

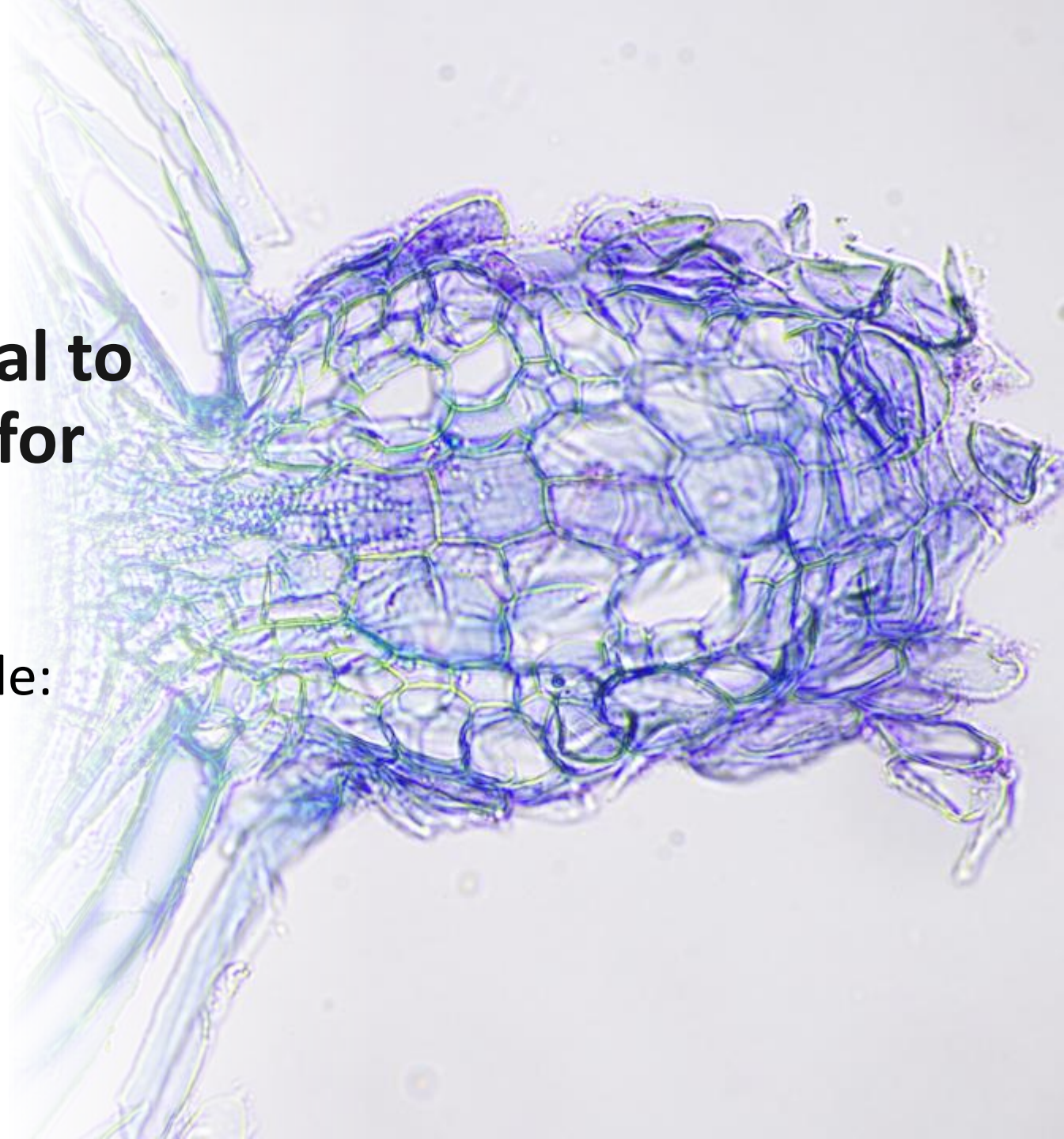
Can we switch from chemical to biological nitrogen fixation for sustainable food security?

From lateral root to functional nodule:
engineering organogenesis in barley

Min-Yao Jhu

2023 Cambridge Global Food Security Symposium

Oldroyd Group



The Vision of ENSA

Engineering Nitrogen Symbiosis for Africa

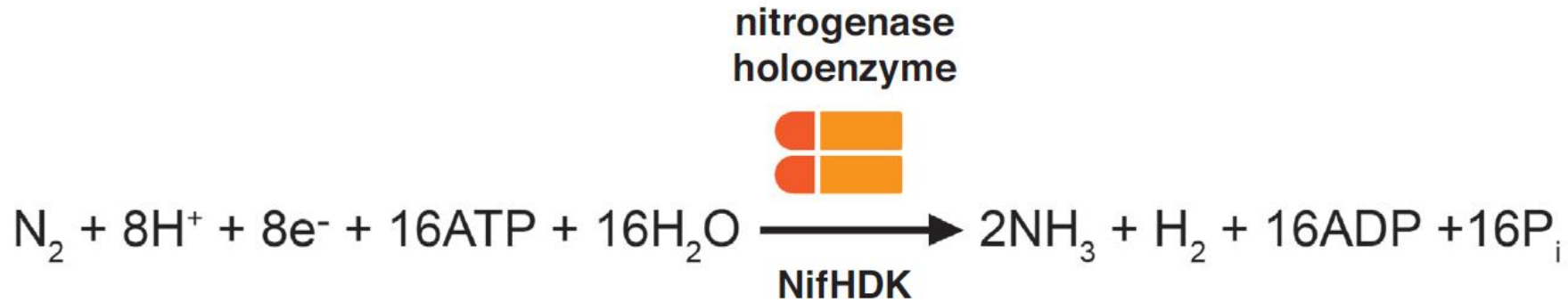
To Sustainably Increase Yields for Small-holder Farmers

- Through a detailed understanding of how plants associate with **beneficial microorganisms**, we aim to broaden their use in agriculture to facilitate sustainable productivity.
- Crop plant productivity is highly dependent on the availability of a **nitrogen source** and farmers generally provide this as **fertilizers**.

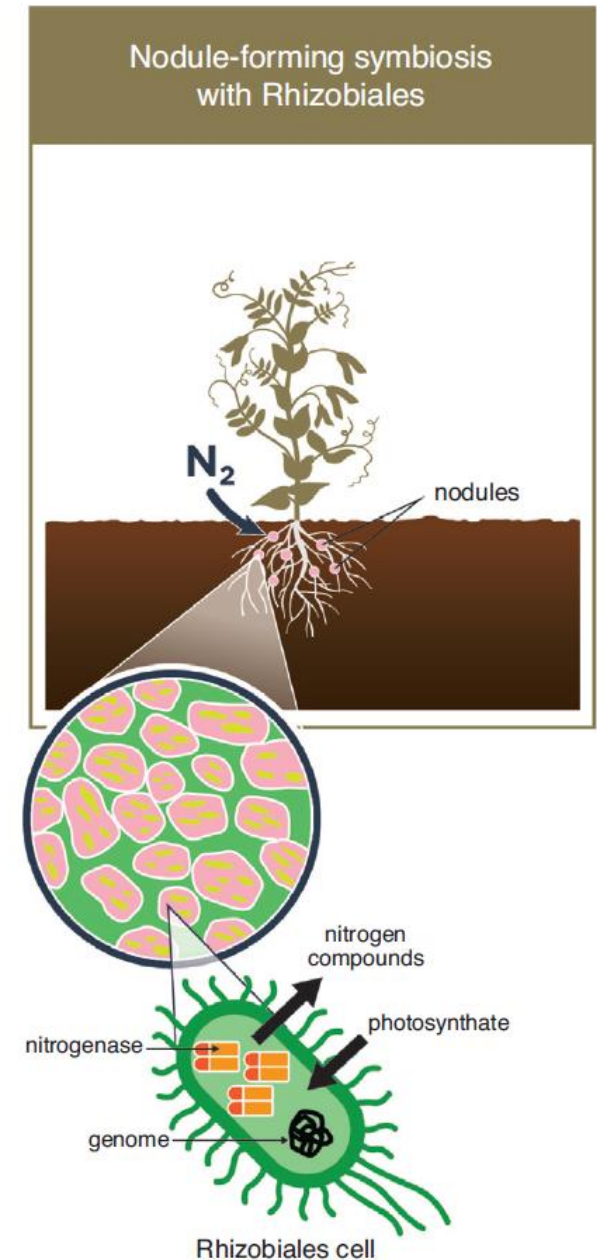


Biological nitrogen fixation

(a)

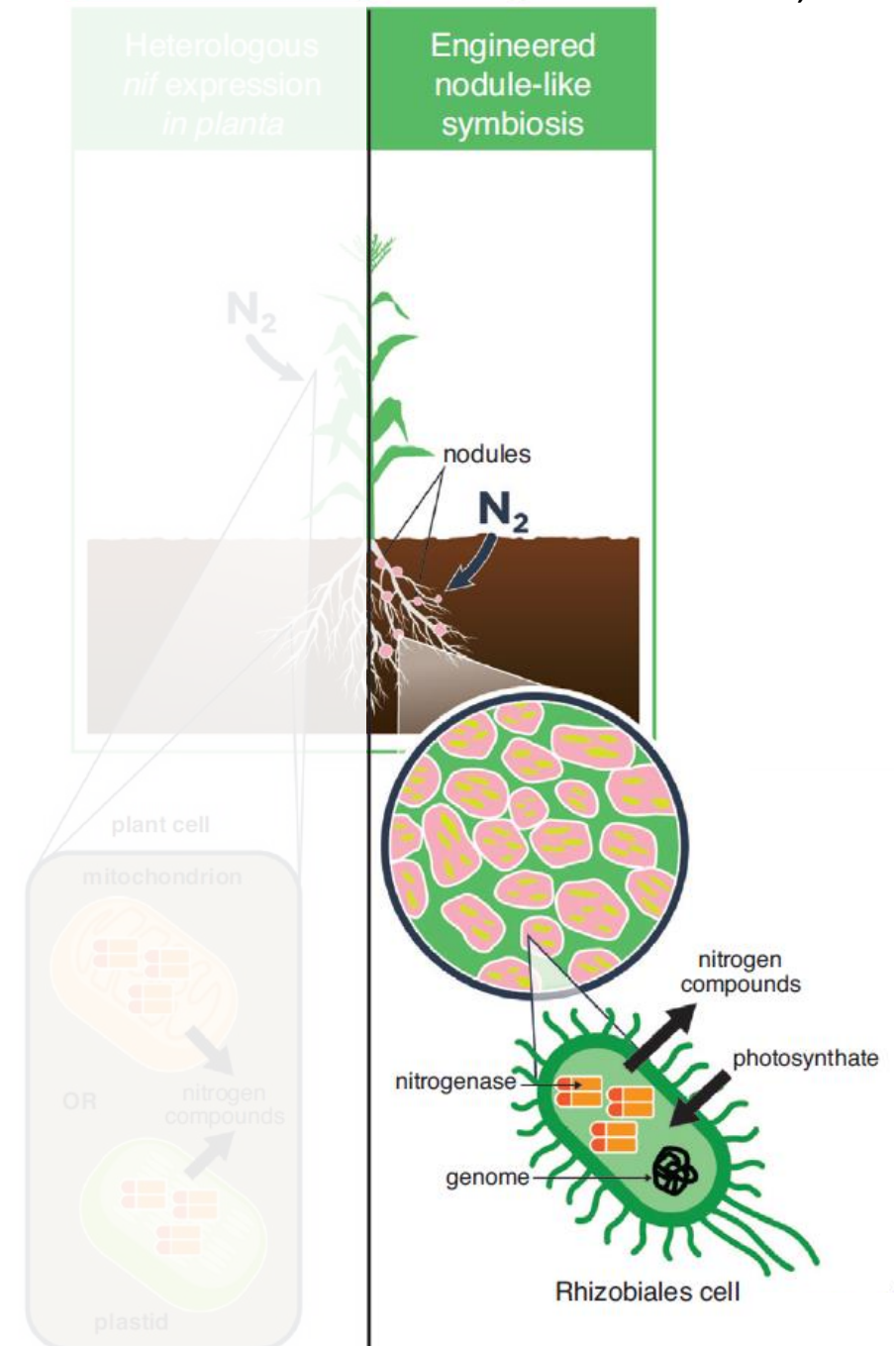
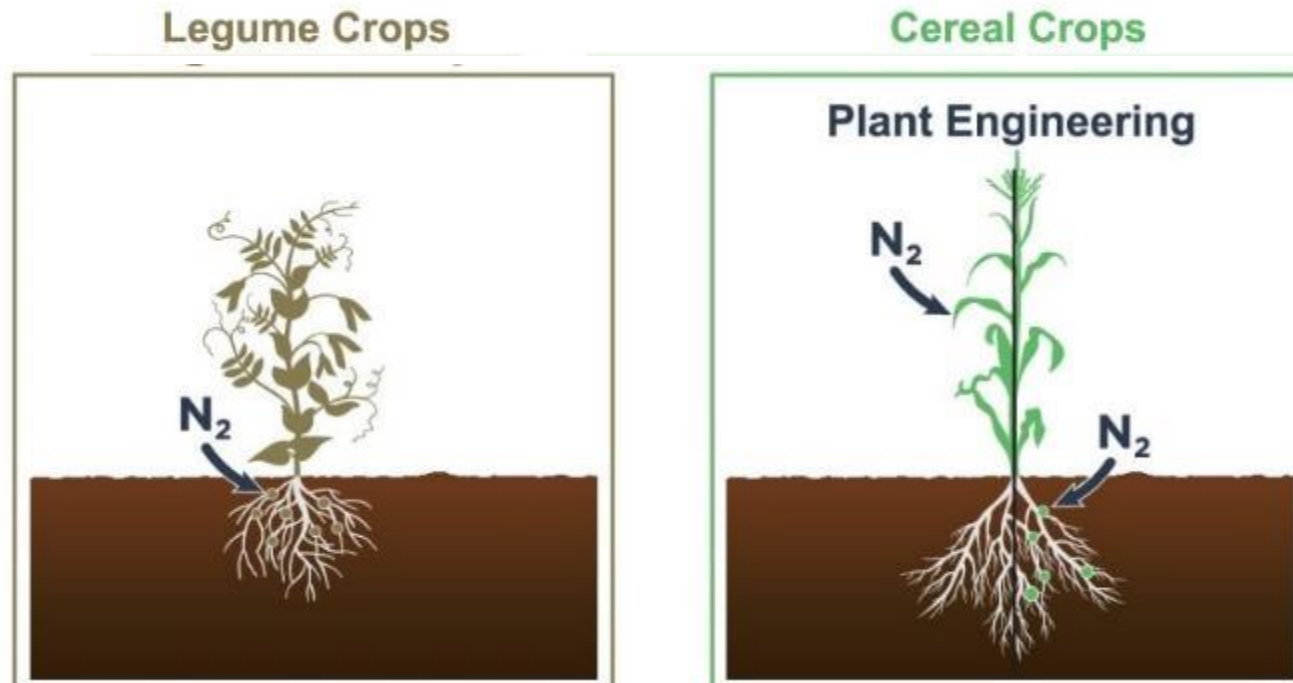


- **Nitrogen-fixing bacteria**
- **Nitrogenase:** convert di-nitrogen to ammonia, a reactive form of nitrogen then can be used in biological processes.
- Legumes form specialized organs on the roots, called **nodules**, that **house the nitrogen-fixing bacteria and provide the suitable oxygen-regulated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



Engineering a Solution

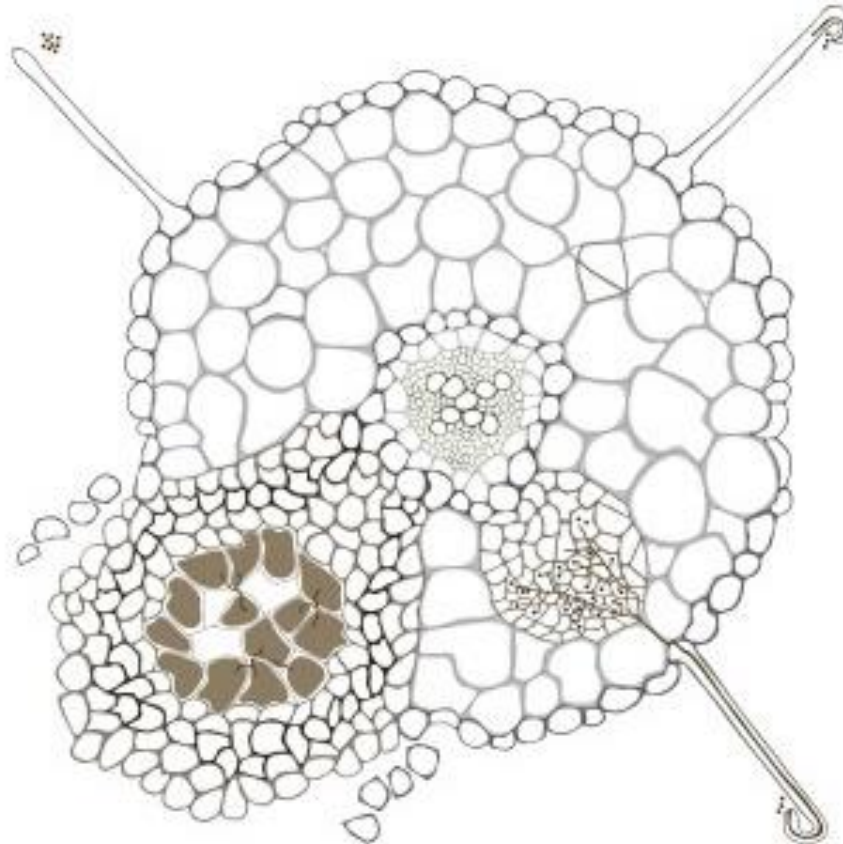
- ENSA: we are attempting to transfer **the capability of associating with nitrogen-fixing bacteria** from legumes to **cereals**.
- **Self-fertilizing cereals**: can support their own productivity without the need to use nitrogenous fertilizers.



The Four Components to Engineering Symbiosis

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

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

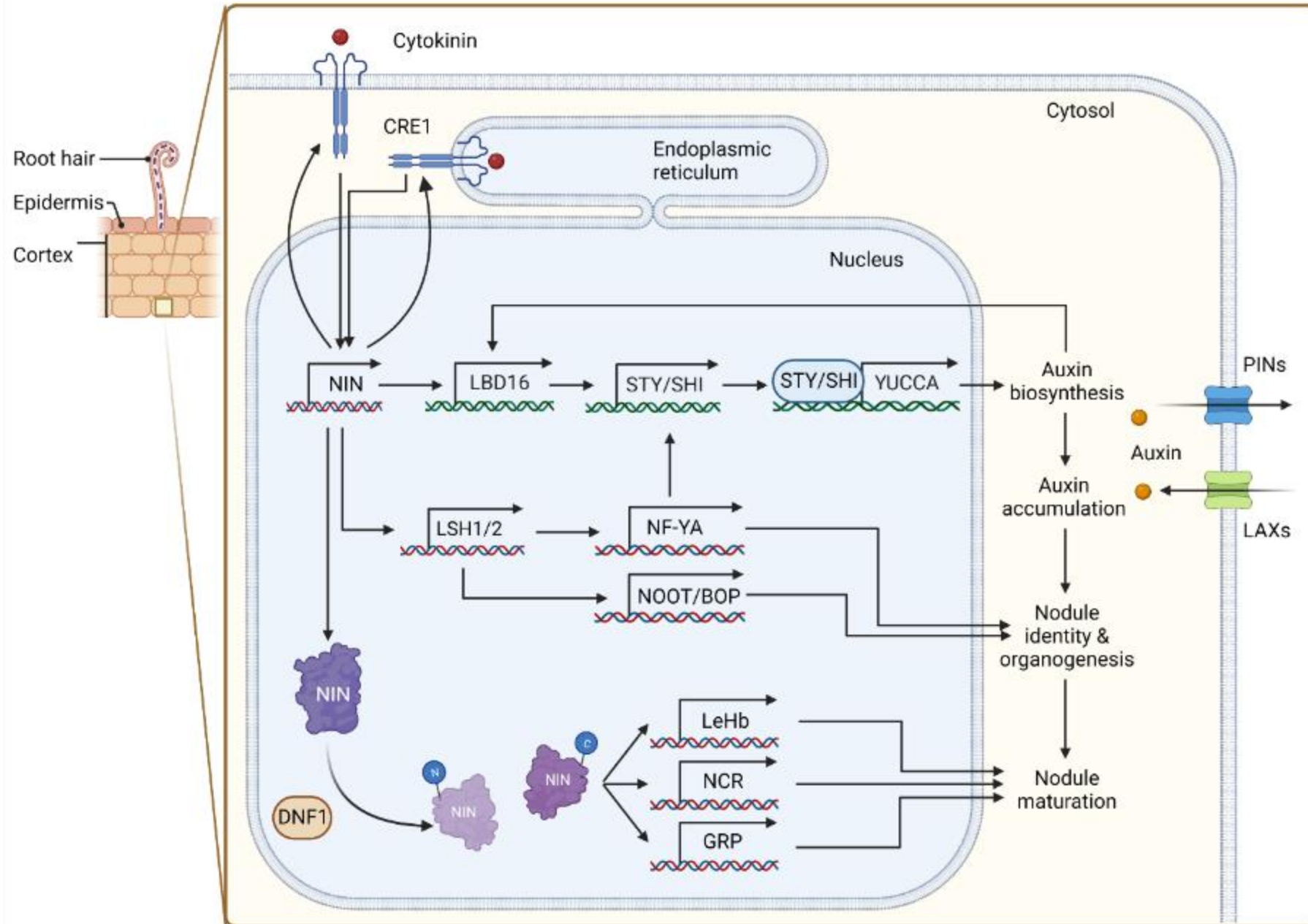


4. **Mature nodule:** Engineer the
Appropriate Environment for
Nitrogen-Fixation within the Nodule

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



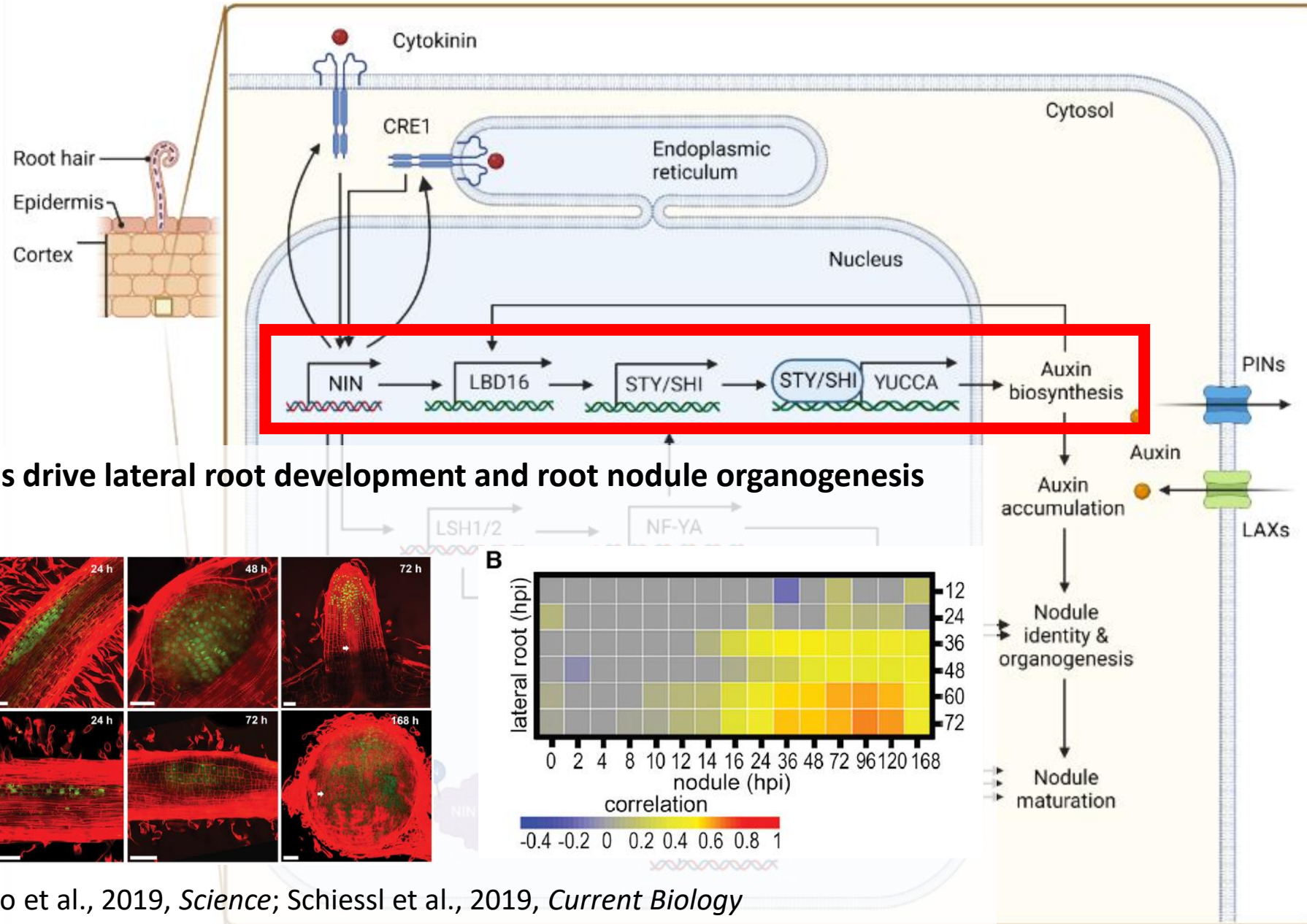
Mechanisms of nodule organogenesis



Mechanisms of nodule organogenesis



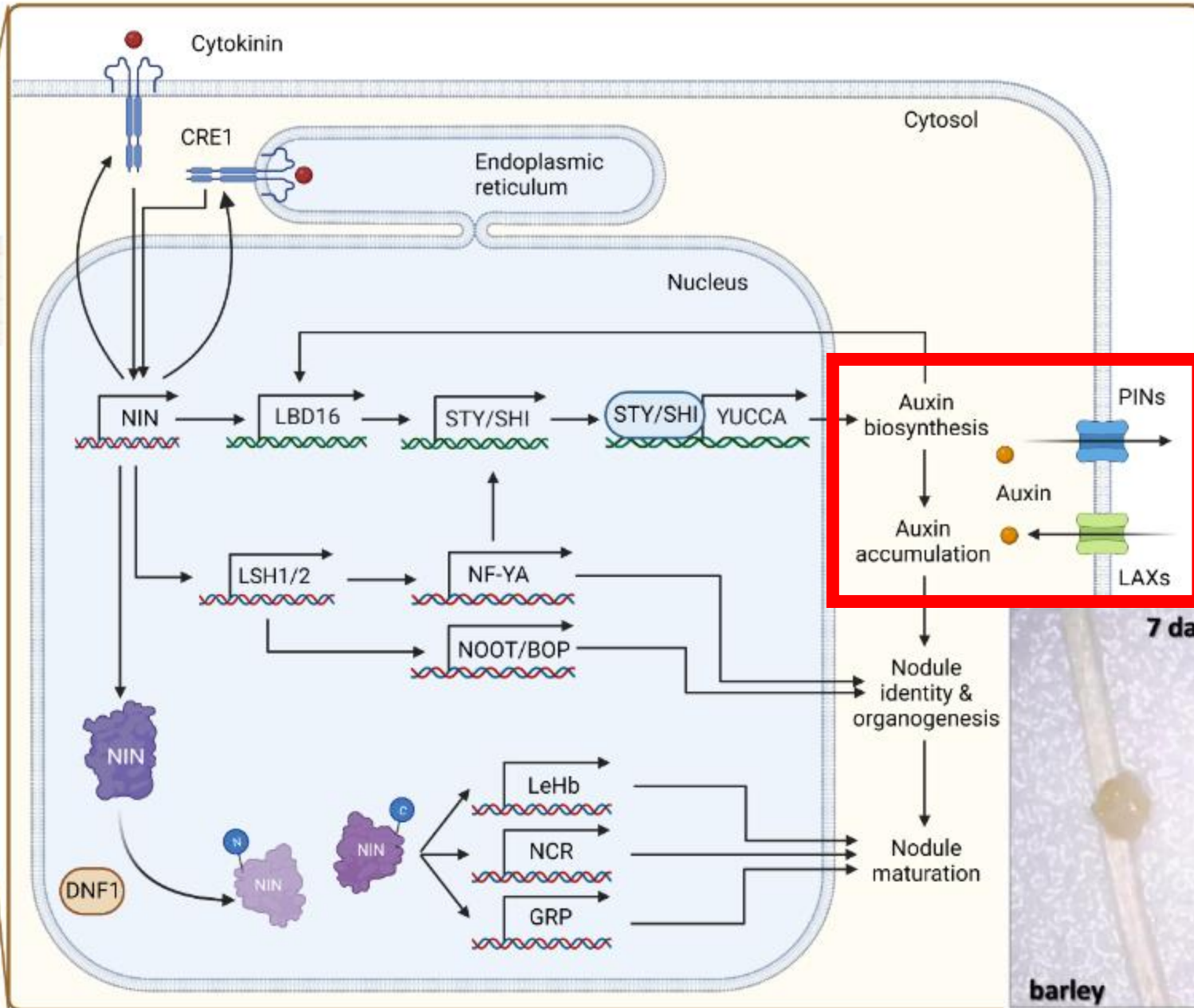
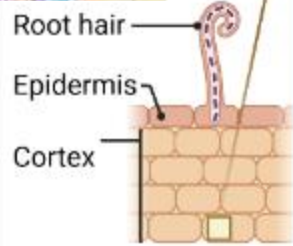
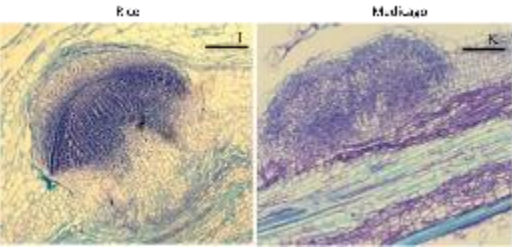
Katharina Schiessl



Soyano et al., 2019, *Science*; Schiessl et al., 2019, *Current Biology*

Jhu & Oldroyd, 2023, *PLOS Biology*

Mechanisms of nodule organogenesis



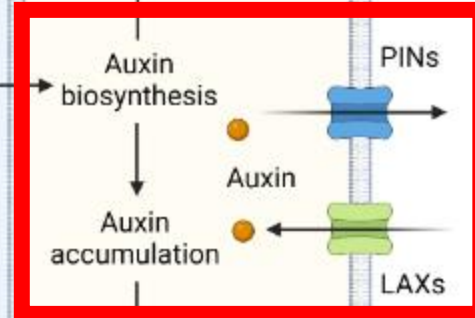
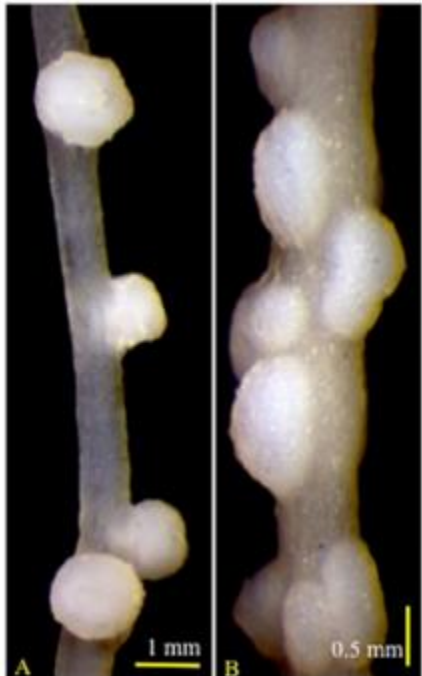
Auxin-Induced Nodule-Like Structures



Mandana Miri

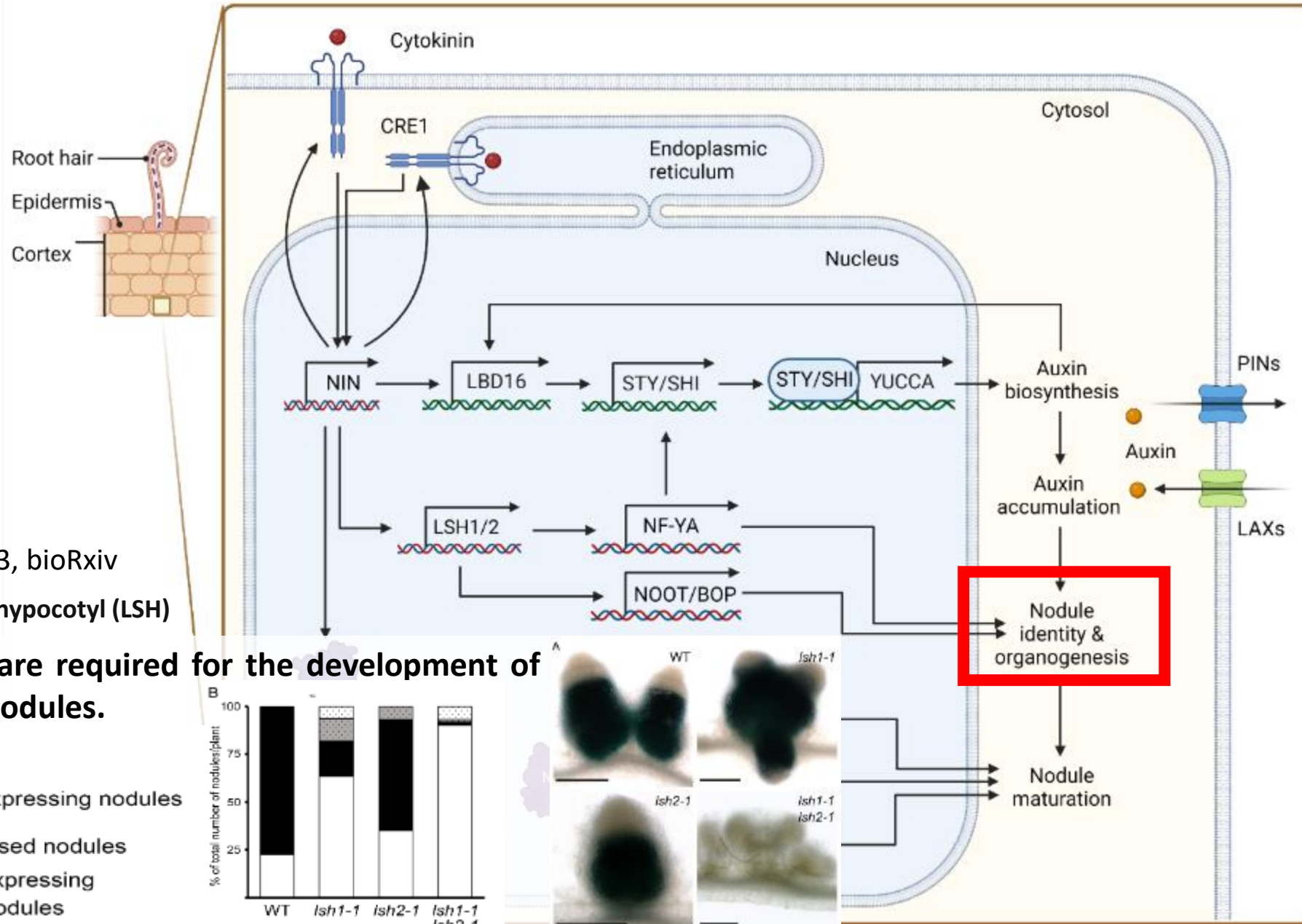
Hiltenbrand et al., 2016

Rice Medicago



barley

Mechanisms of nodule organogenesis



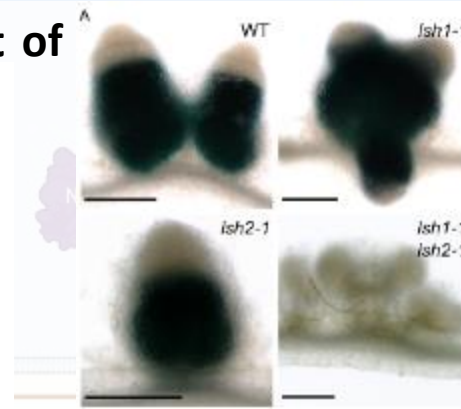
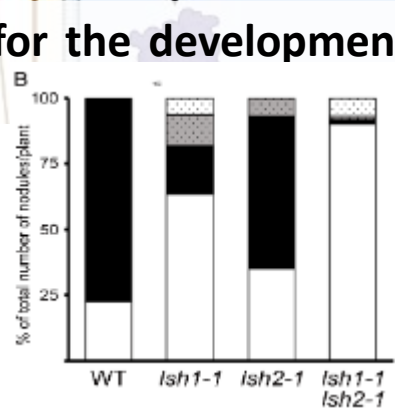
Katharina Schiessl

Schiessl et al., 2023, bioRxiv

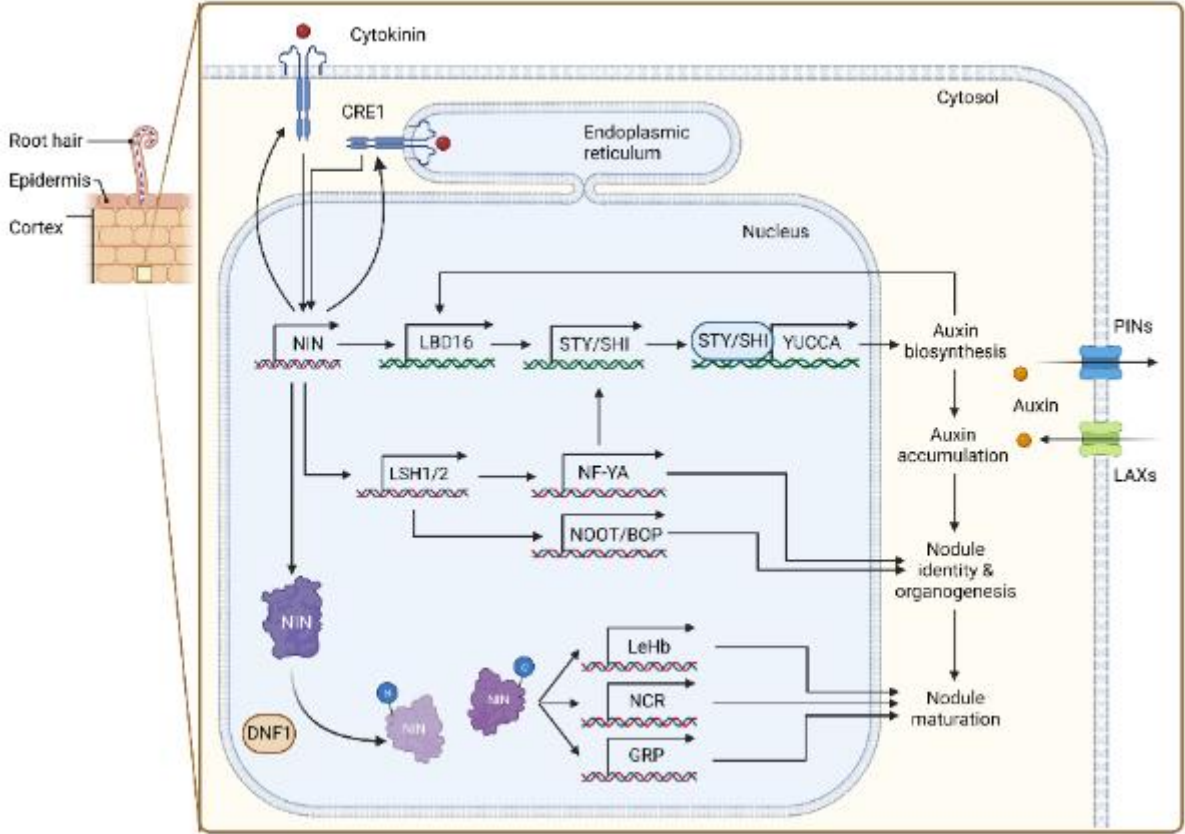
Light sensitive short hypocotyl (LSH)

LSH1 and LSH2 are required for the development of nitrogen fixing nodules.

- white nodules
- blue, *pnifH::GUS* expressing nodules
- ▨ white multilobed/fused nodules
- ▩ blue, *pnifH::GUS* expressing multilobed/fused nodules



From Discovery to Engineering



Test
• Evaluate the phenotypes

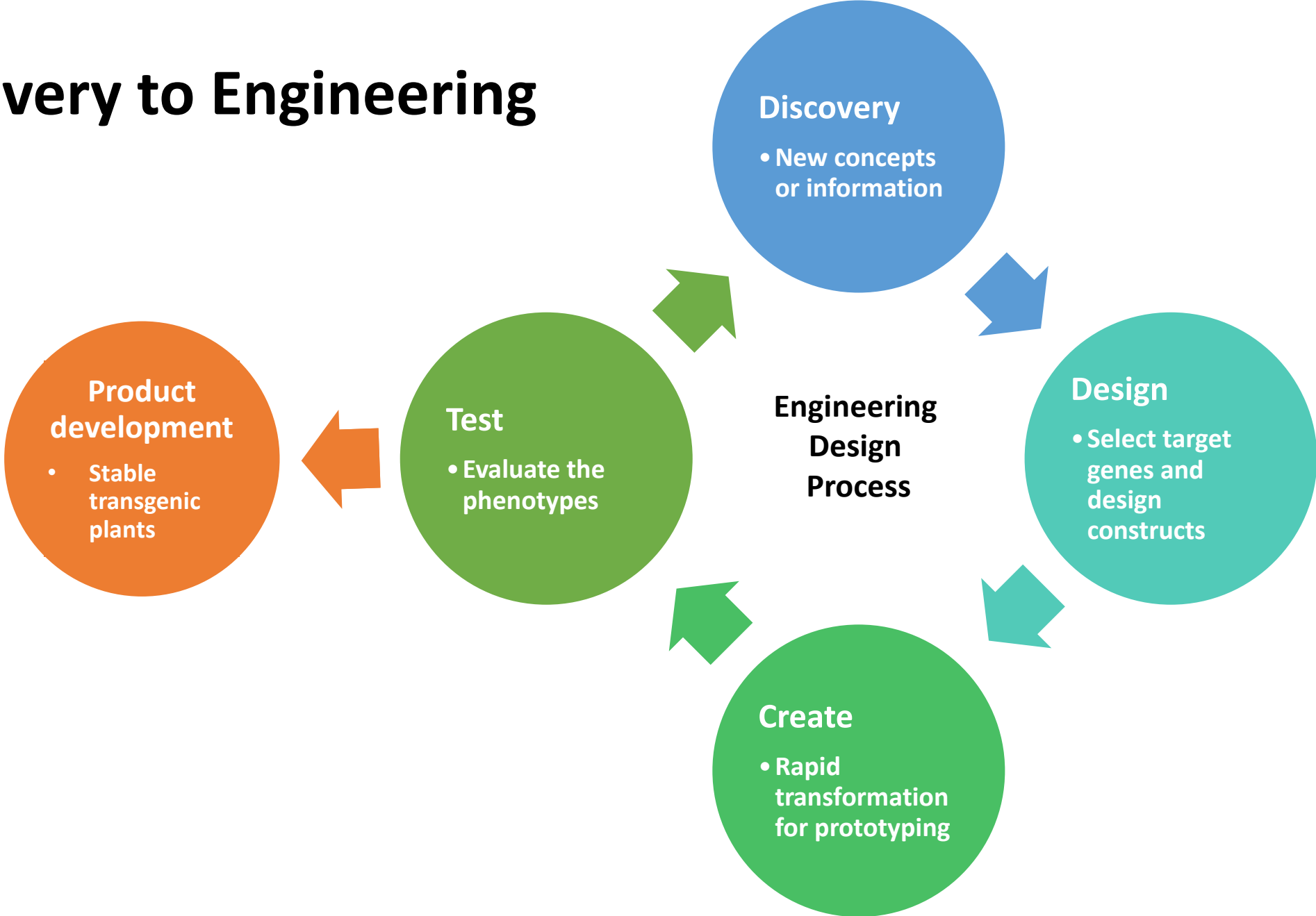
Discovery
• New concepts or information

Engineering Design Process

Design
• Select target genes and design constructs

Create
• Rapid transformation for prototyping

From Discovery to Engineering

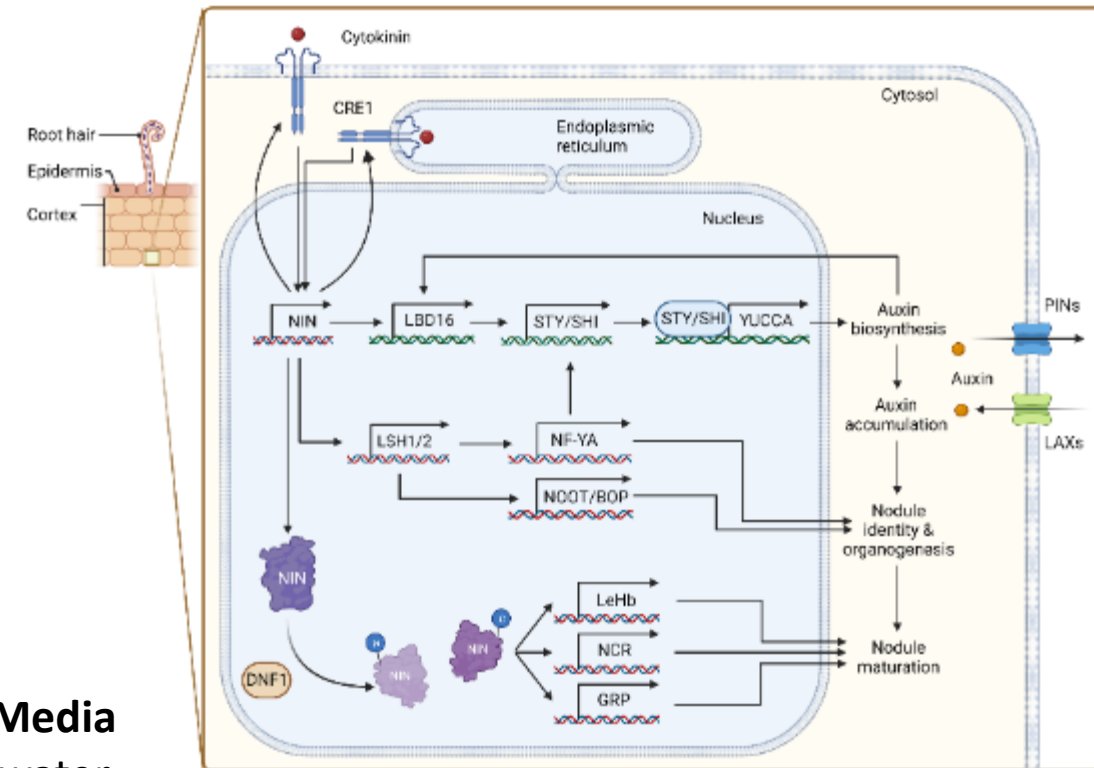
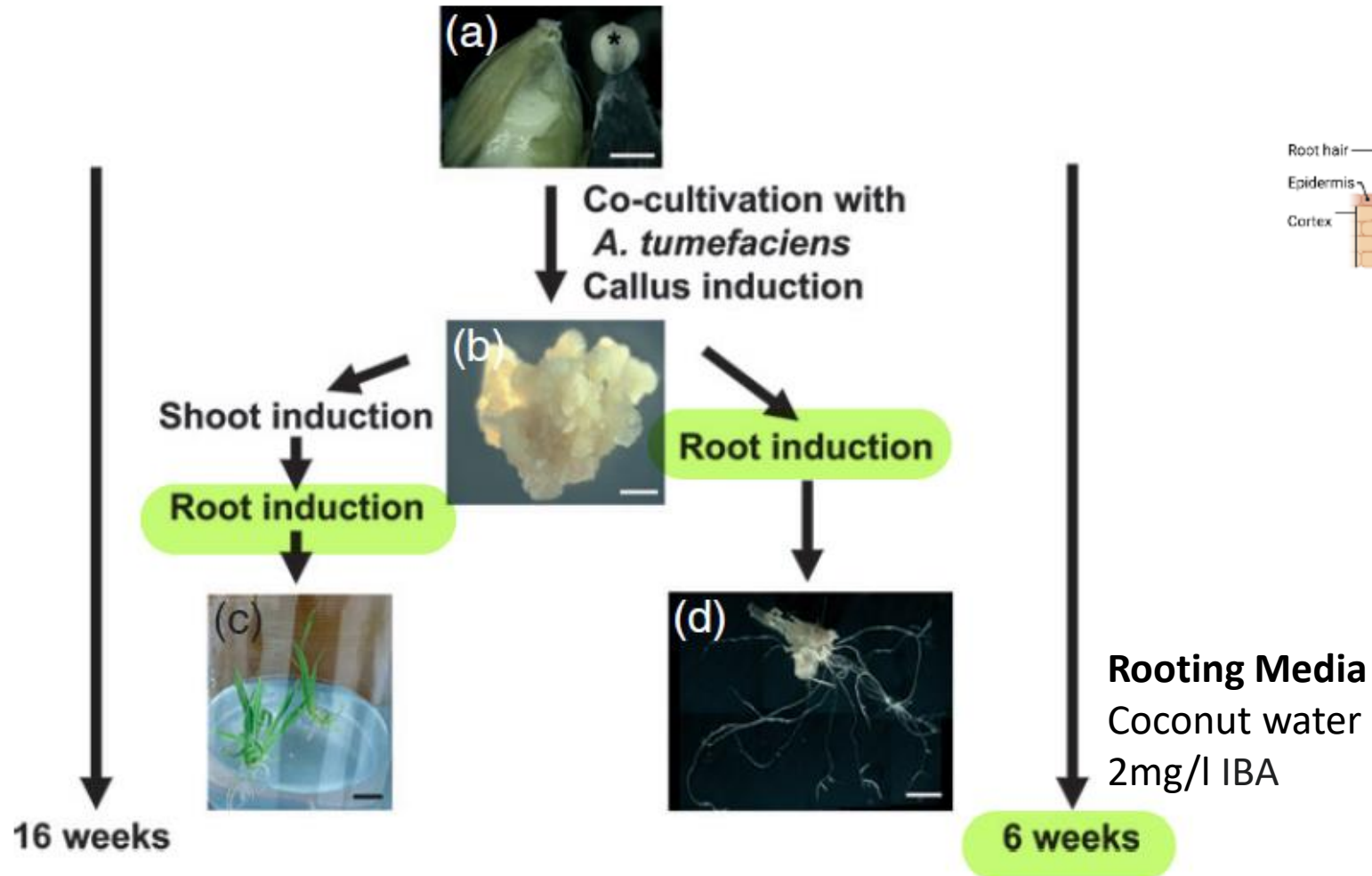


STARTS – A stable root transformation system for rapid functional analyses in barley

Create

- Rapid transformation for prototyping

Imani et al., 2011



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