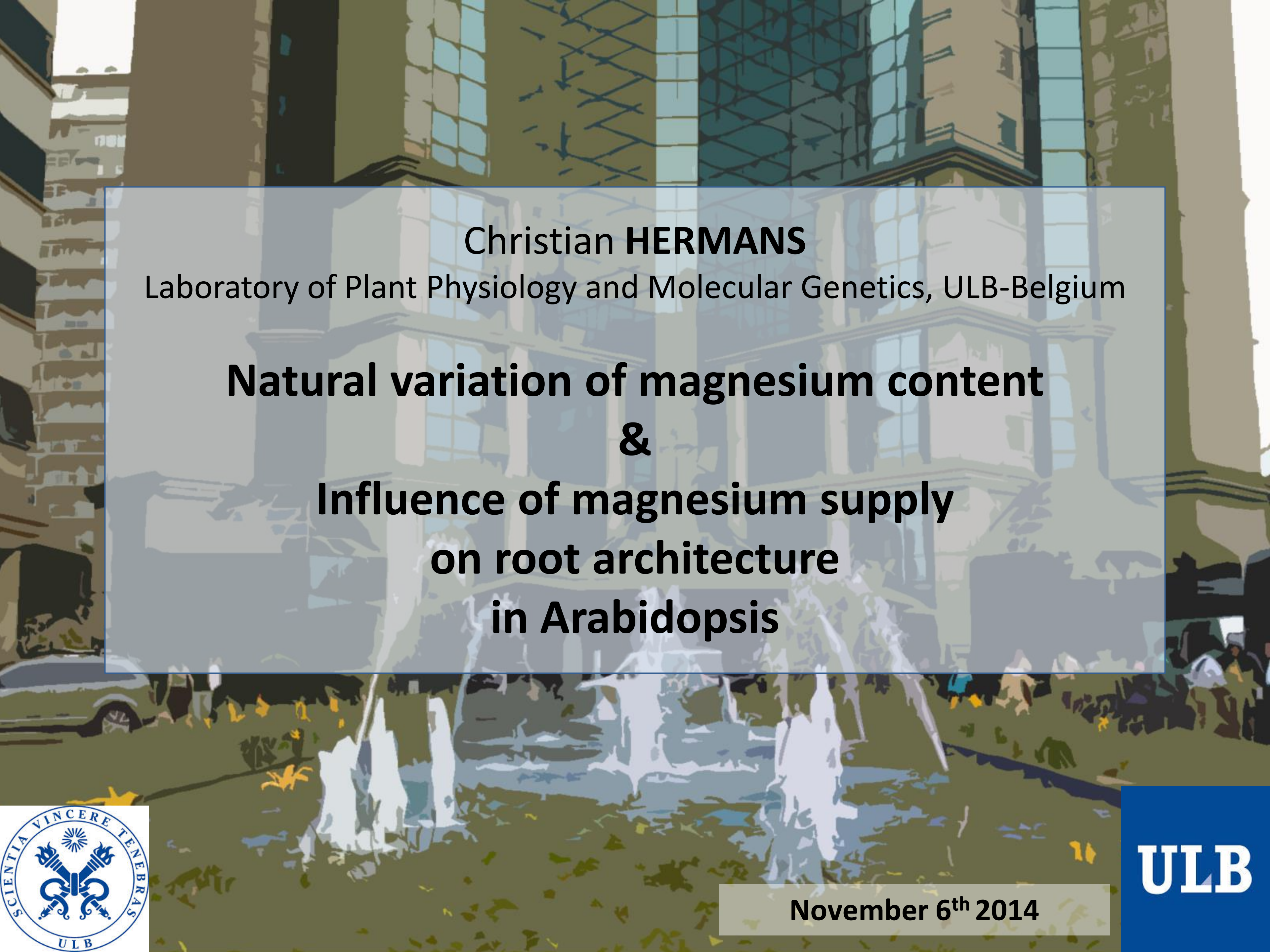
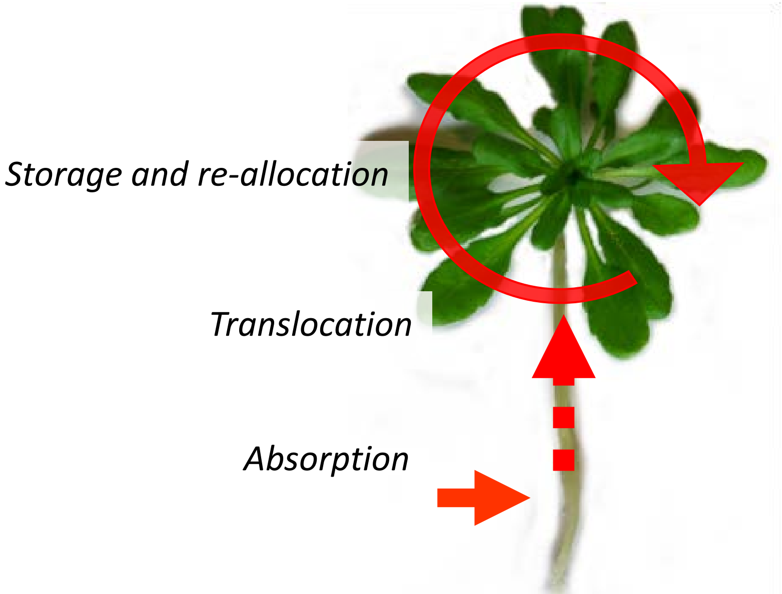


Christian **HERMANS**
Laboratory of Plant Physiology and Molecular Genetics, ULB-Belgium

Natural variation of magnesium content & Influence of magnesium supply on root architecture in Arabidopsis

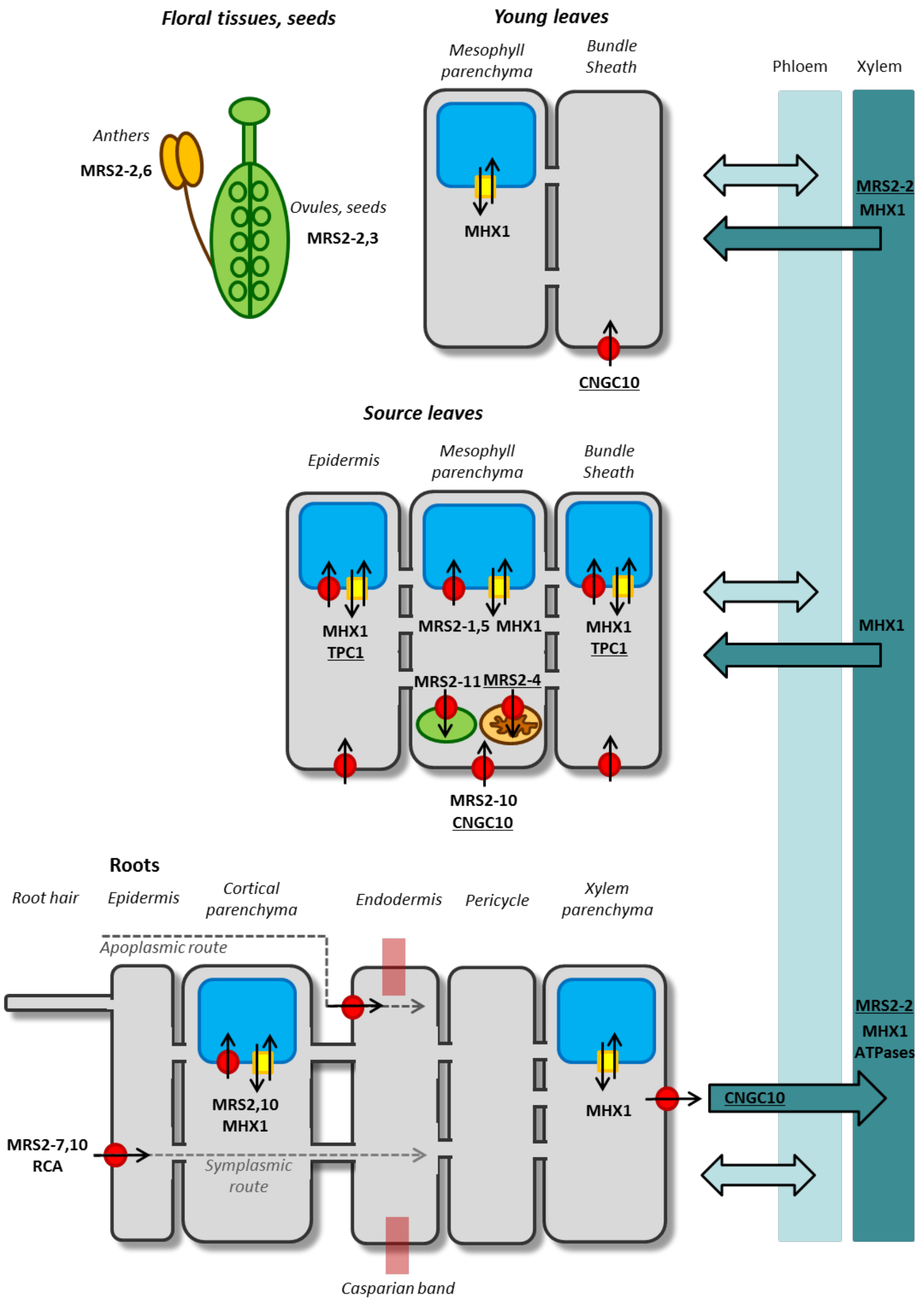
November 6th 2014



Mechanisms of Mg homeostasis are poorly understood in plants

In this session: Julia Dreistein

Transporting magnesium: the MRS2-type Mg^{2+} channels in plants



Hermans et *al.* (2013) *Metallomics* 5: 1170-1183

I. Exploiting ionomic variation to clone genes regulating Mg tissue concentration

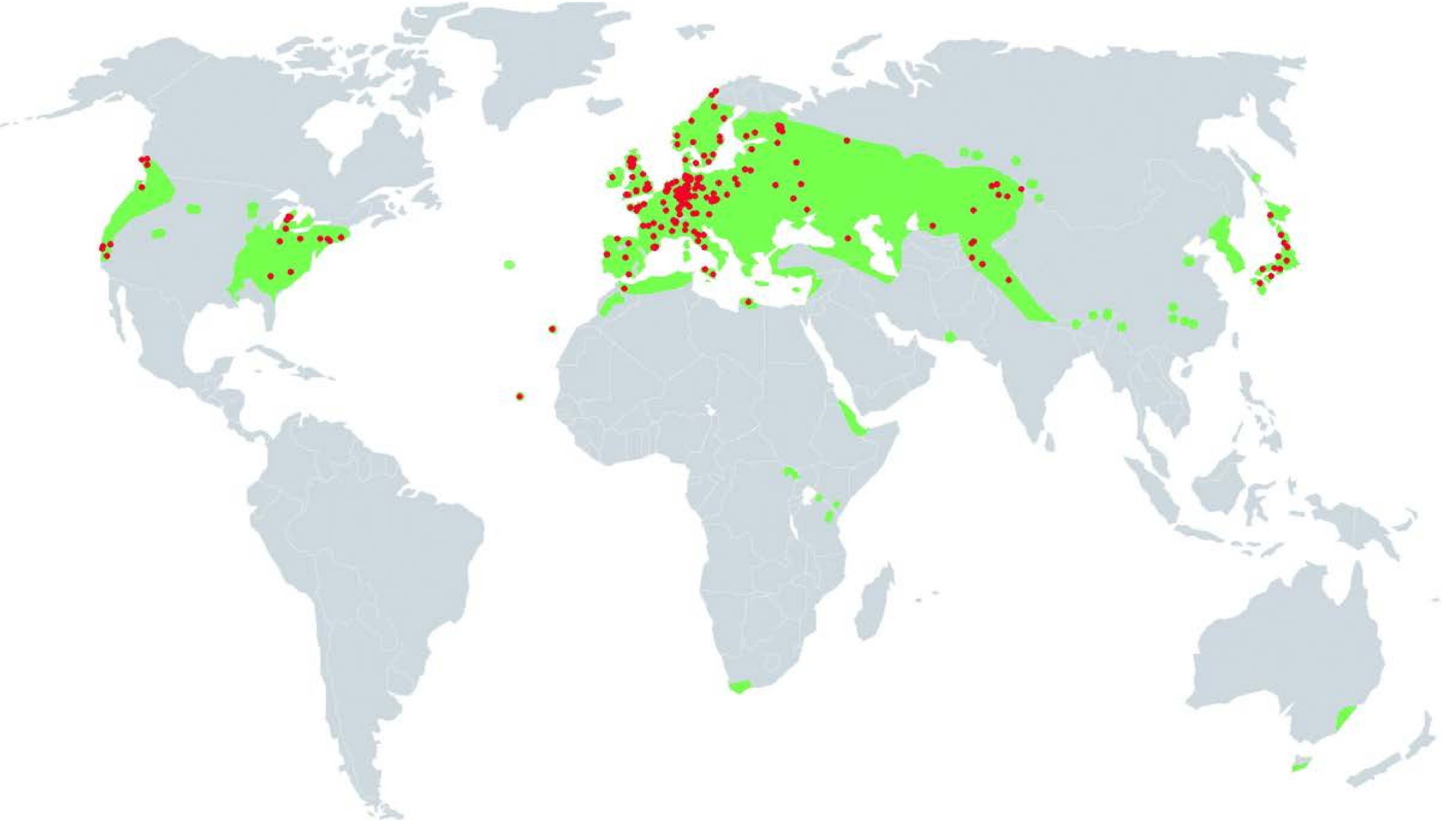
- Mutagenized-induced variation

- Natural variation in accessions/ecotypes

  Screen of experimental populations derived from a cross between two accessions

  Genome-wide screening in large diversity panels

Arabidopsis is globally distributed and consequently subject to varying environments which makes it a useful model for studying adaptation and selection.



EXPERIMENTAL PROCEDURE

Identification of accessions with contrasted Mg concentration in roots and shoots (hydroponics and soil)

① Linkage mapping

Screening [Mg] variation in existing experimental populations (Recombinant Inbred Lines) derived from a cross between 2 accessions (available resources in seed stock centres)

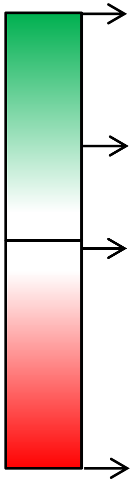
② Bulk segregant analyses

Screening [Mg] variation in newly-generated experimental populations derived from a cross between 2 extreme accessions

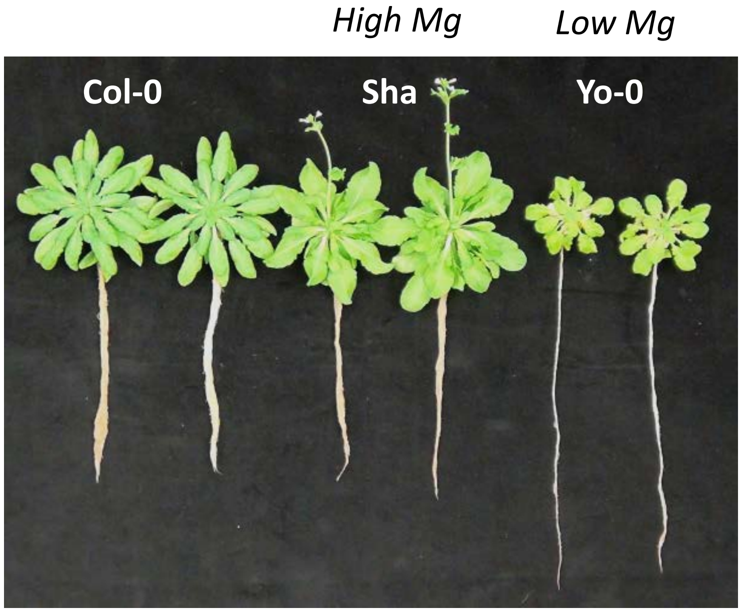
③ Genome association studies

GWAs screening of [Mg] variation in large diversity panels

Nordborg (96) *hydroponics*



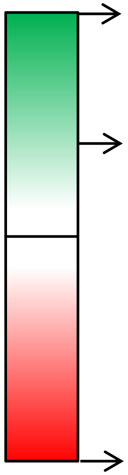
Sha	11.7mg Mg g^{-1} DW
Pro-0	11.6
Ws-2	11.1
Sorbo	10.9
Lp2.2	10.7
Col-0	10.0
Cvi -0	8.5
Lov-5	7.9
Est-1	7.9
Zdr-1	7.7
Pna-17	7.6
Yo-0	7.6



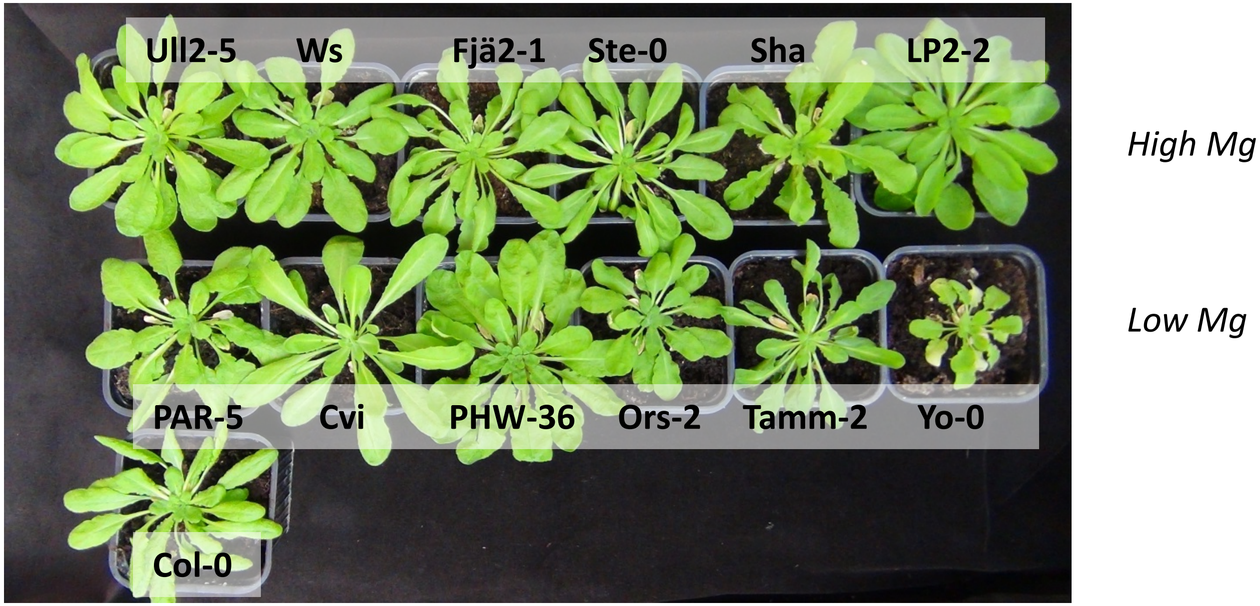
- Baxter et *al*. *Plos ONE 7: e35121*

HapMap (351) *soil*

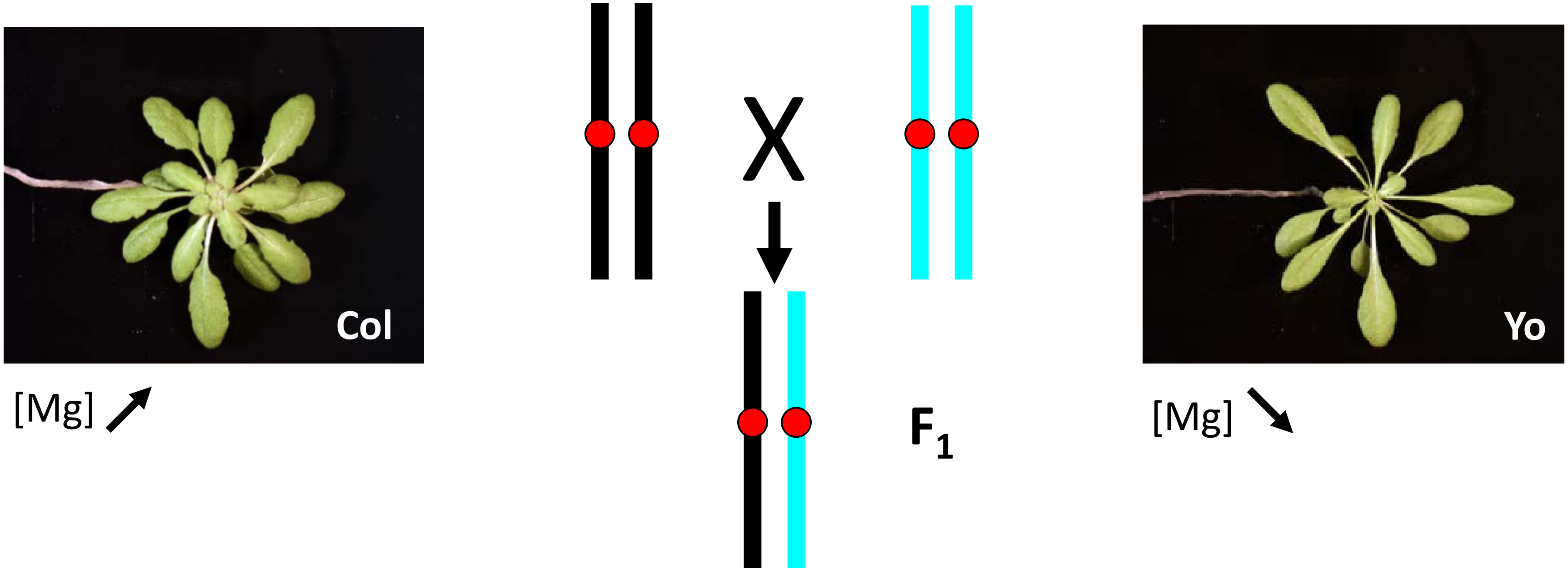
- www.ionomicshub.org/home/PiiMS



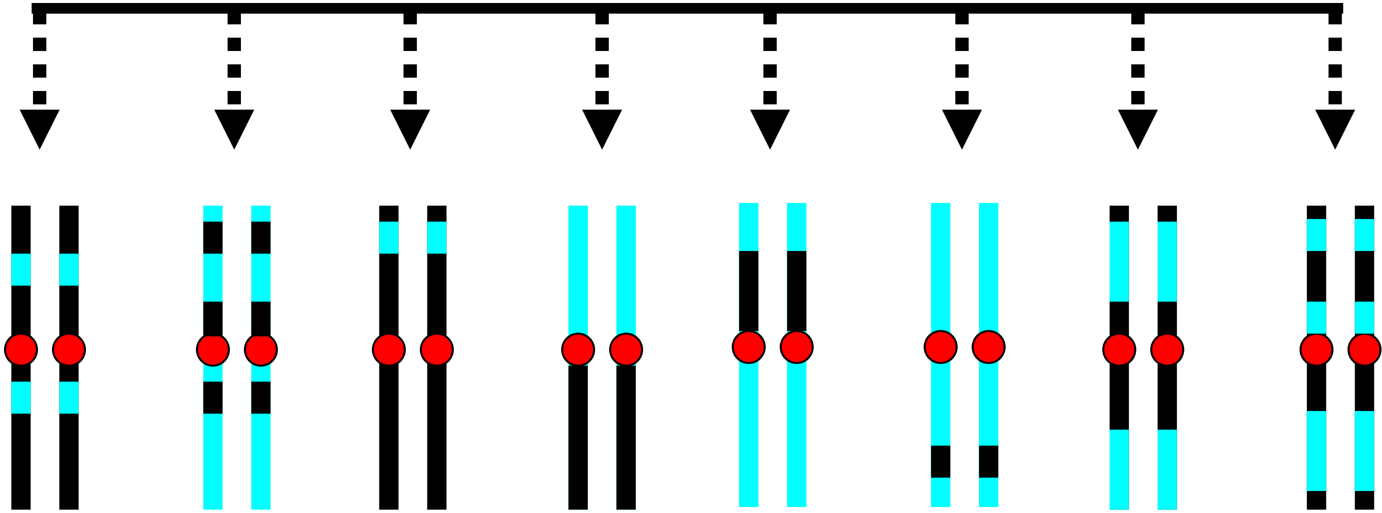
Sg-1	12.6
Ull2-5	12.2
Ws	11.9
Fjä1-2	11.5
Ste-0	11.3
Col-0	9.9
PAR-5	7.8
Cvi-0	7.7
PHW-36	7.7
Ors-2	7.6
Tamm-2	7.6



① Linkage mapping of Mg concentration in tissues



To identify genetic intervals influencing a quantitative trait (e.g. mineral concentration)



***160** Recombinant Inbred Lines*

INRA Versailles

Nutrient solution composition

(Hermans et *al*. 2010)

Macronutrient concentrations (mM)

1.00	$Ca(NO_3)_2$
1.00	$MgSO_4$
0.88	K_2SO_4
0.25	KH_2PO_4

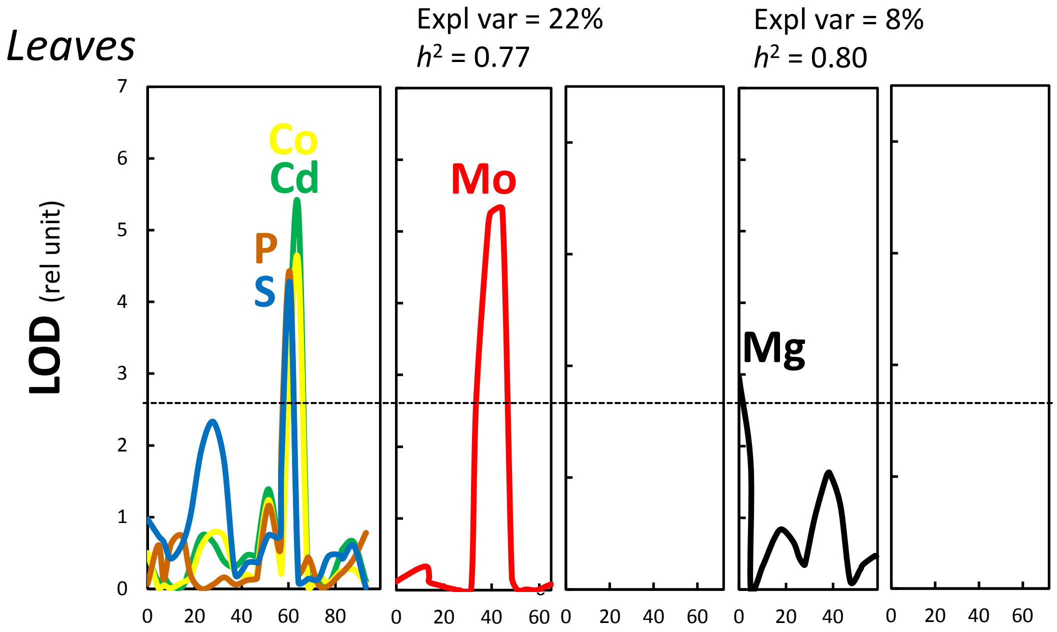
Micronutrient concentrations (µM)

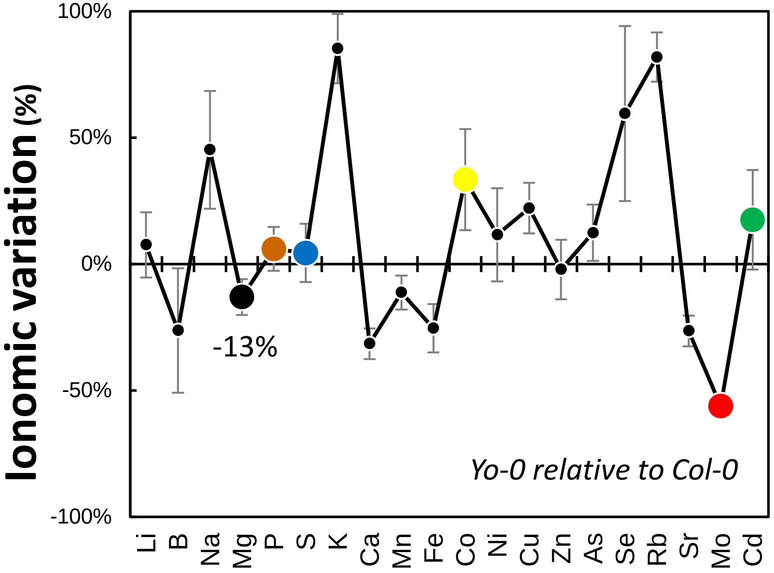
20	FeEDTA
10	NaCl
10	H_3BO_3
1	$ZnSO_4$
1	$MnSO_4$
0.10	$CuSO_4$
0.01	$(NH_4)_6Mo_7O_{24}$

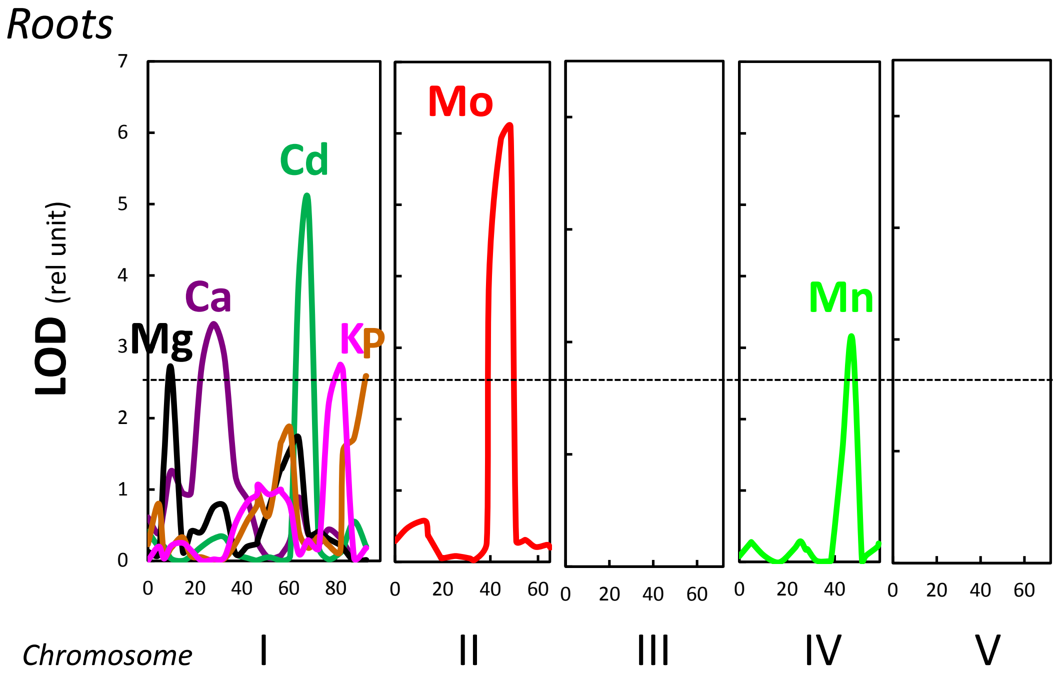
Toxic element cocktail -added one week prior to harvest, at subtoxic concentrations (µM)

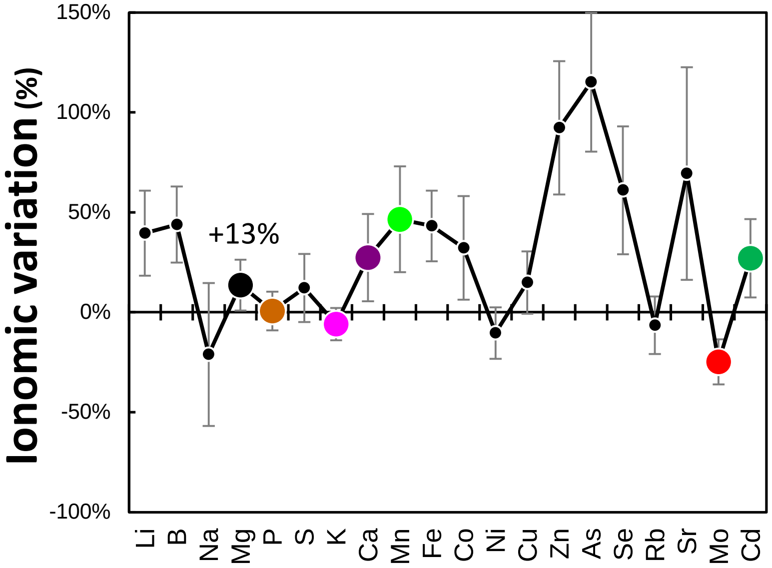
10	$Na_2H\mathbf{As}O_4.7H_2O$	1	$\mathbf{Co}Cl_2.6H_2O$
10	$\mathbf{Pb}(NO_3)_2$	1	$\mathbf{Ni}SO_4.6H_2O$
10	$\mathbf{Li}Cl$	0.50	$K\mathbf{Cr}(SO_4)_2.12H_2O$
		0.10	$\mathbf{Cd}SO_4.8/3H_2O$

Yo-0 X Col-0 (164 RILs tested)

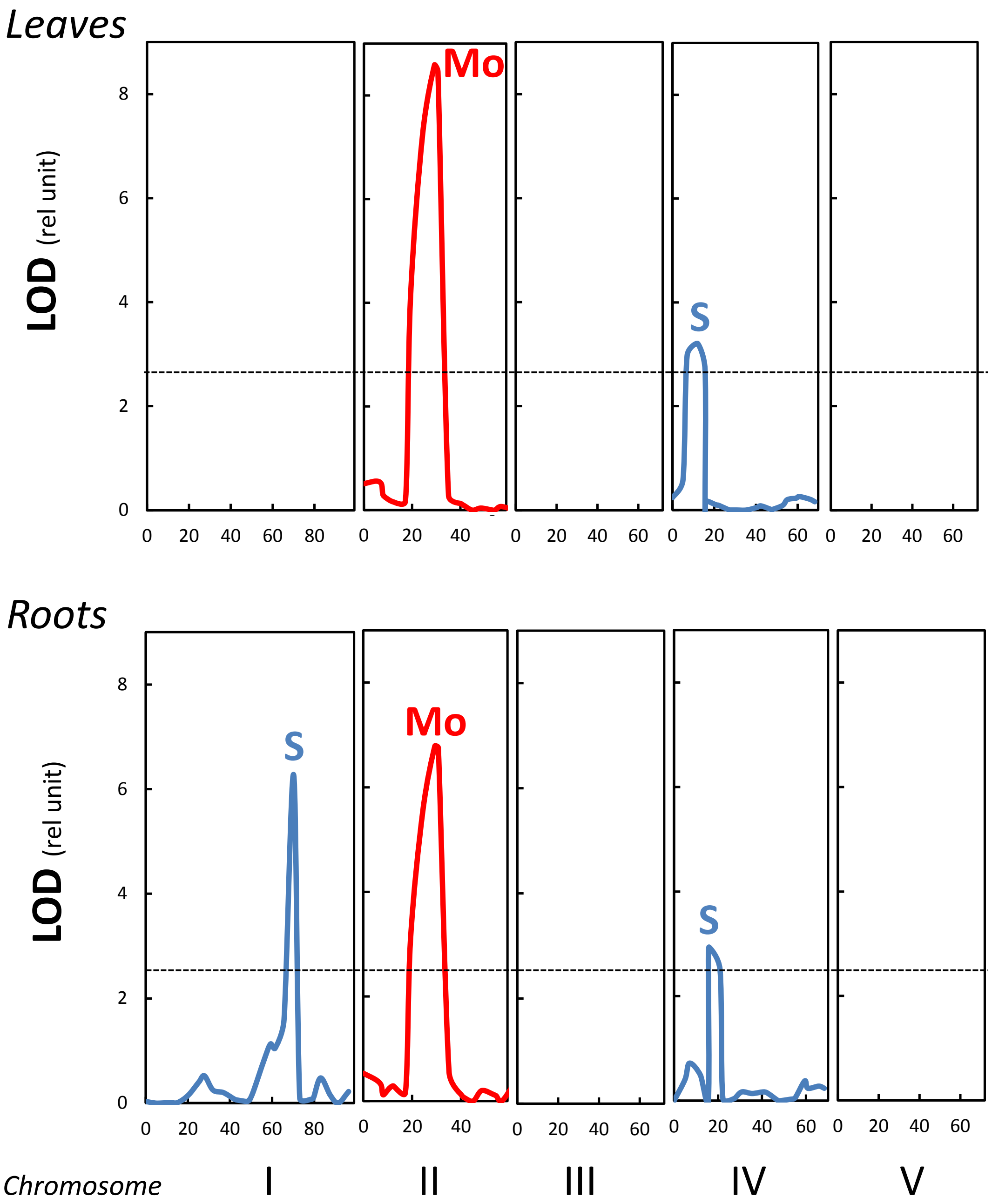


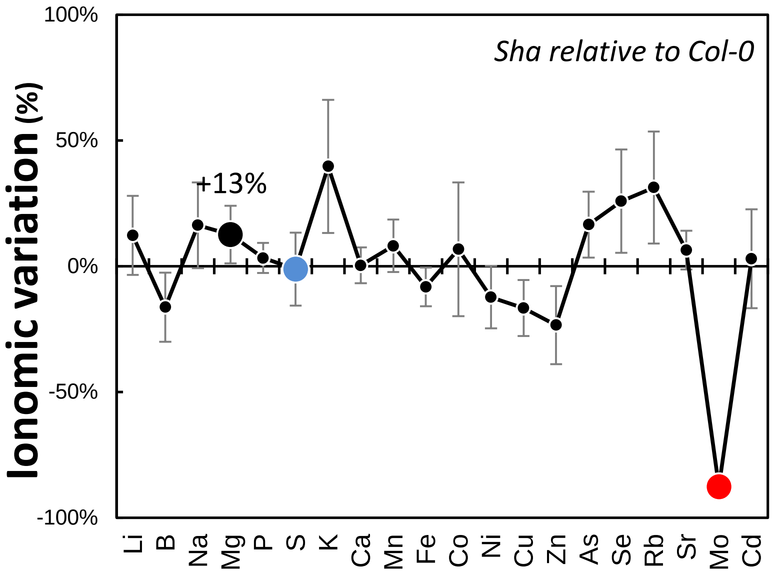


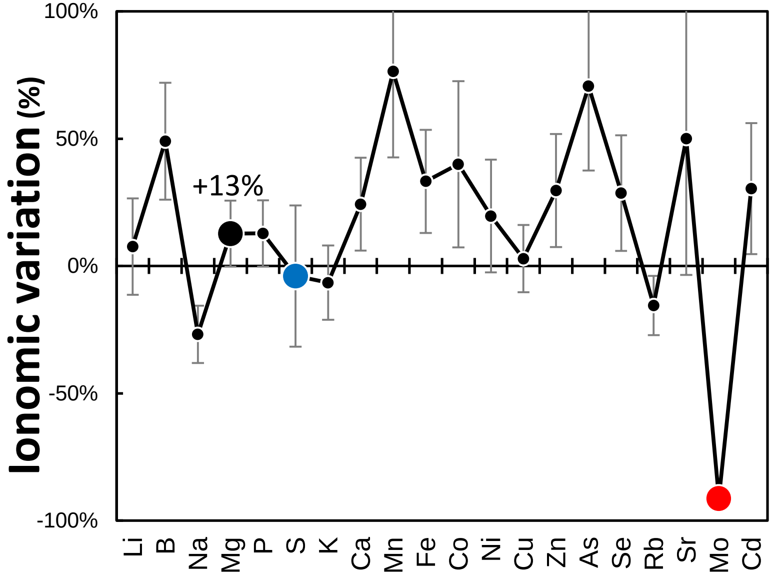




Sha X Col-0 (164 RILs tested)



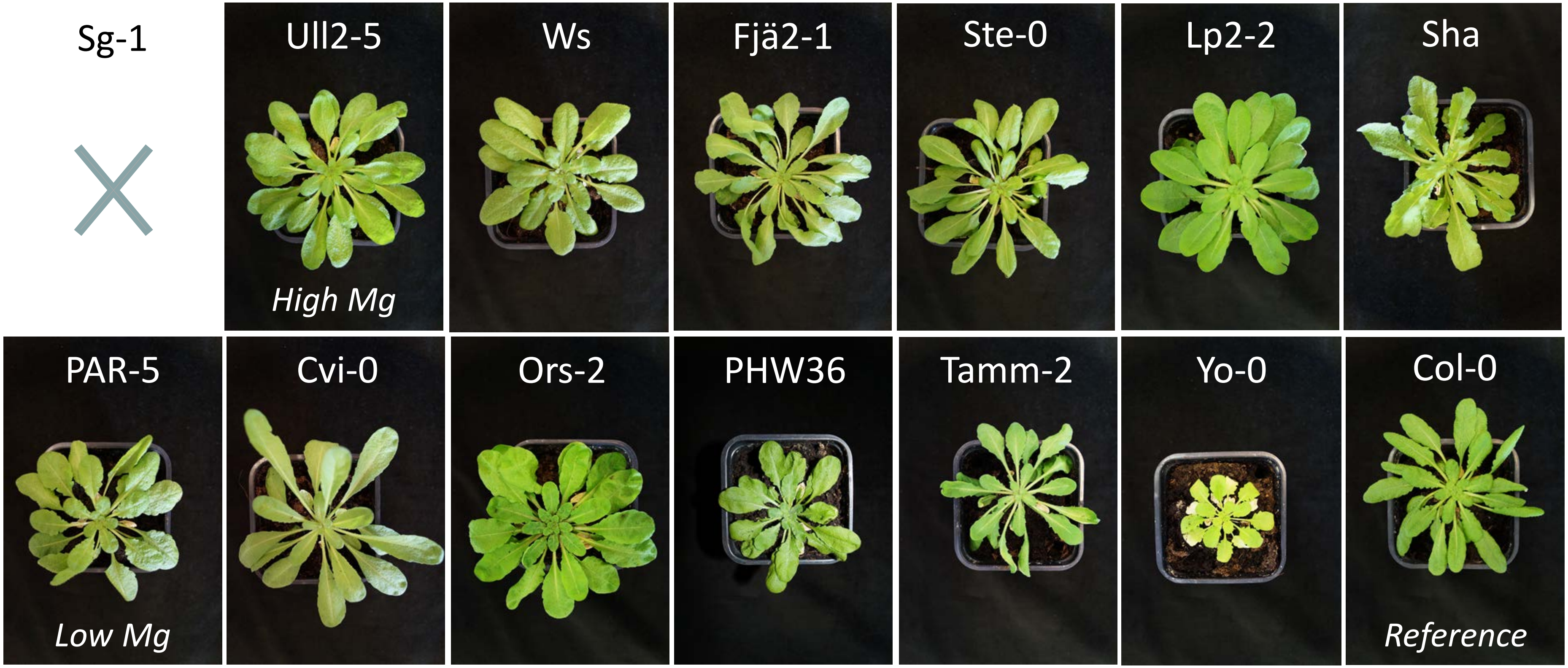




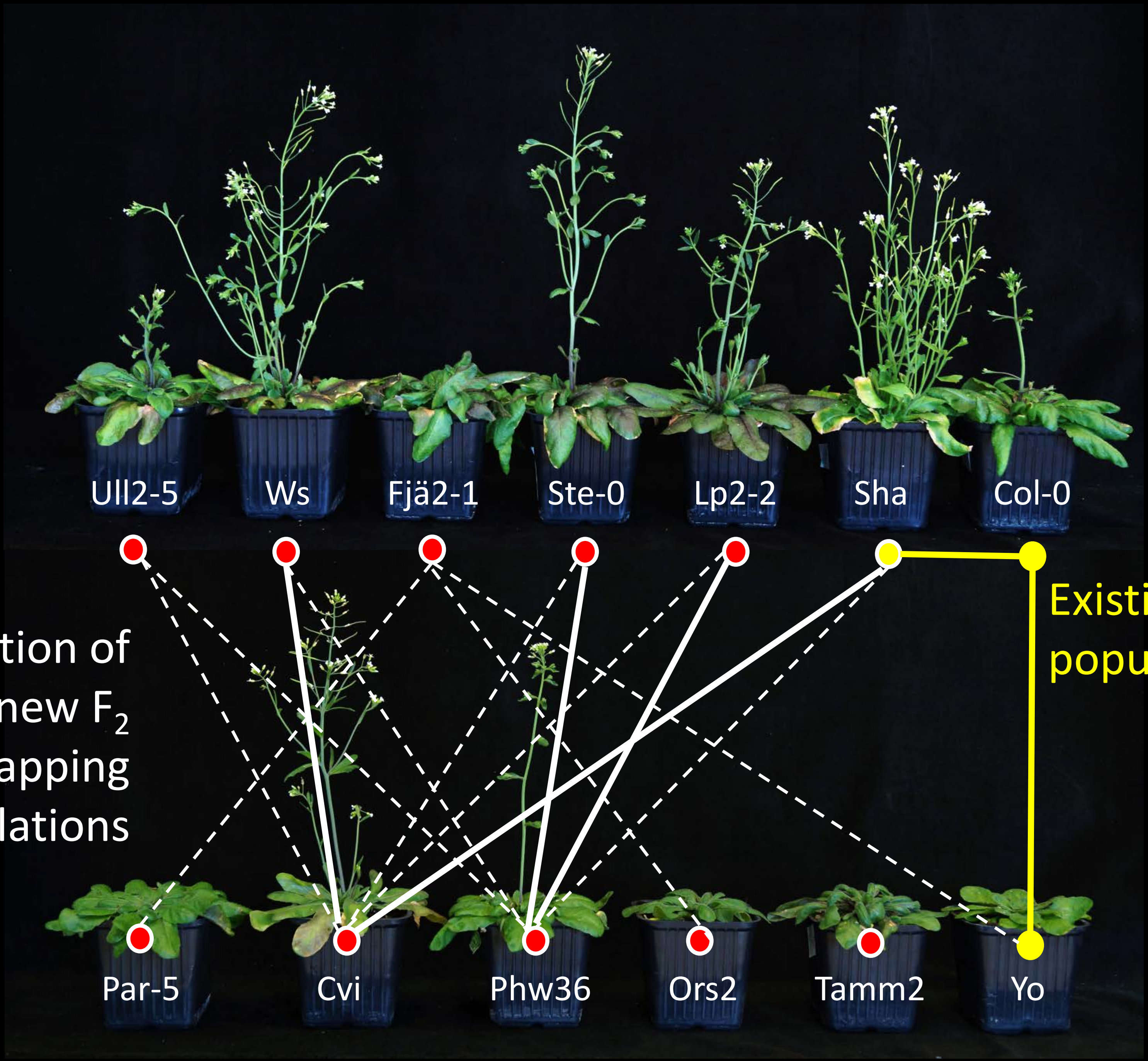
② Creation of new F_2 mapping populations

Drawback to detect more robust Mg-QTL: limited choice of existing RIL families

→ Creation of new mapping populations originating from the crosses between the most contrasted accessions




High Mg
Ull2-5
Sweden
Ws
Russia
Fjä2-1
Sweden
Ste-0
Germany
Lp2-2
Czec Rep.
Sha
Tadjikistan
Col-0
USA
Low Mg
Par-5
France
Cvi
Portugal
Phw36
France
Ors2
Romania
Tamm2
Finland
Yo
USA

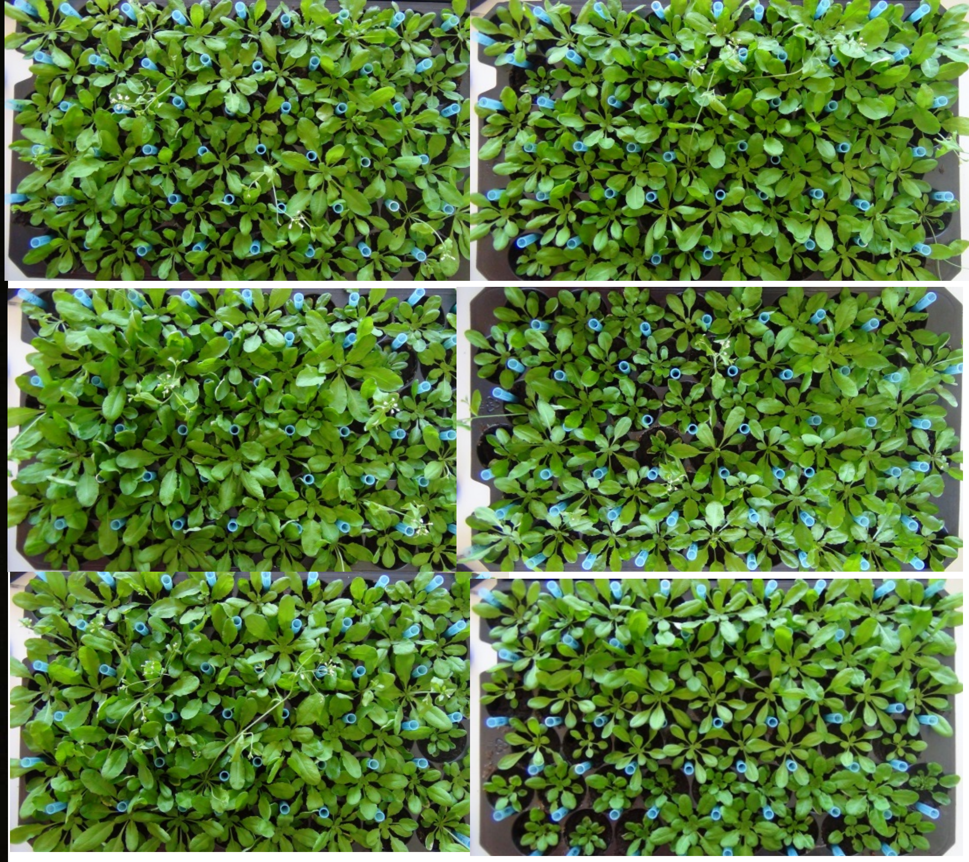


Creation of new F_2 mapping populations

Existing RIL populations

Mapping populations being screened:

Cvi-0 X Ws	254 F_2
Cvi-0 X Sha	103 F_2
PHW36 X Ste-0	170 F_2
Lp2-2 X PHW36	178 F_2

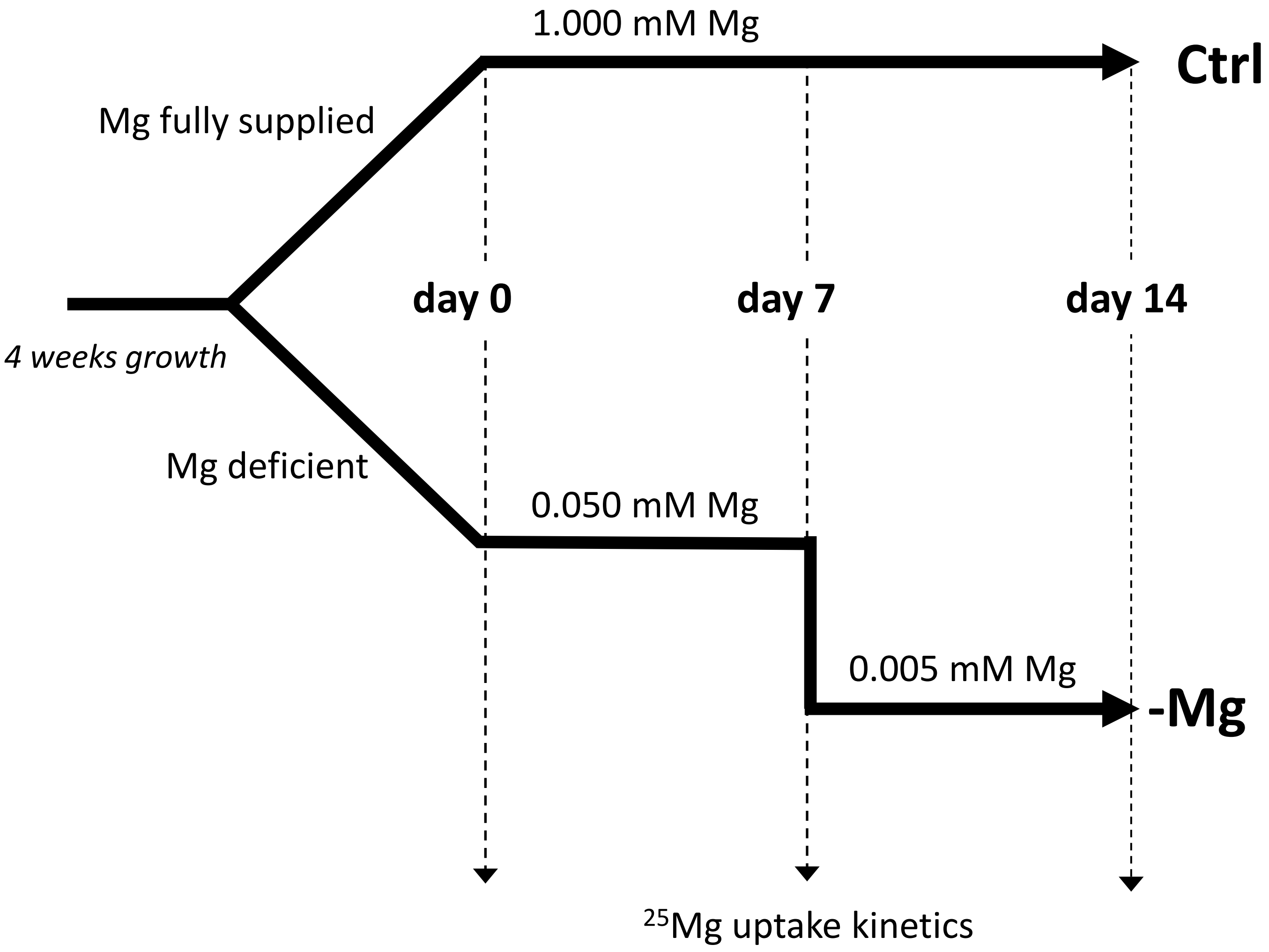


Determining ionomic profile of F_2 individuals

Sequencing 2 DNA pools of individuals with low and high Mg

Calculating allele frequencies (Shoremap pipeline)

③ Physiological characterization of contrasting accessions



1.000 mM Mg

Ctrl

Mg fully supplied

4 weeks growth

day 0

day 7

day 14

Mg deficient

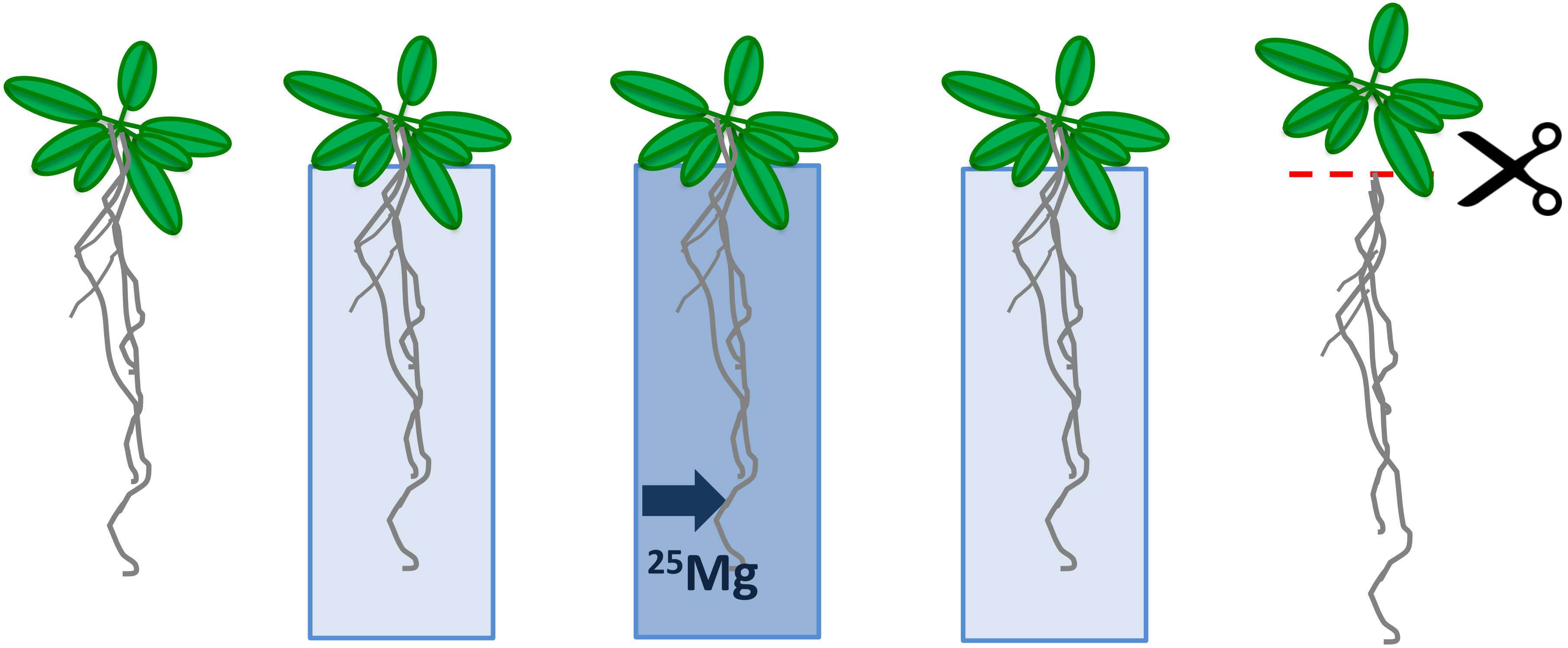
0.050 mM Mg

0.005 mM Mg

-Mg

^{25}Mg uptake kinetics

Experimental procedure to measure ^{25}Mg transport activity



5-week-old plants grown *in hydroponics*
One week treatment at 0.050 and 1.000 mM ^{24}Mg

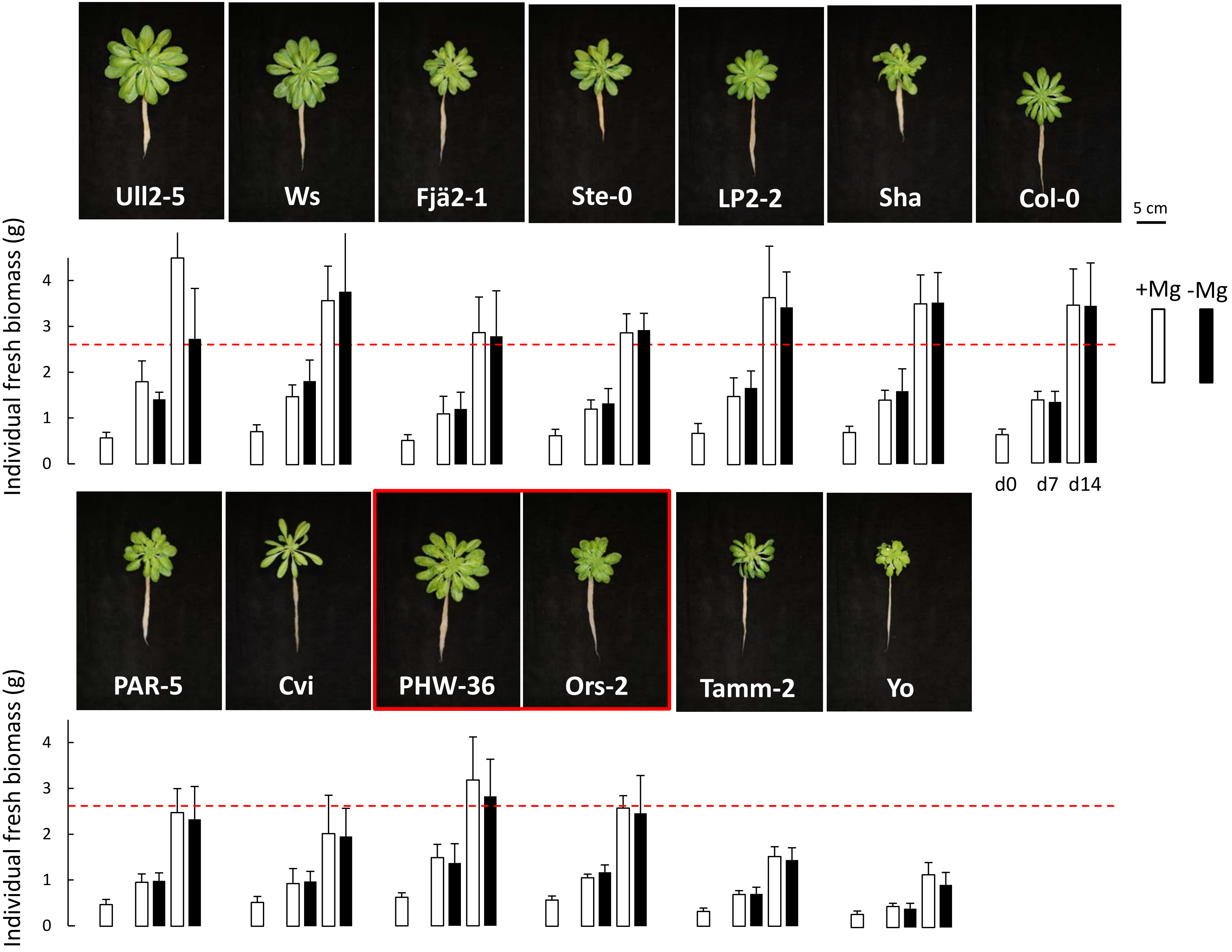
1 min
0.1 mM $CaSO_4$

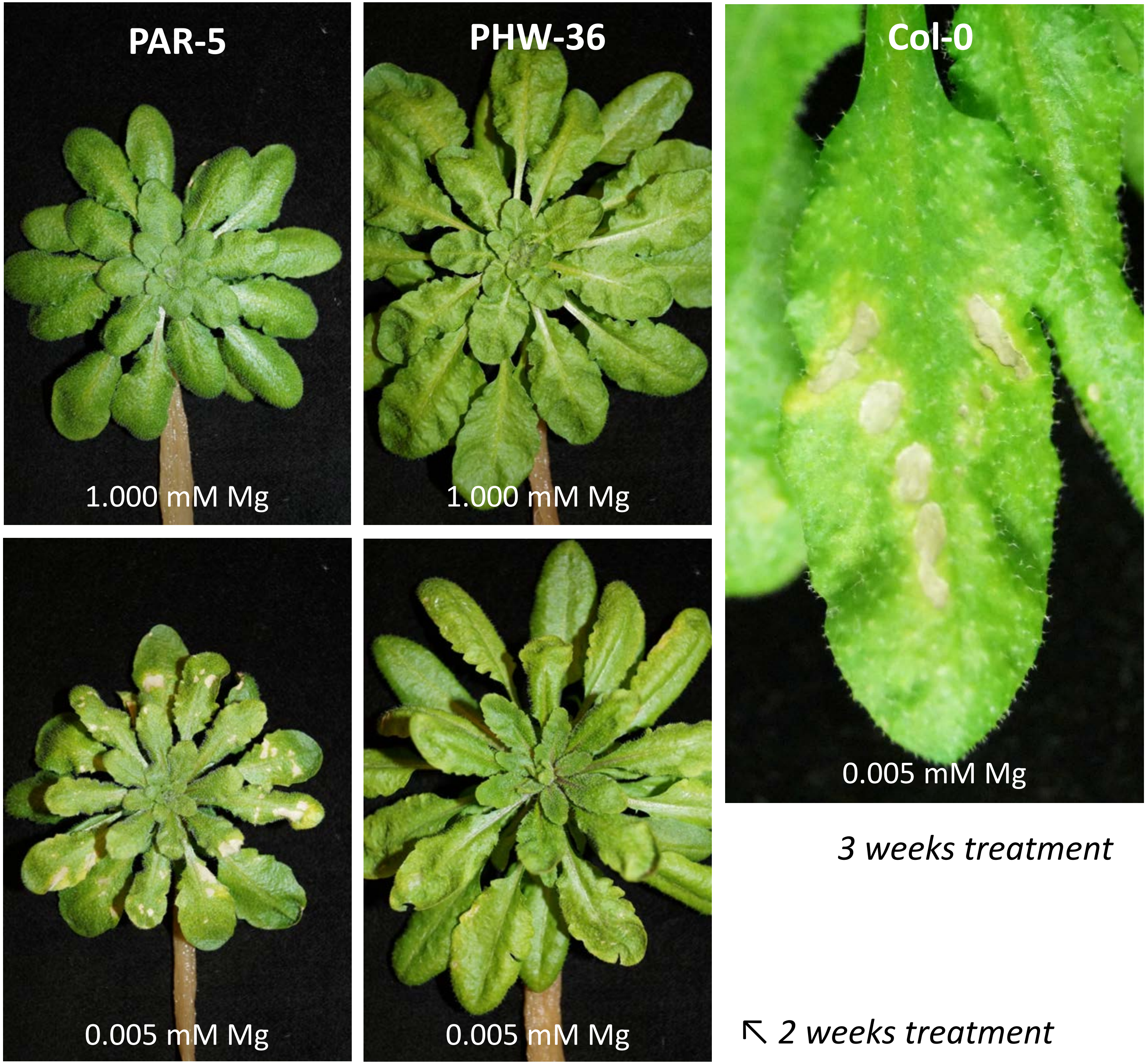
1 min
HAT: 0.050 mM ^{25}Mg
LAT: 1.000 mM ^{25}Mg

5 min
0.1 mM $CaSO_4$

Organs harvesting

HAT: High Affinity Transport
LAT: Low Affinity Transport


Ull2-5
Ws
Fjä2-1
Ste-0
LP2-2
Sha
Col-0
5 cm
Individual fresh biomass (g)
0
1
2
3
4
+Mg
-Mg
d0
d7
d14
PAR-5
Cvi
PHW-36
Ors-2
Tamm-2
Yo
Individual fresh biomass (g)
0
1
2
3
4



3 weeks treatment

↖ *2 weeks treatment*

Conclusions and perspectives

- ✓ Natural variation for [Mg] in tissues exists in *Arabidopsis thaliana*

  (up to 50% difference between most contrasting accesions)

- ✓ [Mg] in plant tissues is a highly heritable trait (h^2 ~ 0.8)

- ✓ Drawback to detect robust Mg-QTL → numerous genes with small additive effects

- ✓ No co-localization with putative Mg transporters (MRS2)

- ✓ Early appearance of –Mg symptoms in some low Mg accessions

- ✓ The identification of loci regulating [Mg] could help drawing Mg biofortification strategies in crops

II. Influence of magnesium supply on root system architecture in *Arabidopsis thaliana*

① Feeding plants with low Mg doses at a very young developmental stage

→ severe reduction of root growth and root biomass production in several species (bean, spinach, maize …)

② Complete Mg starvation at a later growth stage of the plant

→ results in a limited reduction of the root biomass production in comparison to the aerial part in some species (Arabidopsis, rice, sugar beet, pea, Chinese cabbage …) + transcriptome in roots relatively not affected upon -Mg

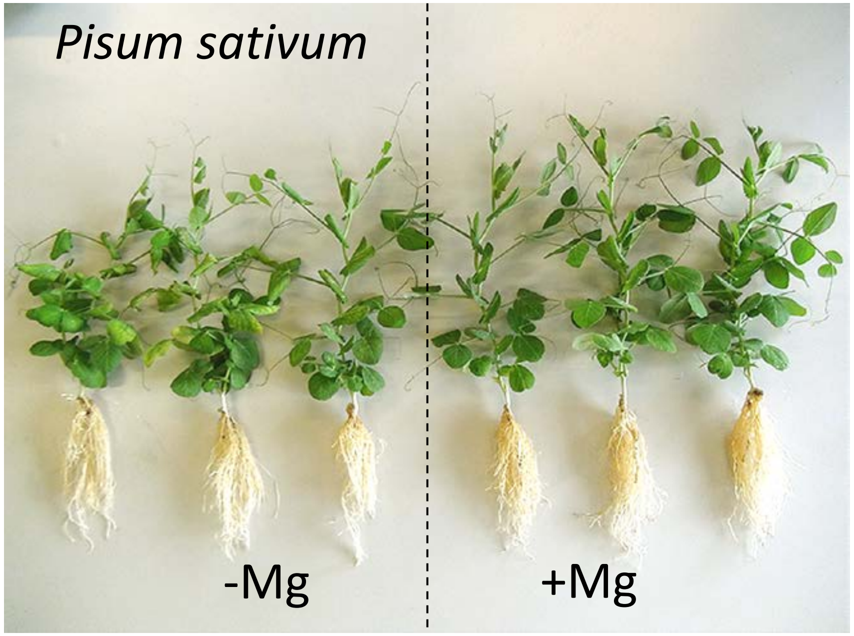
Visual symptoms of magnesium deficiency upon hydroponic culture

In *Arabidopsis thaliana* grown in hydroponics:

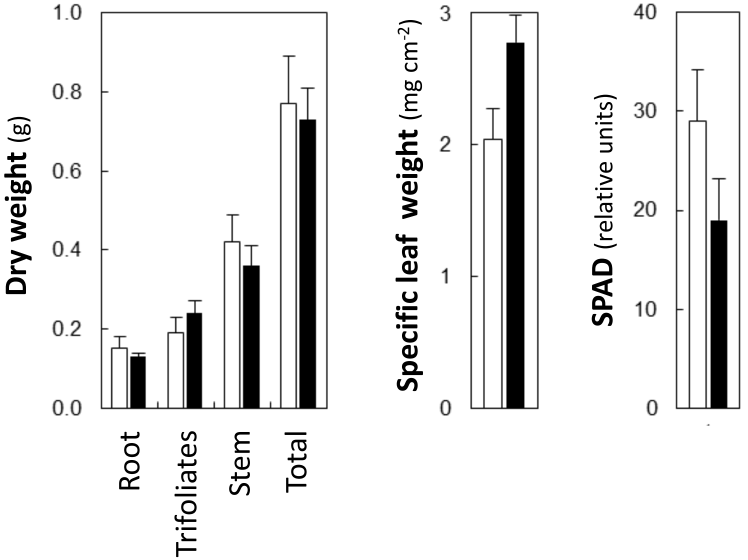
Efficient recycling mechanisms from the above-ground Mg pool to sustain root growth.

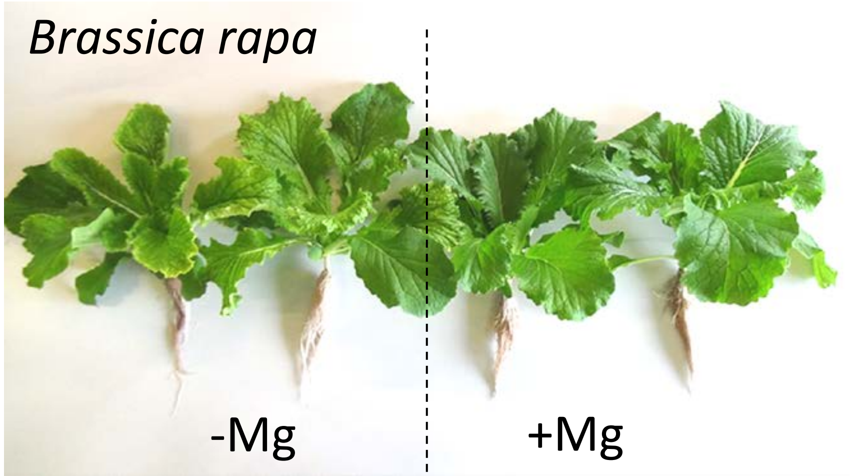
Hermans et *al*. (2010) *New Phytol.* 187: 119-131. (2010) *New Phytol.* 187: 132-144. (2011) *New Phytol.* 192: 428-436.

Visual symptoms of magnesium deficiency upon hydroponic culture

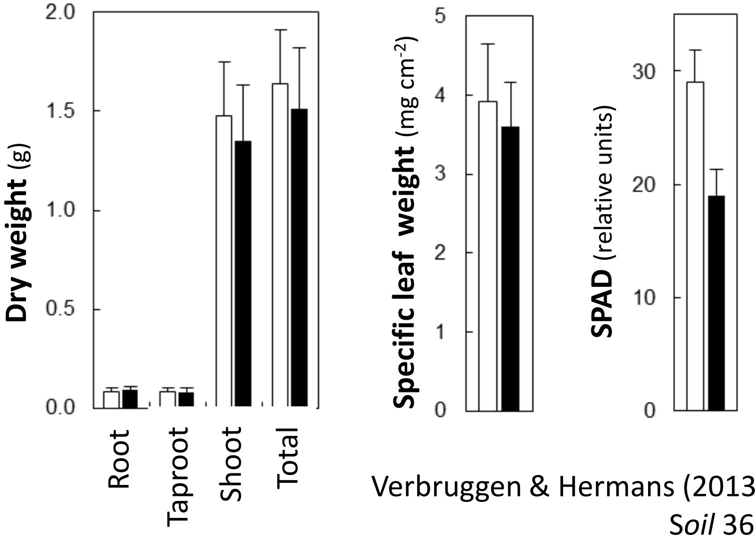


9 d + 2 weeks treatment





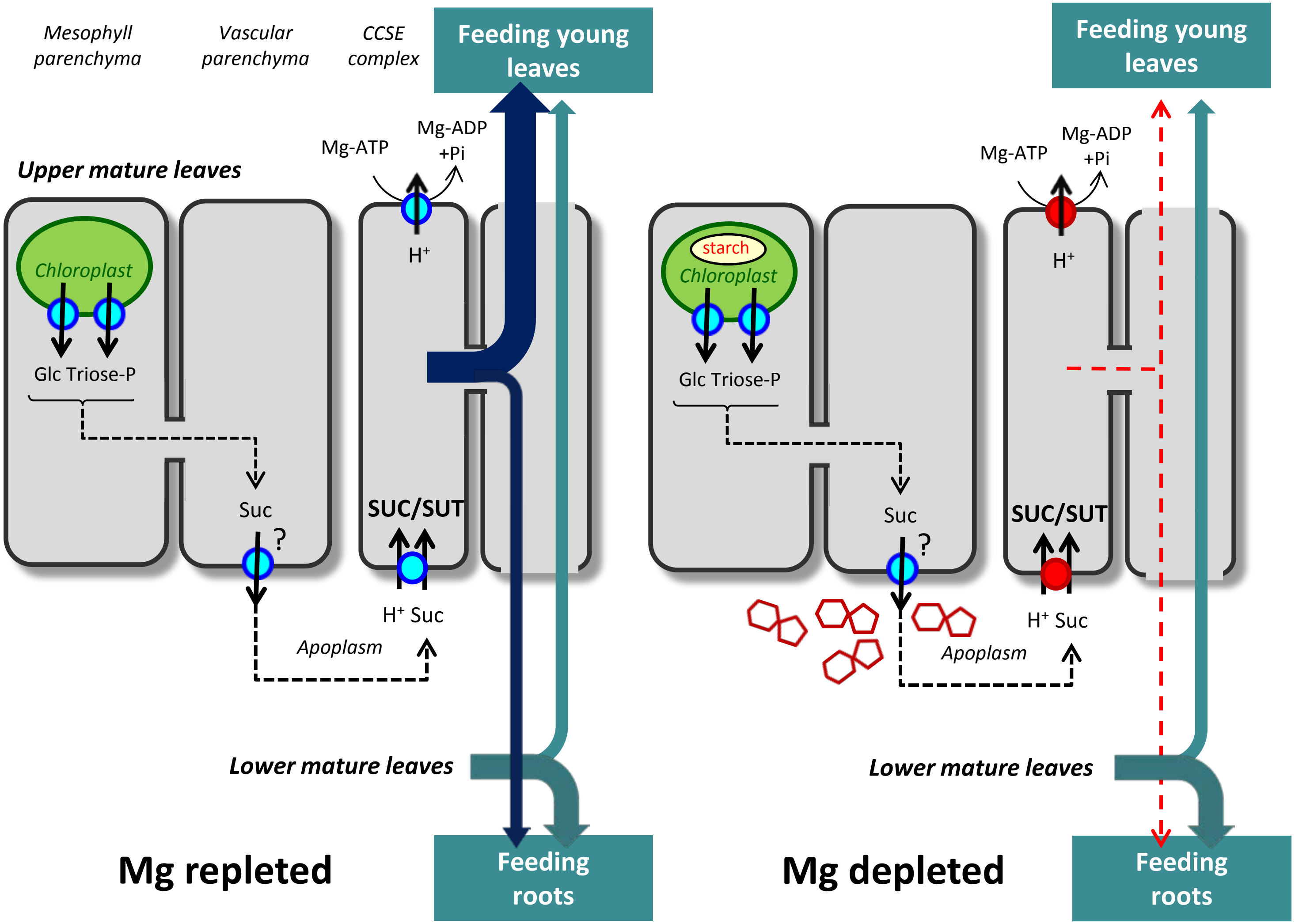
16 d + 2 weeks treatment



Verbruggen & Hermans (2013) *Plant & Soil* 368: 87-99.

A model used to explain a higher biomass allocation in favor of the root emphasizes that sucrose export from the source leaves to the root is proportionally less affected than to the immature leaves at the onset of Mg deficiency

Verbruggen & Hermans (2013) Plant & Soil 368: 87-99



II. Influence of magnesium supply on root system architecture in *Arabidopsis thaliana*

① Setting experimental conditions to observe root morphology in response to Mg supply (*in vitro*)

② Elemental profile upon Mg depletion

③ Influence of Mg supply on lateral root developmental stages

④ Crosstalk with hormones

① Setting experimental conditions to observe root morphology upon Mg depletion

17 days after germination

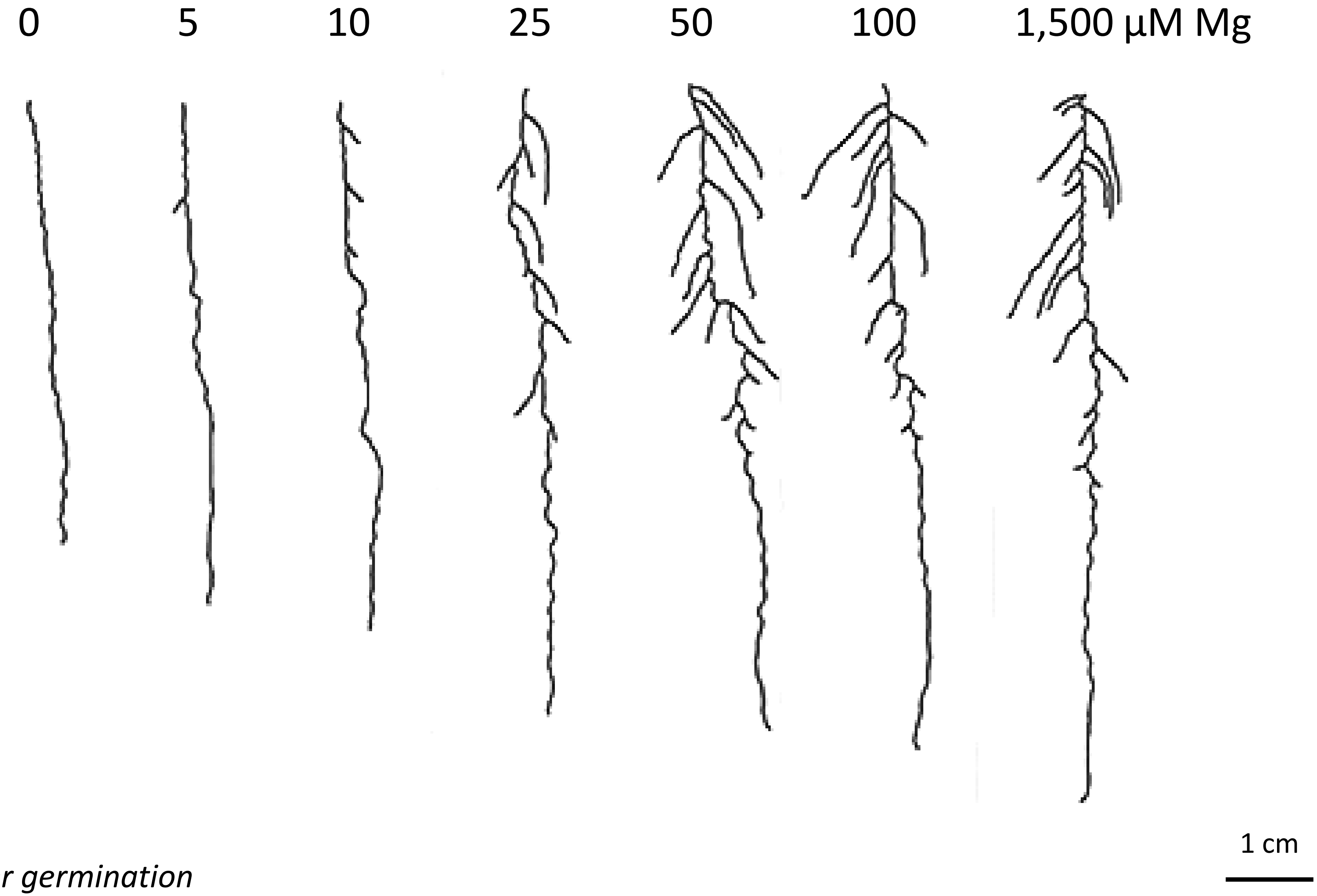
Effect of agar types on root morphology in response to Mg deficiency

Two agar types were used to grow Arabidopsis seedlings:

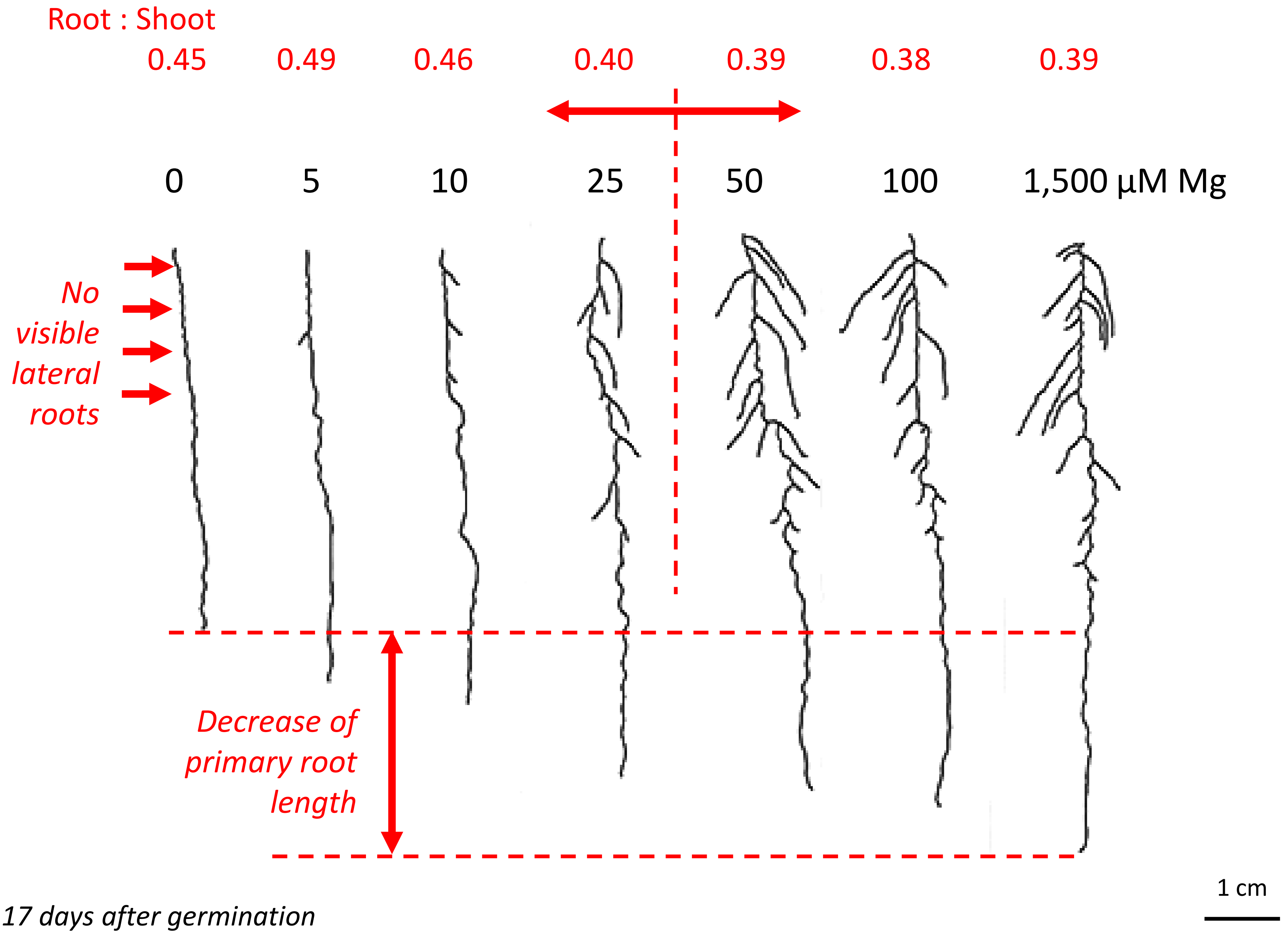
agar 1: plant agar (P1001, Duchefa)

agar 2: high gel strength agar (A9799, Sigma)

Root morphological adaptation to uniformly distributed Mg supply



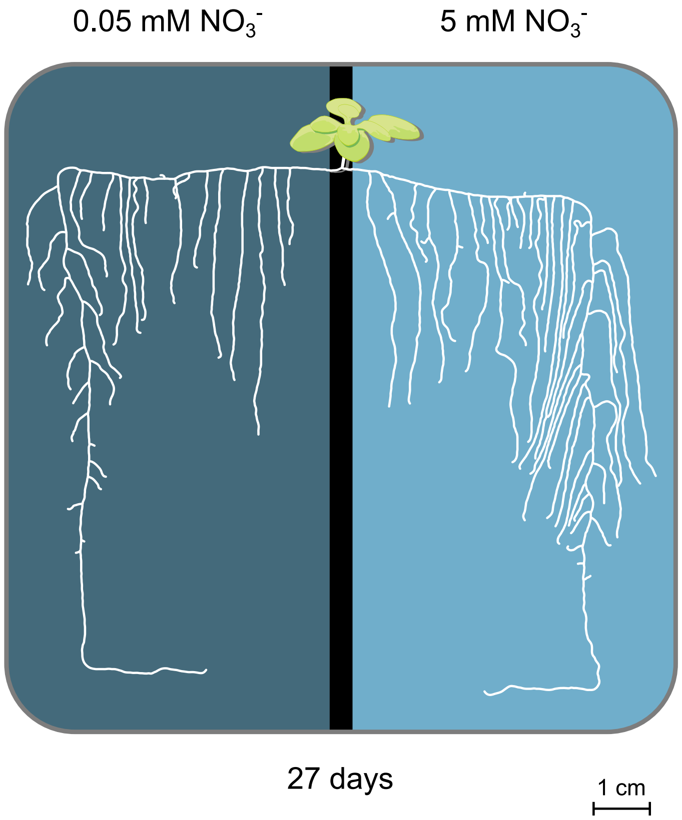
17 days after germination



17 days after germination

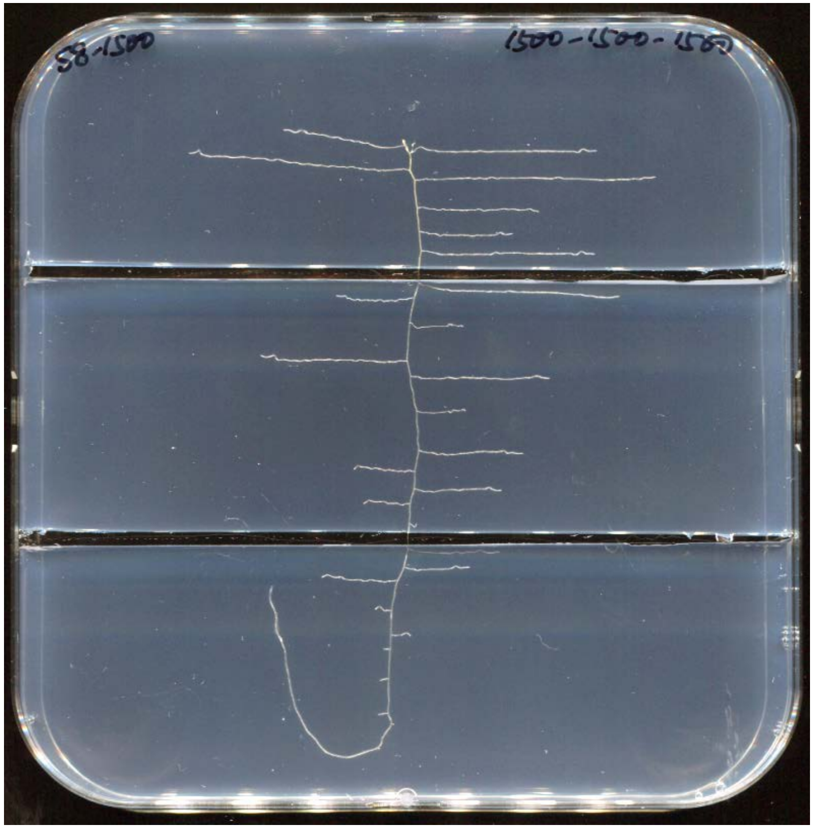
Root morphological adaptation to heterogenous Mg supply

- Nutrient distribution in soil is uneven.
- A stimulatory effect of localized nutritional treatment on root proliferation is frequently documented (e.g. nitrogen). Split-root experiments show a distinct promotion of root growth and mineral uptake in sectors of localized mineral supplies relative to depleted sectors.



Root morphological adaptation to heterogenous Mg supply

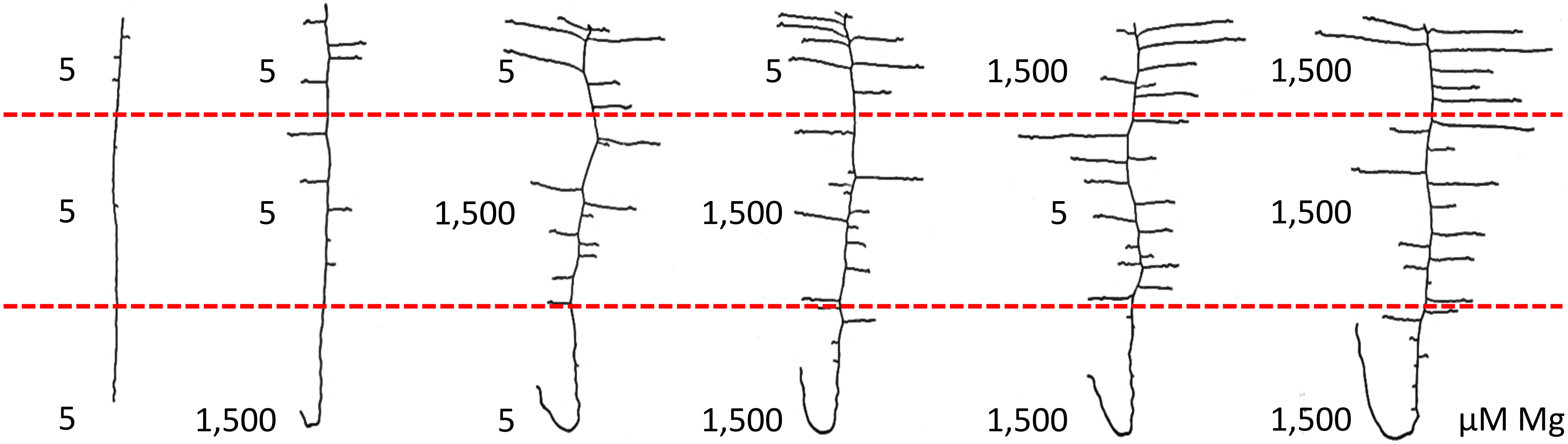
Horizontal strip bands



Vertical ½ - ½

Root morphological adaptation to heterogenous Mg supply

Horizontal strip bands with contrasted Mg concentrations

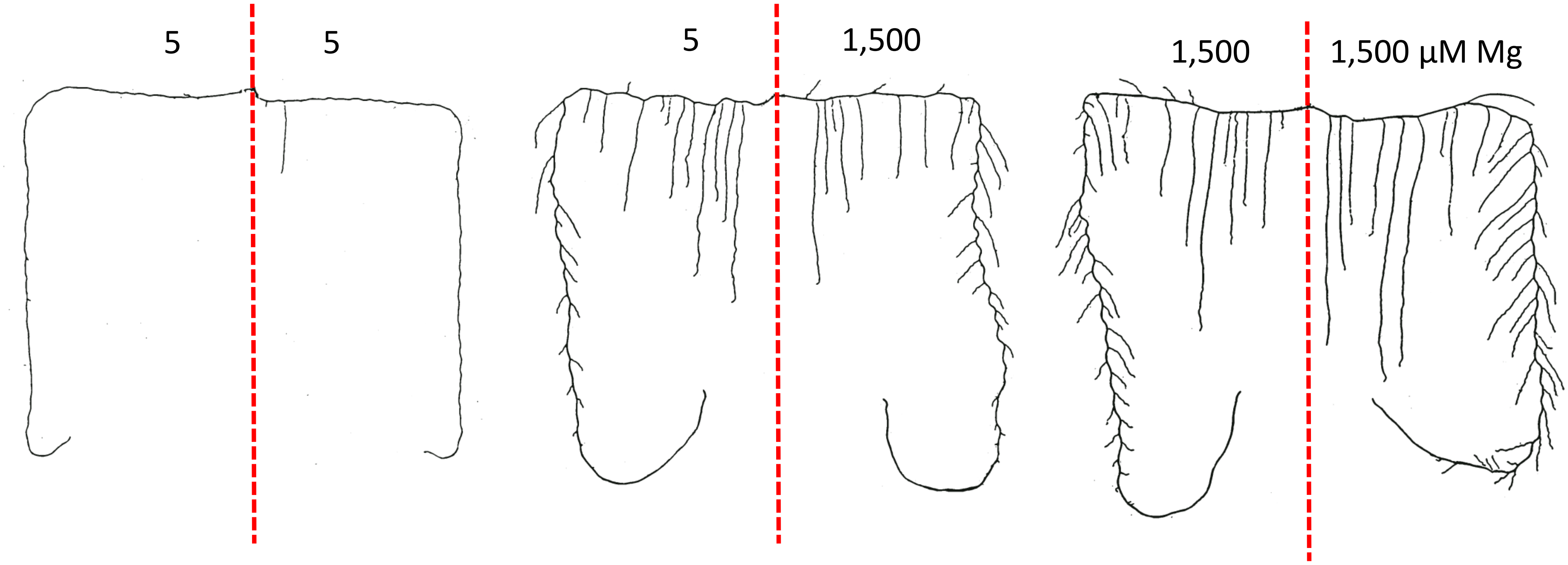


17 days after germination

2 cm

Root morphological adaptation to heterogenous Mg supply

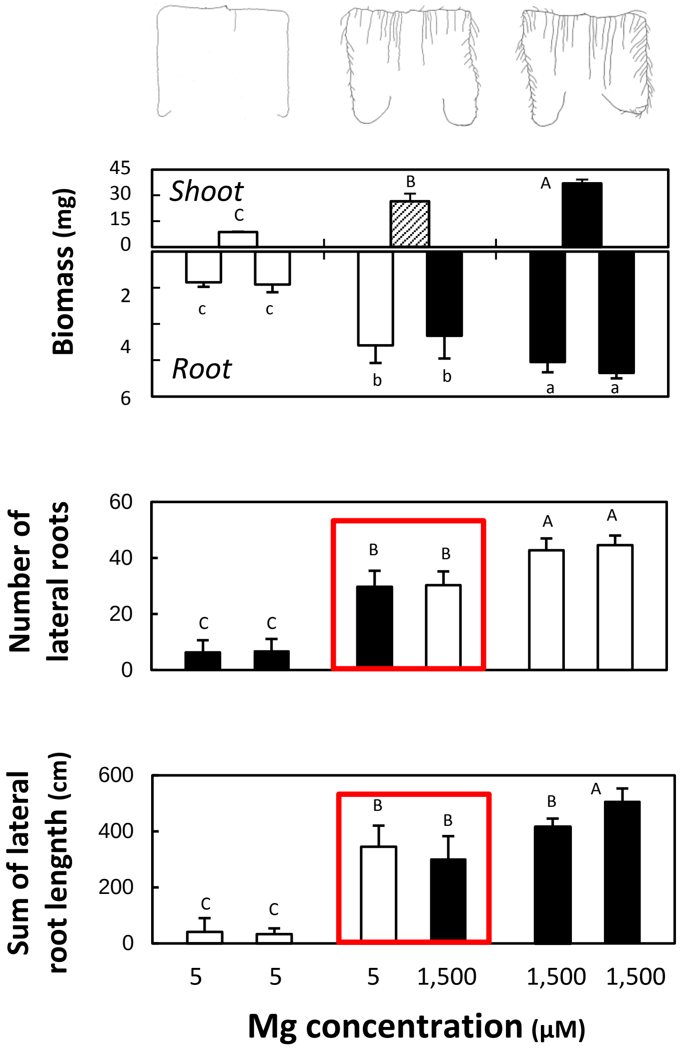
Vertical ½ - ½ split root system



17 days after germination

2 cm

Root morphological adaptation to heterogenous Mg supply



Seemingly, there is no such sensing mechanism to refrain lateral root growth in the unfavorable nutrient zone.

The extra source of the element acquired by the plant in the Mg-rich zone stimulates lateral root outgrowth in the Mg-deprived zone.

II. Influence of magnesium supply on root system architecture in *Arabidopsis thaliana*

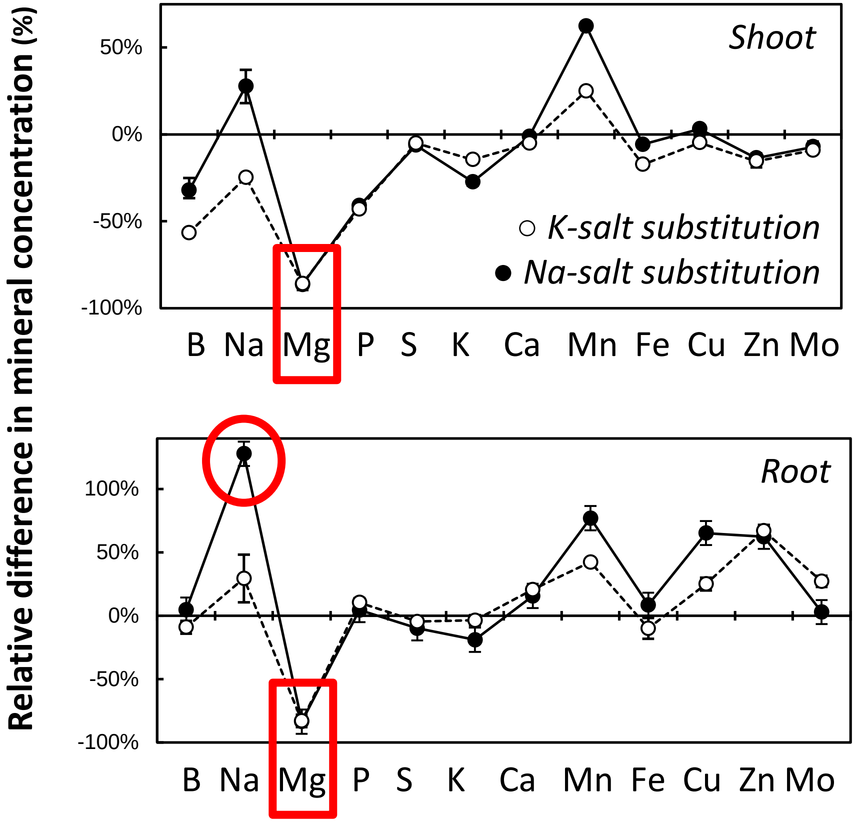
① Setting experimental conditions to observe root morphology in response to Mg supply (*in vitro*)

② Elemental profile upon Mg depletion

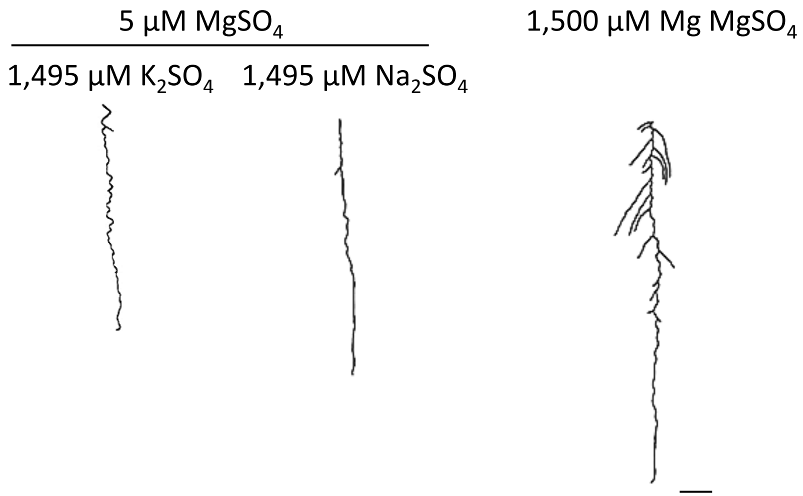
③ Influence of Mg supply on lateral root developmental stages

④ Crosstalk with hormones

② Elemental profile variation in response to magnesium depletion

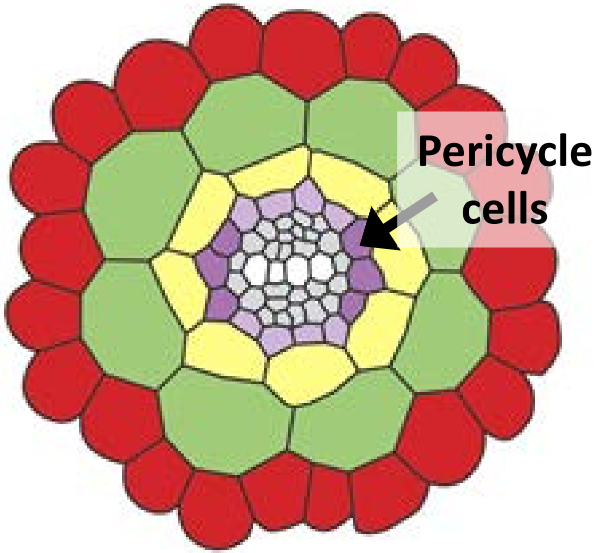


17 dag. *n*=4 (20 pooled organs) ± SE

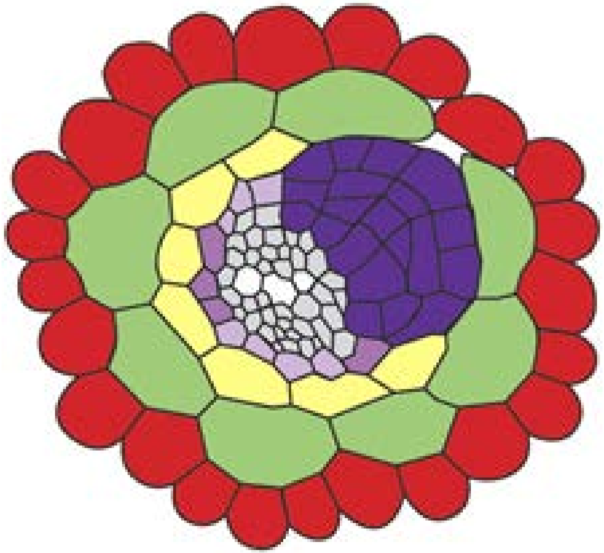


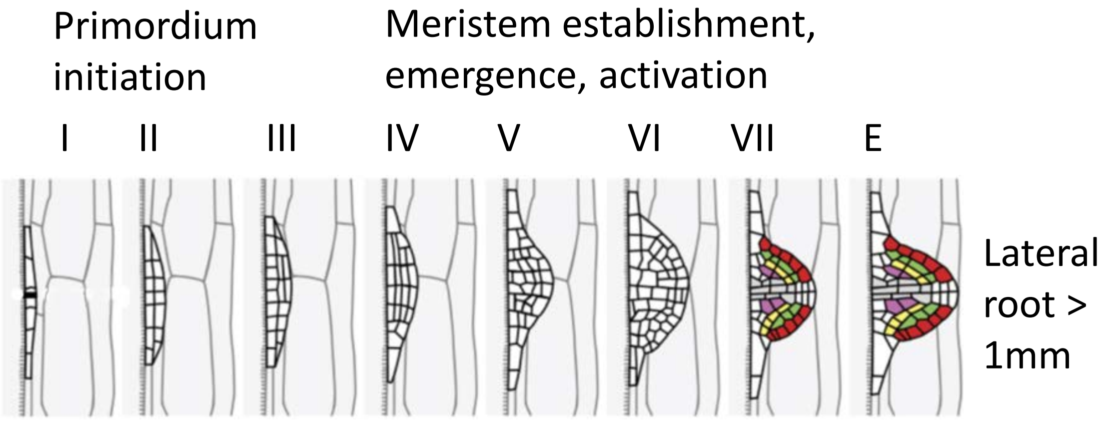
Is Mg deficiency associated with a combination of induced deficiencies and/or toxicities of other elements?

③ Influence of magnesium supply on lateral root developmental stages



Pericycle cells

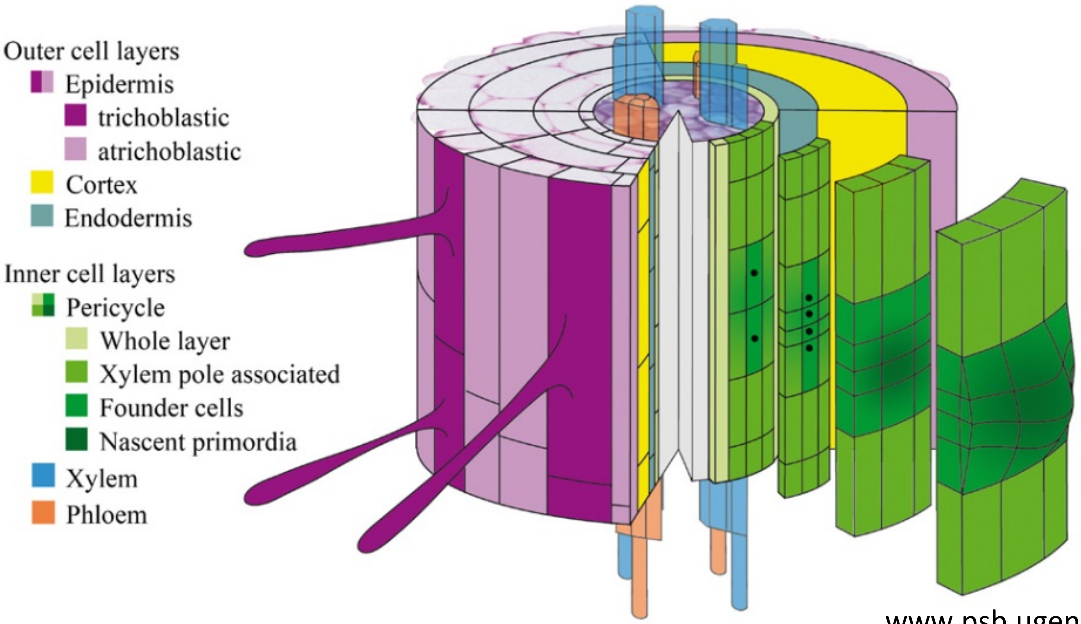




Primordium initiation

Meristem establishment, emergence, activation

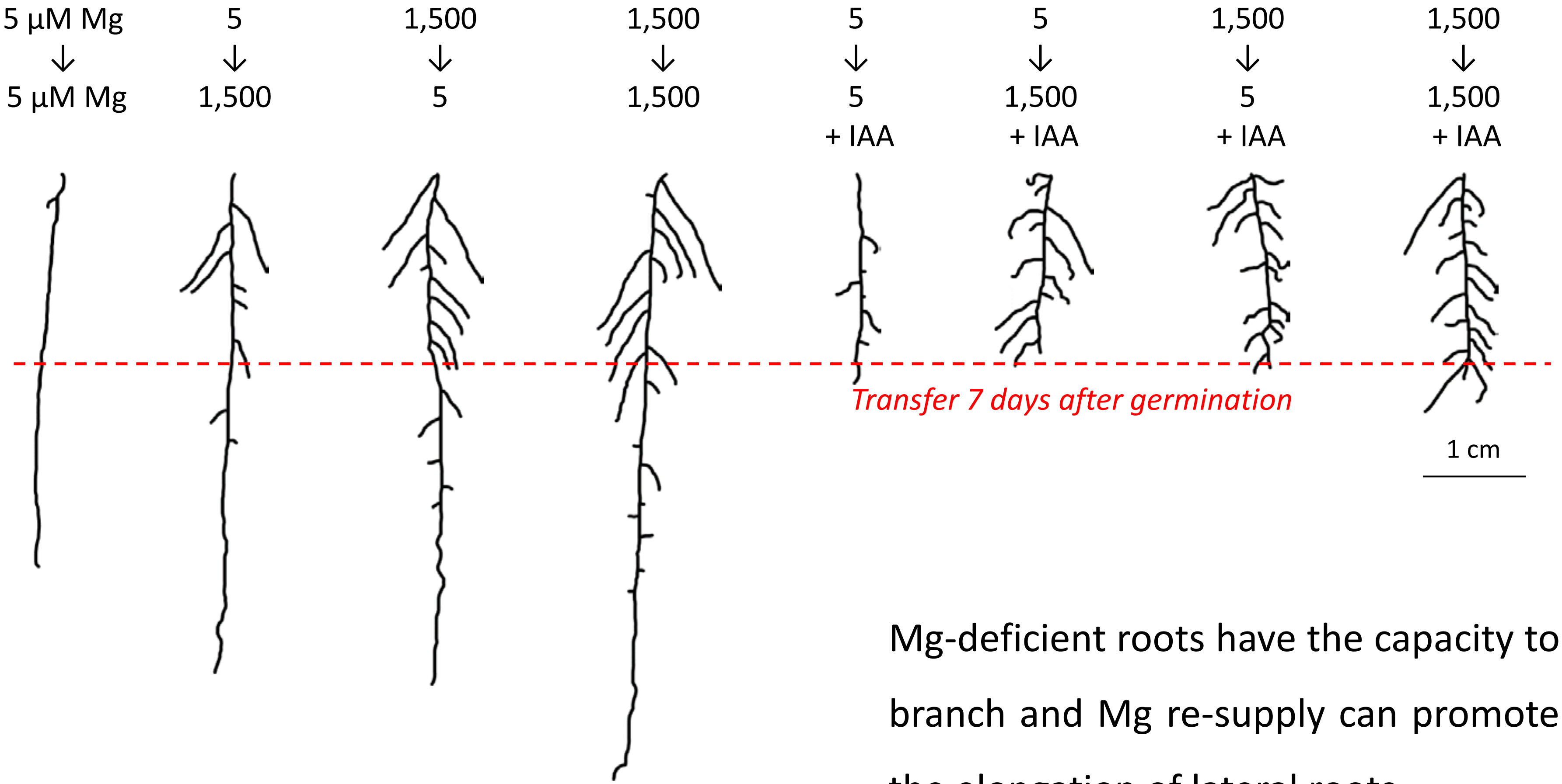
Lateral root > 1mm



www.psb.ugent.be

③ Influence of magnesium supply on lateral root developmental stages

Effect of 3-indole acetic acid (IAA) on lateral root development

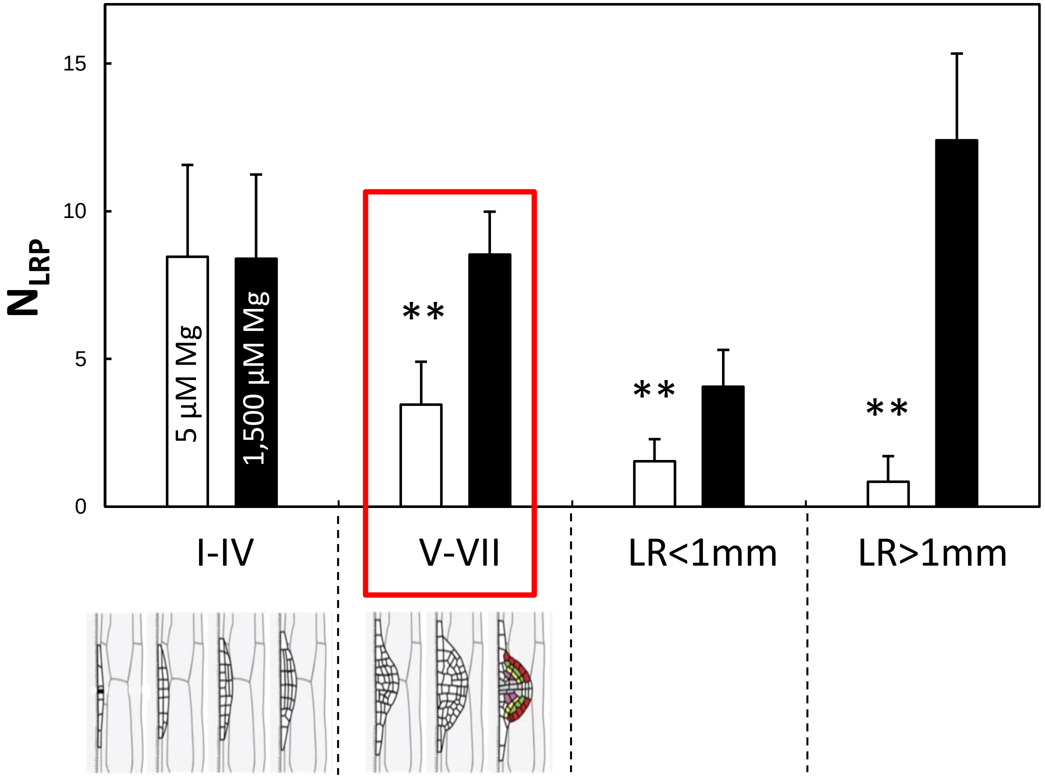


Mg-deficient roots have the capacity to branch and Mg re-supply can promote the elongation of lateral roots.

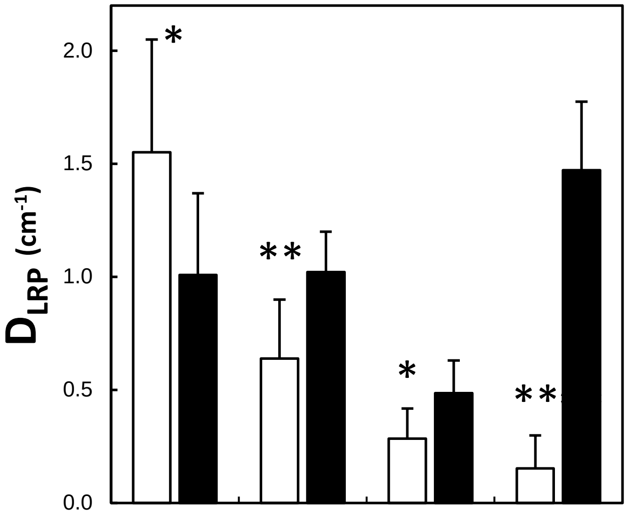
③ Influence of magnesium supply on lateral root developmental stages

Categories of lateral root primordia development in response to Mg deficiency

Number of lateral root primordia



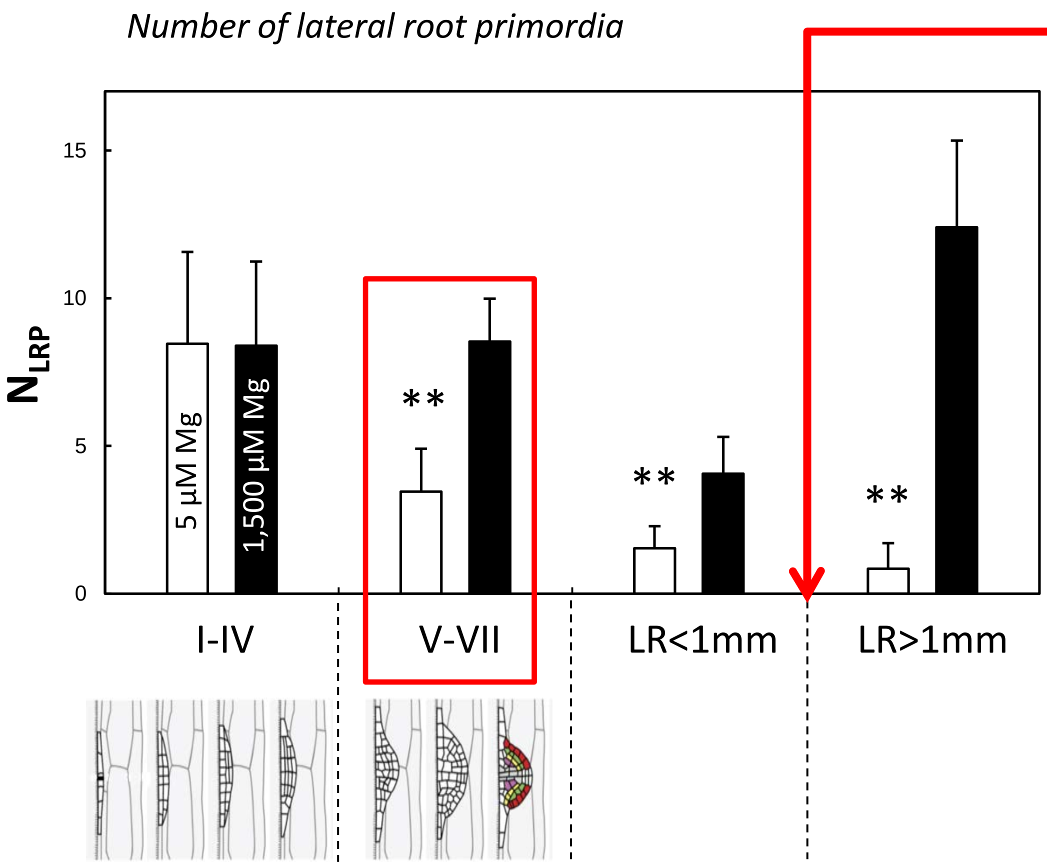
Density of lateral root primordia



17 dag. *n*=15 seedlings ± std. Statistical significance: * (*P*<0.01), ** (*P*<0.001)

③ Influence of magnesium supply on lateral root developmental stages

Categories of lateral root primordia development in response to Mg deficiency

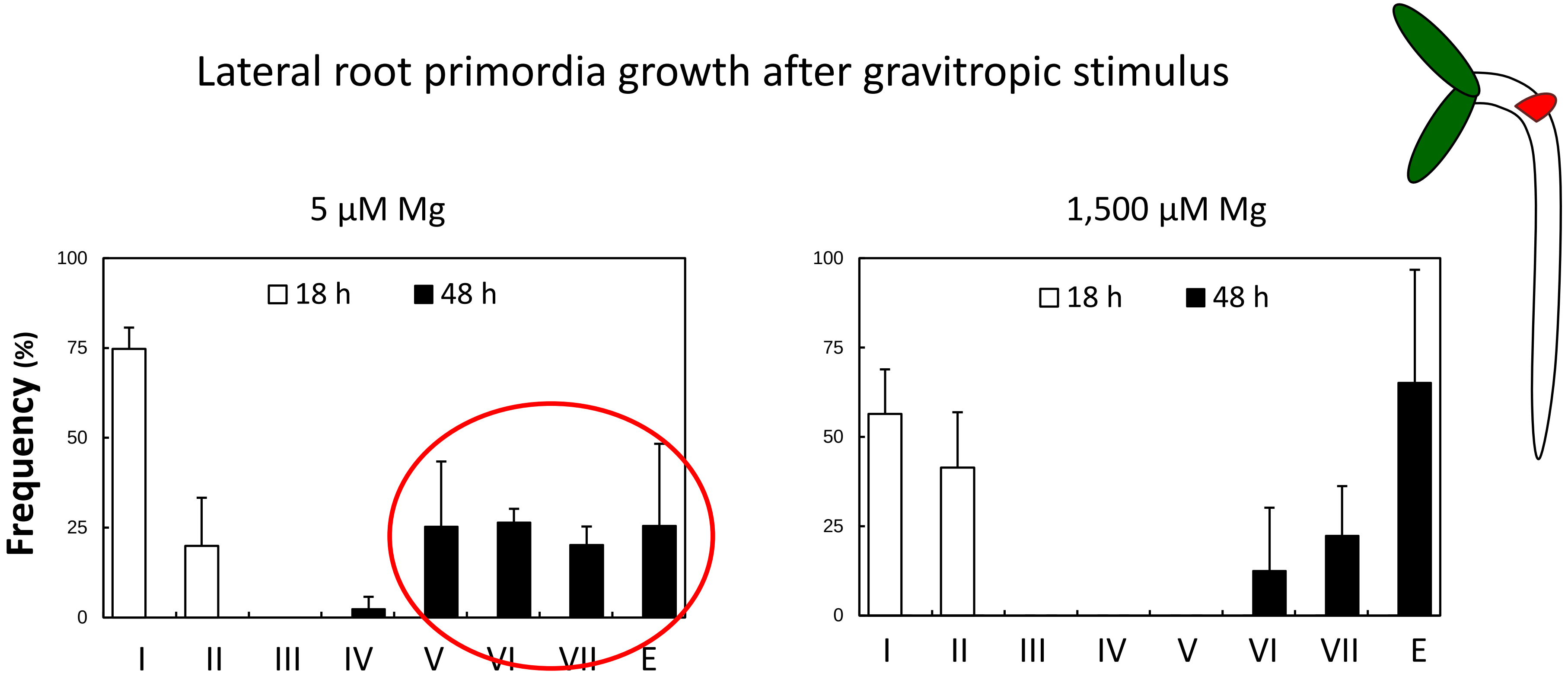


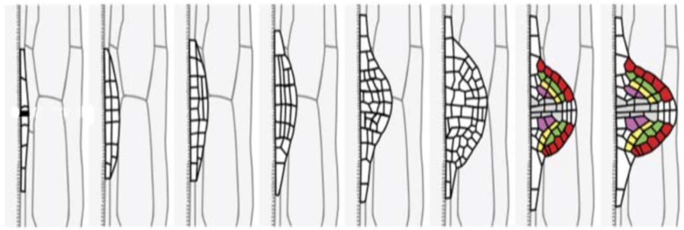
REM: High NO_3^- specifically targets post-emergence

17 dag. n=15 seedlings ± std. Statistical significance: * (*P*<0.01), ** (*P*<0.001)

③ Influence of magnesium supply on lateral root developmental stages

Lateral root primordia growth after gravitropic stimulus





Progression from stage V is affected, in which the primordium undergoes anticlinal divisions and starts to grow through the cortex tissue.

n=15 seedlings ± std

II. Influence of magnesium supply on root system architecture in *Arabidopsis thaliana*

① Setting experimental conditions to observe root morphology in response to Mg supply (*in vitro*)

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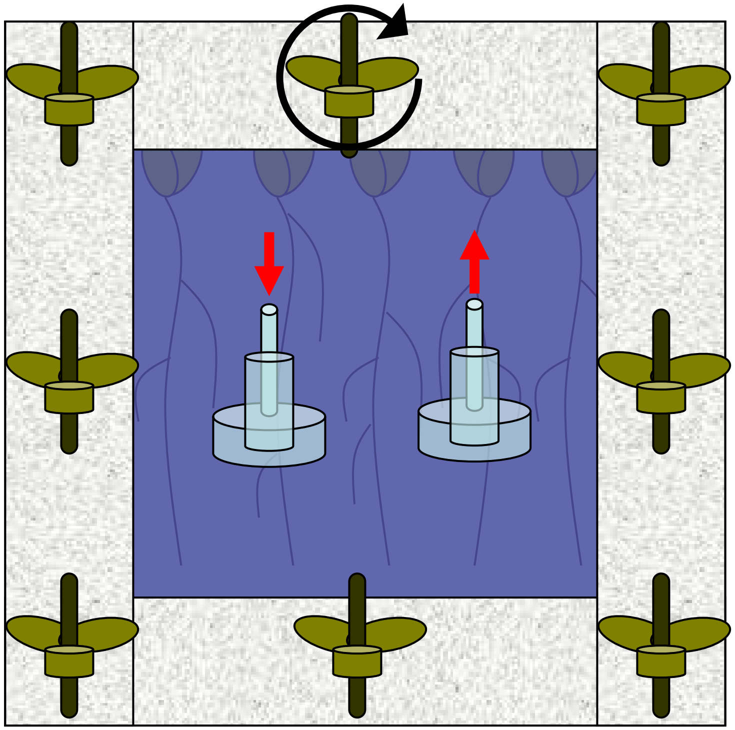
③ Influence of Mg supply on lateral root developmental stages

④ Crosstalk with hormones

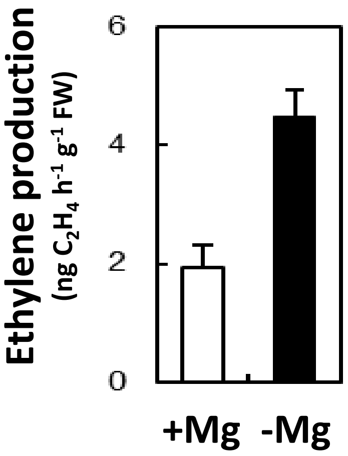
④ Interplay between magnesium nutrition and hormones

New experimental setting for measuring ethylene production in Petri plates.



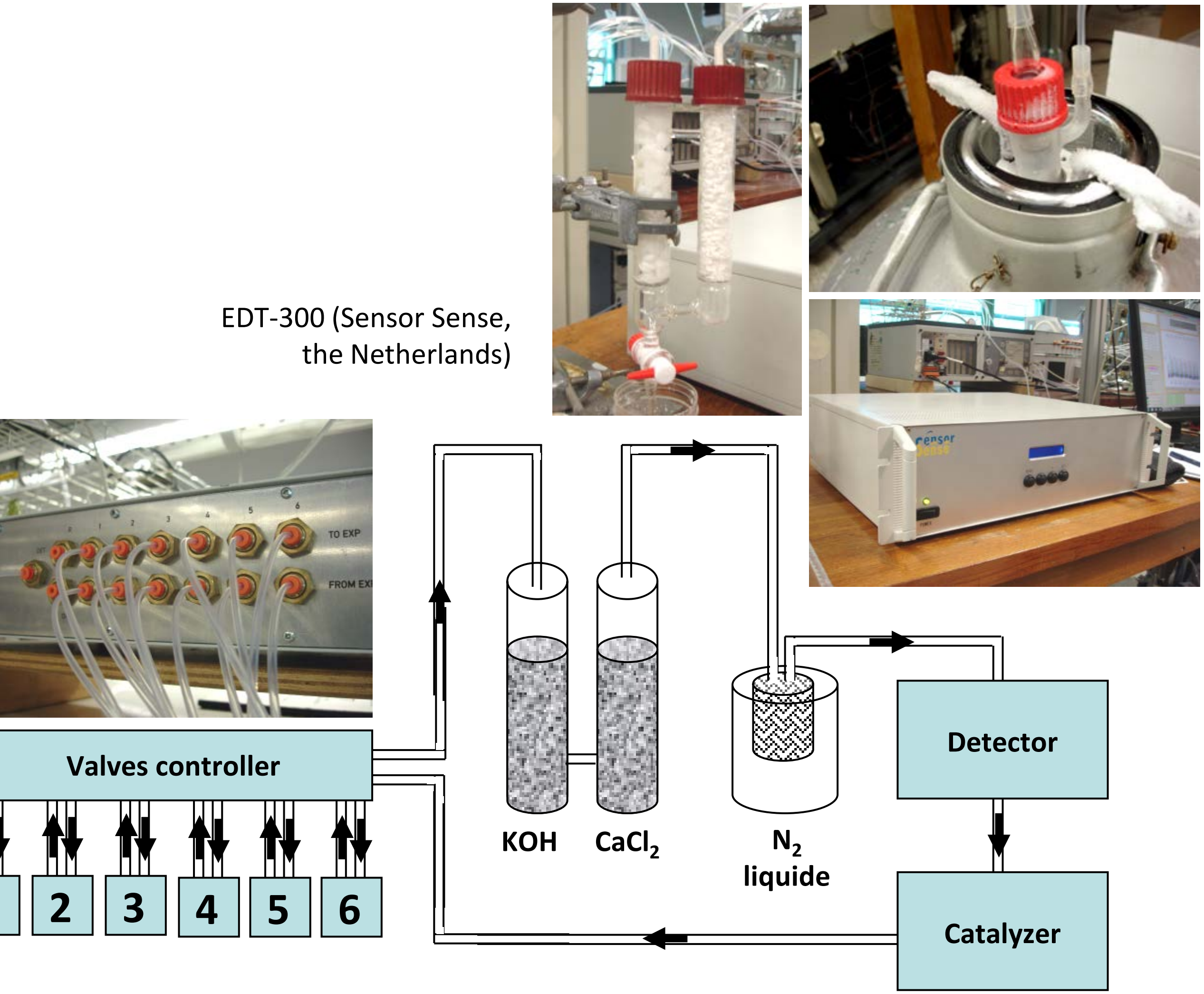
Ethylene

④ Interplay between magnesium nutrition and hormones



Double ethylene emanation by Mg deficient seedlings

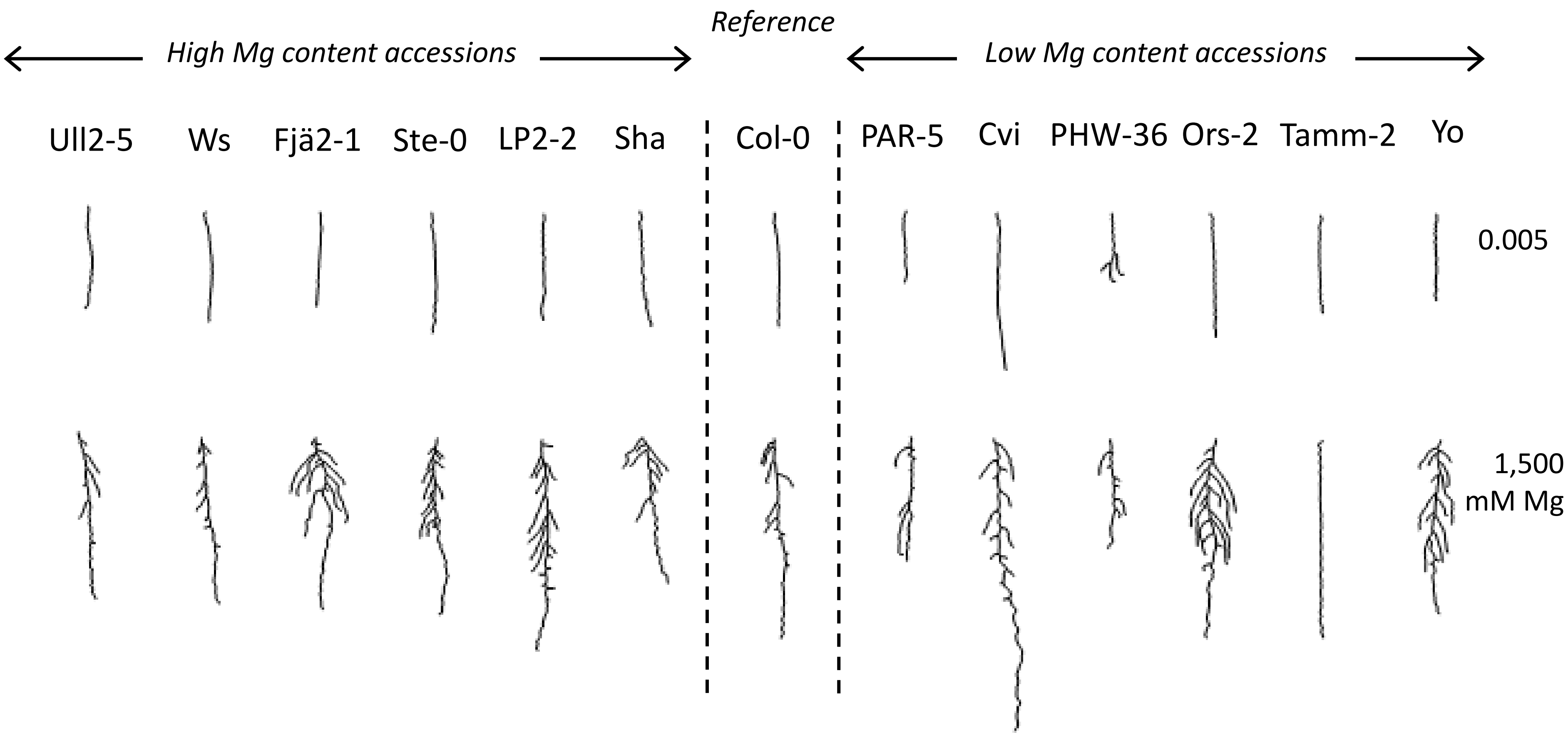
Ethylene



EDT-300 (Sensor Sense, the Netherlands)

Natural variation of root architecture in response to Mg

Does Mg tissue concentration correlate with root architectural features?



Reference

← *High Mg content accessions* →

← *Low Mg content accessions* →

Ull2-5 Ws Fjä2-1 Ste-0 LP2-2 Sha Col-0 PAR-5 Cvi PHW-36 Ors-2 Tamm-2 Yo

0.005

1,500 mM Mg

14 days after germination

1 cm

Conclusions and perspectives

- ✓ Mg depletion noticeably represses lateral root outgrowth, which makes it a remarkable case study.

- ✓ A slowdown in the growth of pre-emerged lateral root primordia was observed upon -Mg (target: stage V). → global transcriptomics upon bending assay.

- ✓ Neither root growth stimulation by localized Mg source, neither repression in Mg-deprived zone was emphasized. Absence of local Mg sensing mechanism in Arabidopsis?

- ✓ Ethylene may play a role in the control on primary root and lateral root elongation upon -Mg.

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Genomics, root development

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