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Phenotyping plant and organisms interactions in the context of sustainable agriculture

Christophe Salon

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Submitted on 5 Jun 2020

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Select the best performing crops..

..“better” yield, environmental « efficiency

Understand plant response to environment
identify genes.

Plant architecture, flowering, senescence

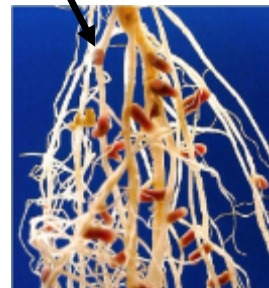
Pods and seeds



Roots and interactions with micro organisms

Valorisation of beneficial biological interactions


Major bottleneck: high-throughput, non-invasive and multiscale phenotyping



Bacteria

Mychoriza

Tools and methods

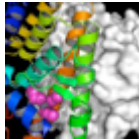


Genetic variability



CONCEPTION of PLANT IDEOTYPES

Characterizing mechanisms and molecular basis



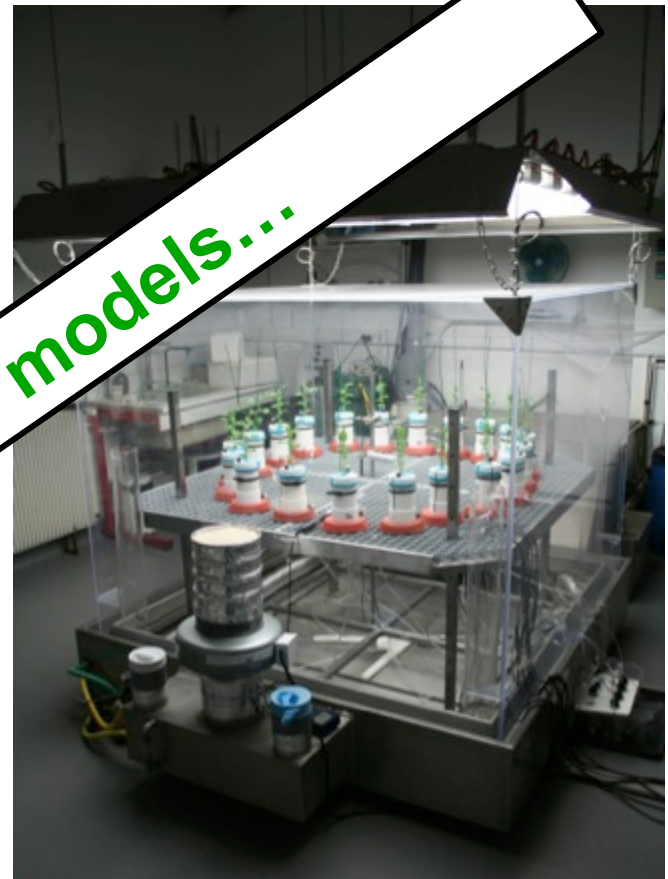
Context	Approach	Phenotypic tools	Examples	Models	Conclusion
		Fluxomic			
		PPHD			
		Rhizotrons			
		Rhizobox			

Characterizing root development



Growth pouches, hydron

C, N, S flux measurement



Labeling chamber $^{13}\text{C}/^{15}\text{N}/^{34}\text{S}$

And also models...



N_2 adapted Isotopic split root

Context	Approach	Phenotypic tools	Examples	Models	Conclusion
		Fluxomic			
		PPHD	Facilities		
		Rhizotrons	Shoot roots etc...		
		Rhizobox	Spielberg's masterpiece		

High Throughput Phenotyping Platform





Building,
greenhouses (240+110m²),
climatic chambers (80m²)
Phenotyping facilities Lemnatec[©]

Context	Approach	Phenotypic tools	Examples	Models	Conclusion
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Fluxomic	
PPHD	Facilities
Rhizotrons	Shoot roots etc...
Rhizobox	Spielberg's masterpiece

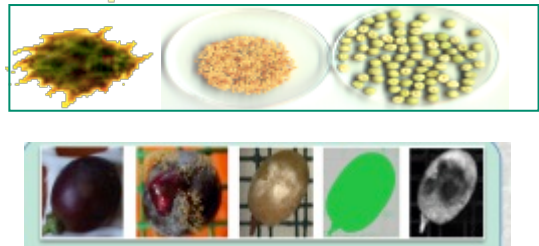
Aerial architecture


20 units/h

**Plant from the
agrosystems**


Organs (seeds...)



Small plants





Germination

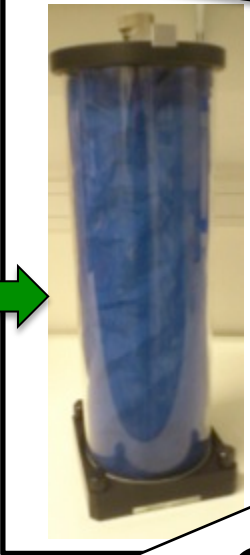


6 units/h

2 units/h

Root system



120 units/h



100 units/h



VIS
NIR

Capacity \approx 1800 plants

Very large capacity

Phenotyping cabinet equipped with cameras and robots

Context	Approach	Phenotypic tools	Examples	Models	Conclusion
		Fluxomic			
		PPHD	Facilities		
		Rhizotrons	Shoot roots etc...		
		Rhizobox	Spielberg's masterpiece		

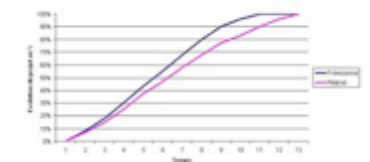
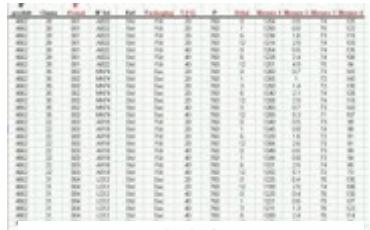
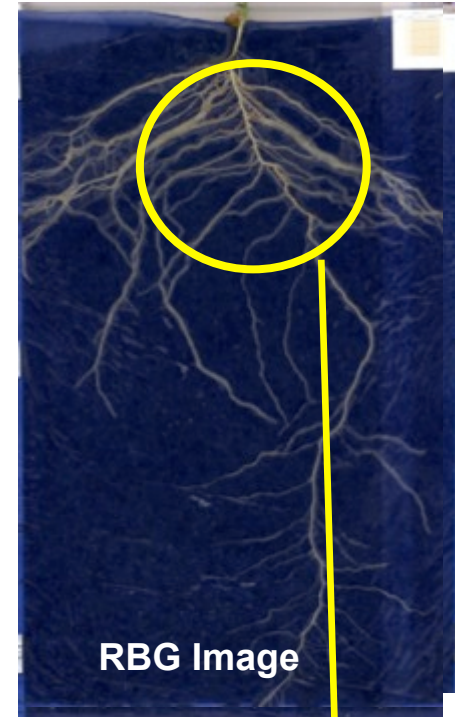
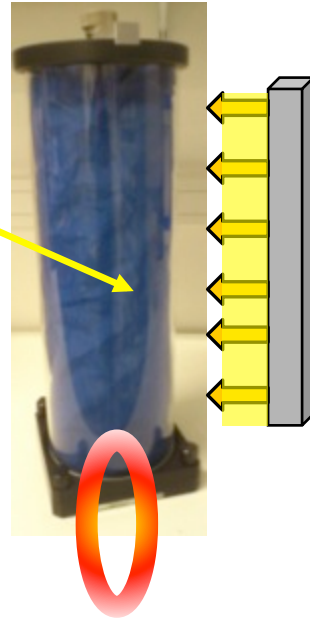
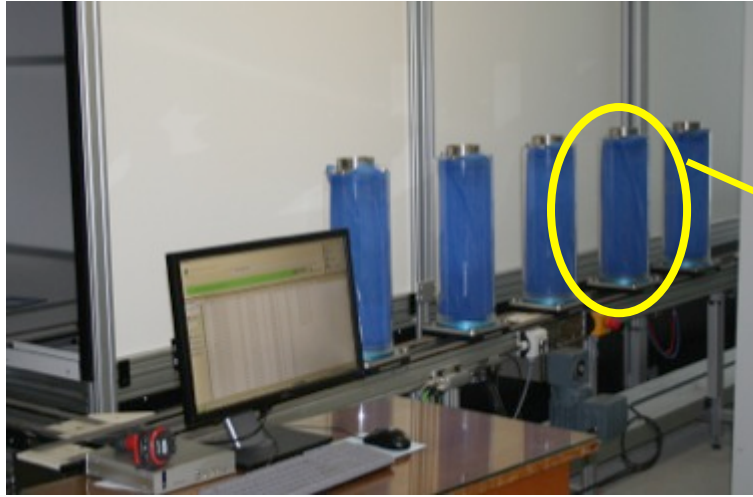




Rhizotrones
(EU Patent INRA-Inoviaflow,
1300 units planed)

Fluxomic
PPHD
Rhizotrons
Rhizobox

Rotating
device



Traits :

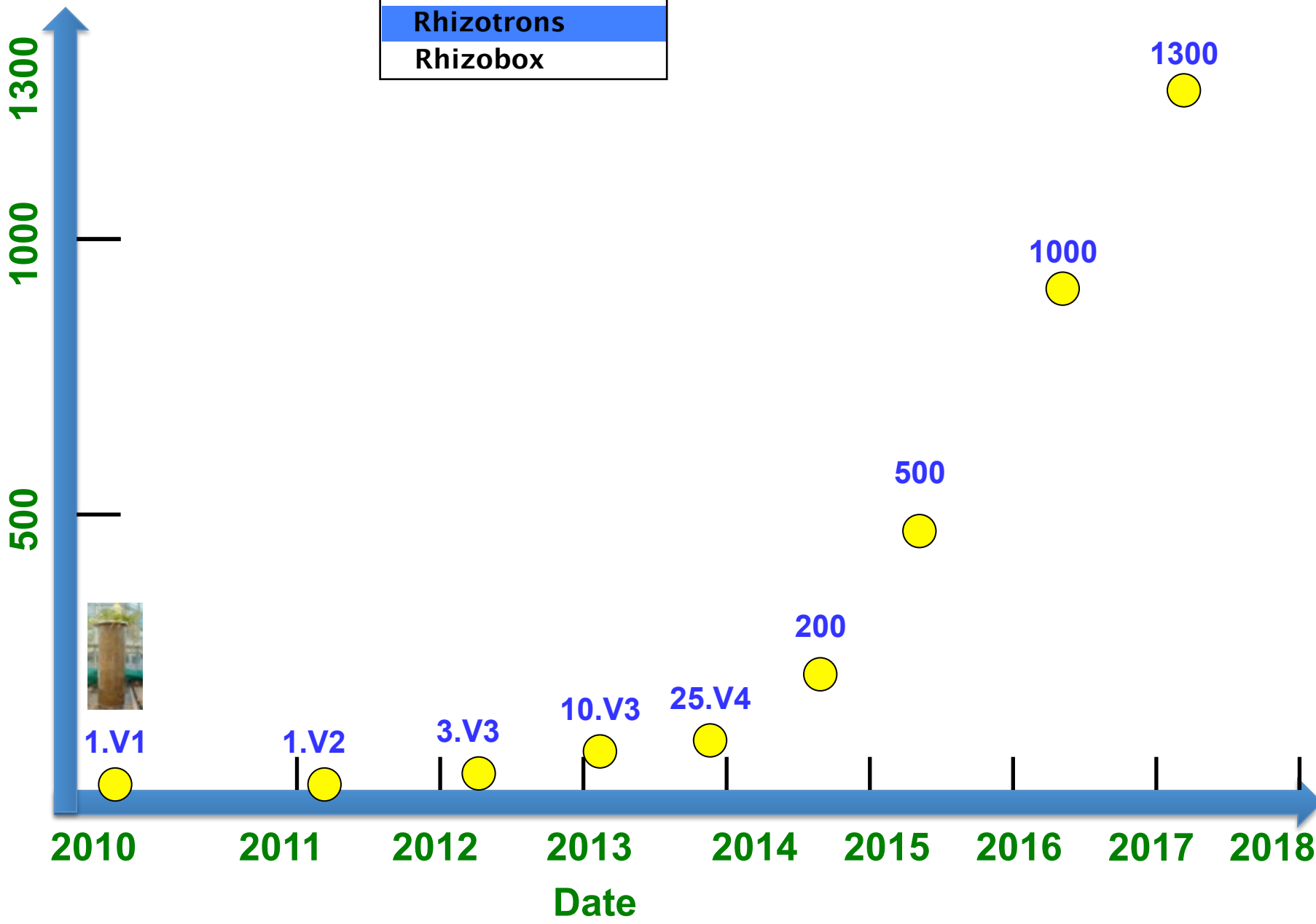
- projected **root** area
- projected **nodule** area
- total **nodule number**
- nodule size** classification
- total root length....



High resolution

Context	Approach	Phenotypic tools	Examples	Models	Conclusion
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Fluxomic
PPHD
Rhizotrons
Rhizobox



2010

2011

2012

2013

2014

2015

2016

2017

2018

Date

1300

1000

500

1.V1

1.V2

3.V3

10.V3

25.V4

200

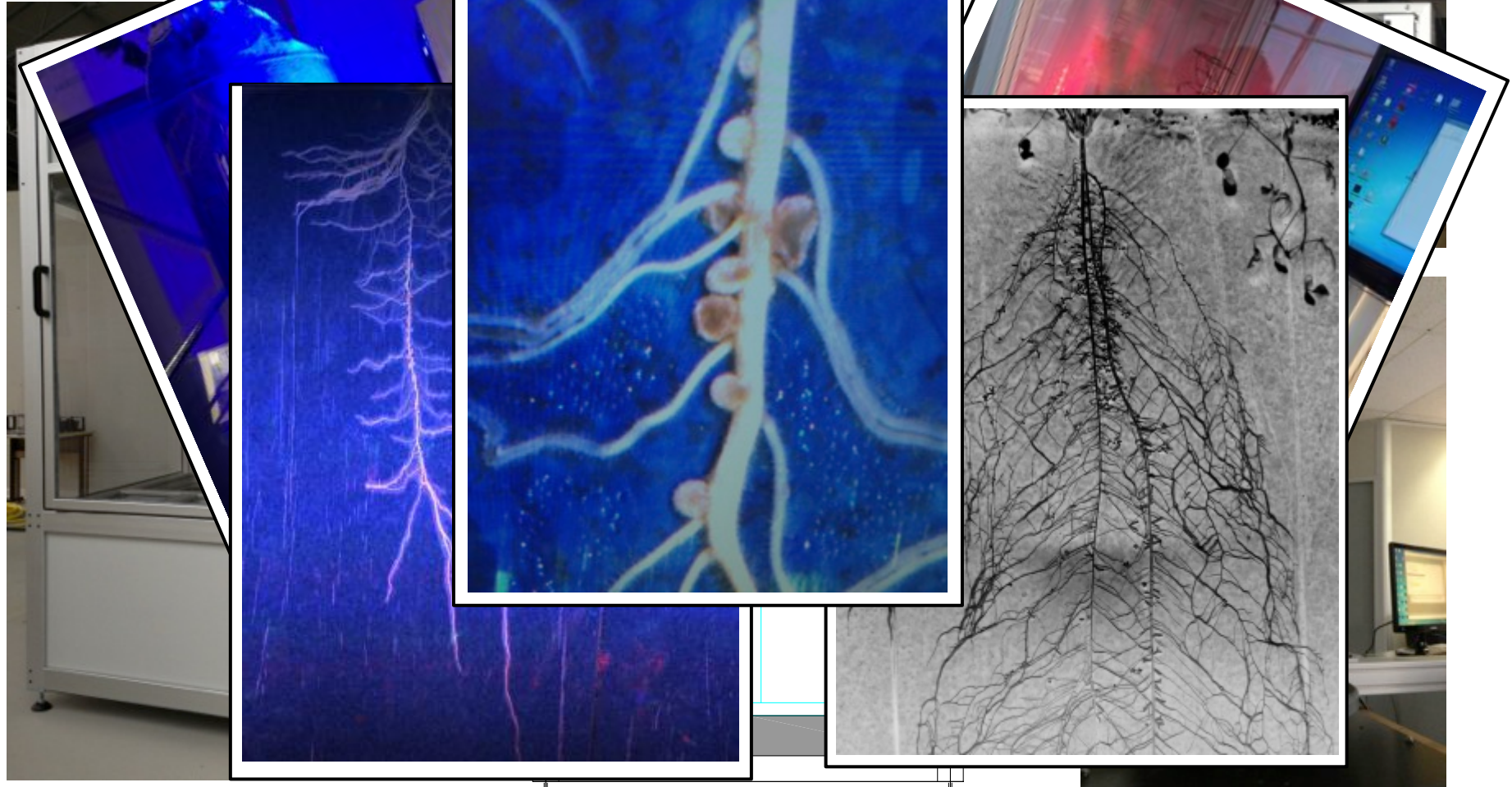
500

1000

1300

Fluxomic
PPHD
Rhizotrons

RhizoBox (INRA-Inoviafl)



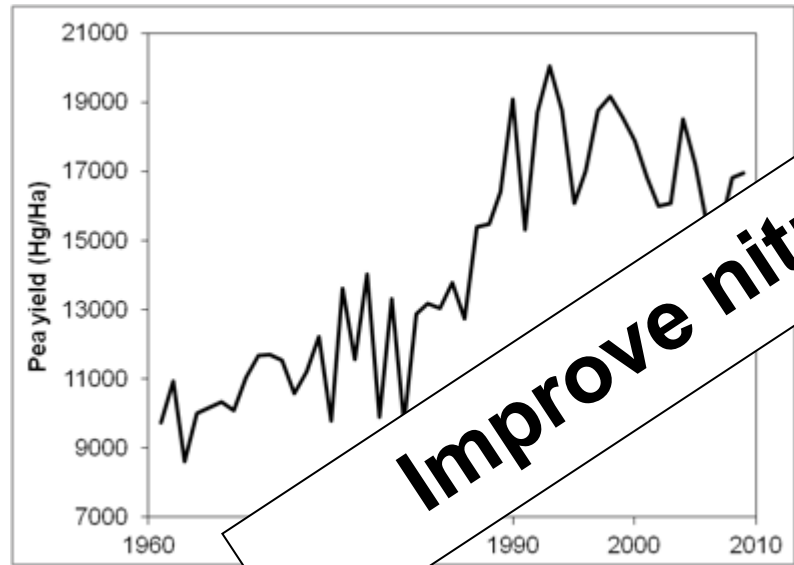
Linear Sensor 12MP, 3 LEDs RVB, precision 50µm
Camera : BASLER racer (Adapter F-Mount V01)

Legumes

Objectives

- Genetic diversity
- Identify a strategy
- Genotype ranking

- Two ways of nitrogen nutrition
- Symbiotic fixation and durability :
 - ↓ fertilizers, fossil energy, GHG emissions, irrigation



But...

Improve nitrogen nutrition

g yields

Sensitivity of symbiotic N₂ fixation to environmental conditions



Regulation of symbiotic N₂ fixation and NO₃ assimilation: determinism, plasticity constituents, optimal root / nodules for maximizing yield?

Context	Approach	Phenotypic tools	Examples	Models	Conclusion
			Legumes		Objectives Genetic diversity Identify a strategy Genotype ranking

Tools and methods



Genetic variability




CONCEPTION of PLANT IDEOTYPES



Characterizing mechanisms and molecular basis



Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

Natural genetic variability

French national collections of pea, fava and lupin (10000 accessions)



Genetic diversity on root architecture



Bourion et al. Annals Bot. 2007

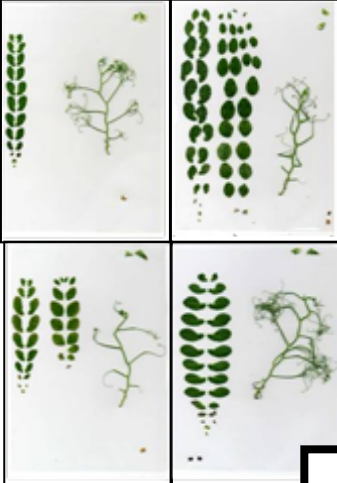
Legumes

Objectives
 Genetic diversity
 Identify a strategy
 Genotype ranking

Recombinant inbred lines (1400 RILs)

STRUCTURE

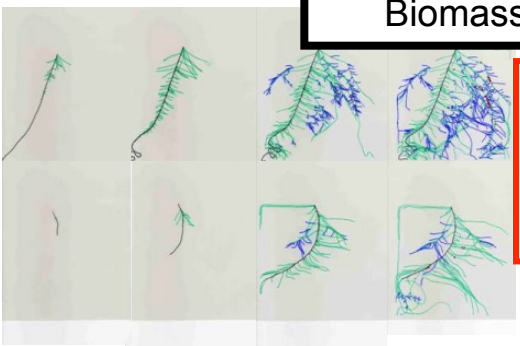
FONCTION



Aerial part :
 Height
 Biomass
 Leaf surface area

Roots:
 Number
 Length
 Biomass

Nodules:
 Number
 Surface
 Biomass



C use efficiency



N uptake efficiency

Bourion et al. TAG 2010

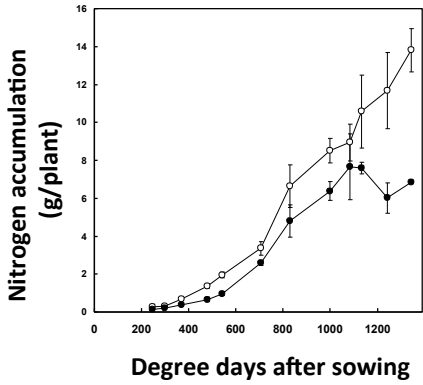
Legumes	Objectives
	Genetic diversity
	Identify a strategy
	Genotype ranking

Induced genetic variability

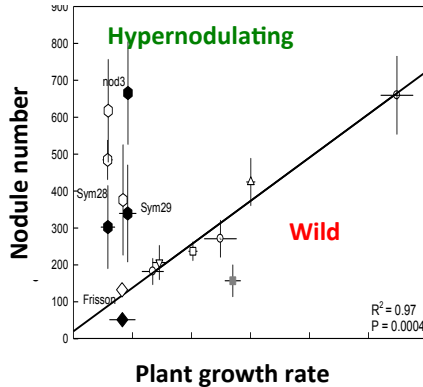
Identify/characterize genes involved in nodulation control or root architecture

Nodule development

Root architecture



Salon et al. Agr 2001

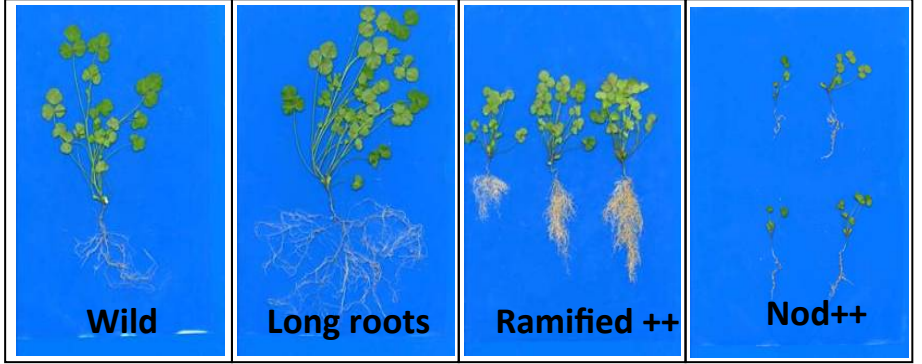


Voisin et al. Plant Soil 2010

Duc et al. 1998

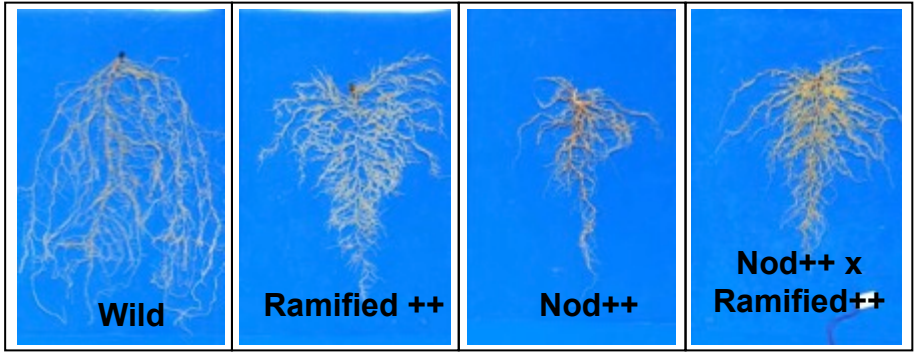
Cazenave et al. Plant Soil 2013

Medicago truncatula, induced by Tnt1



Porceddu et al. BioMed 2008

Pea, induced by EMS



Coll. KK Sidorova

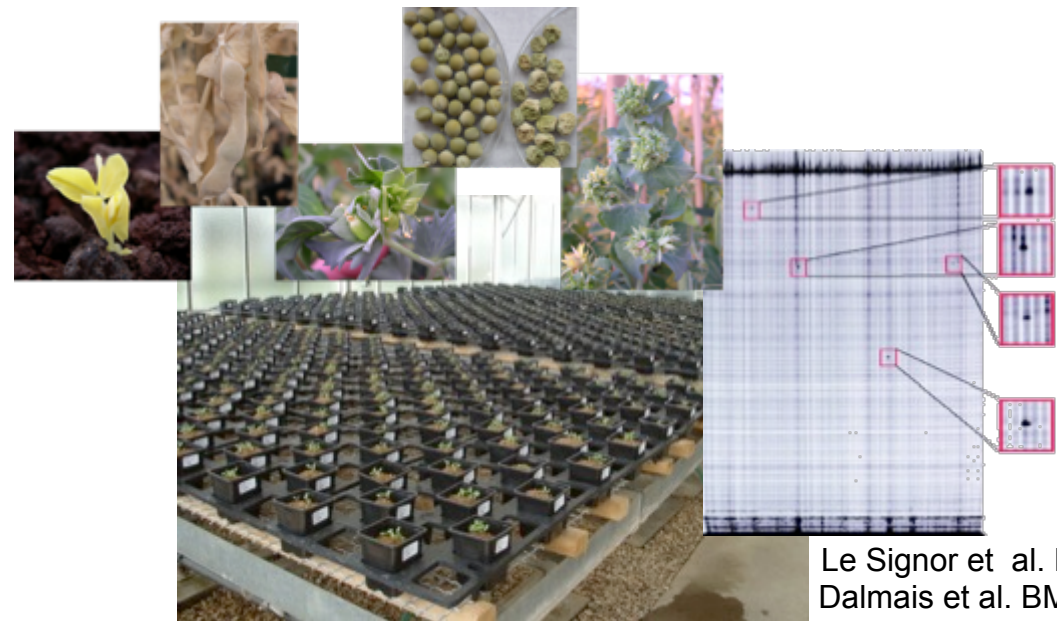
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

TILLING mutant collections

Research mutant in a target gene, analyze the mutation effect

Medicago truncatula Jemalong A17 (9000 M2) and pea (*Pisum sativum*) var. Caméor (5000 M2)



Le Signor et al. Plant Biotechnol 2009
 Dalmais et al. BMC Genome Biol 2008

✓ HTP TILLING platform: ABI 3730 (Contact: lesignor@dijon.inra.fr)

Context	Approach	Phenotypic tools	Examples	Models	Conclusion
			Legumes	Objectives Genetic diversity Identify a strategy Genotype ranking	

Tools and methods



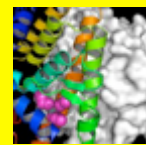
Genetic variability




CONCEPTION of PLANT IDEOTYPES



Characterizing mechanisms and molecular basis

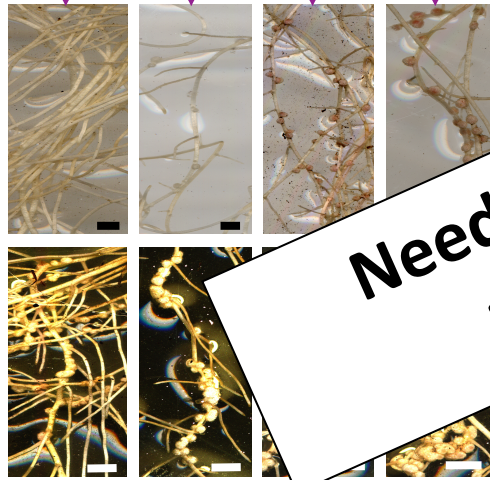
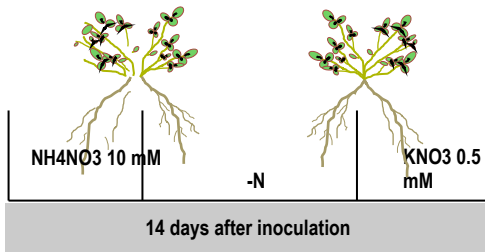


Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

What is the strategy of legume plants faced to a N constraint ?

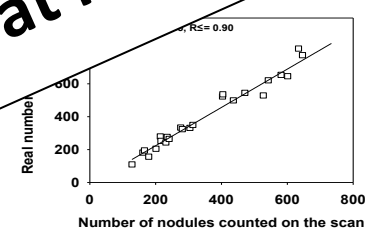
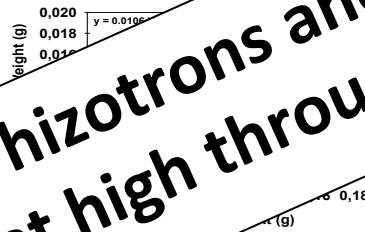
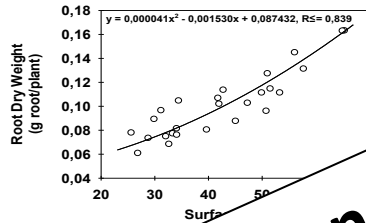
“Low” throughput characterization of nodulated roots



Split roots



Need to use rhizotrons and image analysis at high throughput

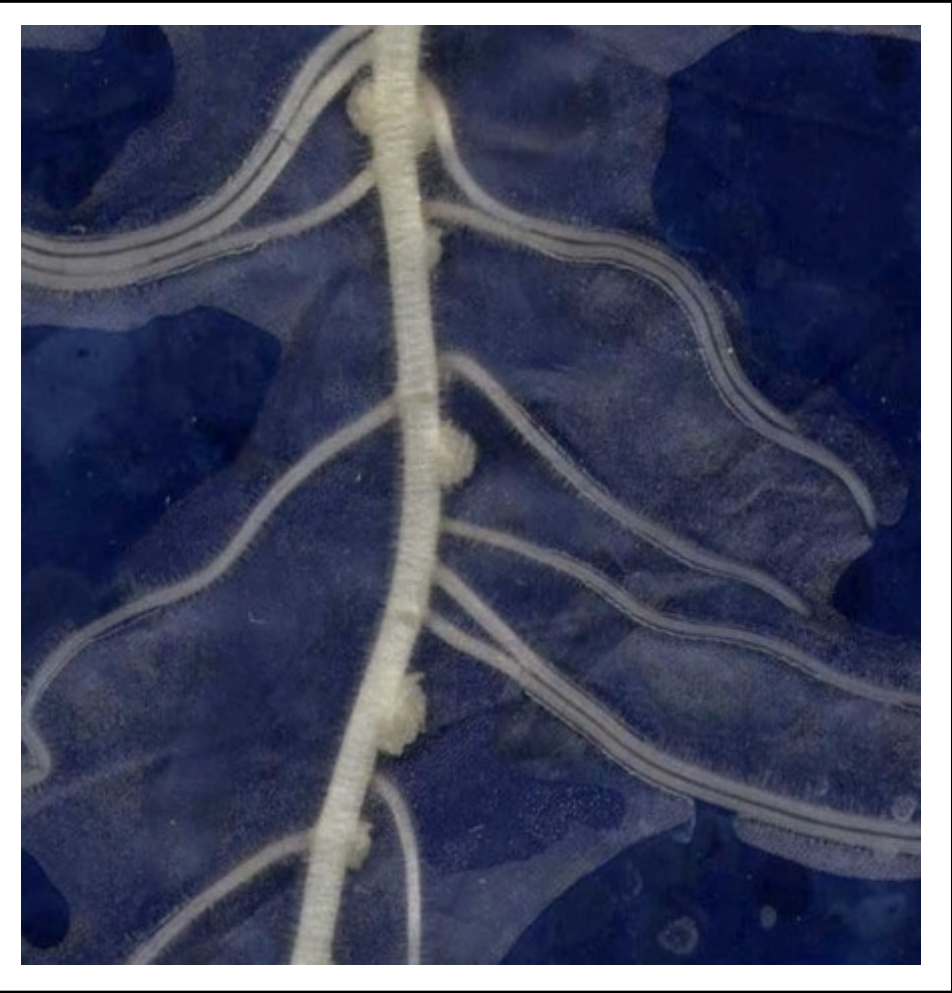


Nodules number and size, appearance

Morphometry versus functional strategy identification

Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

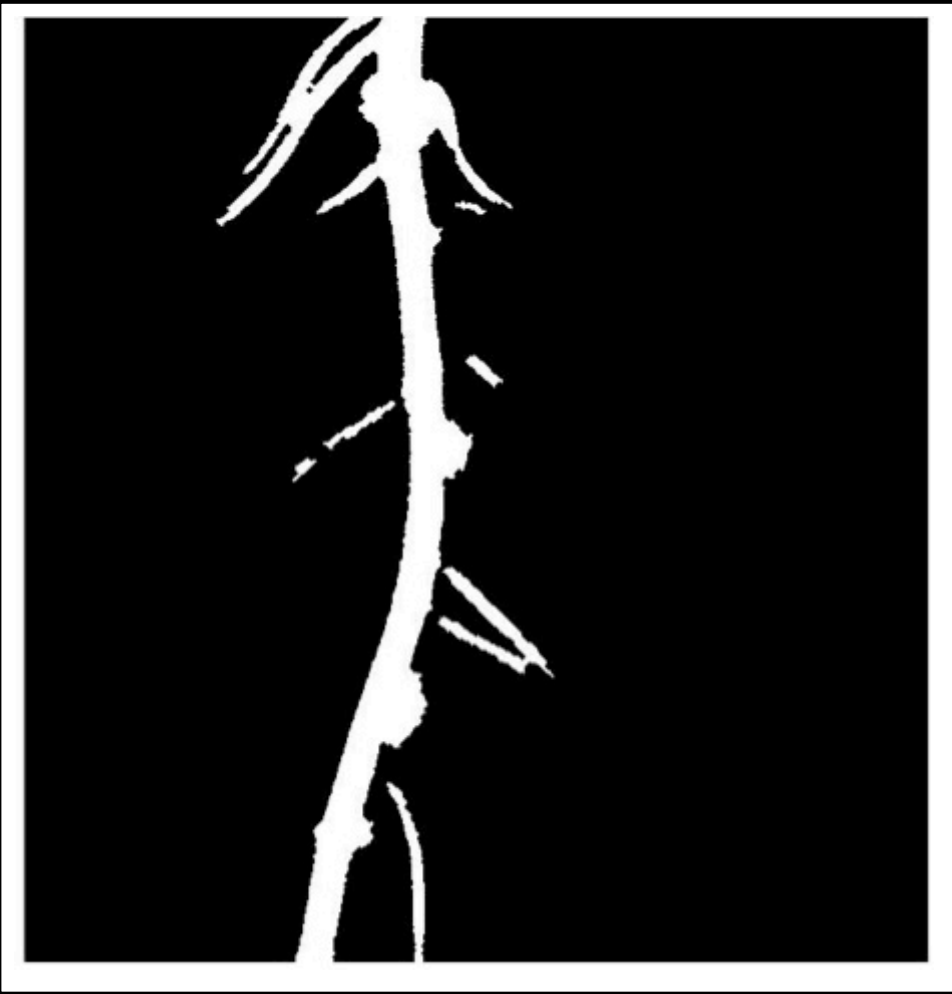
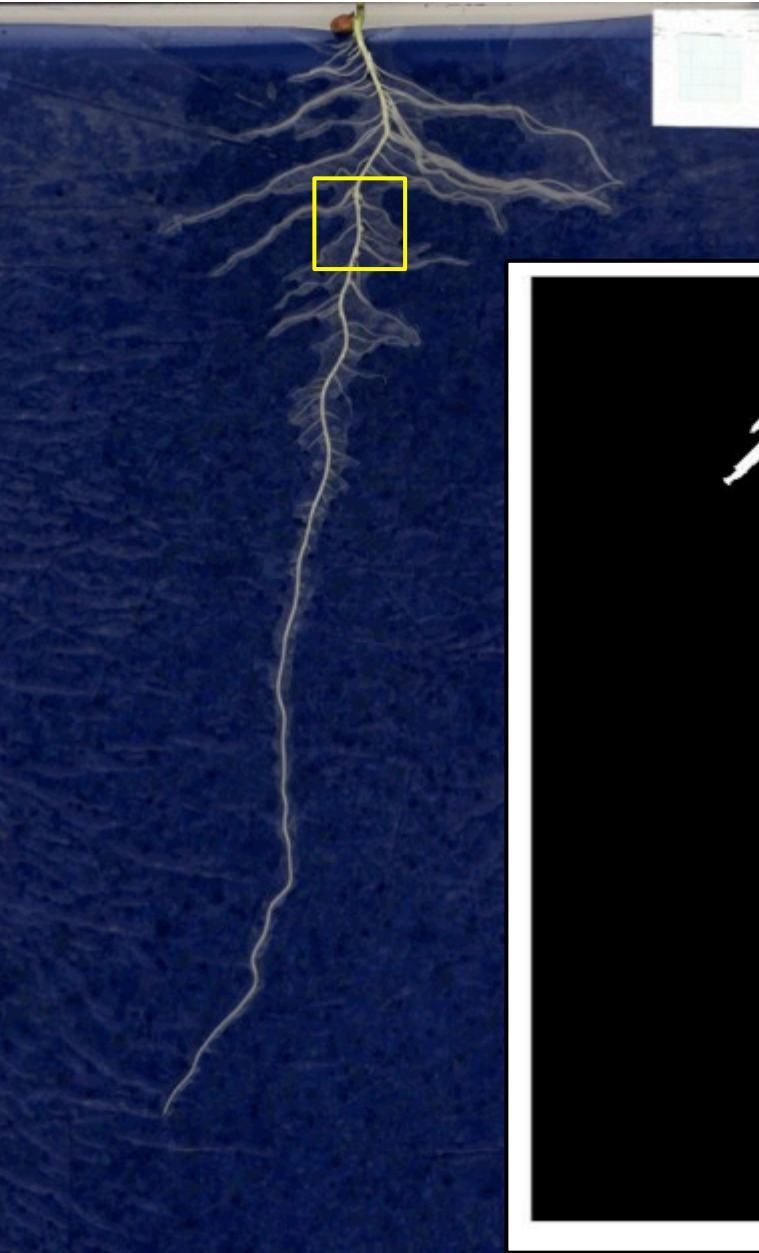


Young plant

Thèse Simeng Han (unpublished)

Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

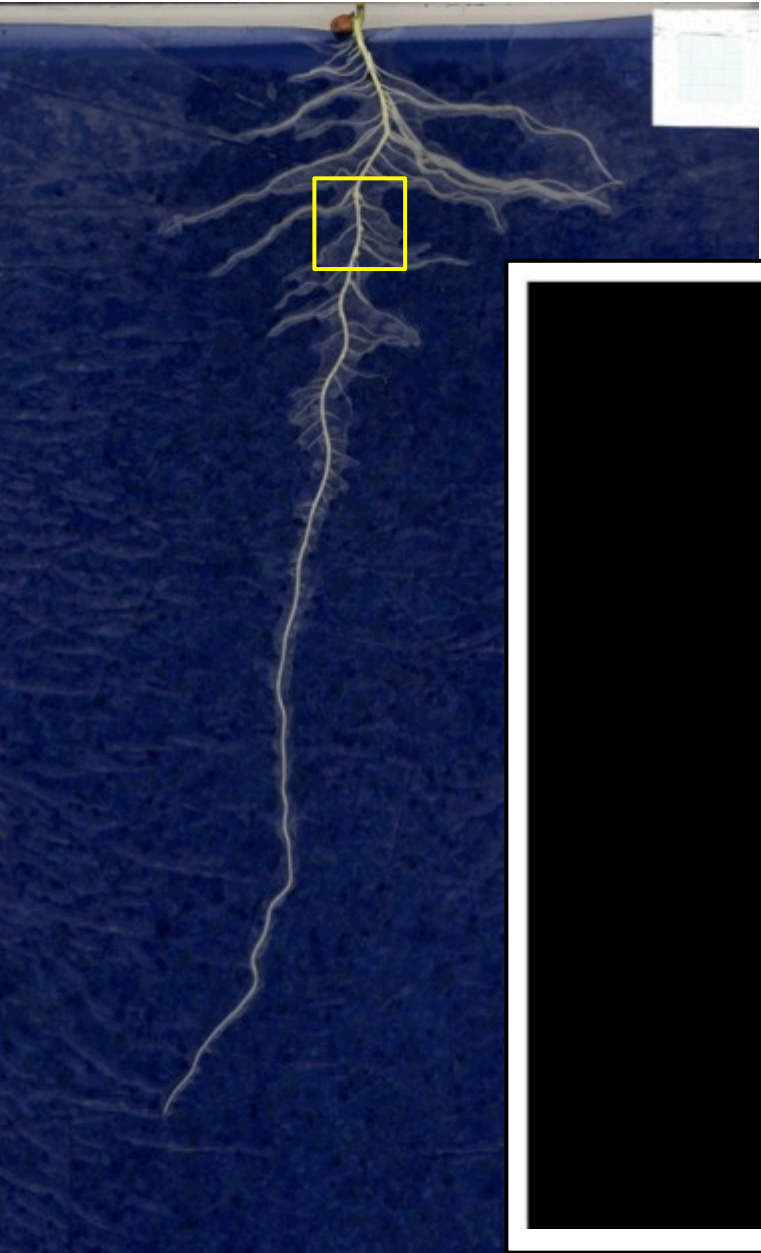


Binary image and threshold

Young plant

Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking



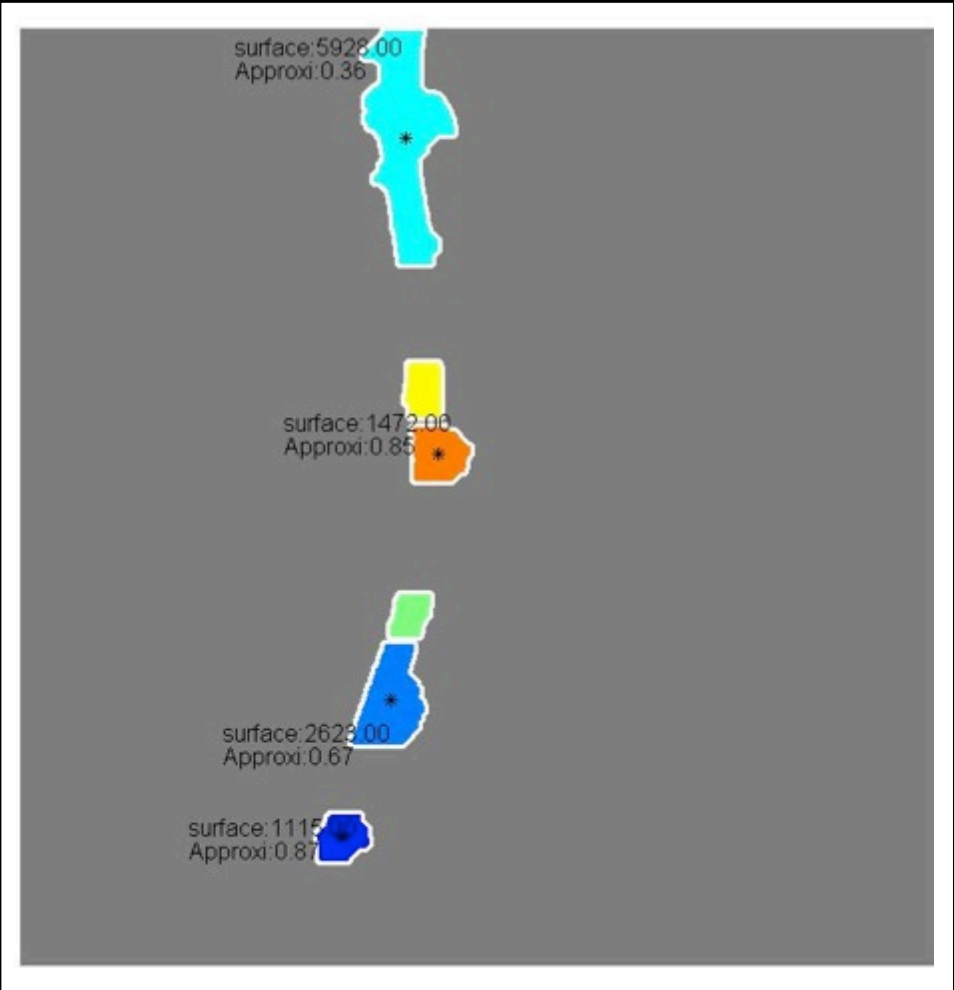
Get rid of unused areas

Smooth the image

Young plant

Legumes

Objectives
 Genetic diversity
 Identify a strategy
 Genotype ranking

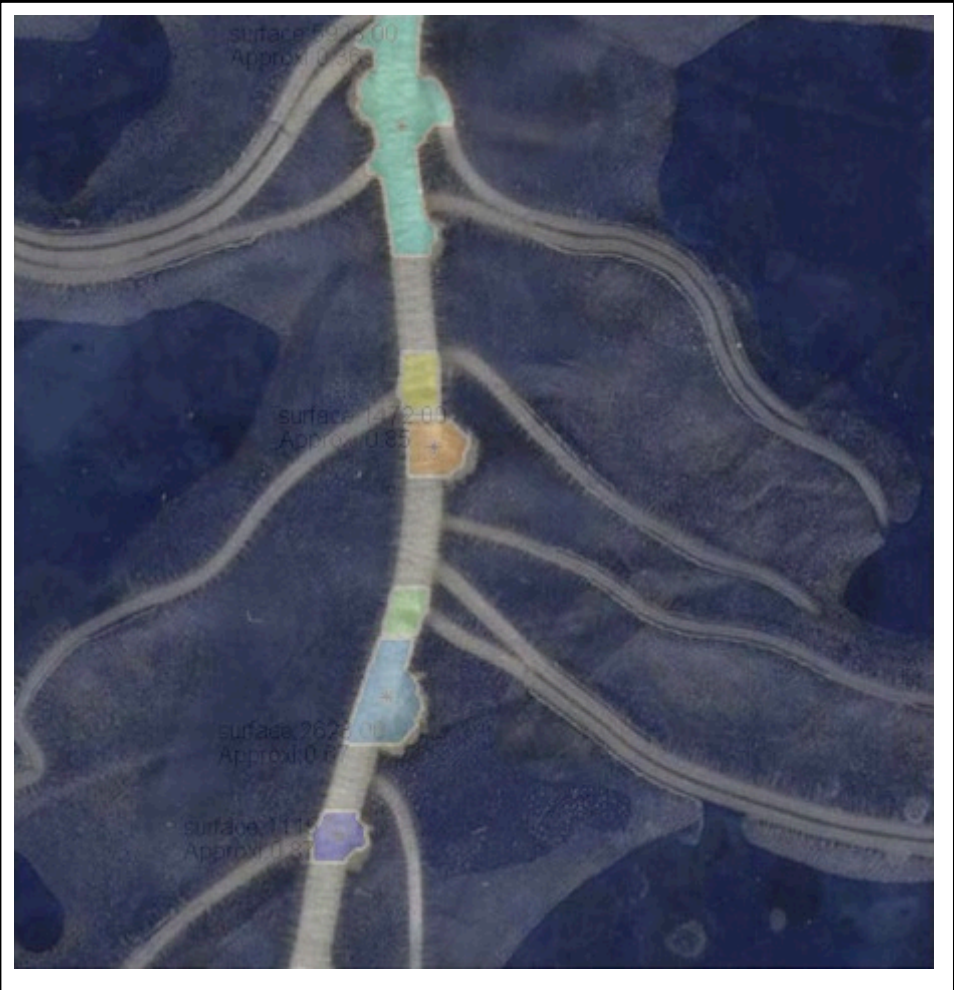


Count nodules with the « circle » form

Young plant

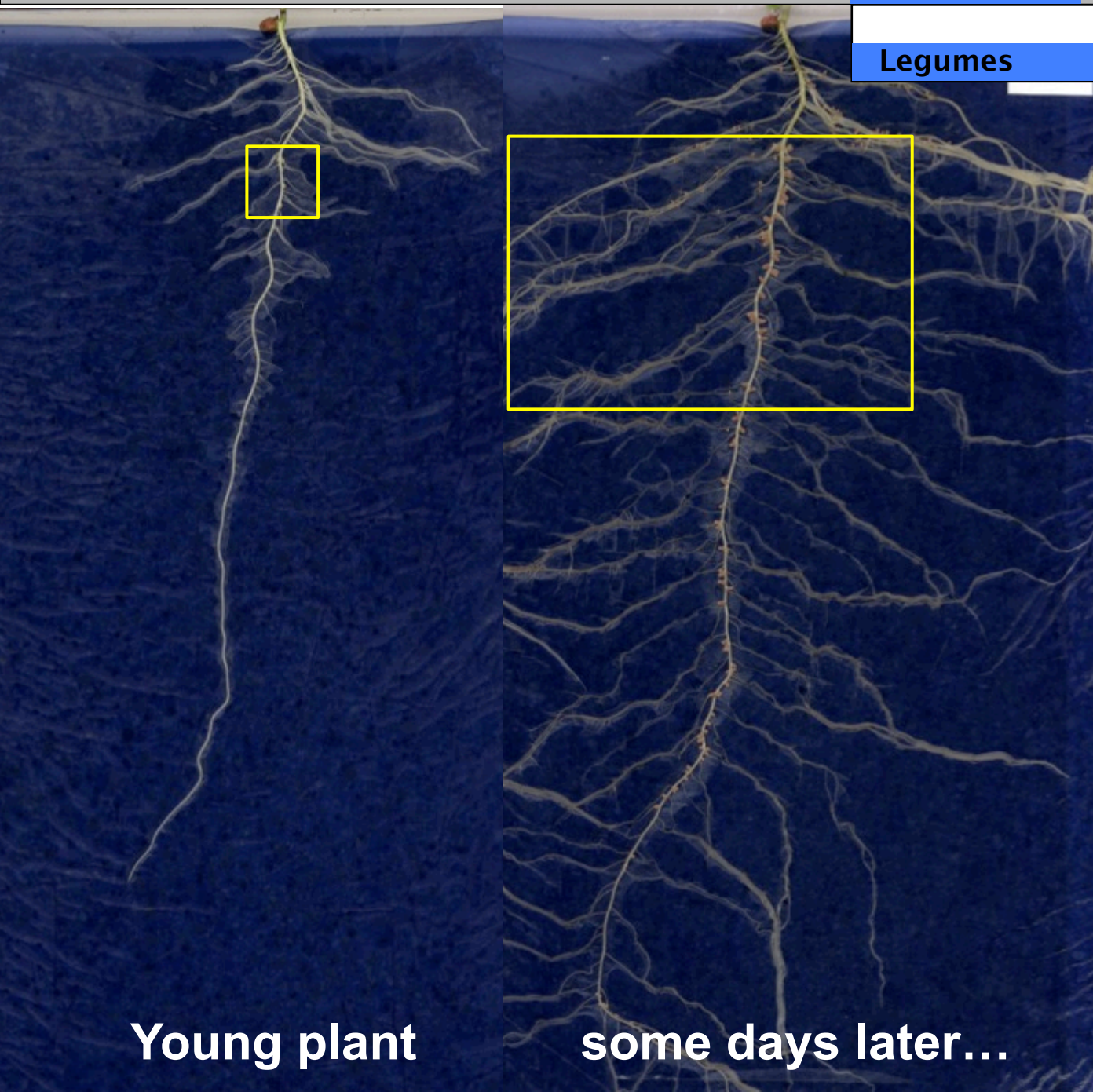
Legumes

Objectives
 Genetic diversity
 Identify a strategy
 Genotype ranking



Original image
 + superposed
 nodules

Young plant



Legumes

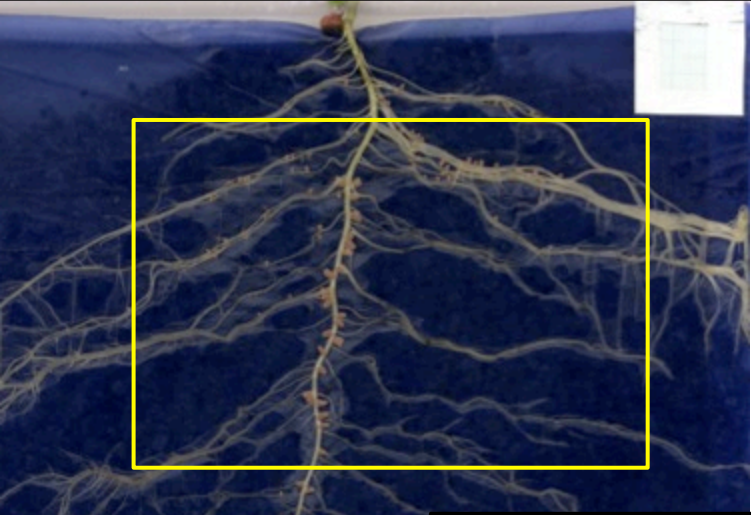
- Objectives
- Genetic diversity
- Identify a strategy
- Genotype ranking

Young plant

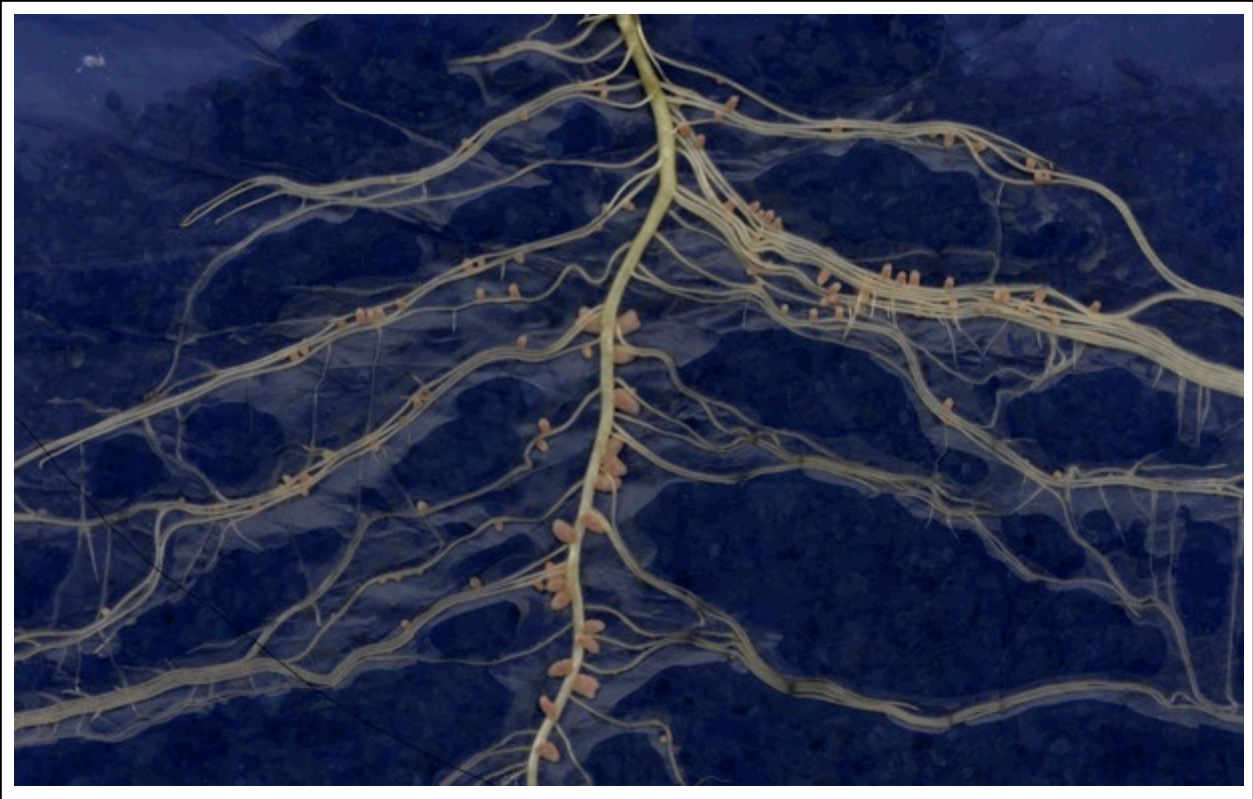
some days later...

Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

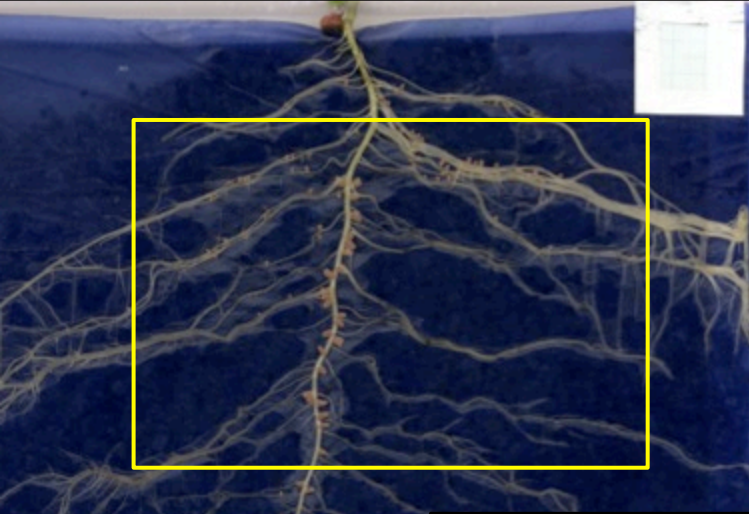


Enlarged image

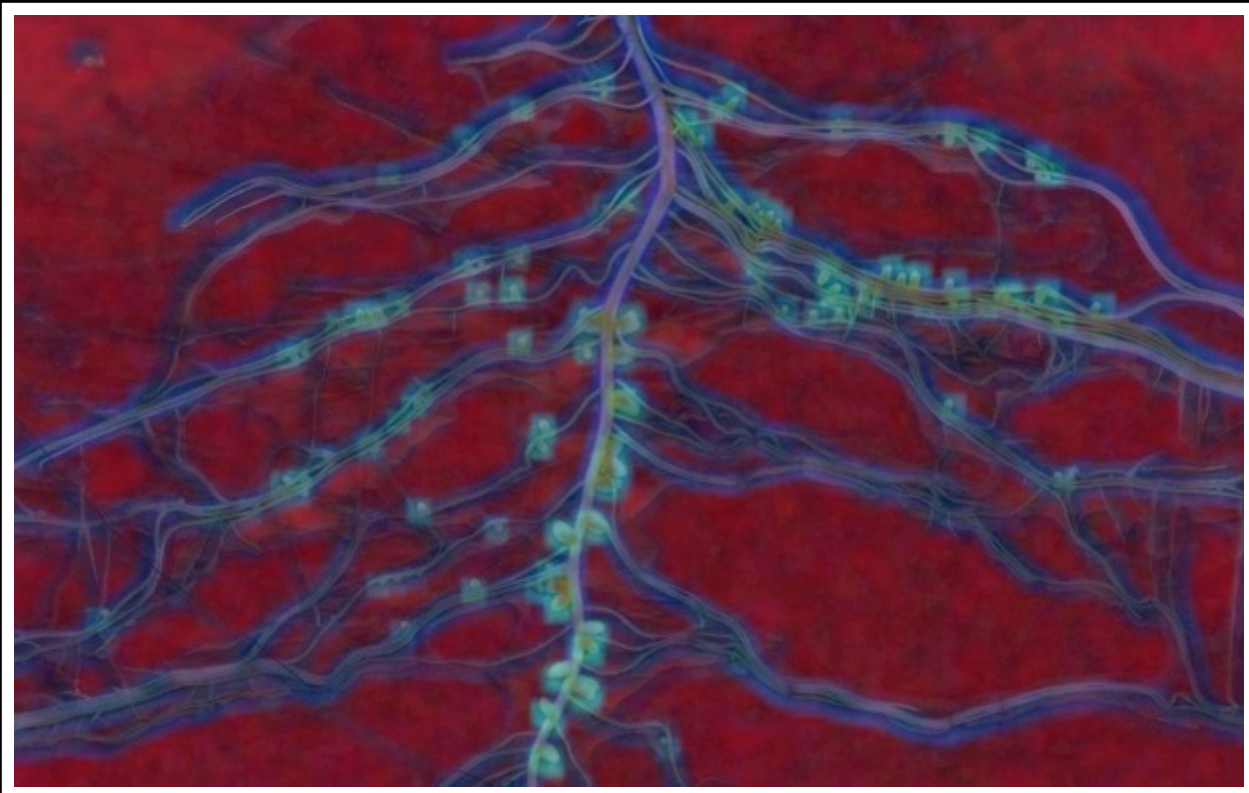


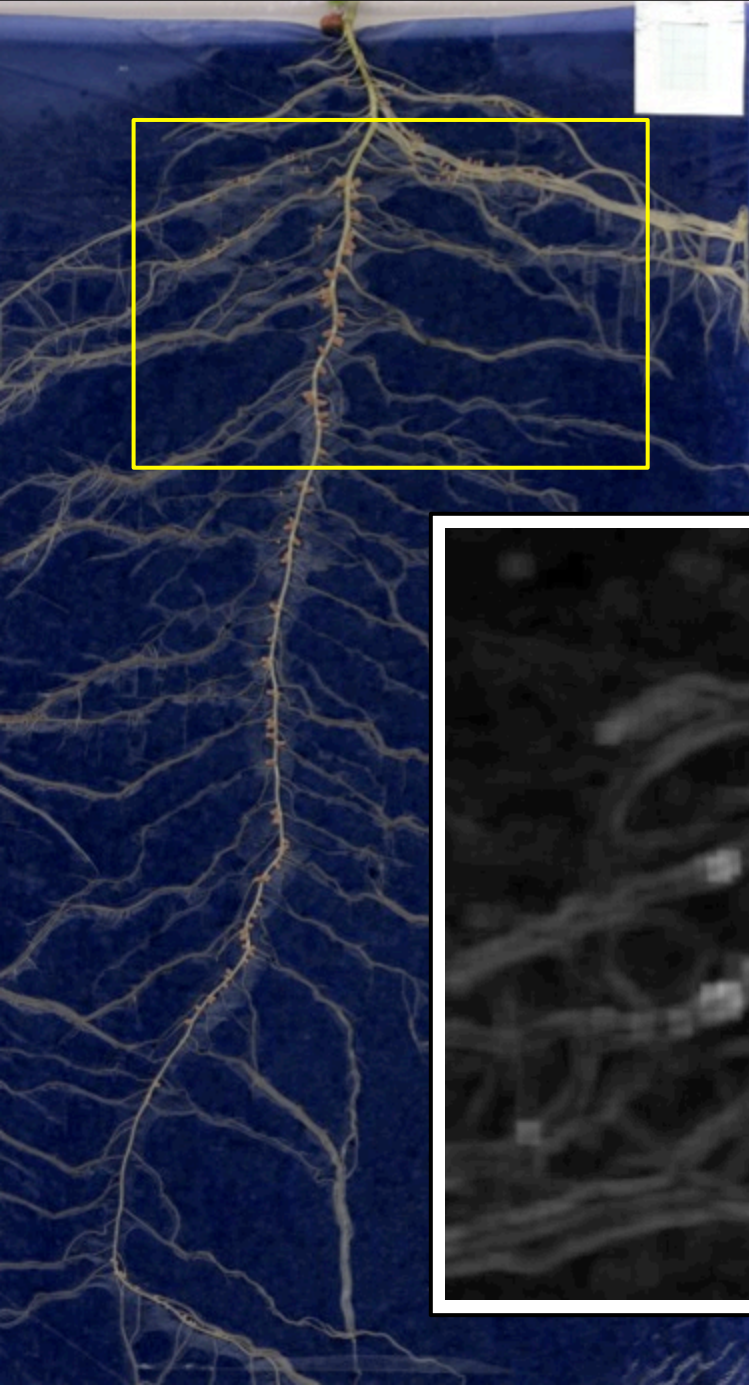
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking



Hybrid space (color + texture)

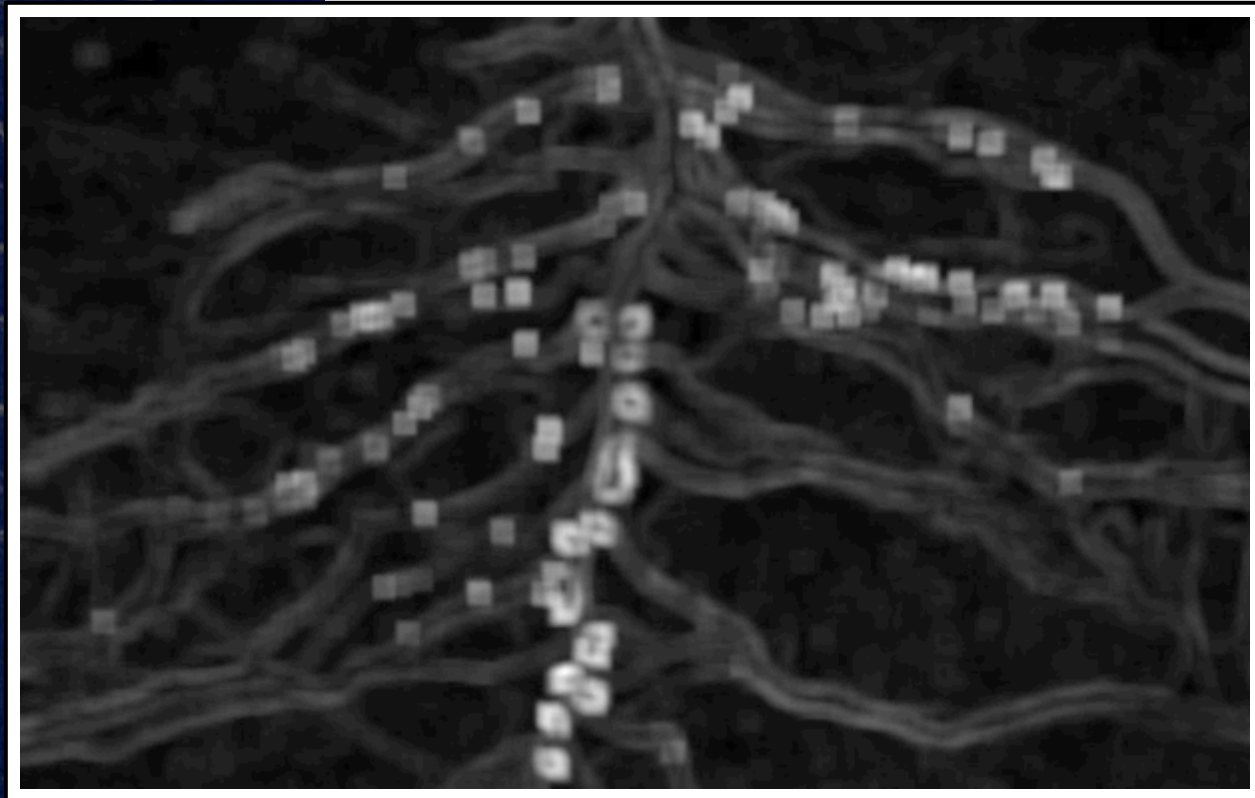


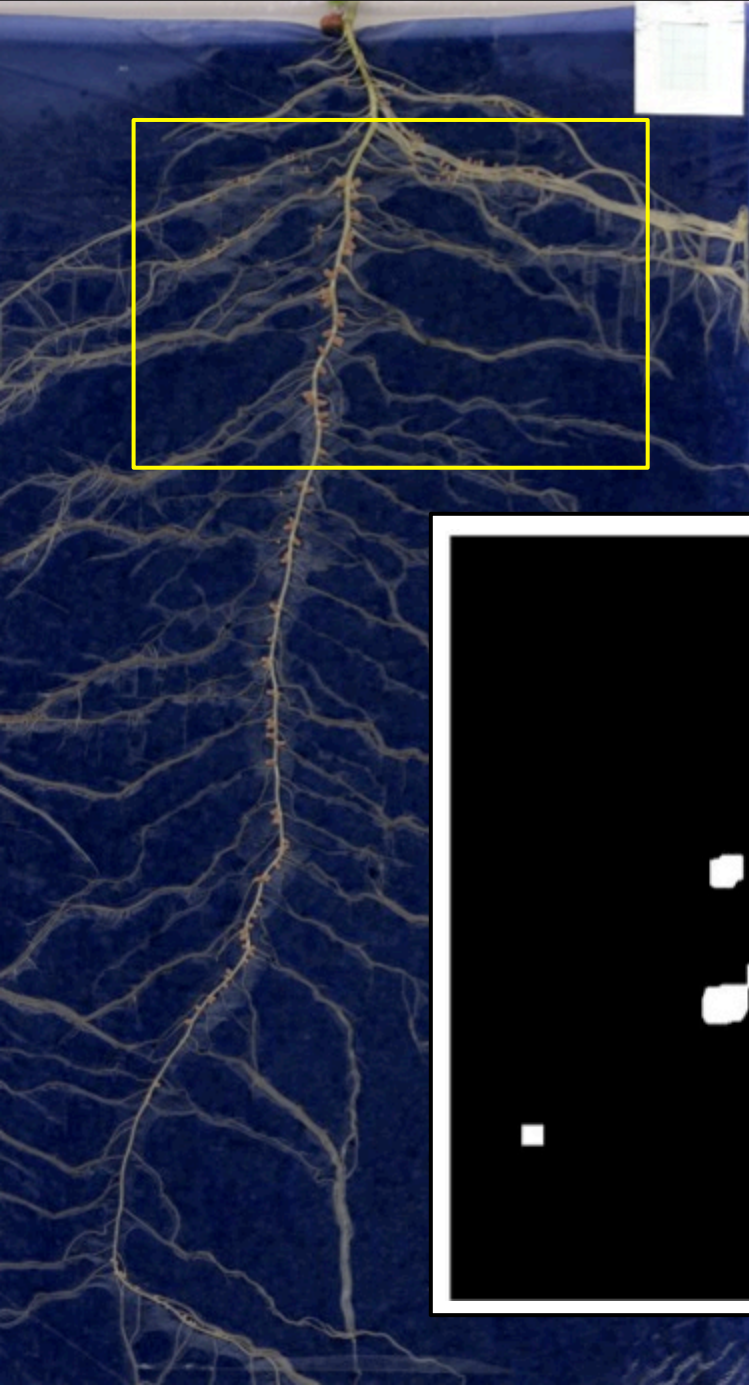


Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

Green band of the RGB image





Legumes

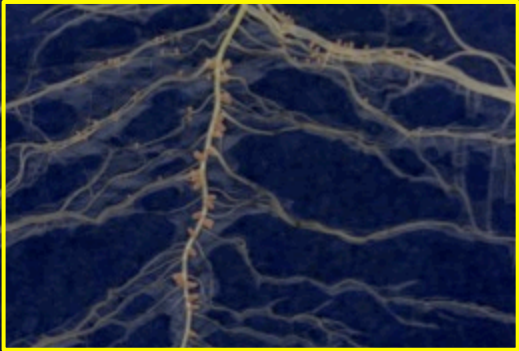
Objectives
Genetic diversity
Identify a strategy
Genotype ranking

Binary image with squared nodules

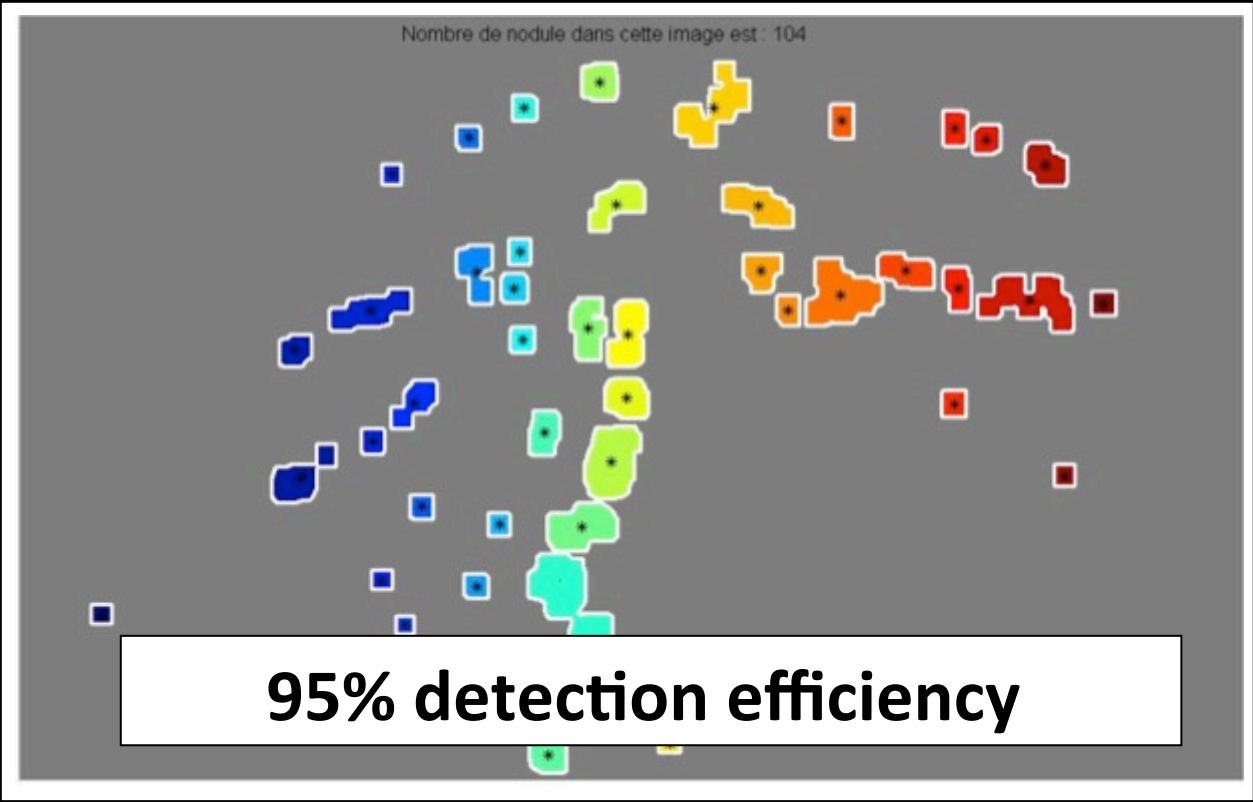


Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

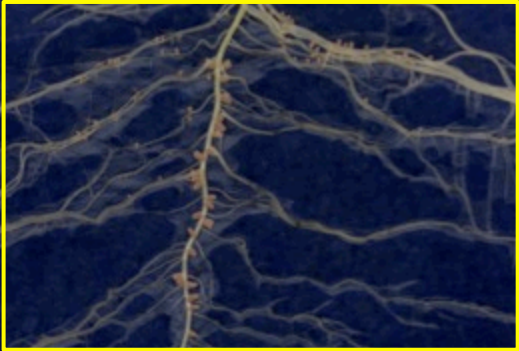


Nodules automatically detected

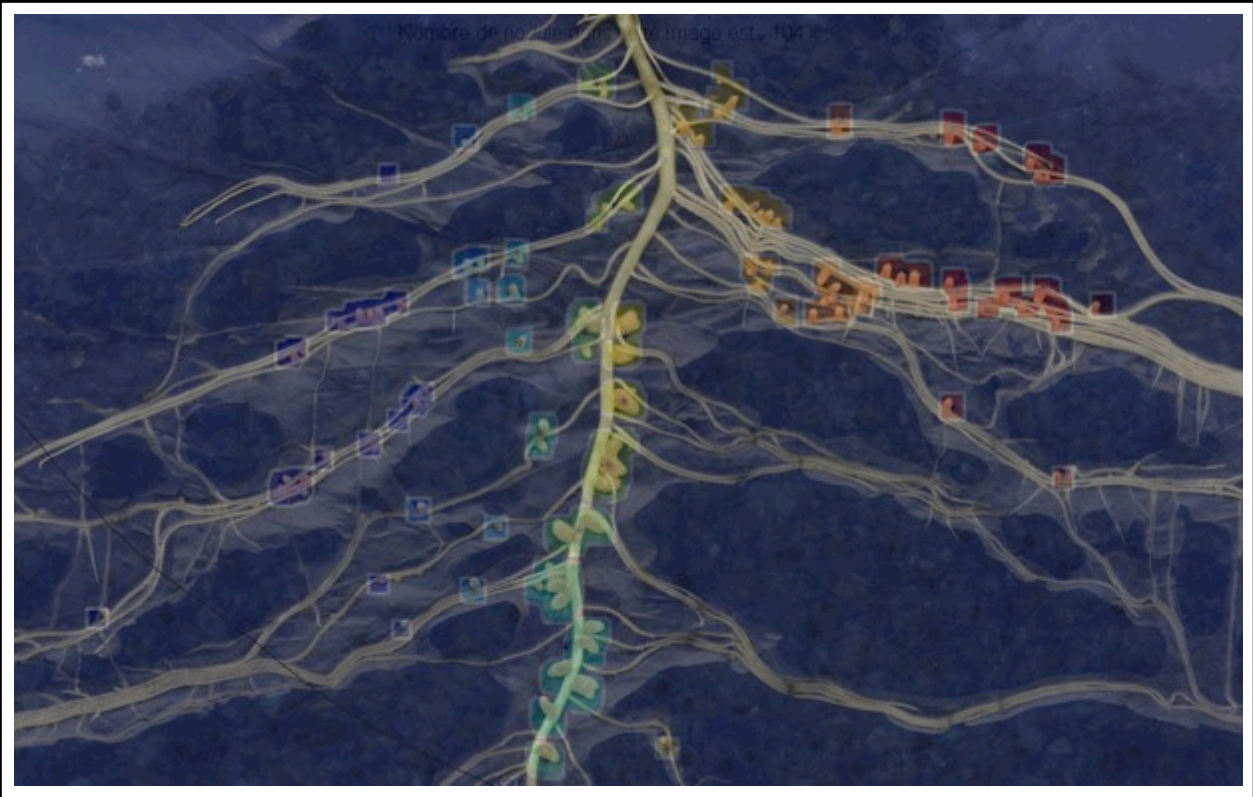


Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking



Original image + superposed nodules

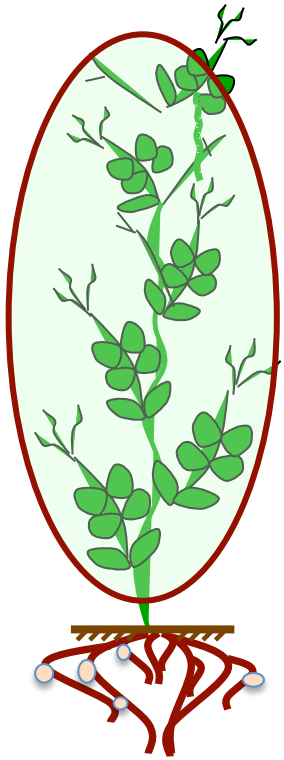
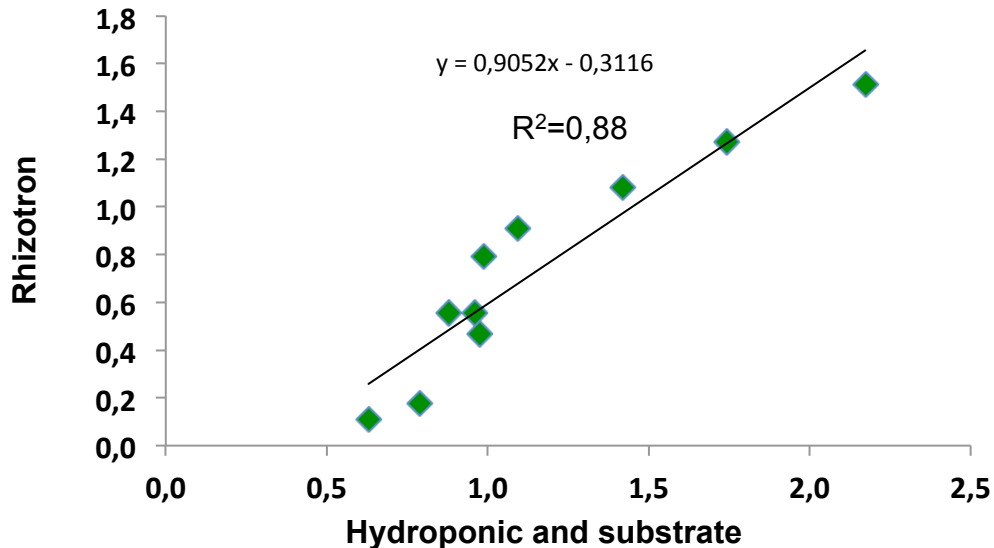
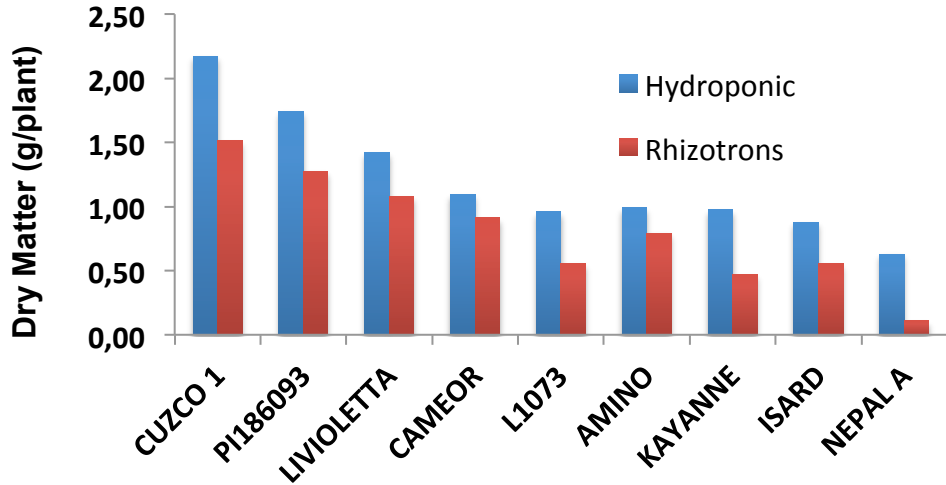


Legumes

Objectives
 Genetic diversity
 Identify a strategy
 Genotype ranking

Ranking pea genotypes: Pea core collection
Hydroponic versus rhizotron

Shoot biomass

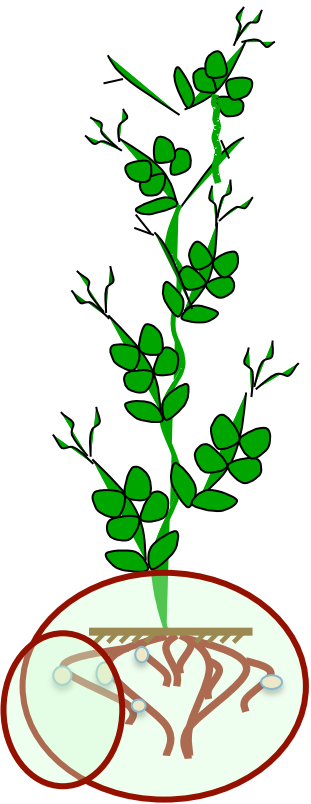


Genotype ranking does not vary

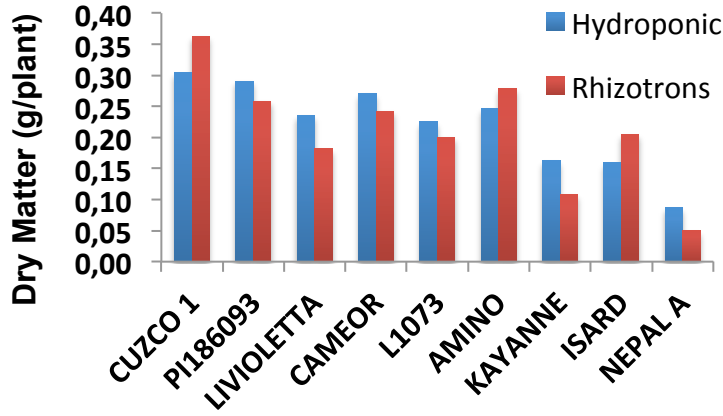
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

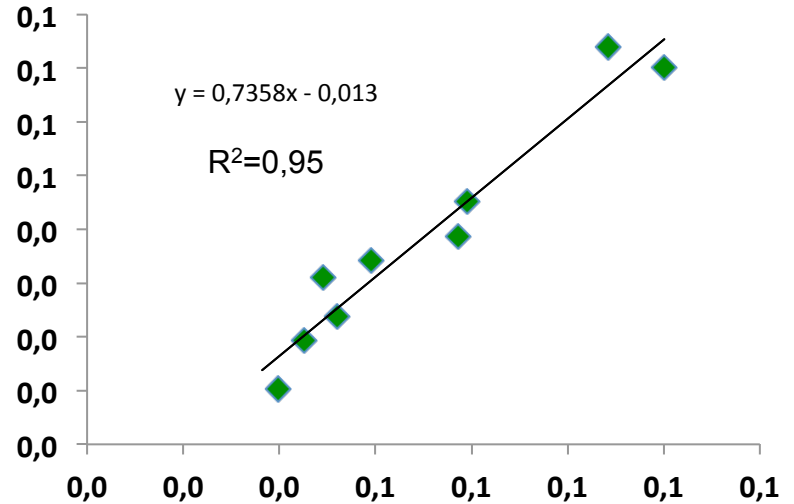
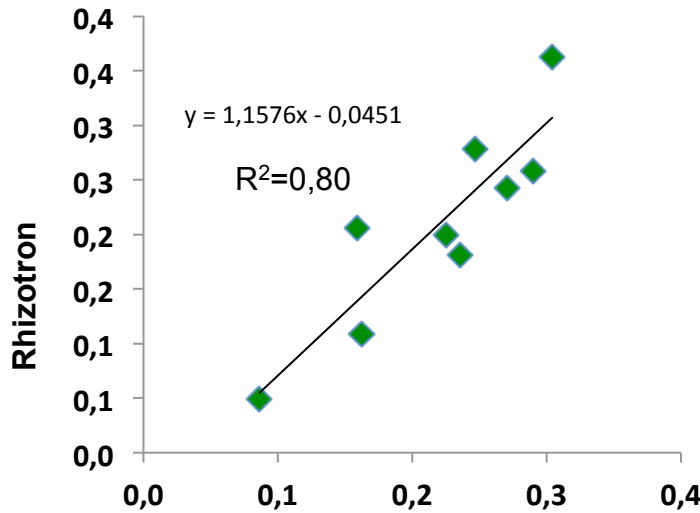
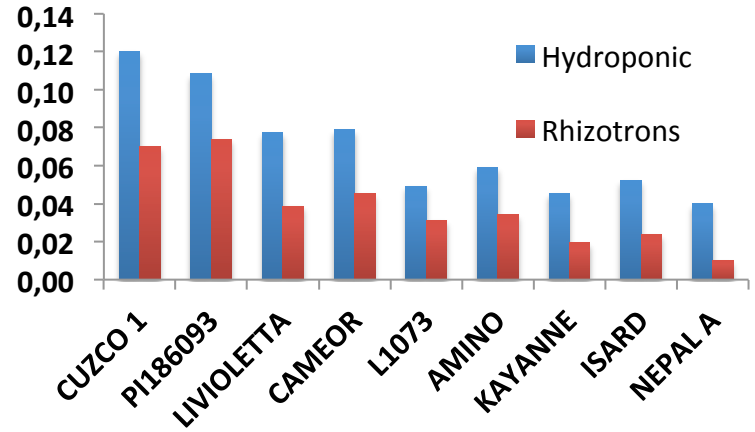
Ranking pea genotypes: Pea core collection



Root biomass



Nodule biomass



Hydroponic and substrate

Context	Approach	Phenotypic tools	Examples	Models	Conclusion
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Ongoing rhizothrone validation

Legumes	Objectives Genetic diversity Identify a strategy Genotype ranking
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Estimate integrative variables :

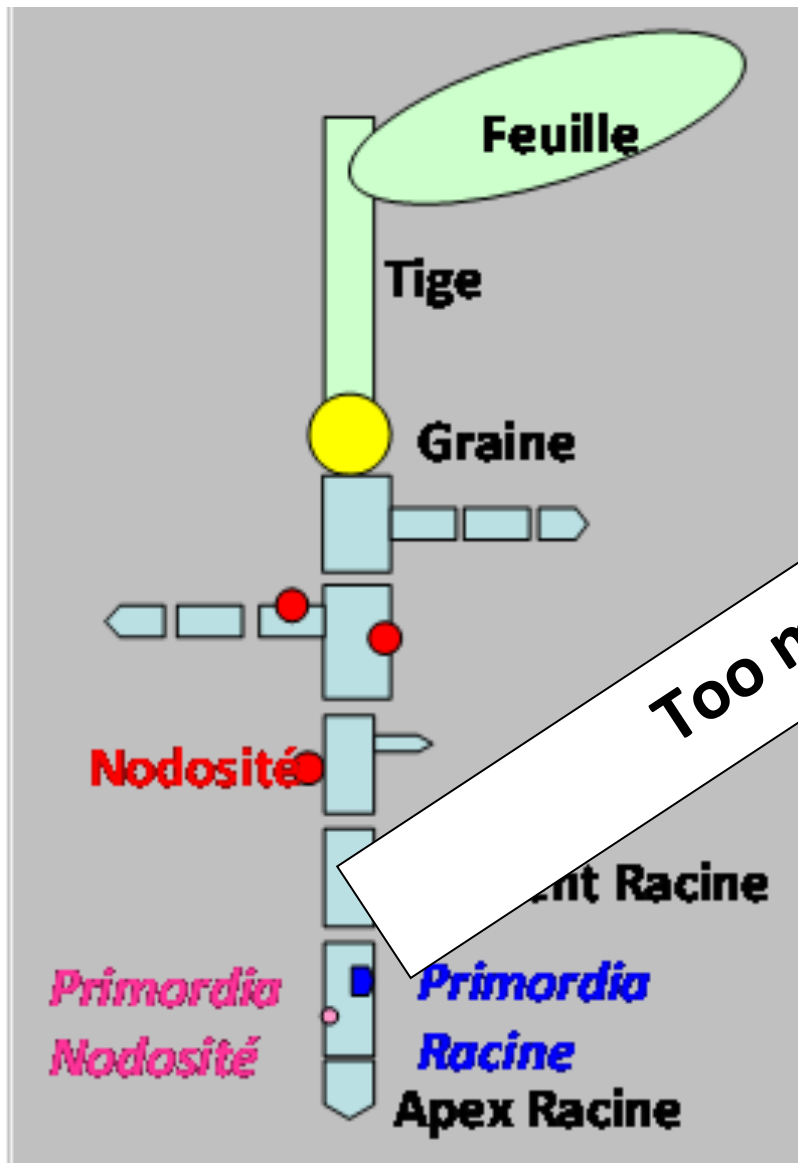
- compare measured/observed variables

Form recognition : nodule and roots number
 Root and nodule surface and biomass

Robustness of traits:

- intraspecific (X genotypes),
- interspecific (Y species),
- modulating the abiotic environment (Z env.)

Mecanistic models : PEA NOD (coll. L Pagès)



Understand process and their interactions

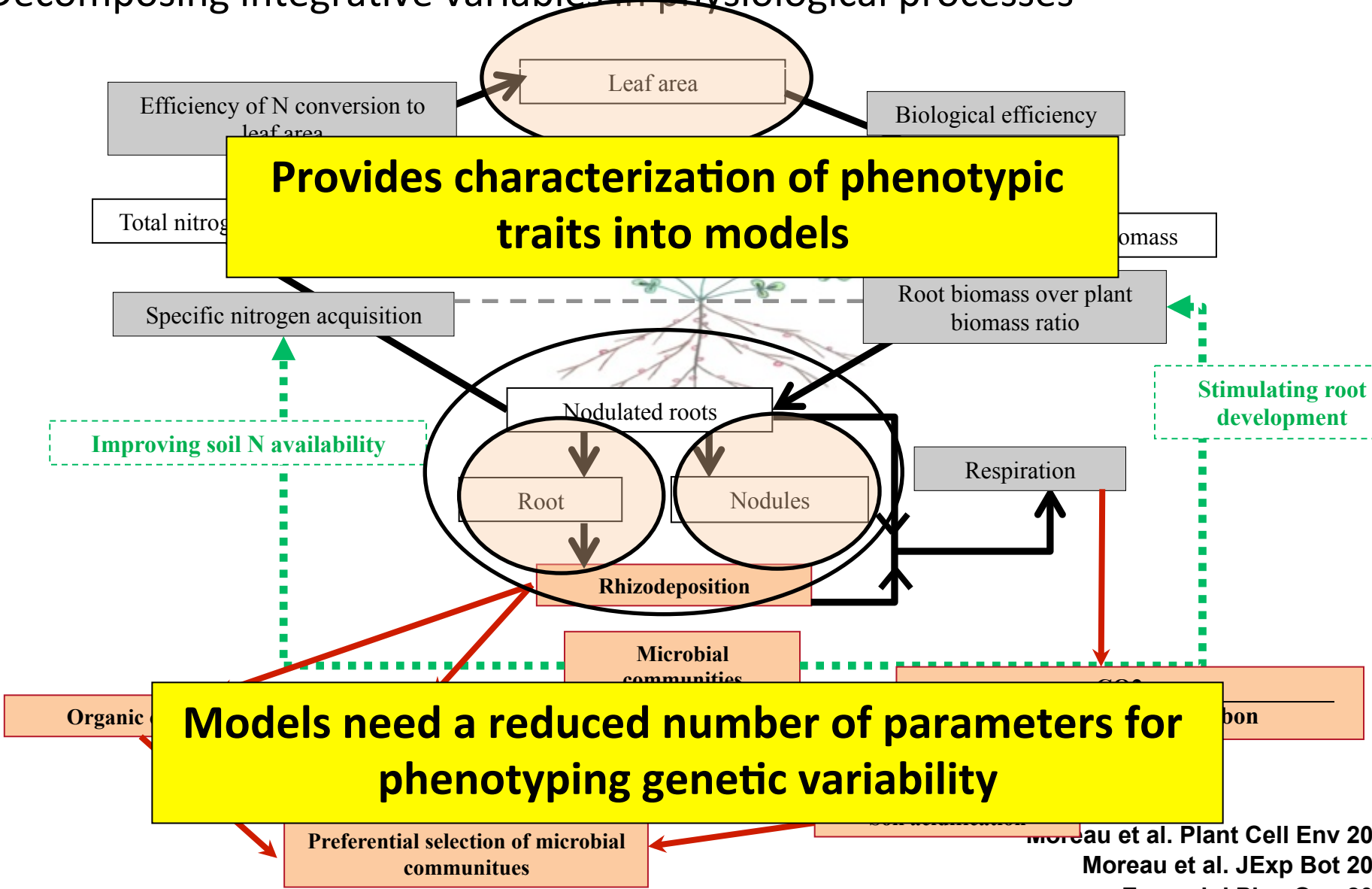
- C and N interaction,
- Root architecture

Too many parameters!!

Naudin et al. Plant & Soil 2011
 Agrophysiologie du pois 2010
 Voisin et al. Plant & Soil 2010
 Salon et al. CR Biologies 2009
 Voisin et al. Annals Bot 2007

Integrative Model: *Medicago*

Decomposing integrative variables in physiological processes



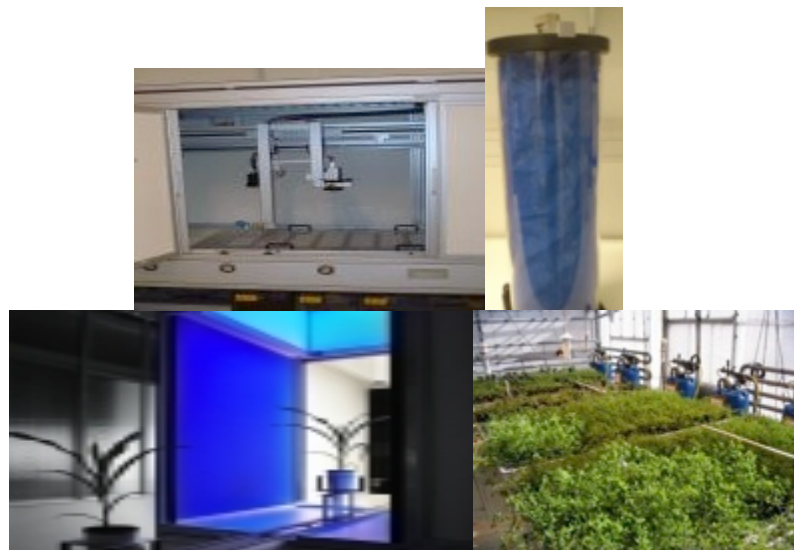
Food for thoughts...

Combine approaches

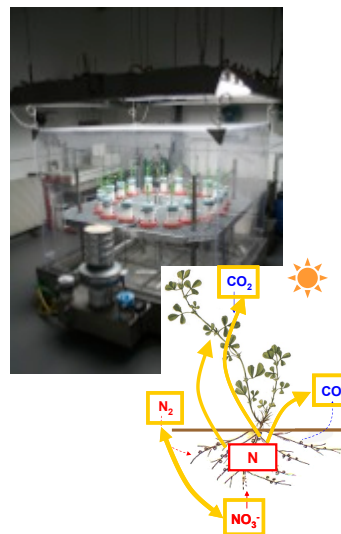
Phenotyping Approach



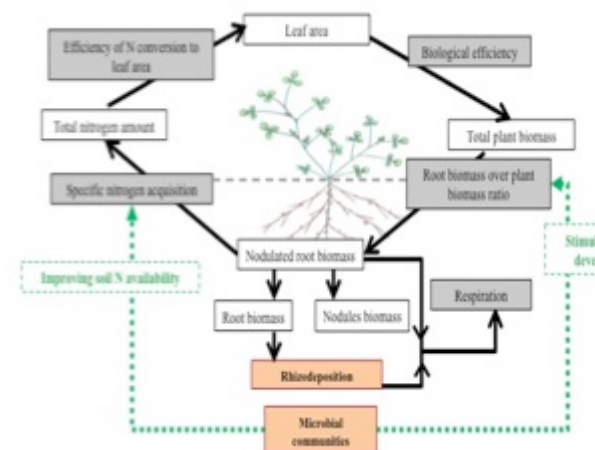
Analytical approach



Identifying differences among genotypes



Modelisation



Interpreting the detected difference

- Towards functional phenotyping (NAAS System)
- Validate to/from the field : Pheno Field Platform in Dijon



Starring...

Christian JEUDY



Céline BERNARD



Jean-Claude SIMON



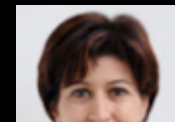
Frédéric COINTAULT



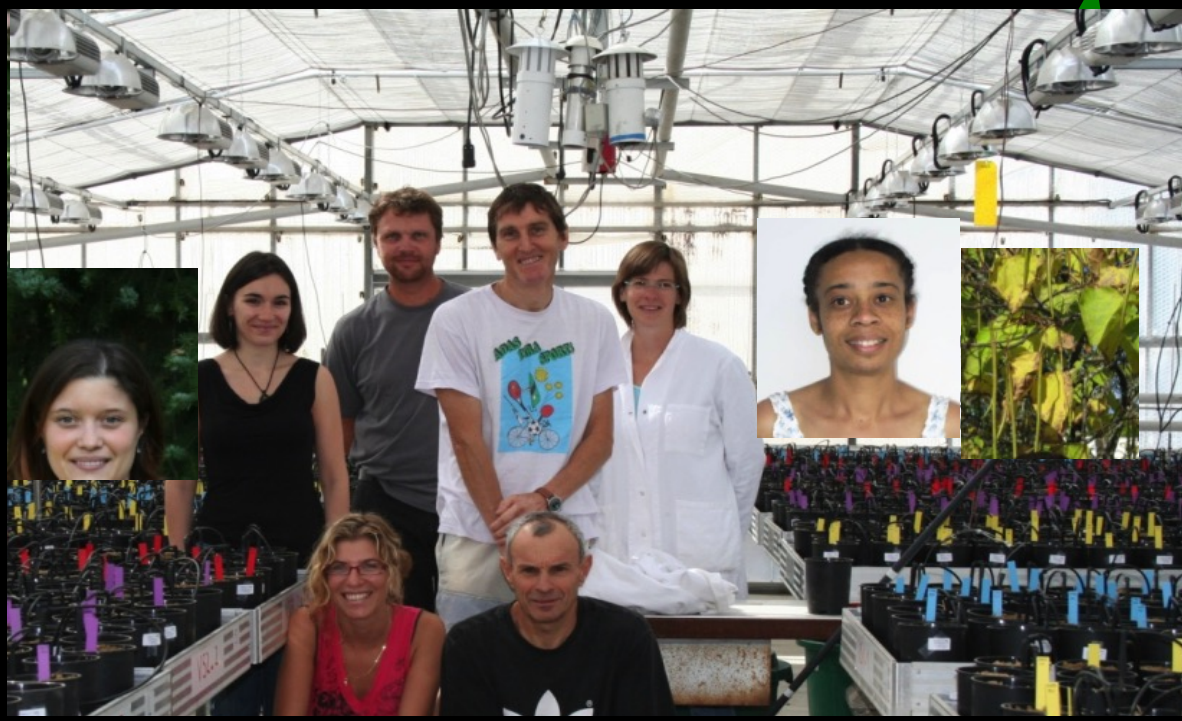
Simeng HAN



Marielle ADRIAN



and also ...



Ecophysiology team

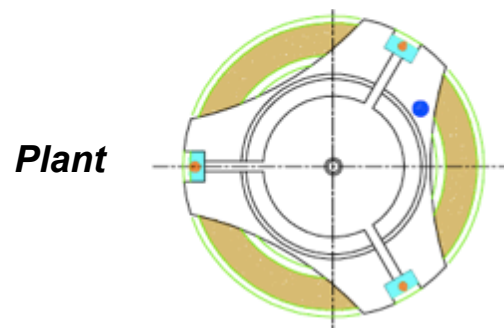
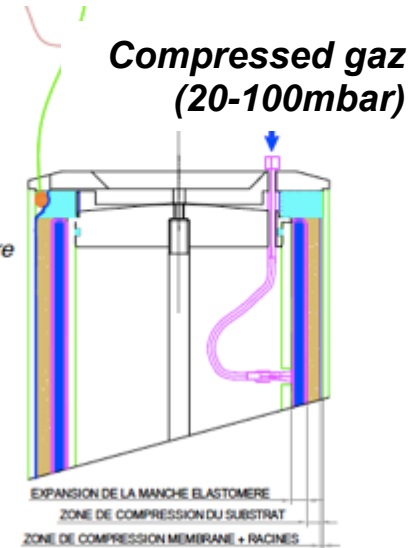
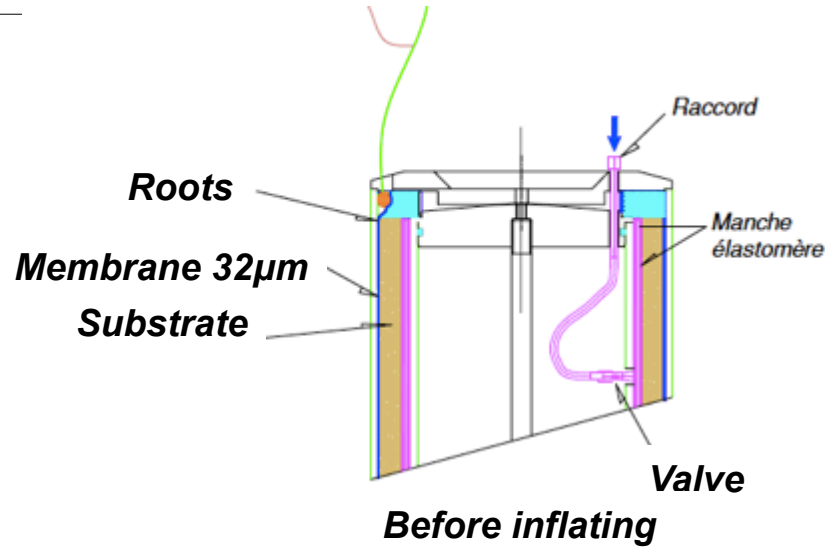
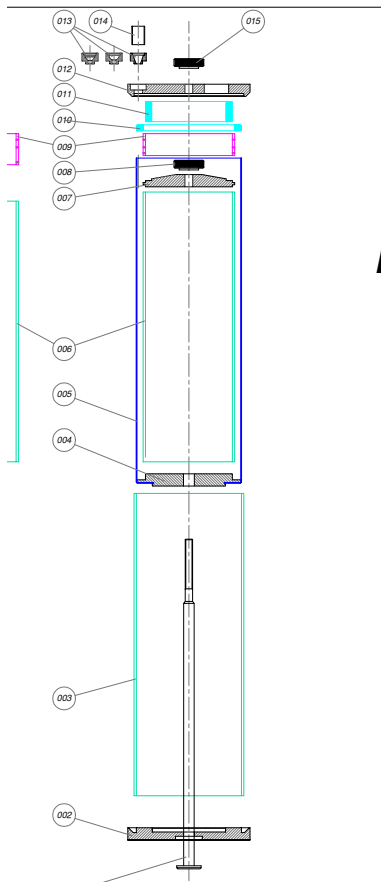


Medicago truncatula team



**Rhizotrones
(EU Licence INRA-
Inoviaflow, 1300 units
planed)**

Fluxomic
PPHD
Rhizotrons
Rhizobox



Context	Approach	Phenotypic tools	Examples	Models	Conclusion
			Grapevine		Objectives
			Legumes		Desease detection

Detecting desease in viticulture

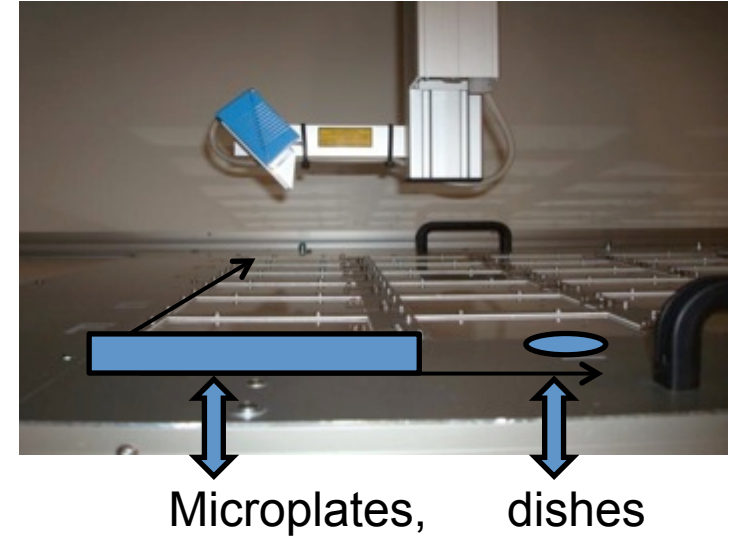
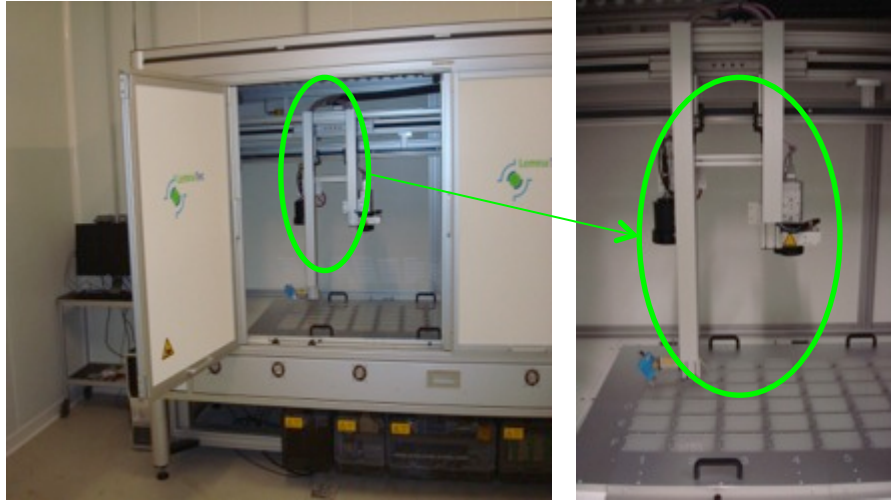
Major problems in viticulture → cryptogamic diseases (oïdium, grey mould) of grapevine



Approaches:

- 1) ↓ number of fongicides treatments and ↓ applied quantity (decision tools) + images (thermography IR...)
- 2) alternative strategies to fongicides

Context	Approach	Phenotypic tools	Examples	Models	Conclusion
			Grapevine		Objectives
			Legumes		Disease detection

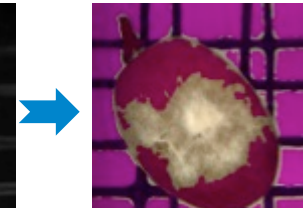
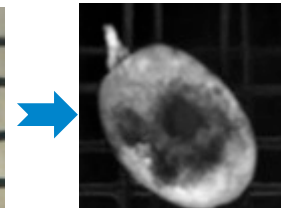
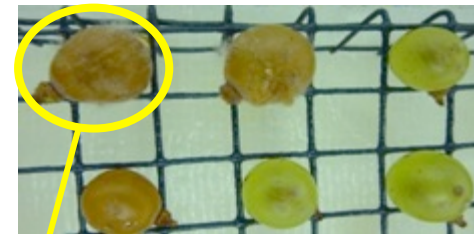


Microplates, dishes

HTS (small biological unit phenotyping)

Colour/texture hybrid spaces

- RGB images in colorimetric spaces that integrates texture,
- object with similar colors may have different textures (so need to practice with biological objects)



Detection/evaluation of the intensity of diseases on grapes/berries