CERTIFICATE OF ATTENDANCE

This certificate certify that:

WIDI SUNARYO

Has attended a Joint Seminar on Bioresources and Technology, conducted by the Faculty of Sciences and Industrial Technology, Prince of Songkla University, (PSU) and International Excellent Program, Mulawarman University (MU)

As a :

SPEAKER

Surat Thani, February 28. 2013

Faculty of Science and Industrial International Excellent Program, MU

Technology, PSU Dean Head

Prince of Songkla University

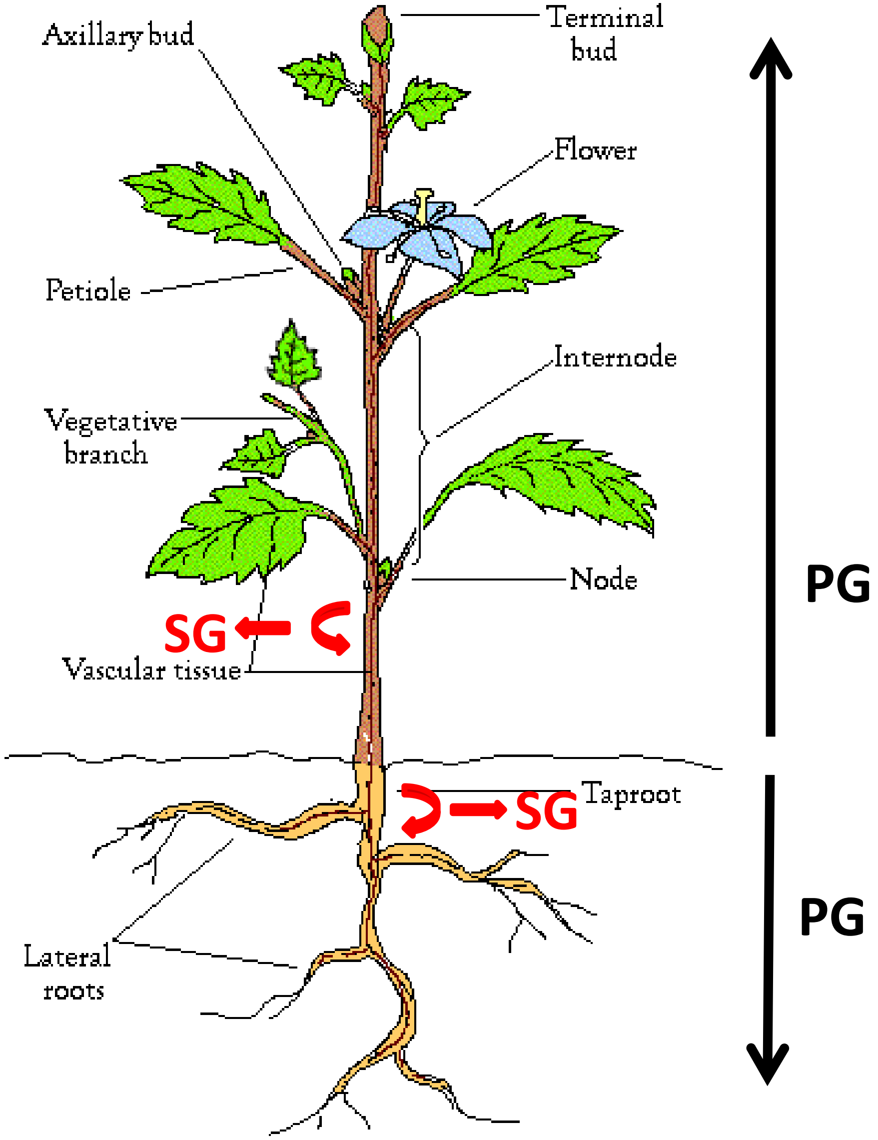
Mulawarman University

How *KNOX* genes drives fiber fromation

Widi Sunaryo

JOINT SEMINAR-PSU-UNMUL, 2013

Secondary growth vs primary growth

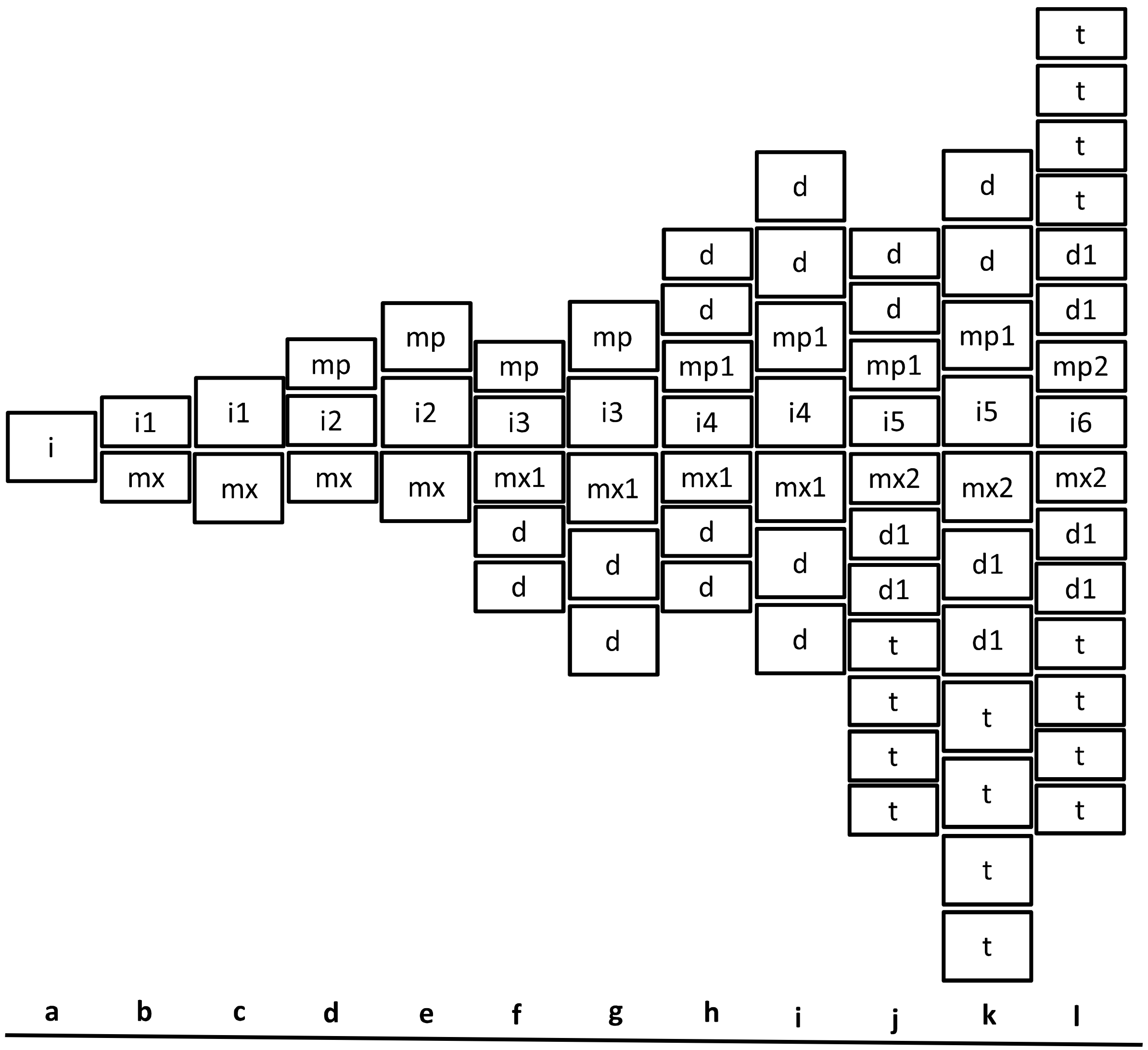


SG: Secondary Growth

PG: Primary Growth

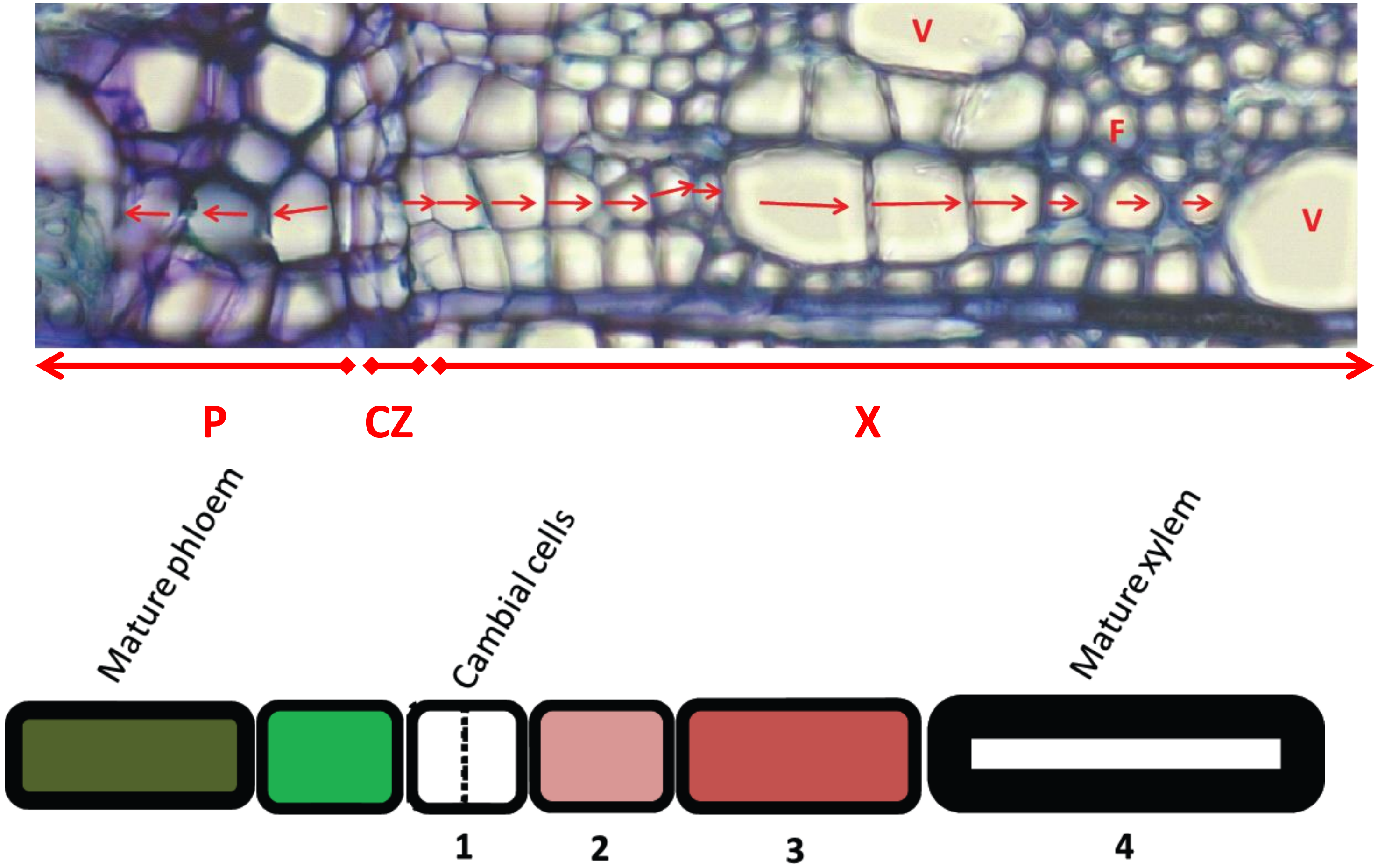
Product of secondary growth

The cambial cell and its derivates



Evert (2006) modified by Sunaryo (2010).

Molecular regulation of secondary growth in plant stems is still little understood



P : Phloem
CZ: Cambial Zone
X : Xylem

1 : Cell division
2 : Cell enlargement
3 : Secondary cell wall deposition
4 : Programmed cell death (mature xylem)

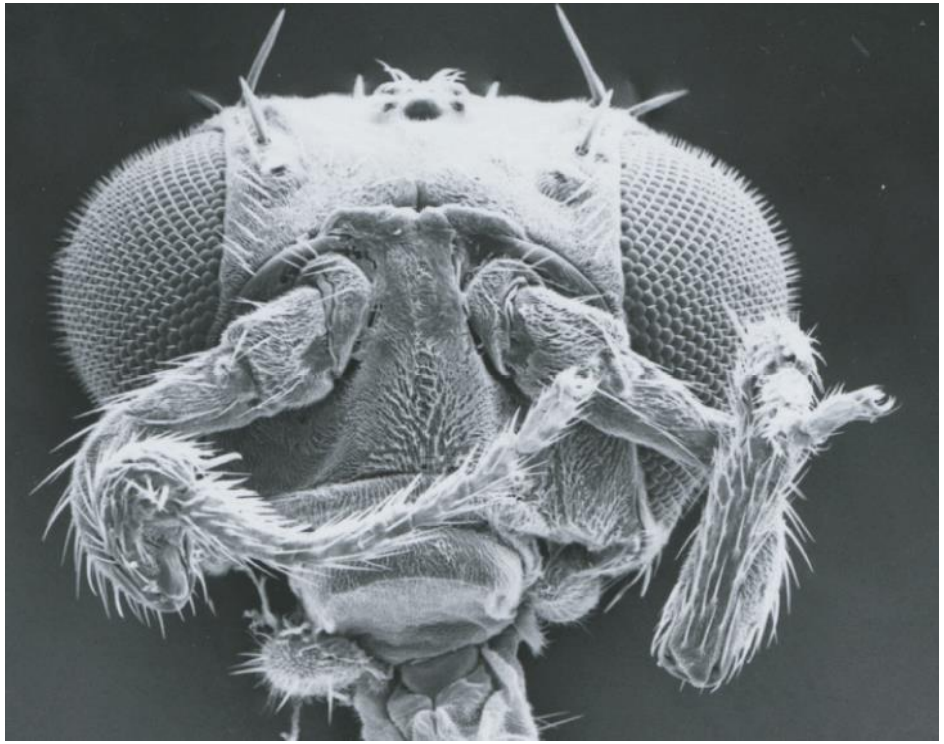
NATURE GENETICS,
VOLUME 39, NUMBER 7
JULY 2007

Mutant of the Month

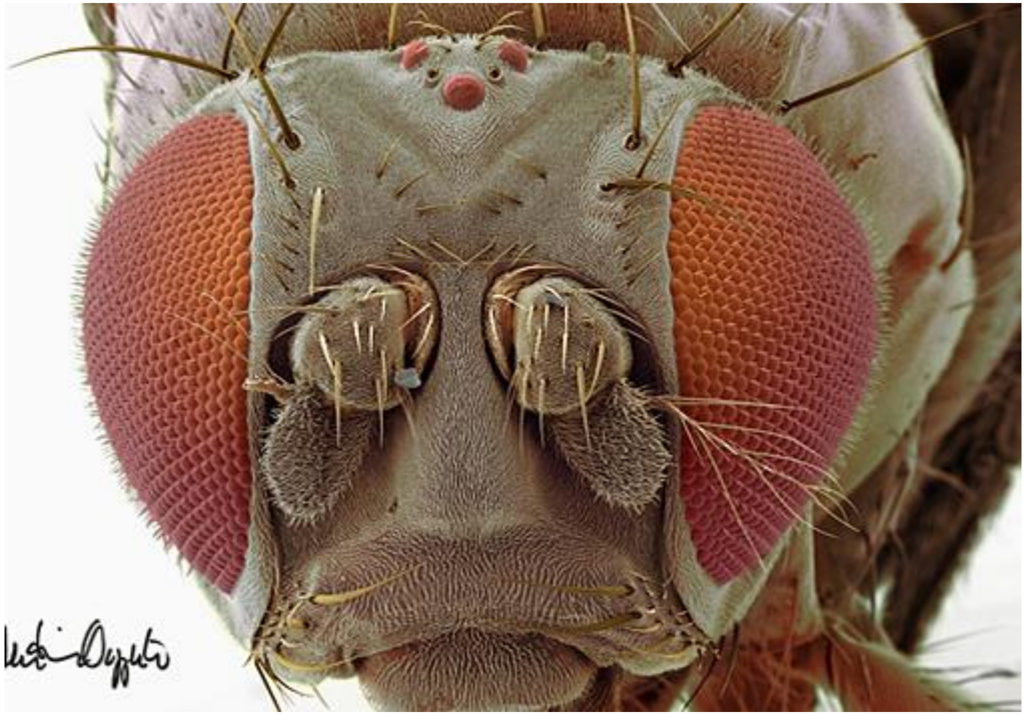

Rudi Turner and FlyBase

This month we honor the spectacular *Drosophila melanogaster* mutant *Antennapedia*. Photos of the *Antennapedia* phenotype are frequently used to capture the interest of biology students (and with good reason—gain-of-function mutations cause a striking antenna-to-leg transformation, as shown above), but lessons learned from this and other homeotic mutations about mechanisms of segmentation and specification of segment identity should not be overlooked. On a basic level, the fact that appendages can be swapped implies that they are homologous and share a common origin. Molecular characterization of the function of homeotic genes, many of which encode transcription factors, has led to insights into the important role of regulation of gene expression in the control of development. And this knowledge is now being used to study evolutionary mechanisms of morphological divergence—quite a legacy for a mutant that at first glance looks like a sci-fi monster come true. *EN*

The spectacular *Drosophila melanogaster* mutant *antennapedia*



***antennapedia* phenotype**



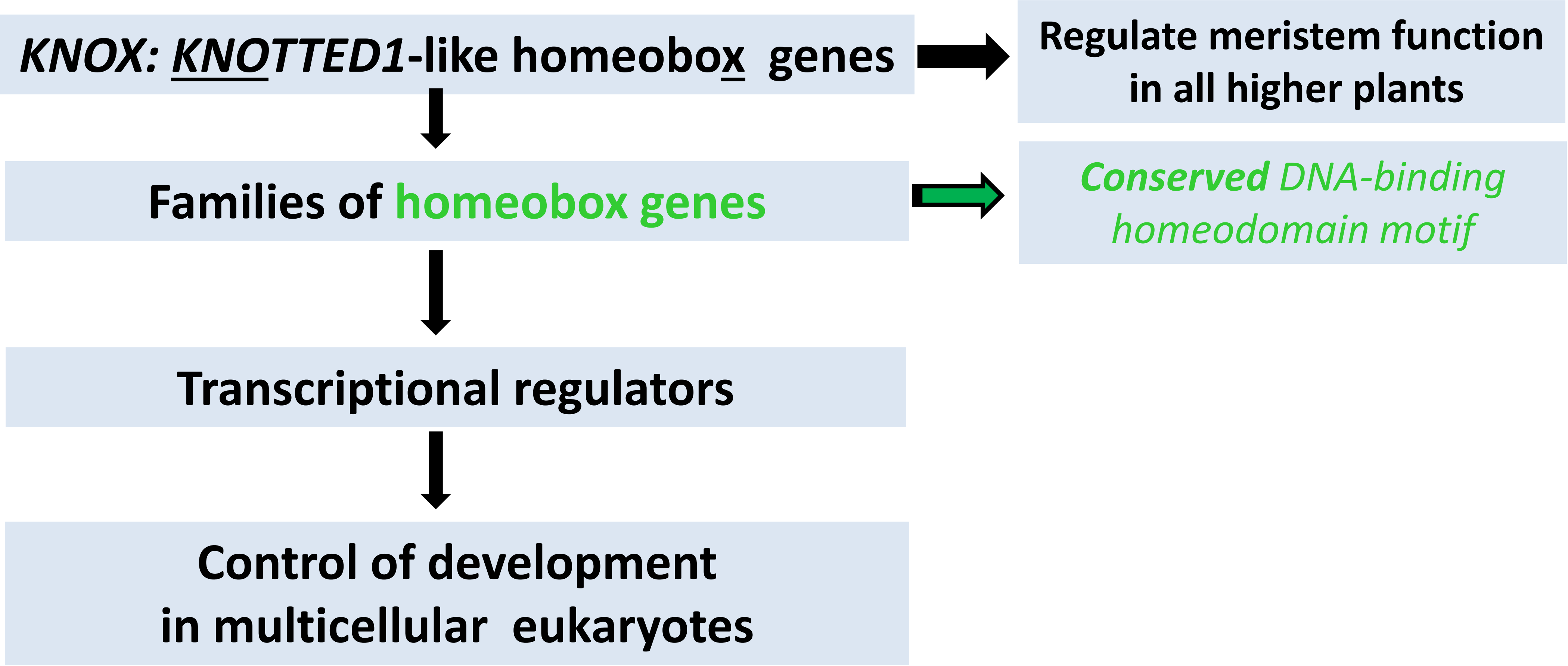
Wild Type

ANTENNAPEDIA?

- *ANTENNAPEDIA* is a member of the homeobox gene family.
- Homeobox genes are genes that encode transcriptional regulators that control development in multicellular eukaryotes
- Homeobox genes encode proteins containing the *conserved DNA-binding homeodomain motif* that is found in transcription factors from all eukaryotes

(Scofield and Murray, 2005)

KNOX Genes?



***KNOX: KNOTTED1*-like homeobox genes**

Regulate meristem function in all higher plants

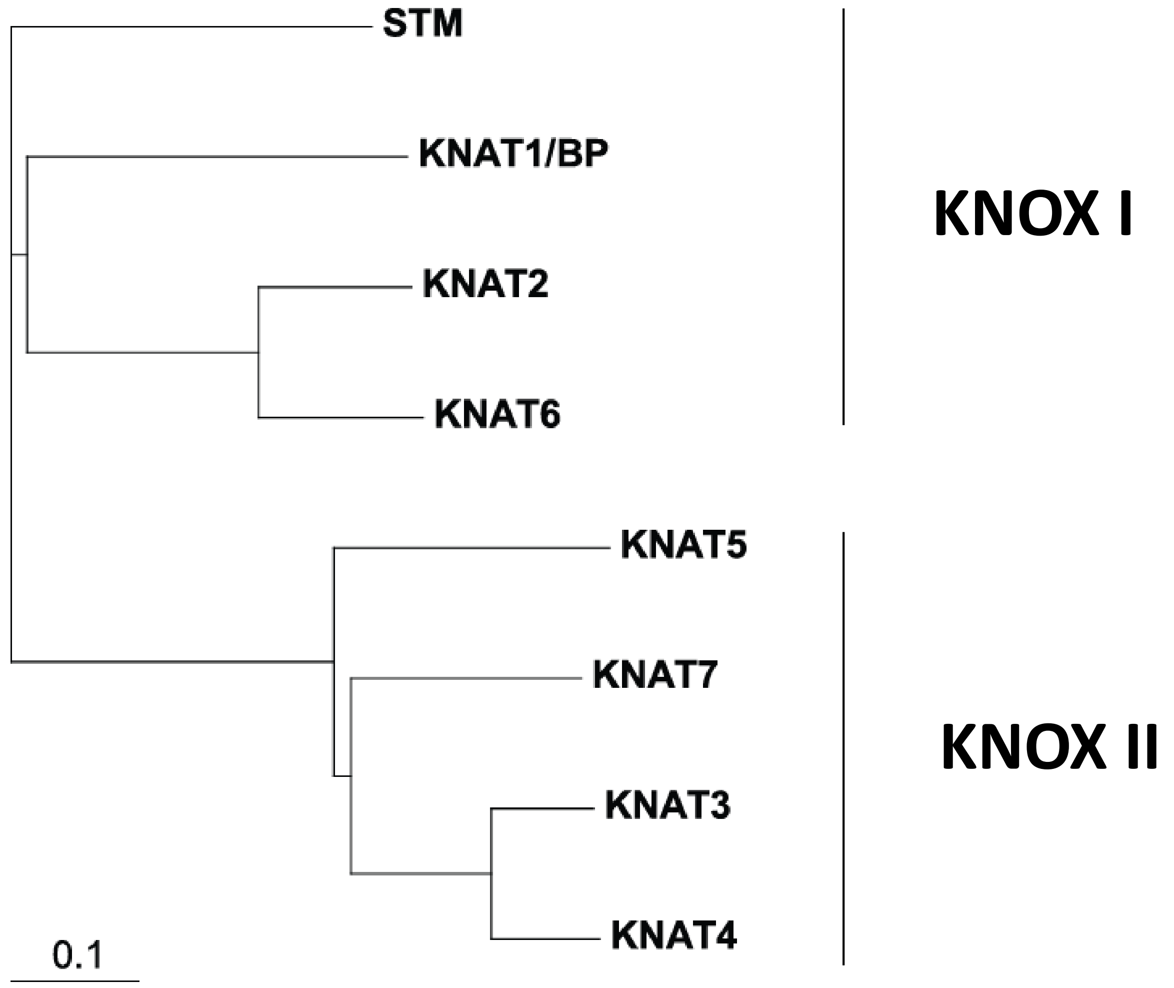
Families of homeobox genes

***Conserved** DNA-binding homeodomain motif*

Transcriptional regulators

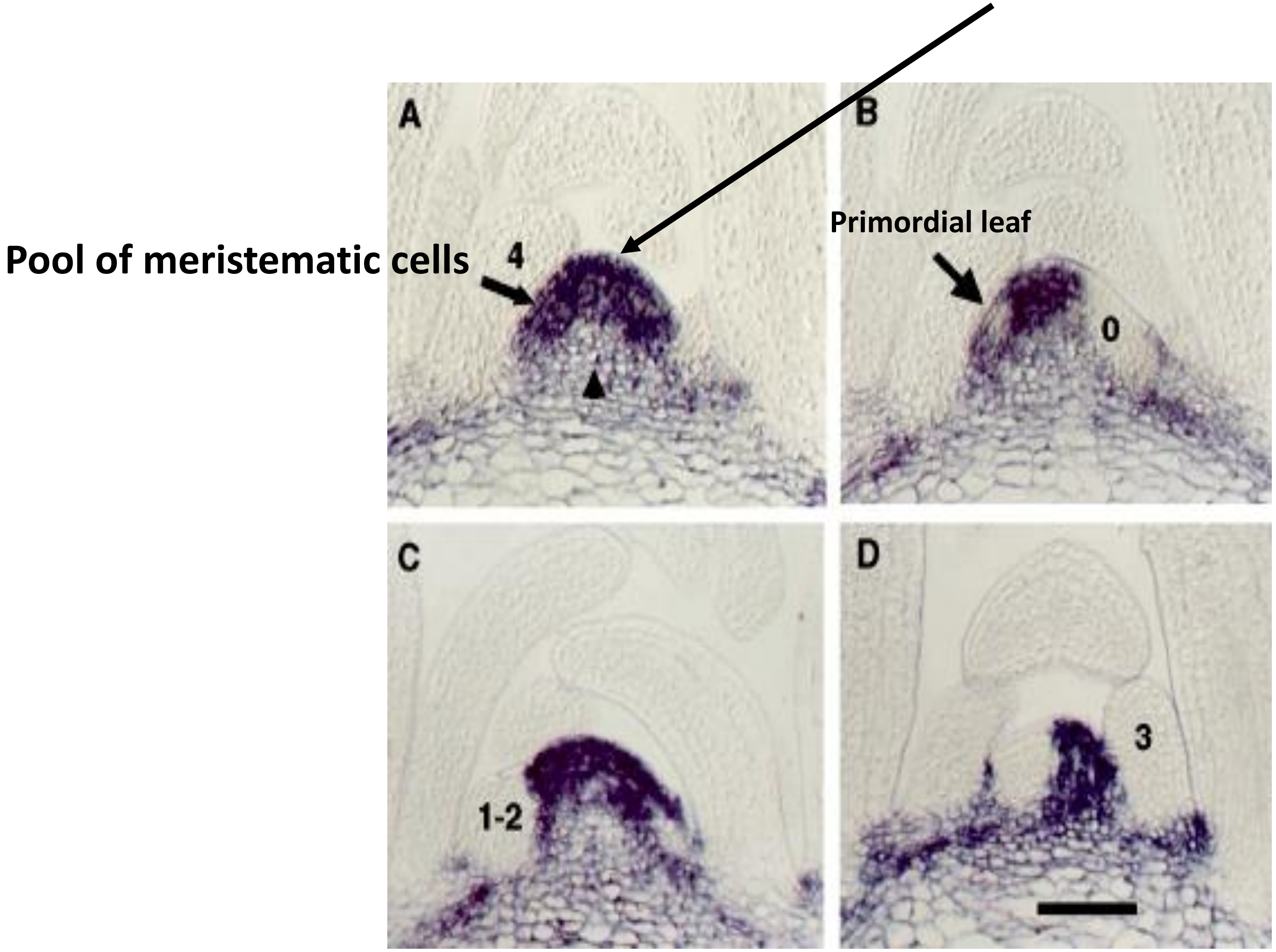
Control of development in multicellular eukaryotes

Phylogenetic relationship of the eight *Arabidopsis* KNOX proteins



Tree was drawn using Treeview (Sunaryo and Fischer , 2009)

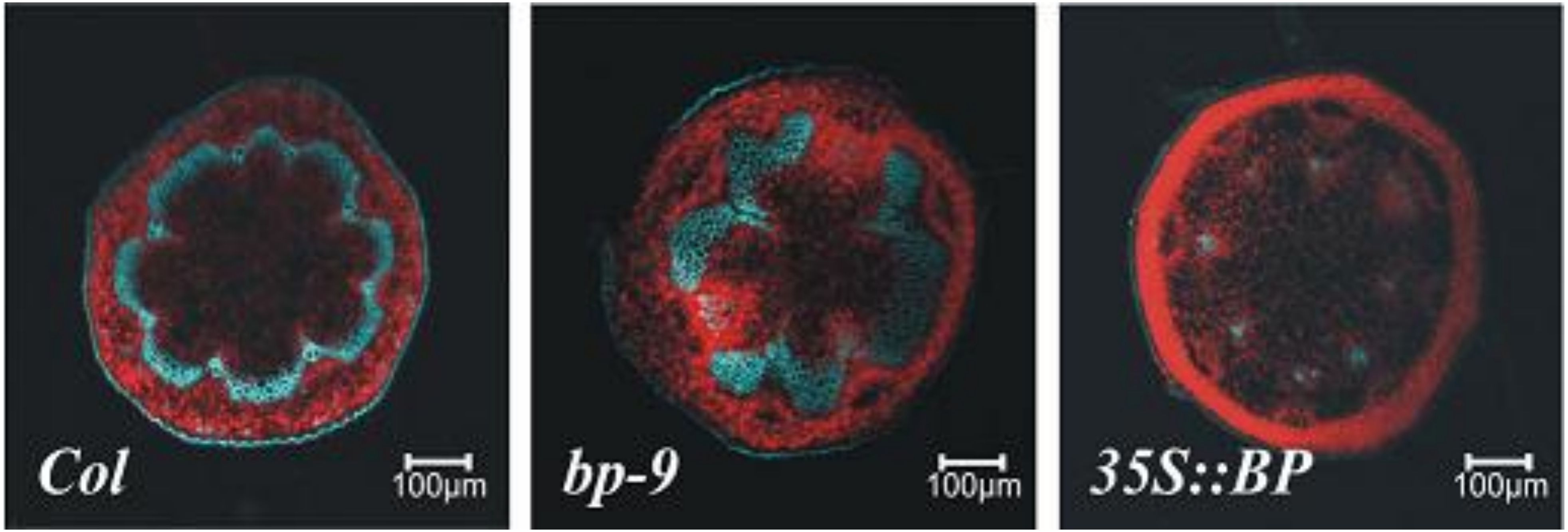
Shoot Meristemless (*STM*) keeps meristematic cells undifferentiated in the shoot apical meristem (SAM) of *Arabidopsis*



(Long and Barton, 2000)

stm phenotype in the seedling

Lignin deposition in *bp-9* mutant of *Arabidopsis thaliana*



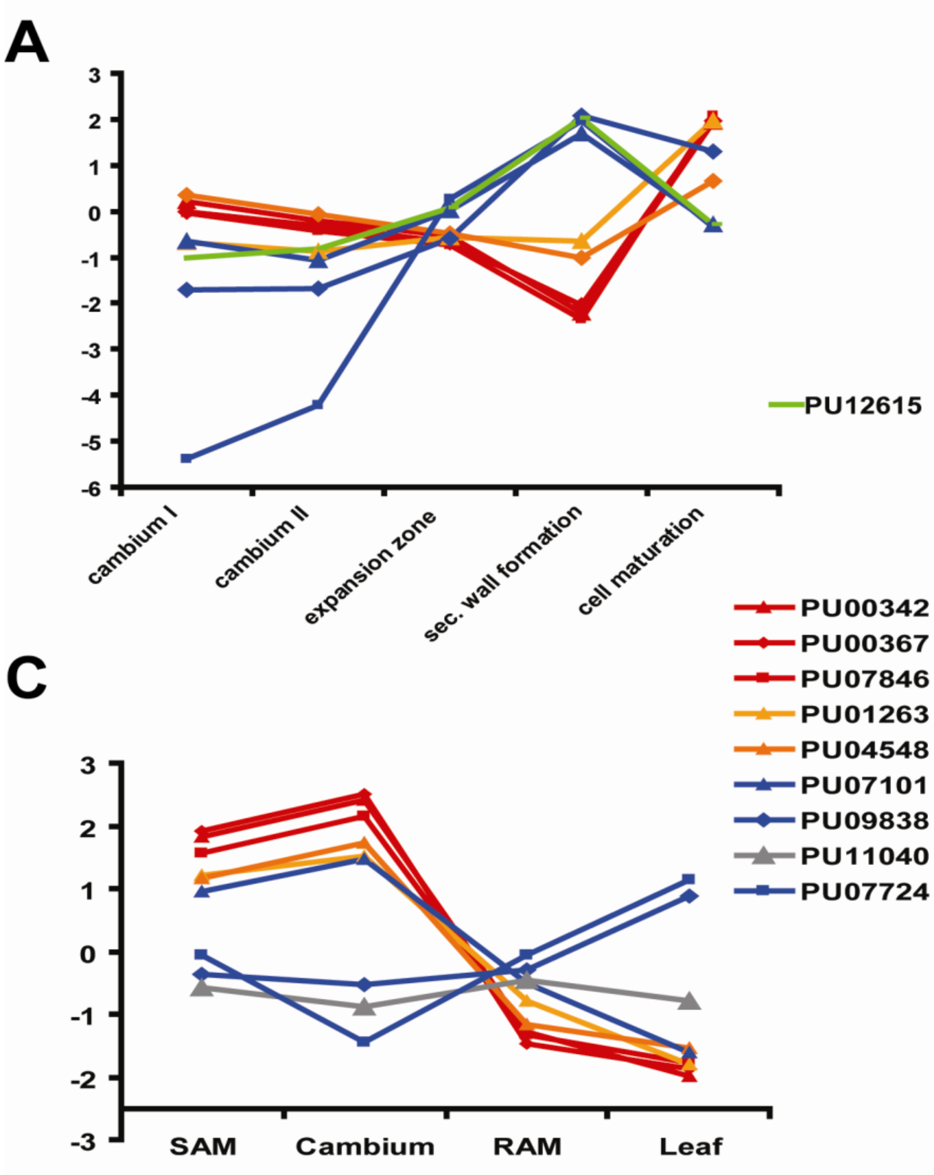
Red : Chlorophyll localization

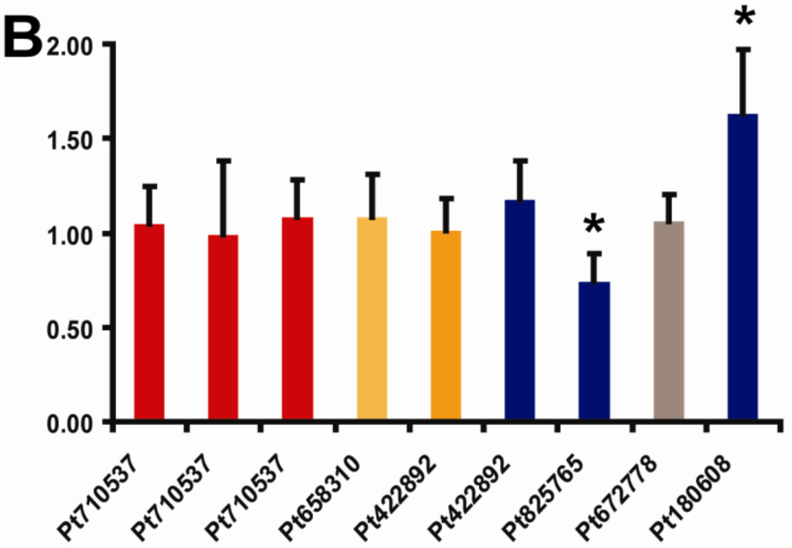
Blue : Lignin deposition

Giovanni Mele et al.. (2003) Genes Dev. 17: 2088-2093

Expression of *KNOX* genes in vascular cambium of poplar

(Sunaryo and Fischer, 2009)





D

EST ID	Protein ID	Clade
PU00342	Pt710537	KNAT1
PU00367	Pt710537	KNAT1
PU07846	Pt710537	KNAT1
PU01263	Pt658310	KNAT6/2
PU04548	Pt422892	KNAT6/2
PU07101	Pt422892	KNAT6/2
PU09838	Pt825765	KNAT3
PU11040	Pt672778	KNAT3/4
PU07724	Pt180608	KNAT7

Model system

Woody plants

- Long generation times
- Slow growth
- Apical-basal gradient
- Seasonal regulation
- Diameter: even > 5 m

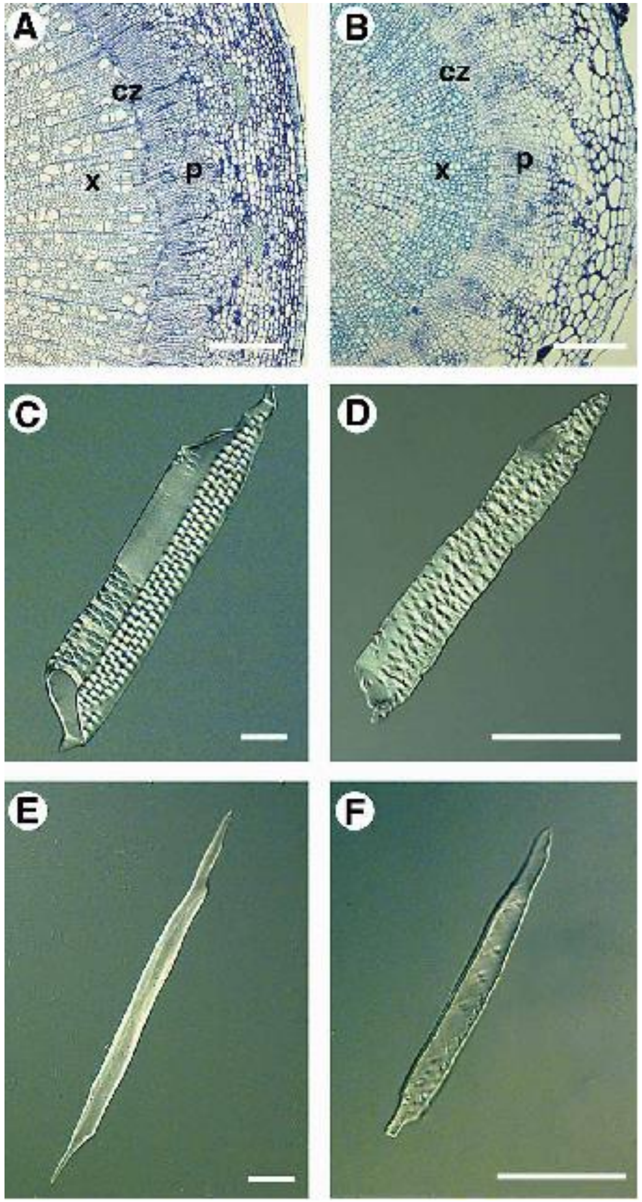
Production of mutants laborious, inefficient and unspecific, genetic approach unfeasible

***Arabidopsis* Hypocotyl**

- Short life cycle
- No apical-basal gradient
- No seasonal regulation
- Diameter: ±1,5mm

Production of mutants and genetic approach feasible

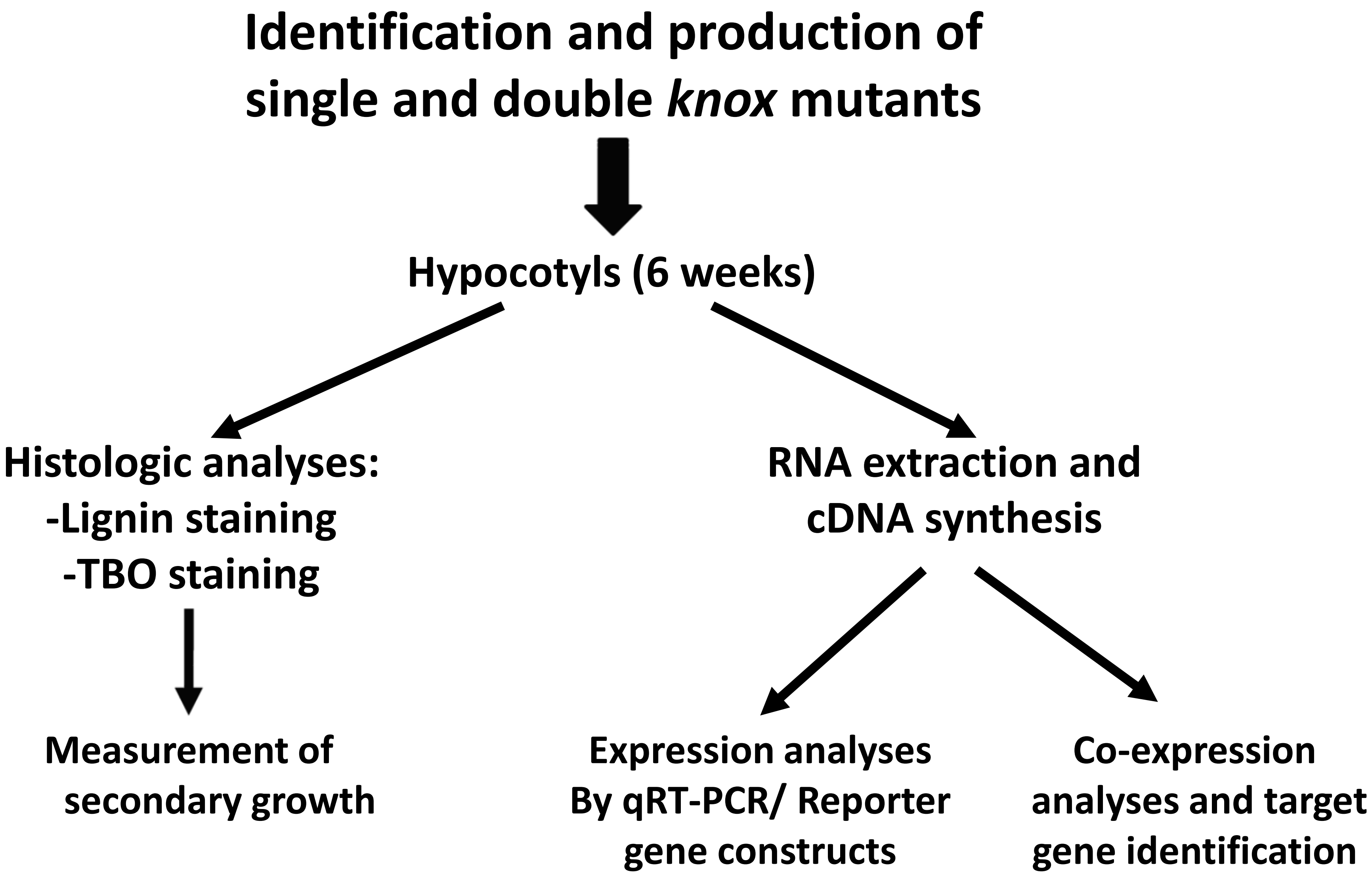
Secondary growth in *Arabidopsis* hypocotyls vs poplar (Chaffey et al. 2002)



Secondary Growth Anatomical Characteristics	*Arabidopsis*	Poplar
Xylem:		
1. Vessel elements	Yes	Yes
2. Fiber 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

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

Strategy



Identification and production of single and double *knox* mutants

Hypocotyls (6 weeks)

Histologic analyses:
-Lignin staining
-TBO staining

Measurement of secondary growth

RNA extraction and cDNA synthesis

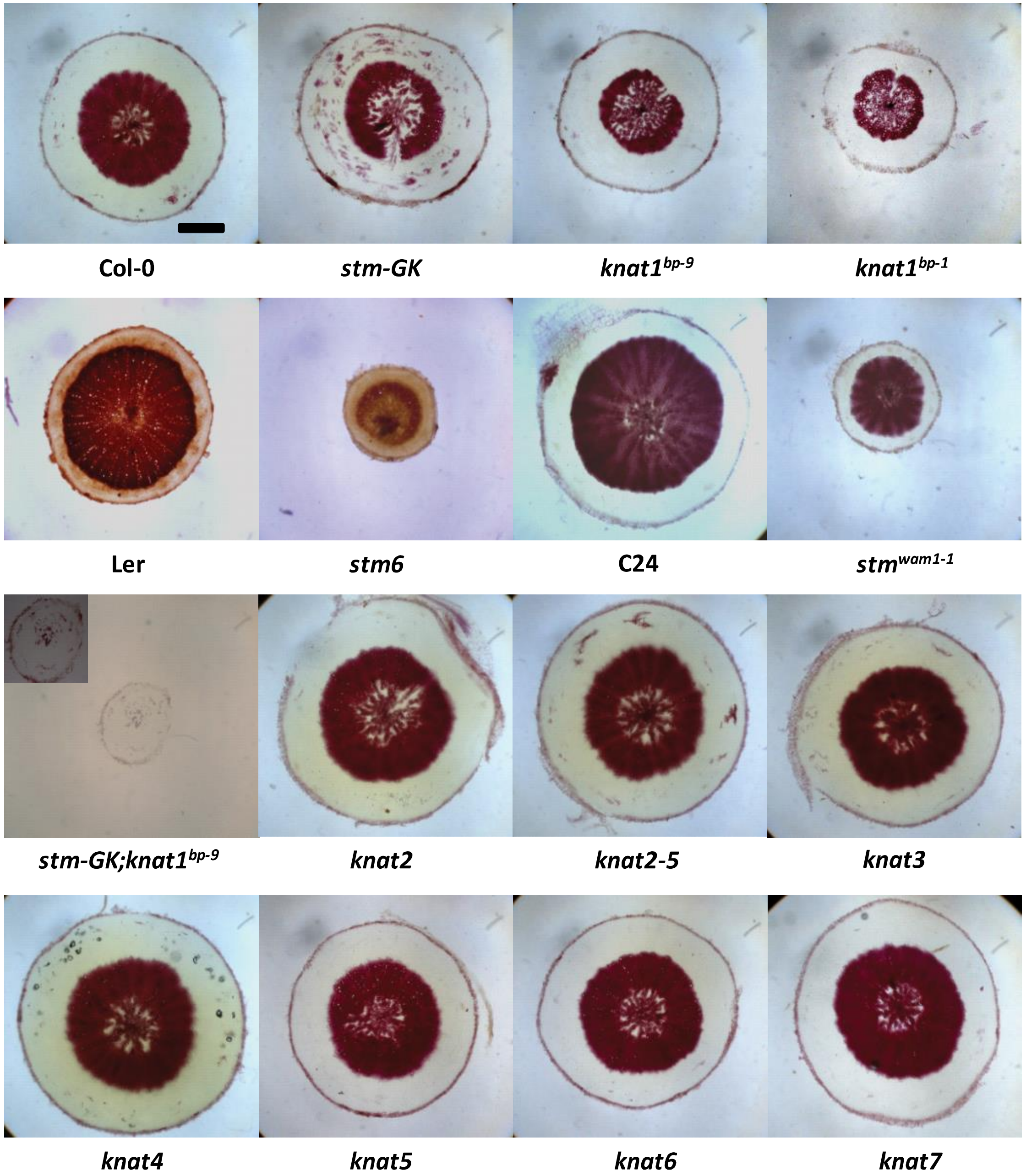
Expression analyses By qRT-PCR/ Reporter gene constructs

Co-expression analyses and target gene identification

Defective hypocotyl diameter in *stm* and *knat1* mutants

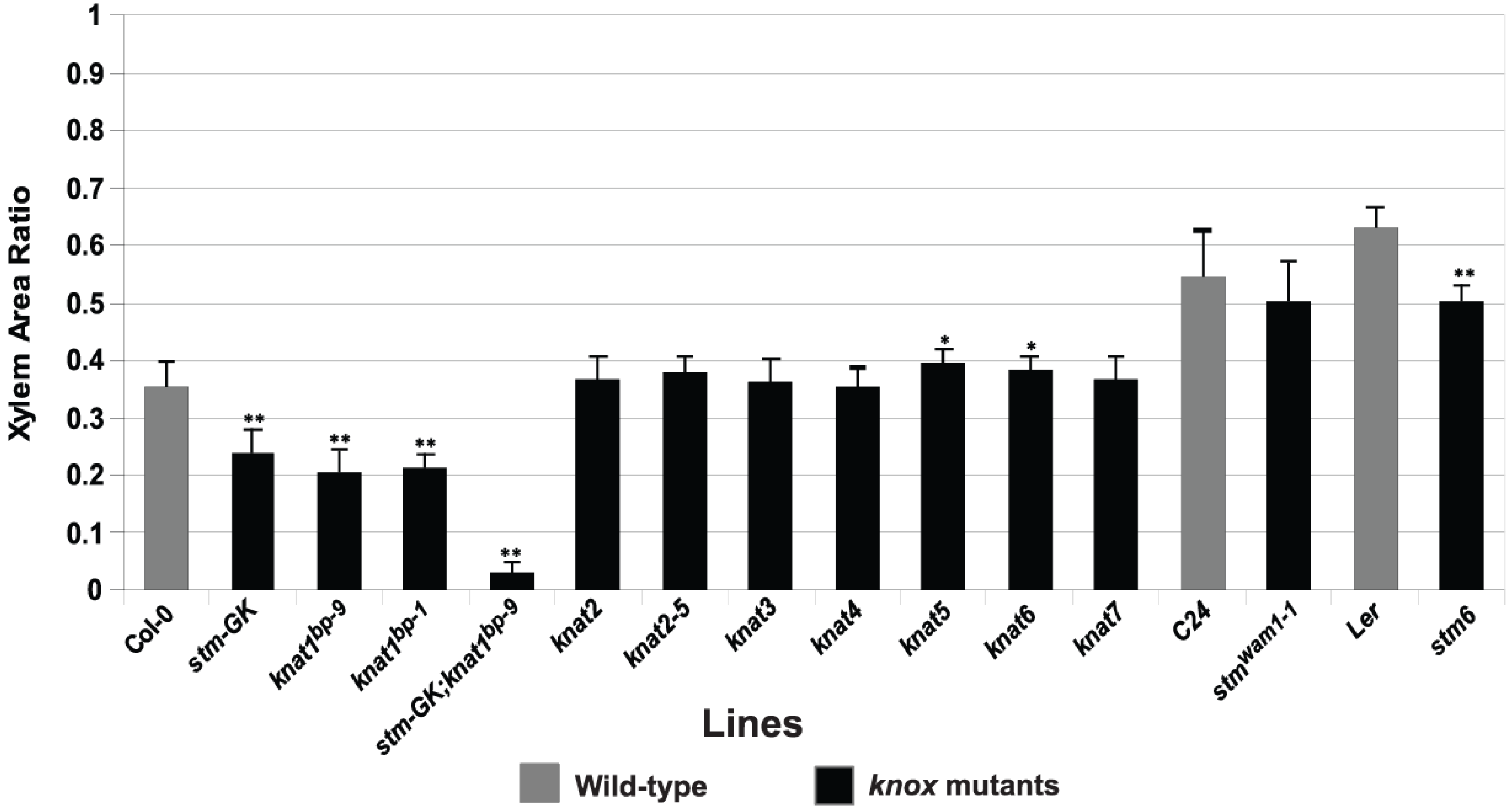
Bar: 1 mm

Defective secondary xylem development in *Arabidopsis* hypocotyls of *stm* and *knat1* mutants



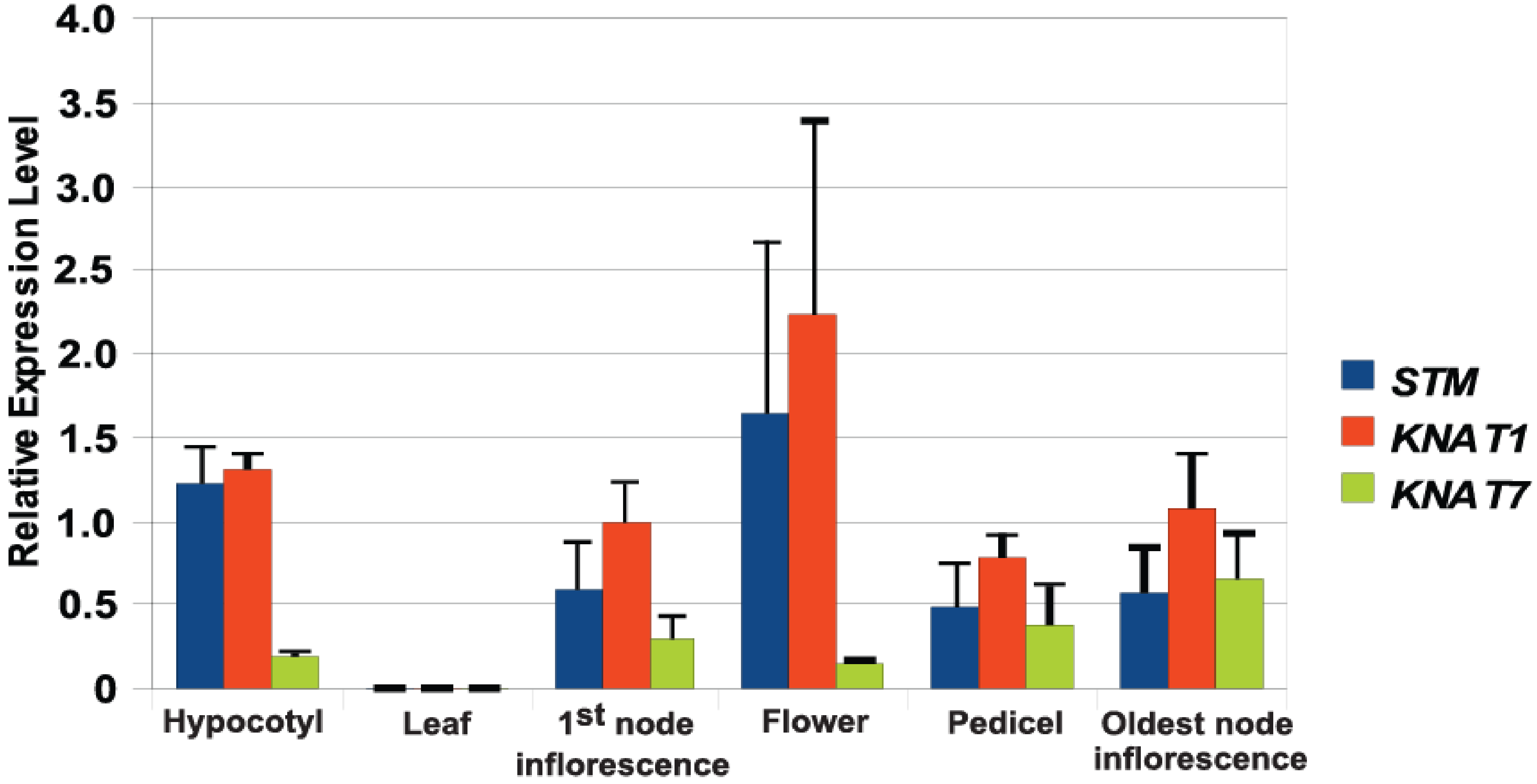
Bar: 200 µm, stained using phloroglucinol HCl

STM and *KNAT1* have overlapping function and act synergistically on secondary growth of *Arabidopsis* hypocotyls



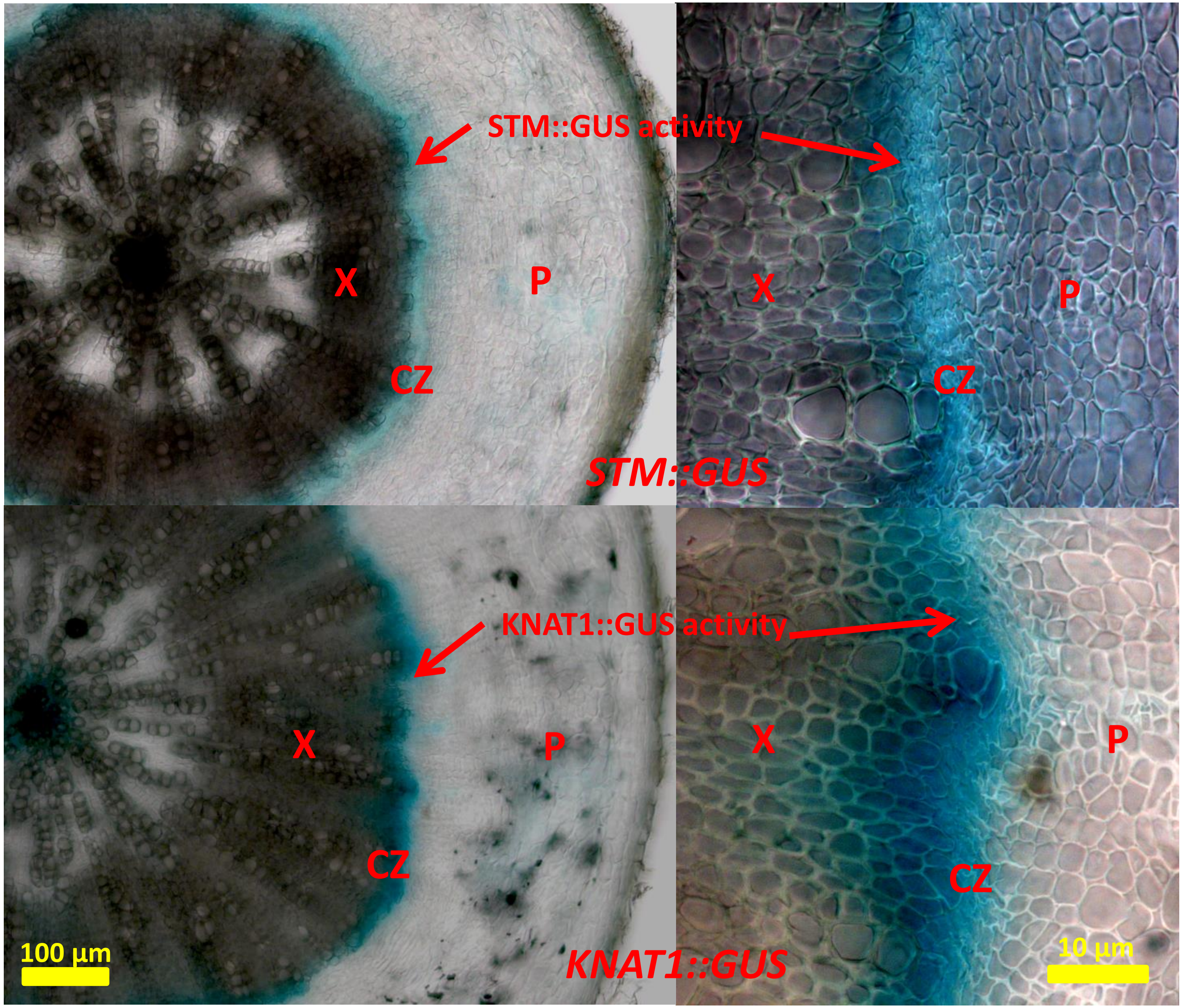
Data was obtained from 3 independent experiments, each with 3 hypocotyls. (**) Significant at p ≤ 0.01, t-test, compared to wild-type, (*) significant at 0.01< p ≤0.05

STM and *KNAT1* are strongly expressed in hypocotyls

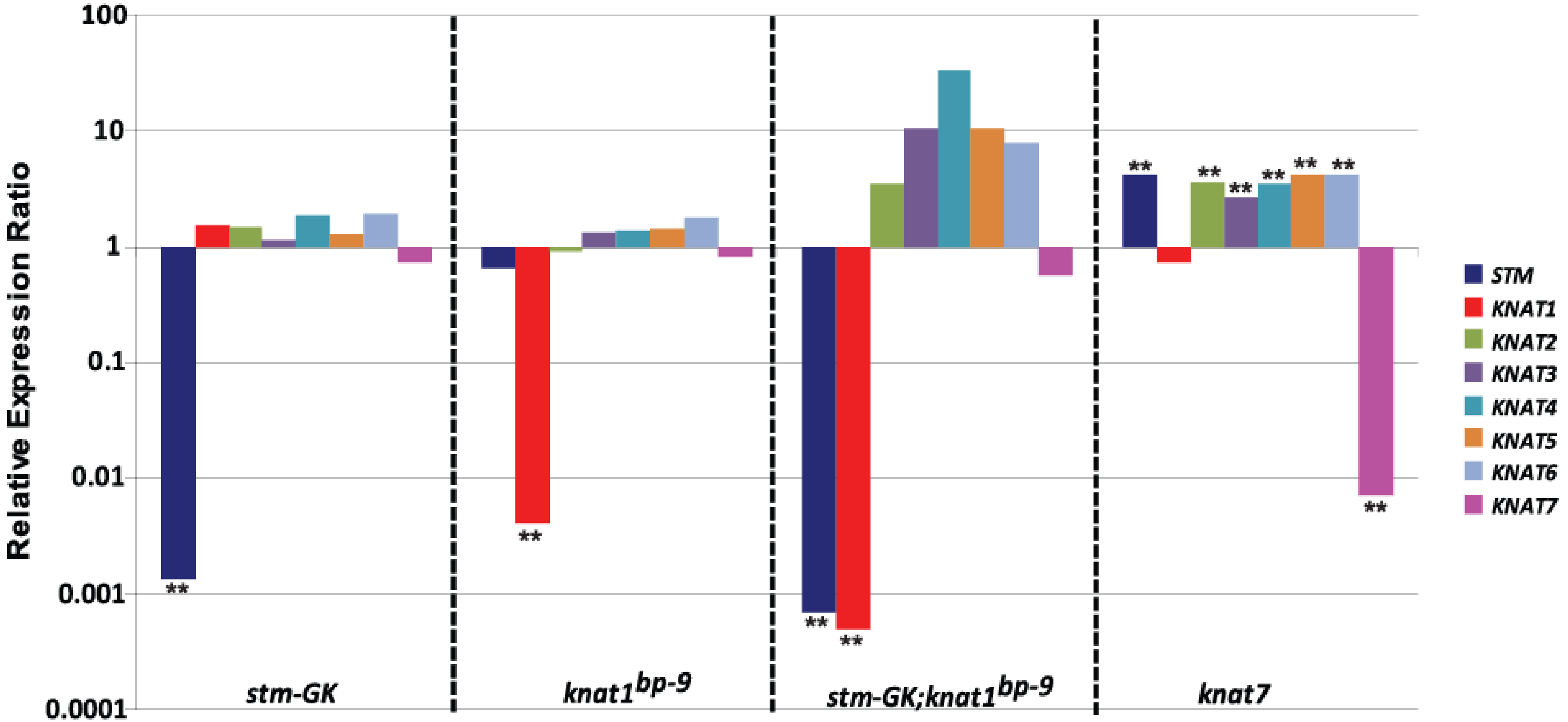


Data were analyzed from 3 biological and 3 technical replicates and normalized to the expression of *ACTIN2*.

STM and *KNAT1* are specifically expressed in the cambial zone

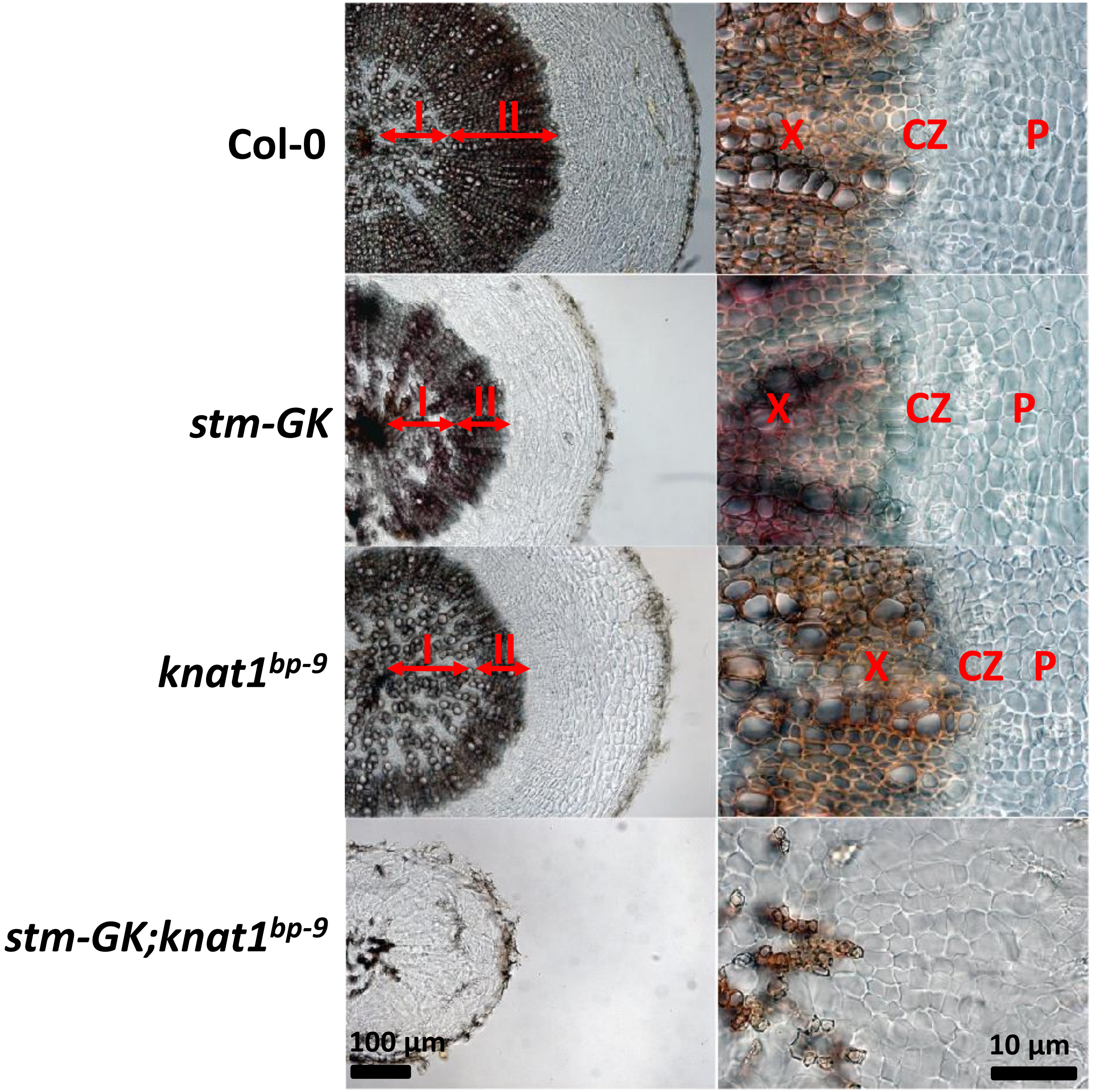


Non-epistatic and synergistic interaction between *STM* and *KNAT1* in *Arabidopsis* hypocotyls



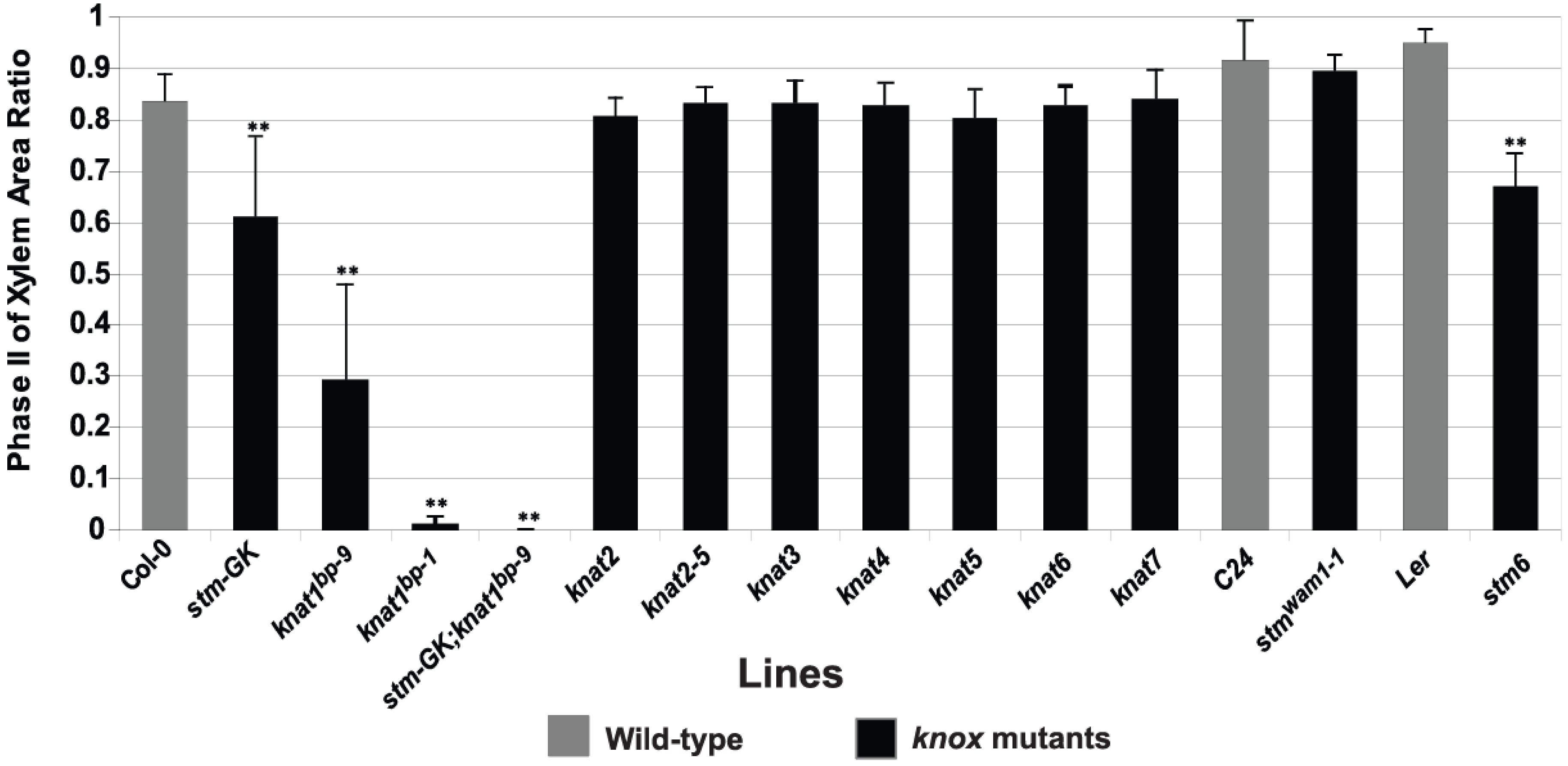
The expression ratio is relative to wild-type (Col-0). Data were analyzed from 3 biological and 3 technical replicates and normalized to the expression of *ACTIN2*

Phase II of secondary xylem development is reduced in *stm* and *knat1* mutants



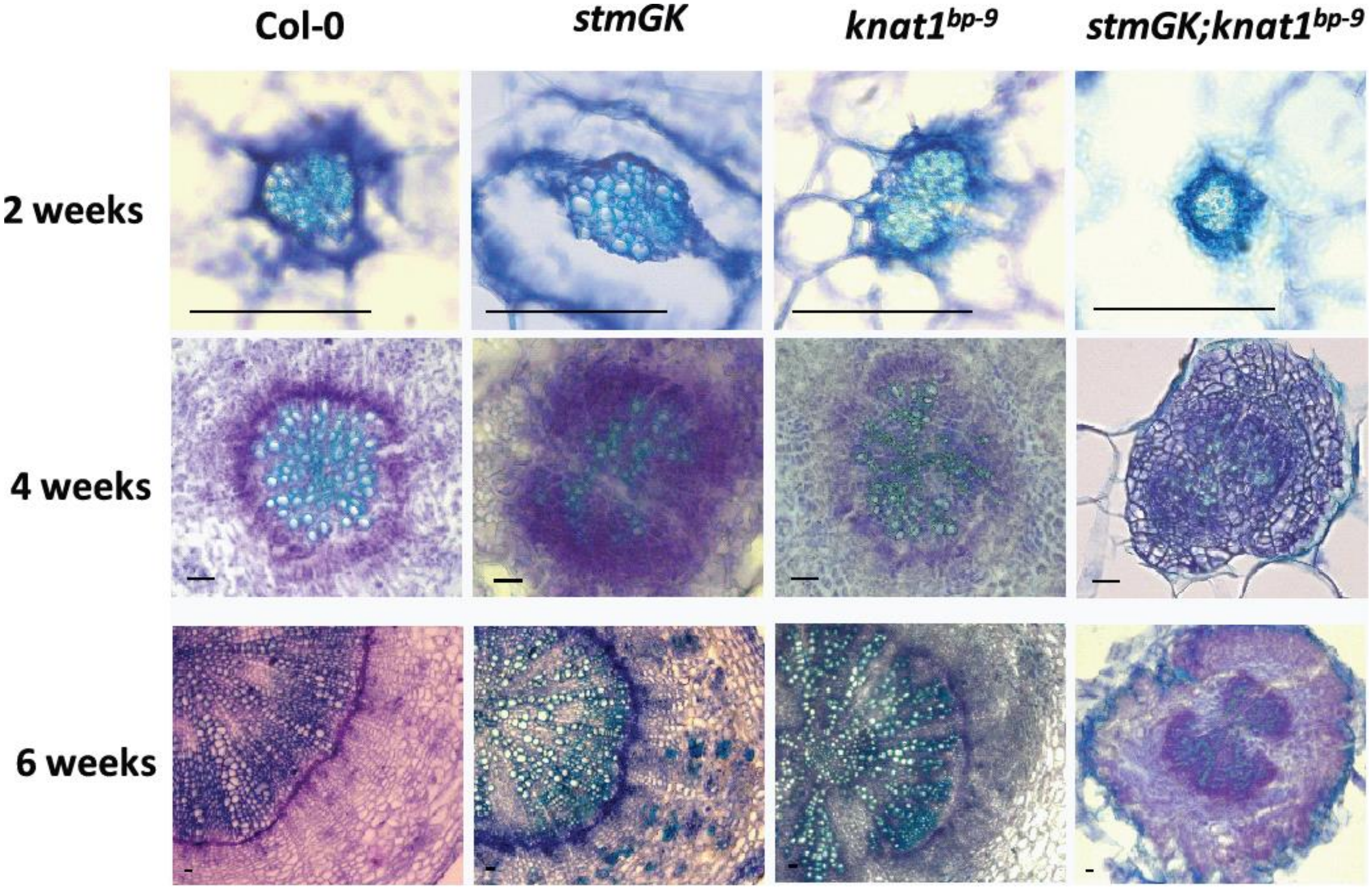
Stained using phloroglucinol-HCl

The phase II of secondary xylem development was significantly decreased in *stm* and *knat1* mutants



Data was obtained from 3 independent experiments, each with 3 hypocotyls. (**) Significant at p ≤ 0.01, t-test, compared to wild-type, (*) significant at 0.01< p ≤0.05.

The development of secondary xylem is inhibited in *stm* and *knat1* mutants



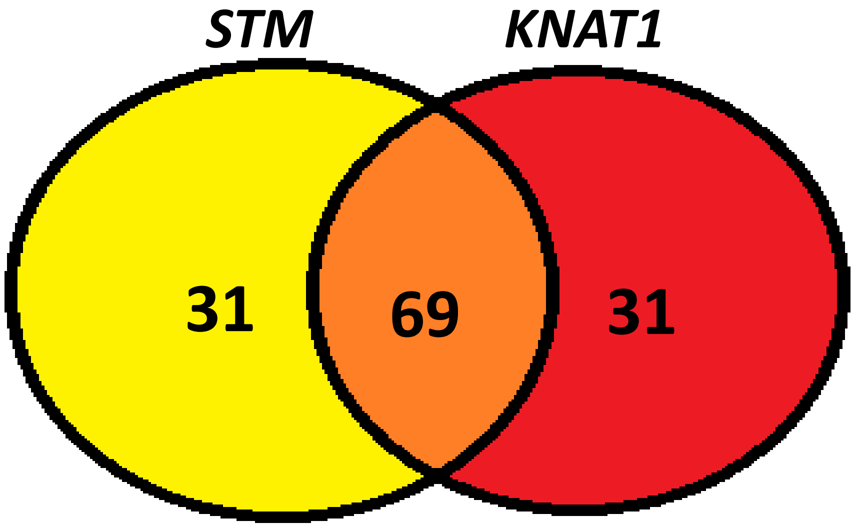
Bar: 50µm, stained using TBO

From 100 genes co-expressed with either *STM* or *KNAT1*, 69 were identical (overlapping)

Co-expression analysis:

	Expression	
STM/KNAT1	⇩ Up	⇧ Down
Target genes	⇩ Up	⇧ Down

Arabidopsis Co-expression Tool (ACT) web server tools
(www.arabidopsis.leedsack.uk/ACT)
21.800 genes from over 300 arrays (Manfield et al. 2006)

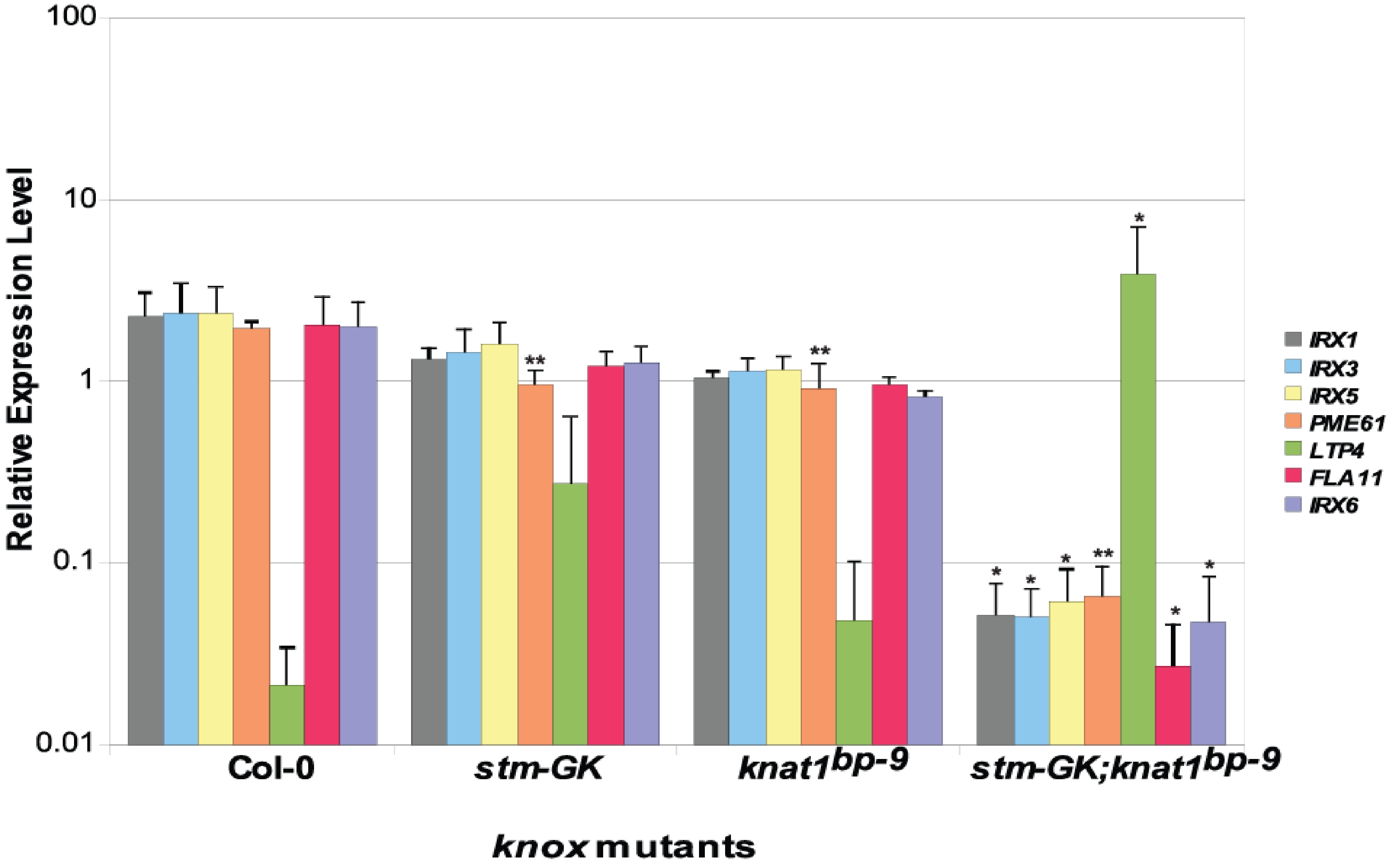


STM/KNAT1 were upstream of genes associated with vascular meristem differentiation, fiber development and secondary cell wall formation

No.	**Gene**	**Function (putative)**	**Fold Change of Expression in *stm-GK;knat1*$^{bp-9}$**	**p value (*)**
1.	*PME61*	Pectin formation	- 44 x	0.0212
2.	*CesA8(IRX1)*	Cellulose biosynthesis	- 30 x	0.0009
3.	*CesA7(IRX3)*	Cellulose biosynthesis	- 186 x	0.0317
4.	*CesA4(IRX5)*	Cellulose biosynthesis	- 76 x	0.0296
5.	*COBL4 (IRX6)*	Arrangement of cellulose microfibrils	- 42 x	0.0248
6.	*ATHB-8*	Vascular identity	- 3 x	0.0150
7.	*SND1*	Xylem fiber identity	N.D.	N.A.
8.	*SND2*	Xylem vessel identity	- 107 x	0.0111
9.	*NST1*	Xylem fiber identity	- 278 x	0.0164
10.	*ARF4*	Auxin signalling	- 3 x	0.0507
11.	*IAA27*	Auxin signalling	- 57 x	0.0005
12.	*GAUT12(IRX8)*	Hemicellulose biosynthesis	- 723 x	0.0009
13.	*LAC4 (IRX12)*	Lignin biosynthesis	- 404 x	0.0116
14.	*CTL2*	Lignin biosynthesis	- 100 x	0.0033
16.	*PAL4*	Lignin biosynthesis	- 3 x	0.0167

Data were analyzed from 4 biological and 2 technical replicates and normalized to the expression of *ACTIN2*. (*) Calculated based on t-test. (N.D) Not detectable, (N.A) not applicable.

STM/KNAT1 were upstream of genes associated with vascular meristem differentiation, fiber development and secondary cell wall formation



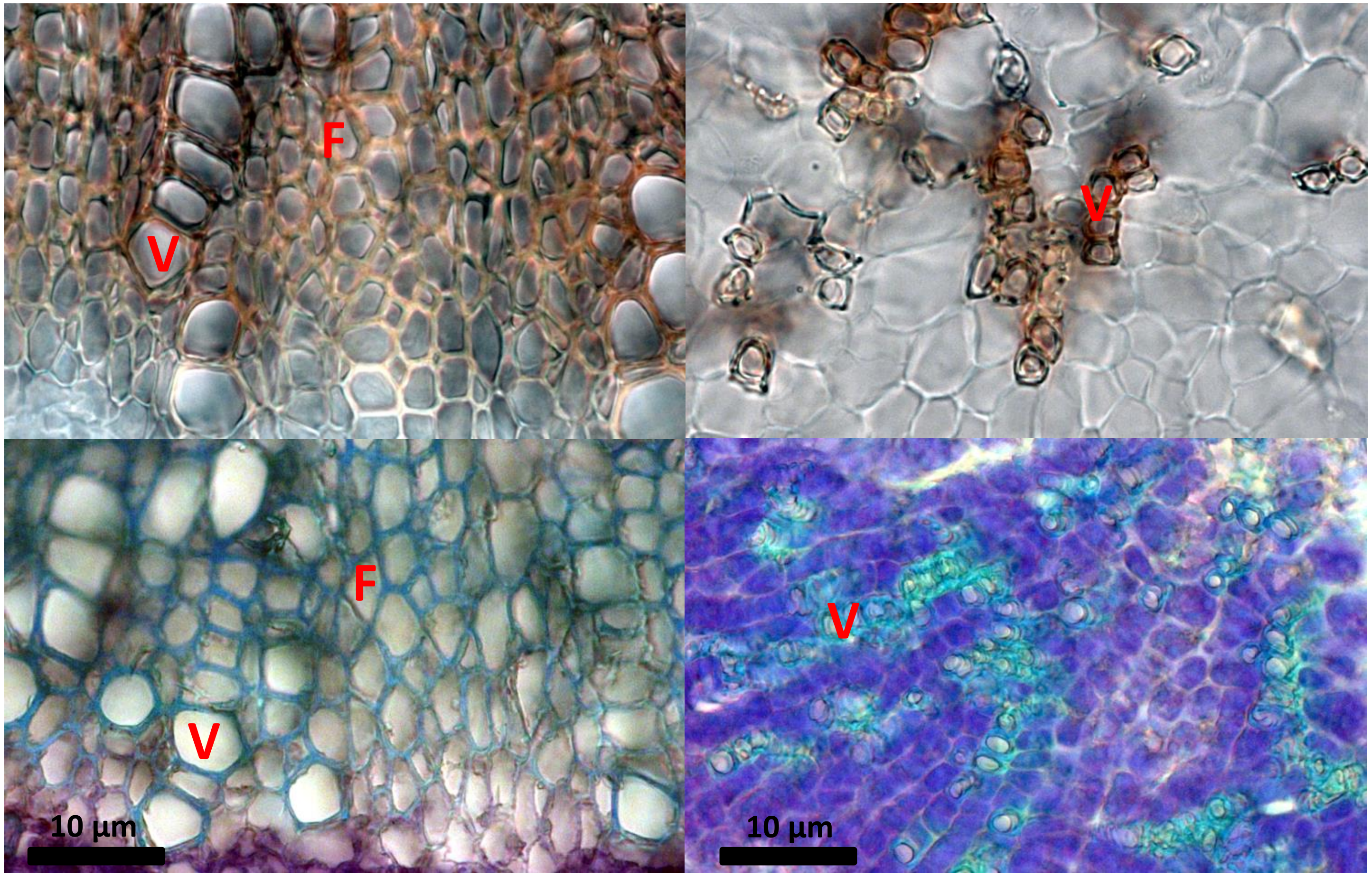
The double mutant of *stm-GK* and *knat1*$^{bp\text{-}9}$ showed a reduced diameter and more angular shape of vessel cells, reminiscent to various *irx* mutants

Col-0

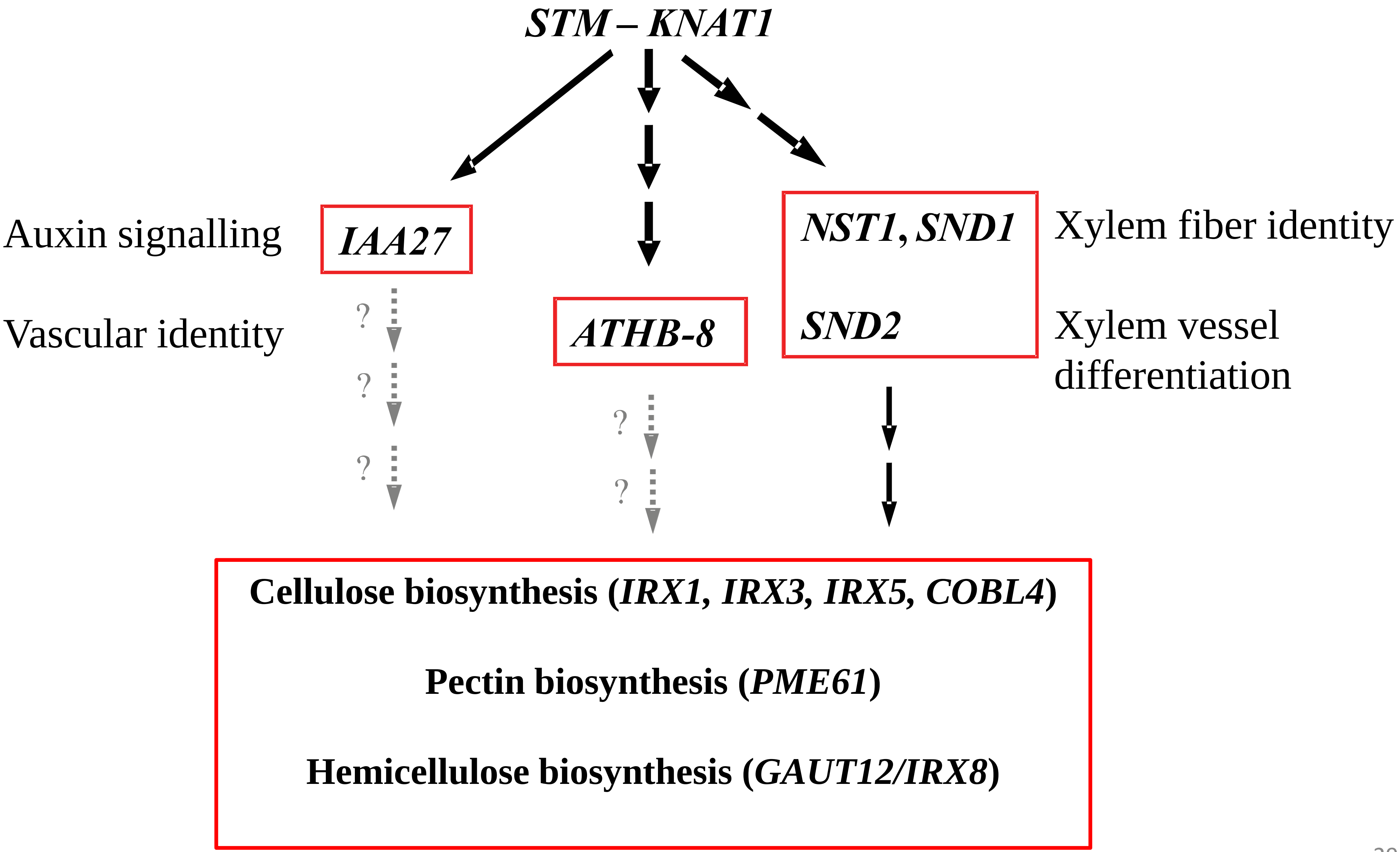
stm-GK;knat1$^{bp\text{-}9}$

Phloroglucinol staining

TBO staining



A working model of *STM* and *KNAT1* action during secondary xylem development



STM – KNAT1

Auxin signalling **IAA27**

Vascular identity

? ? ?

ATHB-8

? ?

NST1, SND1 Xylem fiber identity

SND2 Xylem vessel differentiation

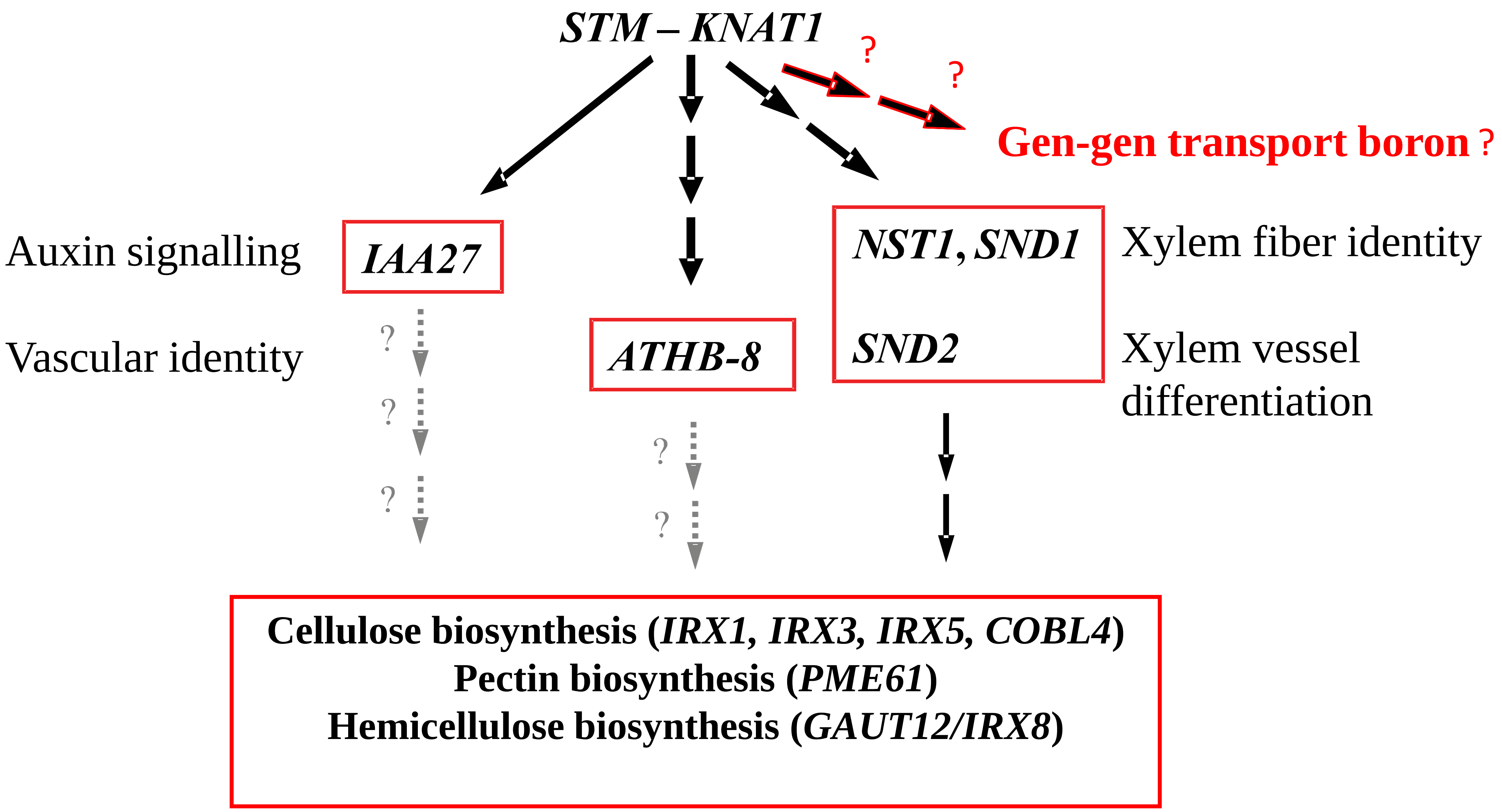
Cellulose biosynthesis (*IRX1, IRX3, IRX5, COBL4*)

Pectin biosynthesis (*PME61*)

Hemicellulose biosynthesis (*GAUT12/IRX8*)

Recent Projects

1. The physiological and molecular studies of the involvement of *STM/KNAT1 in Boron transport associated with deficiency and toxicity responses.*
2. Genetic diversity *of Durio kutejensis* inEast Kalimantan


STM – KNAT1
?
?
Gen-gen transport boron ?
Auxin signalling
IAA27
Vascular identity
?
?
?
ATHB-8
?
?
NST1, SND1
SND2
Xylem fiber identity
Xylem vessel differentiation
Cellulose biosynthesis (IRX1, IRX3, IRX5, COBL4)
Pectin biosynthesis (PME61)
Hemicellulose biosynthesis (GAUT12/IRX8)

Acknowledgements

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- All colle tany and Tree Ph
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  1. DFG (poplar Group)

Thank You

Vielen Dank

Terima Kasih

Leaf rosettes and mis-expression of *knox* mutants compared to wild-type

Col-0

stm-GK

↓ **746 x**
******

knat1^{bp-9}

↓ **243 x**
******

knat2

↓ **318 x**
******

knat3

↓ **42 x**
******

knat4

= 1 x
NS

knat5

↑ **17 x**
*****

knat6

↓ **1967 x**
******

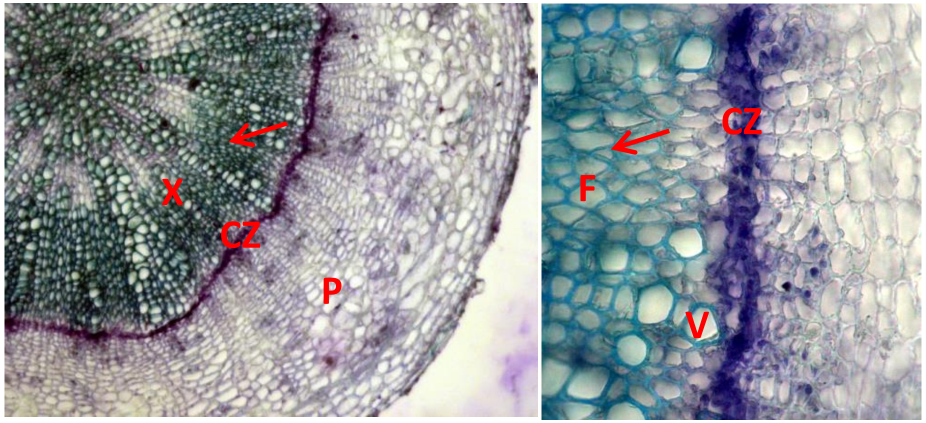
knat7

↓ **140 x**
******

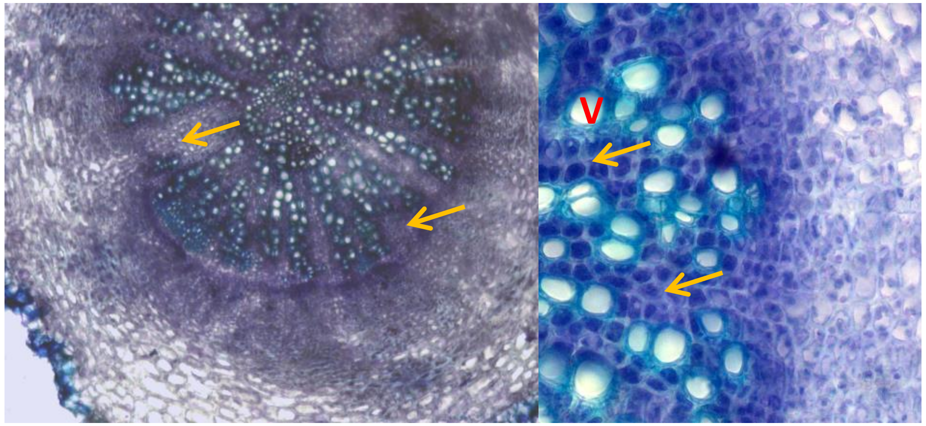
Tangential section of an *Arabidopsis* hypocotyl

STM and *KNAT1* are involved in determination of fiber identity, the formation of phase II xylem and the estasblishment of the cambial zone

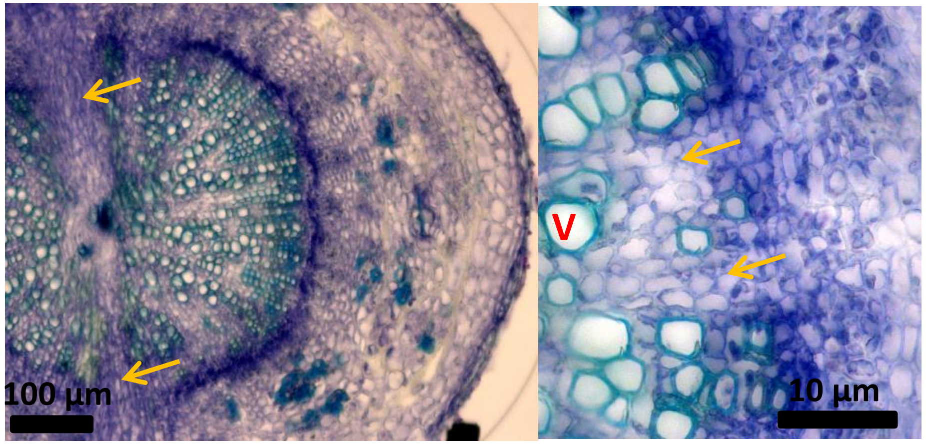
Col-0



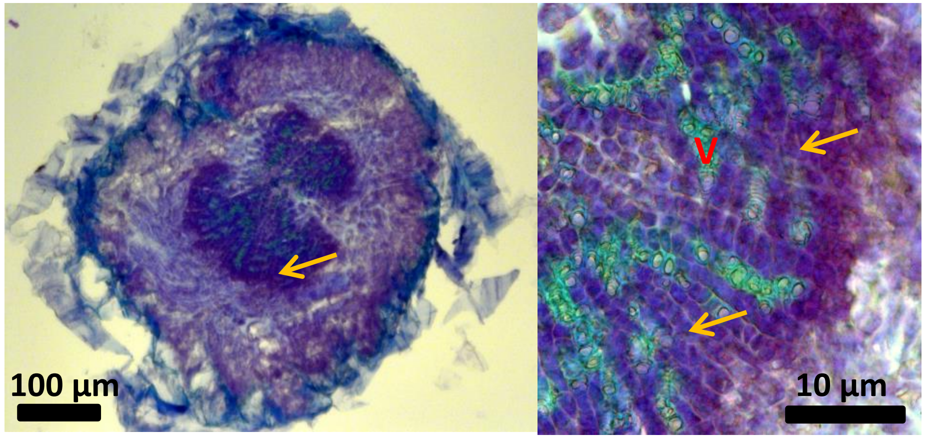
***knat1*$^{bp-9}$**



stm-GK

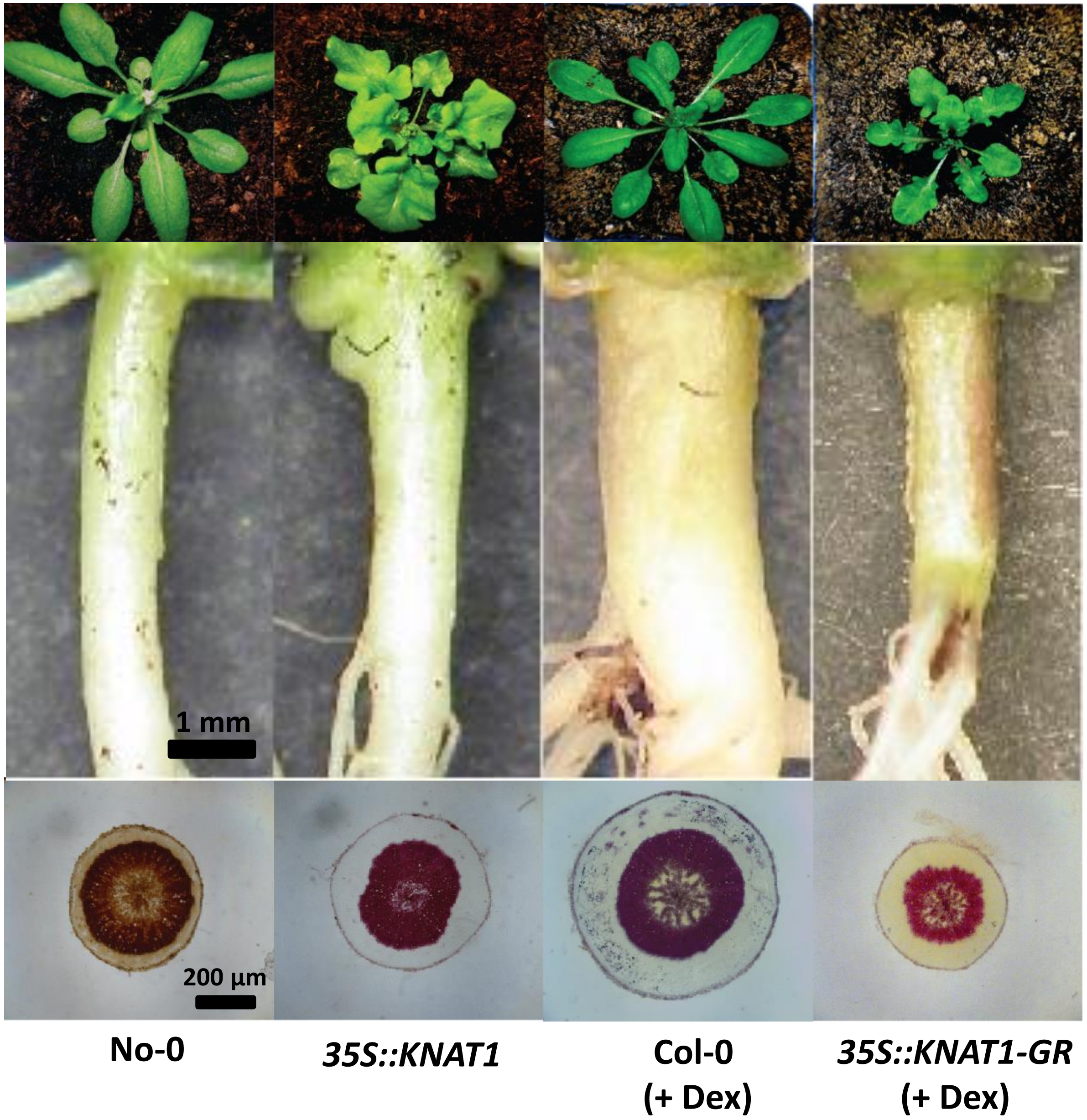


***stm-GK;knat1*$^{bp-9}$**



Red arrows: differentiated xylem cells
Yellow arrows: undifferentiated xylem cells

Secondary growth of *Arabidopsis* hypocotyls was reduced by overexpression of *KNAT1*



Stained using phloroglucinol-HCl

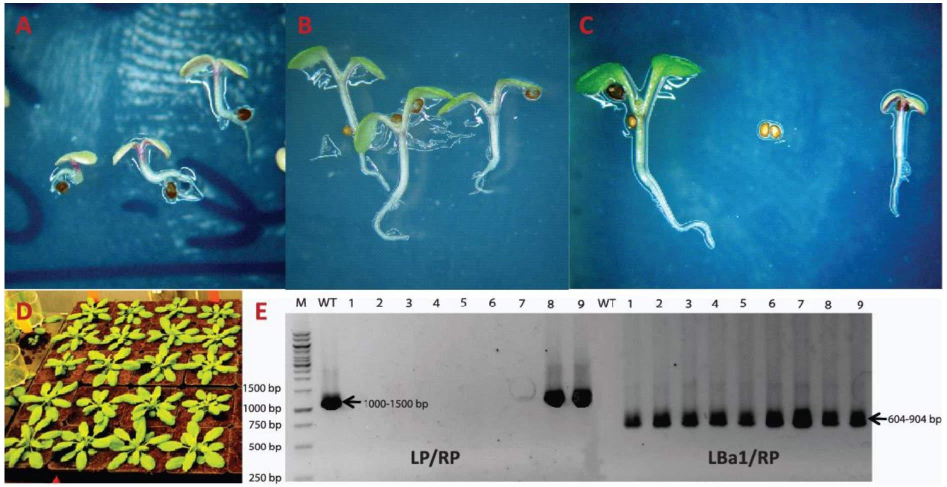
Genes selected from lignin biosynthetic patways reported by Mele et al (2003)

No.	Gene	Locus	Name of Protein	Function (Putative)
1.	At4CL1	AT1G51680	4-Coumarate-CoA Ligase1	Lignin biosynthesis
2.	PAL1	AT2G37040	Phenylalanine Ammonia-Lyase 1	Lignin biosynthesis
3.	CAD1	AT4G39330	Cinnamyl Alcohol Dehydrolase 1	Lignin biosynthesis
4.	PRX	AT3G21770	Peroxidase AtPRXR9GE	Lignin biosynthesis

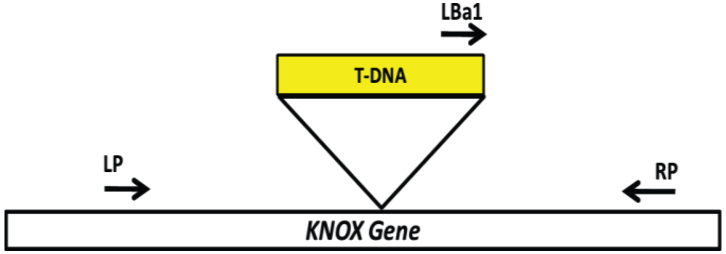
Growth conditions

Grown in long day condition: (16 h light, 8 h dark), fertilized using ½ MS nutrient.

Isolation of *knox* mutants

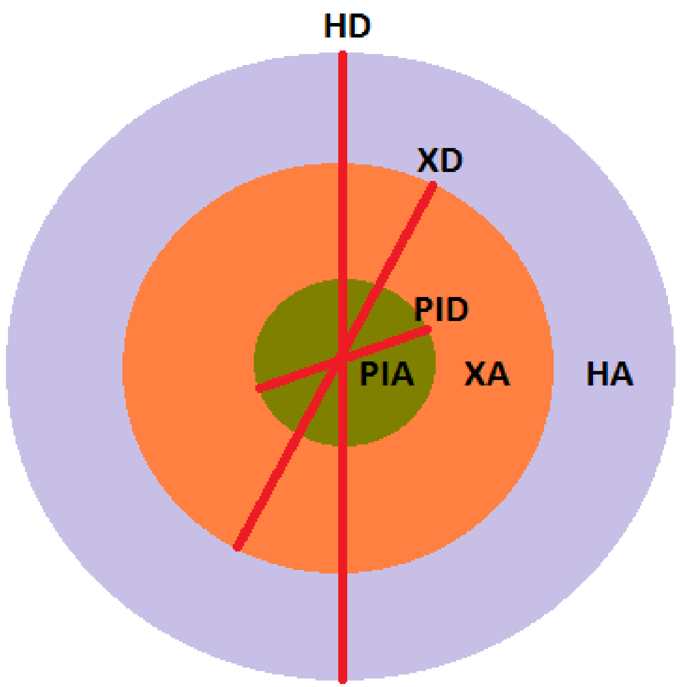


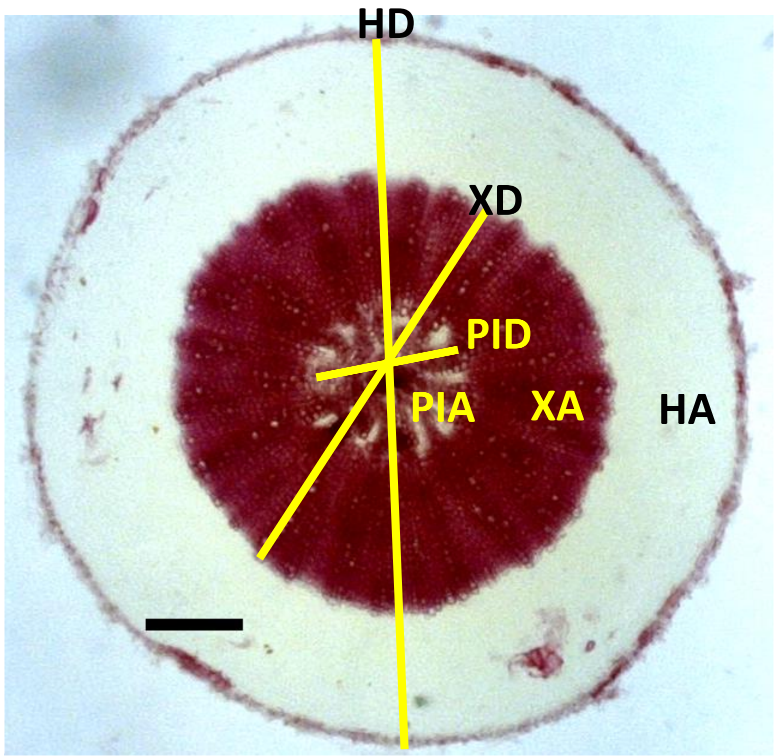
Primer design



(http://signal.edu/tdnaprimers.2.html)

Measurement of secondary growth





$$\text{Xylem Area Ratio} = \frac{\text{Xylem Area } (\mu m2)}{\text{Hypocotyl Area } (\mu m2)}$$

XA = Xylem Area (µm2)
HA = Hypocotyl Area (µm2)
XAR = Xylem Area Ratio

Software: AxioVision rel 4.7.