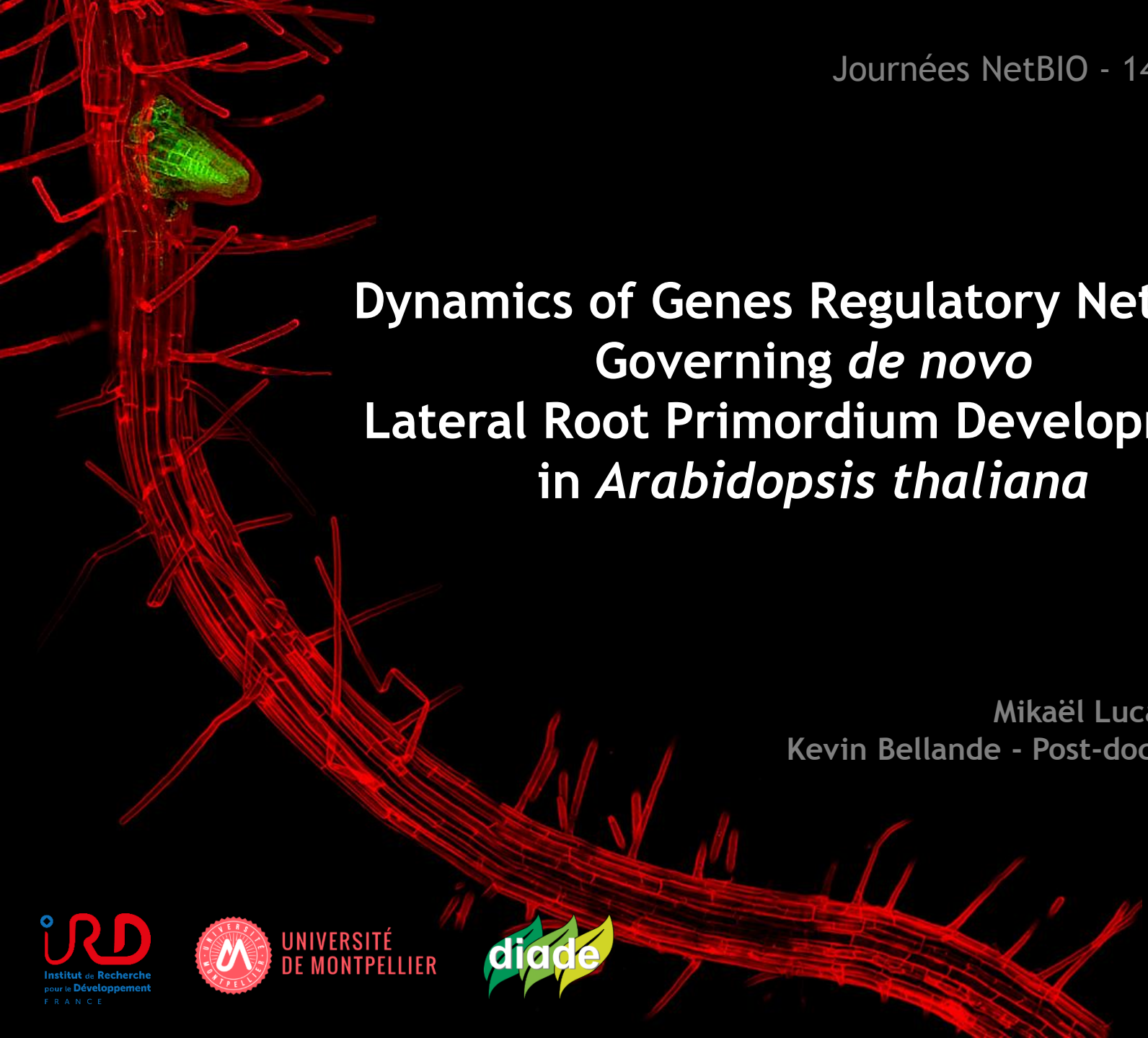


Journées NetBIO - 14/10/2019

Dynamics of Genes Regulatory Network Governing *de novo* Lateral Root Primordium Development in *Arabidopsis thaliana*

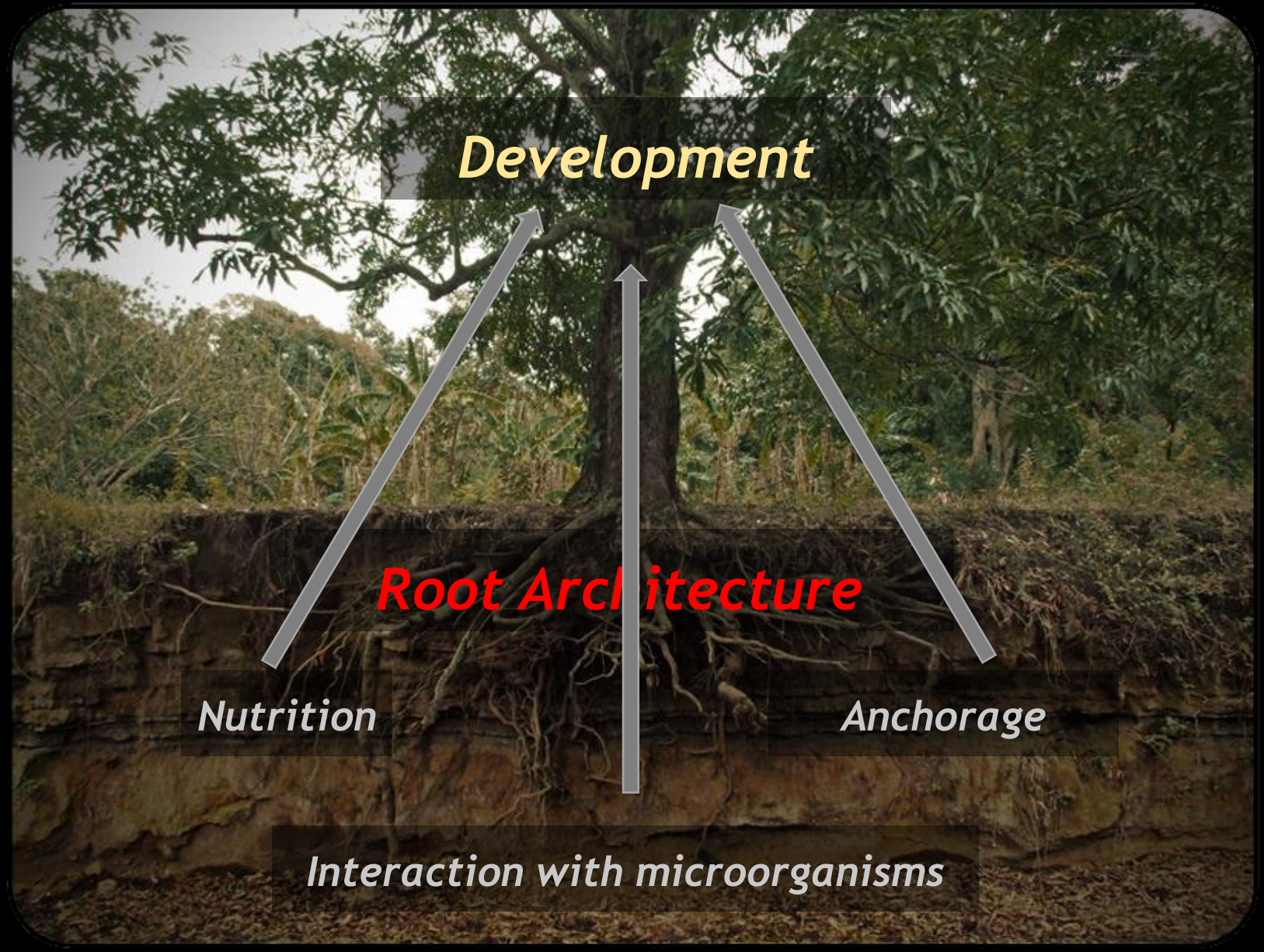
Mikaël Lucas - CR IRD
Kevin Bellande - Post-doctorant IRD

Institut de Recherche pour le Développement FRANCE

UNIVERSITÉ DE MONTPELLIER

diade

Context : Root System

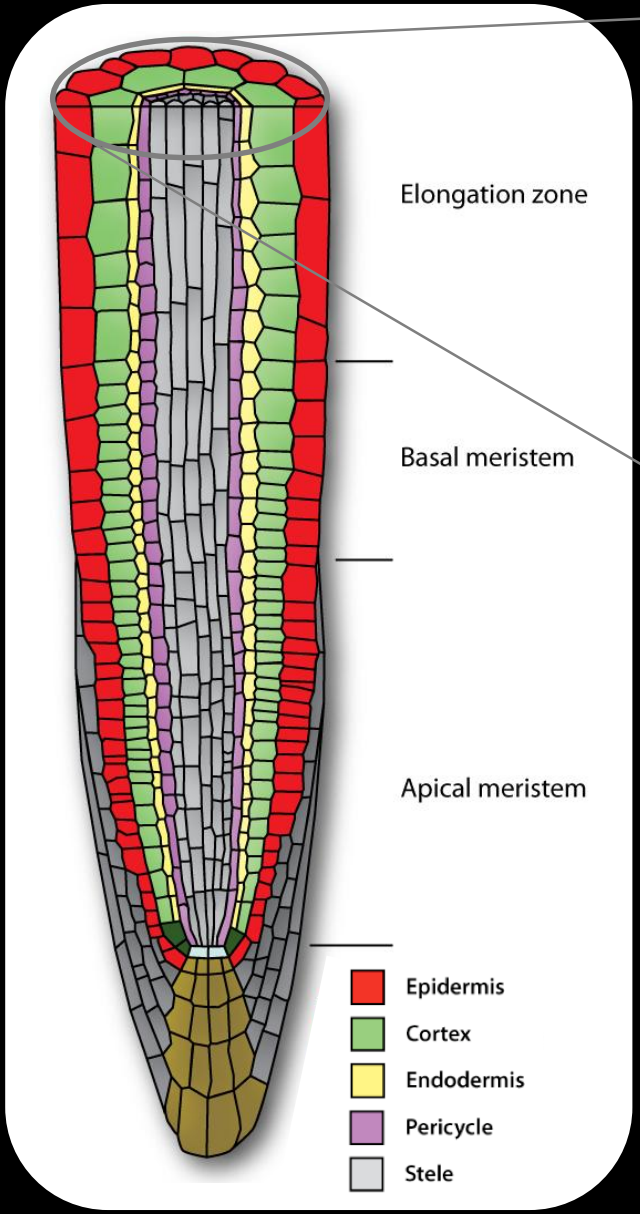


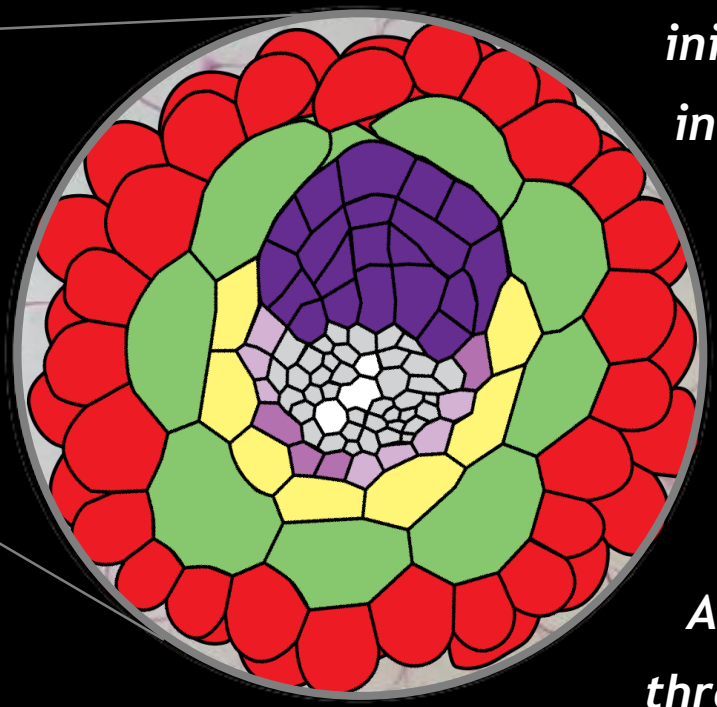
Context : Secondary organogenesis

… to a mature
root system …

… with a complex architecture
resulting from interactions
between genetics and environment.

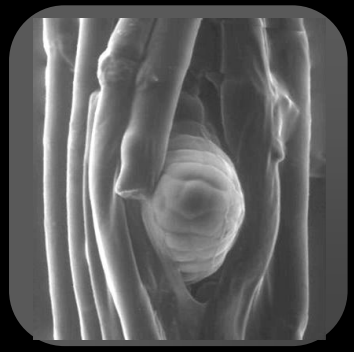
Context : Lateral root organogenesis

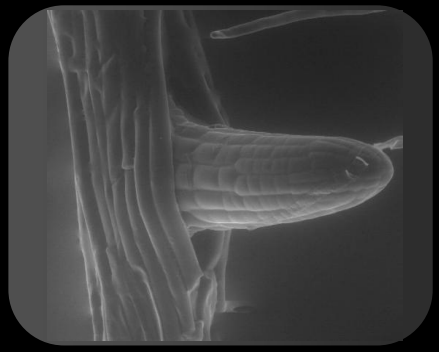




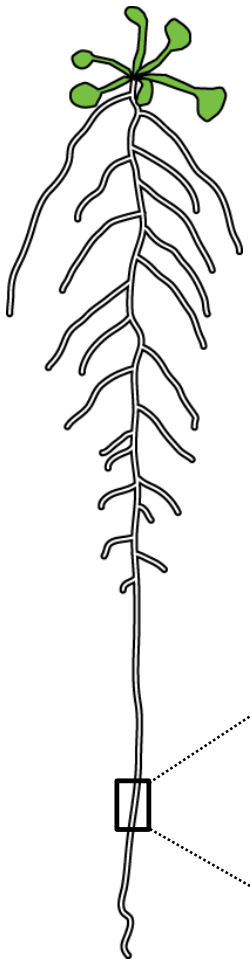
In Arabidopsis, LR initiation occurs in the pericycle

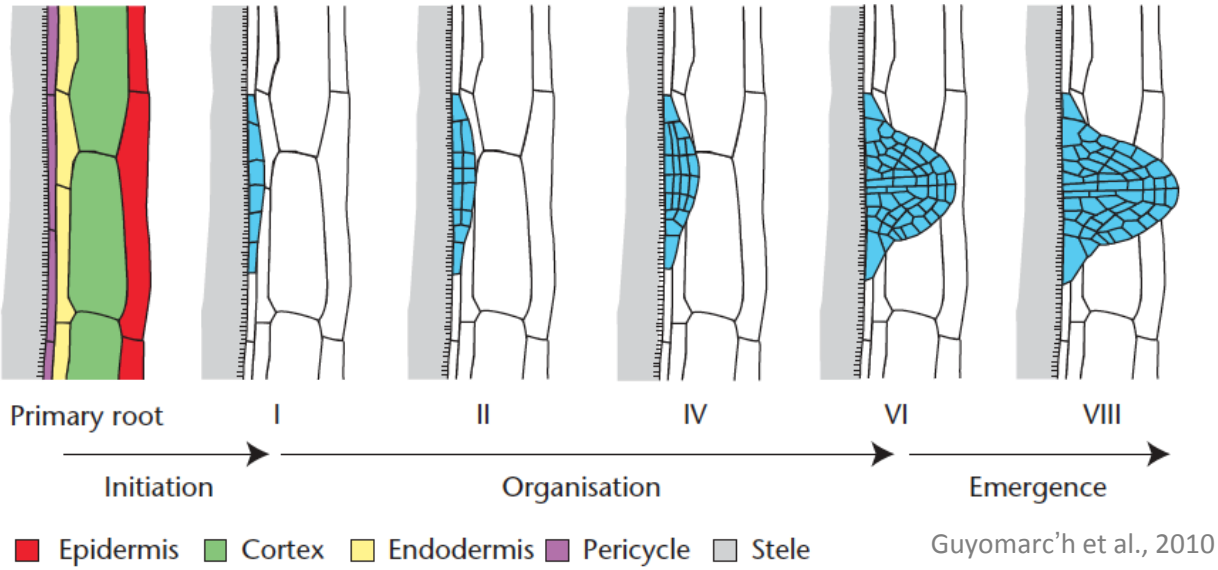
And LR develop through the tissues to finally emerge



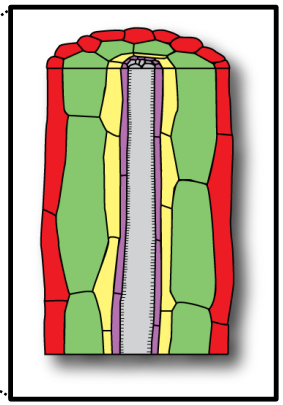


Context : Lateral root organogenesis



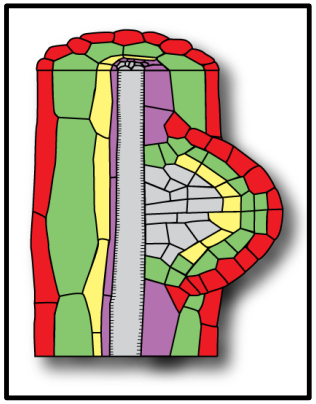


Guyomarc'h et al., 2010



Well described sequence of morphogenesis

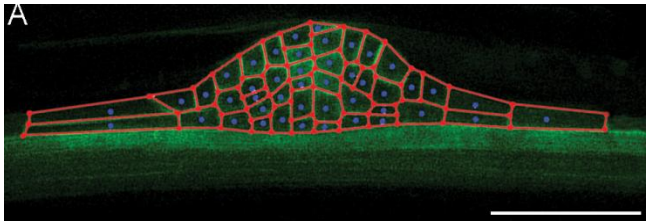
Is this development highly regular ?

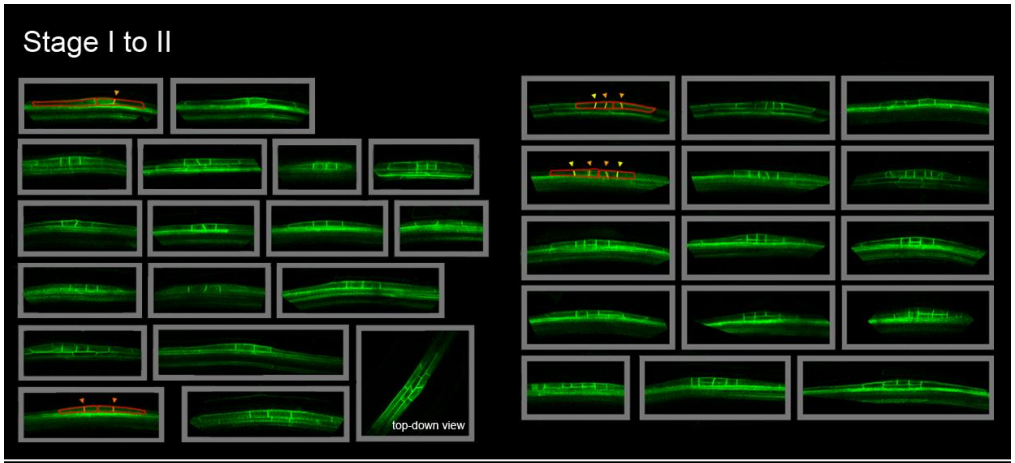


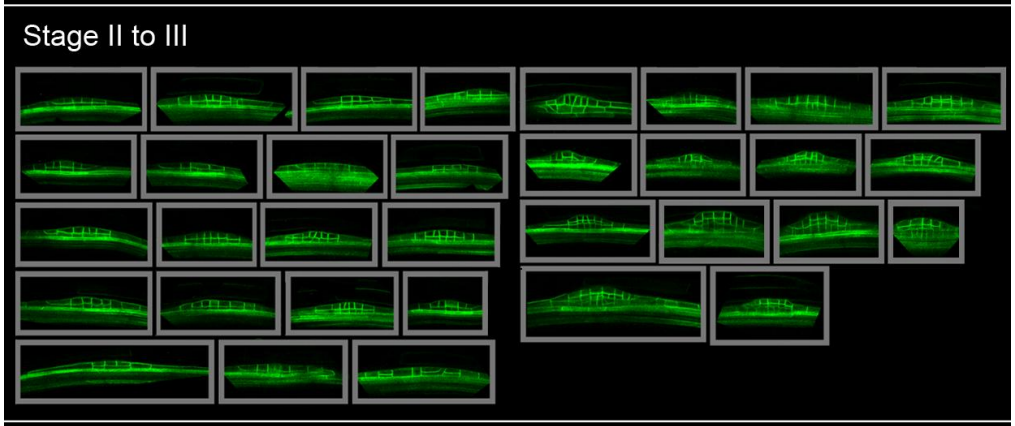
Context : Lateral root organogenesis

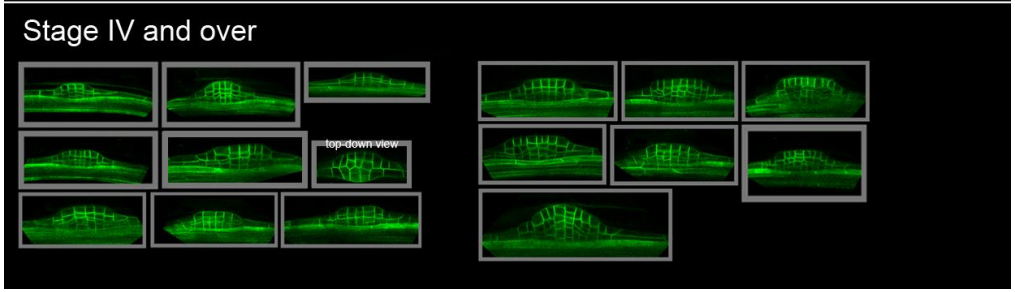
(Lucas et al. 2013)

Let's have a look at lots of LRP





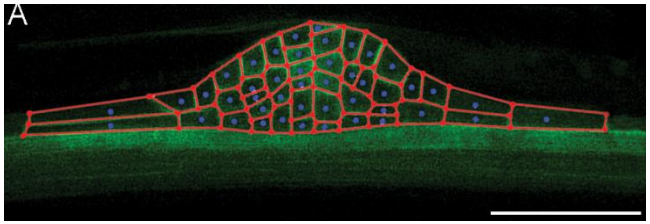


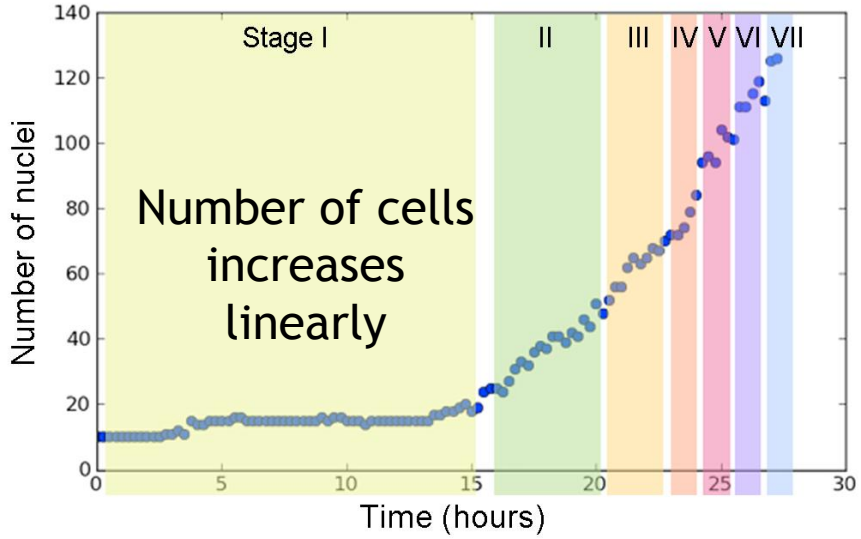


(Lucas et al. 2013)

Context : Lateral root organogenesis

Let's have a look at lots of LRP

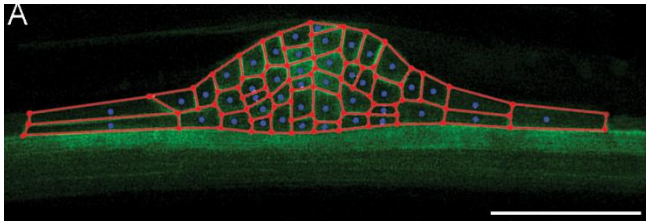




(Lucas et al. 2013)

Context : Lateral root organogenesis

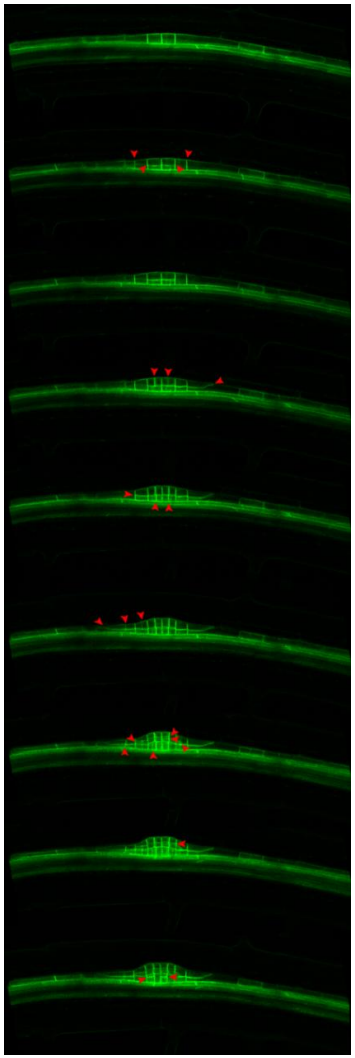
Let's have a look at lots of LRP



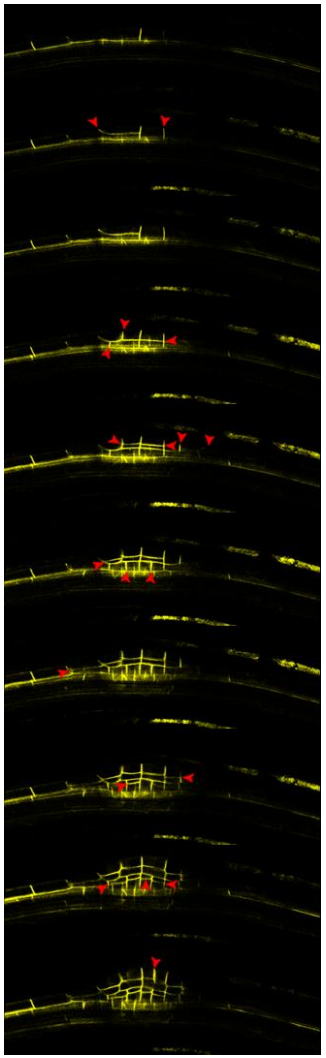
LRP patterning is not stereotypical

Multiple ways of building a LRP

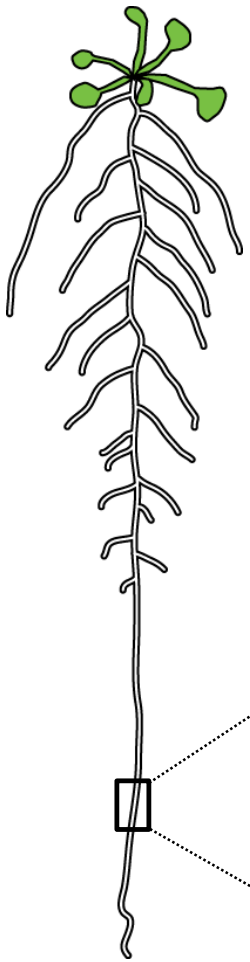
(Lucas et al. 2013)

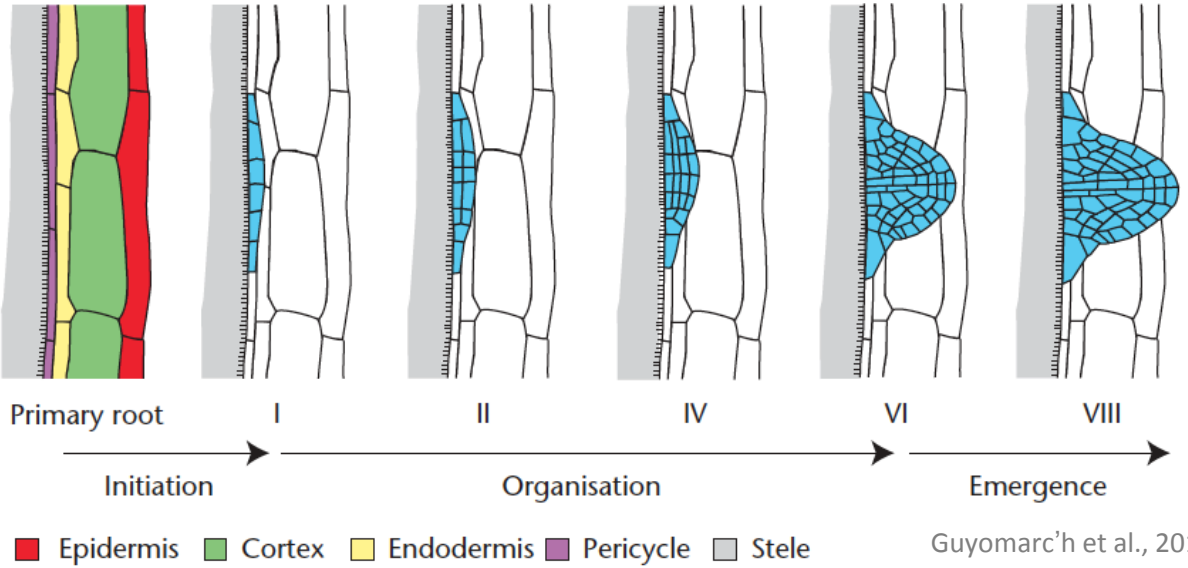


Sequence of division events actually varies between LRP

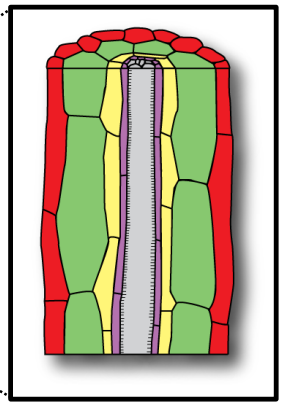


Context : Lateral root organogenesis



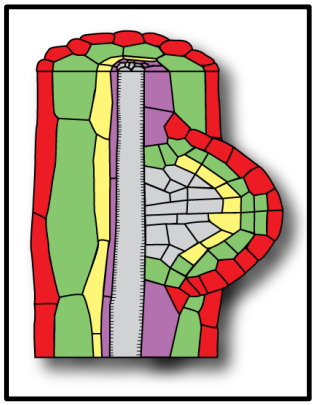


Guyomarc'h et al., 2010



Plastic development, but fixed ending

DYNAMIC PATTERNING ?

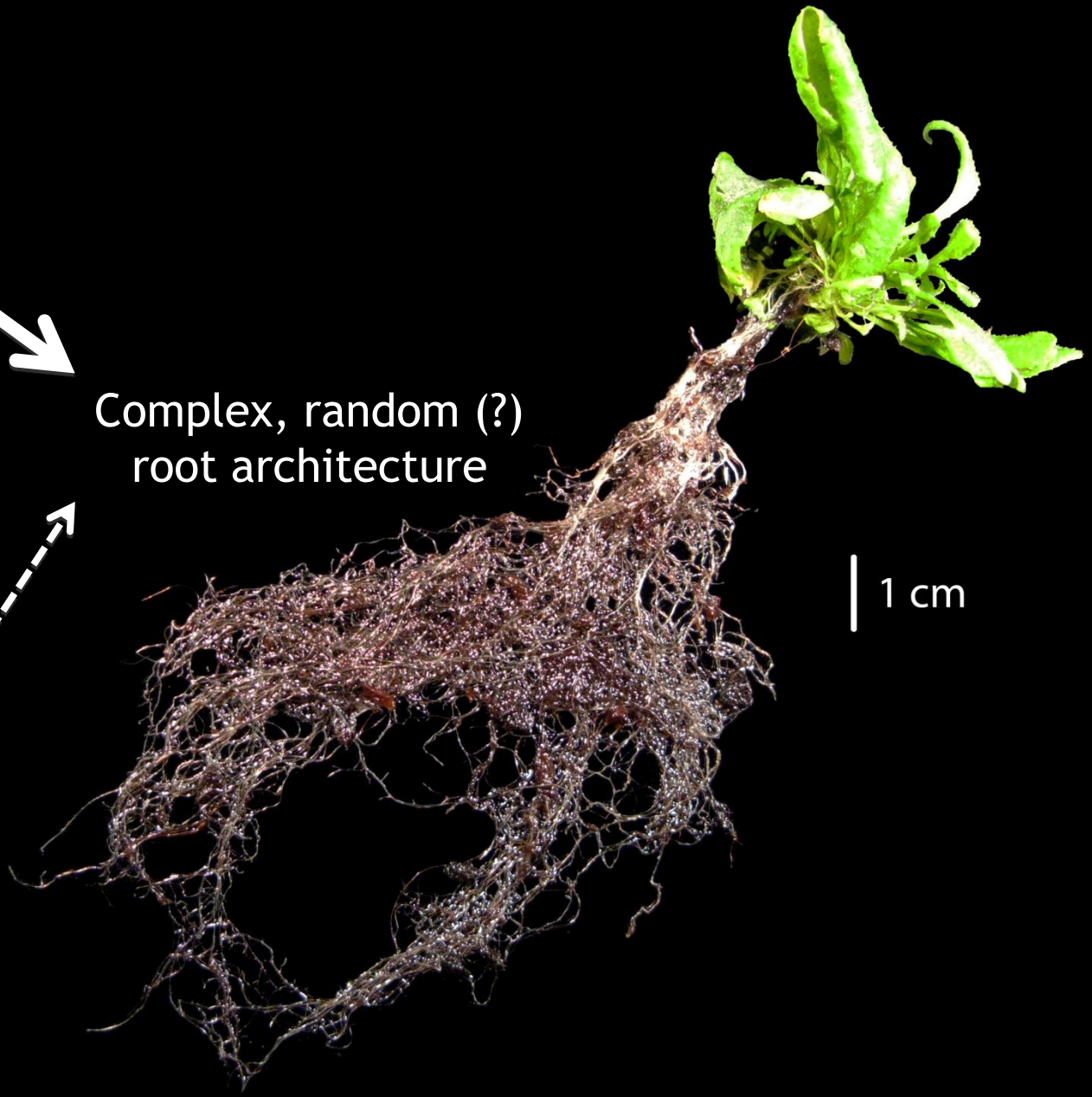


Context : Lateral root organogenesis

Elementary dynamic organogenesis processes

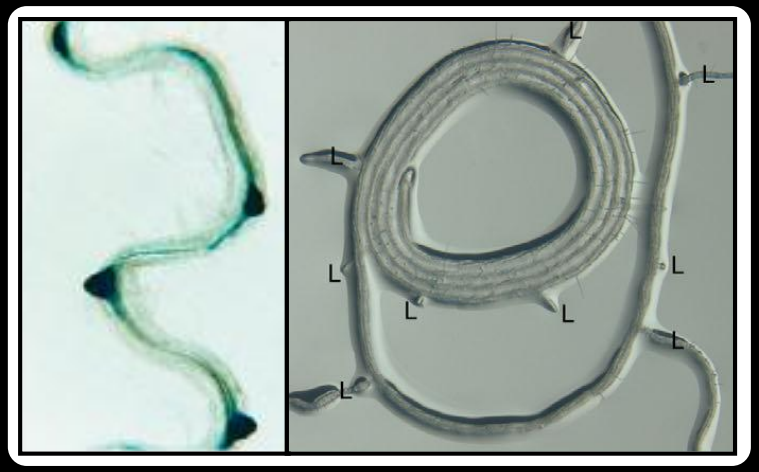
Complex, random (?) root architecture

How to study this process to understand and control RSA ?



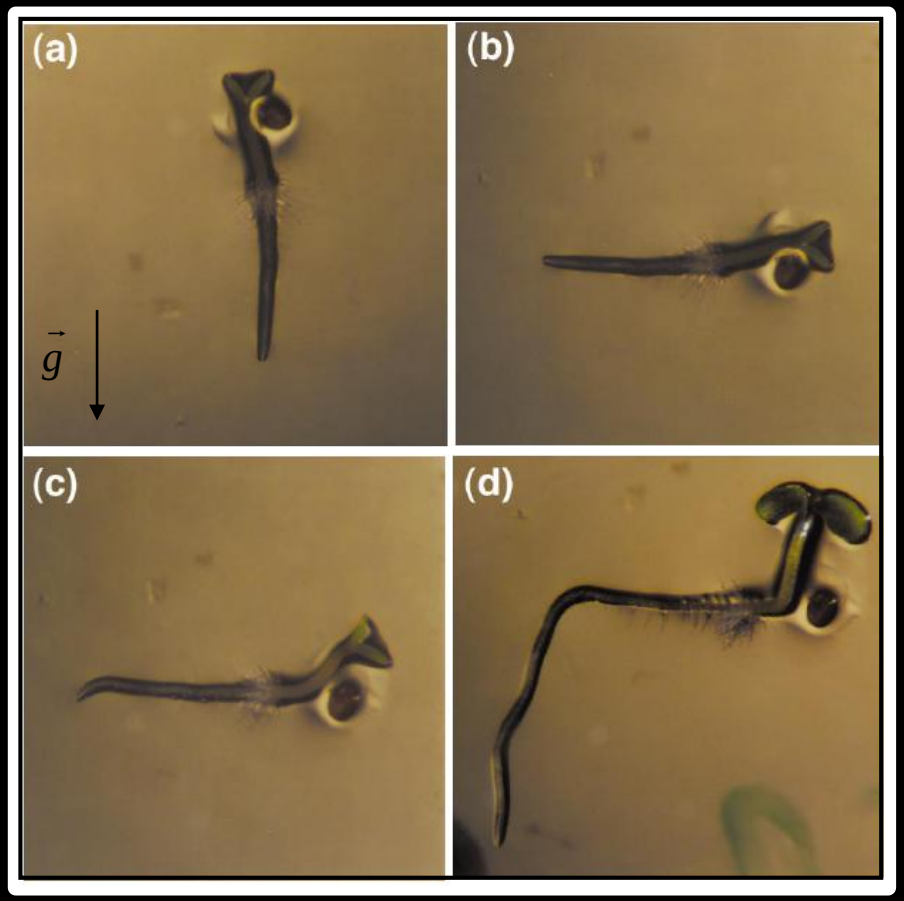
Modulation of lateral root initiation

LRP initiation correlated with root bending



(De Smet et al. 2007)

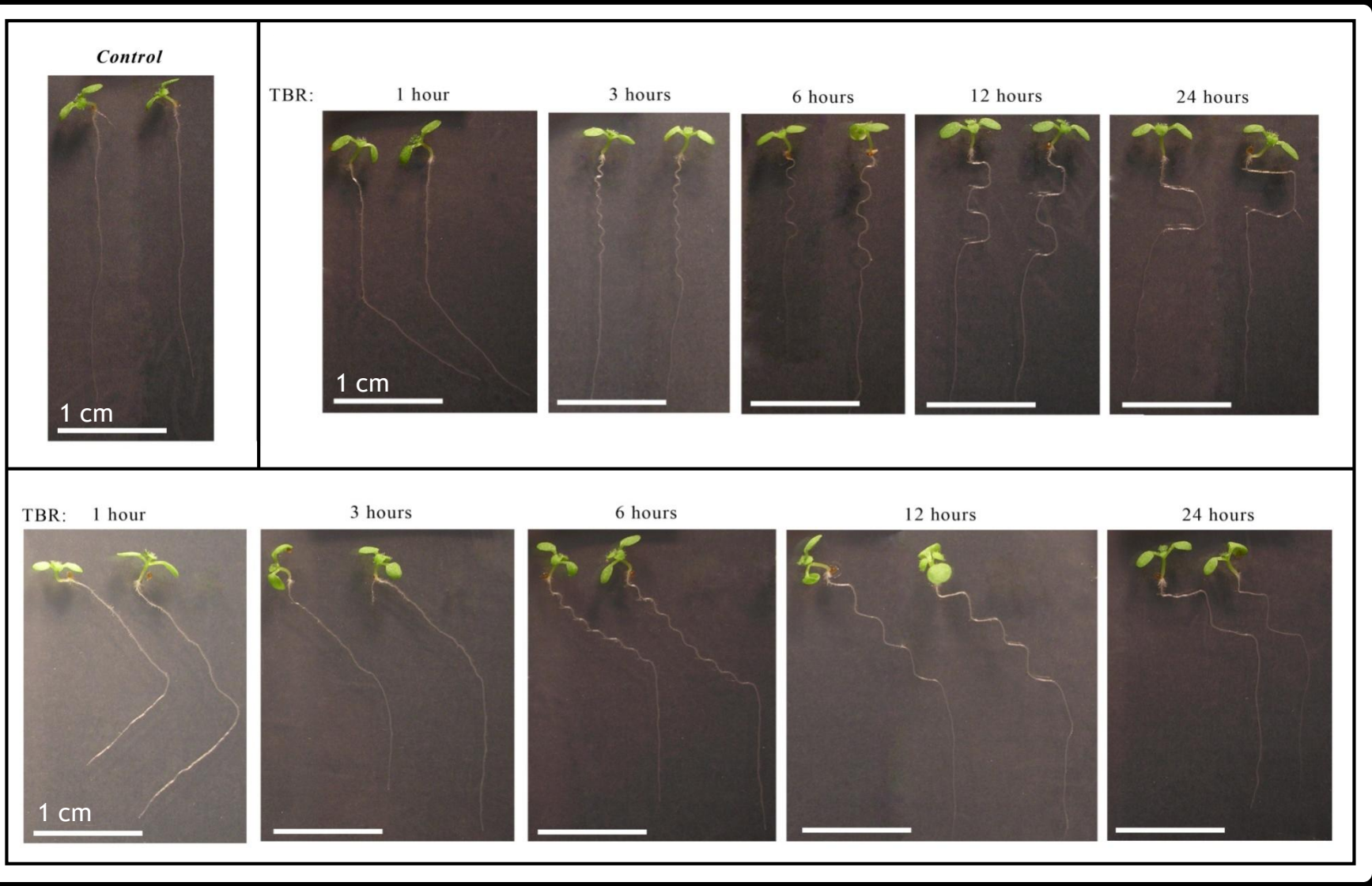
Gravitropism induces root bending



(Rosen, 1999)

Can new lateral roots be induced using gravistimulation ?

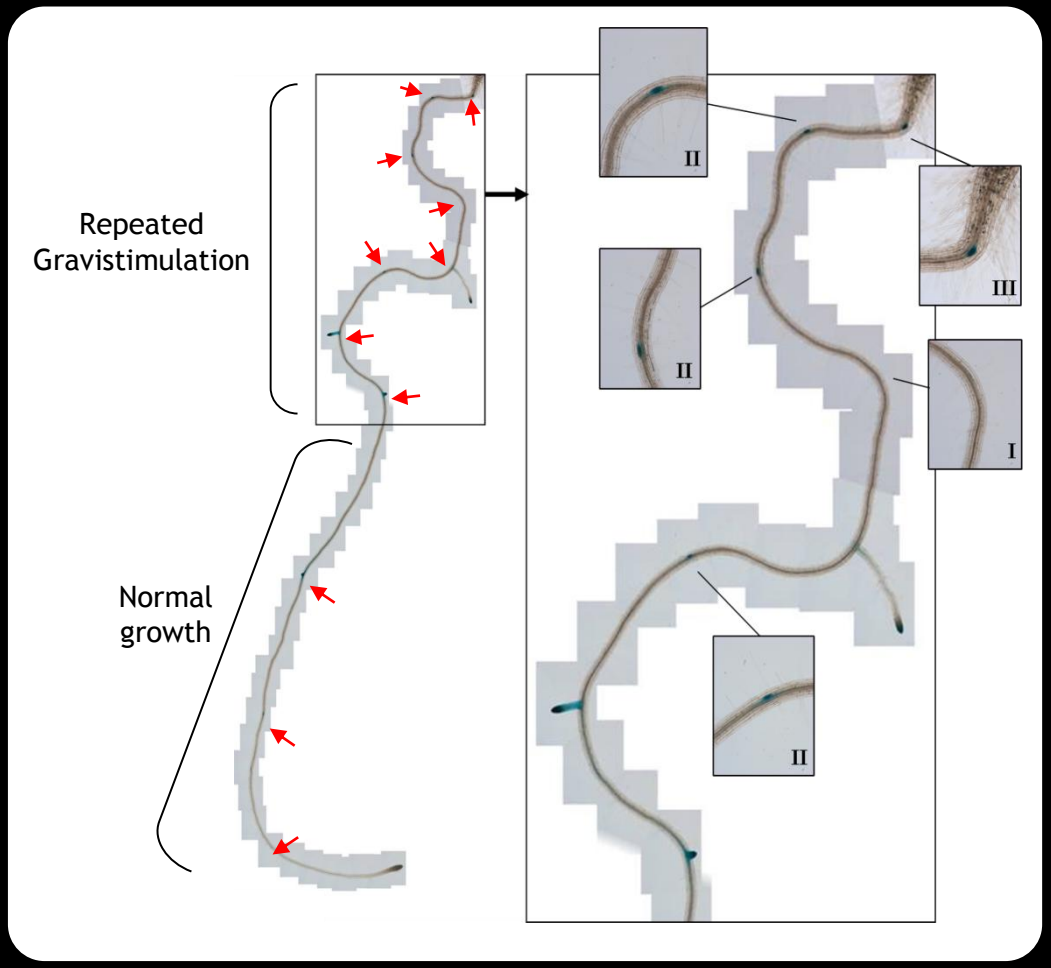
Modulation of lateral root initiation



(Lucas et al. 2008)

Induction of rhizogenesis by gravistimulation

Gravistimulation induces initiation…



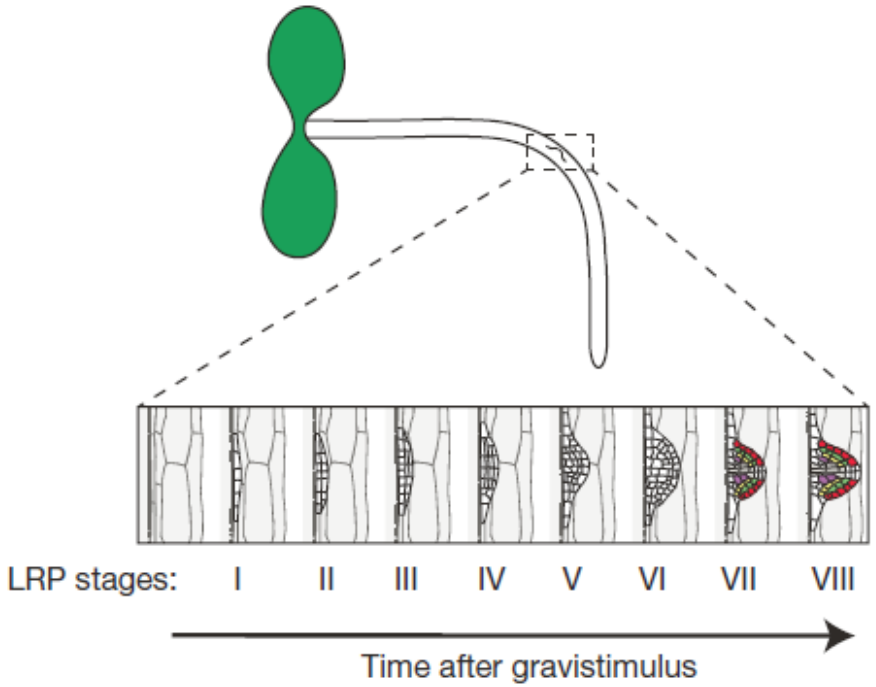
… within a tightly controlled spatio-temporal window

DR5:GFP and propidium iodide

(Lucas et al. 2008)

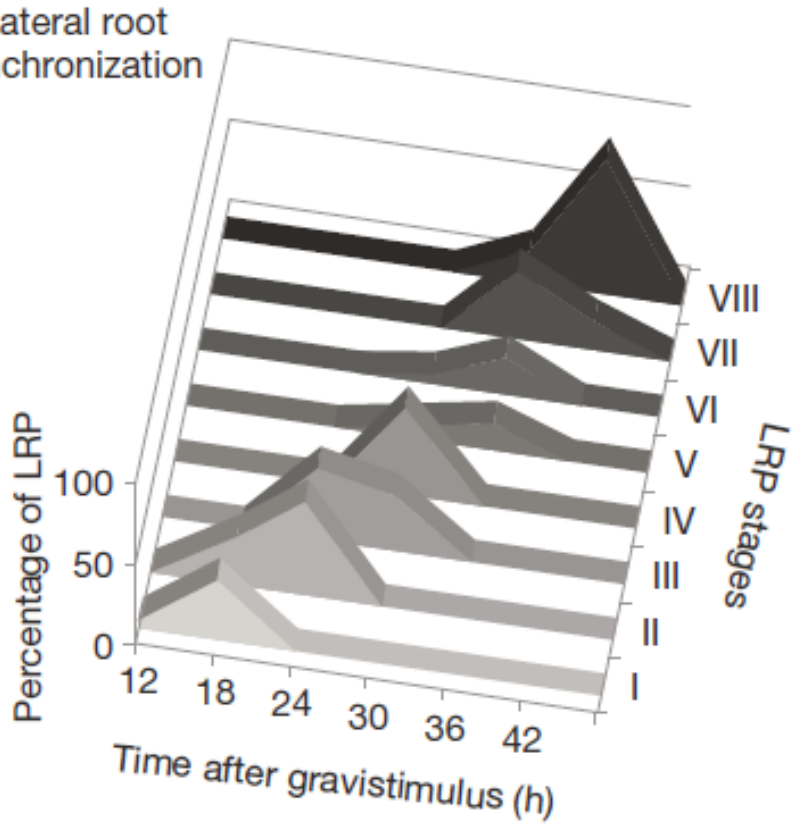
Control of rhizogenesis by gravistimulation

Gravistimulation induces initiation…



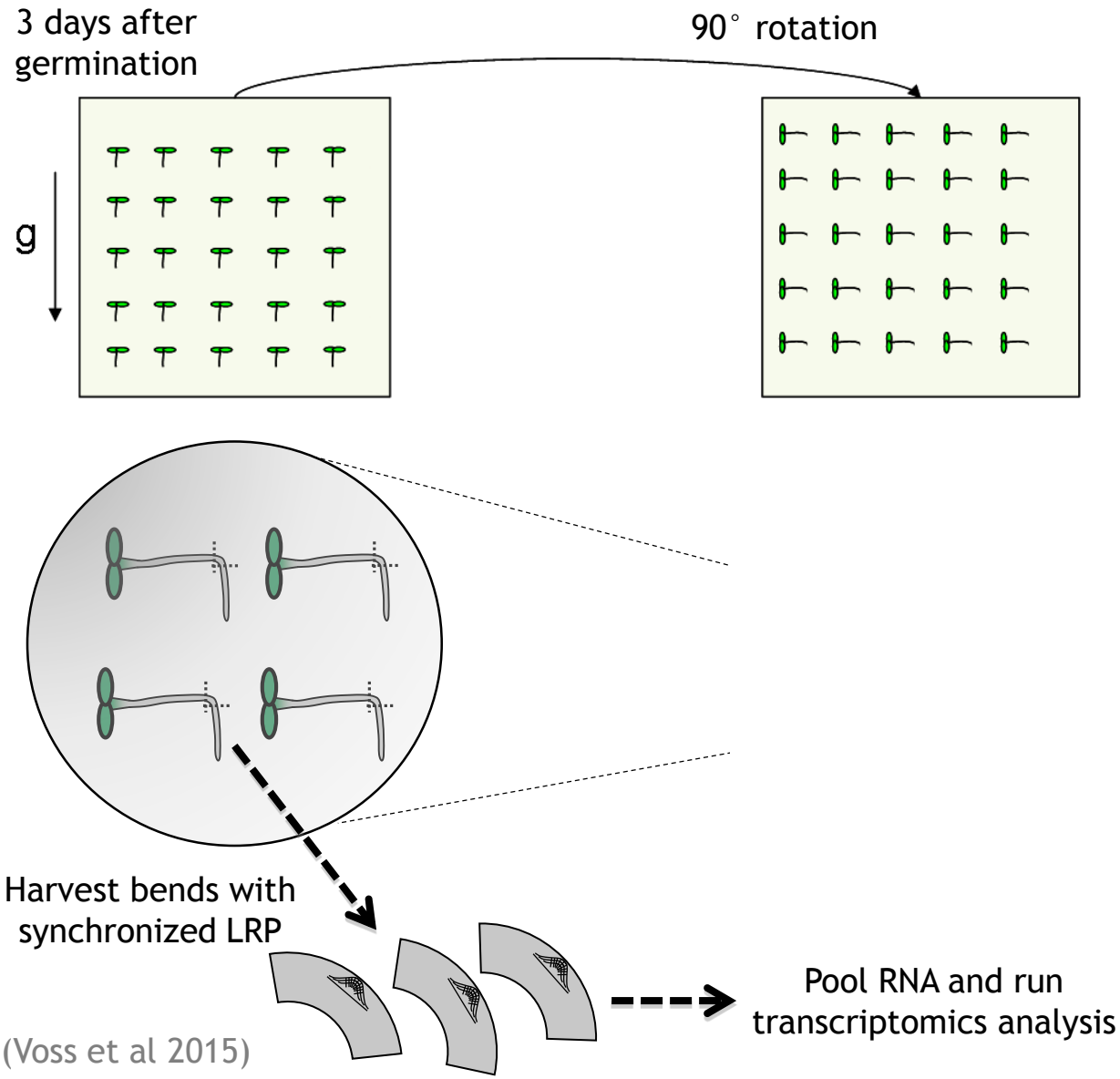
Can we use this to access the dynamics of LR morphogenesis regulation ?

… within a tightly controlled spatio-temporal window



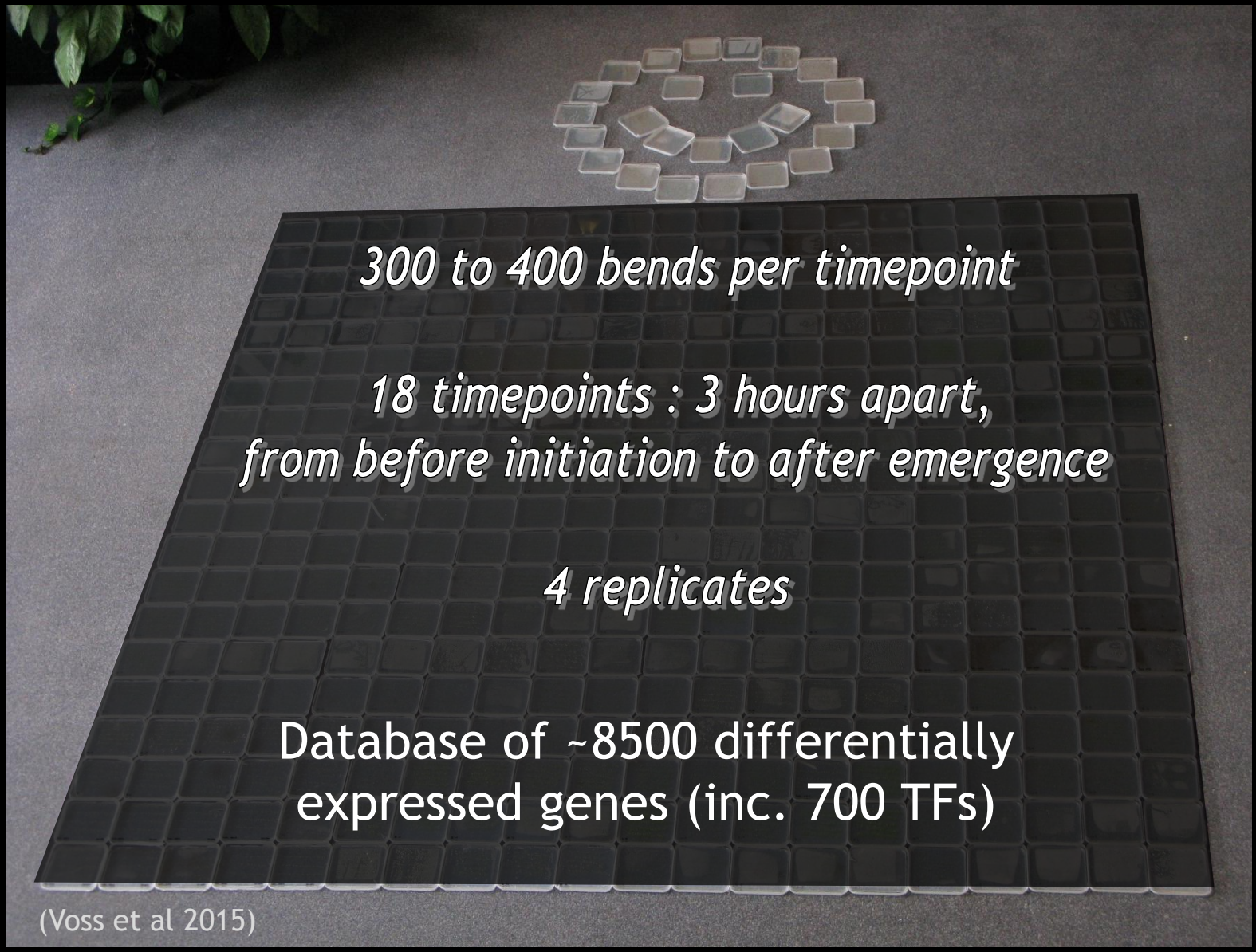
(Péret et al. 2012)

Transcriptomics of LRP development



Transcriptomics of LRP development

Transcriptomics of LRP development



300 to 400 bends per timepoint

18 timepoints : 3 hours apart,
from before initiation to after emergence

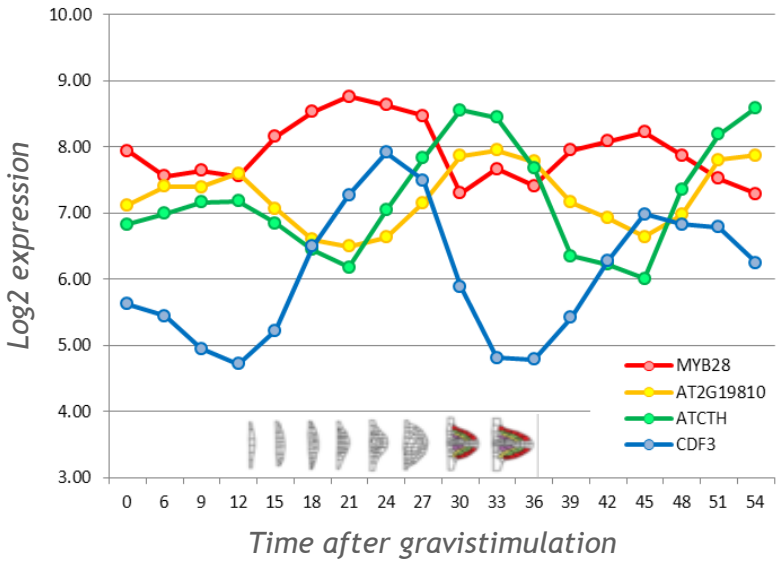
4 replicates

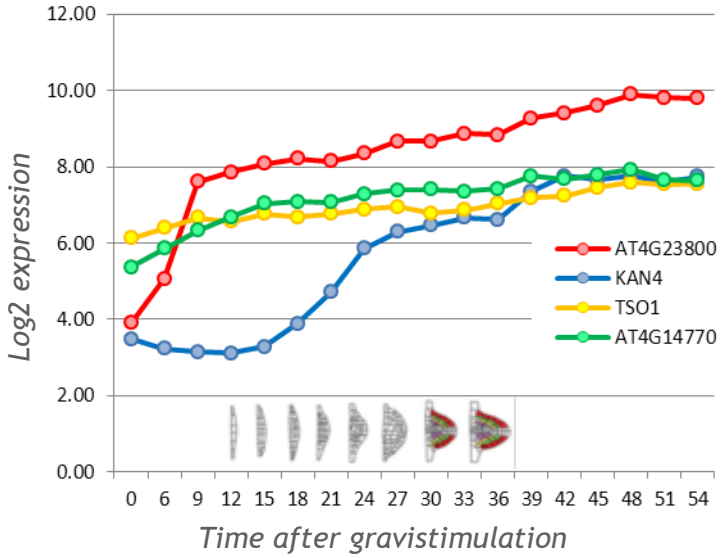
Database of ~8500 differentially expressed genes (inc. 700 TFs)

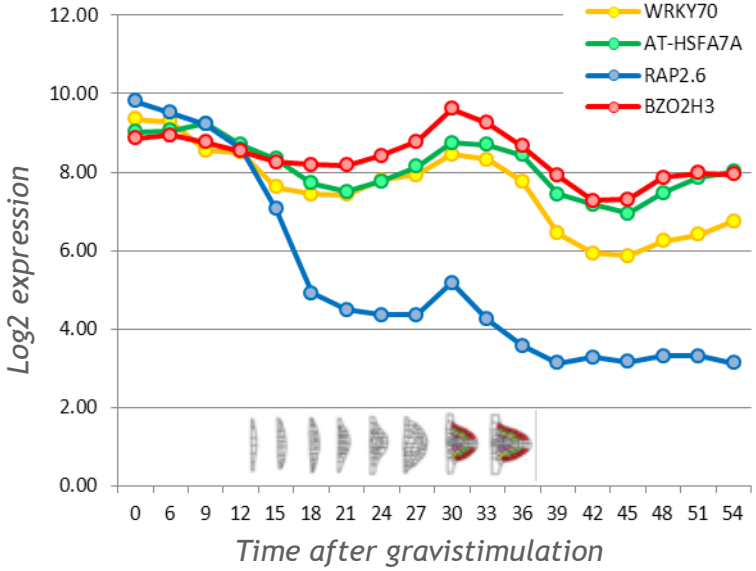
(Voss et al 2015)

Transcriptomics of LRP development

Illustration of some transcription factors expression profiles from the database







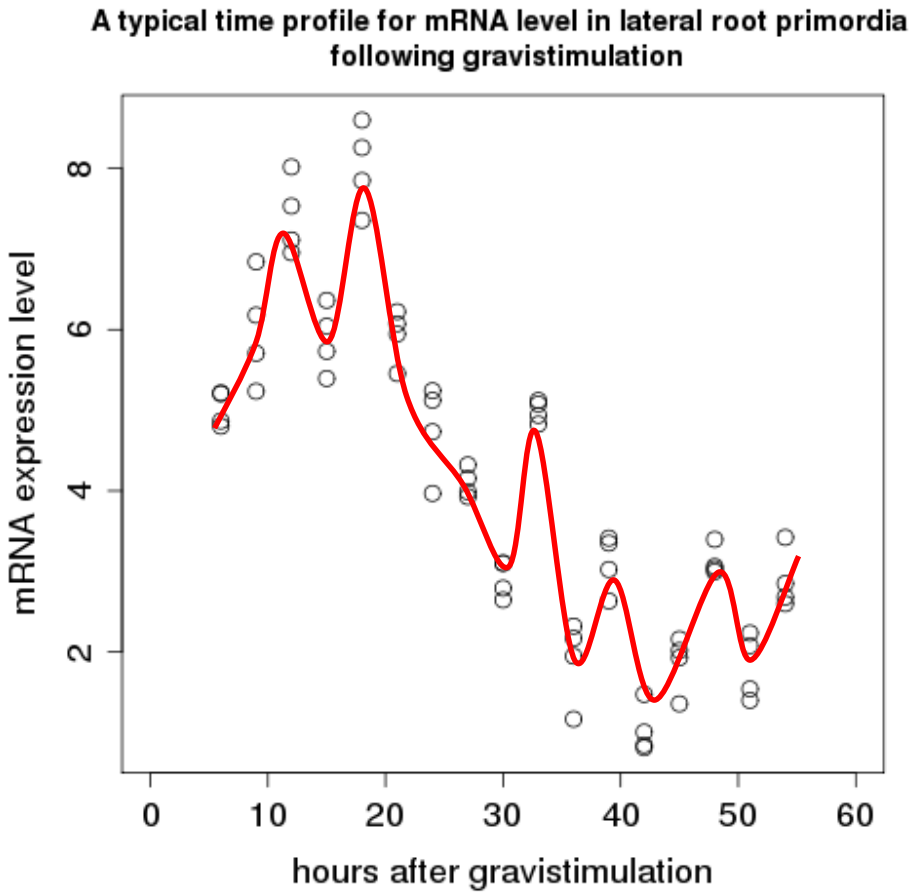
Expression database exploitation

Extracting information from the LR dataset ?

↓

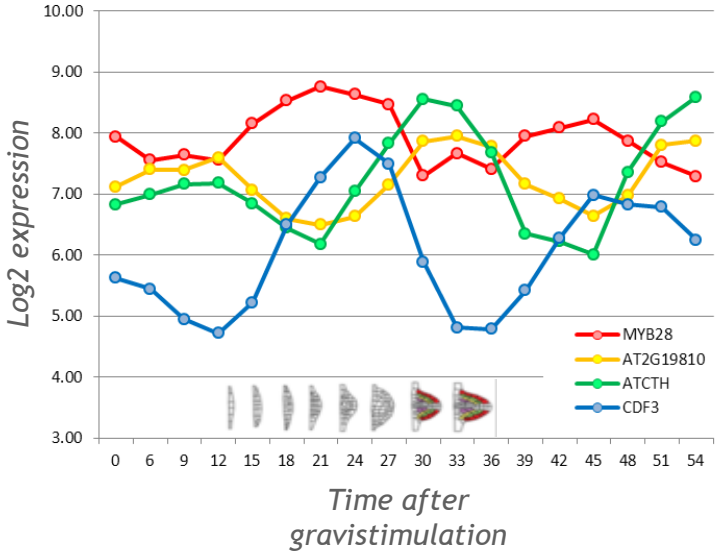
Use statistical network inference methods

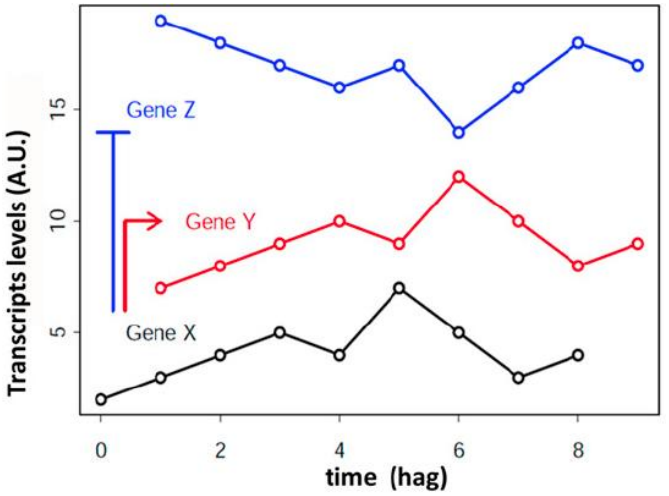
Developed a new algorithm in our lab (J. Lavenus thesis)



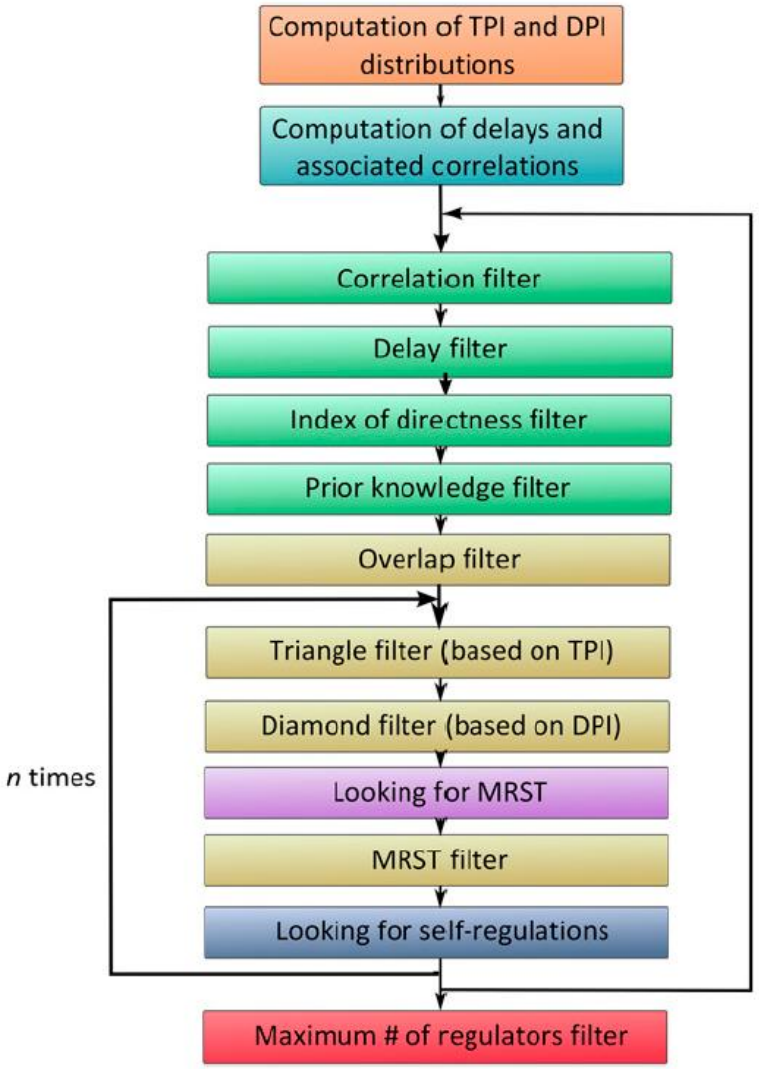
Time Delay Correlation - TDCor

- Implemented in R (CRAN)
- Runs on expression profiles extracted from the LR dataset (or any other transcriptomic kinetics dataset)
- Looks for non-combinatorial linear interactions
- Uses Pearson's correlation with time delay computation to produce a preliminary network
- Uses bootstrap and statistical filters to eliminate false positive and refine the network topology



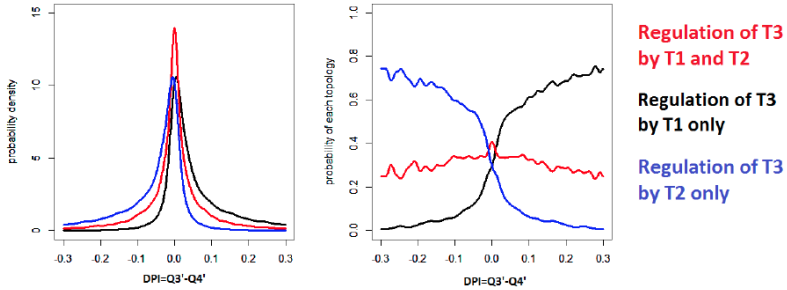


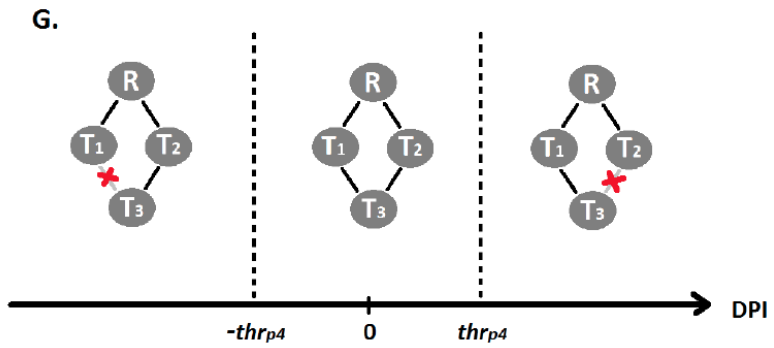
TDCor - data treatment pipeline



TPI= Triangle Pruning Index

DPI= Diamond Pruning Index





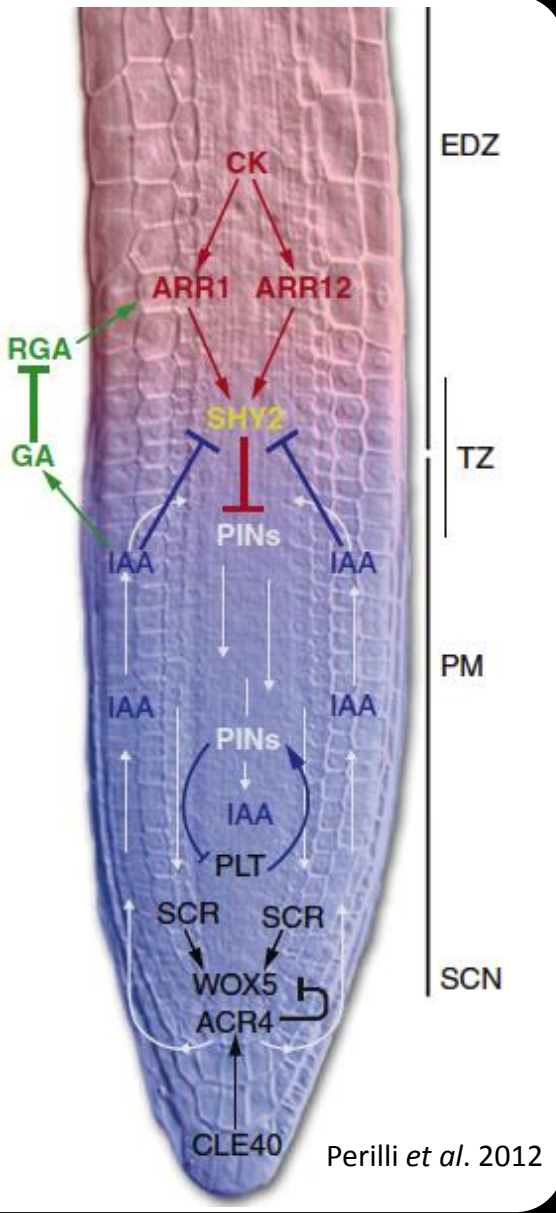
Lavenus et al., Plant Cell 2015

Using TDCor on the LR dataset

Selection of genes involved in

- lateral root formation
- root meristem organization and activity
- hormonal transduction
- cell division
- cell differenciation

Possibility to include any other gene present on the Affymetrix chip (*e.g.* selected because of interesting features of its expression profile …)



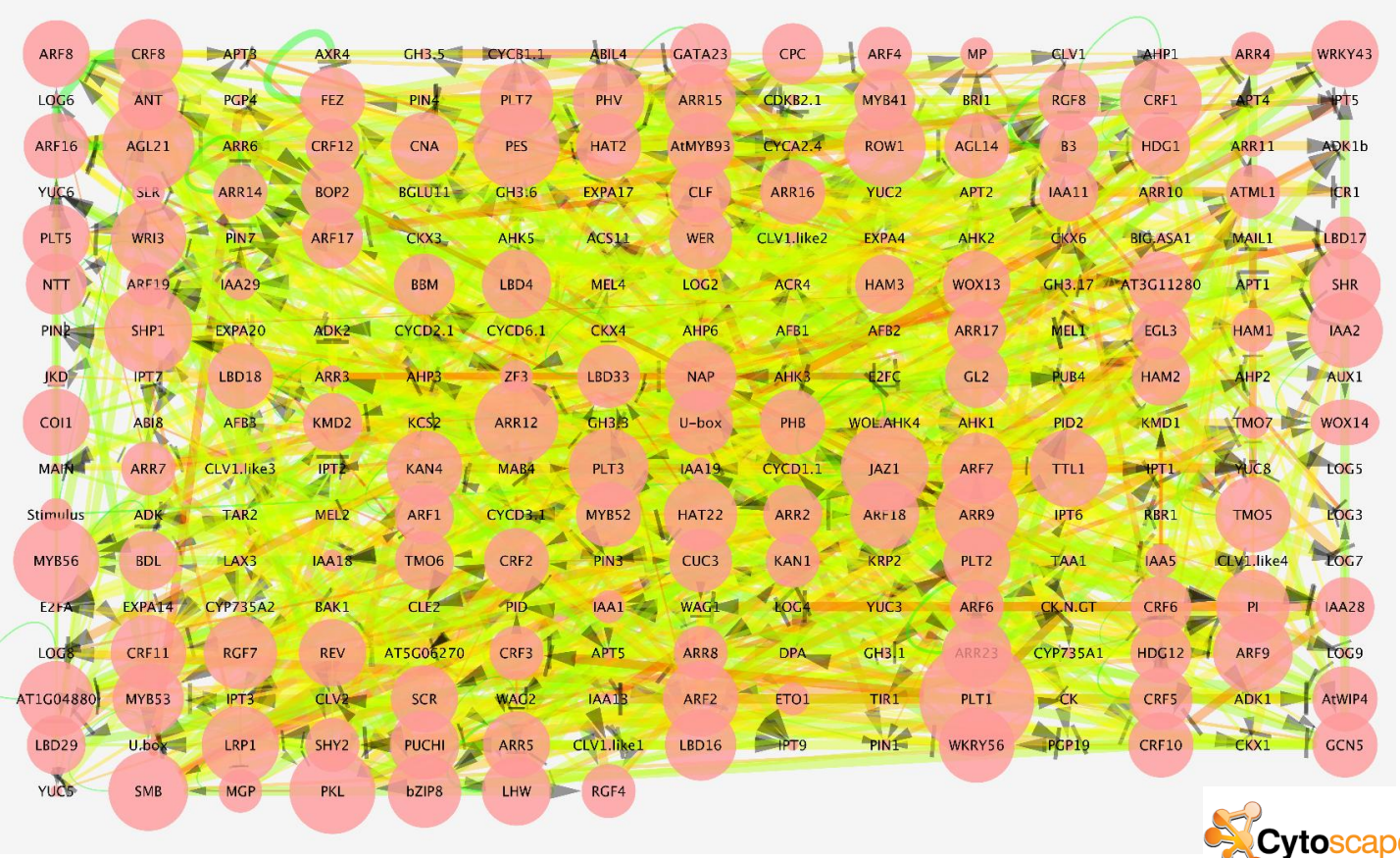
Perilli *et al*. 2012

Using TDCor on the LR dataset

- Selected a list of 261 genes
- Not only transcription factors
- A “prior” data is given to each gene, based on the litterature, to indicate if transcriptional regulation activity has been reported or not
- This “prior” information helps the inference procedure by authorizing or not the algorithm to draw outward edges from the node. However indicating a prior is not compulsory (prior = 2)

Gene	Name	Prior
AT1G02850	BGLU11	0
AT1G03430	AHP5	0
AT1G03840	MGP	2
AT1G04220	KCS2	0
AT1G04240	SHY2	-1
AT1G04550	BDL	-1
AT1G04610	YUC3	0
AT1G04880	AT1G04880	2
AT1G10470	ARR4	-1
AT1G12820	AFB3	0
AT1G15580	IAA5	-1
AT1G15670	KMD2	-1
AT1G16060	WRI3	2
AT1G17950	MYB52	2
AT1G19050	ARR7	-1
AT1G19180	JAZ1	2
AT1G19220	ARF19	2
AT1G19850	MP	1
AT1G20700	WOX14	2
AT1G23080	PIN7	0
AT1G25410	IPT6	0
AT1G25470	CRF12	1
AT1G26680	B3	2
AT1G26870	FEZ	2
AT1G27320	AHK3	0
AT1G27450	APT1	0
AT1G28130	GH3.17	0
AT1G30330	ARF6	1
AT1G30490	PHV	2

Using TDCor on the LR dataset

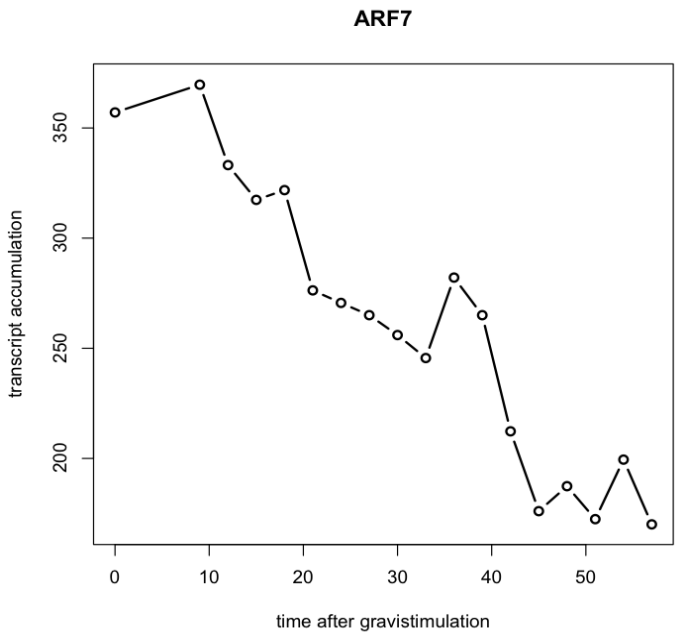


Generated a full network (~3h computation on standard PC)

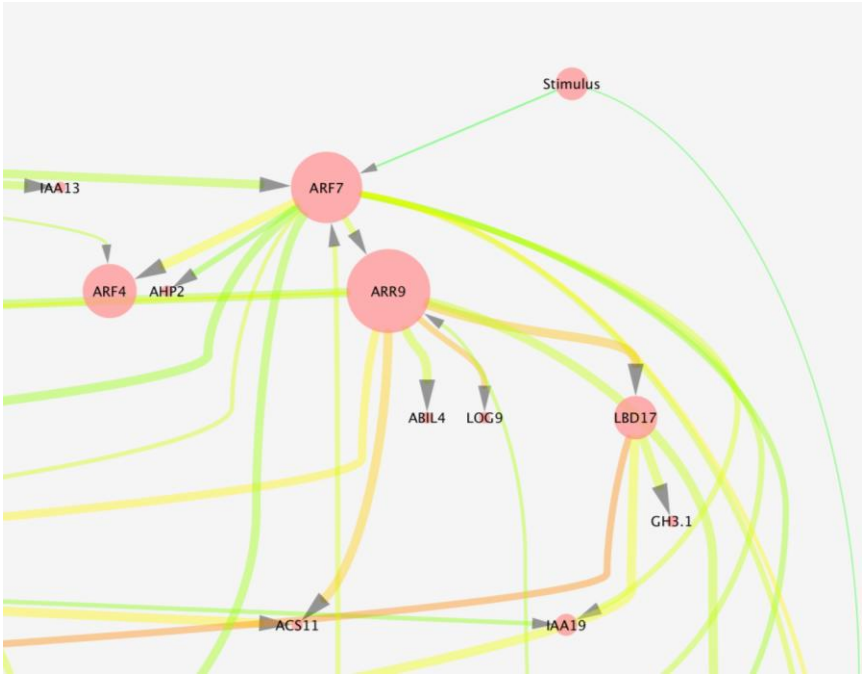
With indices of confidence and directness for each interaction.

But are we confident in the predictions ?

Validating the network – the ARF7 case



Transcription factor ARF7 experimental profile in the LR data set



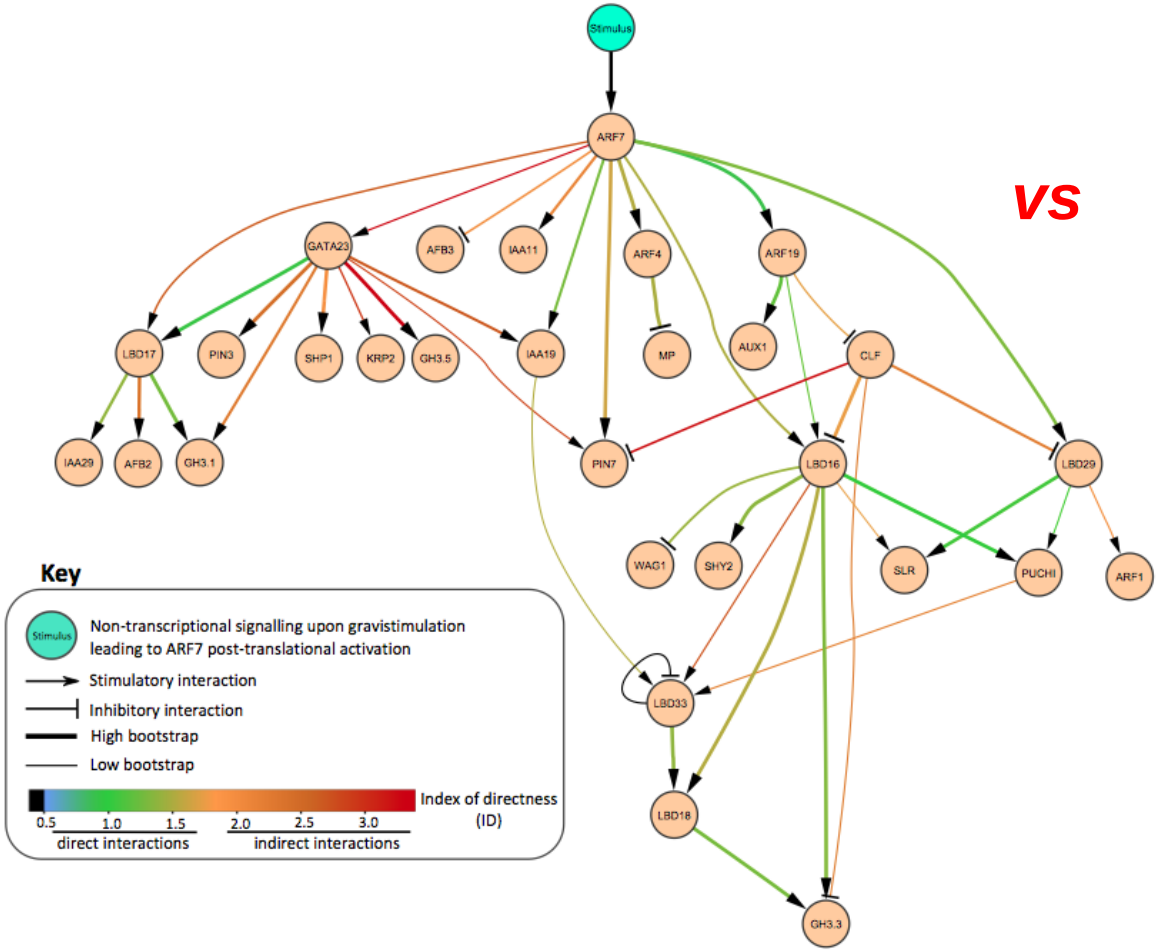
Inference by TDcor algorithm

ARF7 is predicted to occupy a upstream position in the network

ARF7 is predicted to positively regulate a LOB/PUCHI genetic module

Lavenus *et al.*, 2015

Validating the network - the ARF7 case



vs

arf7 arf19
pARF7:ARF7::GR

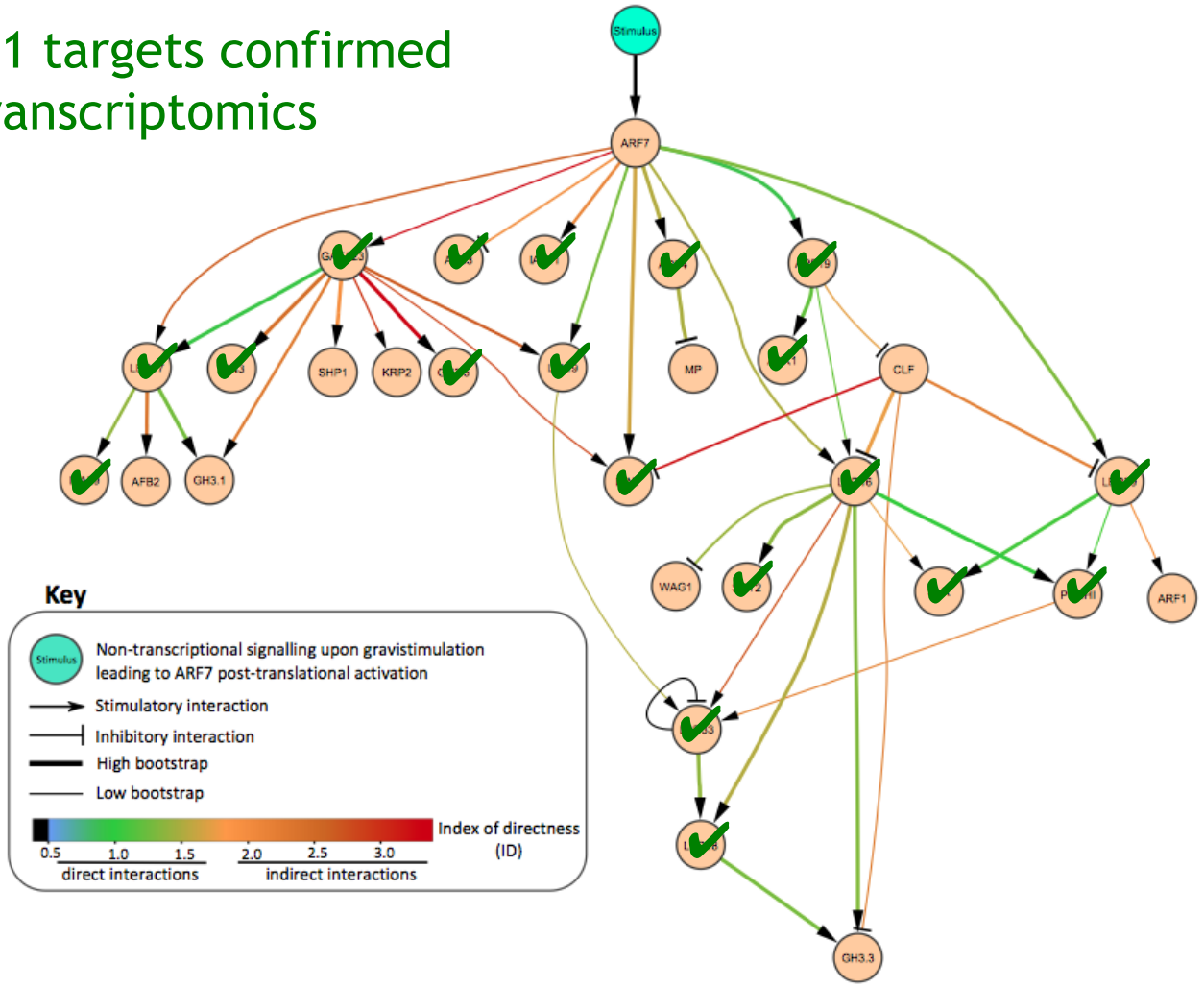
Treated for 4h with :

NAA
DEX
CHX
NAA+DEX
NAA+CHX
DEX+CHX
NAA+DEX+CHX

Lavenus *et al*, Plant Cell 2015

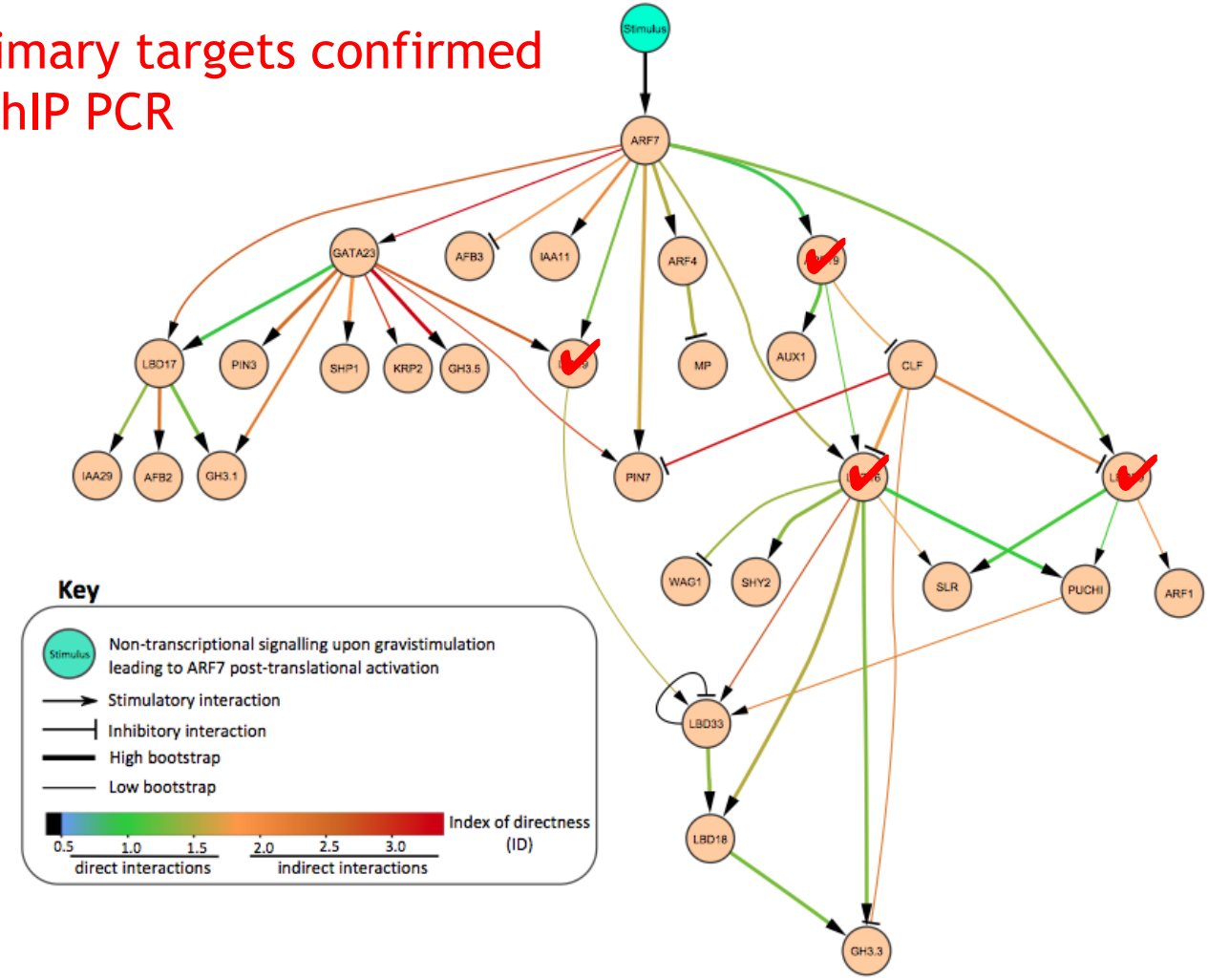
Validating the network - the ARF7 case

22/31 targets confirmed
by transcriptomics

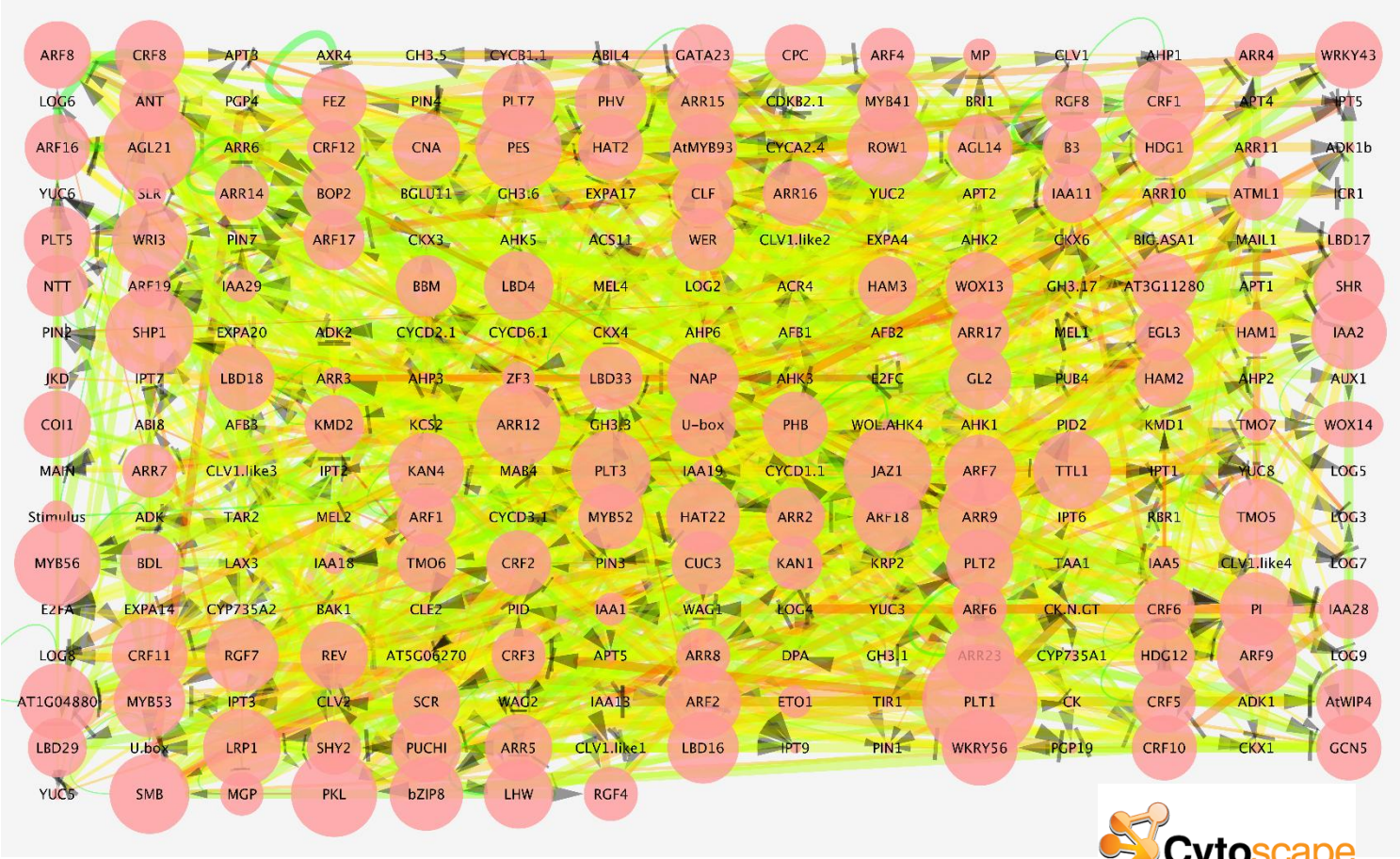


Validating the network - the ARF7 case

4 primary targets confirmed by ChIP PCR

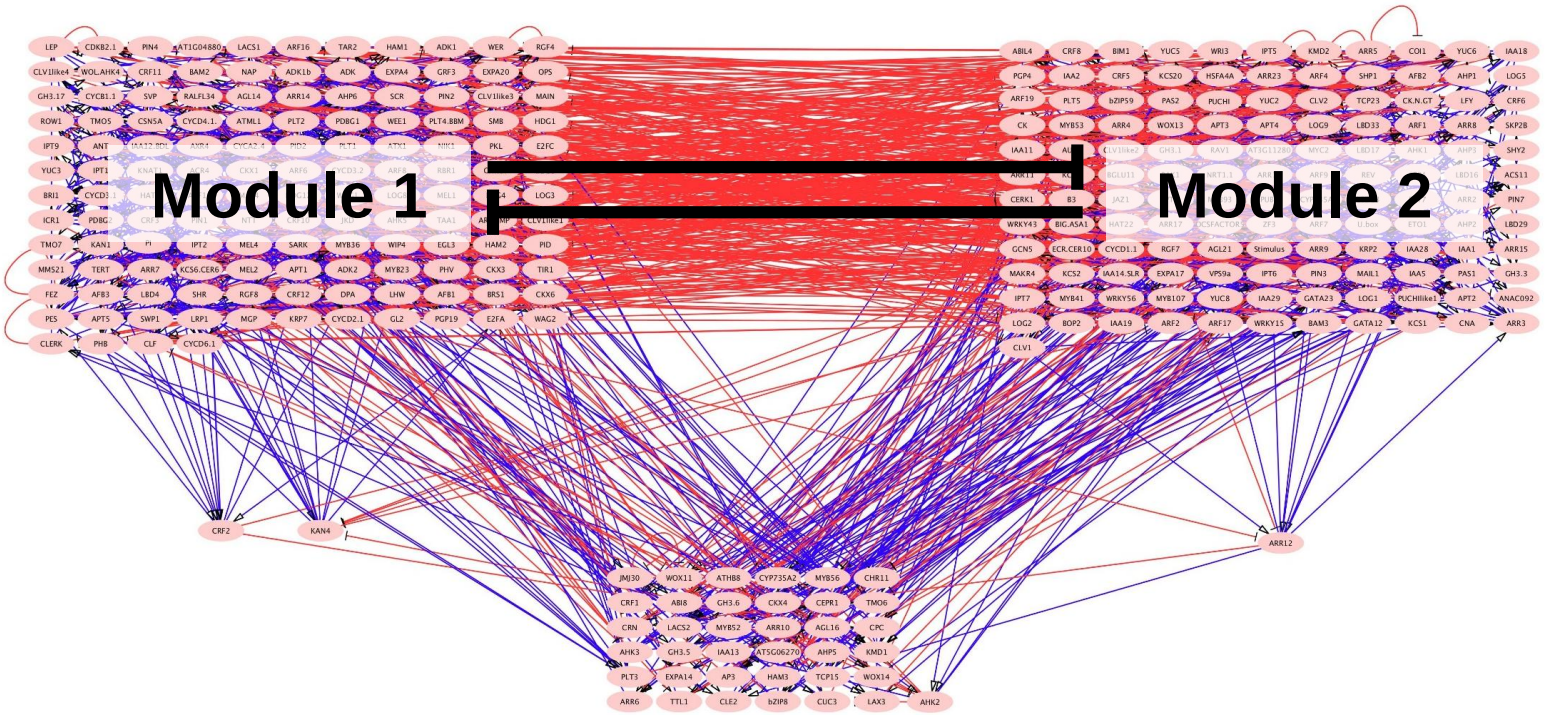


Moving forward with the network



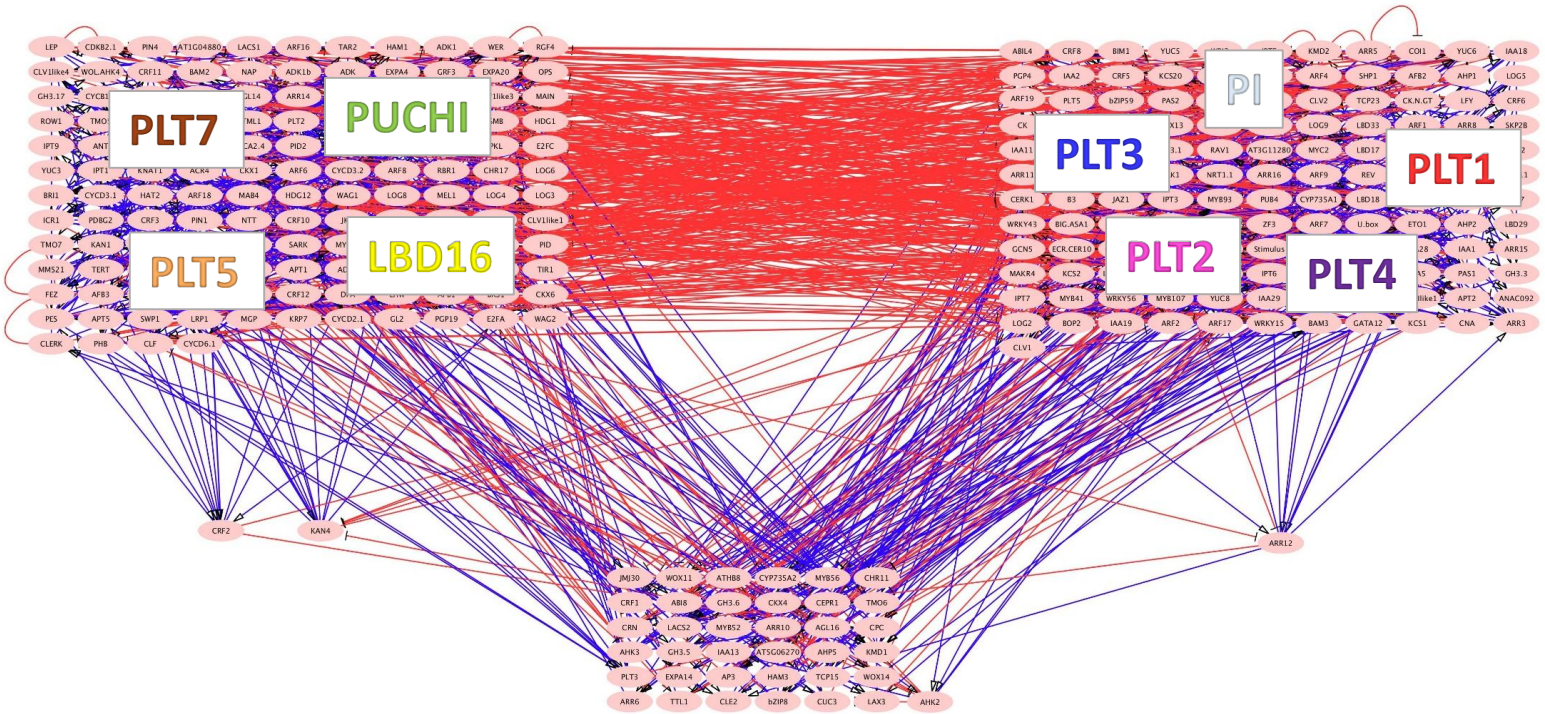
Having validated our inference approach, we went forward with the network exploration

Topology of the LR GRN



Expert (i.e. by hand) analysis of the network structure revealed a modular organisation.

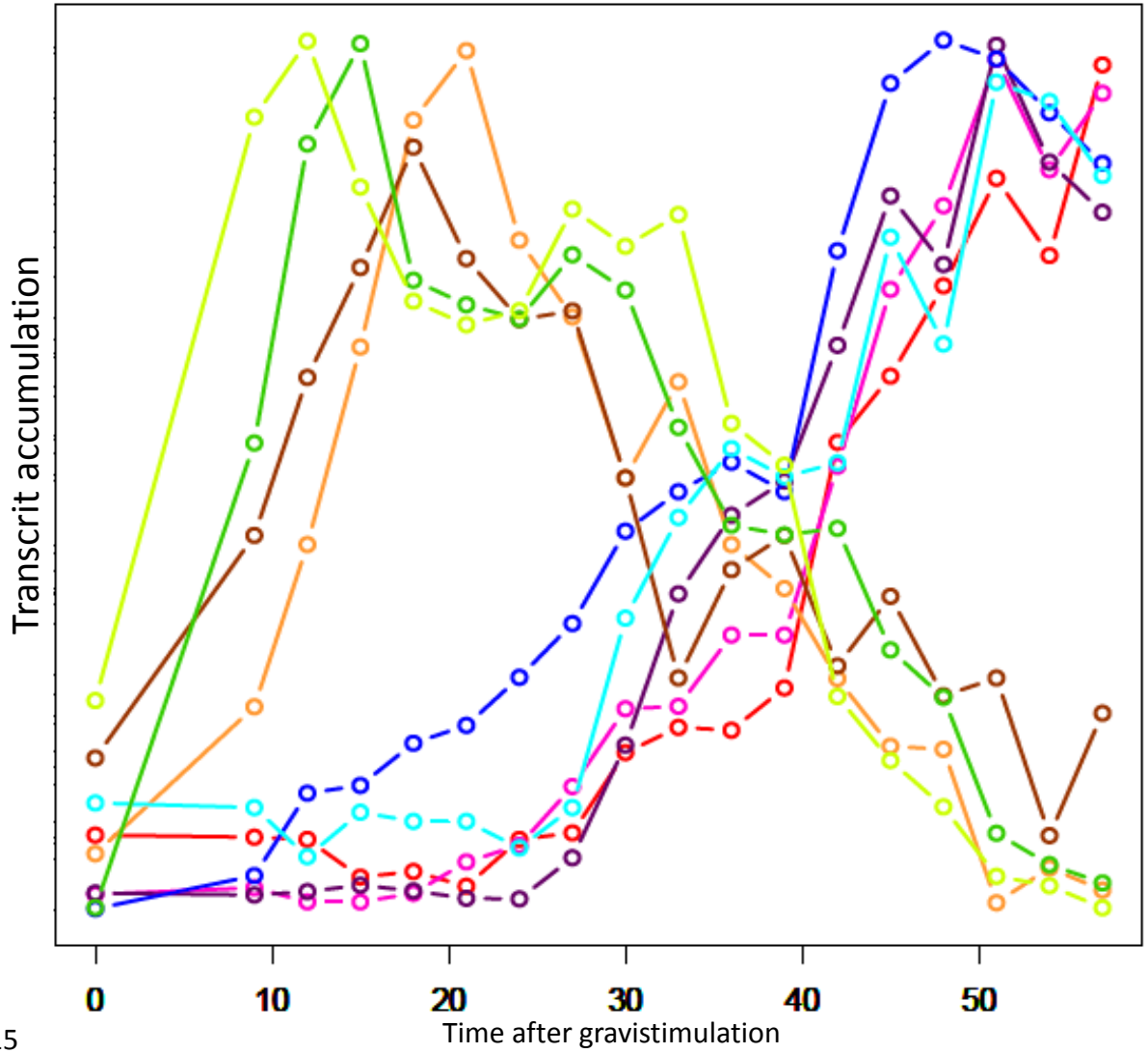
Topology of the LR GRN - biological meaning ?



Having a look at some of the genes in those two modules...

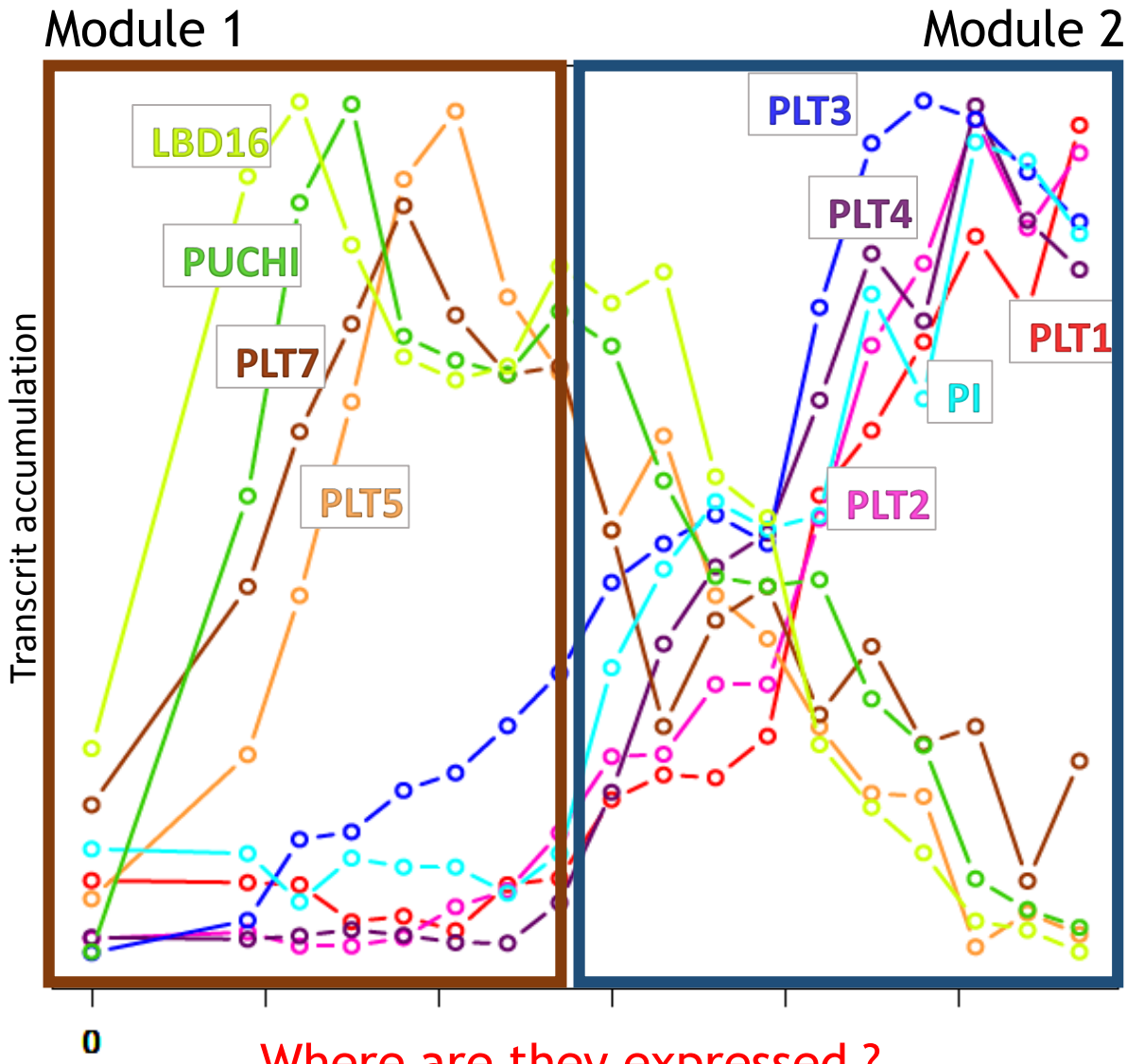
Topology of the LR GRN – biological meaning ?

What are their expression profile like ?



Voß *et al.,* 2015

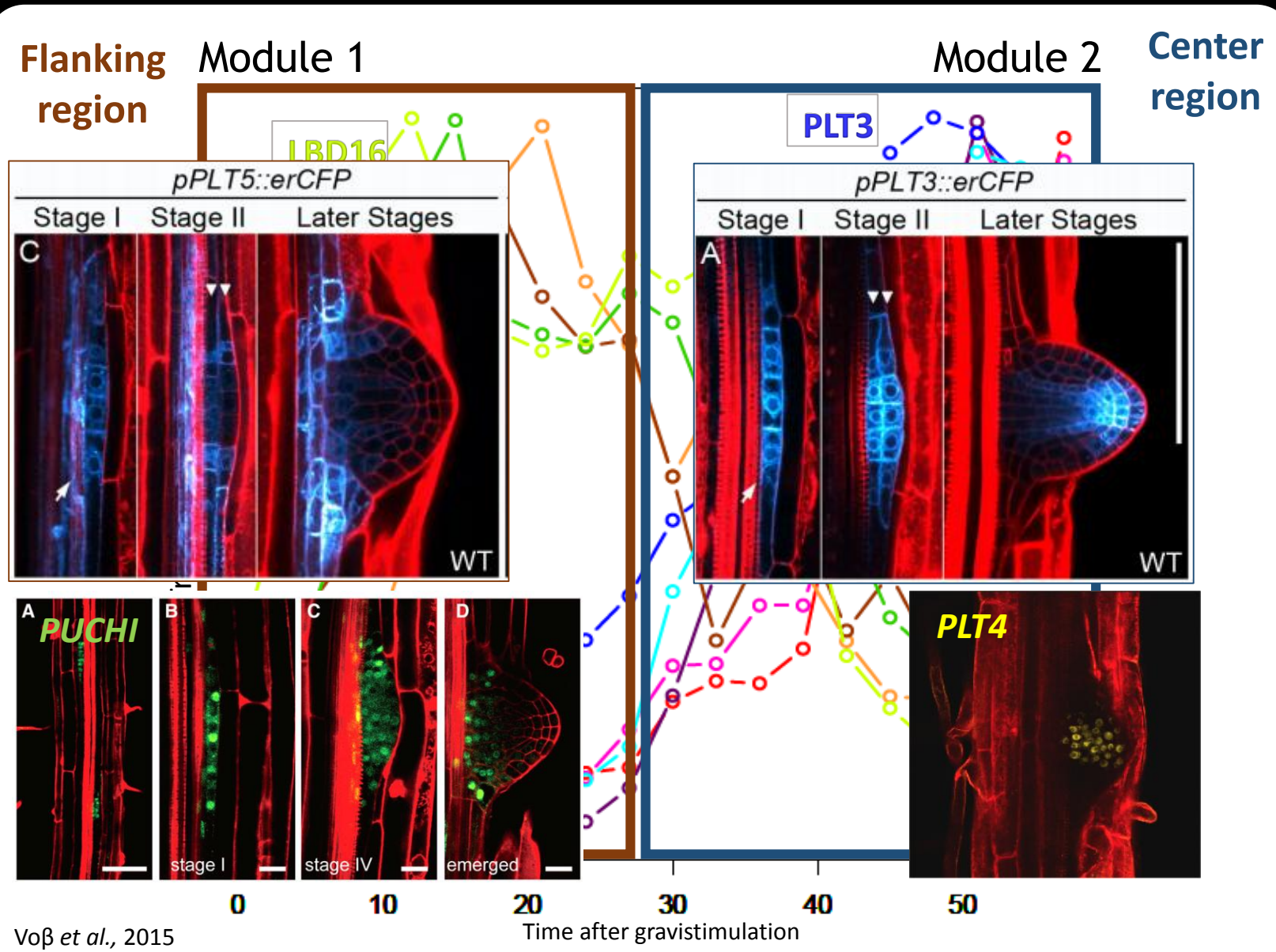
Topology of the LR GRN - biological meaning ?



Where are they expressed ?

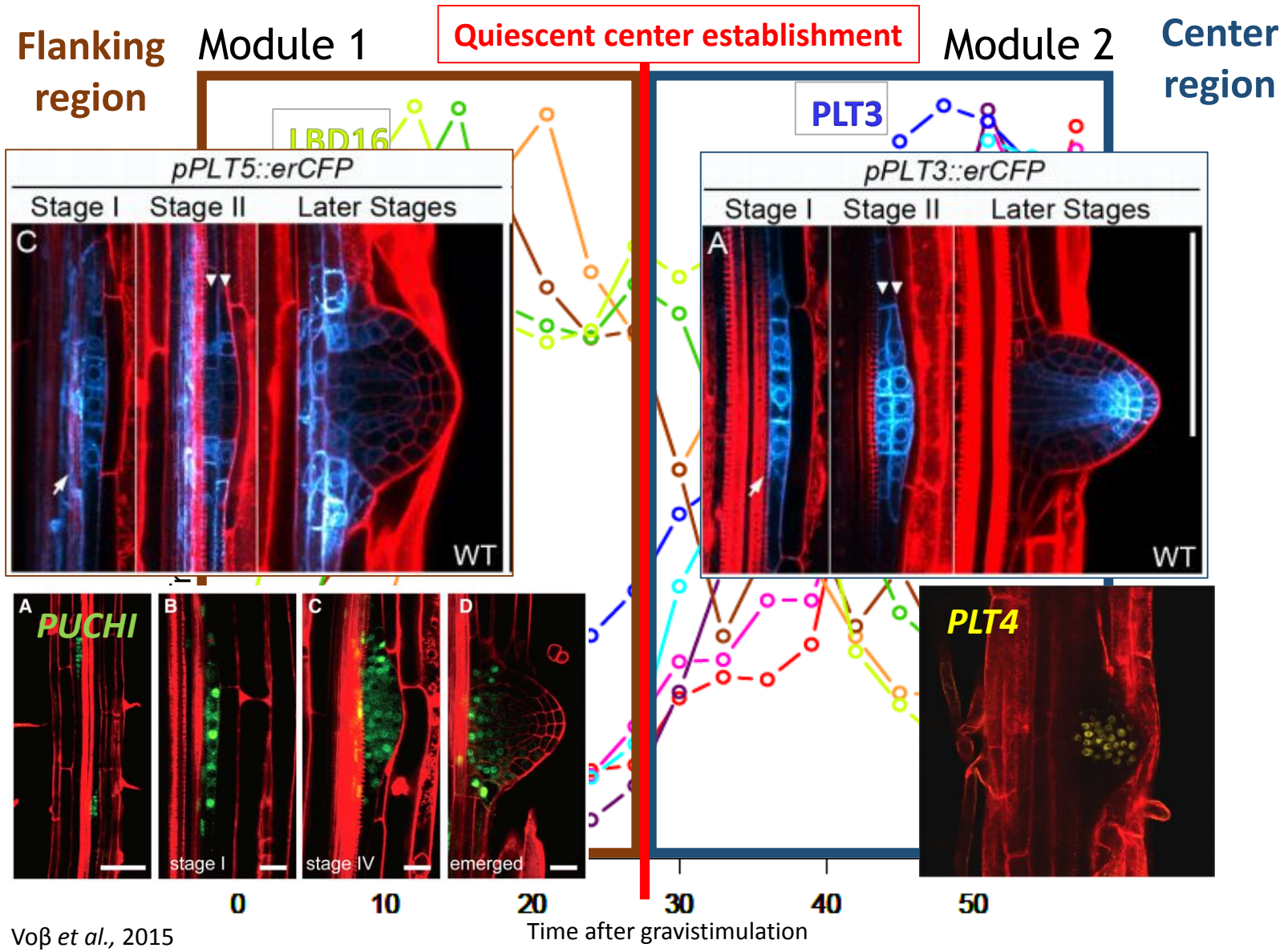
Voß *et al.*, 2015

Topology of the LR GRN – biological meaning ?



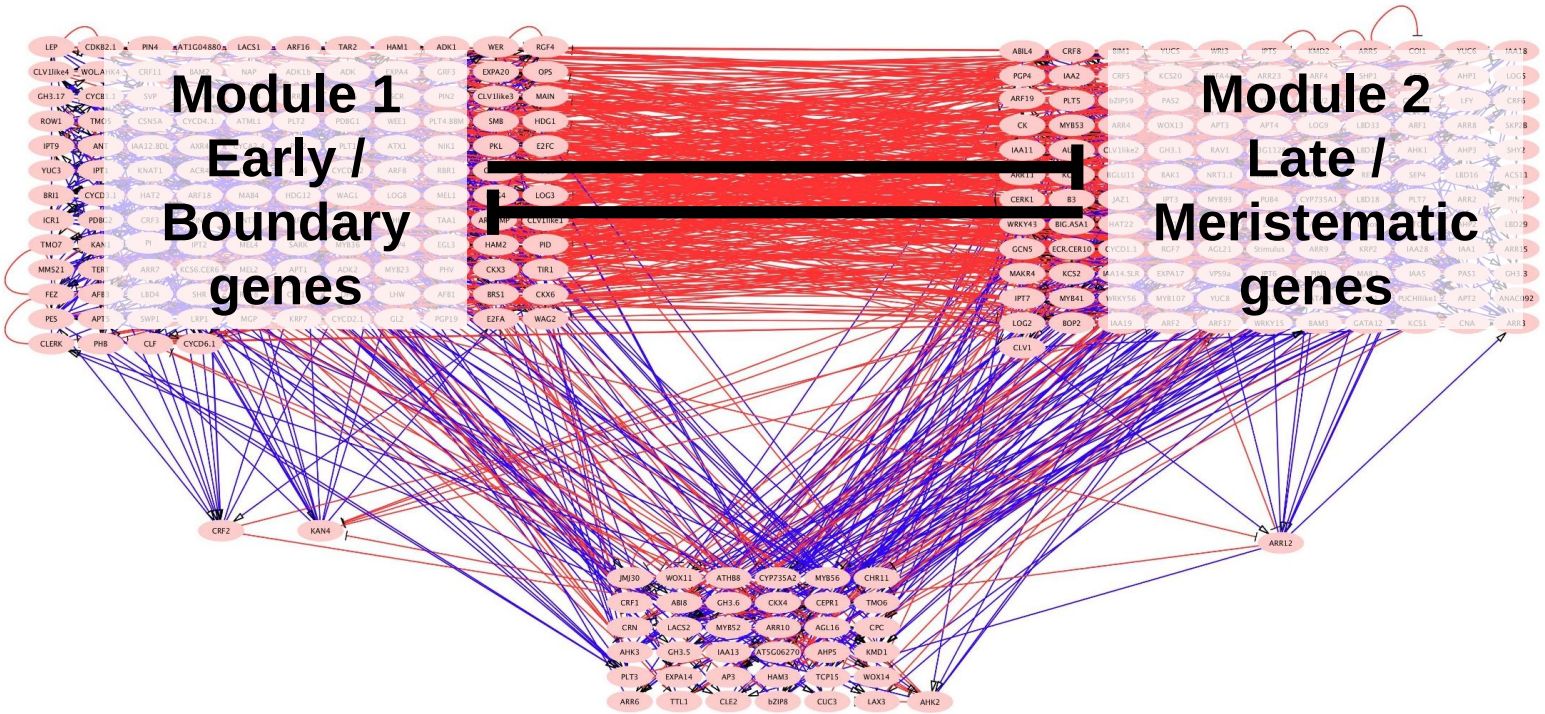
Voß *et al.*, 2015

Topology of the LR GRN - biological meaning ?



Voß *et al.*, 2015

Topology of the LR GRN - biological meaning ?

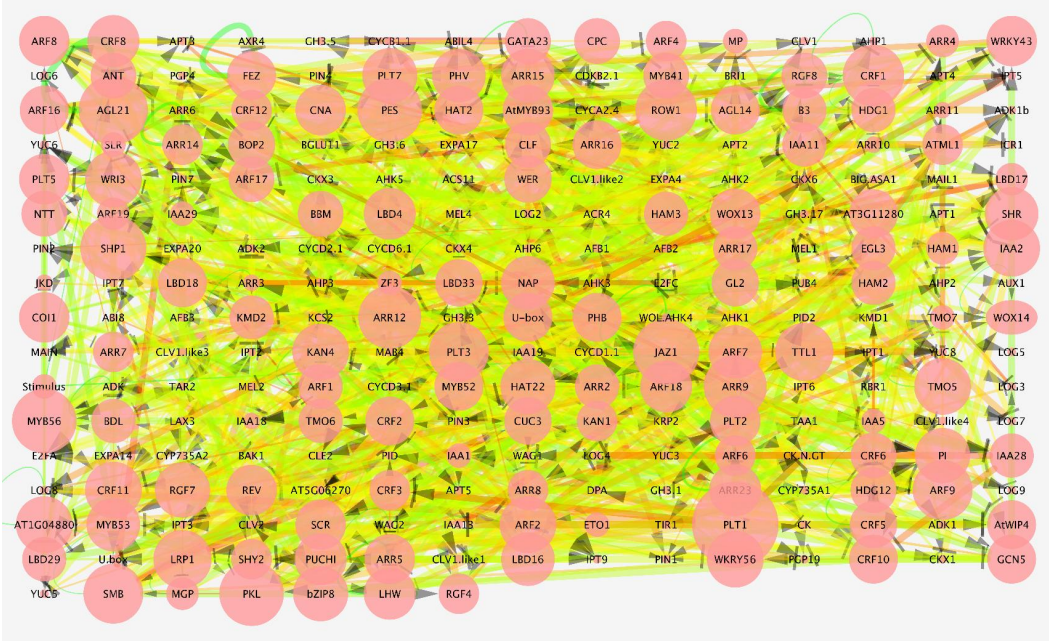


There appears to be biological meaning
behing this modular topology

Can we investigate the dynamics of this patterning event
(establishment of QC / definition of boundary) ?

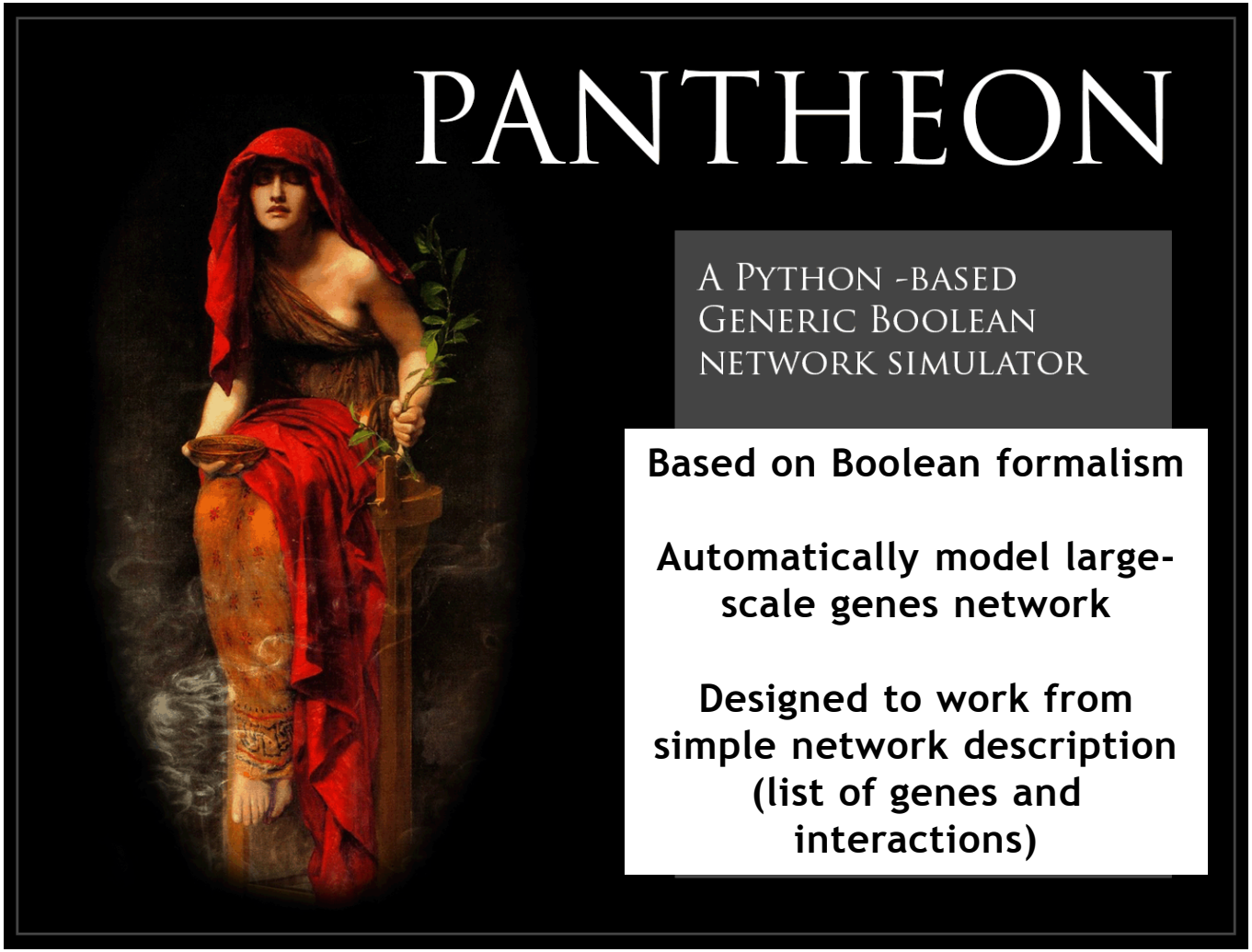
Modeling GRN dynamics - PANTHEON

We wanted to investigate the precise dynamics of our GRN.

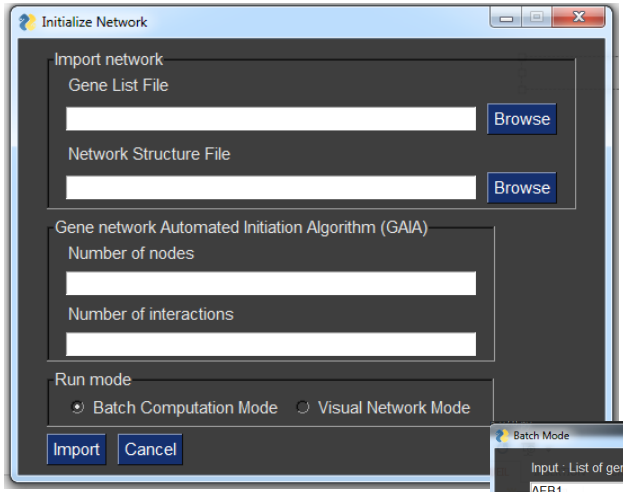


With several hundred of genes and interactions to consider and no already available solution to simulate such a system easily, we opted to develop our own software.

Modeling GRN dynamics - PANTHEON



Modeling GRN dynamics - PANTHEON

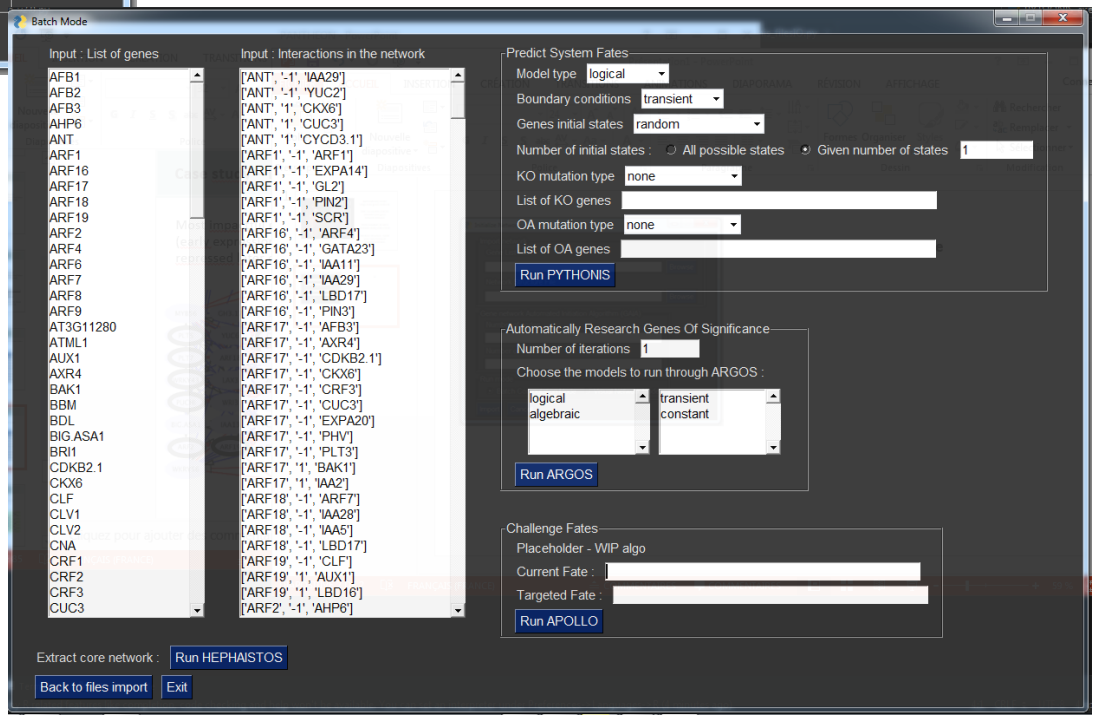


GUI : no need to code to simulate your gene network behavior

Import your network or generate a random one / Export simulation results as csv files

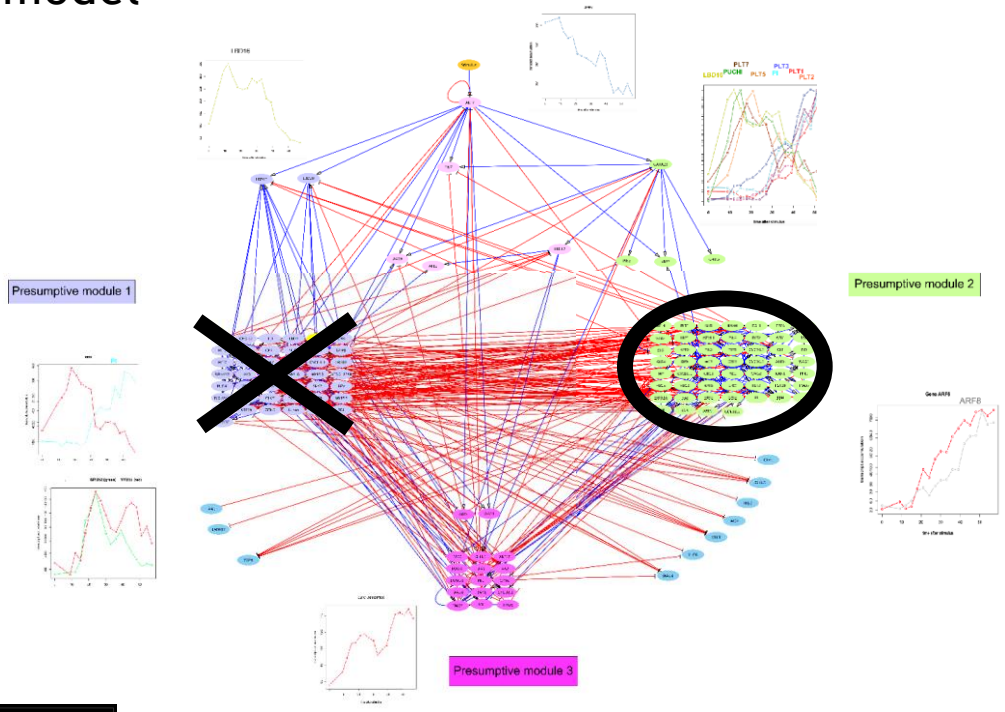
Tools included :
in silico mutants study with a click among other things

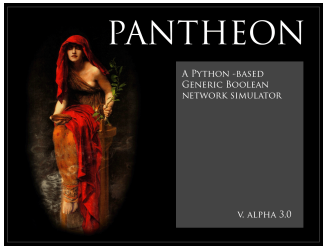
Modular structure :
base library of regulation models can be extended at will with your own



PANTHEON – the LR dataset case study

Working on a subset of 134 genes / 495 interactions, full simulation of the network behavior using pure logical or algebraic model

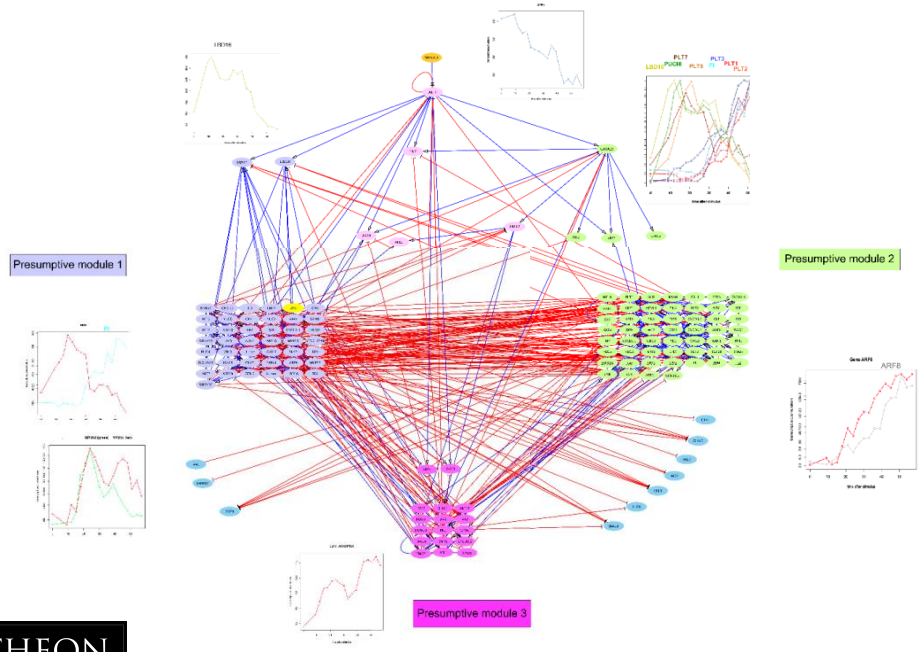


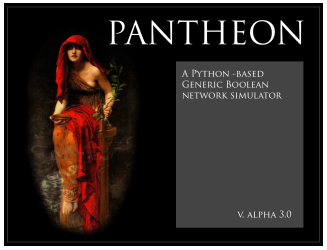


Prediction of majority stable state corresponding to meristematic state (genes from module 2 active, genes from module 1 inactive)

PANTHEON – the LR dataset case study

ARGOS Module – Mass *in-silico* mutagenesis and computation of a score of impact on network behavior for each gene (mean hammond distance between wild-type and mutants stable states for all model and mutation combinations)





Highlight the most important genes for the network behavior with no a priori

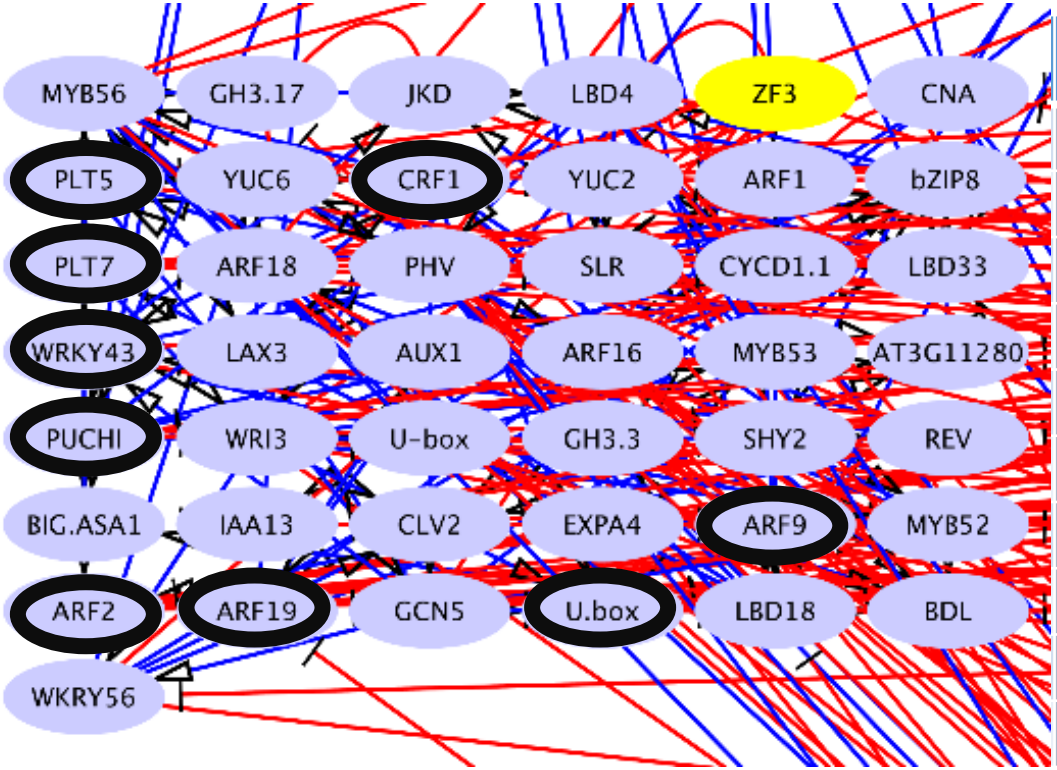
PANTHEON – the LR dataset case study

ARGOS Module – Mass *in-silico* mutagenesis and computation of a score of impact on network behavior for each gene

Genes	mean distance for KO		Genes	mean distance for OA
PLT1	0.168600746		**PLT7**	0.346343284
ARF6	0.136735075		**PUCHI**	0.326567164
LRP1	0.13113806		**CRF1**	0.272742537
PHB	0.124869403		**ARF2**	0.248246269
TMO5	0.103973881		**PLT5**	0.245466418
SHR	0.098656716		**ARF9**	0.226529851
SCR	0.087817164		**ARF17**	0.179328358
SHP1	0.081100746		**U.box**	0.153955224
ATML1	0.063302239		**ARF19**	0.148022388
PID2	0.060970149		**WRKY43**	0.13369403

TOP10 predicted as most significant genes when KO or OA

PANTHEON - the LR dataset case study

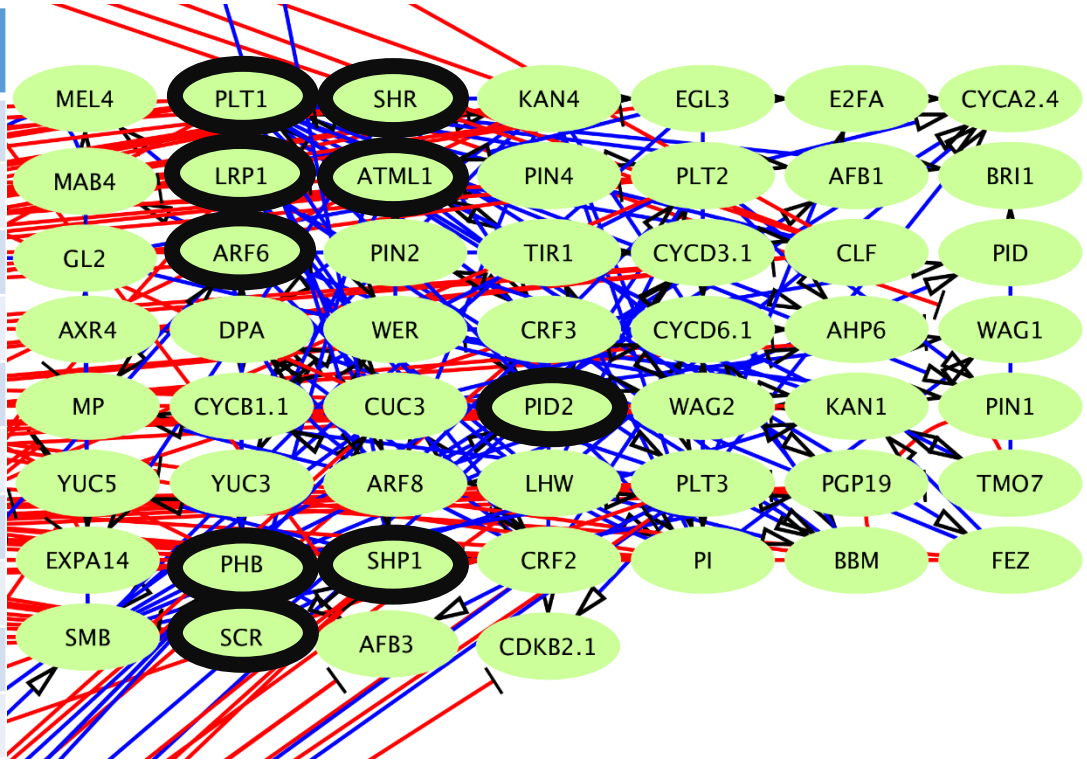


Genes		mean distance for OA
PLT7	✓	0.346343284
PUCHI	✓	0.326567164
CRF1	✓	0.272742537
ARF2	✓	0.248246269
PLT5	✓	0.245466418
ARF9	✓	0.226529851
ARF17		0.179328358
U.box	✓	0.153955224
ARF19	✓	0.148022388
WRKY43	✓	0.13369403

Most impactful genes when OA are in module 1
(early genes which we need to be repressed later on)

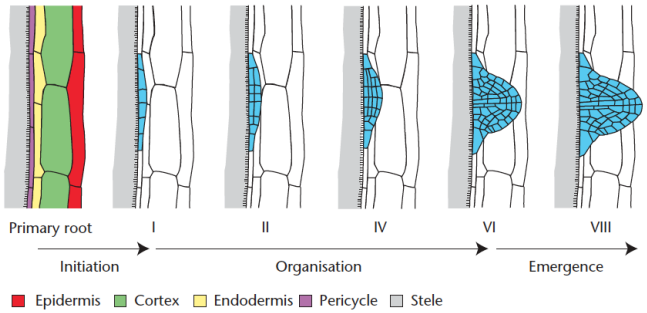
PANTHEON – the LR dataset case study

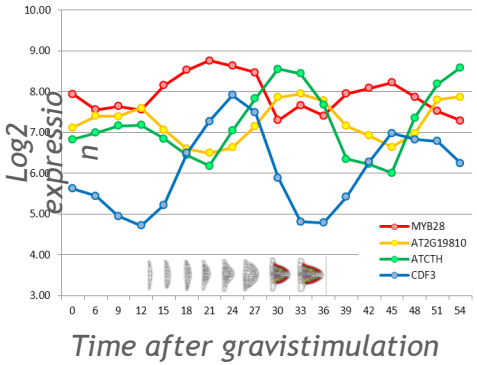
Genes	mean distance for KO
PLT1 ✓	0.168600746
ARF6 ✓	0.136735075
LRP1 ✓	0.13113806
PHB ✓	0.124869403
TMO5	0.103973881
SHR ✓	0.098656716
SCR ✓	0.087817164
SHP1 ✓	0.081100746
ATML1 ✓	0.063302239
PID2 ✓	0.060970149

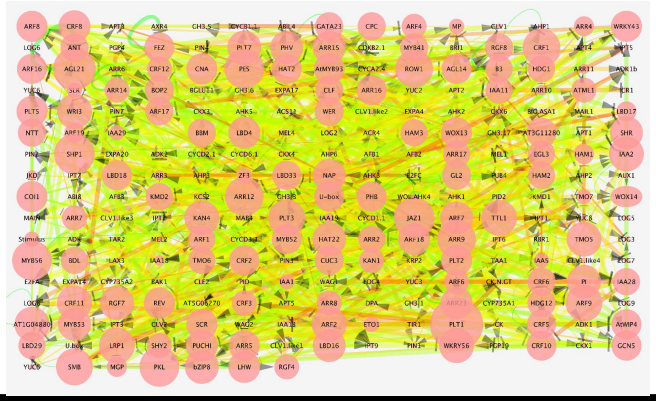


Most impactful genes when KO are in module 2
(late genes which we need to be expressed for LRP development)

LRP morphogenesis - In summary





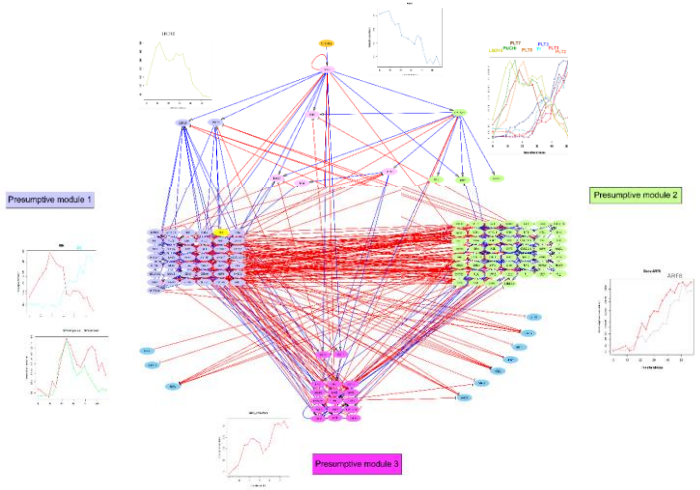


Arabidopsis LR as an excellent model system of organogenesis : simple, controllable, accessible

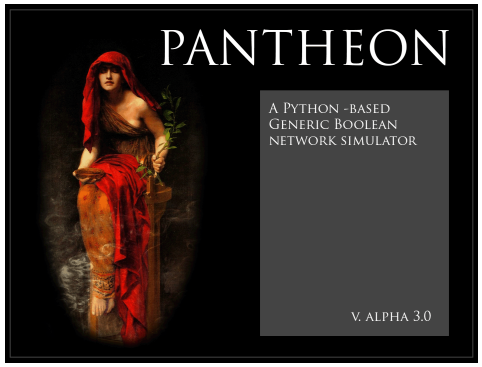
Creation of the LRP database covering the full development of the organ

Creation of the TDCor algorithm and inference of the LRP development GRN

LRP morphogenesis – In summary



Topological analysis revealed a modular structure tied to biological function and a possible bifurcation switch between flank/organizing center identities



Creation of an automated Boolean modeling software which predicted that the topology a the GRN was enough to generate a meristematic identity and was able to retrieve modular organization with no *a priori*

Once now, back to biology to confirm the prediction of the model (i.e. working on generating and characterizing mutants…)

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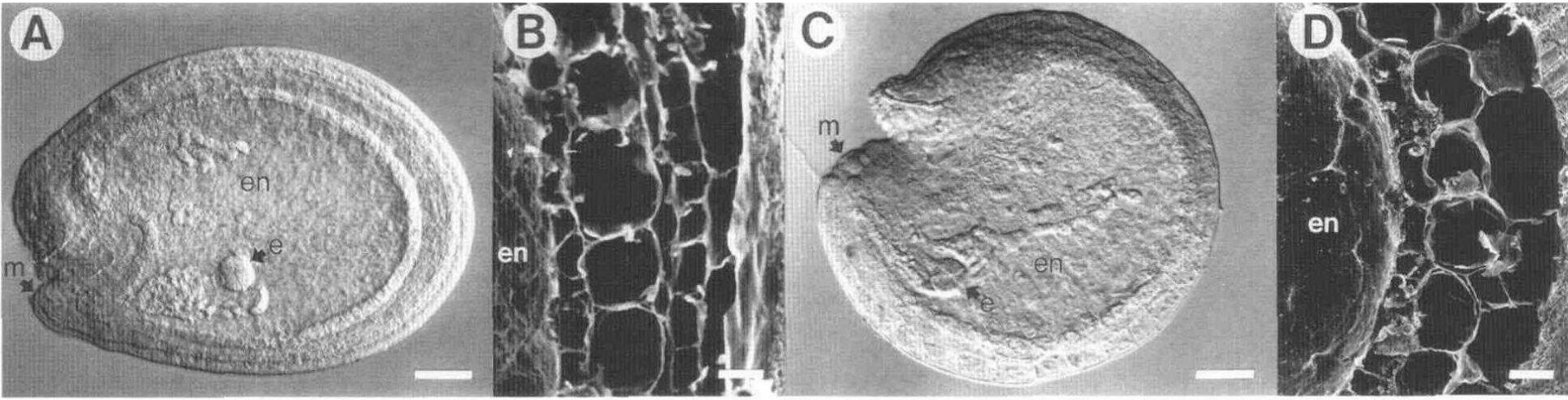
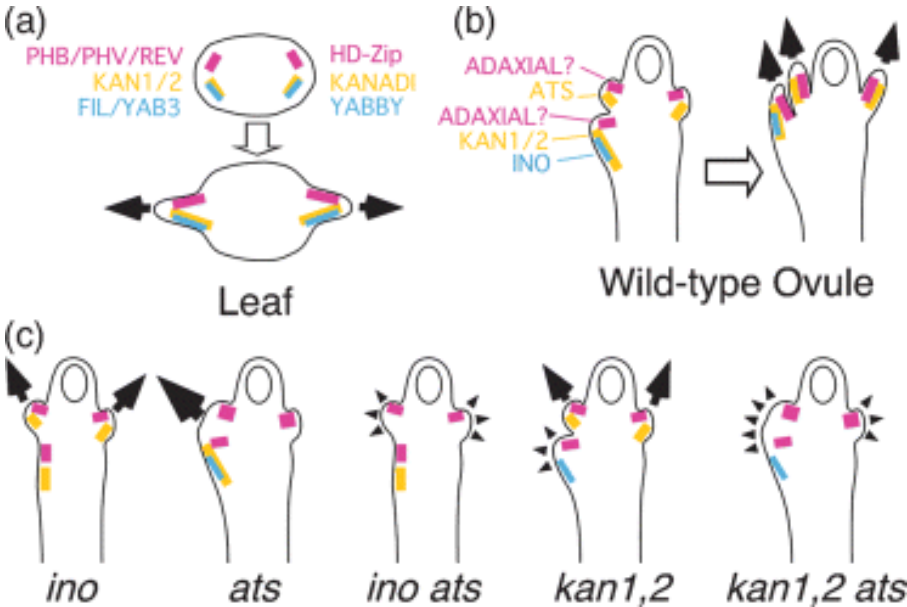
KANADI transcription factor



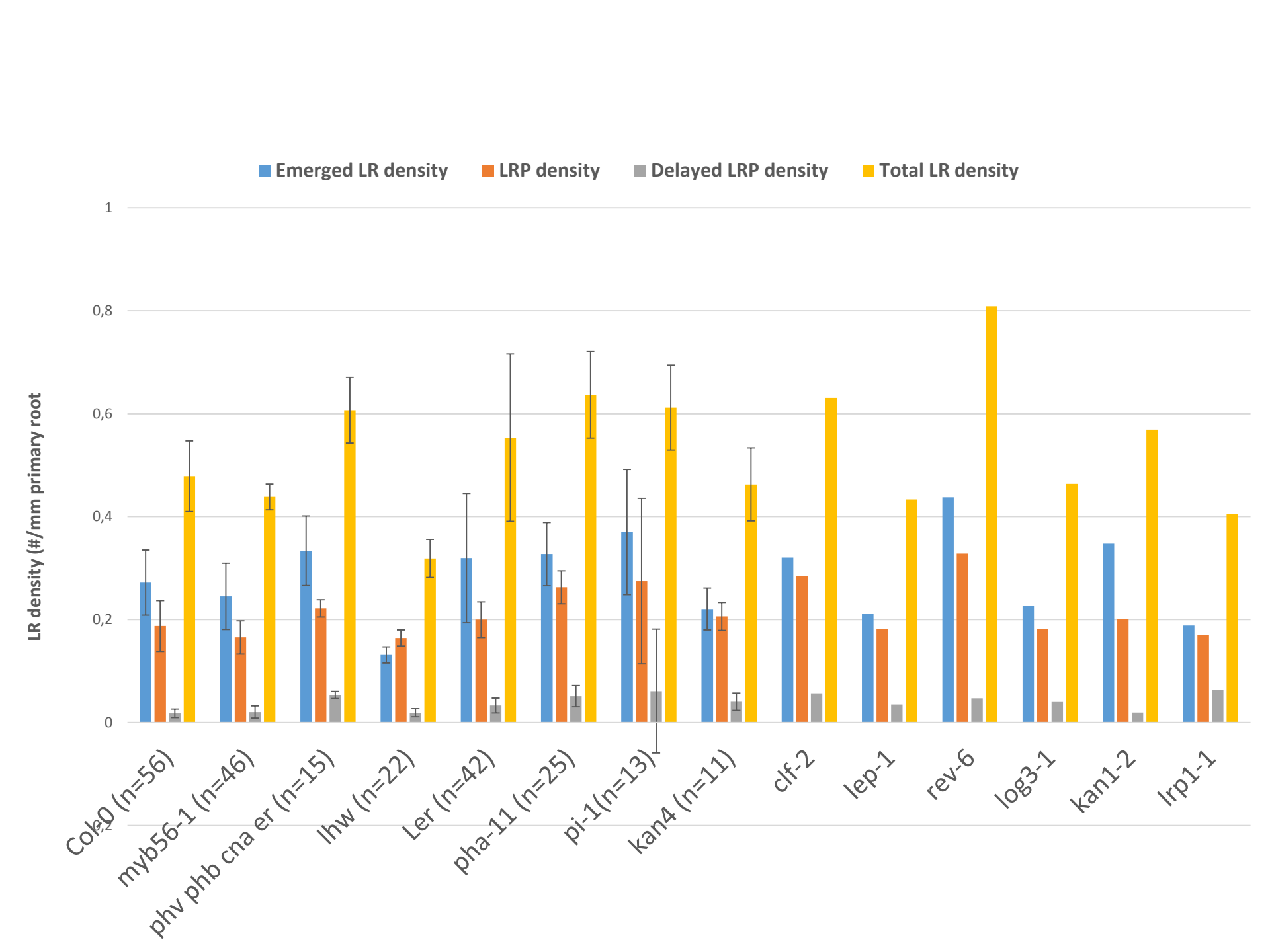
WT *ats/kan4-1*

Roles of polarity determinants in ovule development

- ATS provides boundary maintenance and promotes the laminar growth of the inner ovule integument

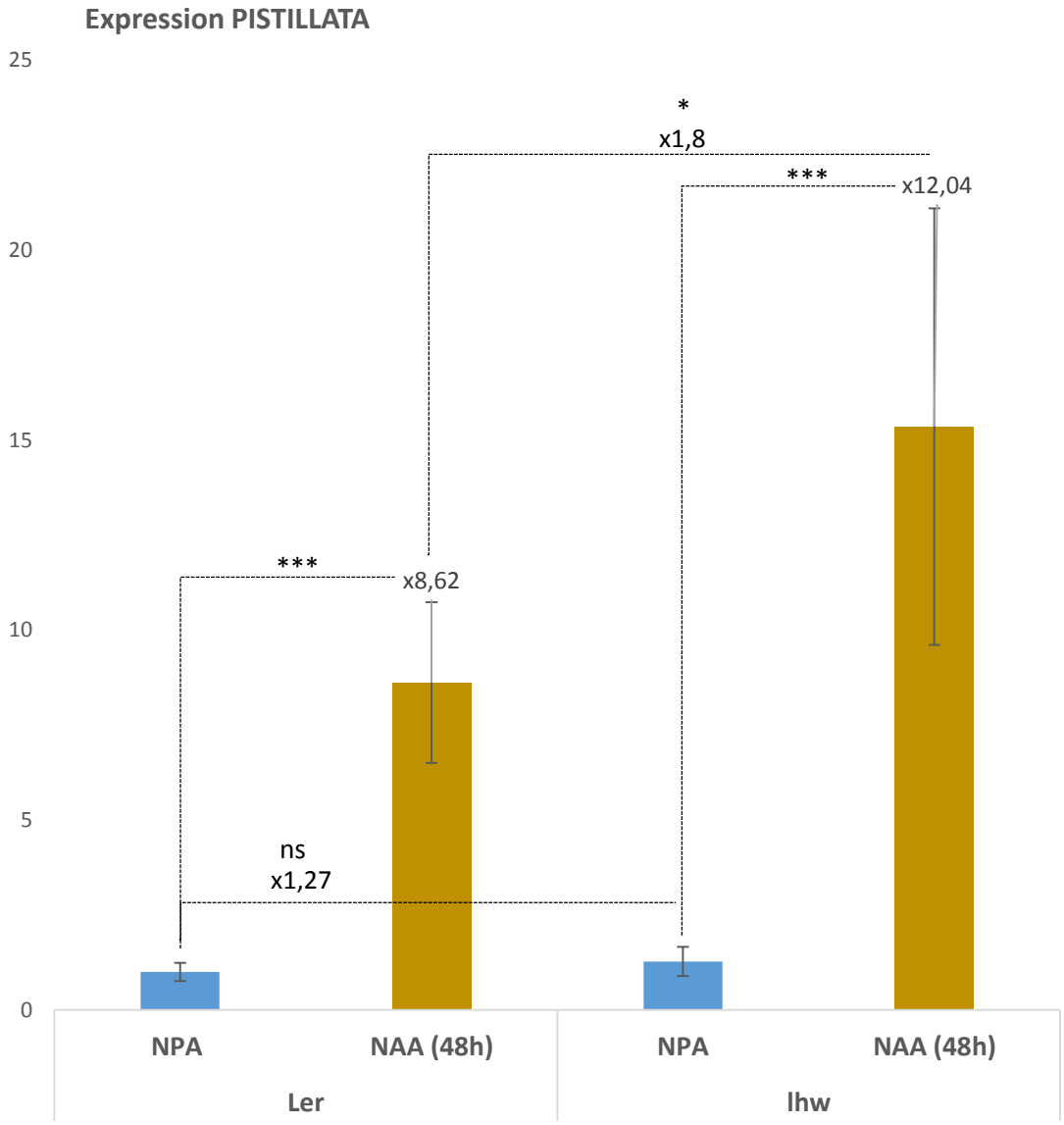


Leon-Kloosterziel, *et al.,* 1994; Mc Abee et al., 2006; Kelley et al., 2009


Emerged LR density
LRP density
Delayed LRP density
Total LR density
LR density (#/mm primary root
1
0,8
0,6
0,4
0,2
0
Col-0 (n=56)
myb56-1 (n=46)
phv phb cna er (n=15)
lhw (n=22)
Ler (n=42)
pha-11 (n=25)
pi-1(n=13)
kan4 (n=11)
clf-2
lep-1
rev-6
log3-1
kan1-2
lrp1-1

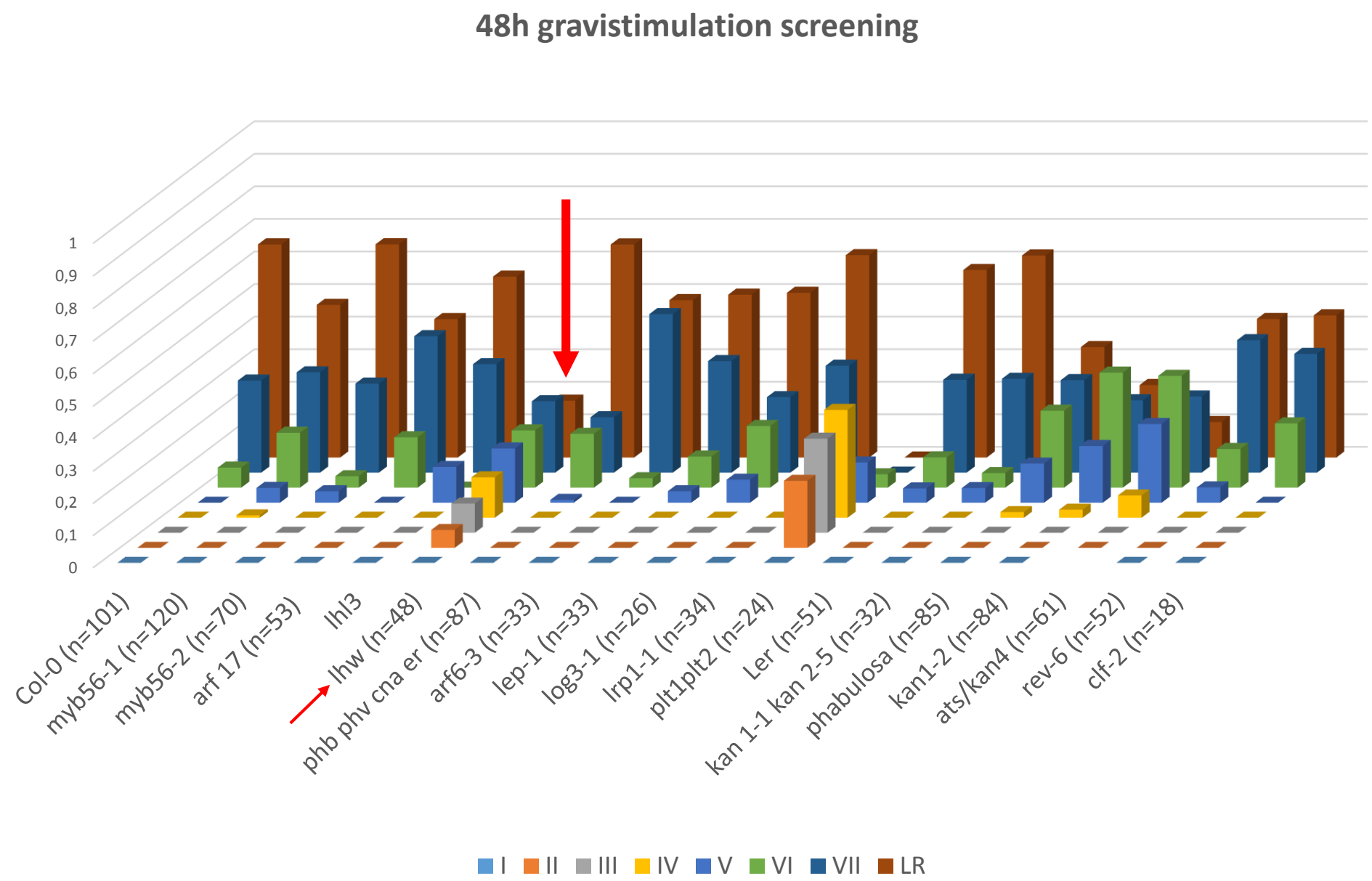
PISTILLATA expression is enhanced in *lhw* mutant

LRIS

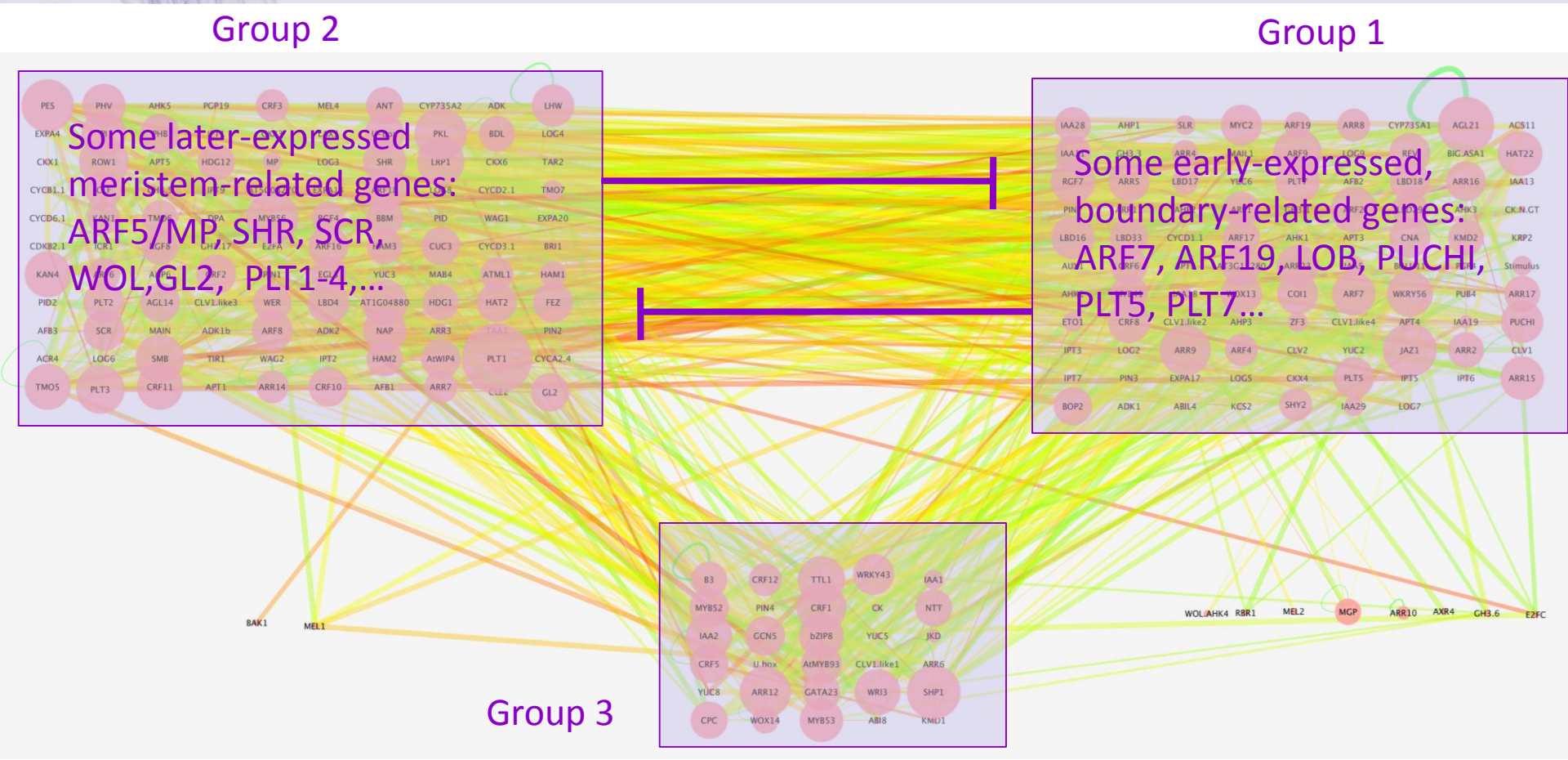


3 independant biological replicats (n>50 seedlings/replicats), Student tests p*** < 0,01

Upstream regulators of PI as candidates genes for QC establishment



Genes are organised into three main groups



Node size

Column	Outdegree
Mapping Type	Continuous Mapping
Current Mapping	100,0 … 0,0 (0.00 – 32.00)

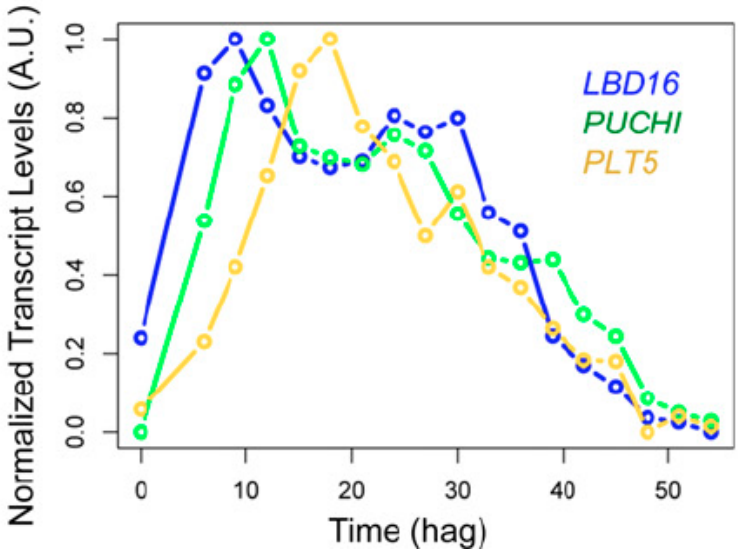
Edge color

Column	index
Mapping Type	Continuous Mapping
Current Mapping	0.00 – 3.86

Edge width

Column	btstp
Mapping Type	Continuous Mapping
Current Mapping	8,0 … 0,0 (0.10 – 100.00)

Activation pattern of the ARF7 module in the primordium

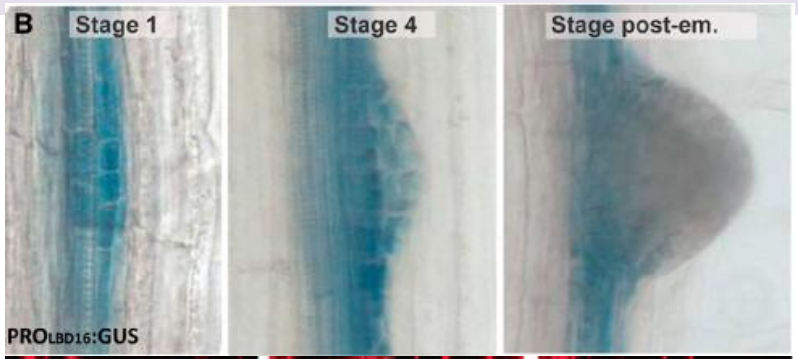


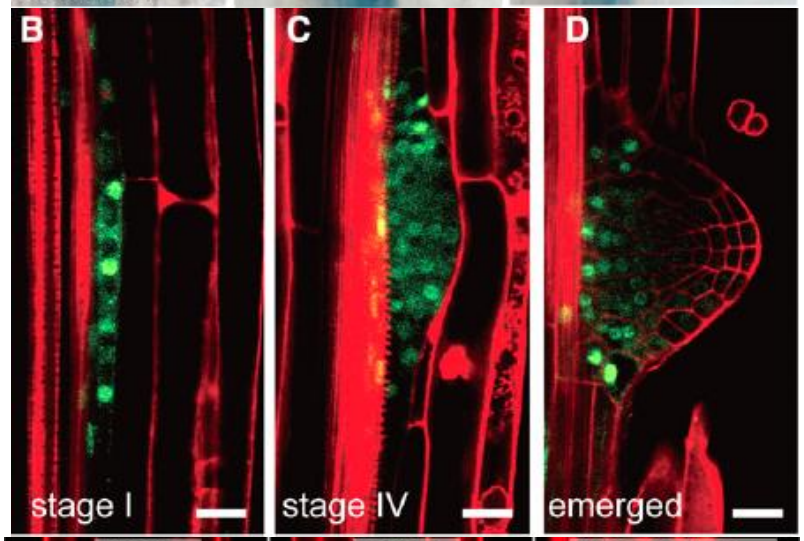
Lavenus et al., 2015
Hirota et al., 2007

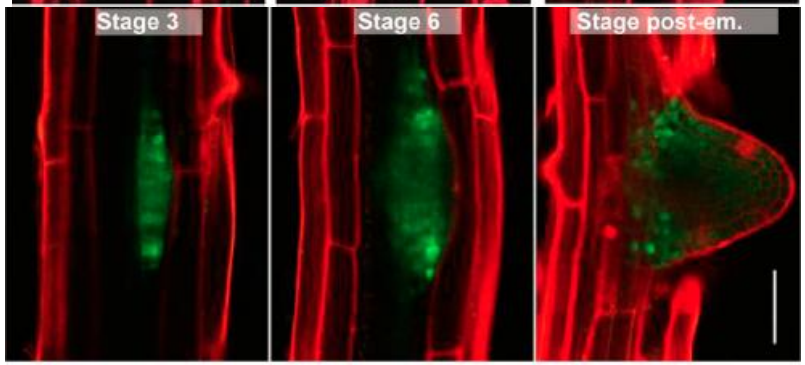
LBD16

PUCHI

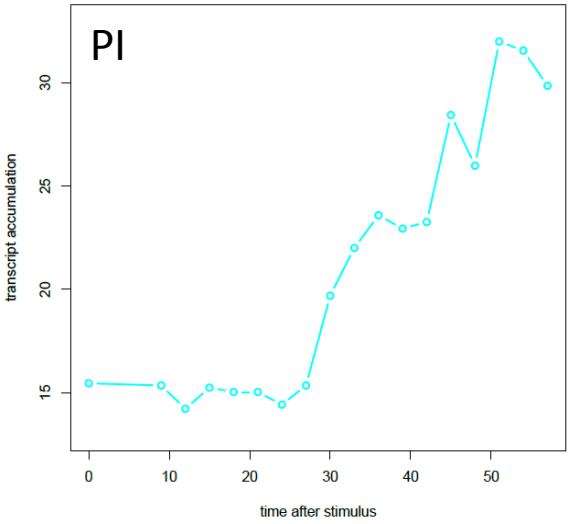
PLT5

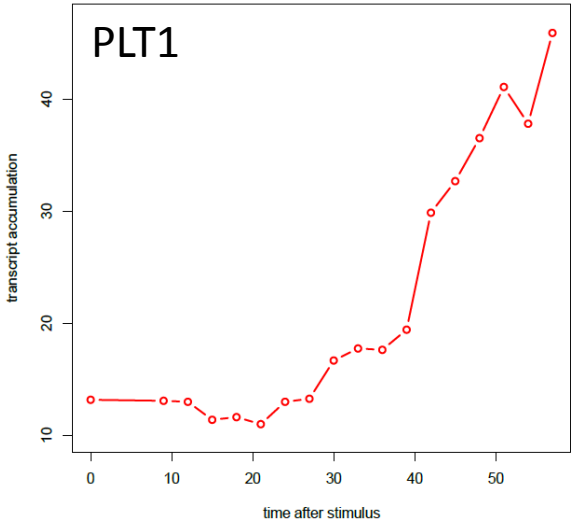


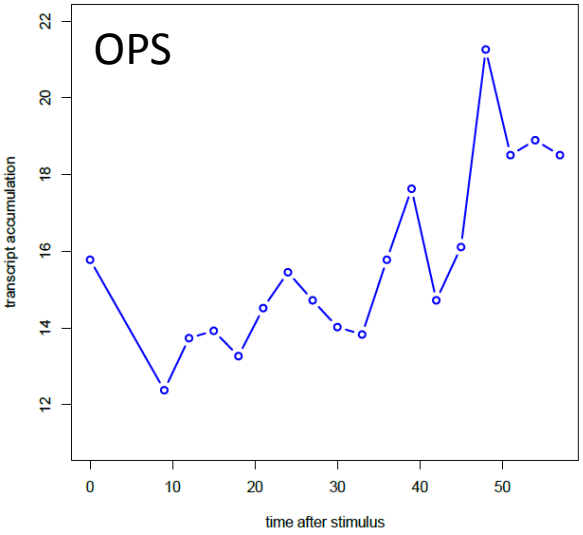


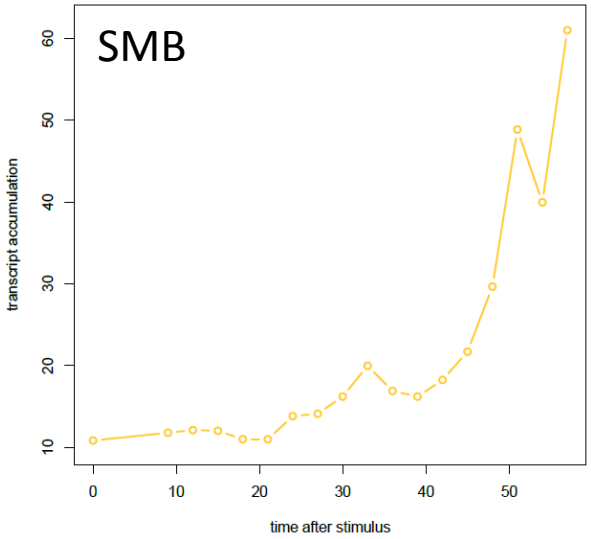


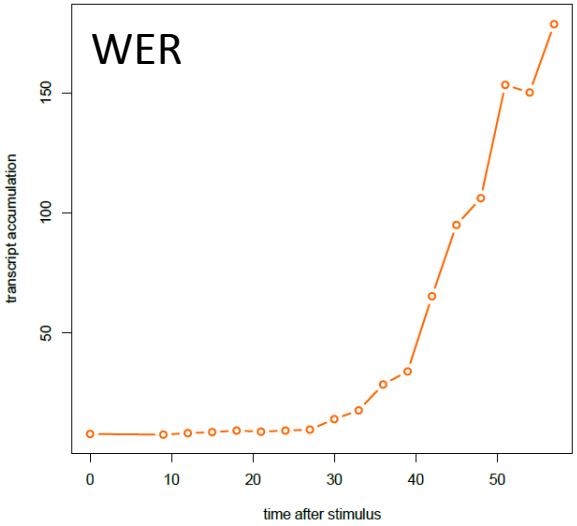
Gene patterns from transcriptomic LR dataset







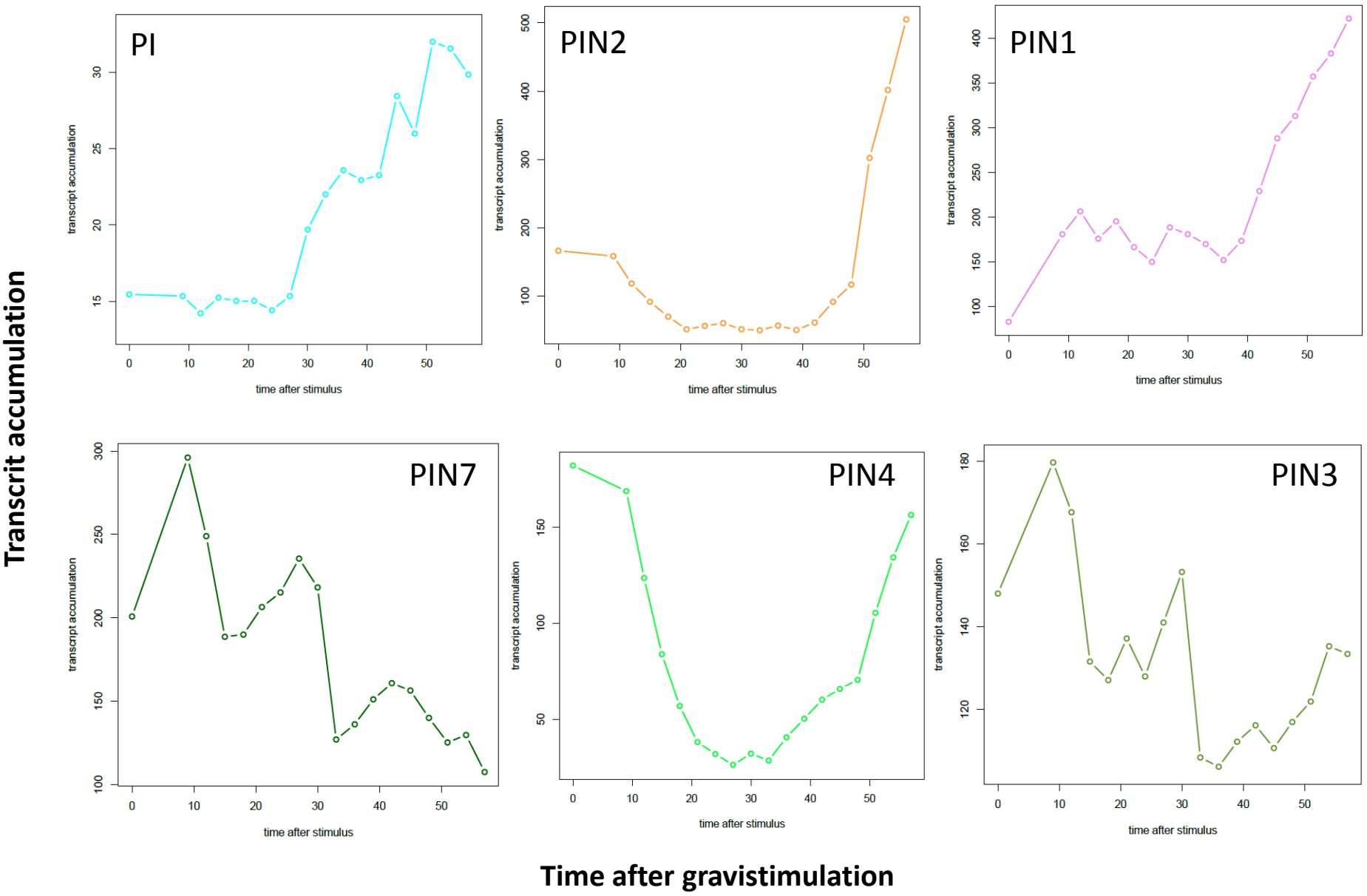




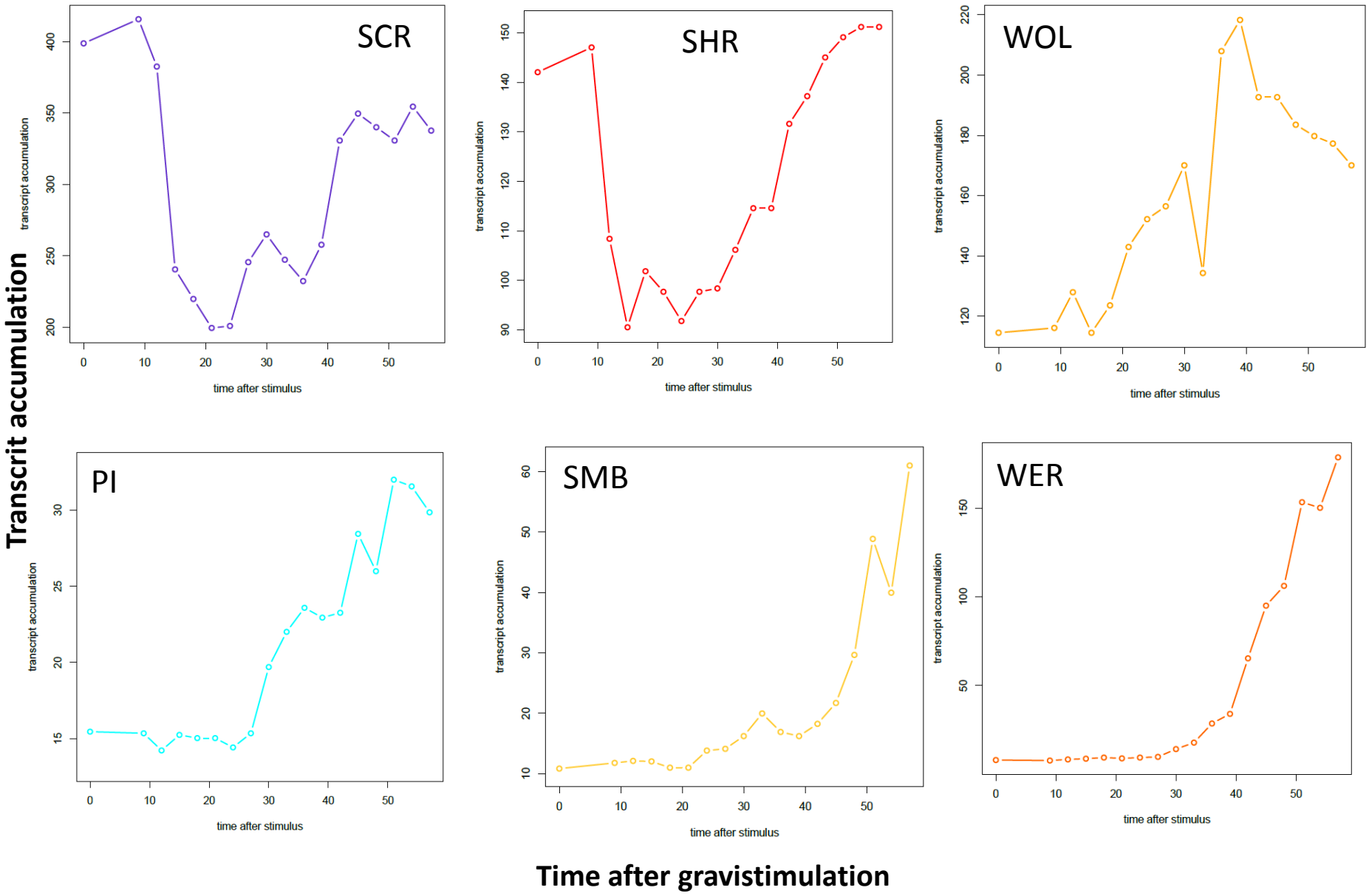
Transcrit accumulation

Time after gravistimulation

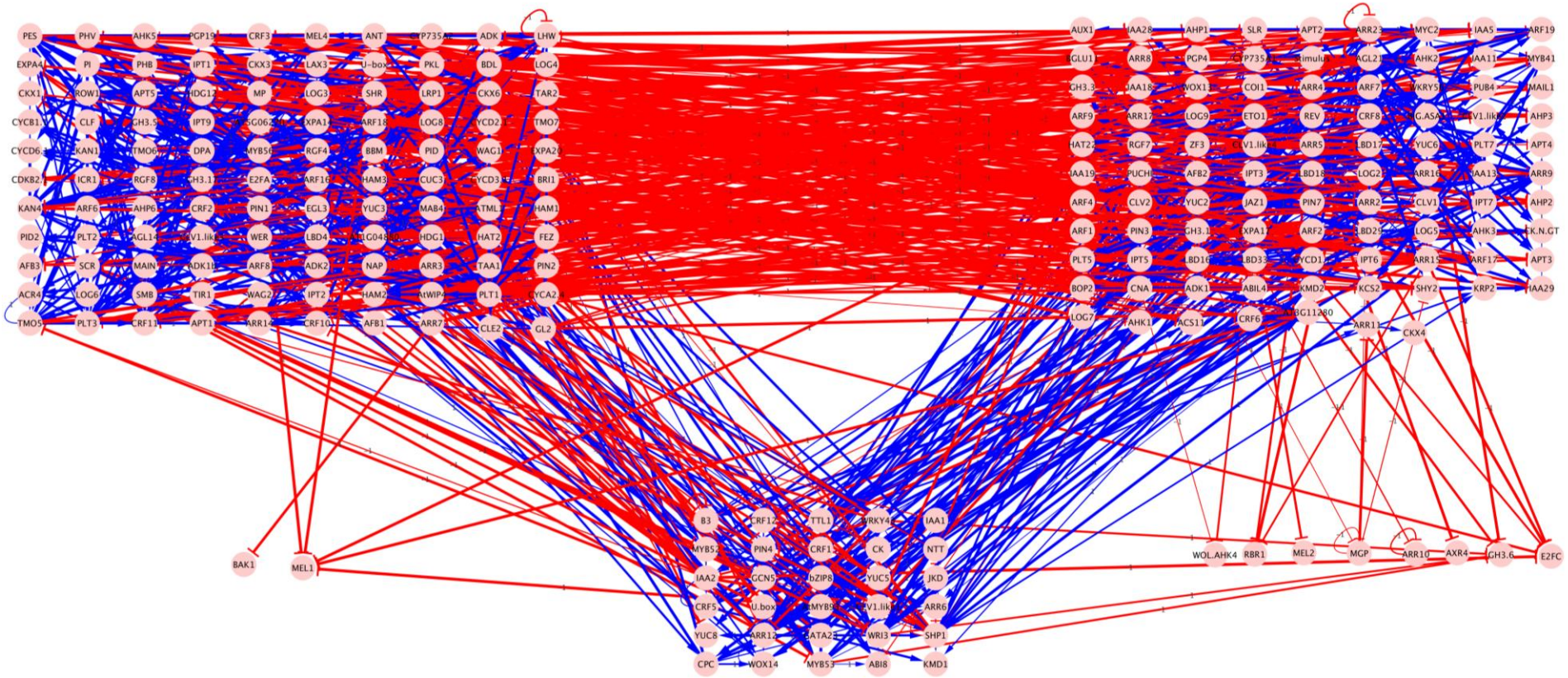
Gene patterns from transcriptomic LR dataset



Gene patterns from transcriptomic LR dataset

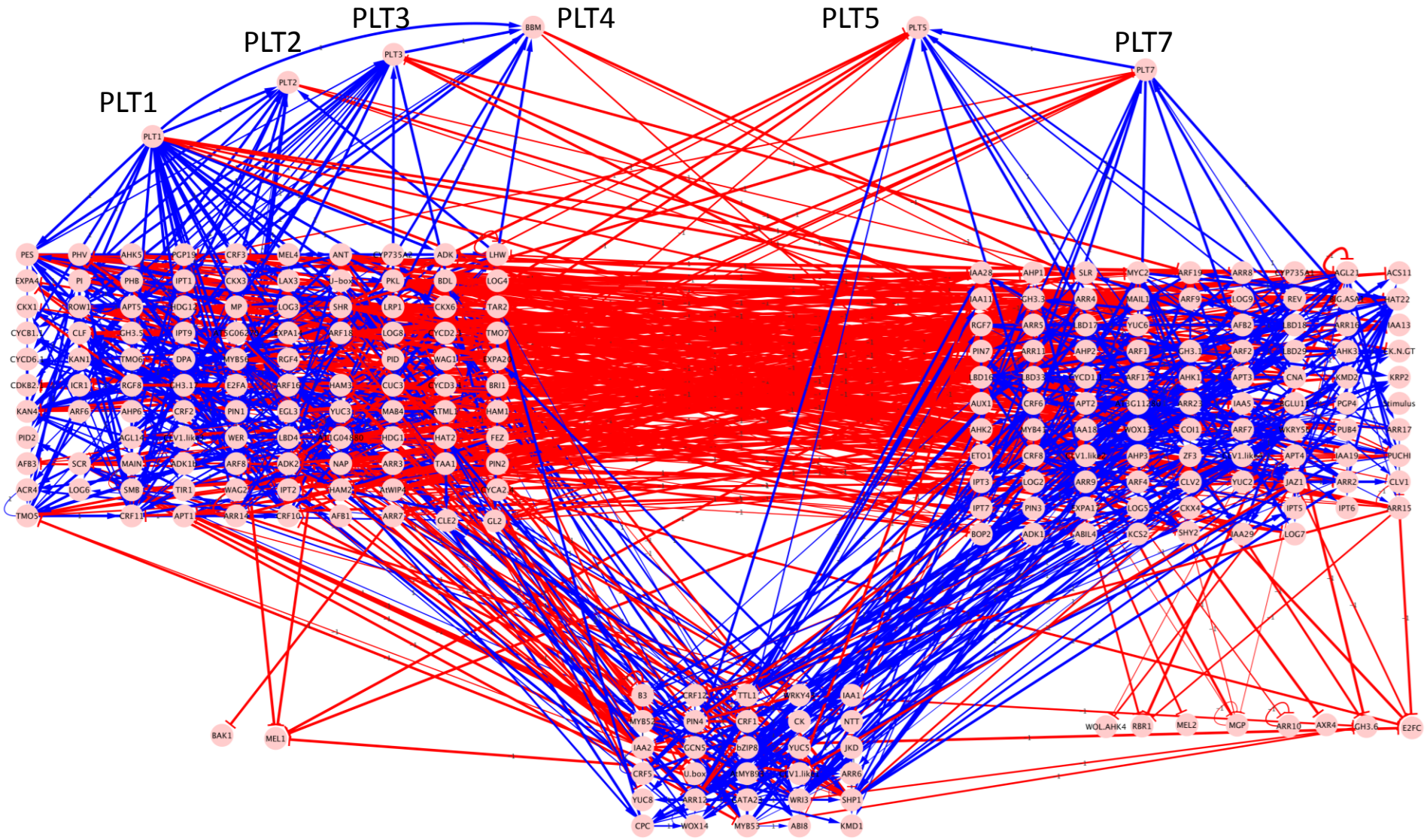


Genes are organised into three main groups



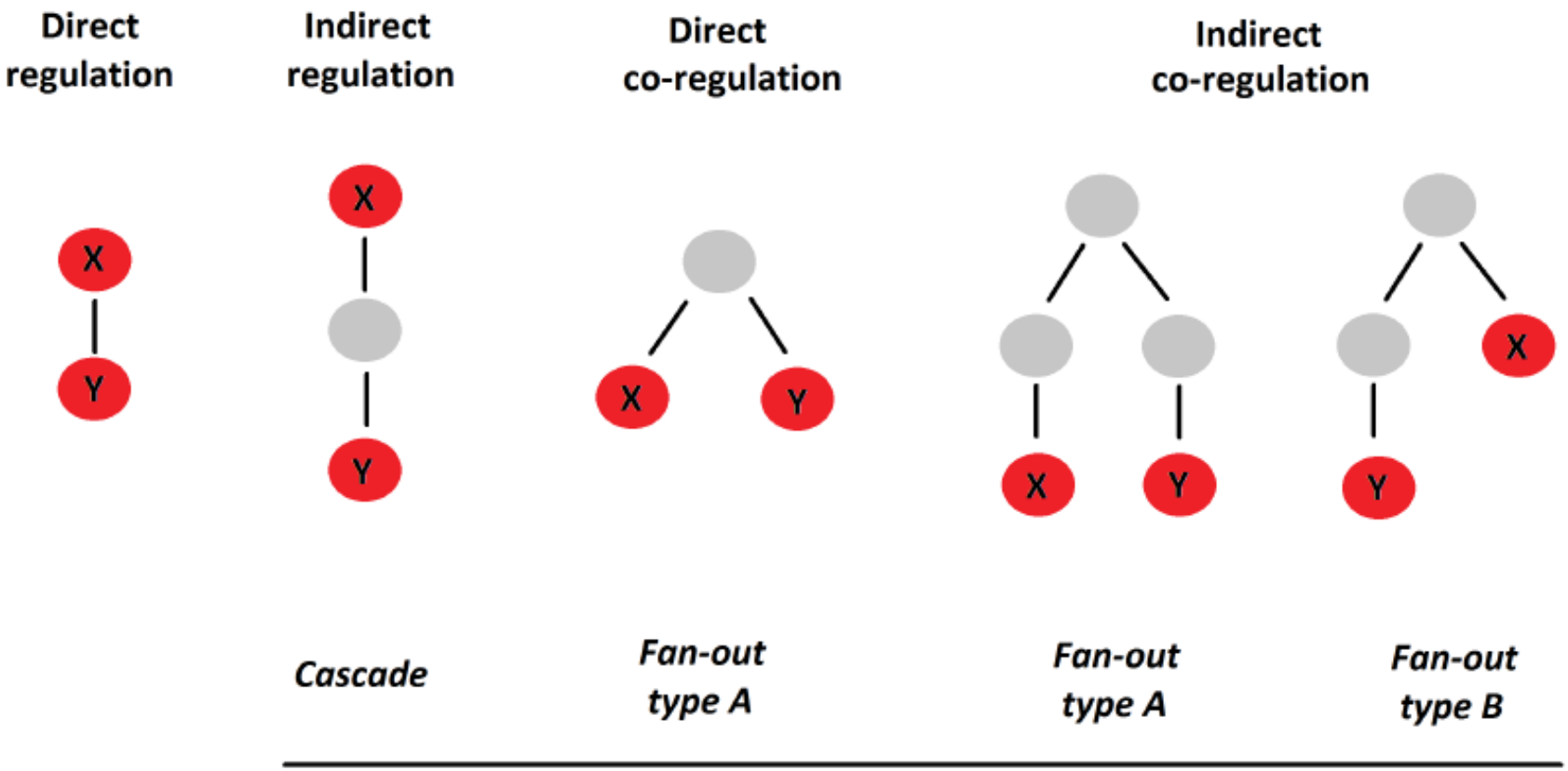
Red edge = inhibition; Blue edge = stimulation

Genes are organised into three main groups: example of PLETHORA family transcription factors



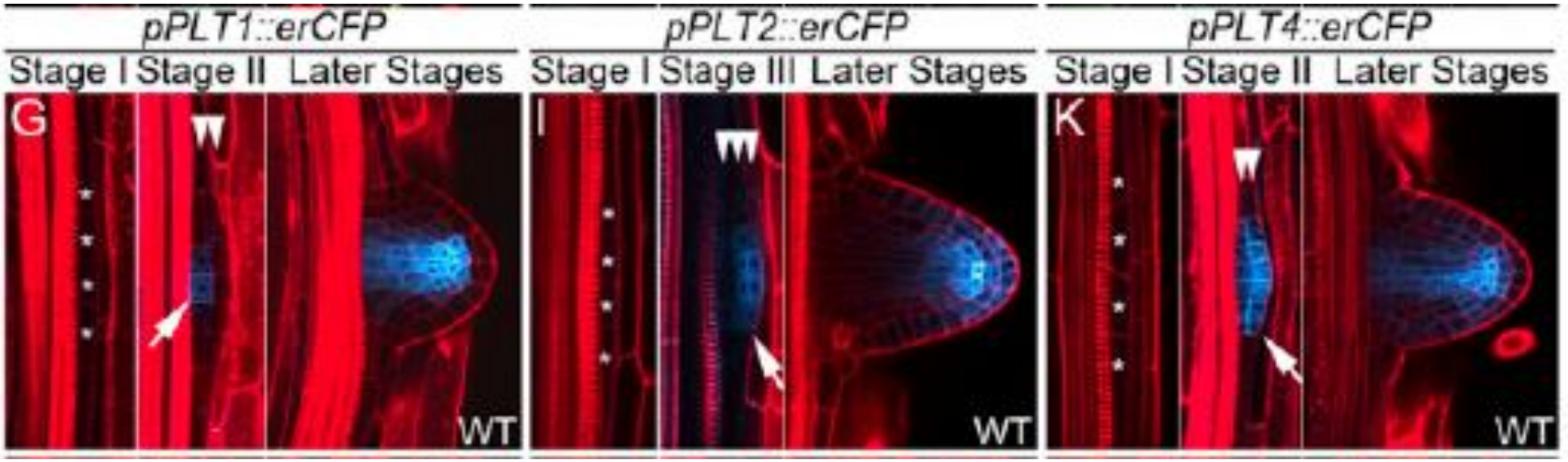
Red edge = inhibition; Blue edge = stimulation

Several network topologies could explain profile correlation



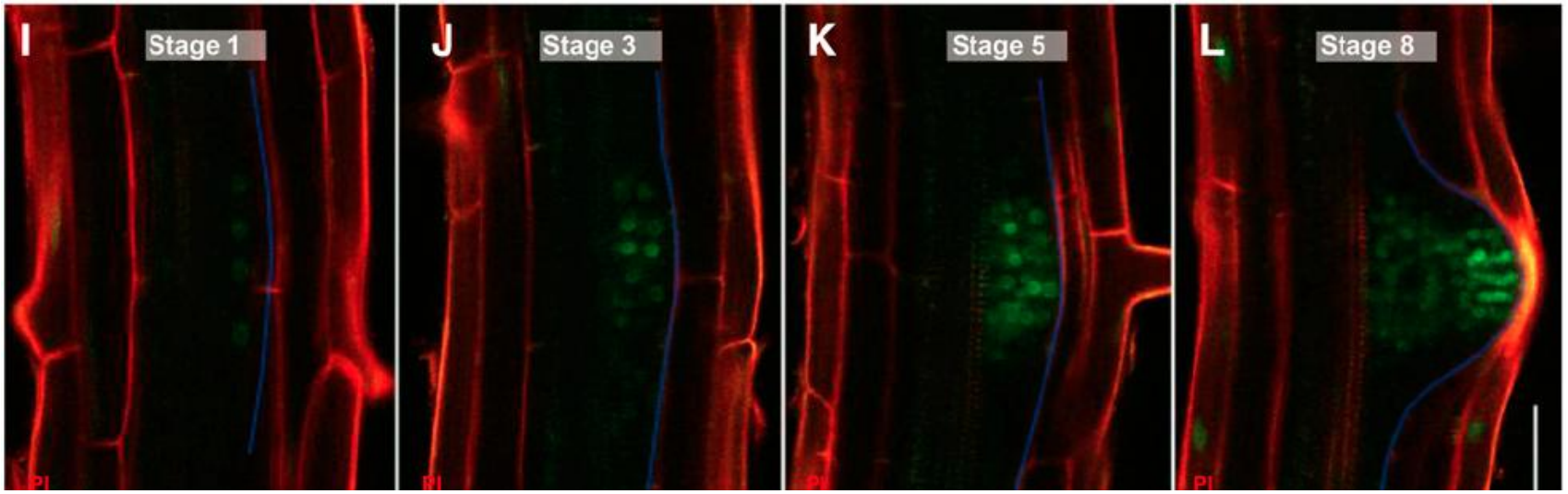
Lavenus *et al*., (2015)

Activation pattern of the ARF5 subnetwork in the LRP



Du & Scheres, 2017

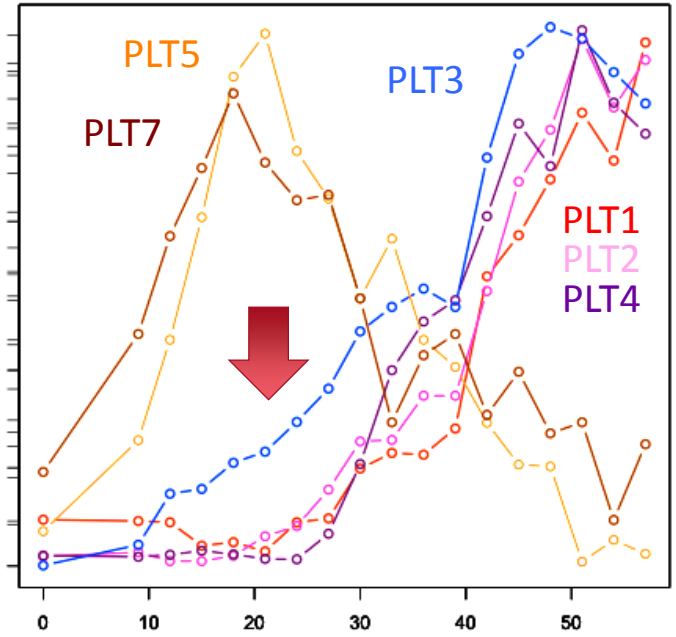
pMP::MP:GFP

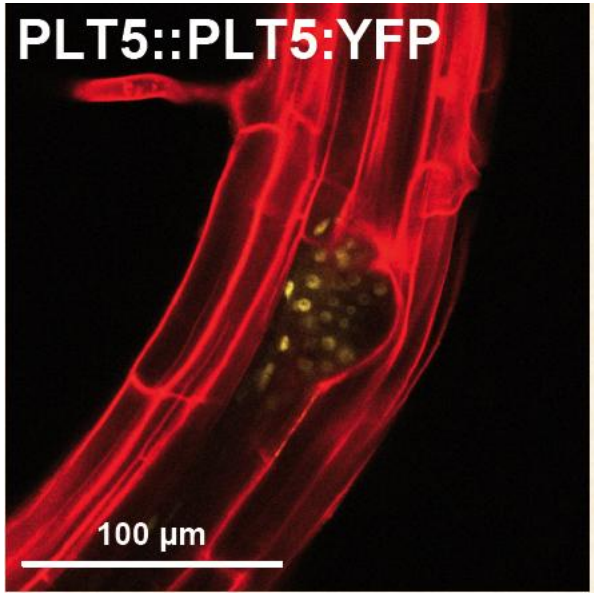


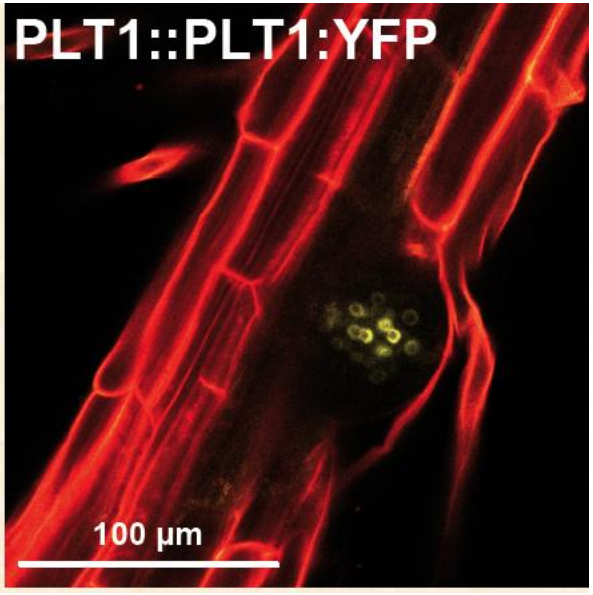
Lavenus et al., 2015

This is consistent with both temporal and spatial patterning in gene expression pattern

Transcript accumulation

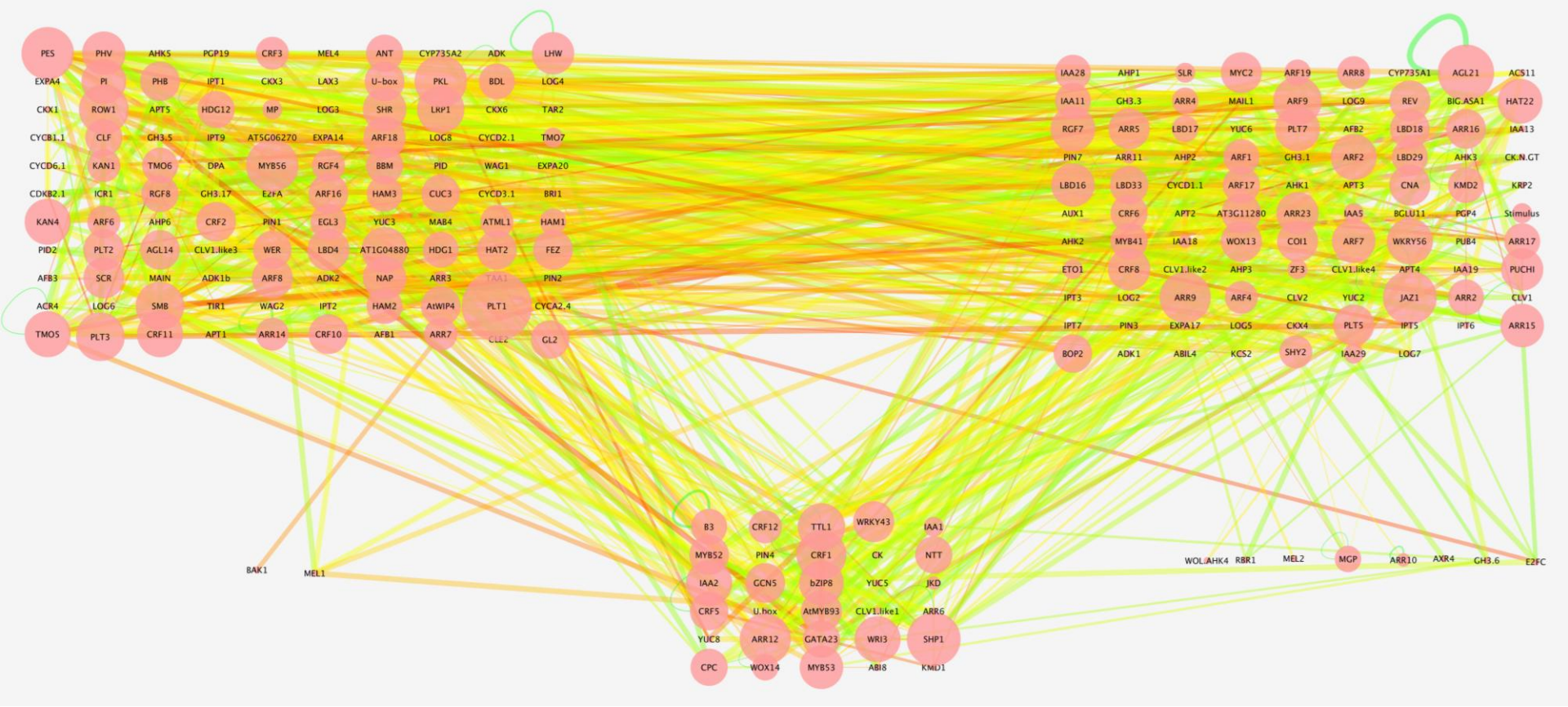






➢ The general topology of the network suggests a toggle-switch mechanism controlling a spatio temporal gene expression pattern in relation with root meristem establishment

Genes are organised into three main groups



Node size

Column	Outdegree
Mapping Type	Continuous Mapping
Current Mapping	100,0 … 0,0 (0.00 – 32.00)

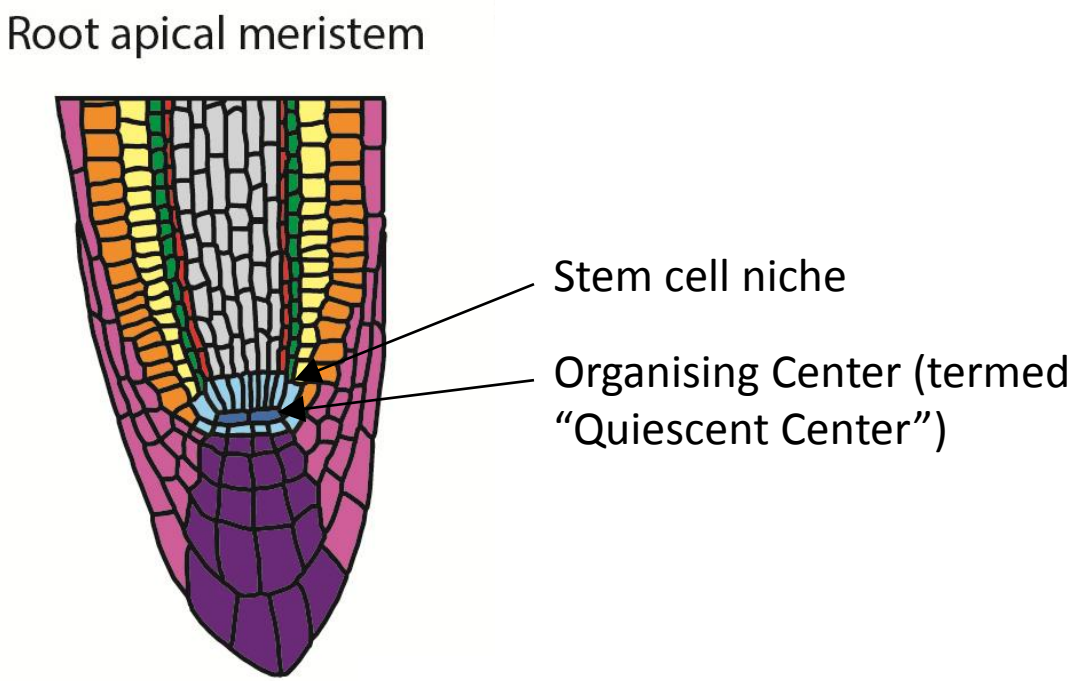
Edge color

Column	index
Mapping Type	Continuous Mapping
Current Mapping	0.00 – 3.86

Edge width

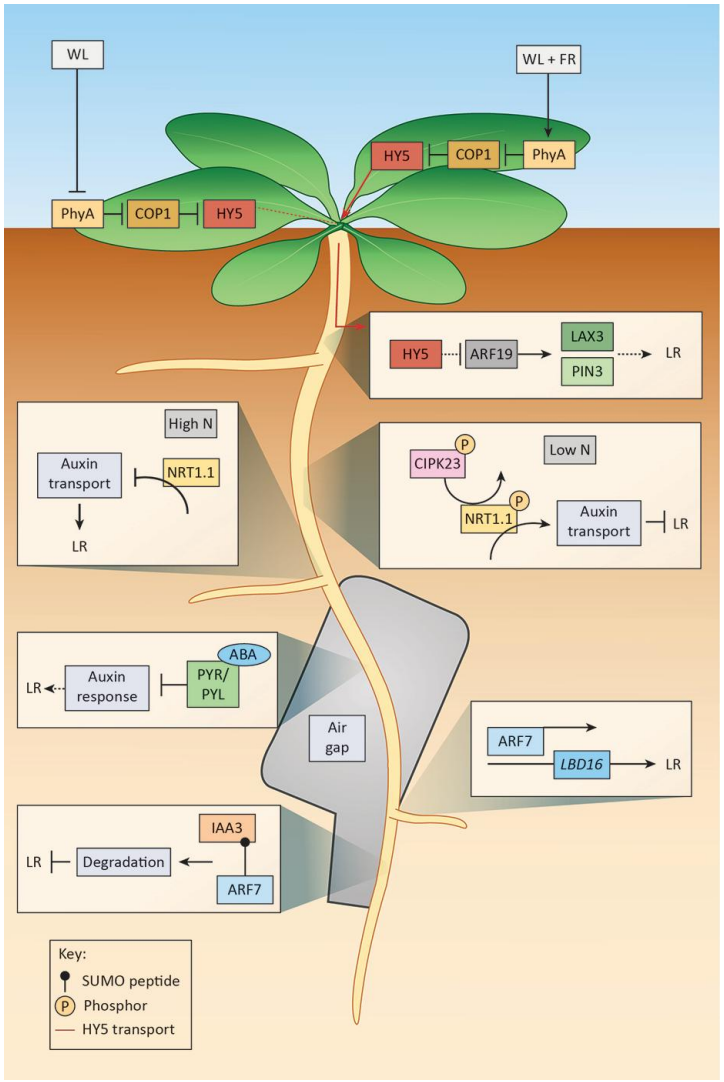
Column	btstp
Mapping Type	Continuous Mapping
Current Mapping	8,0 … 0,0 (0.10 – 100.00)

The root apical meristem generates root primary tissues



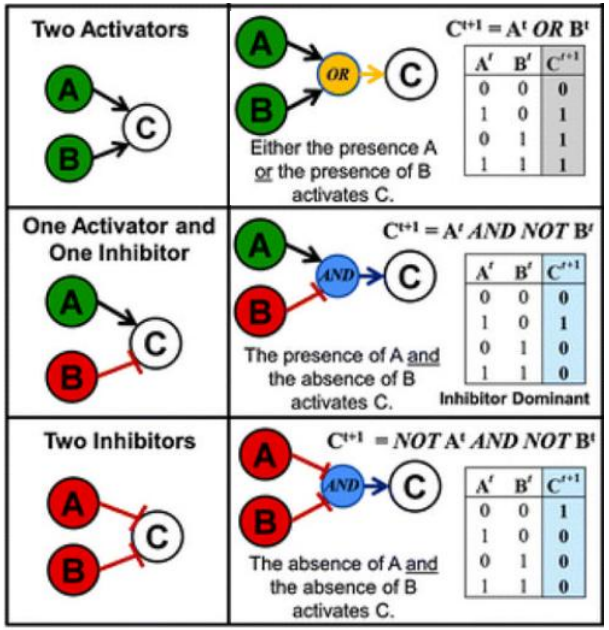
- Primary anatomical organization of roots is stereotyped.
- Root meristem organized around a central stem cell niche
- *Arabidopsis thaliana* as a simple plant model.

The soil and light environment plays a key role in lateral root (LR) positioning



A new simulation algorithm – **PYTHONIS**
(PYTHon-based bOolean Network generIc Solver)

- Uses any given predicted topology of a GRN (e.g. 246 genes, 1069 interactions)
- Boolean modeling as a simplification (various formats)
- Strong biological assumptions
  - *Strong upregulators*
  - *Strong downregulators*
  - *No lazy genes*



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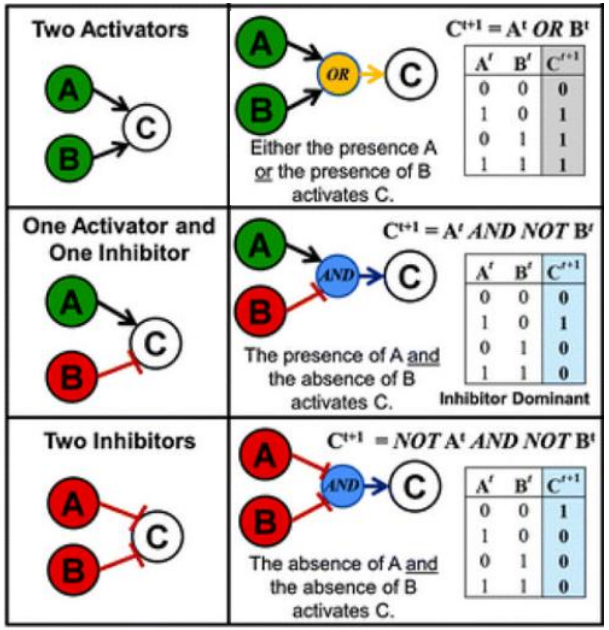

- Automated generation and solving of boolean model for entire network

A new simulation algorithm – **PYTHONIS**

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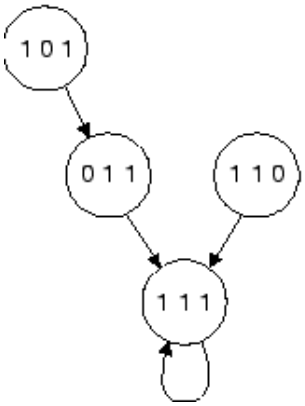
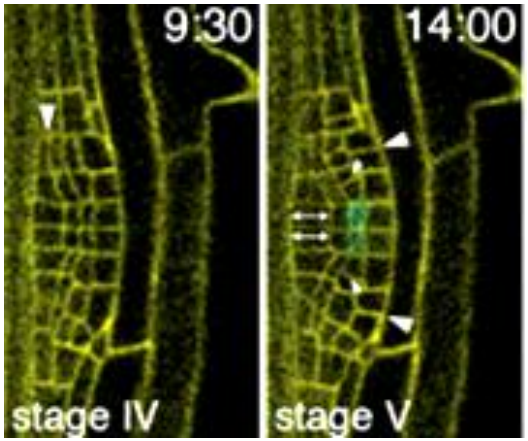
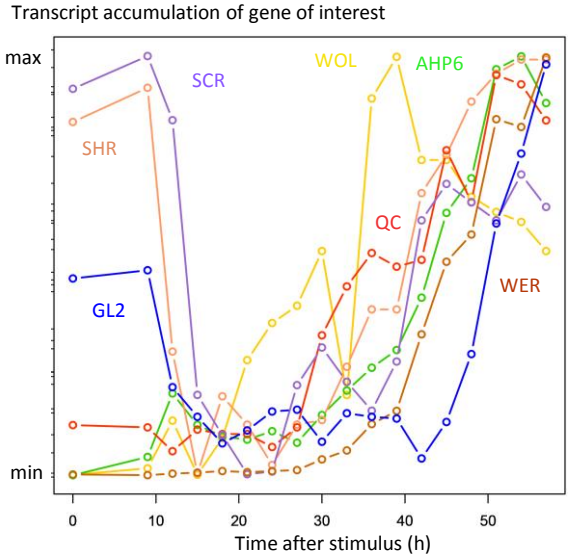
- For any random or given initial state,
  - state flow (deterministic)
  - final states (can be stable states or loops)
  - basins of attraction
  - Hamming distance between basins of attractions
  - => identification of nodes important for cell fate bifurcation?







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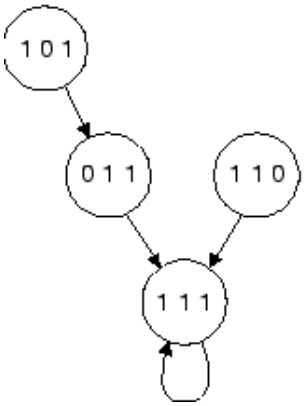

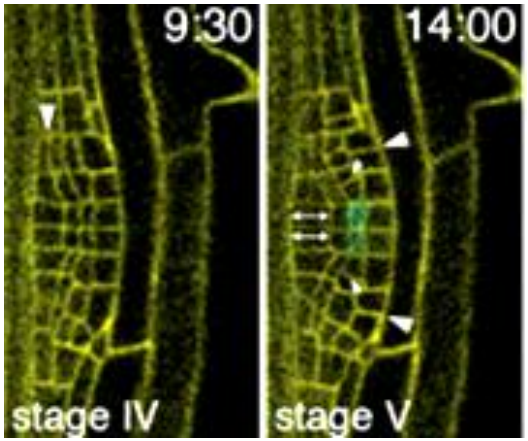

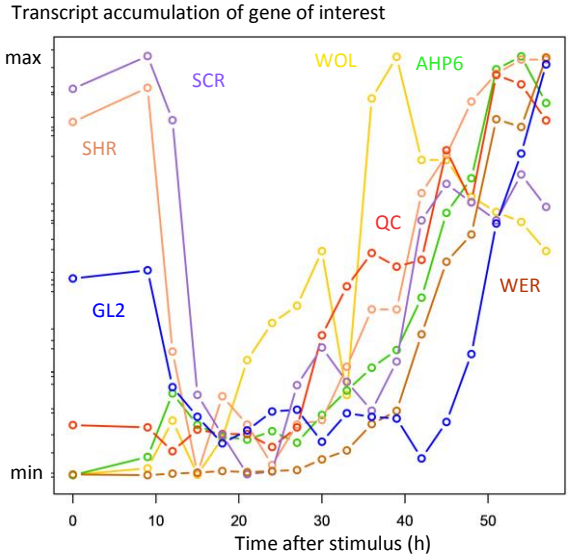

A new simulation algorithm – **PYTHONIS**

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  - state flow (deterministic)
  - final states (can be stable states or loops)
  - basins of attraction
  - Hamming distance between basins of attractions
  - => identification of nodes important for cell fate bifurcation?
- Simulation of the impact of knock-out (always 0) or gain-of-function (always 1) mutations
  - impact on state flow and final states
  - Hamming distance between « mutant » and « wild type » final states
  - => assessing the significance of each node in state flow
- Currently being validated against known regulatory networks

Modeling dynamic properties of the inferred network

➢ Aim : to model dynamically the state flow of the gene network in order to identify gene regulatory cascades, master regulators, attractor states, bifurcation behaviours…

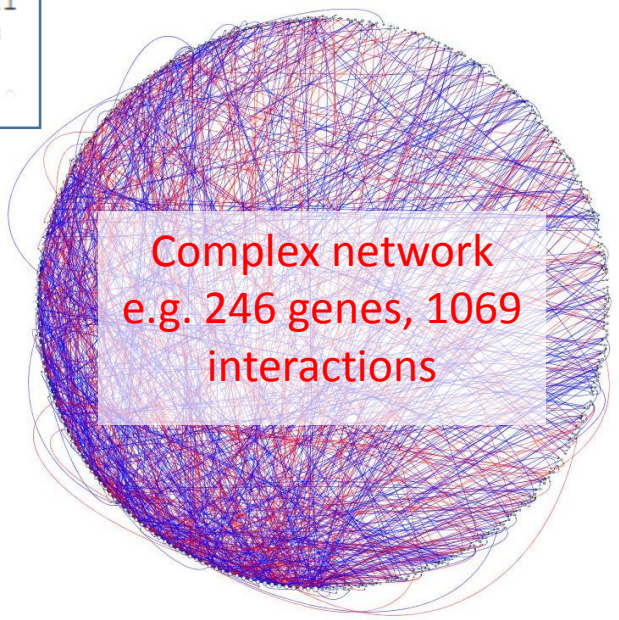
TDCore inputs

1	AT1G02850	BGLU11	0
2	AT1G03430	AHP5	0
3	AT1G03840	MGP	2
4	AT1G04220	KCS2	0
5	AT1G04240	SHY2	-1
6	AT1G04550	BDL	-1
7	AT1G04610	YUC3	0
8	AT1G04880	AT1G04880	2
9	AT1G10470	ARR4	-1
10	AT1G12820	AFB3	0

	V1	V2	V3	V4	V5
1	"AT1G01010"	7.82	7.66	7.57	7.48
2	"AT1G01040"	7.06	7.29	7.27	7.09
3	"AT1G01050"	9.78	9.62	9.8	9.93
4	"AT1G01060"	5.44	5.31	6.01	7.59
5	"AT1G01070"	9.33	9.08	9.06	8.82
6	"AT1G01080"	5.79	5.87	6	5.94
7	"AT1G01090"	9.56	9.6	10.75	11.29
8	"AT1G01100"	10.13	10.61	10.98	11.13
9	"AT1G01110"	4.18	4.33	4	4

TDCor outputs

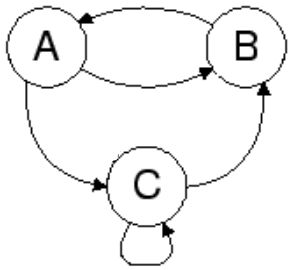
	source	inter	target	btstp	index	?
1	MGP	-1	MGP	2.7	0	0
2	MGP	-1	SHY2	13.7	1.73	1.2
3	MGP	-1	ARR11	92.4	1.004	0.5
4	SHY2	1	ARF1	4.2	1.85	4
5	SHY2	-1	ADK2	17.2	1.677	1.5
6	SHY2	-1	HAM1	100	0.856	0.5
7	SHY2	-1	HAM3	94.9	1.504	0.5
8	BDL	-1	BDL	10.1	0	0
9	BDL	-1	MYC2	38.4	1.004	0.5



Modeling dynamic properties of the inferred network

- Strategy: to use boolean modeling of the network

(a)

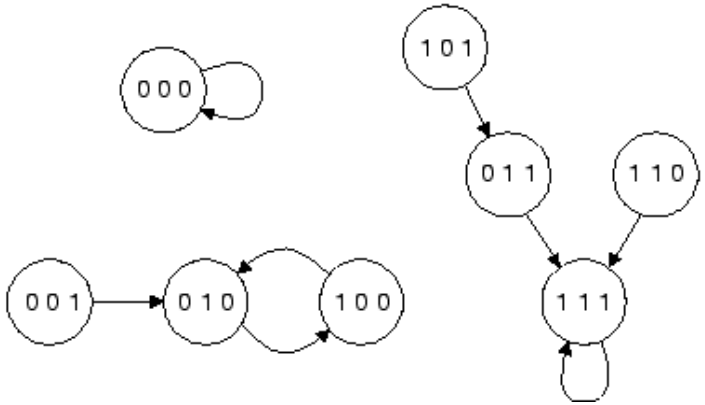


(b) A = B
B = A or C
C = (A and C) or (B and C) or (A and B)

(c)

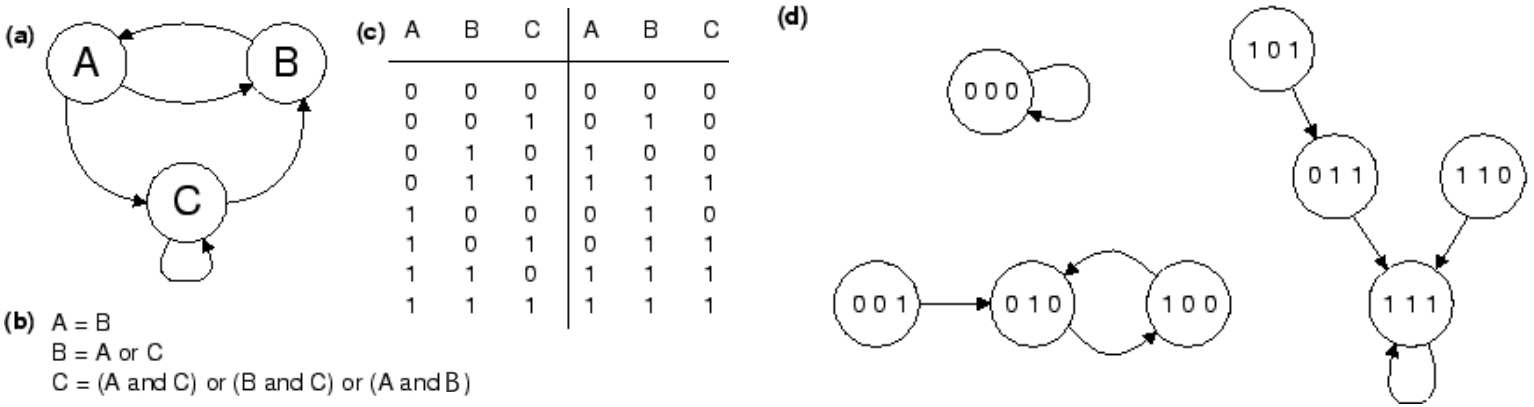
A	B	C	A	B	C
0	0	0	0	0	0
0	0	1	0	1	0
0	1	0	1	0	0
0	1	1	1	1	1
1	0	0	0	1	0
1	0	1	0	1	1
1	1	0	1	1	1
1	1	1	1	1	1

(d)



Modeling dynamic properties of the inferred network

- Strategy: to use boolean modeling of the network



- However most available boolean models require explicit specification of network and of each interaction rules (BooleanNet, NetDS, NetworkToolkitExtended, BooleSim, SimBoolNet, Atalia, …)
  - → Impractical for massive network modelling
  - → Currently developing implementation of automated boolean modelling for large scale networks

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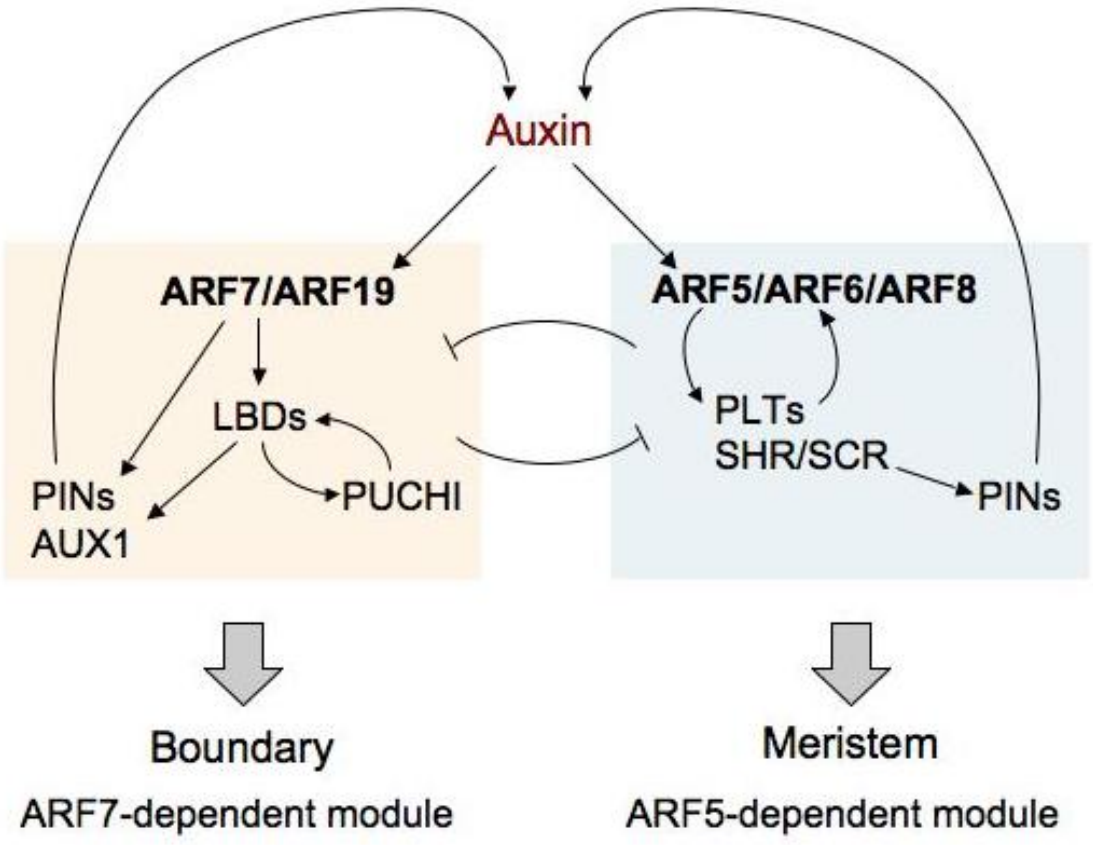
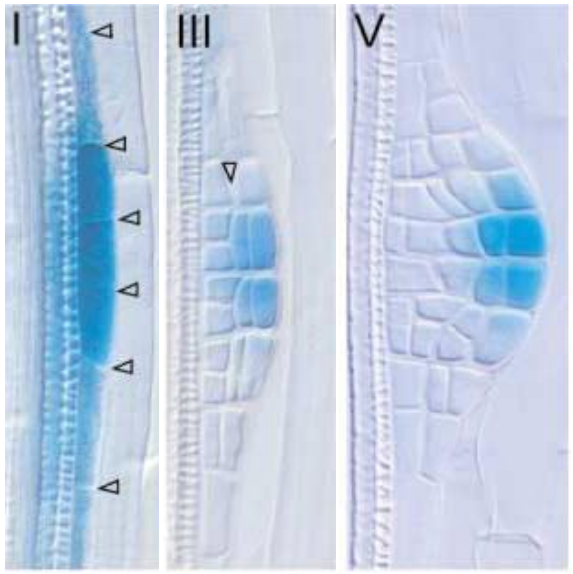
- Boolean modeling as a simplification (various formats)

The LR network is predicted to organize into two subnetworks with distinct crosstalk with auxin



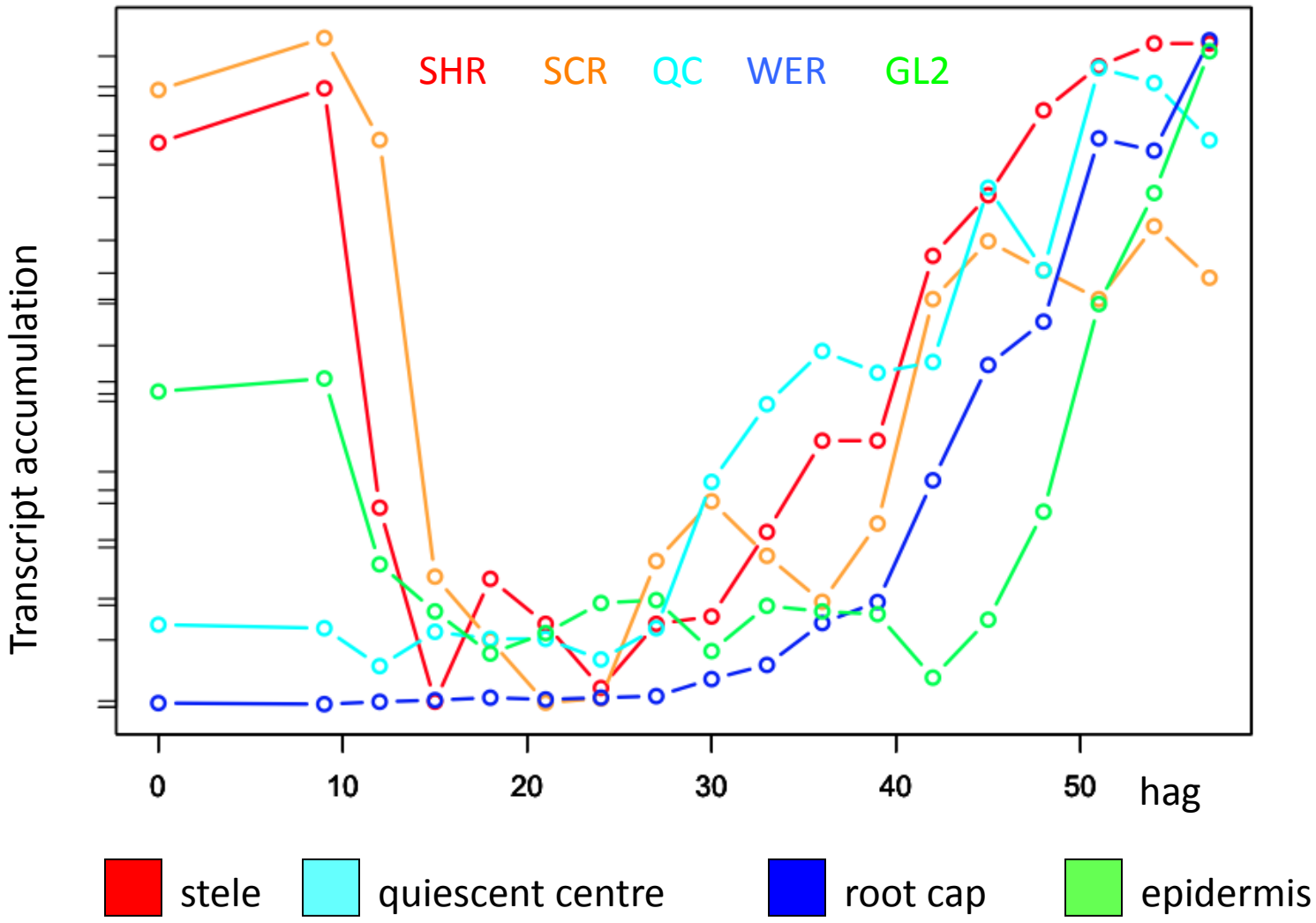
Auxin signaling (DR5::GUS)



Benkova et al., 2003

➢ Distinct crosstalks of each module with auxin distribution and signaling may contribute to progressive patterning of the lateral root primordium.

Use of marker genes to monitor LRP functional patterning



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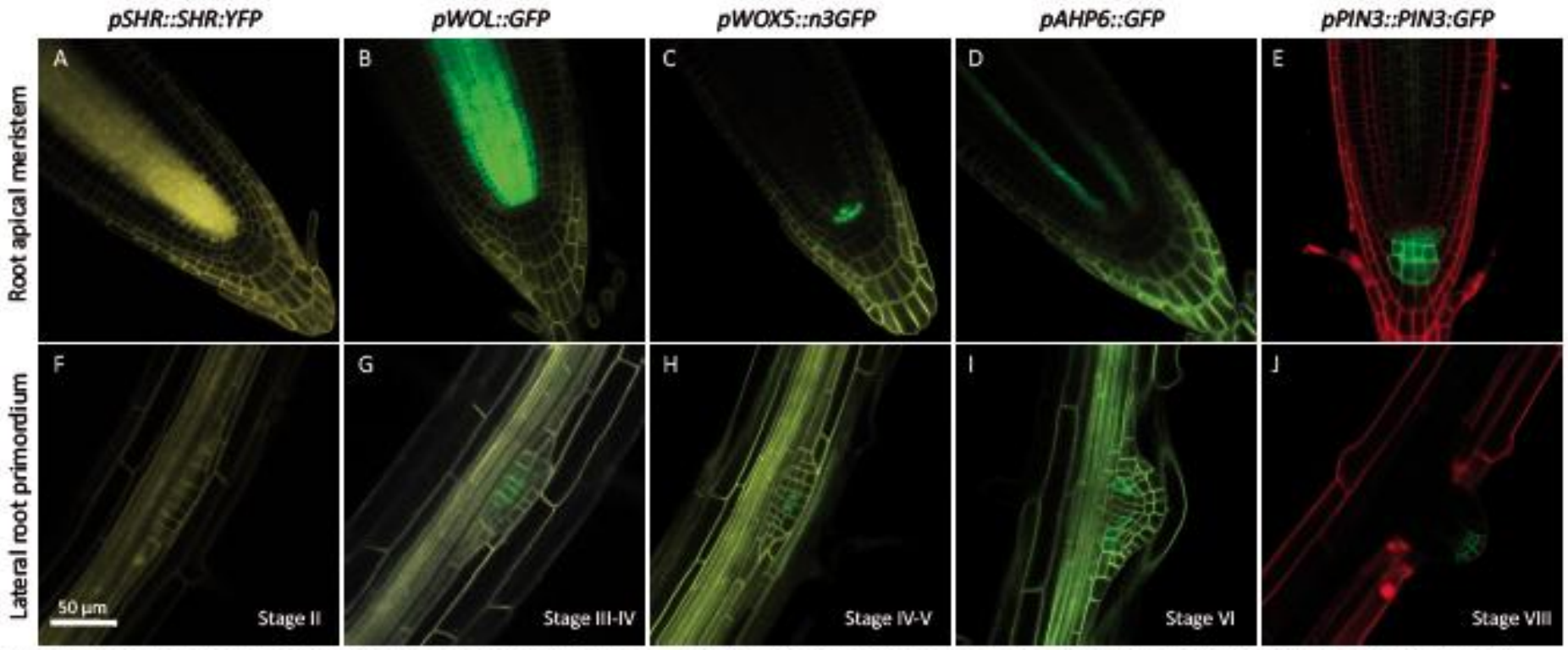
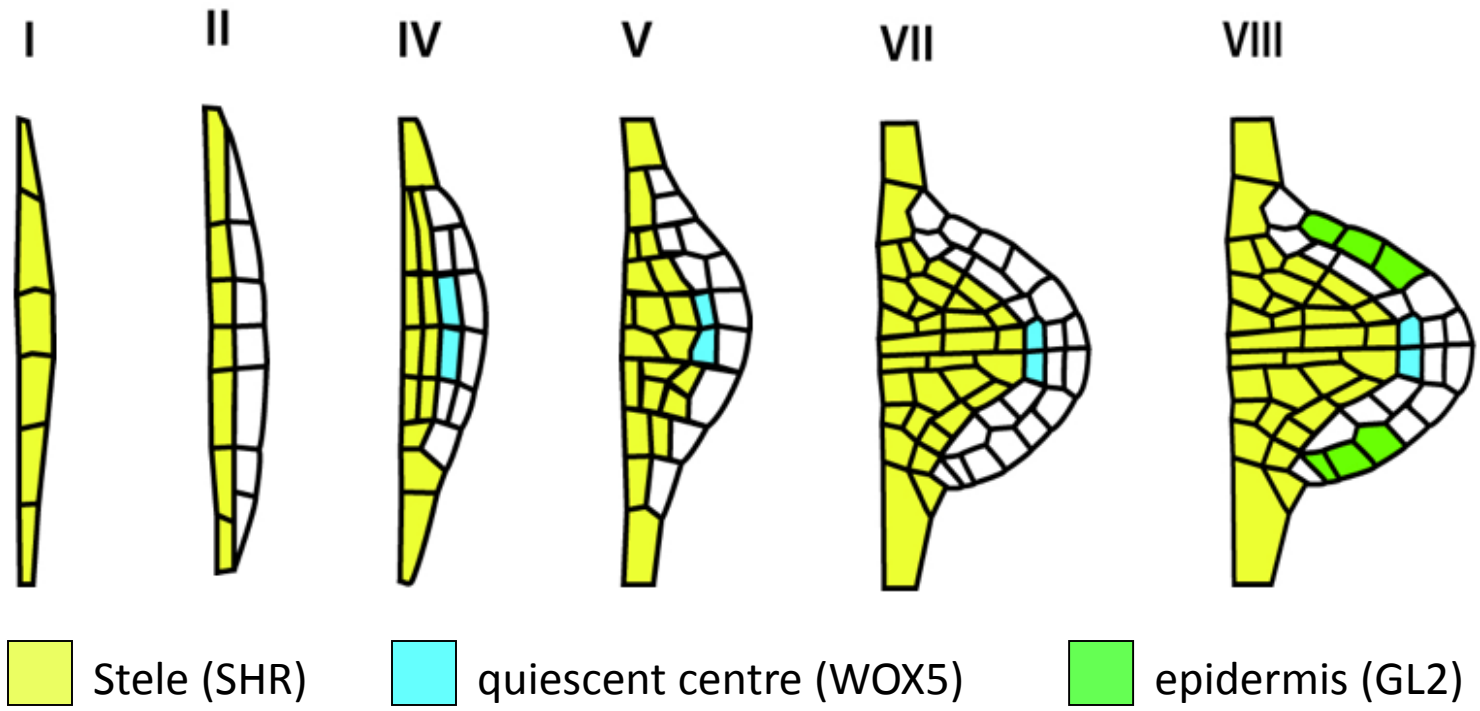


Figure 4. Expression pattern of selected reporter constructs during lateral root formation. Plasma membranes are labelled with WAVE131-YFP (A-D, F-I) or propidium iodide (E,J).

Gradual functional patterning of the LRP

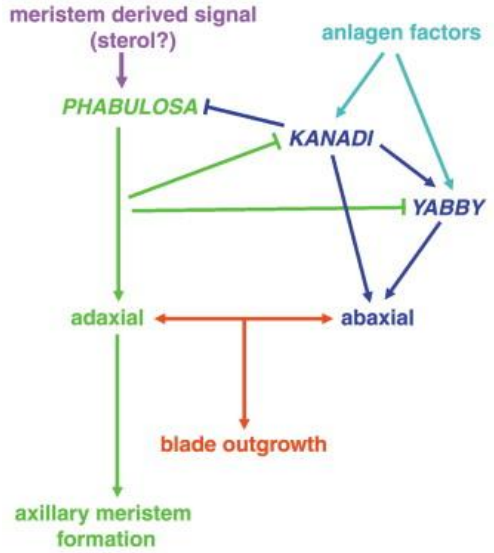


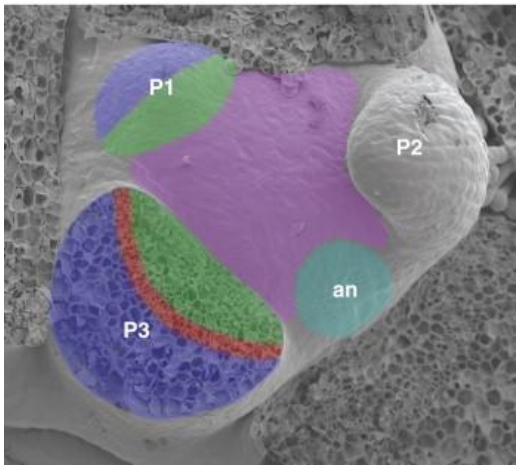
➢ Critical changes in gene expression occur at stage I-II transition and stage IV (meristem formation phase), and early after emergence (expression of epidermis and root cap markers)

HDZIP III, KANADI transcription factors

Eshed *et al.*, 2001, Current Biology

KANADI loss- and gain-of-function alleles suggest that fine regulation of these genes is at the core of polarity establishment. As such, they are likely to be targets of the *PHB*-mediated meristem-born signaling that patterns lateral organ primordia.





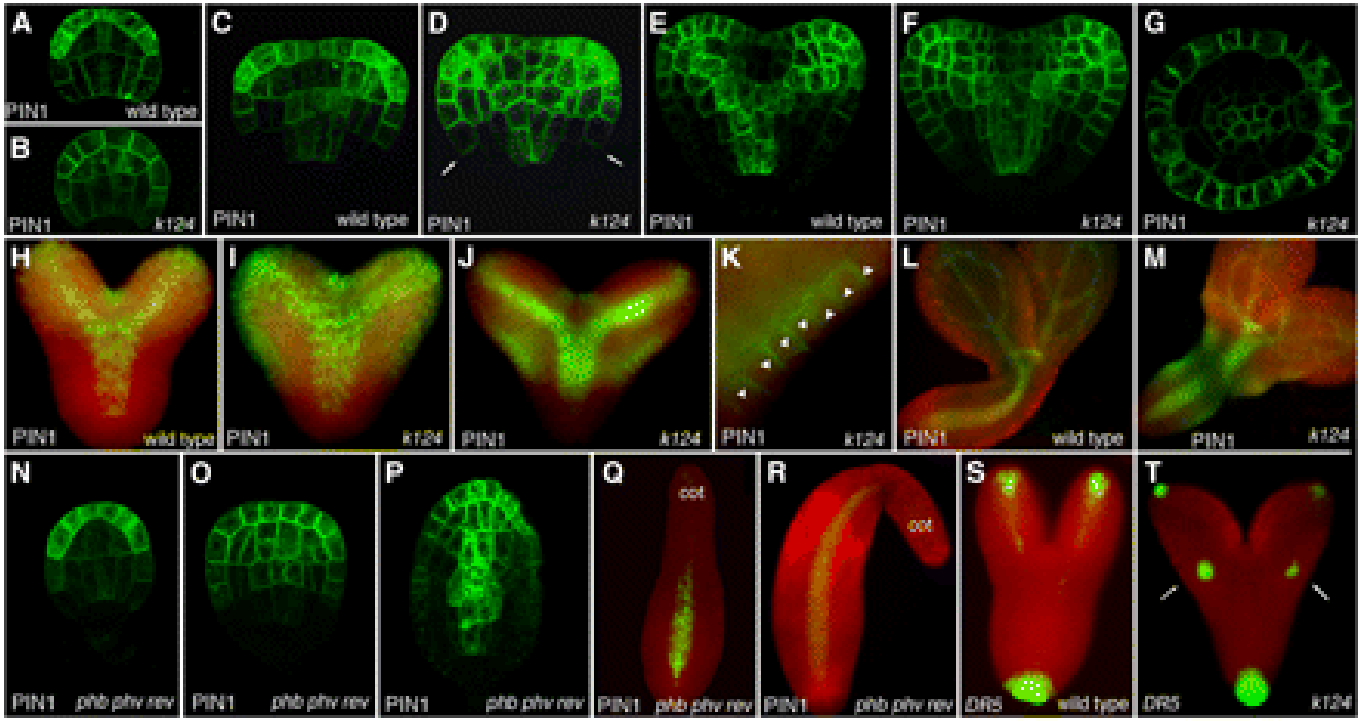
Model of polarity establishment in lateral organs

KANADI transcription factor

Izhaki *et al.*, 2007, Development

KANADI and Class III HD-Zip Gene Families Regulate Embryo Patterning and Modulate Auxin Flow during Embryogenesis in Arabidopsis

Loss of KANADI activity in a Class III HD-Zip mutant background mitigates the defects in bilateral symmetry, implying that the two gene families act antagonistically during embryonic pattern formation.



Materials:

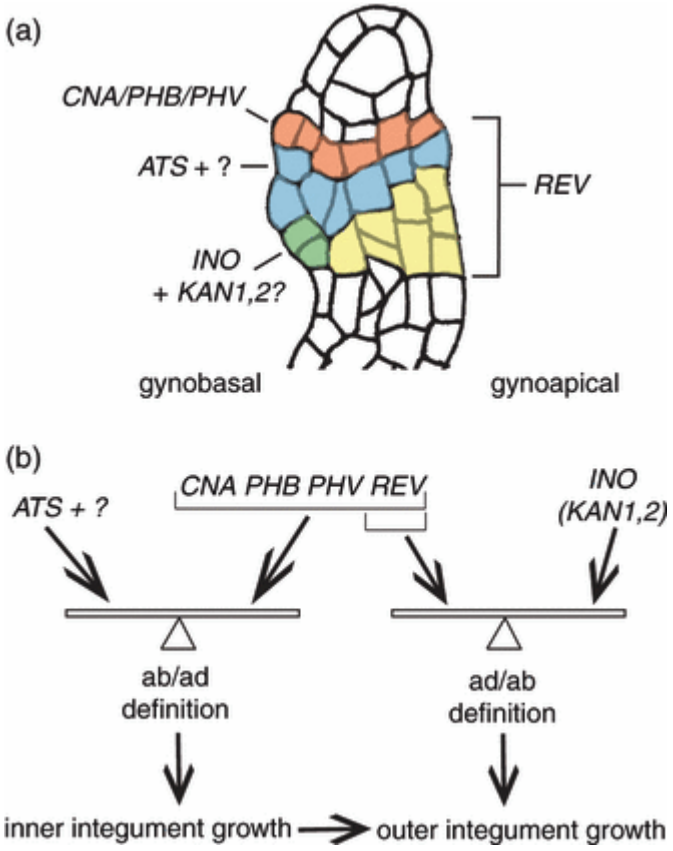
- Mutant lines have been described previously: *kan1-2* (Eshed et al., 1999); *kan2-1* (Eshed et al., 2001); *kan3-1*, *phb-6*, *phv-5*, and *rev-9* (Emery et al., 2003); *kan4-3* (Mcabee et al., 2006)
- *kan1-2 kan2-1 kan3-1 kan4-3* quadruple mutant
- *kan1-2 kan2-1 kan4-3 phb-6 phv-5 rev-9* hextuple mutant

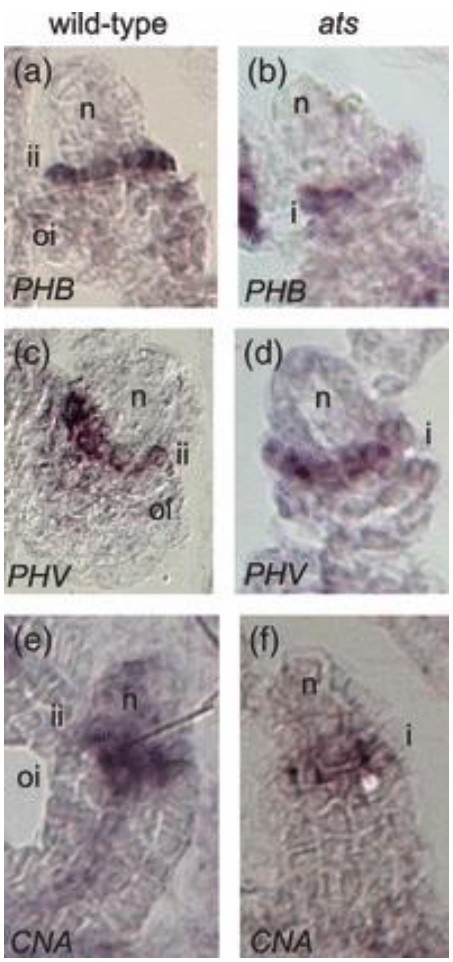
KANADI transcription factor

Kelley *et al.*, 2009, Plant Journal

Roles of polarity determinants in ovule development

Class III homeodomain leucine zipper (HD-ZIPIII) genes *CORONA* (*CNA*), *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*) are expressed adaxially in the inner integument during ovule development, independent of *ABERRANT TESTA SHAPE* (*ATS*, also known as *KANADI4*) activity. Loss of HD-ZIPIII activity can partially compensate for loss of *ATS* activity in the *ats cna phb phv* quadruple mutant, showing that *CNA*/*PHB*/*PHV* act in concert with *ATS* to control integument morphogenesis



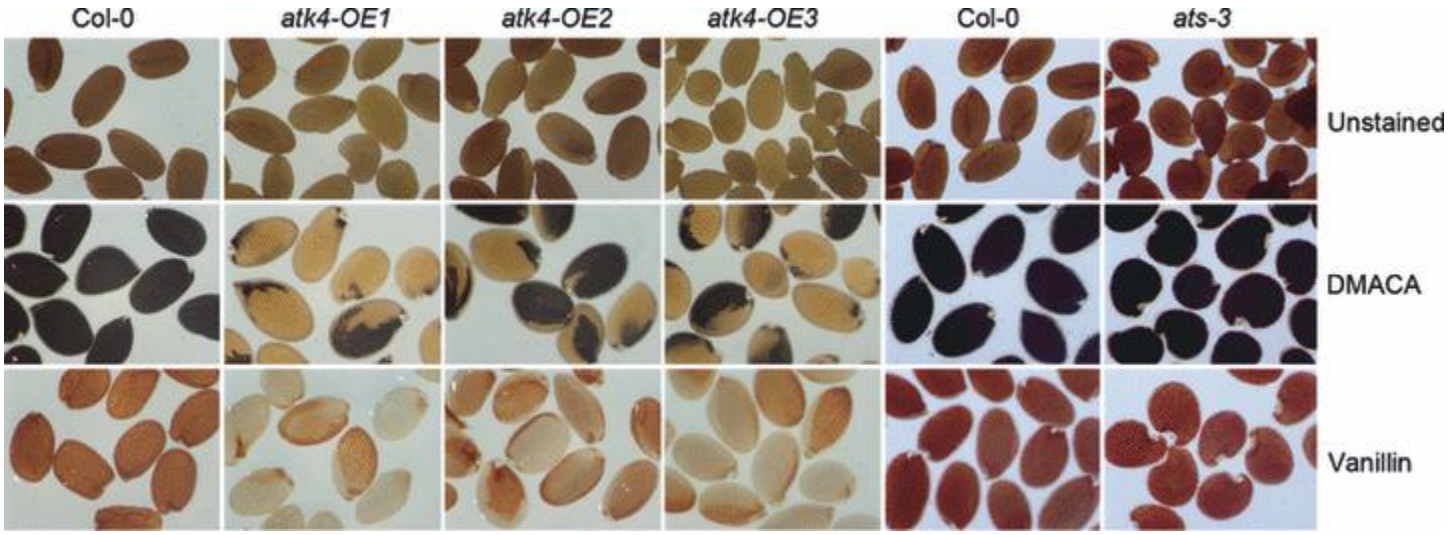


Materials:

- *ats-3 phb-6 phv-5 rev-9/+*

KANADI transcription factor

Gao *et al.*, 2010, Plant Biotech journal
A new dominant Arabidopsis transparent testa mutant, sk21-D, and modulation of seed flavonoid biosynthesis by KAN4



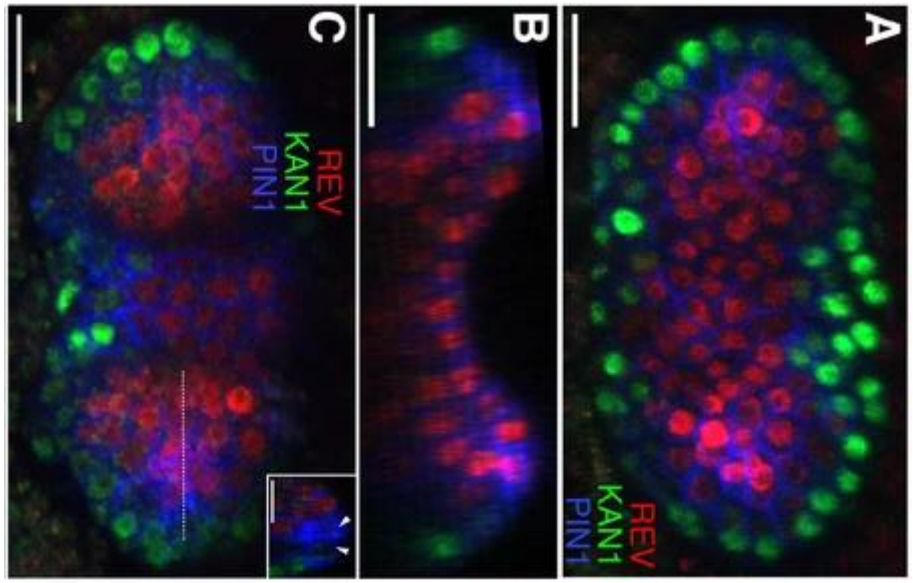
Materials:
- *KAN4* activation over-expression lines

KANADI transcription factor

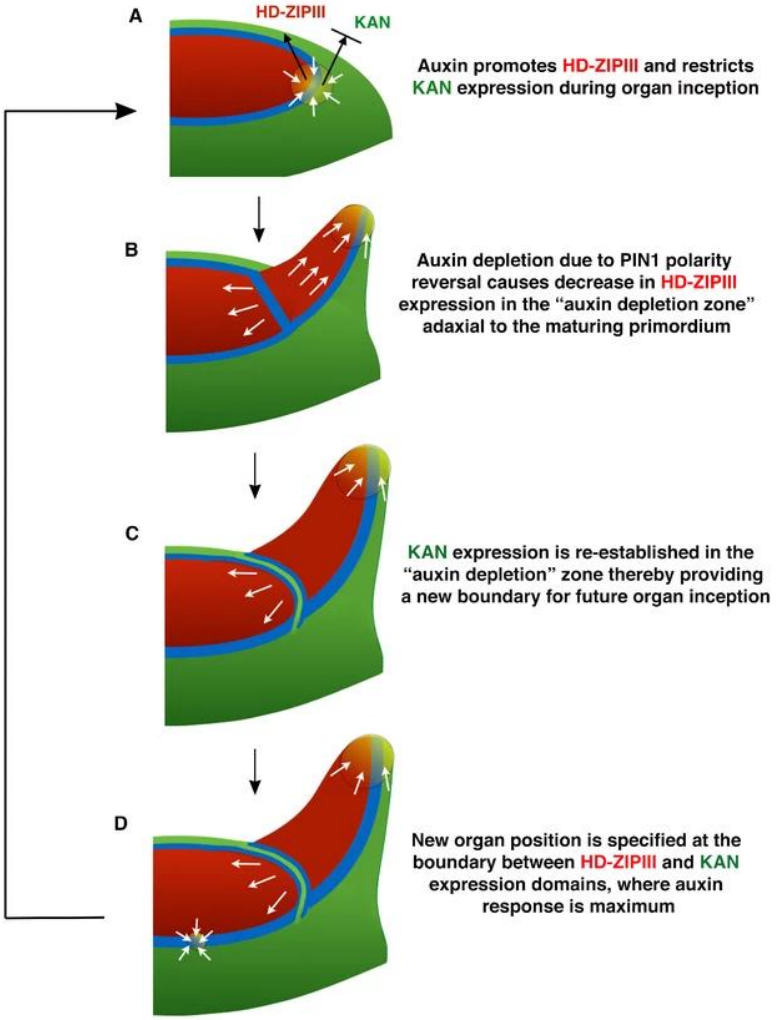
Caggiano *et al.*, 2017, eLife

Cell type boundaries organize plant development

Leaf orientation, morphology and position are pre-patterned by HD-ZIPIII and KAN gene expression in the shoot, leading to a model in which dorsoventral genes coordinate to regulate plant development by localizing auxin response between their expression domains.



vegetative shoot apical meristem



Materials:

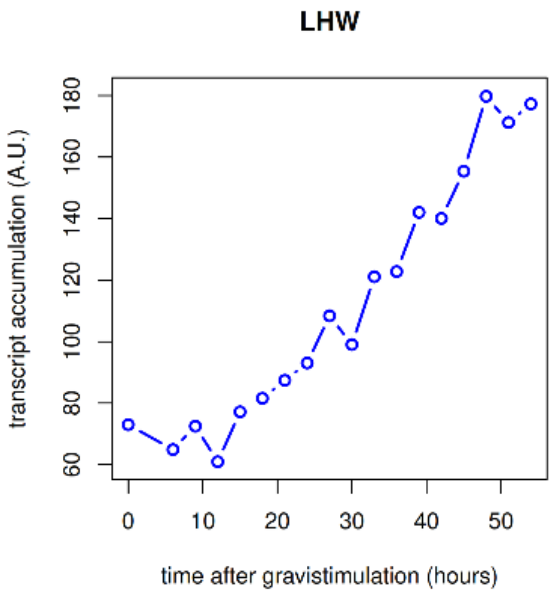
-REV-2 ×YPet (red), PIN1-CFP (blue) and KAN1−2 × GFP (green)

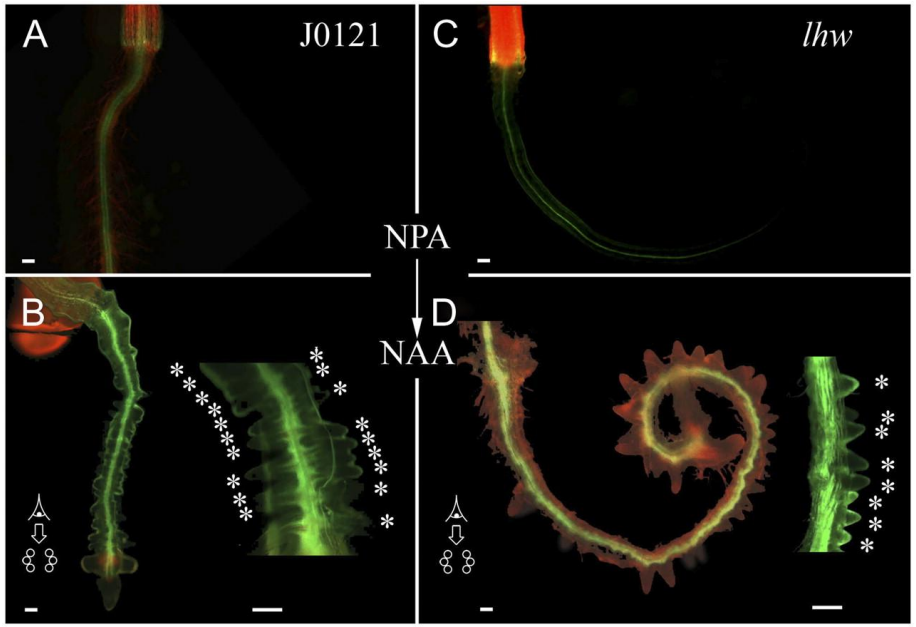
- pREV::REV-VENUS (Heisler et al., 2005)

LONESOME HIGHWAY, bHLH transcription factor

Parizot *et al.*, 2008, Plant Physiology

Quantitative losses in vascular bundle and pericycle heterogeneity appear intimately correlated: There is a concomitant loss of diarch and bilateral structures in *lhw* mutants.





Materials:

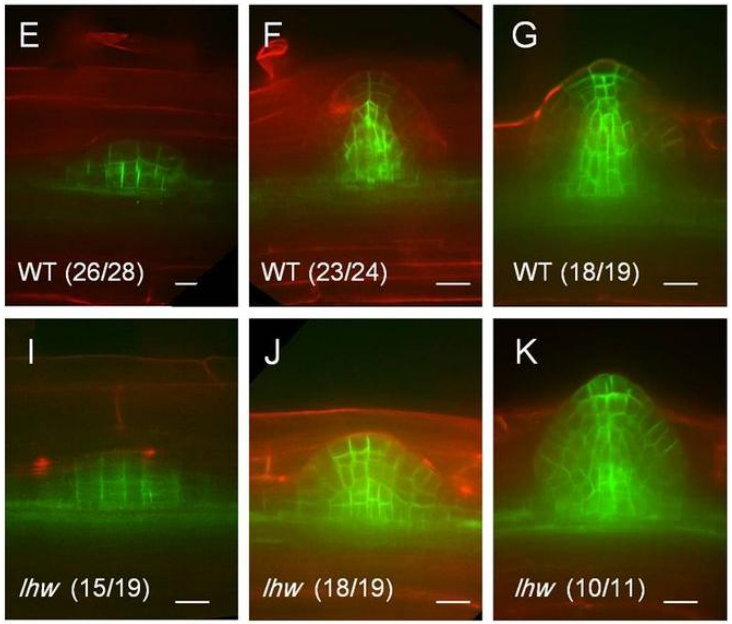
-

LONESOME HIGHWAY, bHLH transcription factor

Ito *et al.*, 2013, Development

LHW is required for proper asymmetric cell division to generate vascular initial cells as well as for the correct expression patterns of components related to auxin flow, such as PIN-FORMED 1 (PIN1), MONOPTEROS (MP) and ATHB-8, and ATHB-8 partially rescues the vascular defects of lhw. These results suggest that LHW functions as a key regulator to initiate vascular cell differentiation in association with auxin regulation.

PIN1::PIN1-GFP



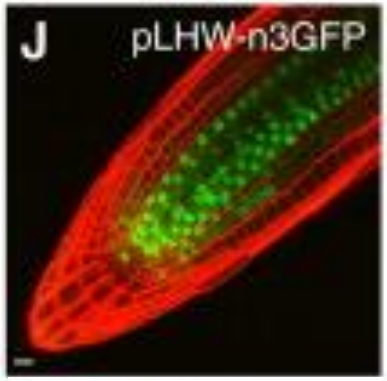
Materials:

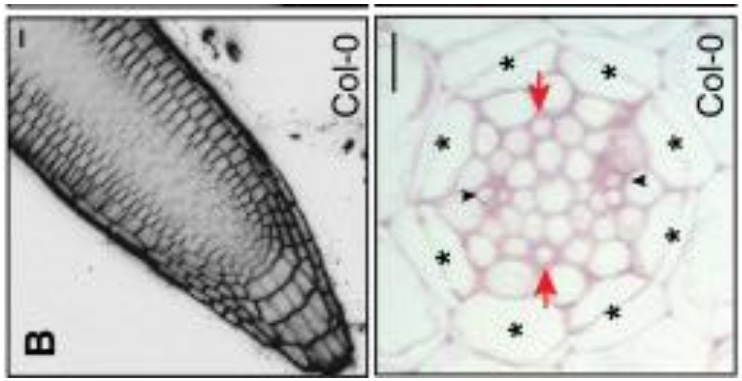
- *lhw (?) expressing PIN1::PIN1-GFP; PIN1::YFP-nls; DR5::GFP*
- estrogen-inducible *LHW expressing DR5::GFP*, *PIN1::YFP-nls* and *MP::YFP-nls (same ?)*

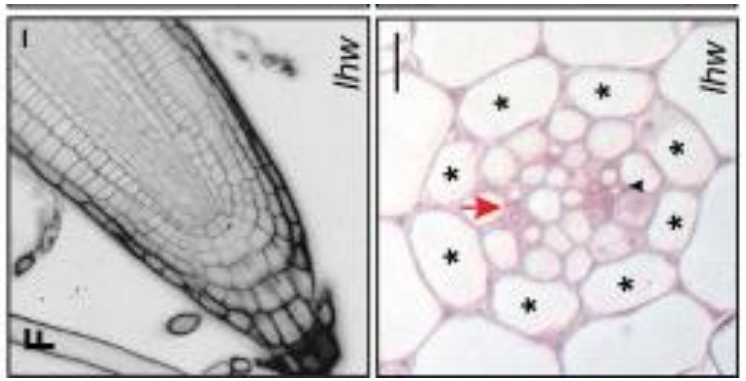
LONESOME HIGHWAY, bHLH transcription factor

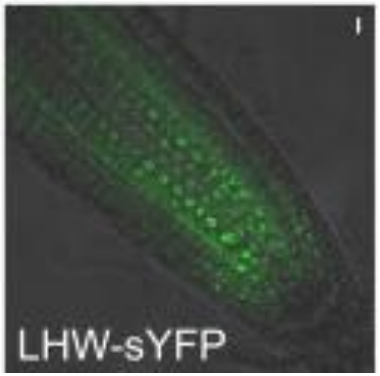
De Rybel *et al.*, 2013, Developmental Cell

A TMO5/LHW bHLH heterodimer controls plant vascular development. The dimer is necessary and sufficient for triggering periclinal cell division. Overlapping transcription patterns localize TMO5/LHW activity







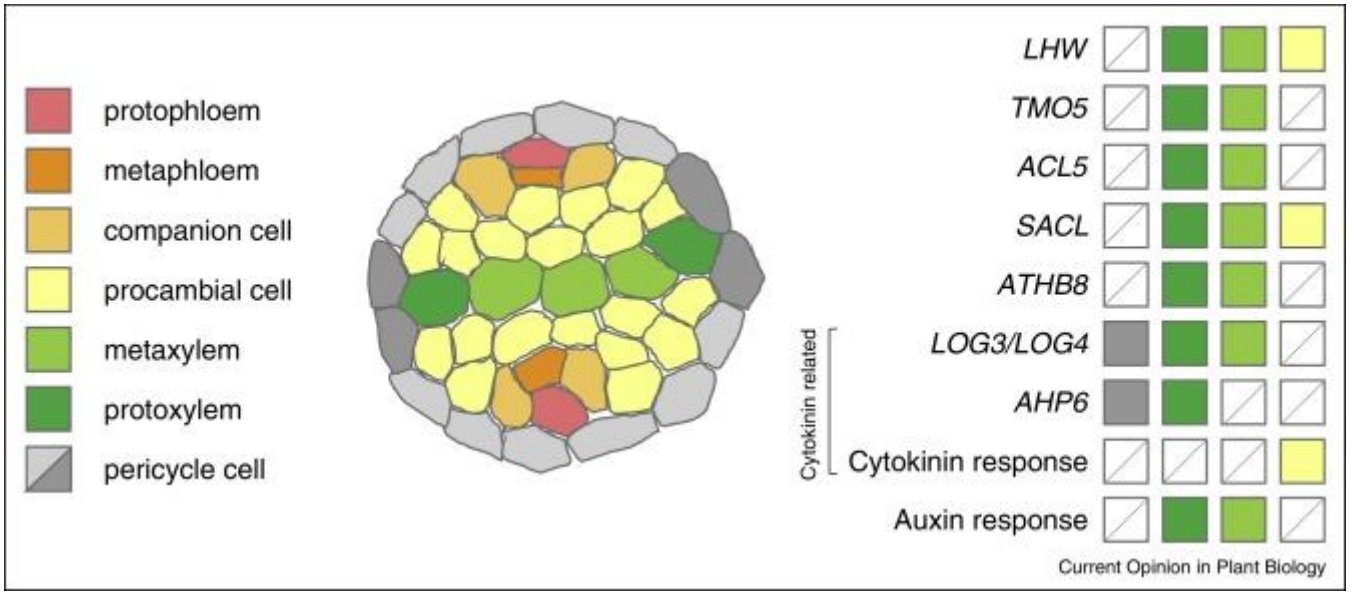


Materials:

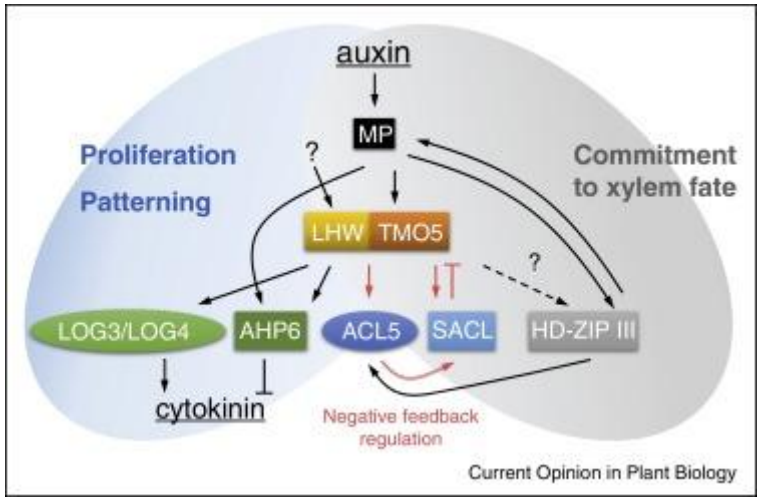
- *pLHW-n3GFP*
- *lhw* (SALK_023629*), and ll1 (*SALK_108940); *lhw ll1* double-mutant
- pLHW-LHW-YFP
- pRPS5A-LHW

LONESOME HIGHWAY, bHLH transcription factor

Vera_Sirera *et al.*, 2016 Current opinion in Plant Bioogy



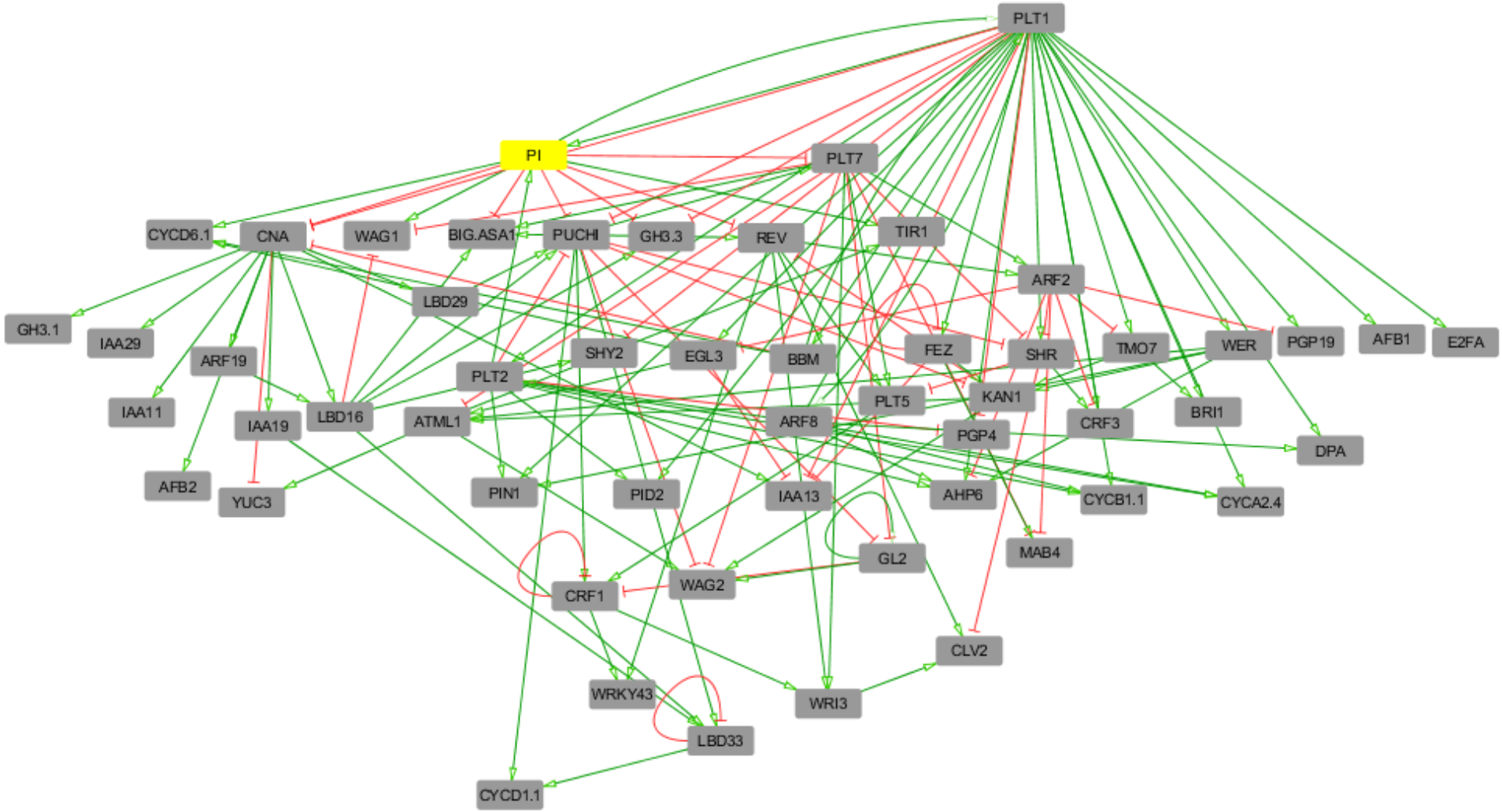
Active domains of key factors for vascular development in an Arabidopsis root apical meristem.



Premières analyses Cytoscape 3.7/
Set de gènes issus du TDCor (sept 2018)

TDCor6.32_output_221018_parallel - Copie_cytoscape_ **Outgoing 2step PI**

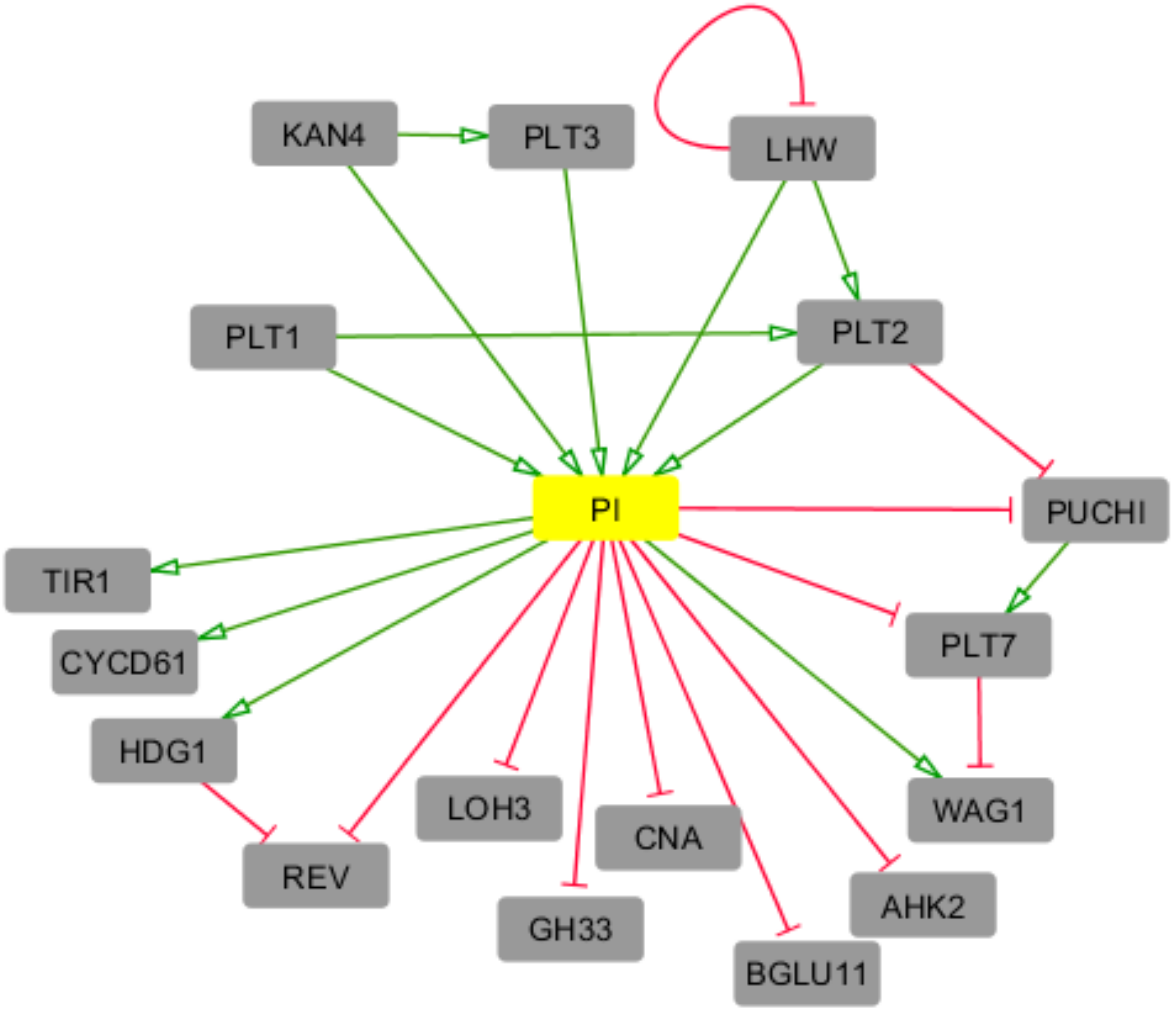
135 nodes, 496 edges ***55 nodes, 136 edges***



Premières analyses Cytoscape 3.7/
Set de gènes issus du TDCor (sept 2018)

TDCor6.32_output_l_gnp-18july29-copy_ **Undirected node PI**

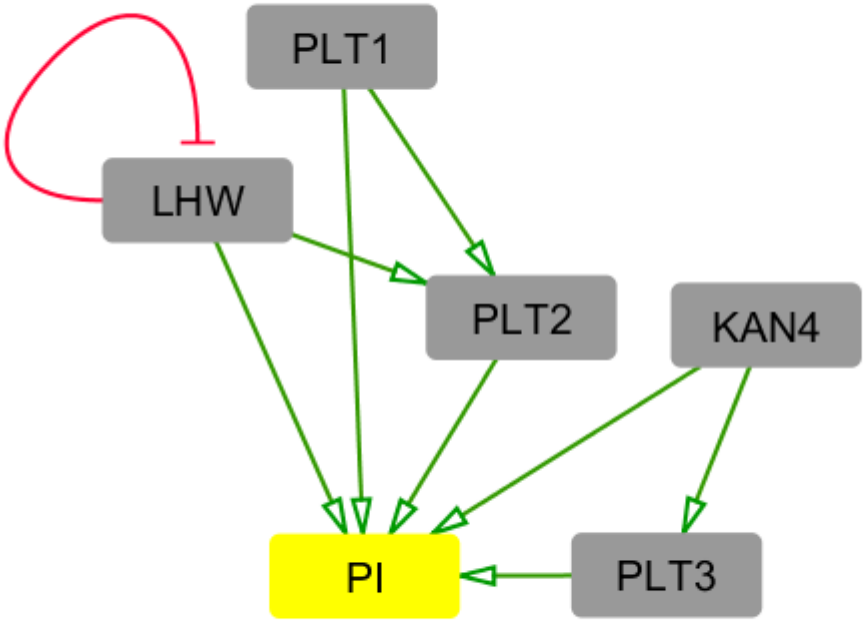
281 nodes, 1216 edges ***18 nodes, 25 edges***



Premières analyses Cytoscape 3.7/
Set de gènes issus du TDCor (sept 2018)

TDCor6.32_output_l_gnp-18july29-copy_ **Incoming 1step PI**

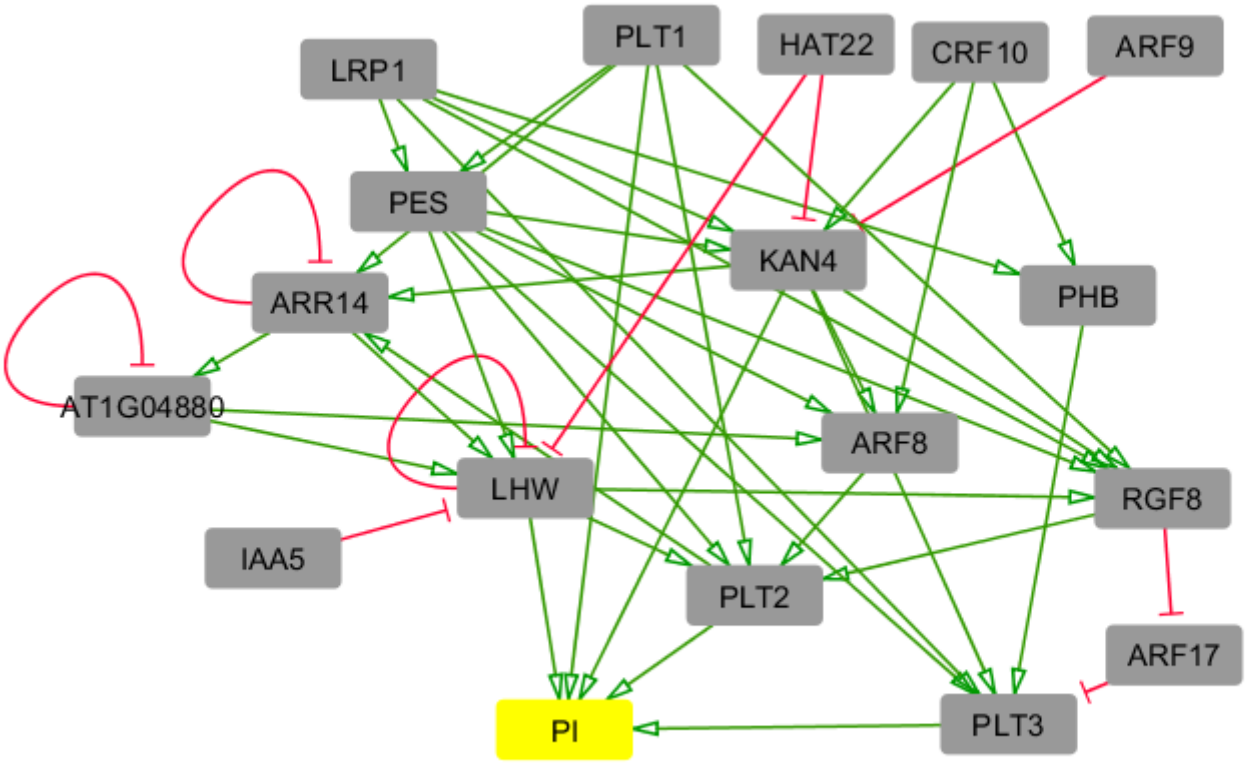
281 nodes, 1216 edges ***6 nodes, 9 edges***



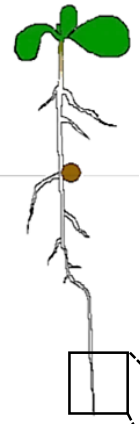
Premières analyses Cytoscape 3.7/
Set de gènes issus du TDCor (sept 2018)

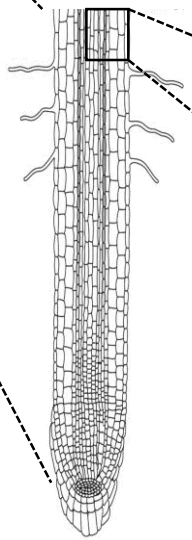
TDCor6.32_output_l_gnp-18july29-copy_ **Incoming 2 step PI**

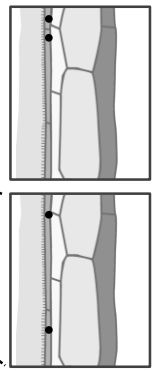
281 nodes, 1216 edges ***18 nodes, 46 edges***



Lateral root development in *Arabidopsis thaliana*







VIII
VII
VI
V
IV
III
II
I

Emergence

Meristem patterning

Quiescent center establishment

LR outgrowth

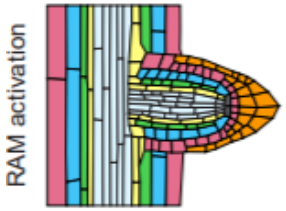
Early morphogenesis phase

Initiation

LRFC specification

Prebranch site

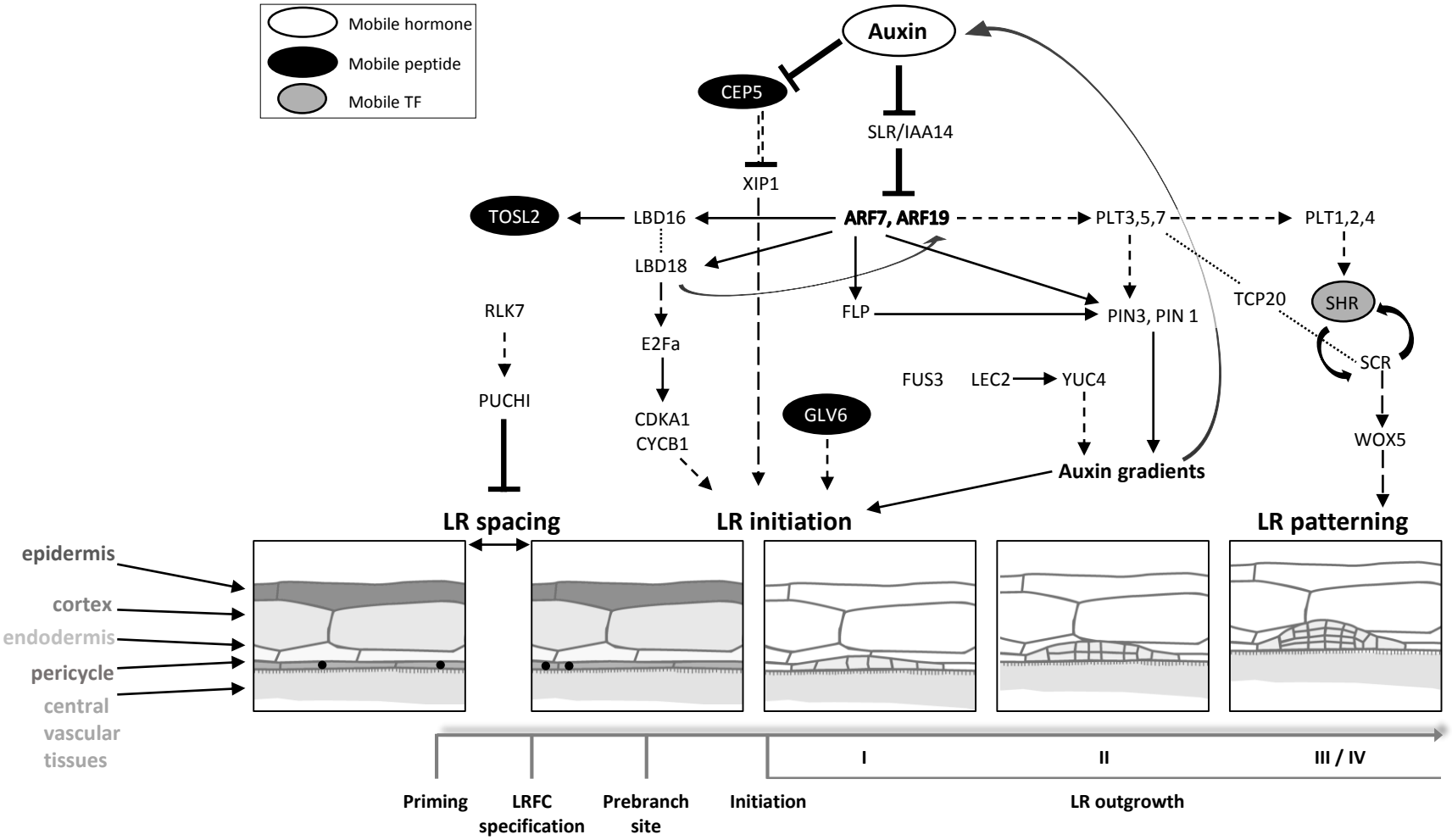
Priming



54h gravistimulation

Mallamy and Benfey, 1997; Casimiro *et al.,* 2003; De Smet *et al.,* 2007; Péret *et al.,* 2012; Lucas *et al.,* 2013; Du and Scheres, 2017; Goh *et al.,* 2016

Feeding GRN with spatial and temporal information result in robust spacing and patterning of developing LRP



Banda *et al*., 2019

The ARGOS module

Reference state flow for a given combination of model parameters (random initial state to final state)

Genes	A	B	C	D	E	…
Initial State 1	0	1	0	1	1	…
Final State 1	0	0	1	0	1	…

The ARGOS module

Reference state flow for a given combination of model parameters (random initial state to final state)

Genes	A	B	C	D	E	…
Initial State 1	0	1	0	1	1	…
Final State 1	0	0	1	0	1	…

Mutate 1 gene (KO or OA) and compute mutant final state

Genes	***A***	B	C	D	E	…
Initial State 1*	**1**	1	0	1	1	…
Final State 1*	**1**	0	0	1	1	…

The ARGOS module

Reference state flow for a given combination of model parameters (random initial state to final state)

Genes	A	B	C	D	E	…
Initial State 1	0	1	0	1	1	…
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Initial State 1*	**1**	1	0	1	1	…
Final State 1*	**1**	0	0	1	1	…

Compute distance between ref final state and mutant final state
e.g. : d = 0,75 (75% different)

The ARGOS module

Reference state flow for a given combination of model parameters (random initial state to final state)

Genes	A	B	C	D	E	…
Initial State 1	0	1	0	1	1	…
Final State 1	0	0	1	0	1	…

Mutate 1 gene (KO or OA) and compute mutant final state

Genes	***A***	B	C	D	E	…
Initial State 1*	**1**	1	0	1	1	…
Final State 1*	**1**	0	0	1	1	…

Compute distance between ref final state and mutant final state
e.g. : d = 0,75 (75% different)

ARGOS does this :

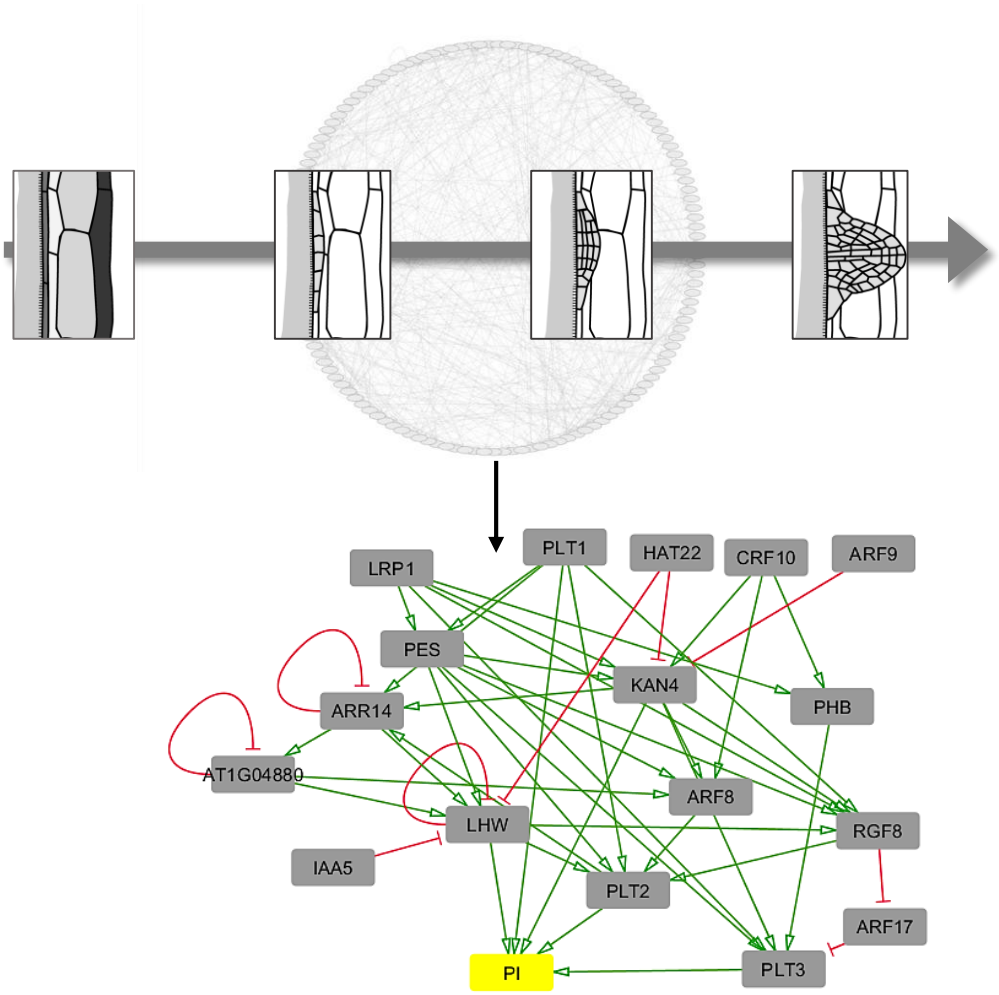
- For each gene,
- For KO and OA,
- For all possible combinations of model parameters,
- For a set of initial states

And return for each gene the mean of all distances for KO and OA

Approach and example of application *in vivo*:

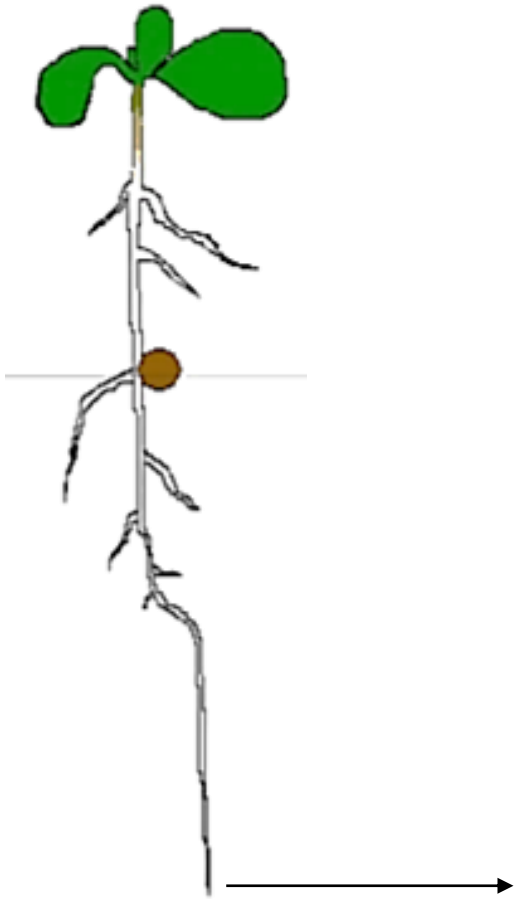
PISTILLATA

Understanding and manipulating functional organisation in developing LR primordia of *A. thaliana*

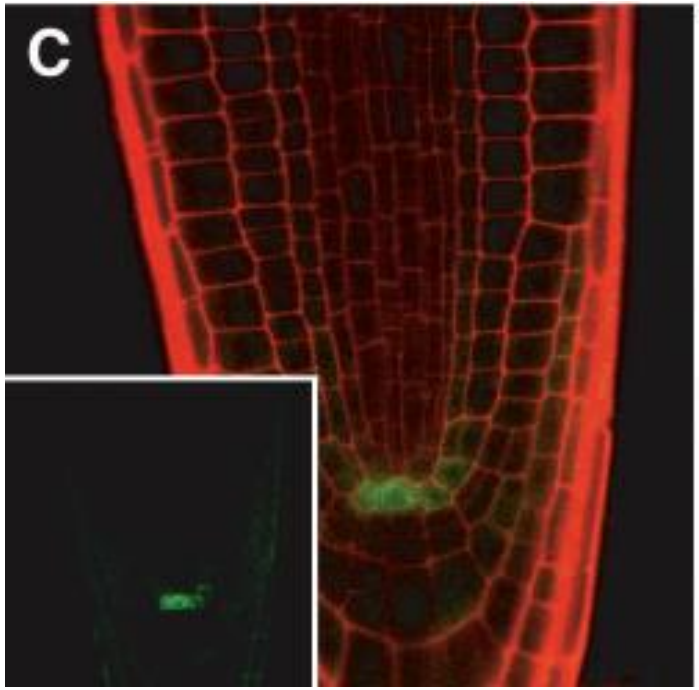


→ Identify and validate new candidates involved in QC formation

Looking for a new quiescent Center marker gene, PISTILLATA:GFP (PI:GFP)



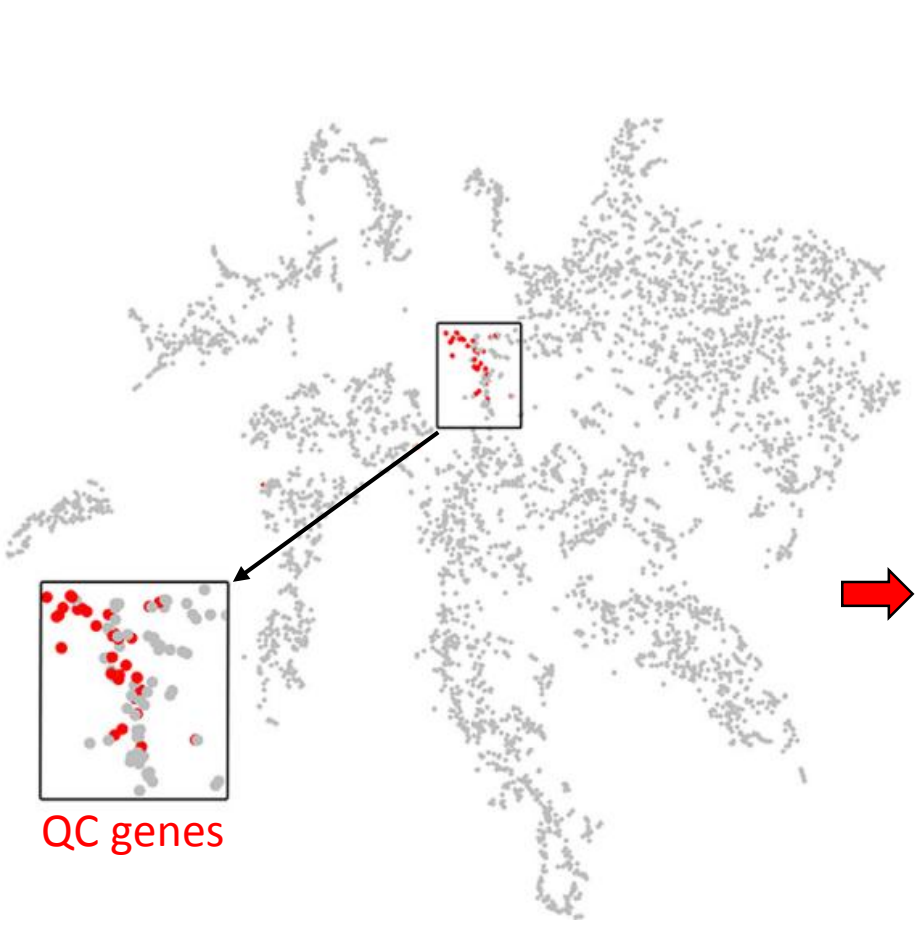
PI:GFP

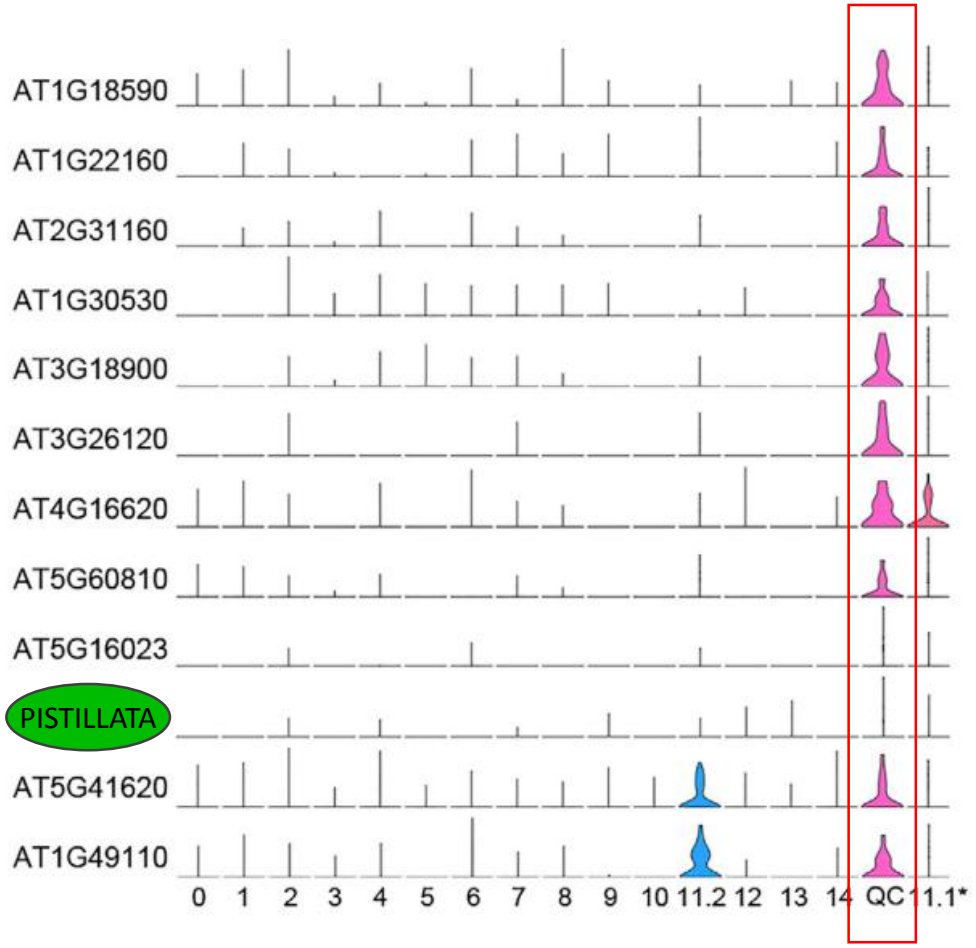


→ **PI : GFP expressed in the QC of primary root**

Nawy *et al.*, 2005

Single-cell RNA Sequencing in root identified 12 quiescent center specific genes

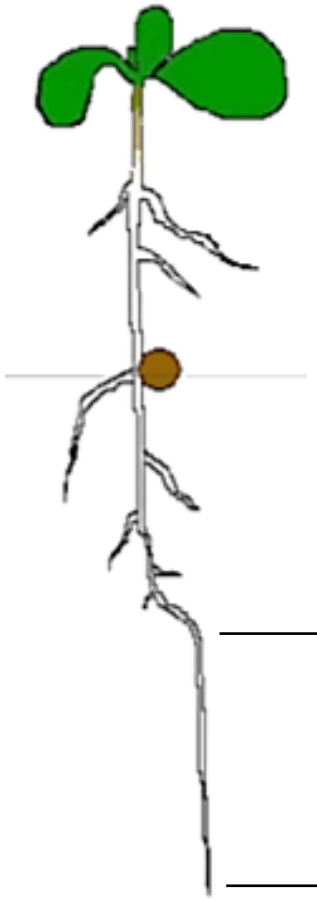


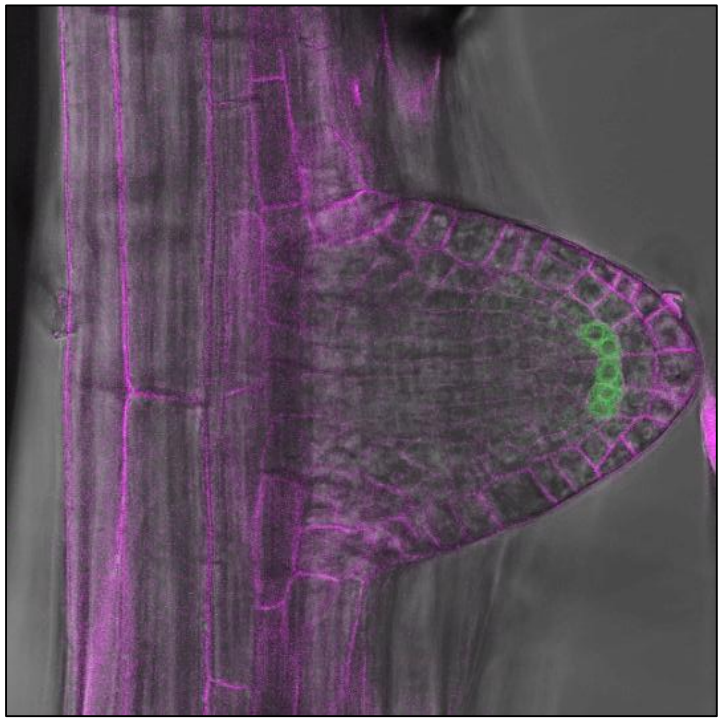


→ **Confirms that PISTILLATA is a QC-specific gene**

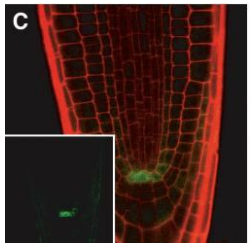
Denyer *et al.,* 2019

PI:GFP expression in the LR primordia ?





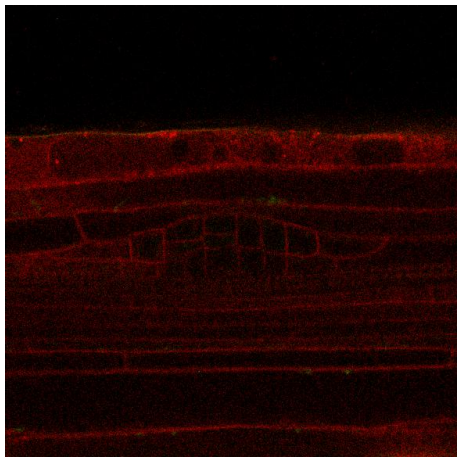
PI::GFP

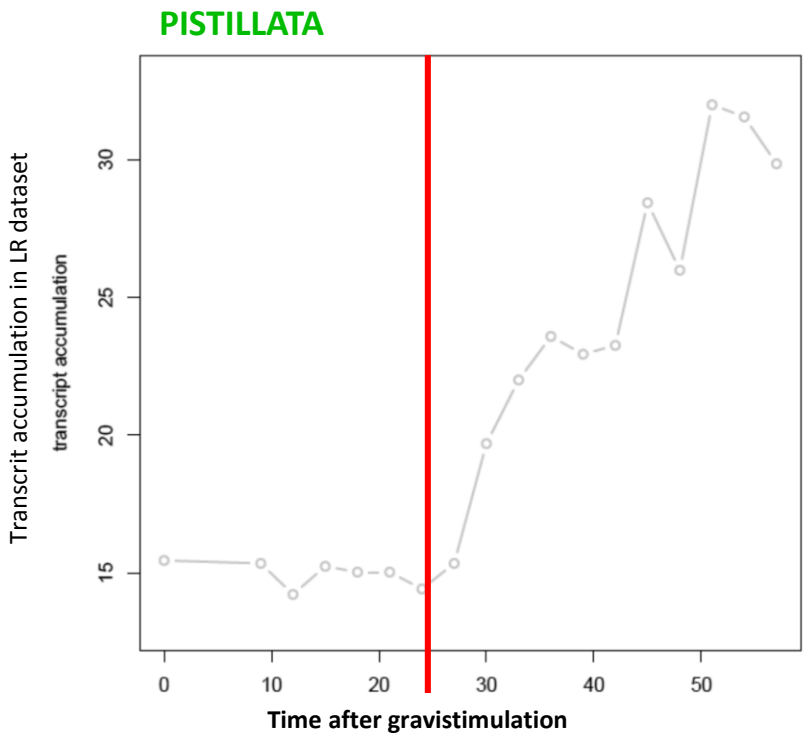


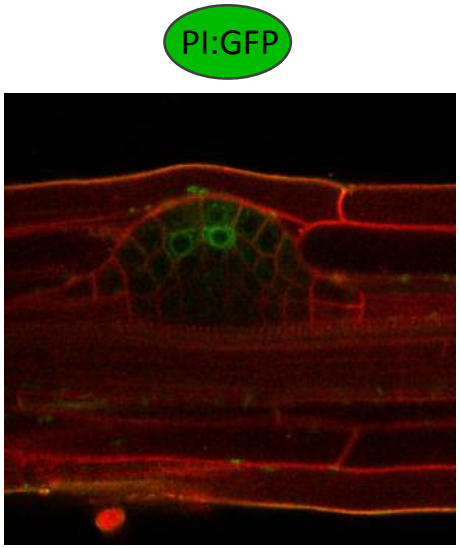
→ **PI::GFP expressed in the QC of primary root AND lateral root**

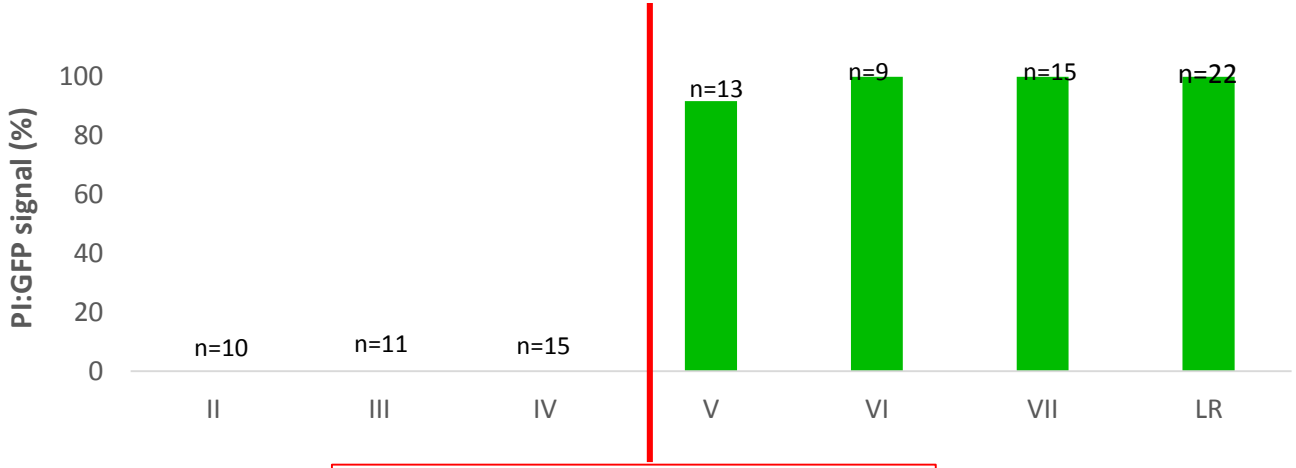
Nawy *et al.*, 2005

PI:GFP signal appears following the quiescent center establishment







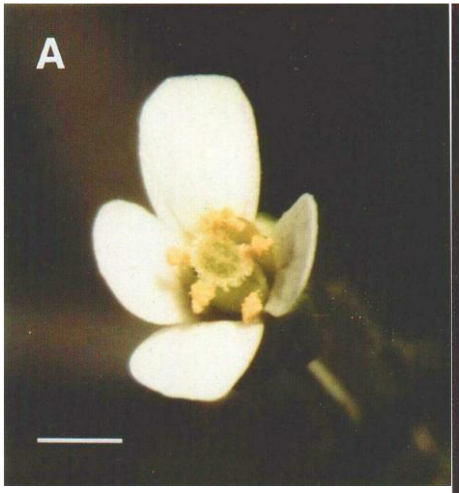


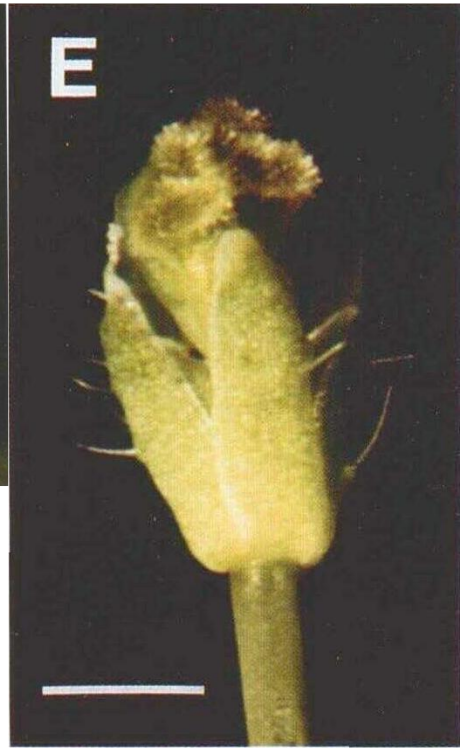
Quiescent center establishment

Voβ *et al.*, 2015

AT5G20240: PISTILLATA (PI)

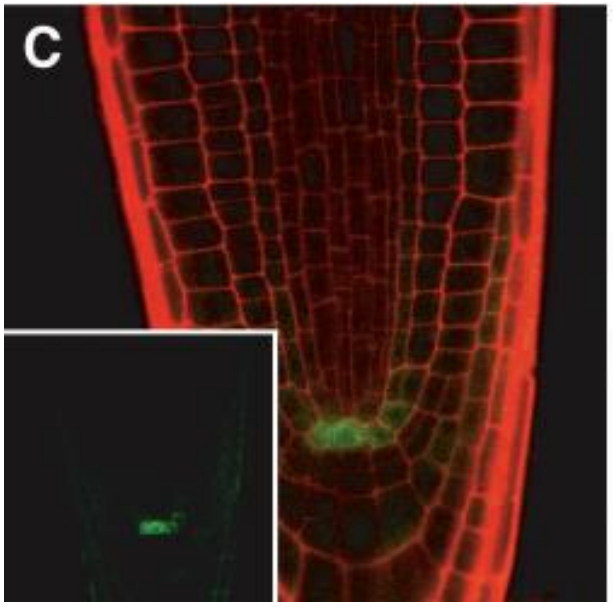
Floral homeotic gene encoding a MADS domain transcription factor. Required for the specification of petal and stamen identities.





pi-1

Class B mutant, outer whorls of sepals and an abnormally large gynoecium

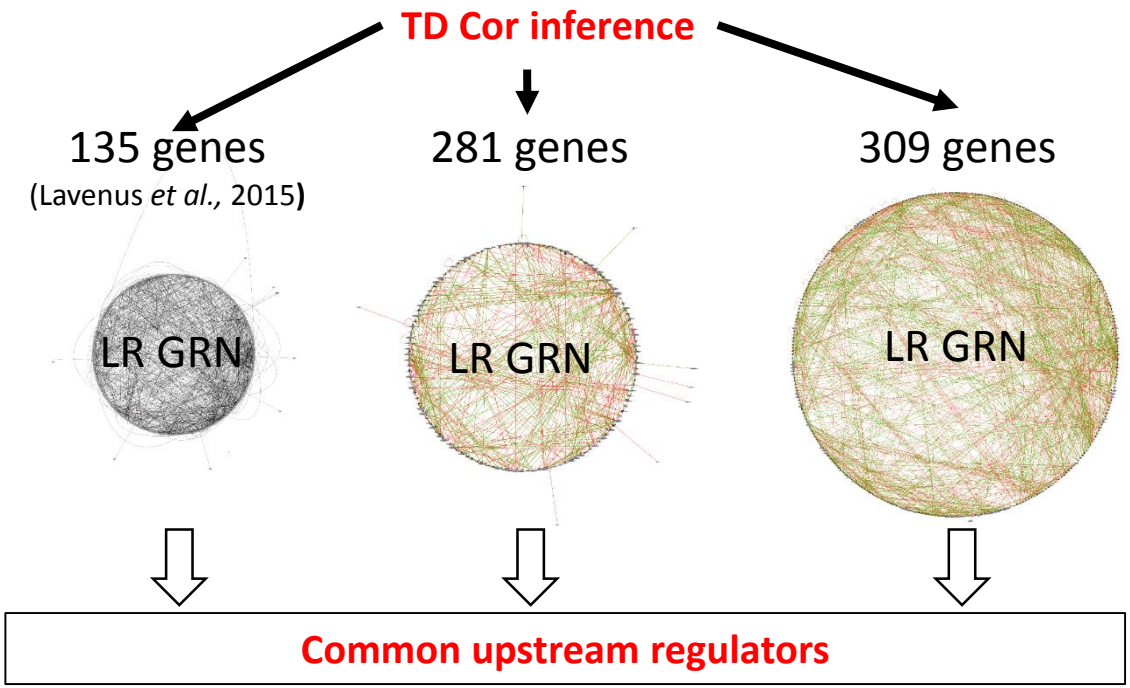


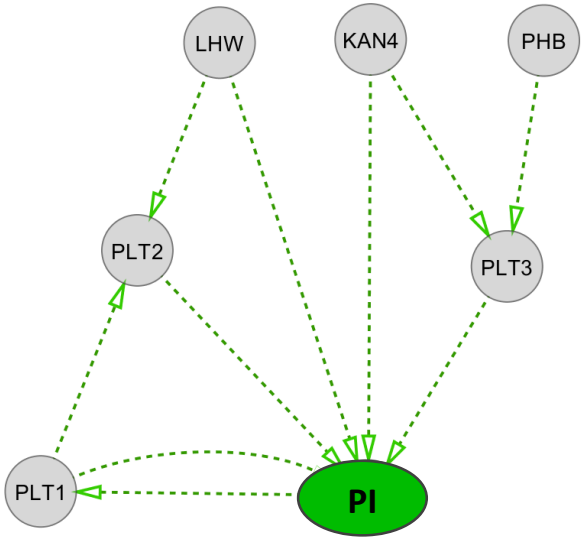
PI : GFP
expressed in the QC of primary root.

No LR phenotype

Bowman *et al.,* 1990; Smyth *et al.,* 1990; Krizek and Meyerowitz, 1996; Nawy *et al.,* 2005

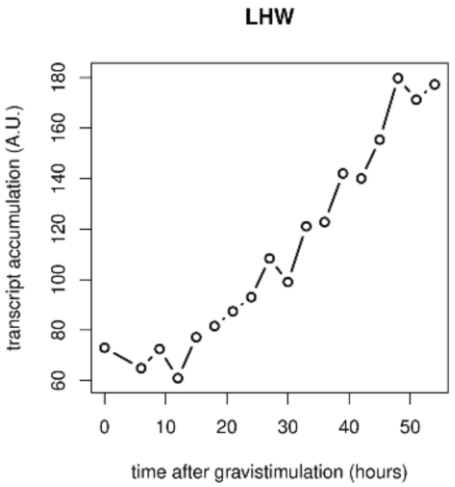
Upstream regulators of PI involved in meristem patterning ?

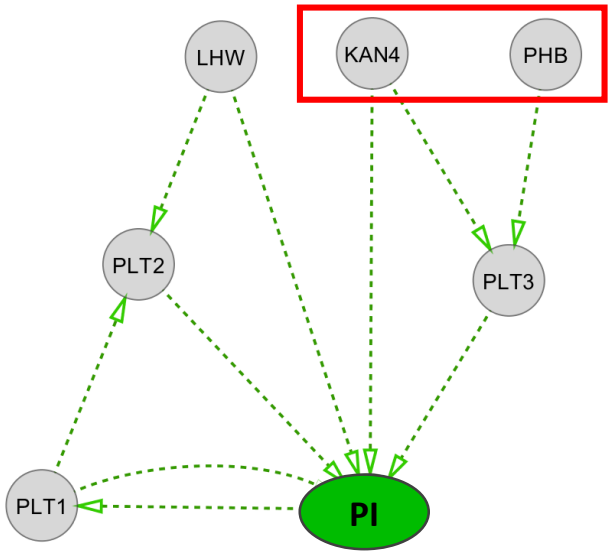


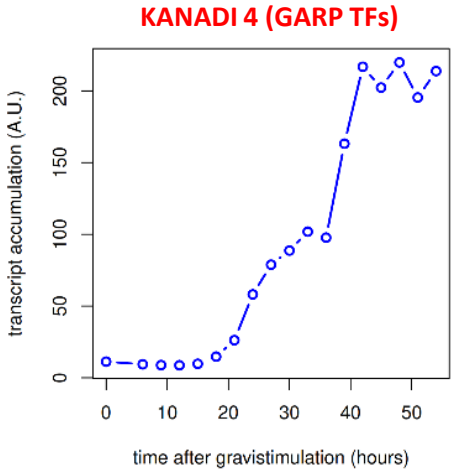


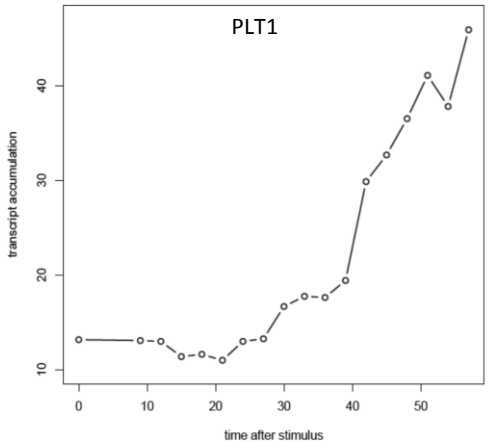
Voβ *et al.*, 2015; Lavenus *et al.*, 2015

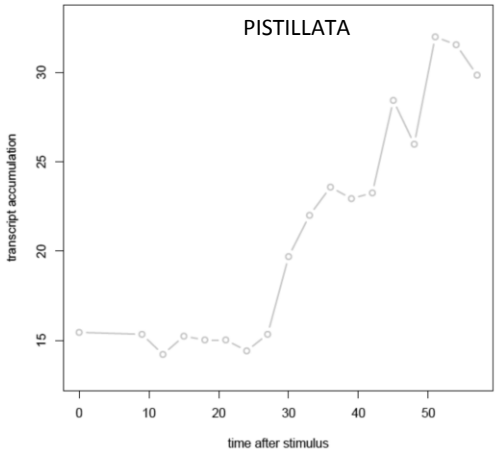
Upstream regulators of PI involved in meristem patterning ?

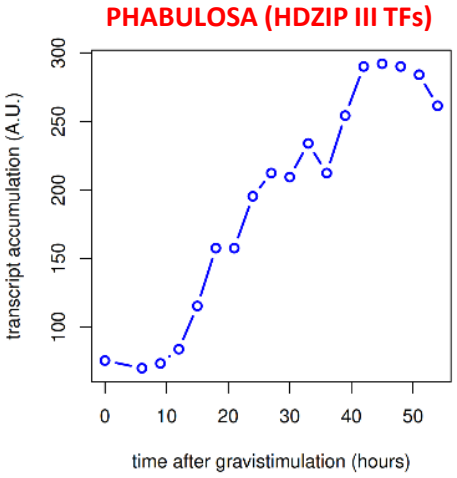












Voβ *et al.*, 2015

KANADI (4) and HDZIP III (5) TFs play antogonists role in development

- Act antagonistically to each other to regulate organs patterning
  - To establish the bilateral symmetry during embryo patterning (auxin)
  - To coordinate leaf dorso-ventral patterning by spatiallized expression domains (auxin)

Leon-Kloosterziel, *et al.,* 1994; Emery *et al.,* 2003; Hawker et al., 2004; Mc Abee et al., 2006; Kelley et al., 2009; Caggiano *et al.,* 2017

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Antagonists role during LR meristem patterning ?

Leon-Kloosterziel, *et al.,* 1994; Emery *et al.,* 2003; Hawker et al., 2004; Mc Abee et al., 2006; Kelley et al., 2009; Caggiano *et al.,* 2017

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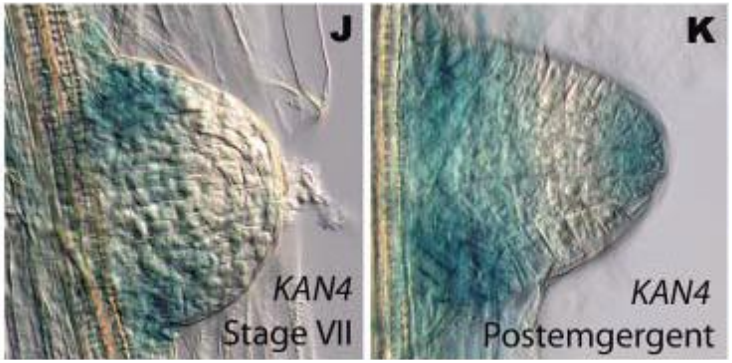
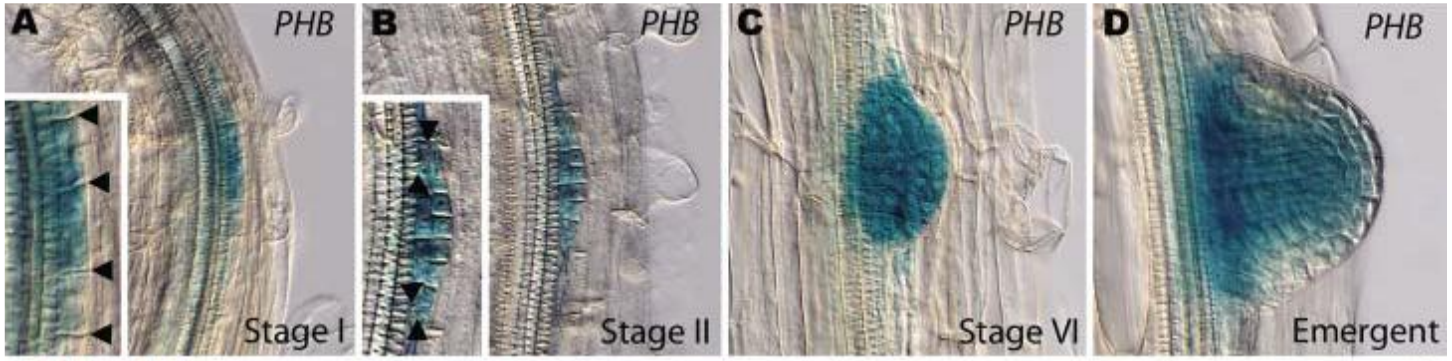
Antagonists role during LR meristem patterning ?

KAN4



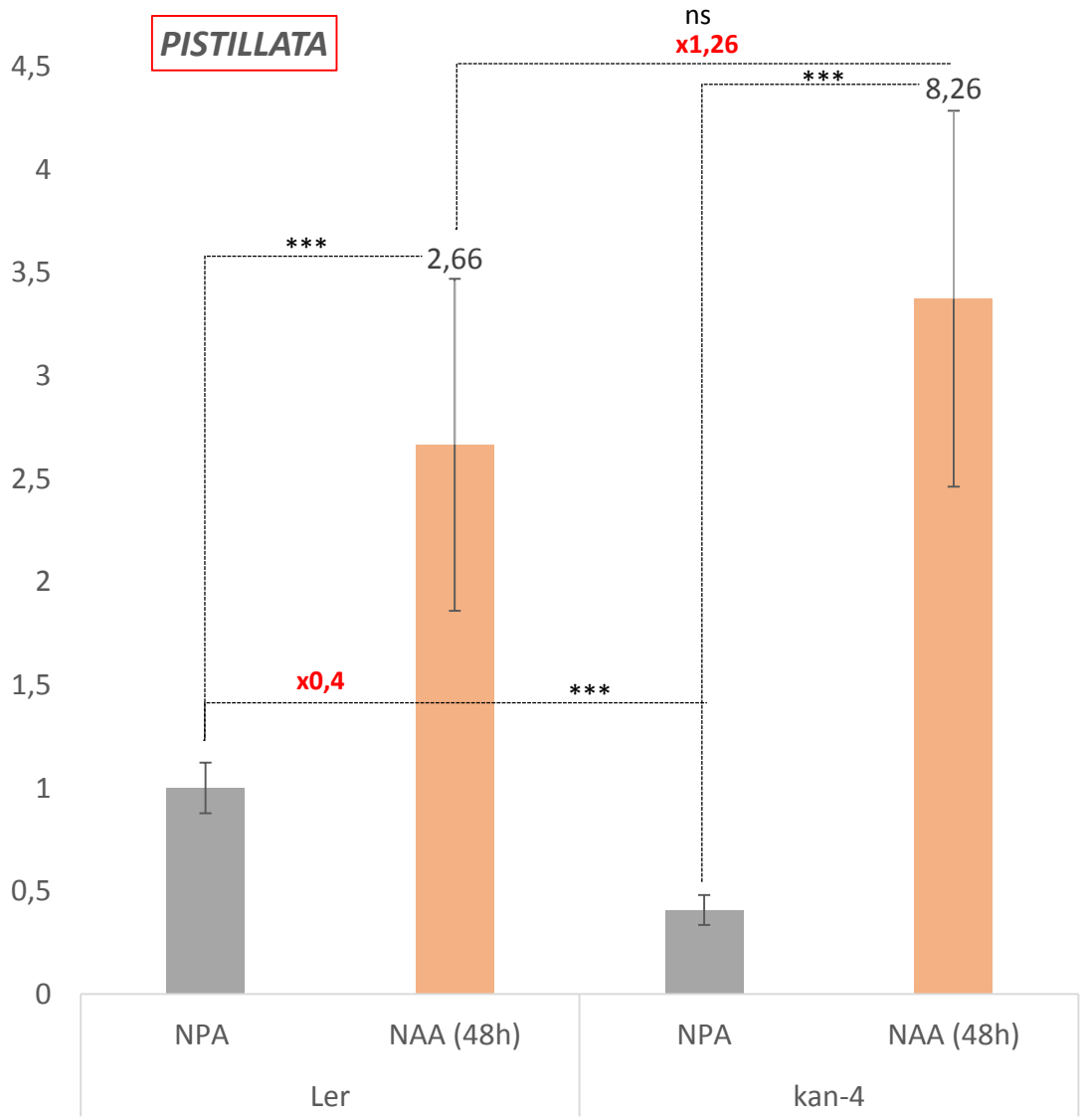
PHB



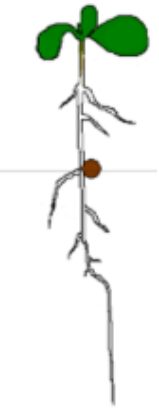
Leon-Kloosterziel, *et al.,* 1994; Emery *et al.,* 2003; Hawker et al., 2004; Mc Abee et al., 2006; Kelley et al., 2009; Caggiano *et al.,* 2017

PISTILLATA expression seems to not be changed in *kan4-1* during LRIS

LRIS: 14d-old seedlings
NPA/NAA (48h)

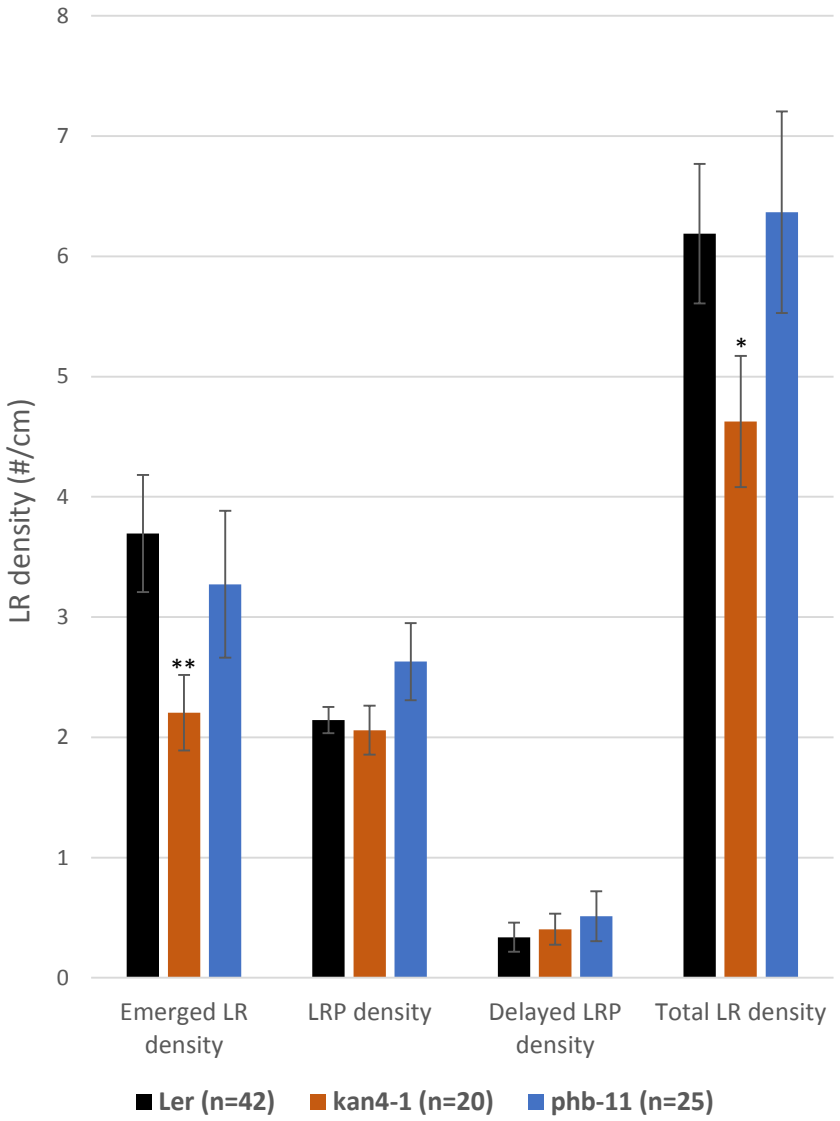


1 biological replicats (n>50 seedlings/ technical replicats), Student tests p*** < 0,01



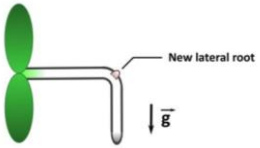
kan4-1 formed less LR primordium

LR Index



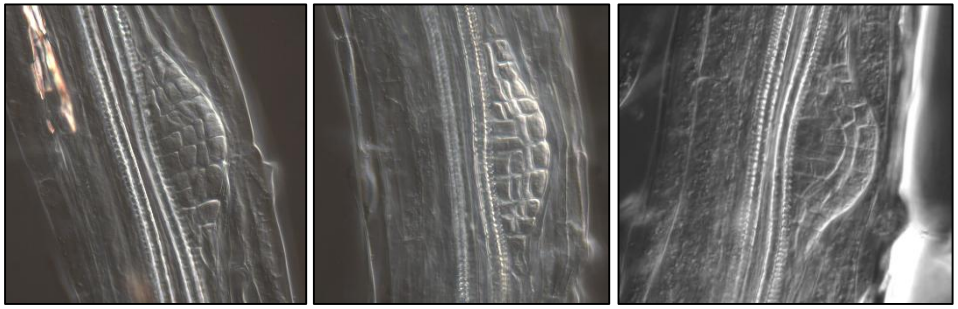
3-5 independant biological replicats (n>20 seedlings), Student tests p** < 0,02

Gravistimulation



kan4-1 and phb-11 show delayed LRPs

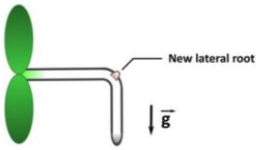
Ler | *kan4-1* | *phb-11*

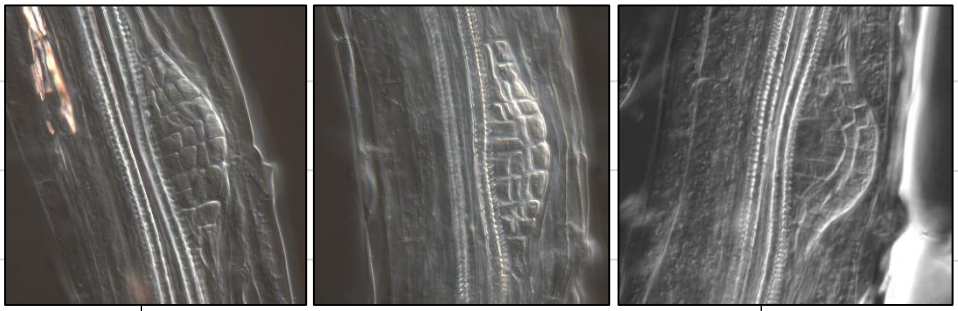


No difference morphologically

Gravistimulation

kan4-1 and phb-11 show delayed LRPs

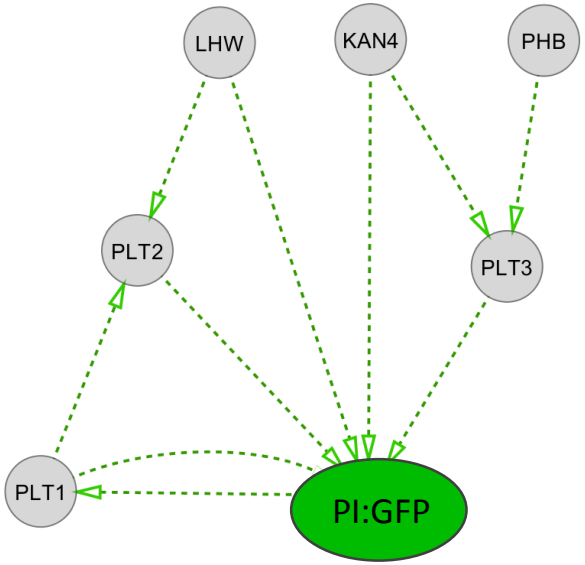


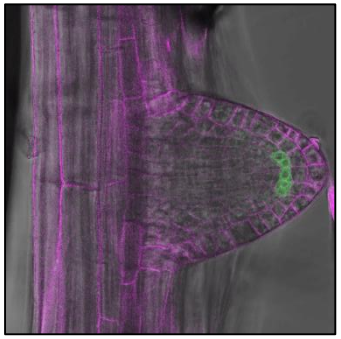


3-5 independant biological replicats, Chi² test p***<0,01

Do kan4, phb, lhw mutations impact QC marker genes expression ?

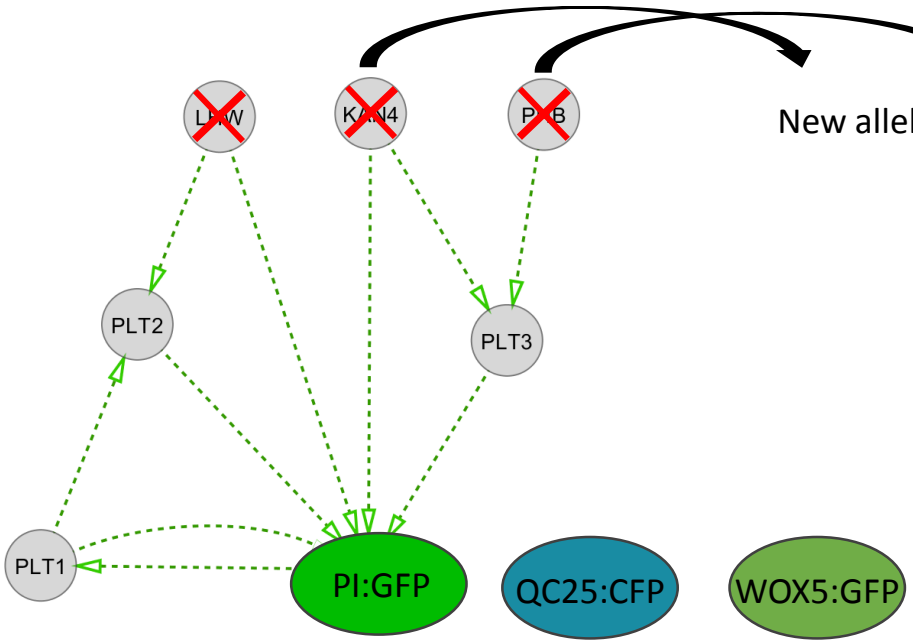
Next steps

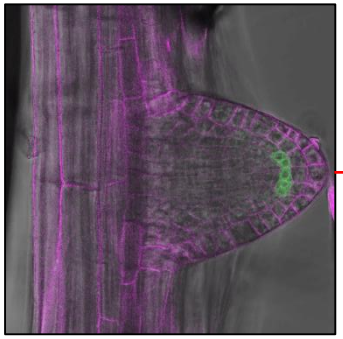




Do kan4, phb, lhw mutations impact QC marker genes expression ?

Next steps





QC ON/OFF *in vivo* ?