High throughput root phenotyping using the “Rhizo” suite

Christophe Salon, Christian Jeudy, Mickaël Lamboeuf, Julien Martinet,
Franck Zenk, Karine Palavioux, Céline Bernard

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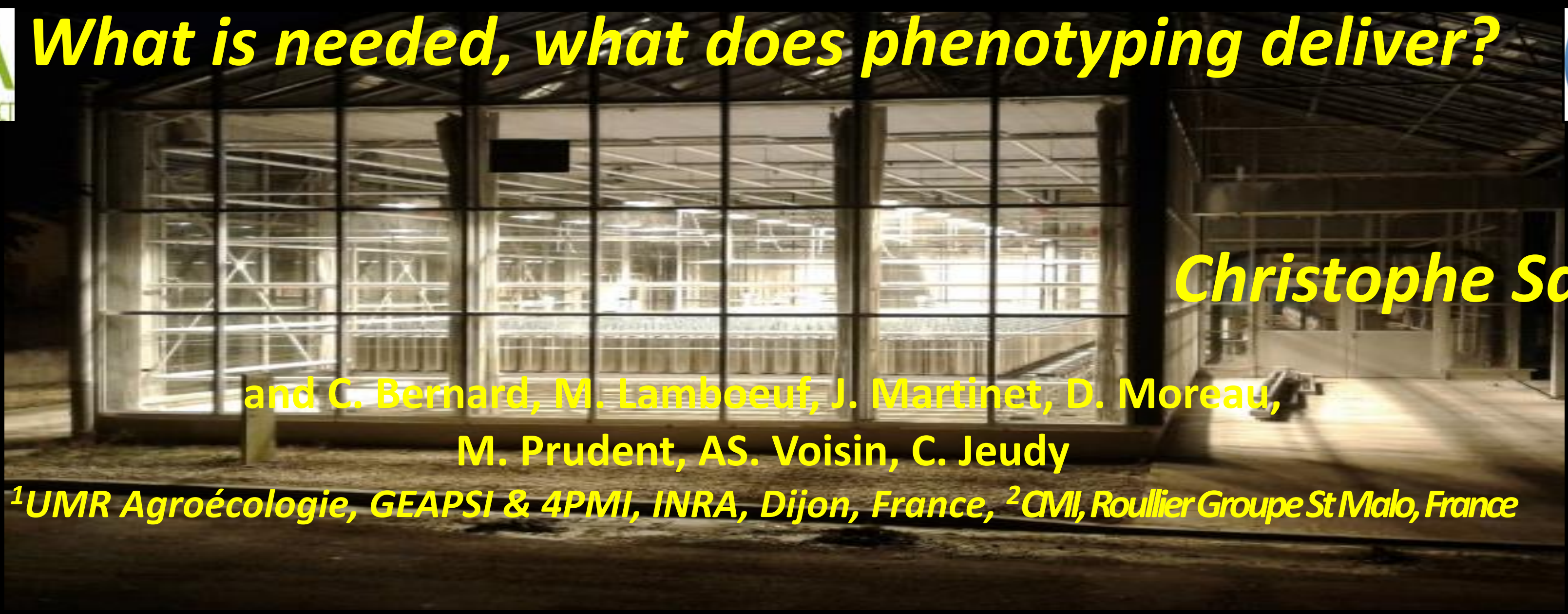
HAL Id: hal-02734173
https://hal.inrae.fr/hal-02734173v1

Submitted on 2 Jun 2020

INRA SCIENCE & IMPACT

What is needed, what does phenotyping deliver?

Agroécologie Dijon Unité de Recherche



Christophe Salon

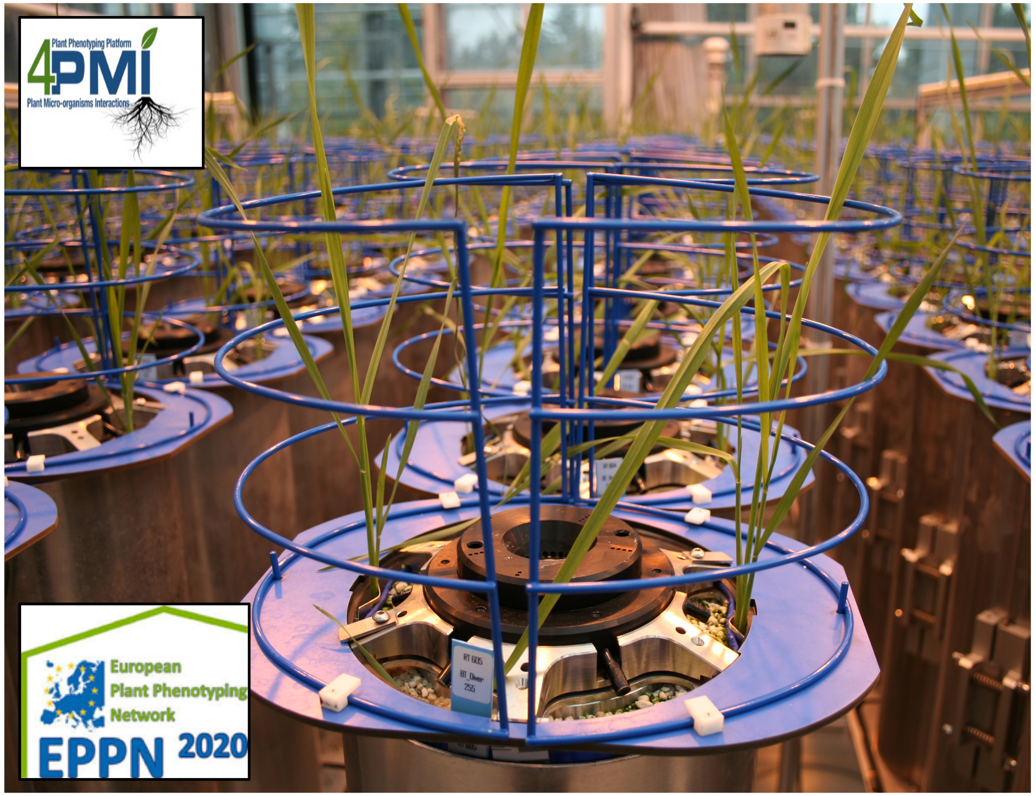
and C. Bernard, M. Lamboeuf, J. Martinet, D. Moreau,
M. Prudent, AS. Voisin, C. Jeudy

[1]UMR Agroécologie, GEAPSI & 4PMI, INRA, Dijon, France, [2]CMI, Roullier Groupe St Malo, France

PHENOME FPPN Réseau Français Phénomique végétale

International Plant Phenotyping Network IPPN

EMPHASIS



4PMI Plant Phenotyping Platform Plant Micro-organisms Interactions

European Plant Phenotyping Network EPPN 2020

Research Unit's Context

- Context
  - Increase of the world population: 2010=6.8 Md→2050~9 Md

    ⇨Increased demand of food provision
  - Decrease of the arable lands: Arable land/person 0.38 ha (1970)→0.23 ha (2000) → 0.15 ha (2050)
  - Growing pressure from input needs
    - ✓fertilizers with limited resources (P) or with high energy cost (NO_3^-, NH_4^+)
    - ✓pesticides with possible threats for agricultural products, soils and water
    - ✓water contributing to geopolitical tensions
  - Acceleration of climate change, to which agriculture is subjected but also contributes.

Lack of sustainability of the current situation

Research Unit’s Context and Challenges

- Necessity for a paradigm change

- Agronomic and environmental challenges
  - ✓Provide agricultural products in high enough quantity and quality
  - ✓Decrease the use of inputs
  - ✓Preserve the environment (sol/water/air)

- Bring together Agronomy and Ecology
  - ✓Design cropping systems : respect and valorize the biodiversity, the regulations and interactions among communities (biotic interactions)
  - ✓Adapt the crop to the environment rather the environment to the crop
  - ✓Realize innovative crop breeding programs

➯ Emergence and promotion of **Agroecology**

Scientific expertises

- Plant communities
  - ✓Cultivated plants and weeds, and their interactions
- Microbial (bacterial and fungal) communities
  - ✓Mutualistic communities
  - ✓Phytopathogenic and human pathogenic communities
  - ✓Functional communities: biogeochemical cycles (C, N), bioremediation,….
- Plant-Microbe interactions
  - ✓Mutualistic (symbiotic or not)
  - ✓Parasites
- Legumes
  - ✓Ecophysiology
- Systemic agronomy (Nicolas Munier Jolain speech)
  - ✓ Designing new cropping systems
  - ✓ Multi-criteria assessment

Organization

Teams

Biology and ecosystemic functions of soils (BIOmE)

F. Martin-Laurent

Genetic and environmental determinisms of the adaptation of plants to innovative CS (GEAPSI)

C. Salon

Mechanisms and management of plants-microorganisms interactions (IPM)

S Jeandroz

Sustainable Management of Arable Weeds (GESTAD)

S. Petit-Michaut

Platforms & Biological Resource Center

GenoSol

S. Mondy

HT Phenotyping Platform

C. Bernard

C. Salon

Biological Resource Center

C. Steinberg

Theme-based scientific workshops

Design of cropping systems

Ni. Munier-Jolain

Plants-microorganisms interactions

C. Salon

D. Wipf

Contribution to the development of leguminous ideotypes valorizing biotic interactions

A.-S. Voisin

Interactions between modeling and experiments/observations

N. Colbach

Research assistance

F. El Ghissassi

Administrative & Resource Management Cell

Support Cells

Objectives

- **Scientific objectives :** Mechanisms underlying the adaptation of plants to agroecosystems ?
  - **multidisciplinary approaches** : genetics, genomics, ecophysiology, molecular physiology
  - **different species** : depending on available genetic and genomic resources

- **Finalized objectives:**
  - Identify **plant ideotypes** for lower input agriculture
  - Improve crop **adaptation and resilience** to environmental constraints
  - Implement **breeding** programs towards these aims

Research topics

- Optimization (time x space) of **soil resource uptake** by legumes in a **context of fluctuating resources** (soil N, S, water) in **connection** with **soil microflora**.

- Understanding of **legume functioning** to **improve** and/or **stabilize yield components**, including **seed composition and quality**, particularly during **heat, water-stress** and **nutrients deficiencies** (N, S).

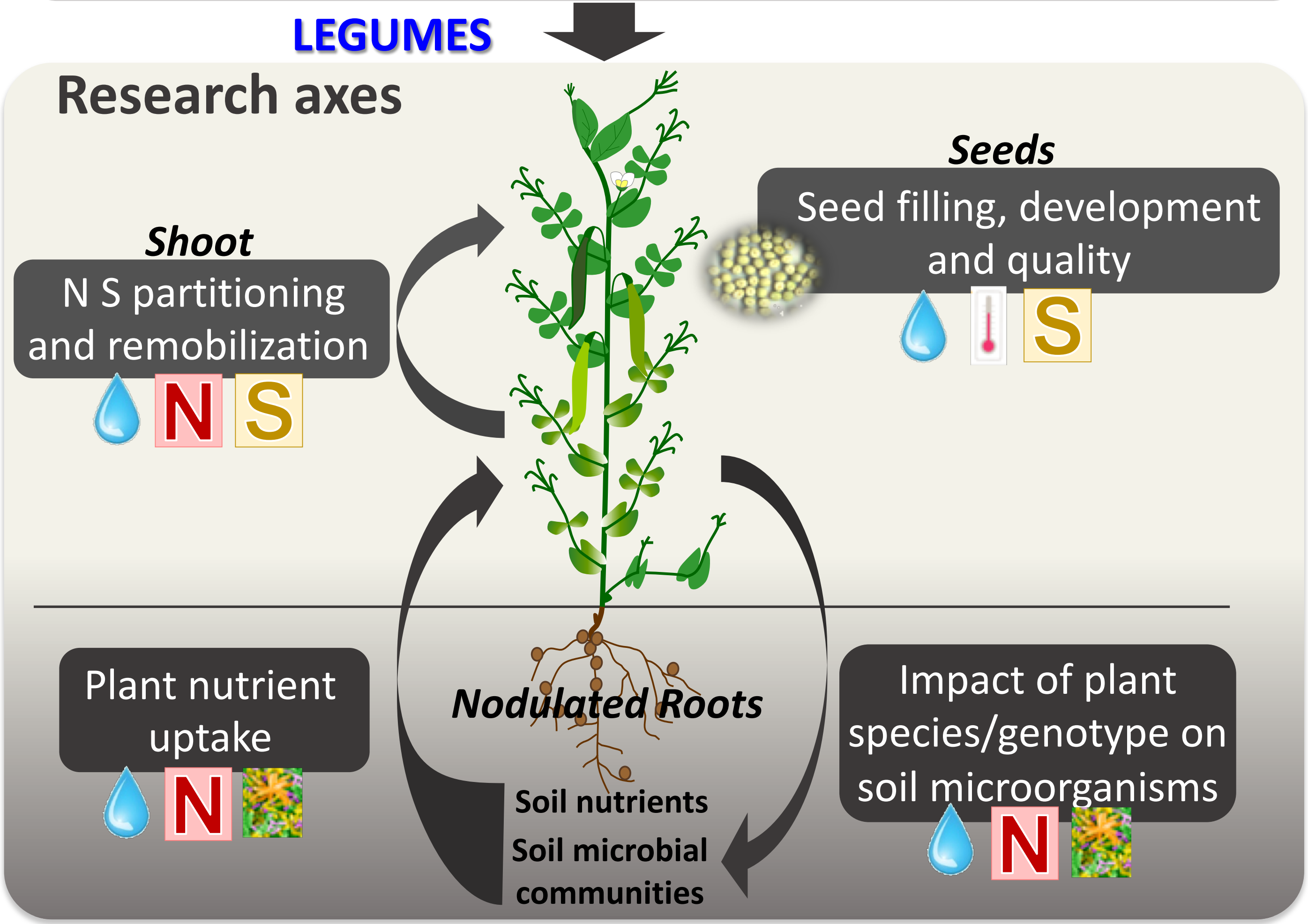
- Study of the **genetic bases** and processes enabling plant **adaptation to agrosystem** habitats.

Conceptual framework

Tools & ressources

- Plant phenotyping tools
- Plant modeling tools
- Genetics and genomic tools
- Legume genetic resources

LEGUMES



Research axes

Environmental factors

Shoot

N S partitioning and remobilization

💧 N S

Seeds

Seed filling, development and quality

💧 🌡 S

Plant nutrient uptake

💧 N ▨

Nodulated Roots

Soil nutrients
Soil microbial communities

Impact of plant species/genotype on soil microorganisms

💧 N ▨

Genetic variation

💧	**H_2O**
🌡	**Temp.**
N	**Nitrogen**
S	**Sulfur**
▨	**Soil microbes**

What is needed?

Genetic variability to work with !

Pea genetic and genomic tools for functional & structural approaches

Recombinant Inbred Lines (1400 RILs)

Mutant collections

✓ Pl

Novel genomic resources open a wide range of applications !

Induced genetic variability

Identify and characterize genes involved in nodulation, root architecture
Benefits (for N) and trade-off (for C) between nodulation and plant growth

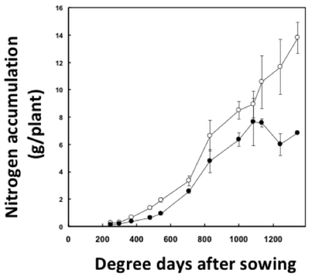
Nodule development and growth

Duc et al. 1998

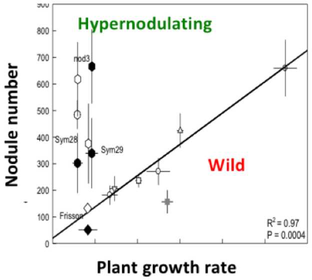
Salon et al. 2001

Cazenave et al. Plant Soil 2013

Voisin et al, A S D 2013, 2015



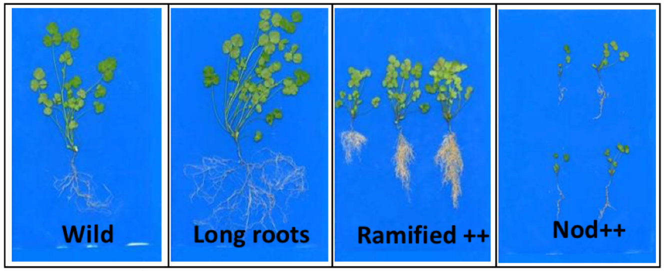
Salon et al. Agr 2001



Voisin et al. Plant Soil 201

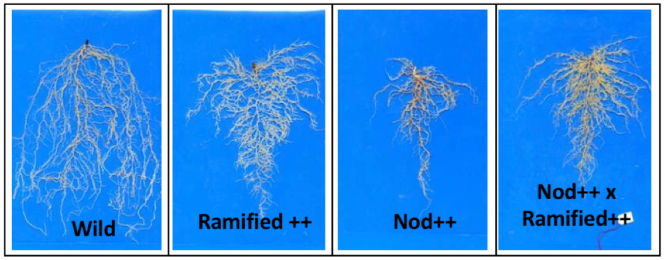
Root architecture

***Medicago** truncatula*, Tnt1



Porceddu et al. BioMed 2008

Pea, EMS



Coll . KK Sidorova

What is needed? Shoots functioning but focus on roots

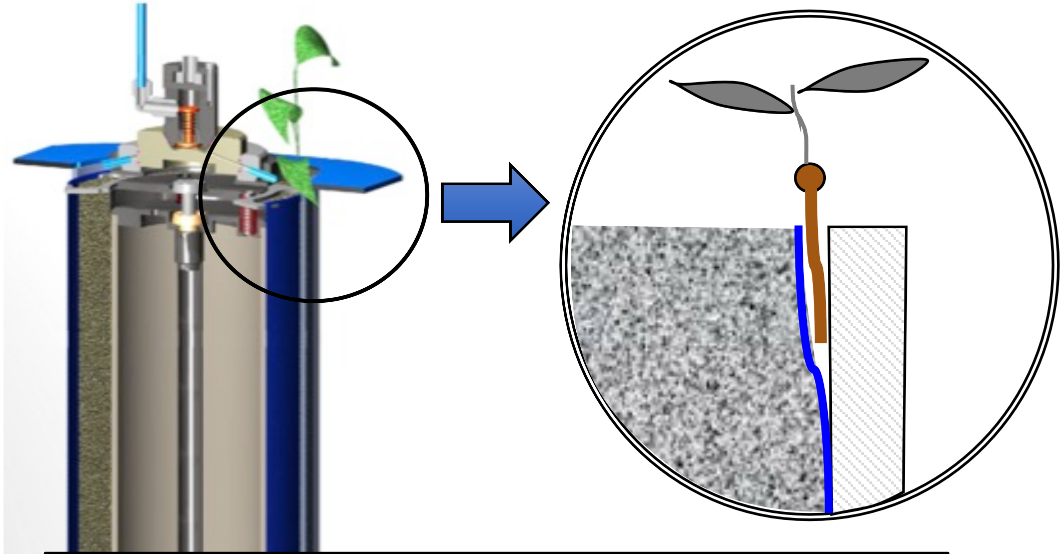
- **Crop breeding programmes**: root traits rarely used as selection criteria, a focus on adaptation to high-input systems,

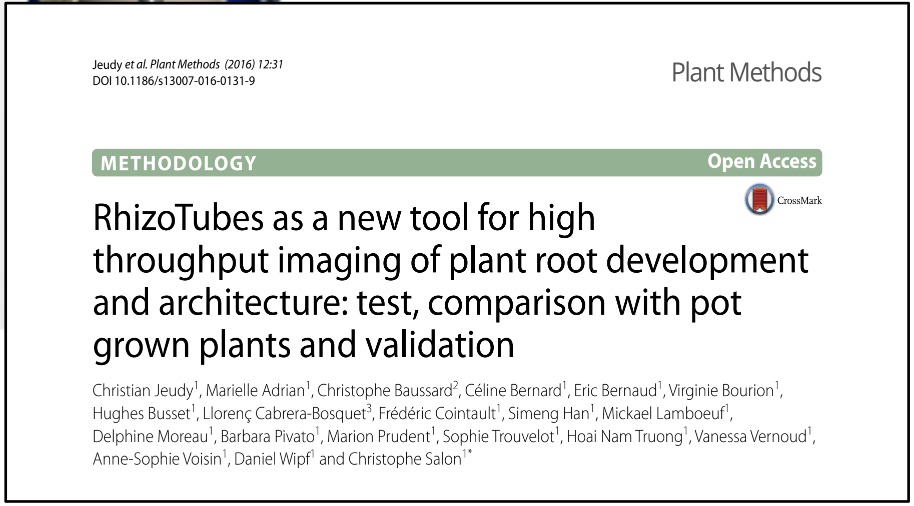
→ Improve crop resource-use efficiency through:
  (i) physiological utilization of acquired resources,
  (ii) resource acquisition

- **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.

What is needed?

Growing plants and looking at roots





Jeudy *et al. Plant Methods* (2016) 12:31
DOI 10.1186/s13007-016-0131-9

Plant Methods

METHODOLOGY **Open Access**

RhizoTubes as a new tool for high throughput imaging of plant root development and architecture: test, comparison with pot grown plants and validation

Christian Jeudy[1], Marielle Adrian[1], Christophe Baussard[2], Céline Bernard[1], Eric Bernaud[1], Virginie Bourion[1], Hughes Busset[1], Llorenç Cabrera-Bosquet[3], Frédéric Cointault[1], Simeng Han[1], Mickael Lamboeuf[1], Delphine Moreau[1], Barbara Pivato[1], Marion Prudent[1], Sophie Trouvelot[1], Hoai Nam Truong[1], Vanessa Vernoud[1], Anne-Sophie Voisin[1], Daniel Wipf[1] and Christophe Salon[1*]

1 to 6 plants

Shading « shell »

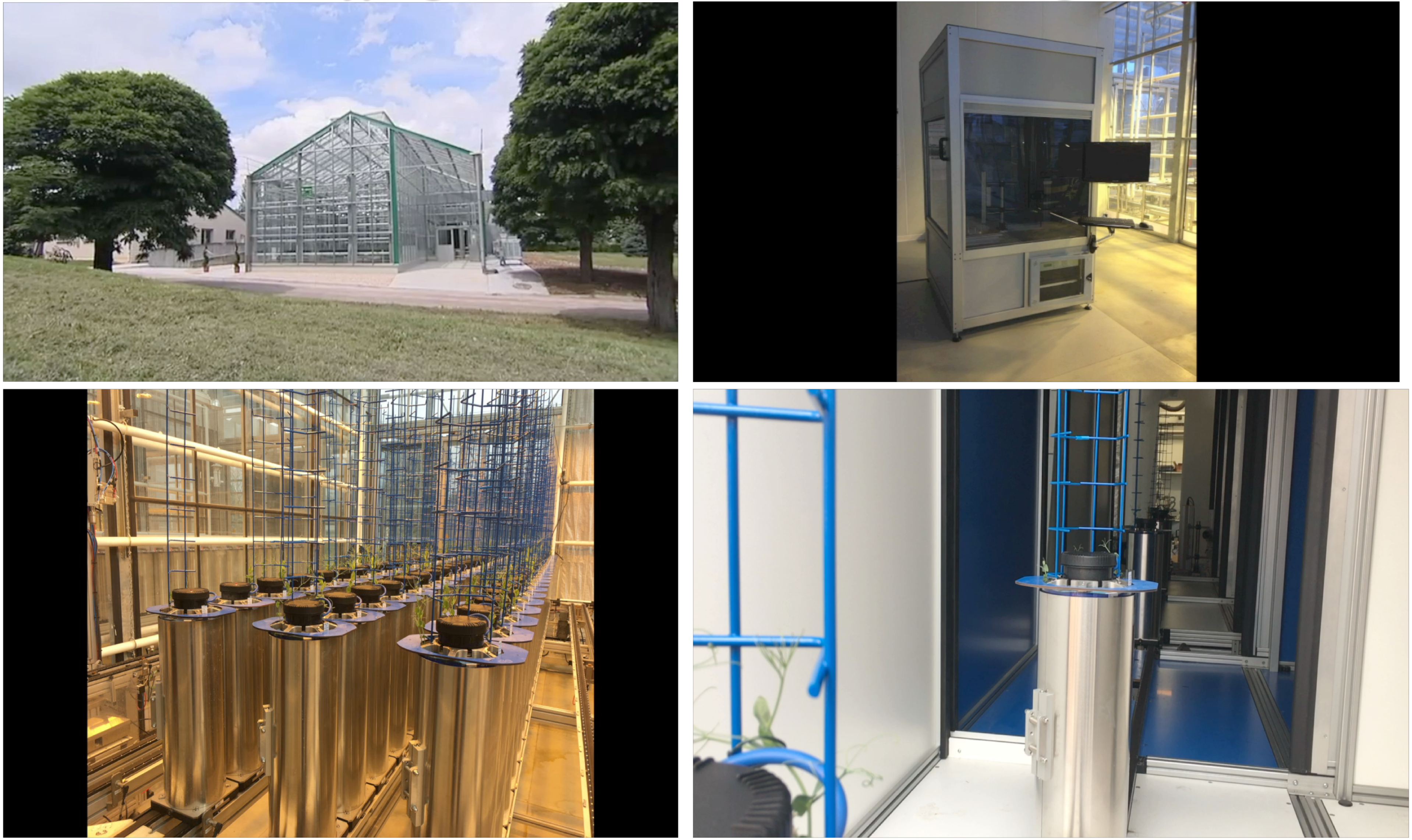
Base for conveyors

PHENOME
Réseau Français Phénomique végétale
F P P N

What is needed?

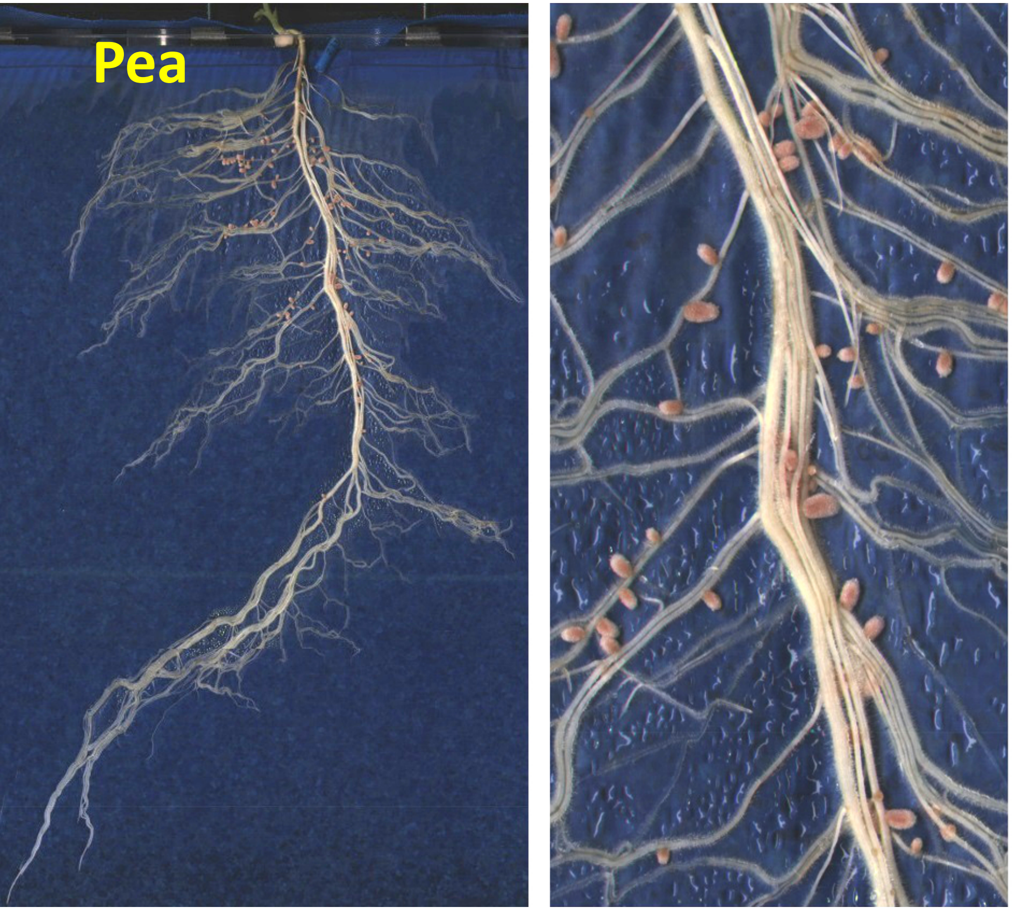
A platform with imaging cabins

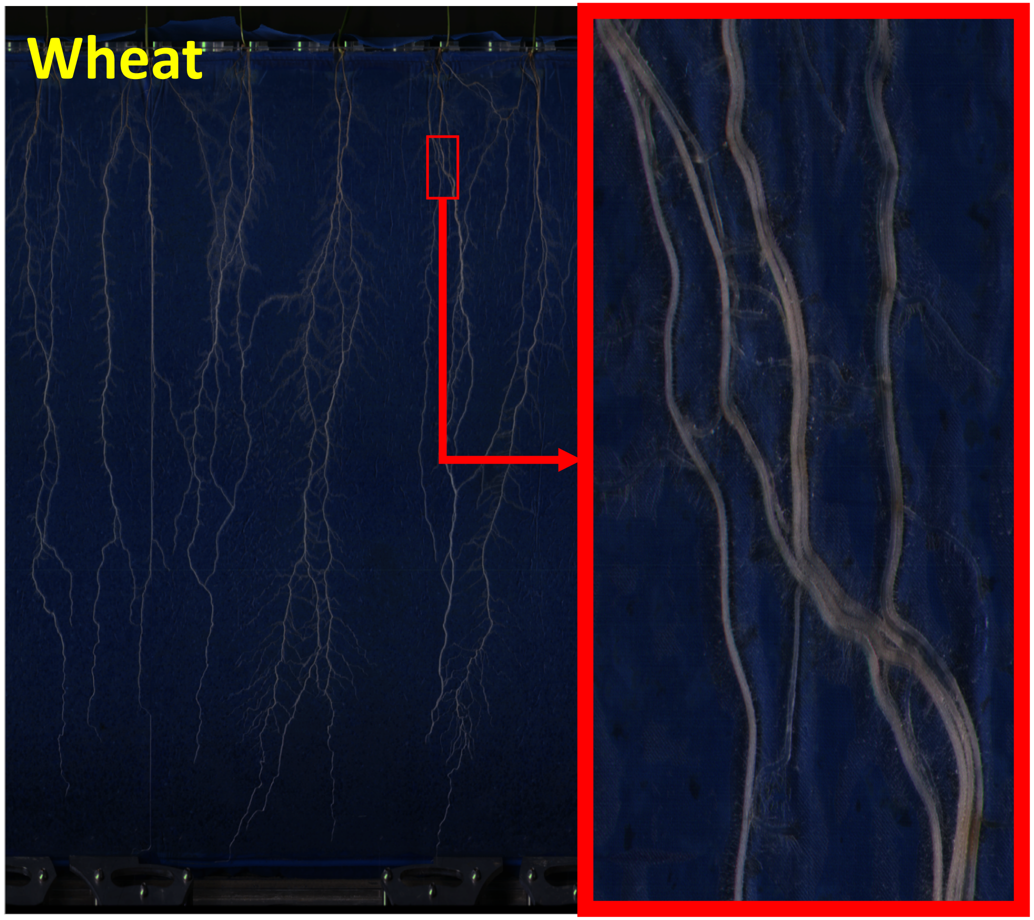
4PMI: Plant Phenotyping Platform for Plant and Microorganisms Interactions



What is needed?

High resolution images





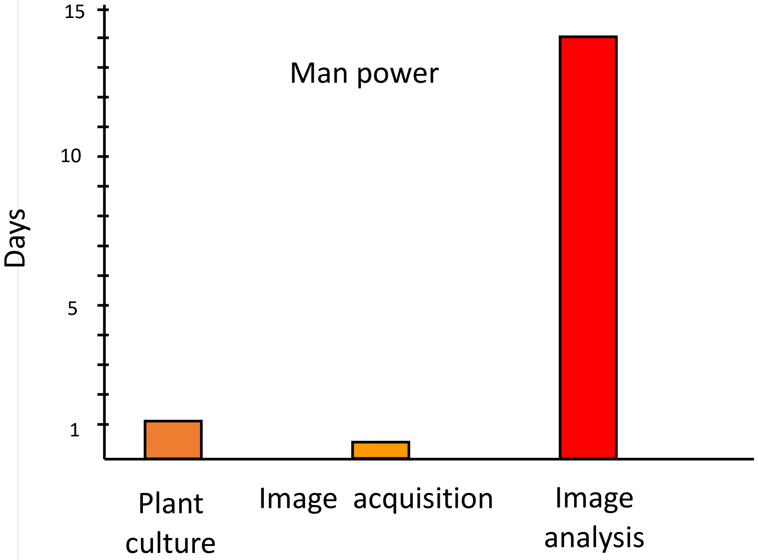
What is needed?

Image analysis

All 142 plant image analysis software solutions:

An online database for plant image analysis software tools
Lobet G., Draye X., Périlleux C.
2013, Plant Methods, vol. 9 (38)
View at publisher | Download PDF

http://www.plant-image-analysis.org/

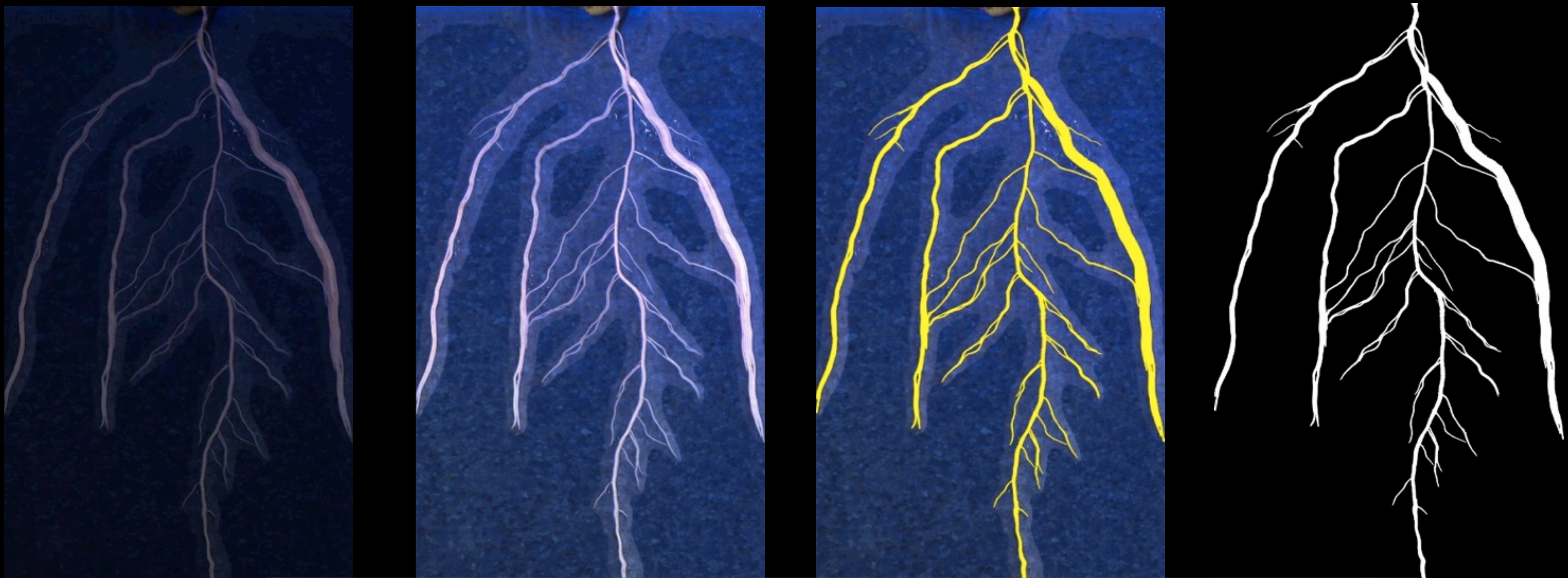


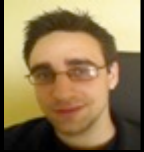
Expert mode

Root system architecture analysis is a bottleneck

What is needed?

Segmentation software



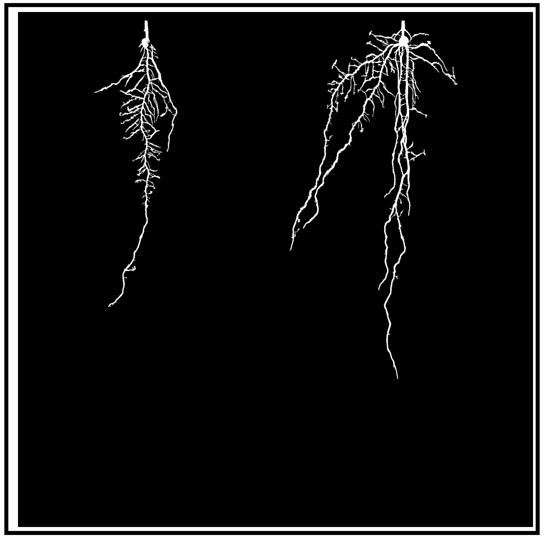


M. Lamboeuf

What is needed?

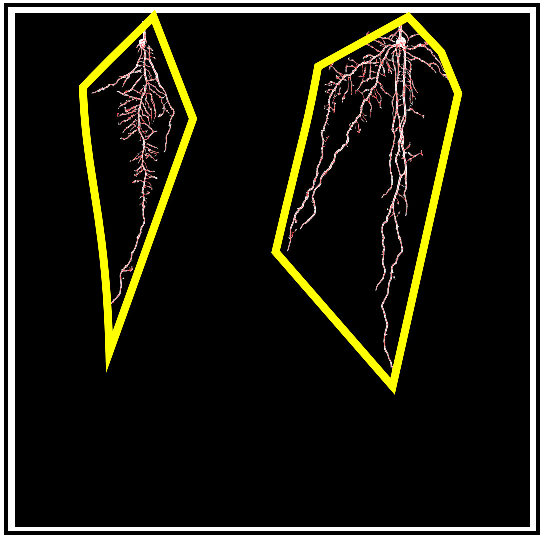
From images to architecture

Original Image

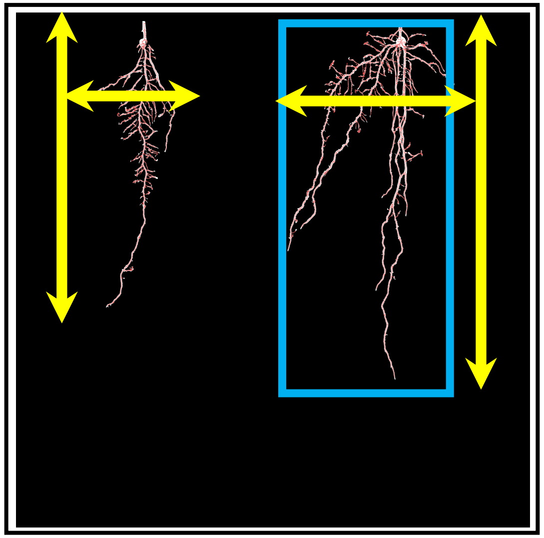


Segmentation

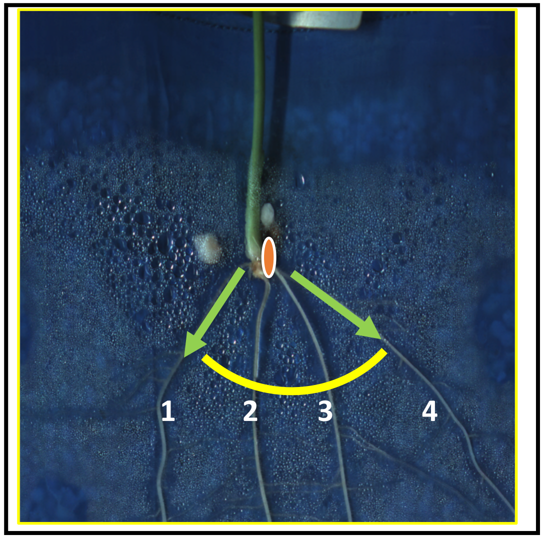
Squeletton



Convex Hull RSA density



Max width, height, box

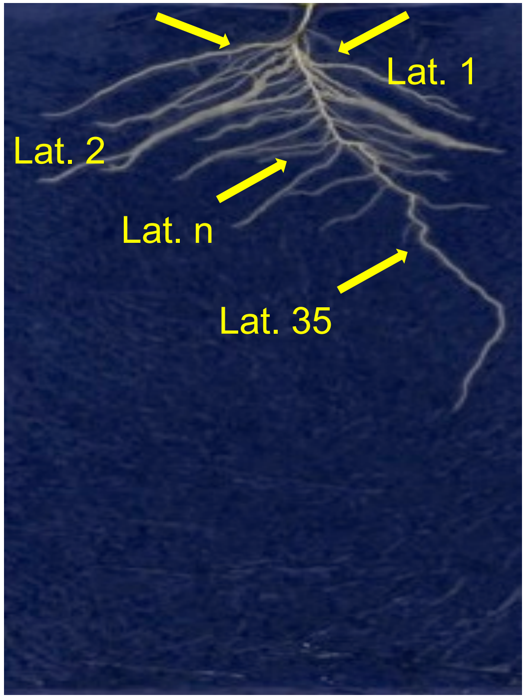


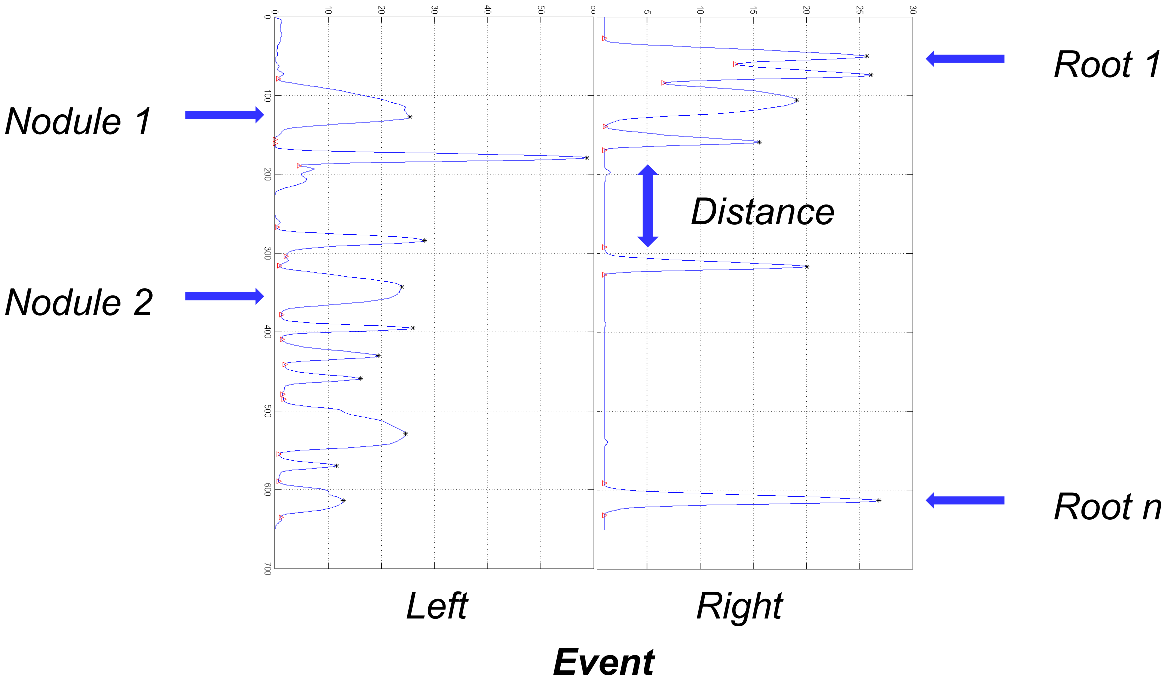
Angle, main roots

What is needed?

Detecting root and nodules “events”

Roots, detect events: lateral roots and nodules detection





Han et al, Machine Vision 2017

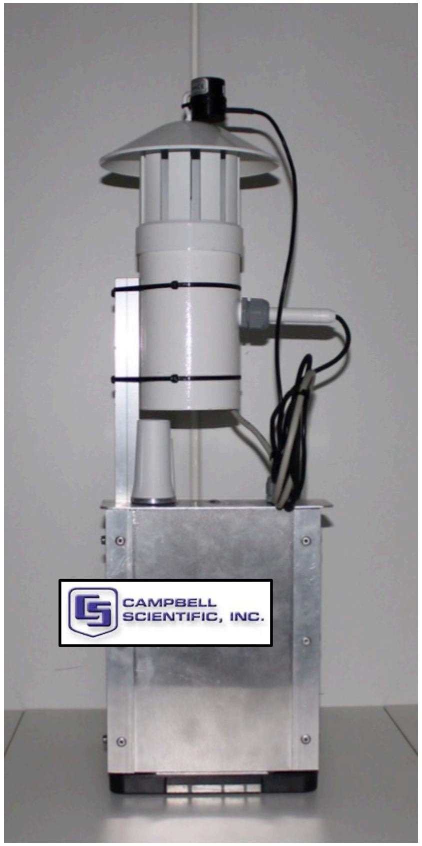
Nodules and lateral roots detection

What is needed?

Environmental conditions

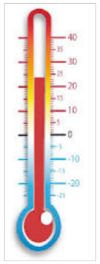
❑ Greenhouse, growth chambers env. conditions fluctuate and are not wholly controlled…

❑ “Phenotype = expression of genotype in a given environment”



Autonomous climate central

As much as possible sensors

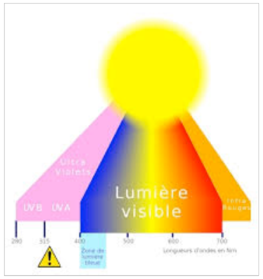


Temperature

(soil, air)

Hygrometry

(soil, air)



PAR sensor

✓ **Mapping of environmental conditions sensed by the plants with (numerous) sensors**

What is needed?

Upstream tools & methods


Automatic production of nutritive solutions


Parts desinfection & cleaning


Mixing substrates

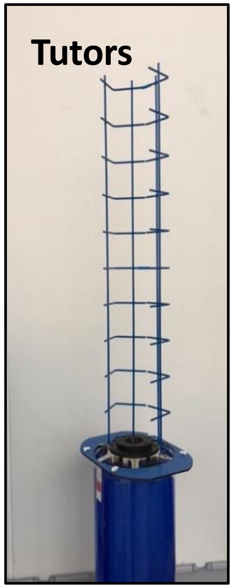

Washing rought substrates

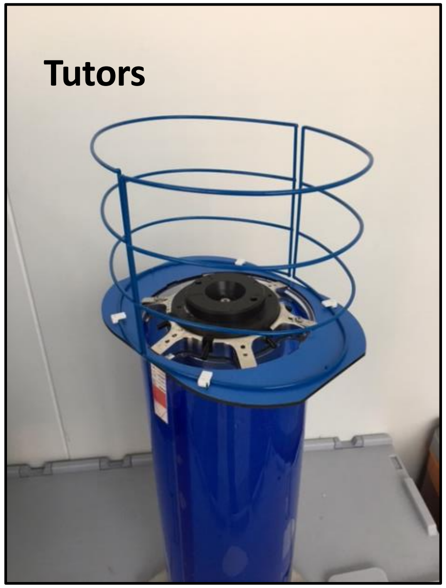
What is needed?

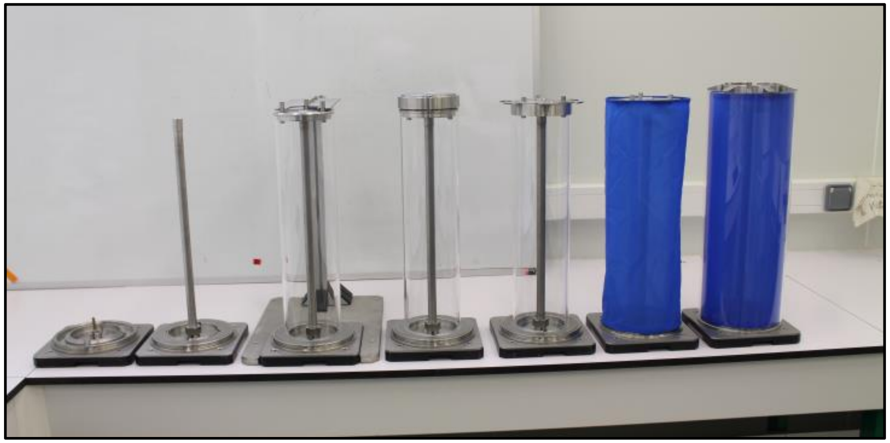
Upstream tools & methods


Assembling RhizoTubes©


Germination chamber


Tutors

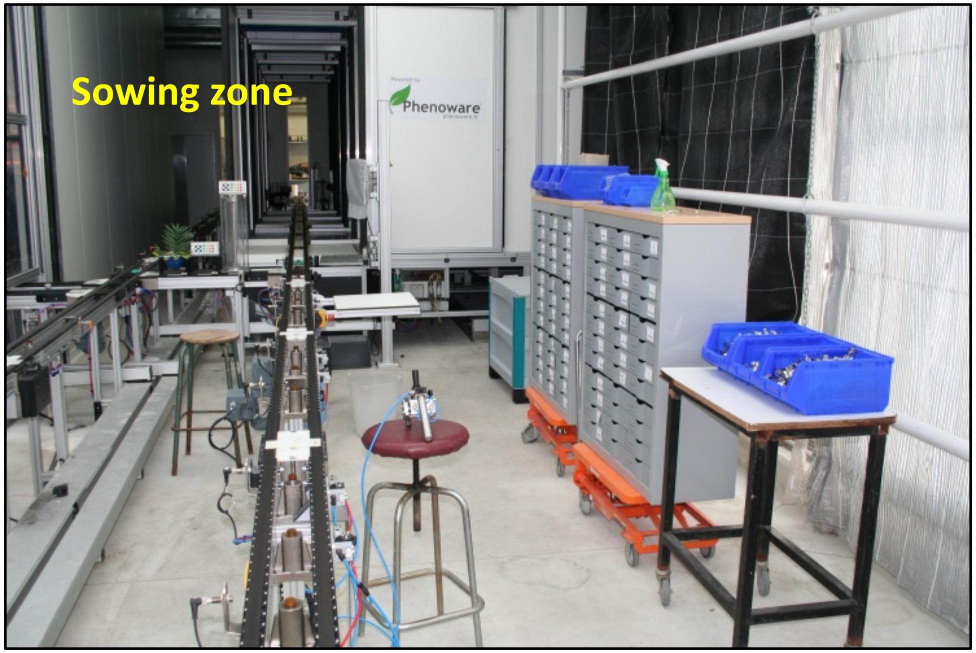

Tutors

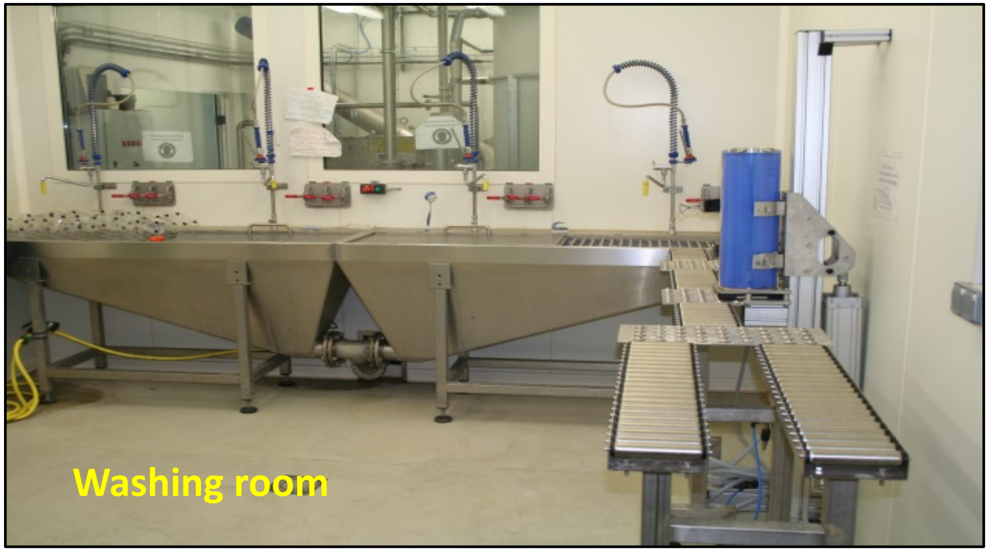


What is needed?

Upstream tools & methods

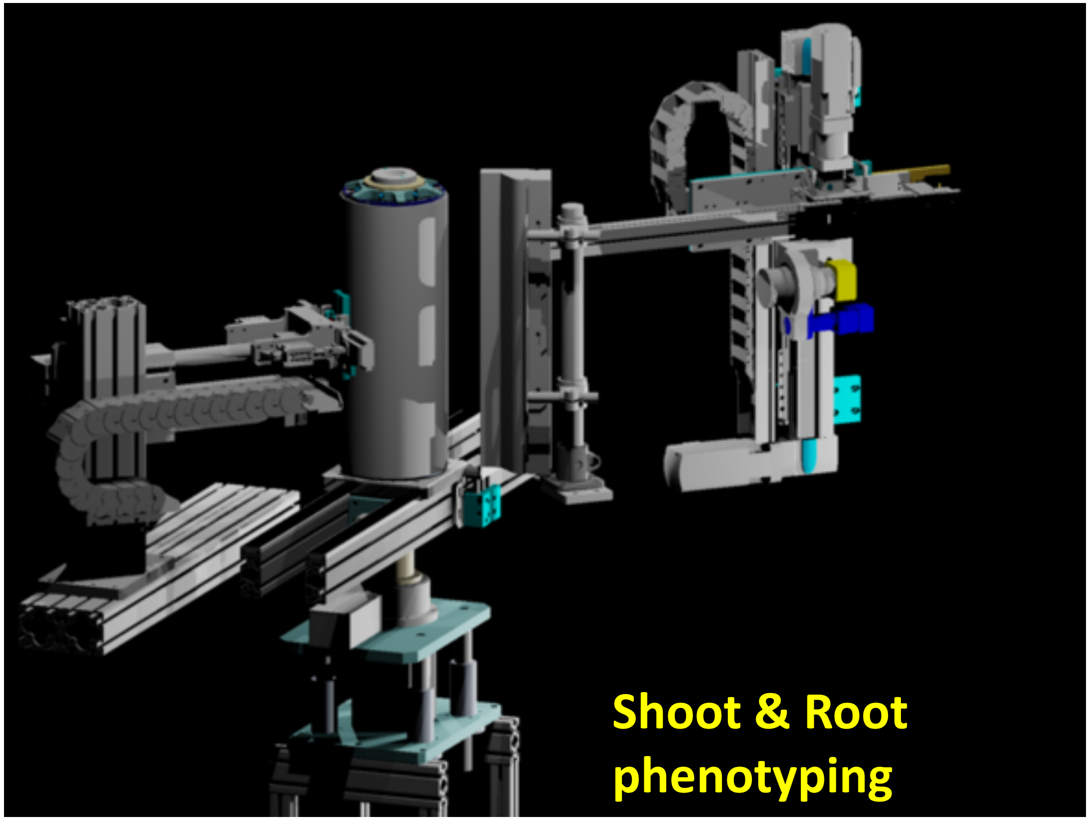

Automatic empoting

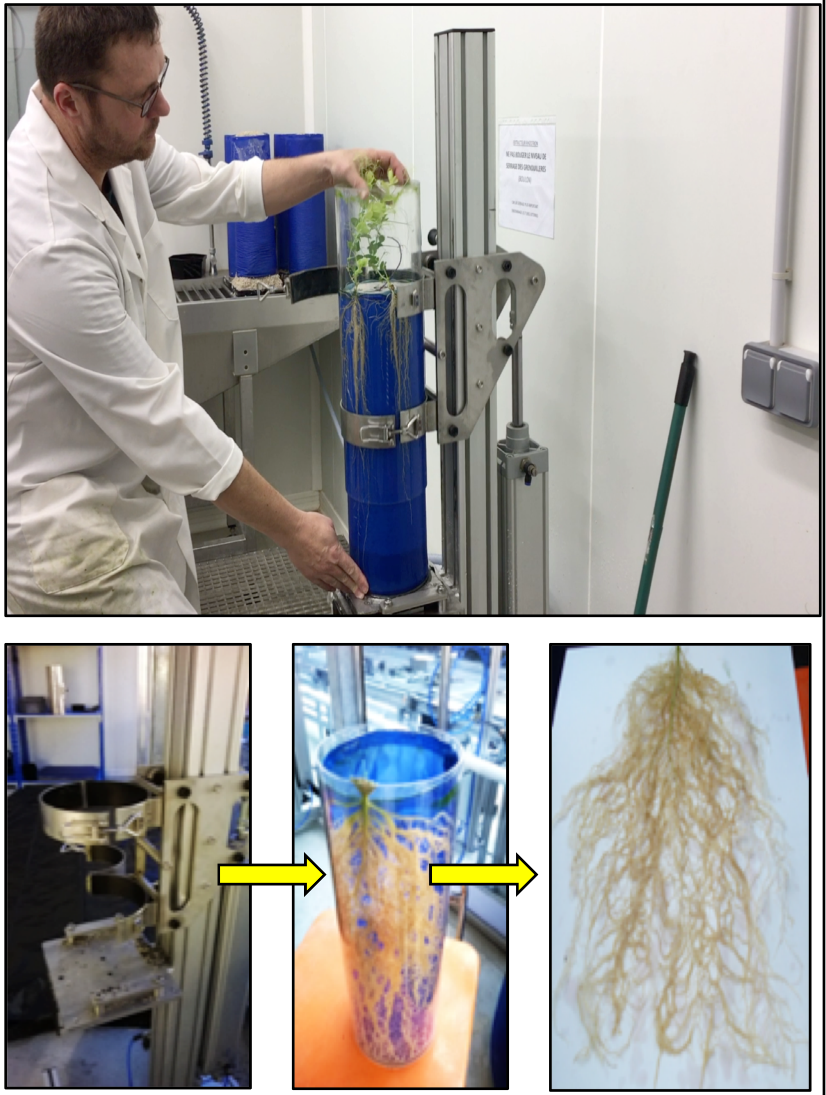

Sowing zone


Washing room

What is needed?

Upstream tools & methods





Root recovery

Take into account the whole chain of high throughput phenotyping !

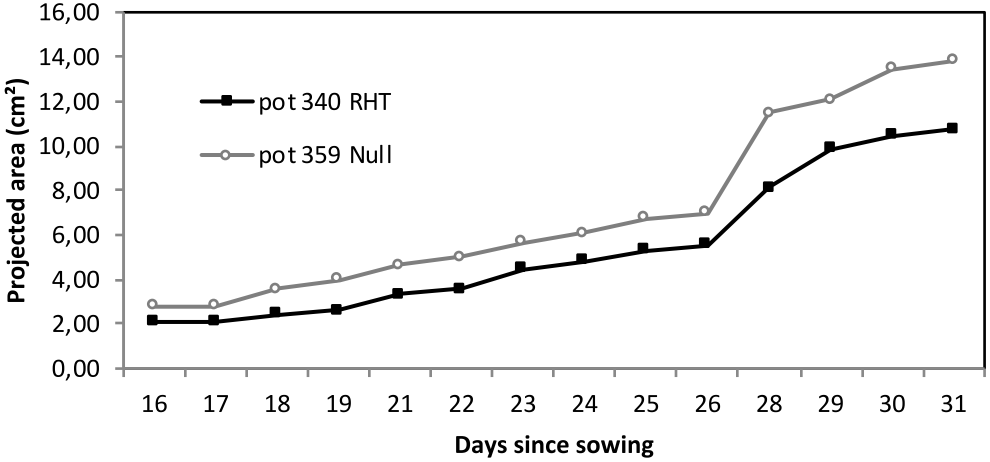
What for ?

Dynamic trait dynamics: wheat exemple

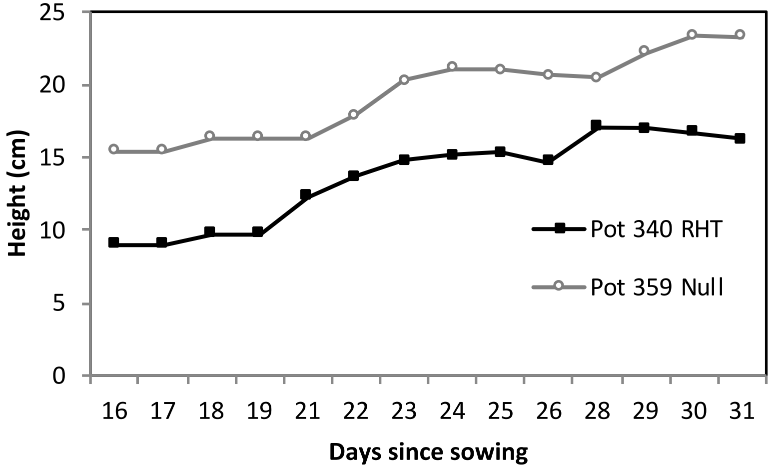

Tall (Null)


Dwarf (RHt)

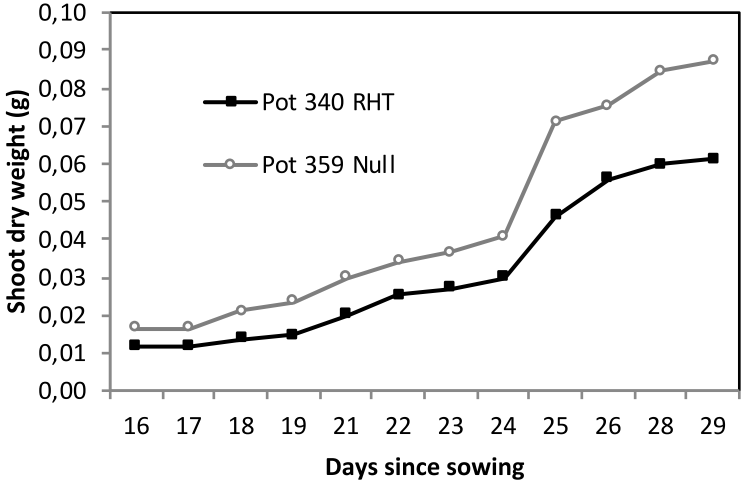
Projected area from images



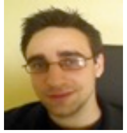
Plant Height from image



Estimated Shoot Dry Weight




Josh Klein

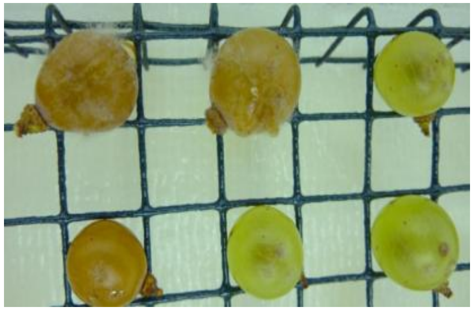

M Lamboeuf

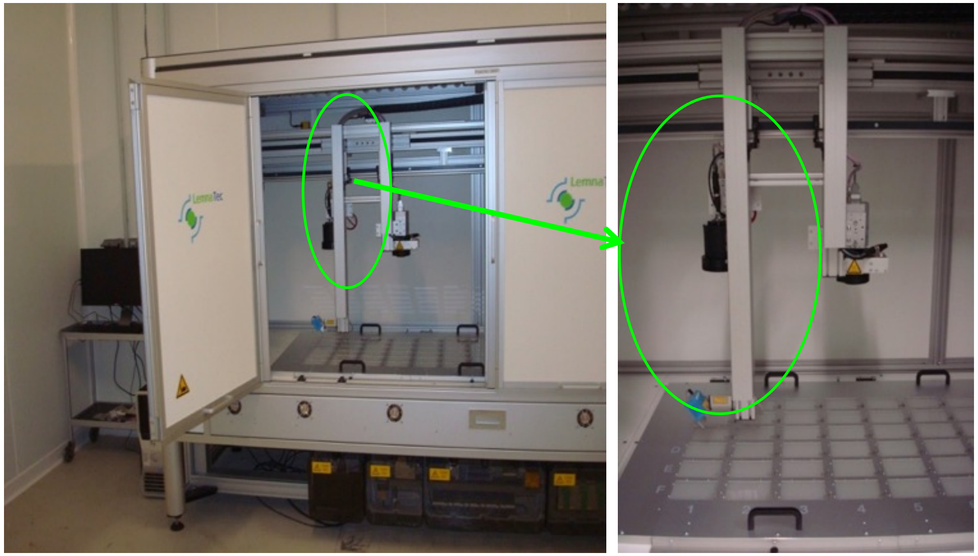
What for ?

Detecting grape deseases

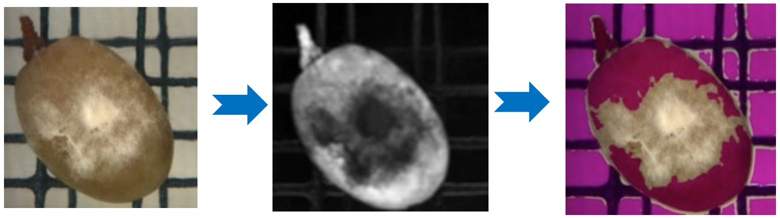
F Cointault
(INRA, Dijon)

Major problem in viticulture ➔ cryptogamic deseases (e.g. oïdium)





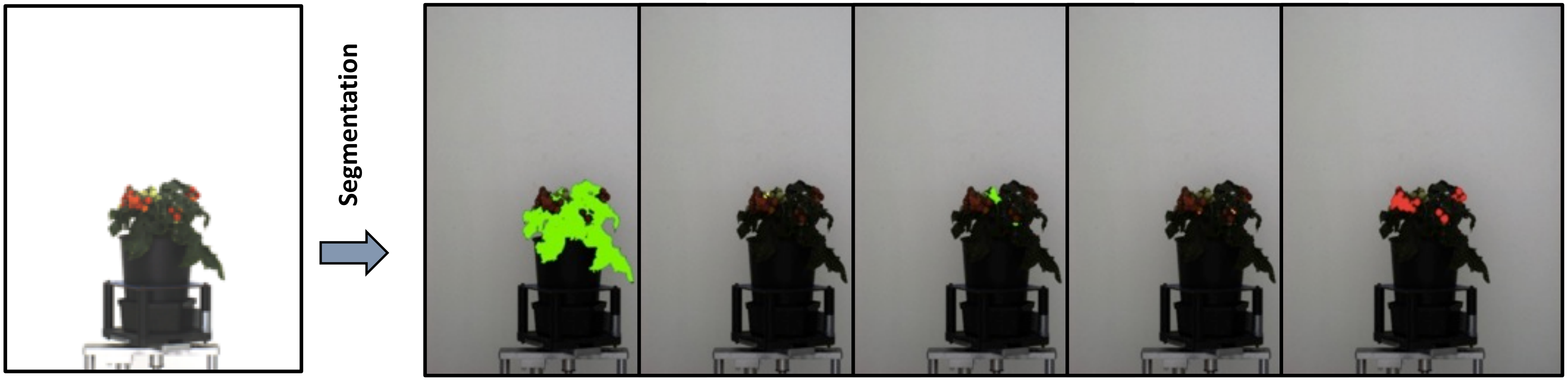
Hybrid spaces : RVB images in colorimetric spaces integrating texture



What for ?

Predicting phenology and fruit maturation

C Rothan
(BFP, INRA)



Original image

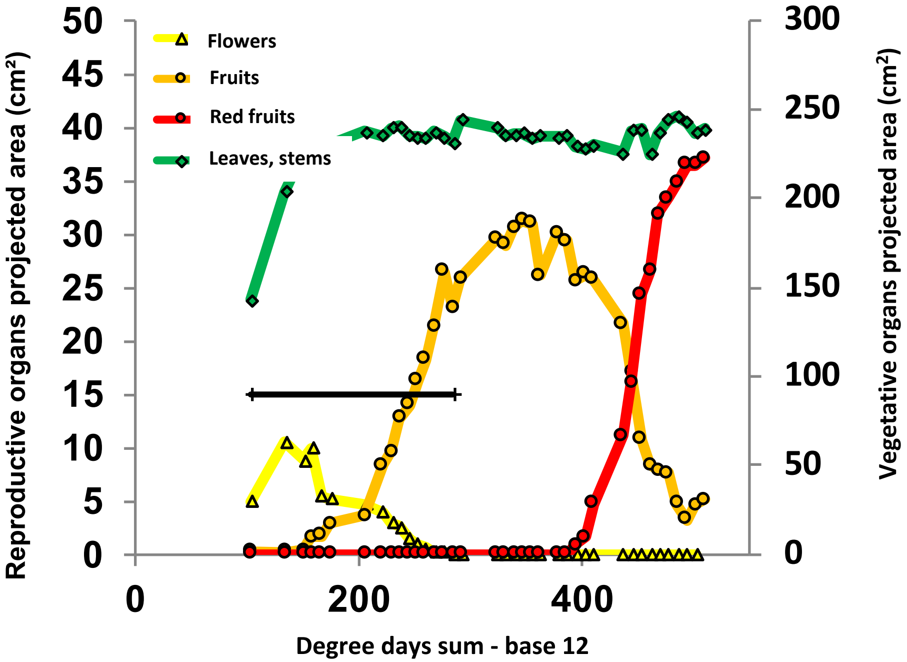
Leaf

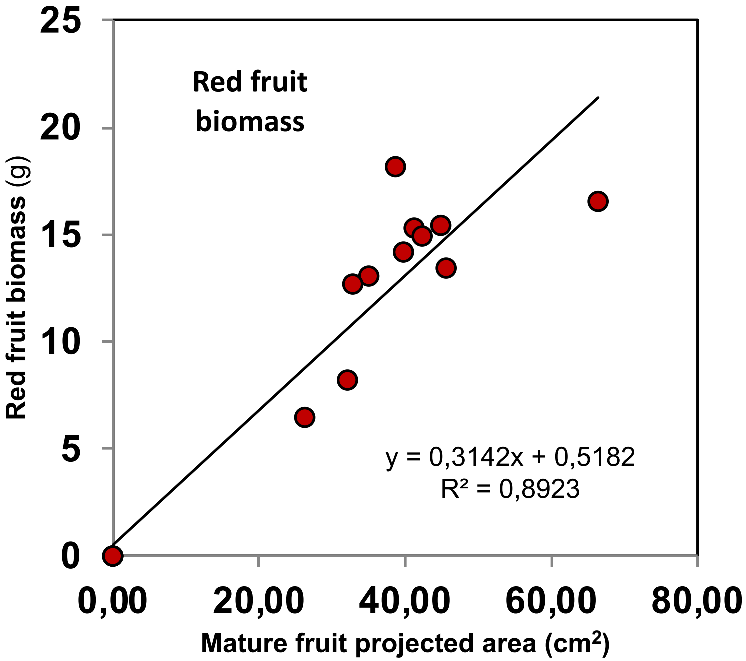
Flowers

Green fruit

Orange fruits

Red fruits

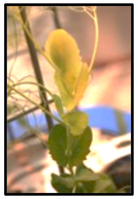


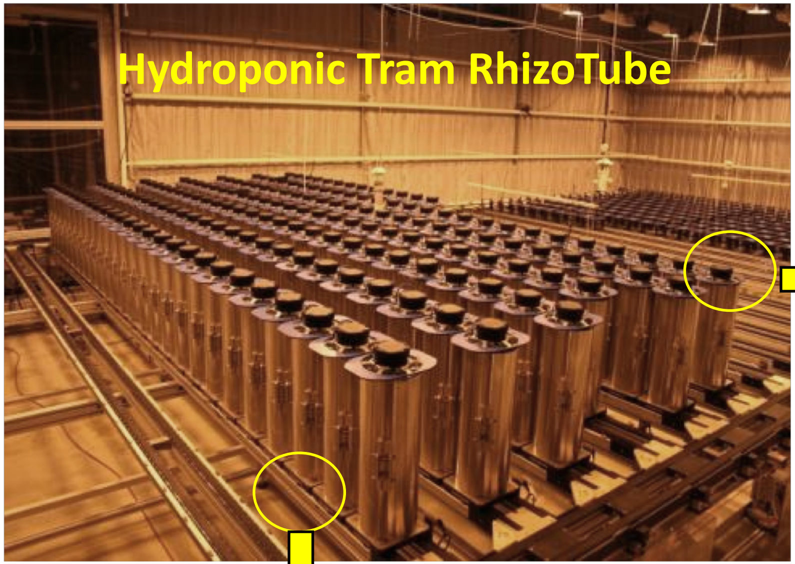


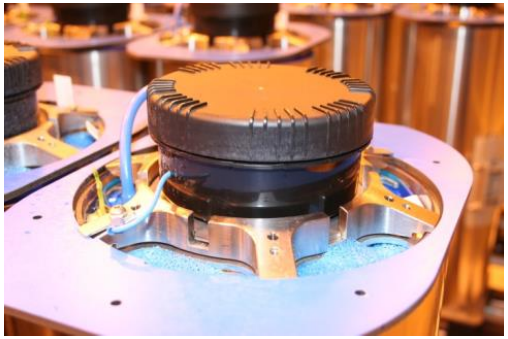
Algorithms adapted to follow phenology and predict fruit maturation

What for ? Assessing genotype capacity to valorize low ressources

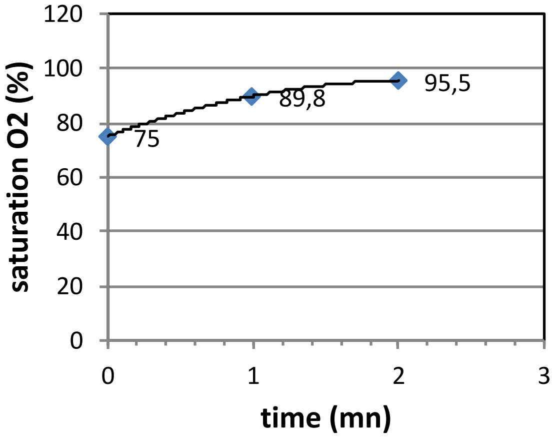
Iron Chlorosis:





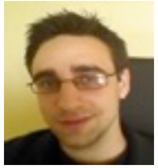


Air bubling pump



Electrified (low tension..) conveyors

C Jeudy
P Declerck



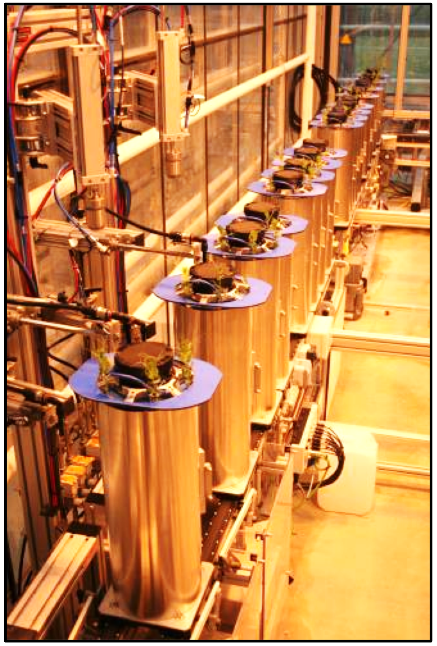
M Lamboeuf

What for ? Assessing genotype capacity to valorize low ressources

Iron Chlorosis:

28 genotypes, 3 Plants per RT (6 replicates/genotype, 30 days (approx. 600°Cj), Shoot phenotyping (3/week)

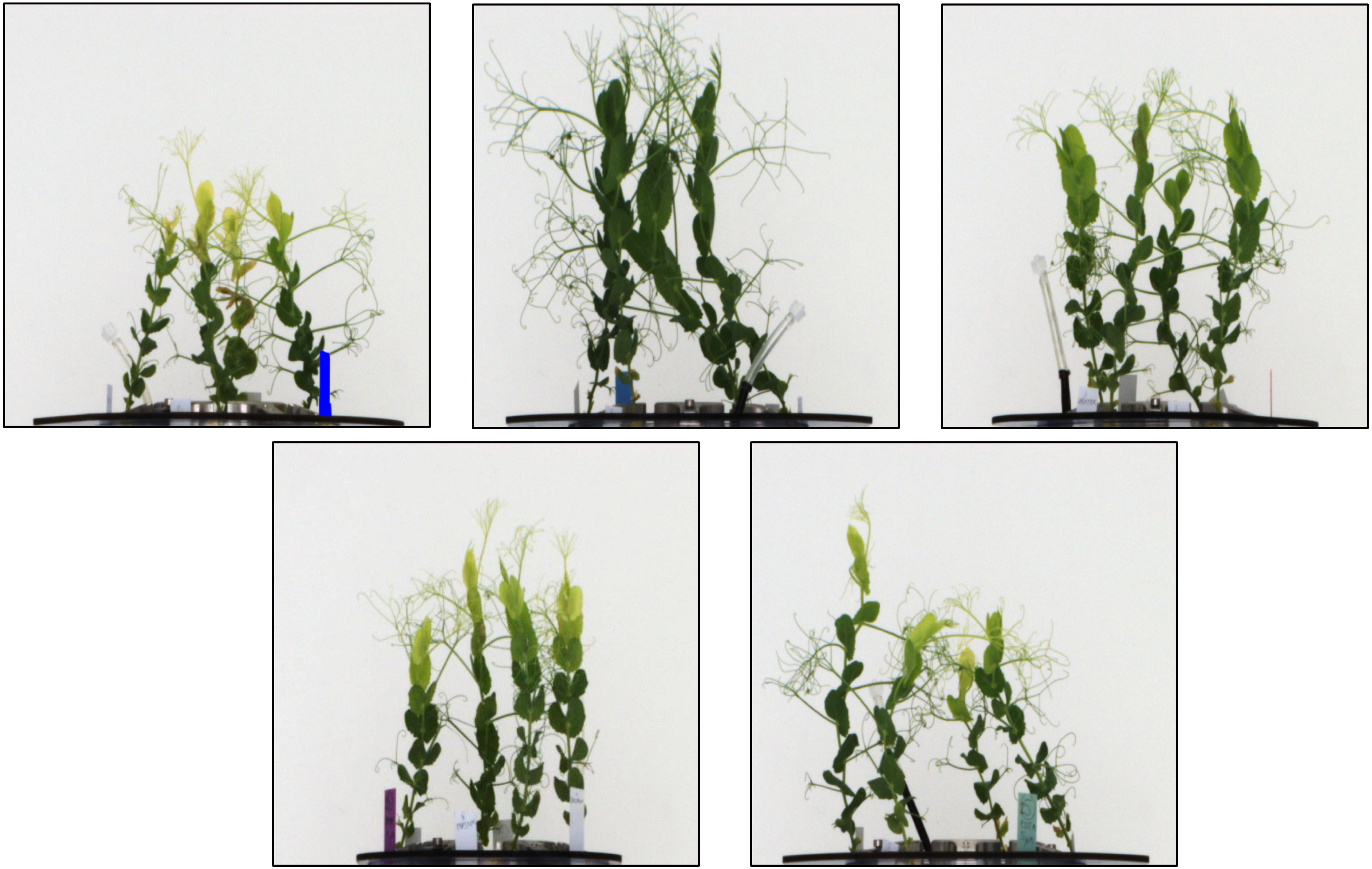
FIPGSO059	FIPGSO140	FIPGSO214	FIPNPZ10	R2NPM088	R2NPM171	GANGSTER
FIPGSO086	FIPGSO170	FIPGSO349	R2NPM015	R2NPM160	BALLTRAP	INDIANA
FIPGSO093	FIPGSO176	FIPGSO352	R2NPM040	R2NPM167	CASPER	ISARD
FIPGSO137	FIPGSO179	FIPGSO399	R2NPM068	R2NPM170	DEXTER	MYSTER



What for ?

Color classes, examples

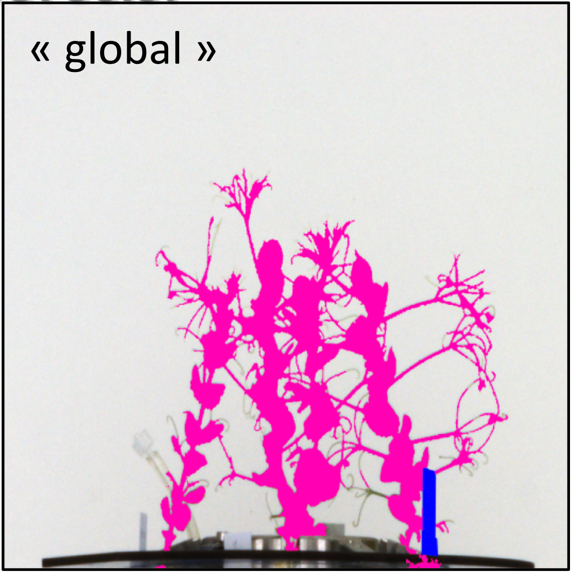
Iron Chlorosis:



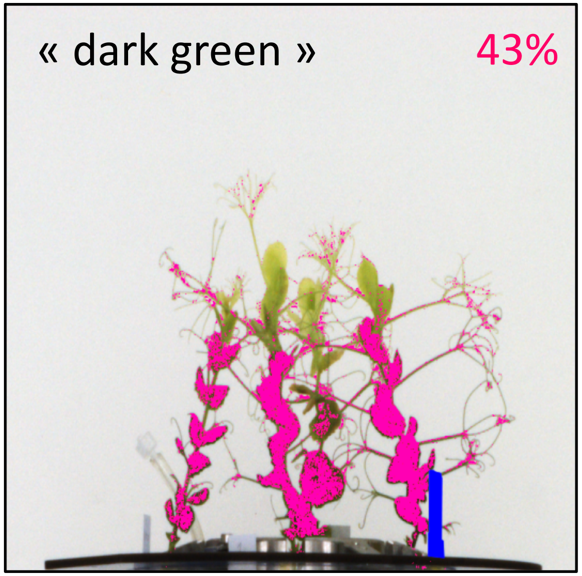
What for ?

Color classes, examples

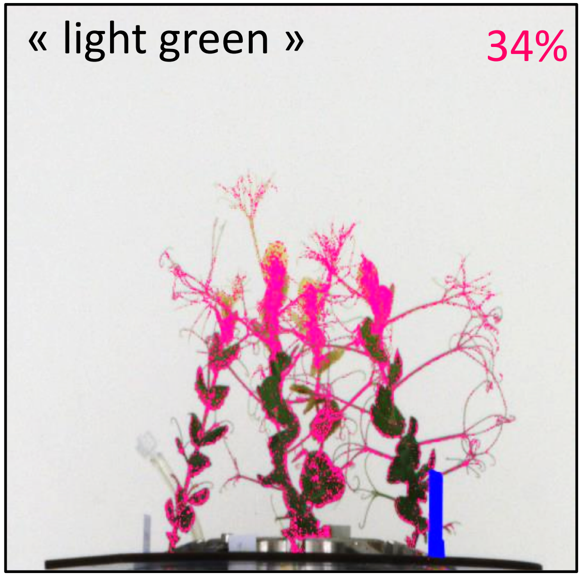
Iron Chlorosis:



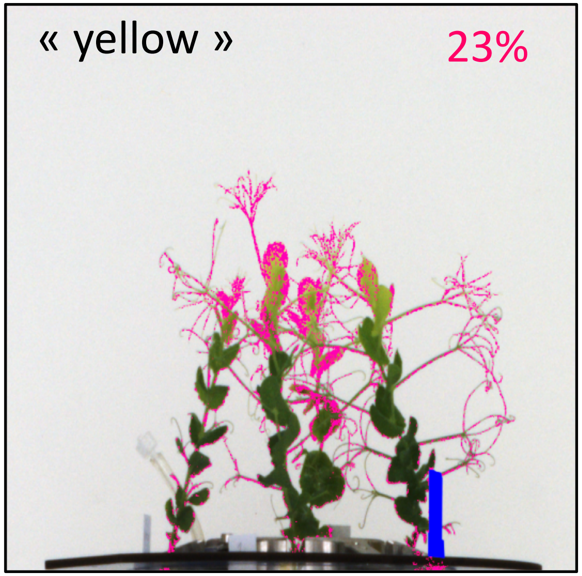
« global »



« dark green » 43%



« light green » 34%



« yellow » 23%

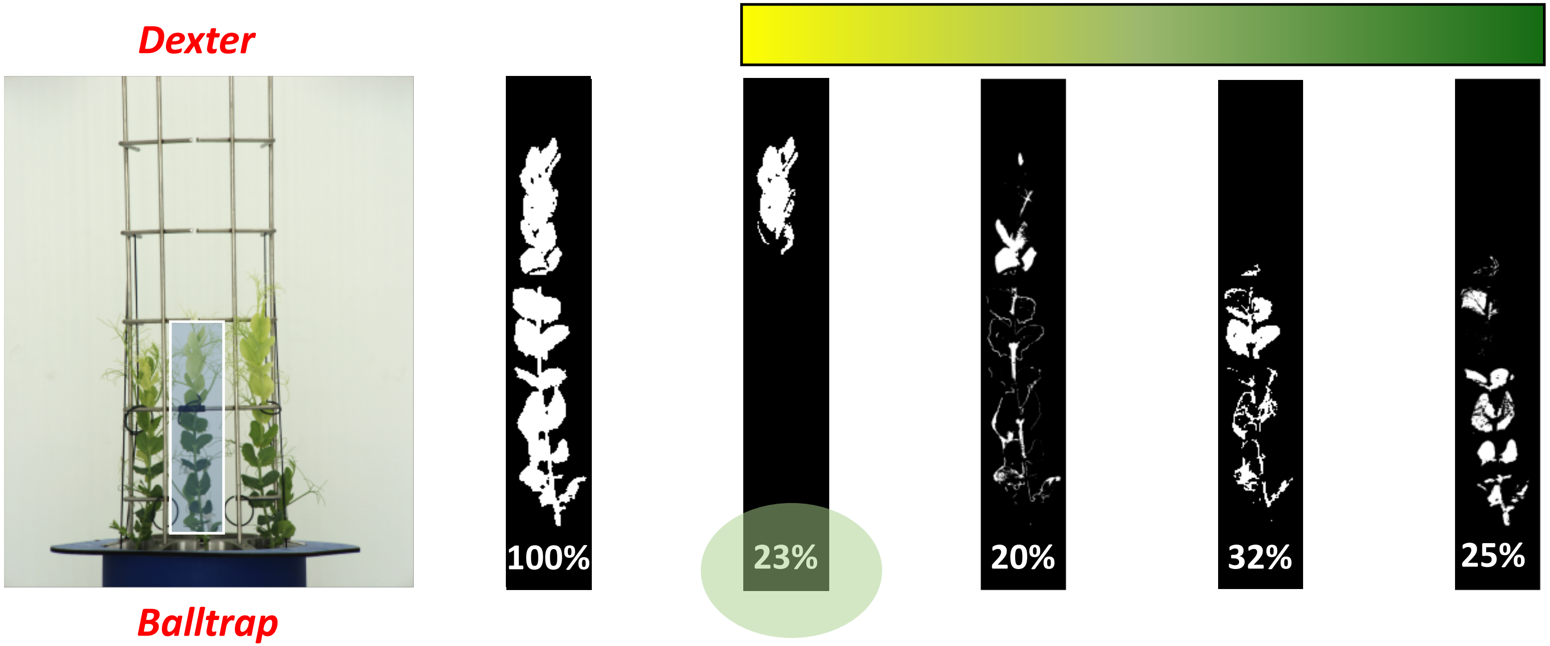
What for ?

Color classes, examples

Iron Chlorosis:

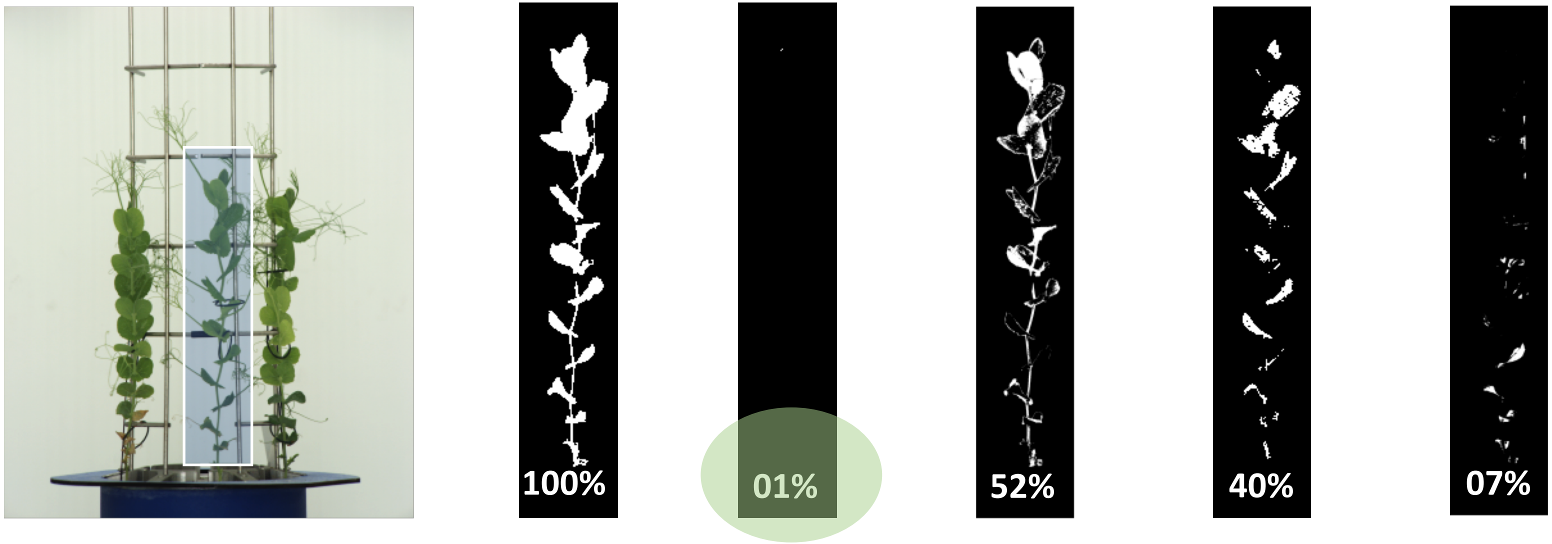
Dexter

Chlorosis Sensible



Balltrap

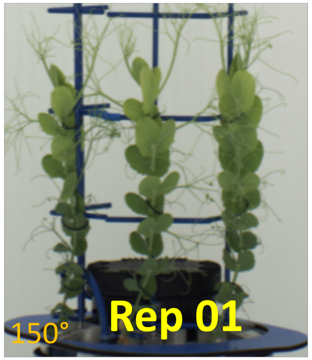
Tolerant

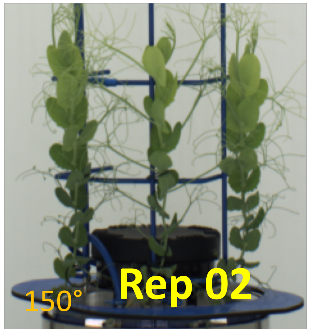


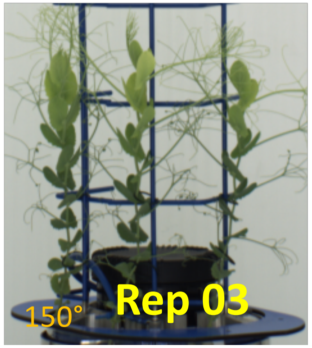
What for ?

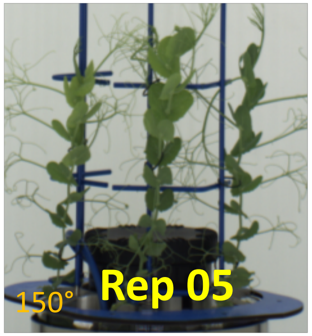
Iron chlorosis identity card

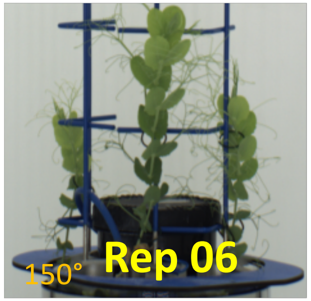
Balltrap





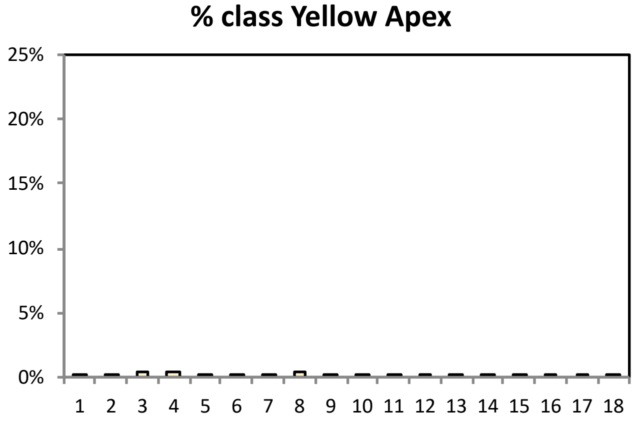


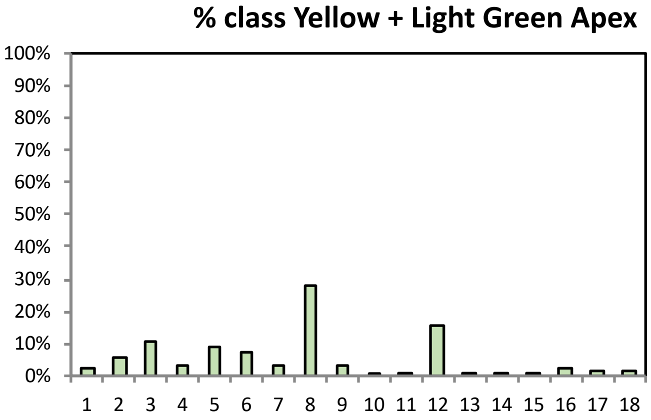




1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0,1%	0,0%	0,3%	0,3%	0,1%	0,1%	0,1%	0,4%	0,1%	0,1%	0,1%	0,3%	0,1%	0,0%	0,0%	0,1%	0,0%	0,1%
2,2%	5,8%	10,4%	3,4%	9,1%	7,2%	3,1%	28,1%	3,5%	0,9%	0,7%	15,5%	0,7%	0,7%	0,3%	2,1%	1,2%	1,6%

	Mean	ET
% Y apex	0,1%	0,1%
% Y+LG apex	5,4%	7,0%

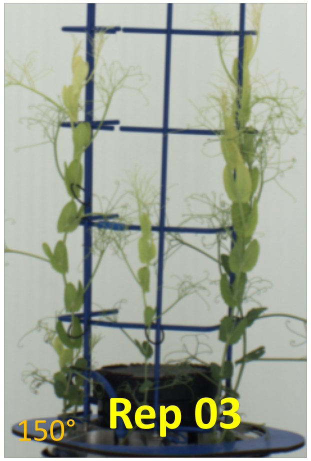


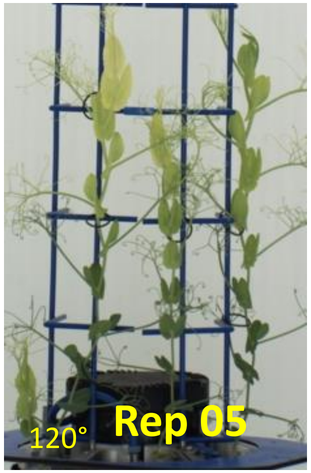


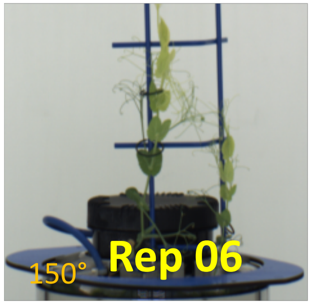
What for ?

Iron chlorosis identity card

FIPGSO093

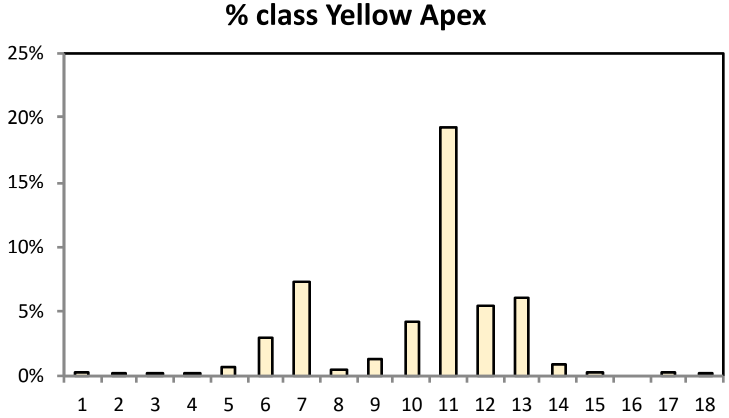


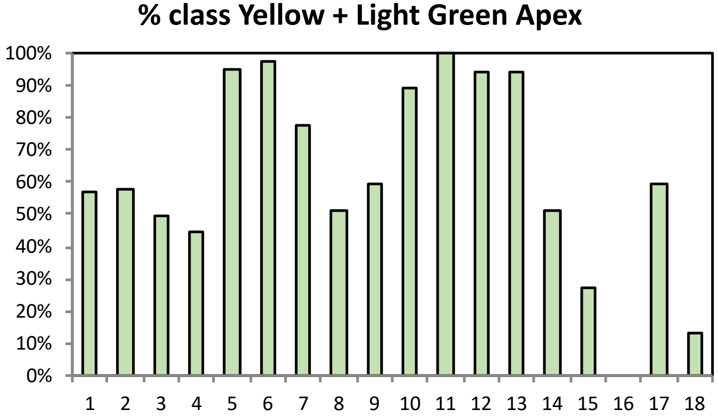




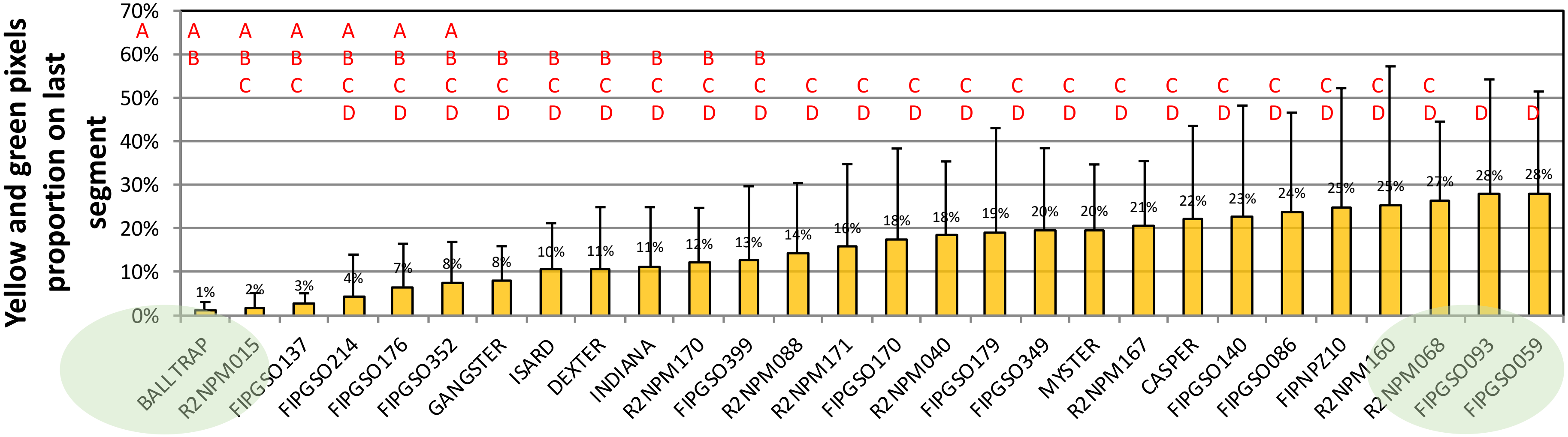
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0,3%	0,1%	0,1%	0,1%	0,7%	3,0%	7,4%	0,6%	1,3%	4,1%	19,2%	5,5%	6,1%	0,9%	0,3%		0,4%	0,1%
57,3%	58,0%	49,3%	44,5%	95,2%	97,2%	78,0%	51,4%	59,4%	88,9%	100,0%	94,0%	94,2%	51,3%	27,5%		59,1%	13,2%

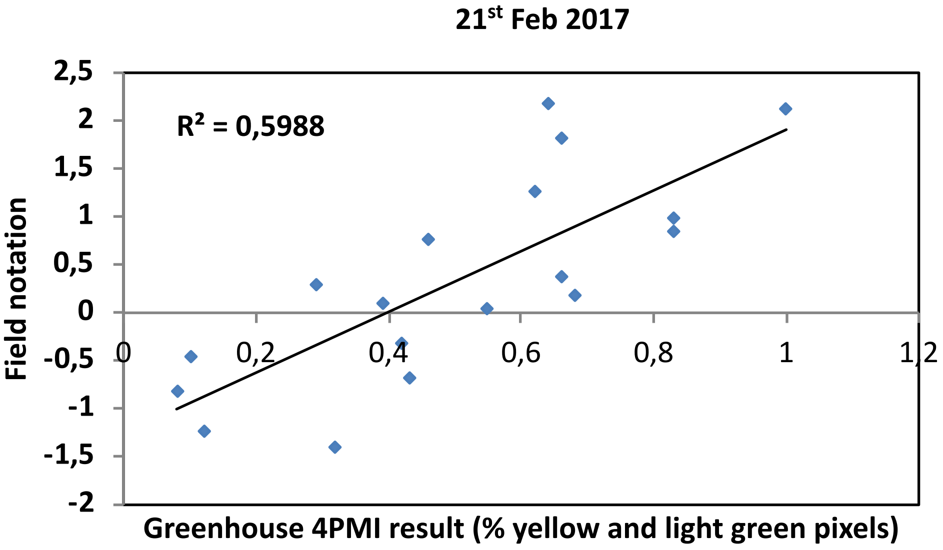
	Mean	ET
% Y apex	2,9%	4,8%
% Y+LG apex	65,8%	26,1%





Field notation vs 4PMI Ranking





80 genotypes were screened, next

- **5 RT repeats (10 plants - 400 RT)**
- **Growth + 6 days**
- **300 genotypes**

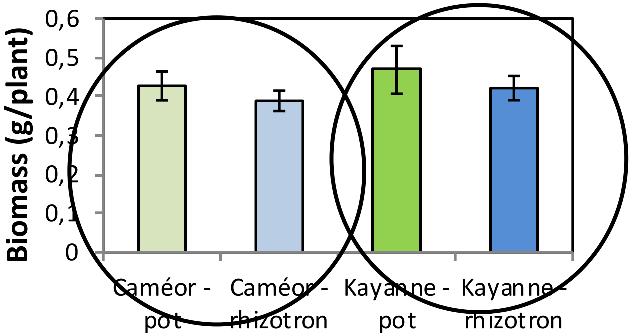
What for ?

Mimicking pot growth

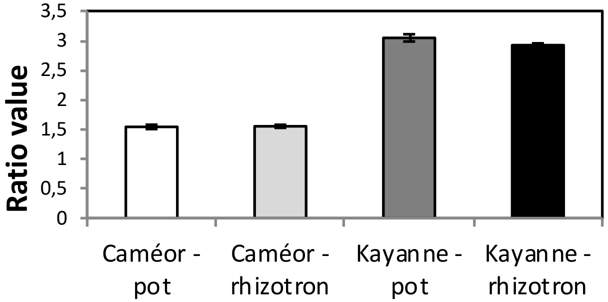
Similar traits either in RT or pots: the pea example

C. Jeudy

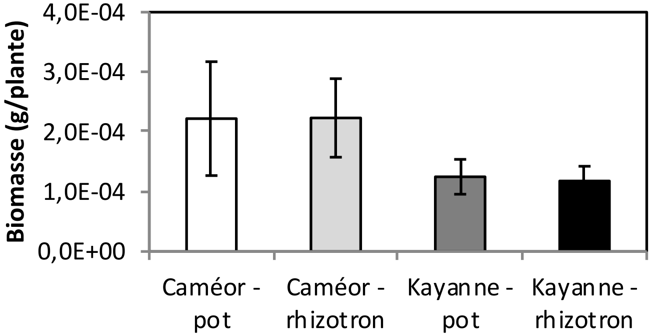
Plant biomass



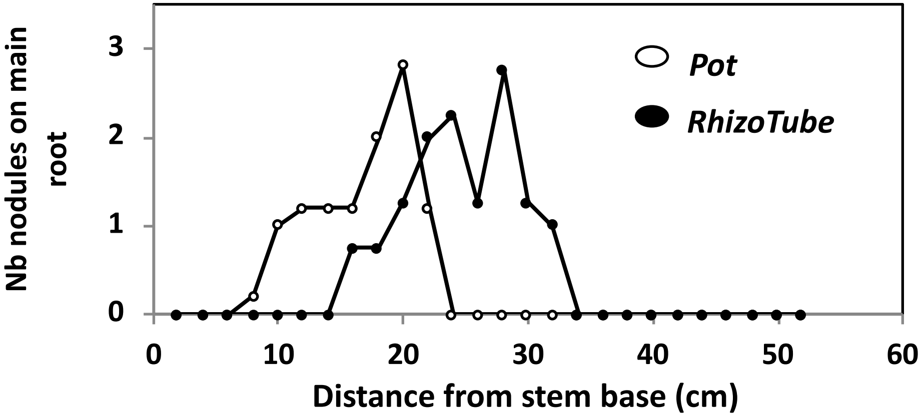
Shoot DW/Root DW

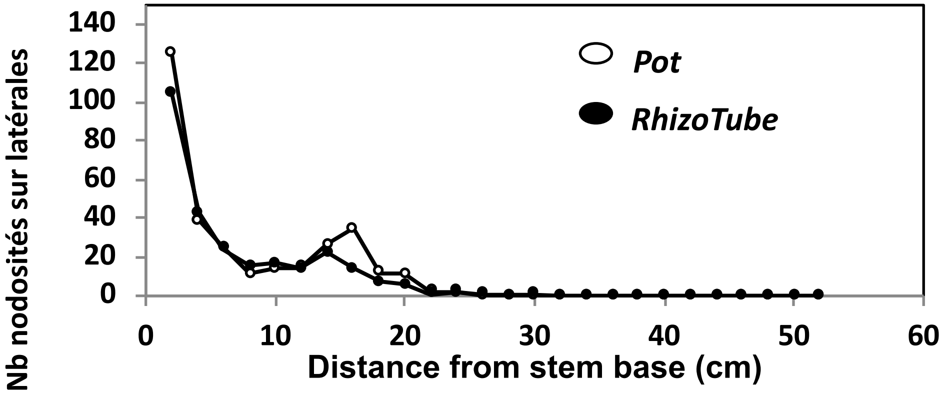


Mean nodule DW



Same distribution profile for noudles

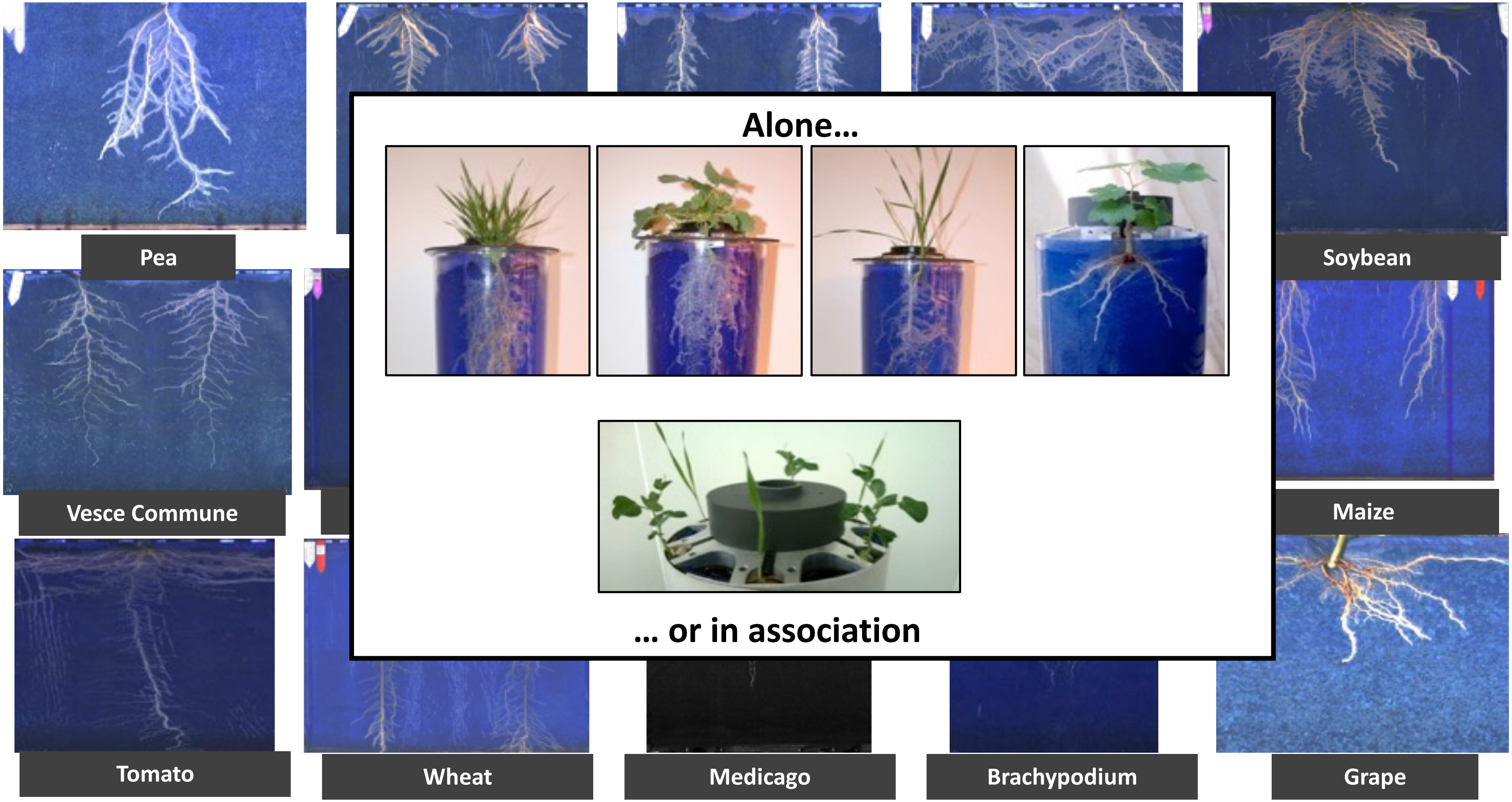




Ok but not relevant to field and each field is different from another !

What for ?

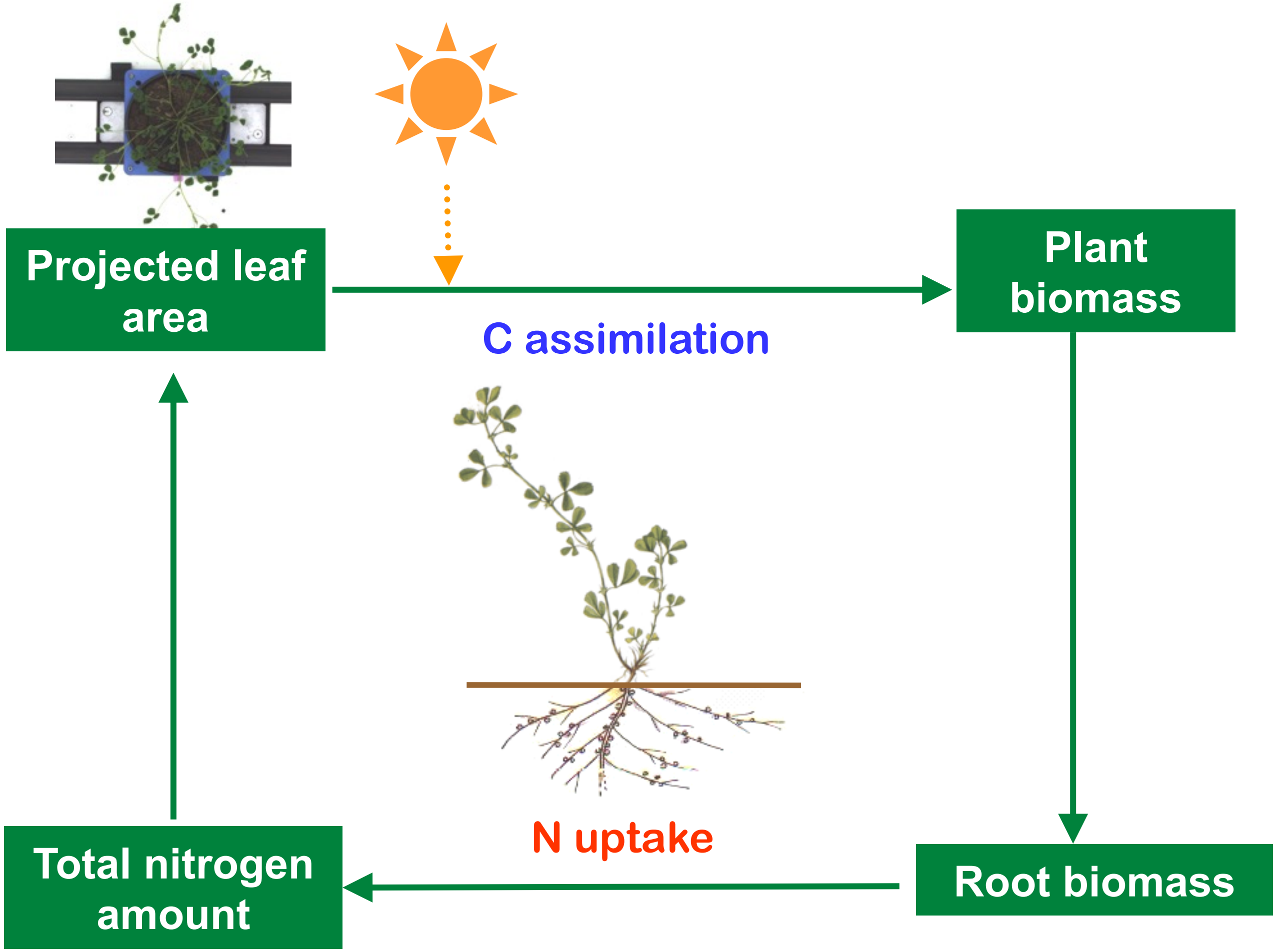
Various species



Alone…

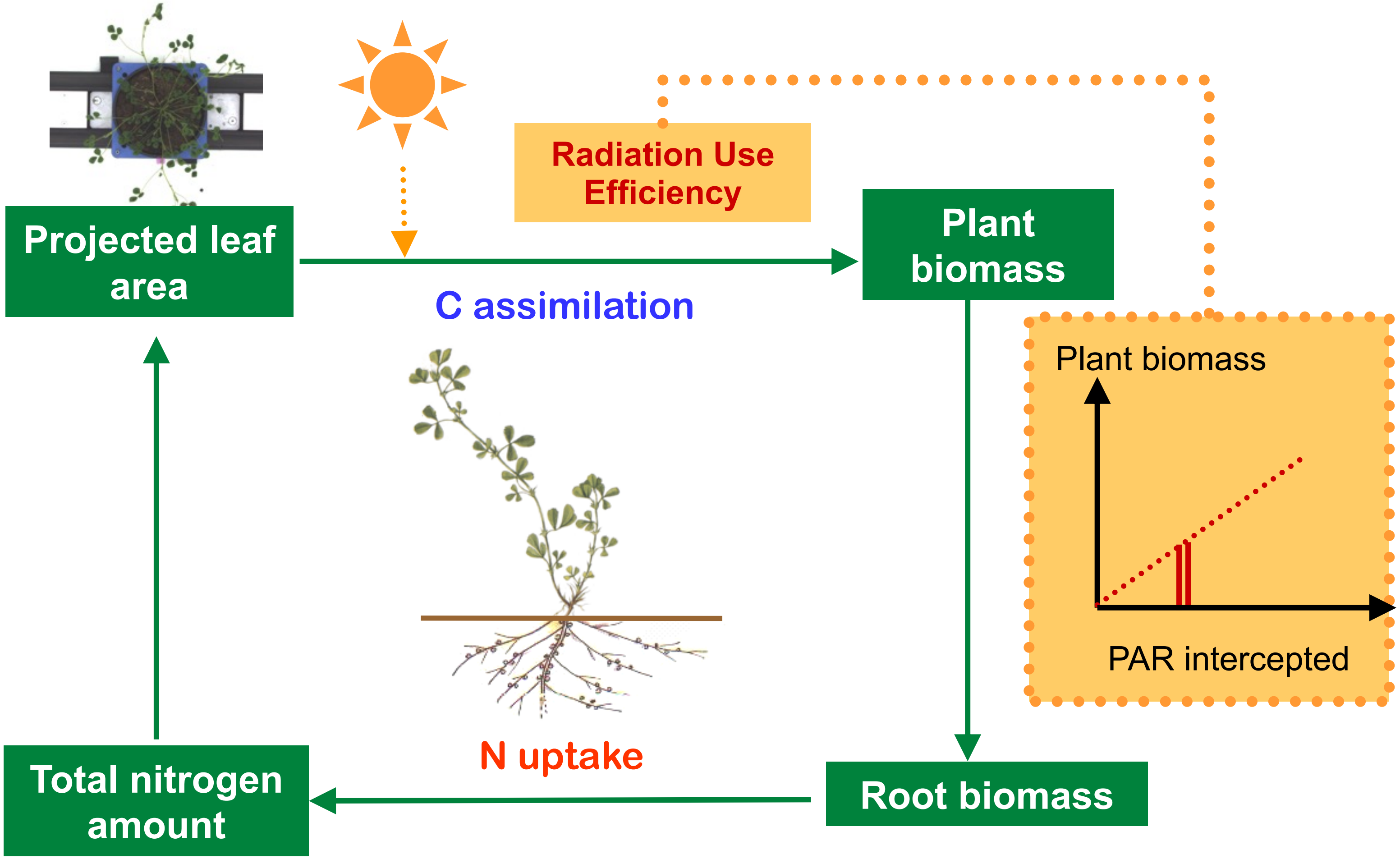
… or in association

What for ?



D Moreau

Model structure



Projected leaf area

Radiation Use Efficiency

Plant biomass

C assimilation

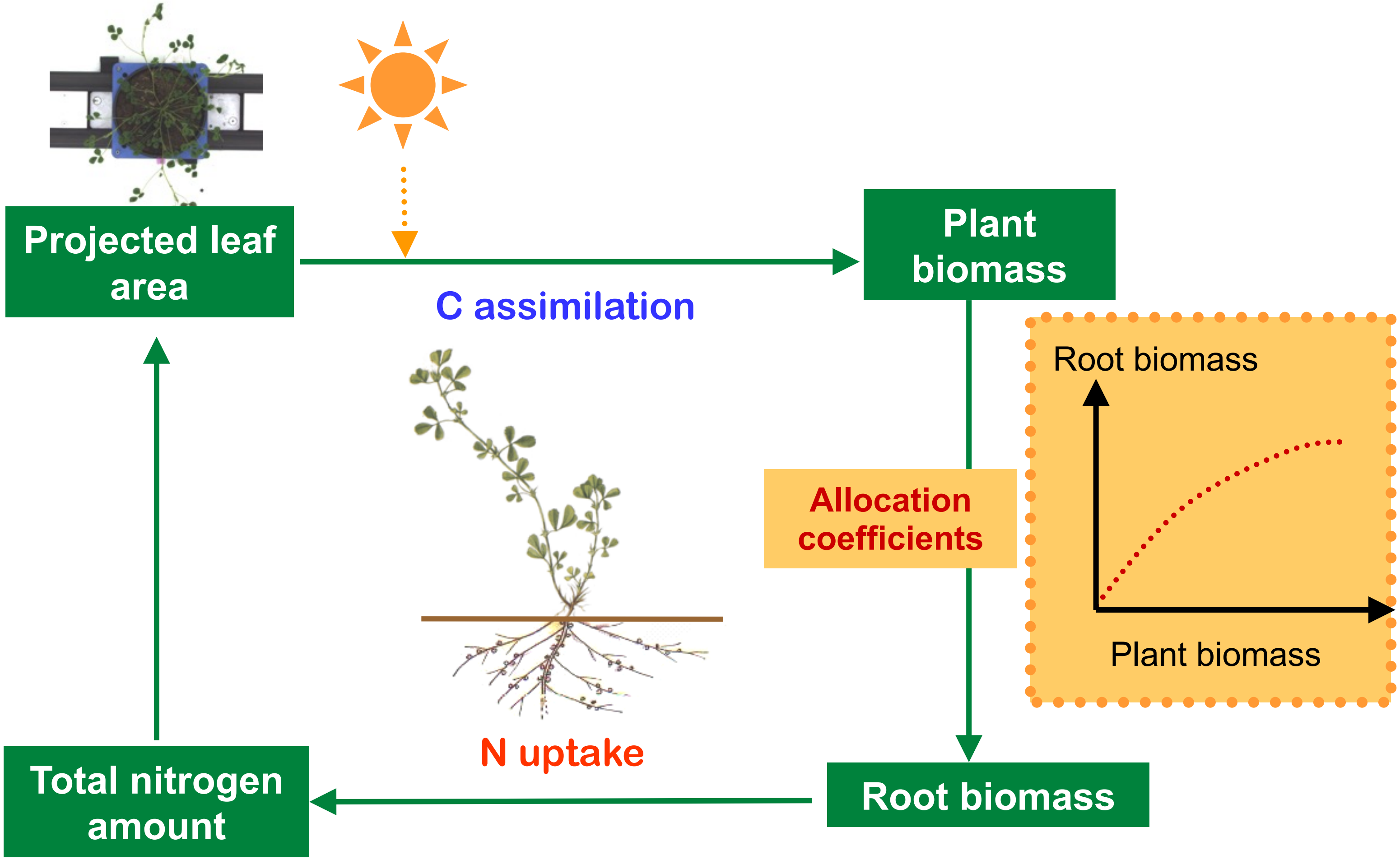
N uptake

Total nitrogen amount

Root biomass

D Moreau

Model structure



Projected leaf area

C assimilation

Plant biomass

Allocation coefficients

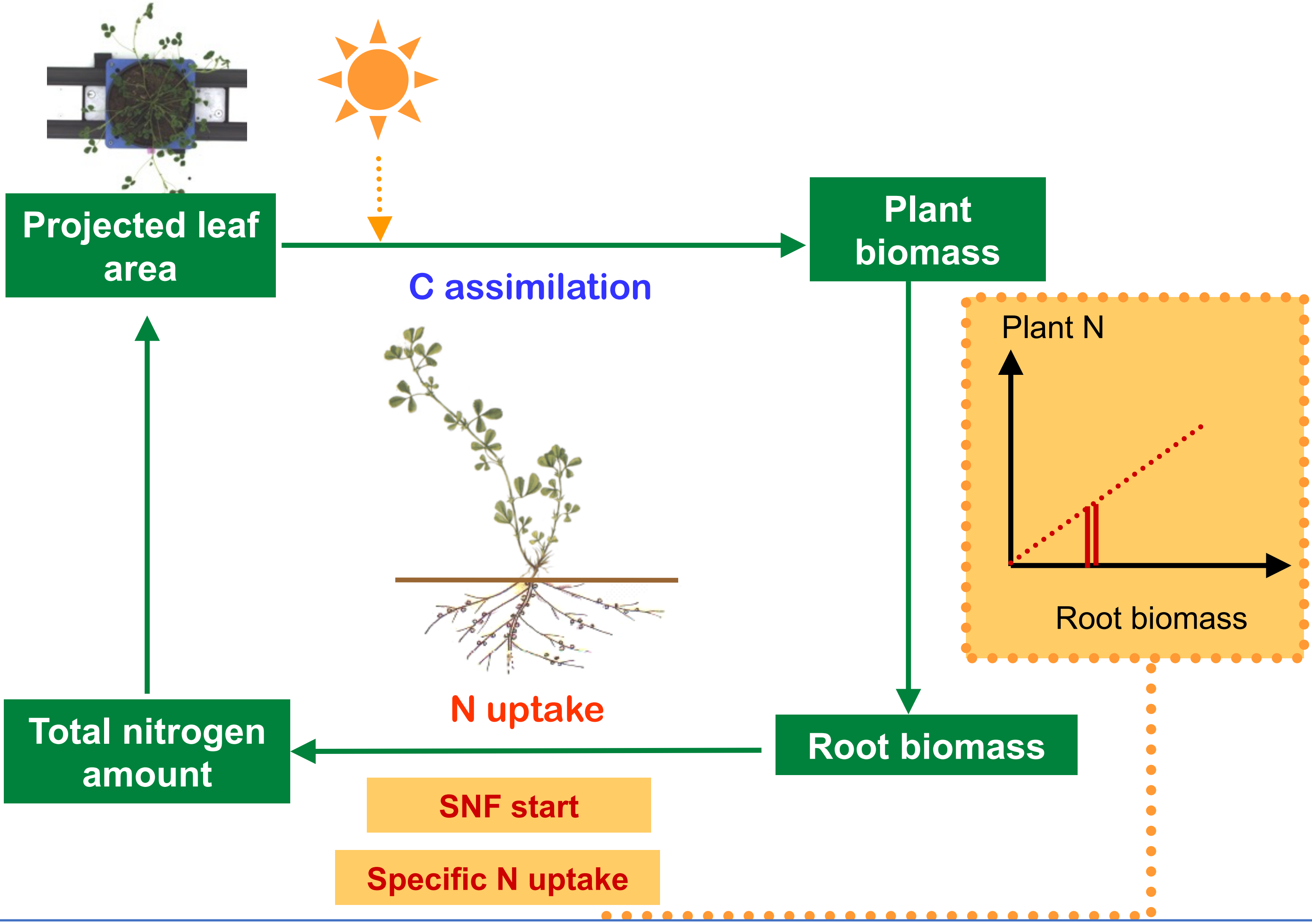
Root biomass

N uptake

Total nitrogen amount

D Moreau

Model structure



Projected leaf area

C assimilation

Plant biomass

N uptake

Total nitrogen amount

Root biomass

SNF start

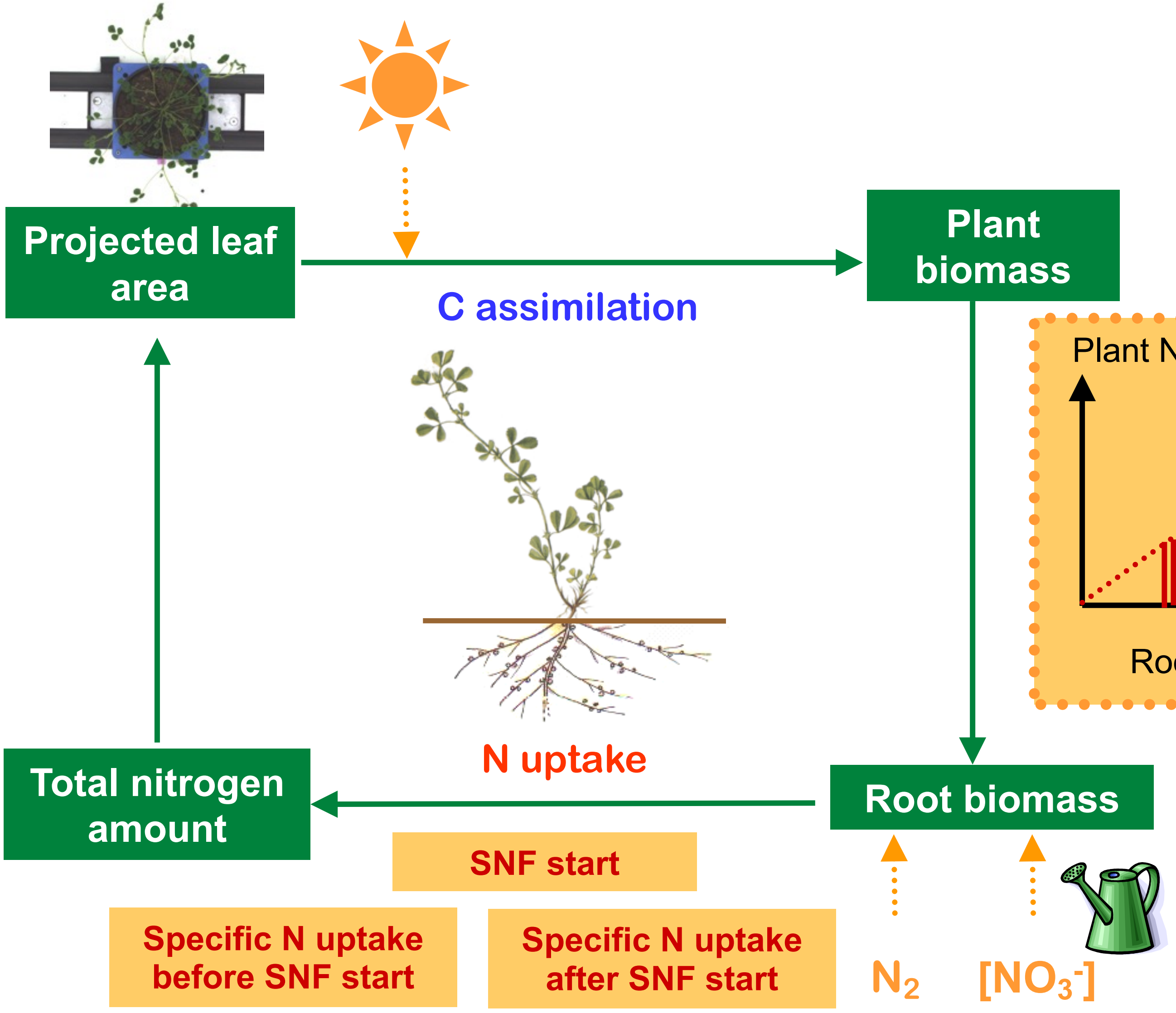
Specific N uptake

D Moreau

What for ?

Combining phenotyping and models

Model structure



Projected leaf area

C assimilation

Plant biomass

Plant N

Root biomass

Root biomass

N uptake

Total nitrogen amount

SNF start

Specific N uptake before SNF start

Specific N uptake after SNF start

N_2

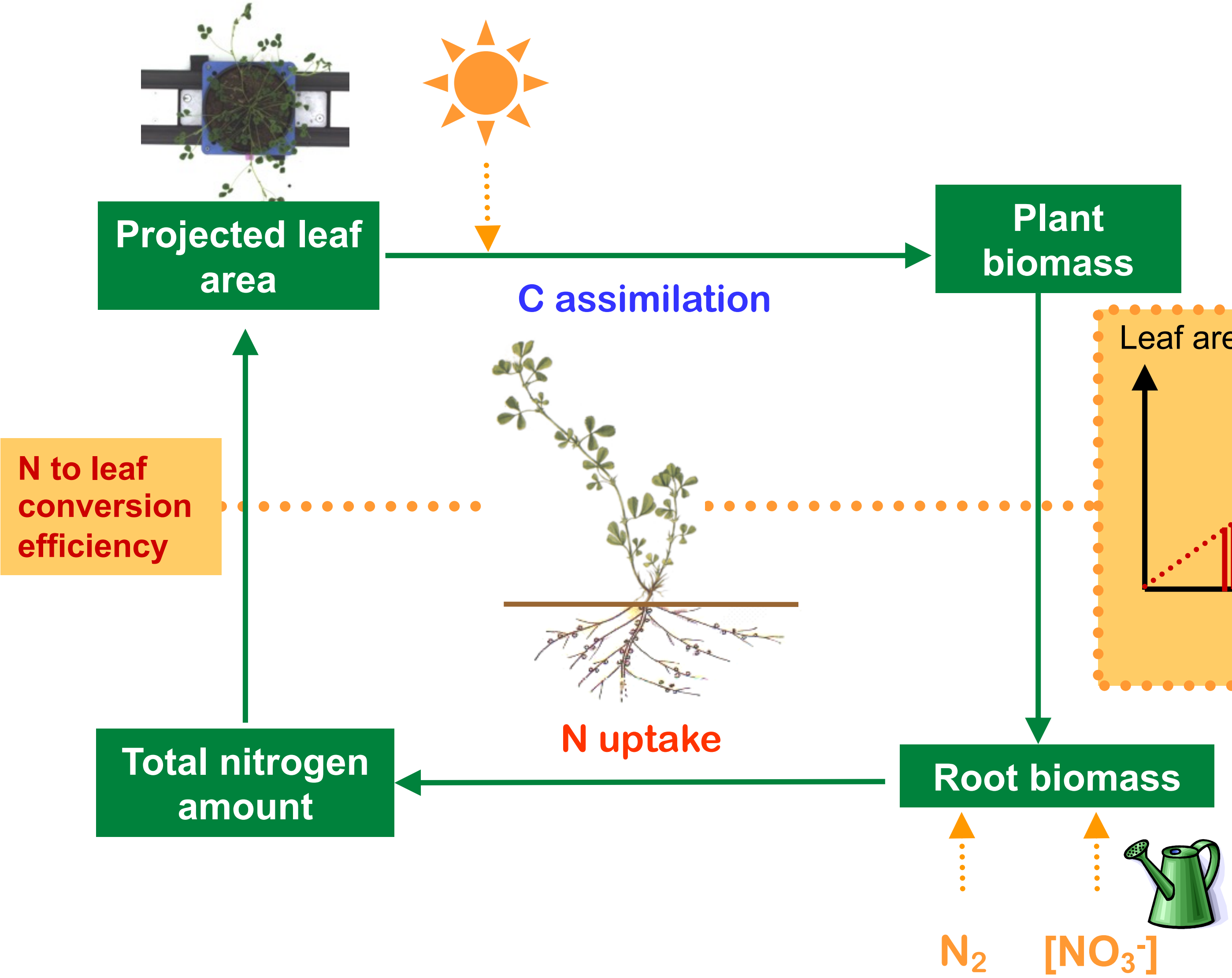
$[NO_3^-]$

D Moreau

What for ?

Combining phenotyping and models

Model structure



Projected leaf area

Plant biomass

C assimilation

N to leaf conversion efficiency

N uptake

Total nitrogen amount

Root biomass

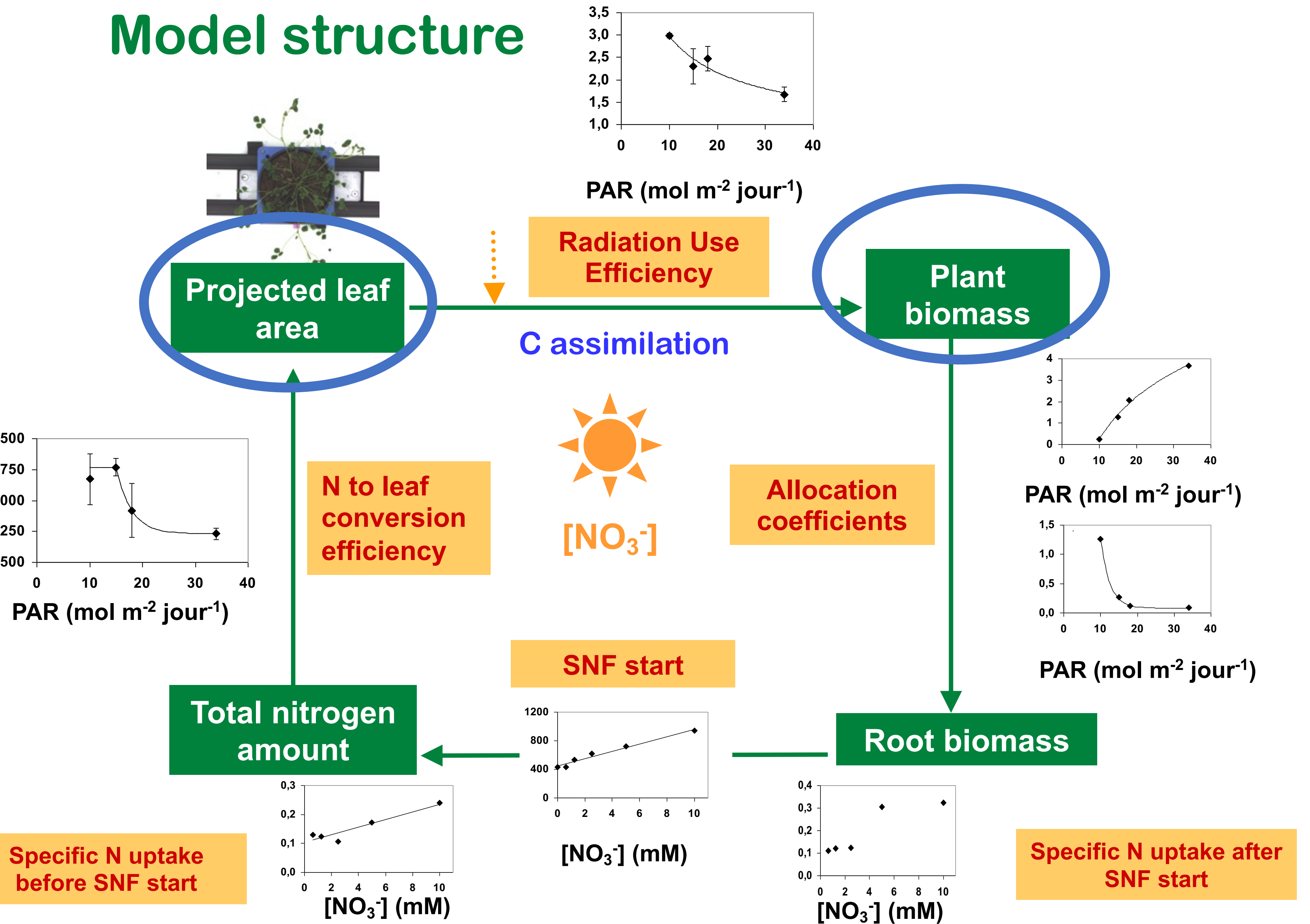
N_2

$[NO_3^-]$

D Moreau

What for ? Combining phenotyping and models

Model structure



Projected leaf area

Radiation Use Efficiency

C assimilation

Plant biomass

Allocation coefficients

Root biomass

SNF start

Total nitrogen amount

N to leaf conversion efficiency

$[NO_3^-]$

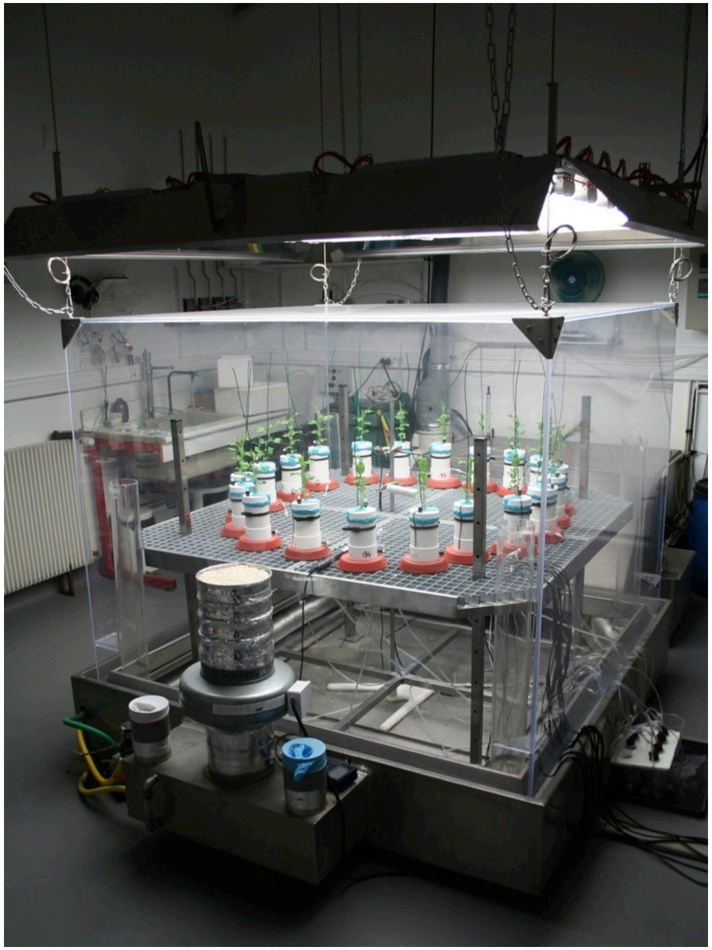
Specific N uptake before SNF start

Specific N uptake after SNF start

D Moreau

And adding fluxomics

Fluxomics (C, N, S)



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

Isotopic split root (N_2)

Salon et al, Journal Exp Bot, 2016

What for ?

Combining phenotyping and models

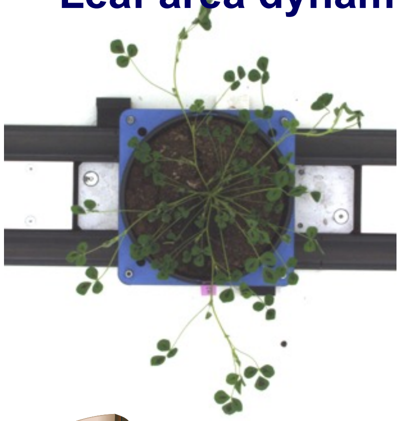
LR4 Population (T. Huguet, CNRS Castanet Tolosan)

Jemalong x DZA315-16   175 RILs

Inoculation with ***R. meliloti*** (strain 2011)

High Throughput Phenotyping

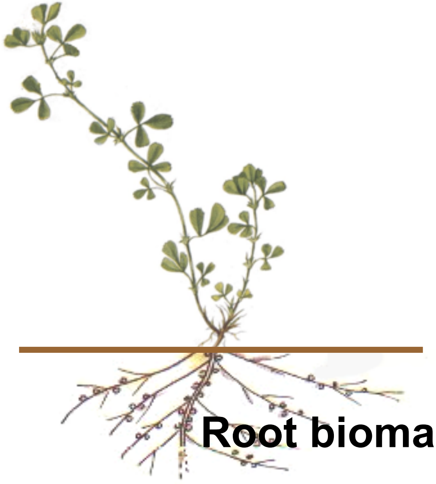
Leaf area dynamics



↓

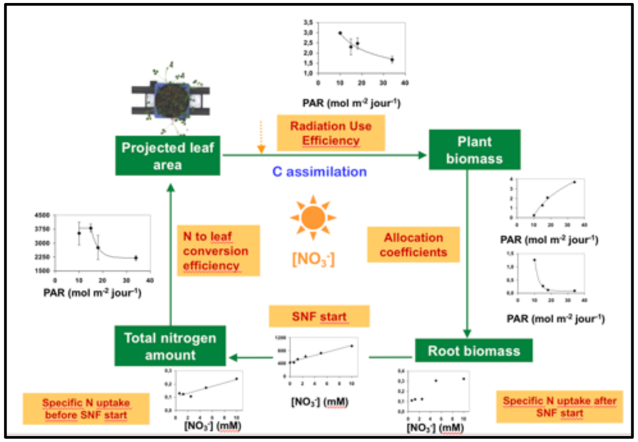
Leaf area

Destructives measurements

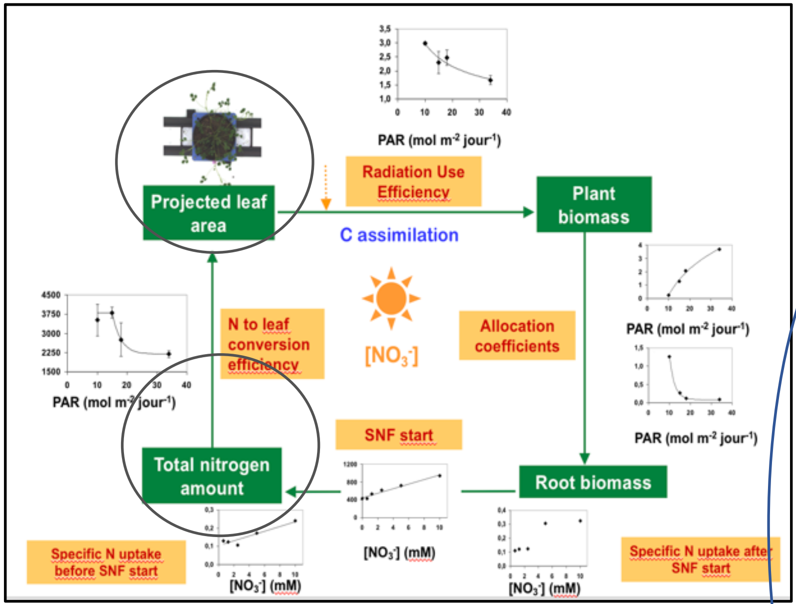


Plant N

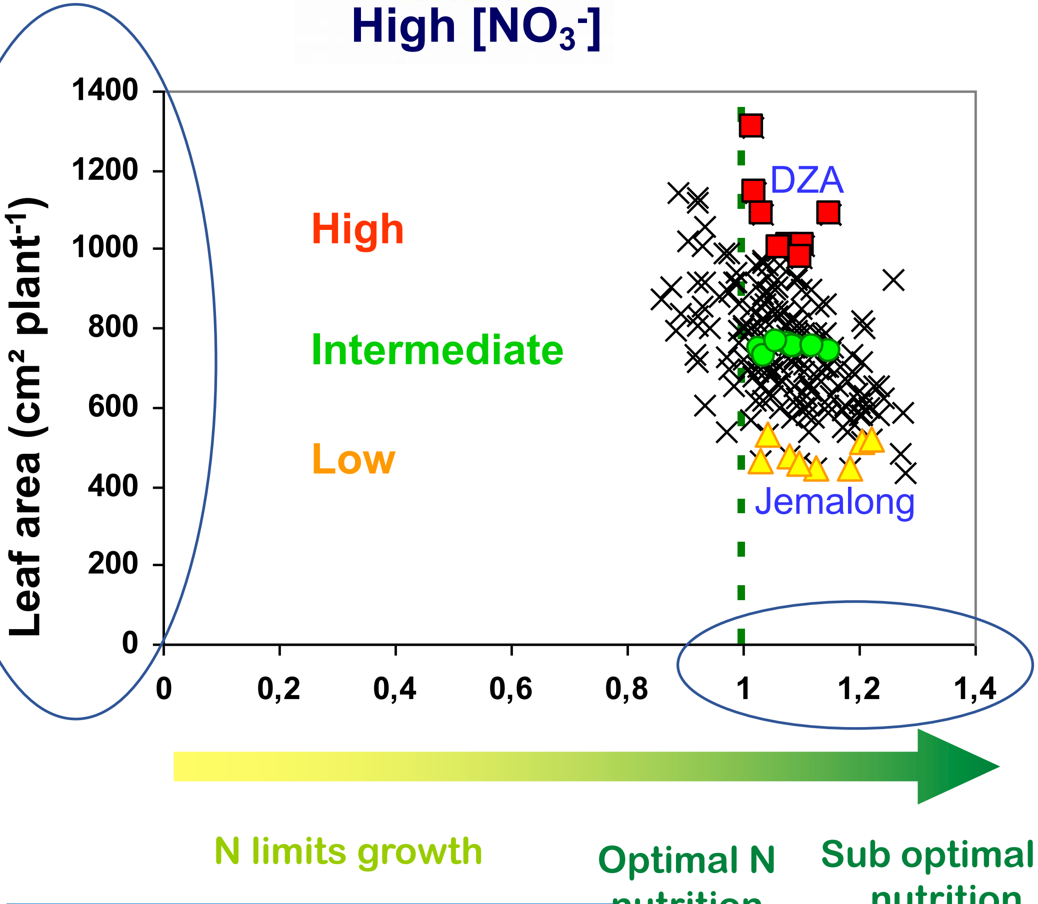
“Grid” to analyze phenotypic differences



What for ? Assessing genetic variability associated to N nutrition



High $[NO_3^-]$

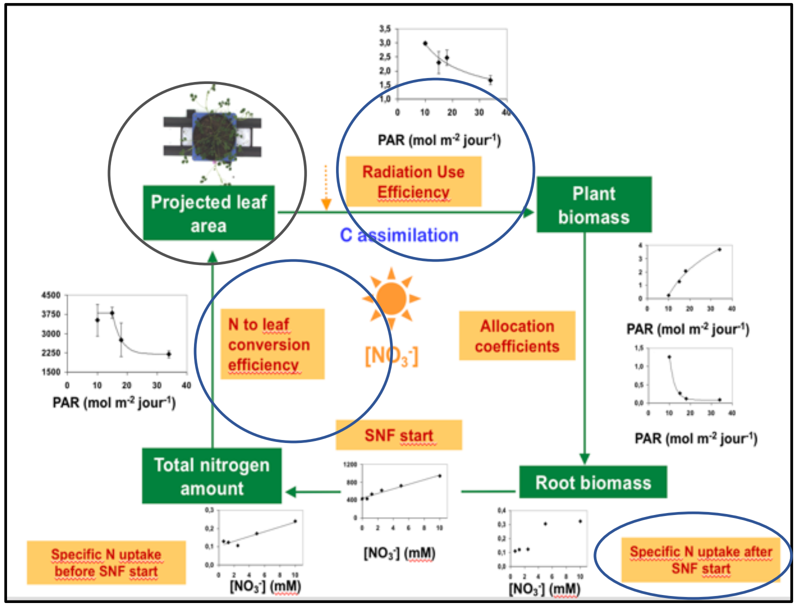


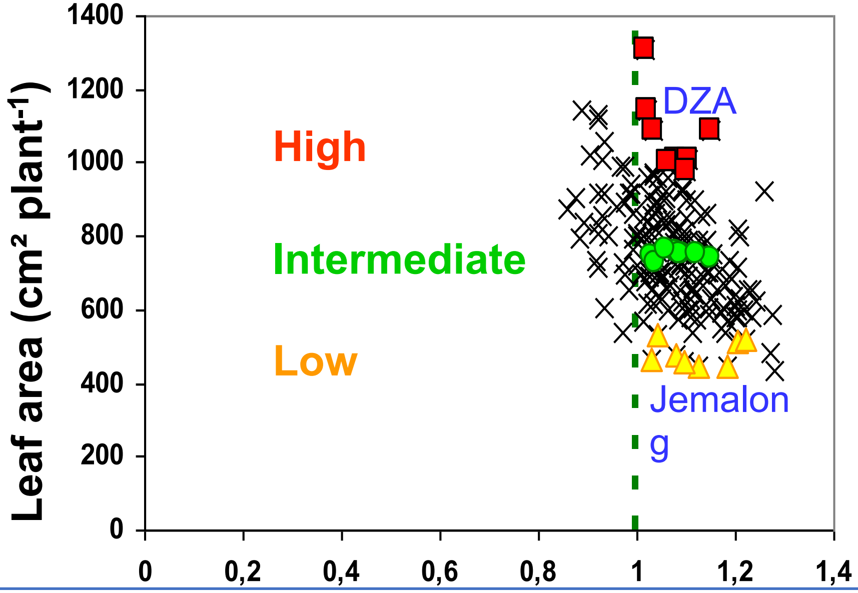
D Moreau

High variability of projected leaf area

Lines not affected on their capacity to assimilate NO_3^-

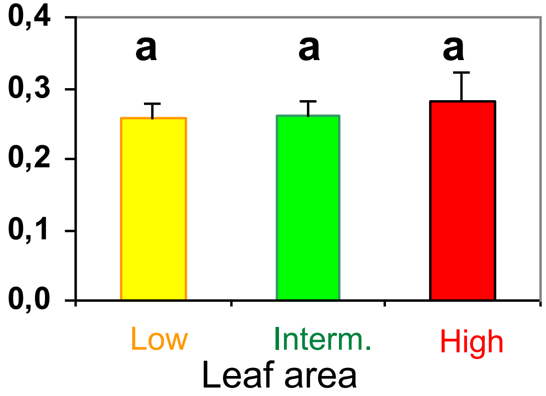
What for ? Assessing genetic variability associated to N nutrition





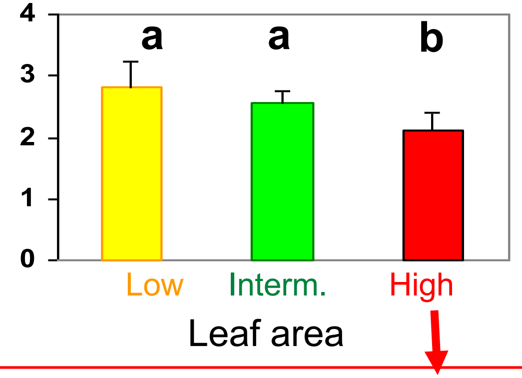
Specific N uptake

(plant N g /root biomass)



RUE

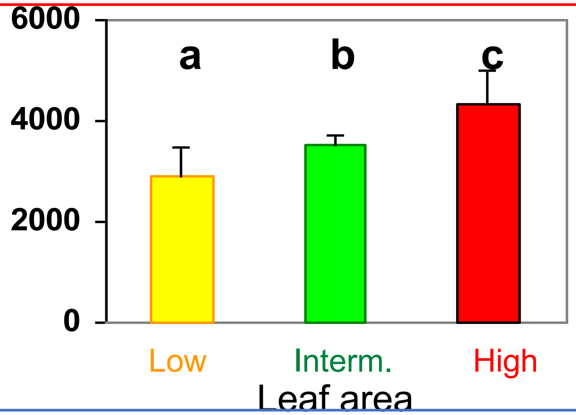
(g of biomass/Interc. PAR MJ)

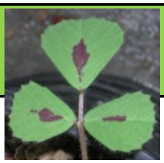


For a given amount of int. PAR, lower accumulated biomass.

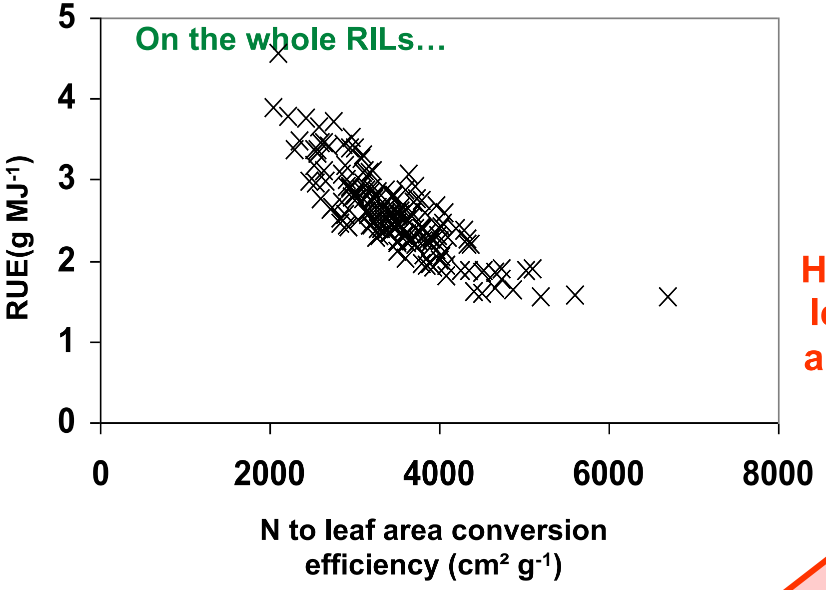
N to leaf area conversion efficiency

(cm² of projected leaf/ Plant N g)



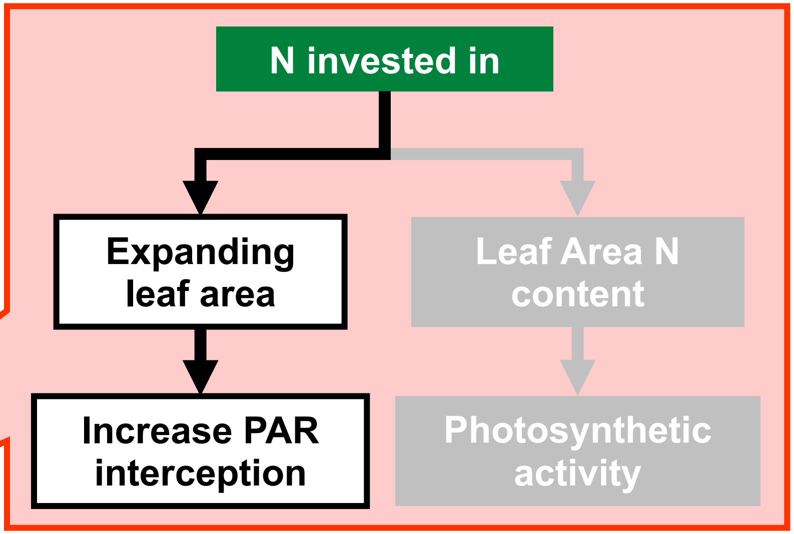


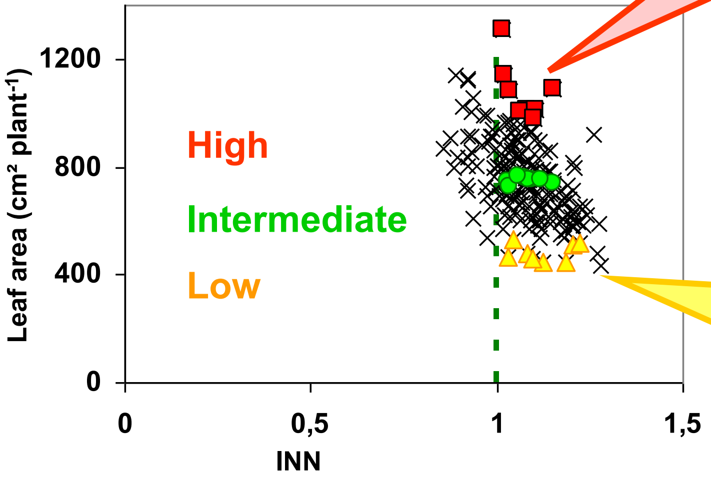
What for ? Assessing genetic variability associated to N nutrition



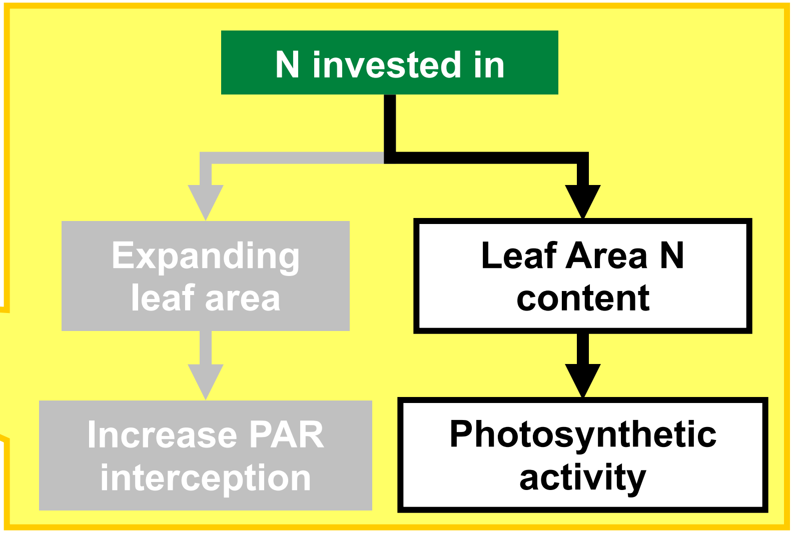
High leaf area

Trade off between N allocation



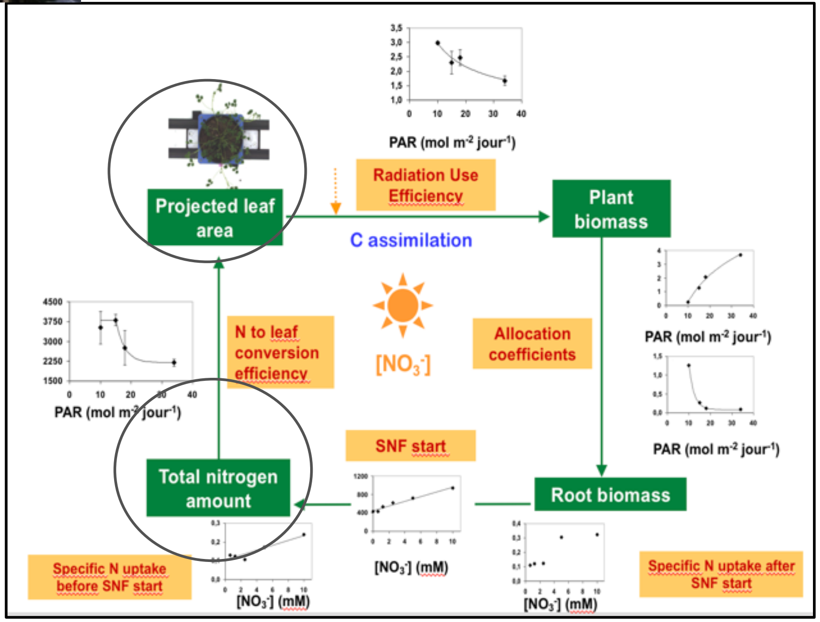


Low leaf area

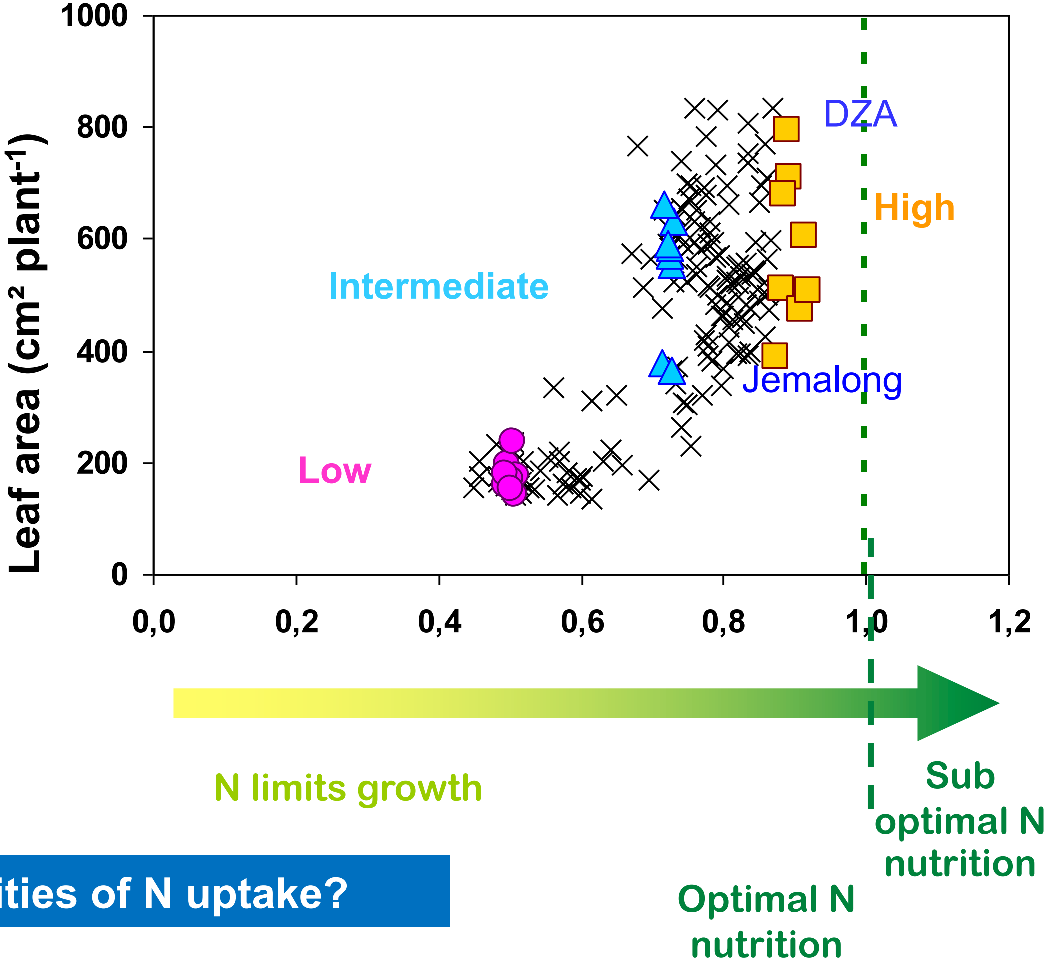


Moreau et al. PSB 2013

What for ? Assessing genetic variability associated to N nutrition

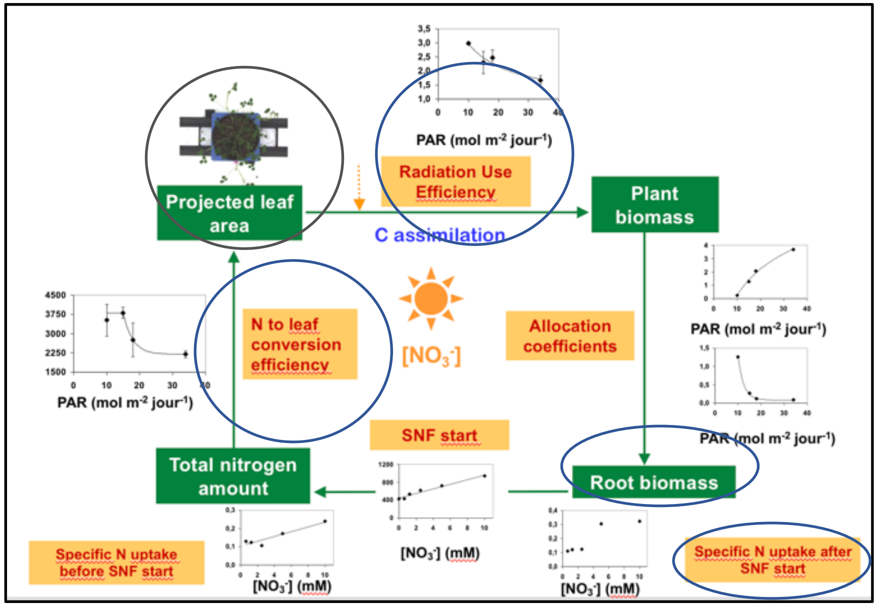


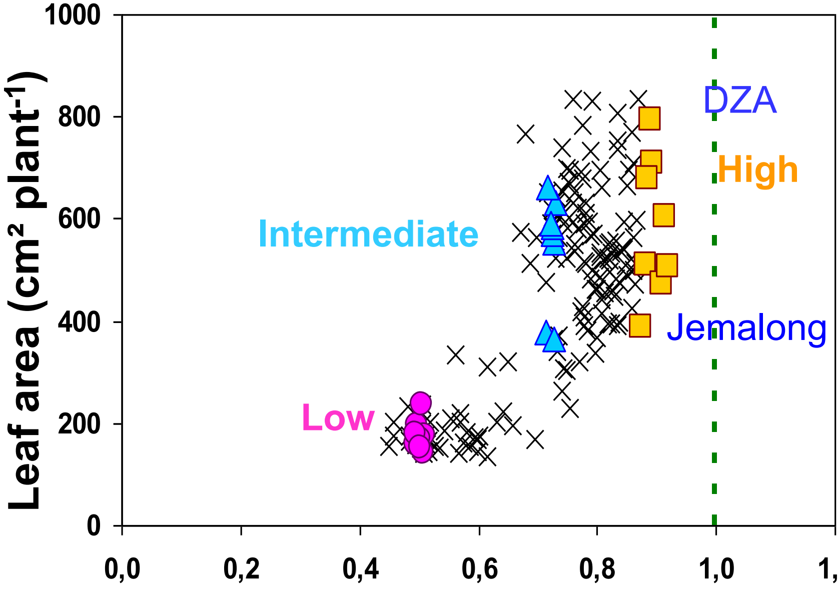
Low [NO_3^-]



Contrasted abilities of N uptake?

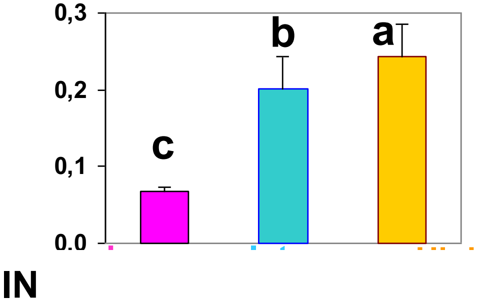
What for ? Assessing genetic variability associated to N nutrition



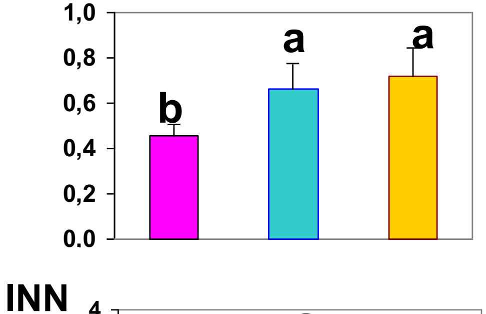


Specific N uptake

(plant N g /root biomass)

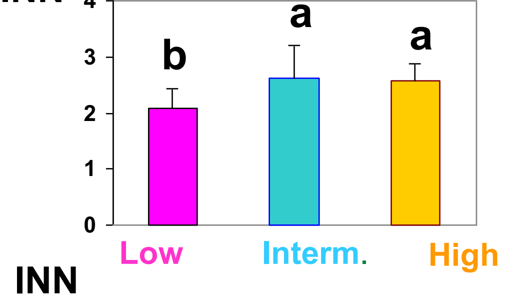


Root biomass
(g per plant)



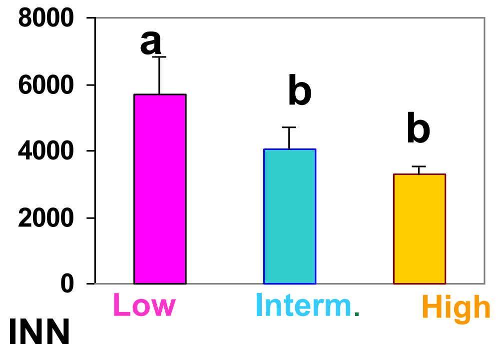
RUE

(g of biomass/Interc. PAR MJ)



N to leaf area conversion efficiency

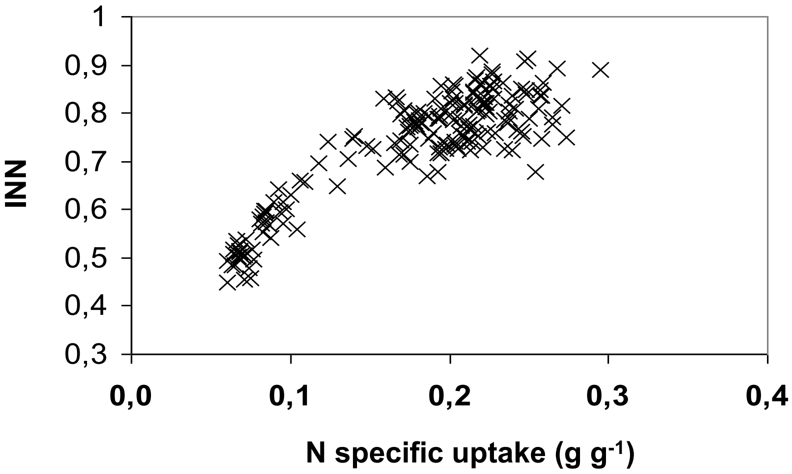
(cm² of projected leaf/ Plant N g)



What for ? Assessing genetic variability associated to N nutrition

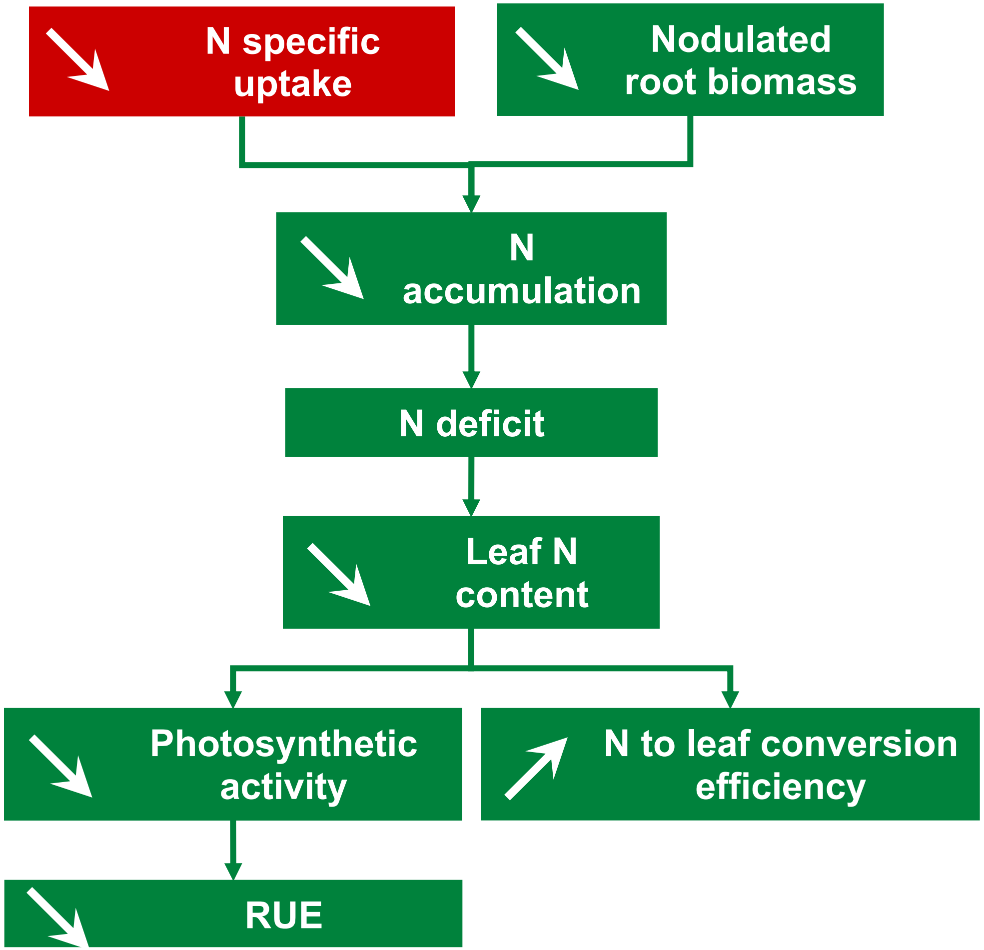
N deficient RILs displayed…

On the whole RILs…



Moreau et al. PSB 2013

Low [NO_3^-]



↘ N specific uptake

↘ Nodulated root biomass

↘ N accumulation

N deficit

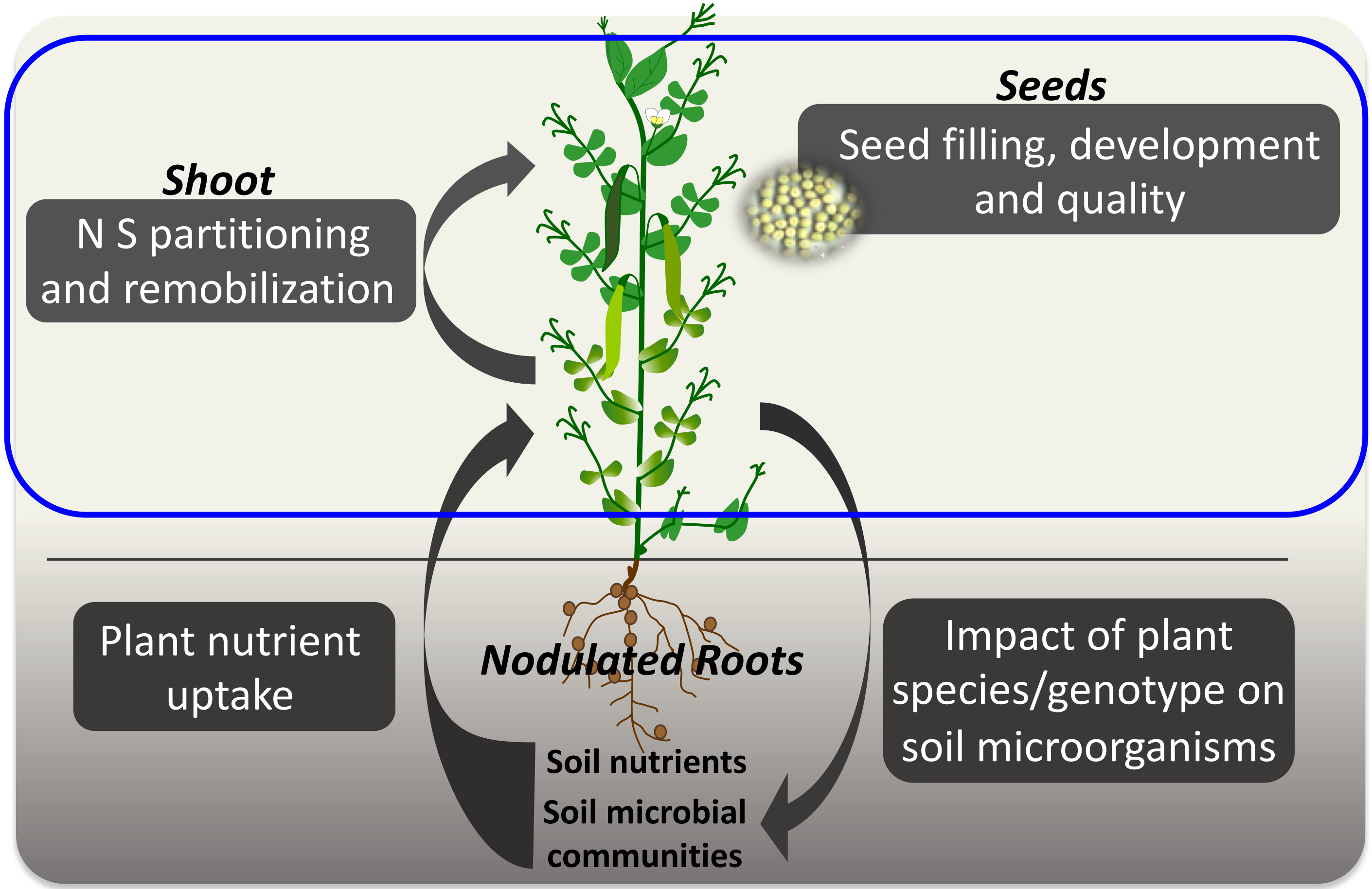
↘ Leaf N content

↘ Photosynthetic activity

↗ N to leaf conversion efficiency

↘ RUE

What for ?



Shoot

N S partitioning and remobilization

Seeds

Seed filling, development and quality

Plant nutrient uptake

Nodulated Roots

Soil nutrients

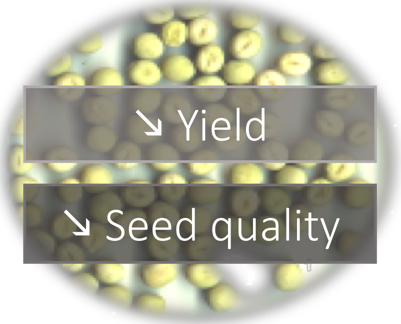
Soil microbial communities

Impact of plant species/genotype on soil microorganisms

(1) Seed quality and legume tolerance to late abiotic stresses

What for ? Combining phenomics with omics: Seed quality

Climate change
Low input systems
Less pollution



↘ Yield

↘ Seed quality

⇨ Molecular targets to increase **stress tolerance**

⇨ Molecular indicators of **plant needs** (water, S)

Nitrate deficiency

Sulfate deficiency

Water deficit

A comparative fluxome and transcriptome analysis of **nitrogen remobilisation between pea and *M. truncatula***

7 N 14

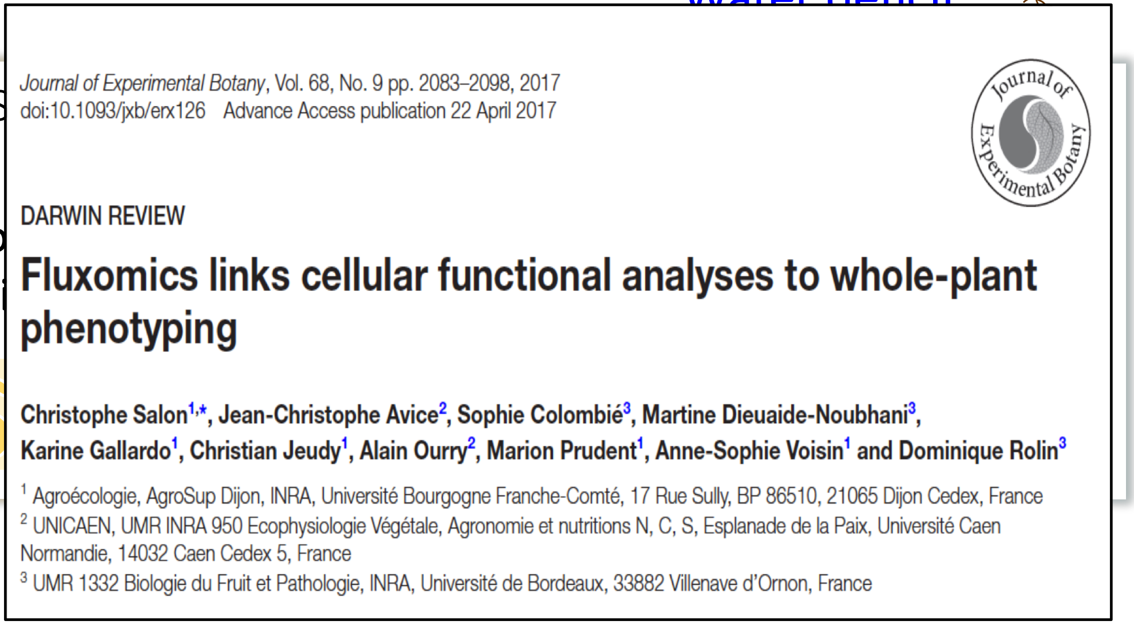
ANR GENOPEA 2010-13

S

Ro

duri

16 S



Journal of Experimental Botany, Vol. 68, No. 9 pp. 2083–2098, 2017
doi:10.1093/jxb/erx126 Advance Access publication 22 April 2017

DARWIN REVIEW

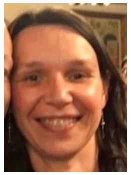
Fluxomics links cellular functional analyses to whole-plant phenotyping

Christophe Salon[1,*], Jean-Christophe Avice[2], Sophie Colombié[3], Martine Dieuaide-Noubhani[3], Karine Gallardo[1], Christian Jeudy[1], Alain Ourry[2], Marion Prudent[1], Anne-Sophie Voisin[1] and Dominique Rolin[3]

[1] Agroécologie, AgroSup Dijon, INRA, Université Bourgogne Franche-Comté, 17 Rue Sully, BP 86510, 21065 Dijon Cedex, France
[2] UNICAEN, UMR INRA 950 Ecophysiologie Végétale, Agronomie et nutritions N, C, S, Esplanade de la Paix, Université Caen Normandie, 14032 Caen Cedex 5, France
[3] UMR 1332 Biologie du Fruit et Pathologie, INRA, Université de Bordeaux, 33882 Villenave d'Ornon, France

K Gallardo

M Prudent

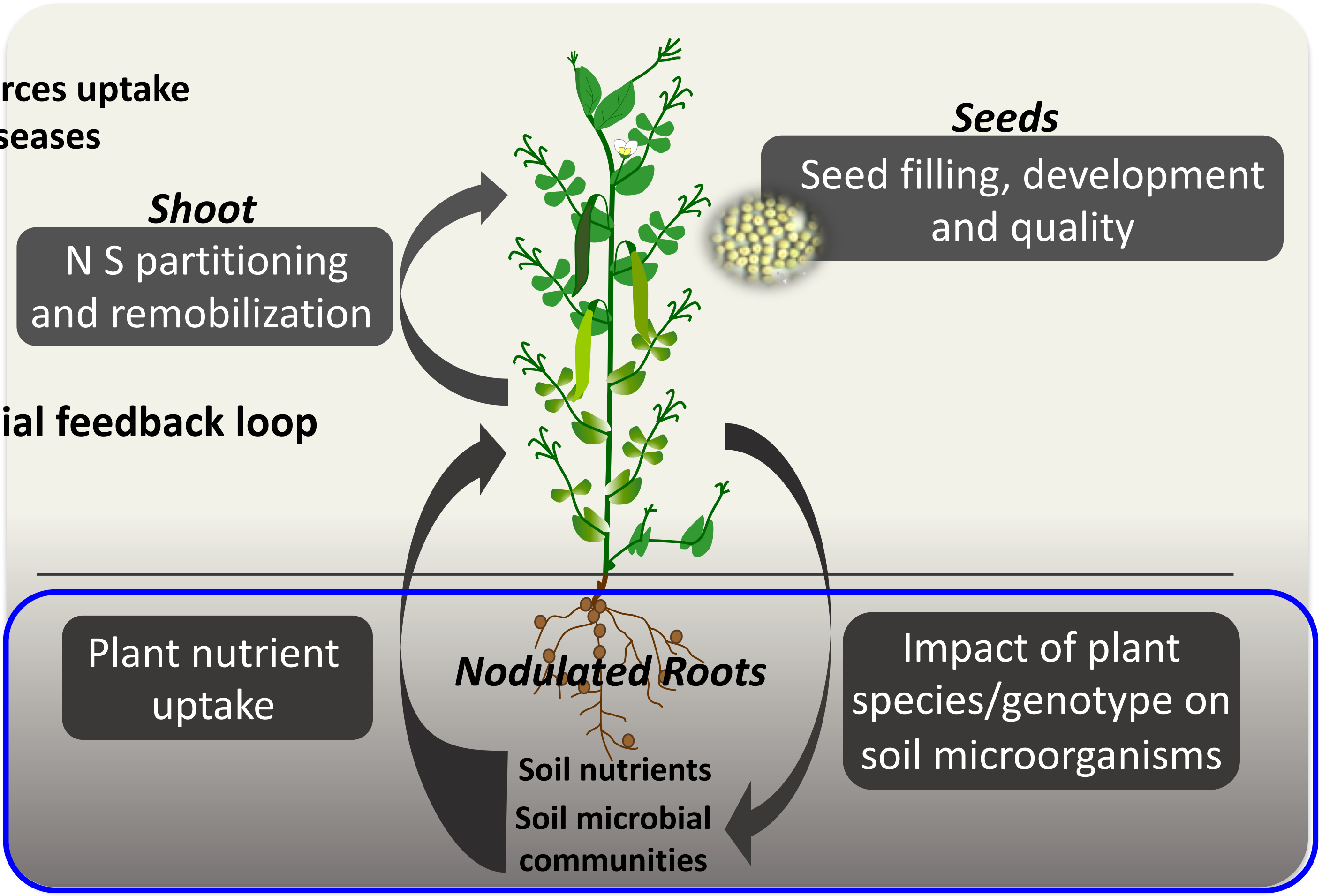


V Vernoud

Phenotyping leaf and roots completes omic's

What for ? Legume-microbe interactions to improve plant nutrition

Facilitating soil resources uptake
Protection against diseases



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Plant – Microbial feedback loop

Plant nutrient uptake

Nodulated Roots

Soil nutrients
Soil microbial communities

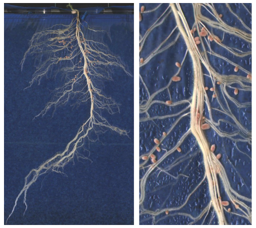
Impact of plant species/genotype on soil microorganisms

(1) Impact of plant genotype on the selection of soil microbes (not only rhizobia : whole microbiome)

(2) Impact of soil microbe diversity on plant growth N nutrition and tolerance to other stresses

Final aim: drive plante-microbe interactions through plant genotype = a new breeding target

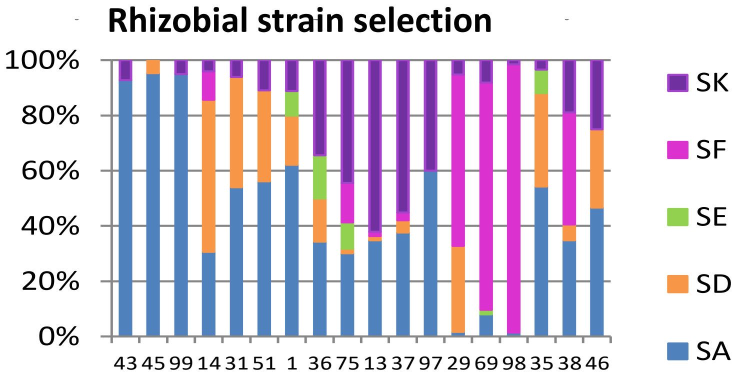
What for ? Legume-microbe interactions to improve plant nutrition



Impact of Pea genotypes on associated Rhizobial symbiotic strains :

104 genotypes inoculated with 5 rhizobial strains :

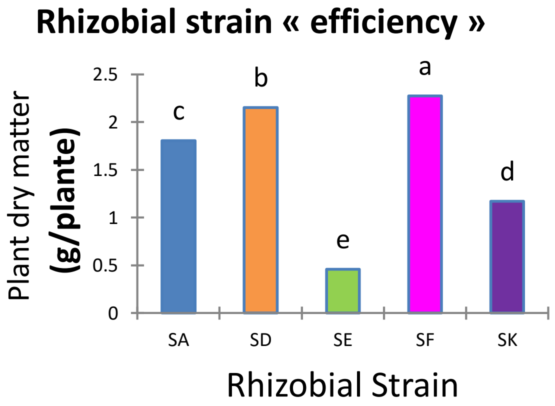
INRA SYMBIOPEA project



Rhizobial strain selection

18 genotypes among the 104 tested

Pea genotypes selected different symbiotic strains.



Rhizobial strain « efficiency »

Symbiotic strains : different efficiencies

On going : identification of plant genetic determinants of rhizobial selection by pea

(Genome Wide Association Study on a wider ranger of plant genetic variability + candidate gene approach)

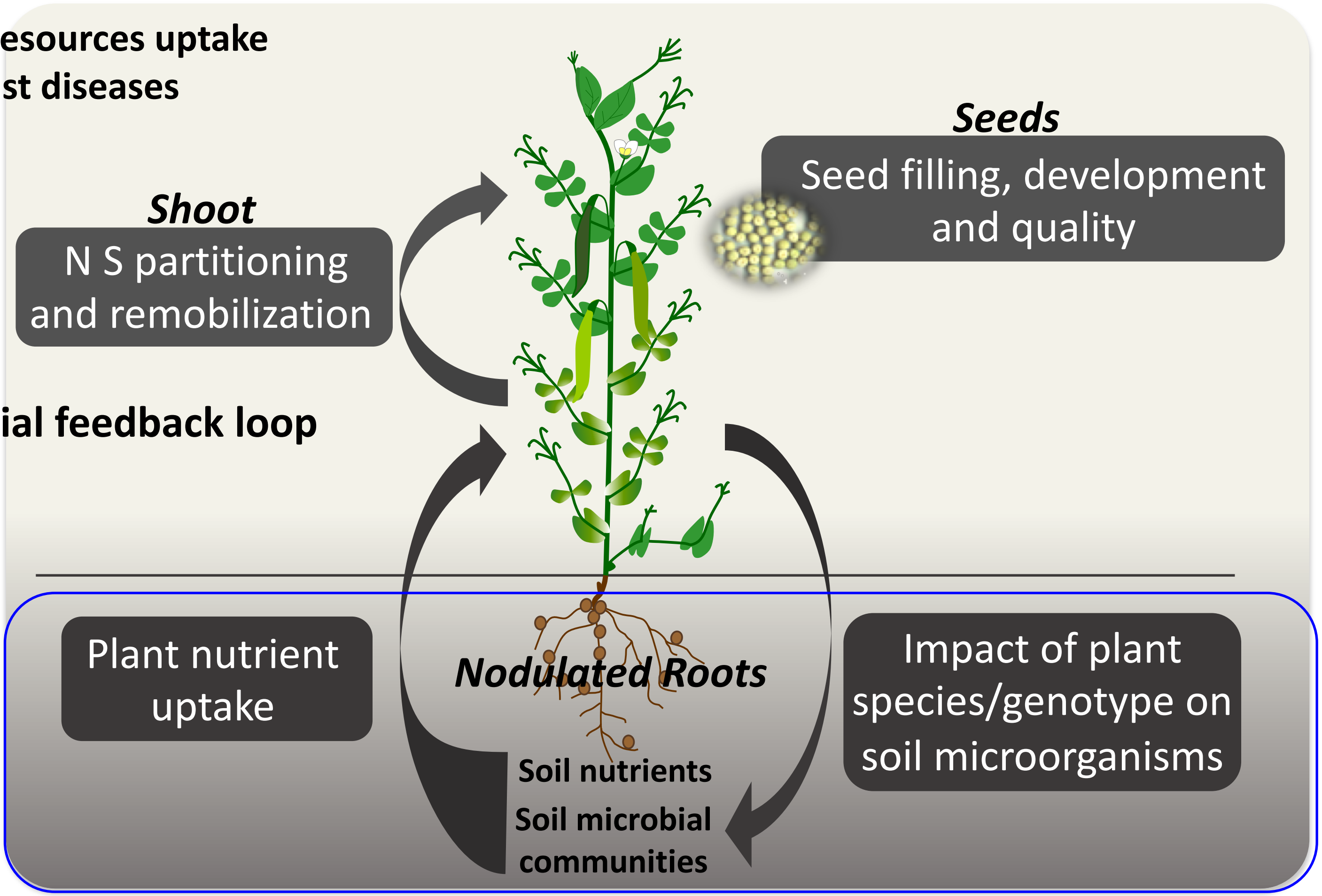
Phenotyping nodulated roots and plant growth

➥ Towards breeding of pea varieties with improved symbiosis for N_2 fixation

What for ? Legume-microbe interactions to improve plant nutrition

Facilitating soil resources uptake
Protection against diseases

Plant – Microbial feedback loop



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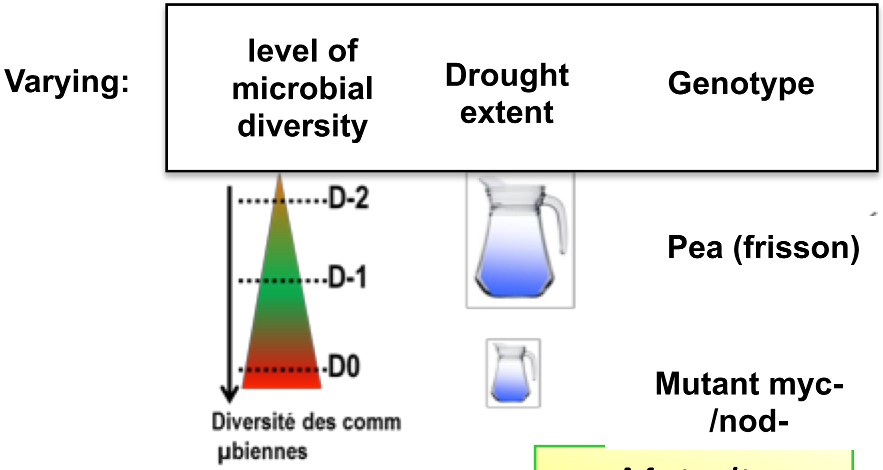
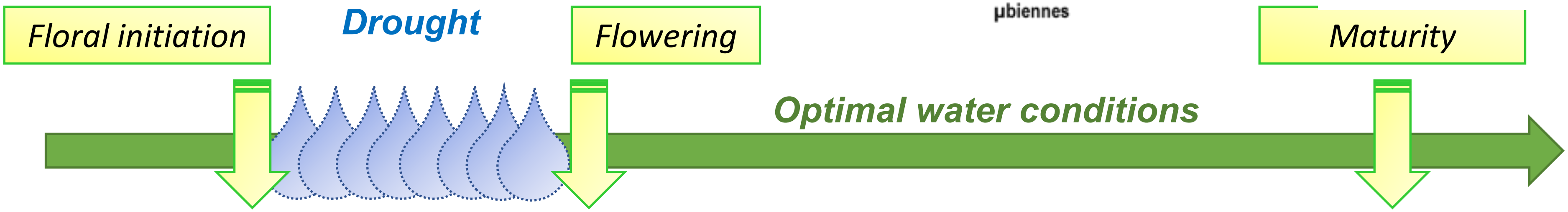
What for ? Legume-microbe interactions to improve plant nutrition

- Impact of diversity level
  of soil microbial communities
  on pea plant response to water stress
- A higher diversity level of soil microbial communities

Varying:



level of microbial diversity	Drought extent	Genotype
D-2 D-1 D0		Pea (frisson)
Diversité des comm µbiennes		Mutant myc-/nod-



no impact on pea drought tolerance… … but provides better pea resilience

Similar response with or without symbioses: non symbiotic communities play a role in this response

On going : Assessing which nodulated root traits are involved

M Prudent

Prudent et al, Plant and Soil, 2015

Key messages, conclusions

- Phenotyping in GH/CC/Fields and models for
  - **Physiological understanding**
  - **Genetic analyses of “morpho traits”**
- Root system architecture analysis is a bottleneck
- Image analysis is a challenge
- Control of environmental conditions is essential
- High throughput phenotyping involves all the chain
- Combine experimental, analytical and modelling approaches

GEAPSI & 4PMI groups


EcoLeg


4PMI


FILEAS

Proteaginous target crop

Thanks to all collaborators!!!

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European Plant Phenotyping Network EPPN 2020
X Draye
R Peruschka
U Schurr
F Tardieu

GROUPE ROULLIER
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U Schurr
F Tardieu

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S Declerck
X Draye
H Freville
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