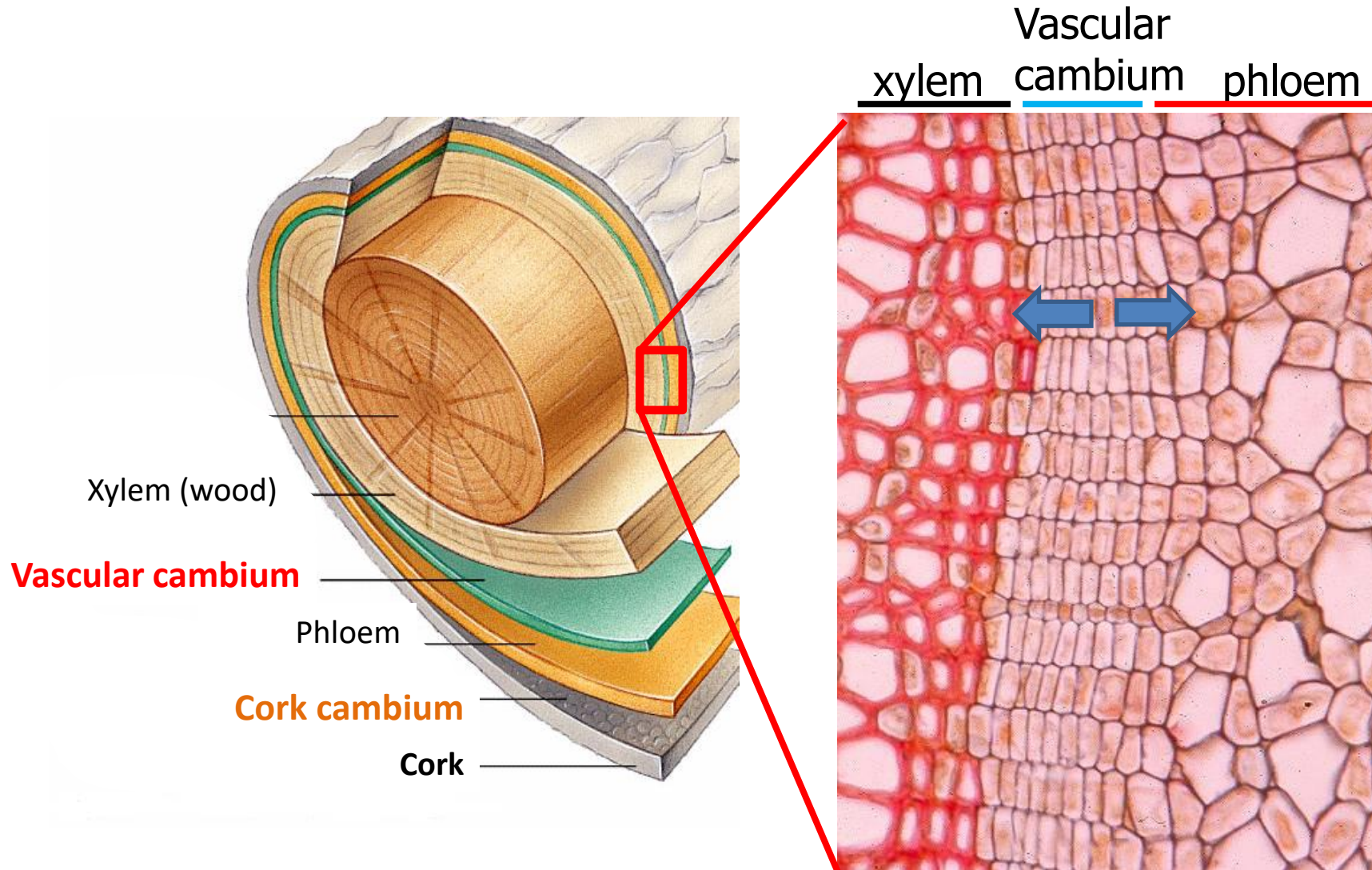


A micrograph of a plant vascular cambium, showing a central ring of cells. The cells are stained with various colors (red, green, blue, yellow) to indicate different levels of activity or specific cellular components. The surrounding tissue is composed of larger, more uniform cells.

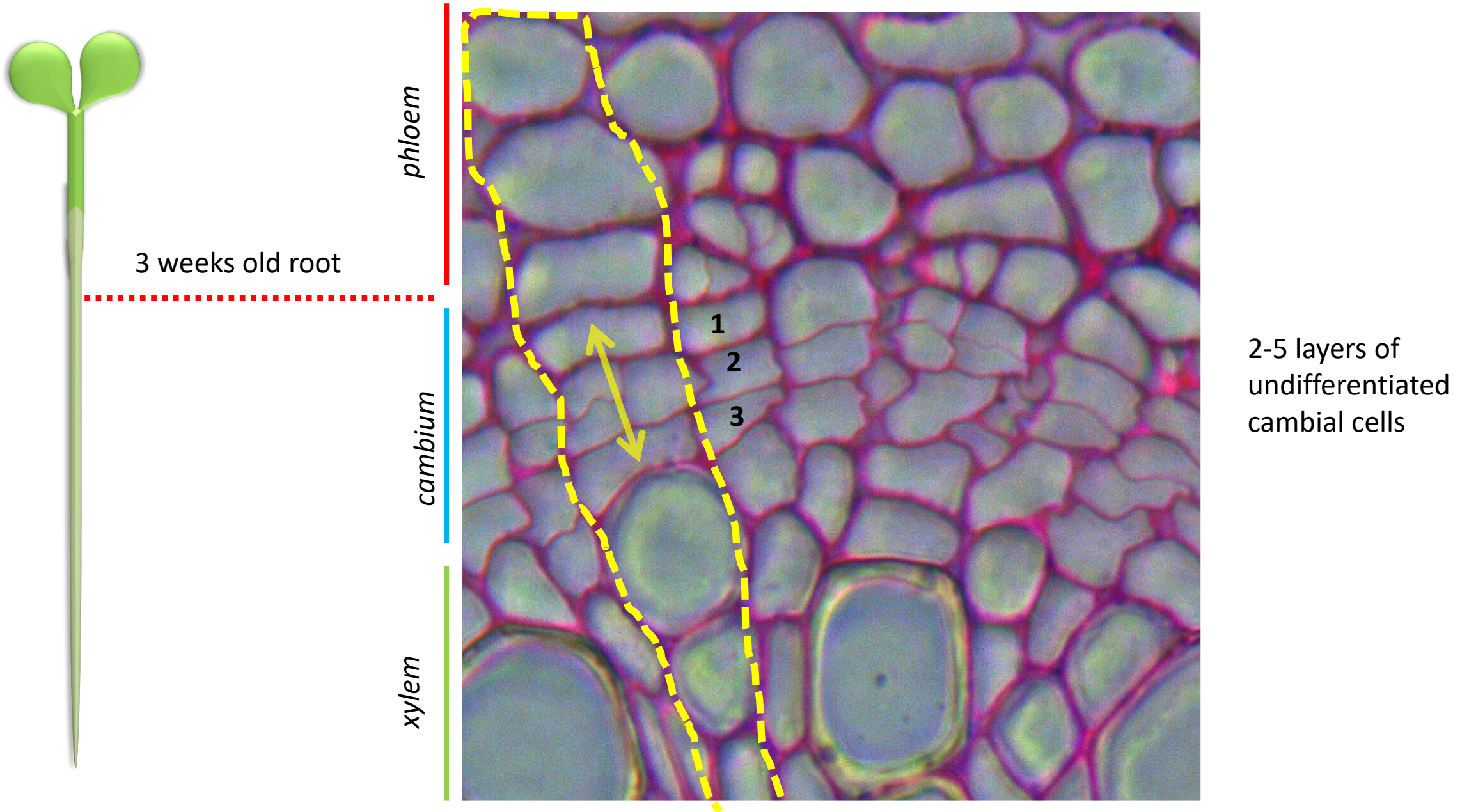
Activity of vascular cambium in Arabidopsis, aspen and cassava

Vascular cambium produces both secondary xylem and secondary phloem

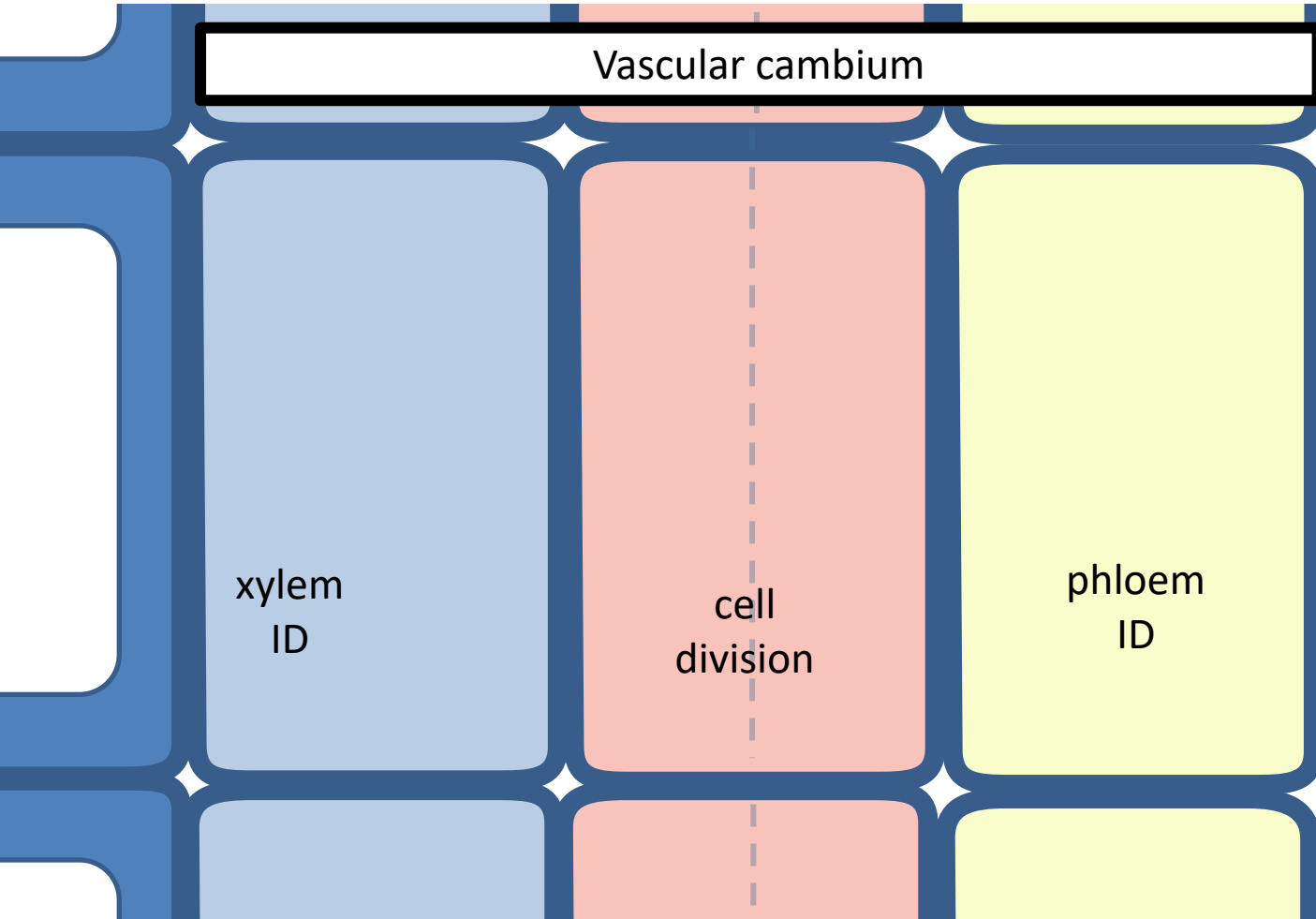


Vascular cambium produces also the secondary tissue of cassava storage root

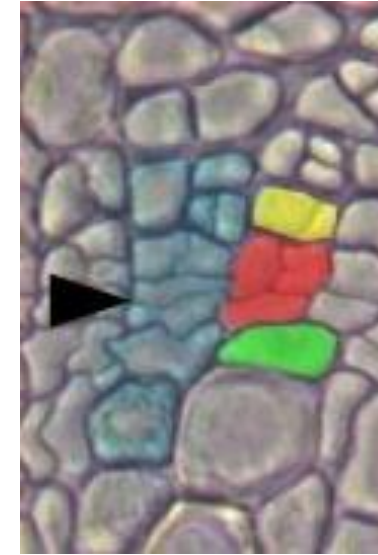
Arabidopsis thaliana root as a model for cambium development



Two types of stem cell divisions

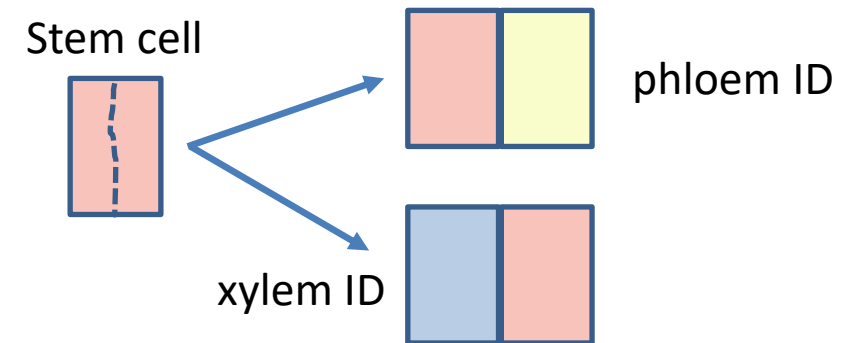


xylem ← stem cell → phloem



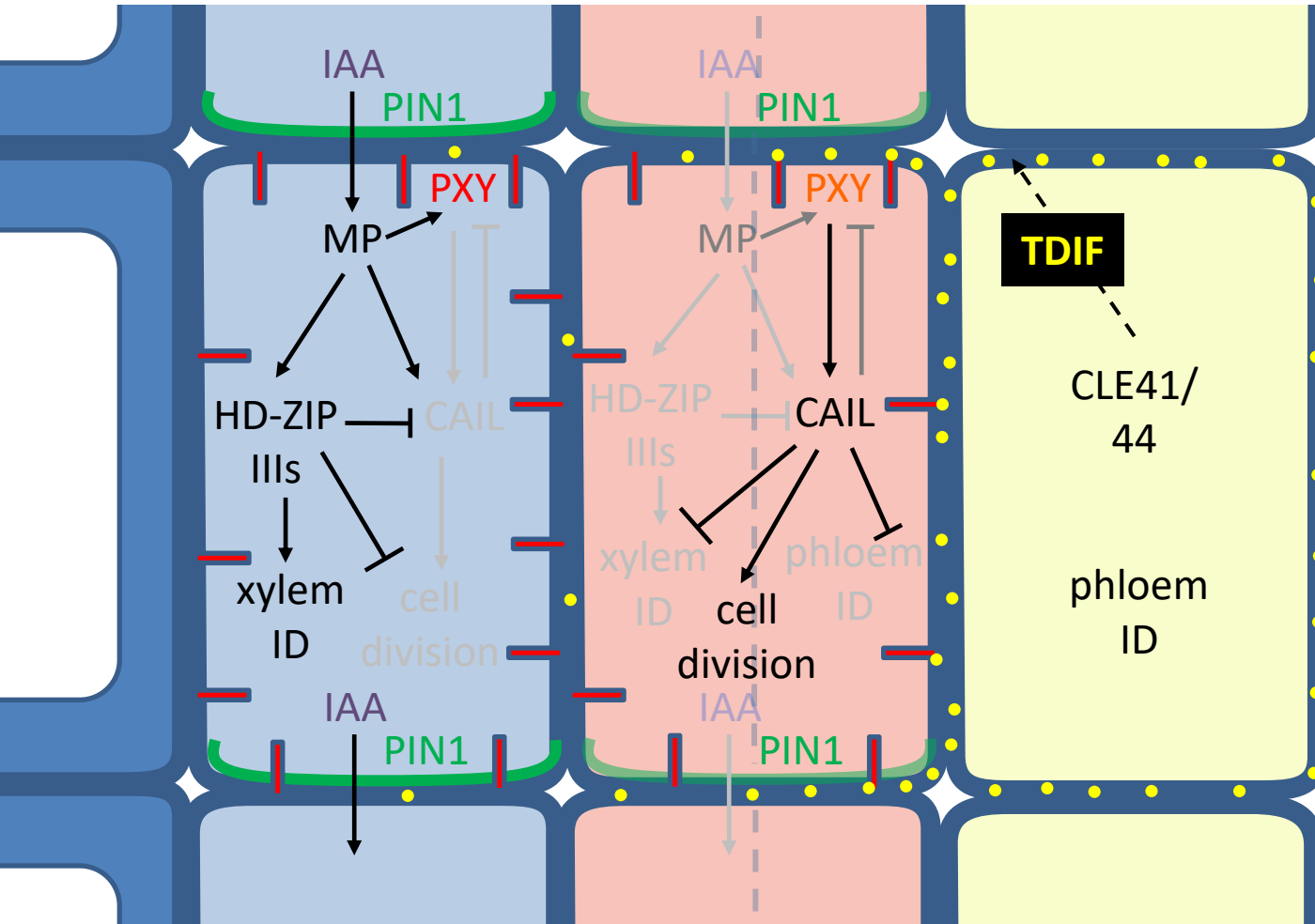
Bifacial stem cell: a single cell produces both phloem and xylem

Stem cell division leading to formation of:

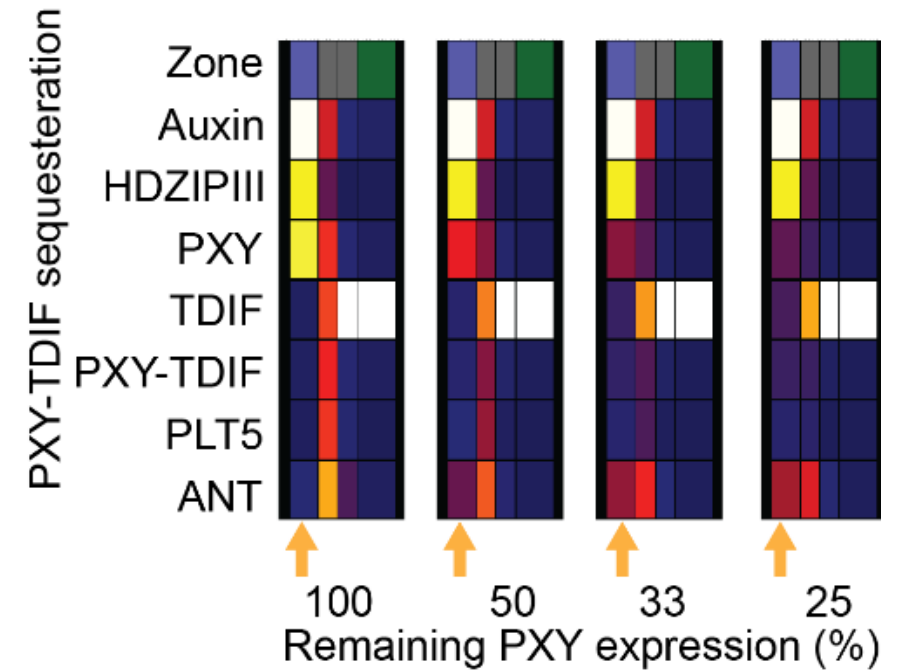


Smetana et al *Nature* 2019

Signalling networks determining the position of stem cells in the vascular cambium

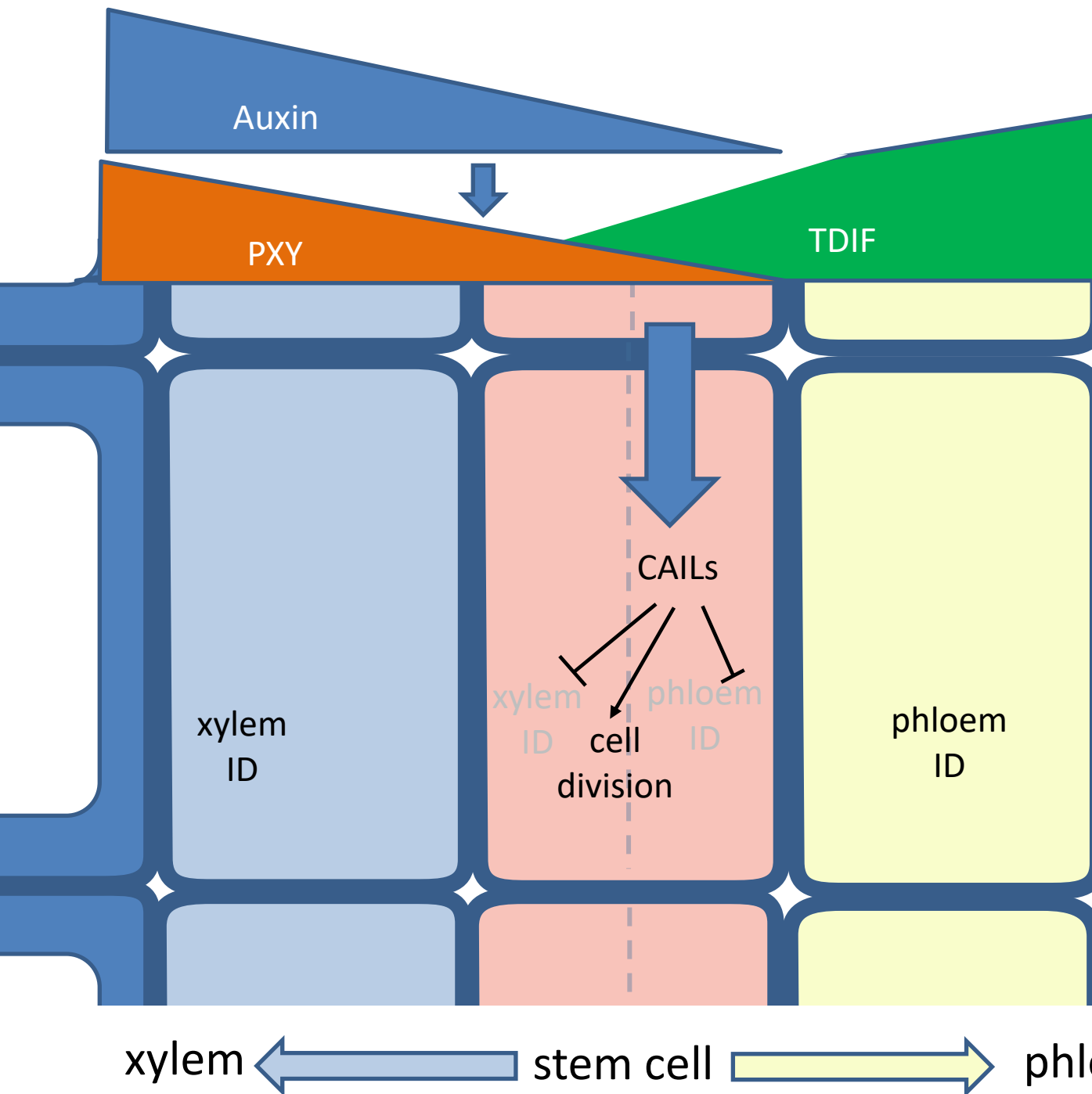


xylem ← stem cell → phloem



Eswaran, Zhang, Rutten et al. *Science* 2024, in collaboration with Peter Etchells' and Kirsten ten Tusscher's labs

Mäkilä et al. *Nature Plants* 2023
Smetana et al. *Nature* 2019



Summary:

- Identification of cambium stem cell factors, CAILs

Auxin accumulates in xylem ID cell to:

1. promote xylem differentiation and xylem tissue patterning
2. activate expression of PXY receptor kinase

- Discovery that TDIF sequestration by PXY prevents further diffusion of TDIF, and thus enables precise CAIL expression and stem cell positioning

- Sequestration mechanism: TDIF binding-induced internalization, signalling and subsequent degradation of TDIF-PXY

- Robust, yet flexible in producing different xylem:phloem ratio

CASS: similar expression patterns of the homologues in cassava storage organ (Wolfgang)

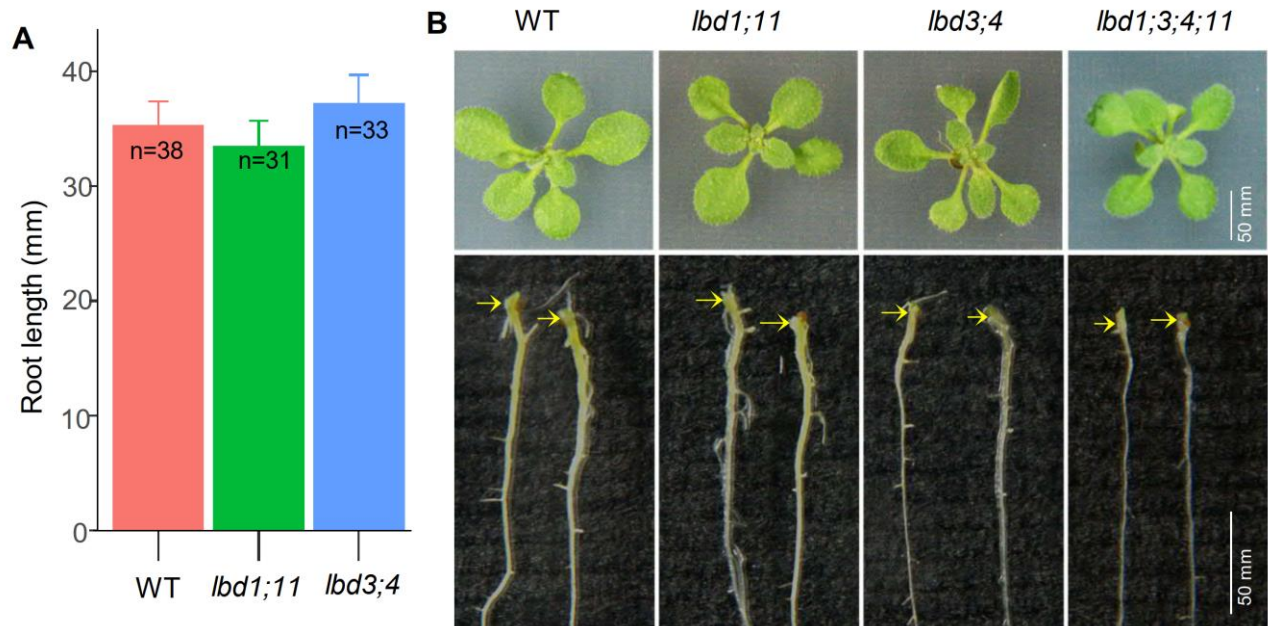
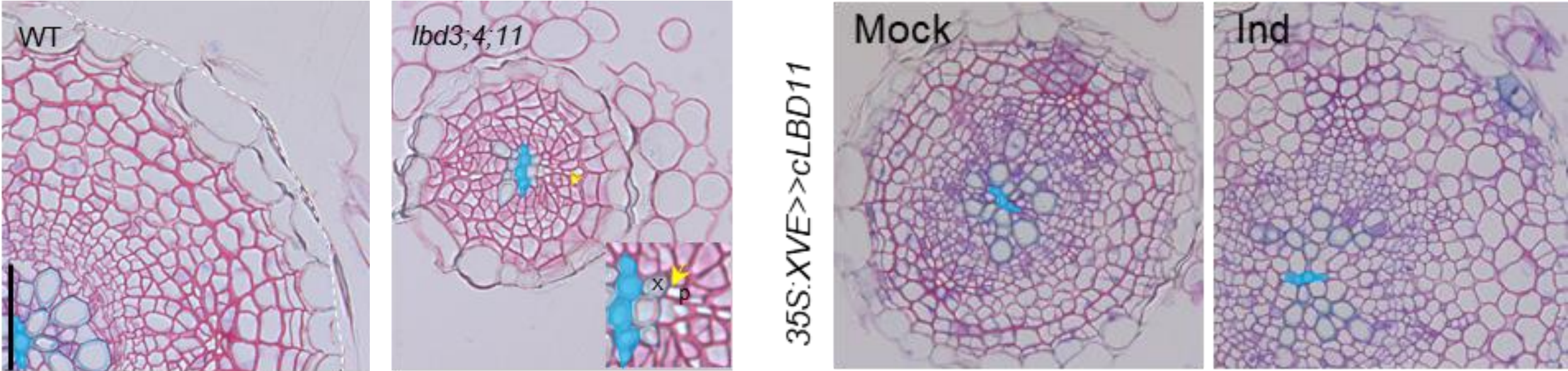
Zhang et al. m/s in preparation

Eswaran, Zhang, Rutten et al. *Science* 2024

Mäkilä et al. *Nature Plants* 2023

Smetana et al. *Nature* 2019

EM2: A set of LATERAL ORGAN BOUNDARIES DOMAIN (LBD) TFs promote specifically radial growth in *Arabidopsis*



Ye et al. *Current Biology* 2021

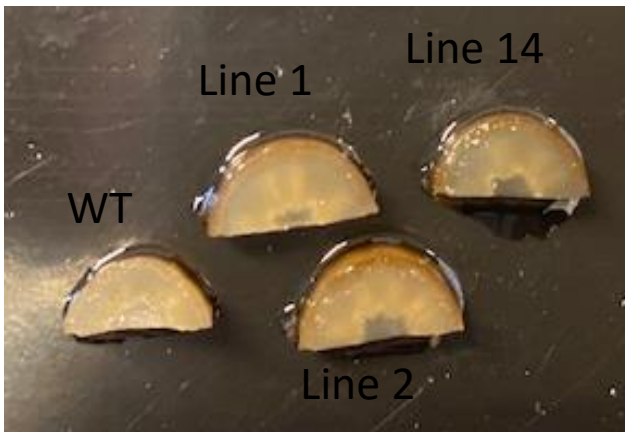
LBDs promote secondary growth by modulating pectin composition via pectate lyase-like genes of primary cell wall (Ye, Wang et al. accepted 'in principle' to *Nature Plants*)

Overexpression of an *AtLBD* leads to enhanced radial growth of hybrid aspen stem

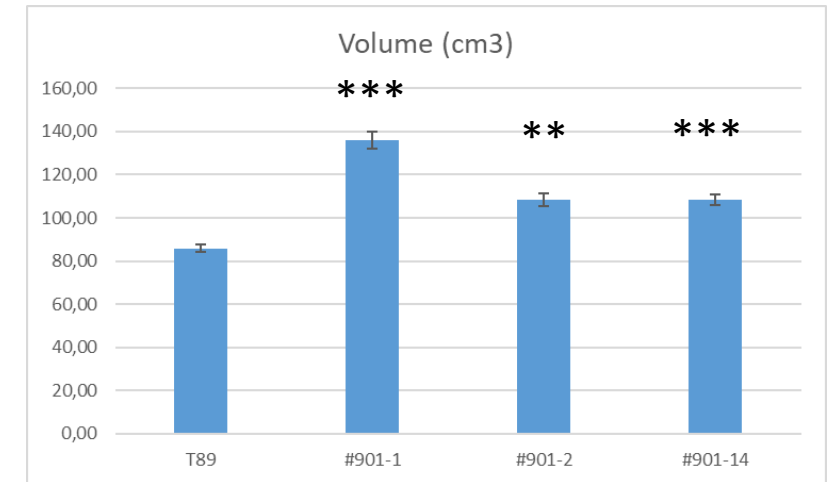
WT Line 11 Line 14 Line 17



pPtLMX5::AtLBD

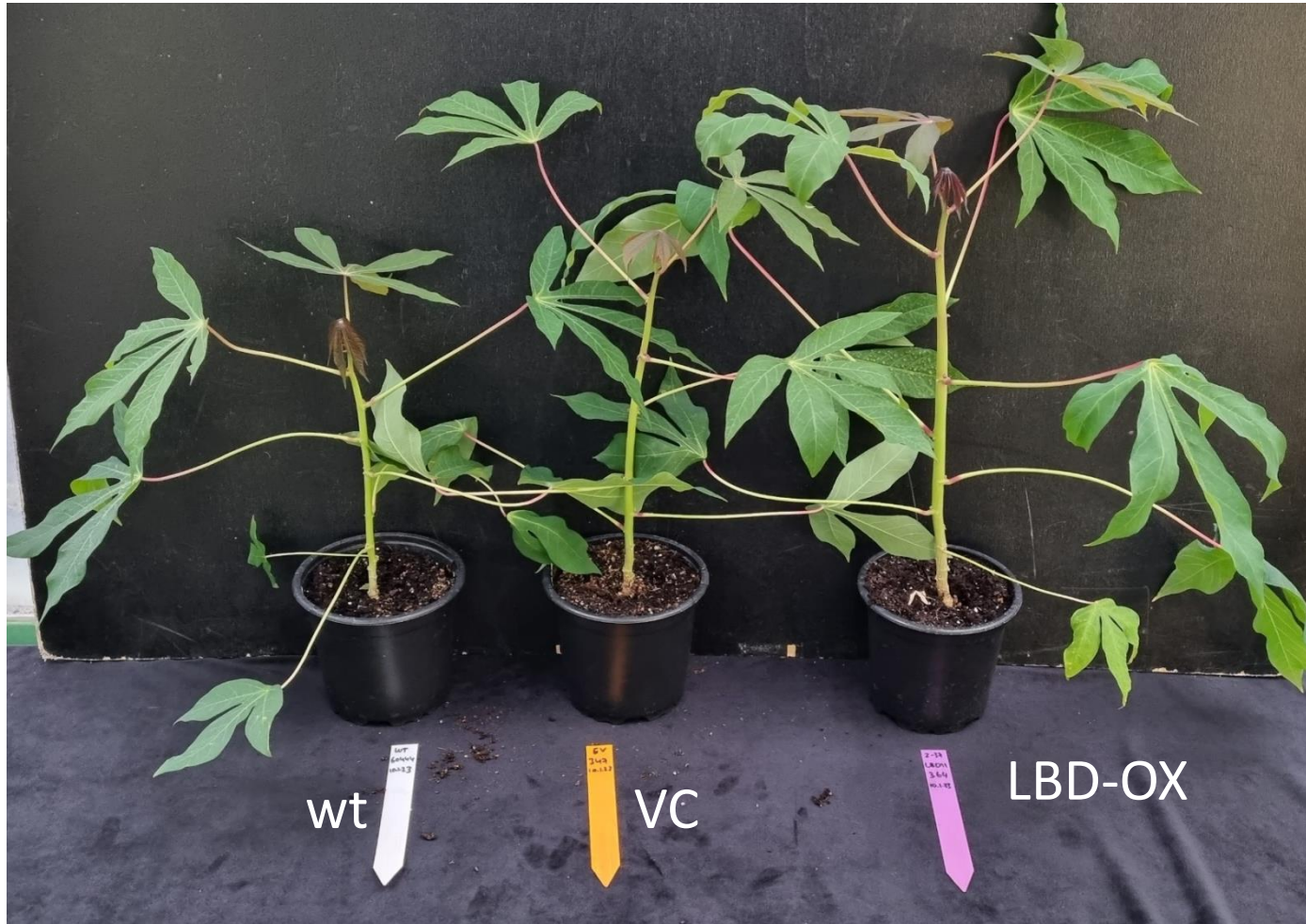


pPtLMX5::AtLBD

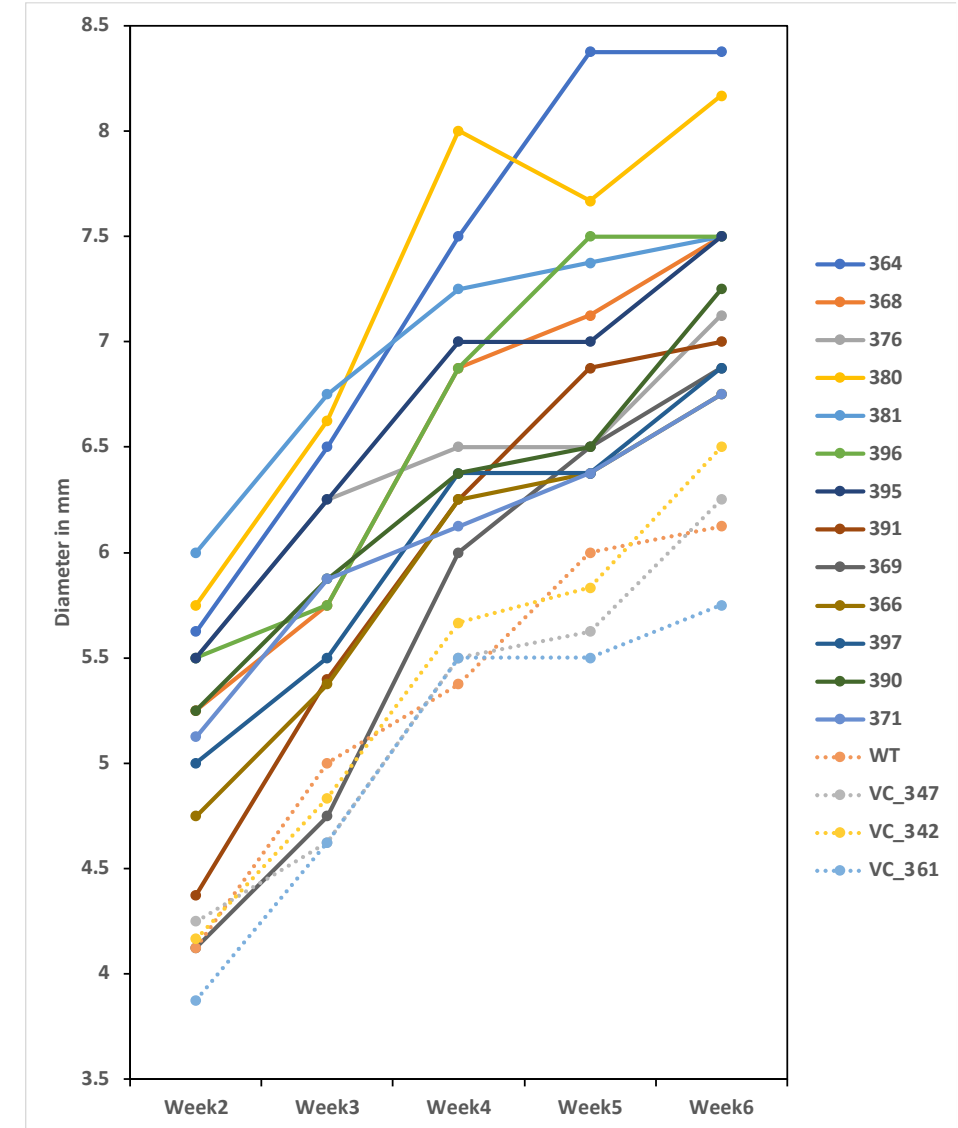


pPtLMX5::AtLBD

Overexpression of an *AtLBD* leads to enhanced radial growth of cassava stem



Next, we overexpress LBDs specifically in storage organs



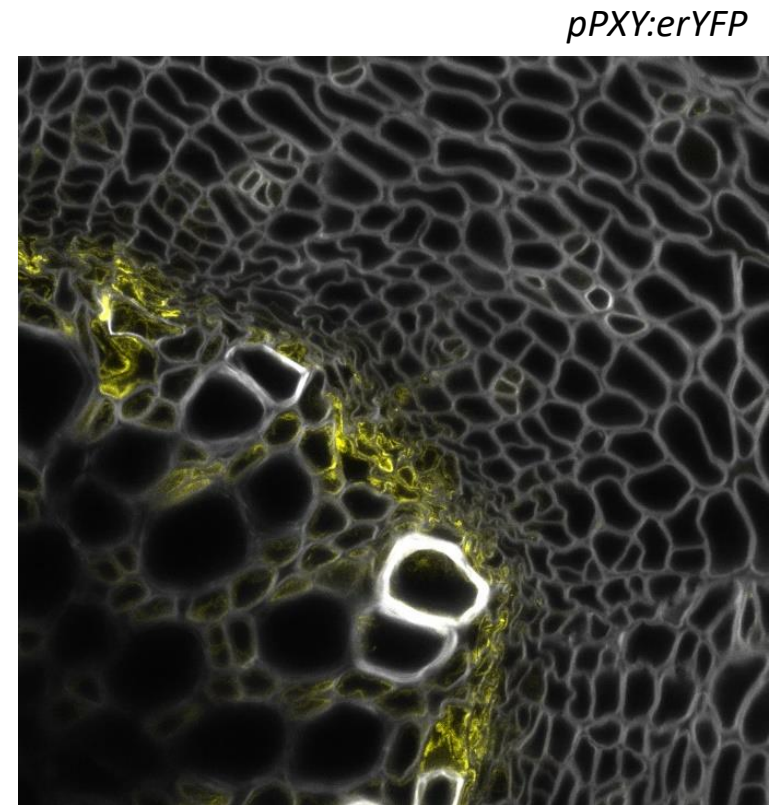
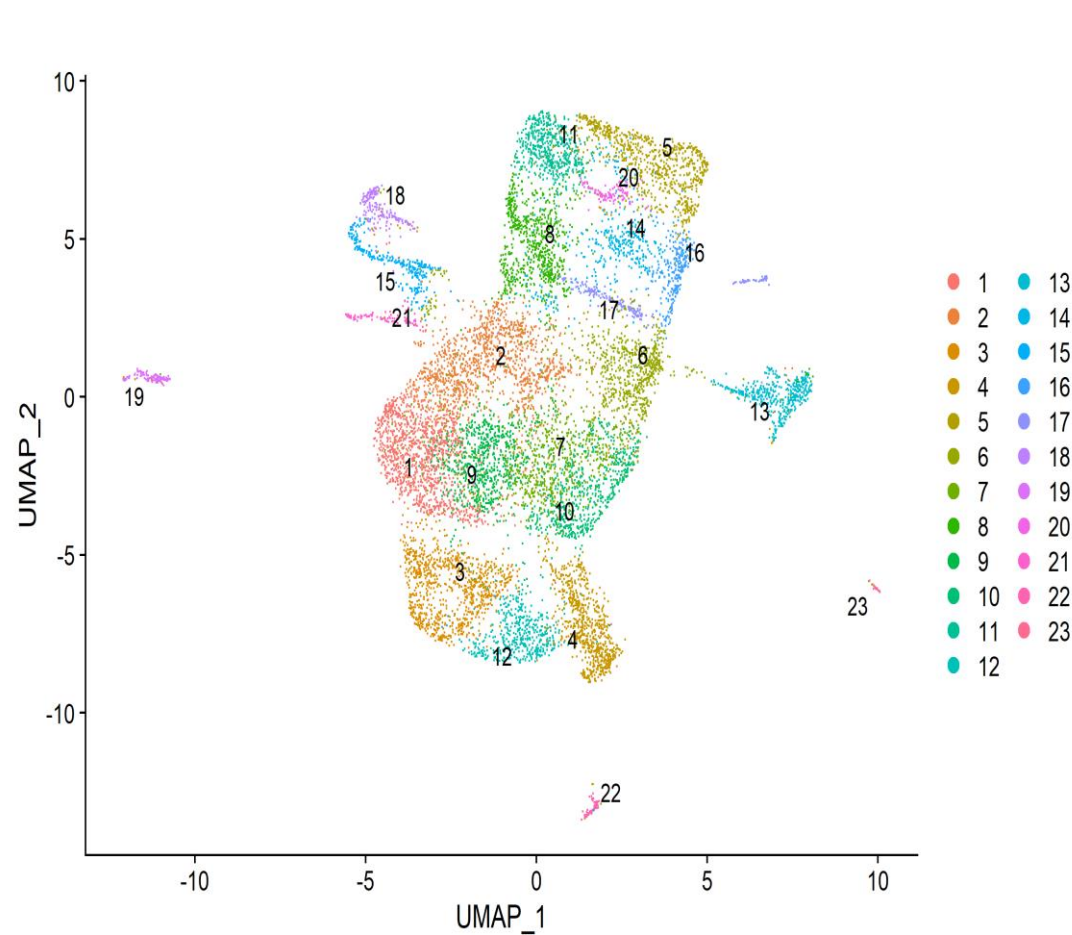
Bill & Melinda Gates
funded CASS3
project

Data from Wolfgang Zierer & Uwe Sonnewald, U Erlangen-Nürnberg

Single cell RNA sequencing in mature Arabidopsis root

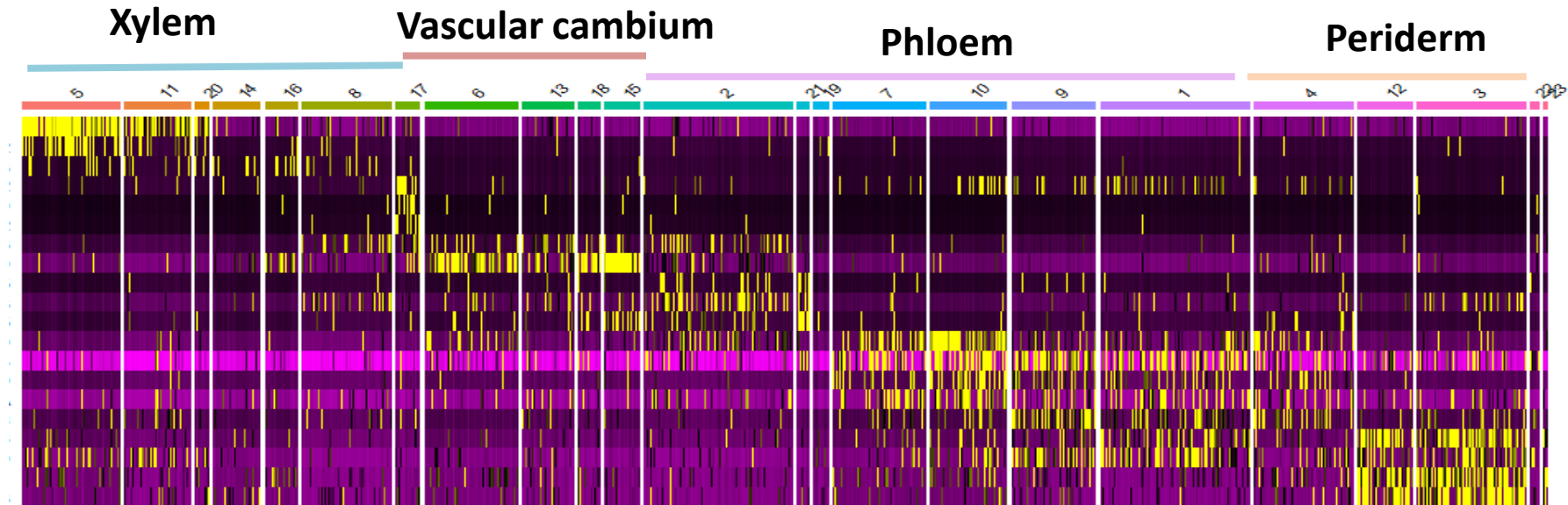
TM4

- 30d-old root, active cork and vascular cambium
- 10xgenomics, initially 23292 cells, after stringent filtering 7349 cells (collaboration with Bert de Rybel and bioinformatics unit, VIB)

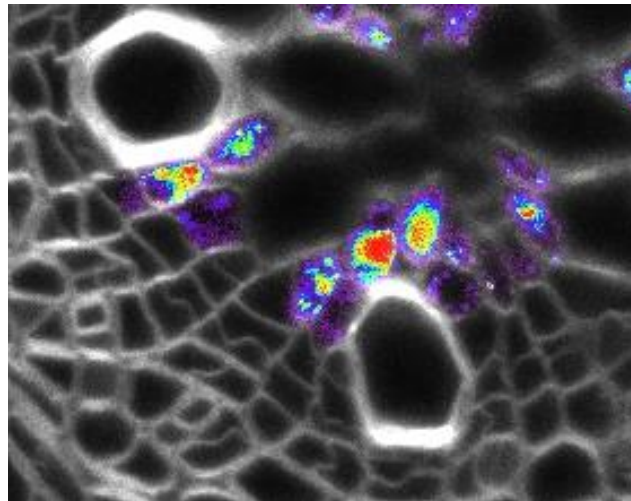


Munan Lyu, Meeri Mäkelä, Hiroyuki Iida

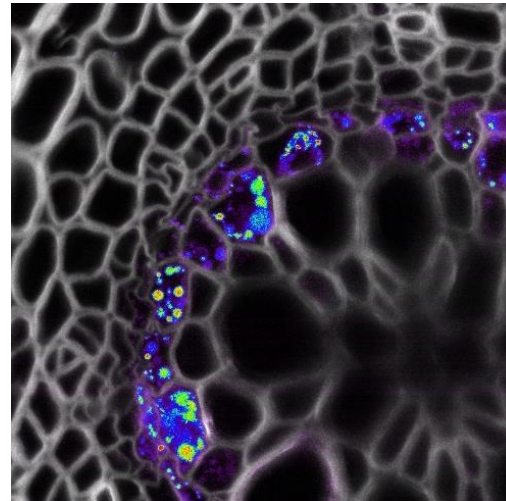
Generation of 93 fluorescent reporters to validate the single cell transcriptome data



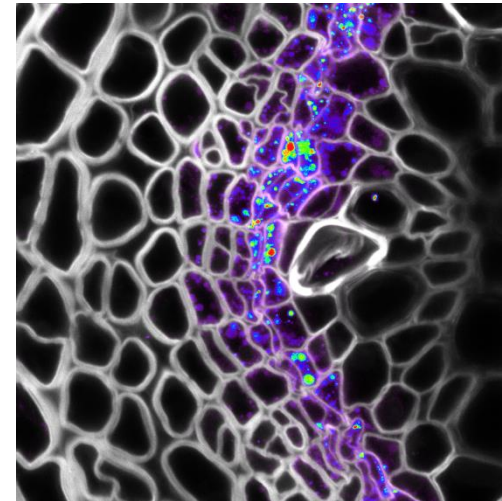
Maturing xylem parenchyma marker



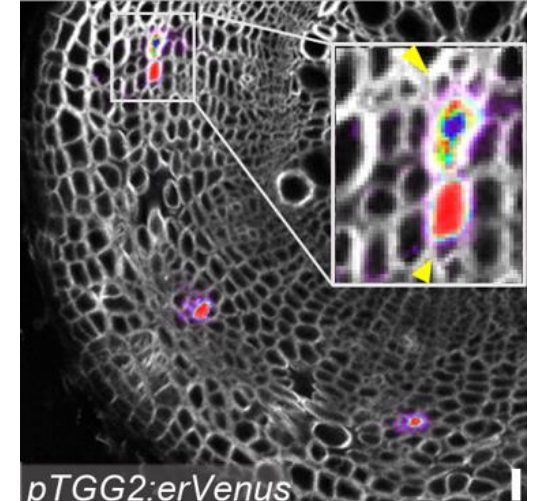
Xylem identity marker



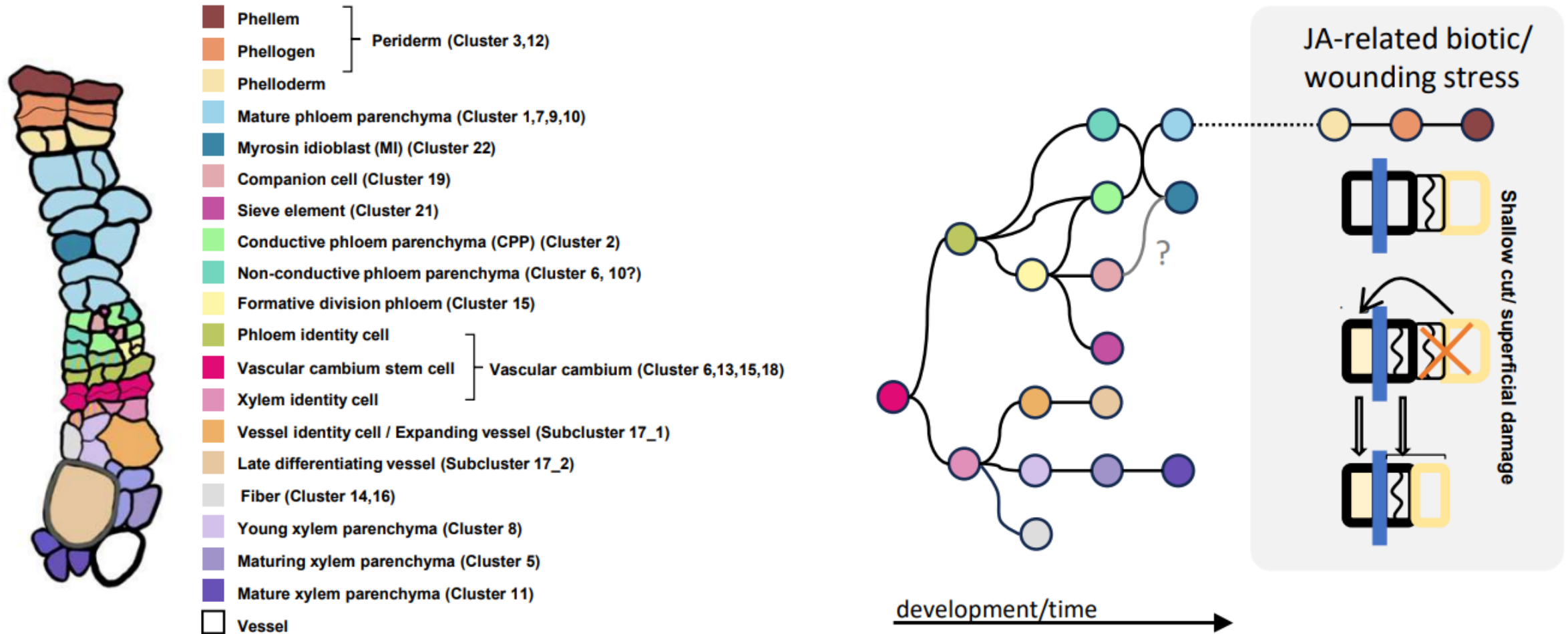
Stem cell marker



Myrosin idioblast



After analyzing 93 reporter lines, we were able to identify 20 different cell types or cell state, and their developmental trajectories

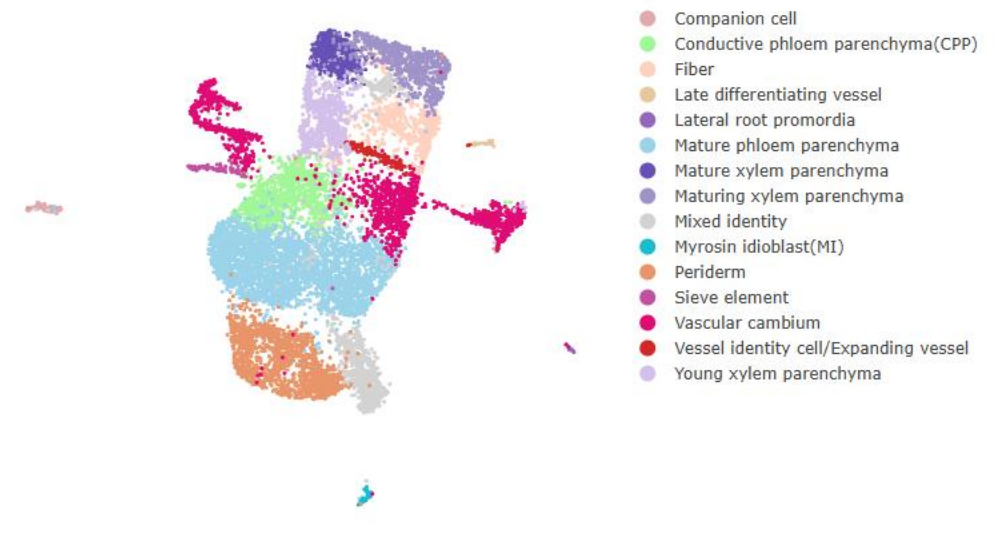
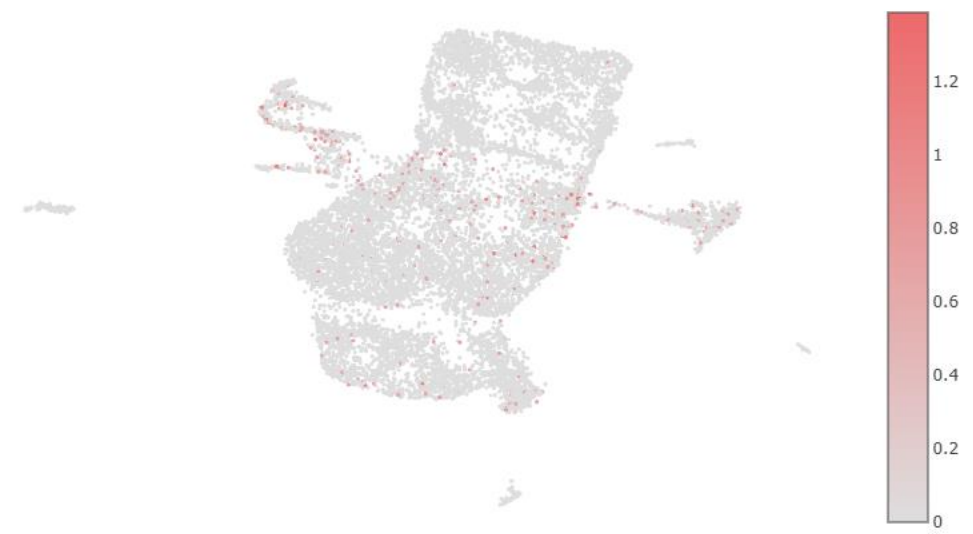


Features AT4G37750-ANT

Metadata Annotation

AT4G37750-ANT

Annotation



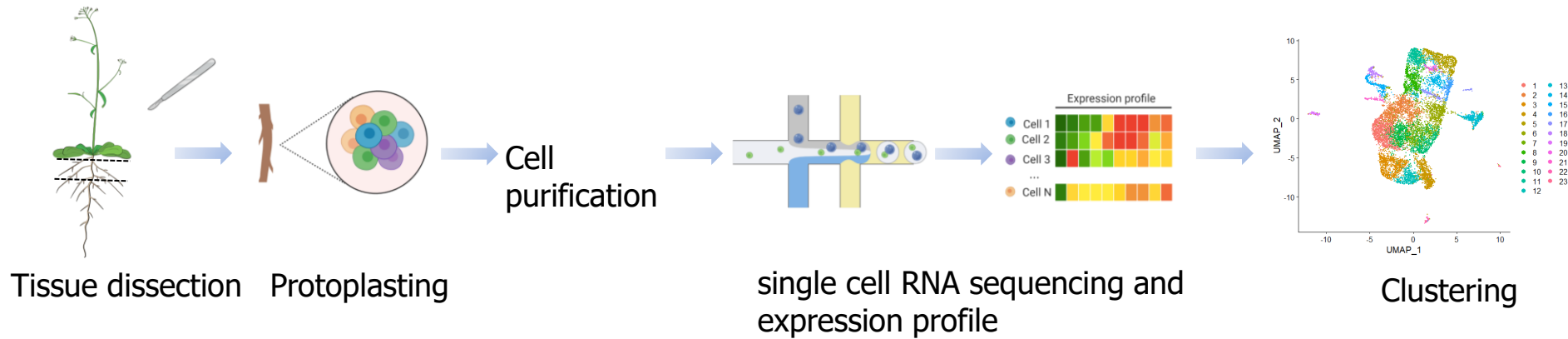
- Vessel
 - Mature xylem parenchyma (Cluster 11)
 - Maturing xylem parenchyma (Cluster 5)
 - Young xylem parenchyma (Cluster 8)
 - Fiber (Cluster 14,16)
 - Late differentiating vessel (Subcluster 17_2)
 - Vessel identity cell / Expanding vessel (Subcluster 17_1)
 - Xylem identity cell
 - Vascular cambium stem cell
 - Phloem identity cell
 - Formative division phloem (Cluster 15)
 - Non-conductive phloem parenchyma
 - Conductive phloem-associated parenchyma (CPP) (Cluster 2)
 - Sieve element (Cluster 21)
 - Companion cell (Cluster 19)
- Vascular cambium (Cluster 6,13,15,18)

Sampo Muranen (UH)

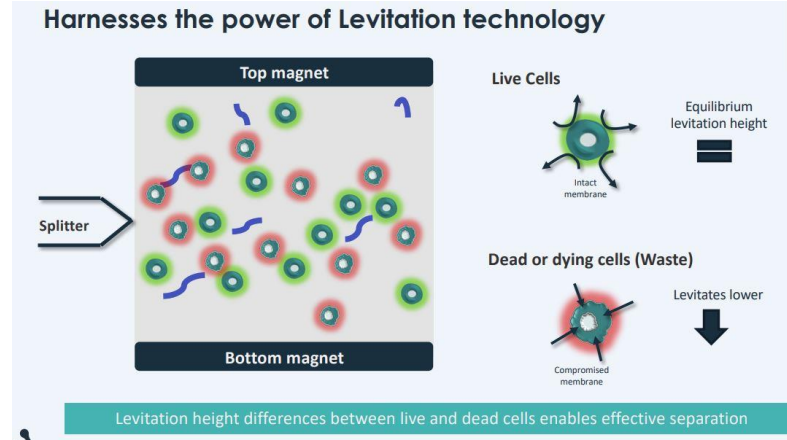
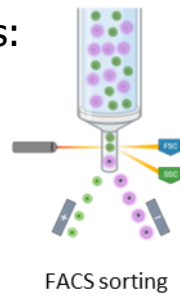
Munan Lyu (UH)

Thomas Eekhout (VIB)

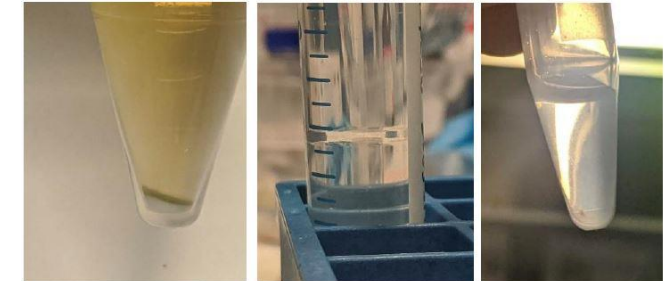
Work plan for Cassava single-cell RNA sequencing



Cell purification options:



LeviCell



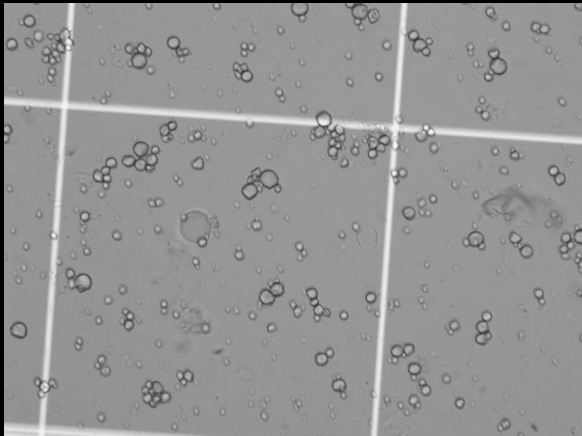
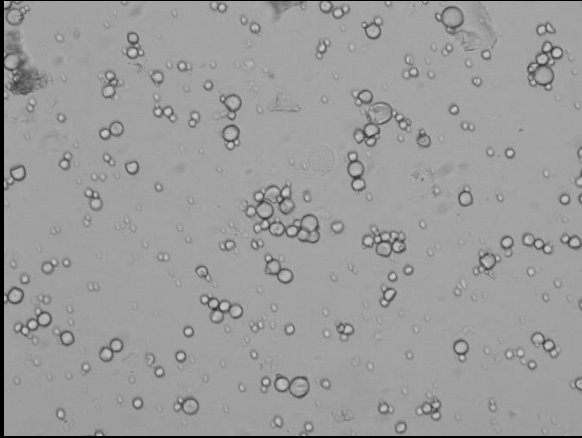
Sucrose gradient-based cell purification

Genotype: 60444—higher transformation efficiency

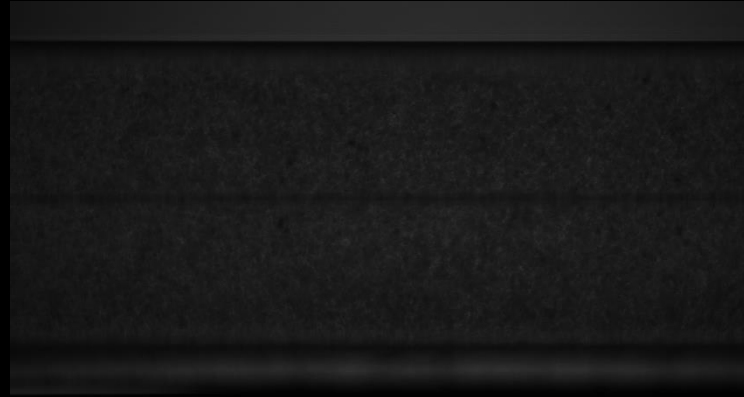
TME7—farmer-preferred genotype

Munan Lyu

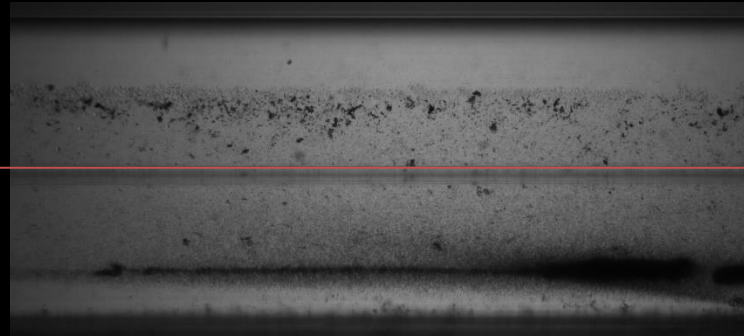
1h protoplasting solution incubation



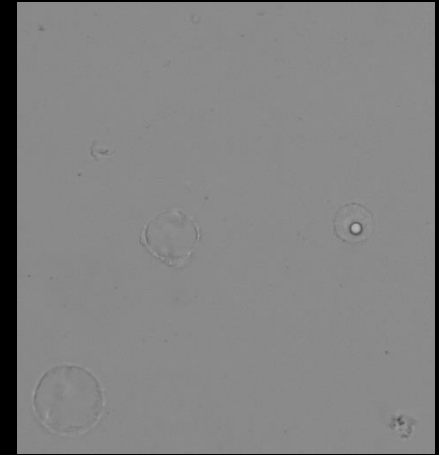
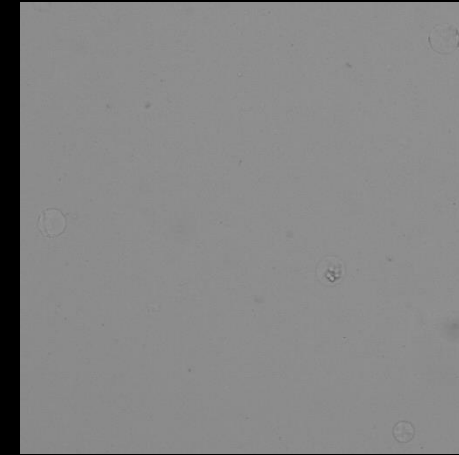
Concentrated cell suspension, full of starch granules, protoplasts were visible



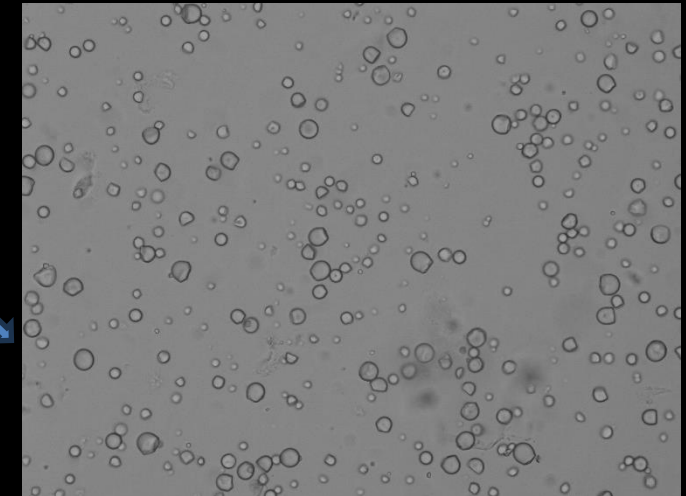
20min cell purification



Cell purification with levicell
Starch granules were clearly removed



There are small number of protoplasts with different sizes, indicating they are from different tissues



Removed starch granules collected from the lower channel

Future plans for single cell RNAseq in cassava

- Optimizing protoplasting protocol to remove starch
- Optimizing single nucleus extraction in parallel
- Possibly combining single cell and single nuclei analysis

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- Peter Etchells, Durham U
- Bert de Rybel, VIB
- Wolfgang Zierer/Uwe Sonnewald, FAU



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