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Challenges and opportunities involving high throughput plant Phenotyping of plant and organisms interactions in PPHD

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Towards sustainable agriculture...

Maintain yields in a context of:

- climate change,

- soil, water resource degradation.



↓ need for high nutrient, water, or pesticides inputs.

Sustainable agricultural practices.



Select the best performing crops..

..“better” yield, environmental « efficiency

Understand plant response to environment
identify genes.

Plant architecture, flowering, senescence

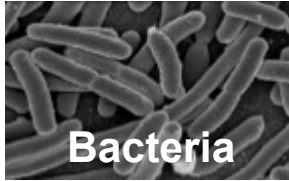
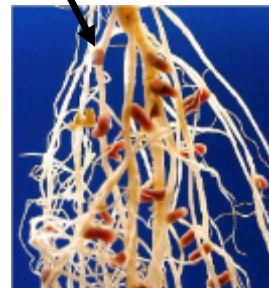
Pods and seeds



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

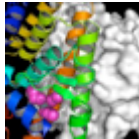


Genetic variability



CONCEPTION of PLANT IDEOTYPES

Characterizing mechanisms and molecular basis



Context	Approach	Phenotypic tools	Examples	Models	Conclusion
		Fluxomic			
		PPHD			
		Rhizotrons			
		Rhizobox			

Characterizing root development

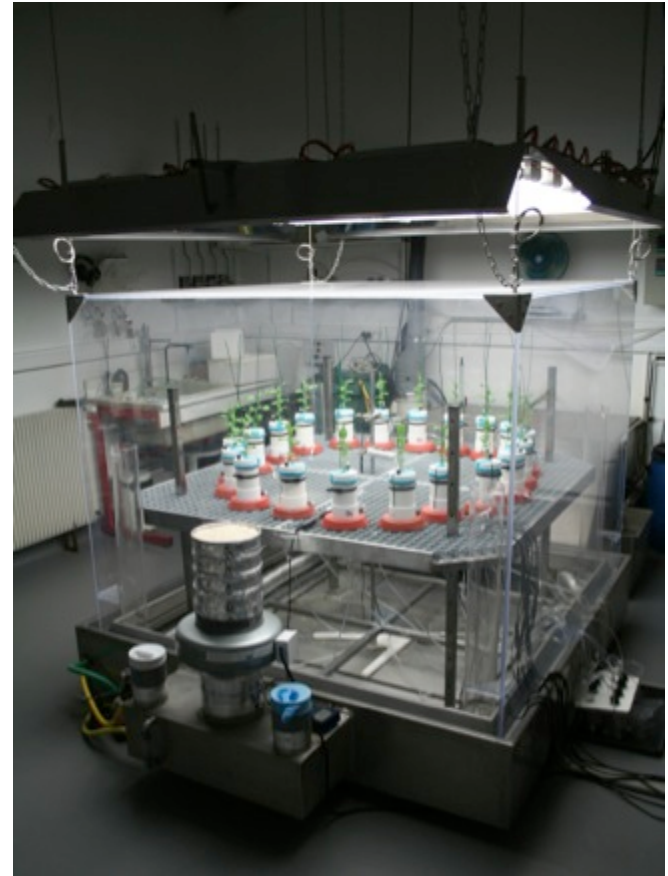


Growth pouches, hydroponics



N₂ adapted Isotopic split root

C, N, S flux measurement



Labeling chamber ¹³C/¹⁵N/³⁴S

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


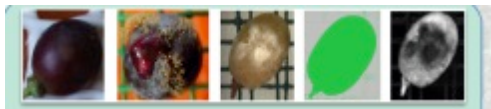
Aerial architecture


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

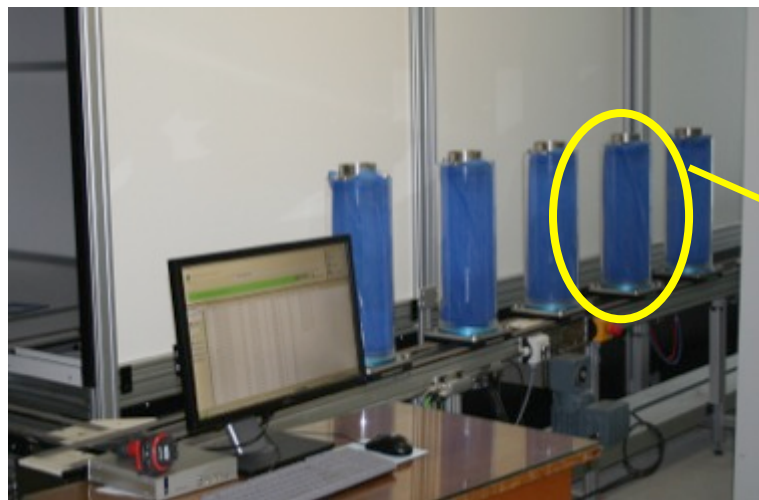
Phenotyping cabinet equipped with cameras and robots

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

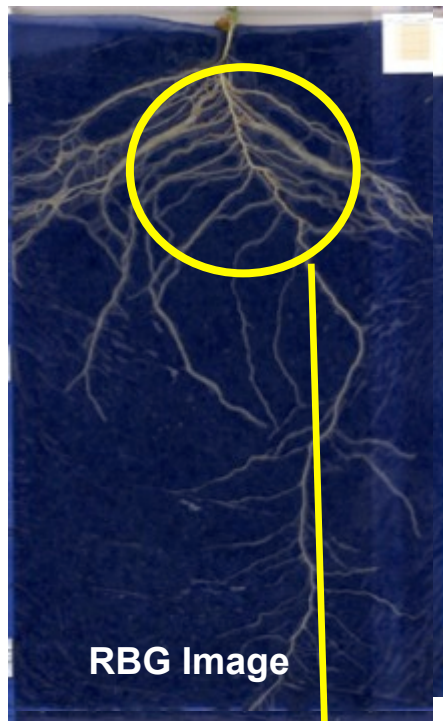
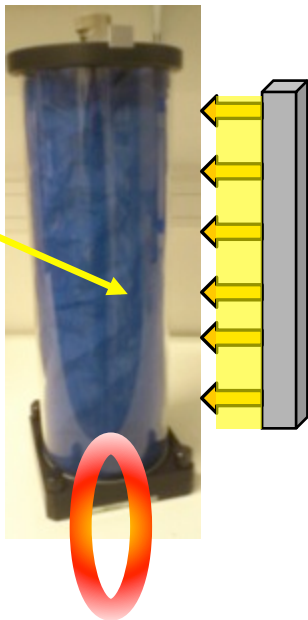


**Rhizothrones
(EU Patent INRA-
Inoviaflow, 1300 units
planned)**

Fluxomic
PPHD
Rhizotrons
Rhizobox



Rotating device

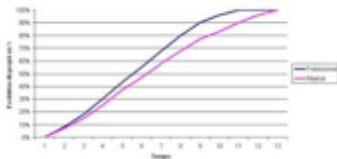
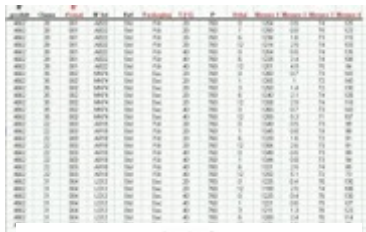


RBG Image



High resolution

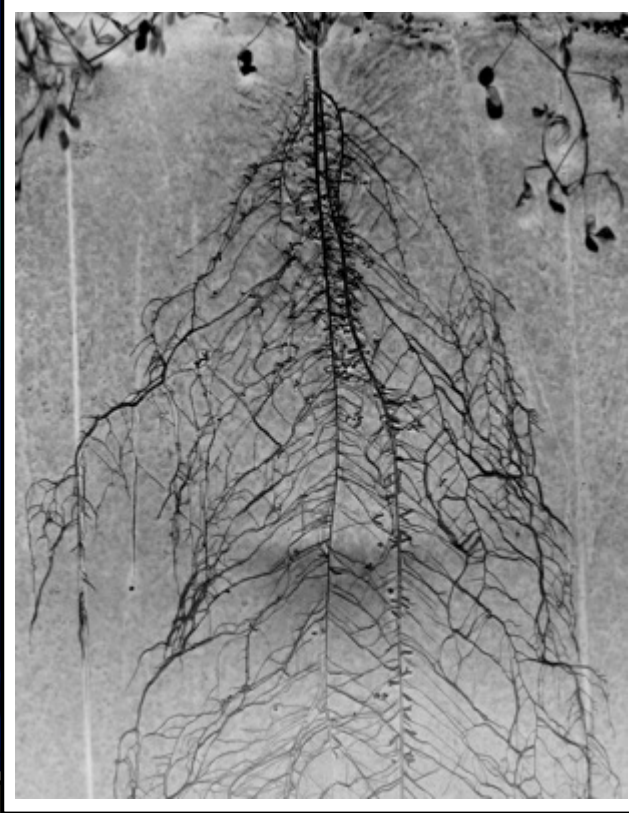
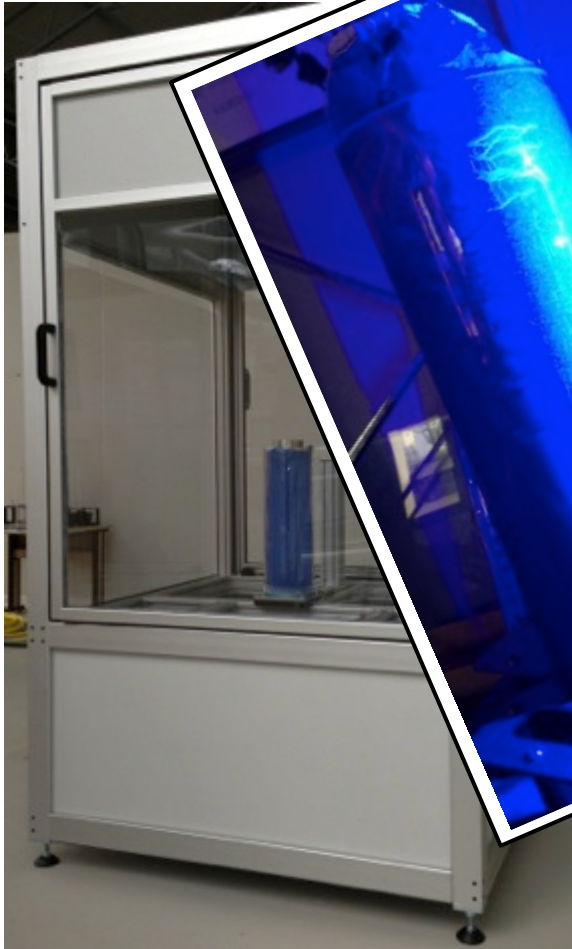
- Traits :
- projected **root** area
 - projected **nodule** area
 - total nodule **number**
 - nodule size** classification
 - total root length....



Context	Approach	Phenotypic tools	Examples	Models	Conclusion
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Fluxomic	PPHD	RhizoBox			
RhizoBox					

RhizoBox (INRA-Inoviaflow)



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

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

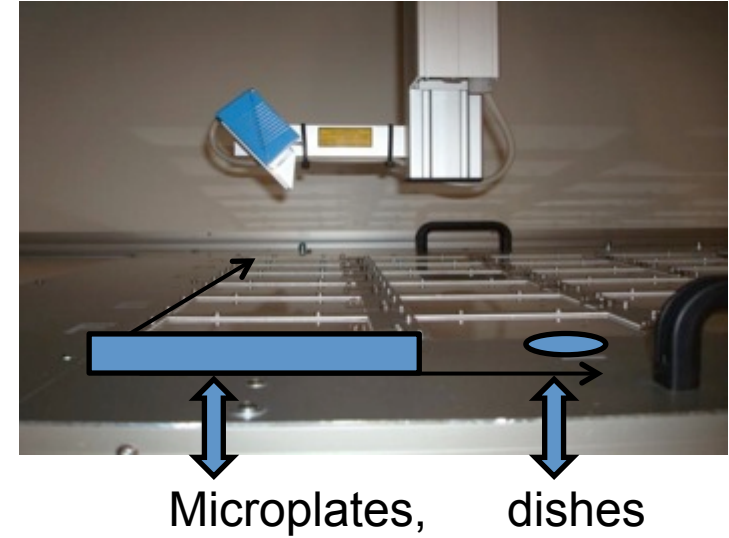
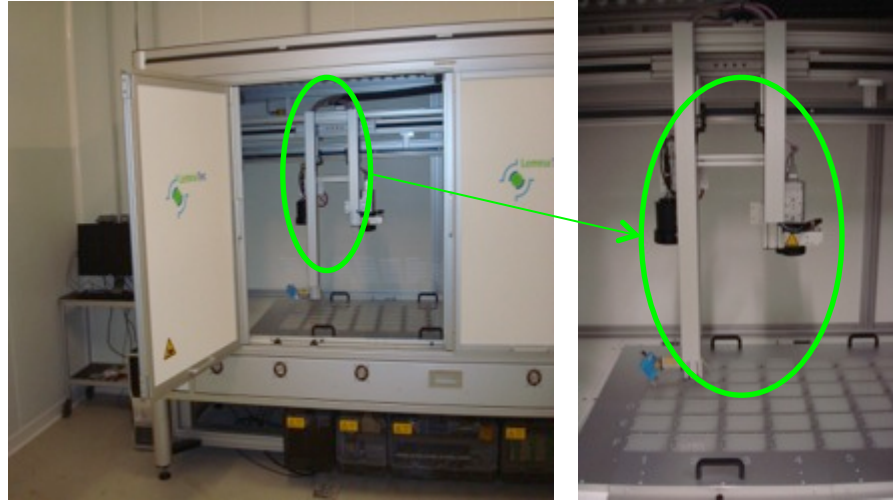


Approaches:

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



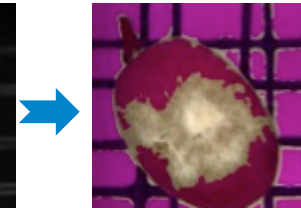
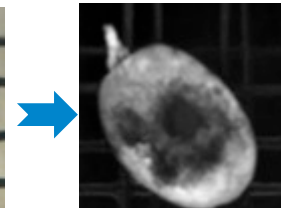
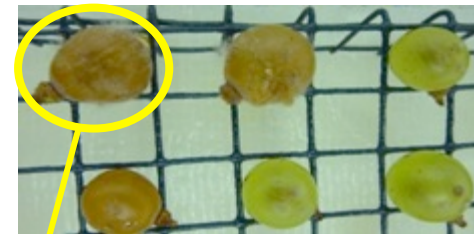
Context	Approach	Phenotypic tools	Examples	Models	Conclusion
			Grapevine		Objectives
			Legumes		Disease detection



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)

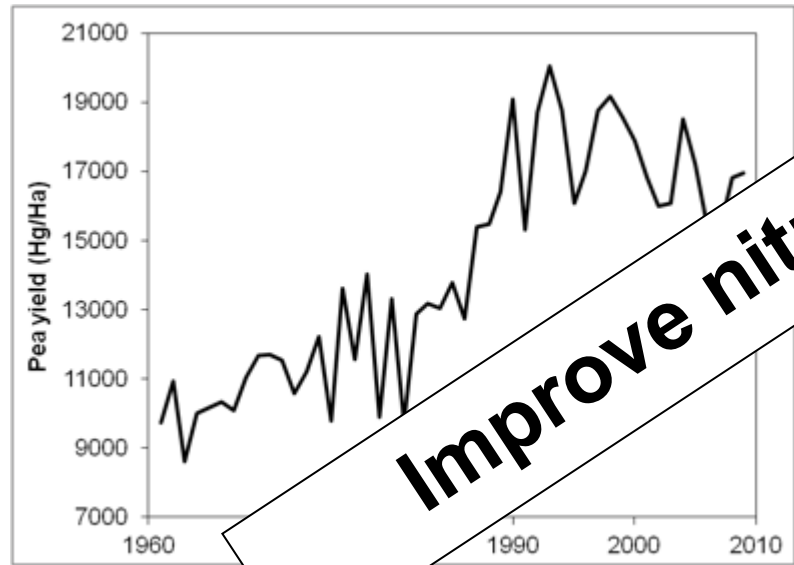


Detection/evaluation of the intensity of diseases on grapes/berries

Grapevine
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
			Grapevine		
			Legumes		Objectives
					Genetic diversity
					Identify a strategy
					Genotype ranking

Tools and methods



Genetic variability




CONCEPTION of PLANT IDEOTYPES



Characterizing mechanisms and molecular basis



Grapevine	
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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

Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

Recombinant inbred lines (1400 RILs)

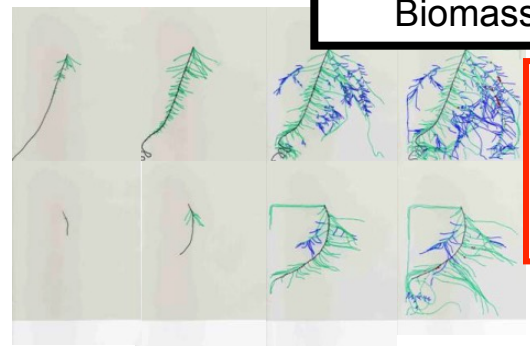
STRUCTURE

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

Grapevine	
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Legumes	
---------	--

Objectives

Genetic diversity

Identify a strategy

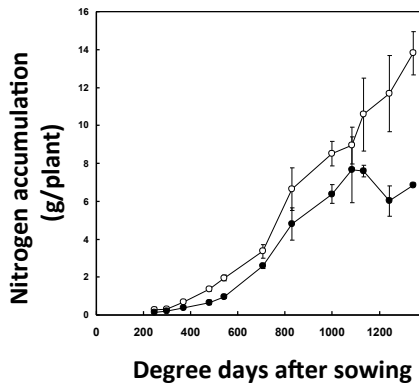
Genotype ranking

Induced genetic variability

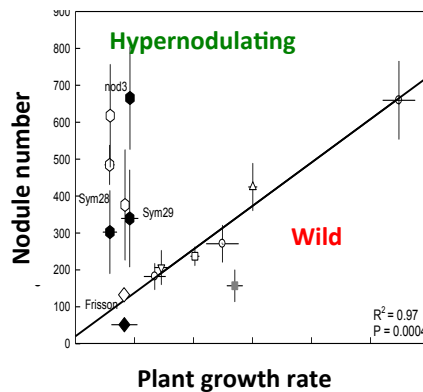
Identify/characterize genes involved in nodulation control or root architecture

Nodule development

Root architecture



Salon et al. Agr 2001

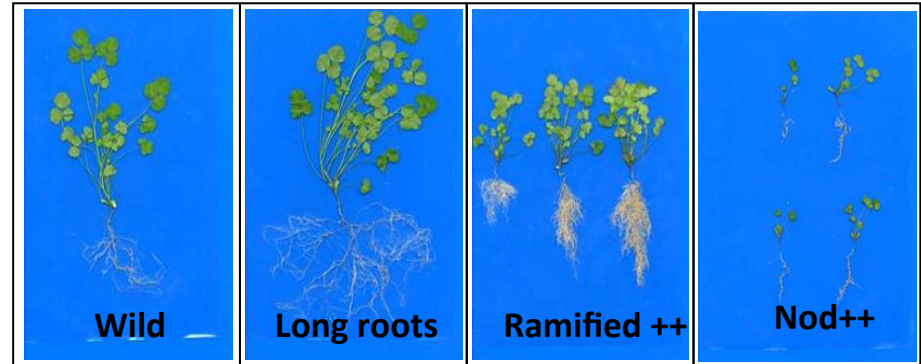


Voisin et al. Plant Soil 2010

Duc et al. 1998

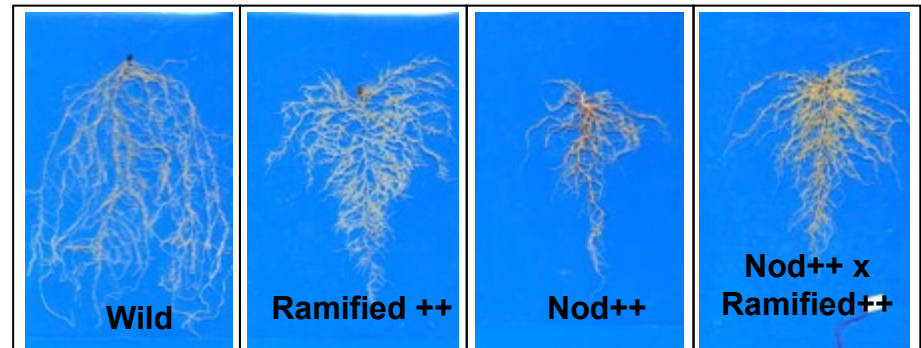
Cazenave et al. Plant Soil 2013

Medicago truncatula, induced by Tnt1



Porceddu et al. BioMed 2008

Pea, induced by EMS



Coll. KK Sidorova

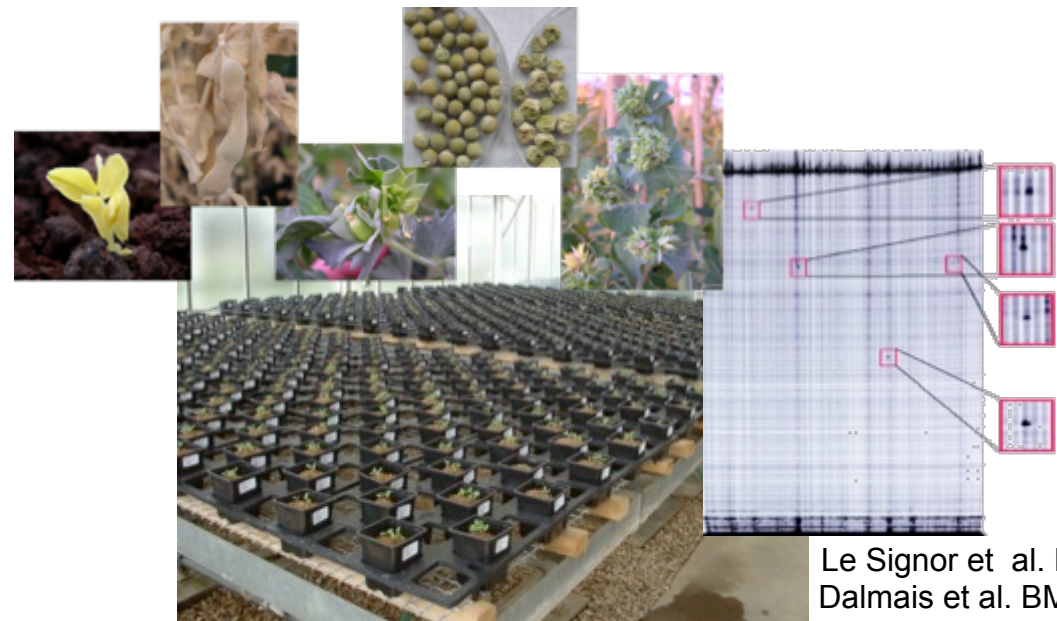
Grapevine
Legumes

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
			Grapevine		
			Legumes		Objectives
					Genetic diversity
					Identify a strategy
					Genotype ranking

Tools and methods



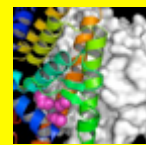
Genetic variability




CONCEPTION of PLANT IDEOTYPES



Characterizing mechanisms and molecular basis

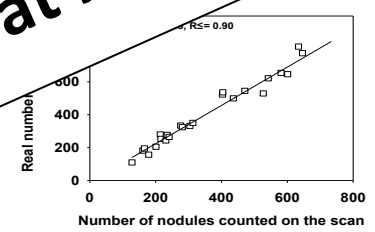
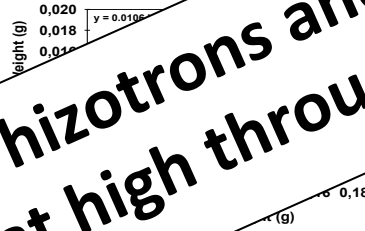
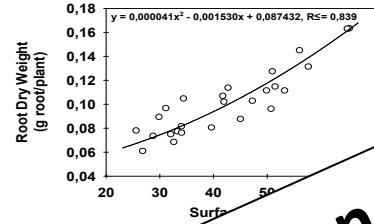
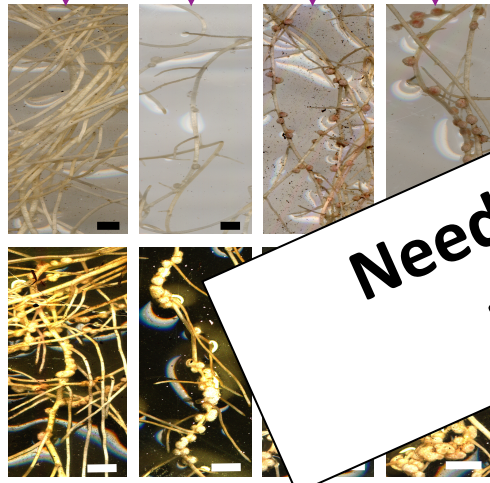
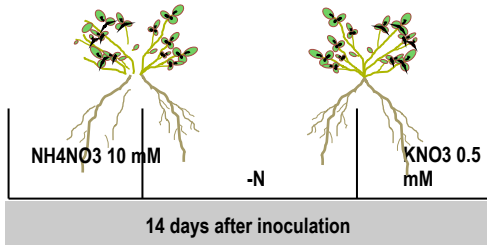


Grapevine
Legumes

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



Need to use rhizotrons and image analysis at high throughput

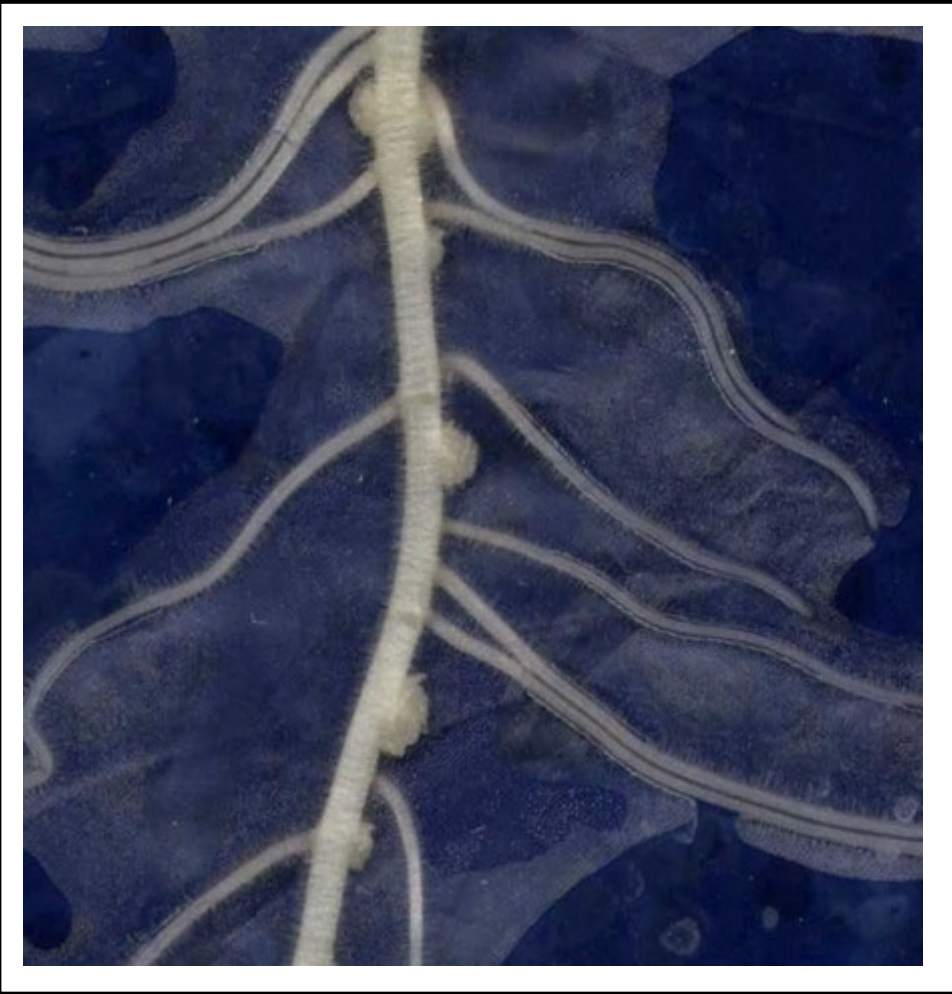
Morphometry versus functional strategy identification

Split roots

Nodules number and size, appearance

Grapevine
Legumes

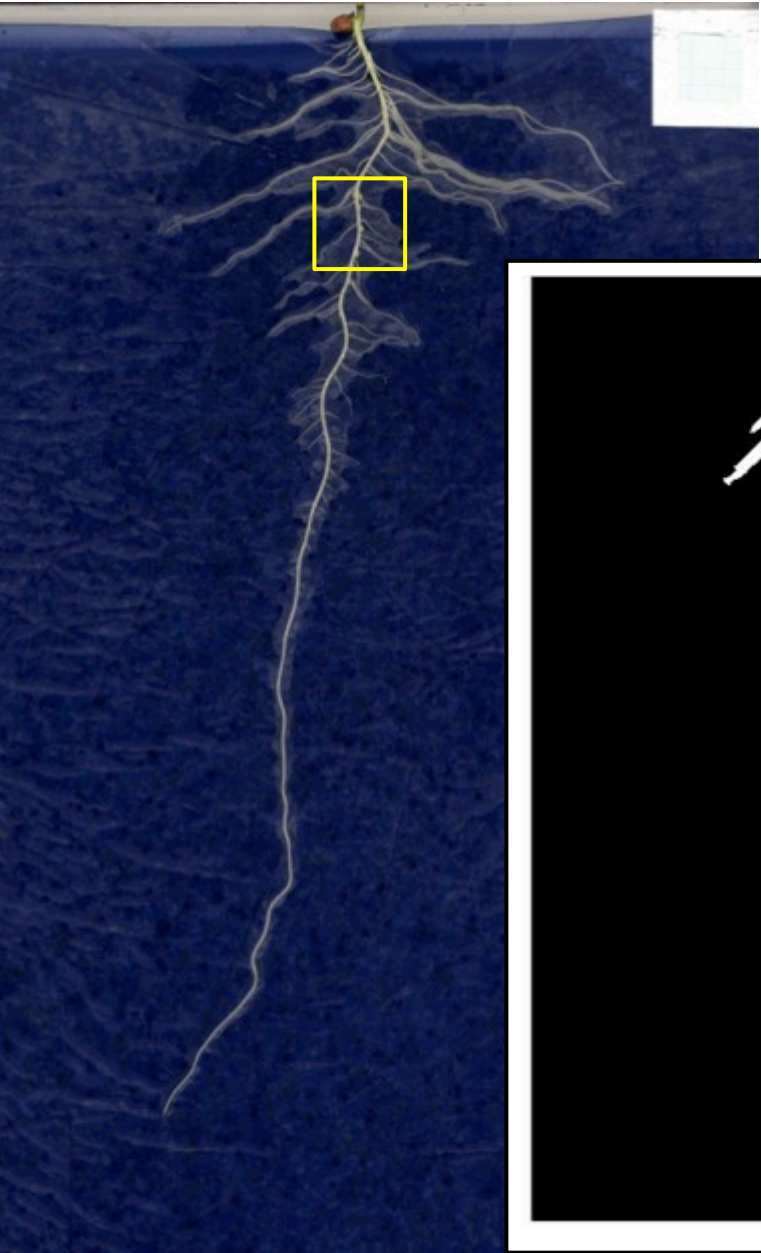
Objectives
Genetic diversity
Identify a strategy
Genotype ranking



Young plant

Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

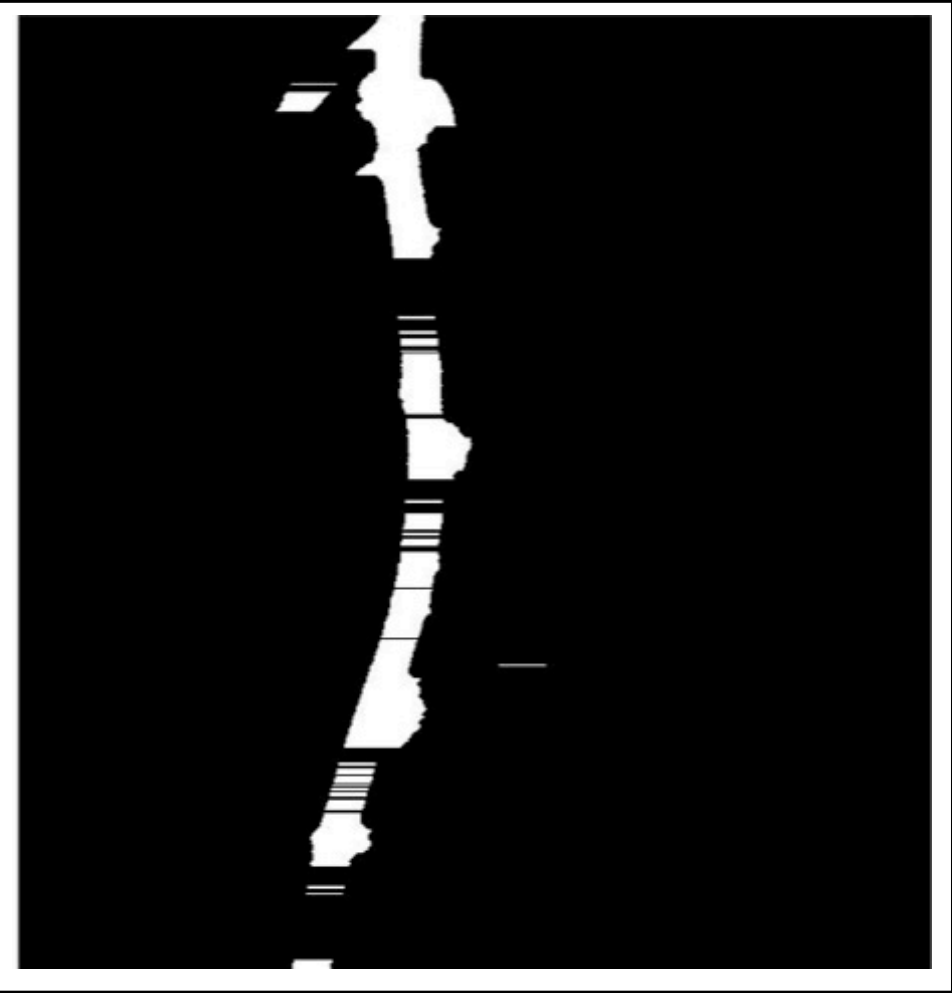


Binary image and threshold

Young plant

Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

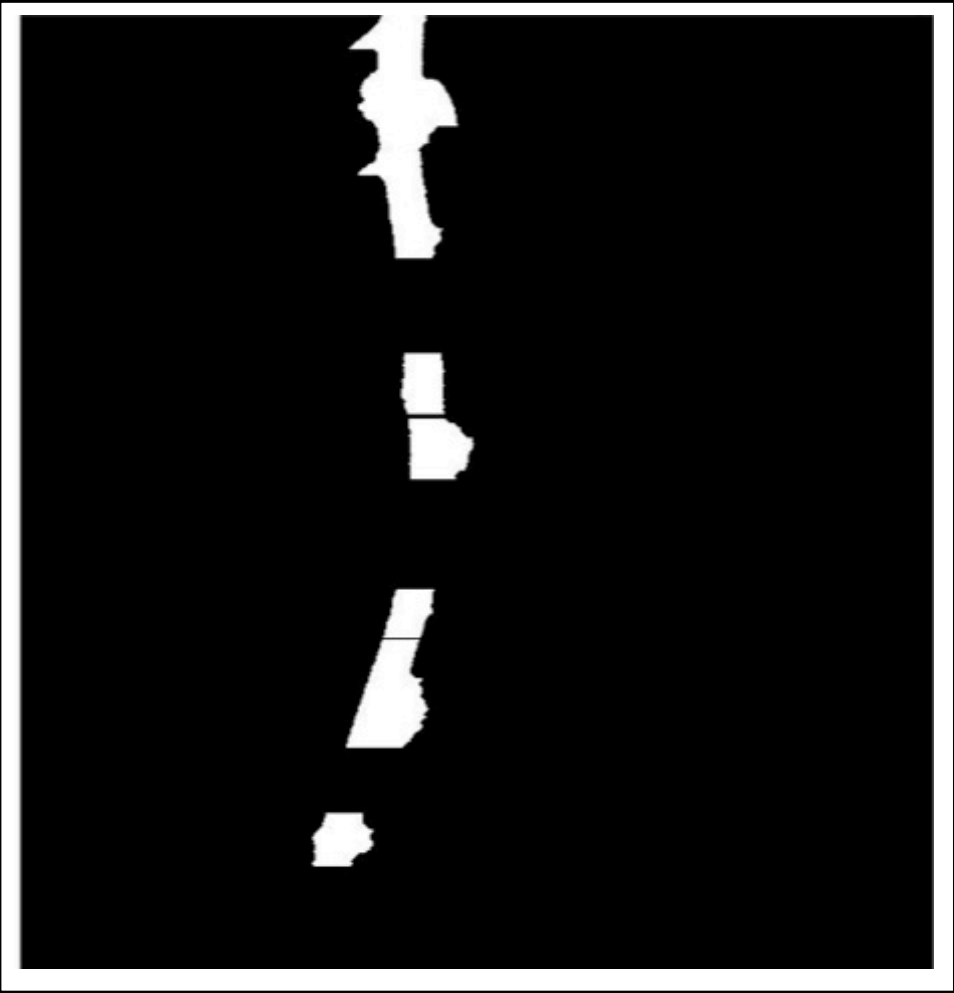


Only keep
nodule
shaped
forms, get
rid of
unused
areas

Young plant

Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

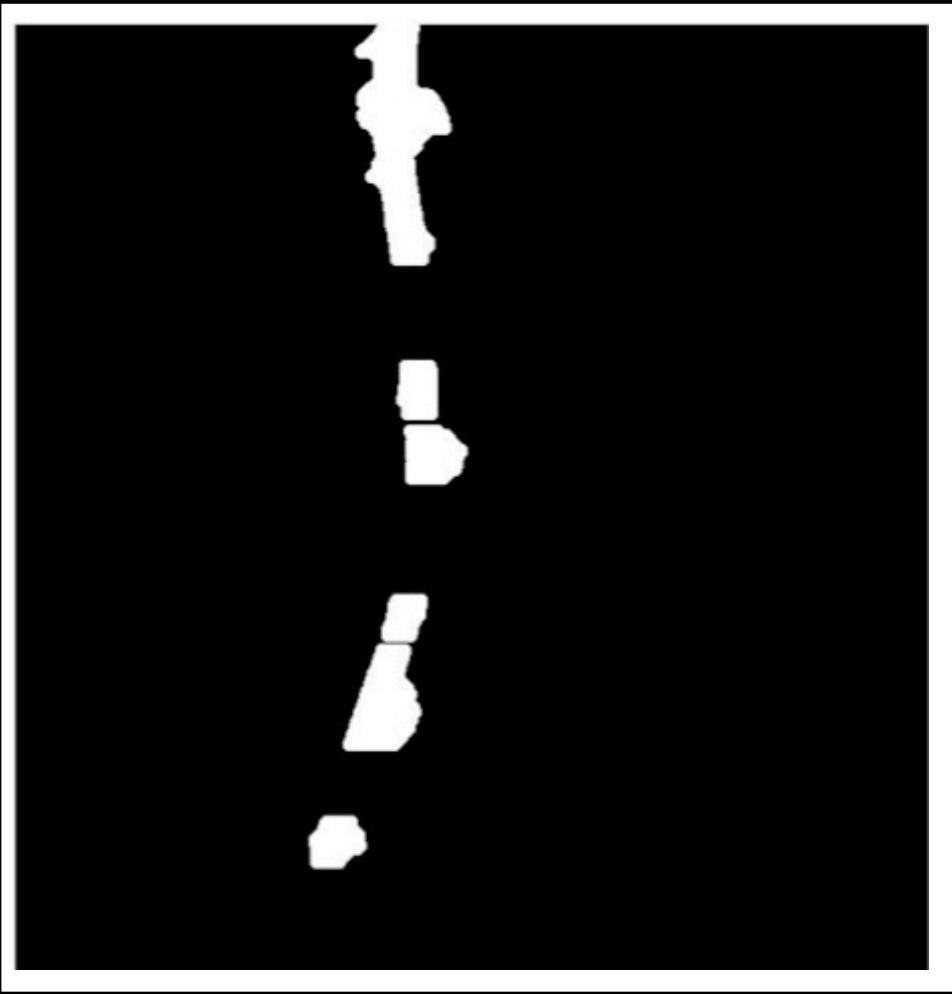


Get rid of unused areas (areaopen)

Young plant

Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

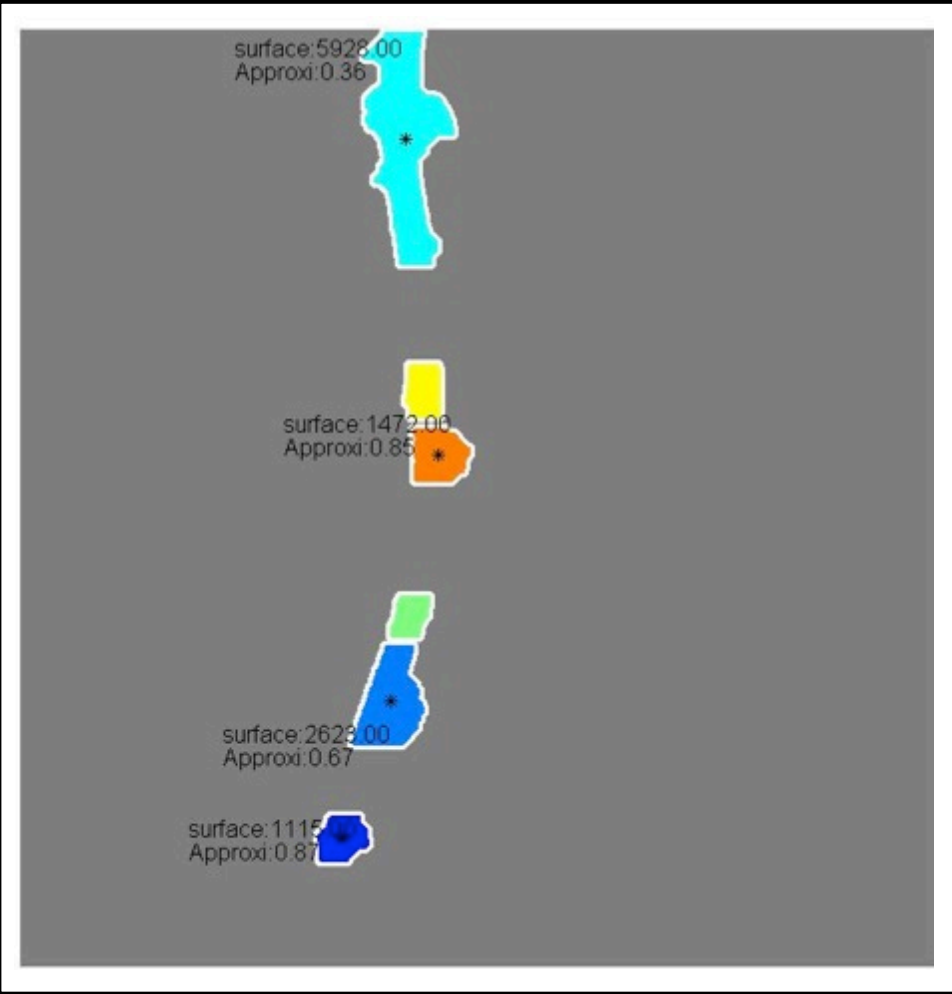
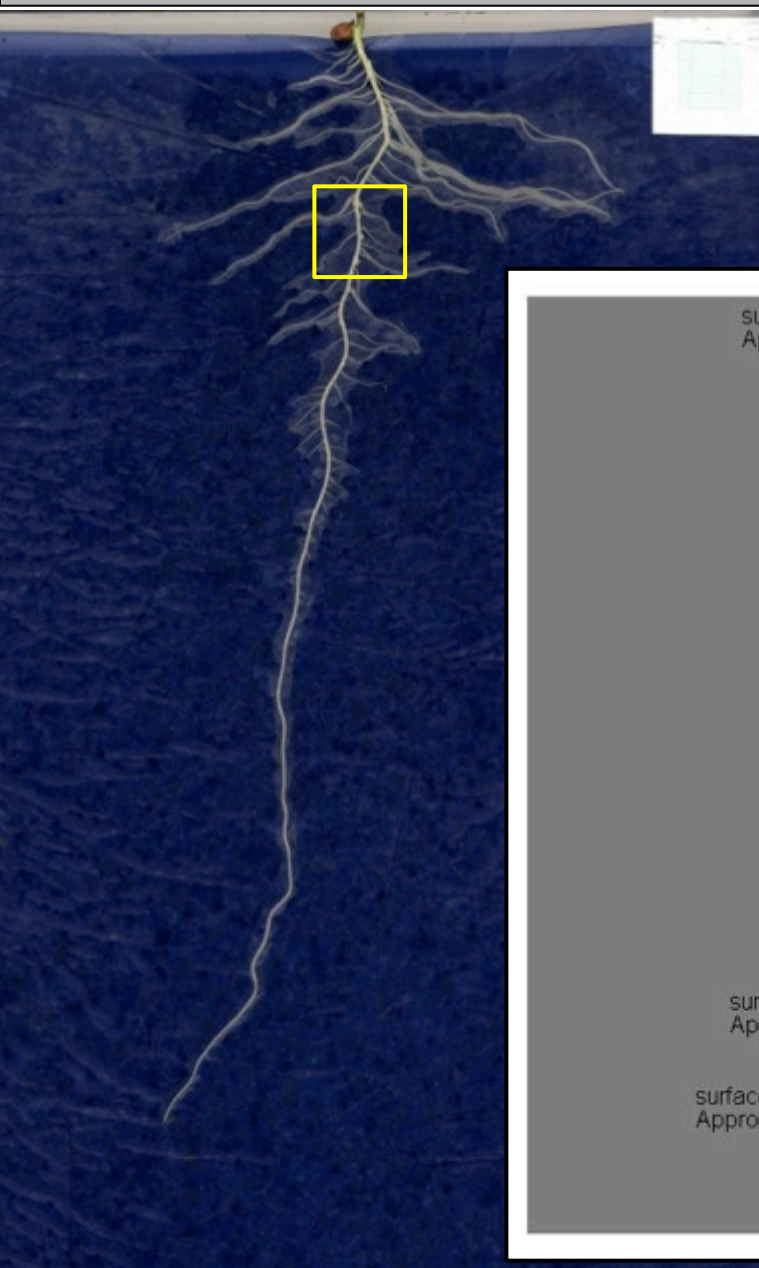


Smooth the image

Young plant

Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking



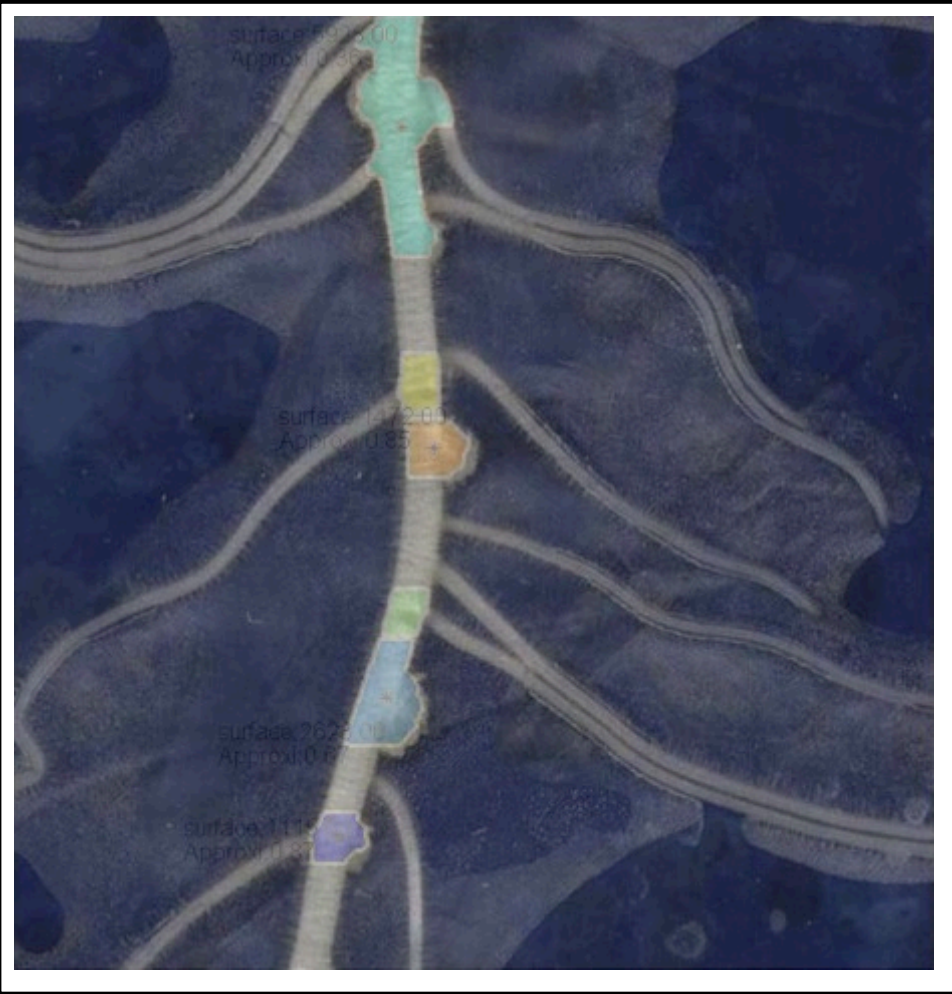
Count nodules with the « circle » form + area on a approximate circle

Young plant

Context	Approach	Phenotypic tools	Examples	Models	Conclusion
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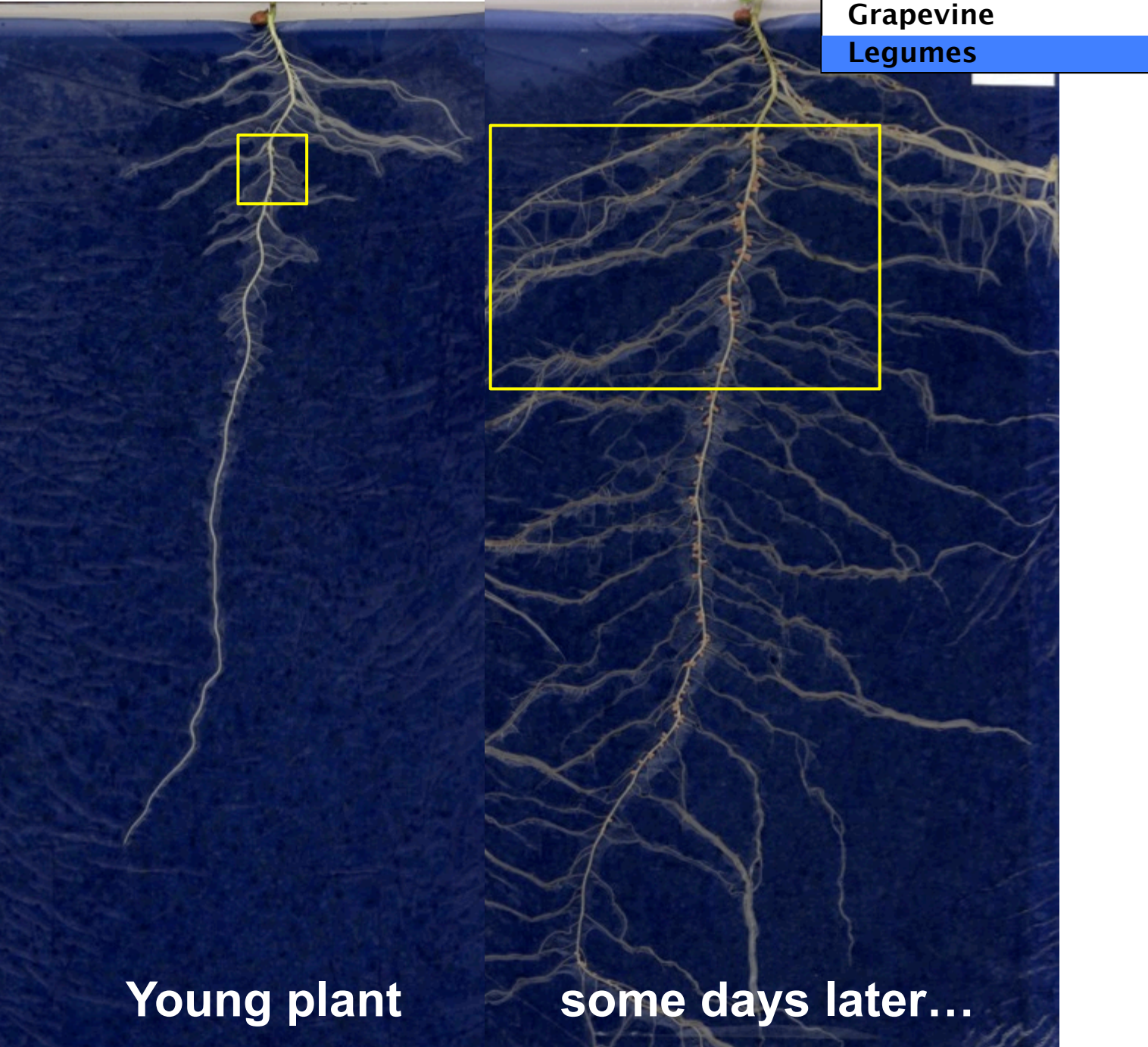
Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking



**Original image
+ superposed
nodules**

Young plant

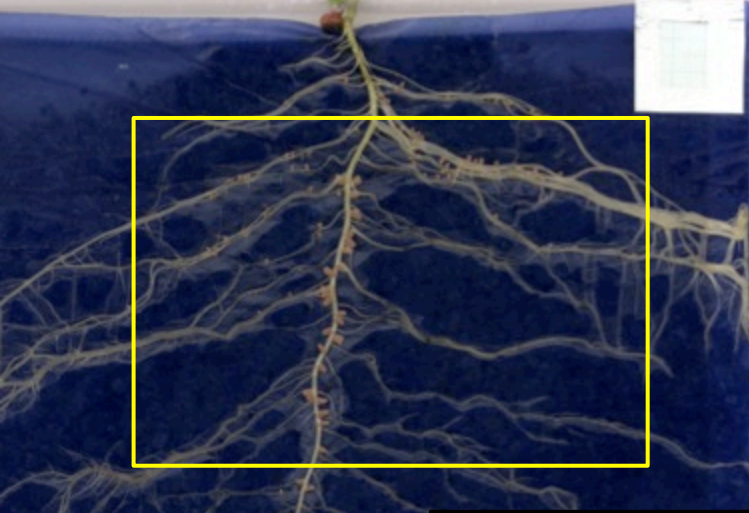
Context	Approach	Phenotypic tools	Examples	Models	Conclusion
			Grapevine		
				<p data-bbox="1526 99 1912 142">Objectives</p> <p data-bbox="1526 142 1912 185">Genetic diversity</p> <p data-bbox="1526 185 1912 228">Identify a strategy</p> <p data-bbox="1526 228 1912 271">Genotype ranking</p>	

Young plant

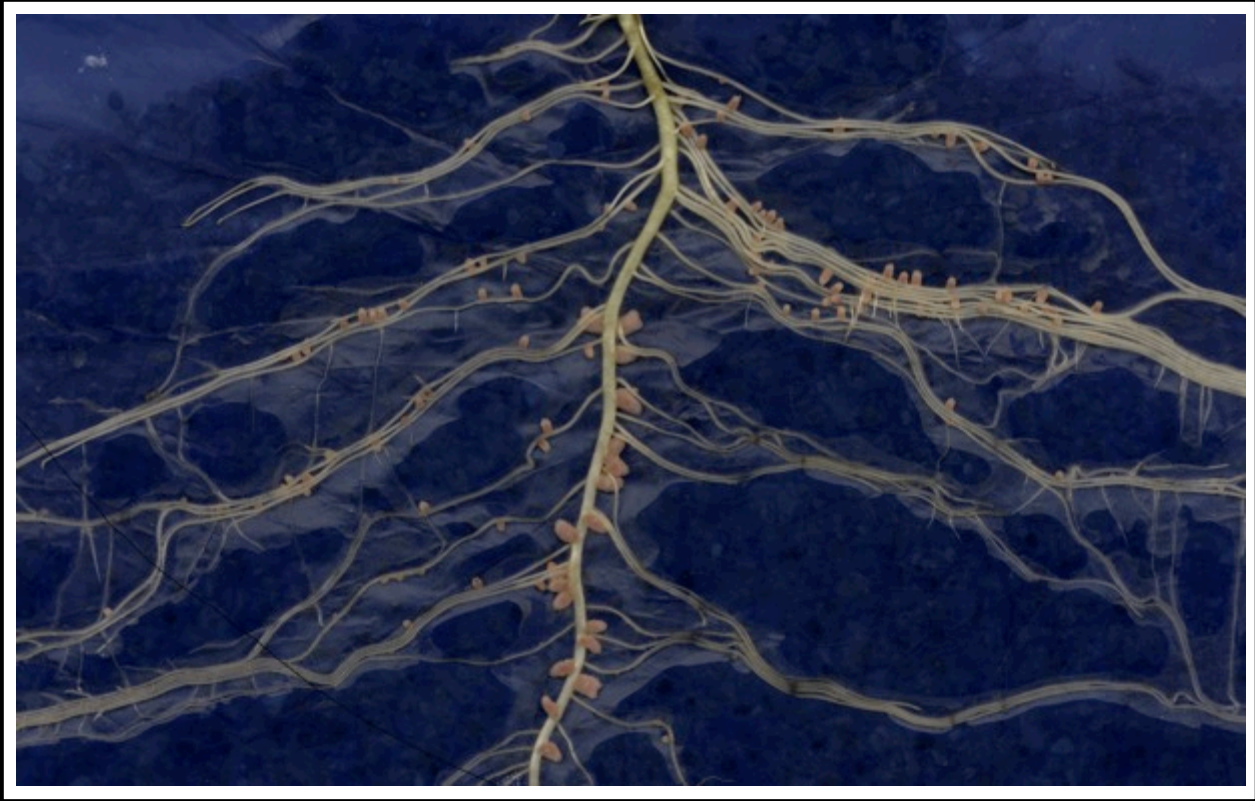
some days later...

Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

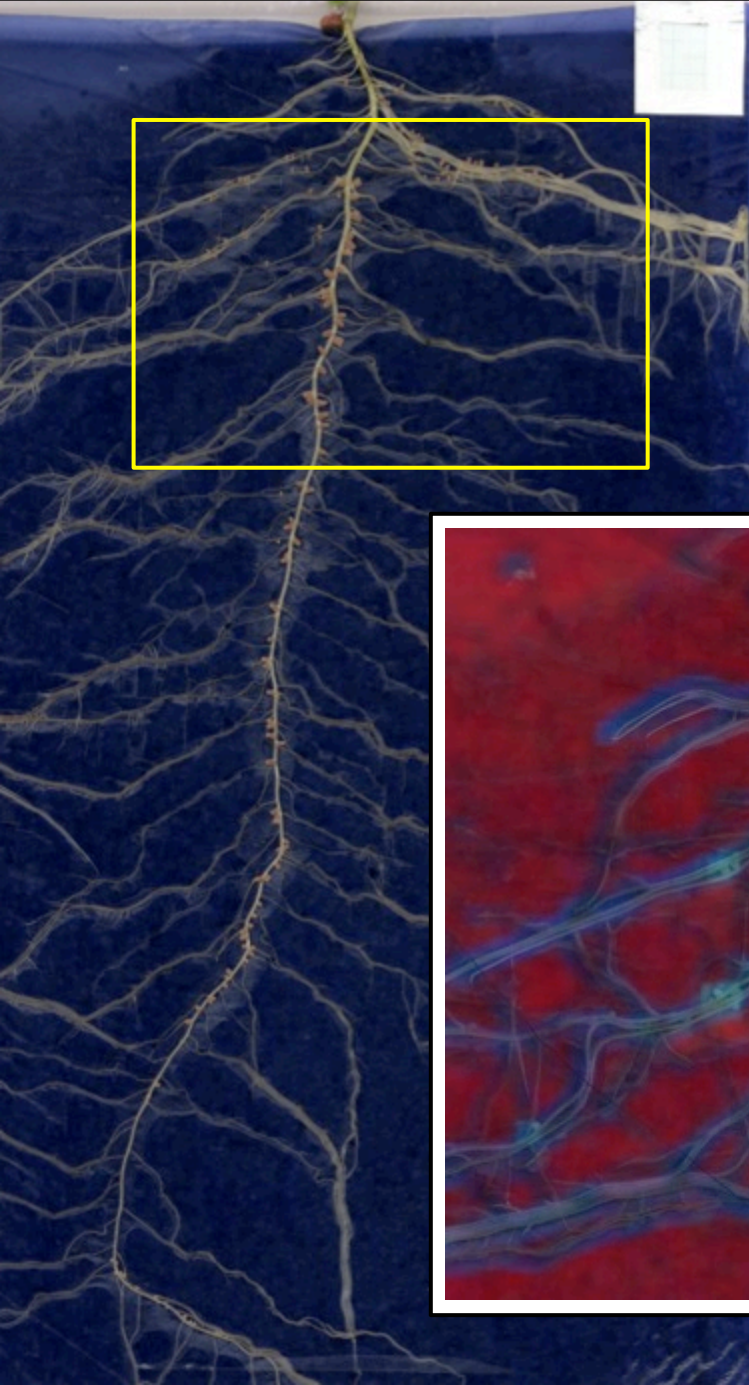


Enlarged image

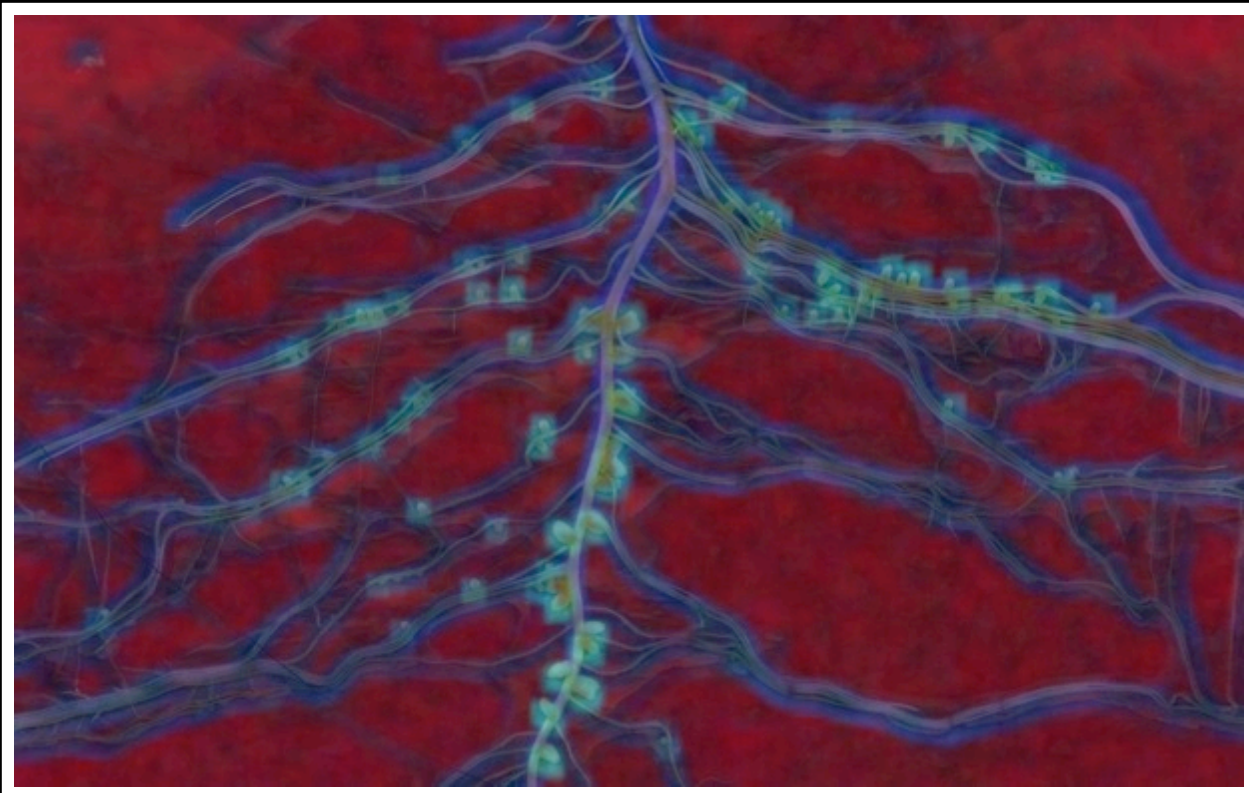


Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

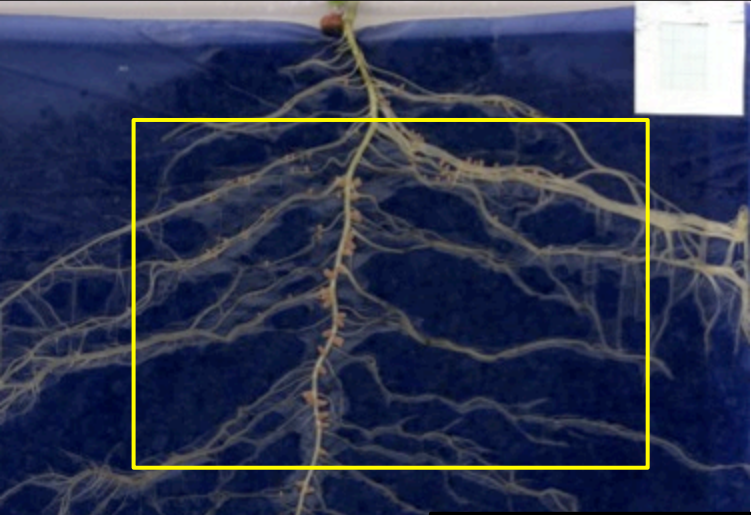


Hybrid space (color + texture)

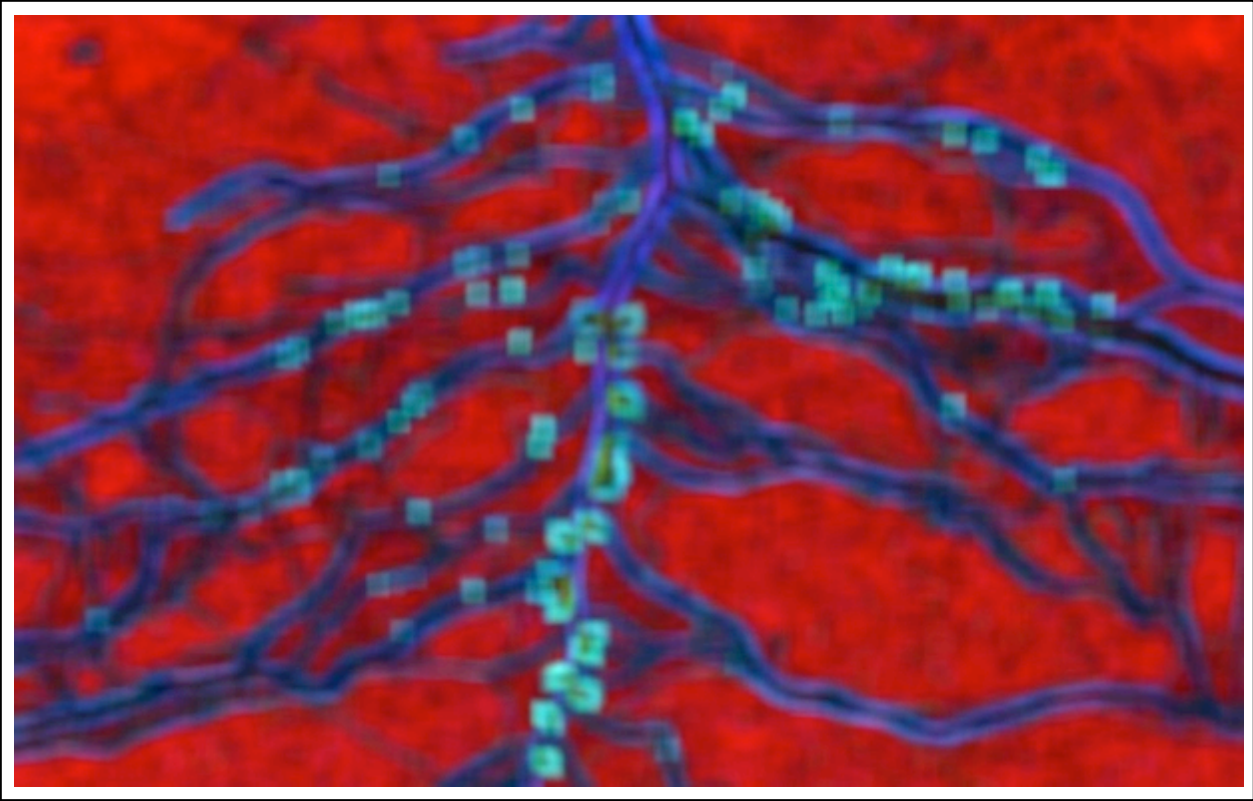


Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

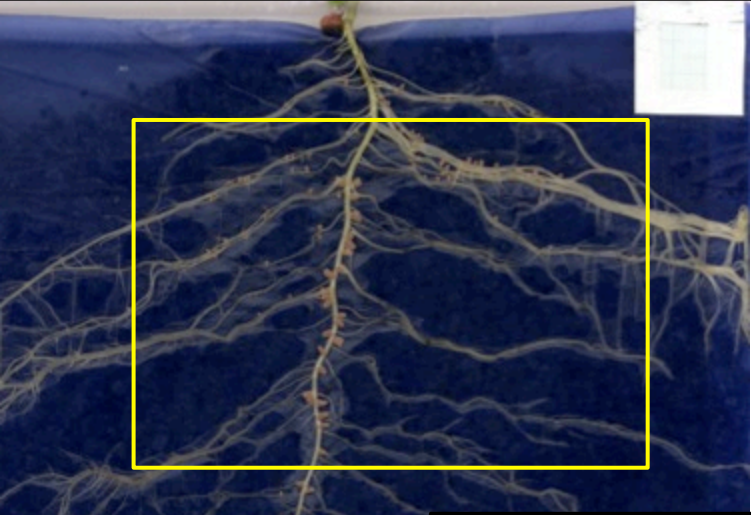


RGB Image of identified nodules

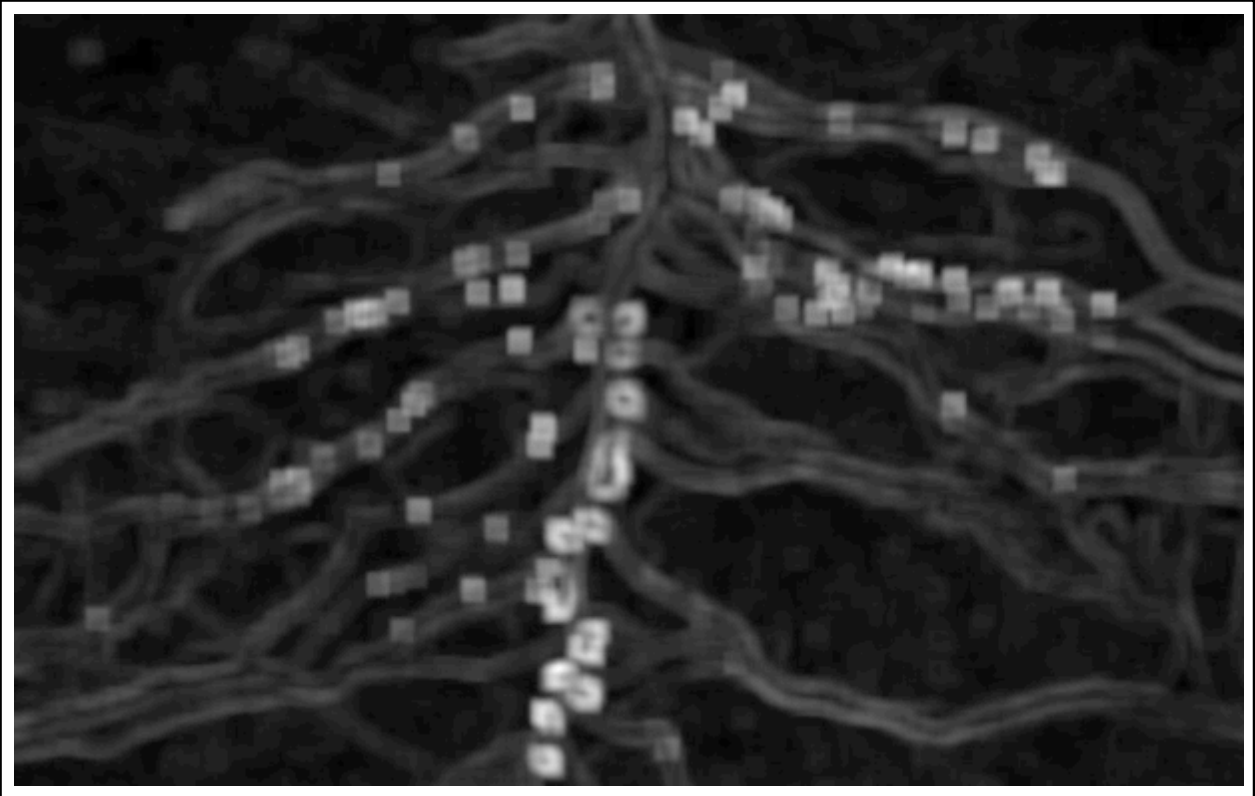


Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

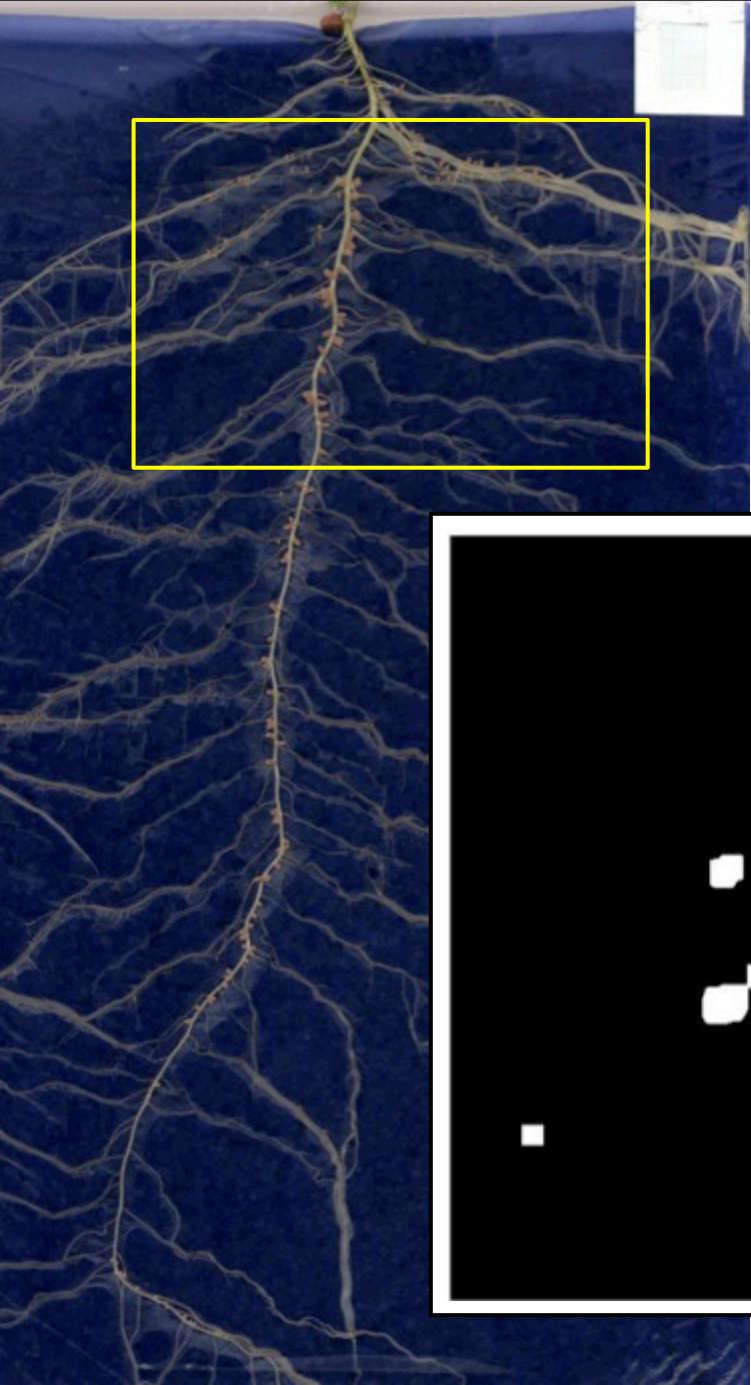


Green band of the RGB image

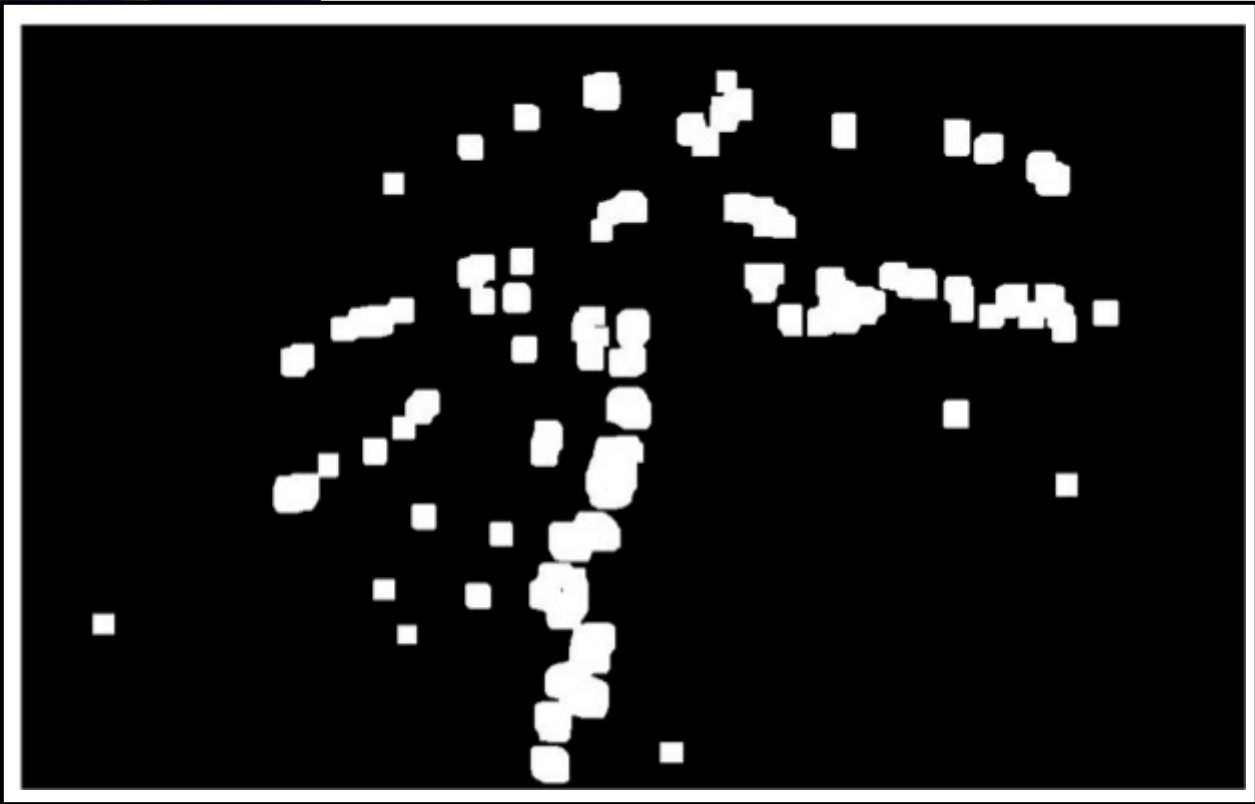


Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

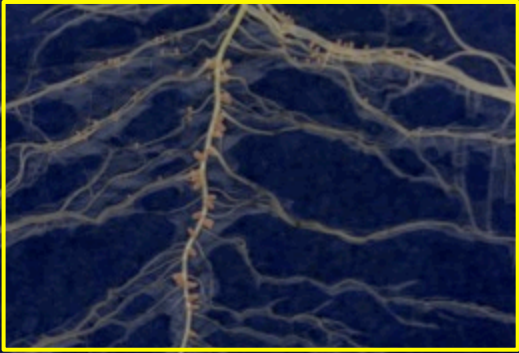


Binary image with squared nodules

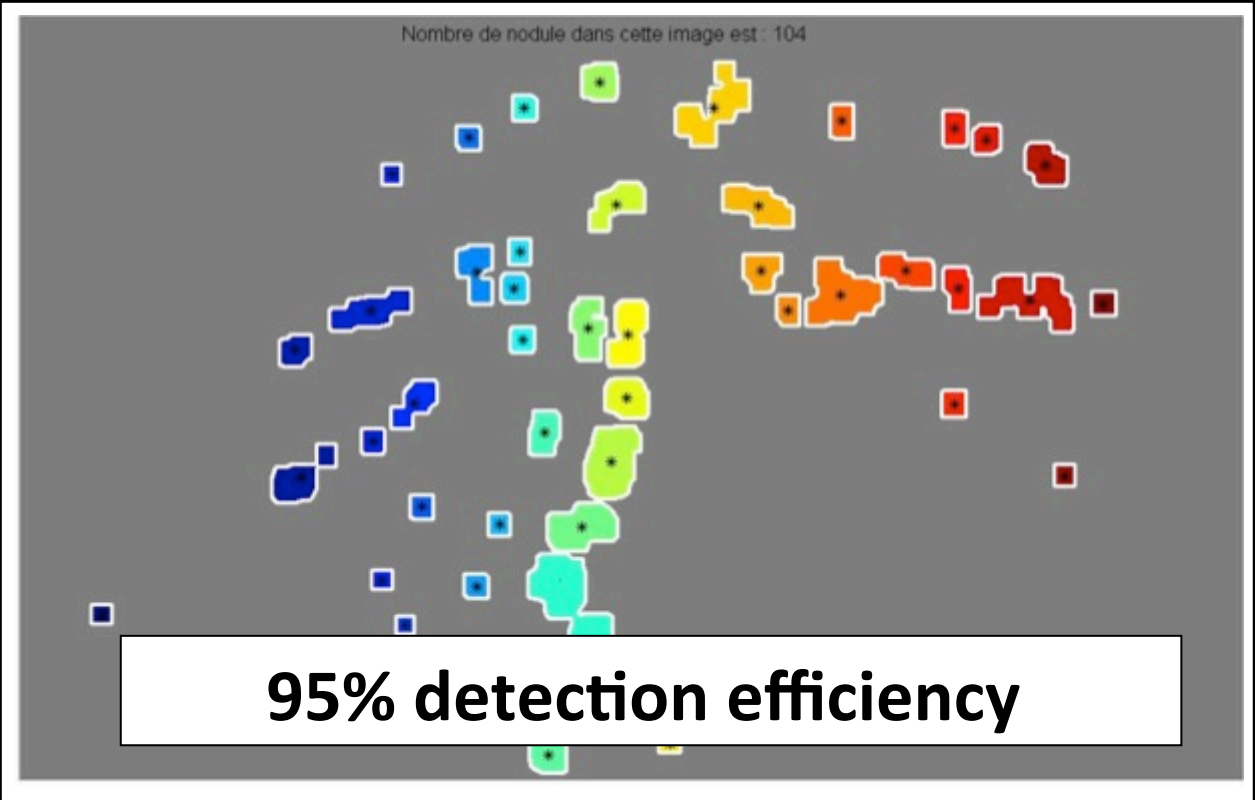


Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking



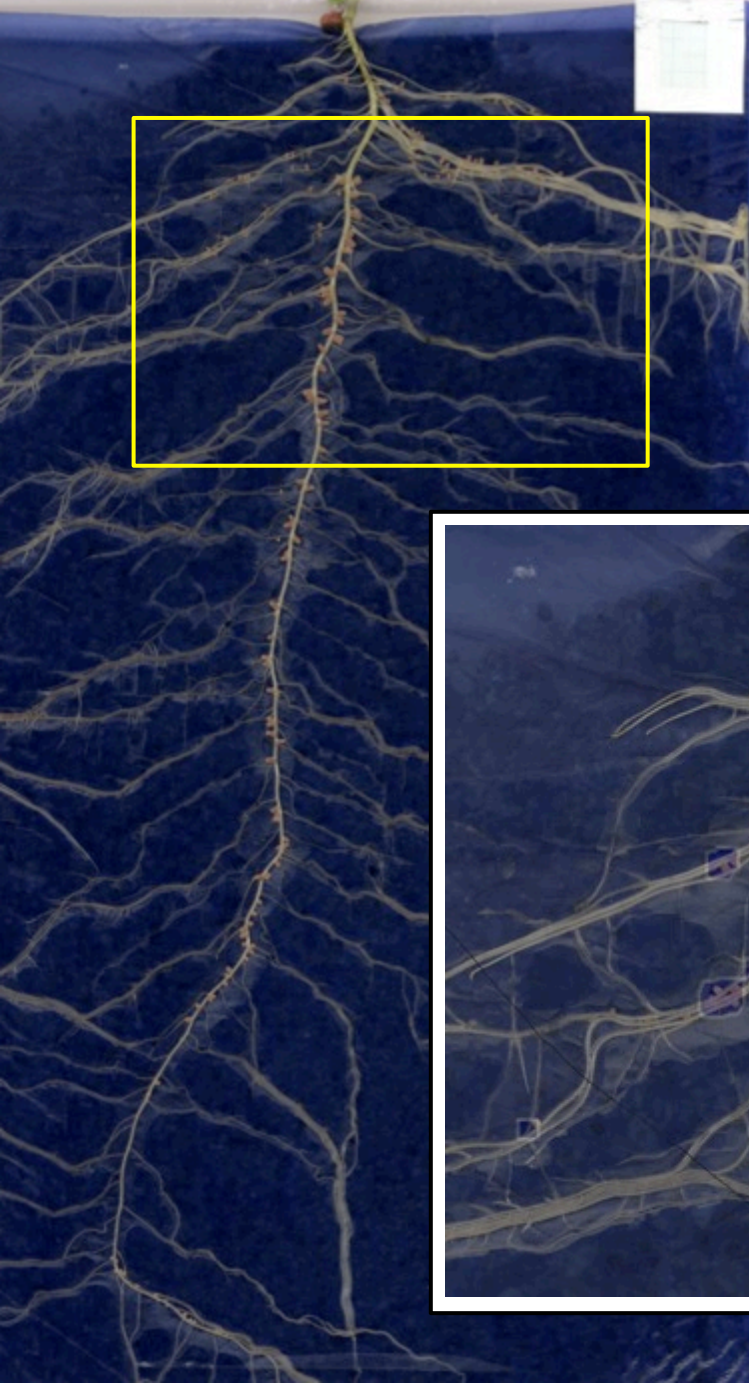
Nodules automatically detected



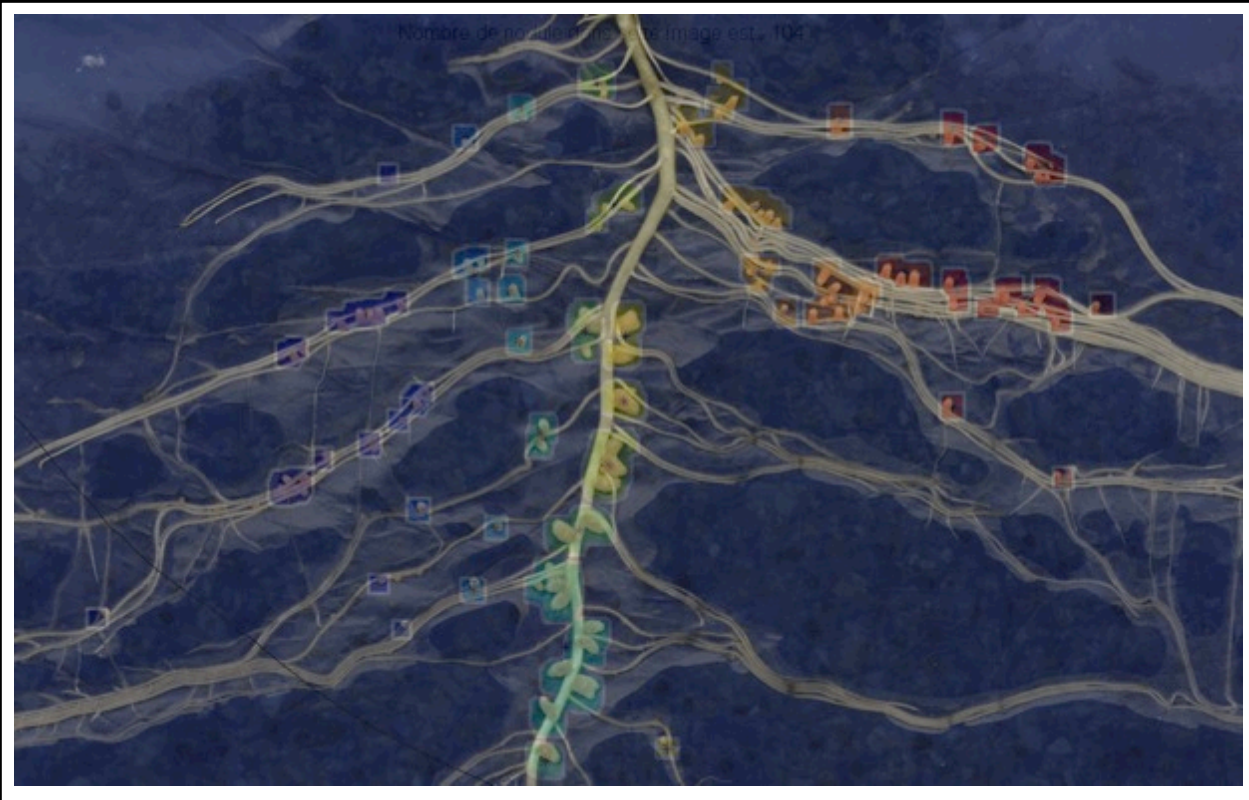
Context	Approach	Phenotypic tools	Examples	Models	Conclusion
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Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking



Original image + superposed nodules

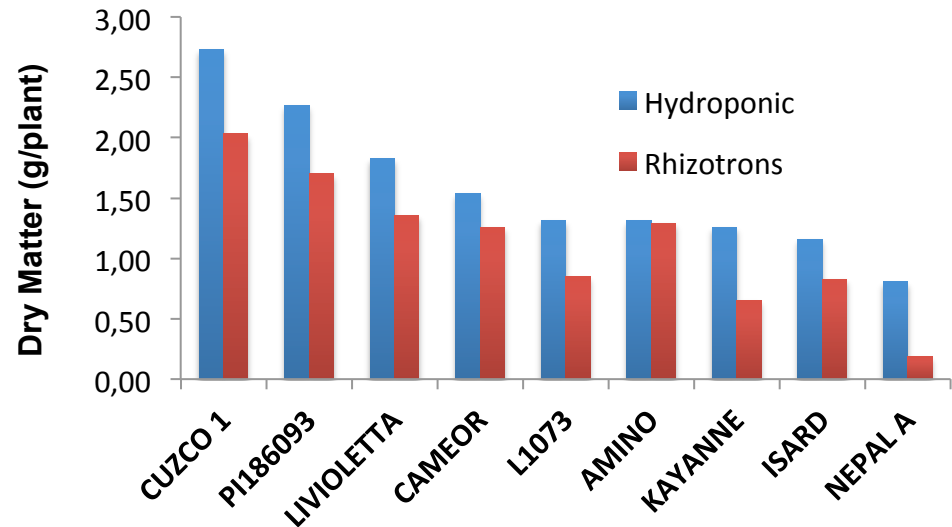
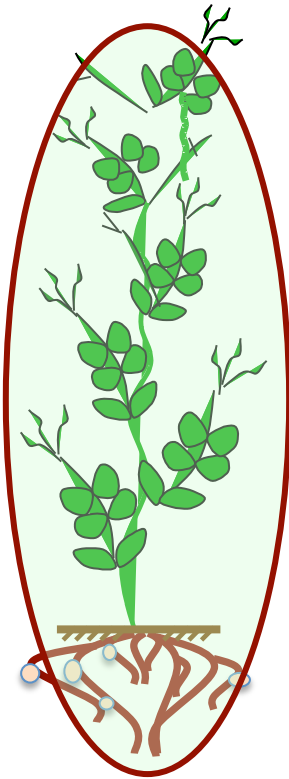


Grapevine
Legumes

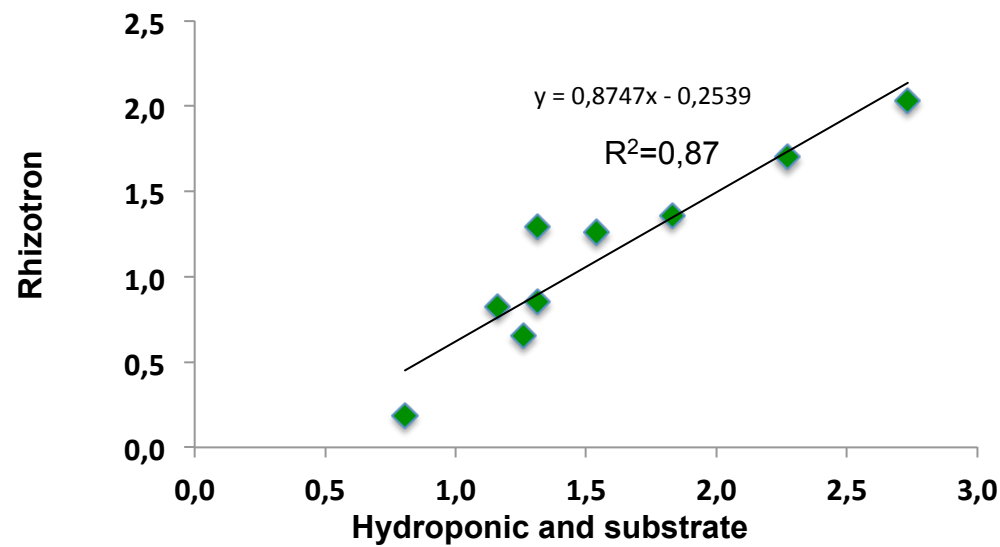
Objectives
Genetic diversity
Identify a strategy
Genotype ranking

Ranking pea genotypes: Pea core collection
Hydroponic versus rhizotron

Plant biomass



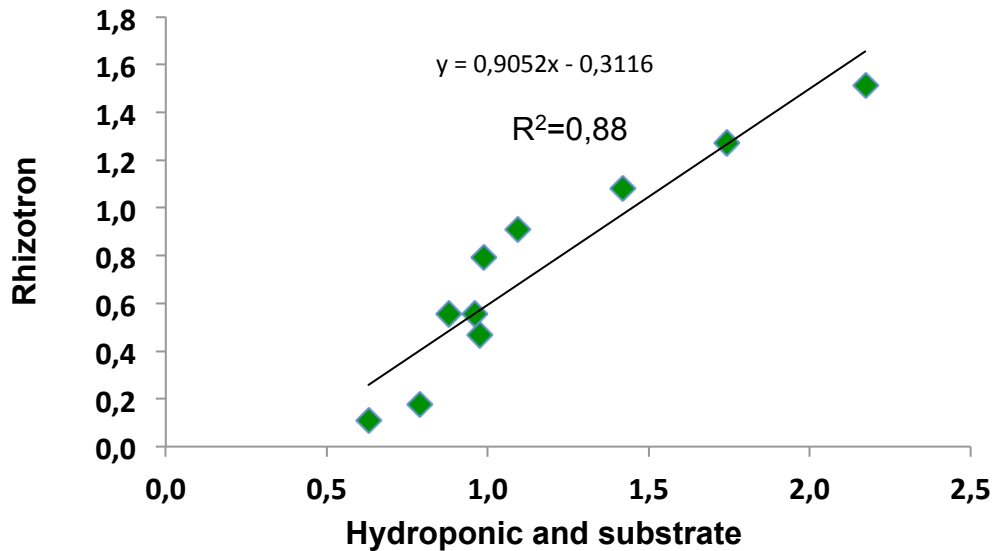
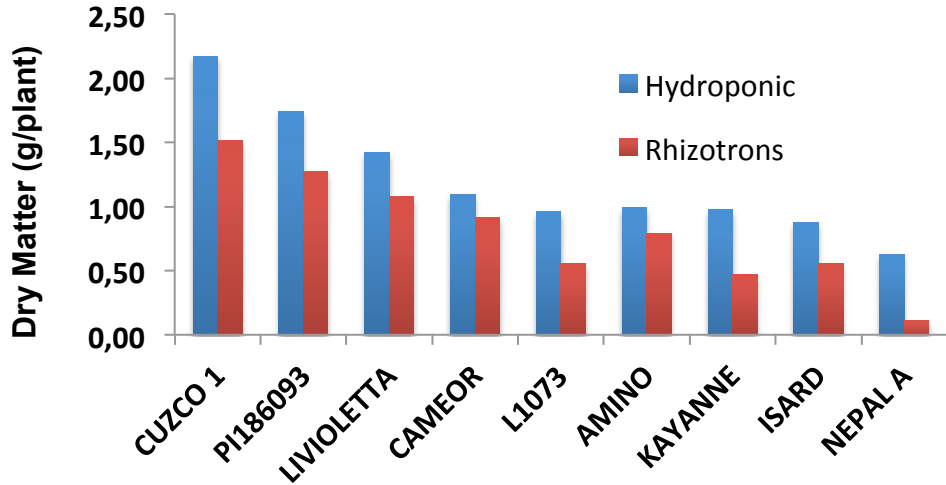
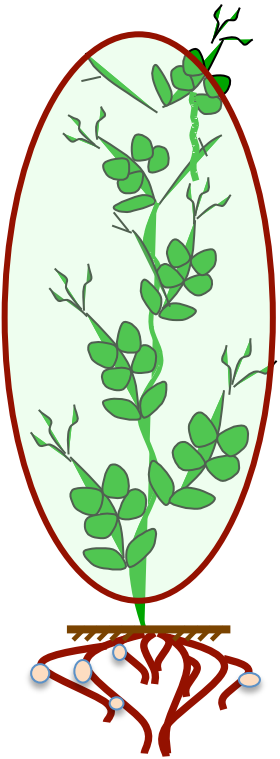
Plant biomass decreases within rhizotrons



Genotype ranking does not vary

Ranking pea genotypes: Pea core collection
Hydroponic versus rhizotron

Shoot biomass

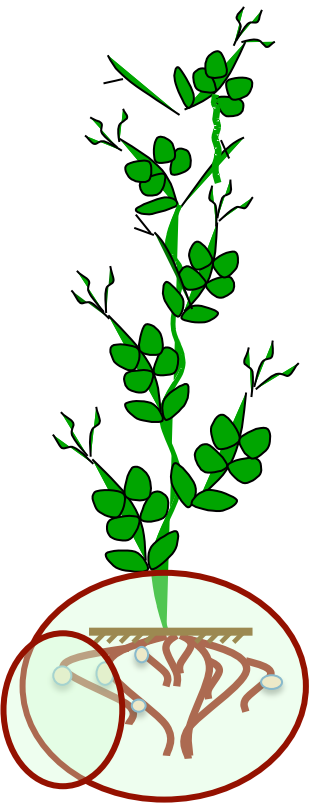


Genotype ranking does not vary

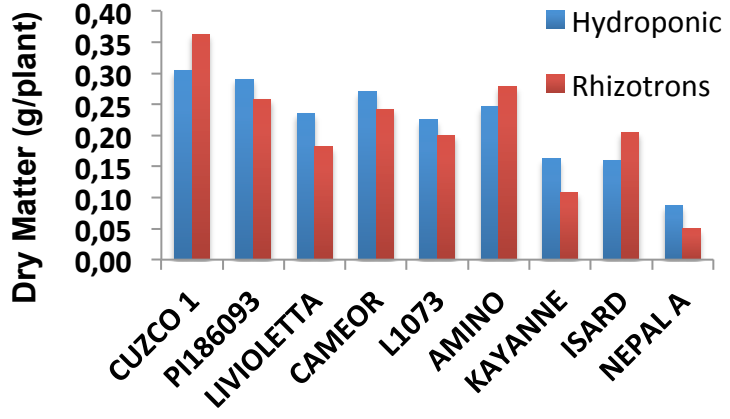
Grapevine
Legumes

Objectives
Genetic diversity
Identify a strategy
Genotype ranking

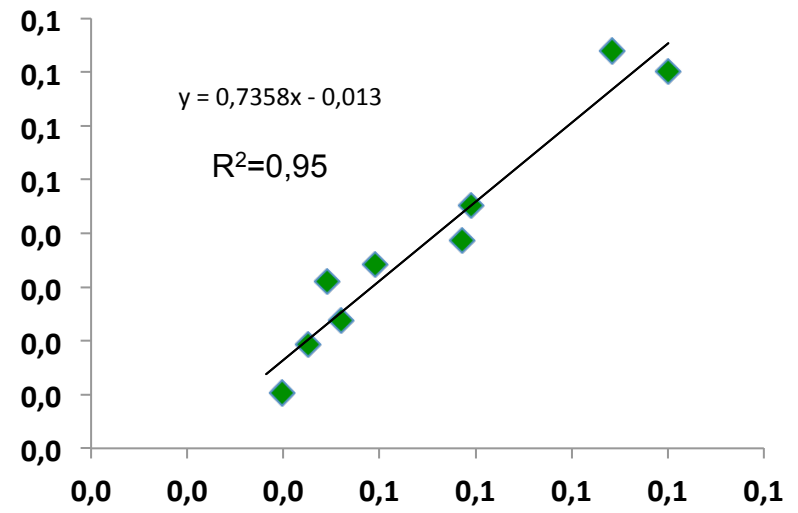
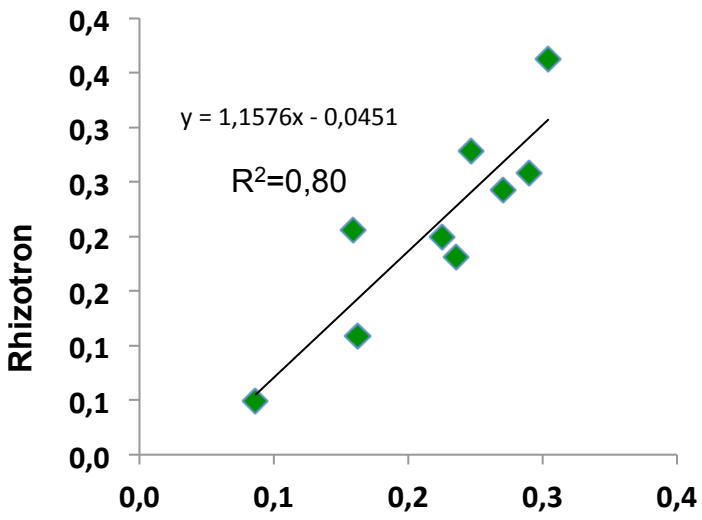
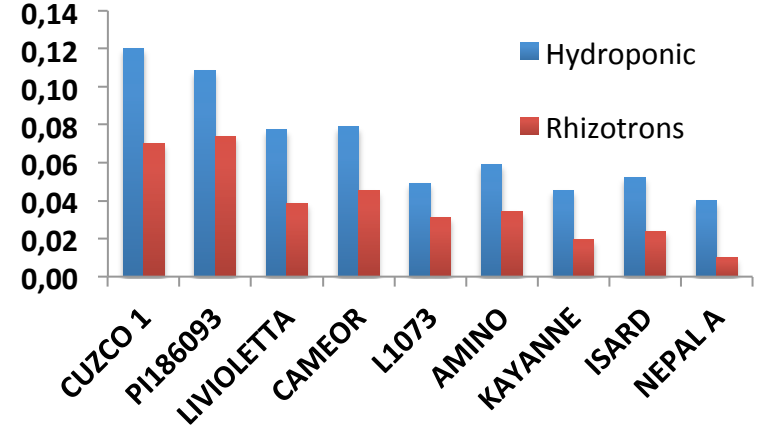
Ranking pea genotypes: Pea core collection



Root biomass



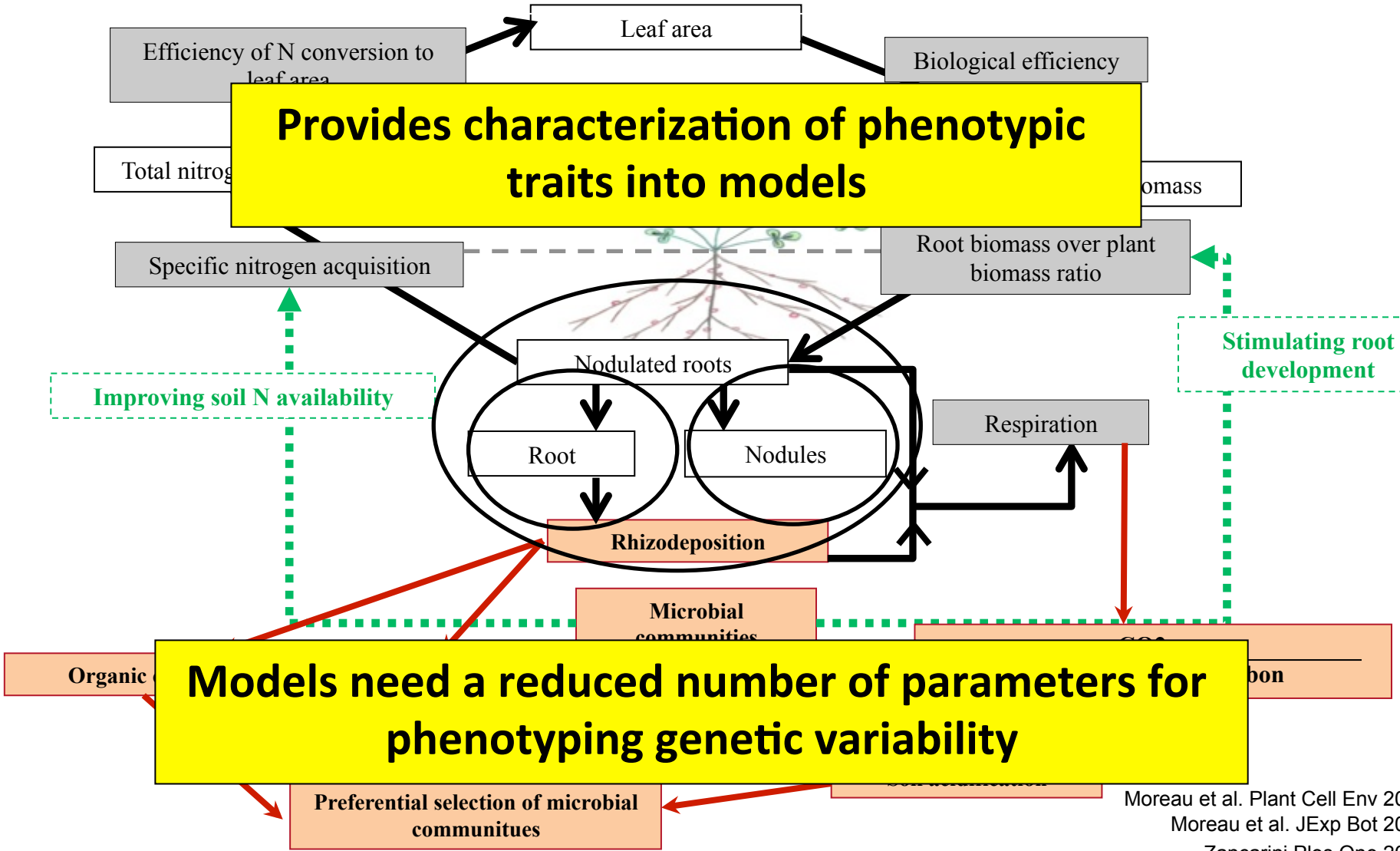
Nodule biomass



Hydroponic and substrate

Integrative Model: *Medicago*

Decomposing integrative variables in physiological processes



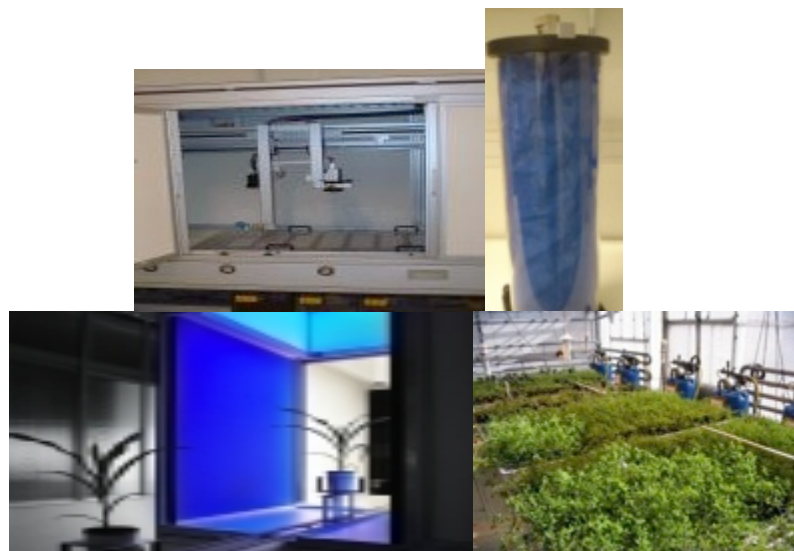
Food for thoughts...

Combine approaches

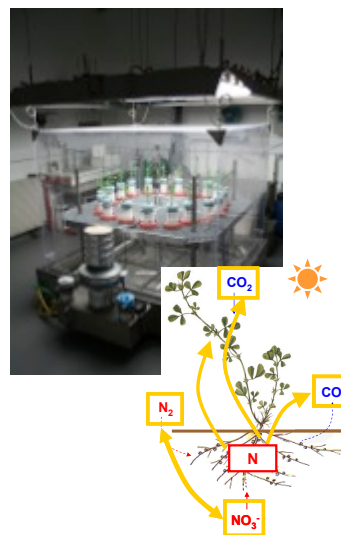
Phenotyping Approach



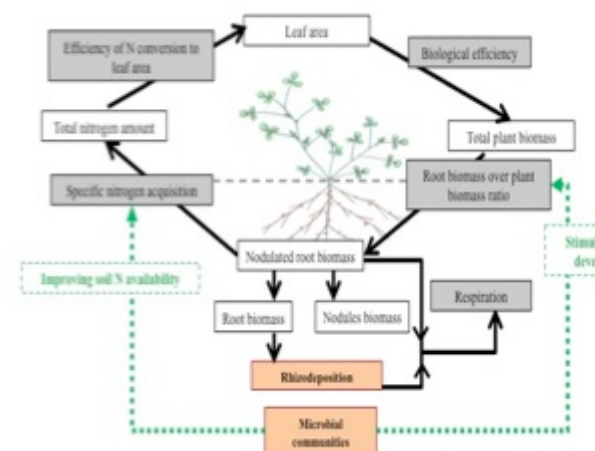
Analytical approach



Identifying differences among genotypes



Modelisation



Interpreting the detected difference

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



Starring...

Christian JEUDY



Céline BERNARD



Jean-Claude SIMON



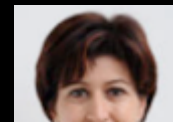
Frédéric COINTAULT



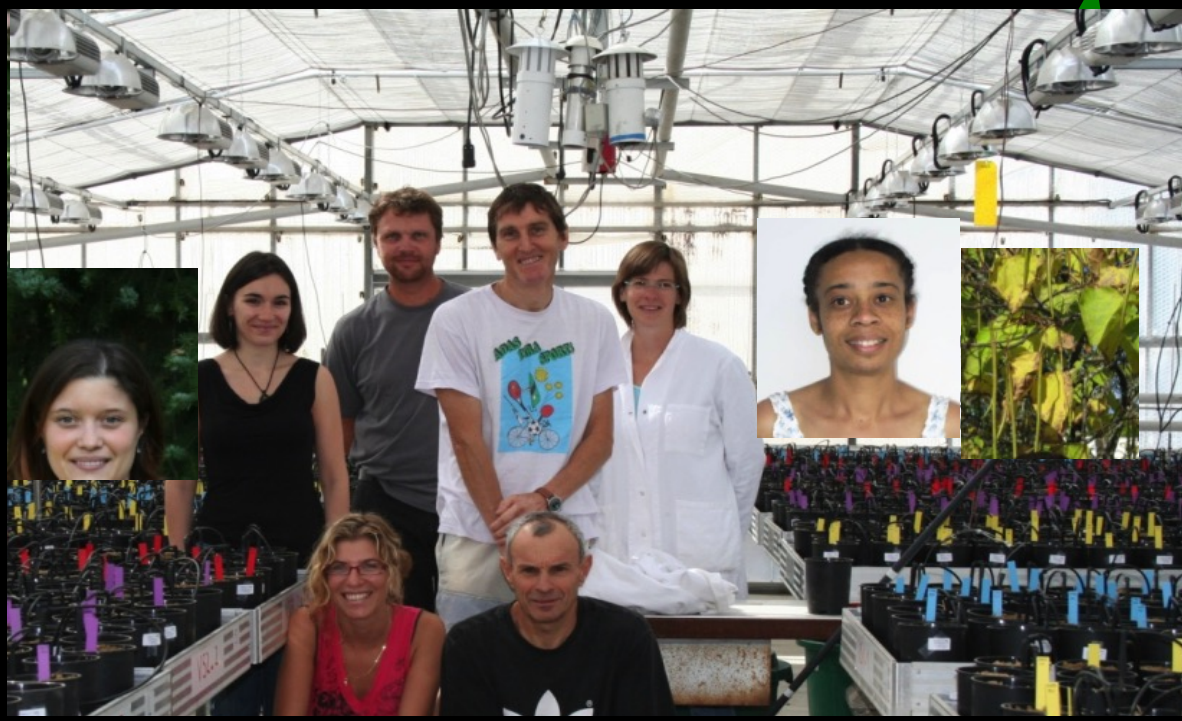
Simeng HAN



Marielle ADRIAN



and also ...



Ecophysiology team



Medicago truncatula team



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

Fluxomic
PPHD
Rhizotrons
Rhizobox

