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..

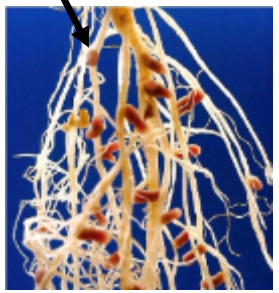
..“better” yield, environmental « effici

Understand plant response to environment identify genes.

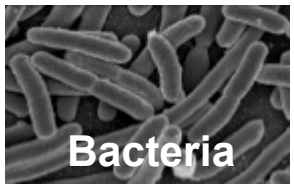
Plant architecture, flowering, senescence

Pods and seeds

Roots and i th micro organisms



Valoris beneficial biological interactions



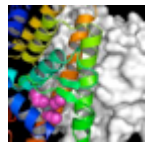
Major bottleneck: high-throughput, non-invasive and multiscale phenotyping

Tools and methods

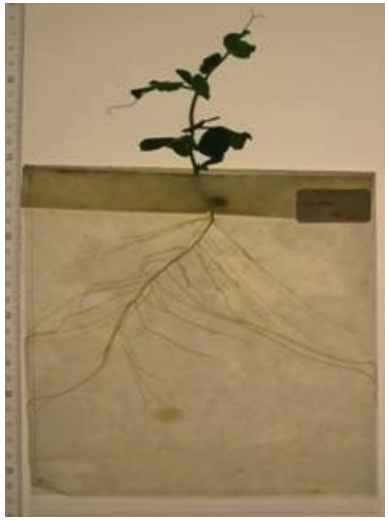
Genetic variability

CONCEPTION of PLANT IDEOTYPES

Characterizing mechanisms and molecular basis



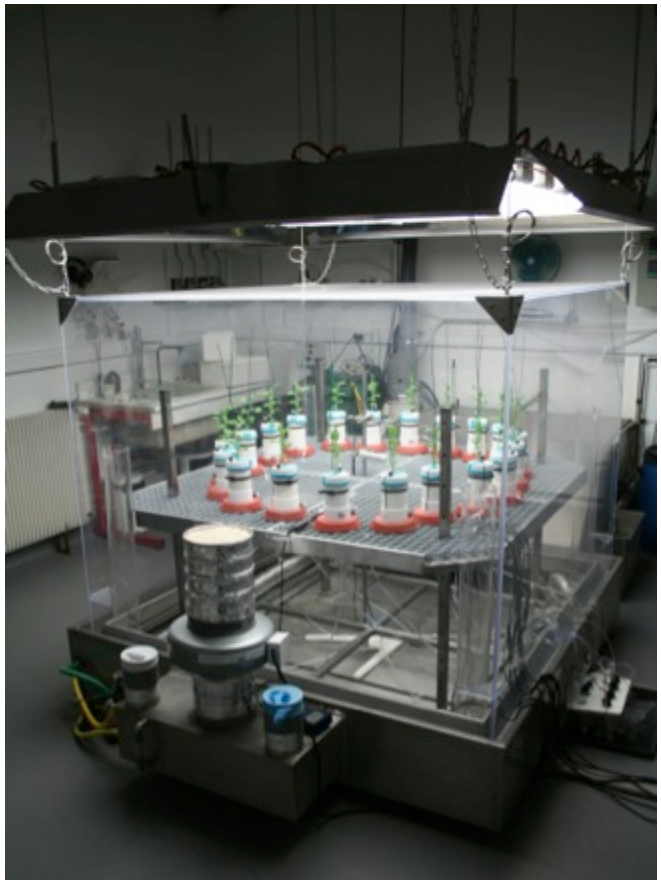
Characterizing root development



Growth pouches, hydroponics

N_2 adapted Isotopic split root

C, N, S flux measurement



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

High Throughput Phenotyping Platform

Building,
greenhouses (240+110m^2),
climatic chambers (80m^2)
Phenotyping facilities Lemnatec©

- Fluxomic
- PPHD
- Rhizotrons
- Rhizobox

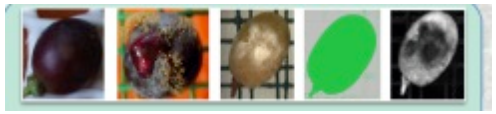
- Facilities
- Shoot roots etc…
- Spielberg's masterpiece

Plant from the agrosystems

Aerial architecture

20 units/h

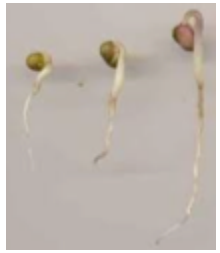
Organs (seeds…)



Small plants

6 units/h

Germination



2 units/h

Root system

120 units/h

VIS

NIR

100 units/h

Capacity ≈ 1800 plants

Very large capacity

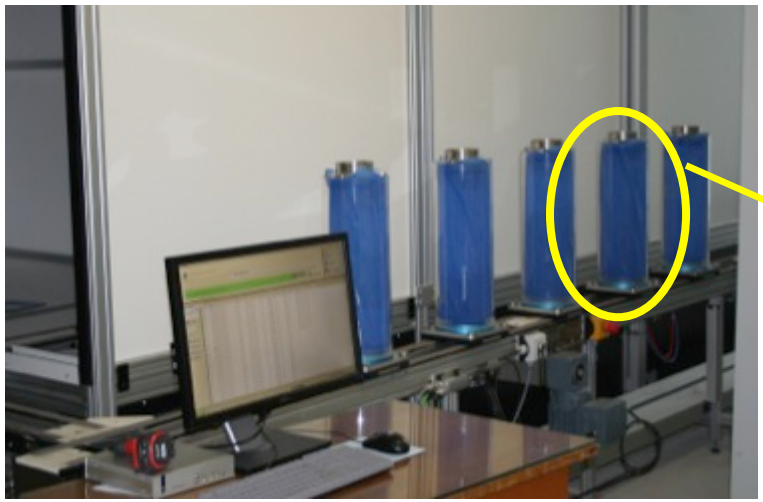
Phenotyping cabi… …ed with cameras and robots

- Fluxomic
- PPHD
- Rhizotrons
- Rhizobox

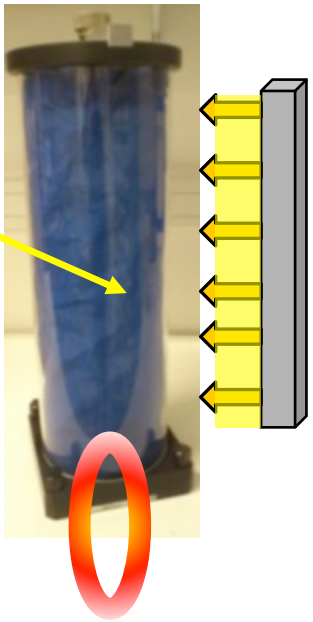
- Facilities
- Shoot roots etc…
- Spielberg's masterpiece

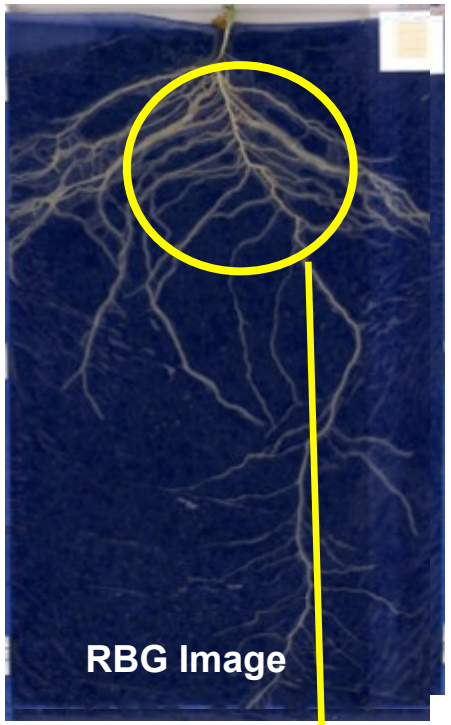
- Fluxomic
- PPHD
- **Rhizotrons**
- Rhizobox

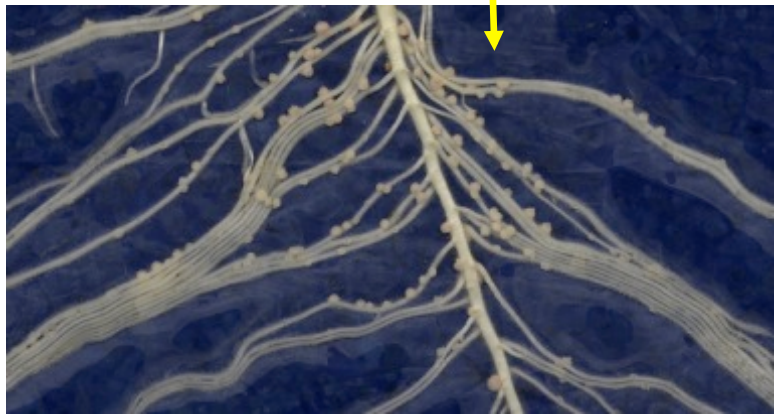
Rhizothrones (EU Patent INRA-Inoviaflow, 1300 units planed)



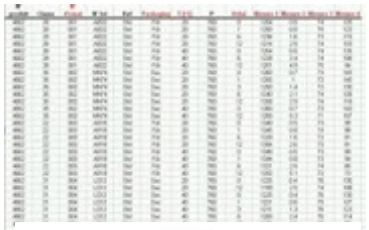
Rotating device

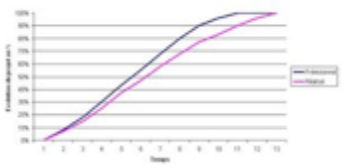






High resolution





Traits :

projected **root** area

projected nodule area

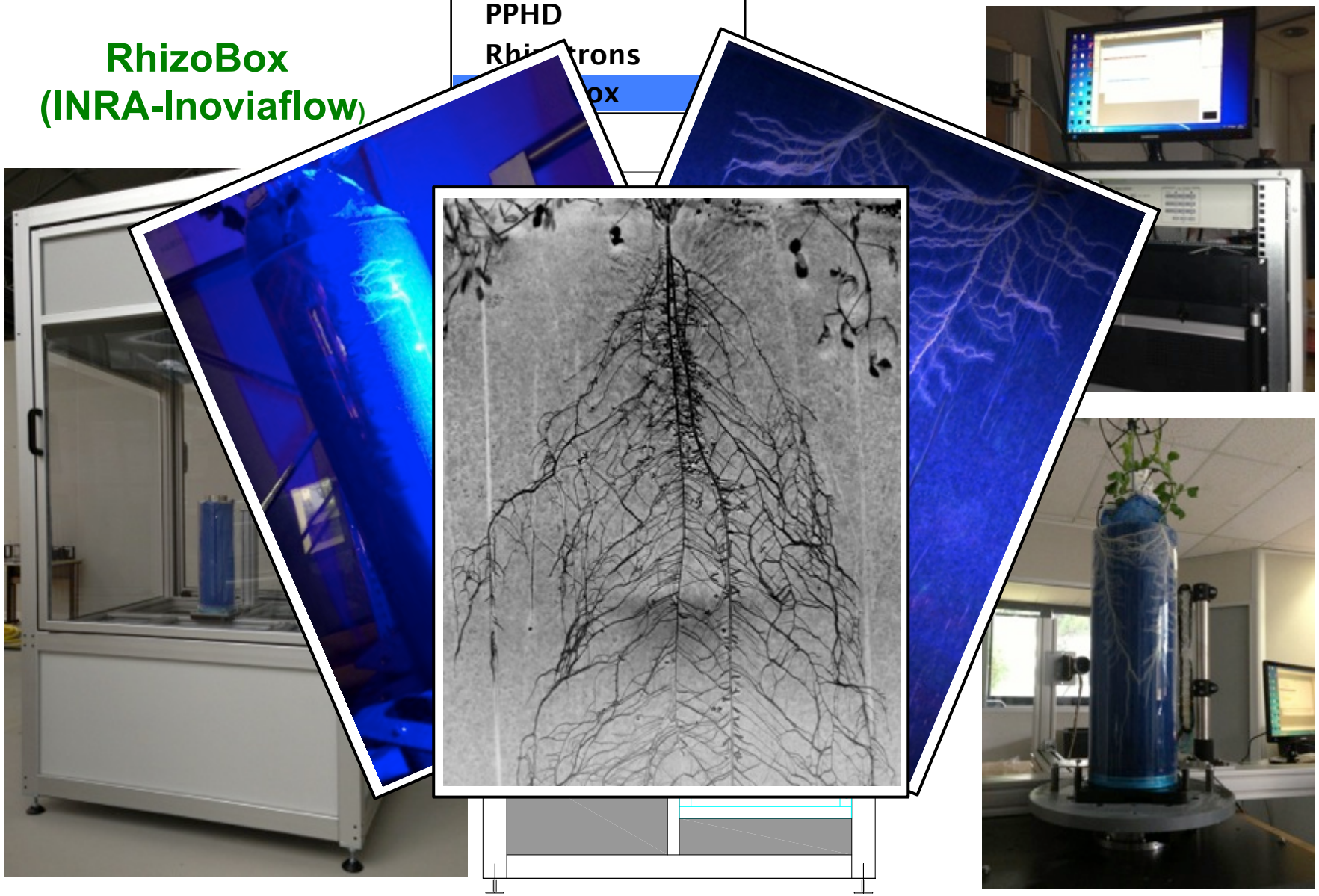
total nodule **number**

nodule size classification

total root length....

- Fluxomic
- PPHD
- Rhi…trons
- …ox

RhizoBox (INRA-Inoviaflow)



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

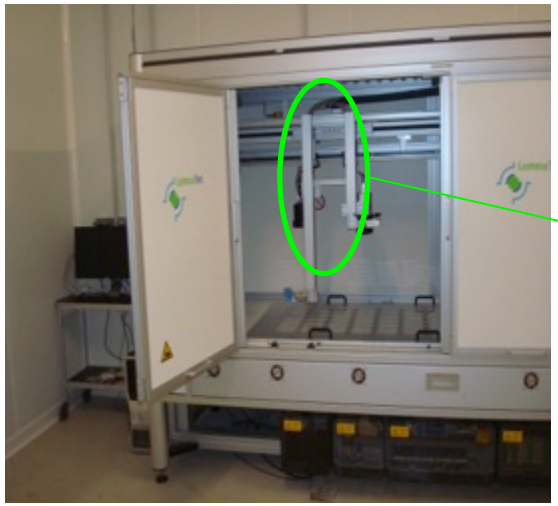
Detecting desease in viticulture

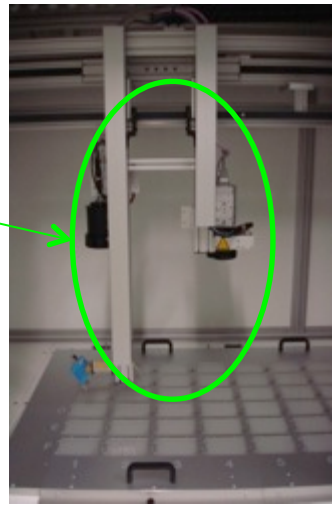
Major problems in viticulture ➔ cryptogamic diseases (oïdium, grey mould) of grapevine

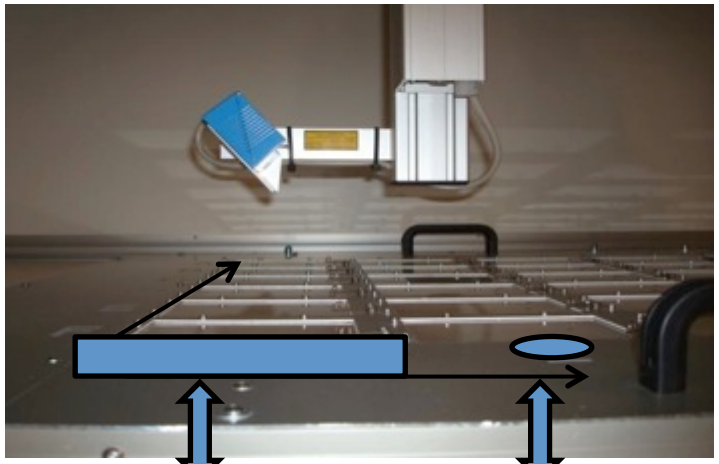
Approaches:

1) 🡻 number of fongicides treatments and 🡻 applied quantity (decision tools) + images (thermography IR…)

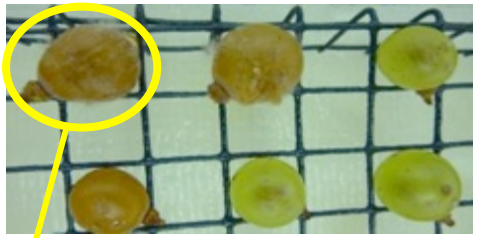
2) alternative strategies to fongicides







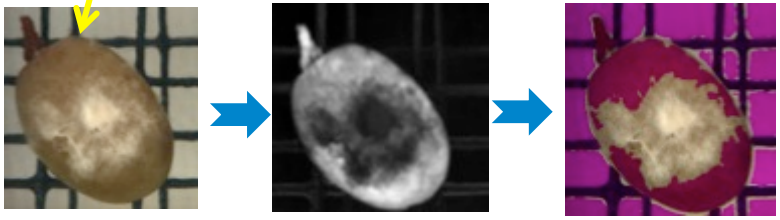
HTS (small biological unit phenotyping)



Colour/texture hybrid spaces

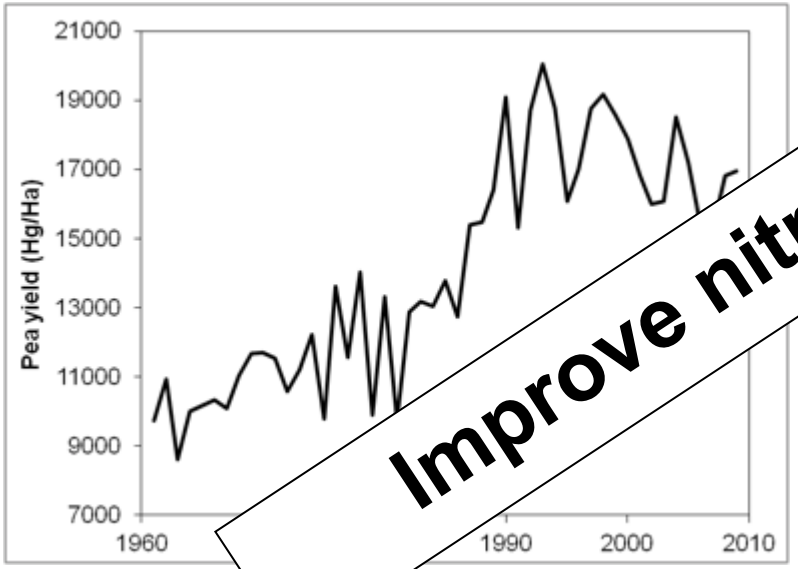
-RGB images in colorimetric spaces that integrates texture,

- 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

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



But…

g yields

Improve nitrogen nutrition

Sensitivity of symbiotic N_2 fixation to environmental conditions

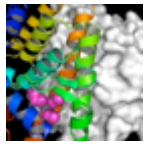
Regulation of symbiotic N_2 fixation and NO_3 assimilation: determinism, plasticity constituents, optimal root / nodules for maximizing yield?

Tools and methods

Genetic variability

CONCEPTION of PLANT IDEOTYPES

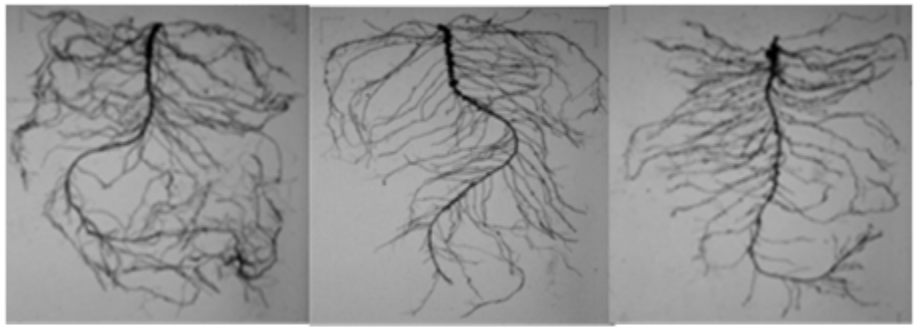
Characterizing mechanisms and molecular basis



Natural genetic variability

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

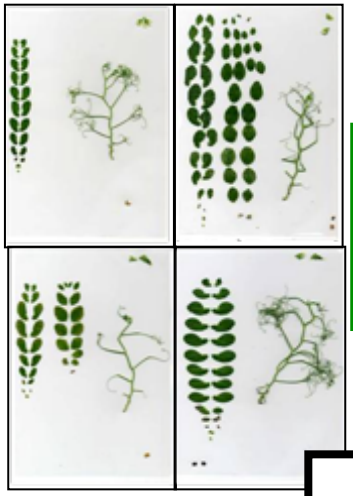
Genetic diversity on root architecture



Bourion et al. Annals Bot. 2007

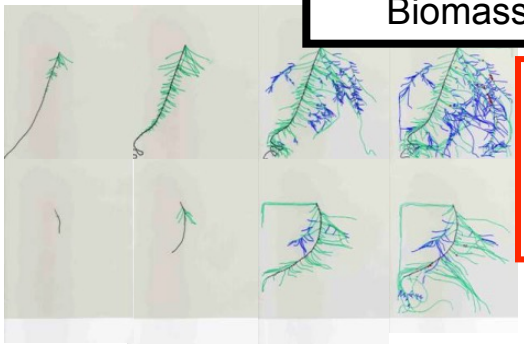
Recombinant inbred lines (1400 RILs)

STRUCTURE



Aerial part :
Height
Biomass
Leaf surface area

Roots:
Number
Length
Biomass



Nodules:
Number
Surface
Biomass

FONCTION

C use efficiency

N uptake efficiency

Bourion et al. TAG 2010

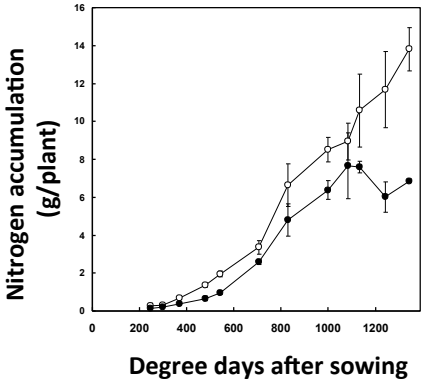
Induced genetic variability

Identify/characterize genes involved in nodulation control or root architecture

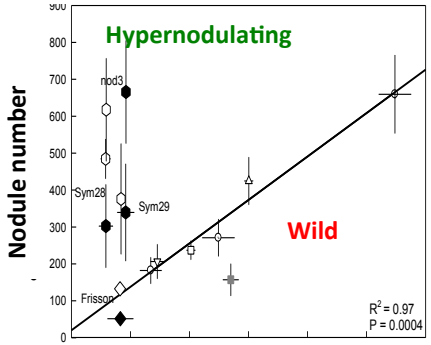
Nodule development

Duc et al. 1998

Cazenave et al. Plant Soil 2013



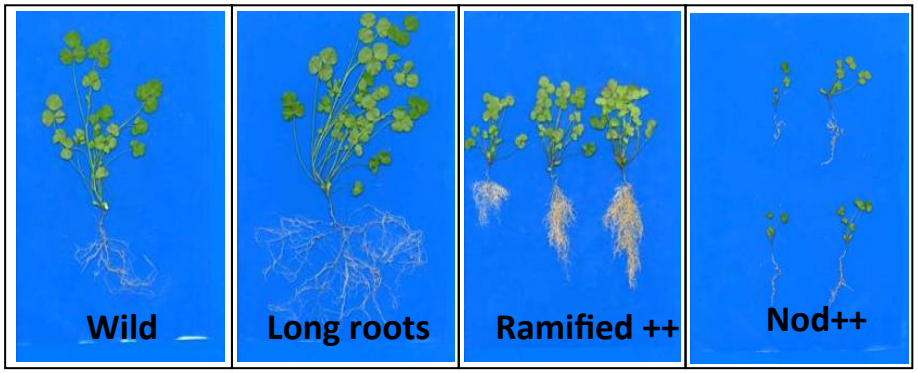
Salon et al. Agr 2001



Voisin et al. Plant Soil 2010

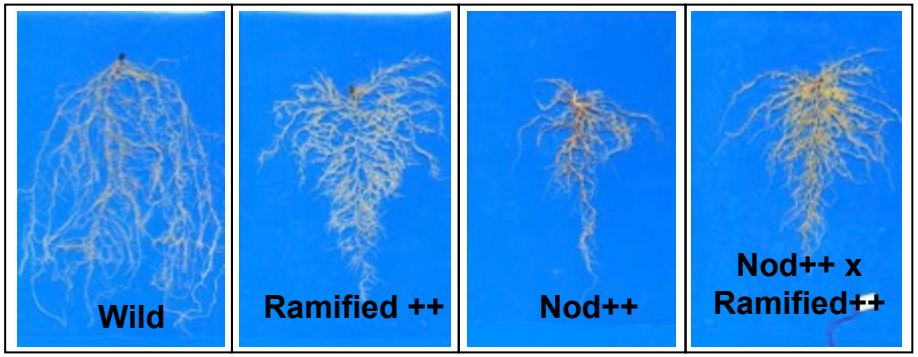
Root architecture

***Medicago truncatula*, induced by Tnt1**



Porceddu et al. BioMed 2008

Pea, induced by EMS

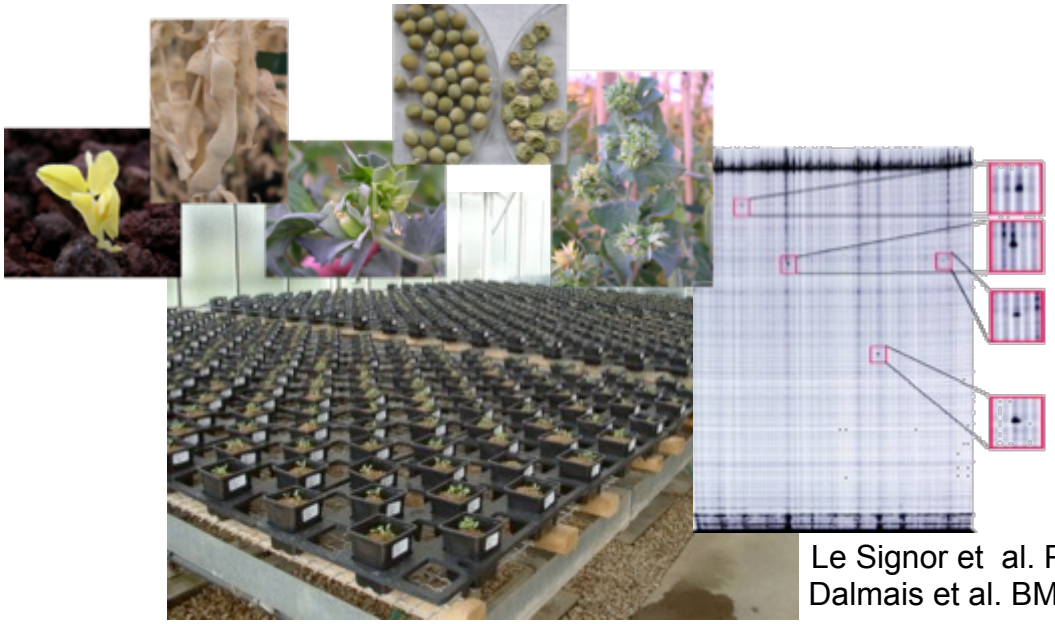


Coll . KK Sidorova

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

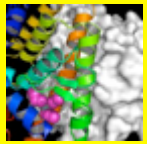
✓ **HTP TILLING platform: ABI 3730 (Contact: lesignor@dijon.inra.fr)**

Tools and methods

Genetic variability

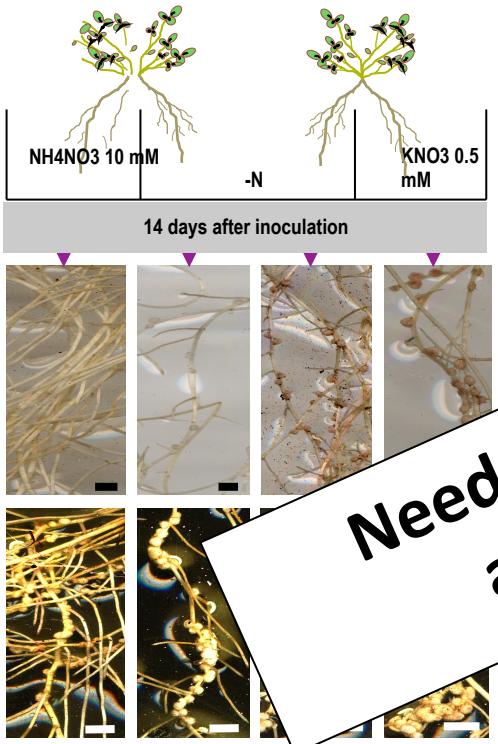
CONCEPTION of PLANT IDEOTYPES

Characterizing mechanisms and molecular basis

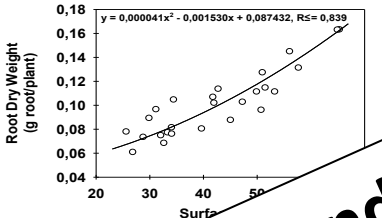


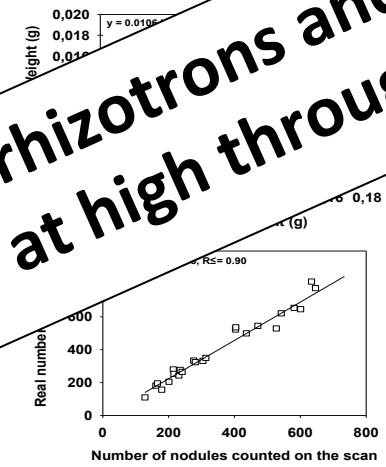
What is the strategy of legume plants faced to a N constraint ?

"Low" throughput characterization of nodulated roots



Split roots





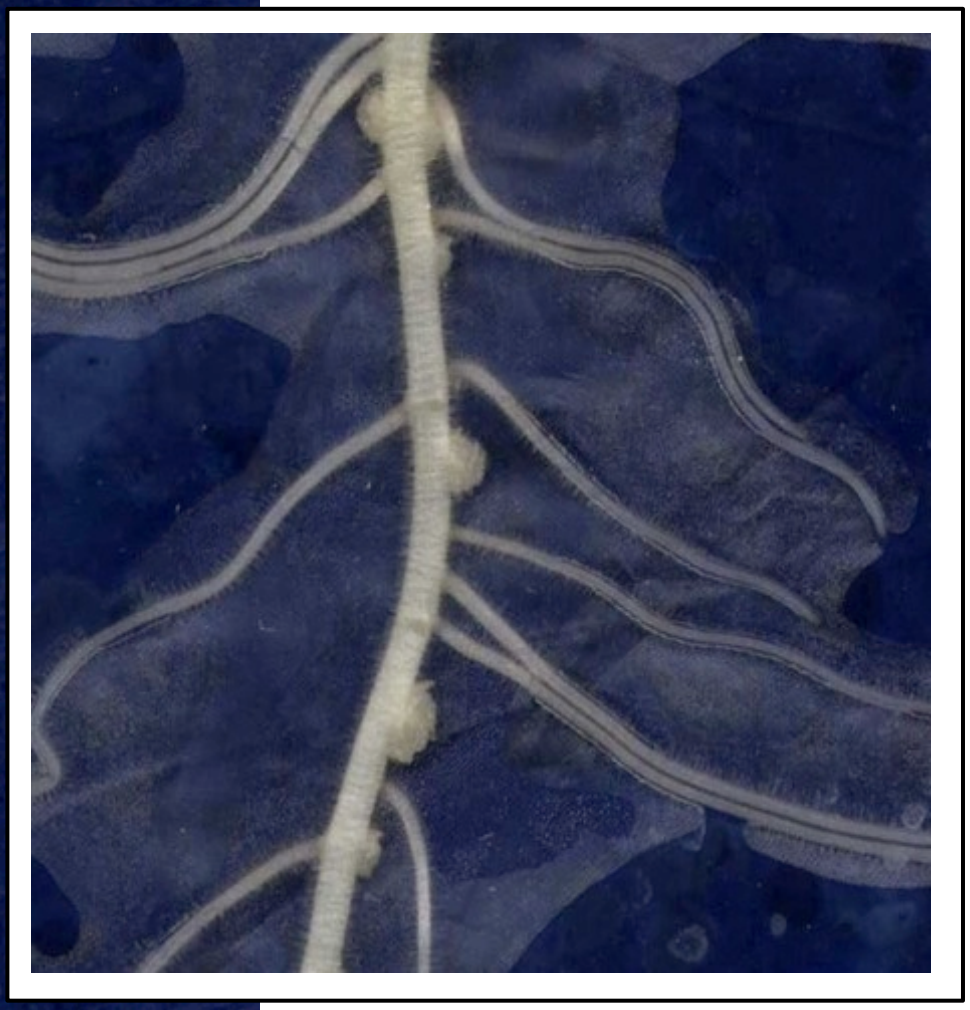
Nodules number and size, appearance

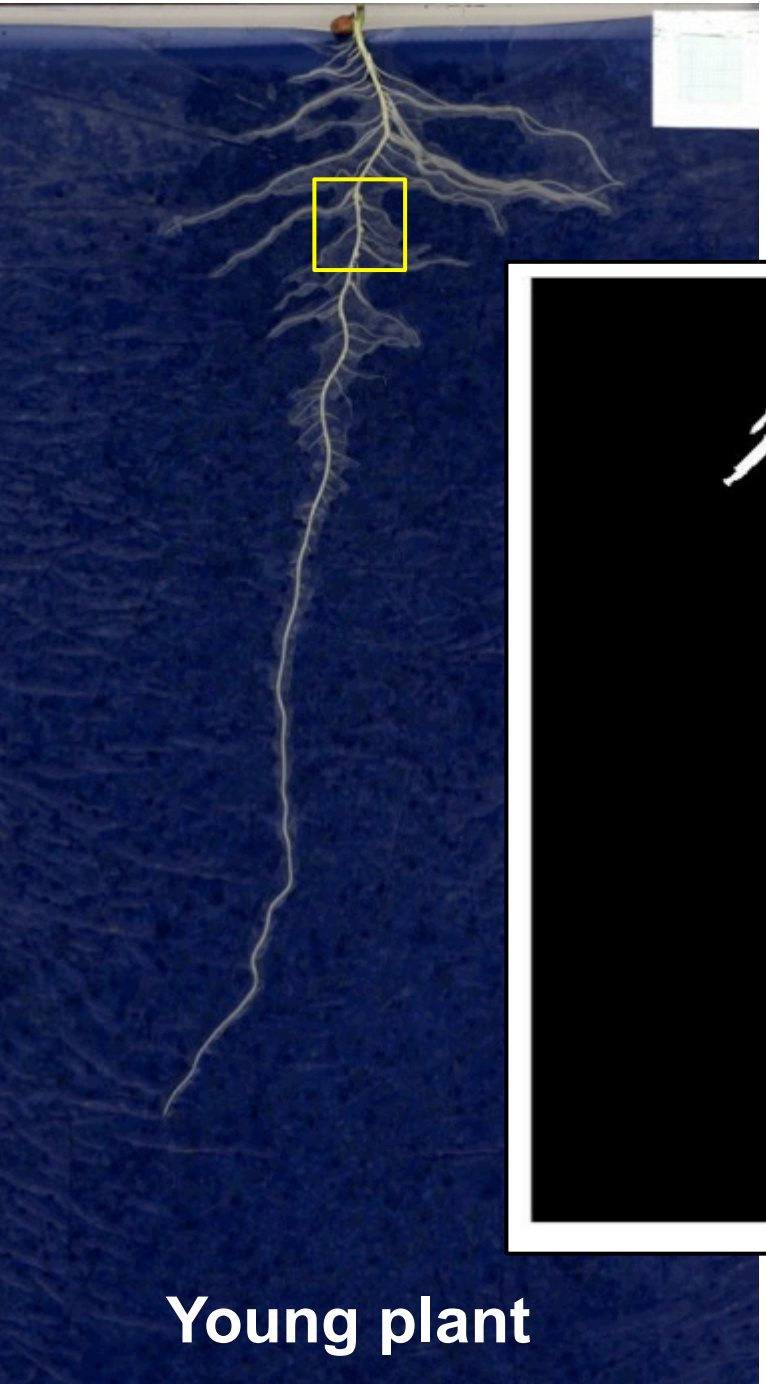
Need to use rhizotrons and image analysis at high throughput

Morphometry versus functional strategy identifcation

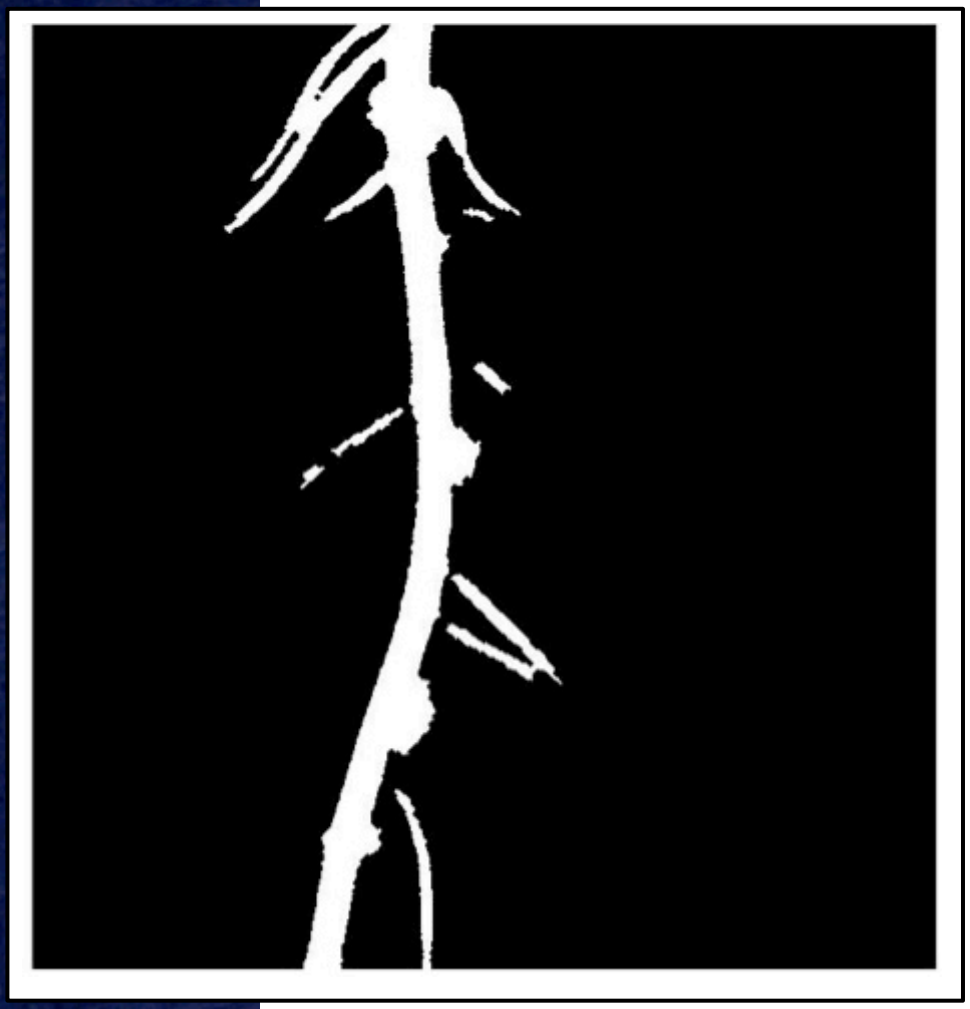
Ruffel et al. (2008), Plant Physiol. 146: 2020-2035.
Salon et al. (2009), CRAS, 332 :1022-1033.
Jeudy et al. (2010), New Phytol, 185:817-828.


Young plant

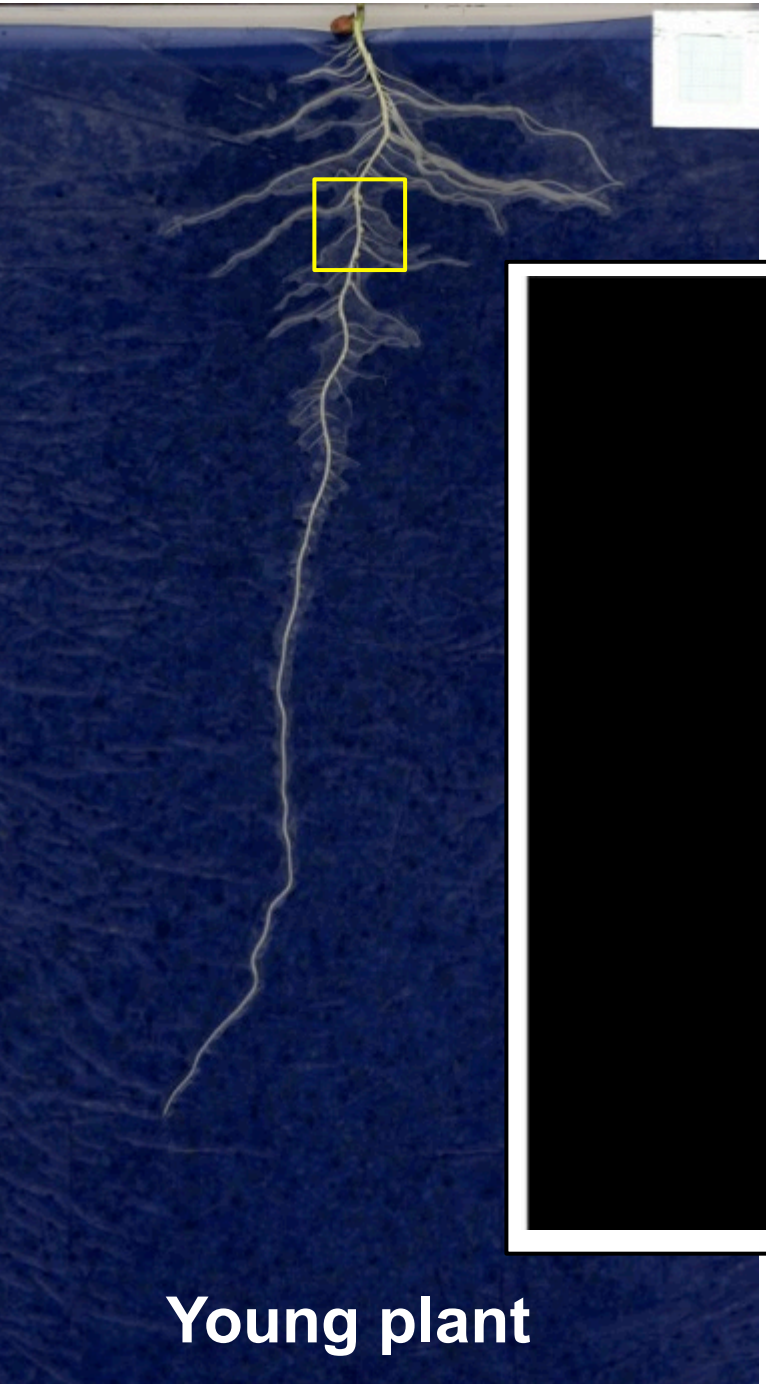


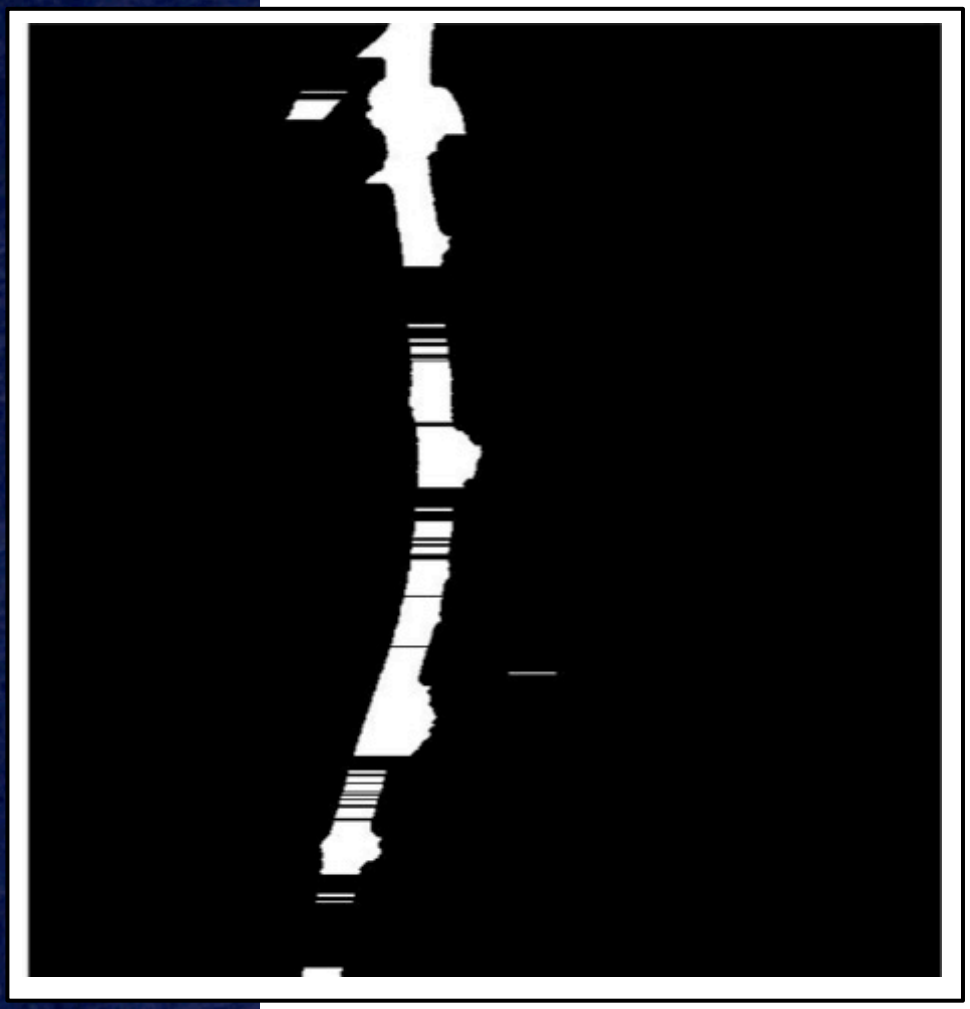


Young plant

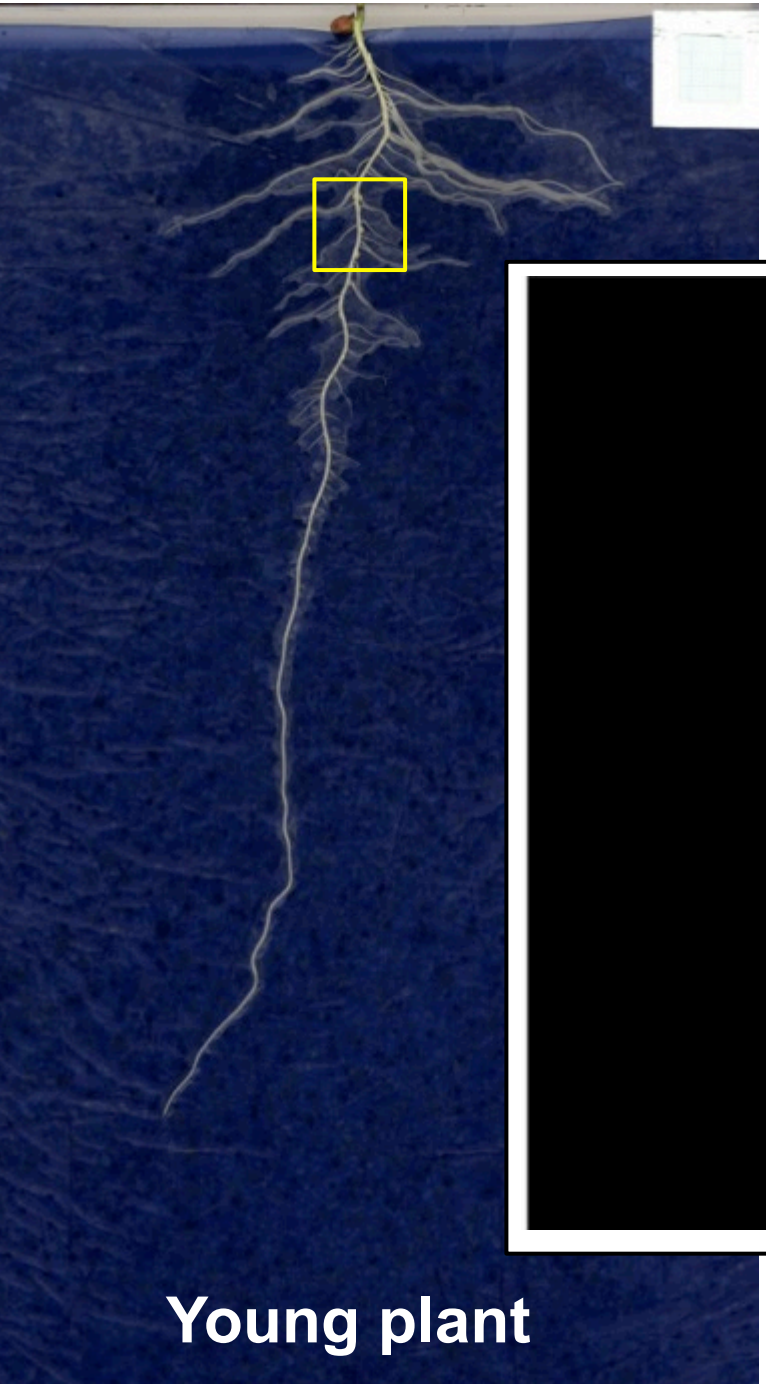


Binary image and threshold

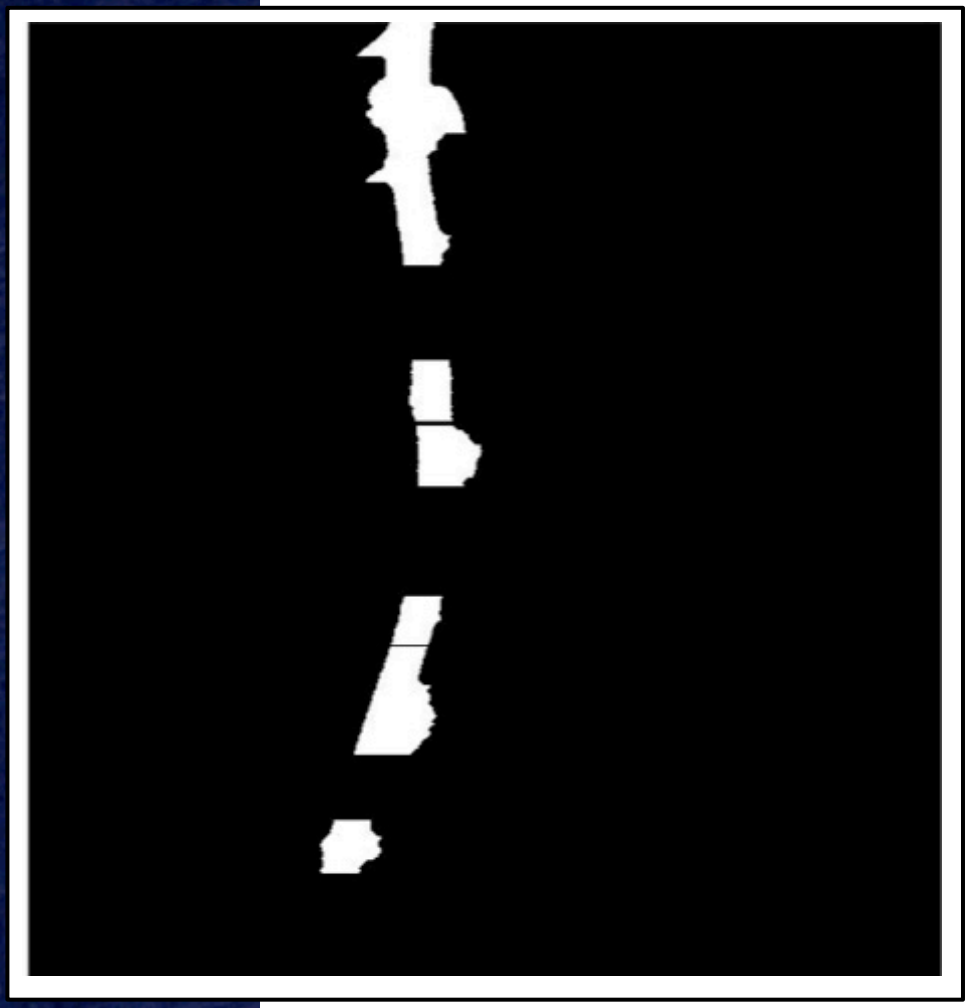




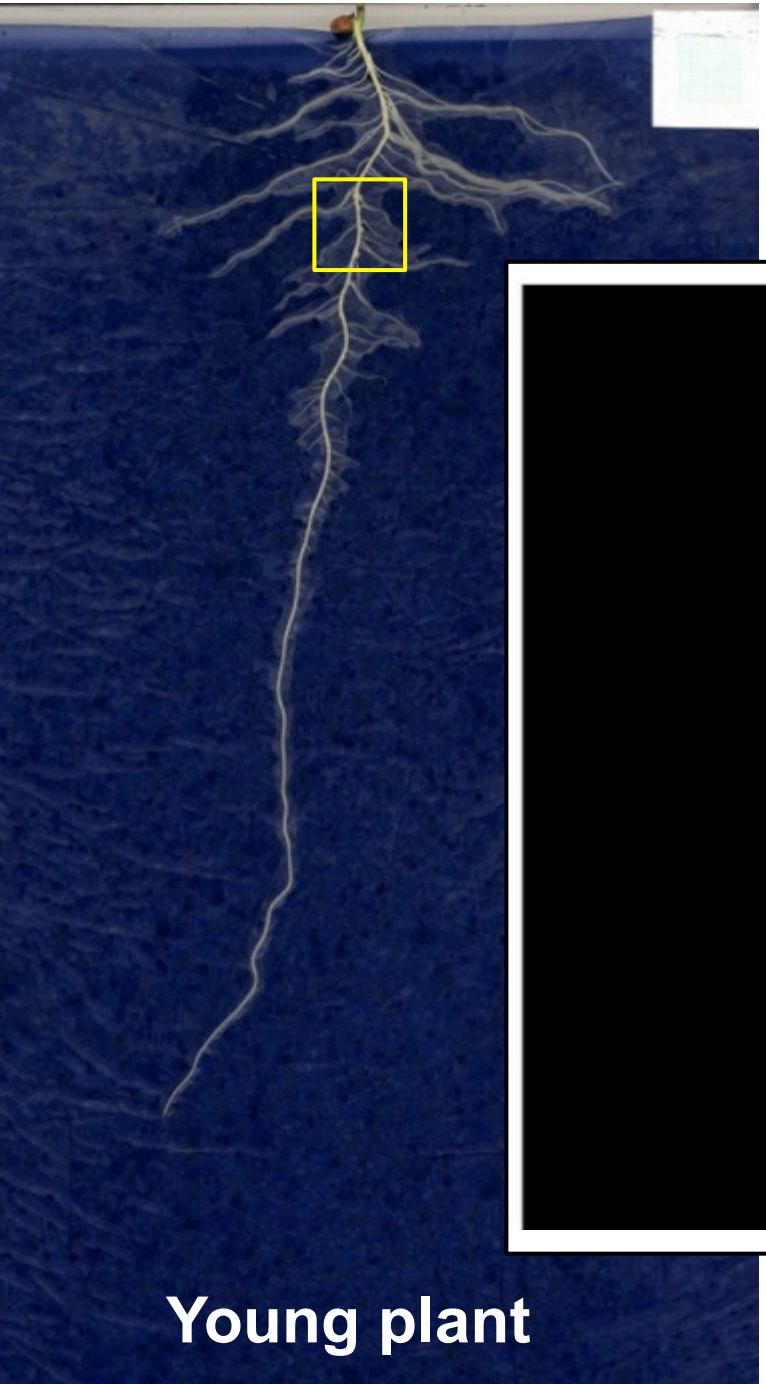
Only keep nodule shaped forms, get rid of unused areas



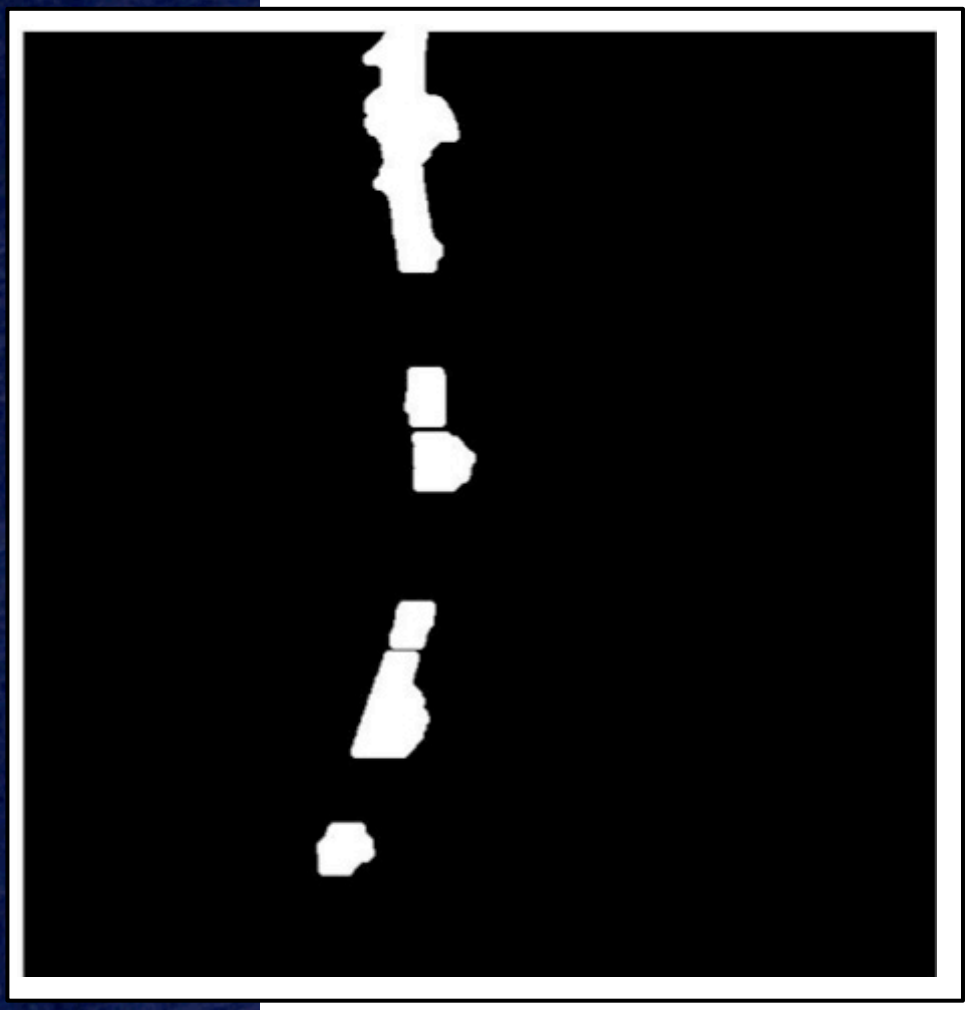
Young plant



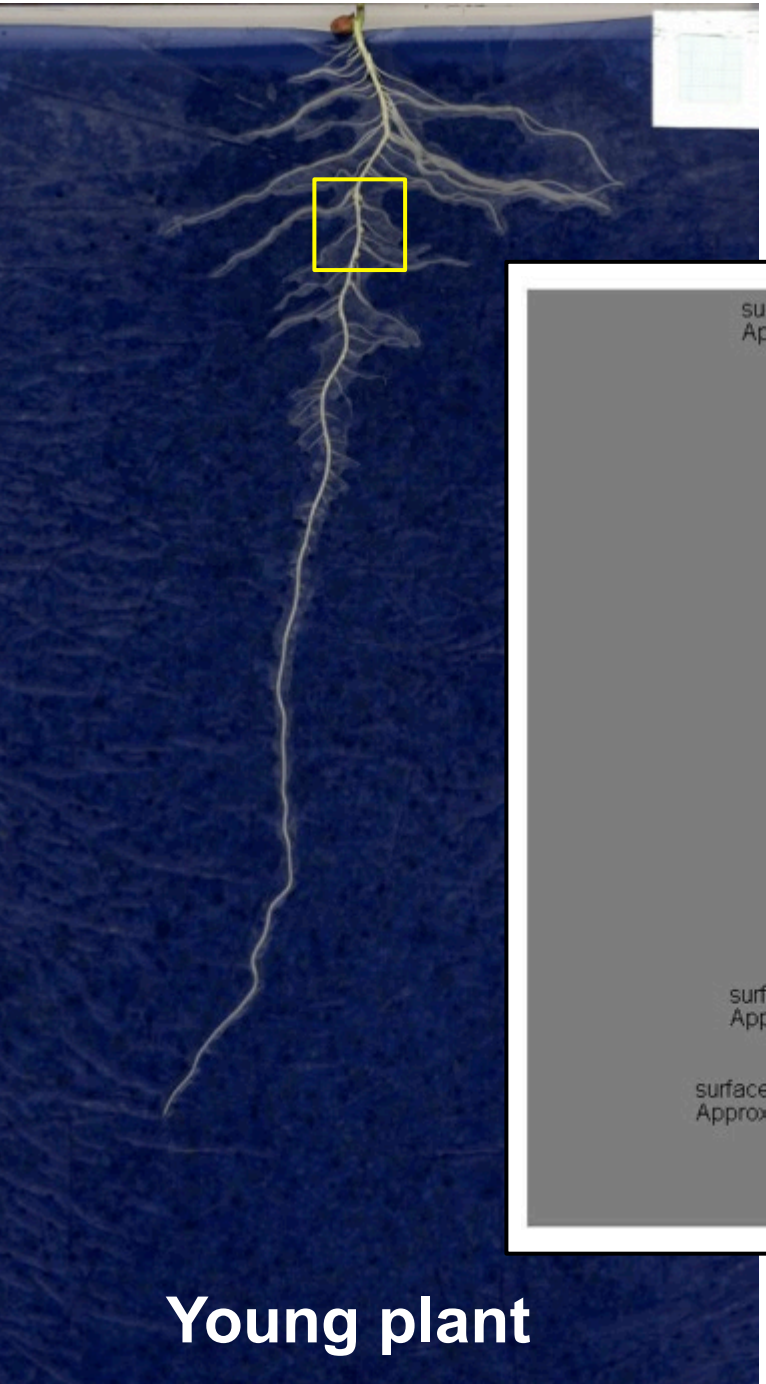
Get rid of unused areas (areaopen)

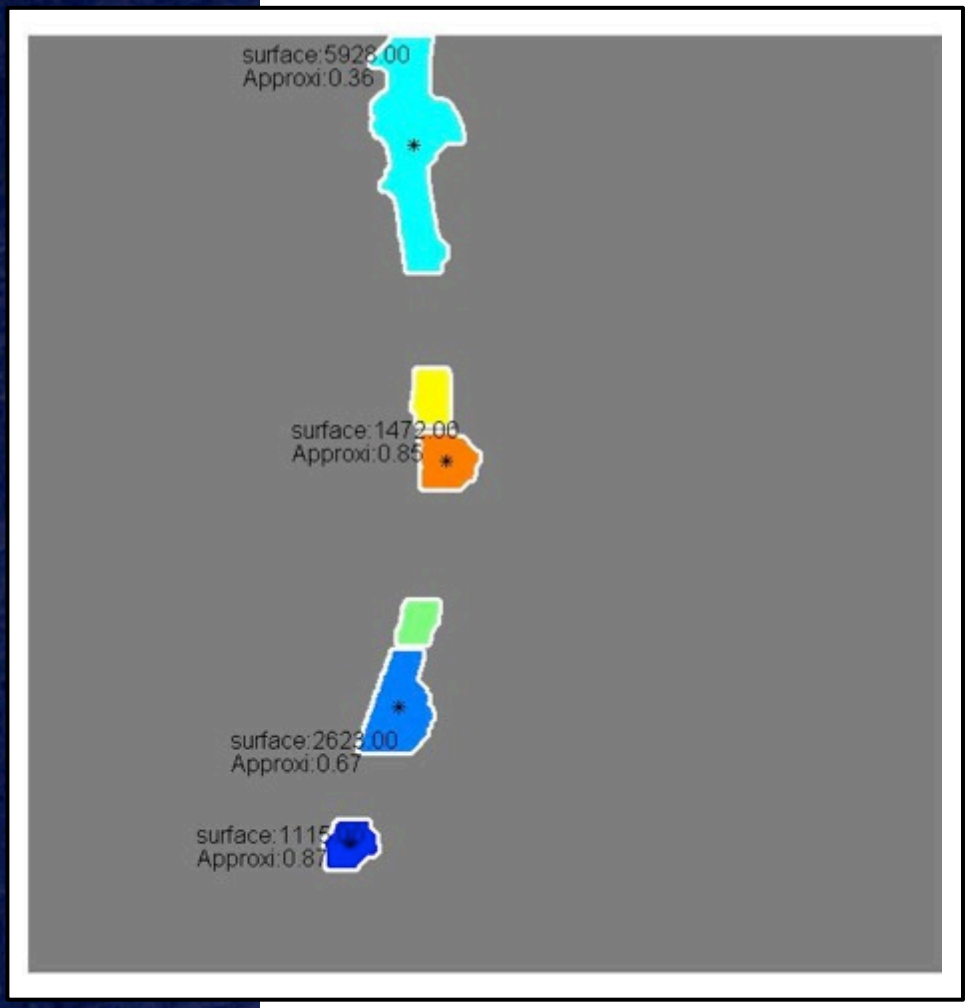


Young plant



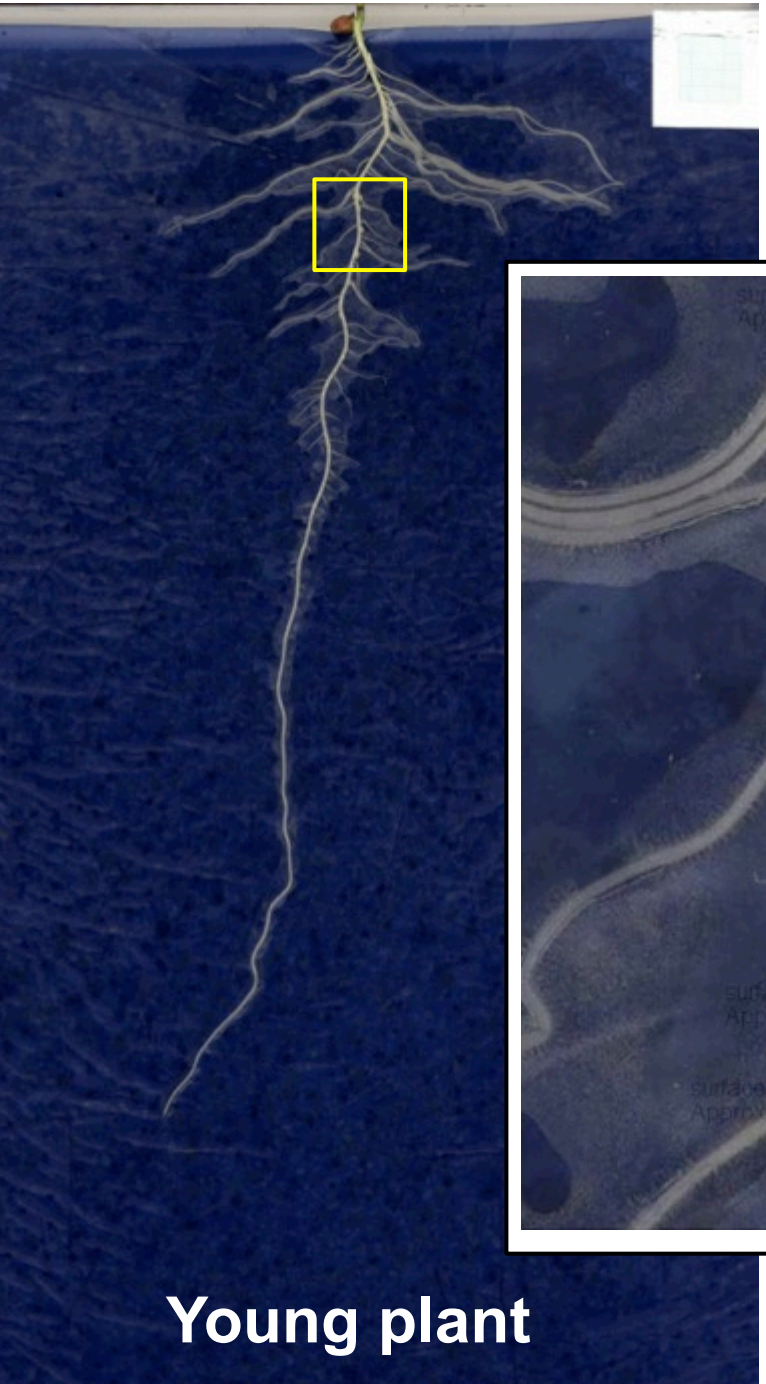
Smooth the image



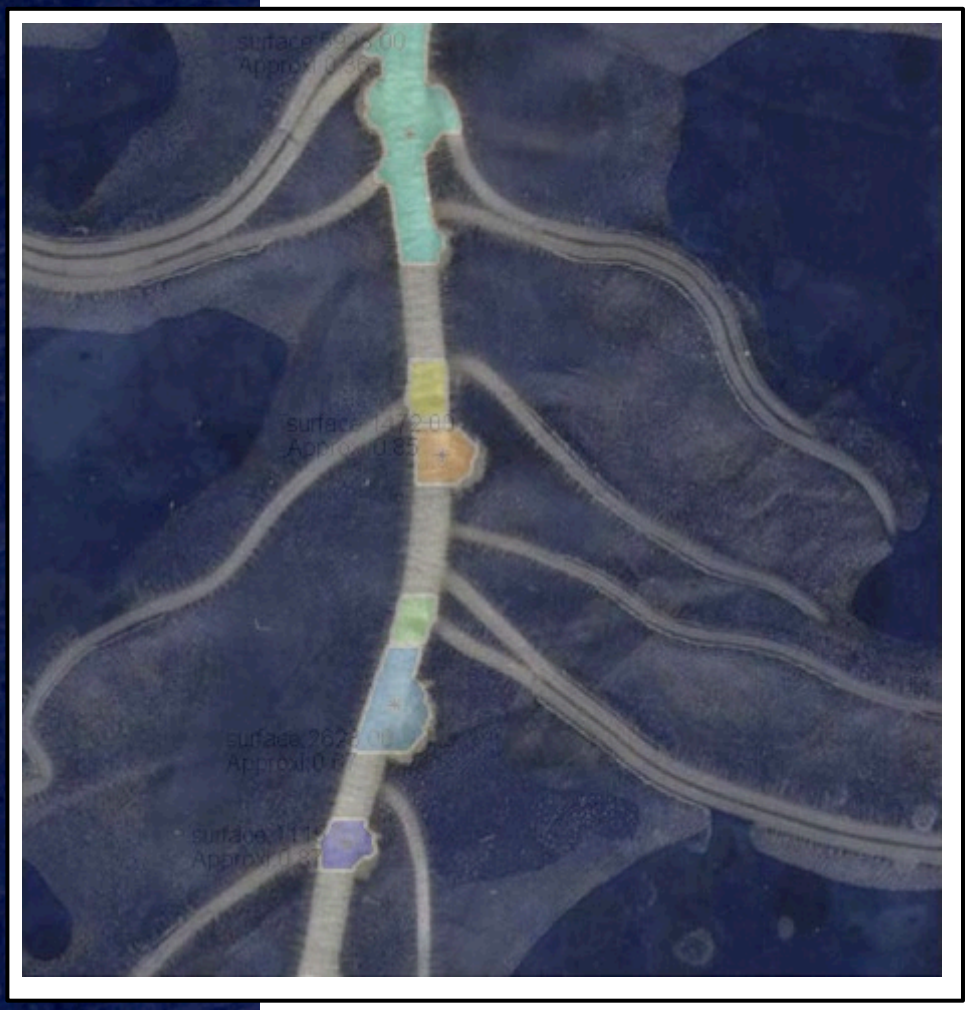


Count nodules with the « circle » form + area on a approximate circle

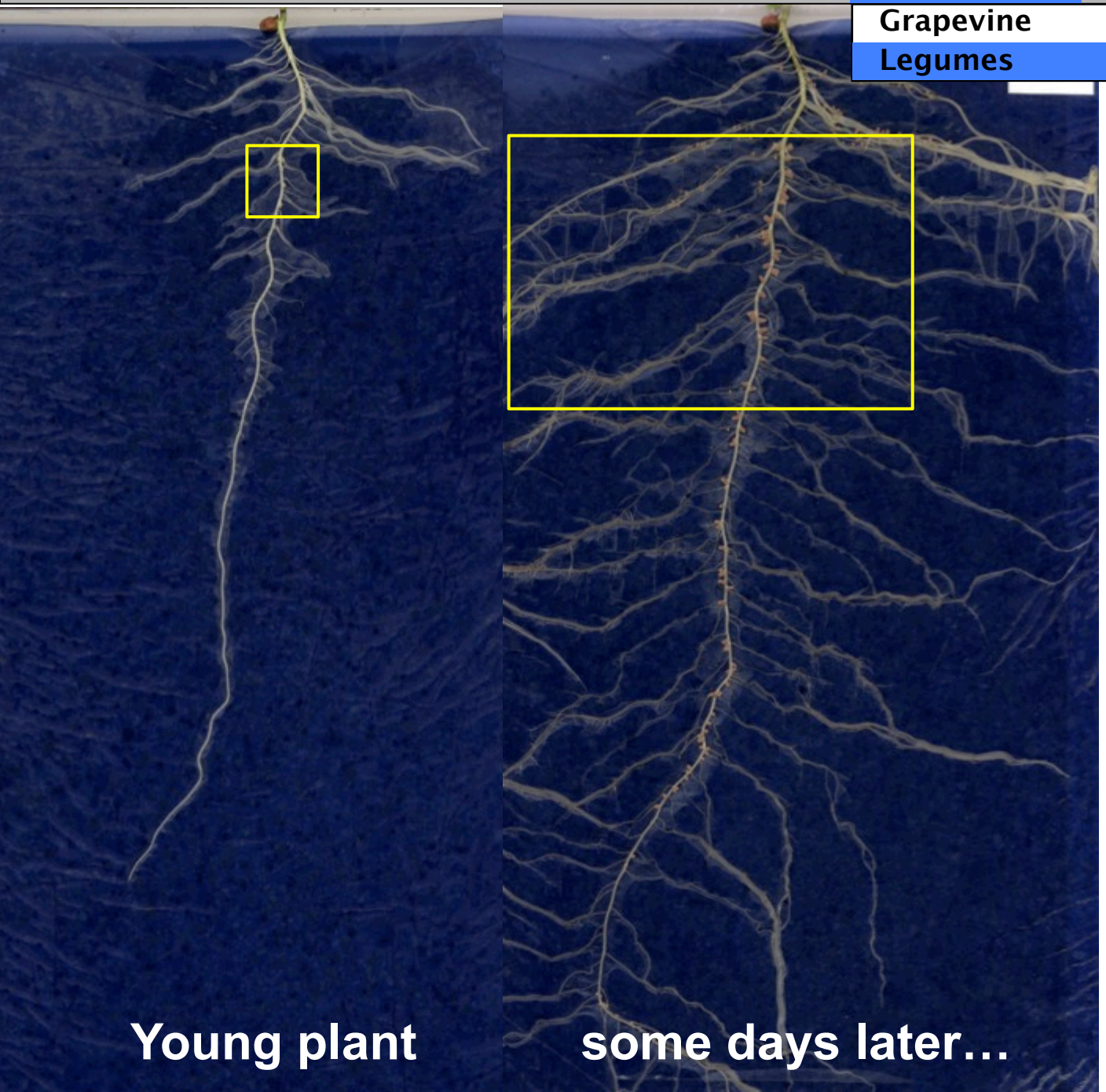
Young plant

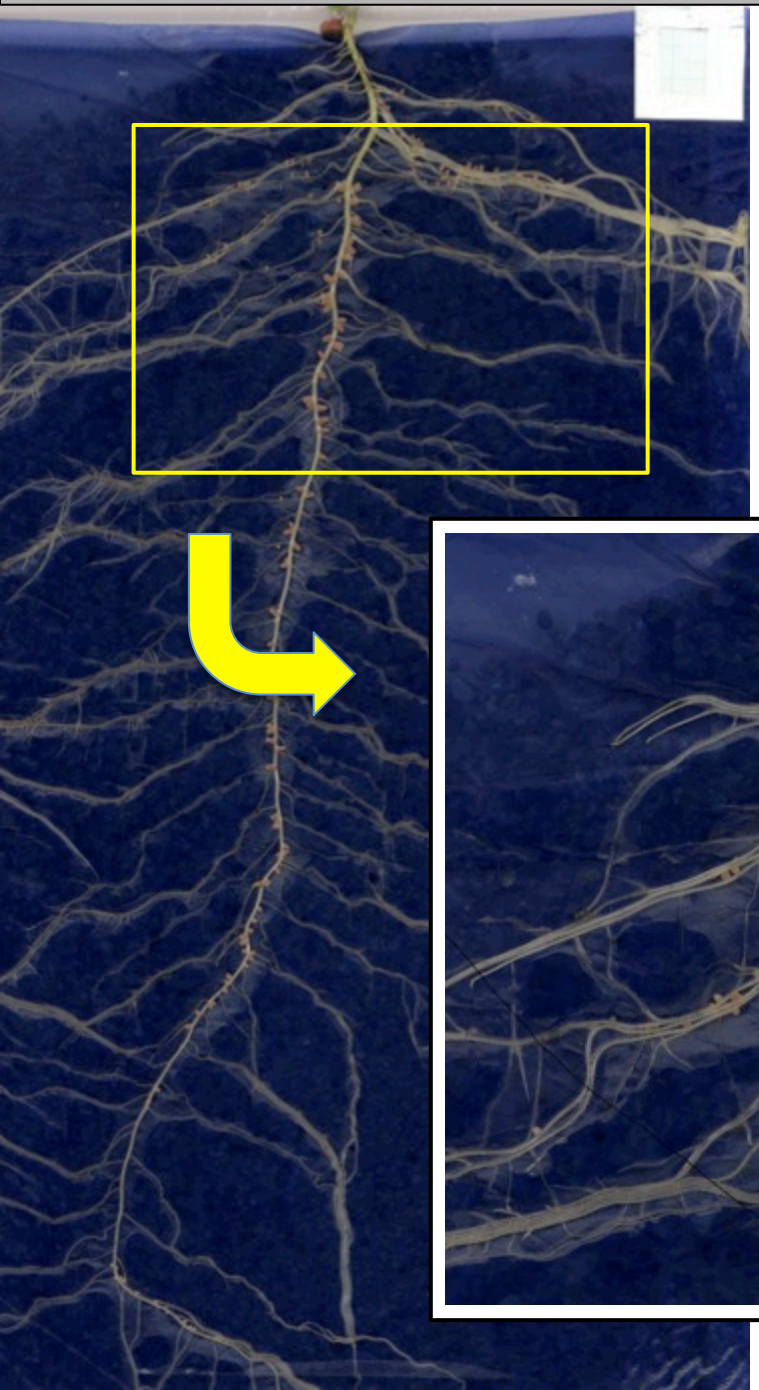


Young plant

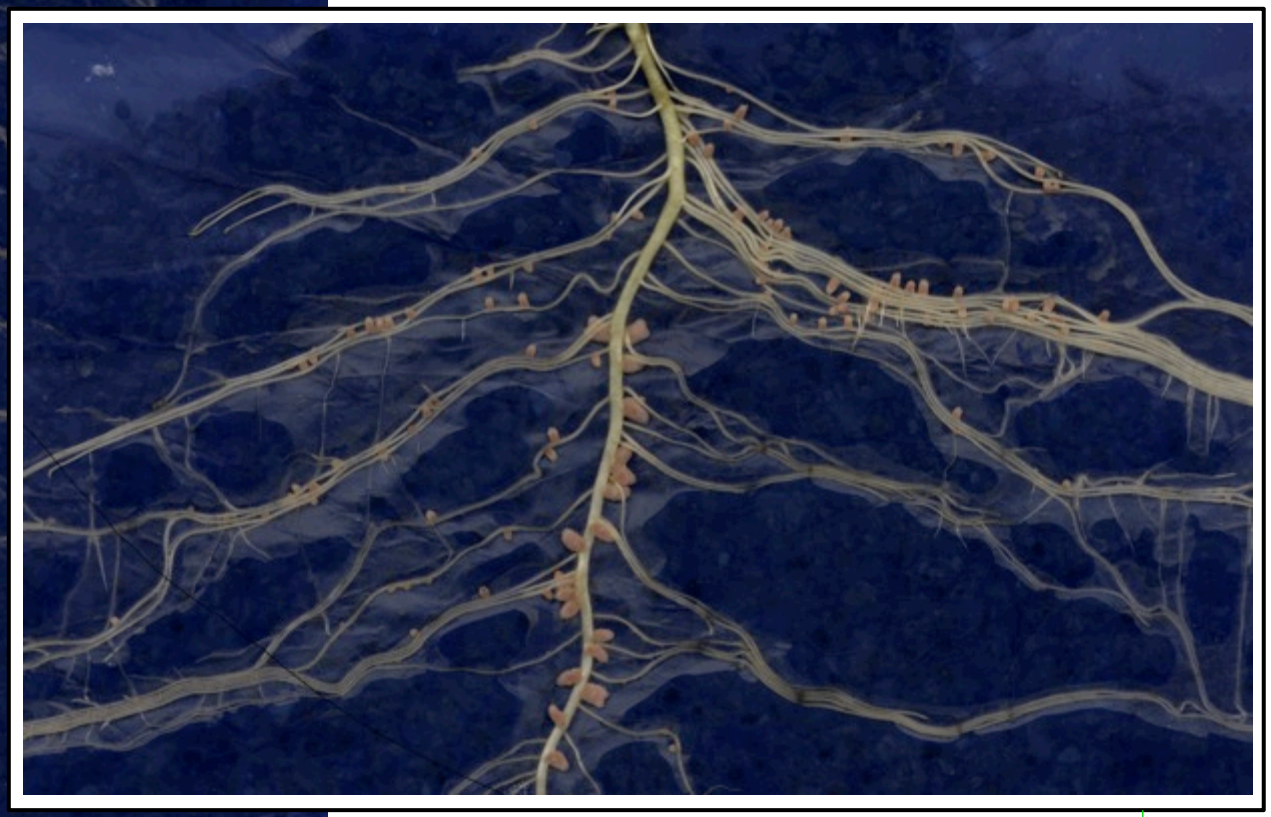


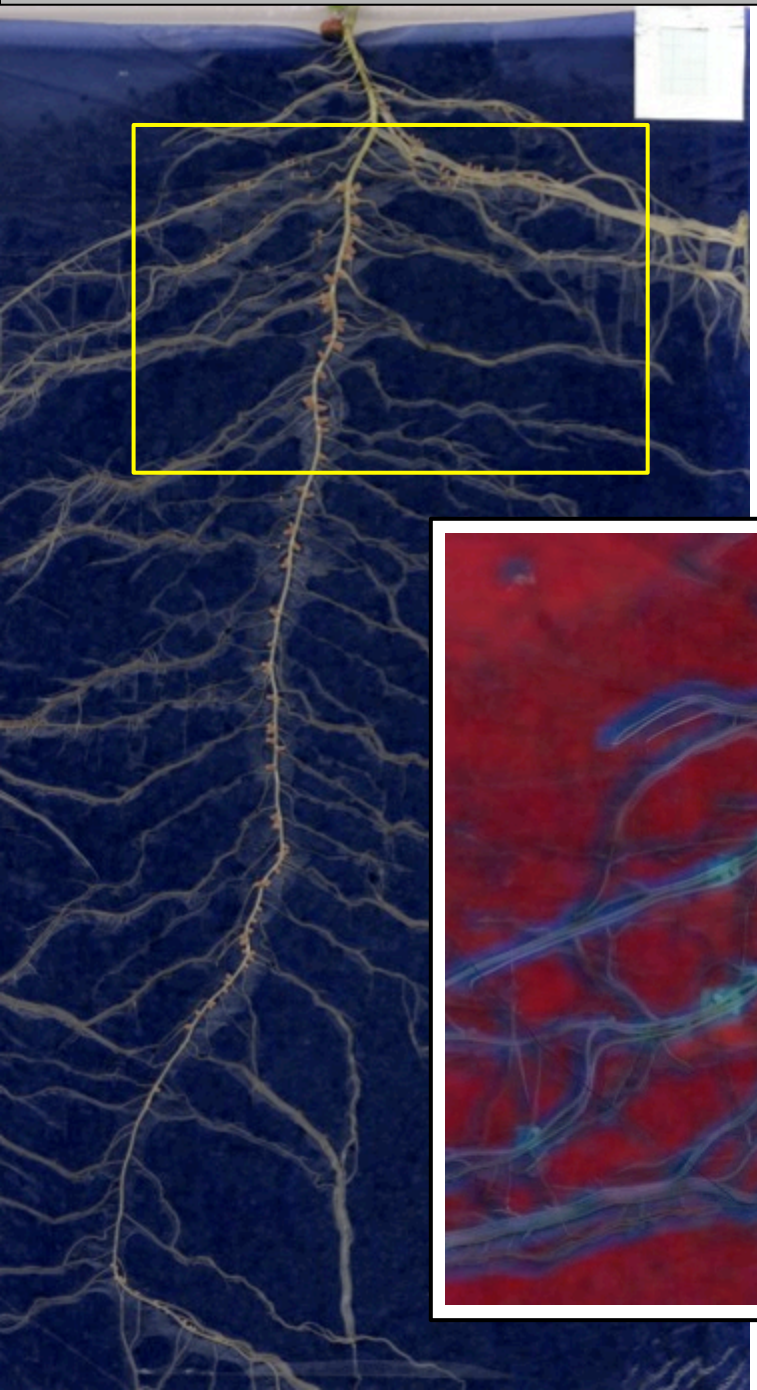
Original image + superposed nodules


Young plant
some days later…

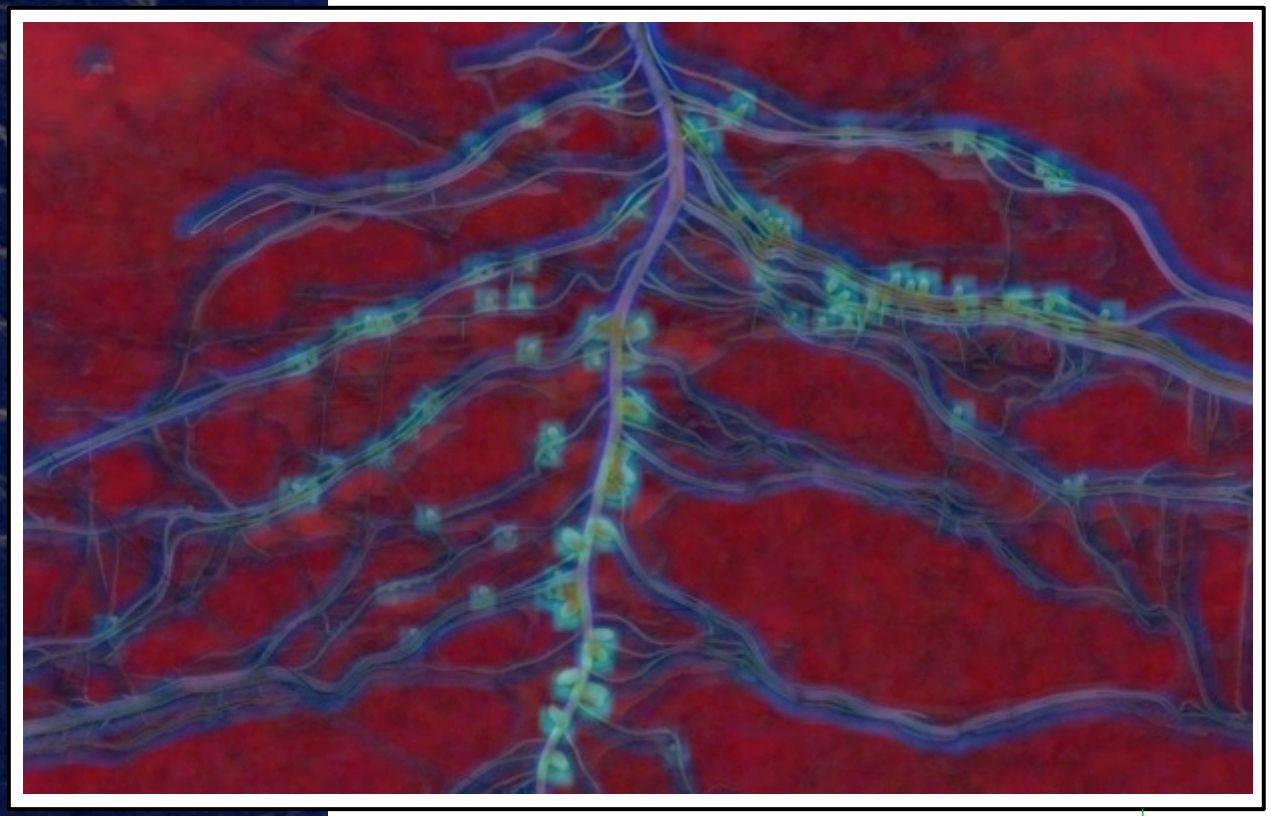


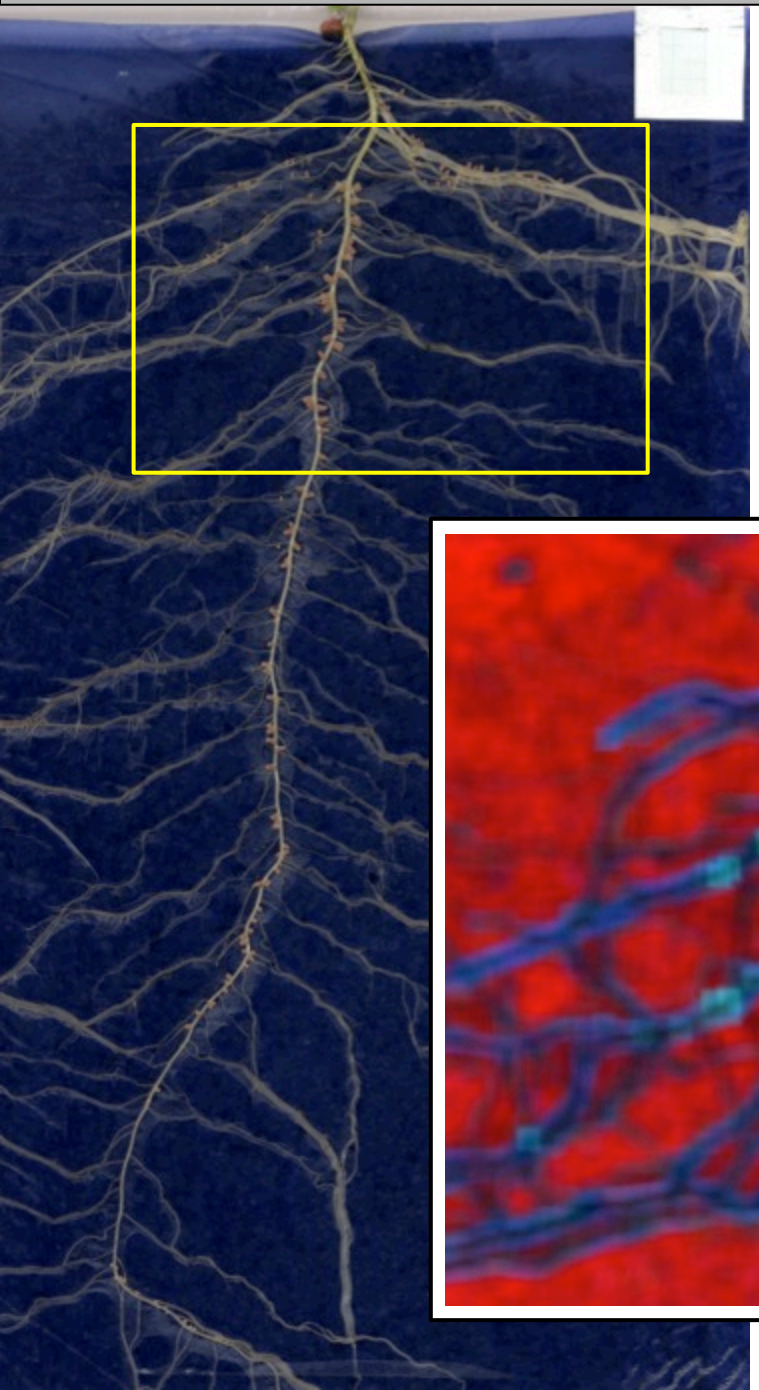
Enlarged image



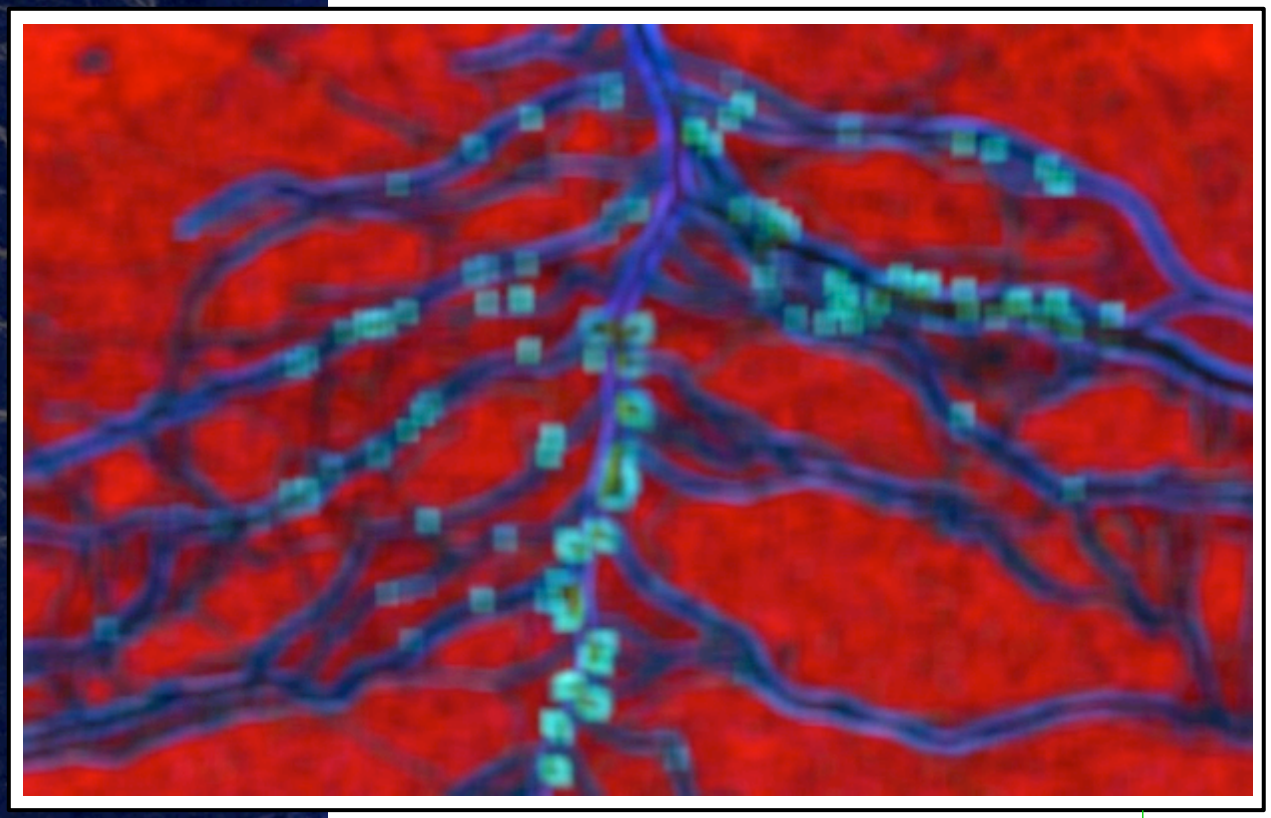


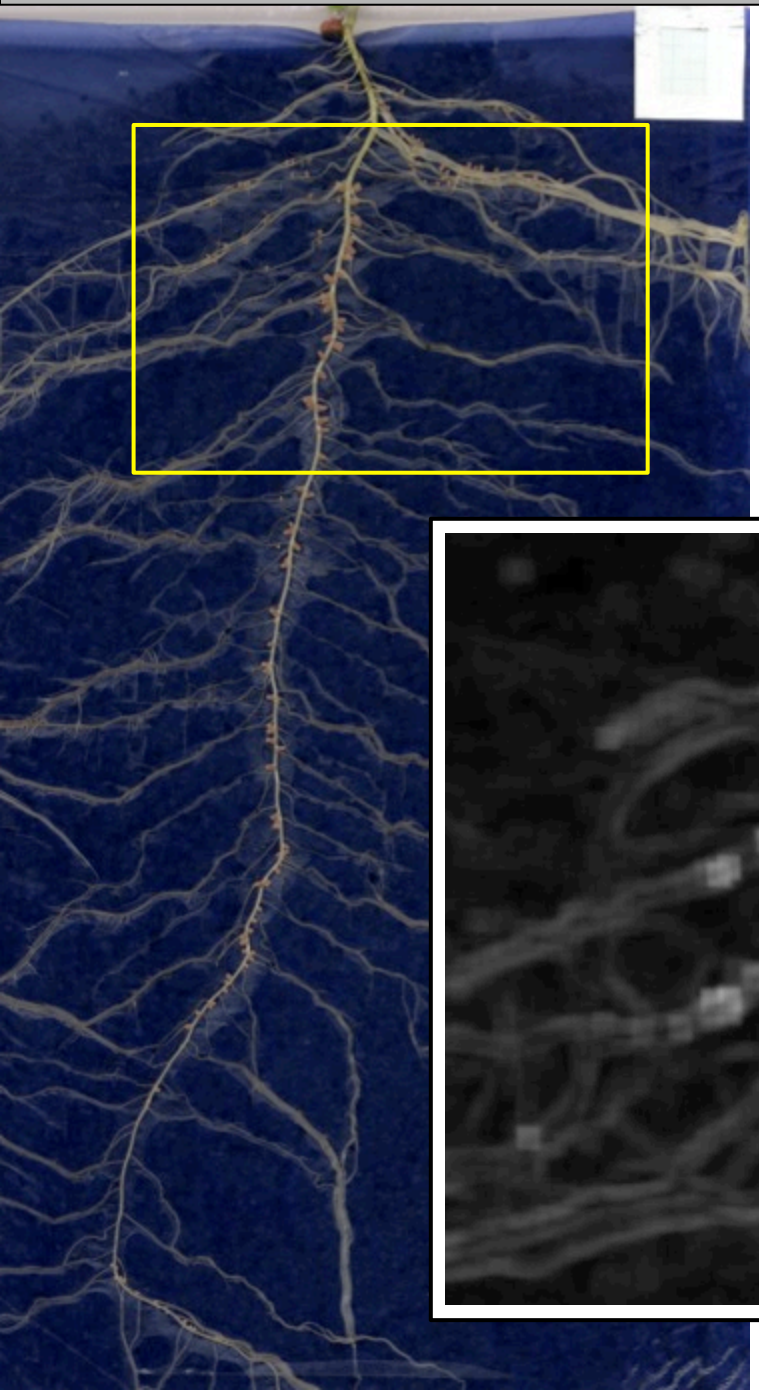
Hybrid space (color + texture)



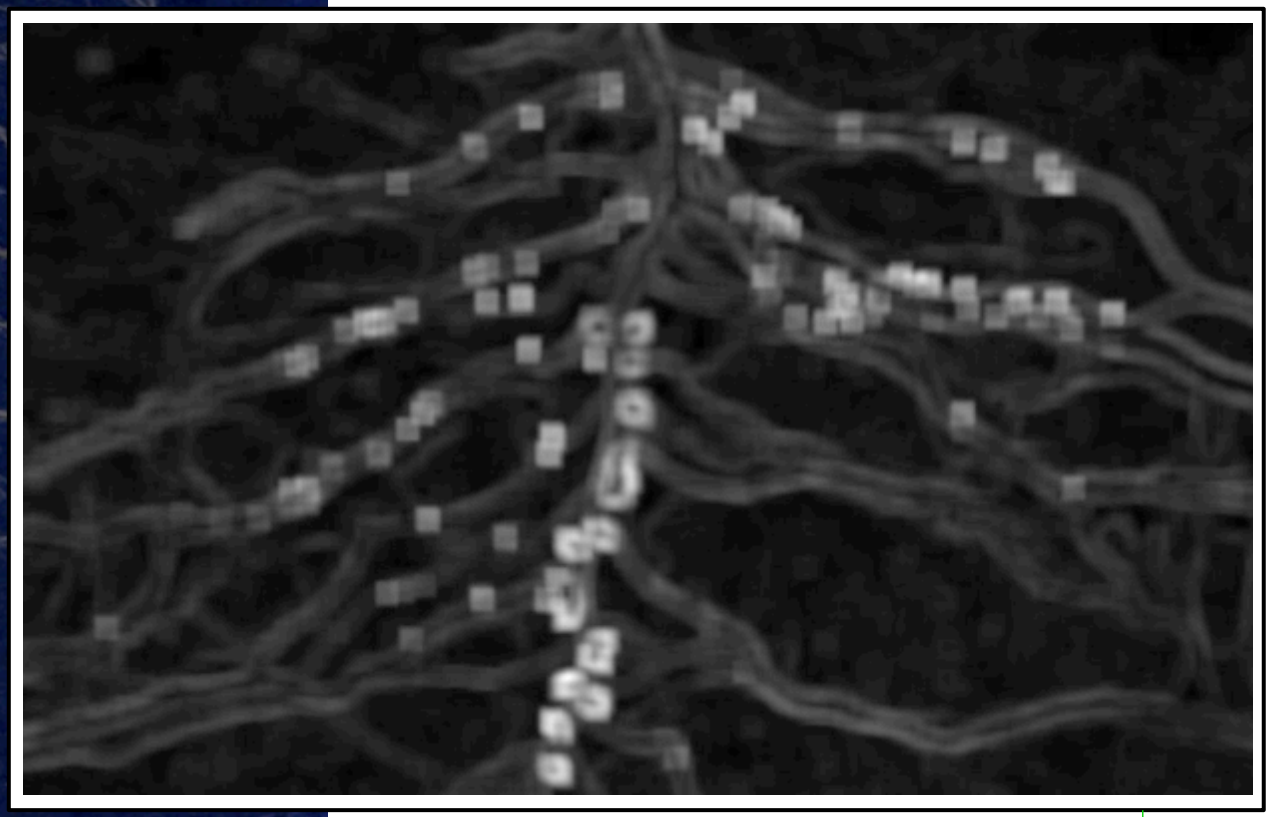


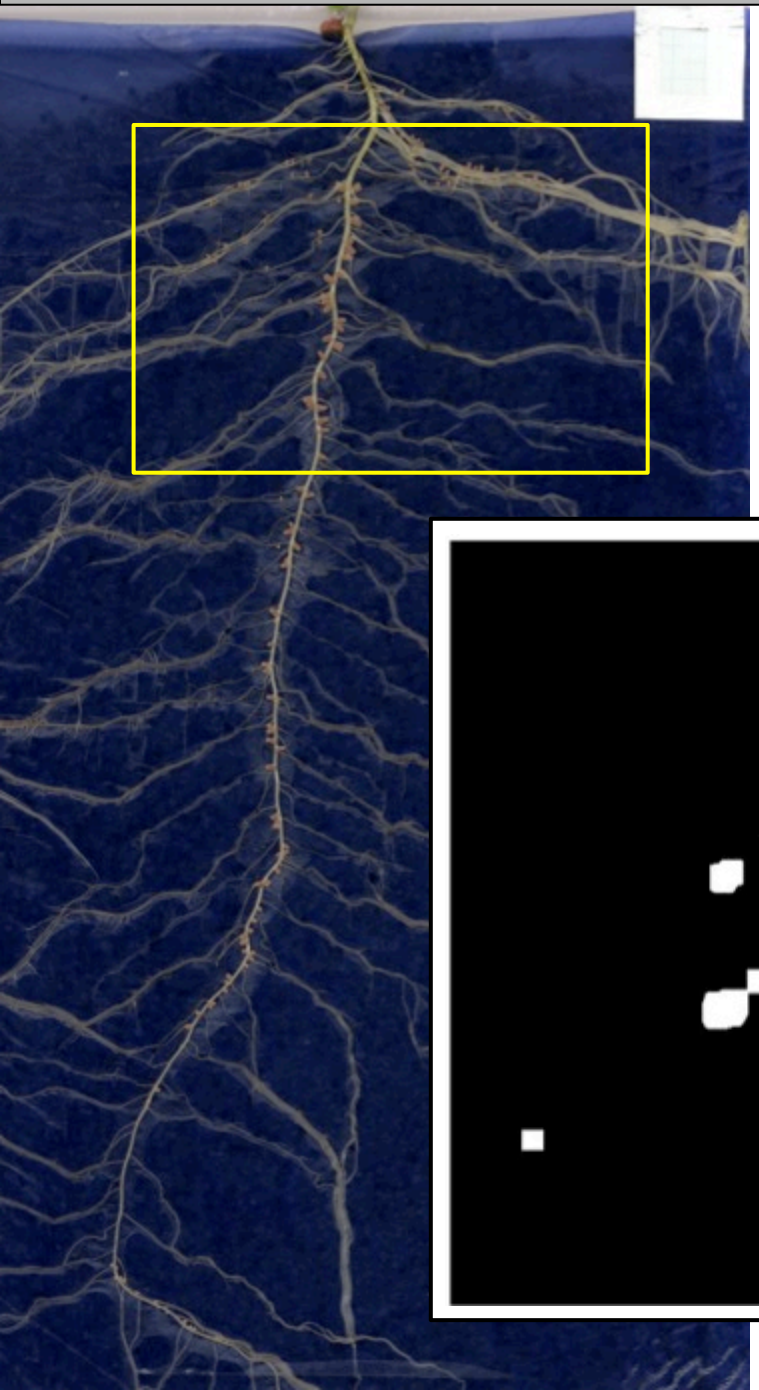
RGB Image of identified nodules



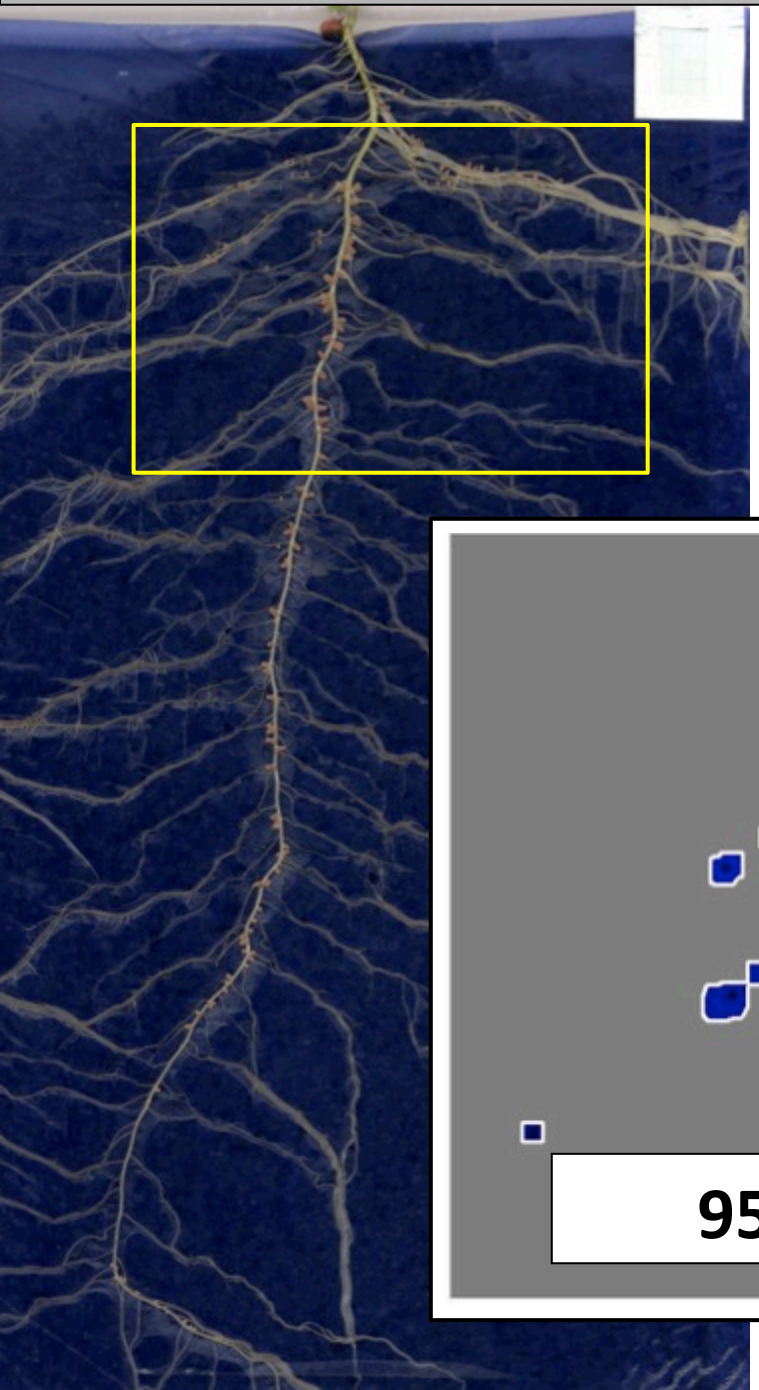


Green band of the RGB image

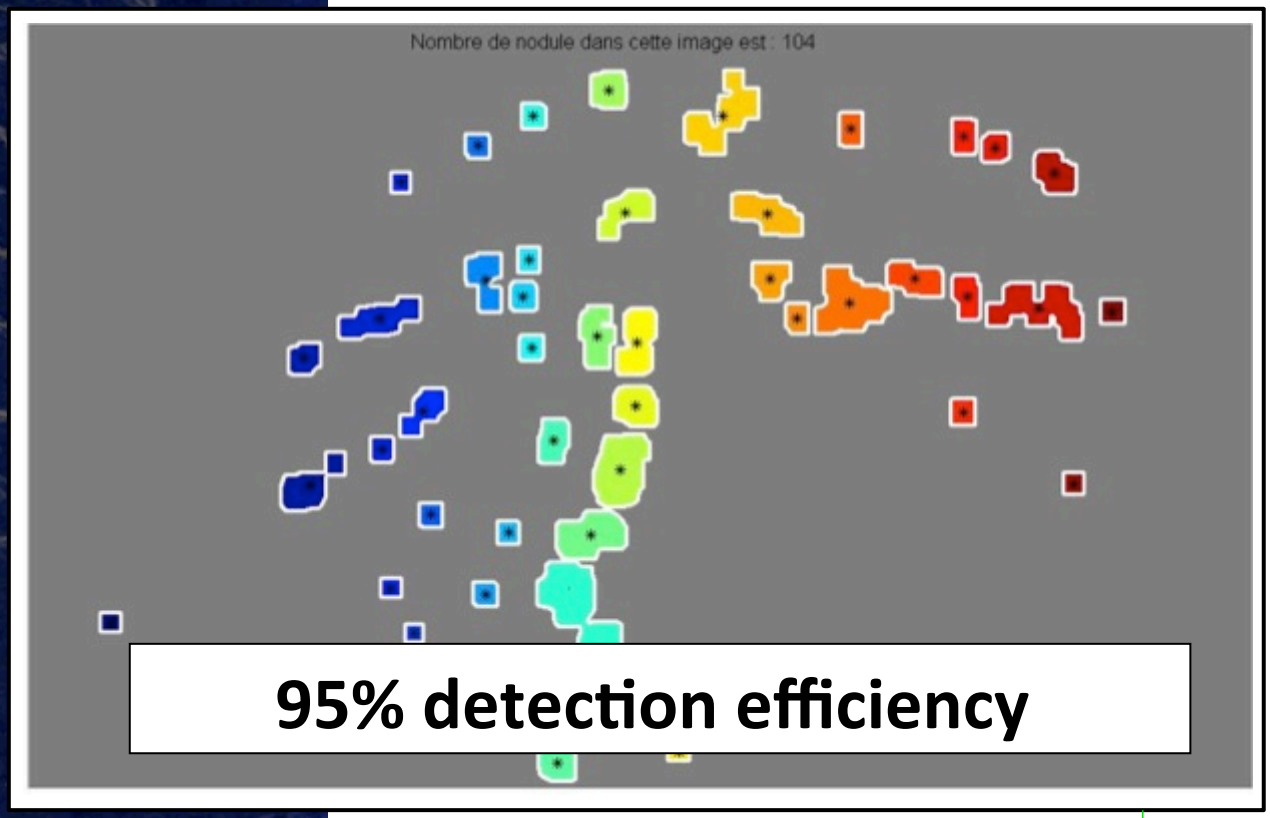




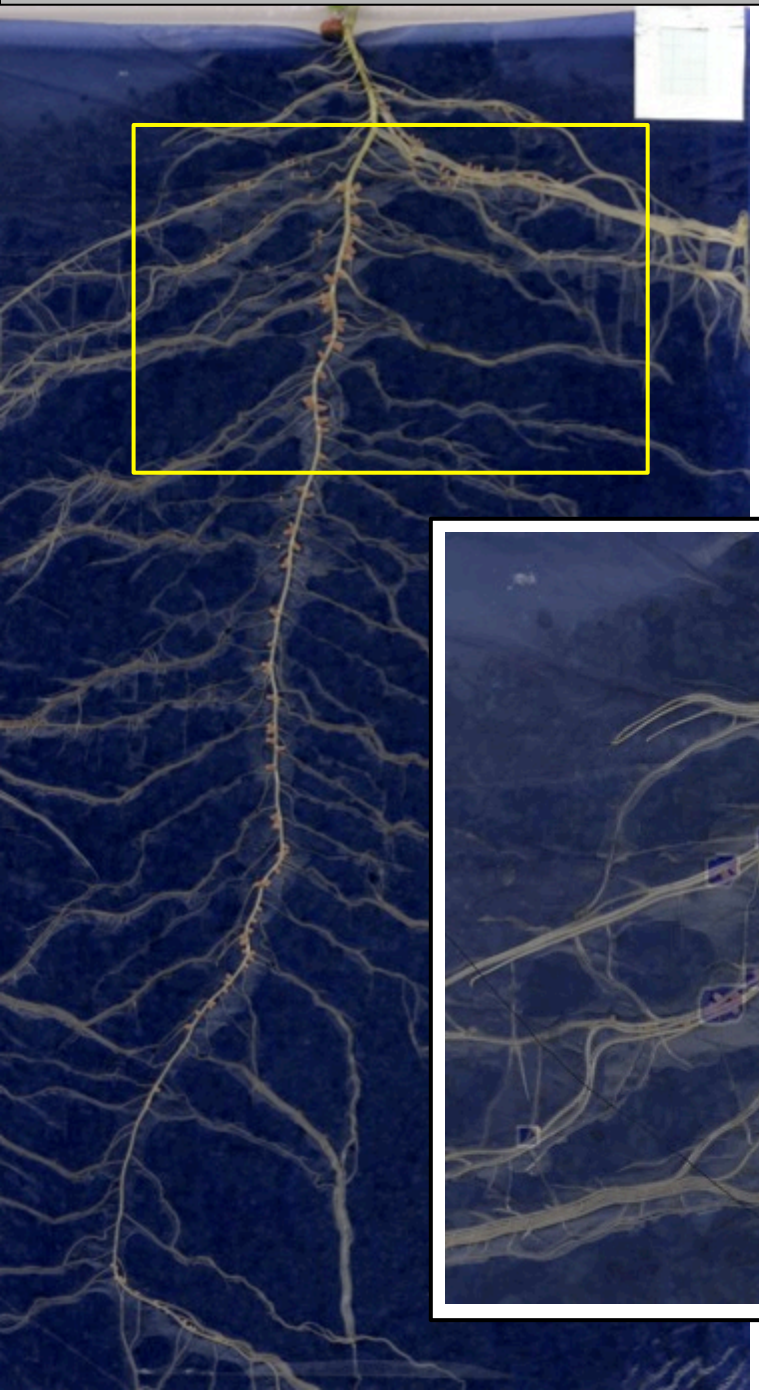
Binary image with squared nodules



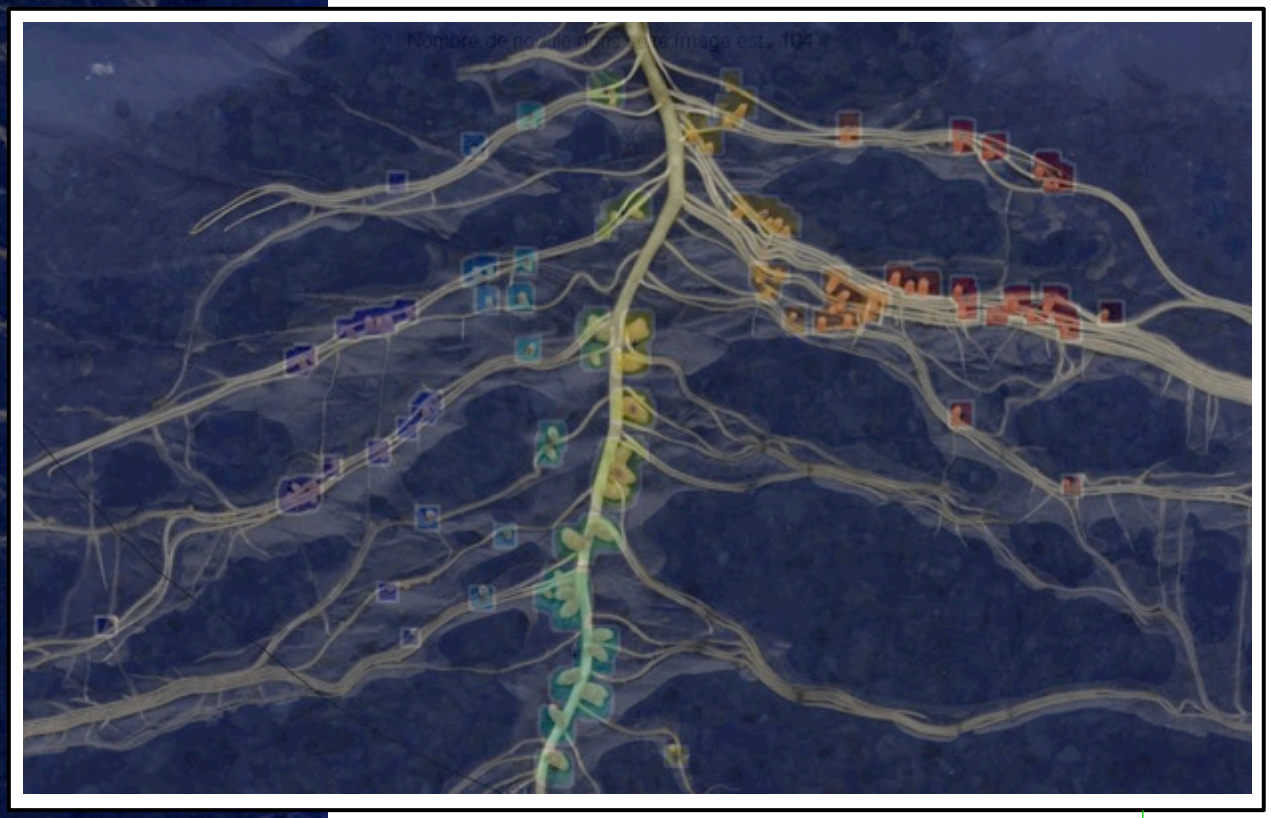
Nodules automatically detected



95% detection efficiency

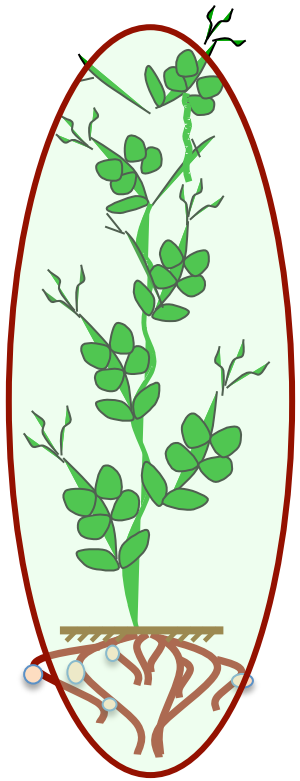


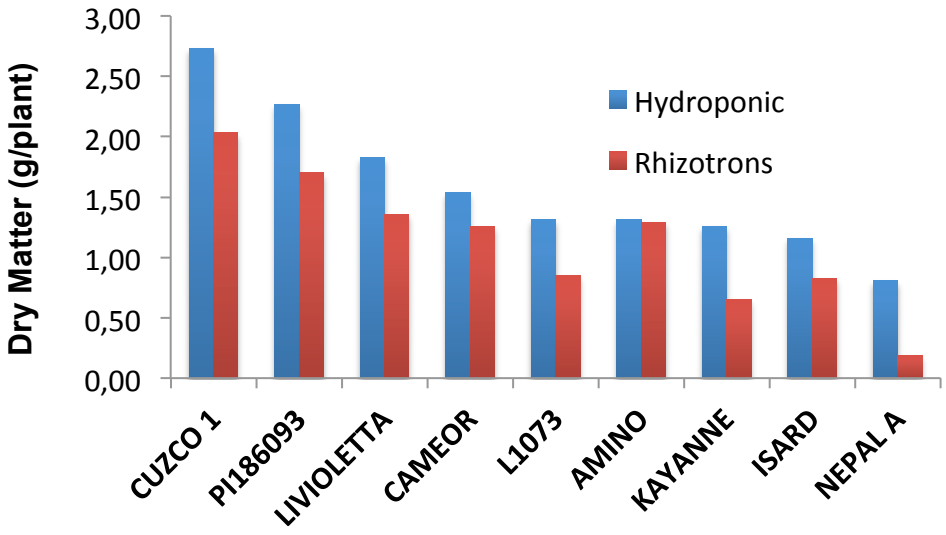
Original image + superposed nodules



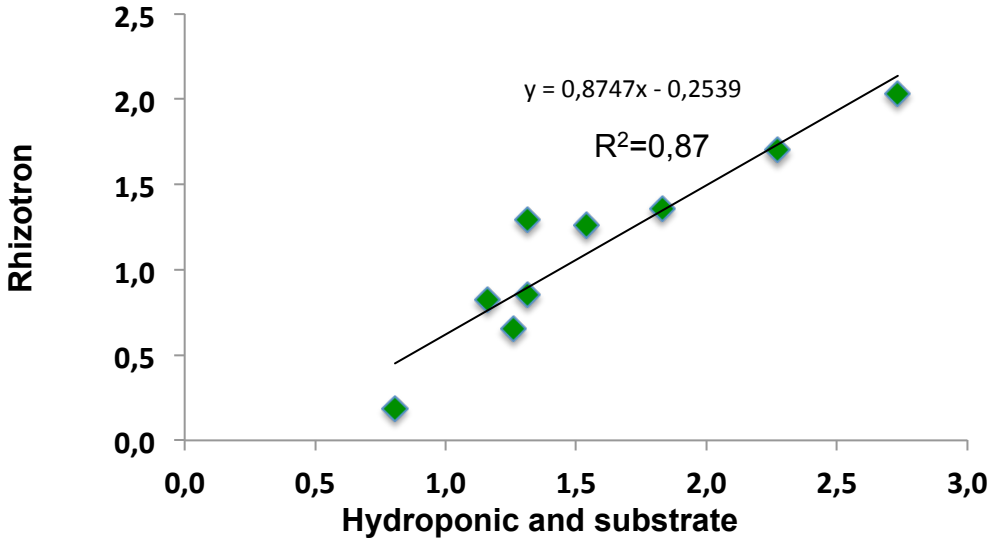
Ranking pea genotypes: Pea core collection
Hydroponic versus rhizotron

Plant biomass





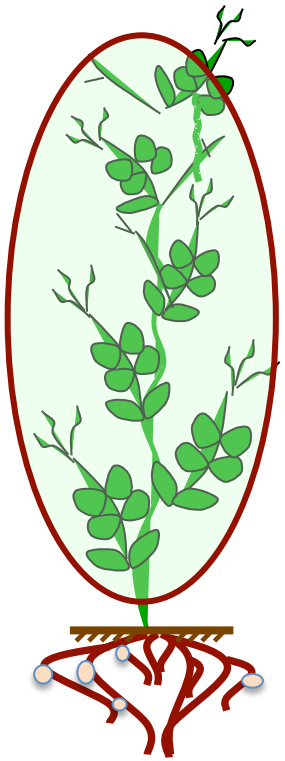
Plant biomass decreases within rhizotrons

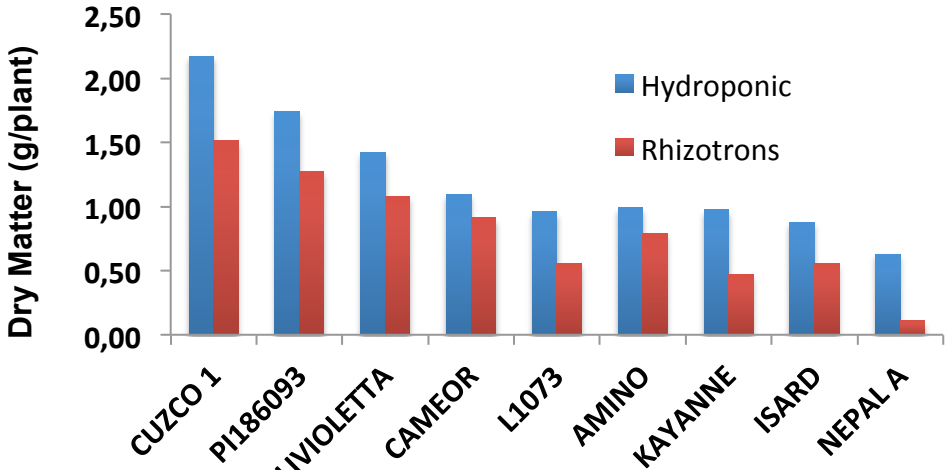


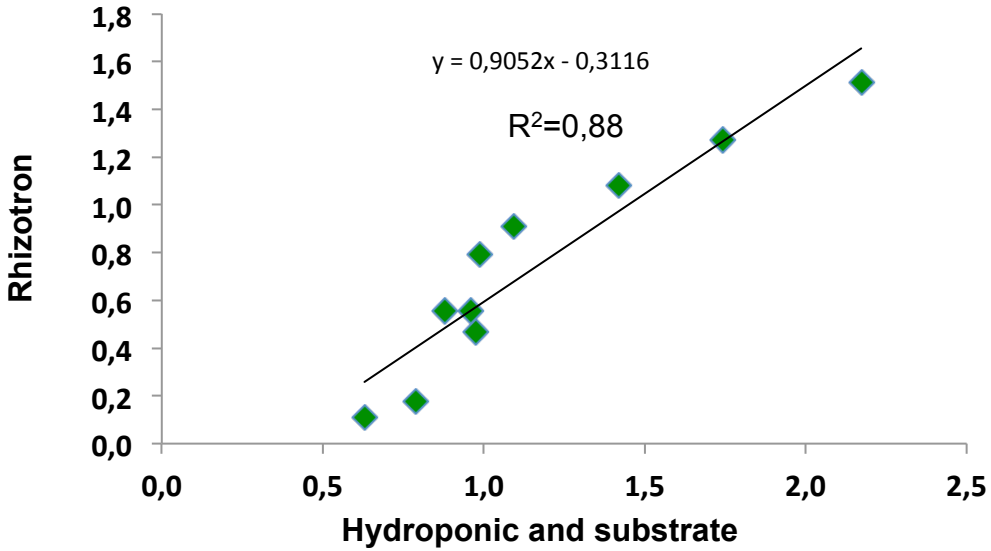
Genotype ranking does not vary

Ranking pea genotypes: Pea core collection
Hydroponic versus rhizotron

Shoot biomass

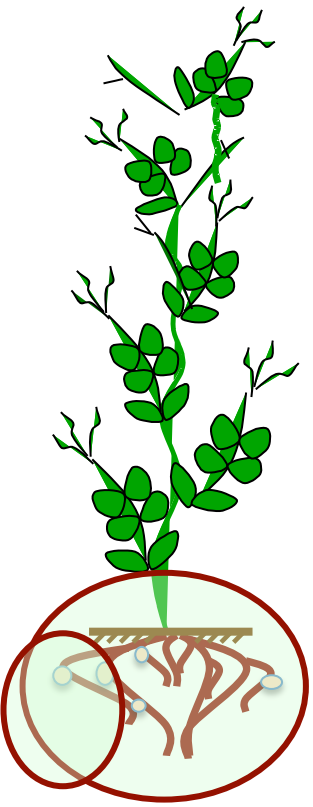




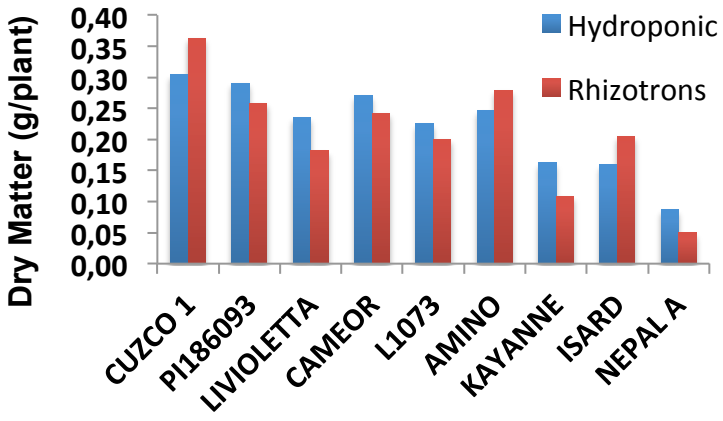


Genotype ranking does not vary

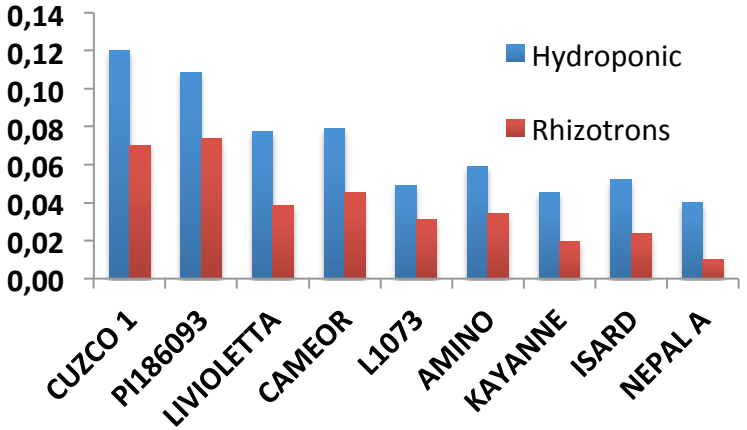
Ranking pea genotypes: Pea core collection

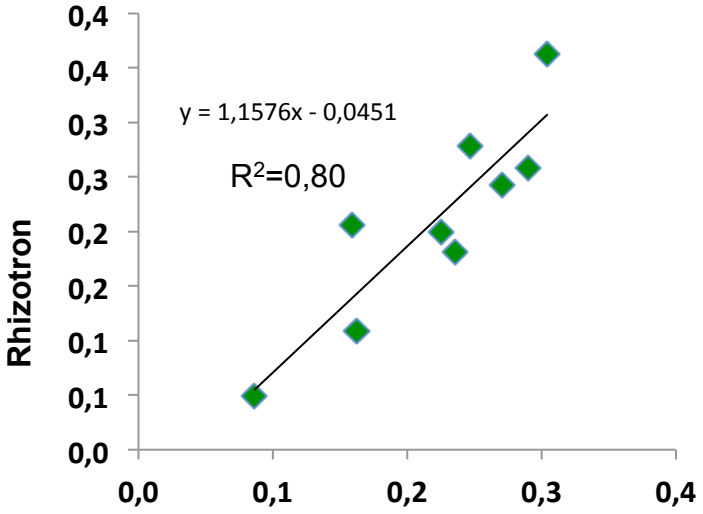


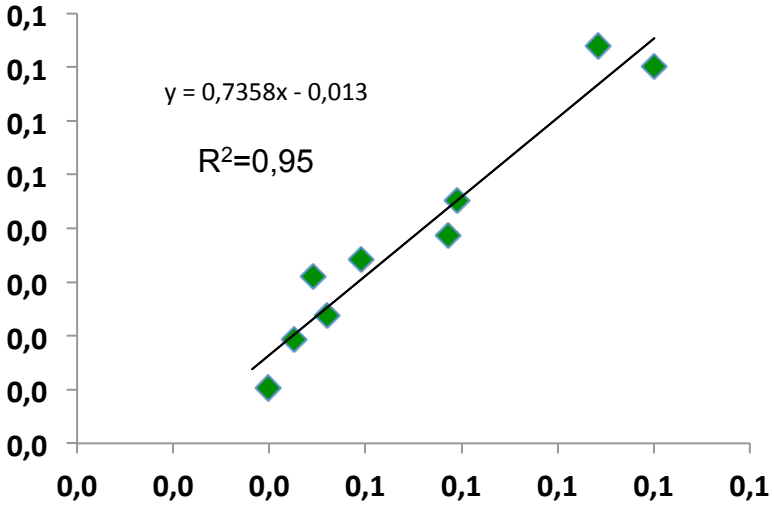
Root biomass



Nodule biomass



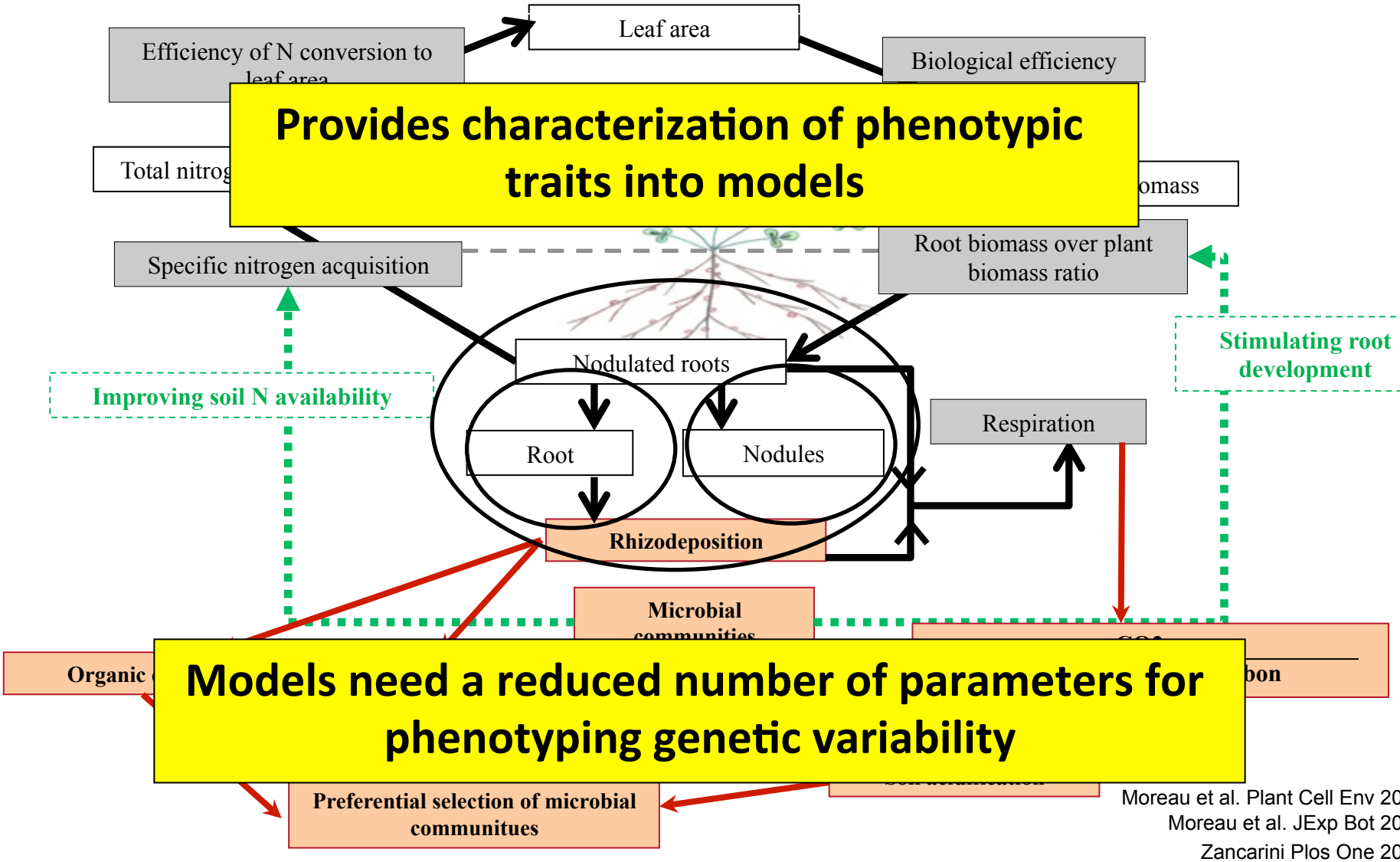




Hydroponic and substrate

Integrative Model: *Medicago*

Decomposing integrative variables in physiological processes



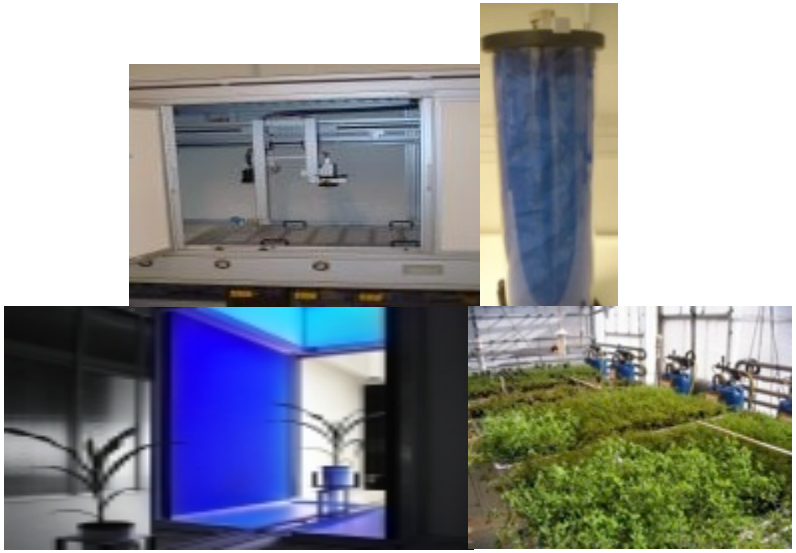
Provides characterization of phenotypic traits into models

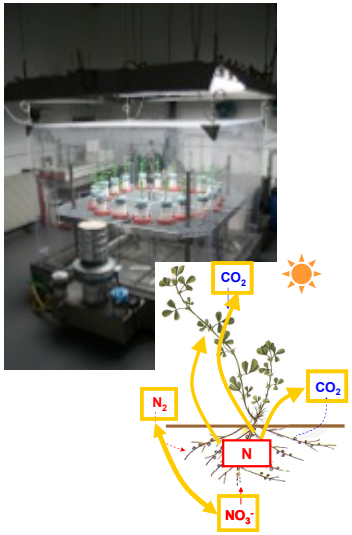
Models need a reduced number of parameters for phenotyping genetic variability

Moreau et al. Plant Cell Env 2006
Moreau et al. JExp Bot 2008
Zancarini Plos One 2013

Food for thoughts… Combine approaches

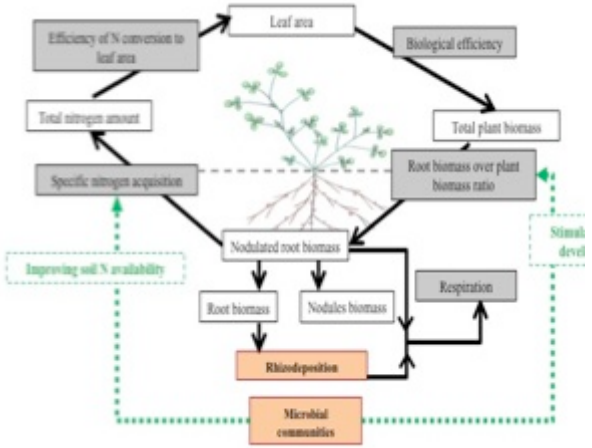
Phenotyping Approach + **Analytical approach**





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

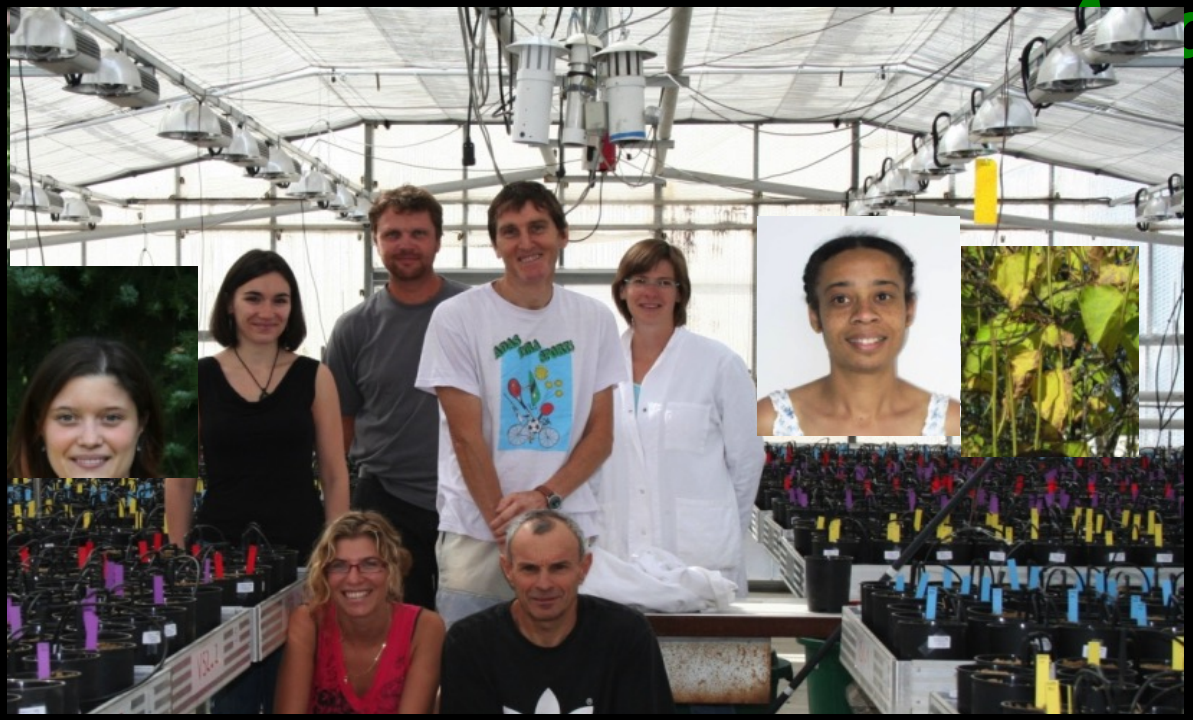
Jean-Claude SIMON

Frédéric COINTAULT

Simeng HAN

Marielle ADRIAN

d also …



Ecophysiology team

Medicago truncatula team

- Fluxomic
- PPHD
- **Rhizotrons**
- Rhizobox

Rhizothrones (EU Licence INRA-Inoviaflow, 1300 units planed)