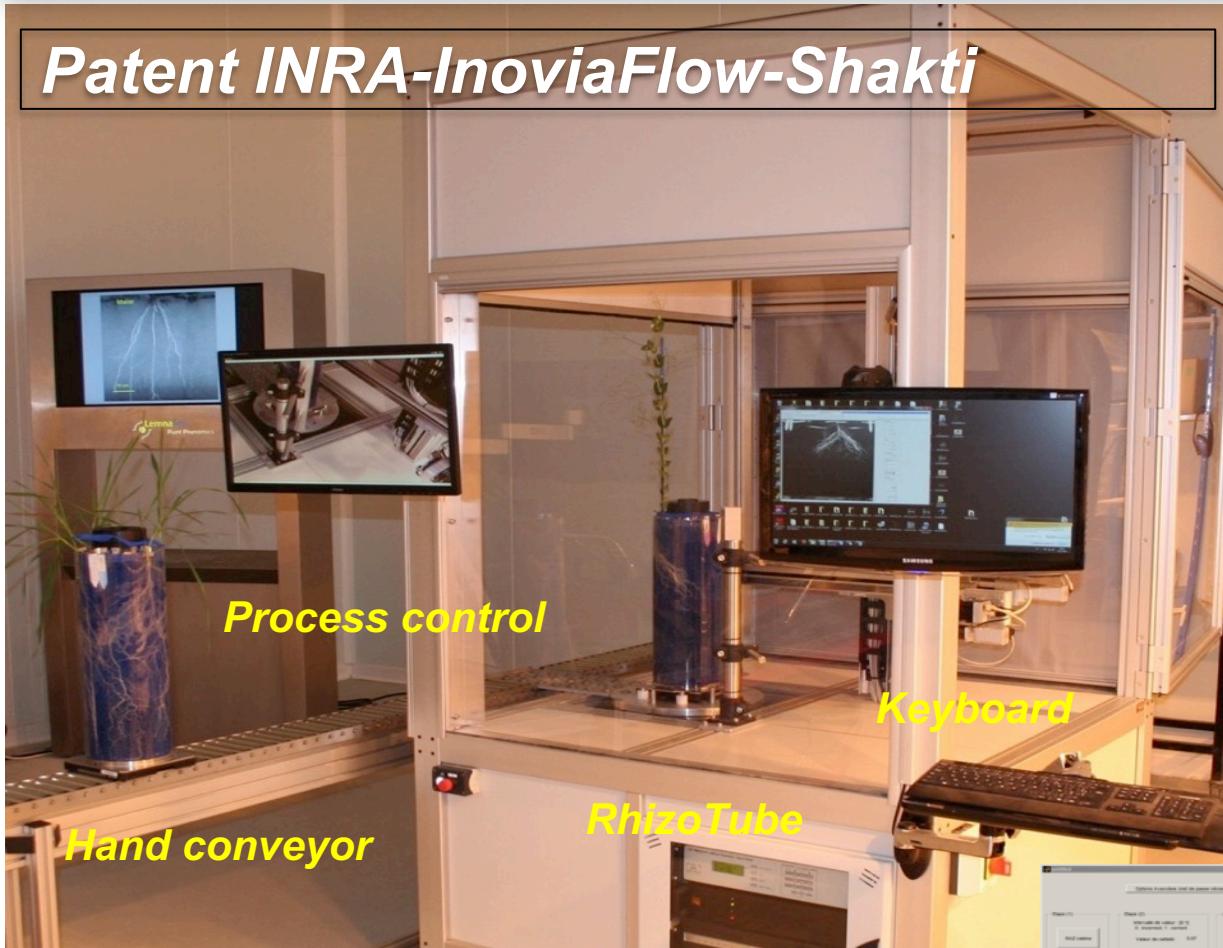
I give up counting slides...

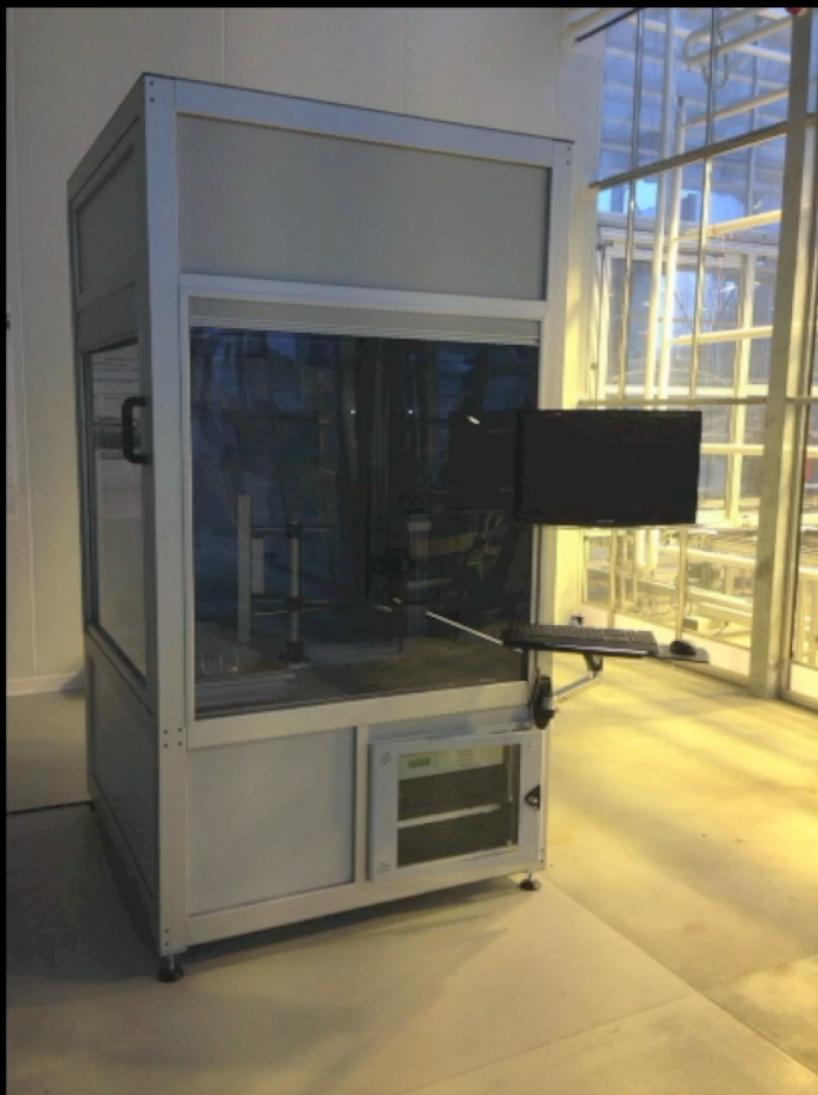
# RhizoTube: the concept

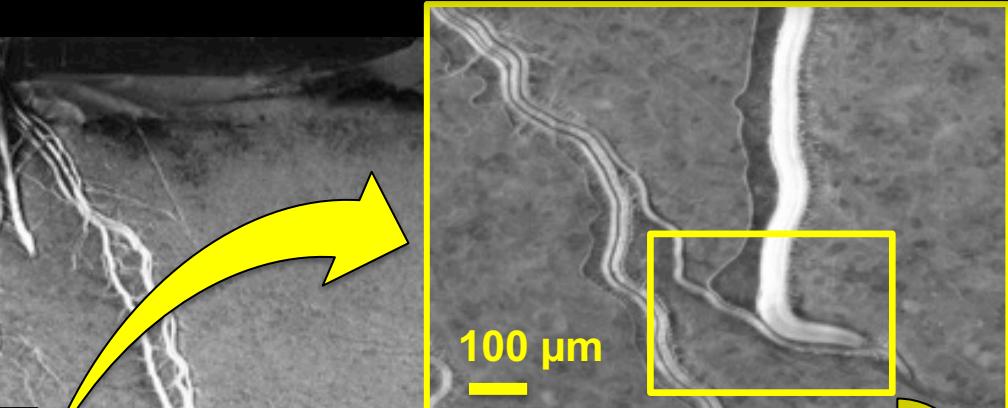
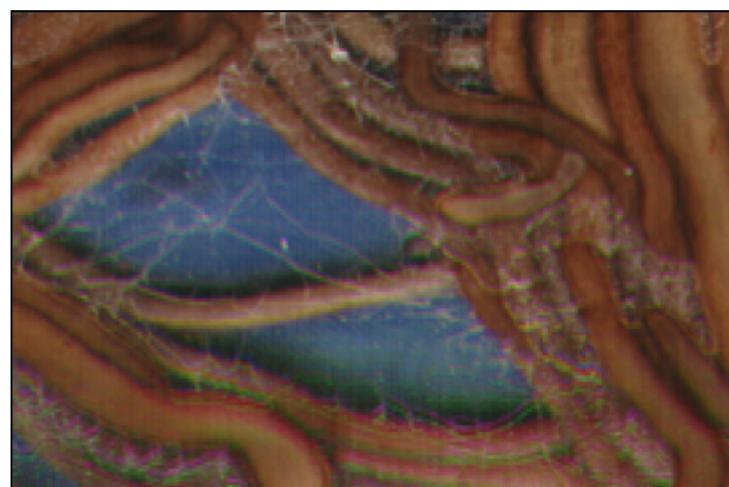




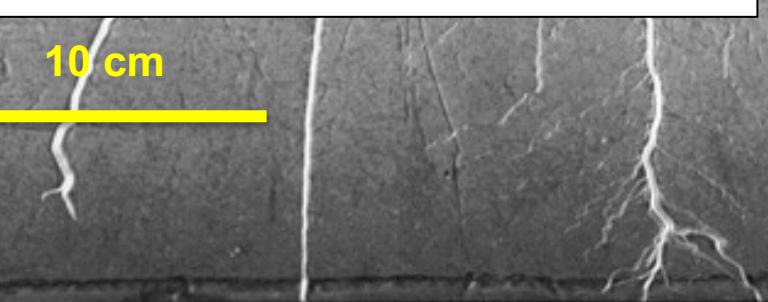
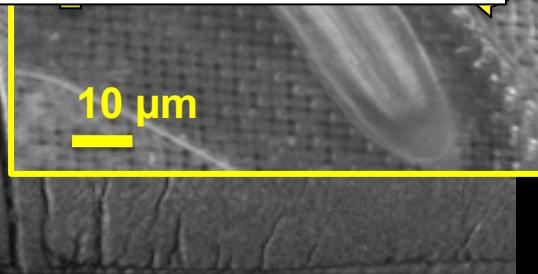
## Patent INRA-InoviaFlow-Shakti

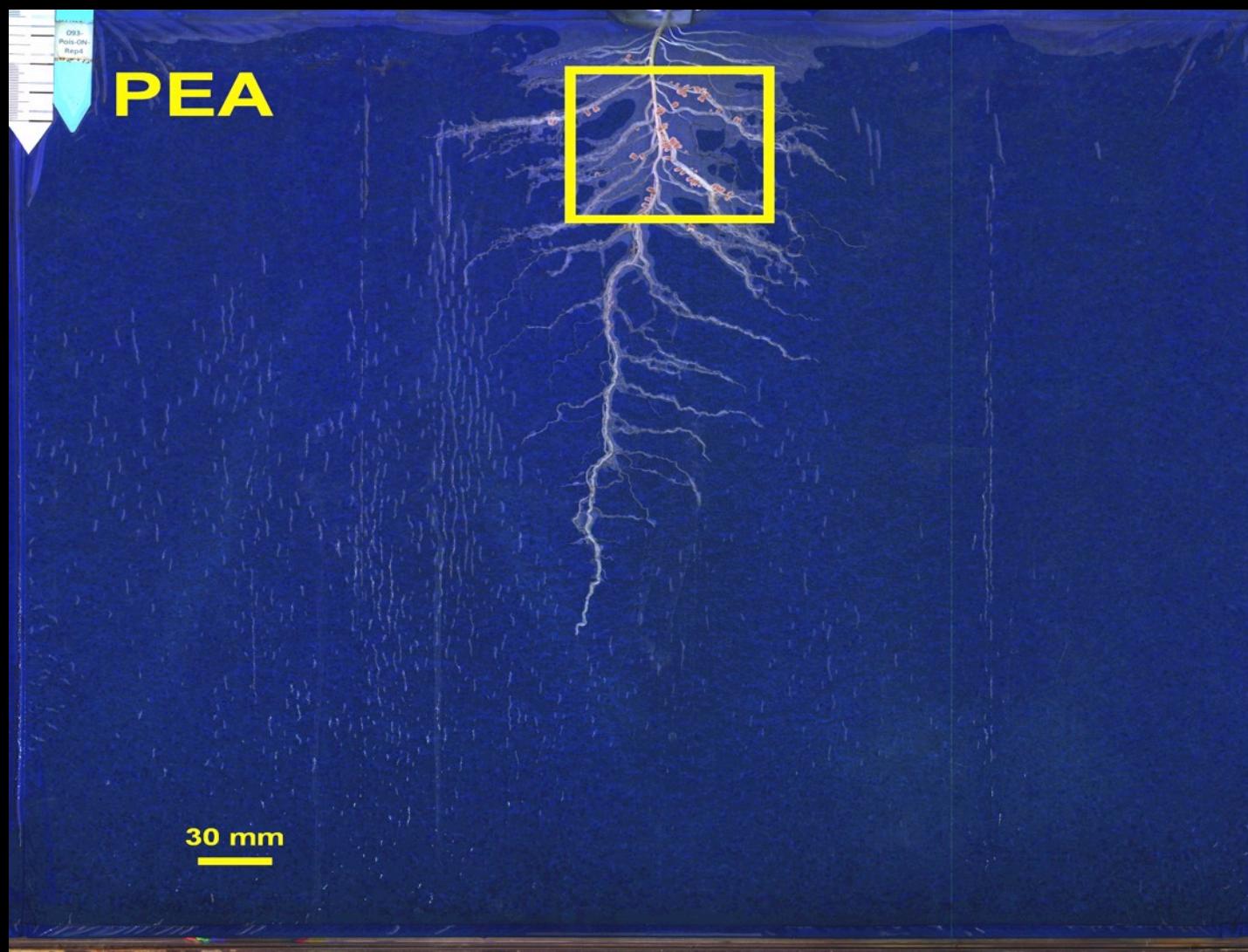


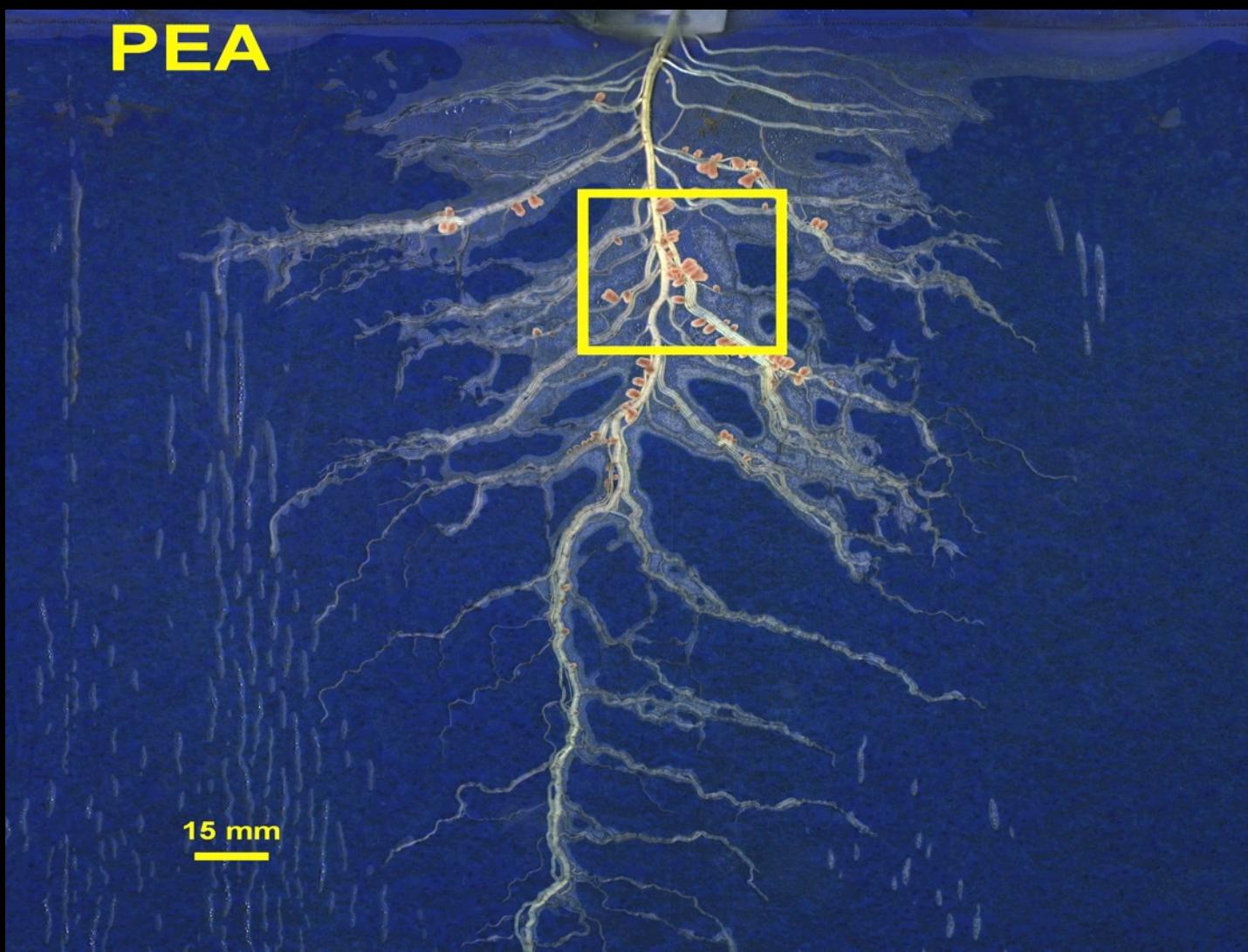


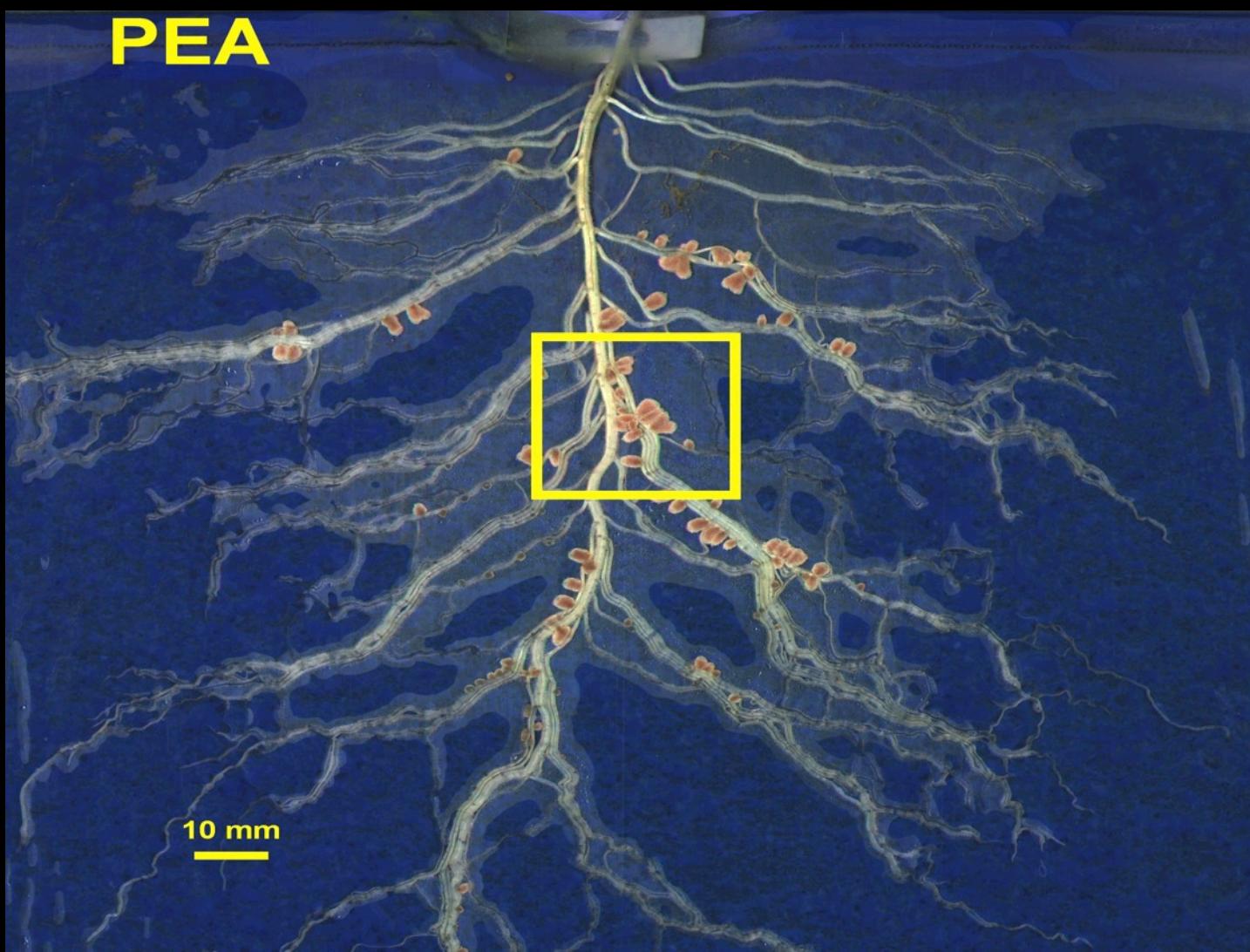
**Maize****Nodules**100  $\mu\text{m}$ **Hyphae**

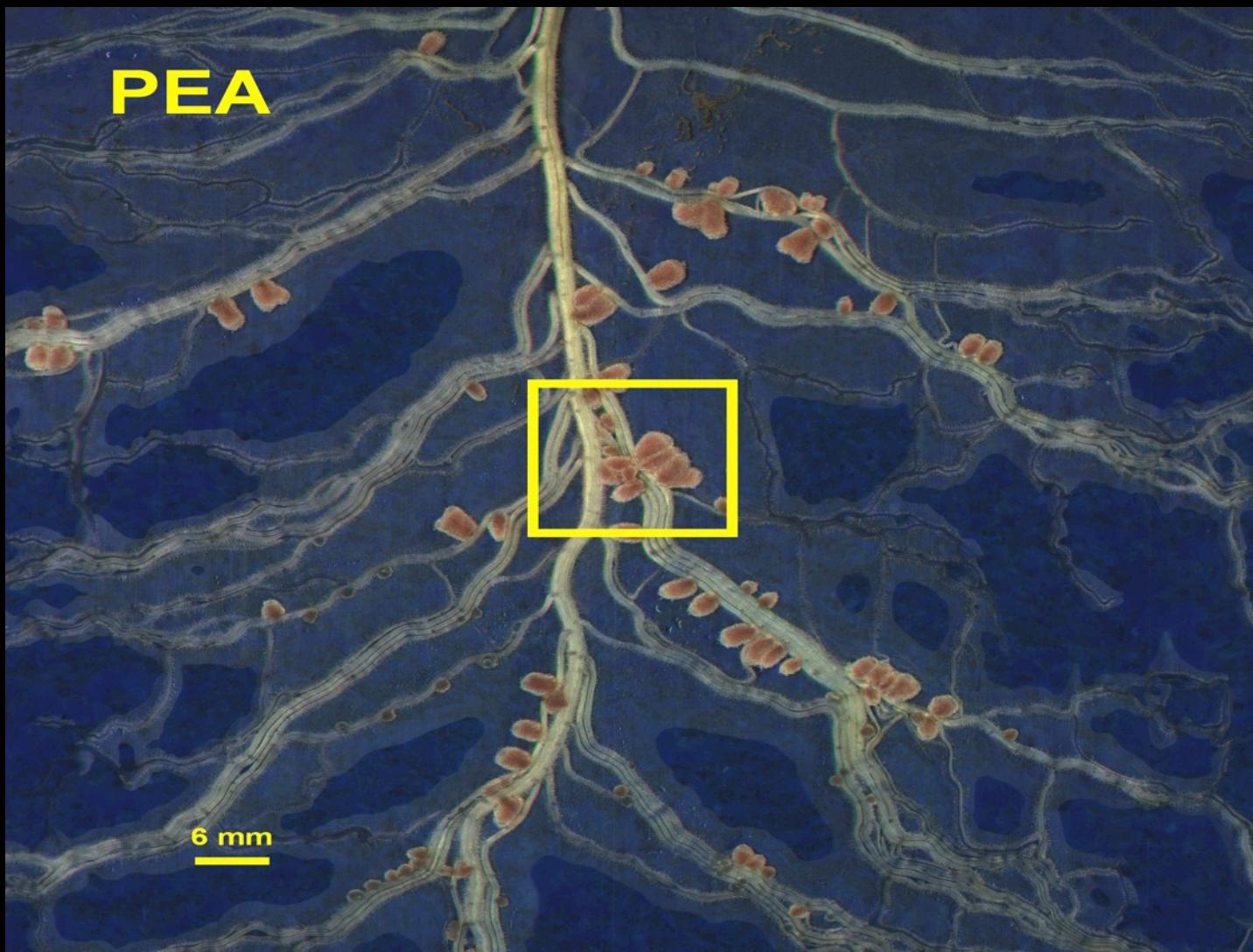
10 cm

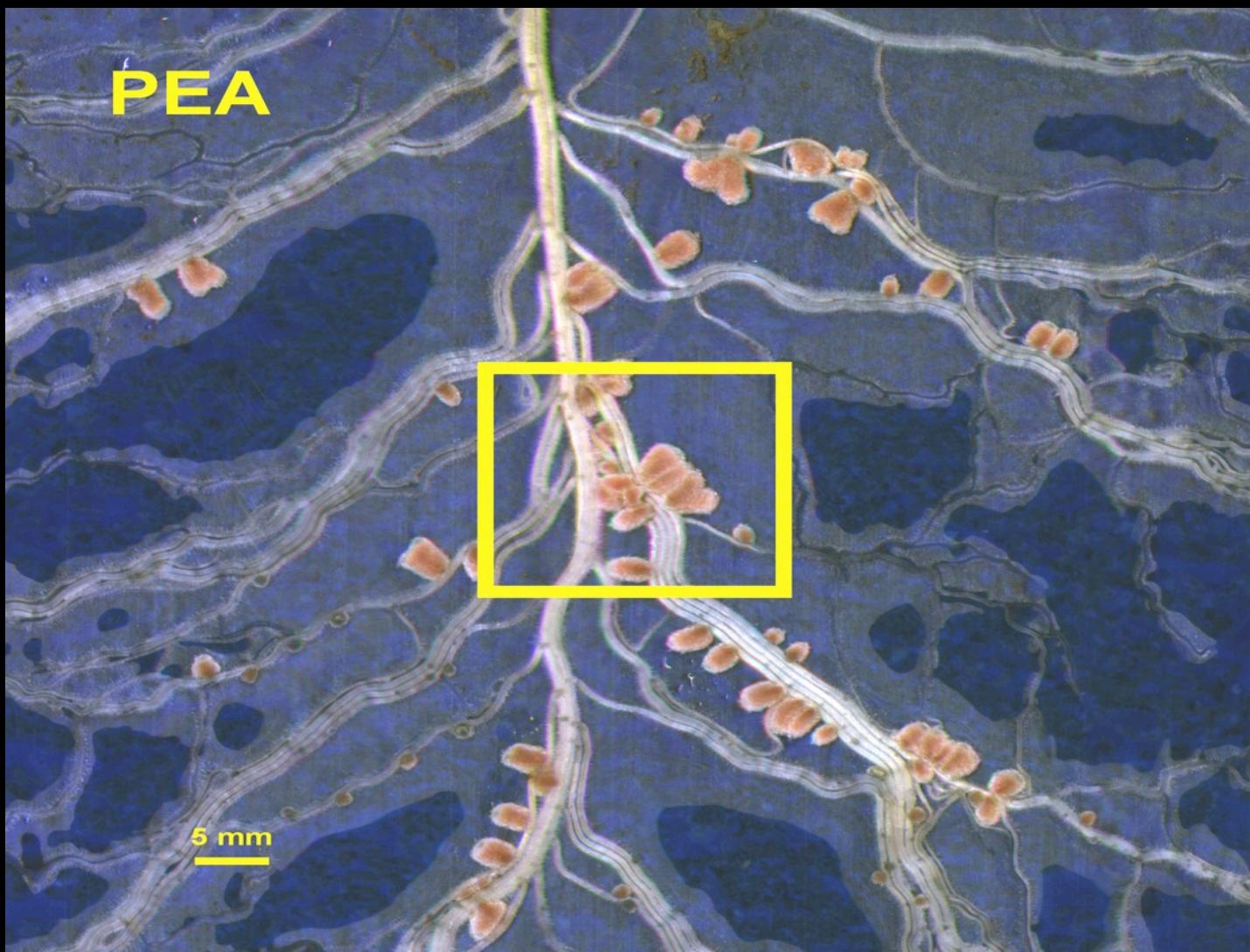
10  $\mu\text{m}$ 

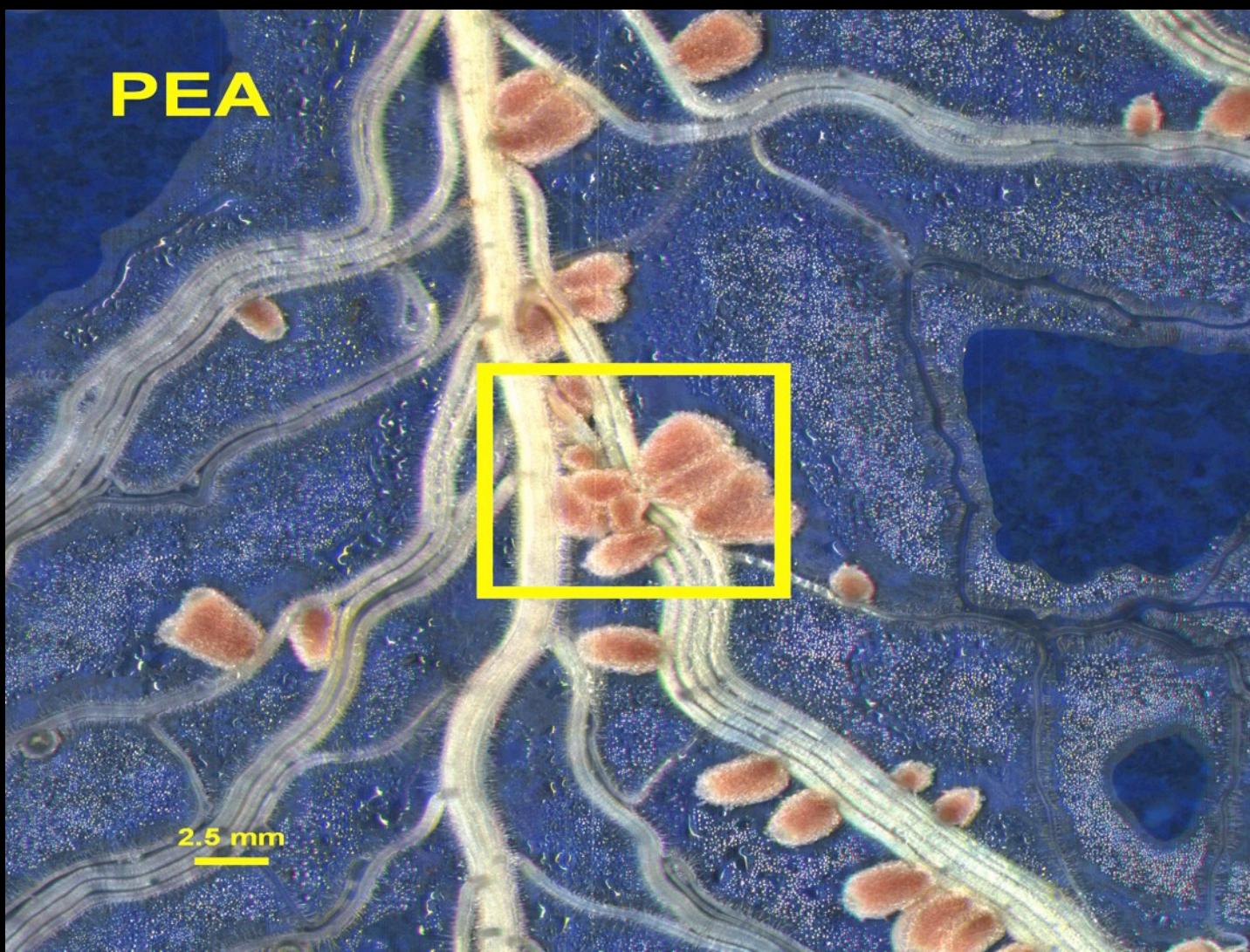








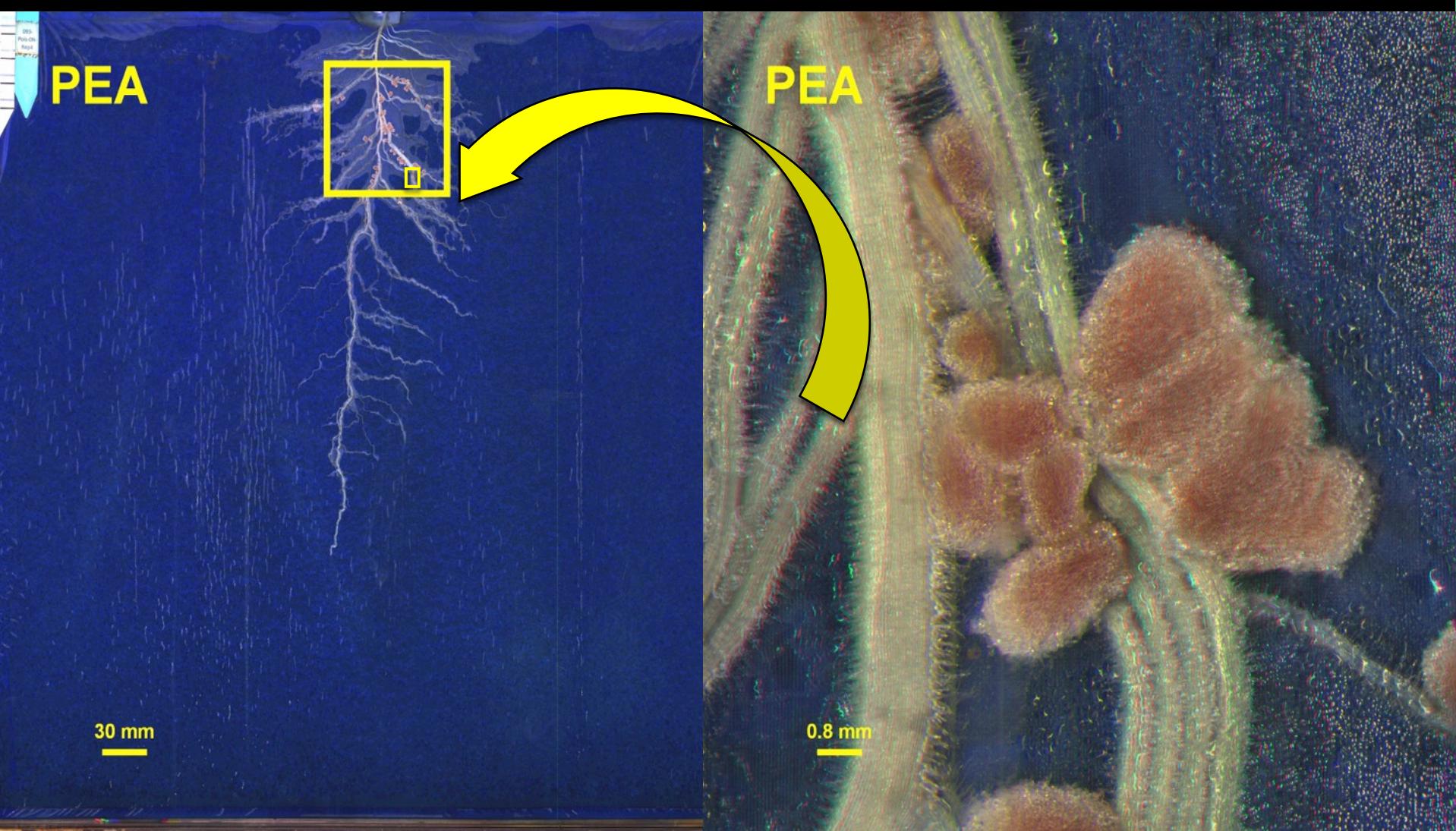














World wide  
distribution



PhenoTrait

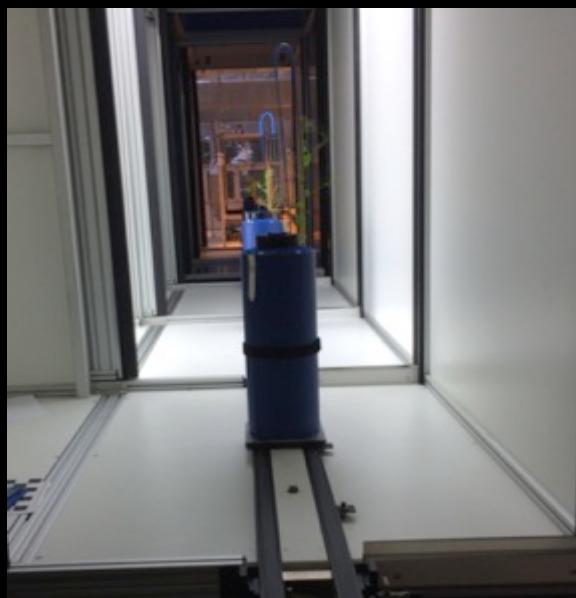
Trademark





## RhizoCab HR

Medium throughput:  
100 RT/Day, 5-60s/day/WL  
Very high resolution (7  $\mu\text{m}$ )

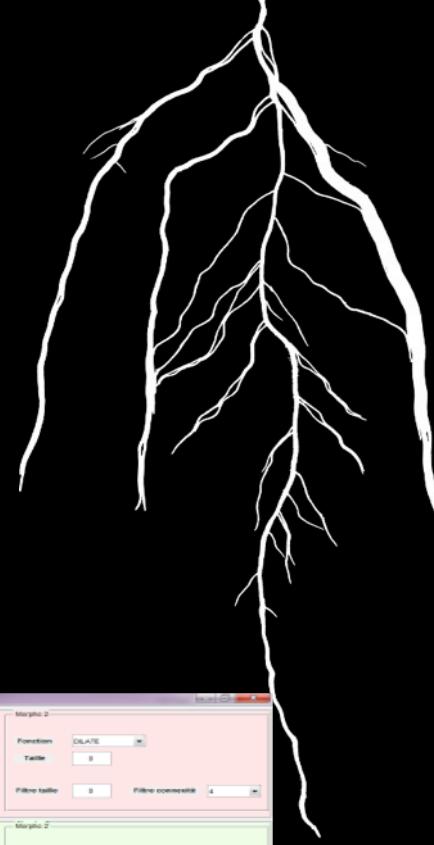
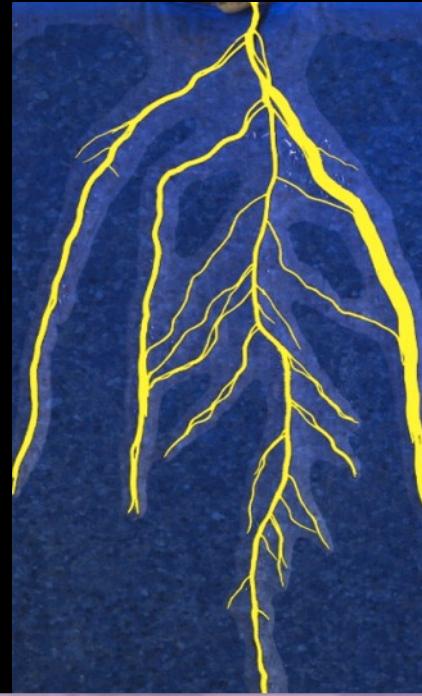


## RhizoCab HT

High throughput:  
1000 RT/day, 5s/RT/WL  
Medium resolution (42  $\mu\text{m}$ )



# Segmentation software

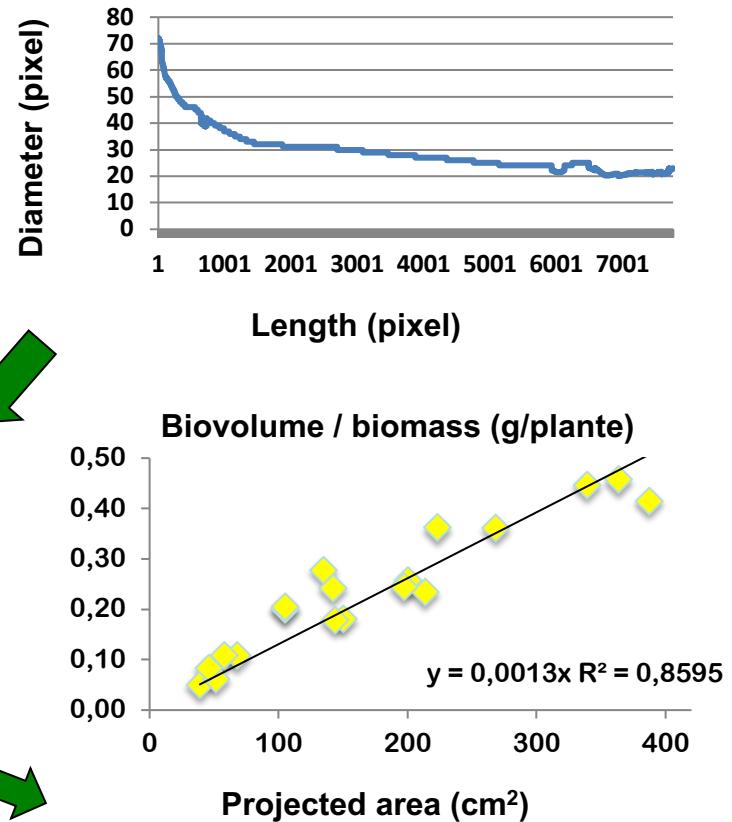
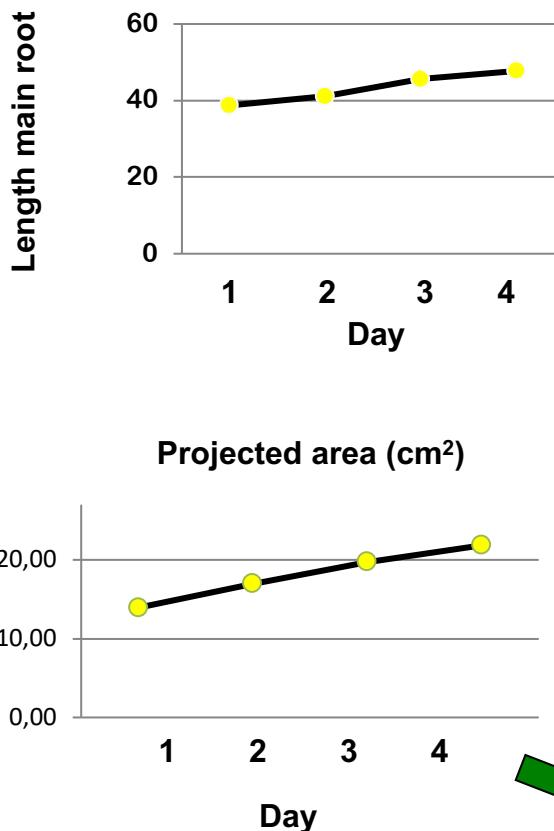
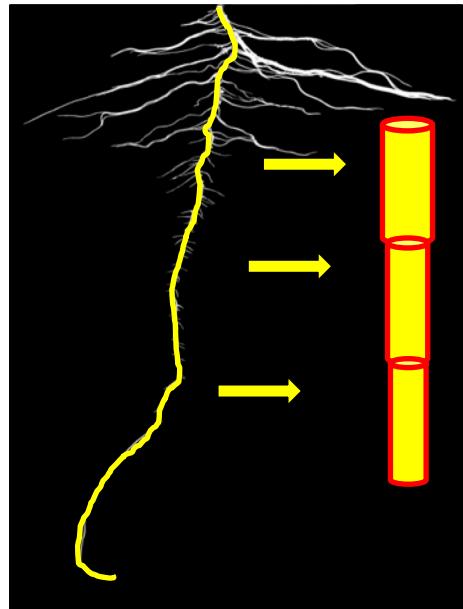


The screenshot shows the Adhèse - Application d'Analyse Adhésive (beta 1) software interface. The main window displays three panels for segmentation: Zone 1 (Latéral), Zone 2 (Latéral), and Zone 3 (Latéral). Each panel includes a color calibration bar, a coordinate system (x\_min, x\_max, y\_min, y\_max), and morphological processing controls (Morpho 1 and Morpho 2) with parameters like Taille and Filtre connectif. Below these panels is a 'Contrôle' section with checkboxes for 'Utiliser Zone 2', 'Utiliser Zone 3', 'Canal', 'IRIS', 'Fluorescence', and 'Visible'. At the bottom left are buttons for 'Importer des paramètres de segmentation', 'Exporter des paramètres de segmentation', 'Import des données d'analyse', and 'Reset Analyse'. On the right side, there are buttons for 'Segmenter', 'Analyser', 'Segmenter > Analyser', and a large blue button labeled 'En attente'.



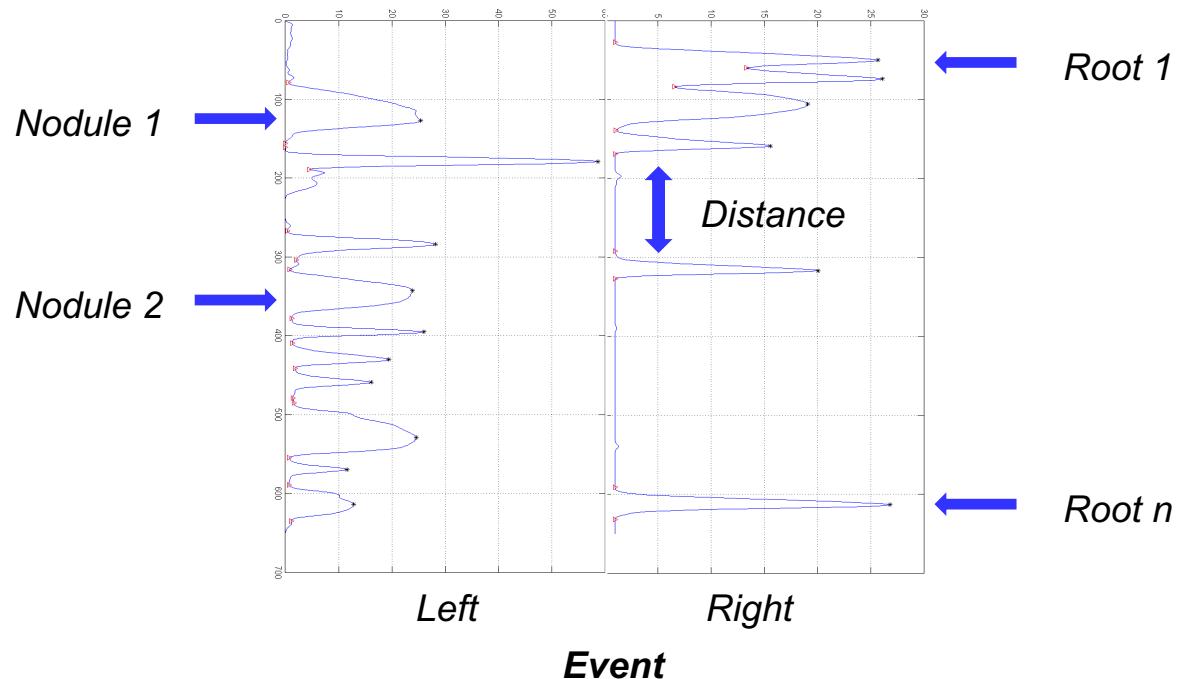
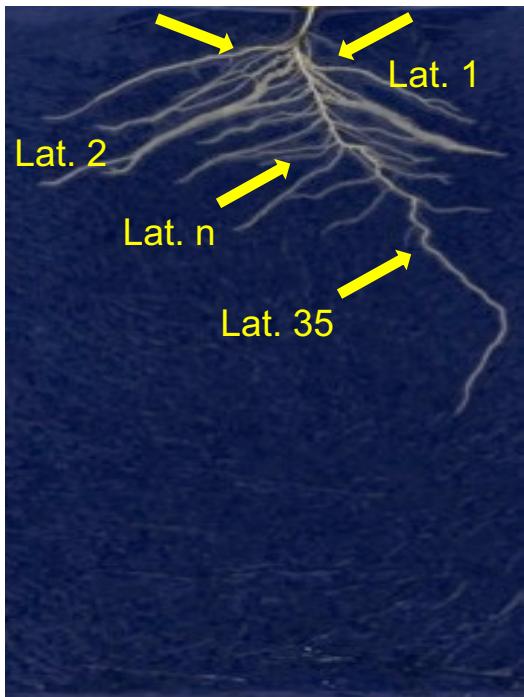
M. Lamboeuf

Roots: Length, diameter => projected area => biovolume



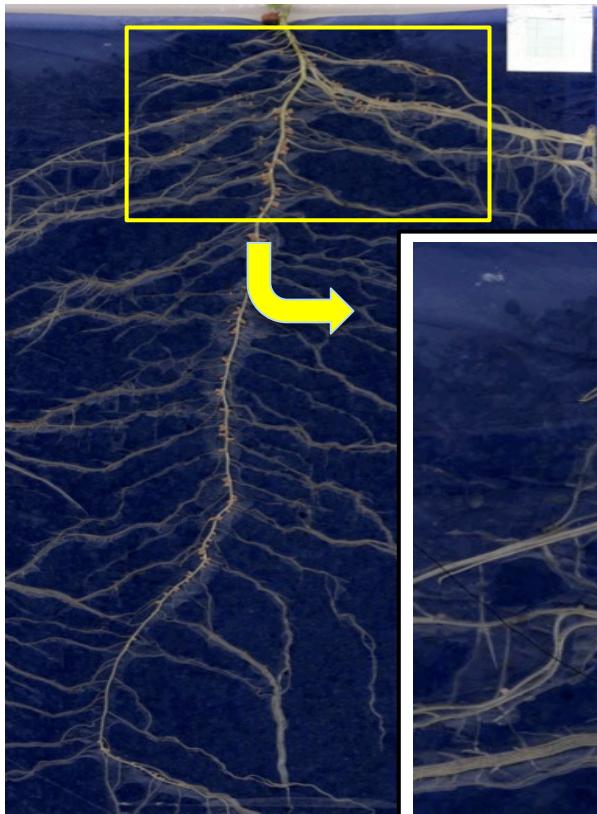
**Dynamic trait characterization**

## Roots, detect events: lateral roots and nodules detection

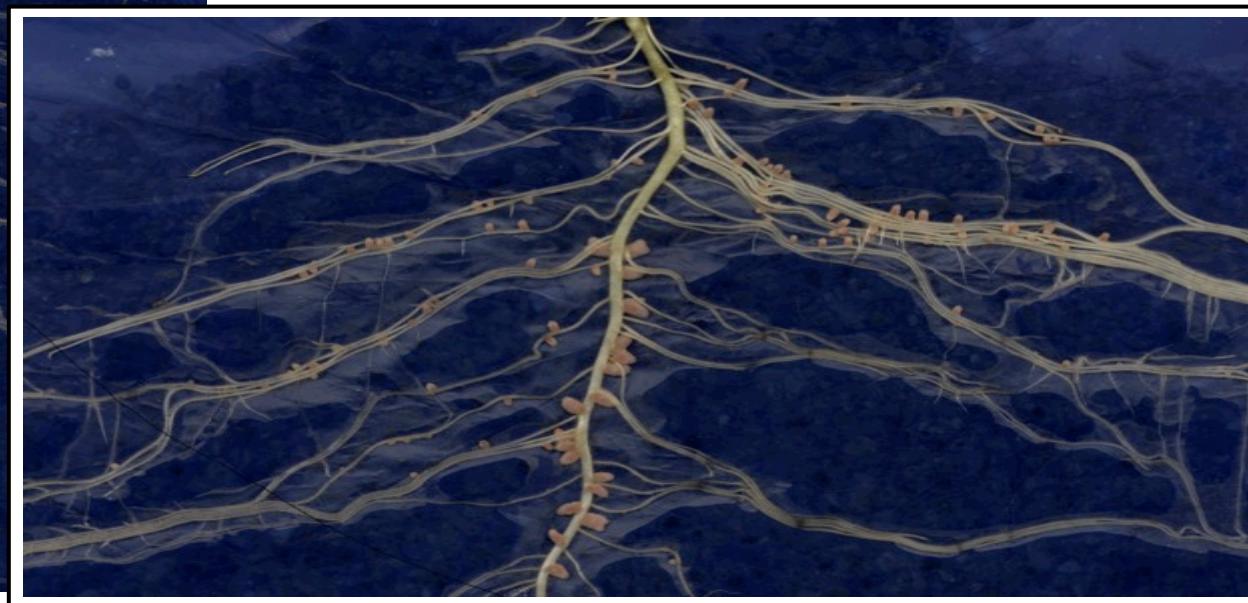


**Nodules and lateral roots detection**

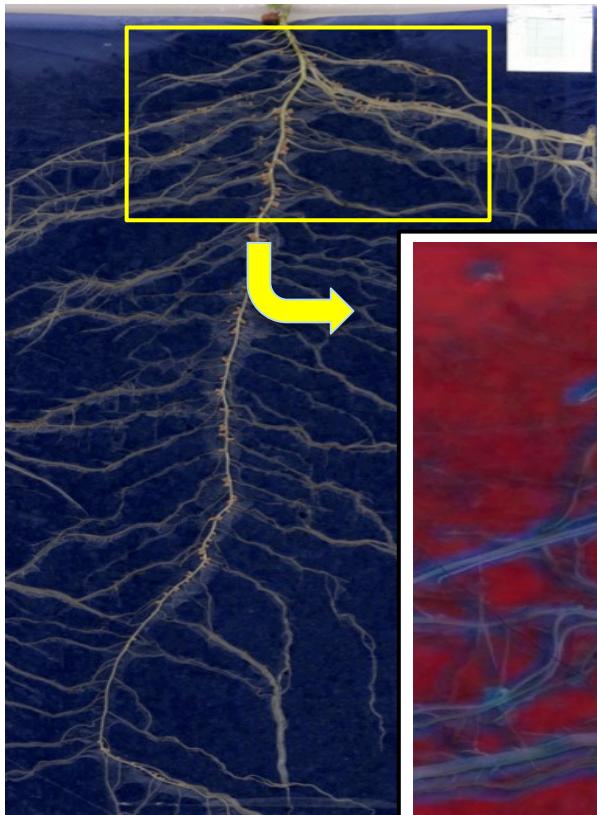
Nodules: Number, projected surface, position, color



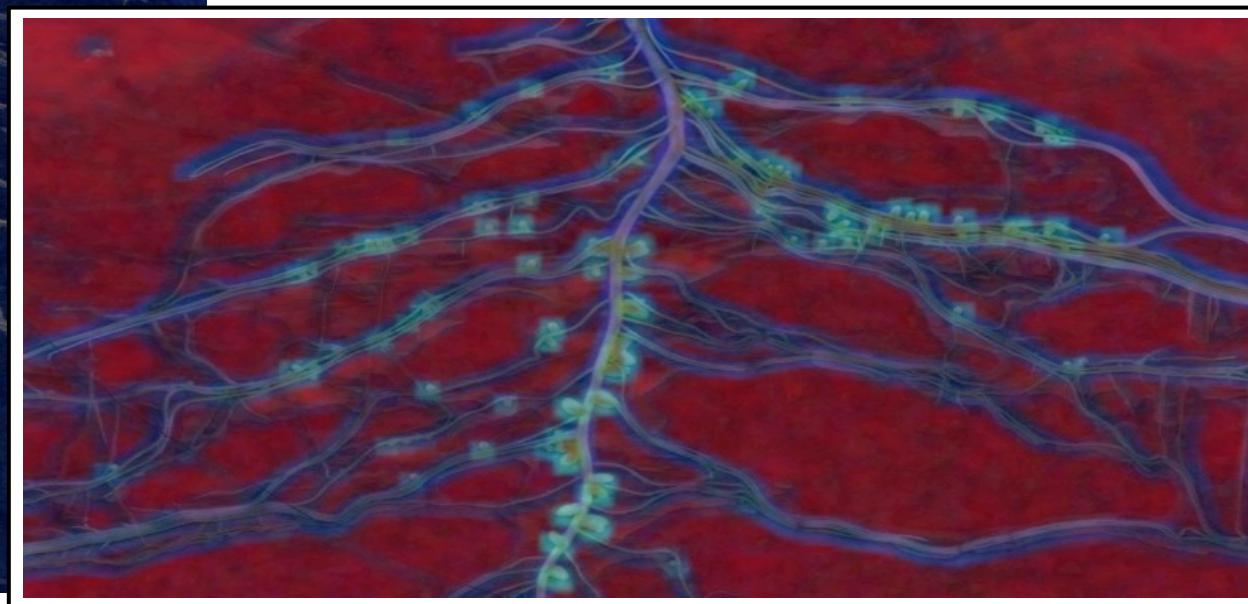
Focus on image



Nodules: Number, projected surface, position, color



**Hybrid spaces (color + texture)**  
(Cointault et al, 2008)



Nodules: Number, projected surface, position, color

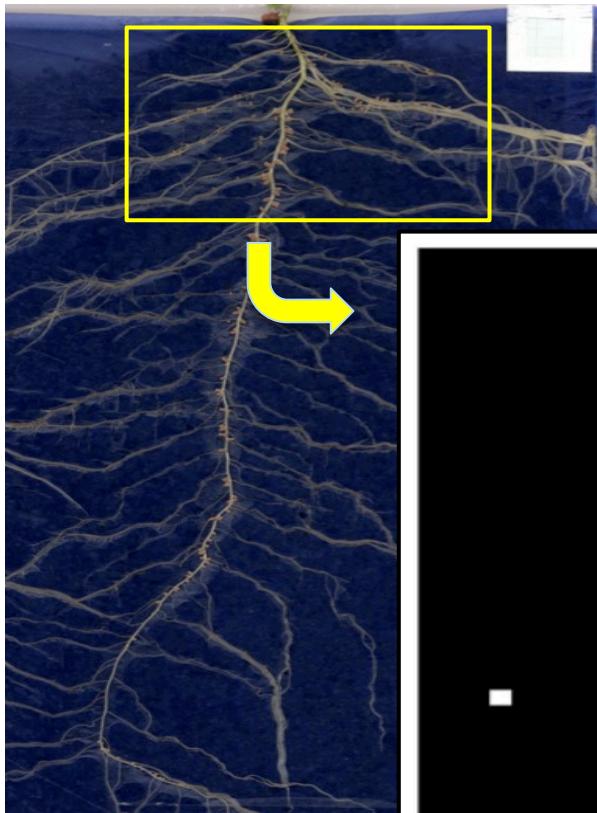
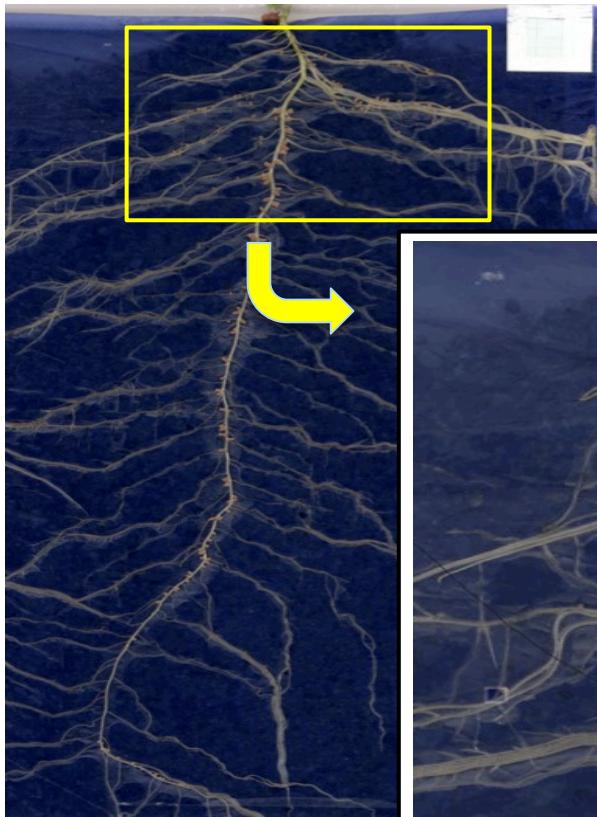


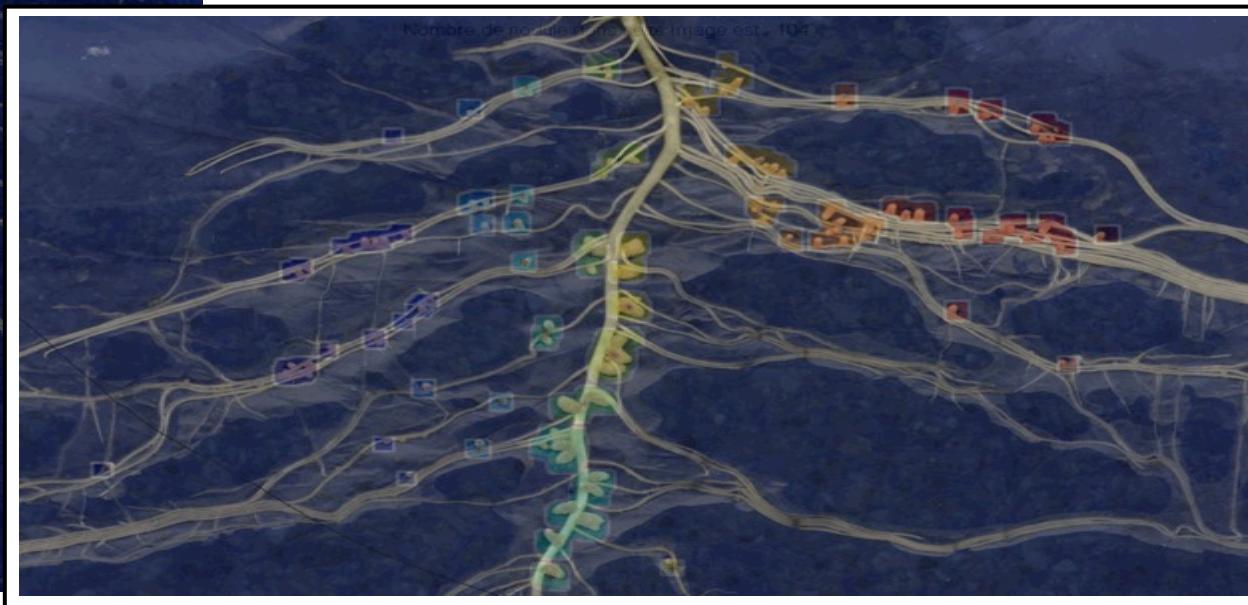
Image with nodules



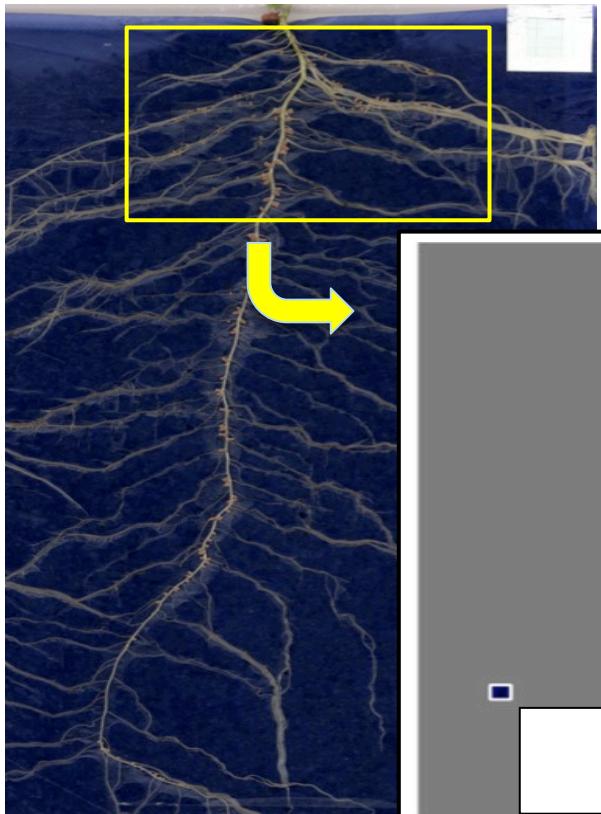
Nodules: Number, projected surface, position, color



Original image + superimposed nodules

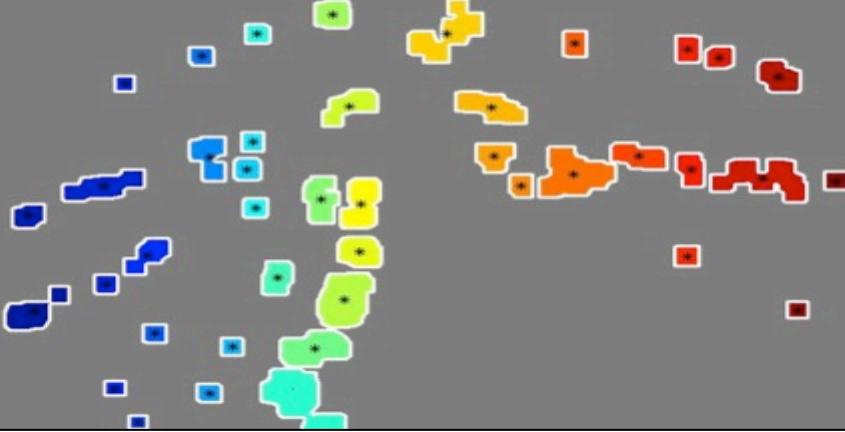


Nodules: Number, projected surface, position, color



Nodules detected

Nombre de nodule dans cette image est : 104

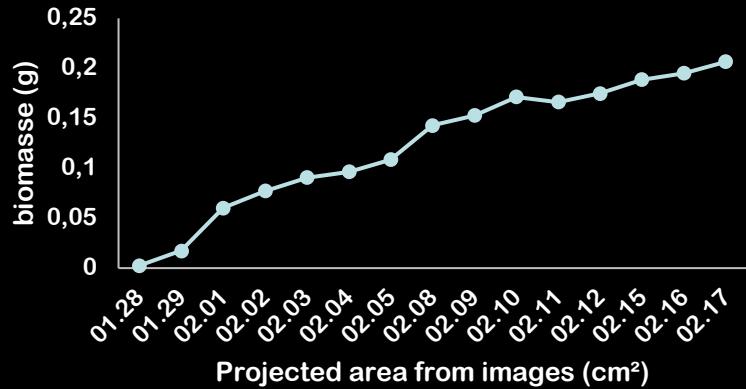


95% detection efficiency

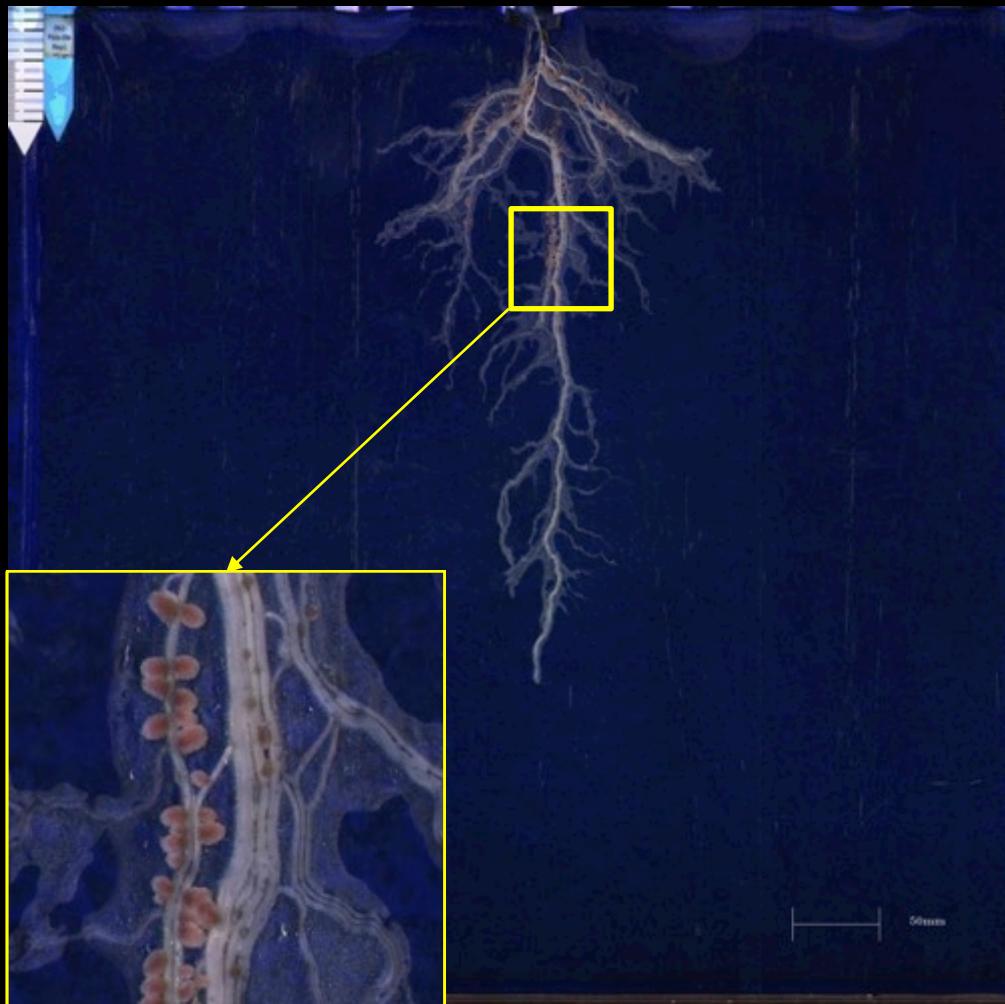
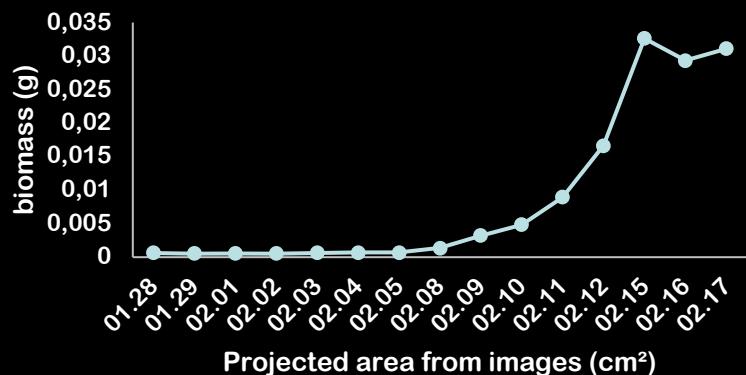


## A simple example: root and nodule dynamics

Root biomass



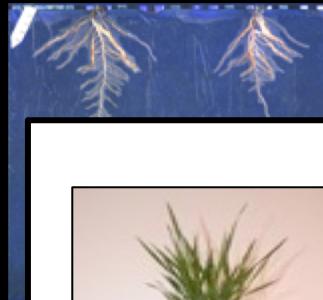
Nodule biomass



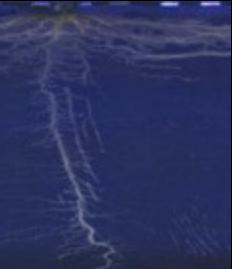
# Which species?



Pea



Vesce Commune



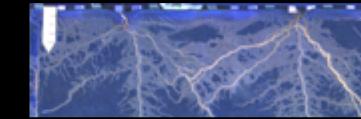
Tomato



Wheat



Medicago



Brachypodium

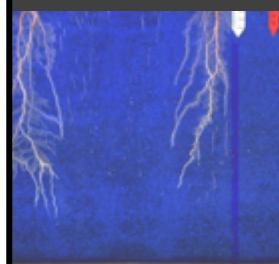


Grape

Alone...



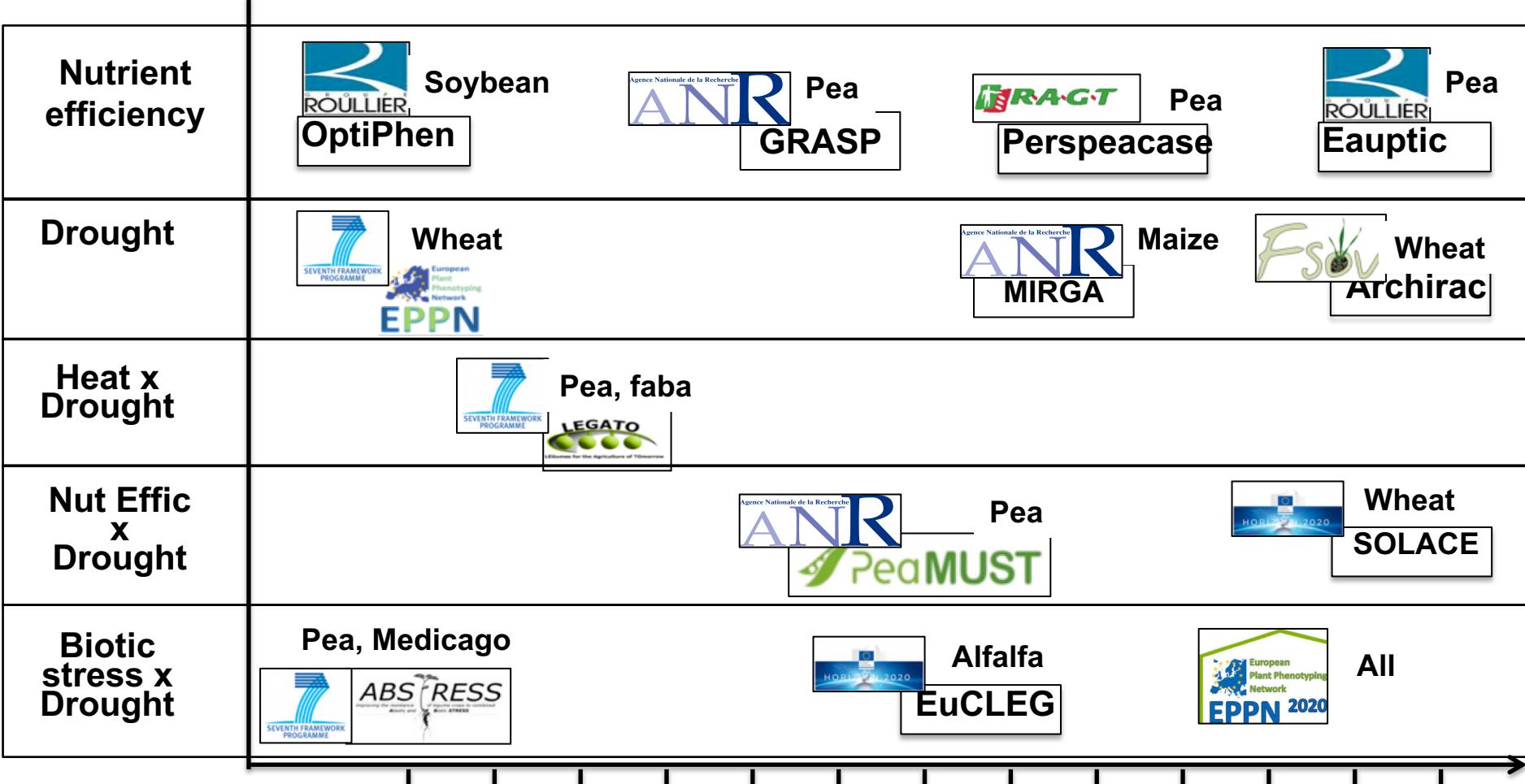
Soybean



Maize



... or in association



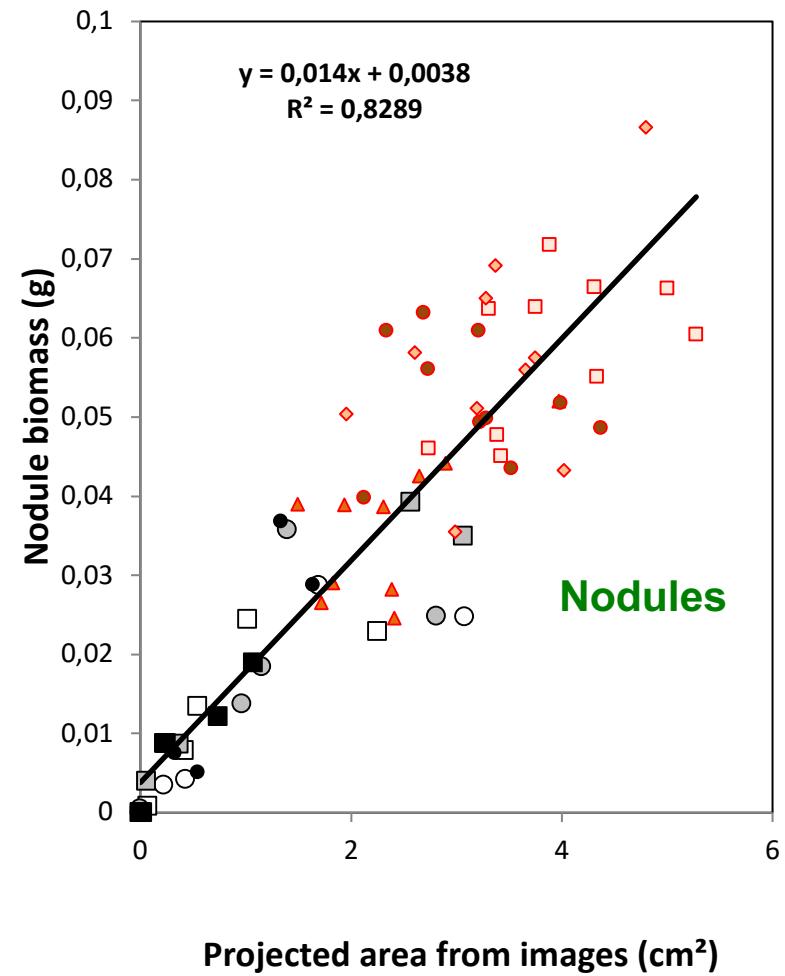
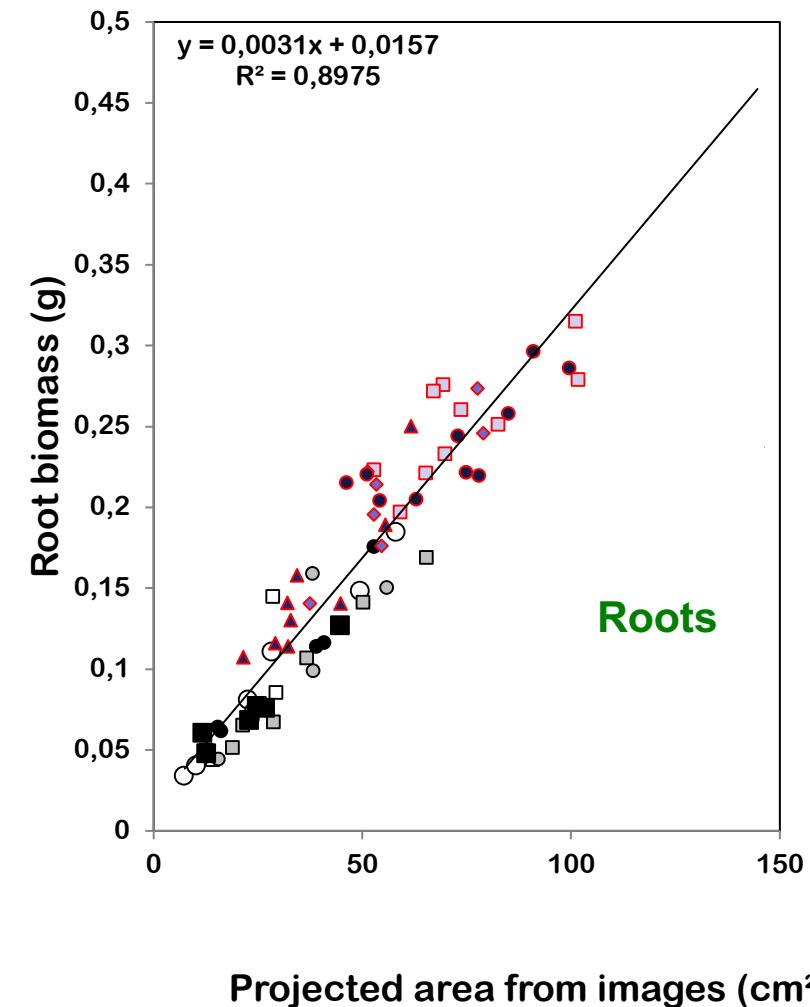
500

1000

Throughput (rhizotube number)

# Some results: Pea

## Genotypes with contrasted architectures: pea



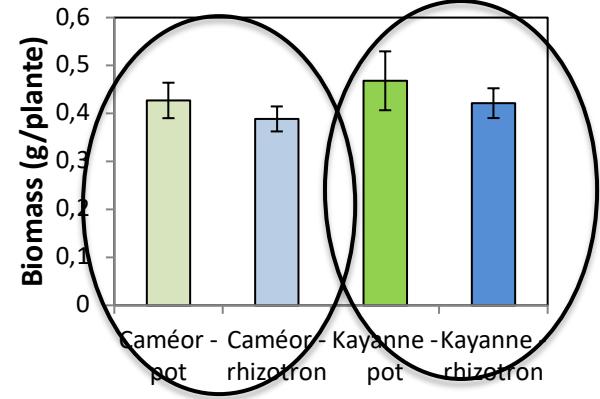
# RhizoTubes vs Pots ?

Similar traits in pots and RhizoTubes: pea

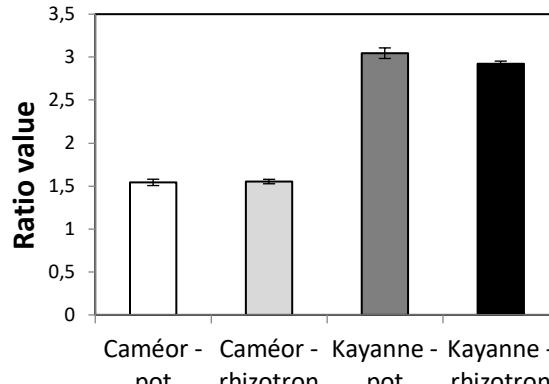


C. Jeudy

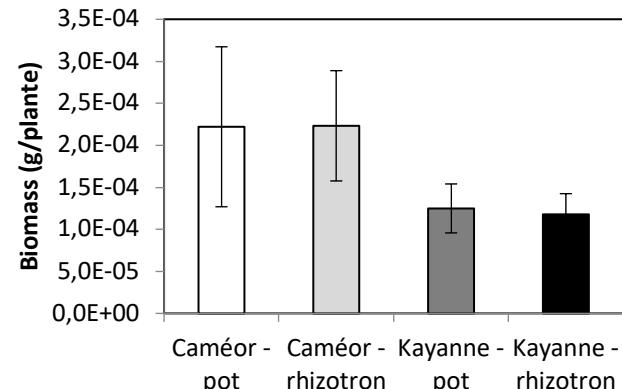
Plant biomass



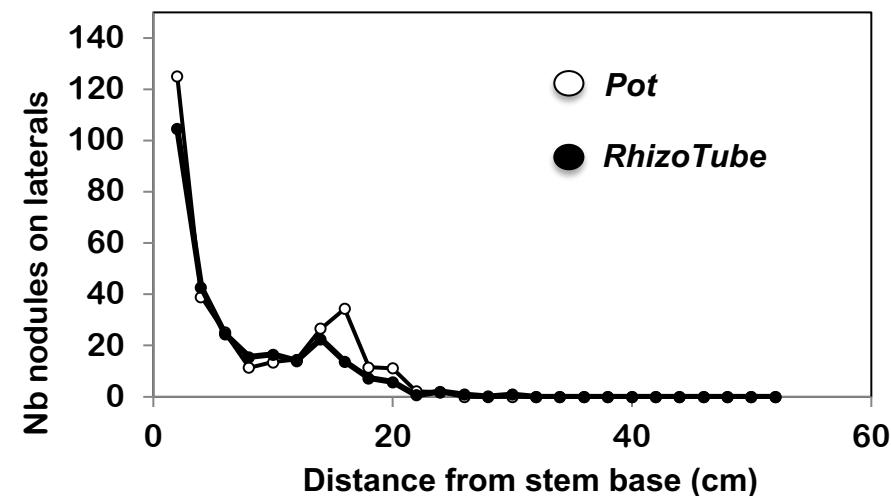
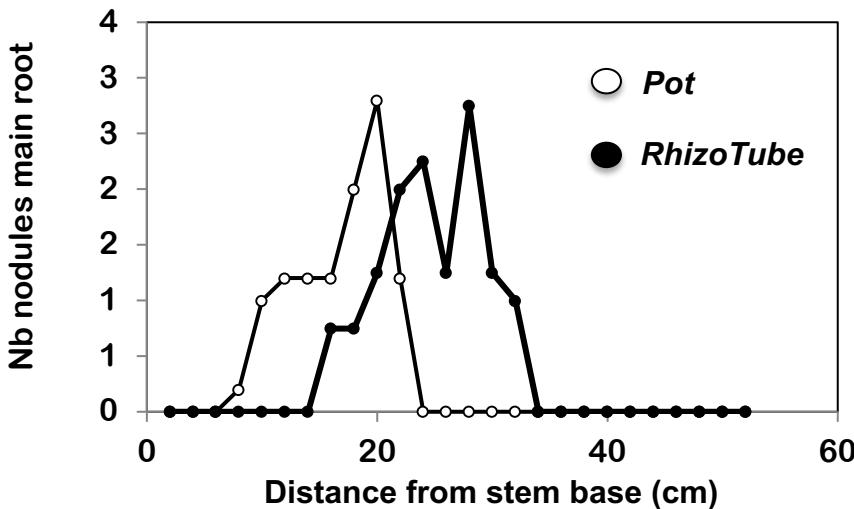
Shoot/root biomass



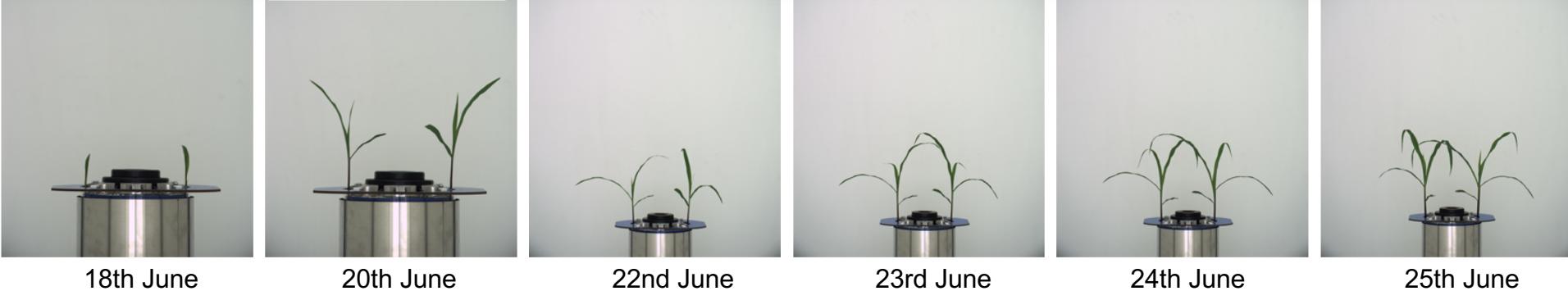
Mean nodule biomass



Same distribution profile!



# Some results: Maize (MIRGA)



18th June

20th June

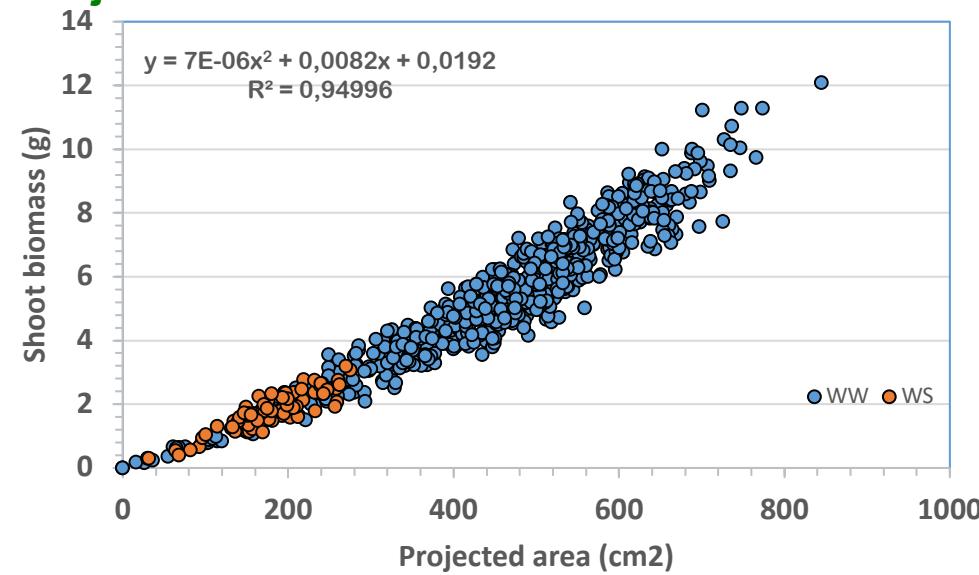
22nd June

23rd June

24th June

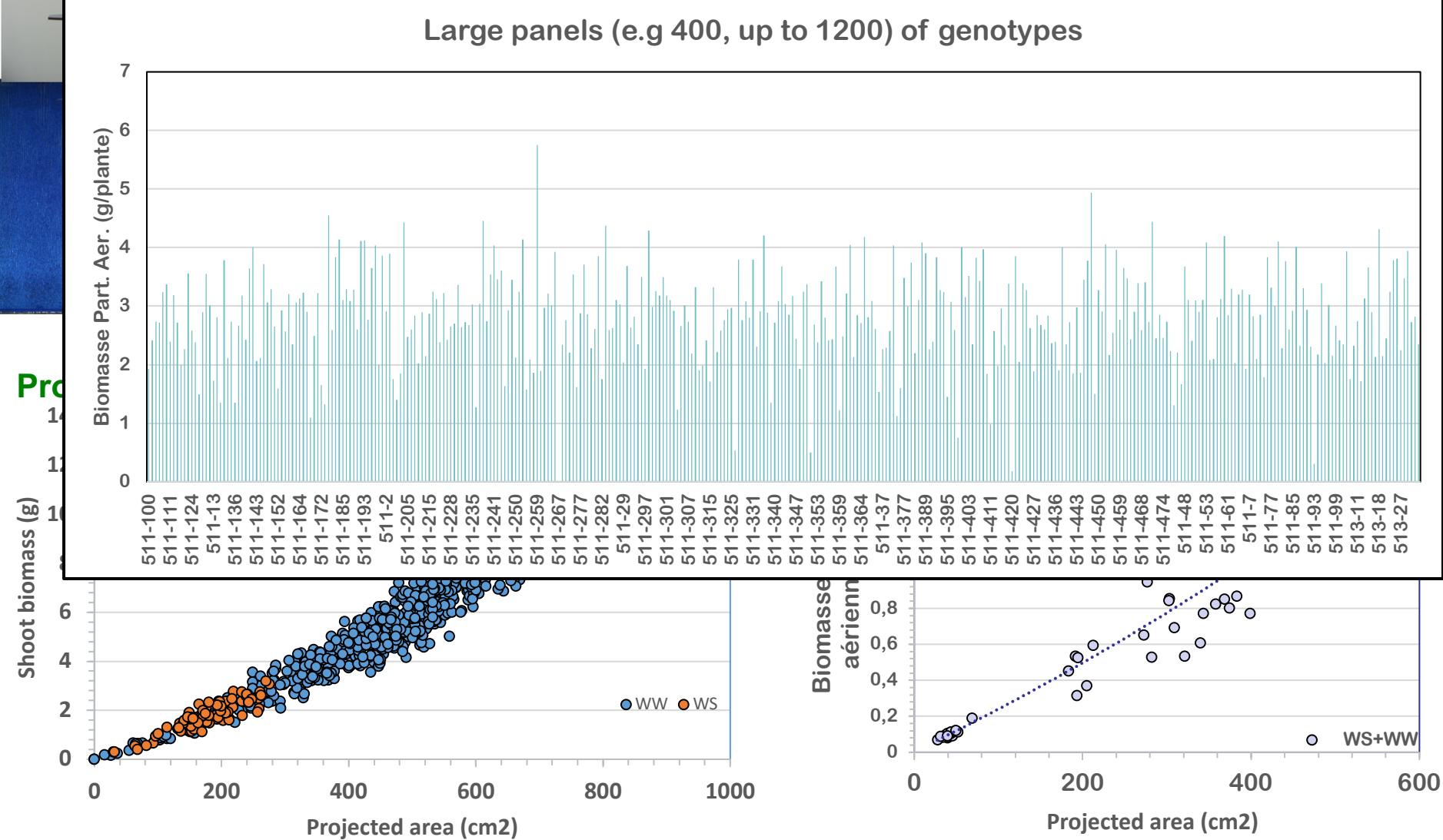
25th June

## Projected area vs shoot biomass



# Some results: Maize (MIRGA)

Large panels (e.g 400, up to 1200) of genotypes





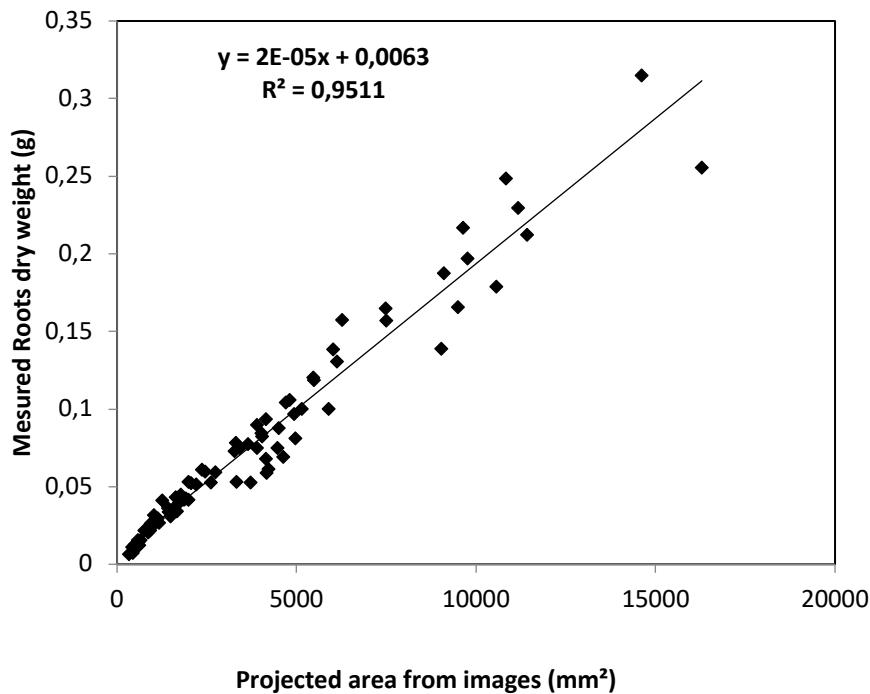
## Projected area vs root biomass and length: wheat

*EPPN Project, Josh Klein AARO Volcani Israel*

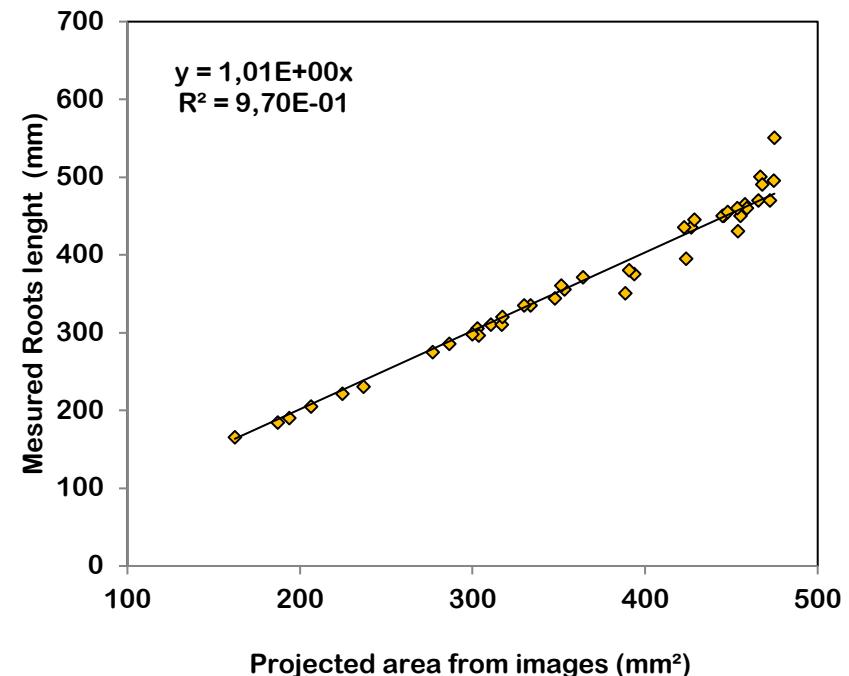


**Josh Klein**

**Root dry matter**



**Root length**



# **Christmas wishes...and what you'll get under the tree**

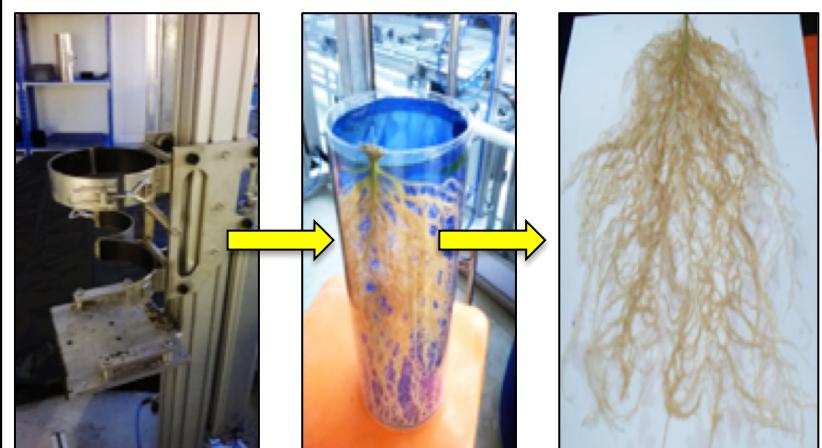
Automatic trait quantification	Done	Nearly done	... soon
Root projected area	X		
Total root length	X		
Root convex hull	X		
Root exploration dynamics (H and V)	X		
Root density		X	
Root number (incl. typology)		X	
Root angle		X	
Root diameter		X	
Nodule projected area	X		
Nodule number (inc.typology)	X		
Nodule biovolume (inc. classes)		X	
Nodule number, position on each root			X
Nodule efficiency			X
Mycorrhizes, hyphae		X	
Germination checkup	X		



**Mounting**



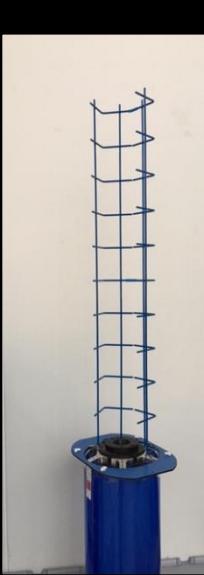
**Empotting**



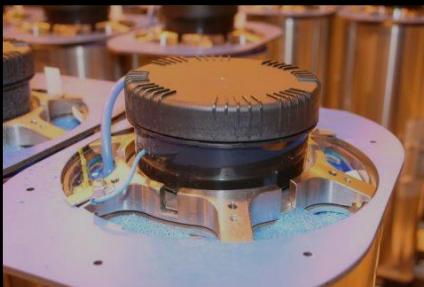
**Fast root recuperation  
(allows 'omics)**



**Germination chamber**



**Tutors**



**Bubbling pump**



**Energy base**



# Food for thoughts...



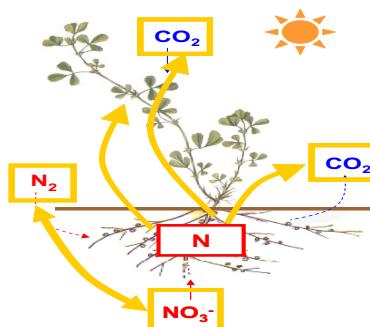
## Combine approaches

Phenotyping  
Approach

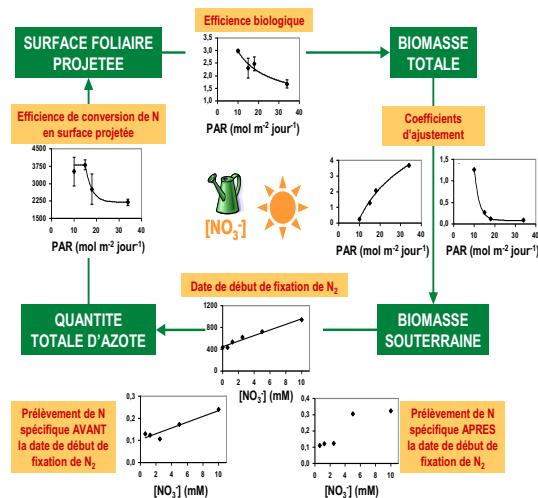


Analytical  
approach

(e.g.  
fluxomics)



Models



Identifying differences  
among genotypes

Interpreting the  
detected difference



**C Bernard**



**C Jeudy**



**J Martinet**



**K Palavioux**



# The 4PMI Group...



**S Han**



**JC Simon**



**F Cointault**

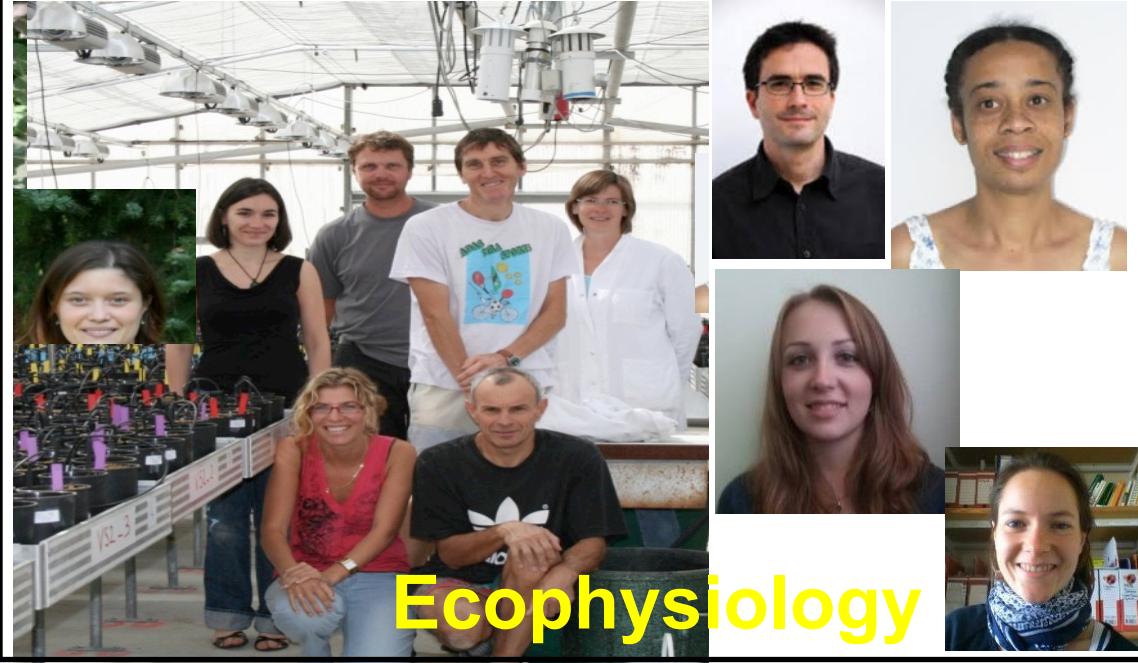


**M Lamboeuf**



**C Baussard**

# *The GEAPSI Group...*



## FILEAS



## Proteaginous target crop

