Certificate of Appreciation

We hereby express our sincere appreciation to:

Widi Sunaryo, Ph.D.

as:

Oral Presenter

in the International Joint Symposium of
ASEAN Youth Exchange on Biotechnology of Biomass Utilization
for ASEAN Development

Samarinda, July 27th, 2015

Assoc. Prof. Hunsa Punnapayak
Chulalongkorn University

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Mulawarman University

Genetic perpectives of wood formation explained by a weed

Presented at The International Joint Symposium of ASEAN Youth Exchange on Biotechnology of Biomass Utilization for ASEAN Development between Mulawarman University, Indonesia and Culalongkorn University, Thailand

Samarinda, 27 July 2015

Widi Sunaryo, Ph.D

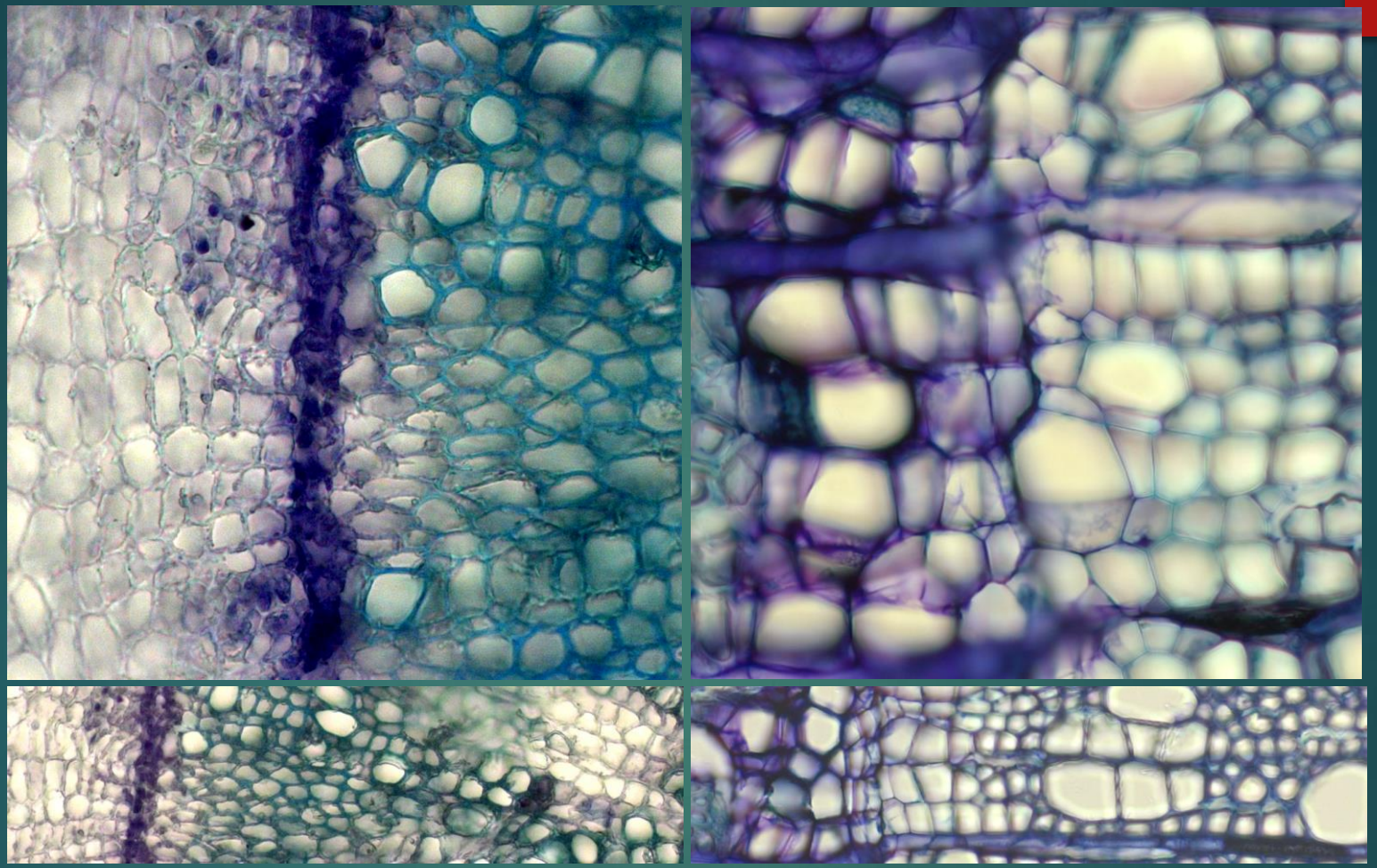
Laboratory of Plant Biotechnology
Faculty of Agriculture, Mulawarman University

Is Arabidopsis the better tree?

A weed: *Arabidopsis thaliana*

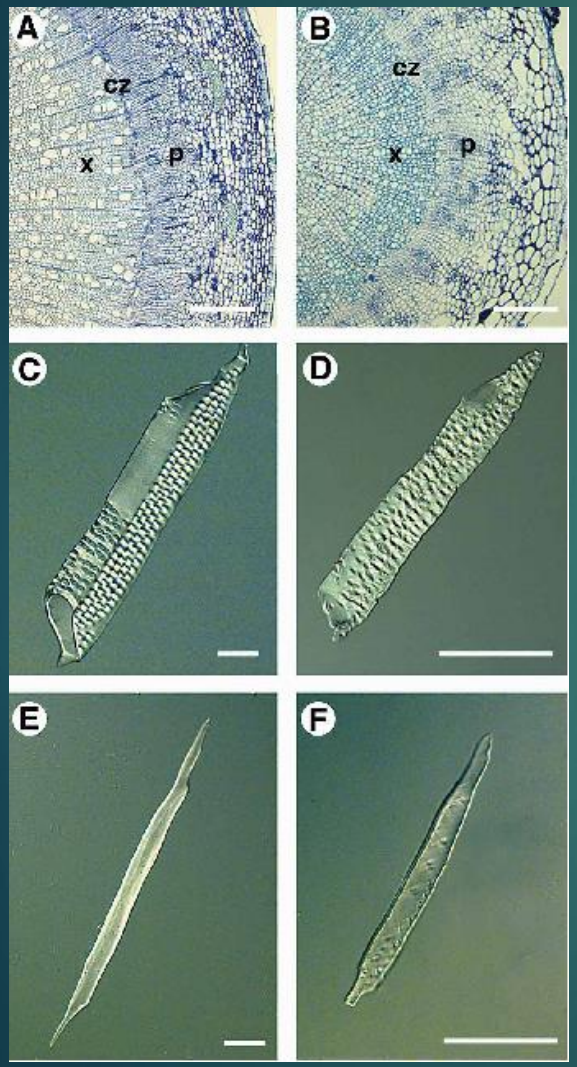
Angiosperm tree

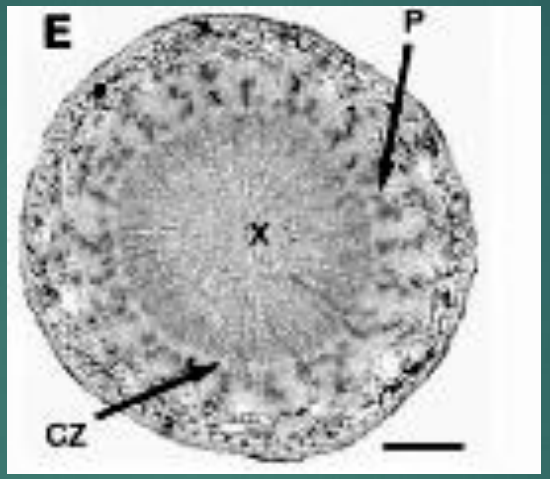
Arabidopsis hypocotyl vs poplar stem

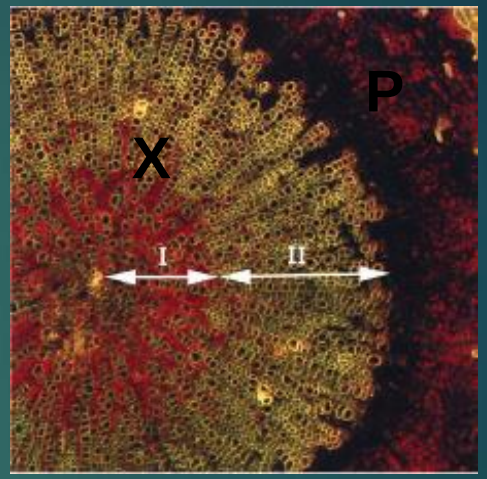


(Sunaryo, 2010)

Vascular cambium and secondary growth in *Arabidopsis* hypocotyls are a model for wood formation (Chaffey et al. 2002)



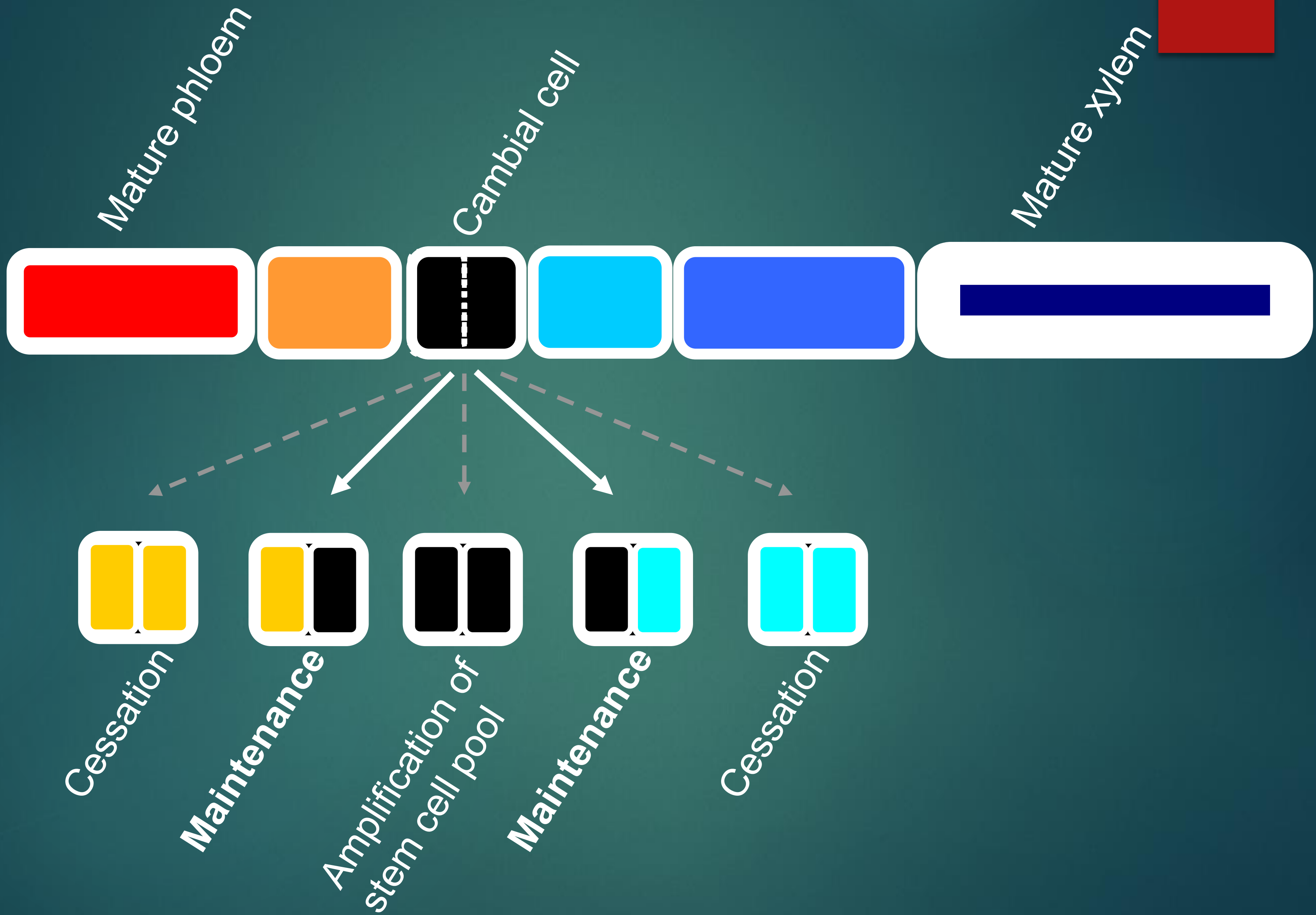




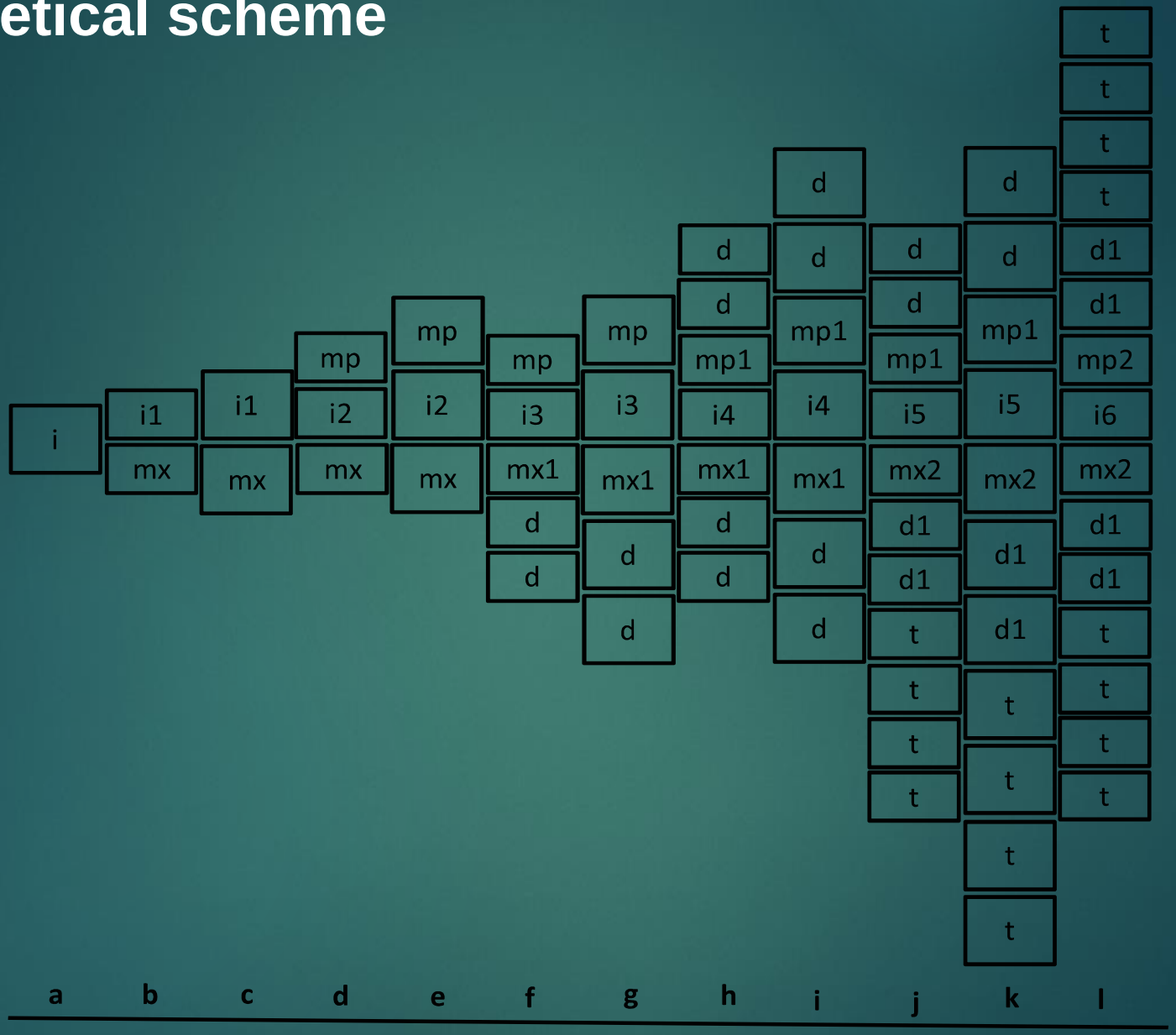
CZ: Cambial Zone, X: Xylem, P: Phloem

Secondary Growth Anatomycal Characteristics	*Arabidopsis*	*Poplar*
Xylem:		
1. Vessel elements	Yes	Yes
2. Fibre cells	Yes	Yes
2. Parenchyma cells	Yes	Yes
2. Rays	No	Yes
Phloem:		
1. Sieve tube elements	Yes	Yes
2. Companion Cells	Yes	Yes
3.Parenchyma cells	Yes	Yes

Fate determination



Formation of secondary xylem and phloem in a theoretical scheme



The cambial cell and its derivates. Redrawn and modified Sunaryo (2010) rom Evert (2006). (i) Initials, (mx) mother cells of xylem, (mp) mother cells of phloem, (d) daughter cells, (t) tissue cells derived from daughter cells. (a-l) time events.

Functional genomic study in tree

↓

Major obstacles:
-Slow growth
- Long generation times

↓

Production of mutants is laborious, inefficient, and unspecific.

↓

Profound understanding of molecular and genetic control regulating secondary xylem development is still lacking

Functional genomic study

Basic strategy:

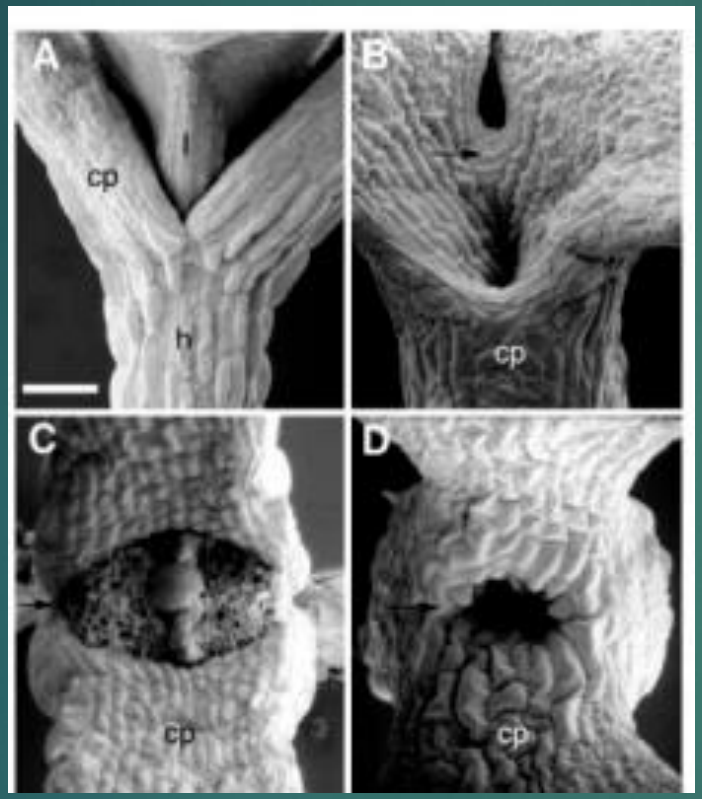
Down regulation/mutation

Upregulation/ Overexpression

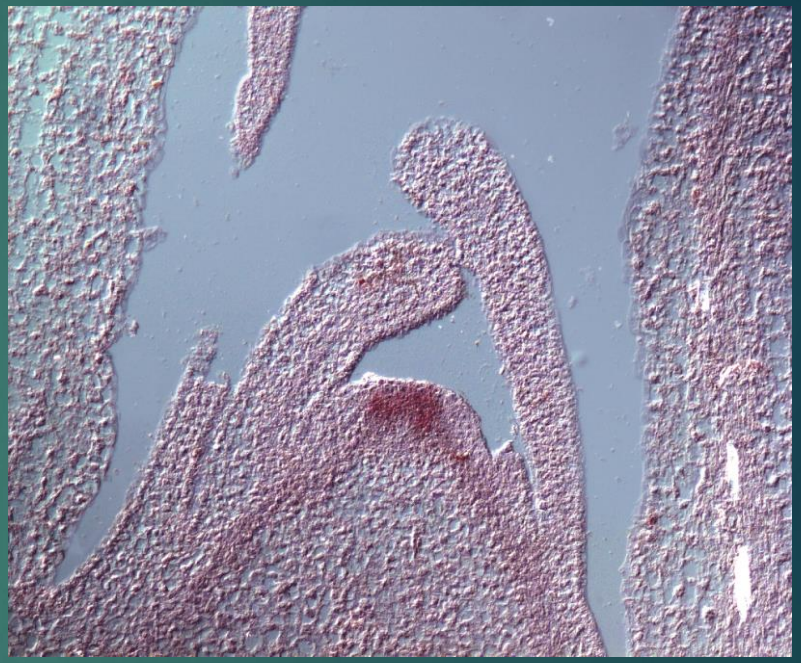
Production of mutants is laborious, inefficient, and unspecific.

Profound understanding of molecular and genetic control regulating secondary xylem development is still lacking

Shoot Meristemless, *STM,* keeps cells undifferentiated

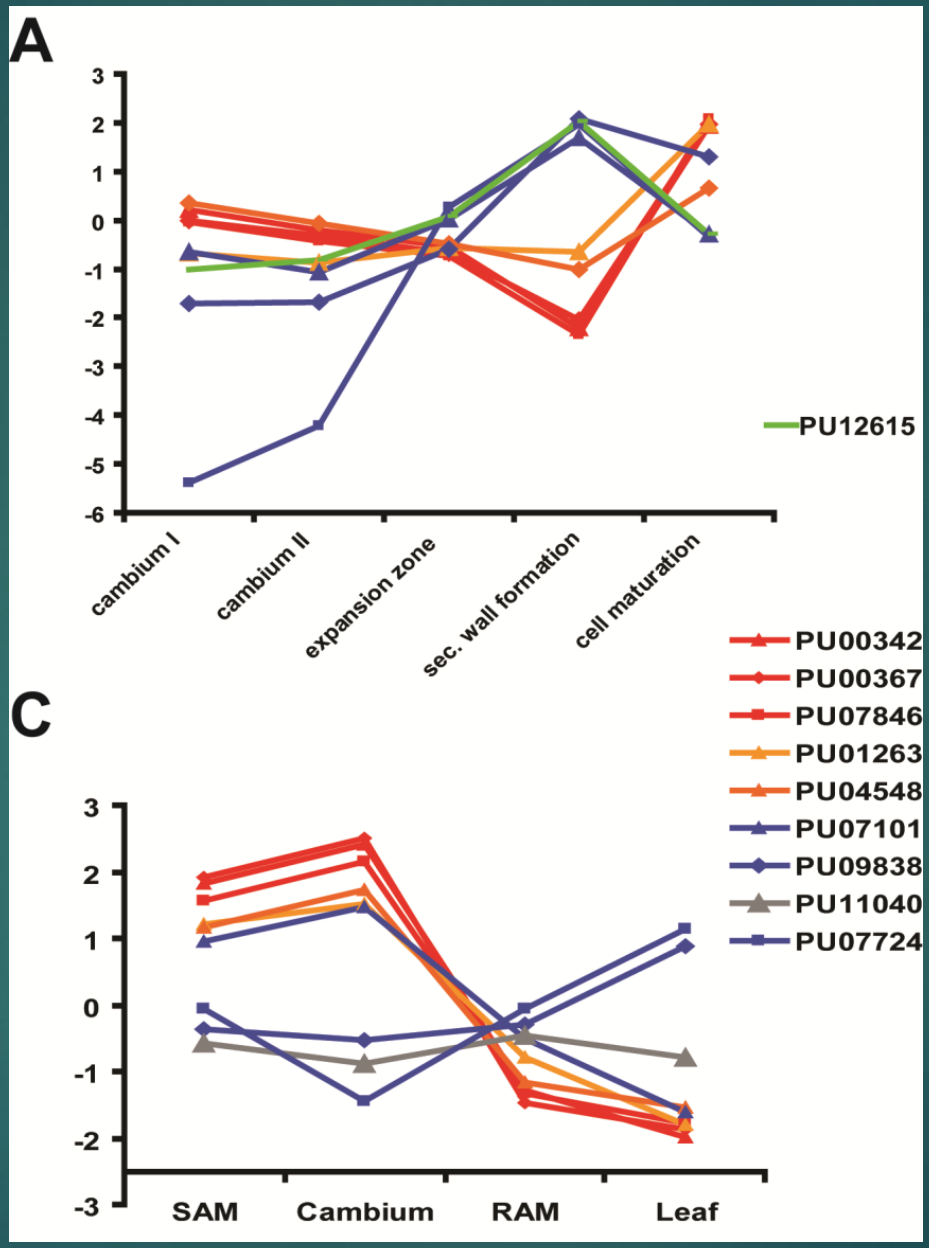


Long and Barton, 1996

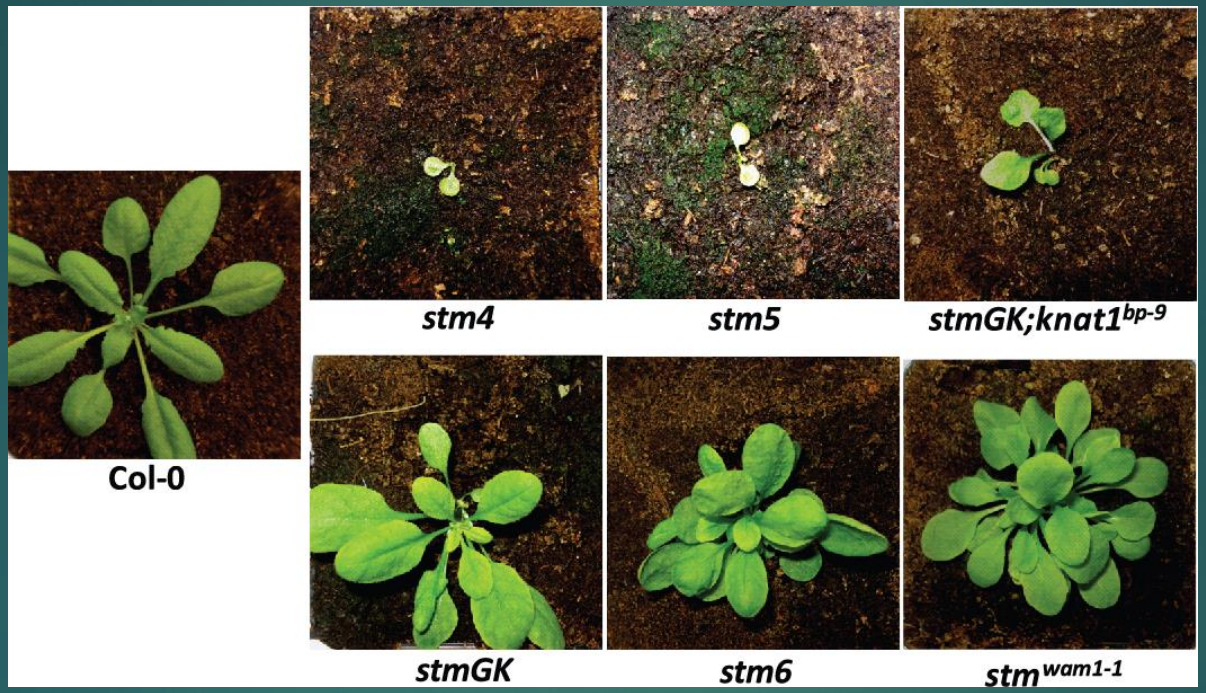


Homeodomain transcription factor

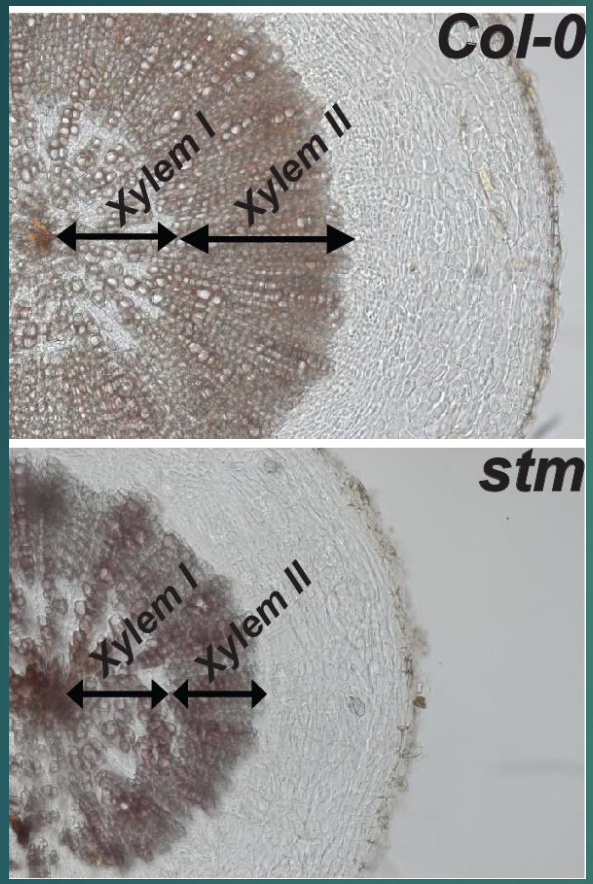
STM homologs expressed in poplar cambium

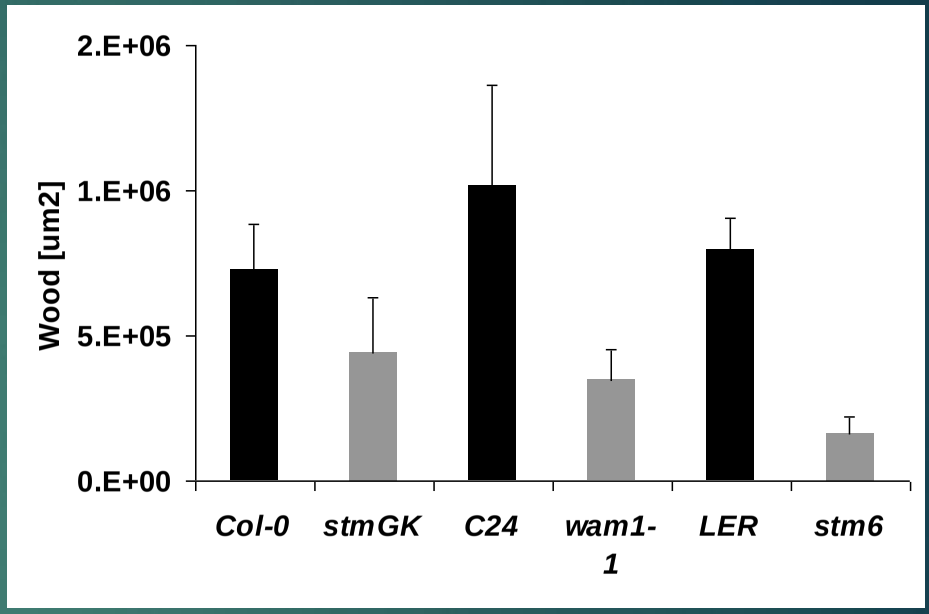


Weak *stm* mutants with similar total biomass than WT

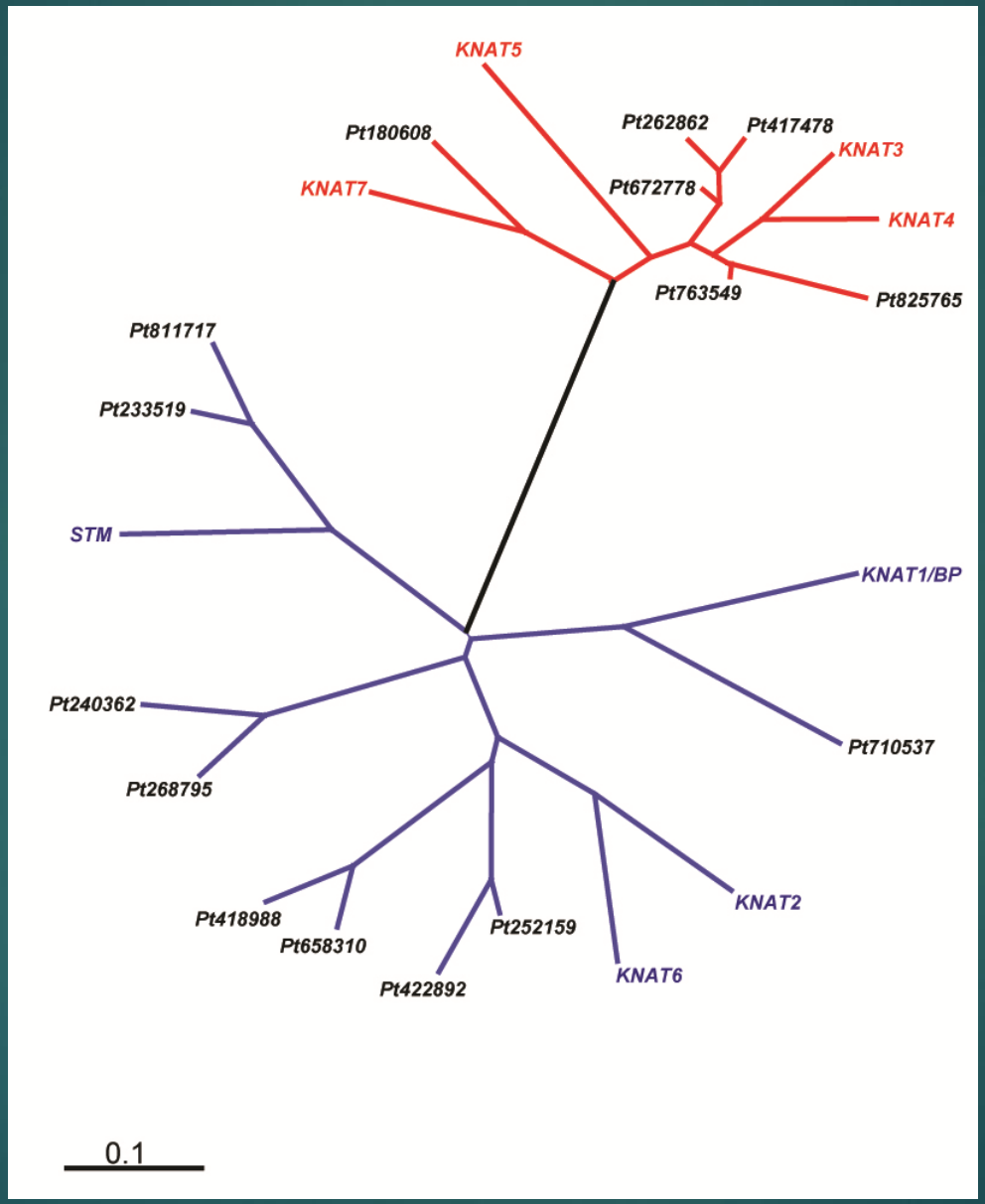


Less wood in weak *stm* mutants



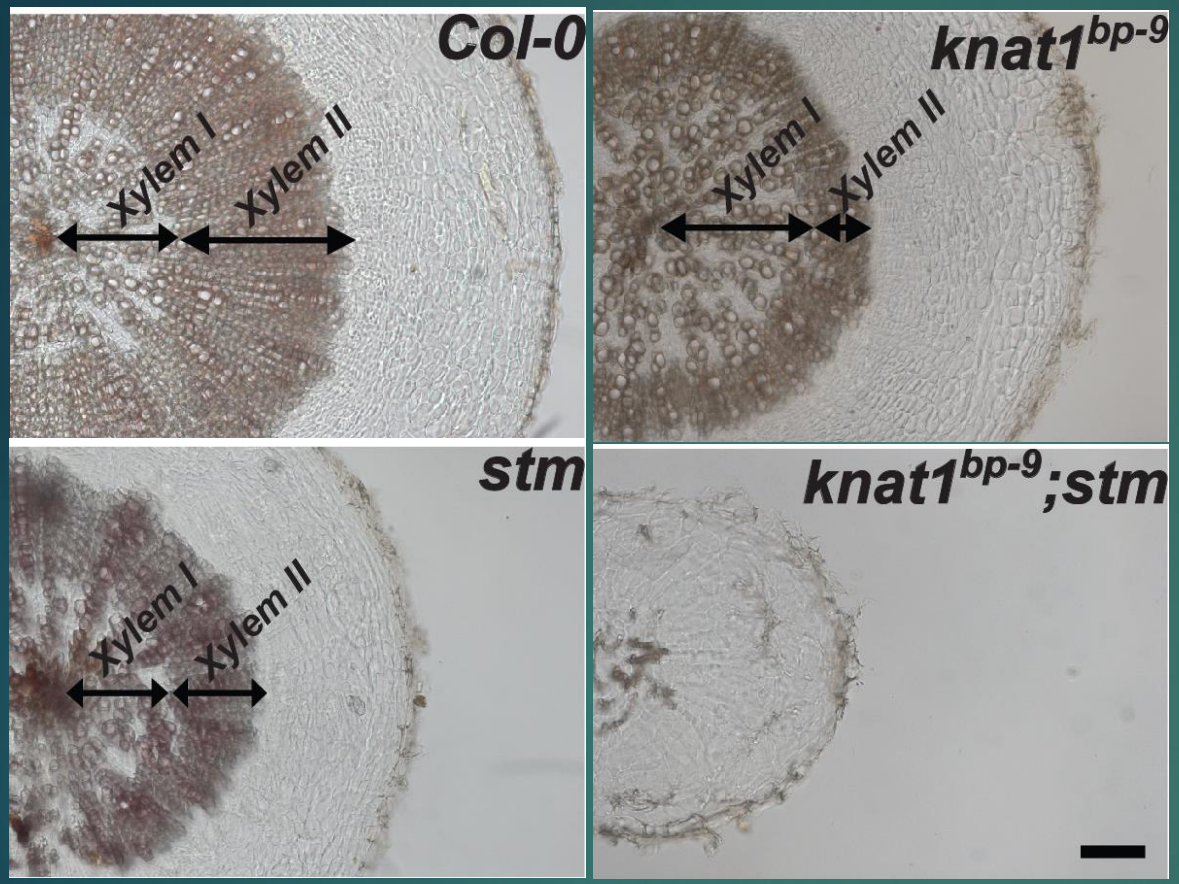


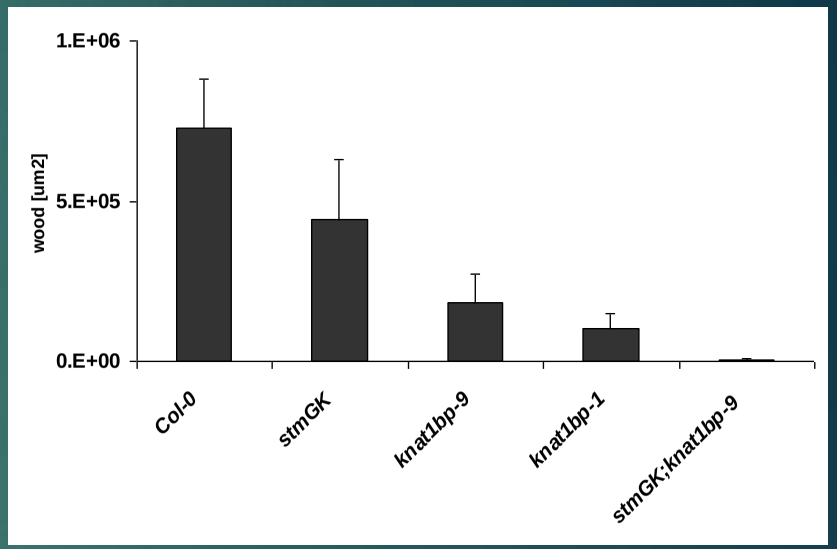
Redundancy?


KNAT5
Pt180608
Pt262862
Pt417478
KNAT3
KNAT7
Pt672778
KNAT4
Pt763549
Pt825765
Pt811717
Pt233519
STM
KNAT1/BP
Pt240362
Pt710537
Pt268795
KNAT2
Pt418988
Pt658310
Pt252159
KNAT6
Pt422892
0.1

knat1 mutants with phenotype similar to *stm*

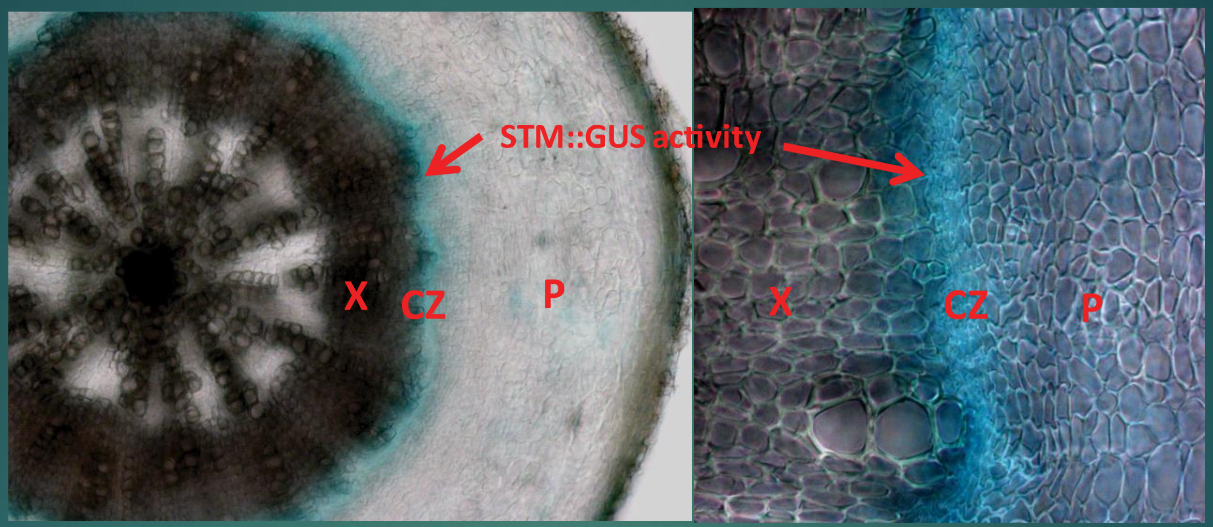
Overlapping functions for *KNAT1* and *STM*



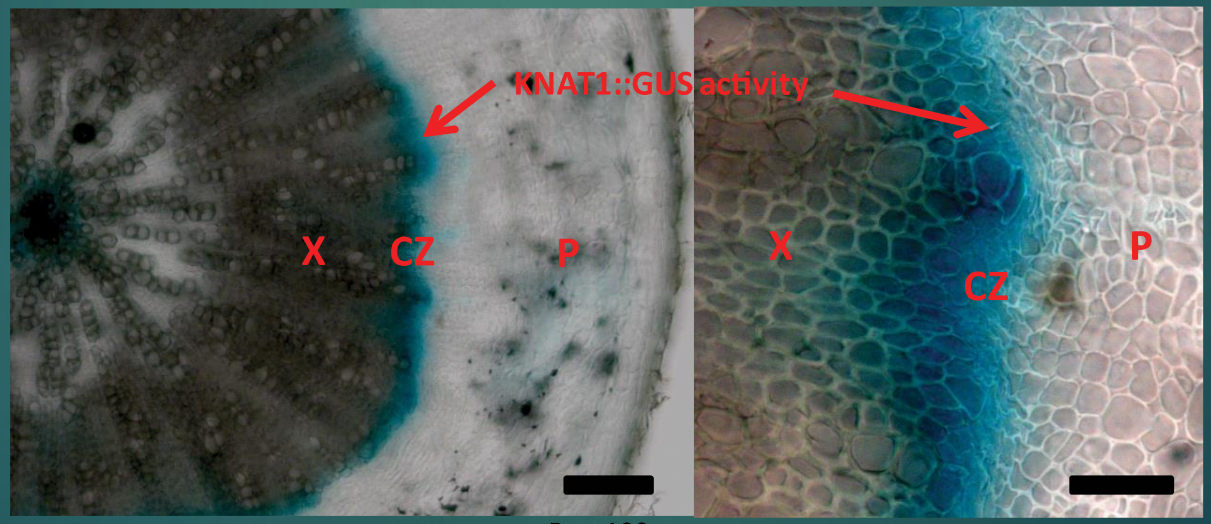


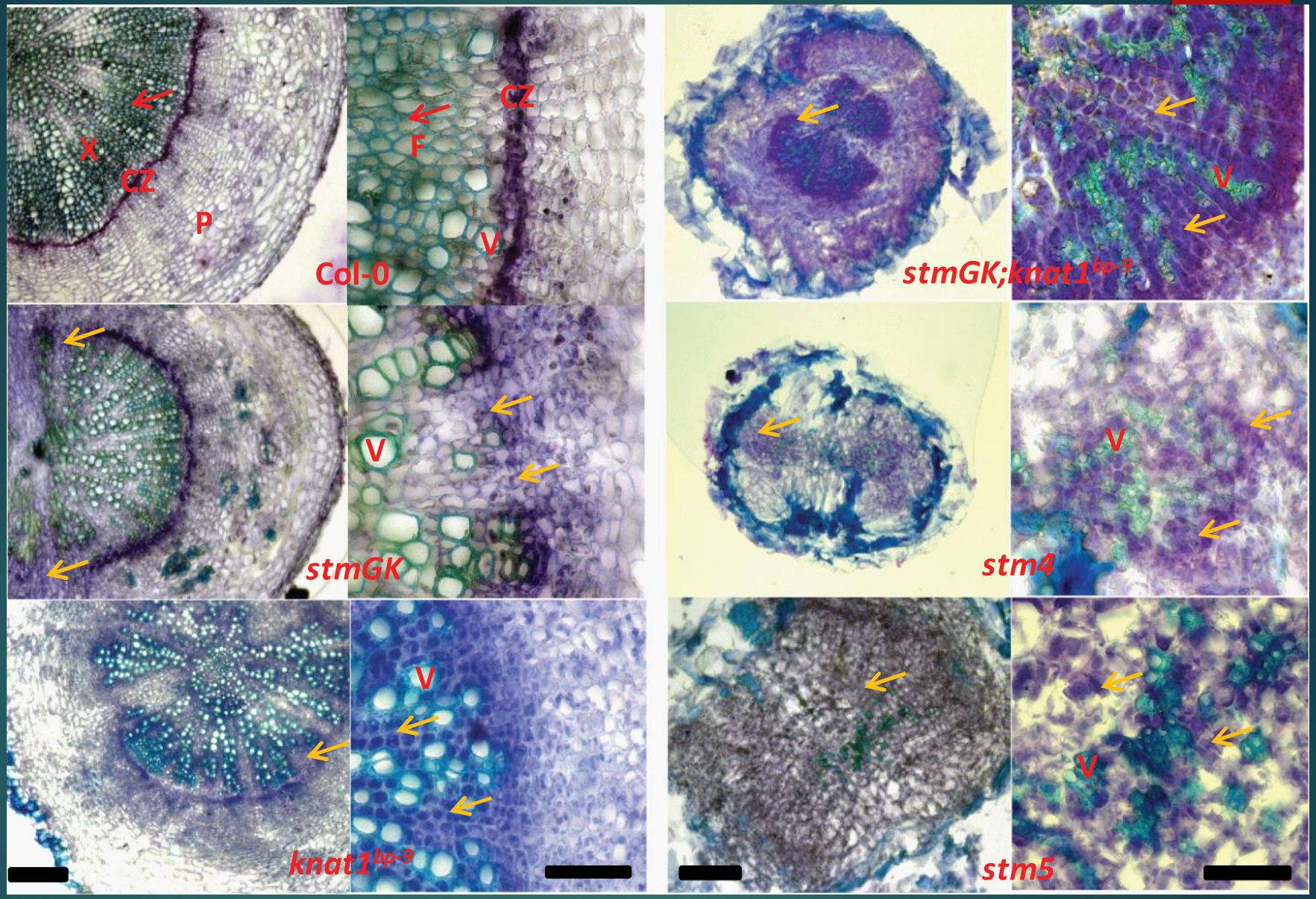
KNAT1 and *STM* expression in cambium and developping xylem

STM::GUS

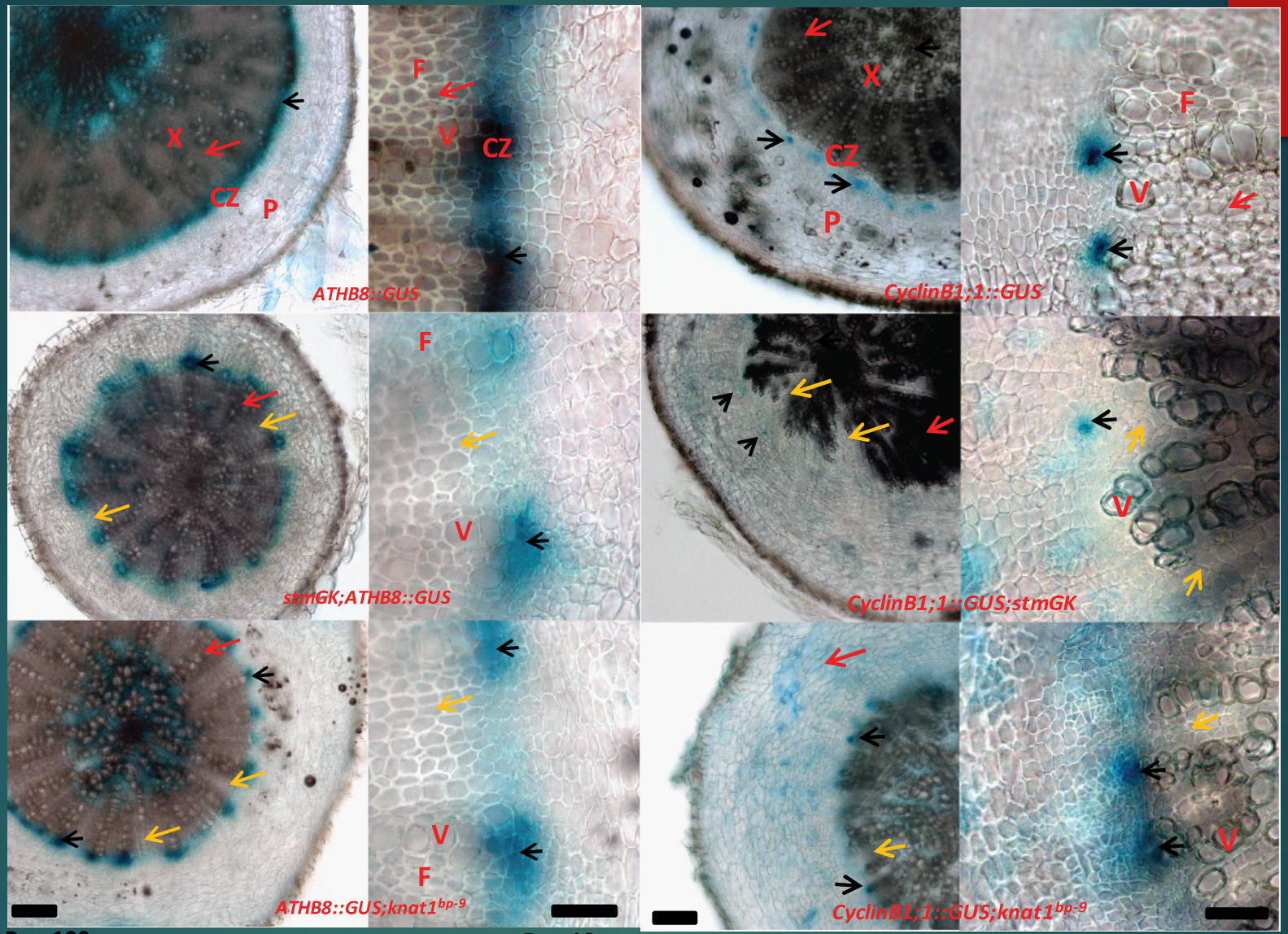


KNAT1::GUS





KNAT1 and *STM* are required xylem fiber cell formation and maintenance of cambial cells



KNAT1 and *STM* are required for early differentiation in xylem fiber cell files

STM – KNAT1

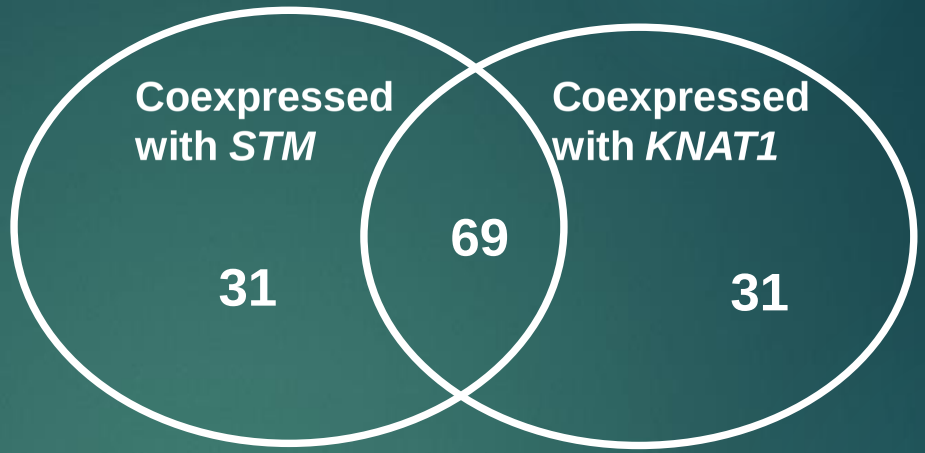
↓

Maintenance of cambial cells

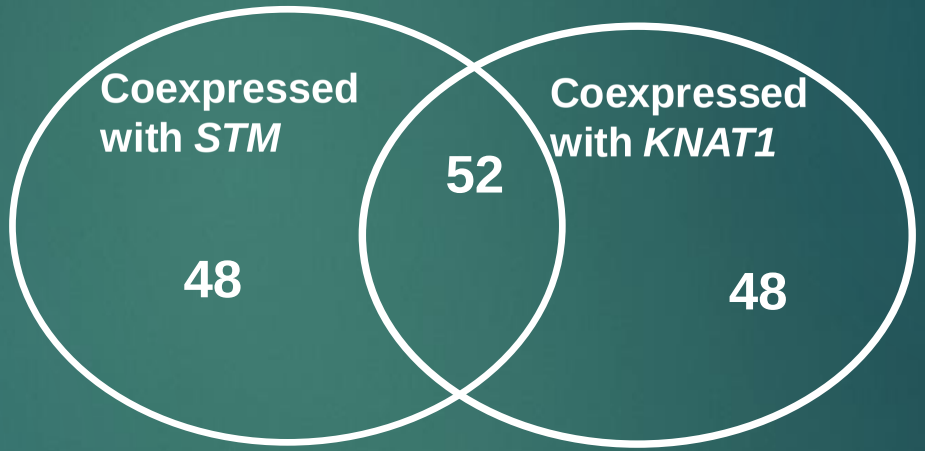
Xylem fiber cell differentiation

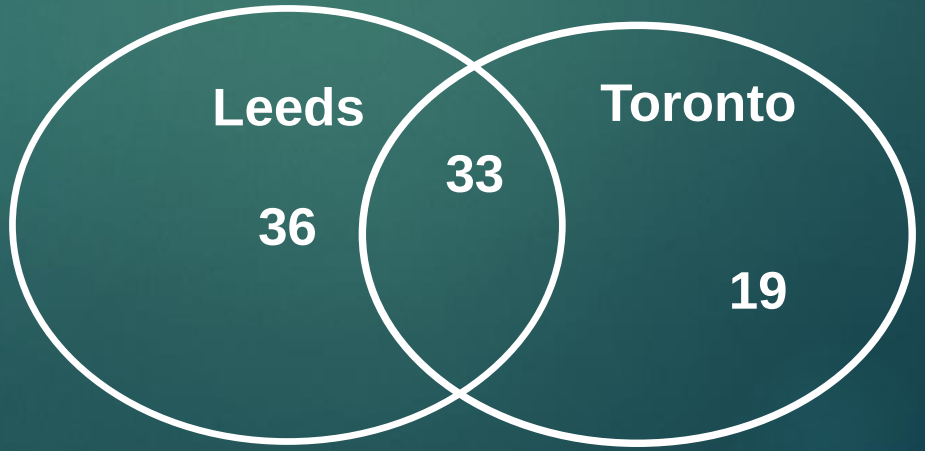
Downstream tragets?

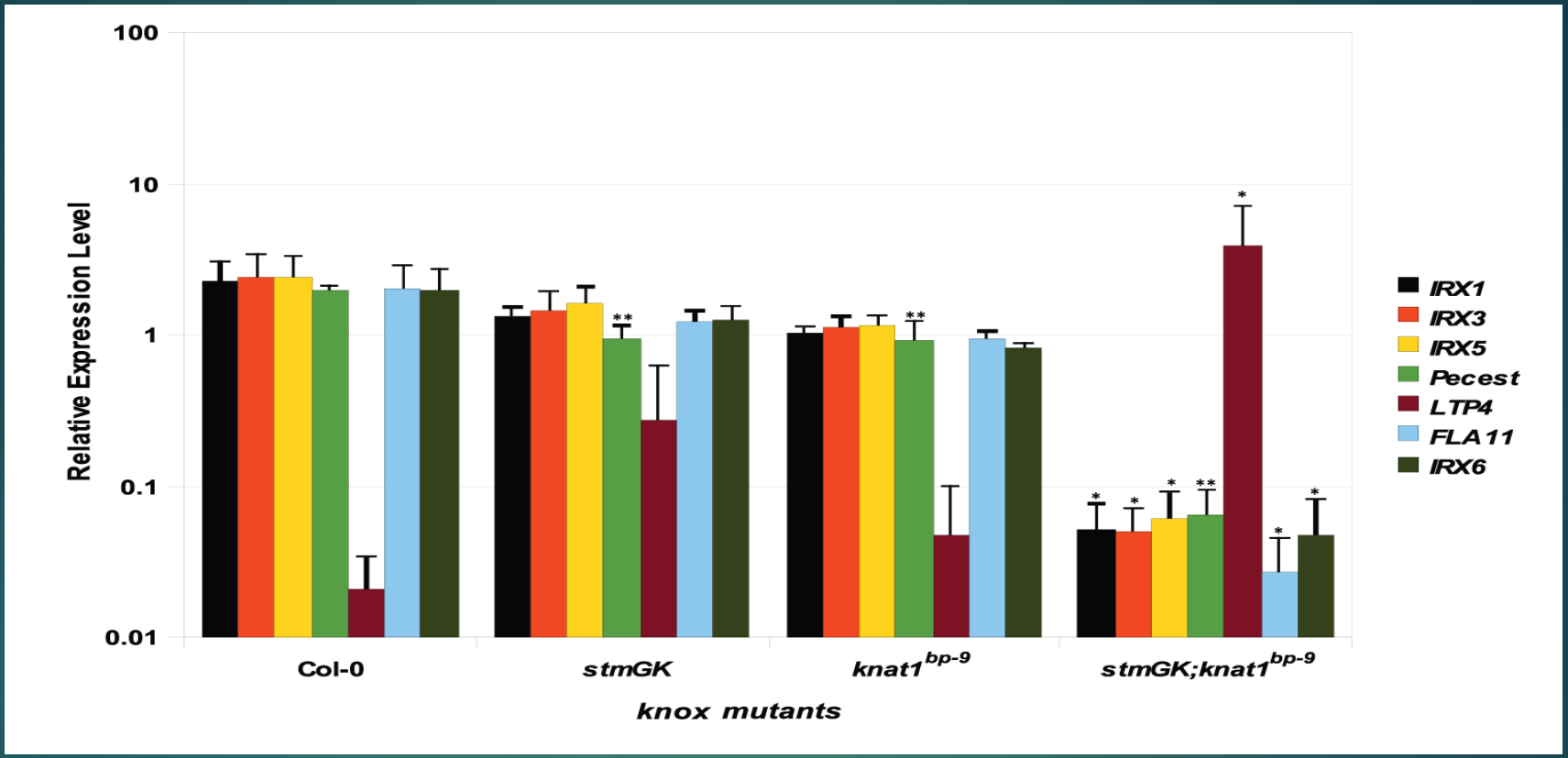
„Leeds“ database
+300 datasets
+21 000 genes
+Top 100



„Toronto“ database
+392 datasets
+21 000 genes
+Top 100



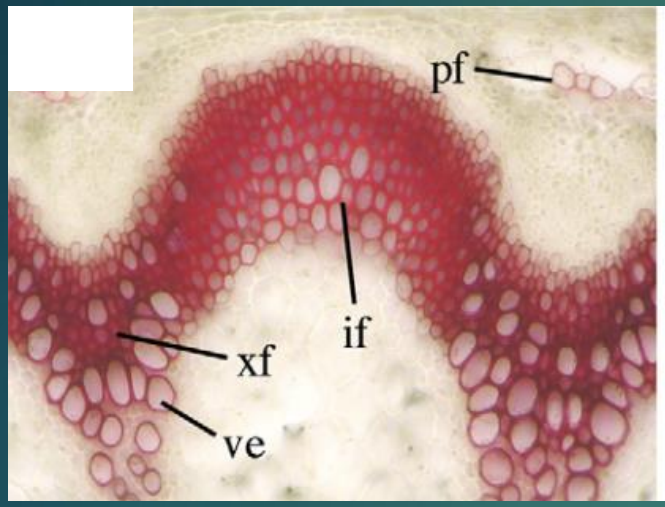


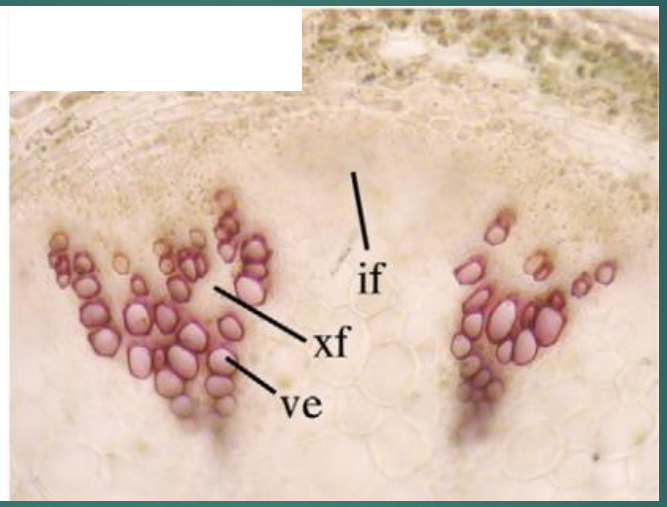

100
10
1
0.1
0.01
Relative Expression Level
Col-0
stmGK
knat1bp-9
stmGK;knat1bp-9
knox mutants
IRX1
IRX3
IRX5
Pecest
LTP4
FLA11
IRX6

STM/KNAT1 are required for the expression of...

NST1	***nst1***	Transcription factor
SND1	***snd1***	Transcription factor
SND2	***snd2***	Transcription factor
ATHB8	***ATHB8-ox***	Transcription factor
IAA27	*iaa27*	Transcription factor
CESA8	***irx1***	Cellulose synthase
CESA7	***irx3***	Cellulose synthase
CESA4	***irx5***	Cellulose synthase
COBRA-LIKE4	***irx6***	Fibril organization
PME61		Pectin methylesterase
GAUT12	***irx8***	Galacturonosyltransferase
LAC4	***irx12***	Laccase
CTL2		Chitinase-like
PAL4	*pal4*	Phenylalanine ammonia-lyase
FLA11		GPI anchor

NST1 and SND1 are switches for xylem fiber cell identity

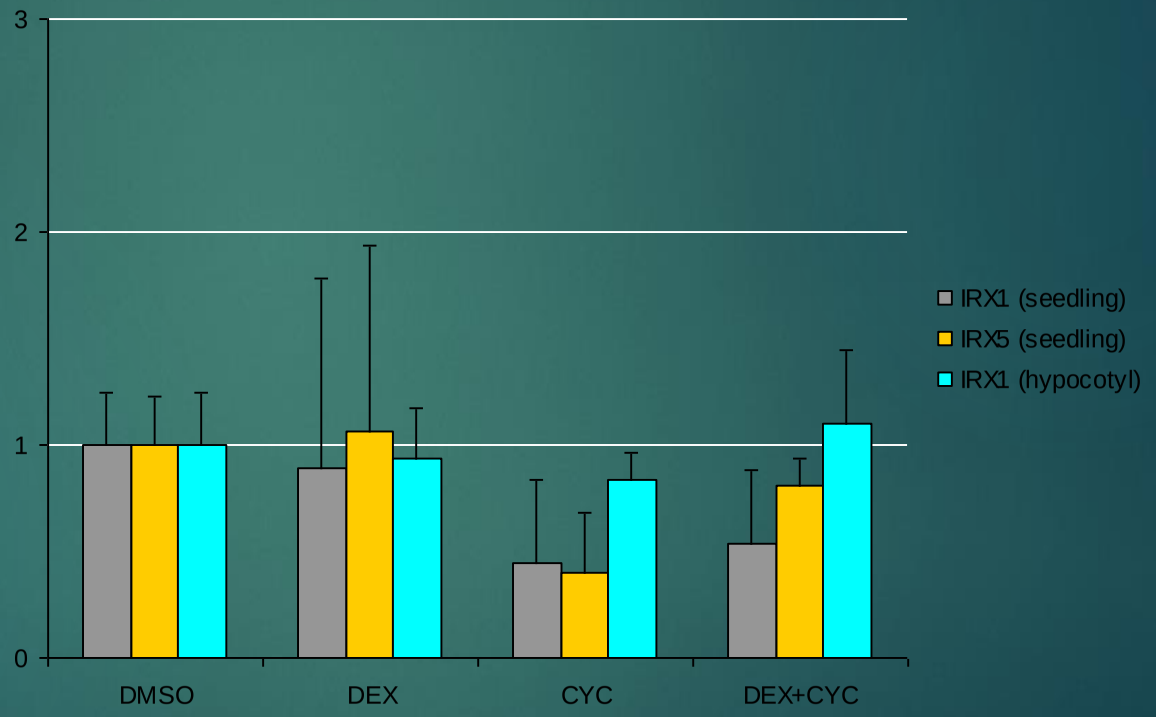




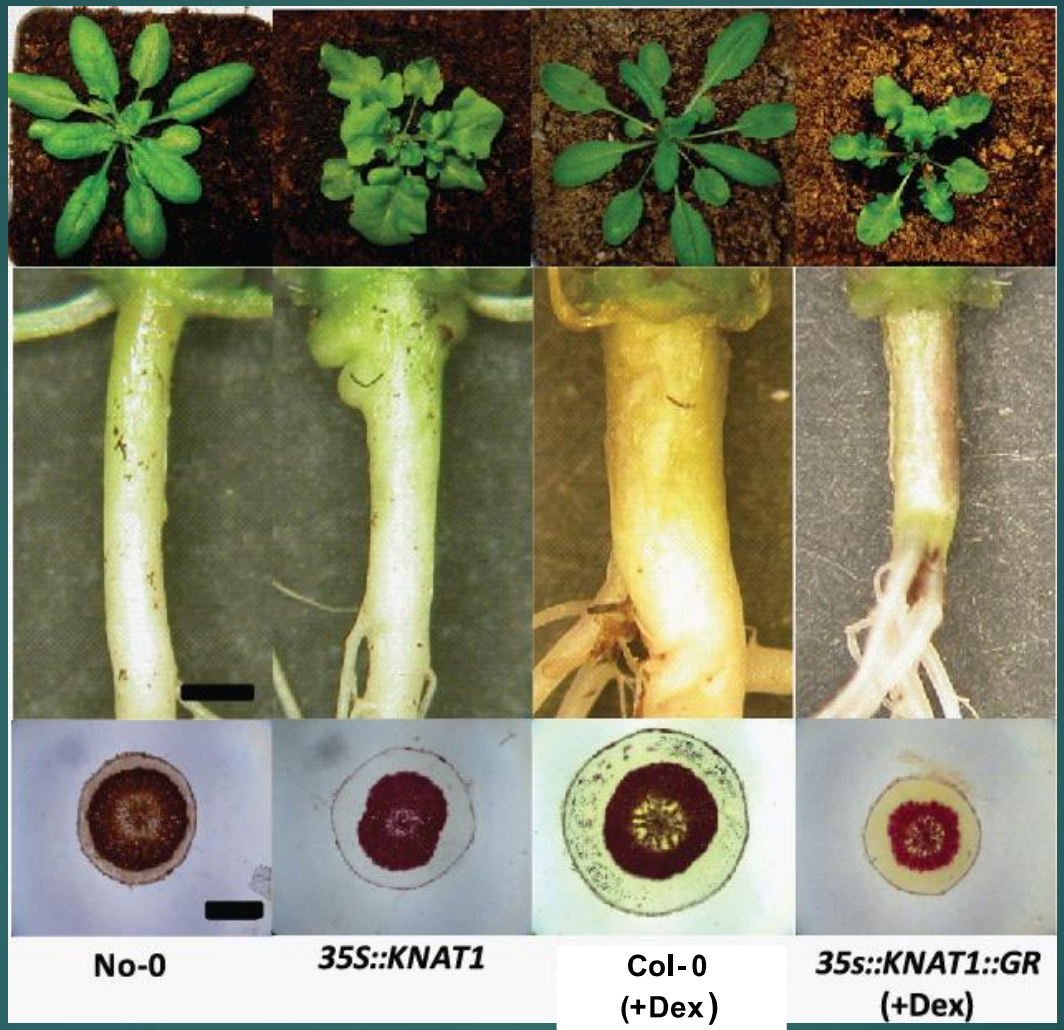
Zhong and Ye, 2007

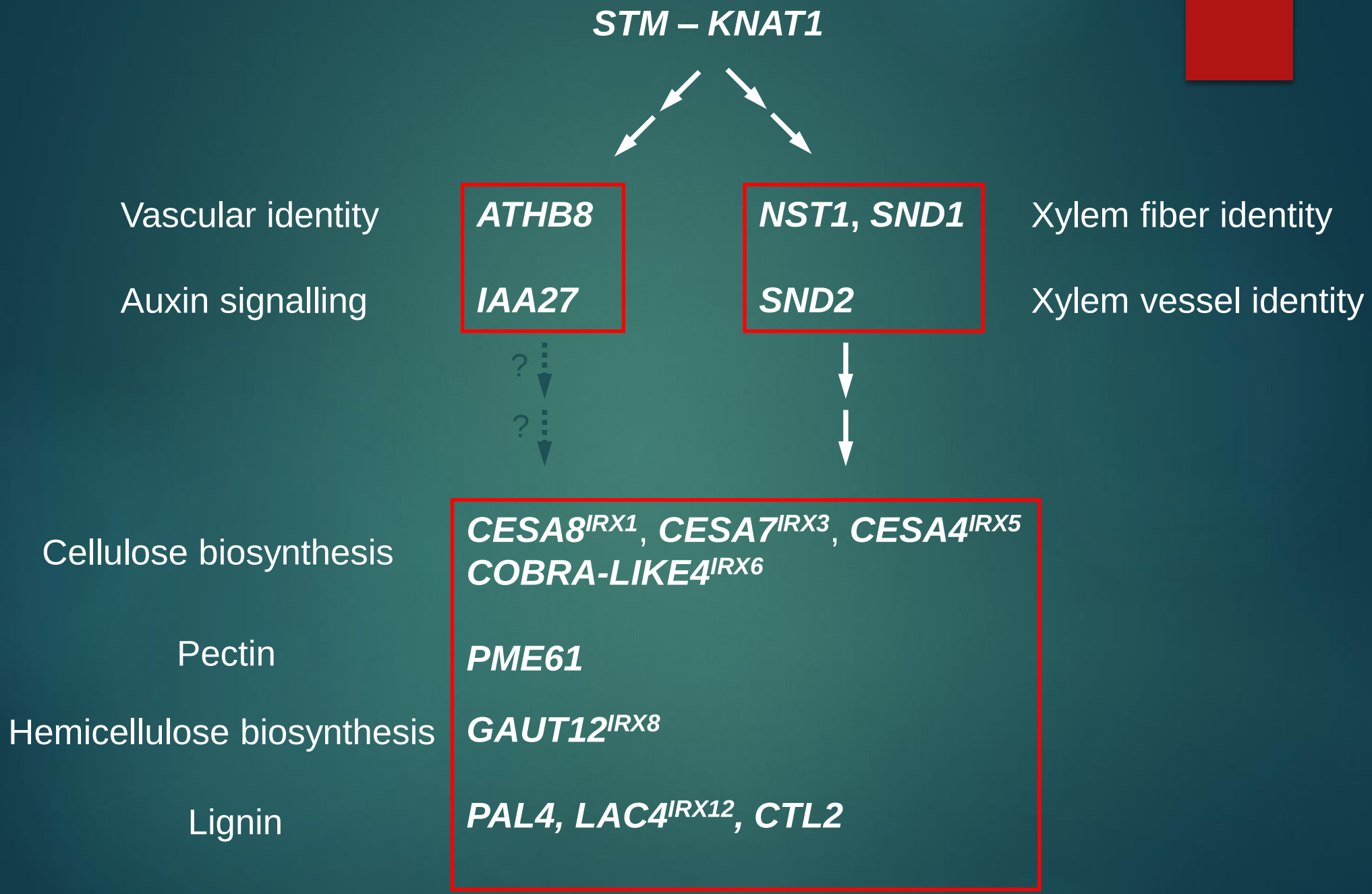
Direct tragets of STM/KNAT1 action?

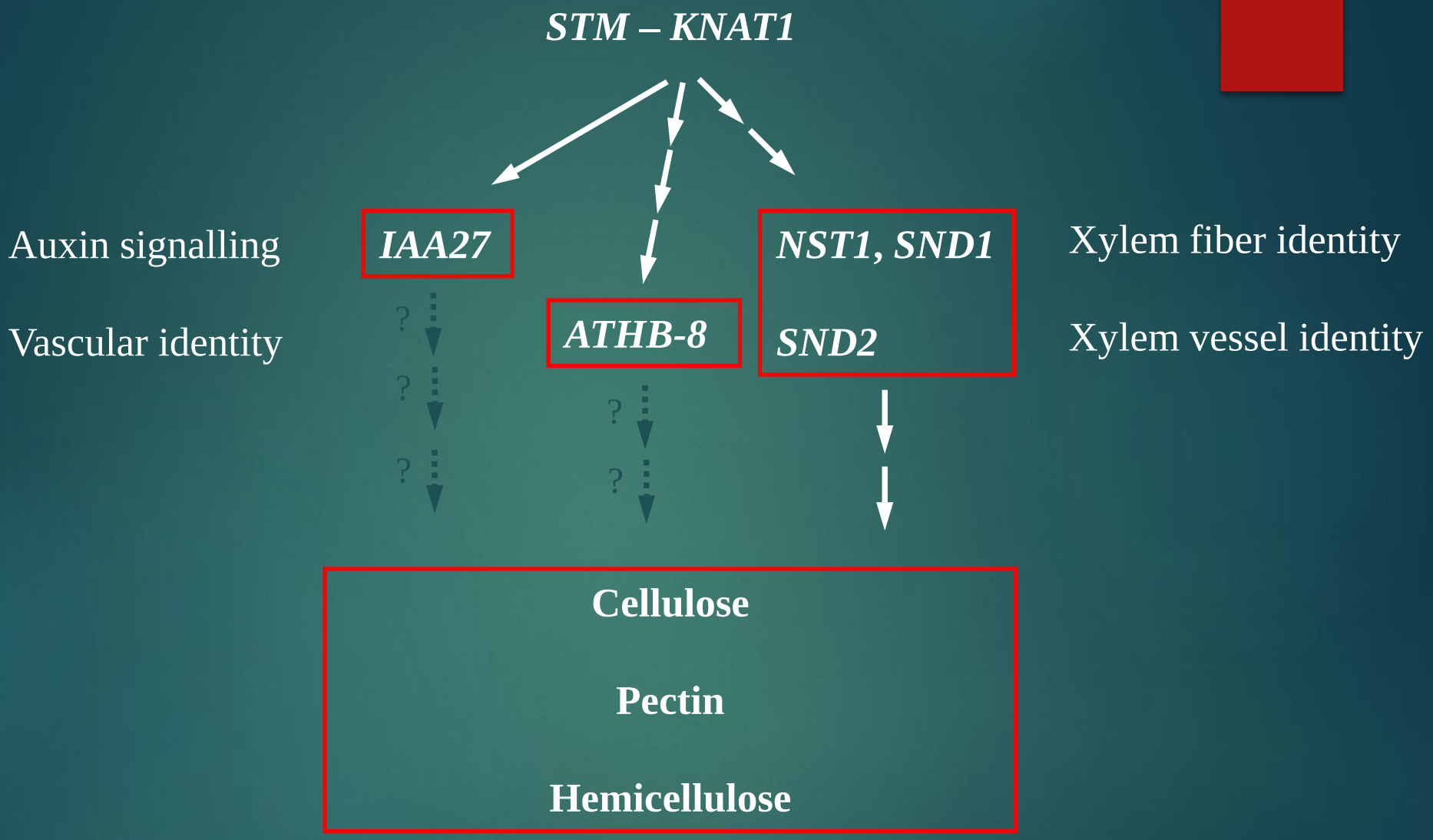
35S::*KNAT1-GR*



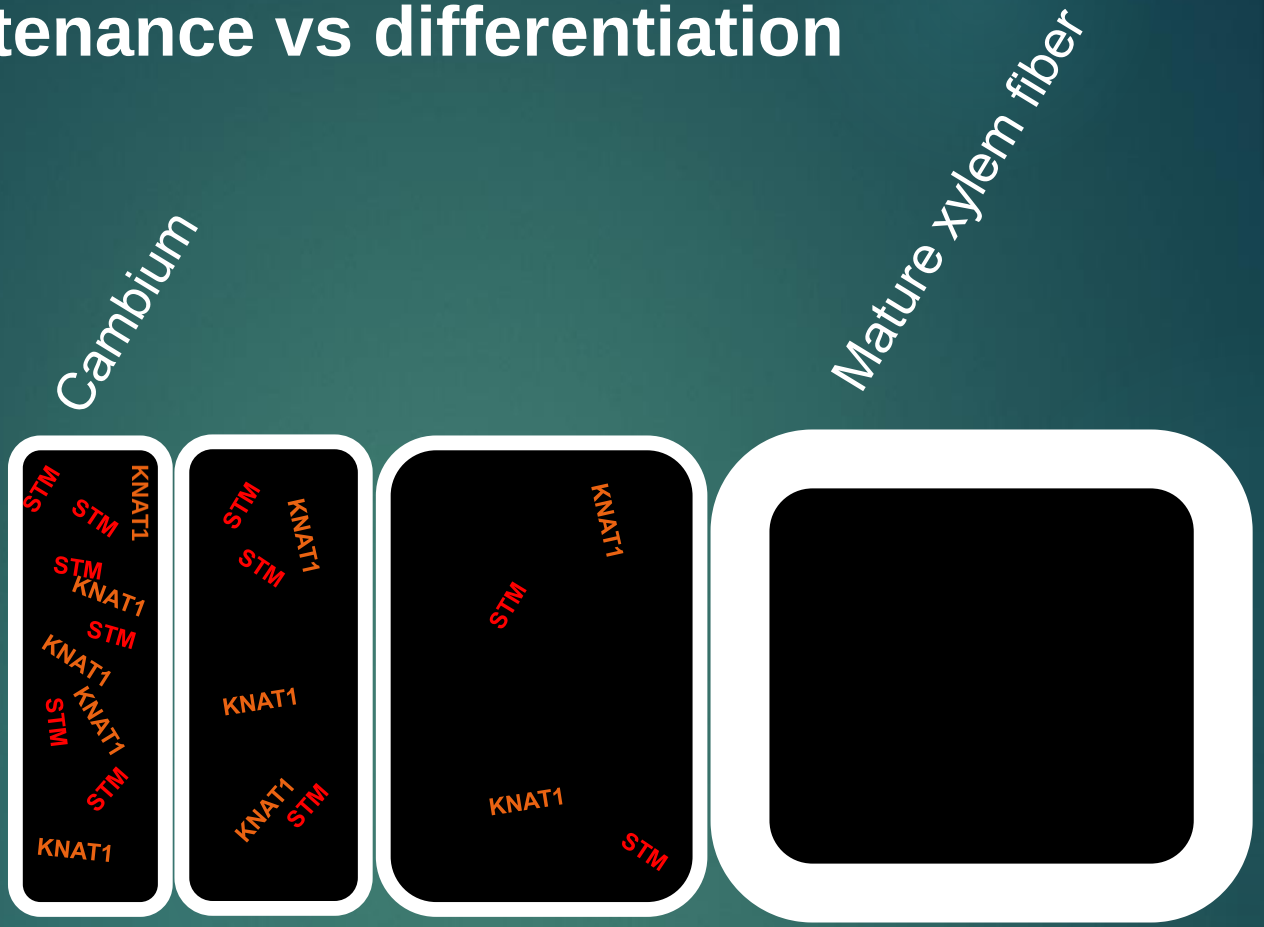
35S::*KNAT1-GR* is functional

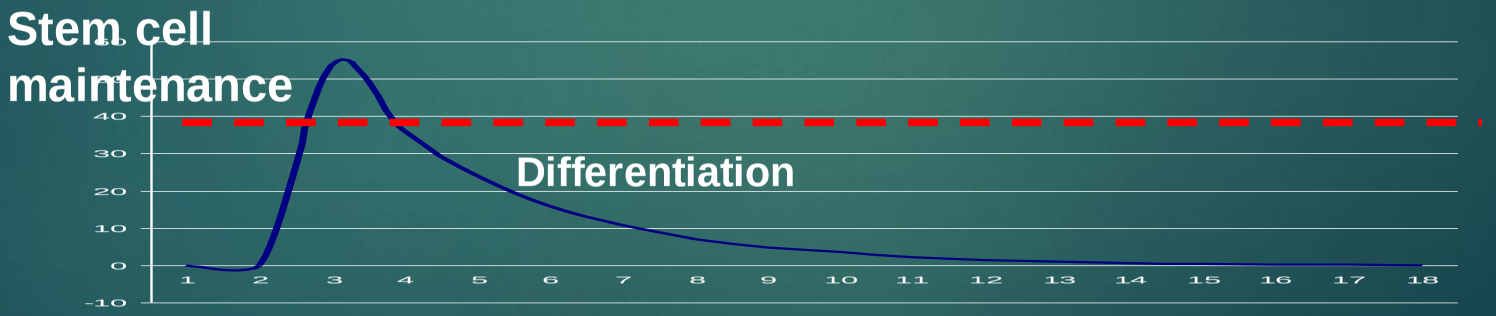



STM – KNAT1
Vascular identity
ATHB8
Auxin signalling
IAA27
NST1, SND1
Xylem fiber identity
SND2
Xylem vessel identity
?
?
Cellulose biosynthesis
CESA8IRX1, CESA7IRX3, CESA4IRX5
COBRA-LIKE4IRX6
Pectin
PME61
Hemicellulose biosynthesis
GAUT12IRX8
Lignin
PAL4, LAC4IRX12, CTL2

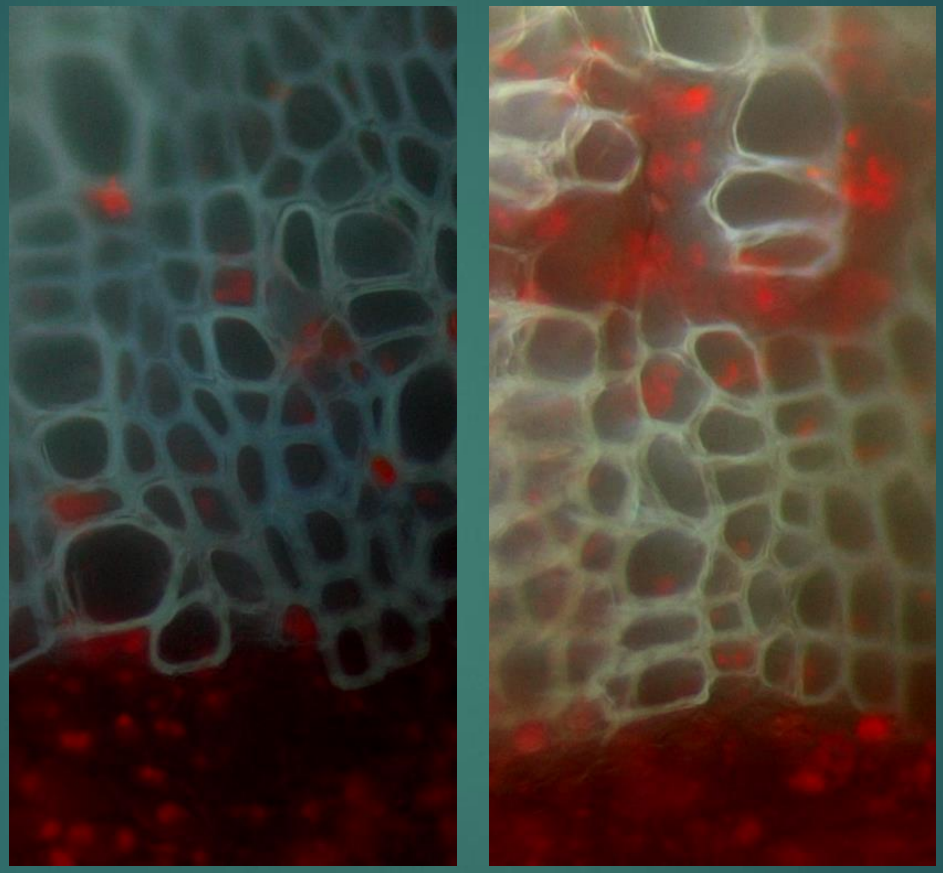

STM – KNAT1
Auxin signalling
IAA27
NST1, SND1
Xylem fiber identity
Vascular identity
ATHB-8
SND2
Xylem vessel identity
?
?
?
?
?
Cellulose
Pectin
Hemicellulose

Stem cell maintenance vs differentiation



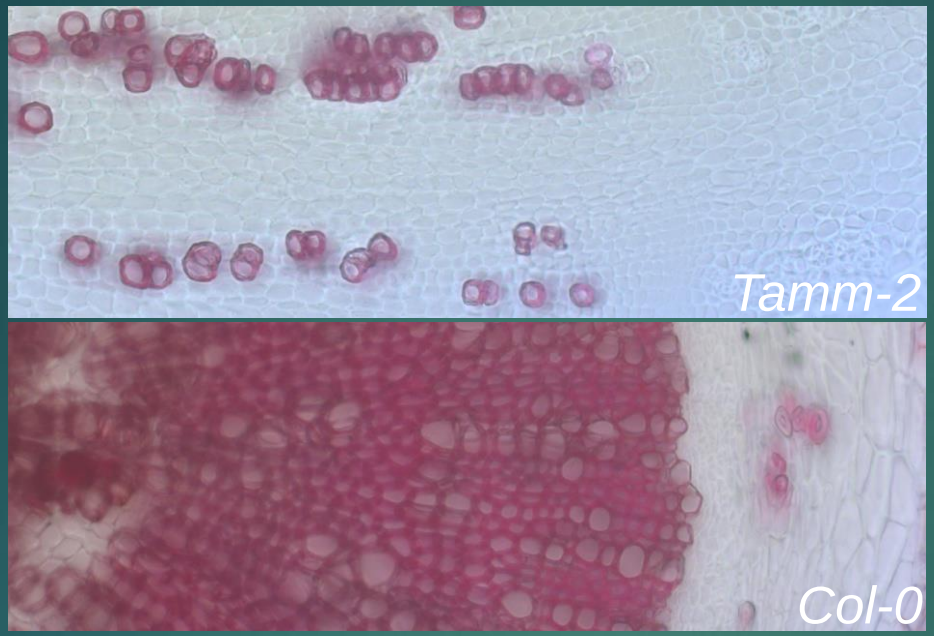


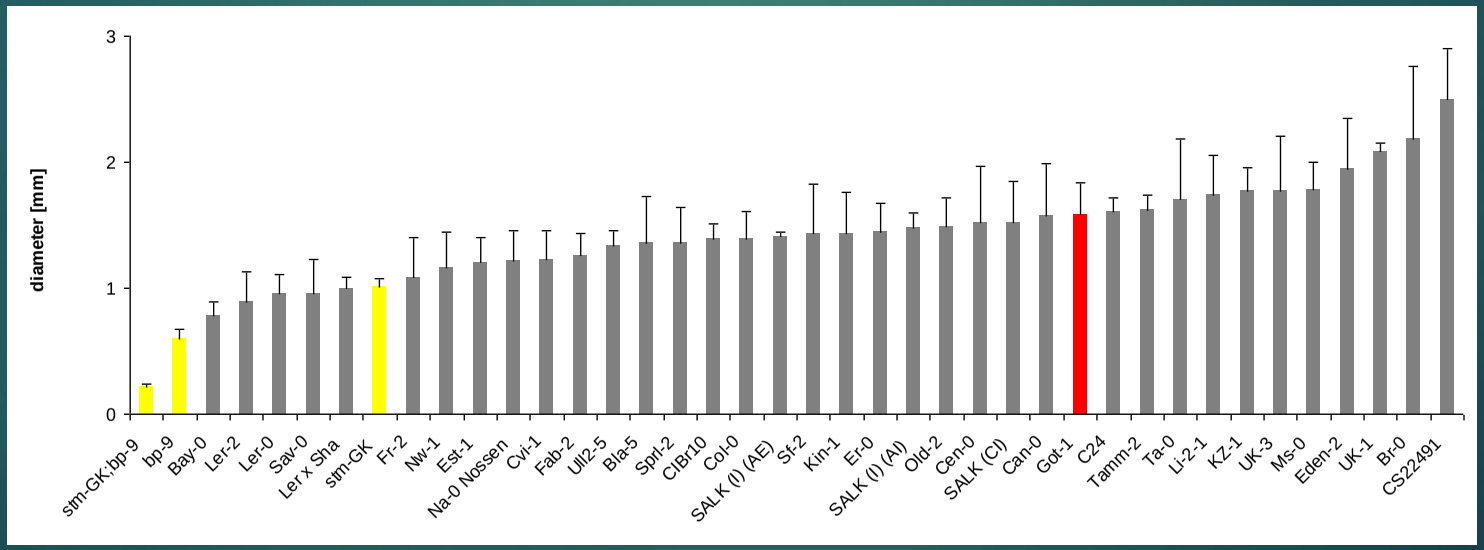
More reverse genetics...



WT ***bor1-N537312***

Natural variation





Acknowledgments

Widi Sunaryo
Xu Jin
Benjamin Faust

Andrea Polle

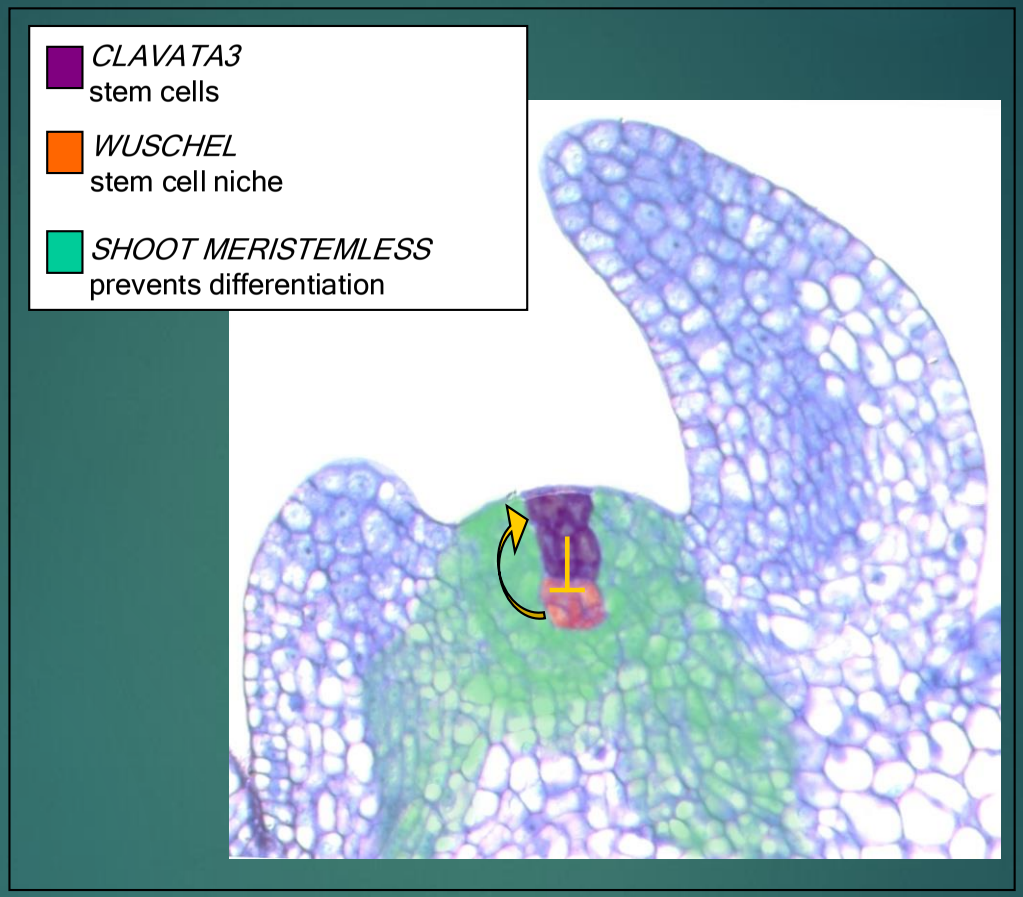
Caroline Gutjahr
University of Lausanne

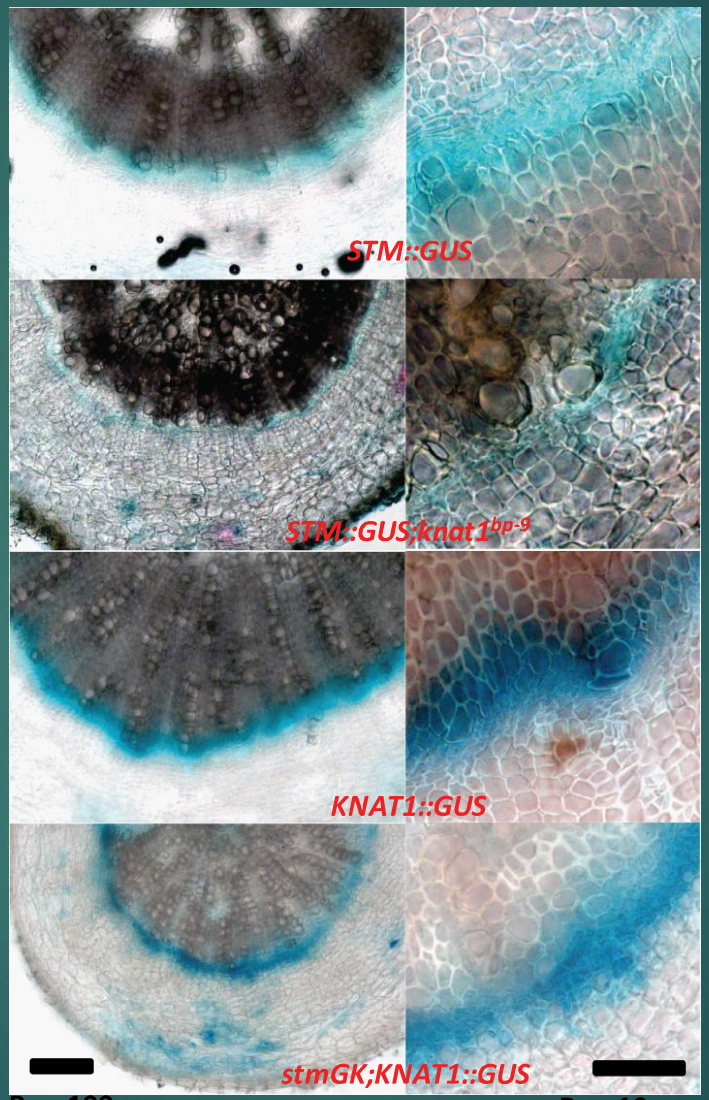
Seed stocks:

Peter Doerner, University of Edinburgh, UK
Angela Hay, University of Oxford, UK
Wolfgang Werr, University of Cologne, Germany

Nottingham Arabidopsis Stock Centre, UK

DFG Pappelgruppe, DFG Fi1668


CLAVATA3
stem cells
WUSCHEL
stem cell niche
SHOOT MERISTEMLESS
prevents differentiation


STM::GUS
STM::GUS;knat1bp-9
KNAT1::GUS
stmGK;KNAT1::GUS
Bar: 100 µm
Bar: 10 µm