A multispecies GWAS approach uncovers root responses to phosphate deficiency

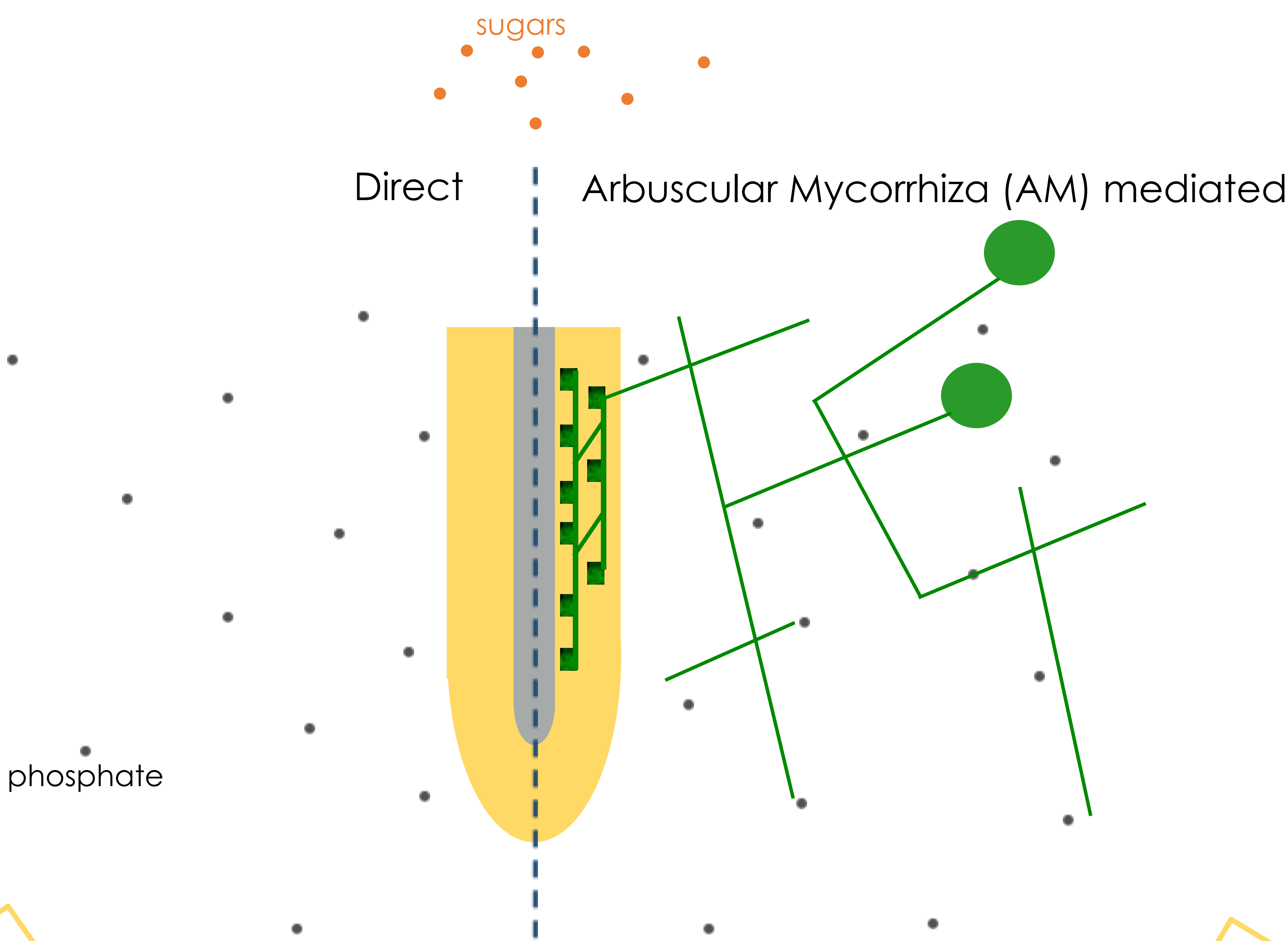
Marco Giovannetti
Busch/Dagdas group
2nd Monday Seminar

GREGOR MENDEL INSTITUTE OF MOLECULAR PLANT BIOLOGY
ÖSTERREICHISCHE AKADEMIE DER WISSENSCHAFTEN

Nature, 2009

- **Phosphate** is one of the main limiting factors for plant growth
- A limited resource
- Its price is expected to increase year by year
- Much of what is applied is leaching to soil

plant phosphate uptake



SINCE 450 MYA

phosphate, water & other nutrient

1000 fungal species → 200000 plant species

sugars & lipids

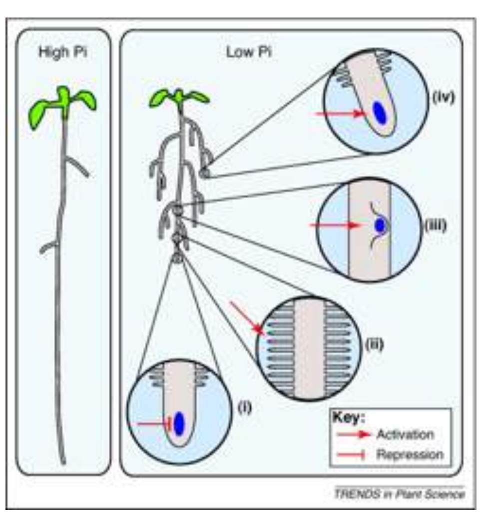
NOT ARABIDOPSIS

Phosphate puzzle pieces

Rarely mycorrhizal phosphate is considered

Single plant species approach

Developmental responses



Nutritional changes

Phosphate transport, recovery and recycling

Regulation of mycorrhizal phosphate transfer

Single plant species approach

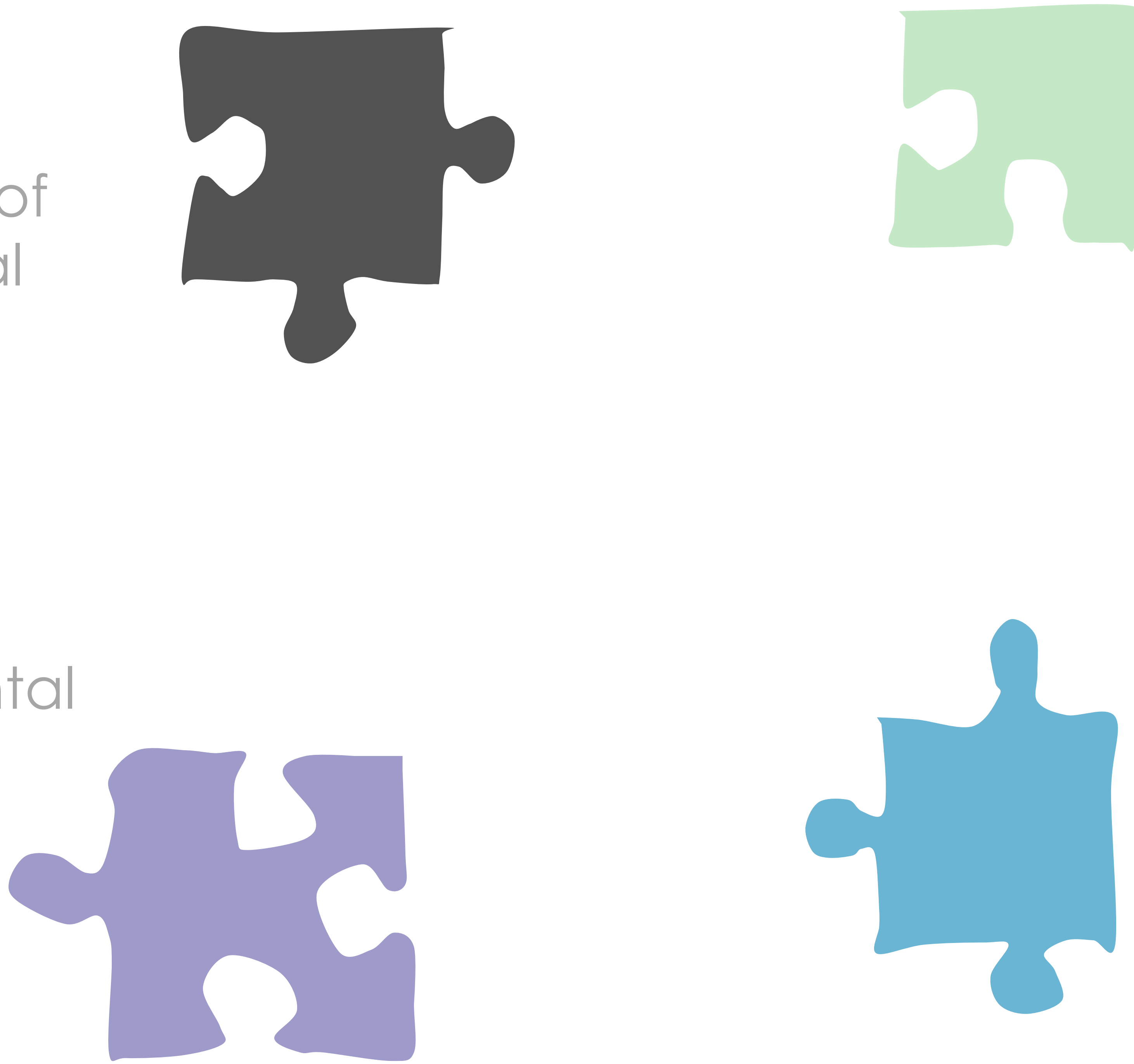
How can we solve the puzzle?

Developmental responses

Nutritional changes

Phosphate transport, recovery and recycling

Phosphate puzzle to be solved



Regulators of mycorrhizal symbiosis

Multiple plant species approach

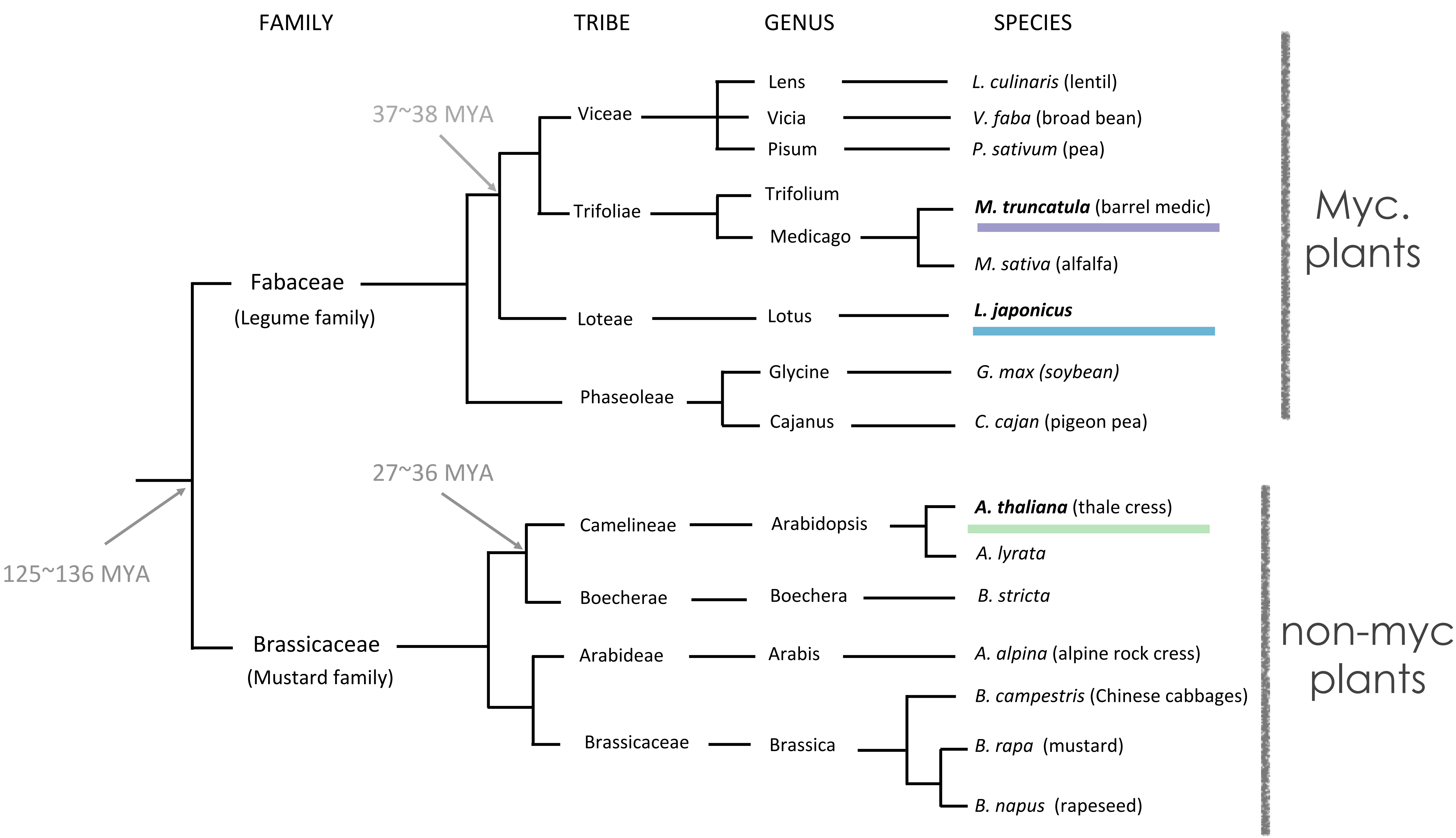
Developmental responses

Nutritional changes

High Throughput Root Phenotyping

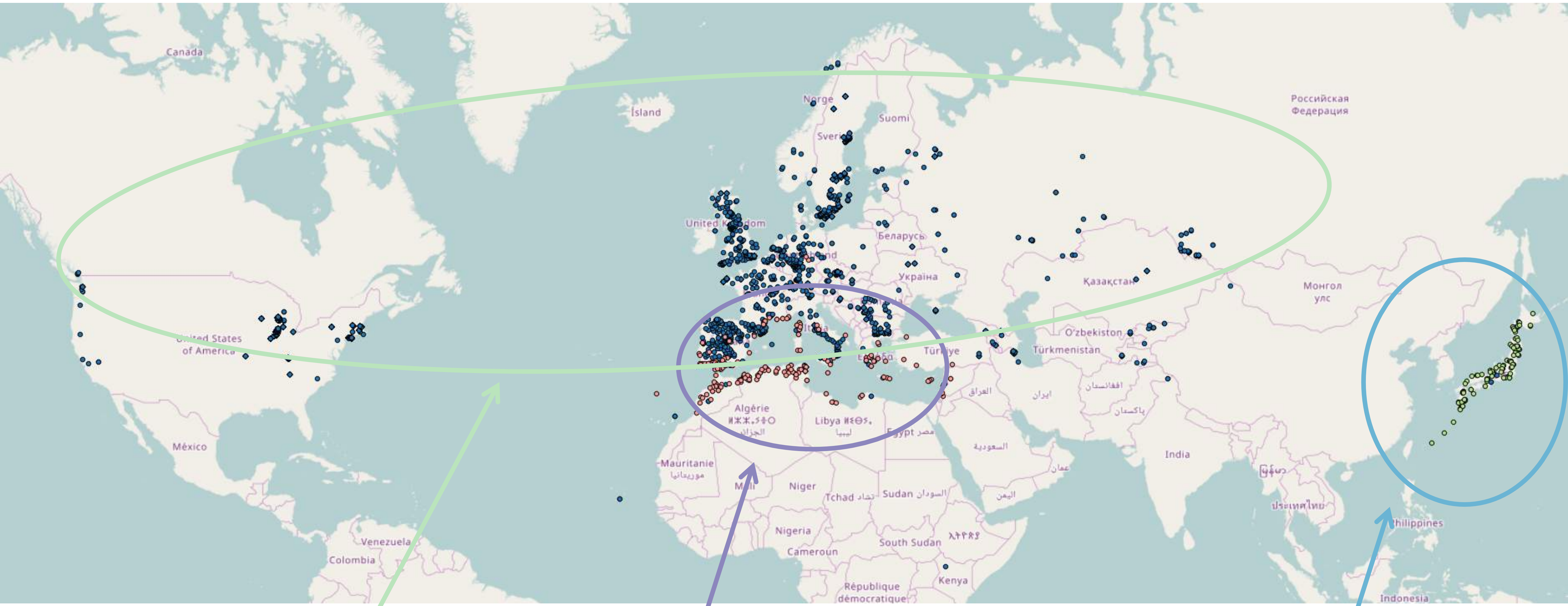
Tissue Phosphate quantification

Multiple plant species



modified from Hyung et al., 2014

Multiple plant genotypes



231 A. thaliana

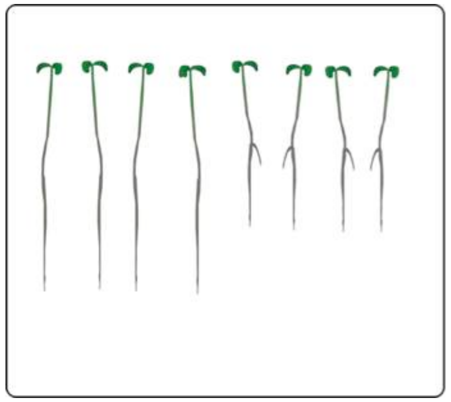
140 M. truncatula

130 L. japonicus

accession = genotype

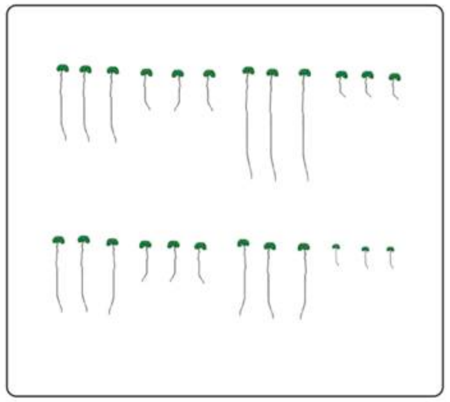
Multiple phosphate-related traits

- Automatic root trait quantification over low (20 µM) and high (750 µM) phosphate condition of natural populations (→ 16 traits, 2 conditions, each day)



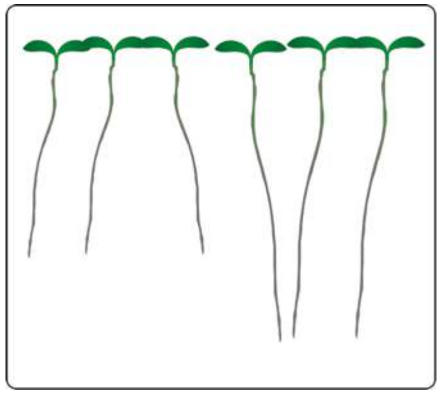
130 *Lotus japonicus*

vs.



230 Arabidopsis thaliana
(from Santosh Satbhai)

vs.

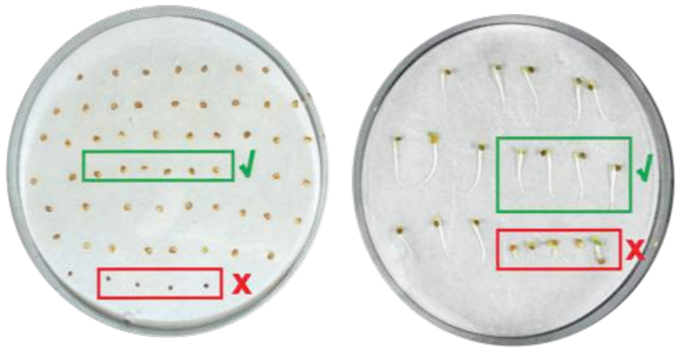


140 Medicago truncatula

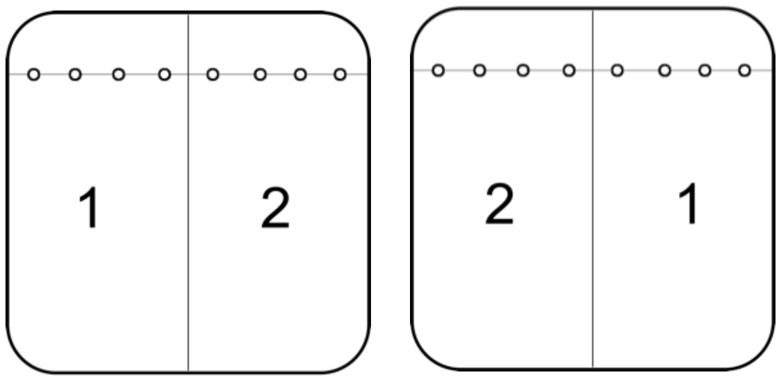
Multi plant species approach

High Throughput Root Phenotyping

High-throughput root phenotyping

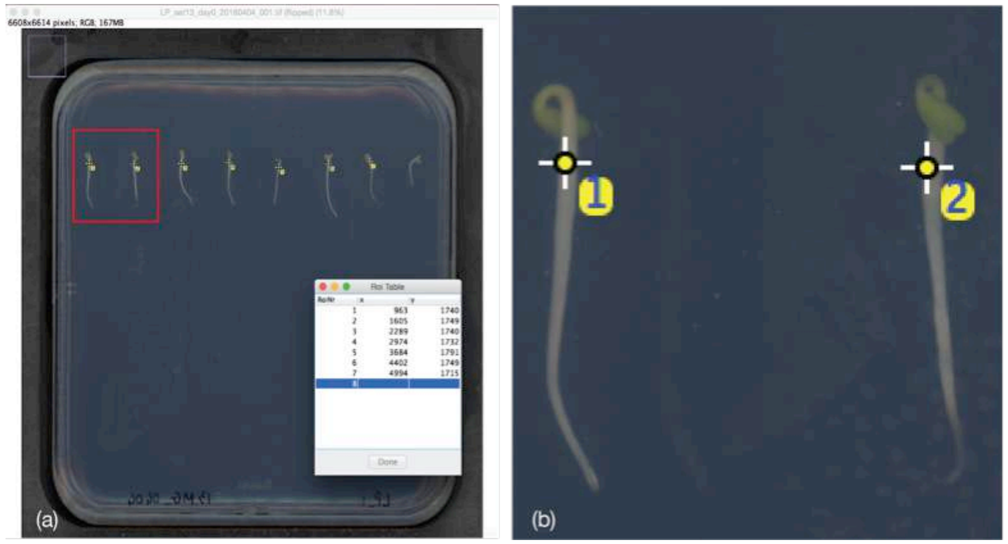


1. pre-germination

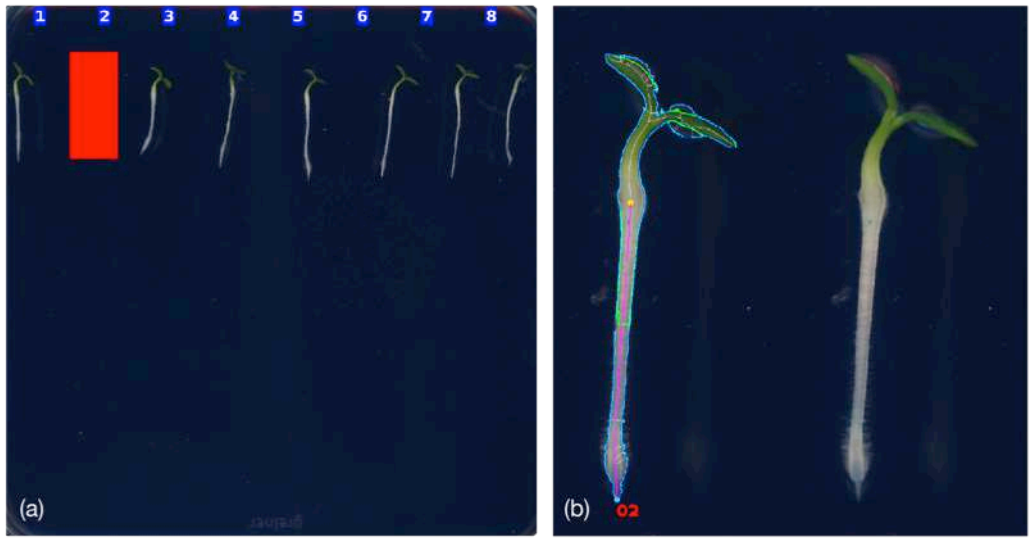


2. plant growth (8 reps)

3. HT image acquisition



4. root identification by user

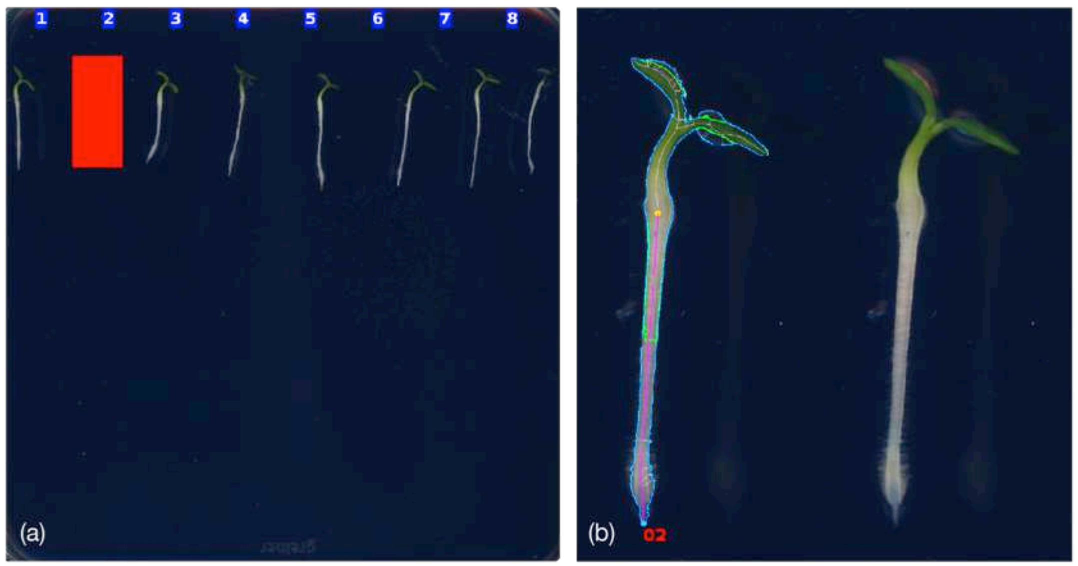


5. root segmentation

~2800 images, ~380 GB, 7 weeks

Slovak et al., 2014; Giovannetti et al., 2017

Each root gives 16 traits each day



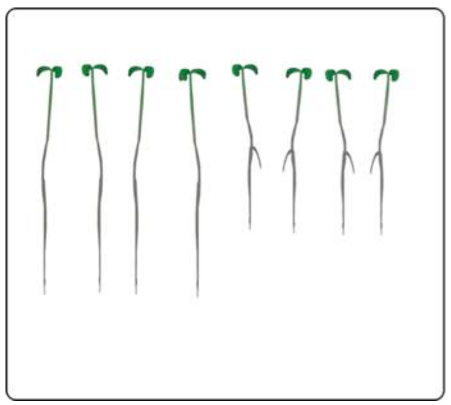
Root segmentation

Total length
Euclidian length
Root tortuosity
Root growth rate
Relative root growth rate
Root angle
Root direction index
Root horizontal index
Root vertical index
Root linearity
Average root width
Root width 20
Root width 40
Root width 60
Root width 80
Root width 100

9x
(days)

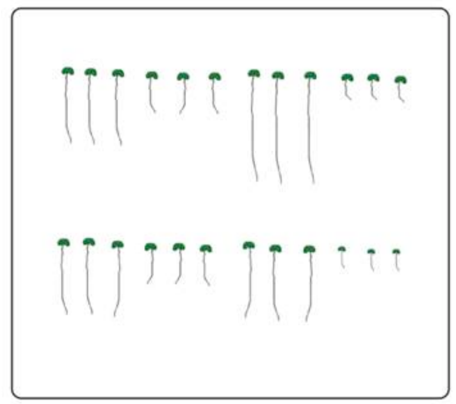
Multiple phosphate-related traits

- Automatic root trait quantification over low (20 µM) and high (750 µM) phosphate condition of natural populations (→ 16 traits, 2 conditions, each day)



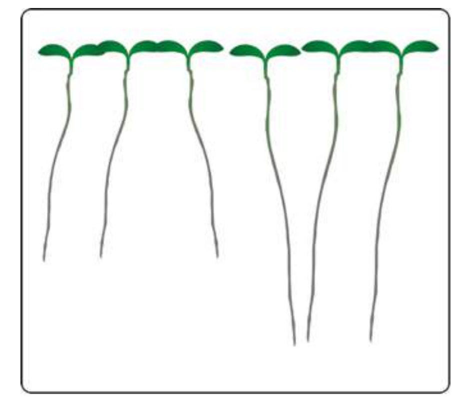
130 *Lotus japonicus*

vs.



230 *Arabidopsis thaliana*
(from Santosh Satbhai)

vs.



140 *Medicago truncatula*

- Root and shoot phosphate quantification (ion chromatography) in 130 Lotus accessions

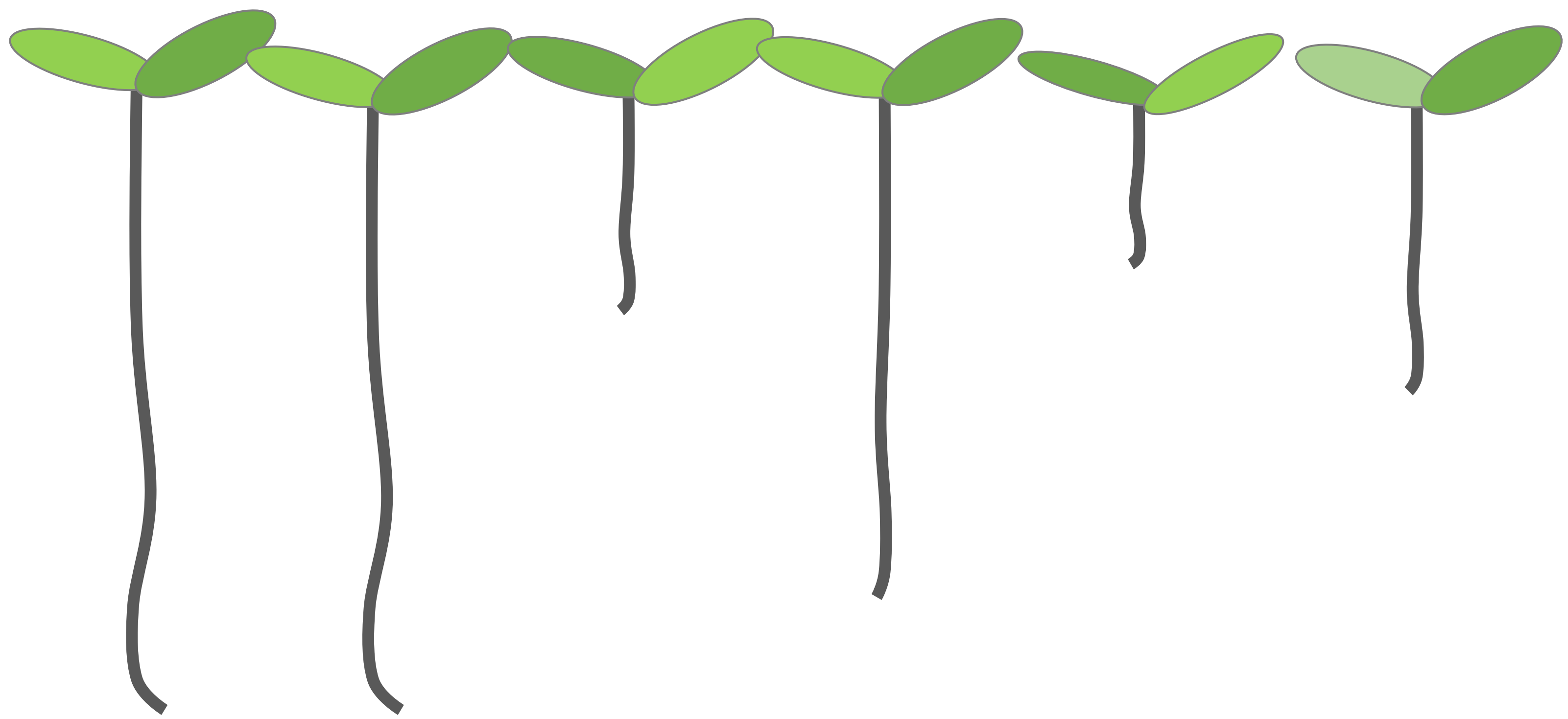
High Throughput Root Phenotyping

Multi plant species approach

Tissue Phosphate quantification

Genome Wide Association Study (GWAS)

Root length

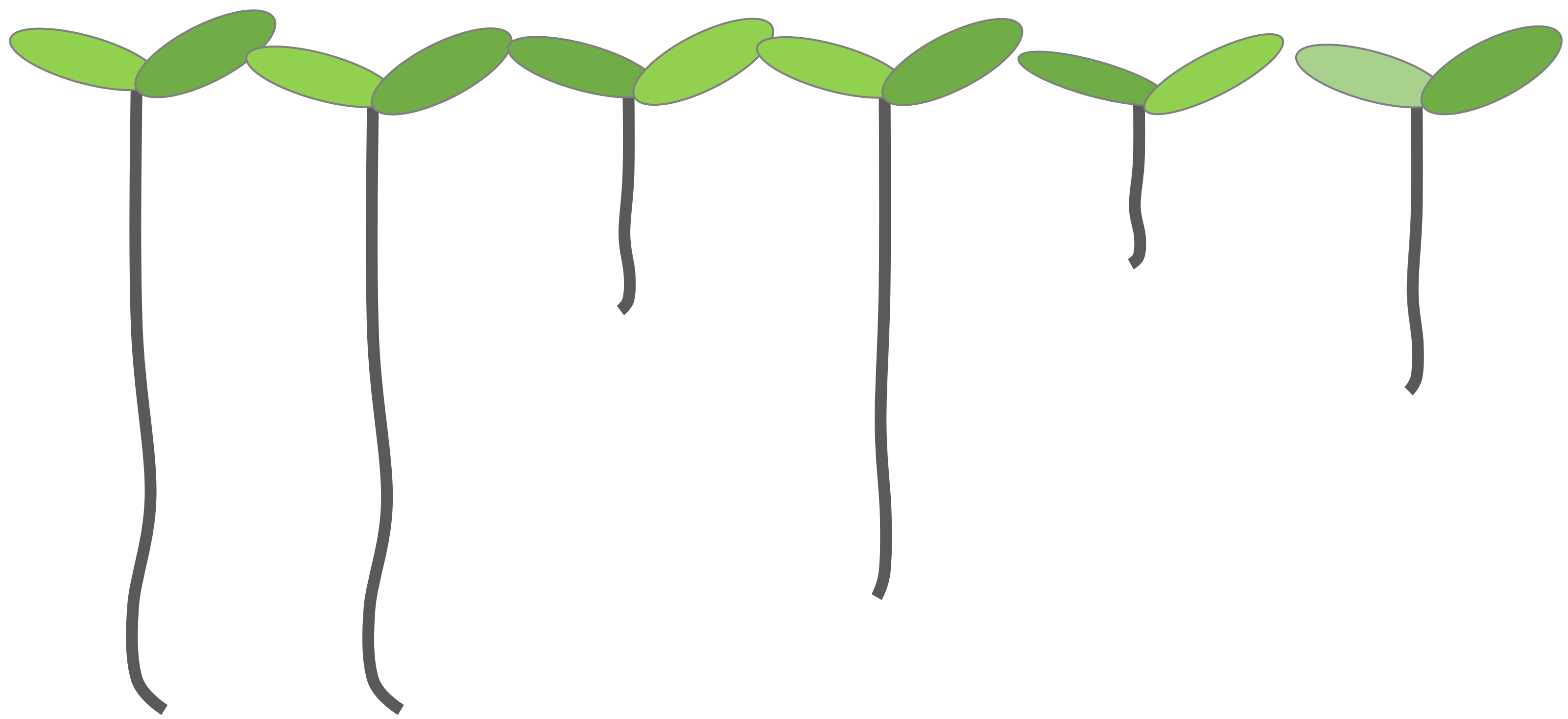


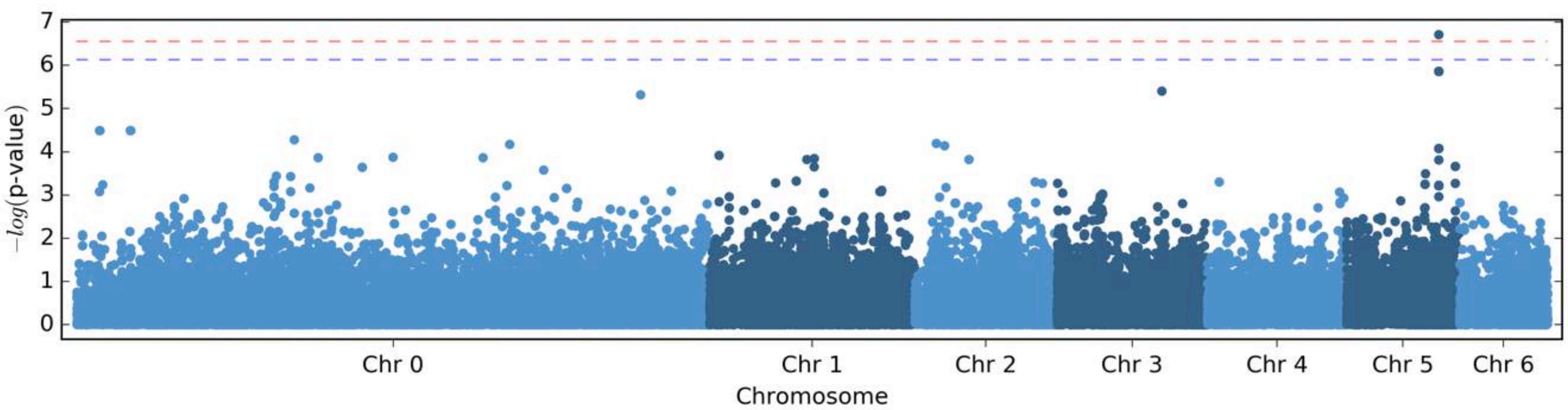
SNPs

A	A	A	A	A	A
T	T	T	T	T	T
T	T	T	T	T	T
C	C	C	C	C	C
G	G	G	G	G	G
C	C	C	C	C	C
T	T	T	T	T	T
A	A	A	A	A	A
G	G	G	G	G	G
C	C	C	C	C	C

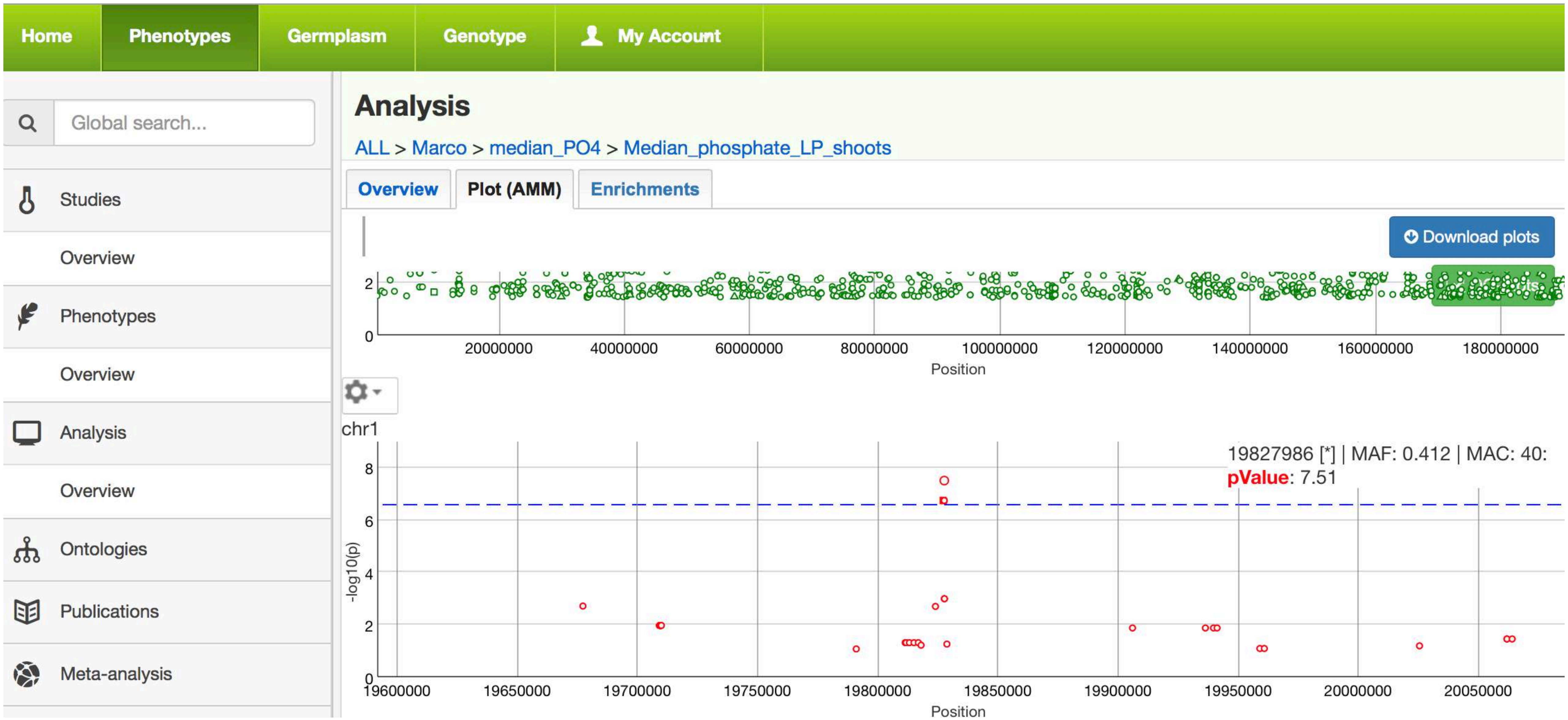
Genome Wide Association Study (GWAS)

Root length





GWAPP: A web application for GWAS in Arabidopsis and Lotus (lotus.au.dk/gwas)



Project of Stig Andersen and Uemit Seren

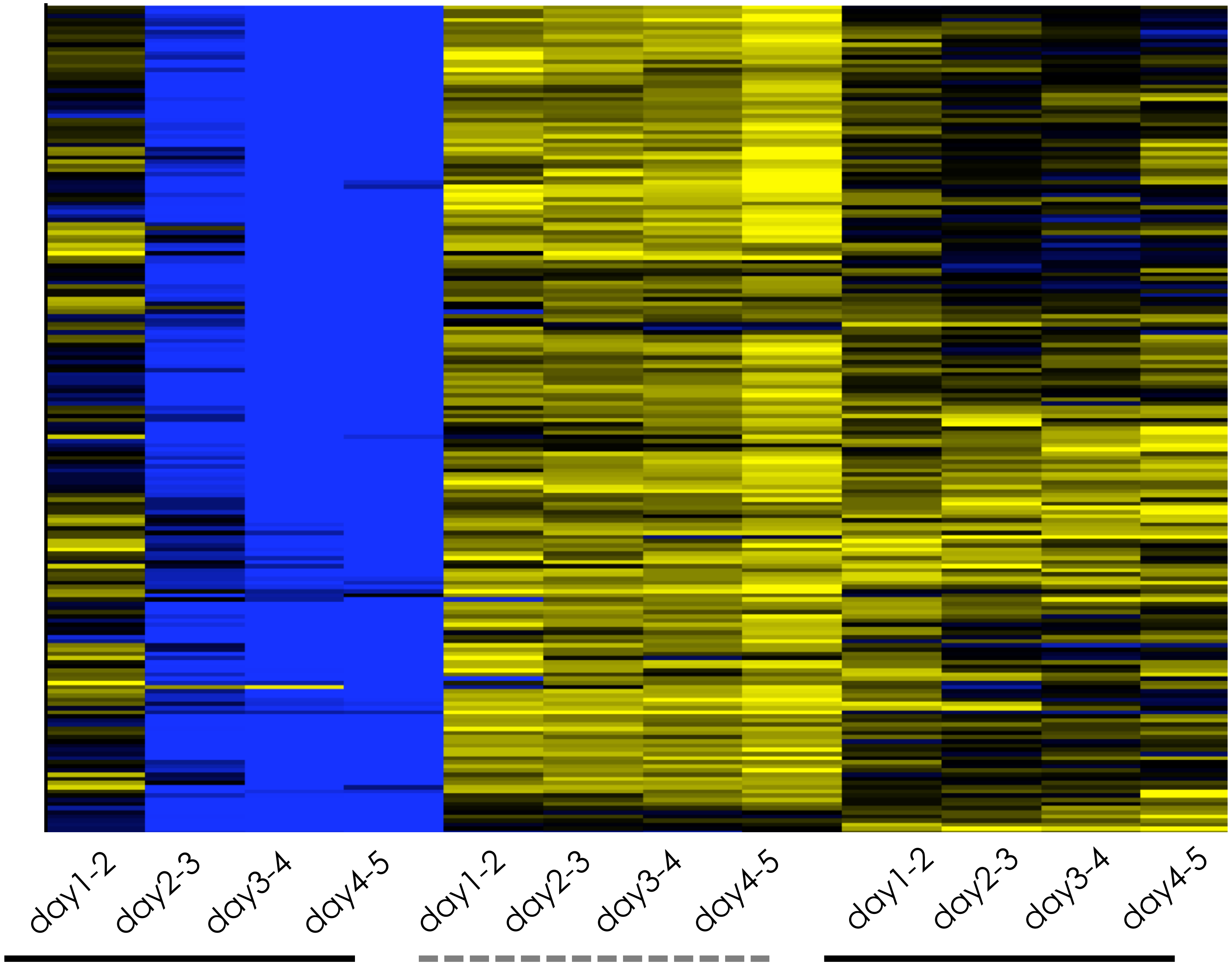
Aims

1. Are mycorrhiza forming plant species responding to phosphate deficiency in a manner similar to *Arabidopsis thaliana* ?

2. Are root growth traits correlated to nutrient accumulation capacity?

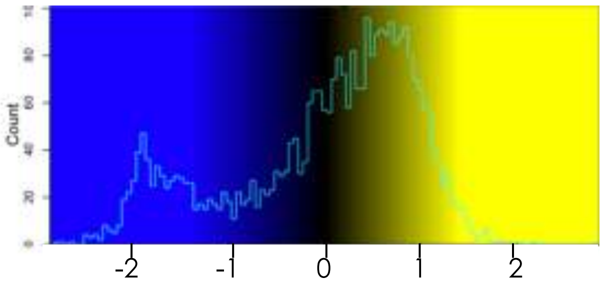
3. Are variants of orthologous genes associated with P-related traits in different species?

Low phosphate has a unique bimodular effect on Arabidopsis root growth



Growth rate

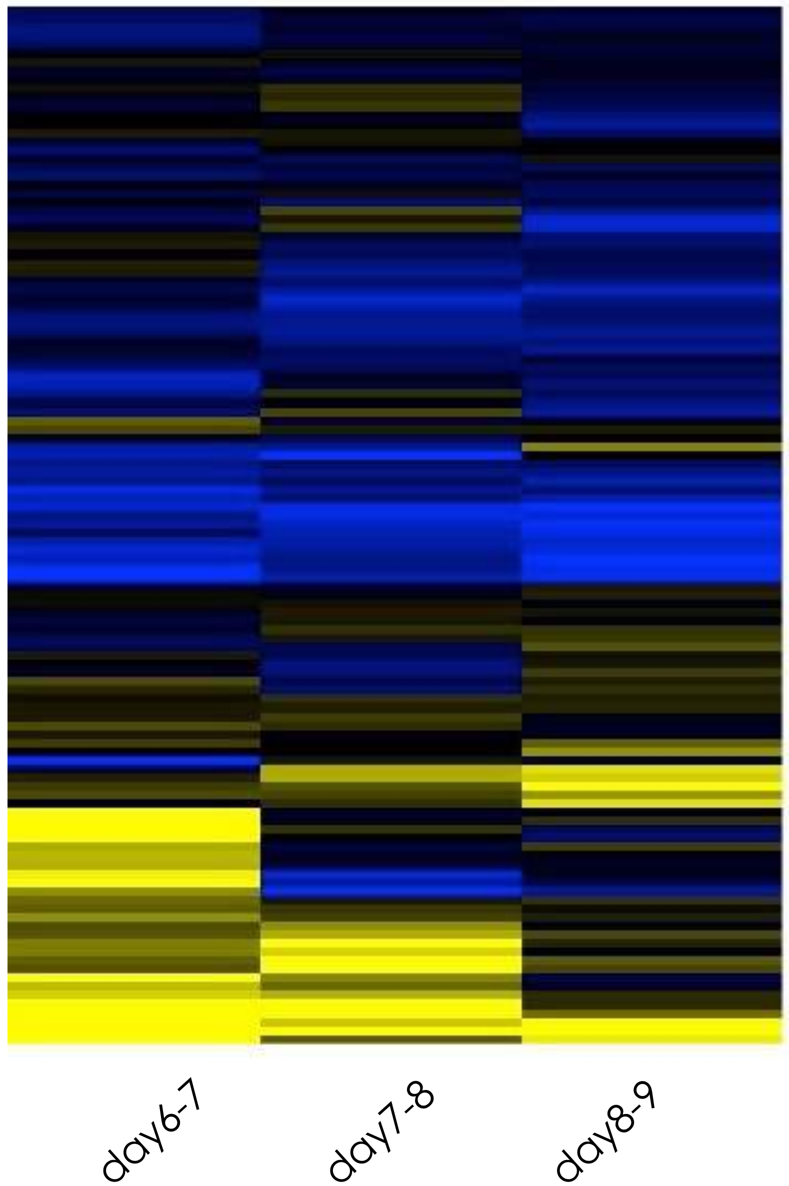
231 *A. thaliana* accessions



data from Santosh Satbhai

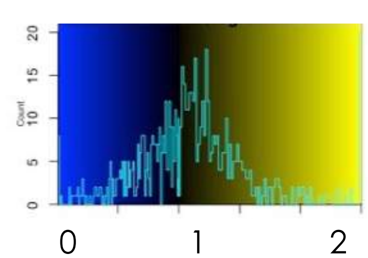
Both Medicago and Lotus have a genotype-dependent bimodular responses to low phosphate

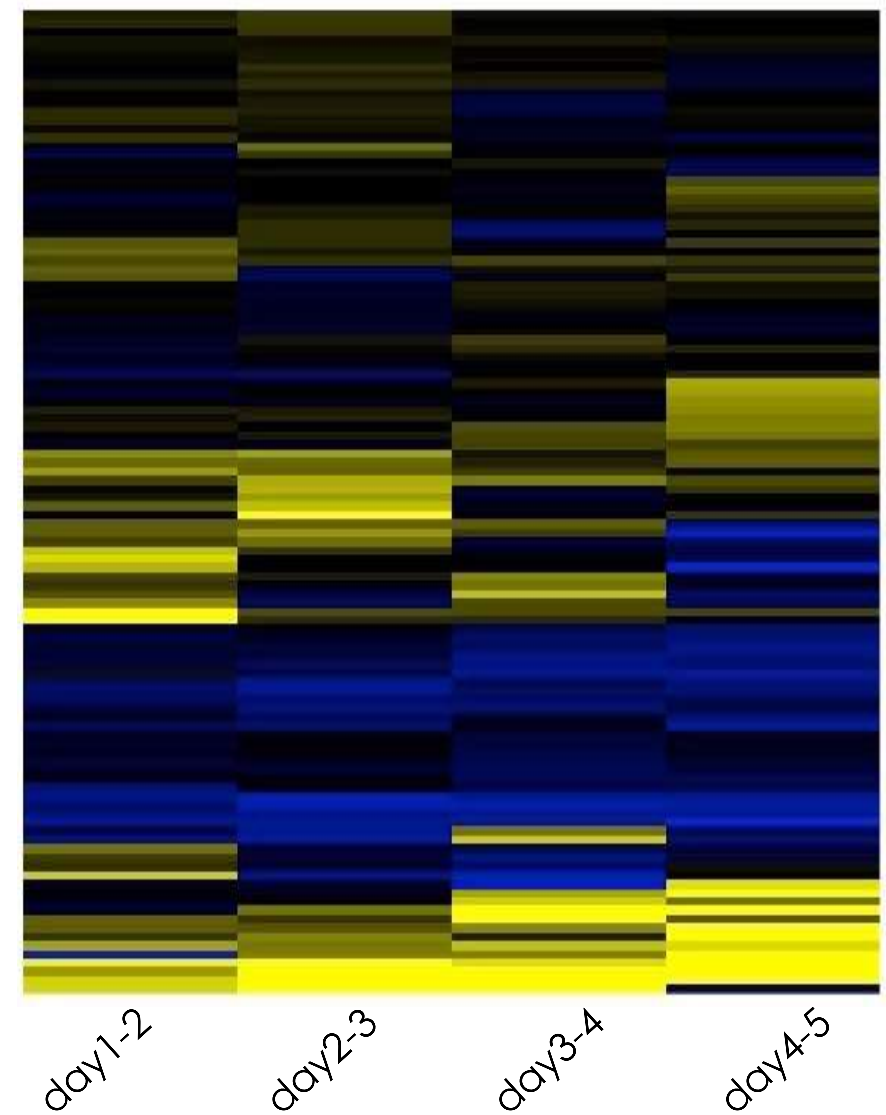
130 Lotus accessions



Growth rate

140 Medicago accessions





Growth rate

Aims

1. Are mycorrhiza forming plant species responding to phosphate deficiency in a manner similar to *Arabidopsis thaliana* ?

2. Are root growth traits correlated to nutrient accumulation capacity?

3. Are variants of orthologous genes associated with P-related traits in different species?

High Throughput
Root Phenotyping

Tissue Phosphate
quantification

2. Are root growth traits correlated to nutrient accumulation capacity?

Lotus shoot and root
phosphate concentration

?

Lotus

Total root width

Root length

Root direction

Hypocotyl width

Root tip width

Root tortuosity

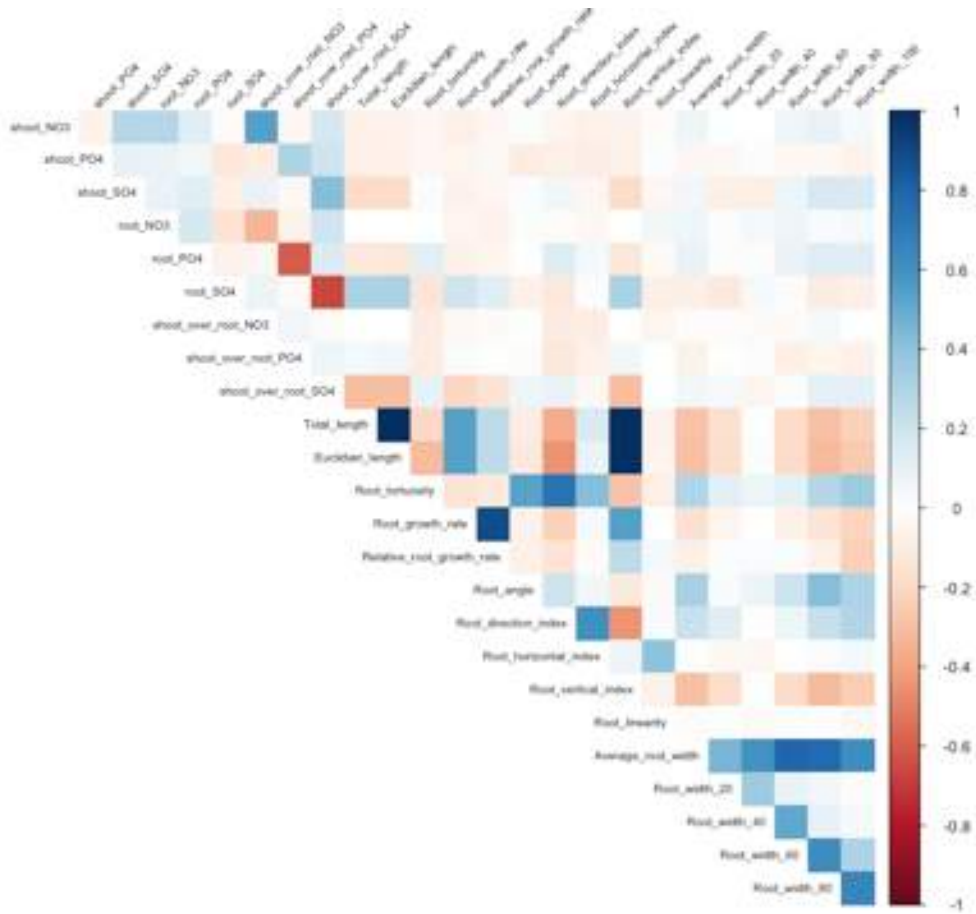
Root growth rate

High Throughput
Root Phenotyping

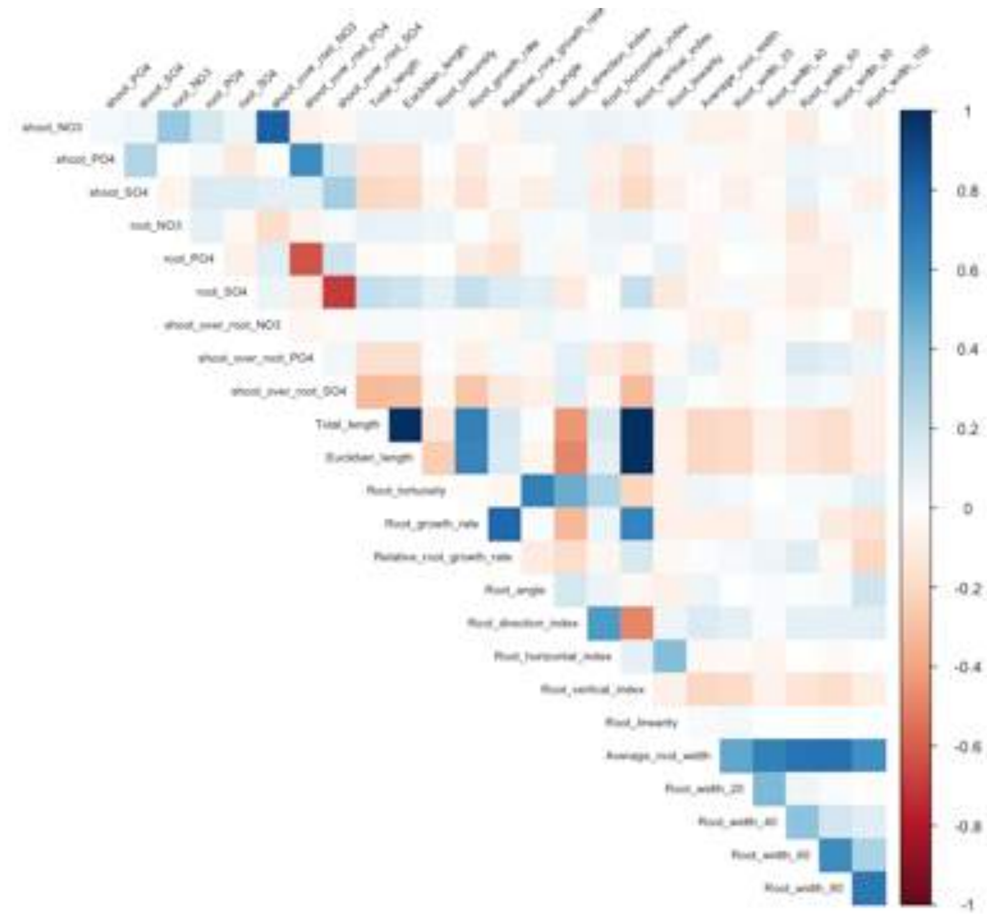
Tissue Phosphate
quantification

In Lotus most of correlations among traits do not depend on P levels

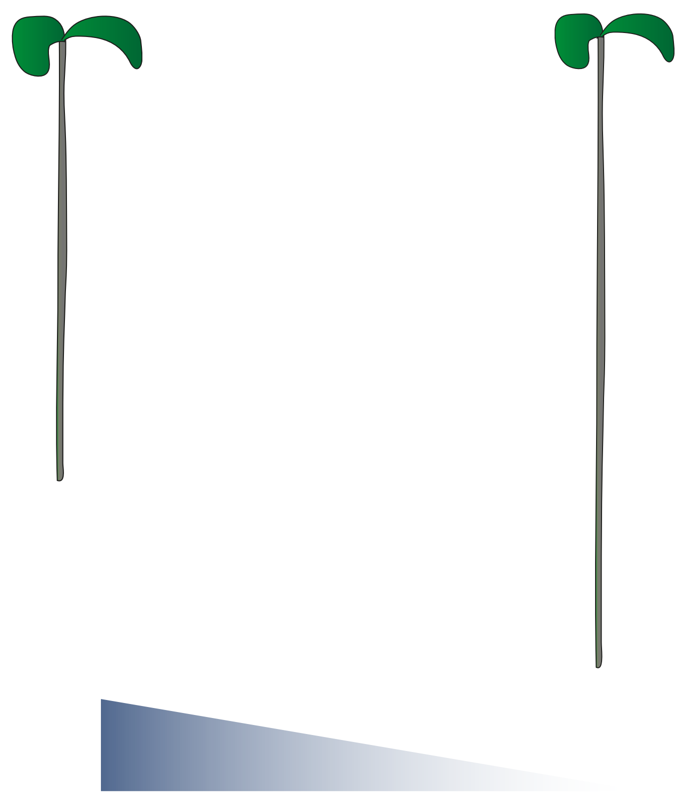
Traits over LP

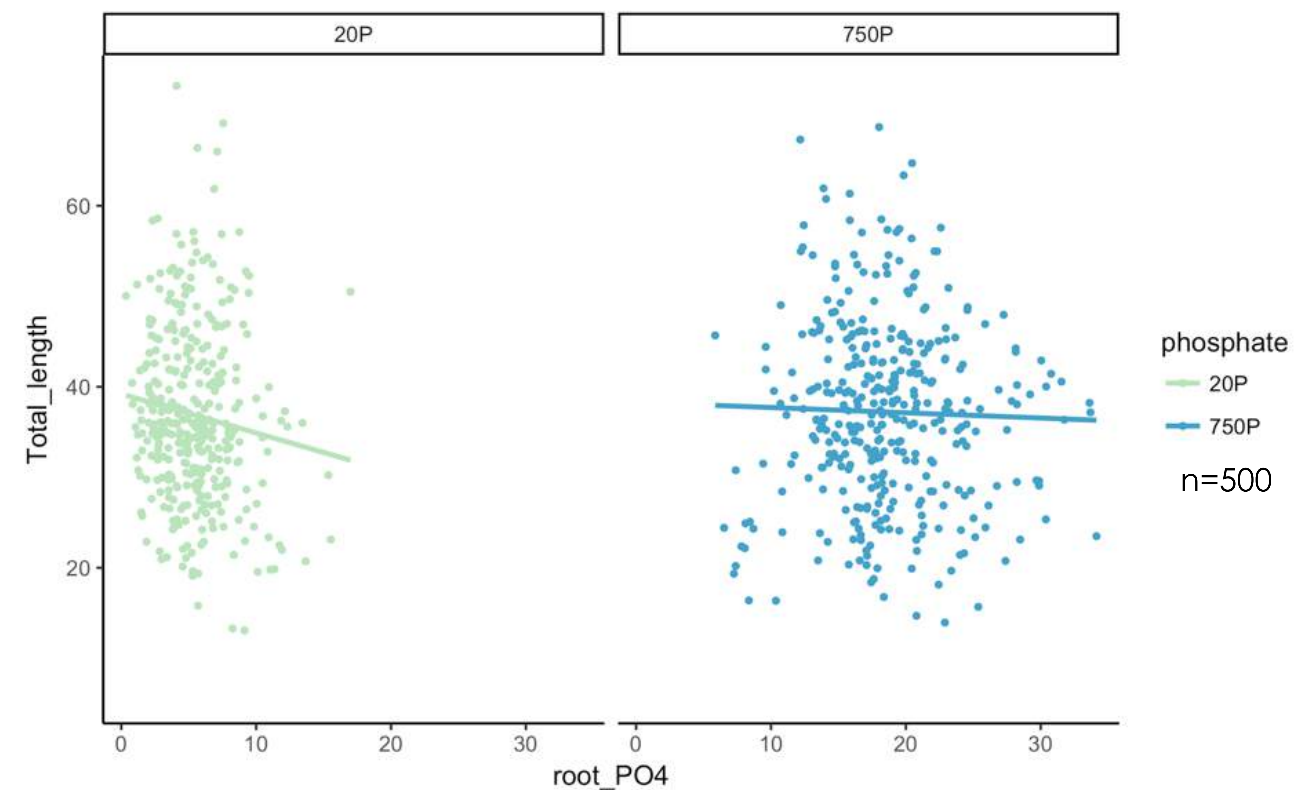


Traits over HP

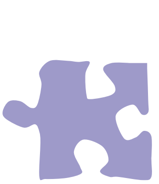


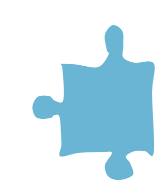
Only in low phosphate medium, root length negatively correlates with phosphate concentration



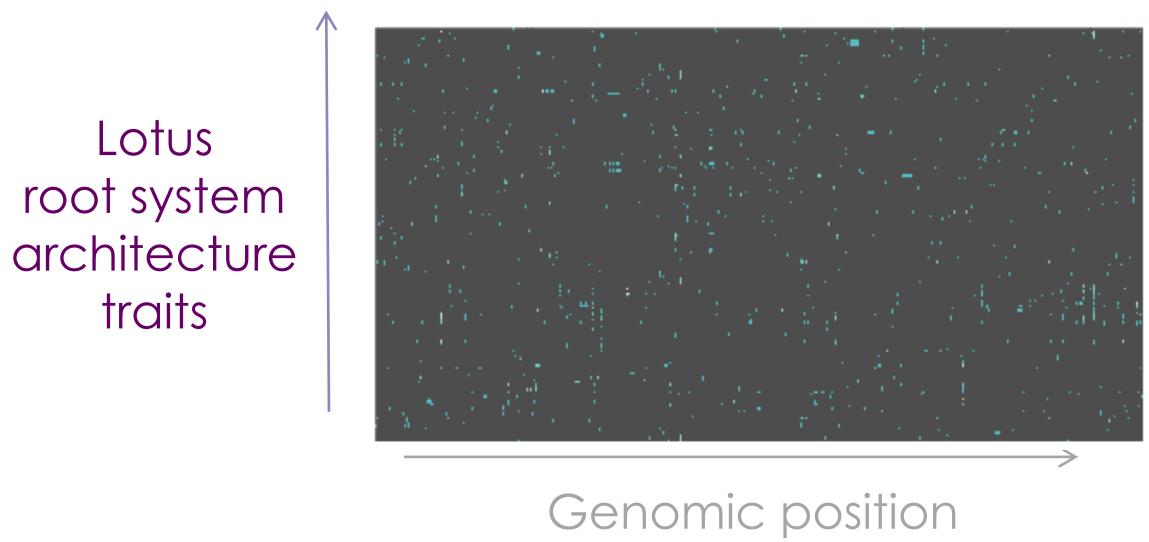


r= -0.12 p=0.03 r= -0.02 p=0.59

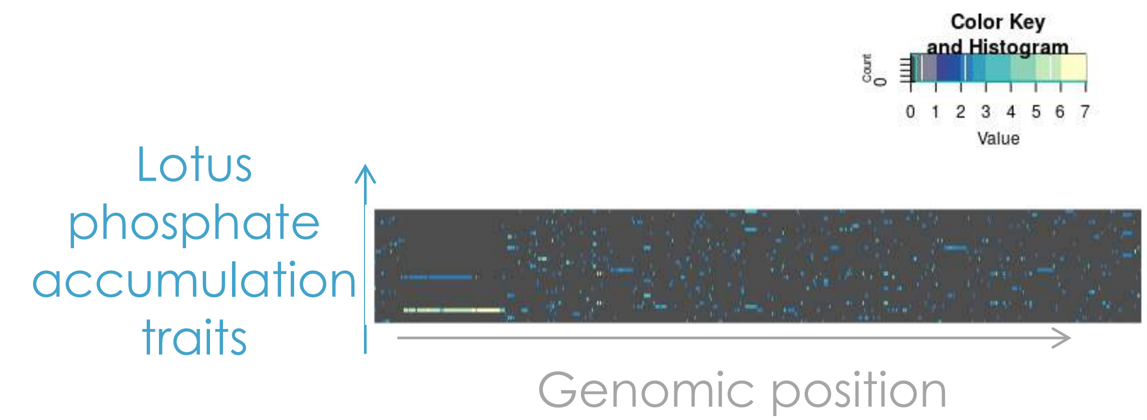


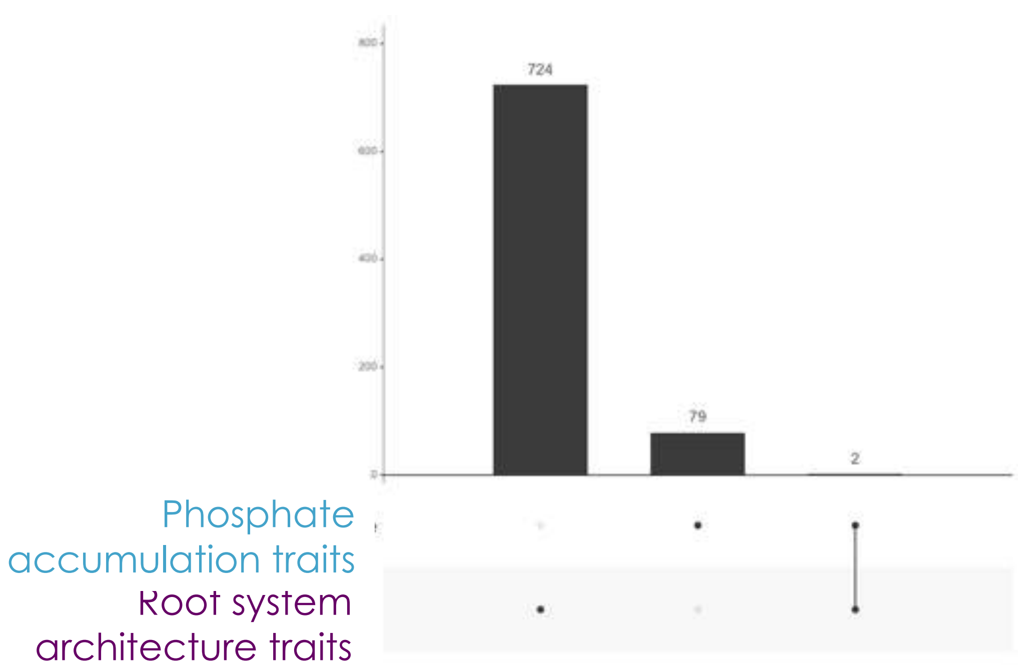


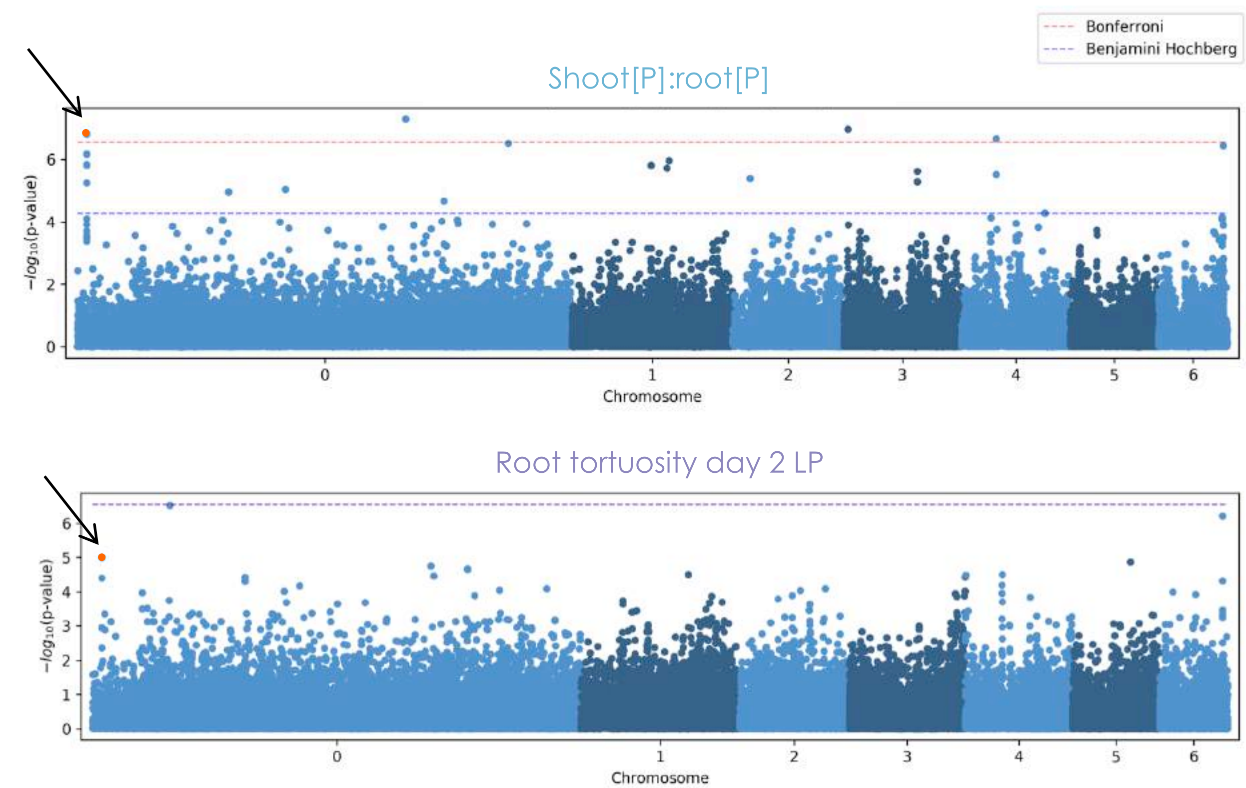
Overlapping Lotus GWAS from different traits pinpoint to a single locus



vs.





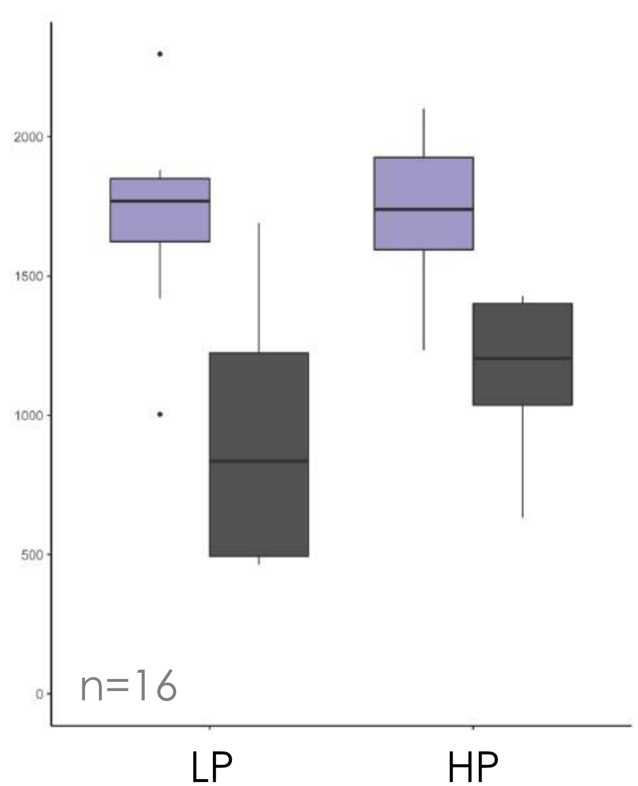


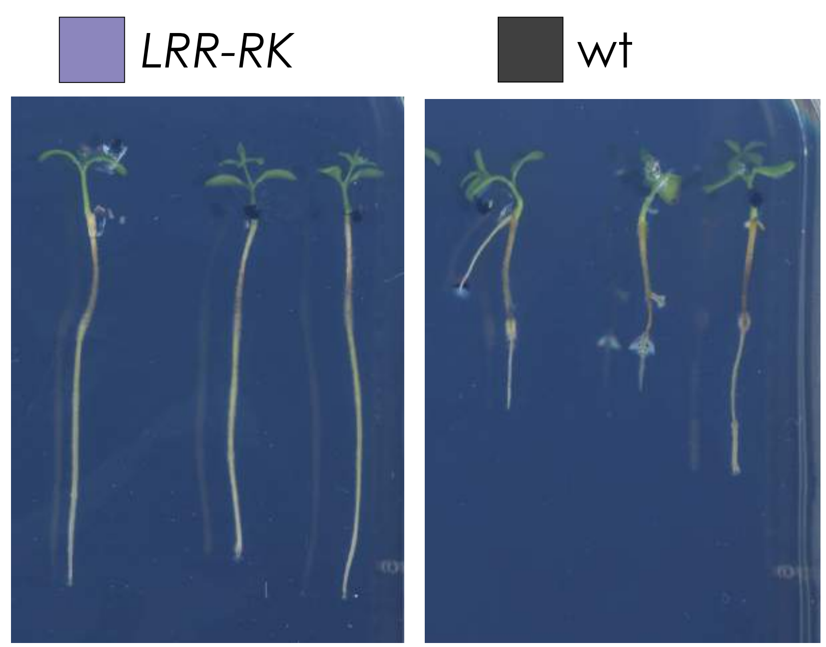
High Throughput Root Phenotyping

Tissue Phosphate quantification

A cell surface receptor, LRR-RK, associated with different P-dependent traits, controls primary root growth and phosphate accumulation in Lotus leaves

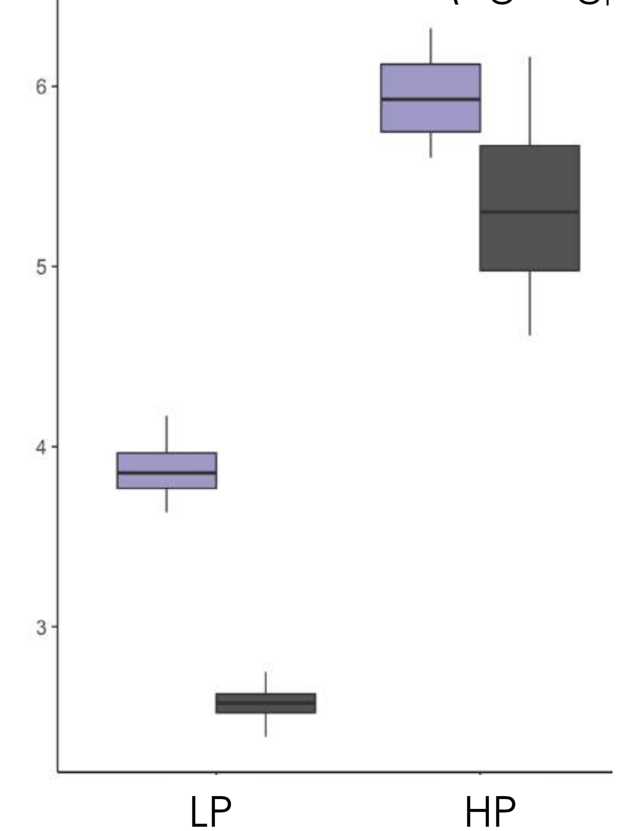
Primary root length (px)





LP day 10

Leaf phosphate concentration (ng/mg_{FW})



- A possible receptor of root to shoot phosphate signaling?
- Low level of expression (hard to spot with other approaches)

High Throughput Root Phenotyping

Tissue Phosphate quantification

Aims

1. Are mycorrhiza forming plant species responding to phosphate deficiency in a manner similar to *Arabidopsis thaliana* ?

2. Are root growth traits correlated to nutrient accumulation capacity?

3. Are variants of orthologous genes associated with P-related traits in different species?

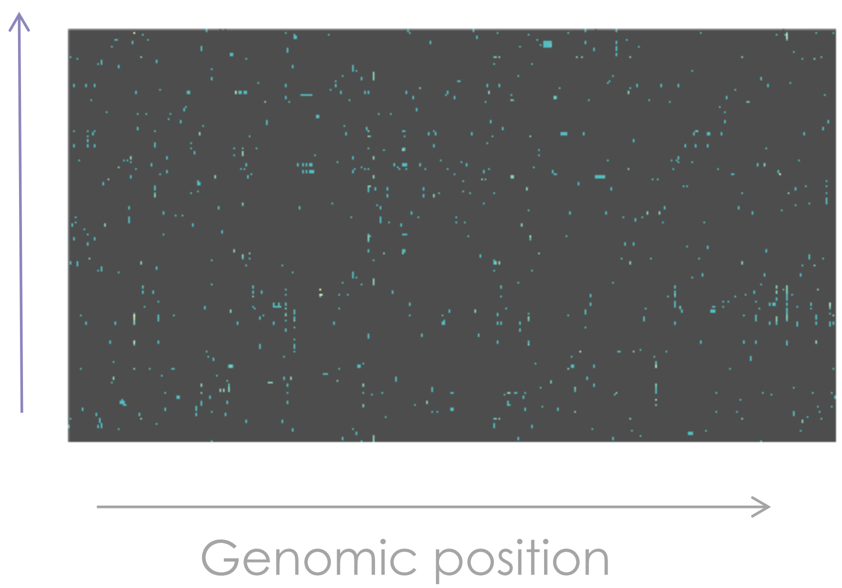
Multi plant species approach

High Throughput Root Phenotyping

Tissue Phosphate quantification

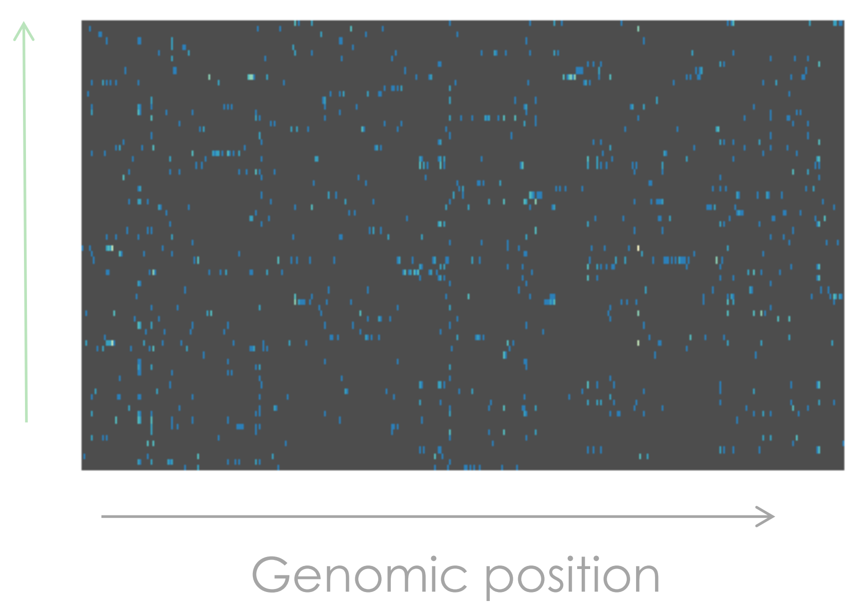
Overlapping GWAS from different plant species pinpoints to a few loci

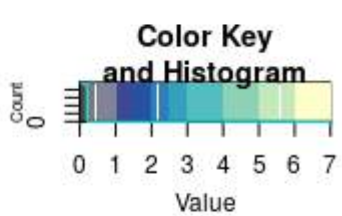
Lotus
Root system
architecture
traits



Lotus
phosphate
accumulation
traits

Arabidopsis
Root system
architecture
traits



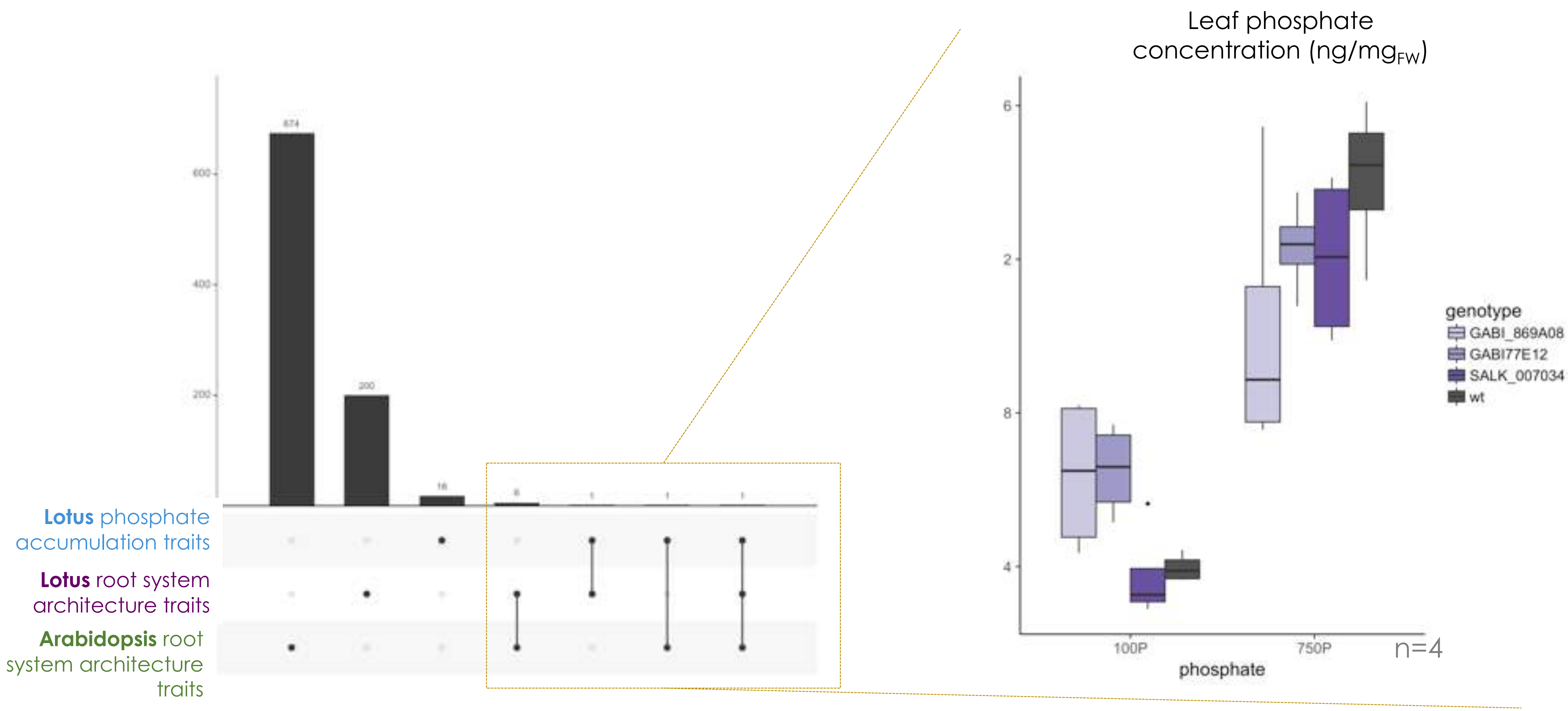


Multi plant
species
approach

Tissue Phosphate
quantification

High Throughput
Root Phenotyping

Variation in orthologous genes in Arabidopsis and Lotus is associated with P responses less than expected by chance

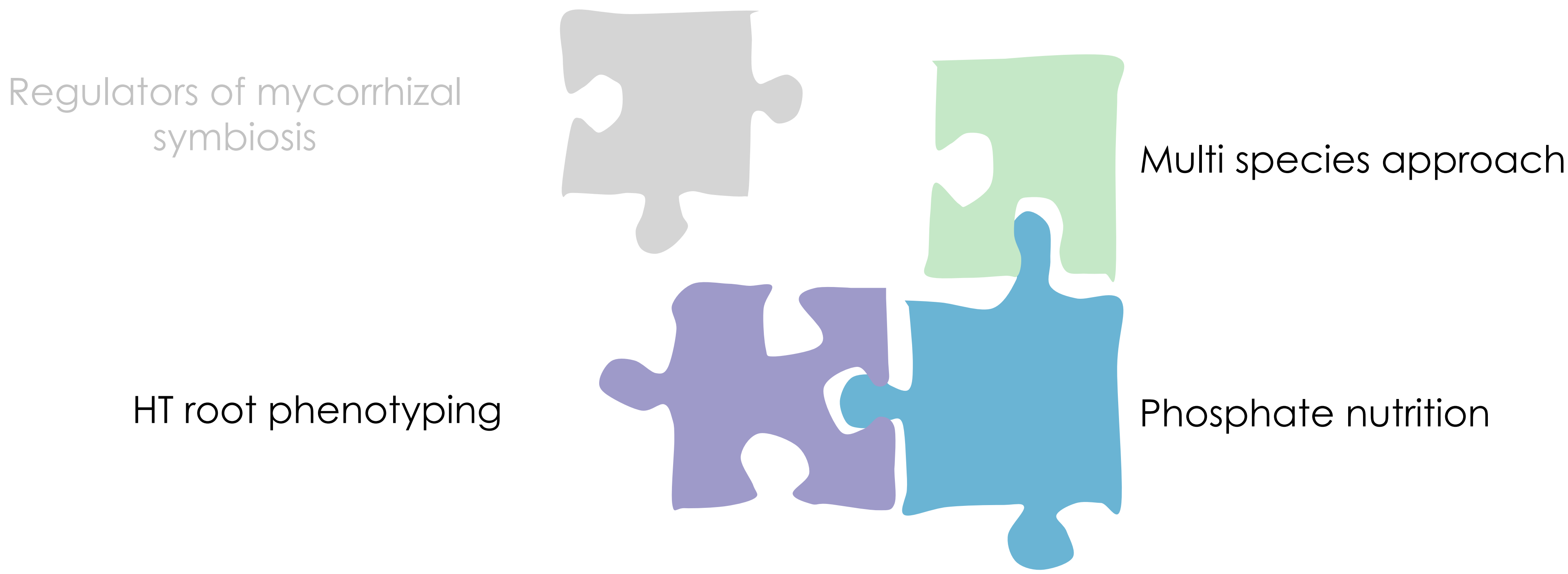


-The number of overlaps among plant species is less than what expected by chance (10000 permutation test on the same gene lists)

-3 out 3 genes selected are responsible for different phosphate accumulation in Arabidopsis (based on available data for 300 mutants, p=0.02 to get it by chance).

- 1 mutant line available in Lotus shows the same phenotype

Conclusions



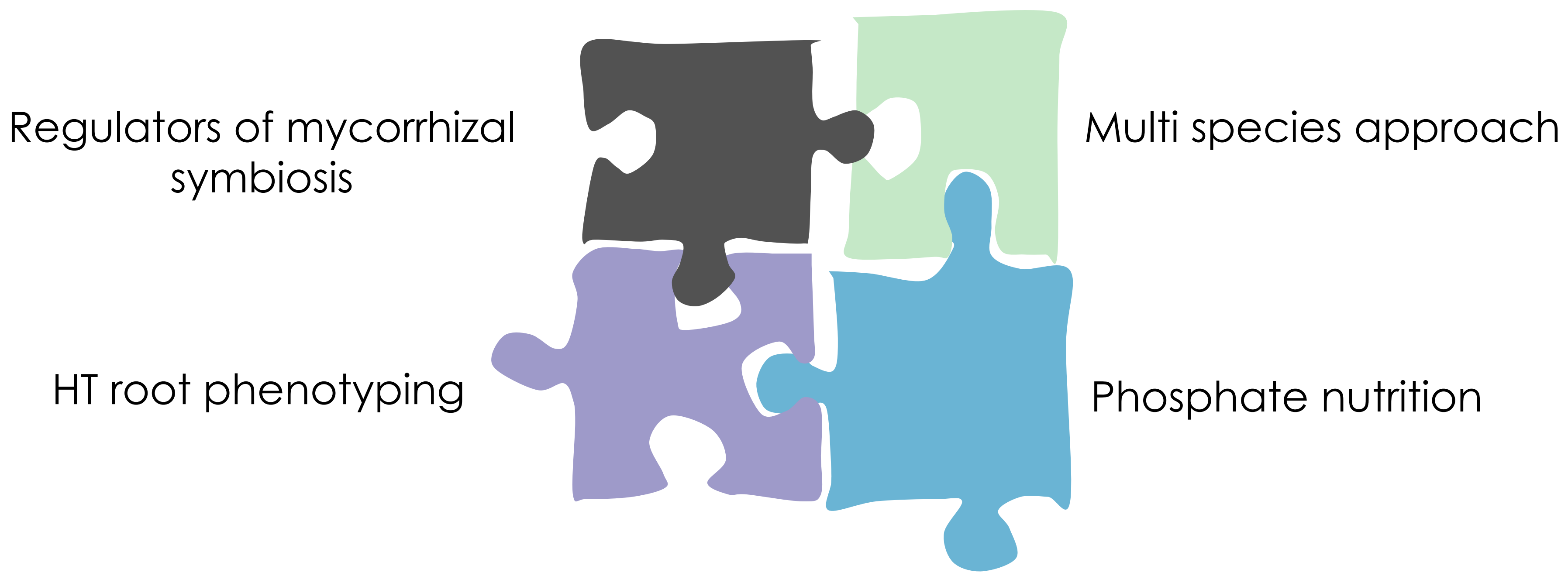
- Lotus is a feasible plant for HT root phenotyping

- Integration of phosphate accumulation and root growth highlights true positive genes in phosphate

- A cell surface receptor, Leucine-Rich Repeat Receptor Kinase, regulates root growth and phosphate accumulation in Lotus

- Comparing GWAS hits from different plant species, we identified three genes involved in phosphate accumulation

What’s next?



- Understand at which level the cell surface protein is regulating phosphate levels in Lotus (root to shoot signal?)

- Comparing Lotus vs. Medicago GWAS overlap (phospholipase)

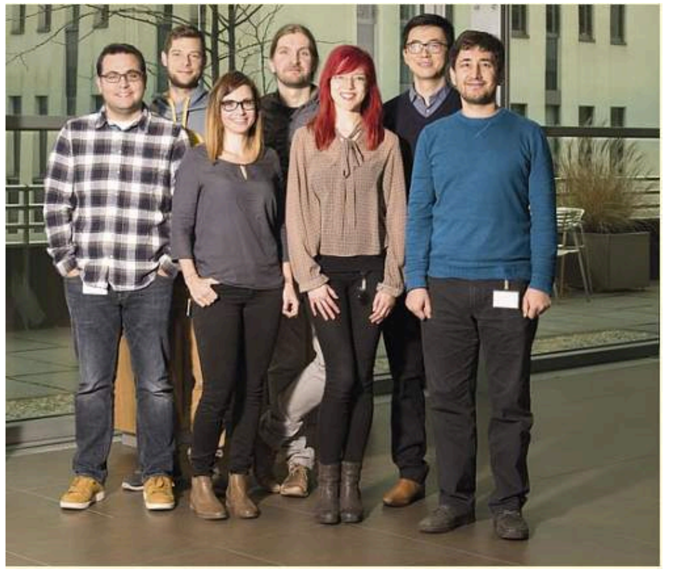
- Dissecting Lotus mutants for their capacity of forming mycorrhizal interaction over different phosphate levels

- Set up a root phenotyping system for later stages of growth

Acknowledgements

Wolfgang Busch
Christian Goeschl
Alexander Bindeus
Santosh Satbhai
Anna Małolepszy
Katharina Jandrasits
Samantha Krasnodebski
and the group

Yasin Dagdas & group



Nordborg group
Uemit Seren
Rahul Pisupati

Stanislav Kopriva
University of Cologne

Stig Andersen
University of Aarhus

Medicago Hapmap project

VBCF Plant Facilities

Lab Service

MARIE CURIE ACTIONS

GMI GREGOR MENDEL INSTITUTE OF MOLECULAR PLANT BIOLOGY

ÖAW ÖSTERREICHISCHE AKADEMIE DER WISSENSCHAFTEN

Vienna BioCenter

Thank you for your attention!

Lotus japonicus as a model plant

Sequenced genome (v3.0)

Self pollinating plant species

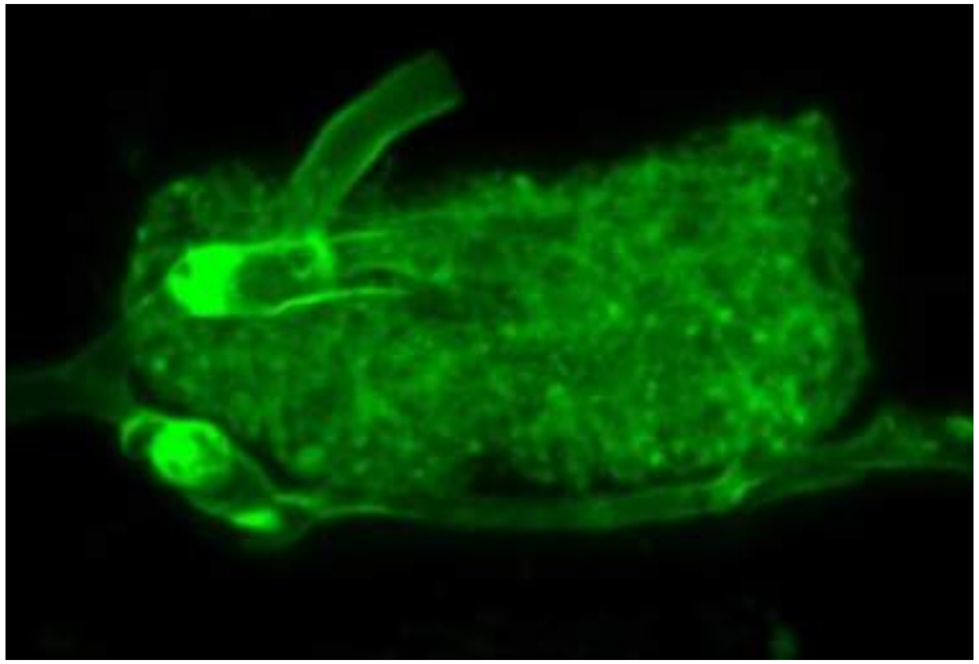
More than 100k insertional mutant lines with 640k annotated insertions

Lotus Base (lotus.au.dk): an integrated online platform

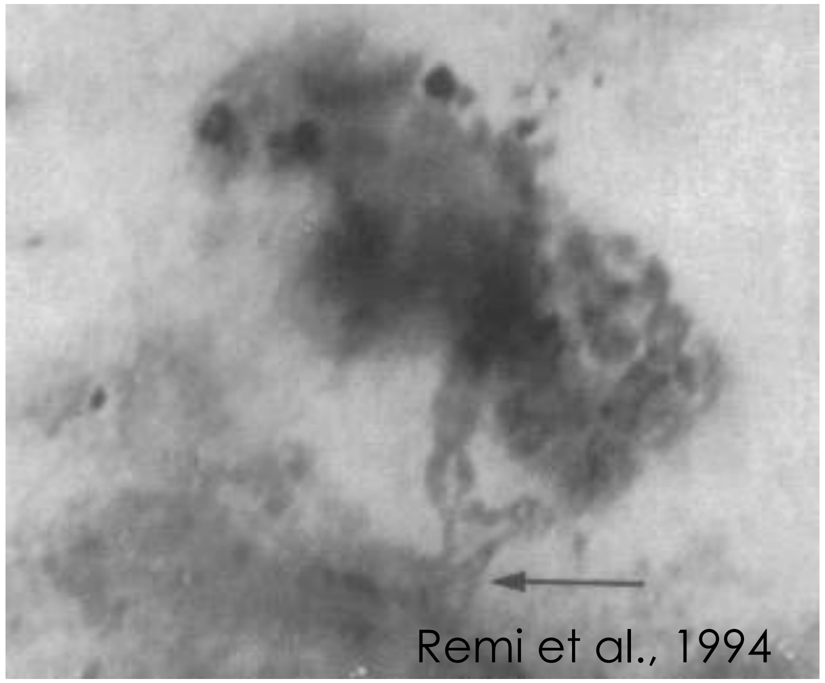
User-friendly online GWAS platform (lotus.au.dk/gwas)

Malolepszy et al., 2016. Mun et al., 2016

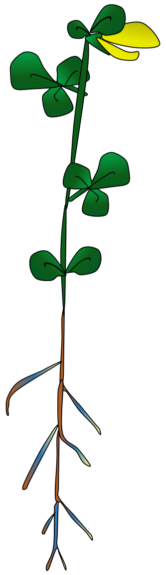
Now and then



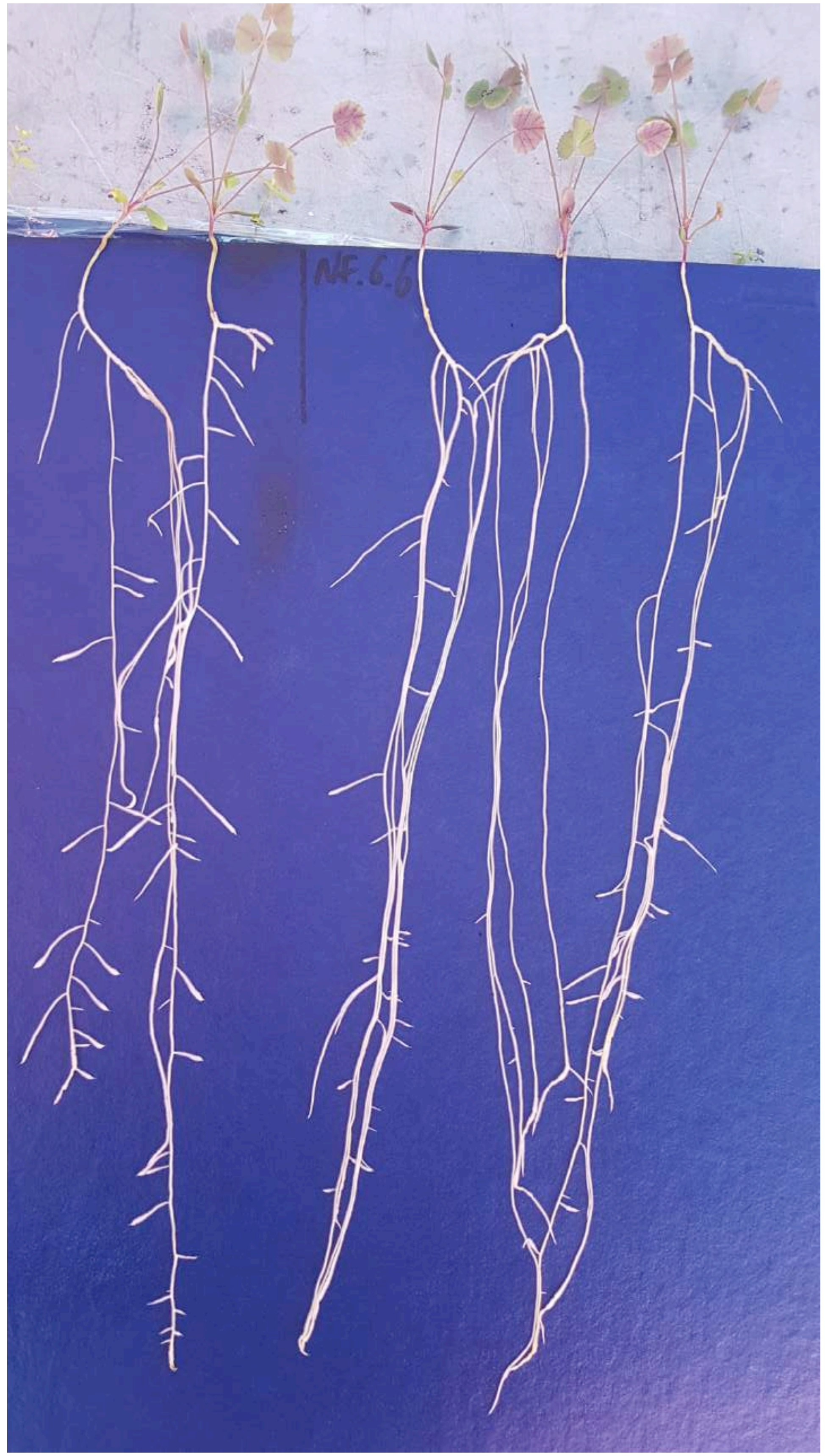
Today



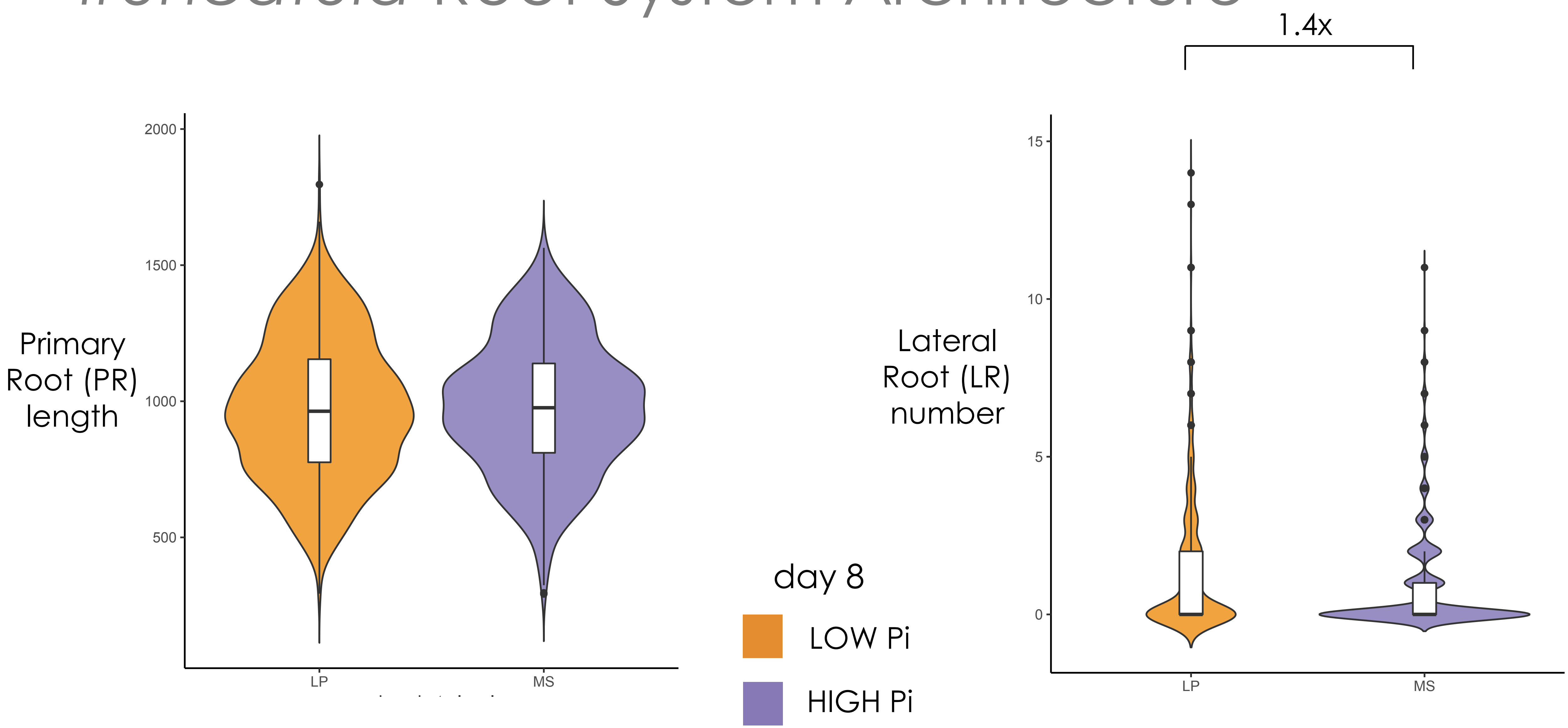
Some 400 MYA



Aglaophyton major


NF.6.6

Effects of phosphate deficiency on *Medicago truncatula* Root System Architecture

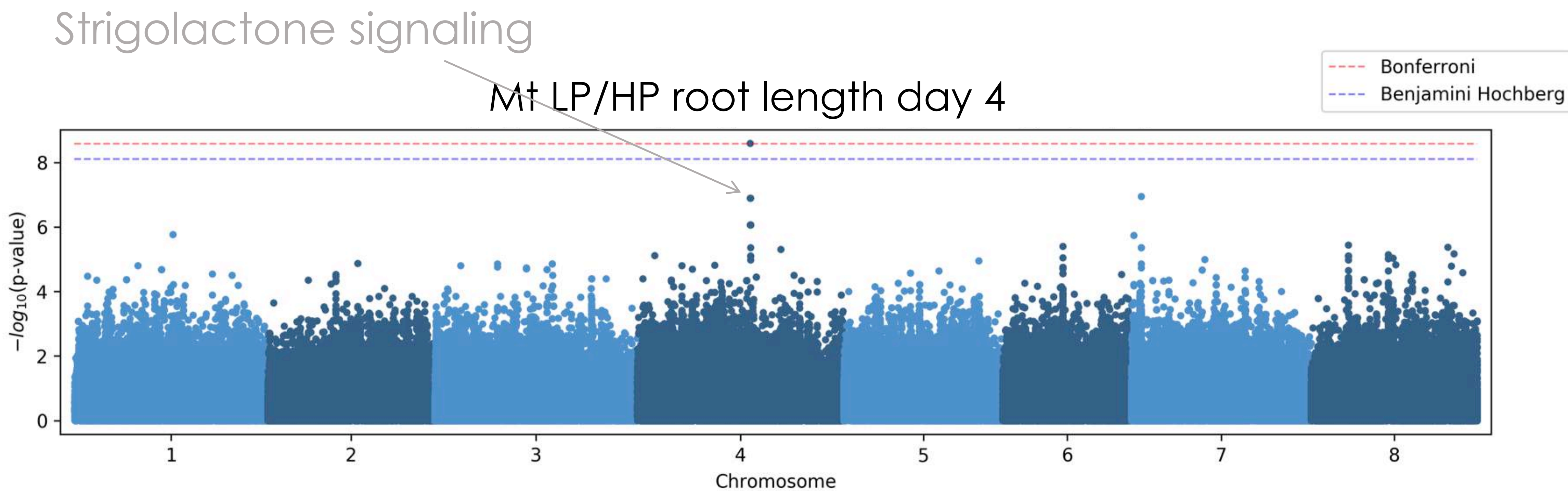


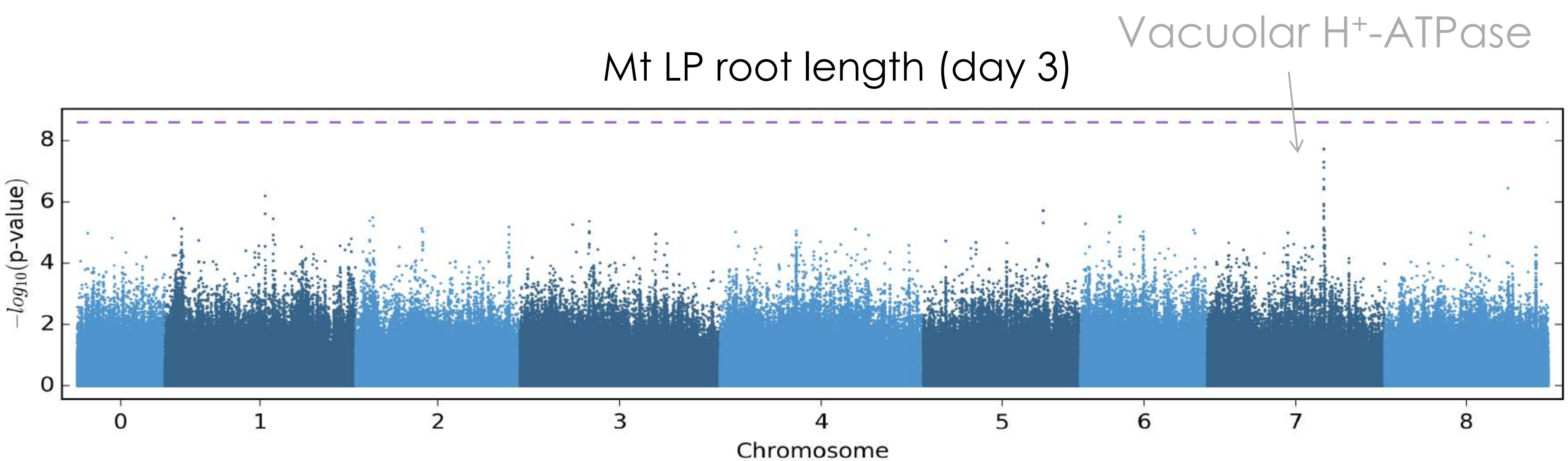
	Effect on PR length (p-value)?
phosphate	0.65
genotype	$<10^{-16}$
phosphate*genotype	$<10^{-16}$

	Effect on LR number (p-value)?
phosphate	$<10^{-16}$
genotype	$<10^{-16}$
phosphate*genotype	$<10^{-16}$

n = 1600

Candidate genes regulating root growth in Medicago

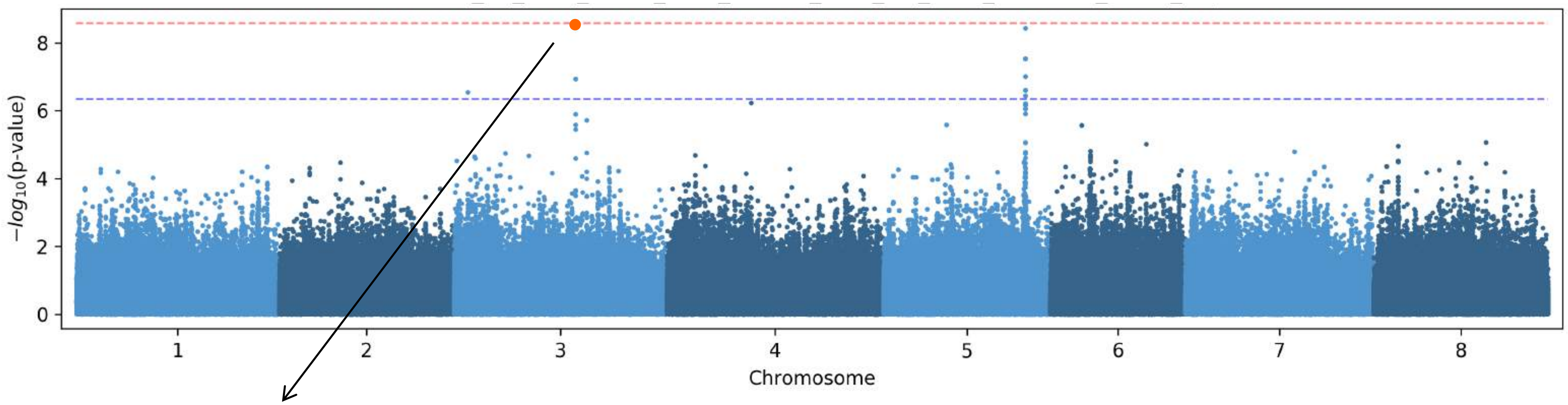




Based on an imputation of Medicago genome (40M SNPs) with Beagle

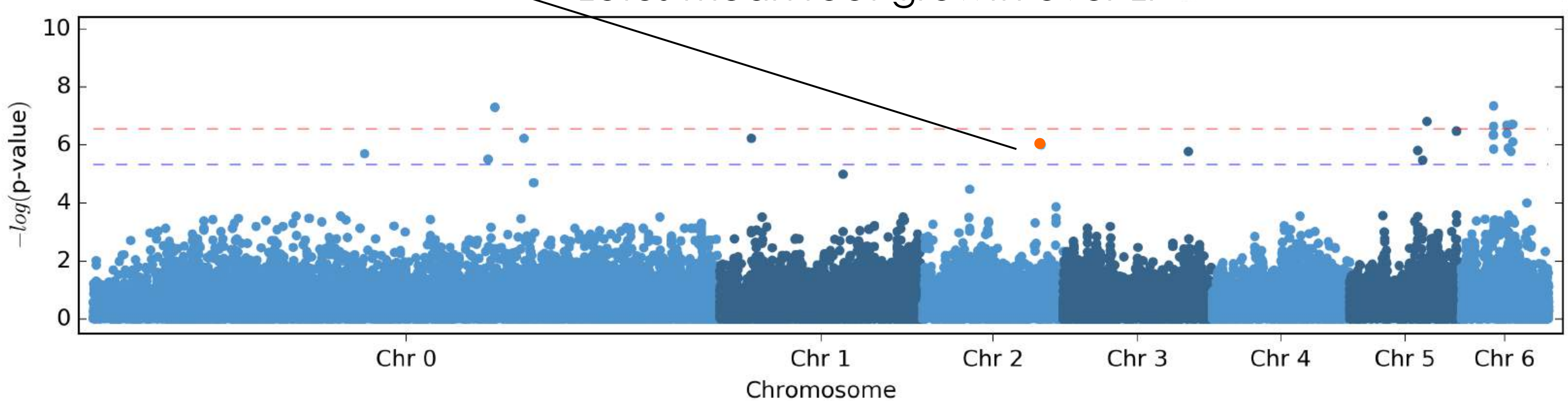
Orthologue loci in Lotus and Medicago are both associated with variation in root responses to phosphate

Medicago ratio(LP/HP) Root direction day 2 (and 3)

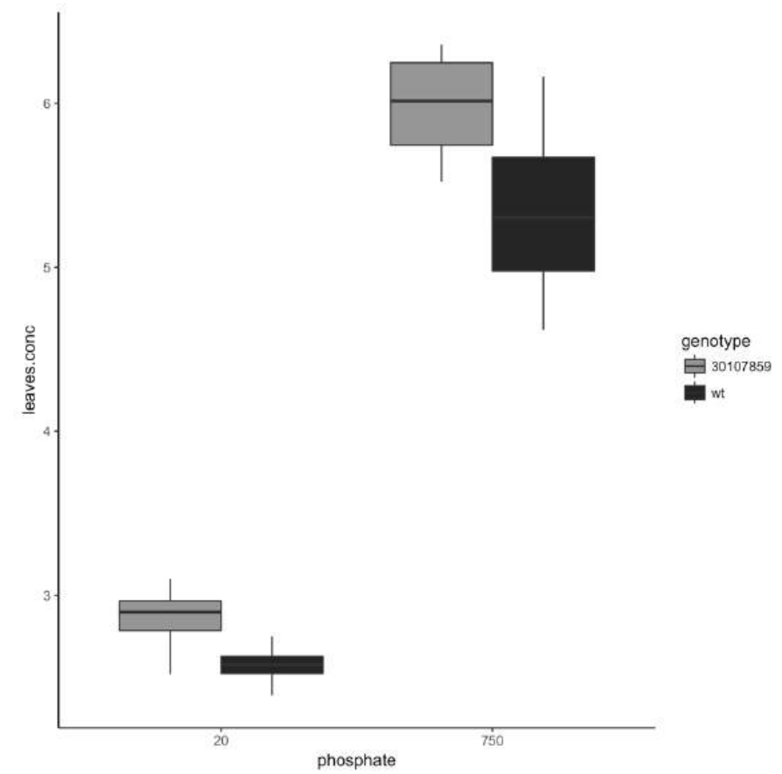


phosphoinositide-specific phospholipase C

Lotus mean root growth over LP

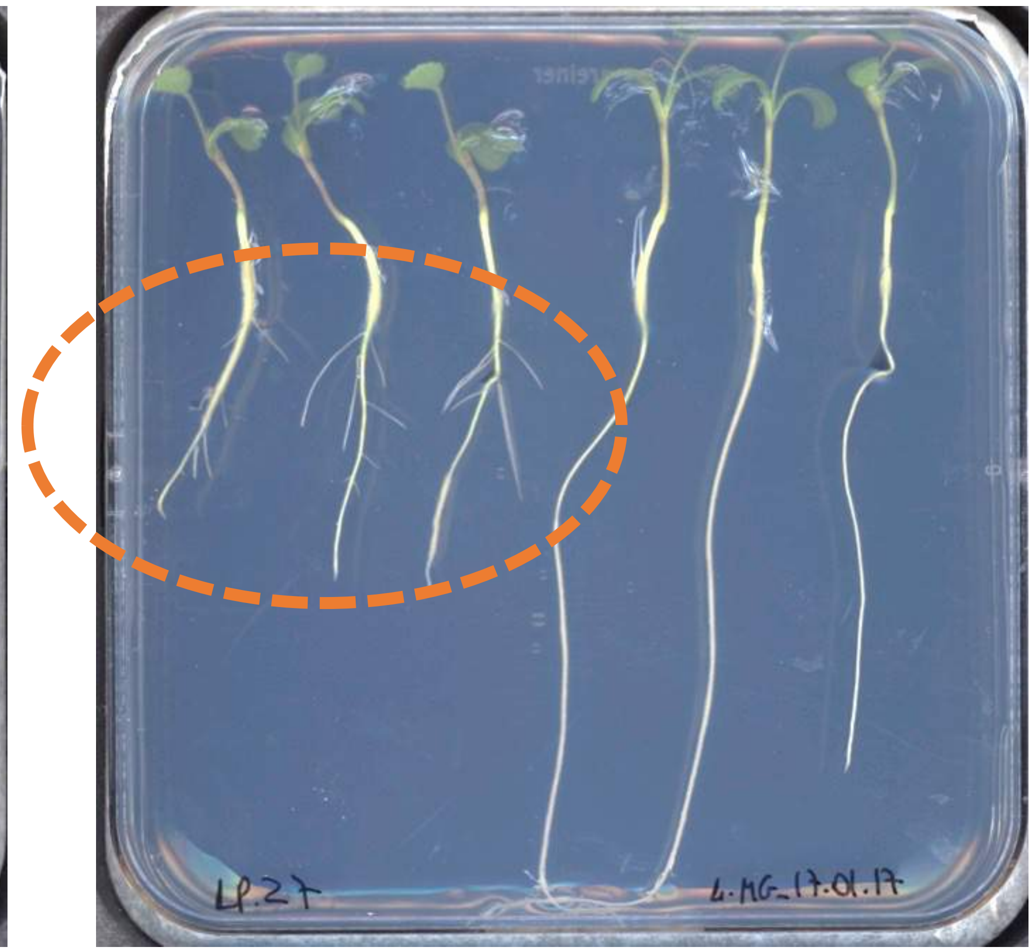


Both involved in lipid changes upon phosphate stress?
Or lipid signaling…


leaves.conc
6
5
4
3
20
750
phosphate
genotype
30107859
wt

Low phosphate is increasing lateral root number exclusively on certain genotypes

phosphate	750 µM	20 µM

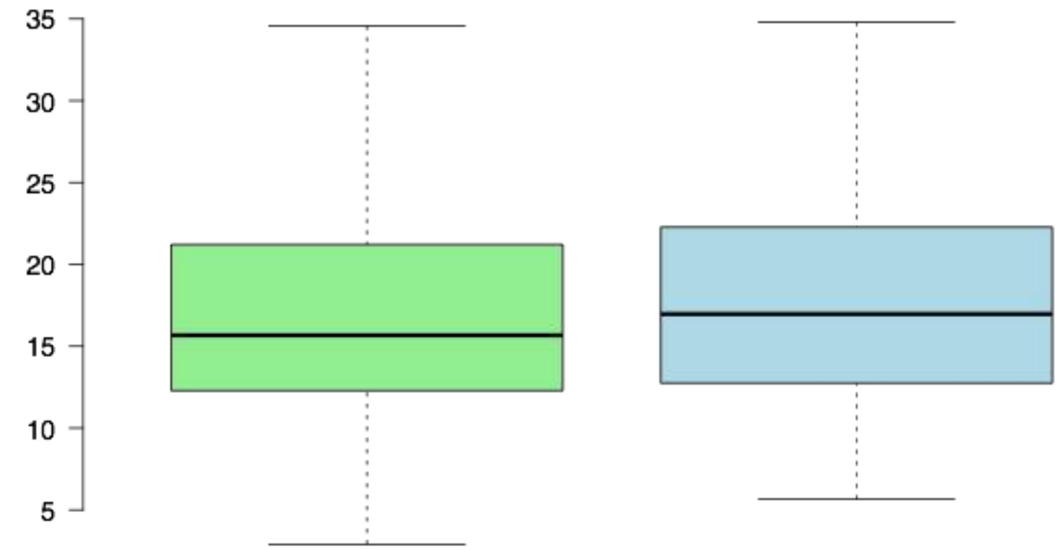


genotype	A	B	A	B

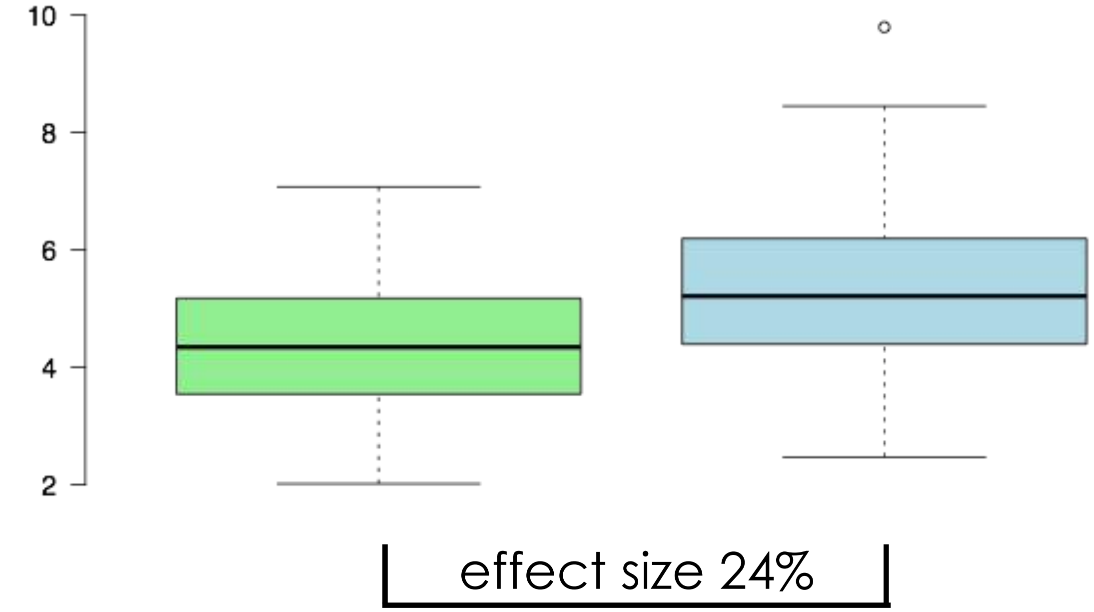
Day 7

Shoot anion concentration (ng/mg$_{FW}$)

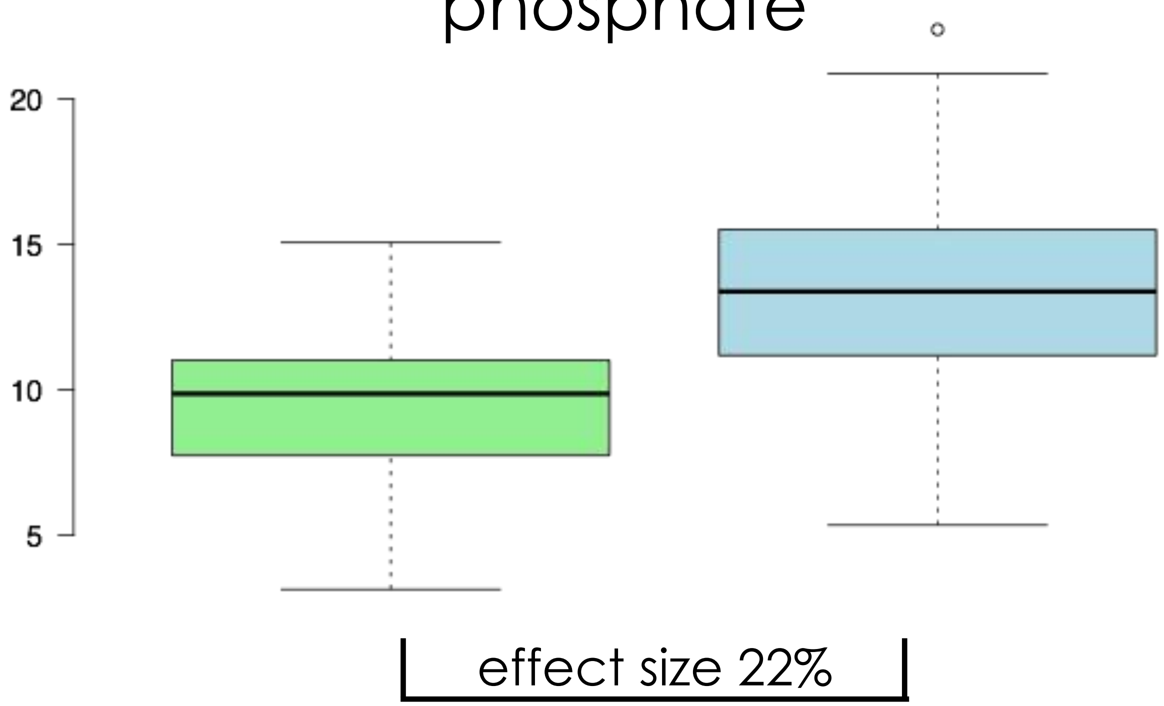
nitrate

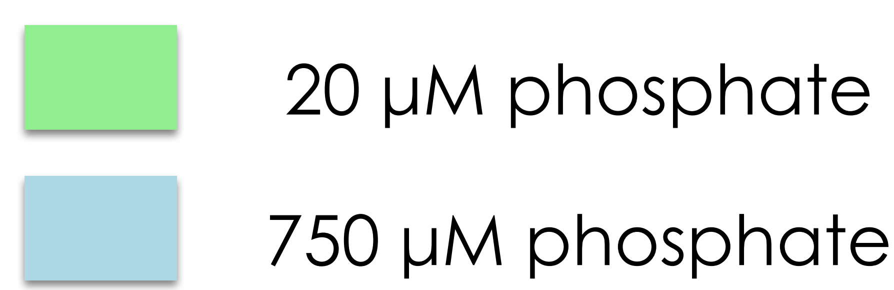


sulfate

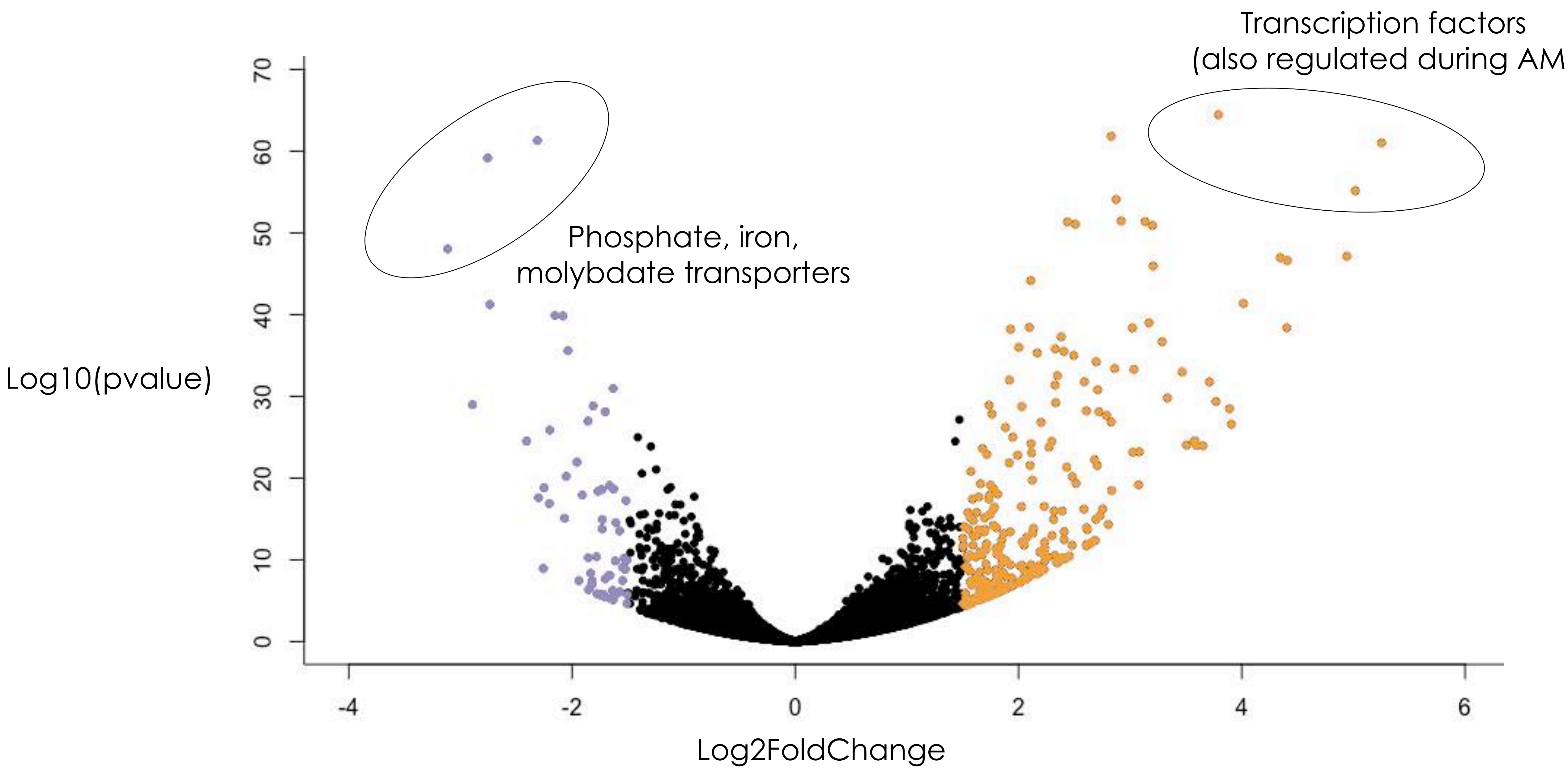


phosphate



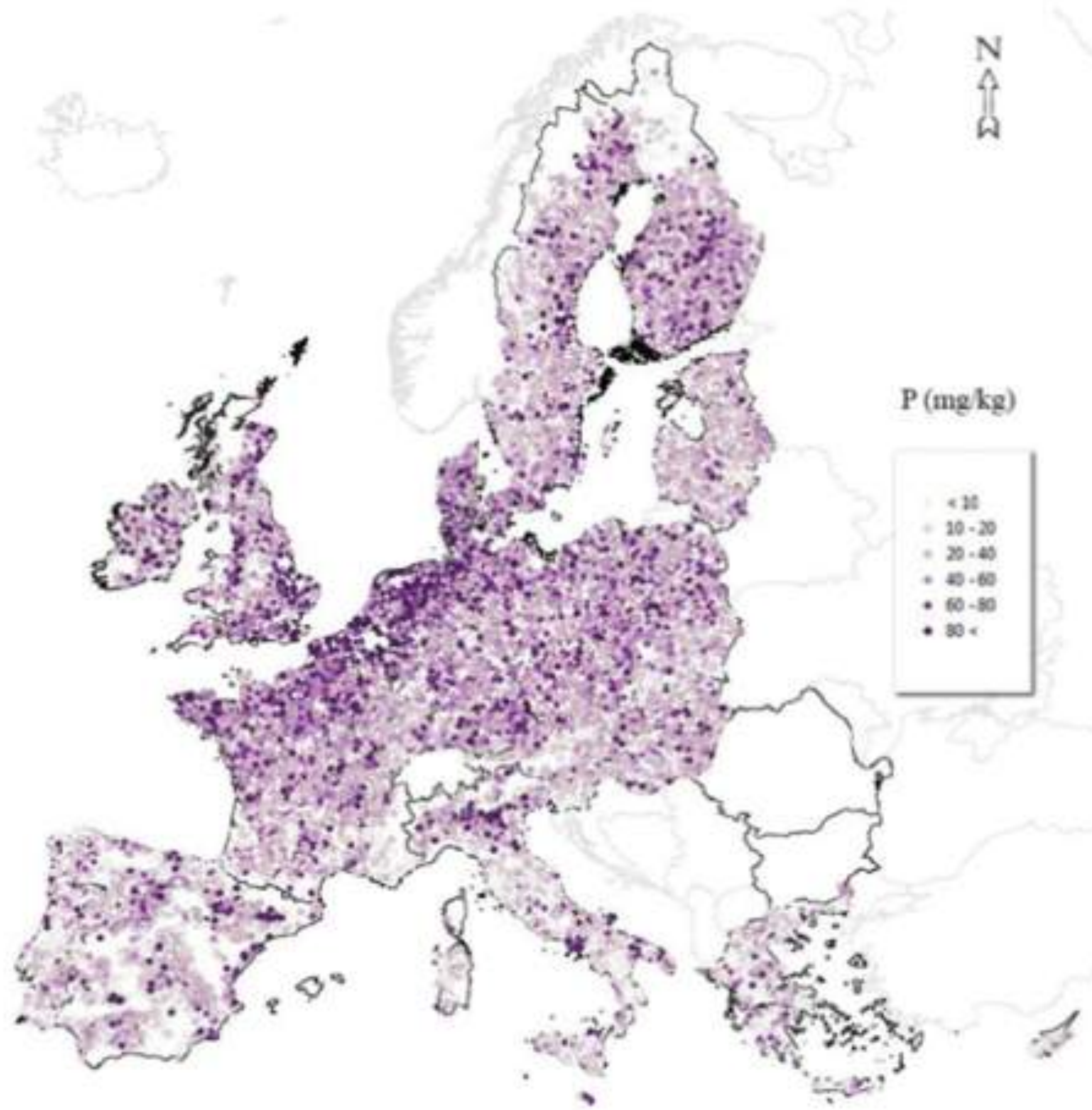


RNAseq reveals genes that are expressed in roots and influenced by phosphate levels

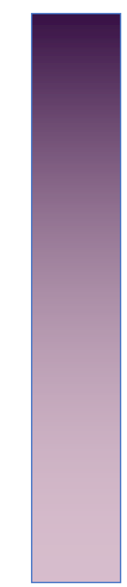


More than 60 downregulated and 285 upregulated genes
(Log2fold change >1.5 or <-1.5)
Perfect Atlas for following GWAS candidate genes

Top soluble phosphorous concentration



P (mg/kg)



> 80

< 20

Different levels of phosphate are a selective pressure for specific alleles?

Ballabio et al., 2016

High Phosphate		Low Phosphate	
$Ca(NO_3)_2$	1.5 mM	$Ca(NO_3)_2$	1.5 mM
KH_2PO_4	0.75 mM	KH_2PO_4	20 µM
KNO_3	1 mM	KNO_3	1 mM
$MgSO_4$	0.75 mM	$MgSO_4$	0.75 mM
KCl	-	KCl	0.70 mM

\+ *0.8% agarose*
\+ *MES 0.8 g/L*
pH 5.7

EUROPEAN UNION

RI AT CZ

Interreg
EUROPEAN UNION
Austria-Czech Republic
European Regional Development Fund