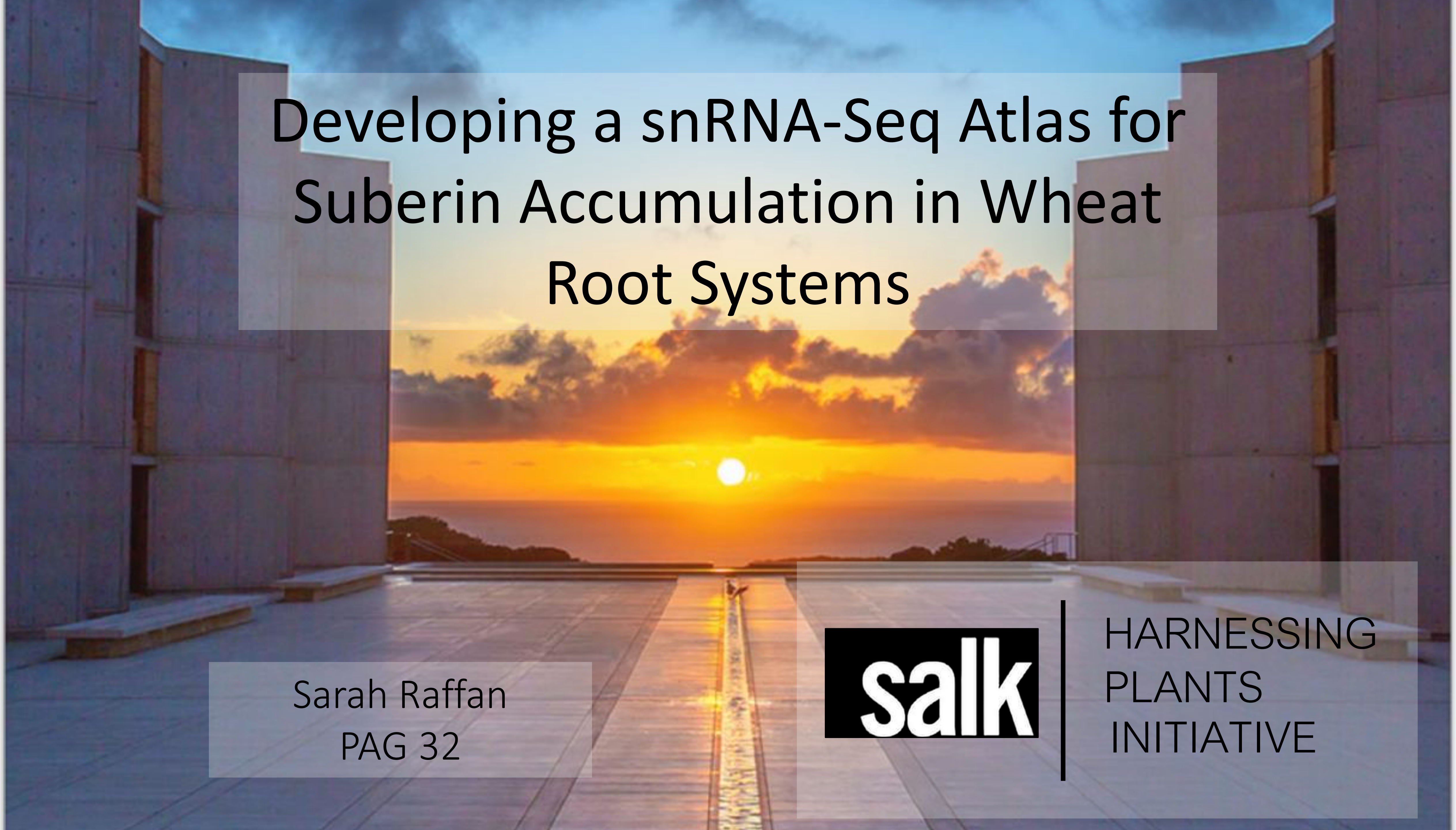
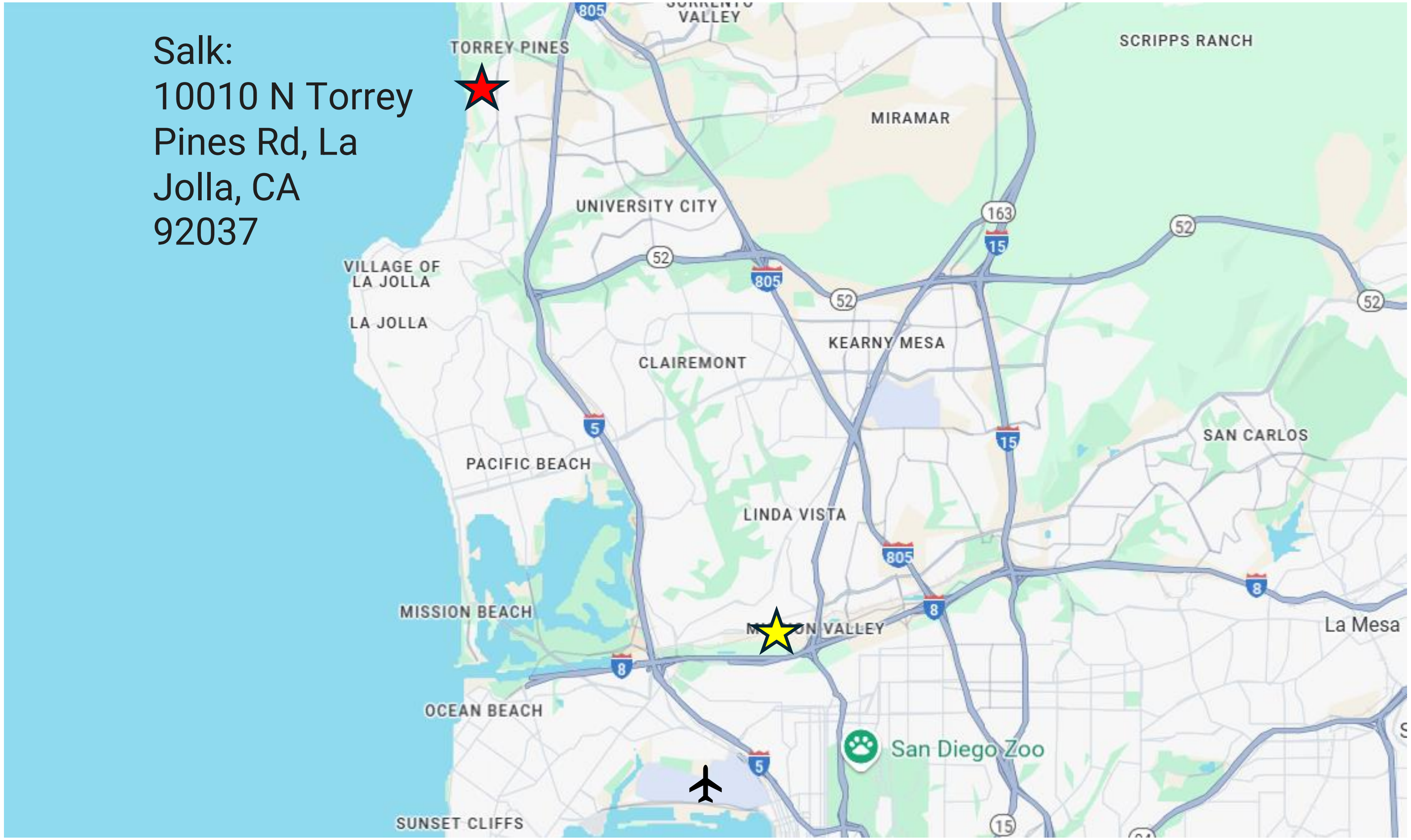


Developing a snRNA-Seq Atlas for Suberin Accumulation in Wheat Root Systems

Sarah Raffan
PAG 32

salk
HARNESSING
PLANTS
INITIATIVE

The Salk Institute for Biological Studies



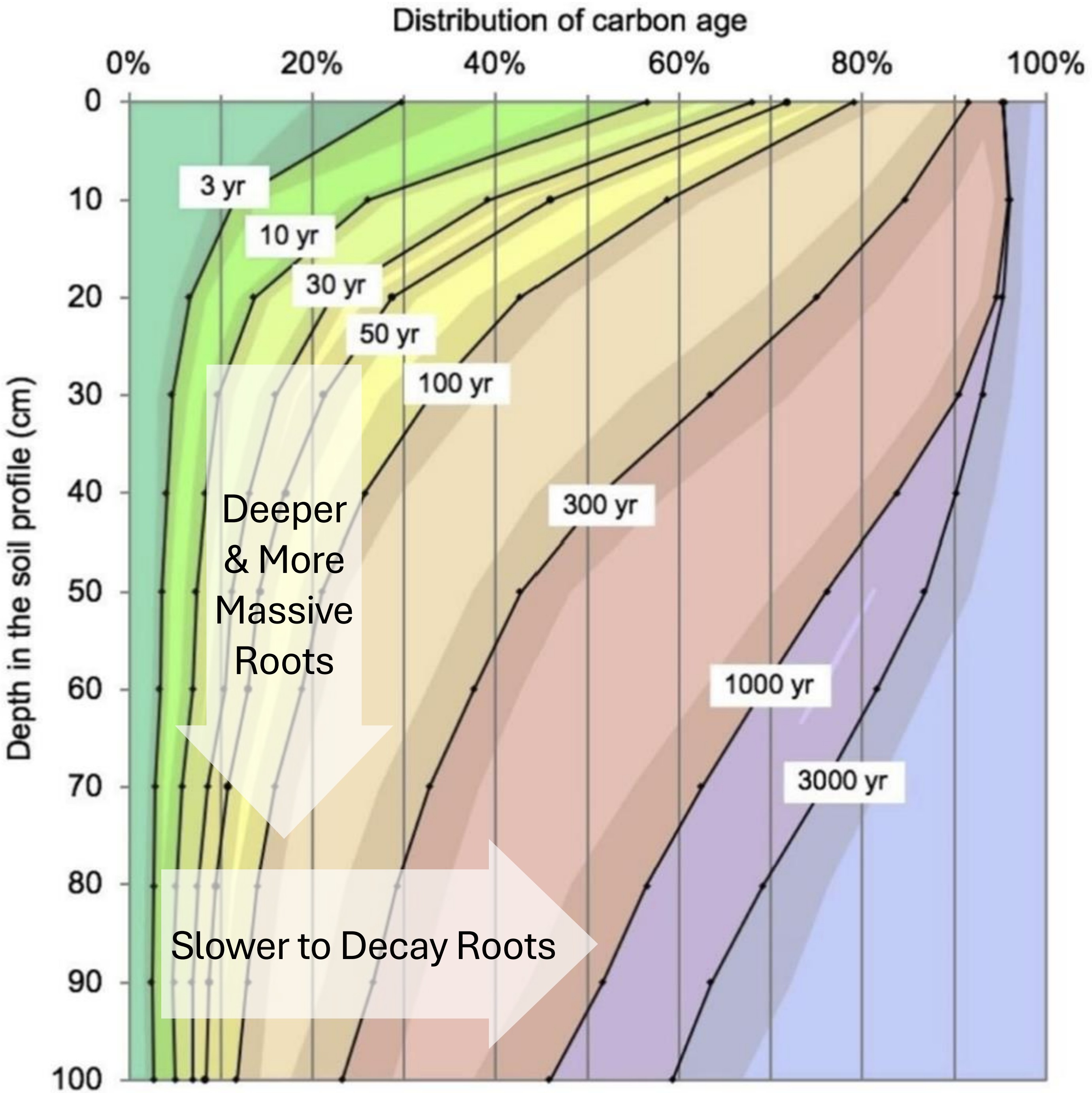
Salk:
10010 N Torrey Pines Rd, La Jolla, CA 92037

The Salk Harnessing Plants Initiative

CRoPS: Carbon Removal on a Planetary Scale Enhanced Carbon Sequestering Cultivars to Mitigate Climate Change

CPR: Coastal Plant Restoration – Restoring our Wetlands for Carbon Sequestration

Focused on plant-based carbon sequestration



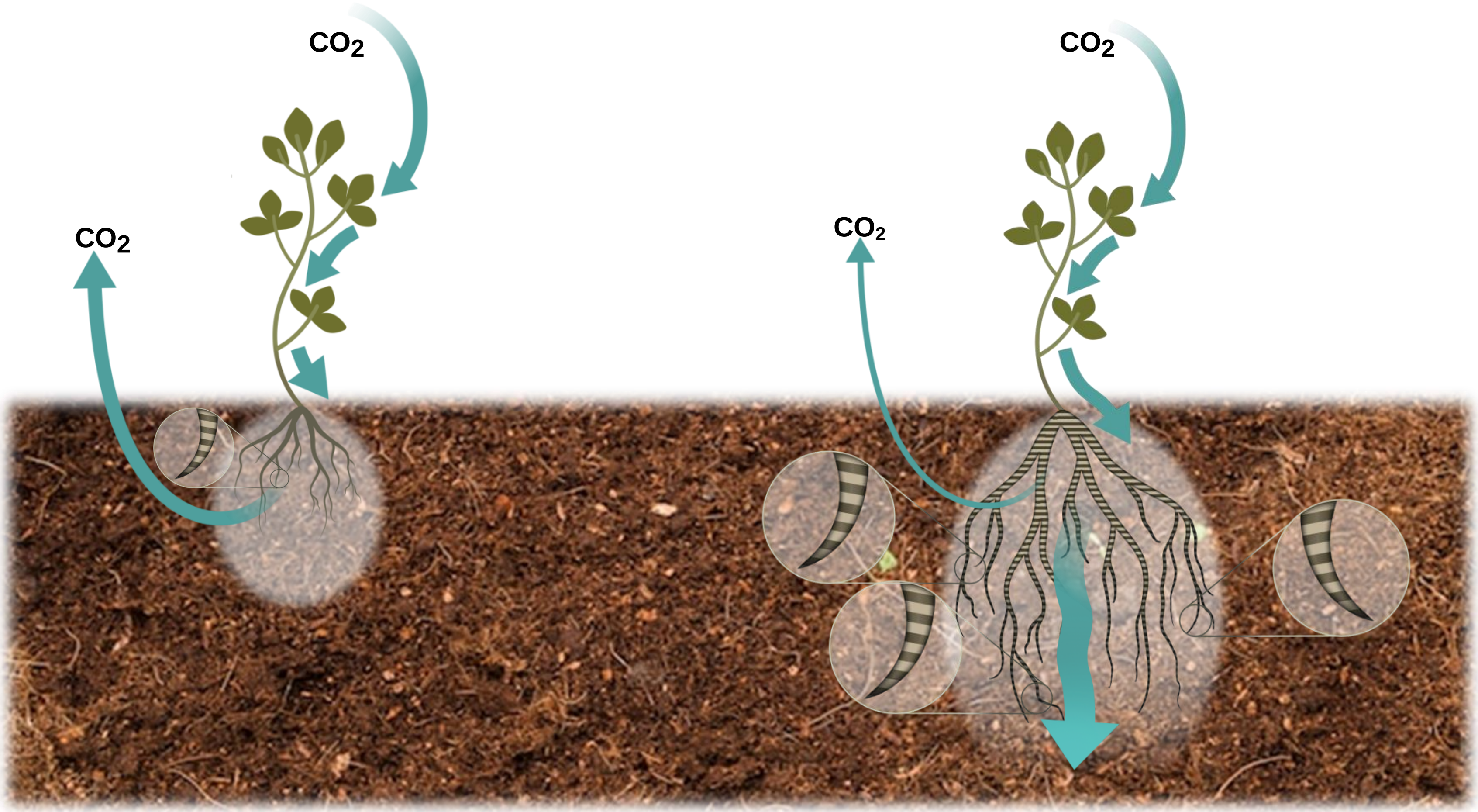
Deeper & More Massive Roots

Slower to Decay Roots

Balesdent et al. (2018) Atmosphere–soil carbon transfer as a function of soil depth. Nature

HPI ideotype plants

3 Characteristics to Enhance Plant Based Carbon Sequestration



Normal Plants

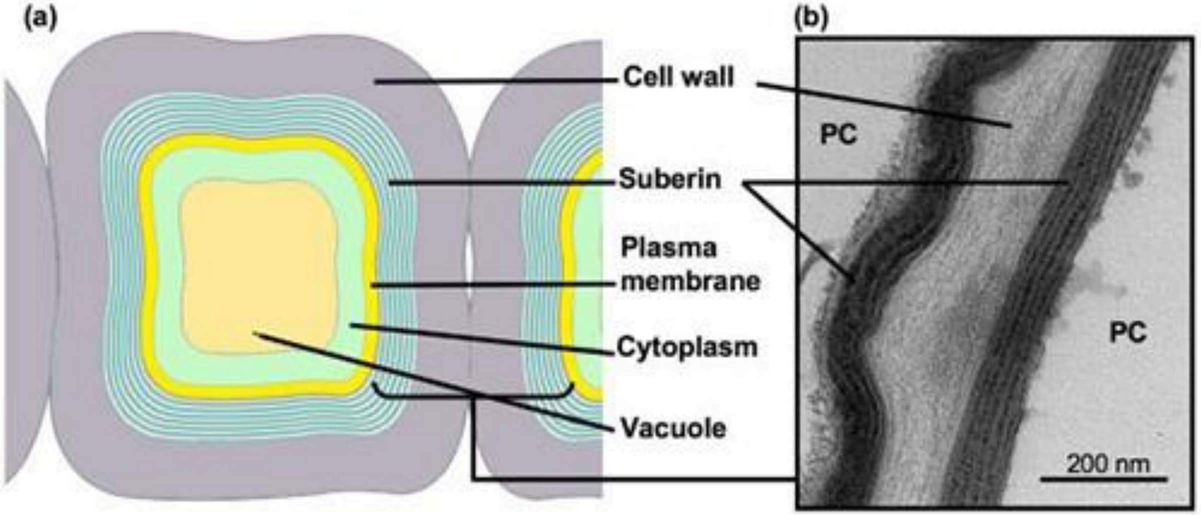
HPI Ideotype Plants

- ⬆ Root depth
- ⬆ Root biomass

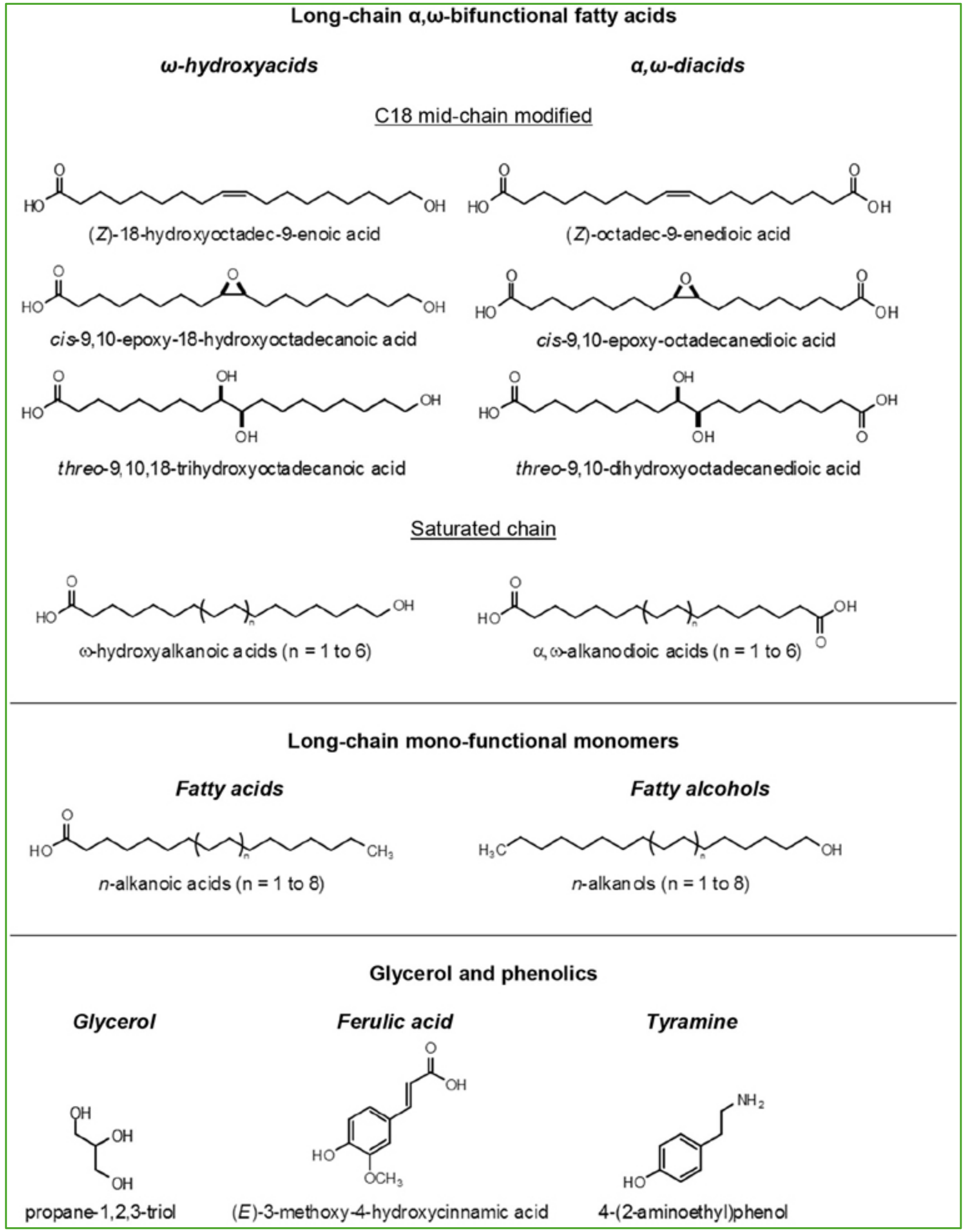
- ⬆ Suberin

What is suberin?

- Complex polyester biopolymer that is composed of long-chain fatty acids and polyaromatics.
- Exists naturally in plant roots and helps reduce water loss and limit the invasion of pathogens.

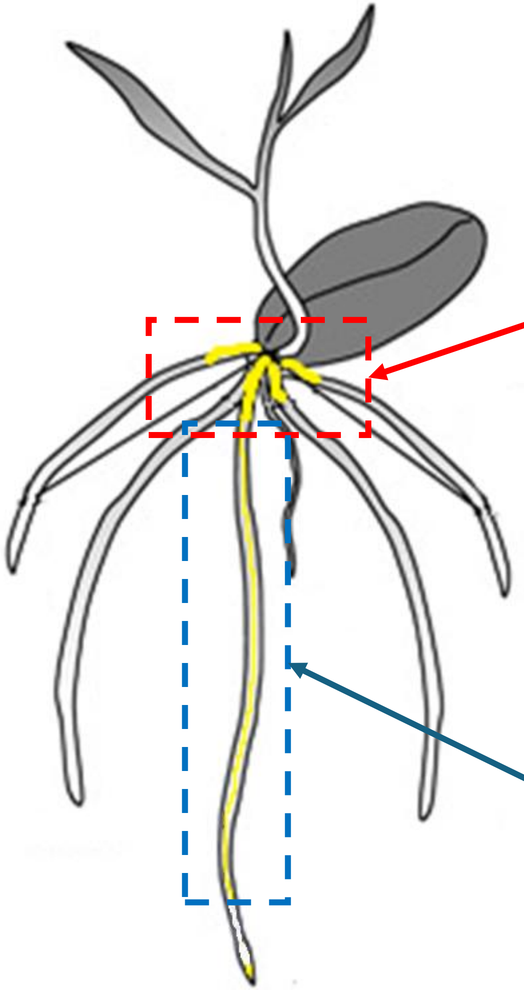


Graça, J. and Santos, S. *Macromolecular bioscience*, *7*(2), pp.128-135. (2007)



Graça, J. *Frontiers in chemistry*, *3*, p.62. (2015)

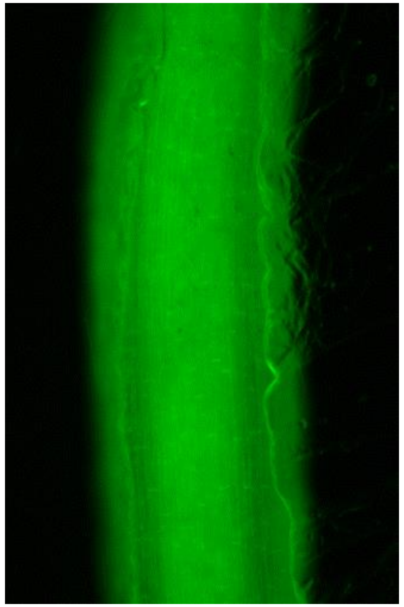
Where is suberin in the roots in wheat?

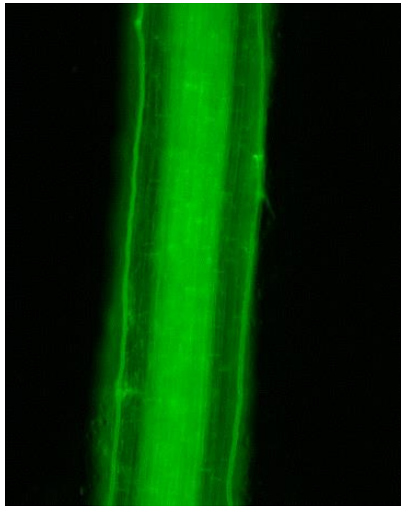


A suberized exodermis forms in the top 10-15% of the root

- forms after ~14 days
- more in the nodal roots

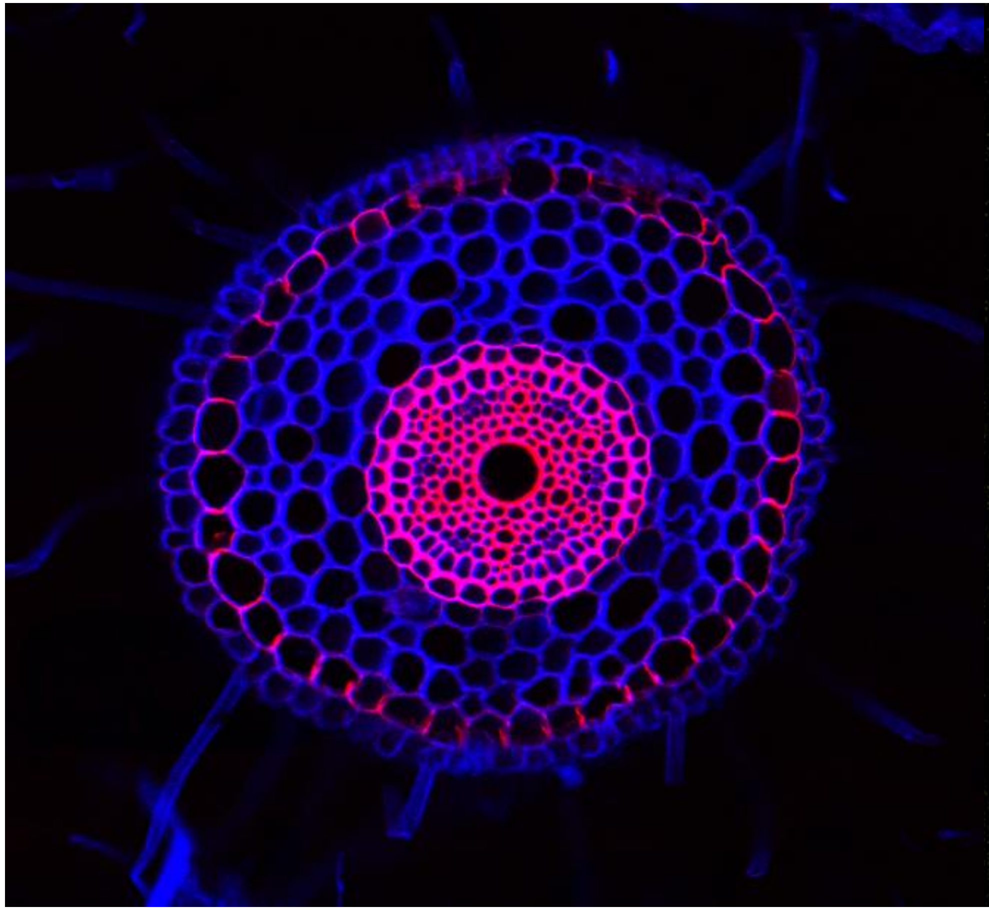
Wheat has a suberized endodermis that spans most of the root



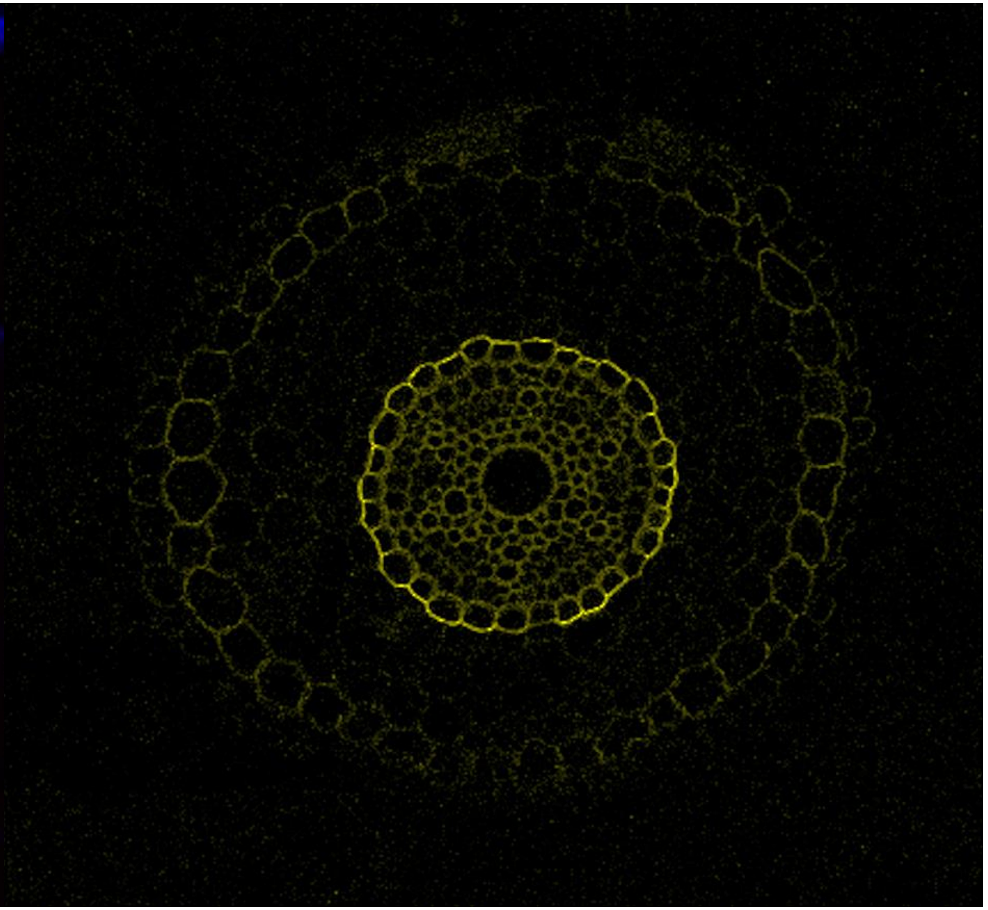


Modified from Sanguineti, M. C., et al. *Annals of Applied Biology* 151.3 (2007).

Visualizing suberin in wheat roots



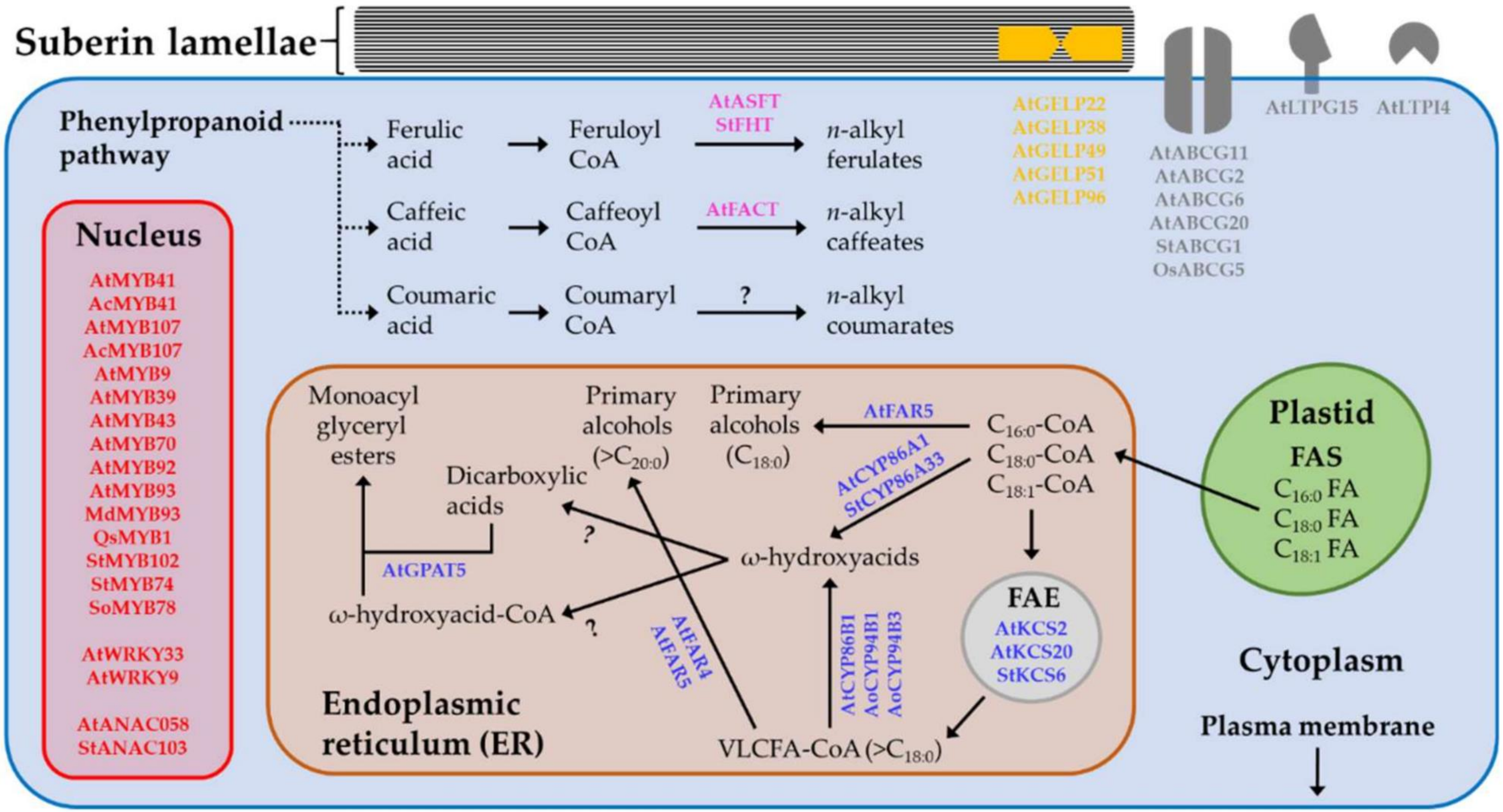
BF + CW



FY

Method from Sexauer, M. et al., Development, 148(23) (2021)

Biosynthesis of suberin



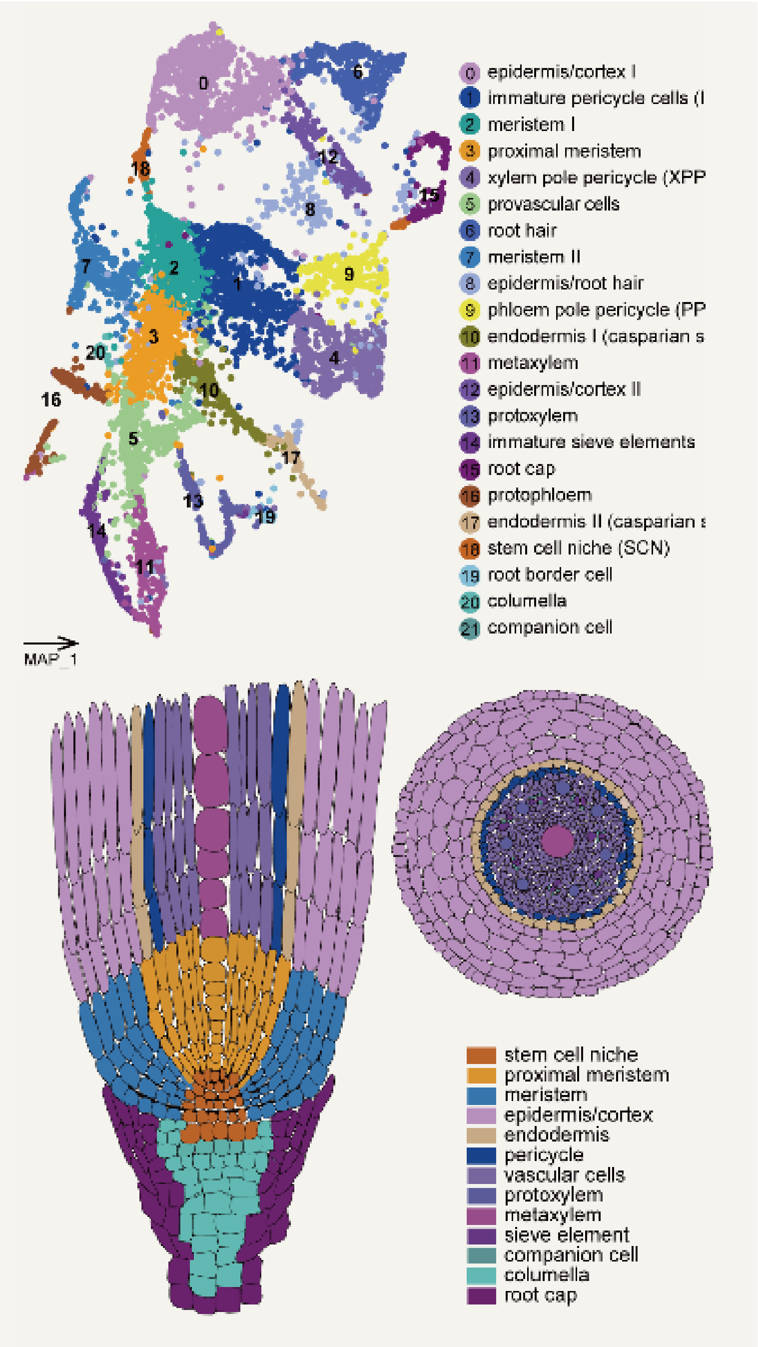
Nomberg, G., et al. *Plants*, *11*(3), p.392 (2022).

Wheat single nuclei atlas

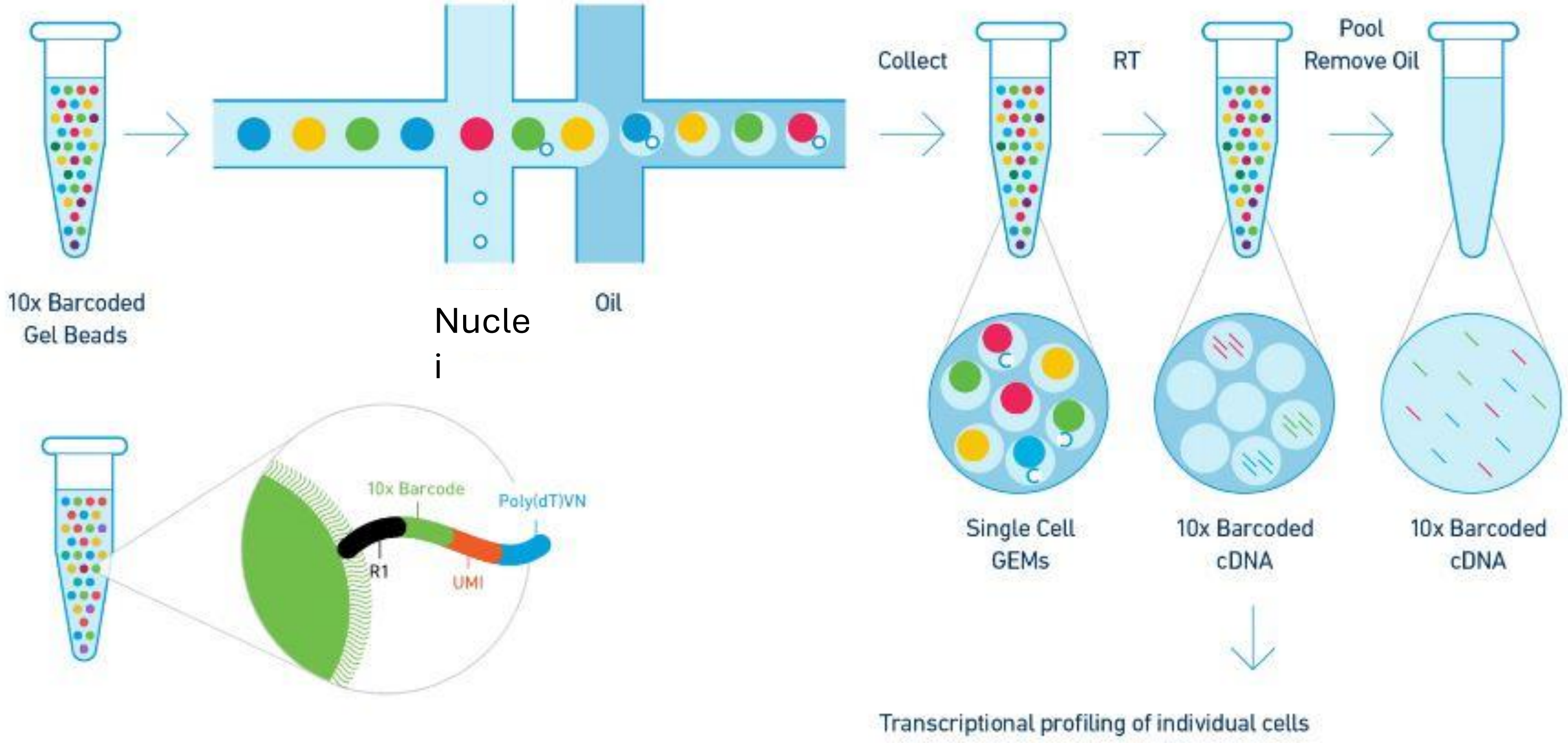
- Asymmetric gene expression and cell-type-specific regulatory networks in the root of bread wheat revealed by single-cell multiomics analysis.

Zhang, L., *et al. Genome Biol* **24**, 65 (2023).

- 0.5cm of the root tips of 3-day old roots were analyzed
- 6875 cells

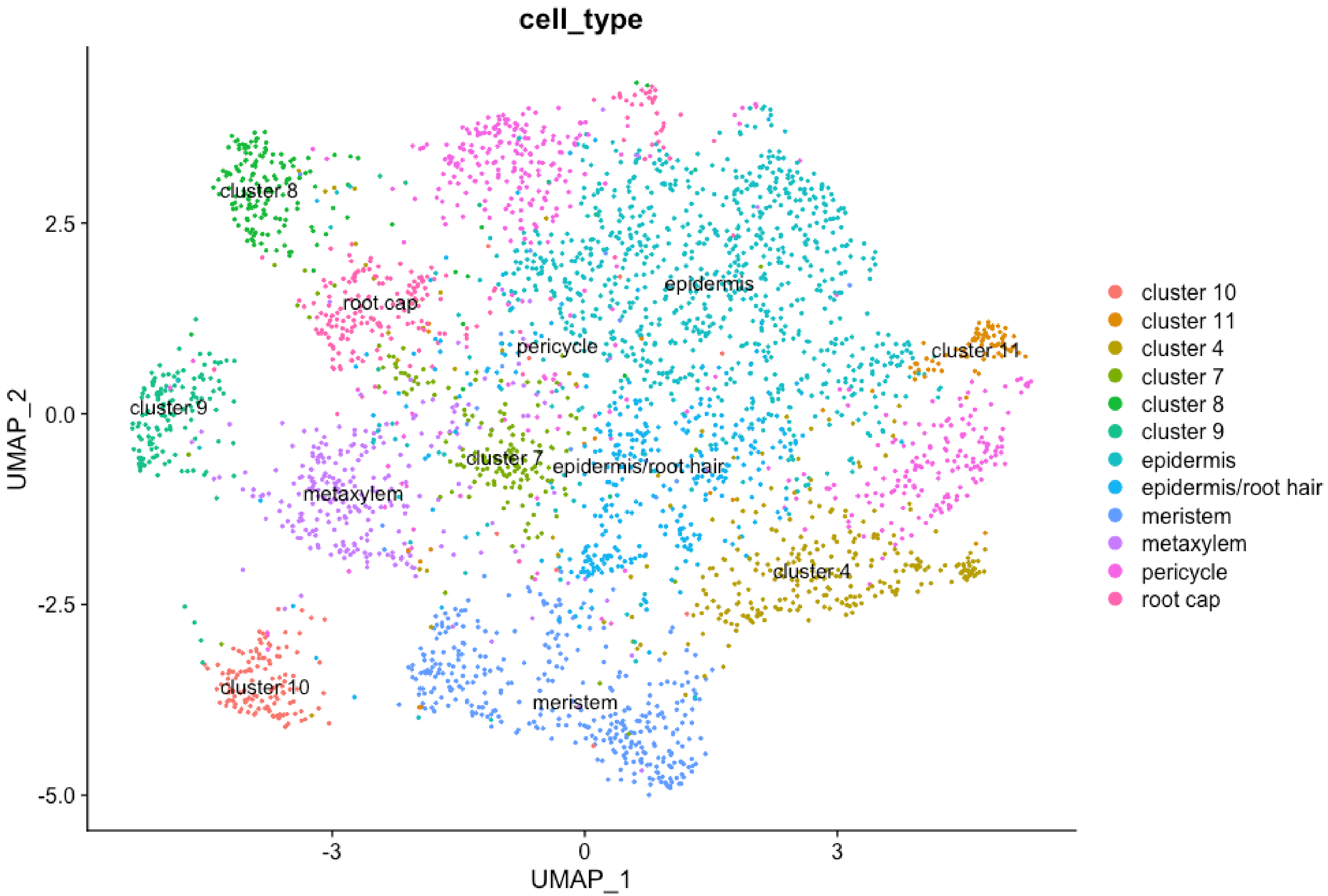


Single nuclei sequencing of wheat roots



10x Genomics

Wheat single nuclei atlas



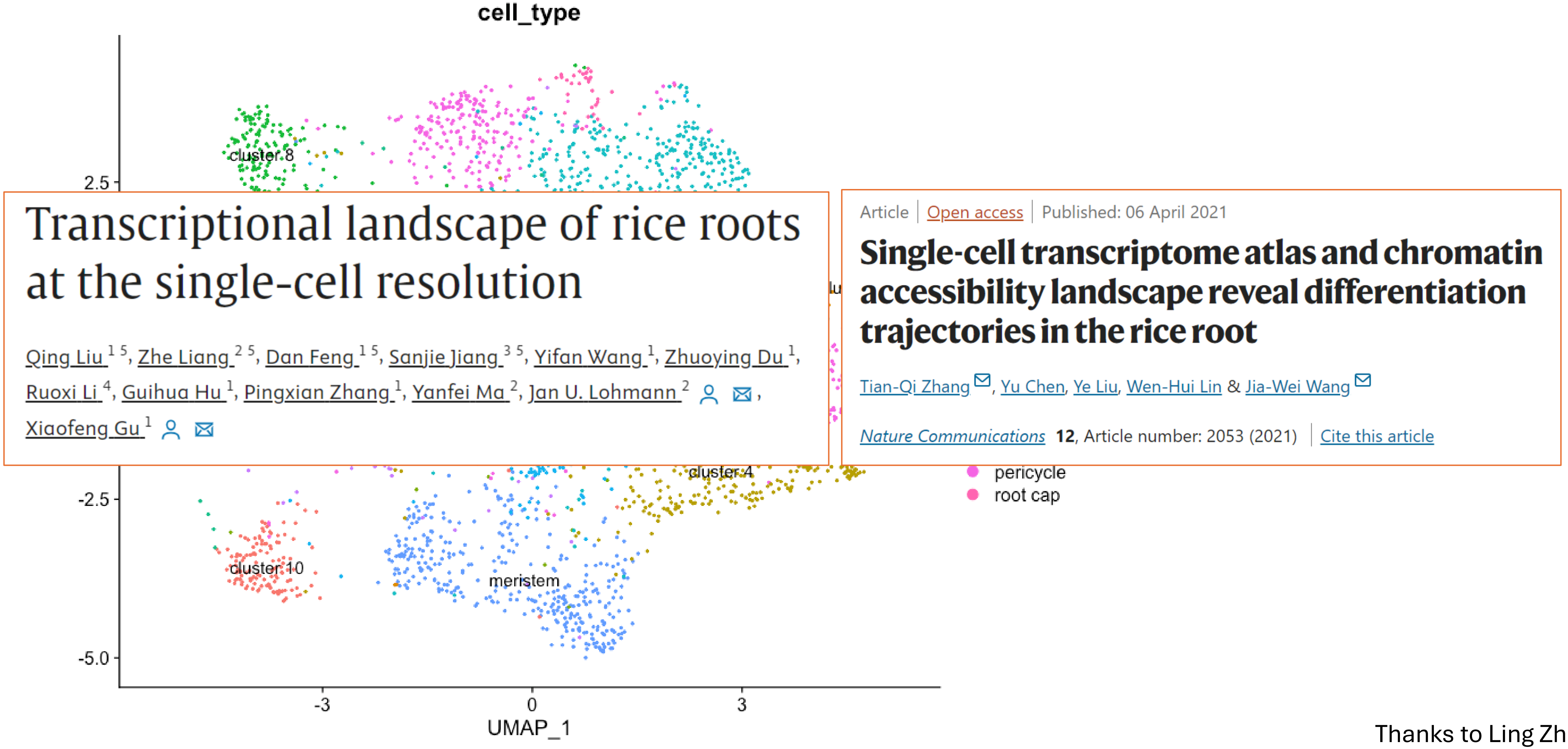
10-day old whole root samples

3465 nuclei

Cluster annotation from Zhang, L., et al. *Genome Biol* 24, 65 (2023).

Thanks to Ling Zhang

Wheat single nuclei atlas



Transcriptional landscape of rice roots at the single-cell resolution

Qing Liu[1 5], Zhe Liang[2 5], Dan Feng[1 5], Sanjie Jiang[3 5], Yifan Wang[1], Zhuoying Du[1], Ruoxi Li[4], Guihua Hu[1], Pingxian Zhang[1], Yanfei Ma[2], Jan U. Lohmann[2], Xiaofeng Gu[1]

Article | Open access | Published: 06 April 2021

Single-cell transcriptome atlas and chromatin accessibility landscape reveal differentiation trajectories in the rice root

Tian-Qi Zhang, Yu Chen, Ye Liu, Wen-Hui Lin & Jia-Wei Wang

Nature Communications **12**, Article number: 2053 (2021) | Cite this article

Thanks to Ling Zhang

Cluster annotation from rice orthologues

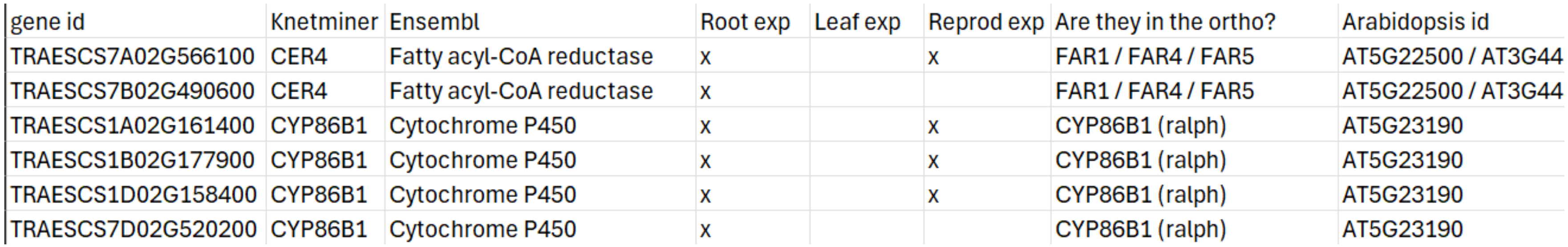
- Two datasets have 2 genes in common
  - OsCESA7 = Os10g0467800 or LOC_Os10g32980 (AT5G44030)
  - OsCESA9 = Os09g0422500 or LOC_Os09g25490 (AT5G17420)
- Xylem vs epidermis
- Both root tips 5-day vs 3-day
- Different cultivars used – ZH11 vs Nipponbare and 93-11

- Neither helped with the cluster annotation for wheat

Thanks to Ling Zhang

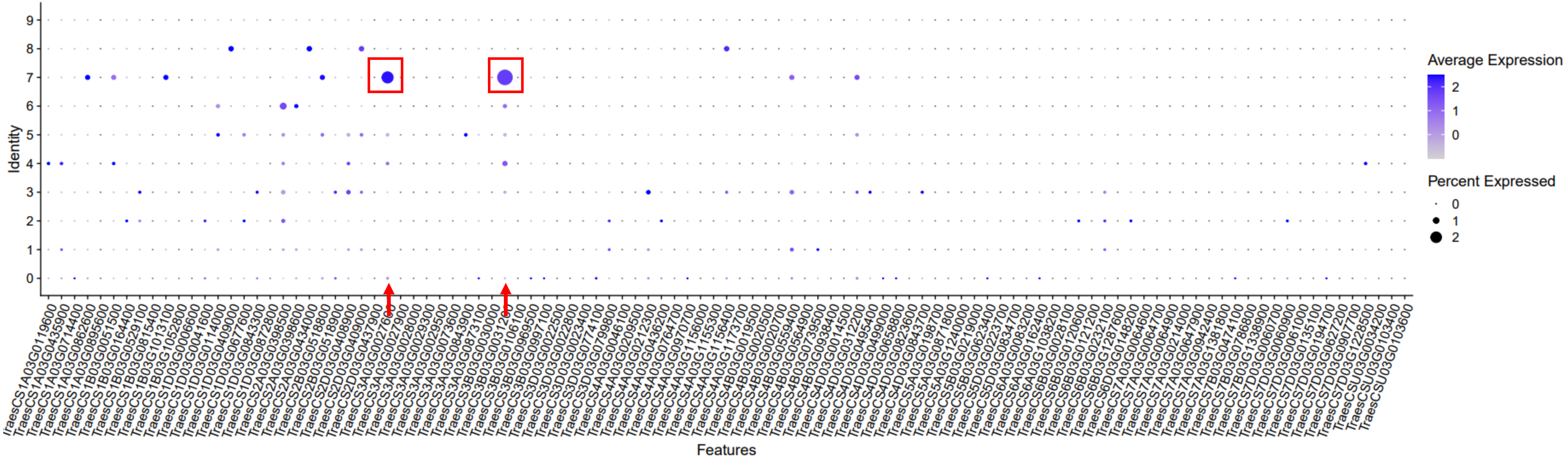
‘Suberin biosynthesis’ genes in wheat

- Orthologs from list of Arabidopsis genes from literature – 109 wheat genes
- Knetminer – 79 genes
  - Using ConceptID:2745187 (suberin biosynthetic process), ConceptID:2849625 (suberin network) and ConceptID:1379979 (suberin biosynthesis)



gene id	Knetminer	Ensembl	Root exp	Leaf exp	Reprod exp	Are they in the ortho?	Arabidopsis id
TRAESCS7A02G566100	CER4	Fatty acyl-CoA reductase	x		x	FAR1 / FAR4 / FAR5	AT5G22500 / AT3G44
TRAESCS7B02G490600	CER4	Fatty acyl-CoA reductase	x			FAR1 / FAR4 / FAR5	AT5G22500 / AT3G44
TRAESCS1A02G161400	CYP86B1	Cytochrome P450	x		x	CYP86B1 (ralph)	AT5G23190
TRAESCS1B02G177900	CYP86B1	Cytochrome P450	x		x	CYP86B1 (ralph)	AT5G23190
TRAESCS1D02G158400	CYP86B1	Cytochrome P450	x		x	CYP86B1 (ralph)	AT5G23190
TRAESCS7D02G520200	CYP86B1	Cytochrome P450	x			CYP86B1 (ralph)	AT5G23190

Suberin biosynthesis genes



FAR1 / FAR4 / FAR5 genes

Fatty Acyl-Coa reductase

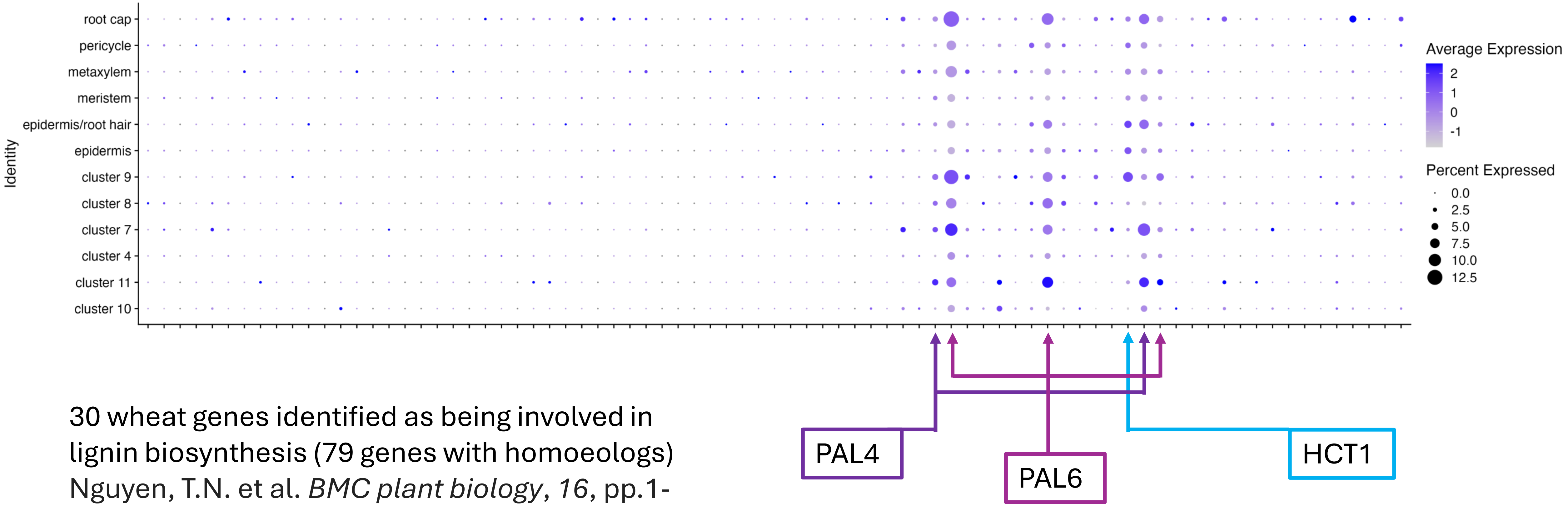
AT5G22500 / AT3G44540 / AT3G44550 genes

TraesCS3A02G013800

TraesCS3B02G016500

Thanks to Ling Zhang

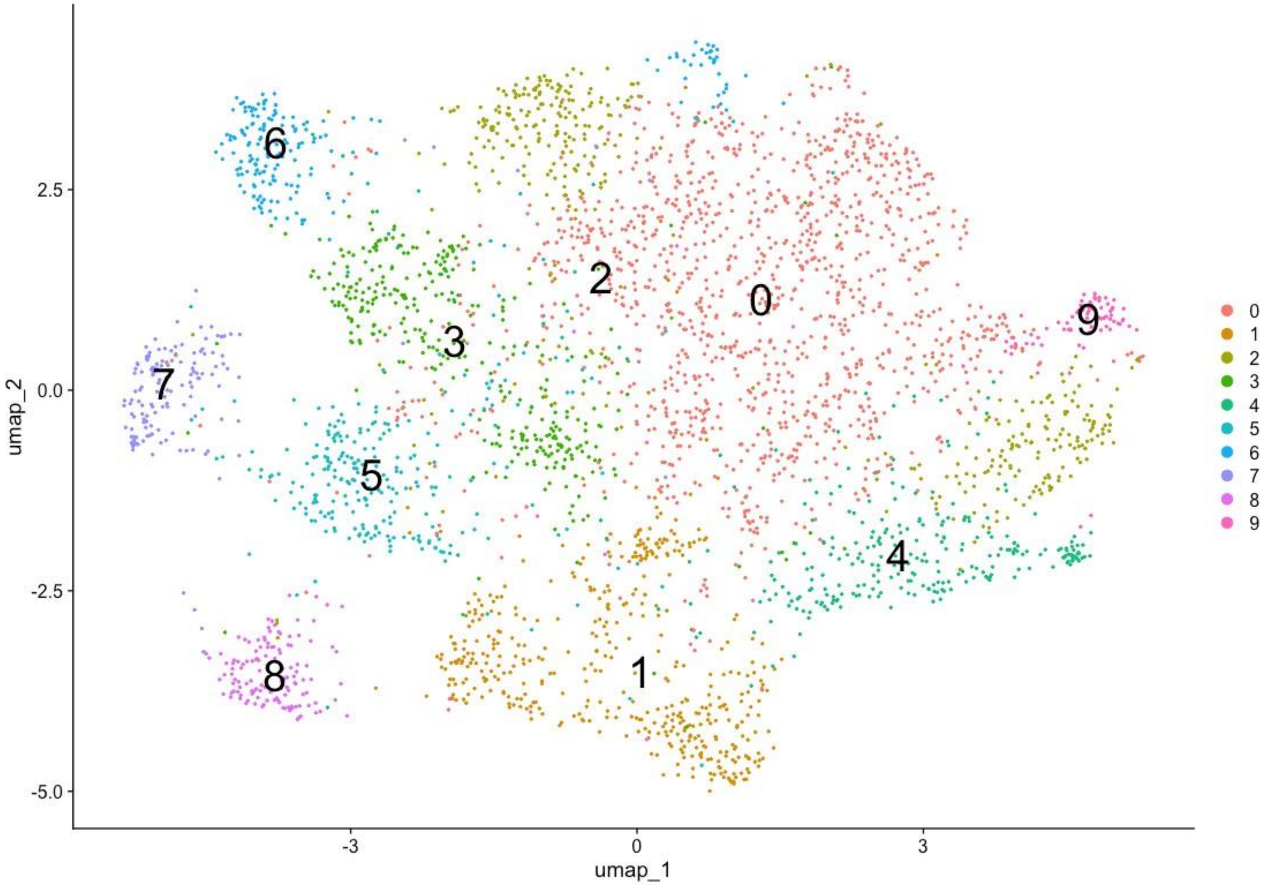
Lignin biosynthesis genes



30 wheat genes identified as being involved in lignin biosynthesis (79 genes with homoeologs)
Nguyen, T.N. et al. *BMC plant biology*, *16*, pp.1-16 (2016)

Thanks to Ling Zhang

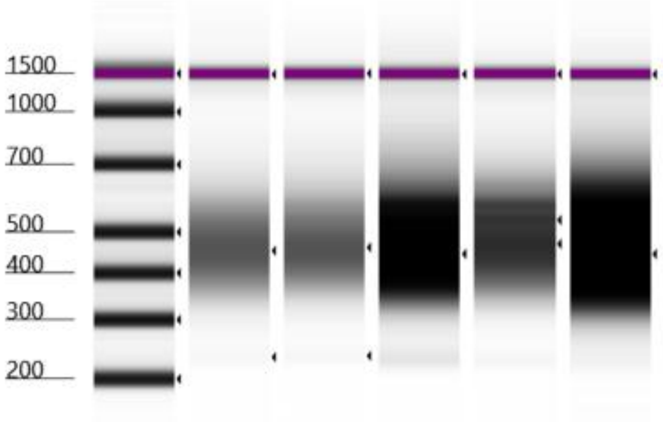
Wheat single nuclei atlas

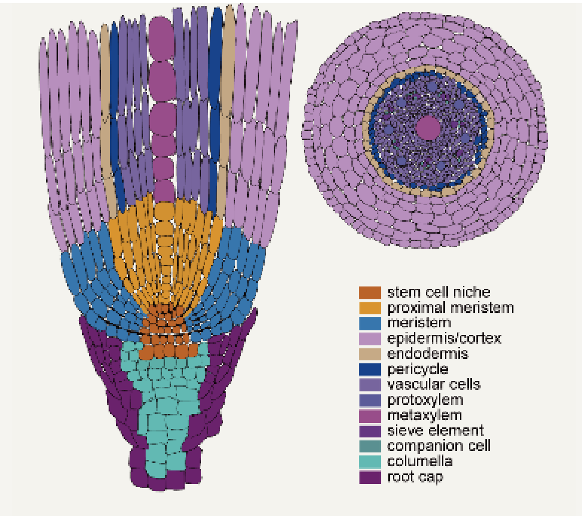


Thanks to Ling Zhang

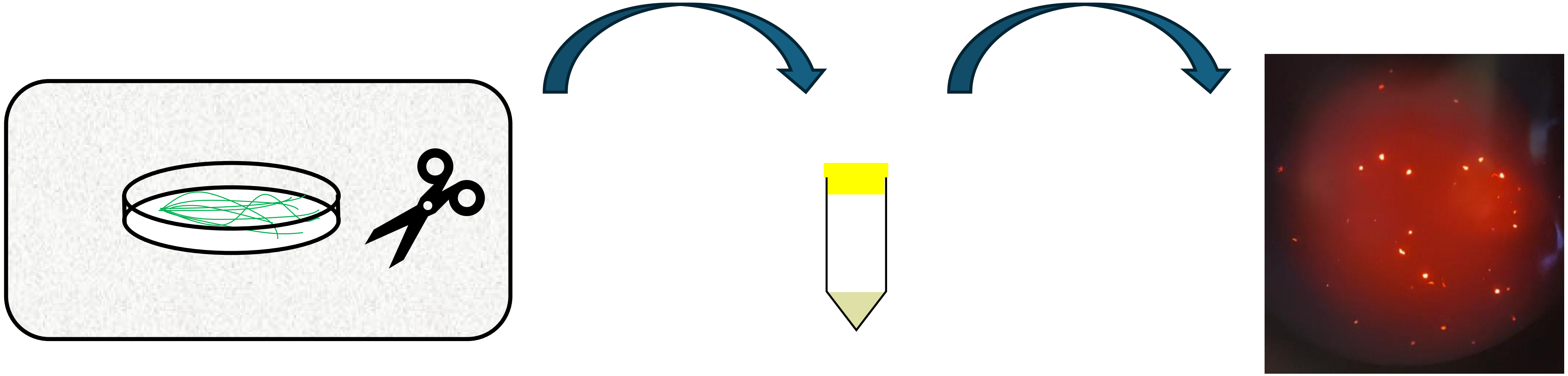
Optimized nuclei extraction

- Modified protocol from Zhang, L., et al. *Genome Biol* 24, 65 (2023).
- 18-day fresh wheat plants
- Estimated number of nuclei extracted between 20,000 – 60,000





Optimized nuclei extraction



Cut samples on ice

Filter samples

FACS

Optimized nuclei extraction

	Previous	**Current**
Sample preparation	Frozen at -80°C	Fresh
Number of plants	10-20 whole root systems	1 root system per sample
Time to extract	3 hours	1 hour
Extracted nuclei	< 10,000	20,000 – 60,000 *

* Also optimized FACs protocol

Acknowledgements

Busch Lab

Wolfgang Busch
Charlotte Miller
Sean Jarrell-Hurtado
Sara Ye

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Caz O’Connor