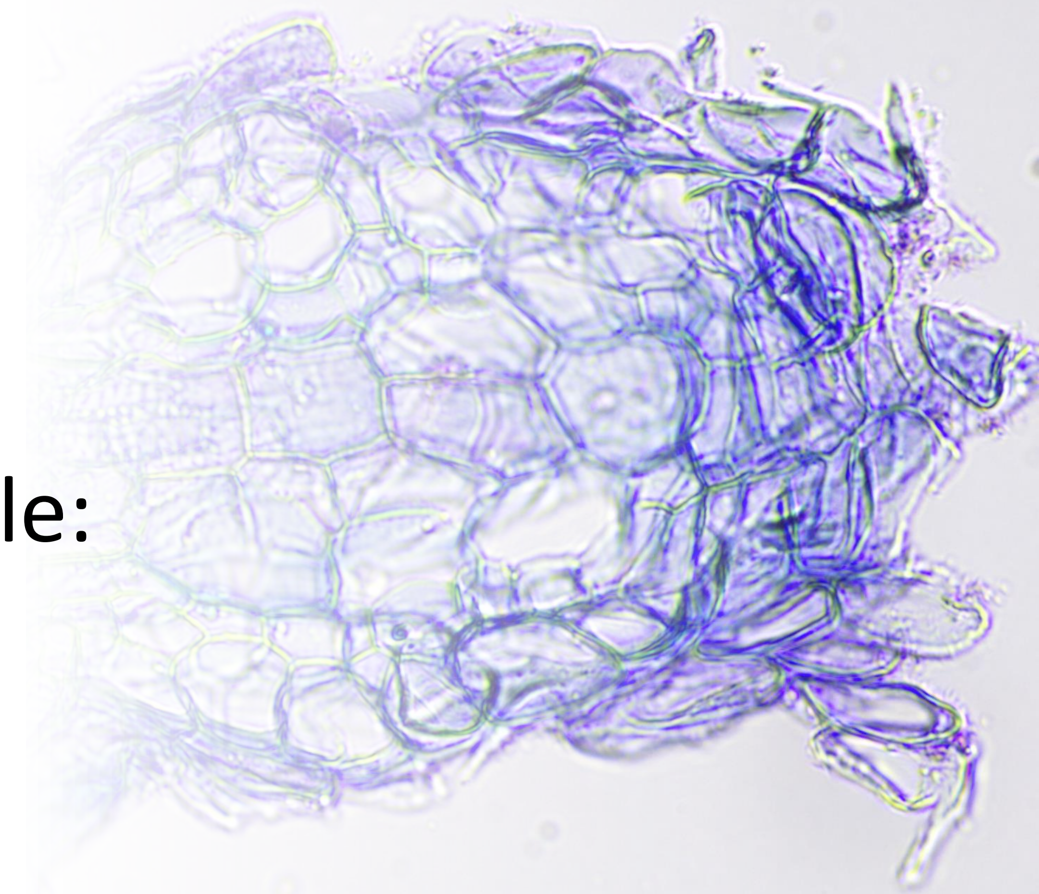
Sustainable Crop Nutrition

From lateral root to functional nodule: engineering organogenesis in barley



Min-Yao Jhu

2022 Global Food Security Coffee Break Seminars

Oldroyd Group

- **Sustainable crop nutrition – Giles Oldroyd**

Through a detailed understanding of how plants associate with **beneficial microorganisms**, we aim to broaden their use in agriculture to facilitate sustainable productivity.

The Vision of ENSA
Engineering Nitrogen Symbiosis for Africa

To Sustainably Increase Yields for Small-holder Farmers

- Crop plant productivity is highly dependent on the availability of a **nitrogen source** and farmers generally provide this as **fertilizers**.

Biological nitrogen fixation

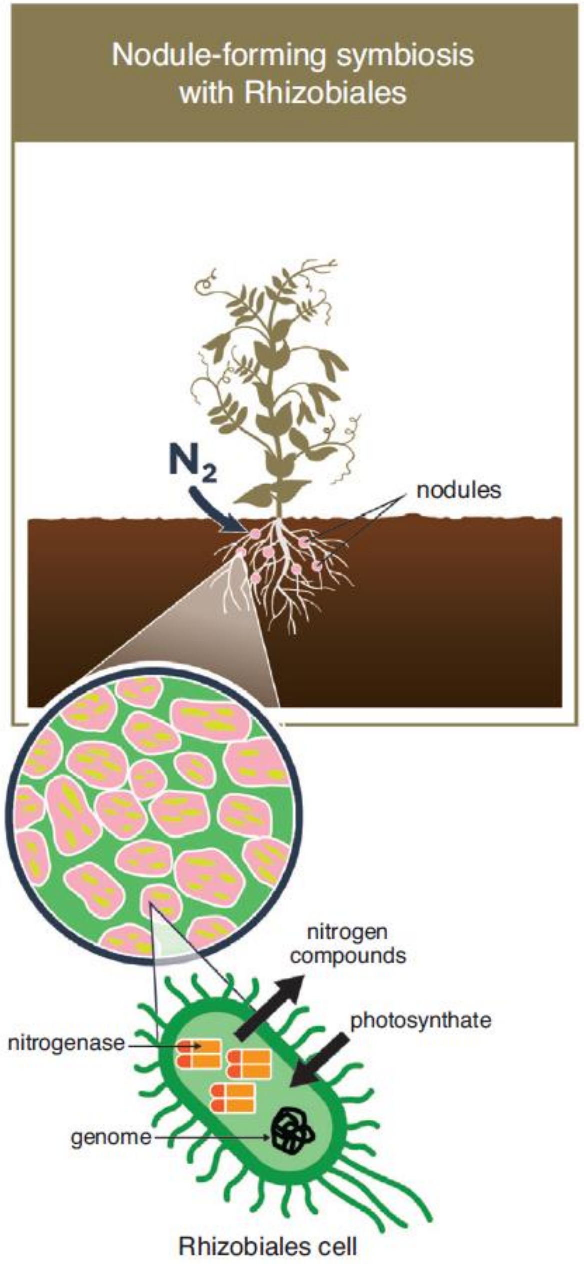
(a)

$$N_2 + 8H^+ + 8e^- + 16ATP + 16H_2O \xrightarrow[\text{NifHDK}]{\text{nitrogenase holoenzyme}} 2NH_3 + H_2 + 16ADP + 16P_i$$

- **Nitrogen-fixing bacteria**
- **Nitrogenase**: <u>convert di-nitrogen to ammonia</u>, a reactive form of nitrogen then can be used in biological processes.
- Legumes form specialized organs on the roots, called **nodules**, that **house the nitrogen-fixing bacteria and provide the suitable oxygen-regulated environment for nitrogen fixation to occur.**

(b) Legume Crops

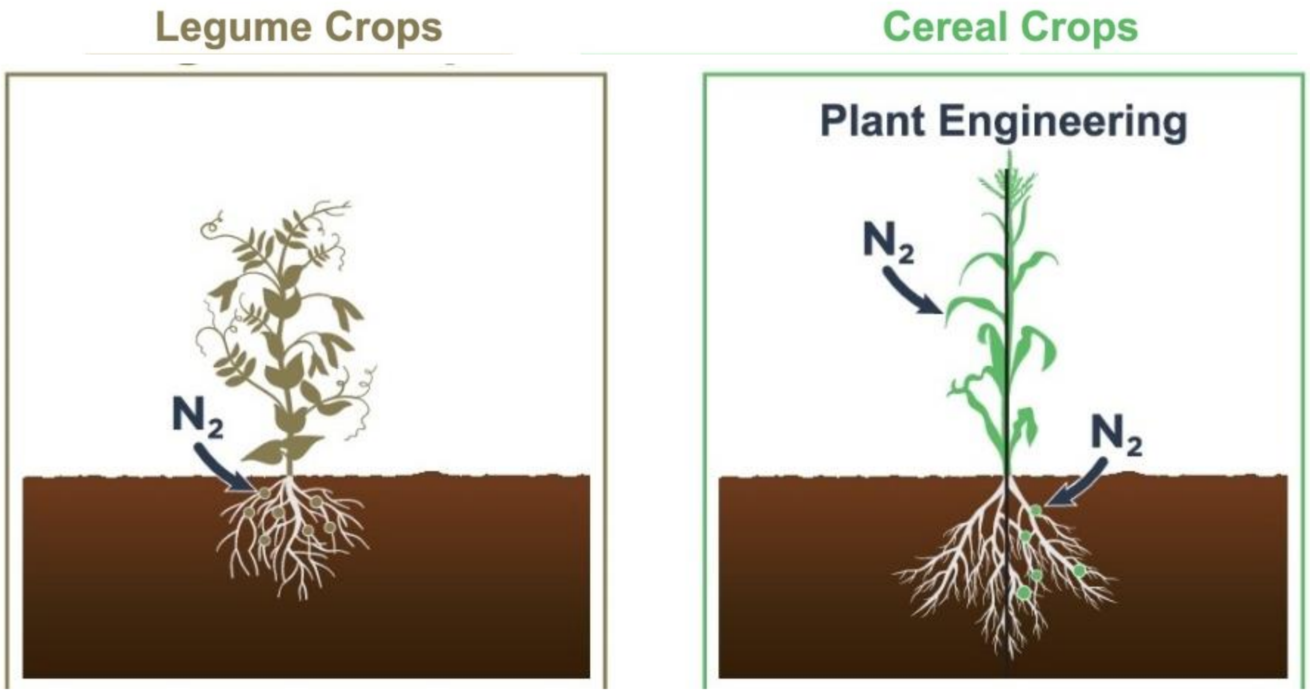
Bloch et al., 2020

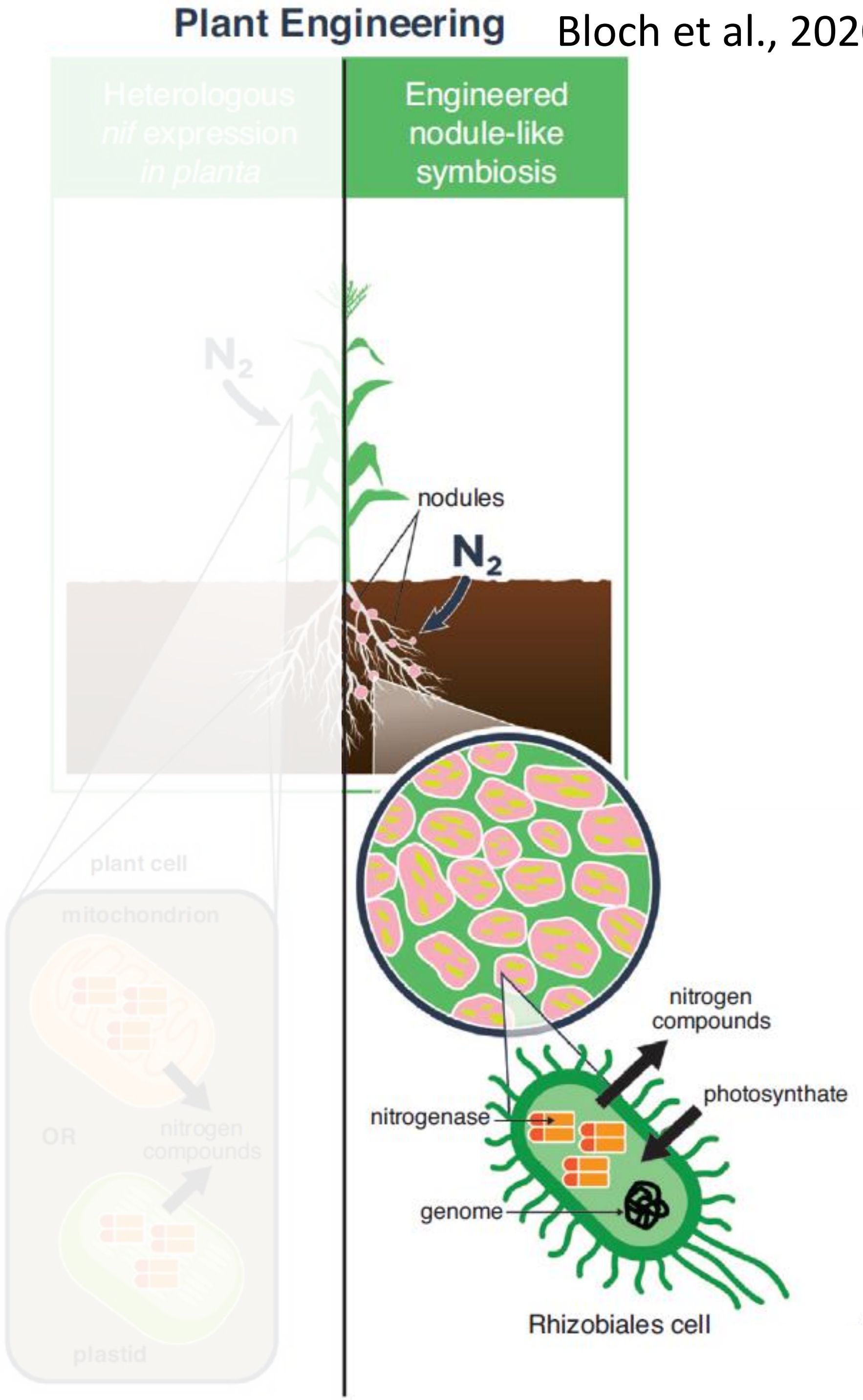


Bloch et al., 2020

Engineering a Solution

- ENSA: we are attempting to transfer **the capability of associating with nitrogen-fixing bacteria** from legumes to **cereals**.
- **Self-fertilizing cereals**: can support their own productivity without the need to use nitrogenous fertilizers.

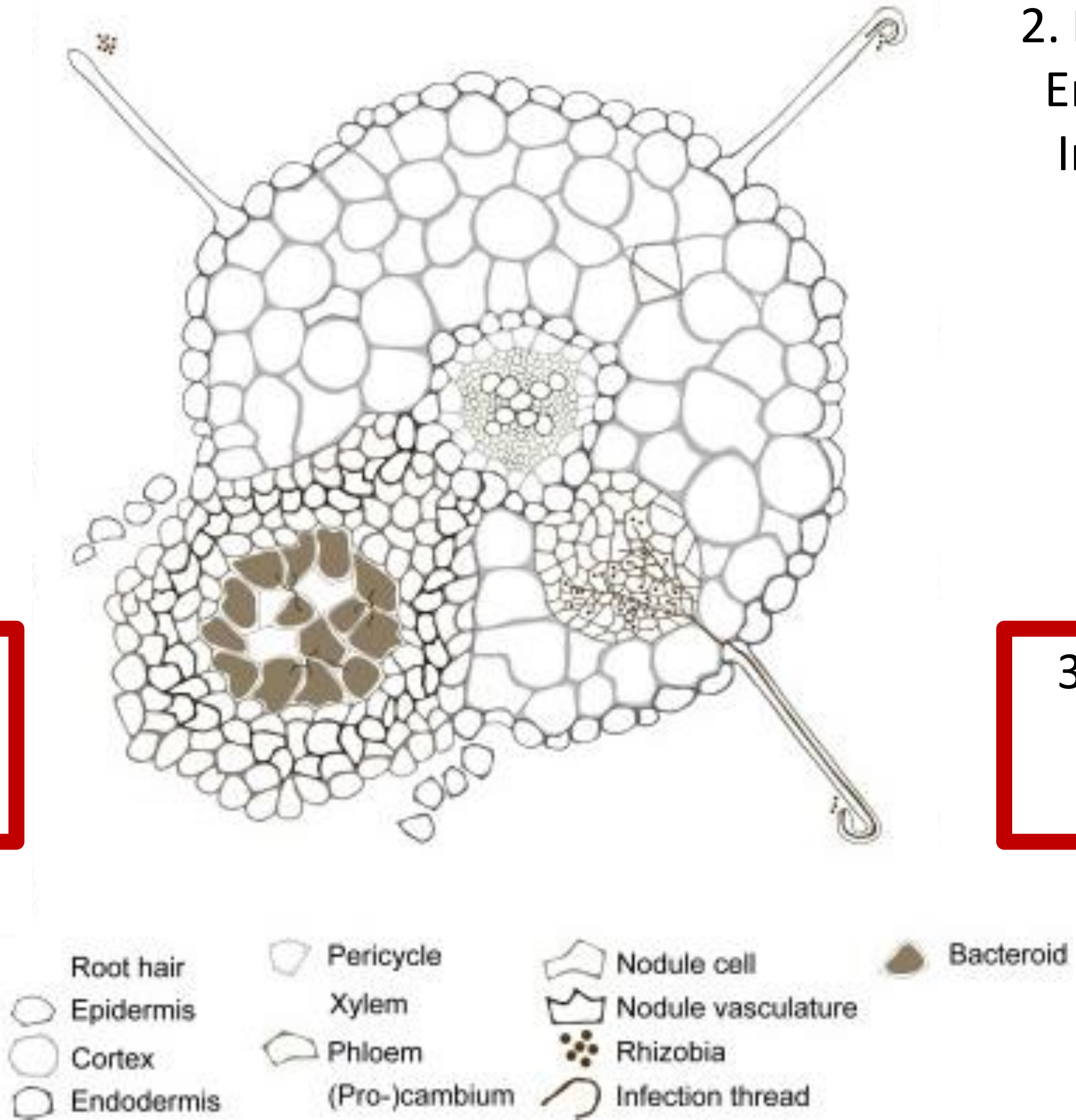




The Four Components to Engineering Symbiosis

1. **Pre-infection**:
Engineer Perception of Nitrogen Fixing Bacteria

2. **Nodule initiation**:
Engineer Bacterial Infection Process



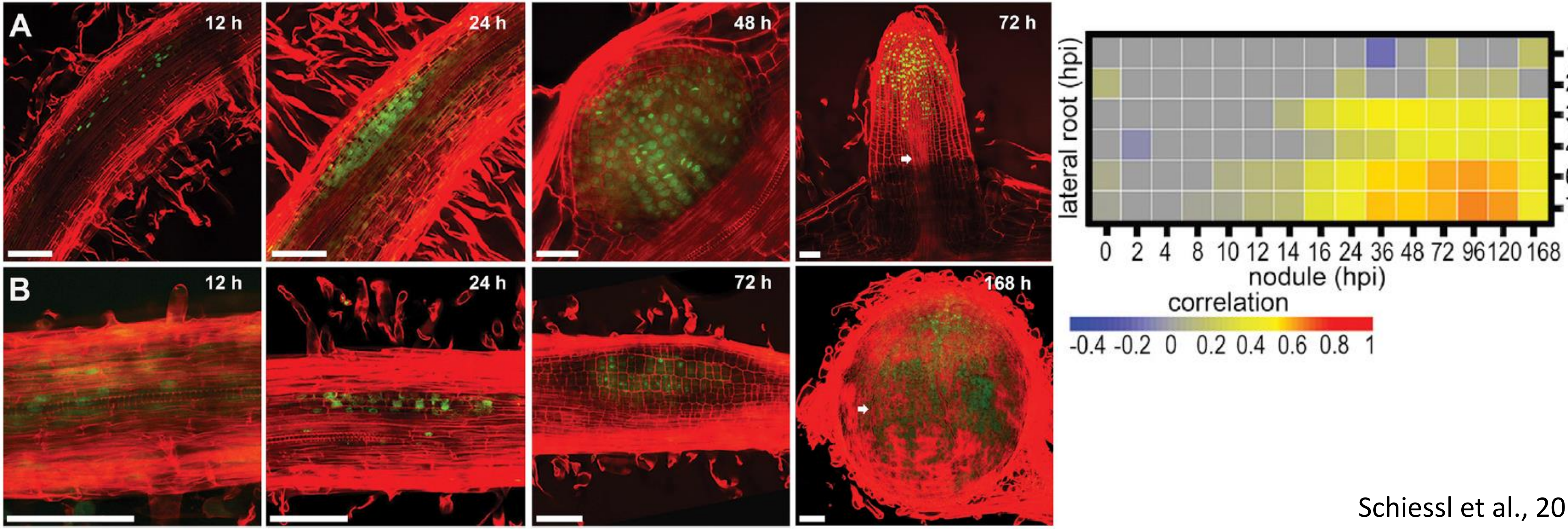
4. **Mature nodule**: Engineer the Appropriate Environment for Nitrogen-Fixation within the Nodule

3. **Nodule primordia**:
Engineer Nodule Organogenesis

Lin et al., 2020

Shared genes drive lateral root development and root nodule symbiosis pathways

- Nodule and lateral root initiation converges on a local accumulation of the plant hormone **auxin** and a set of **auxin**-responsive regulators.



Schiessl et al., 2019

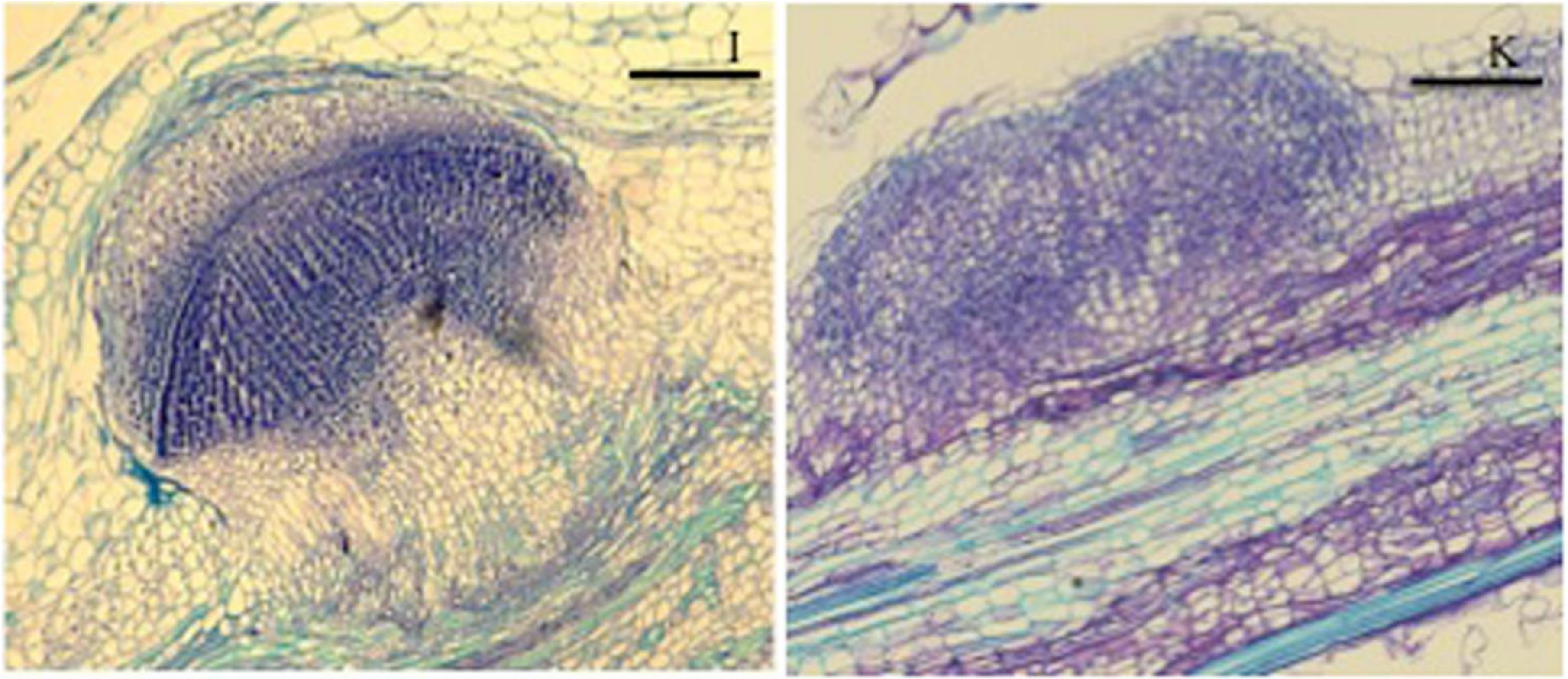
Auxin-Induced Nodule-Like Structures in rice and Medicago

- **Auxins** induce the formation of **nodule-like structures (NLSs)** on legume roots in the absence of rhizobia.
- **NLSs** appear to be structurally similar in rice and Medicago roots.

Hiltenbrand et al., 2016

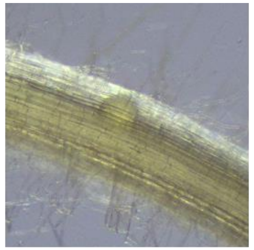
Rice Medicago

Rice Medicago



Research aims

- **Engineer Nodule Organogenesis**
  - How to engineer nodule organogenesis from **existing signaling and developmental mechanisms** in **barley lateral root or nodule like structure**?
  - How to engineer the following **cell differentiation** and introduce **nodule identity** to promote the formation of **functional nodules** in barley?



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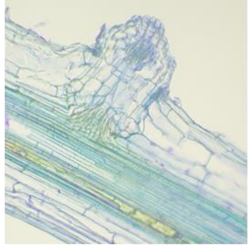

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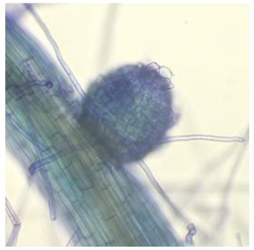

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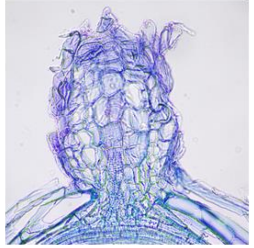
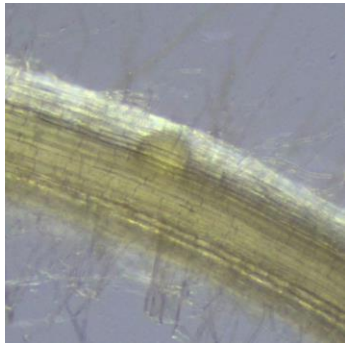


4. Spatiotemporal engineering: Cell-type specific promoters with STARTS

Outline



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A comparison of lateral root patterning among dicot and monocot plants

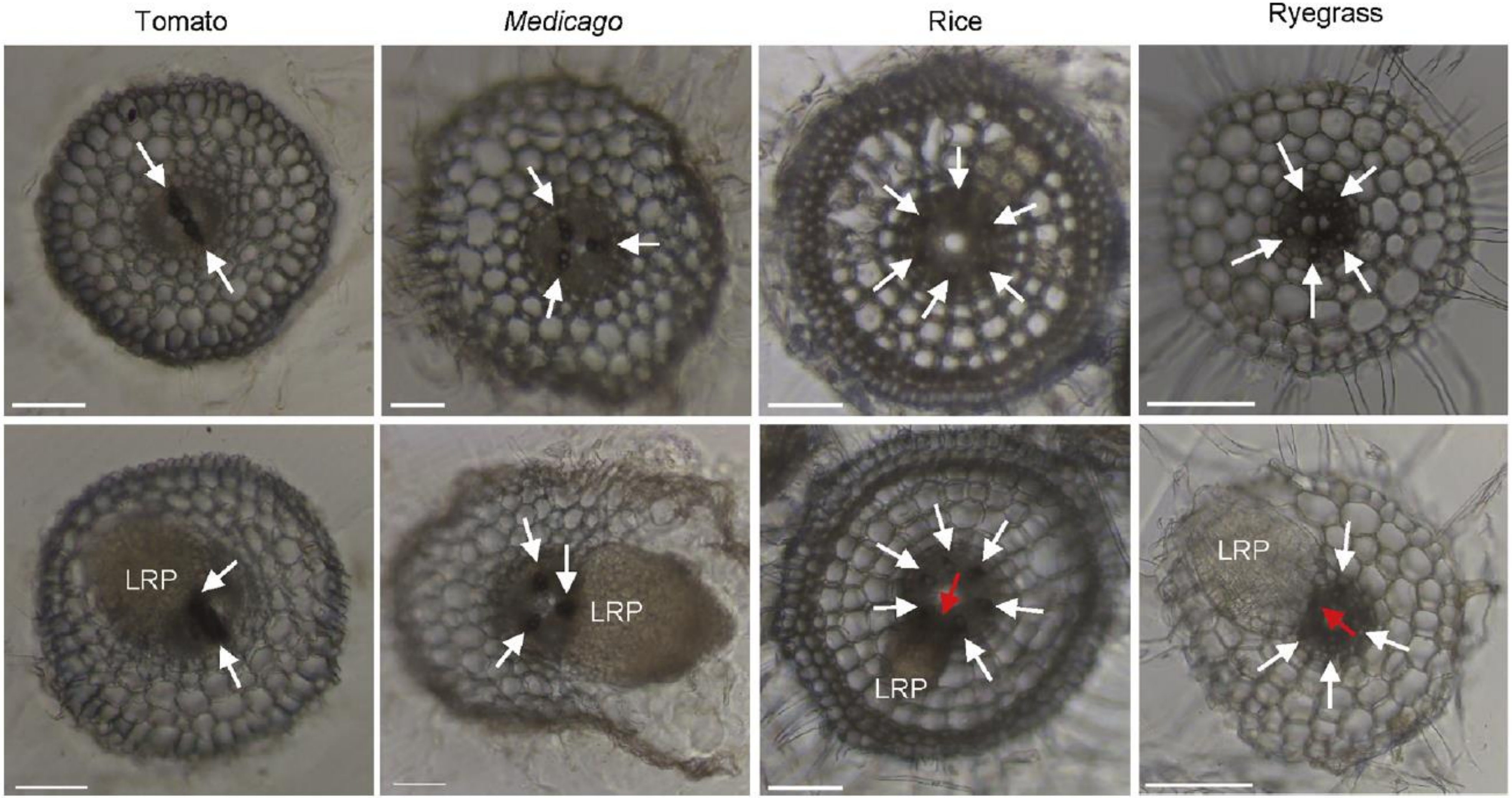
Chen et al., 2018

- Tomato and Medicago:

Lateral root primodium initiates from **xylem pole pericycle.**

- Rice and ryegrass:

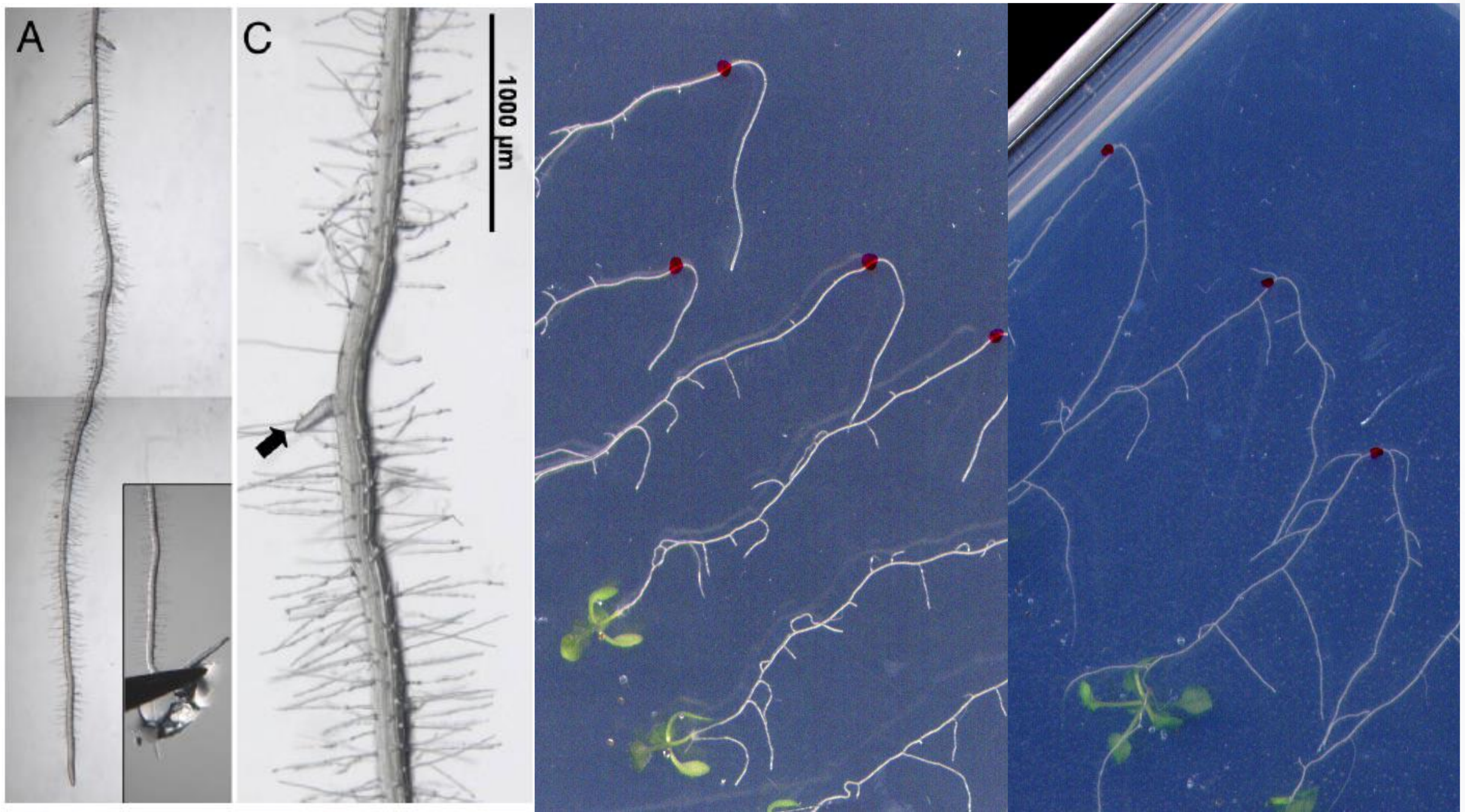
Lateral root primodium initiates from **phloem pole pericycle.**



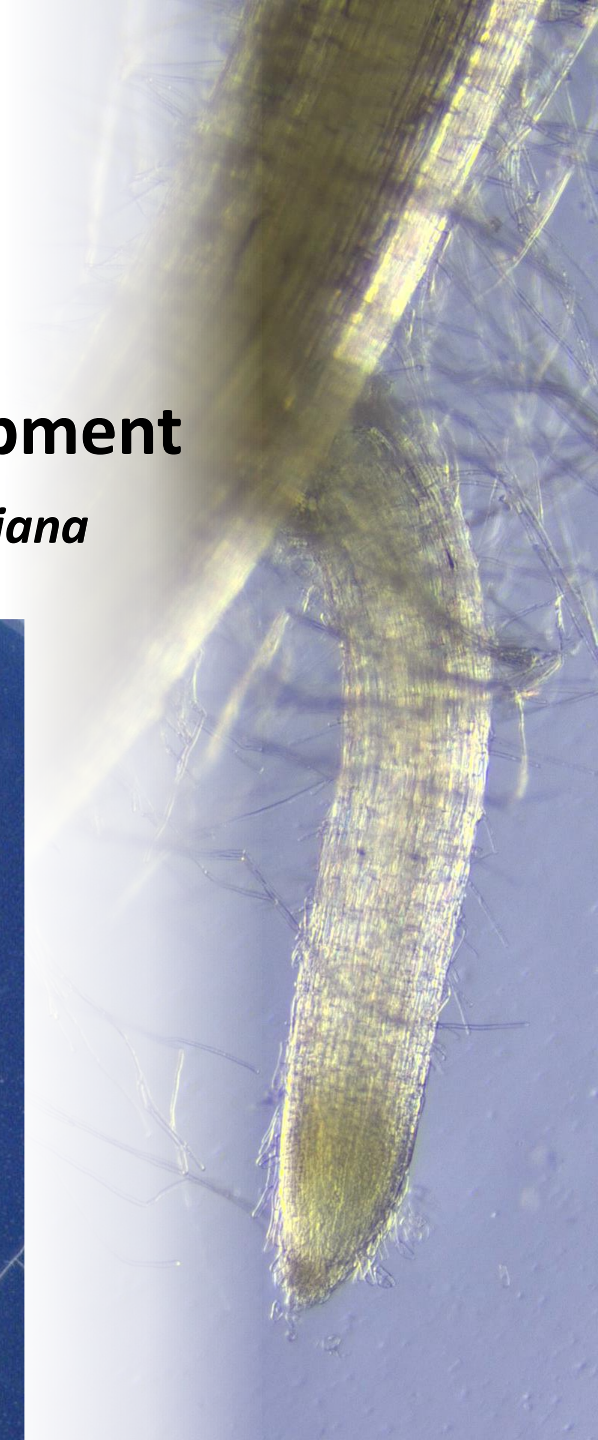
Research approaches

1. Temporal understanding on barley lateral root development

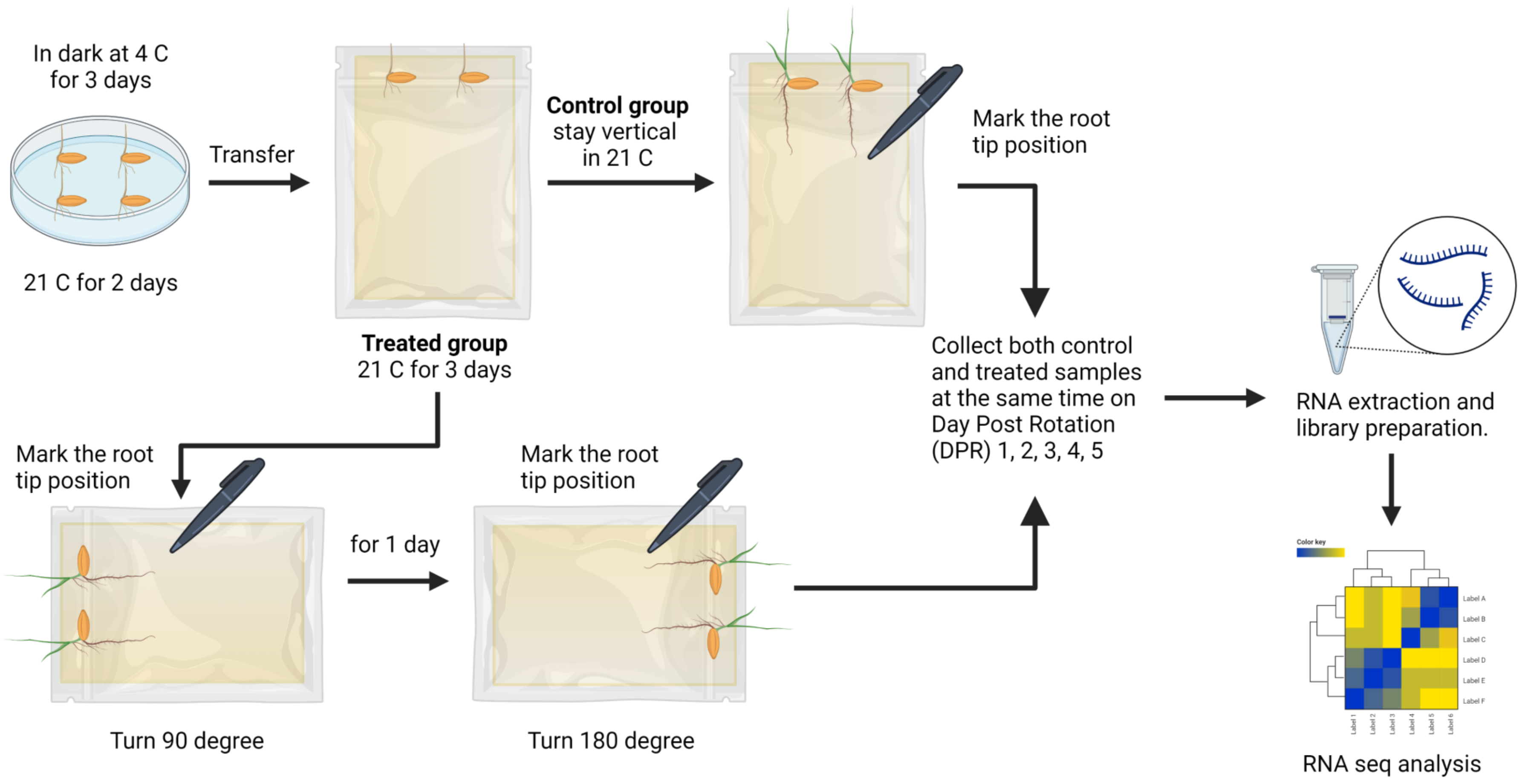
Gravity and mechanical induction of lateral root initiation in *Arabidopsis thaliana*

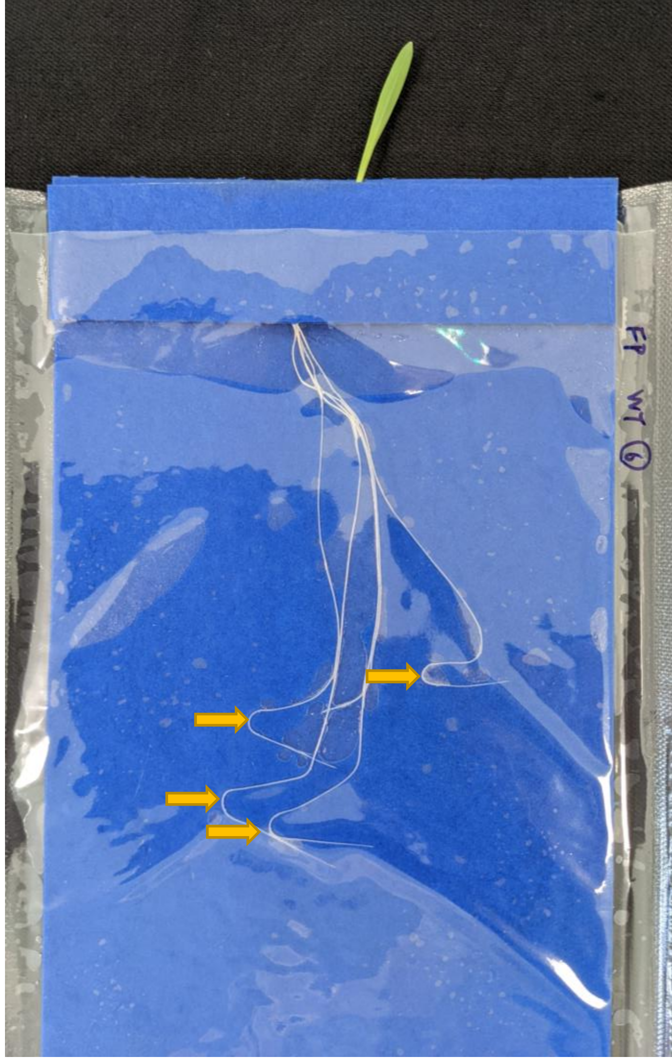


Ditengou et al., 2008



Time course RNA-Seq analysis on gravity induction of lateral root initiation in barley





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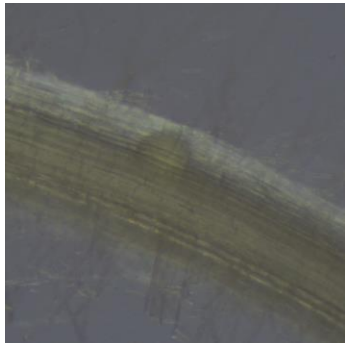

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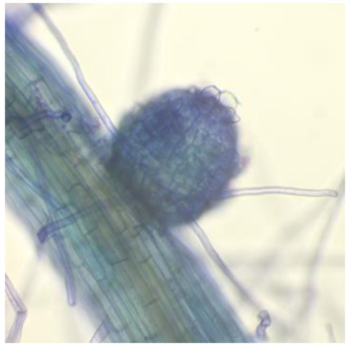

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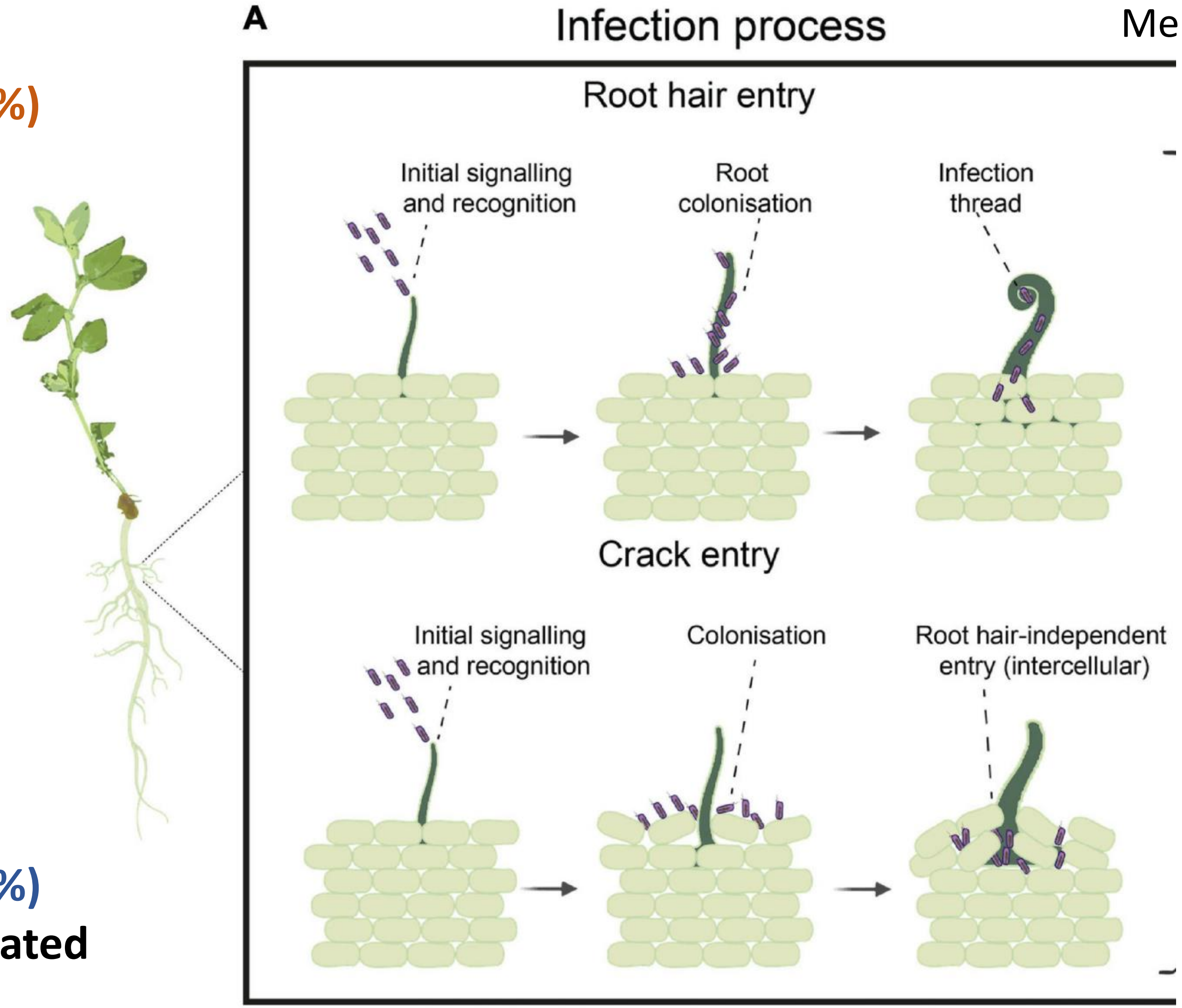
3. Visium **spatial transcriptome** on barley lateral root and nodule-like structure

4. **Spatiotemporal engineering**: Cell-type specific promoters with STARTS

Introduction

Mendoza-Suárez et al., 2021

- Intracellular infection (75%)



- Intercellular infection (25%)
- Ancient and less sophisticated

Research approaches

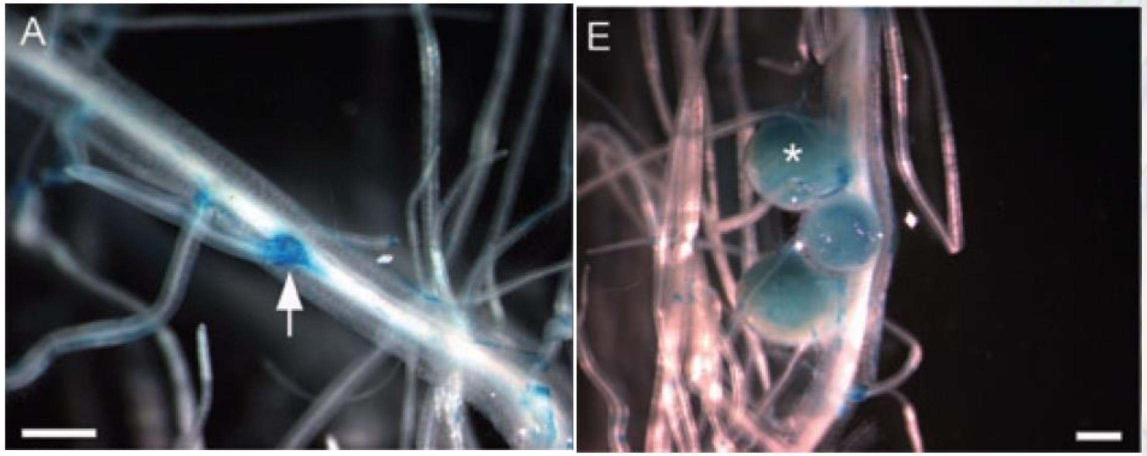
2. Temporal understanding on Rhizobium-induced nodule-like structure in barley

***Rhizobium* IRBG74**

- The first confirmed **legume-nodulating** symbiont from the **Rhizobium (Agrobacterium)** clade.
- **Induced nodulation** in legumes
- **Crack entry**.
- **Intercellular infection.**
- Plant growth-promoting hormones.

7 dai

15 dai

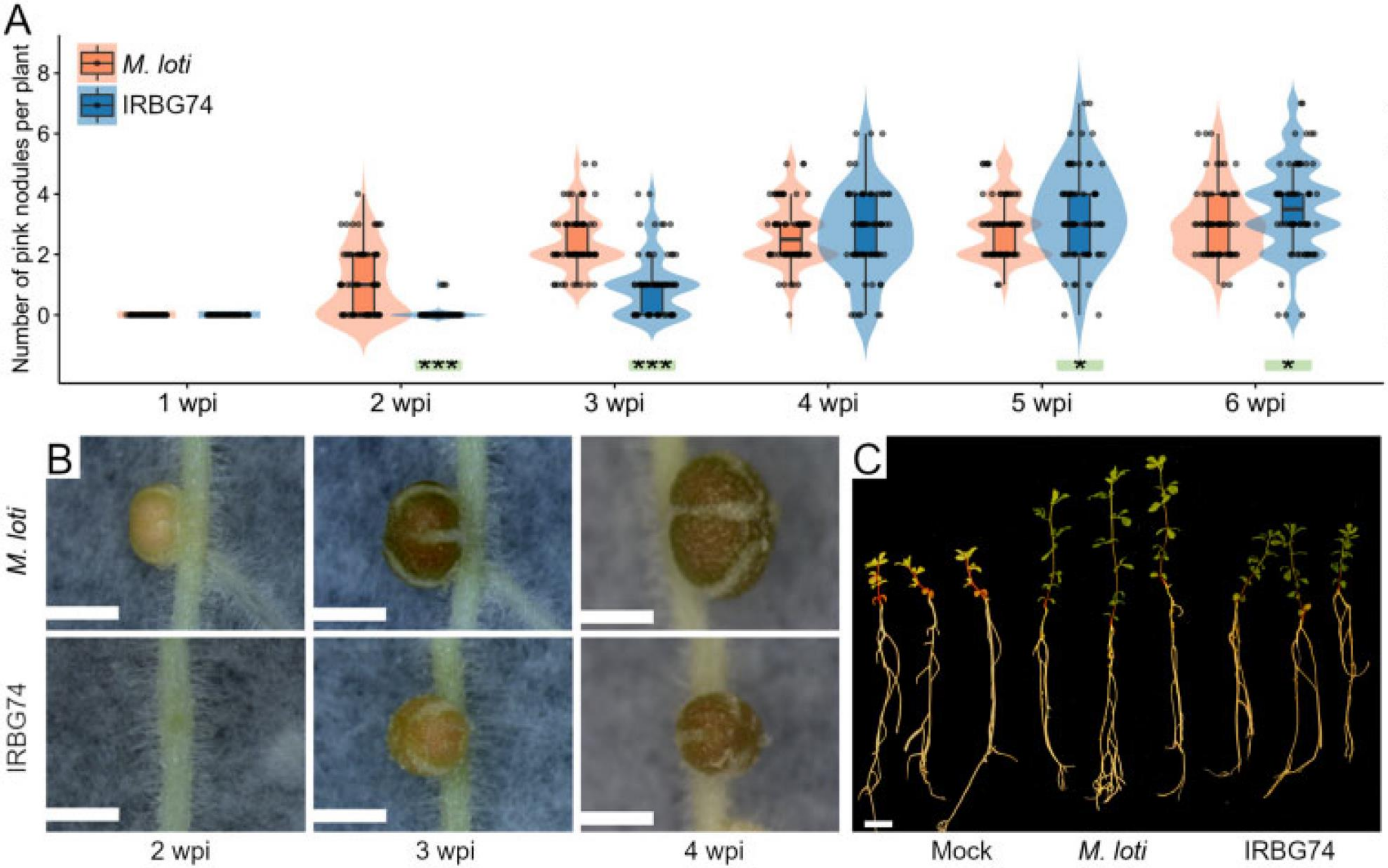


Cummings et al., 2009

Distinct signaling routes mediate intercellular and intracellular rhizobial infection in *Lotus japonicus*

- **Intracellular infection**
- ***Mesorhizobium loti* (*M. loti*)**

- **Intercellular infection**
- **IRBG74**

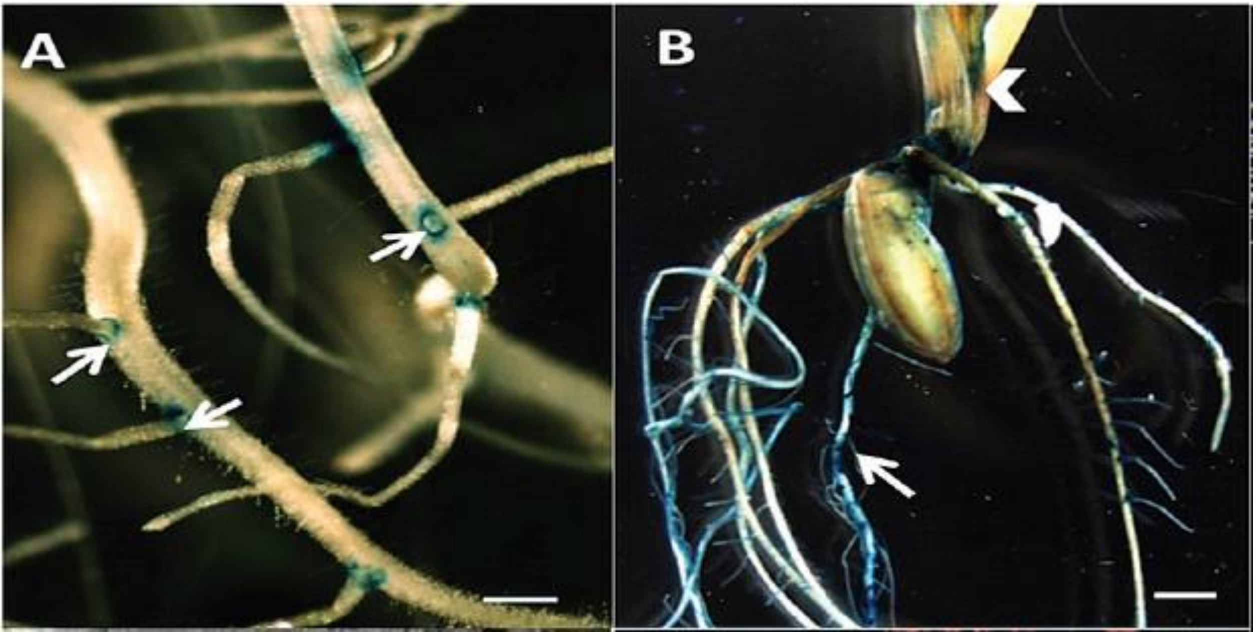


Montiel et al., 2021

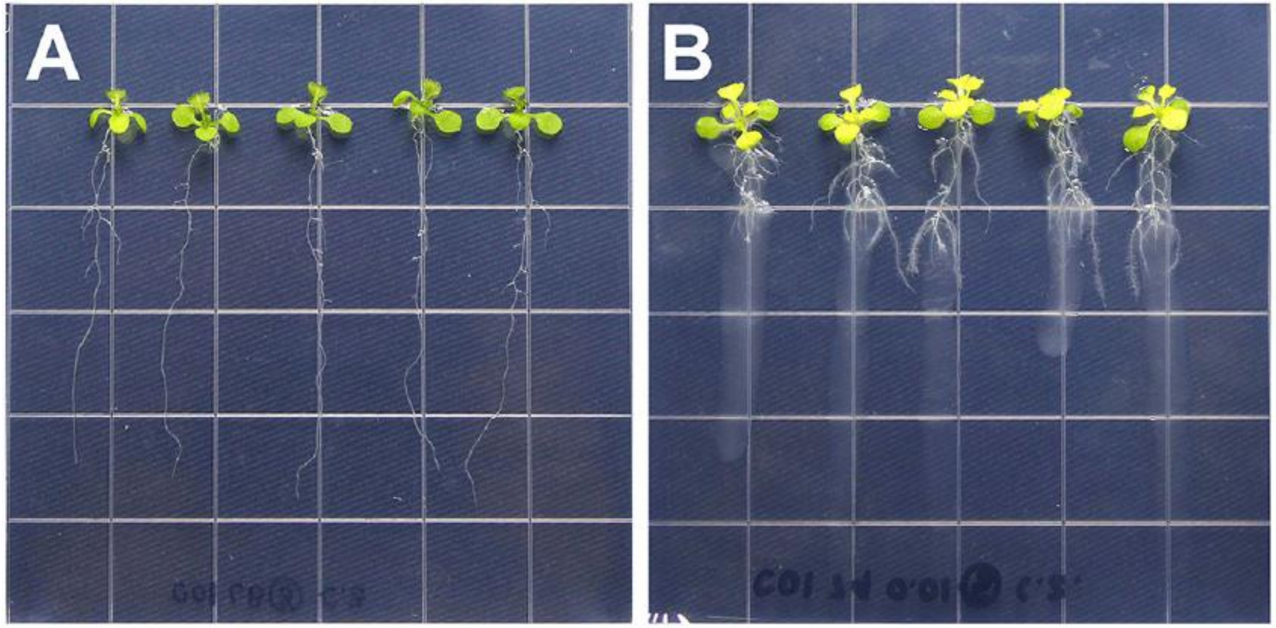
IRBG74 is an efficient plant growth-promoting rhizobacteria (PGPR)

- IRBG74 has a particular affinity for establishing beneficial interactions with **flooded plants**, both legumes and cereals.
    - IRBG74 stimulated early rice growth resulting in **increased yields at maturity.**
    - IRBG74 **promotes lateral root formation** in *Arabidopsis*.

Mitra et al., 2016



Zhao et al., 2018

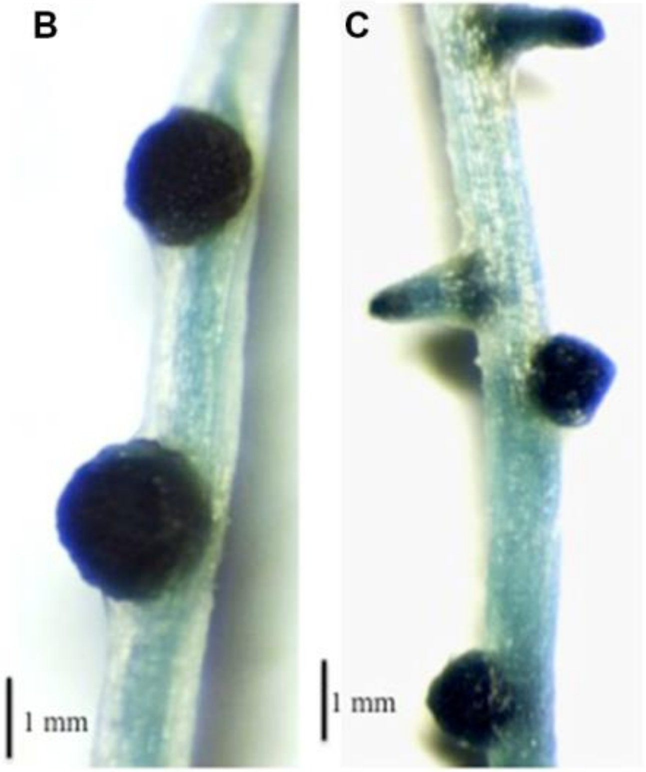


Auxin-Induced Nodule-Like Structures (NLS) in rice and Medicago

- *Azorhizobium caulinodans* is a known nitrogen-fixer and can colonize rice roots (Gopalaswamy et al., 2000; Dixon and Kahn, 2004).
- *A. caulinodans* can colonize rice **NLS** and **lateral roots** as well.

Rice Medicago

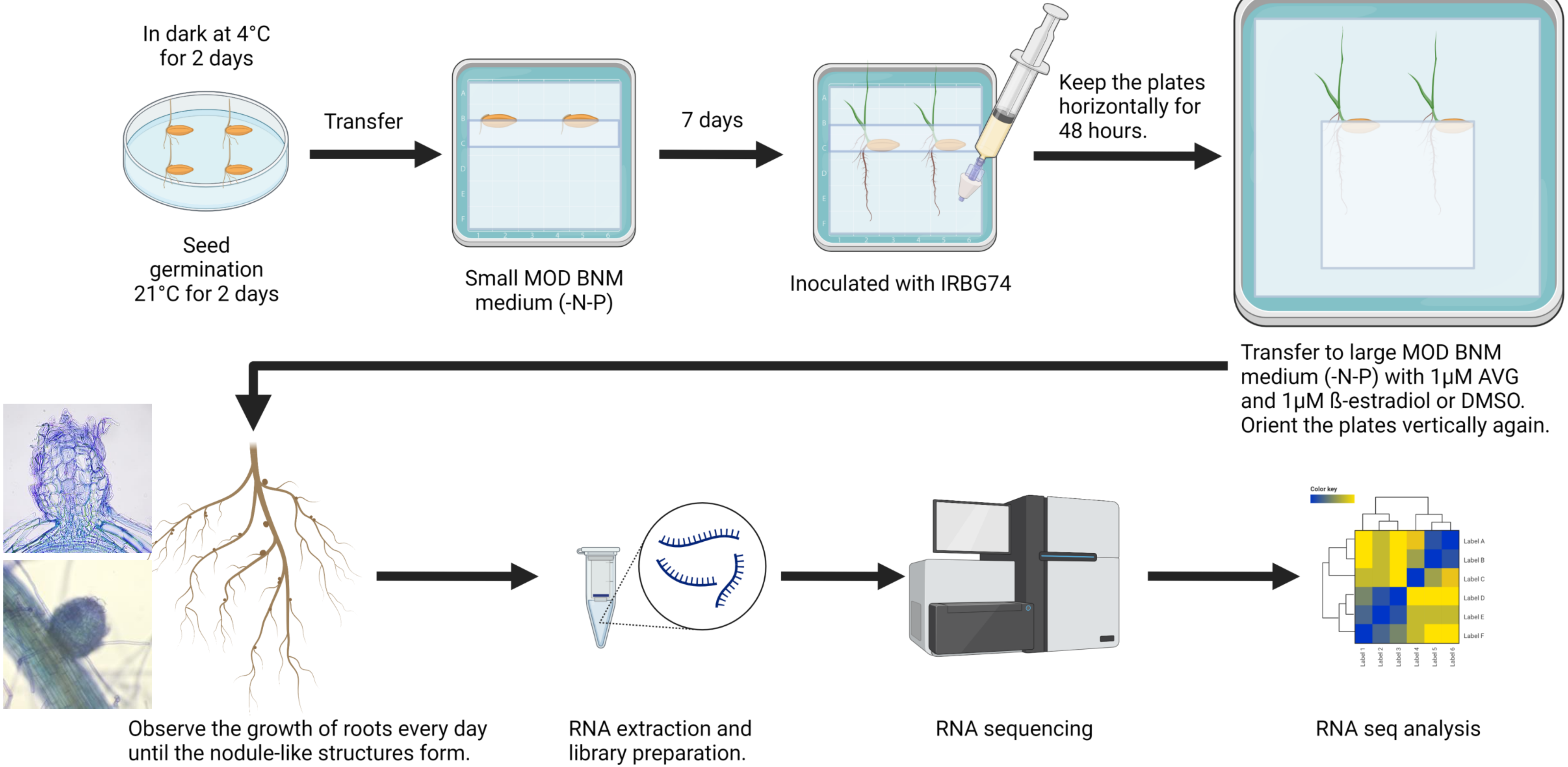
Rice



Hiltenbrand et al., 2016

Rhizobium-induced Nodule-Like Structures in Barley

T2 homozygous



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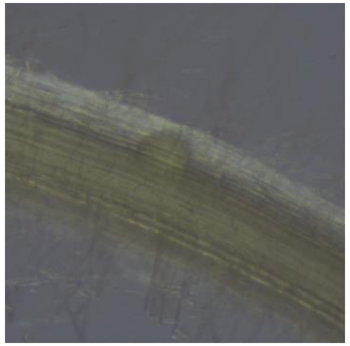

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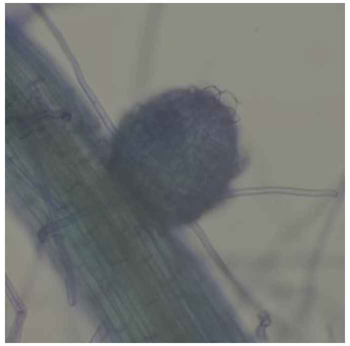

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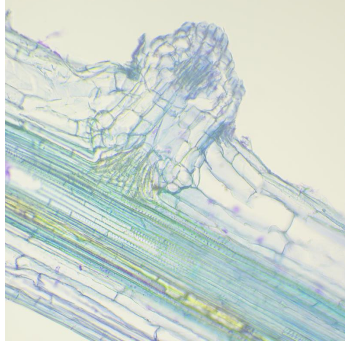

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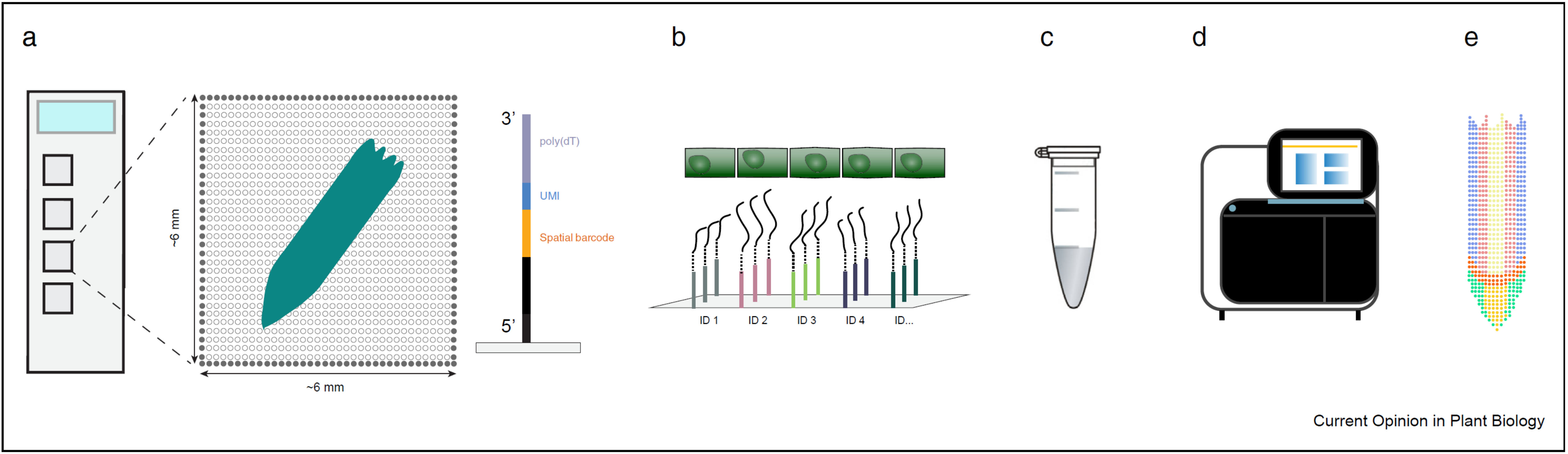
What is Spatial Transcriptome?

Visium Spatial Gene Expression: Map the whole transcriptome within the tissue context.

Giacomello, 2021



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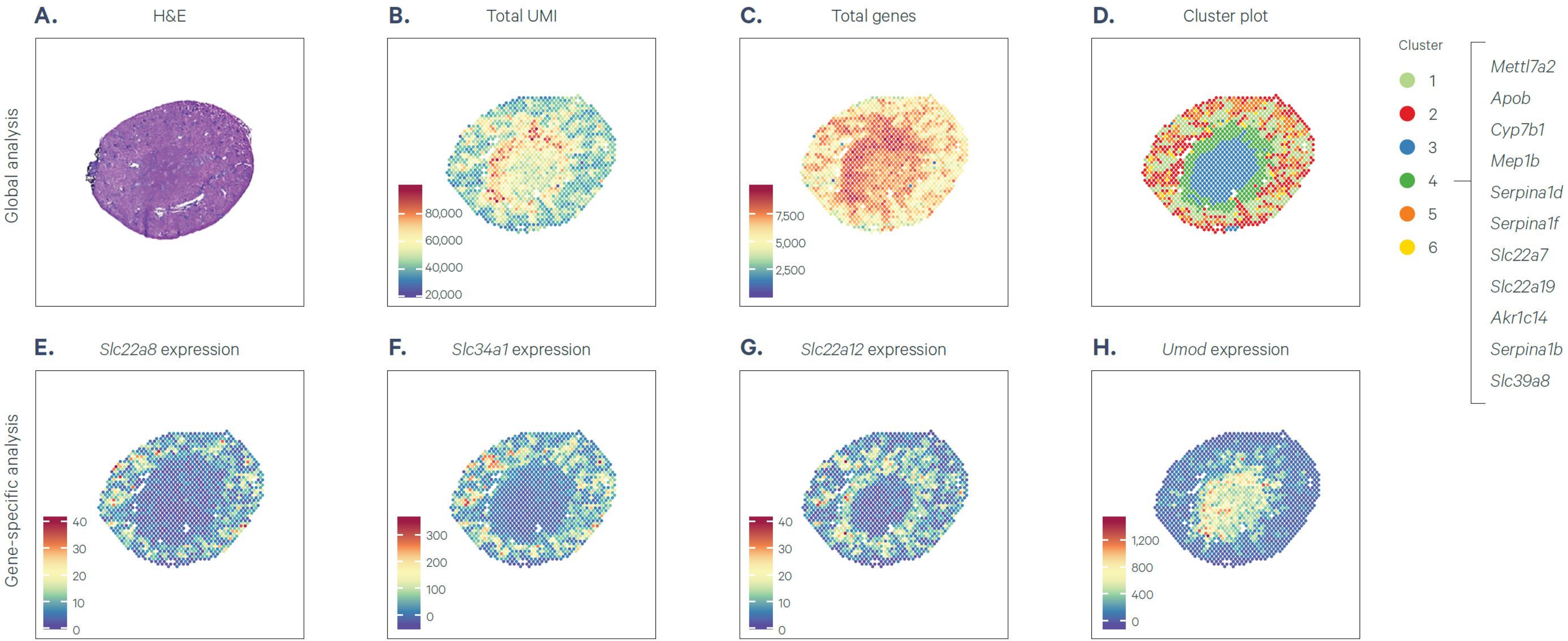
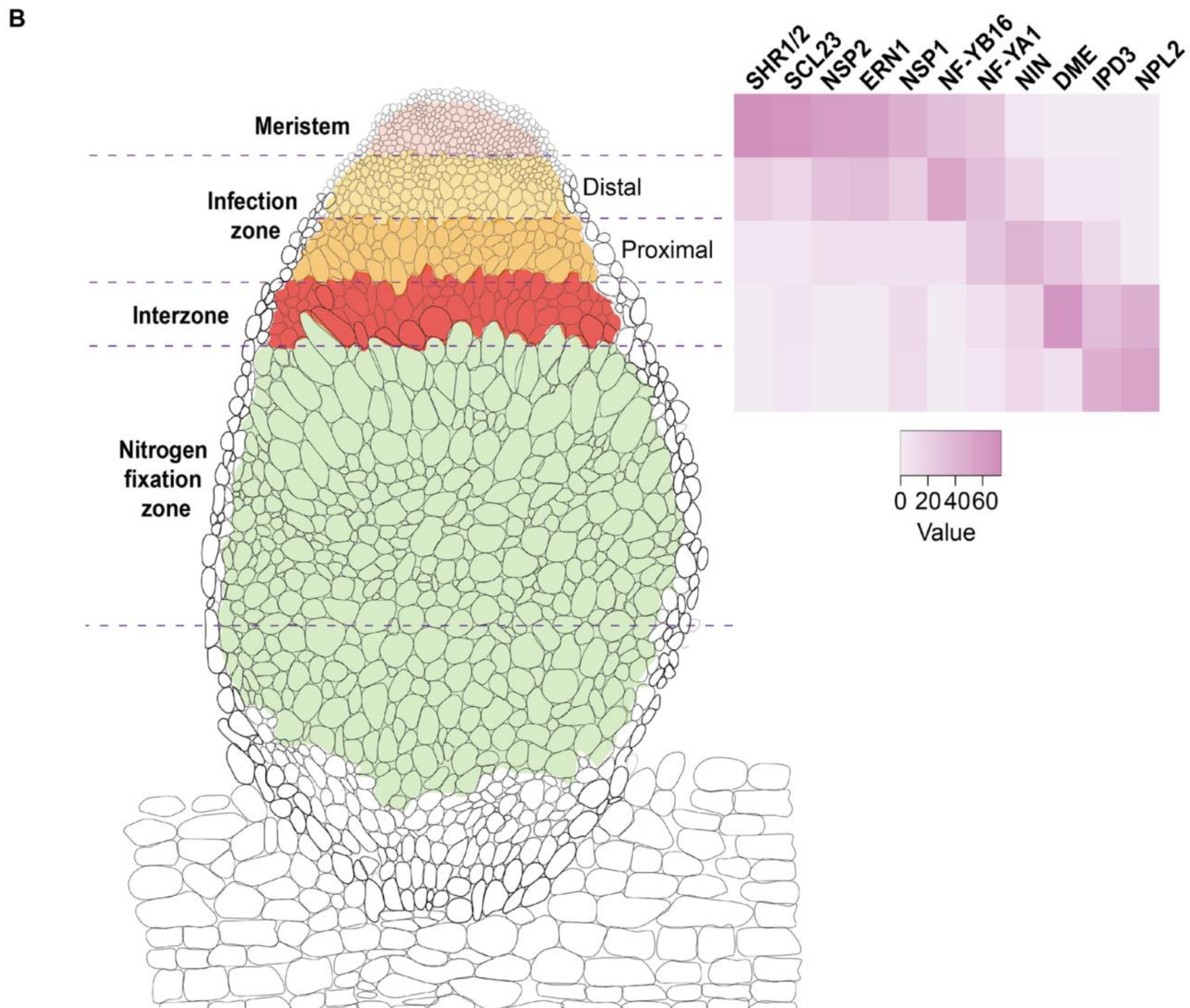


Why do we need Spatial Transcriptome?

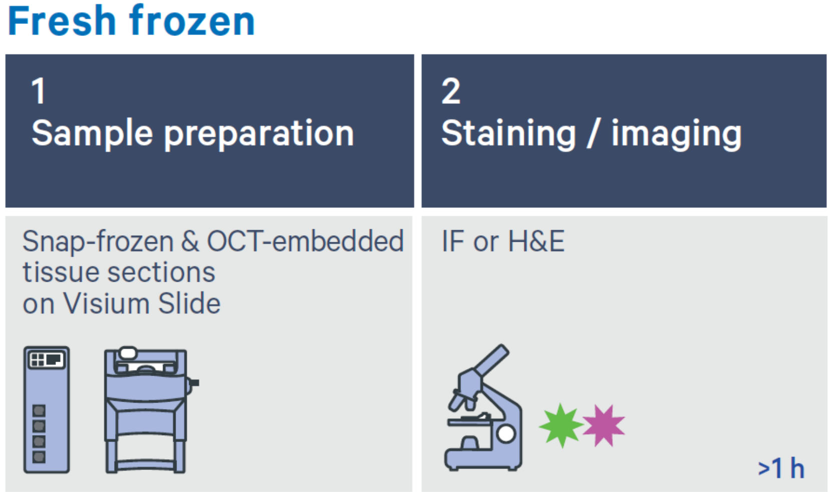
Wang et al., 2022

- Identify **spatiotemporal gene expression patterns** **in barley nodule-like structures and lateral roots**
- Gain a complete view of **development complexity**
- Discover **new biomarkers or cell-type specific promoters**

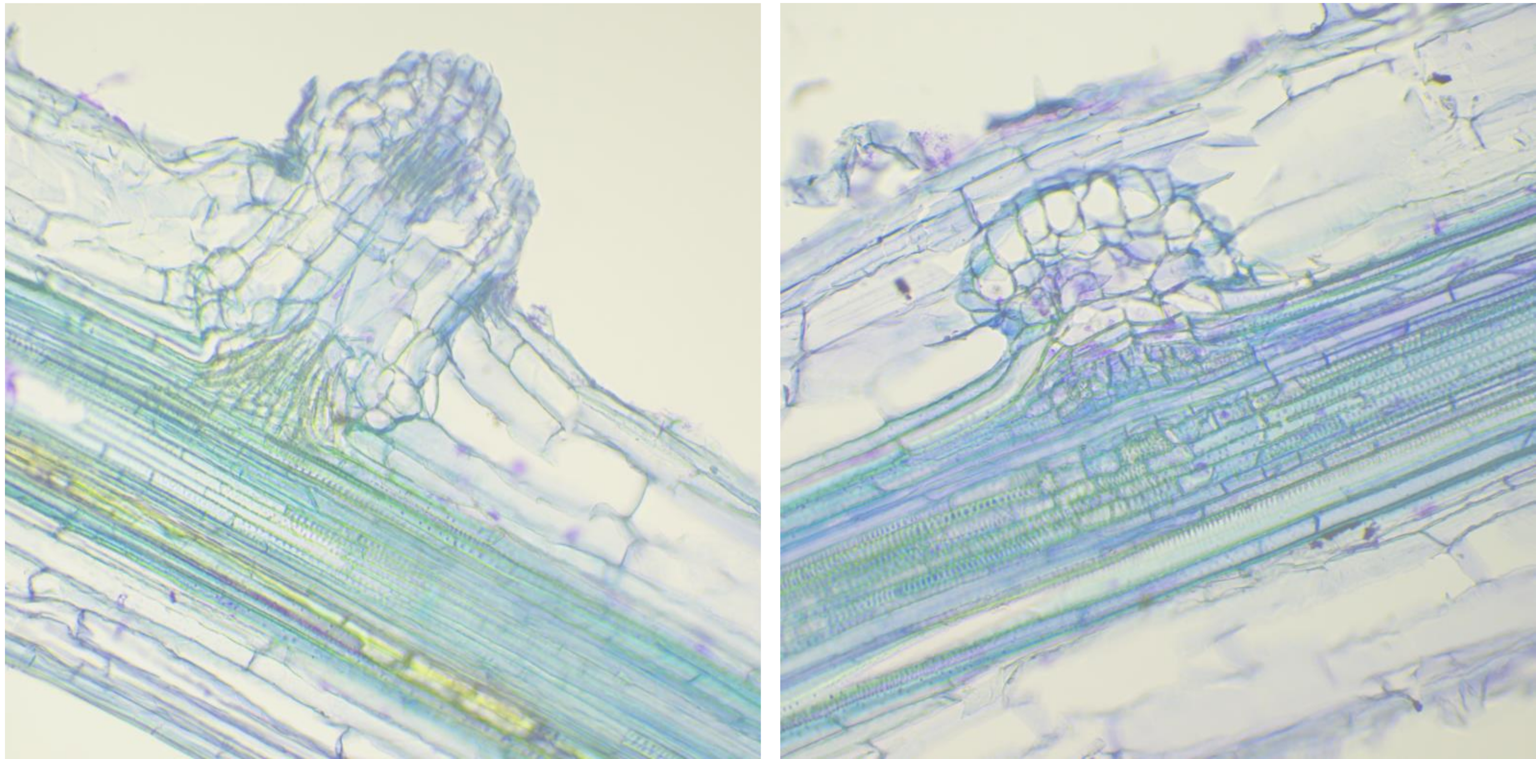


Current progress & experiment plans

1. **Cryosectioning optimization for barley emerging lateral roots** and nodule-like structures
2. Visium Spatial Tissue Optimization
3. Prepare Spatial Transcriptomics sequencing libraries
4. Data analysis



Stained with Toluidine blue



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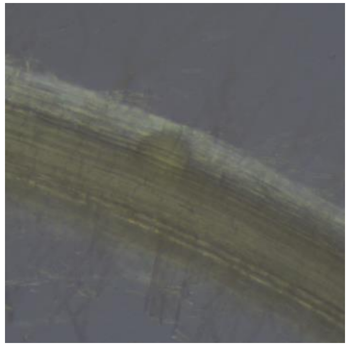

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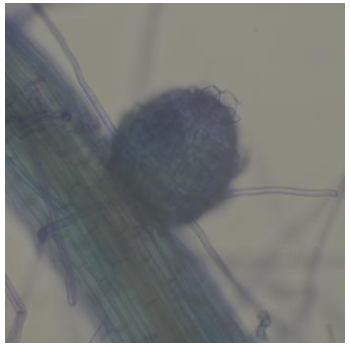

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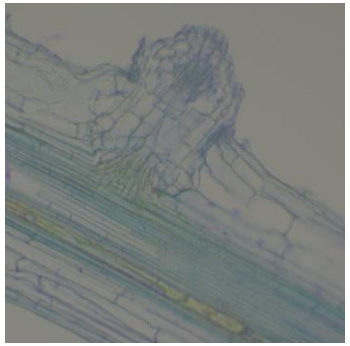

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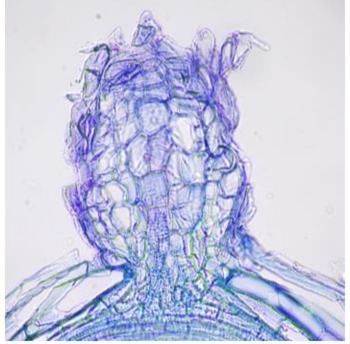
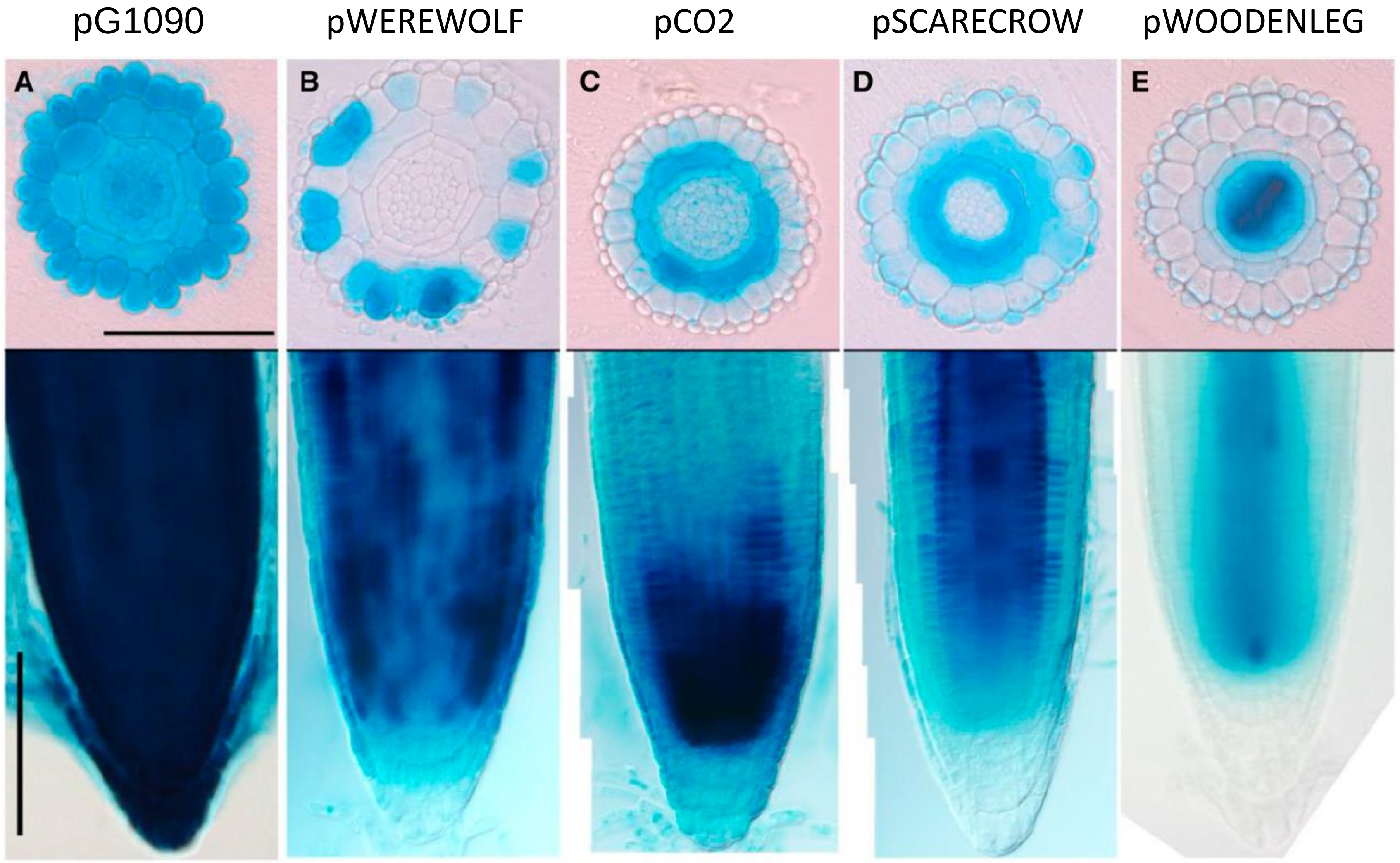


4. Spatiotemporal engineering: Cell-type specific promoters with STARTS

Root Cell Type-Specific Genes in Arabidopsis

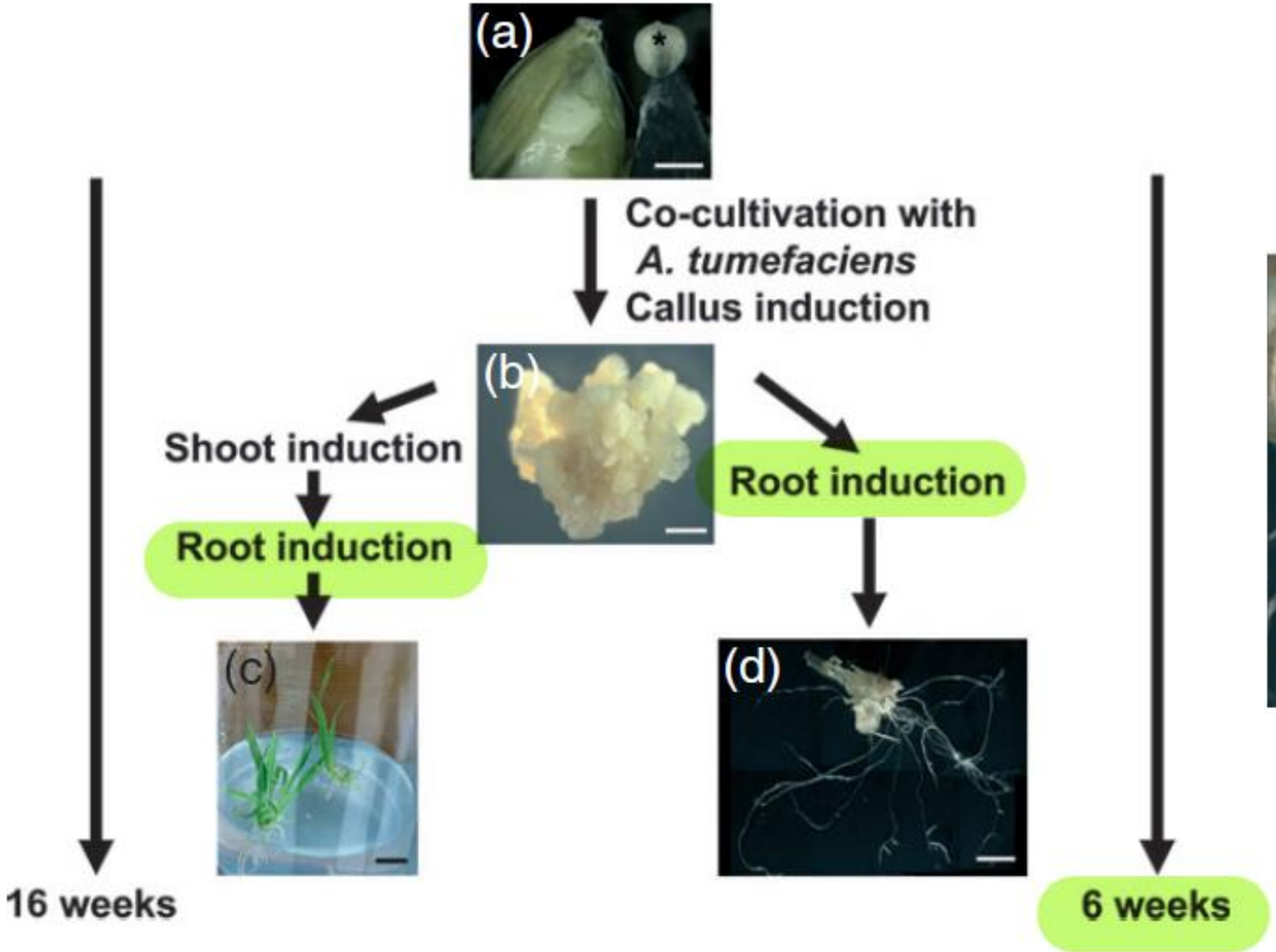
- Cell-type specific promoter
    - Epidermis: WEREWOLF (**WER**)
    - Cortex: CORTEX2 (**CO2**)
    - Endodermis: SCARECROW (**SCR**)
    - Vascular bundle, shoot apical meristem: WOODENLEG/CRE1/AHK 4 (**WOL**)



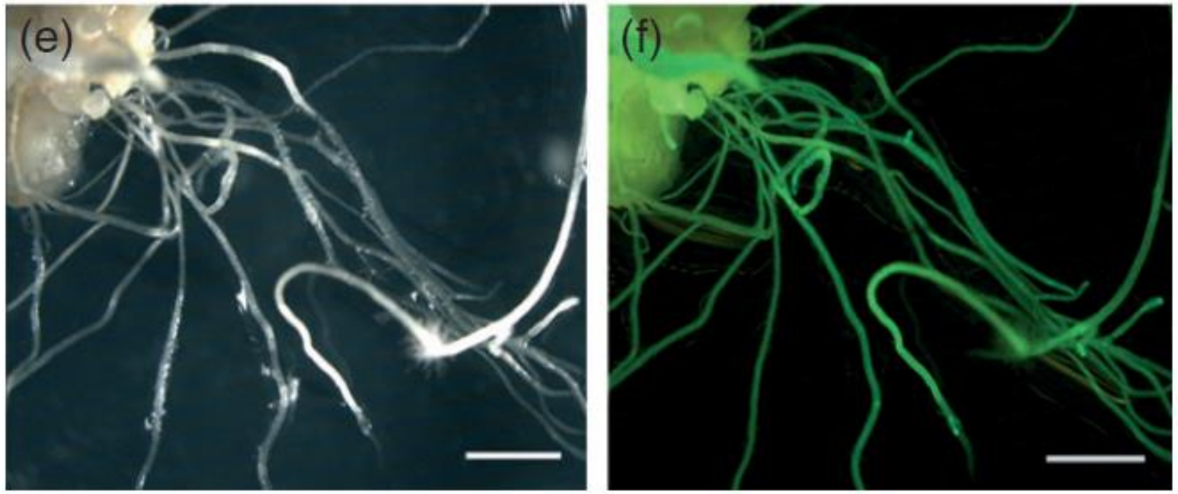
Siligato et al., 2016

STARTS – A stable root transformation system for rapid functional analyses in barley

Imani et al., 2011



- Overexpression of synthetic green fluorescent protein (sGFP) in barley roots by STARTS.



Summary

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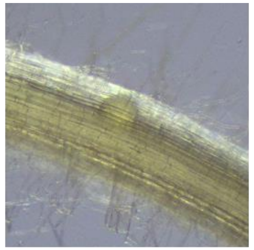

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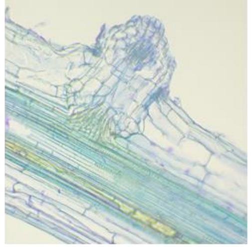

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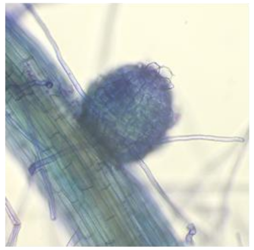

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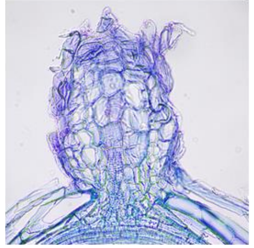

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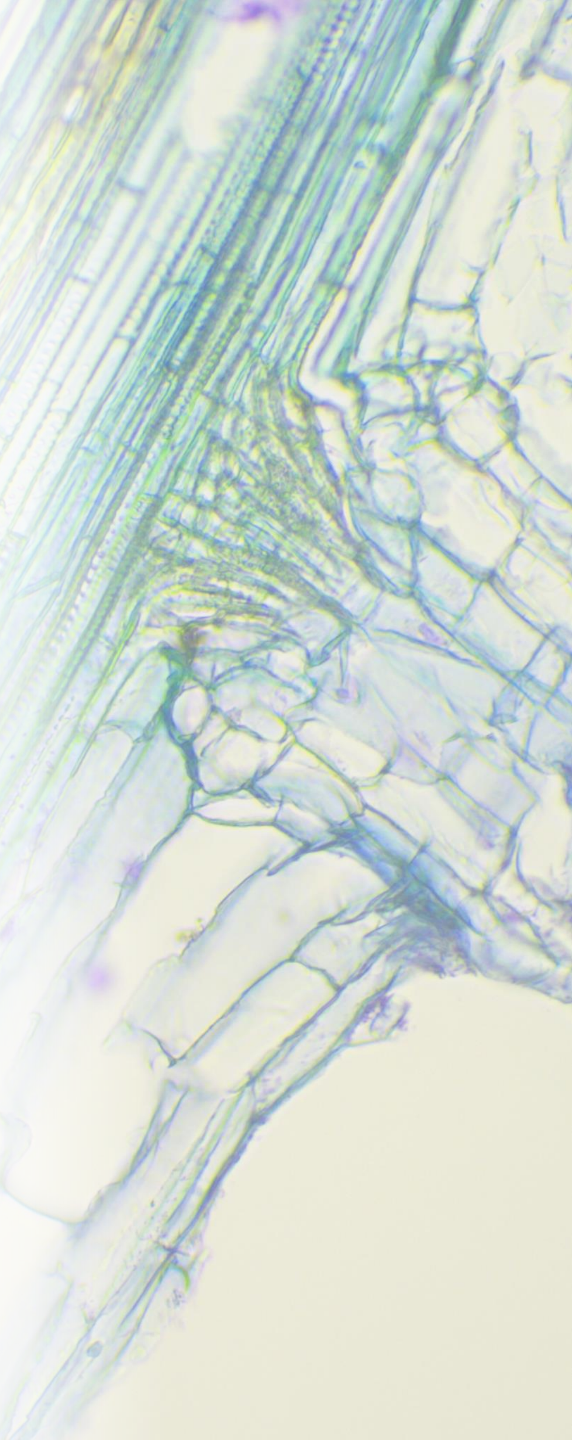

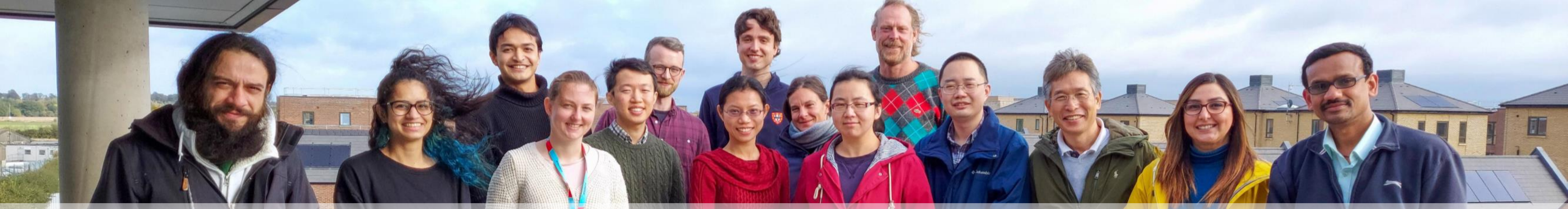

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