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

From lateral root to functional nodule: engineering organogenesis in barley

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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

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.

\[
\text{N}_2 + 8\text{H}^+ + 8\text{e}^- + 16\text{ATP} + 16\text{H}_2\text{O} \xrightarrow{\text{NifHDK}} 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{P}_i
\]
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

3. Nodule primordia: Engineer Nodule Organogenesis

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

Lin et al., 2020
Mechanisms of nodule organogenesis

Jhu & Oldroyd, 2023, PLOS Biology
Mechanisms of nodule organogenesis

Shared genes drive lateral root development and root nodule organogenesis

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

Jhu & Oldroyd, 2023, PLOS Biology
Mechanisms of nodule organogenesis

Hiltenbrand et al., 2016

Rice Medicago

Auxin-Induced Nodule-Like Structures

Mandana Miri
Mechanisms of nodule organogenesis

Schiessl et al., 2023, bioRxiv

Light sensitive short hypocotyl (LSH)

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

Jhu & Oldroyd, 2023, PLOS Biology
From Discovery to Engineering

Discovery
• New concepts or information

Design
• Select target genes and design constructs

Engineering Design Process

Test
• Evaluate the phenotypes

Create
• Rapid transformation for prototyping
From Discovery to Engineering

**Discovery**
- New concepts or information

**Design**
- Select target genes and design constructs

**Engineering Design Process**

**Create**
- Rapid transformation for prototyping

**Product development**
- Stable transgenic plants

**Test**
- Evaluate the phenotypes
STARTS – A stable root transformation system for rapid functional analyses in barley

Imani et al., 2011

Create

• Rapid transformation for prototyping

Rooting Media
Coconut water
2mg/l IBA

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