Towards synthetic nitrogen-fixing symbioses in grasses

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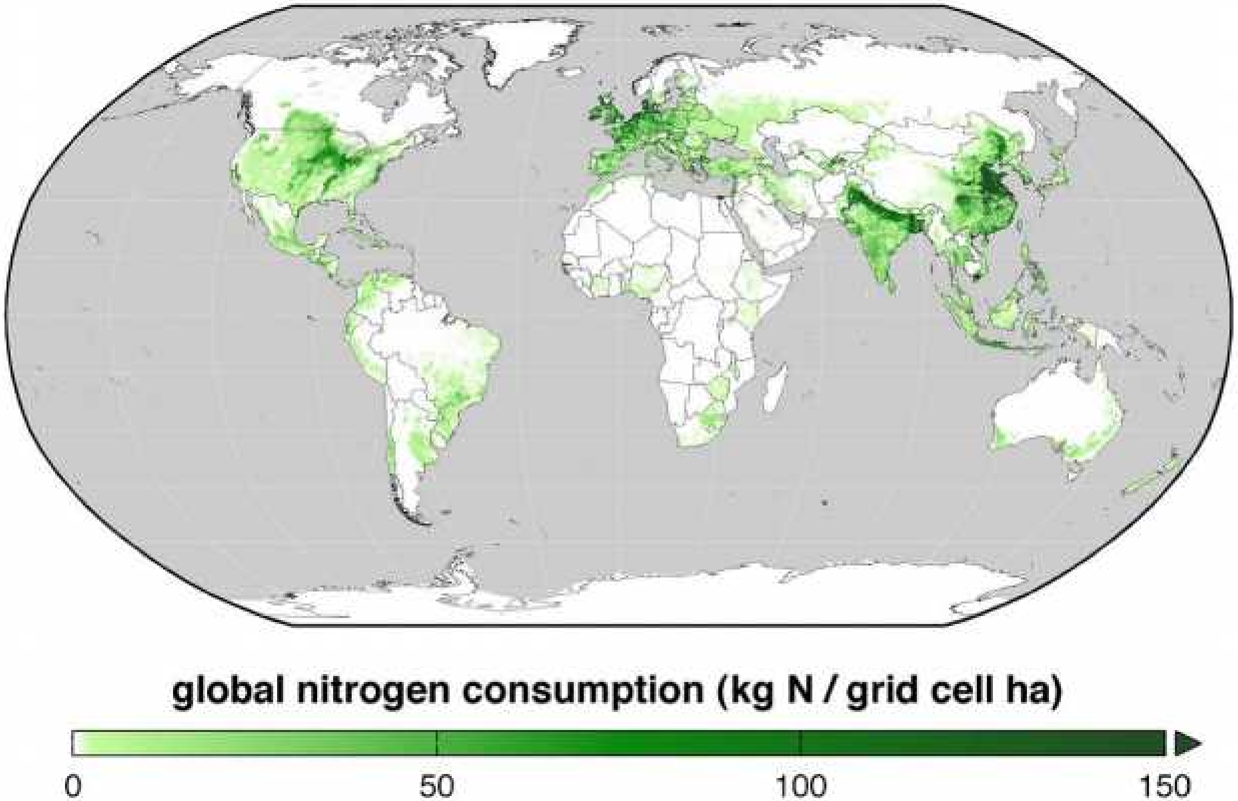
The Nitrogen Fertiliser Problem



Mueller et al 2012, *Nature* **490**: 254-257

Problems: Over-use in many regions, under-use in others

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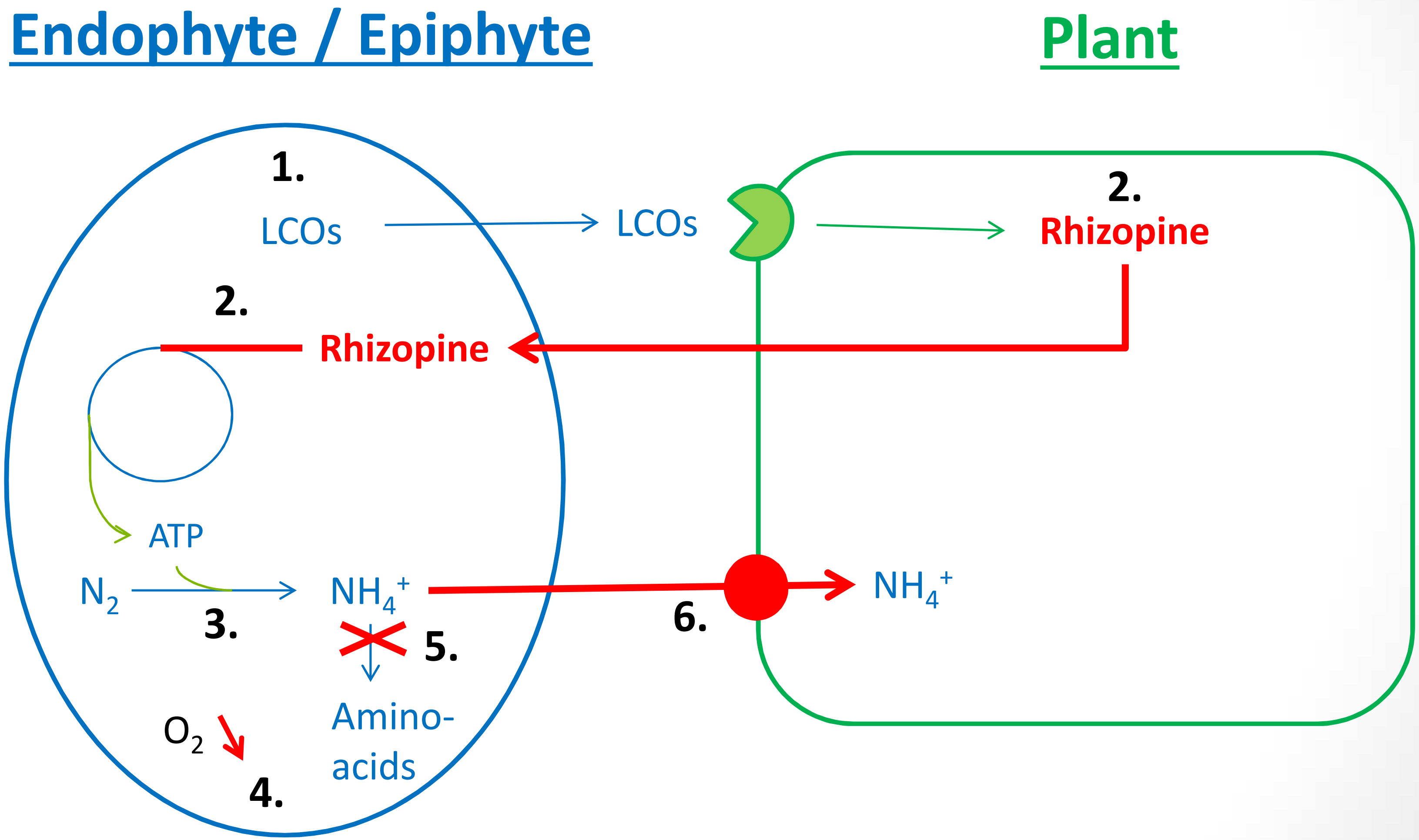

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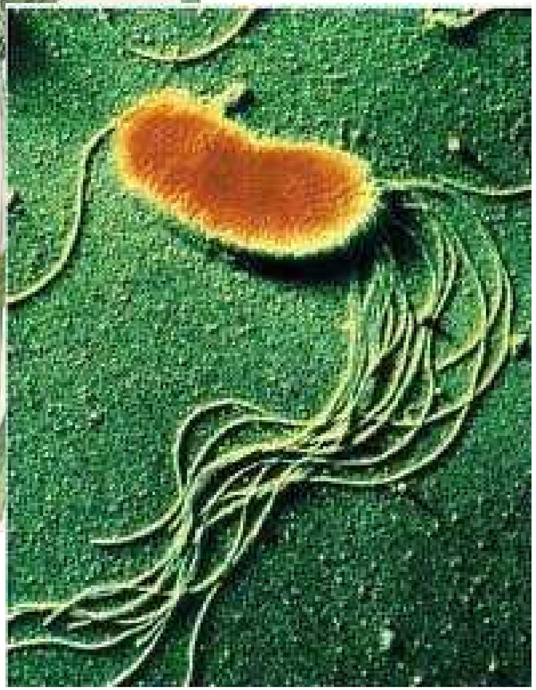
Model and crop systems for modifying associative interactions

Setaria viridis
Barley
Maize



Pseudomonas florescens Pf5*:* epiphyte

Rhizobium sp. IRBG74*:* endophyte

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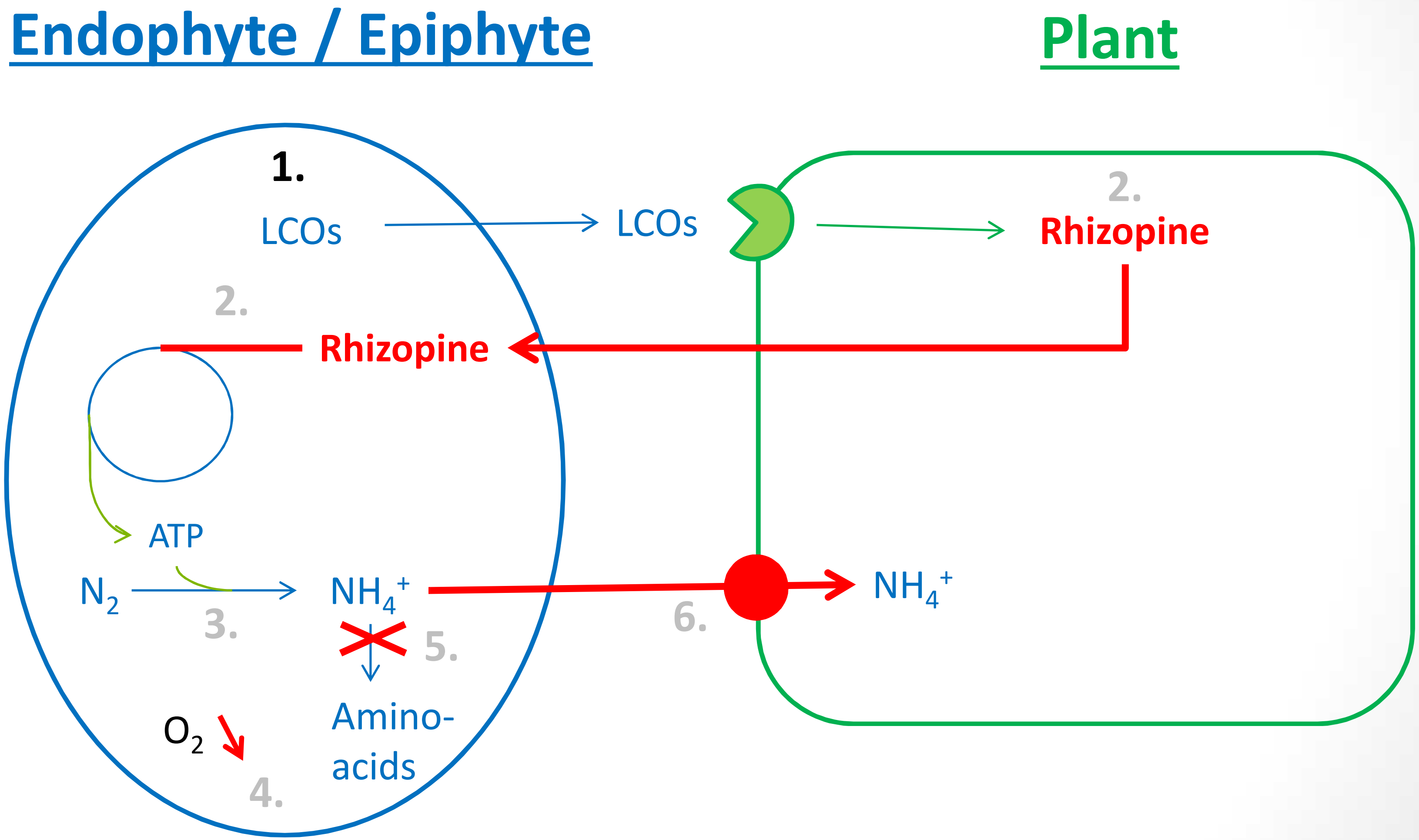

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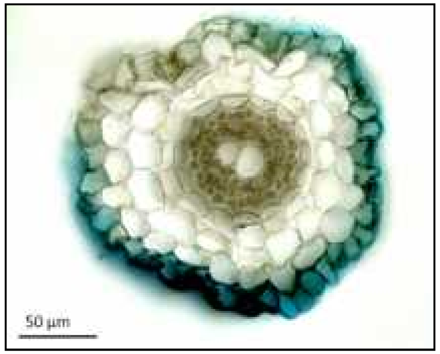
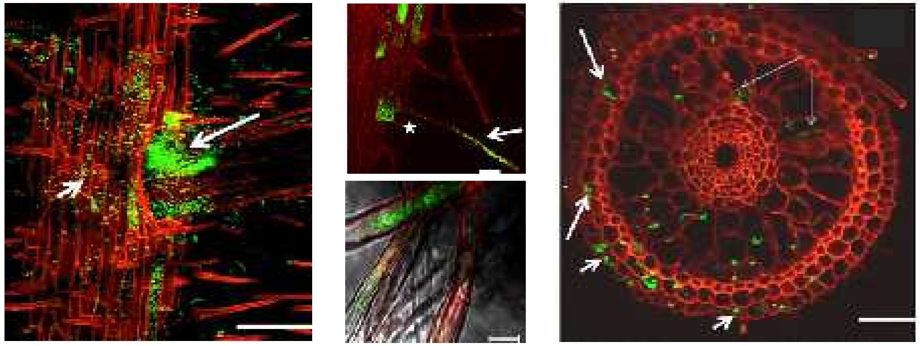
1. Colonization of various cereals by *Rhizobium* sp. IRBG74

- *Rhizobium* sp. IRBG74 is an efficient colonizer of Setaria and rice

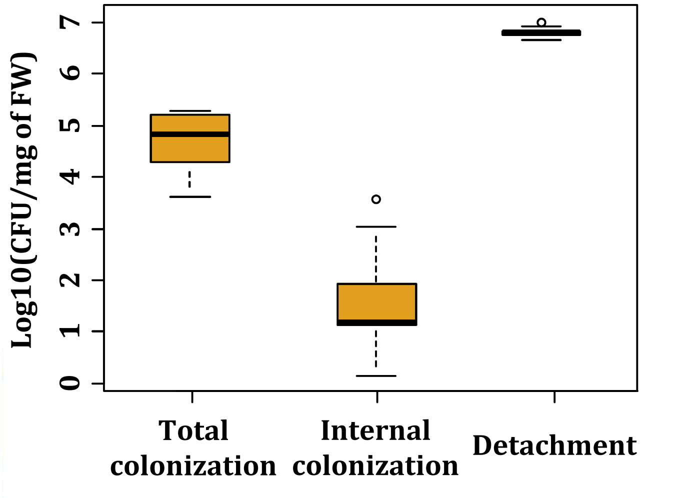


Setaria viridis



Oryza sativa

- *Rhizobium* sp. IRBG74 is also an efficient colonizer of *Hordeum vulgare*



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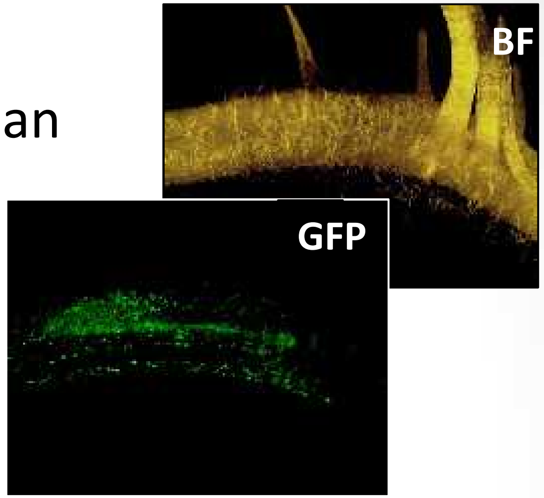
Colonization of *Zea mays* by *Rhizobium* sp. IRBG74

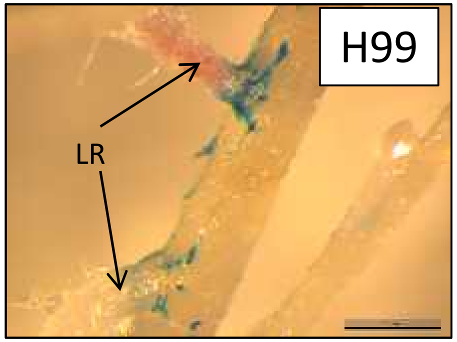


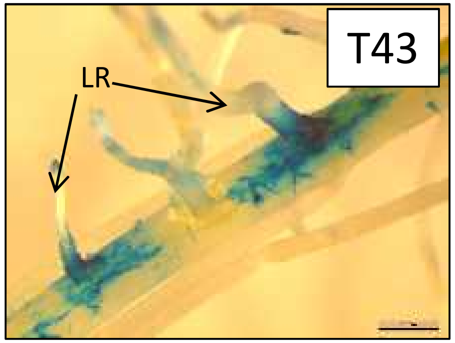
- *Rhizobium* sp. IRBG74 does not seem to be an efficient colonizer of B73 *Zea mays*

<u>But</u>

- B73 *Zea mays* can be colonized by several nitrogen-fixing bacteria

- Other *Zea mays* germplasm (H99 and T43) can be colonized by *Rhizobium* sp. IRBG74





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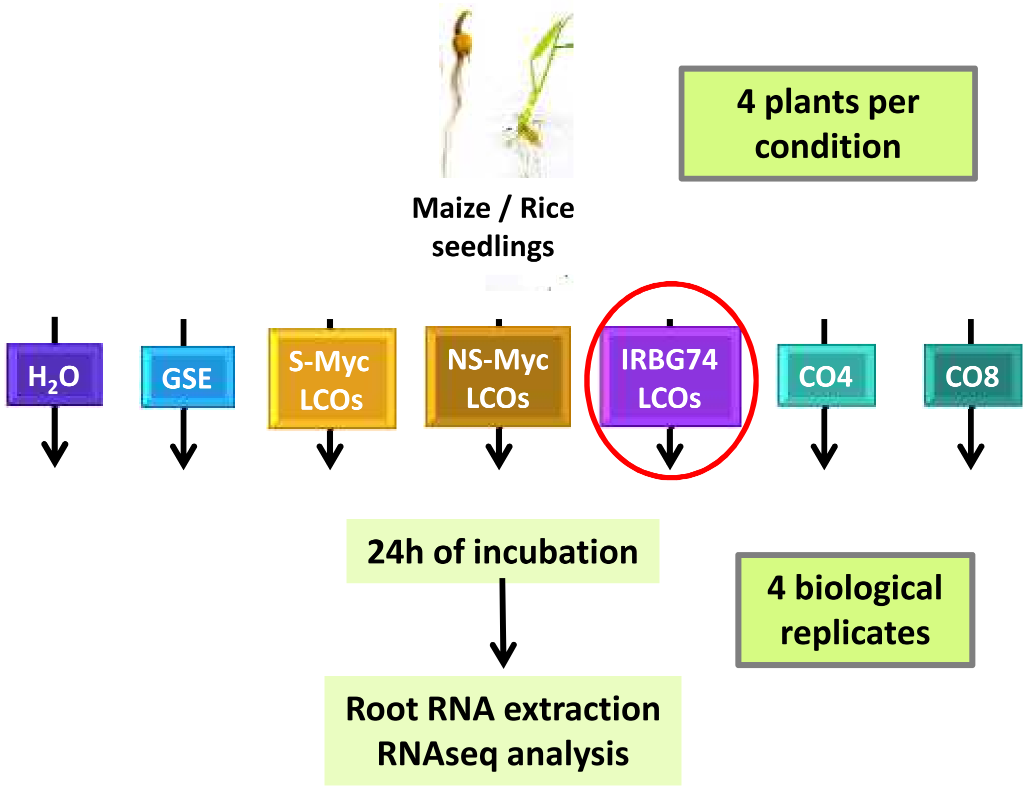
Identification of genes regulated in response to *Rhizobium* sp. IRBG74 LCOs

- Incubation of rice and maize (B73) seedlings with diffusible microbial signals, including those from IRBG74



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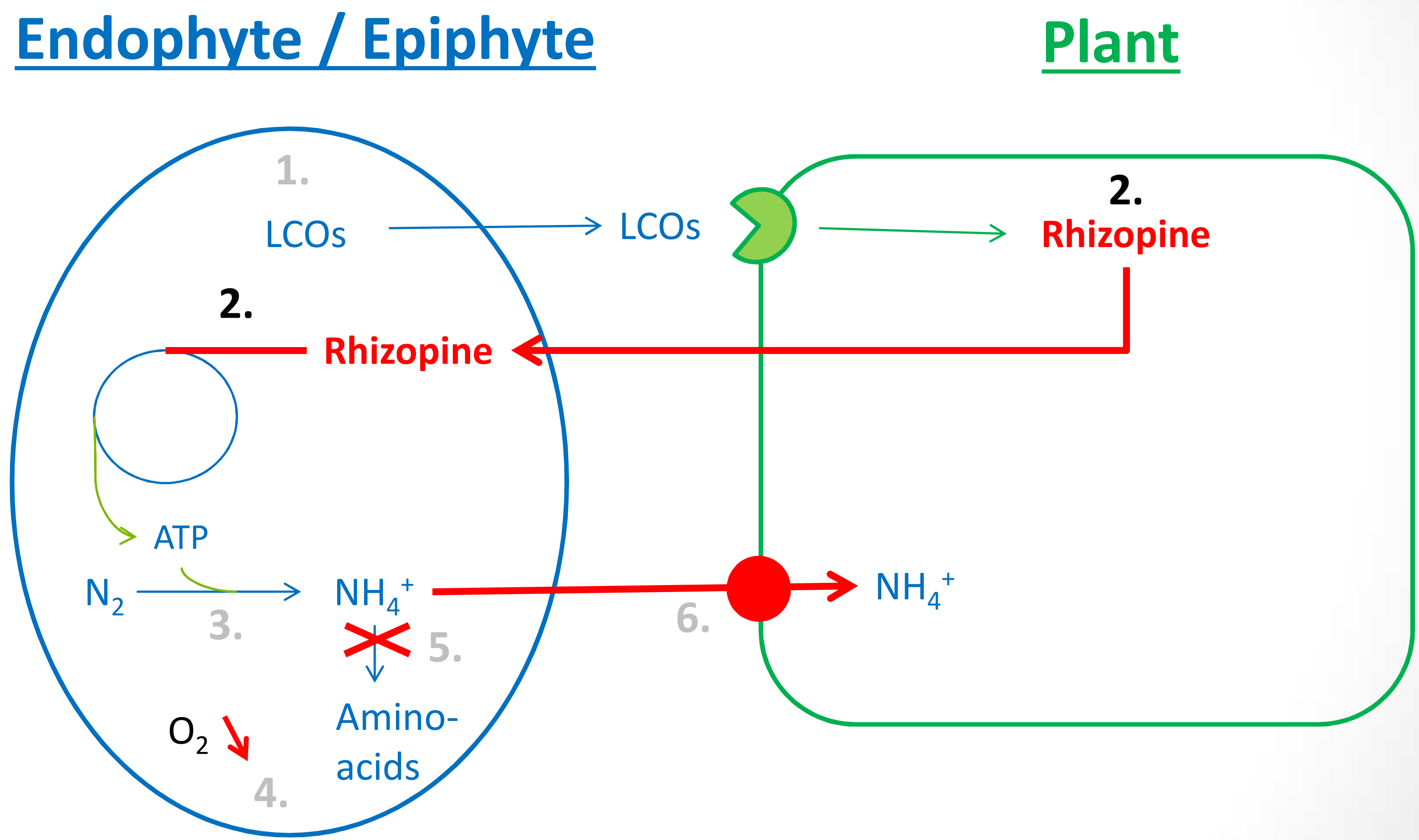

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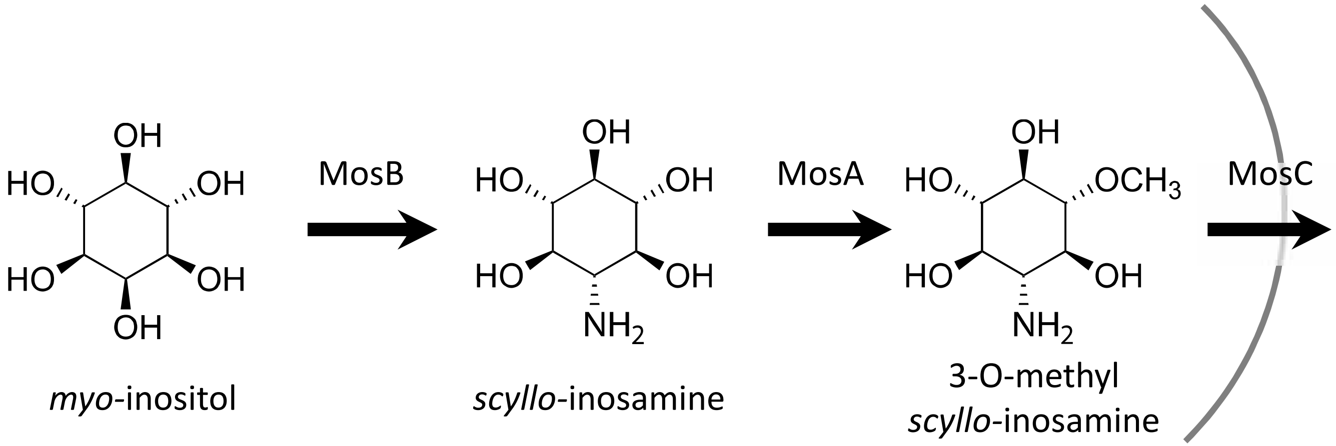
2. Rhizopine synthesis

- Literature pathway of rhizopine synthesis



myo-inositol

scyllo-inosamine

3-O-methyl *scyllo*-inosamine

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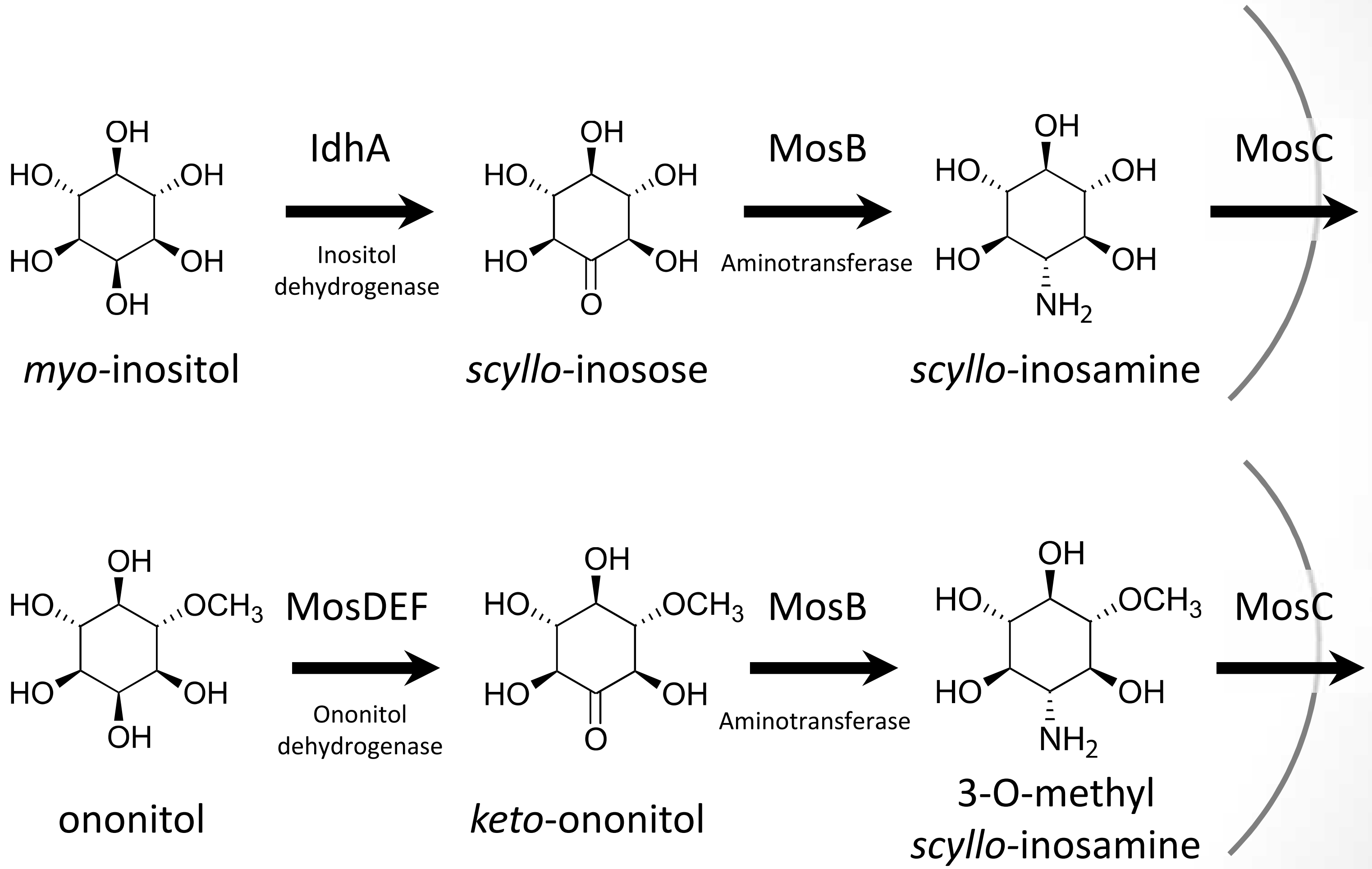
Proposed novel pathway of rhizopine synthesis



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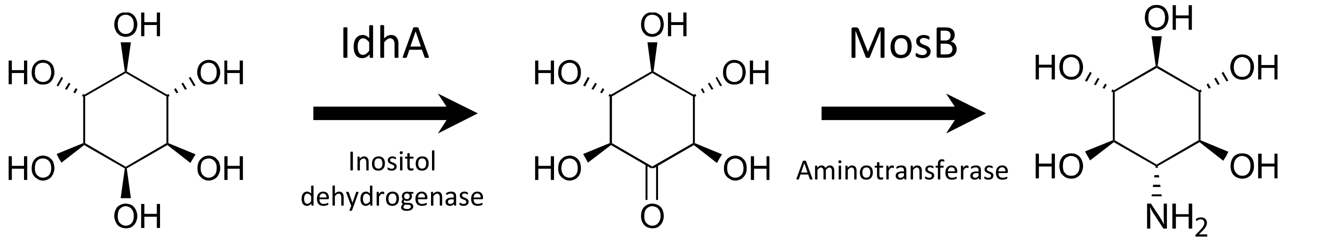
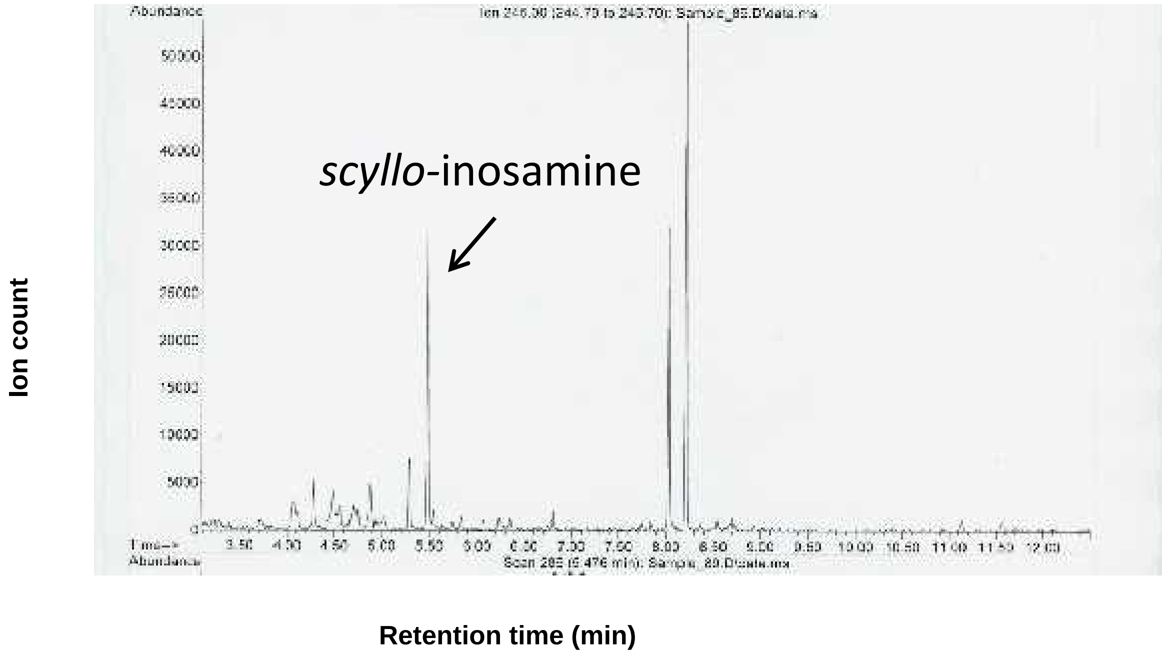
Tobacco transient transformation



myo-inositol *scyllo*-inosose *scyllo*-inosamine



Oldroyd Lab. Ponraj Paramasivan

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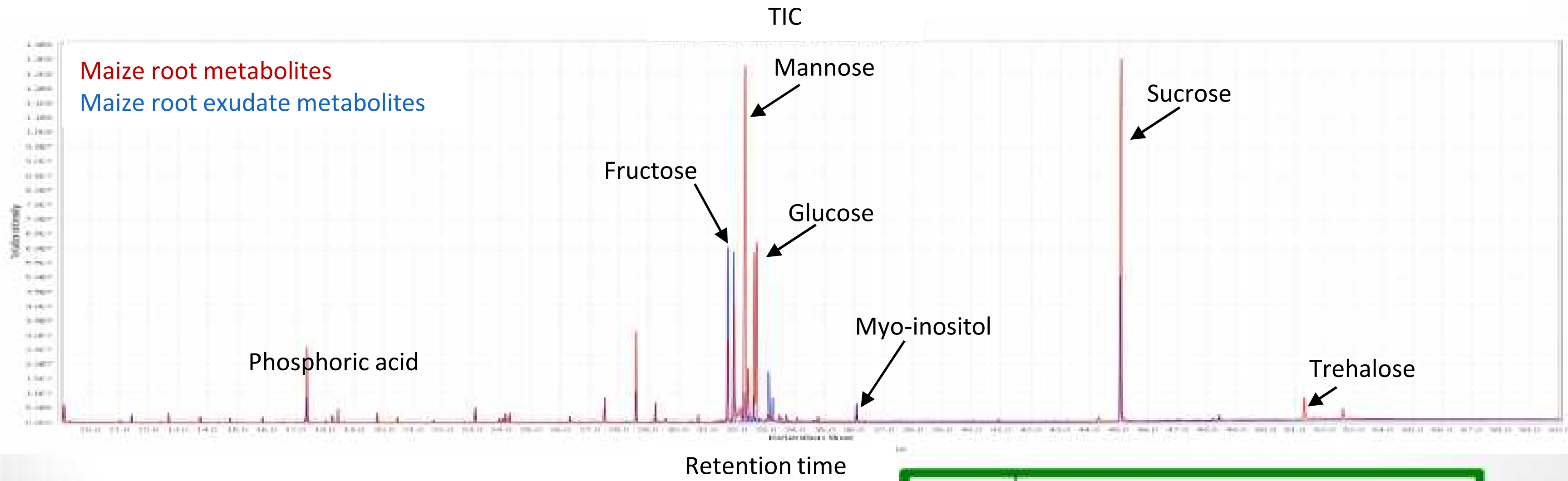
Optimize carbon supply from roots to bacteria

- Identify and quantify metabolites in *S. viridis* and maize roots and root exudates by GC-MS

- Among the metabolites identified were sugars, organic and amino acids, sugar alcohols, etc.



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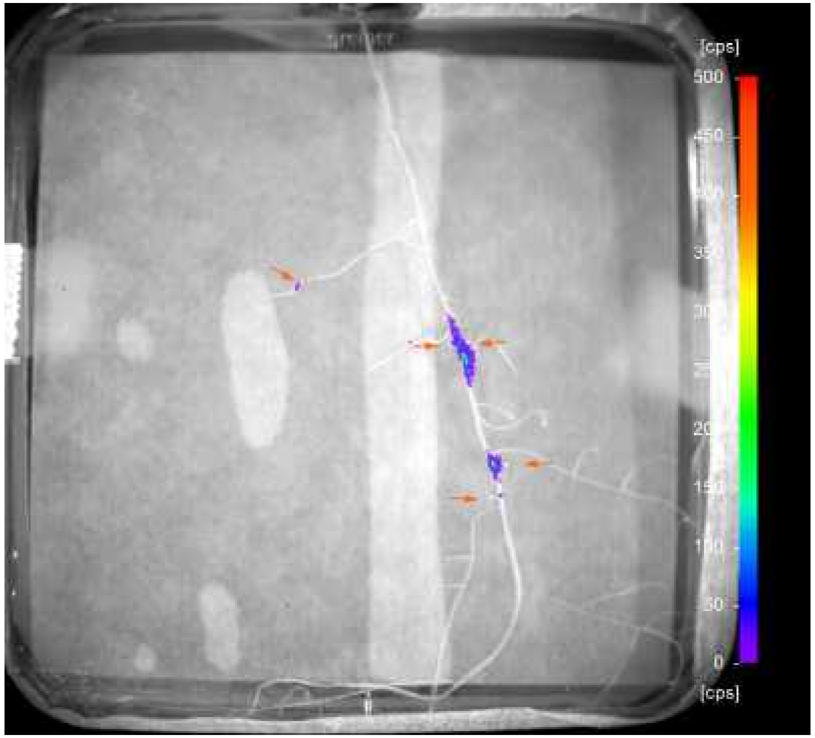
Novel rhizopine biosensor demonstrates rhizopine secretion



M. sativa nodulated by rhizopine-producing *S. meliloti* L5-30. Rhizopine biosensor on root surface

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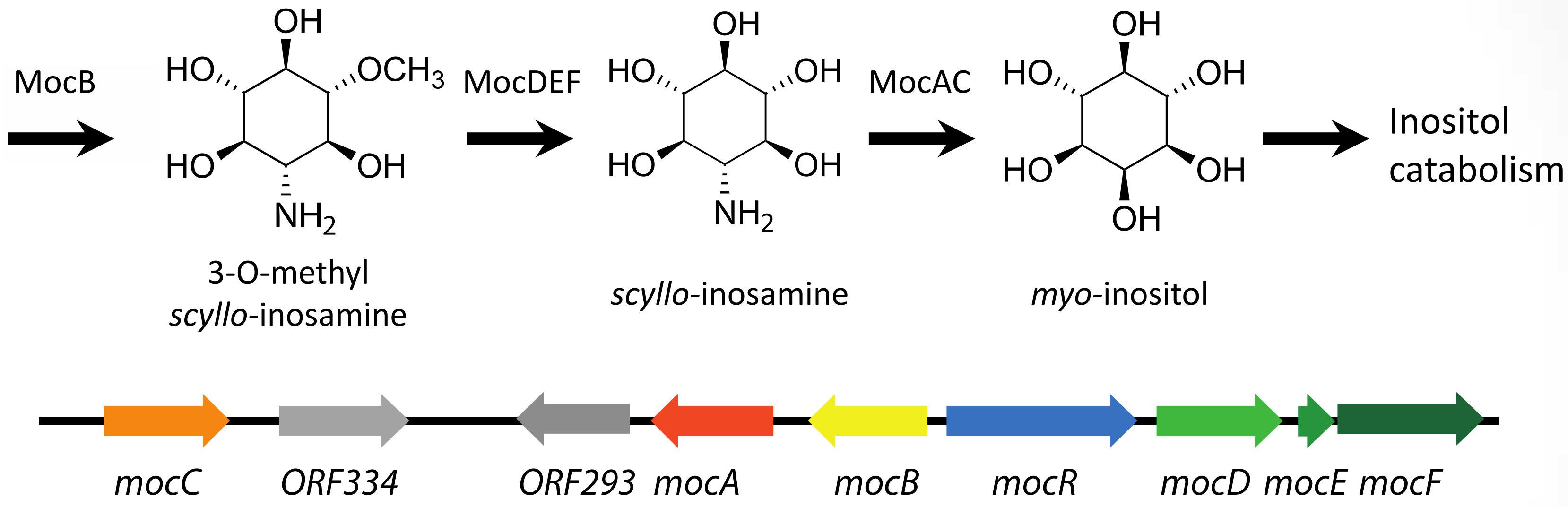
Engineering rhizopine catabolism



- Successfully transferred rhizopine catabolism to *Rhizobium* sp. IRBG74

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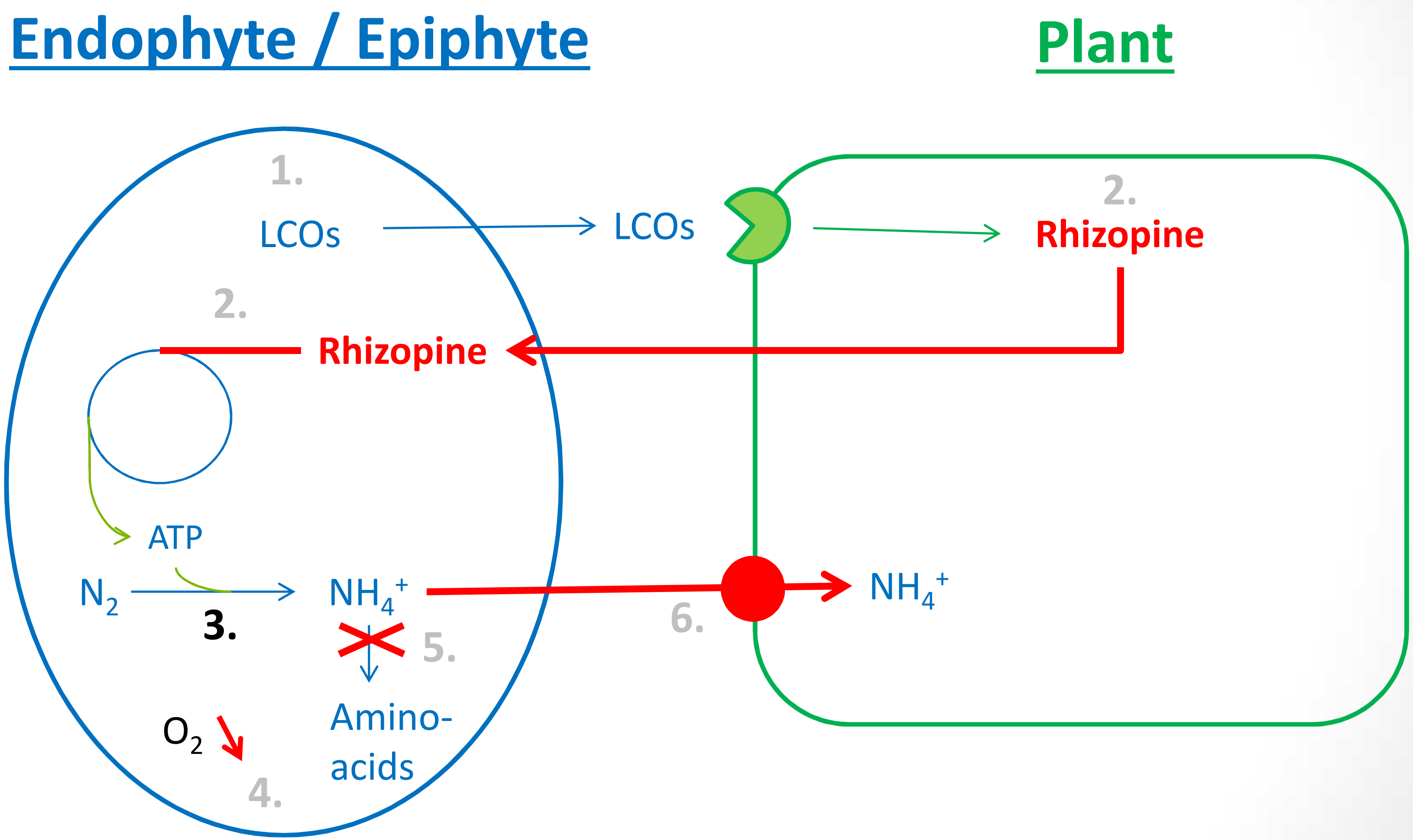
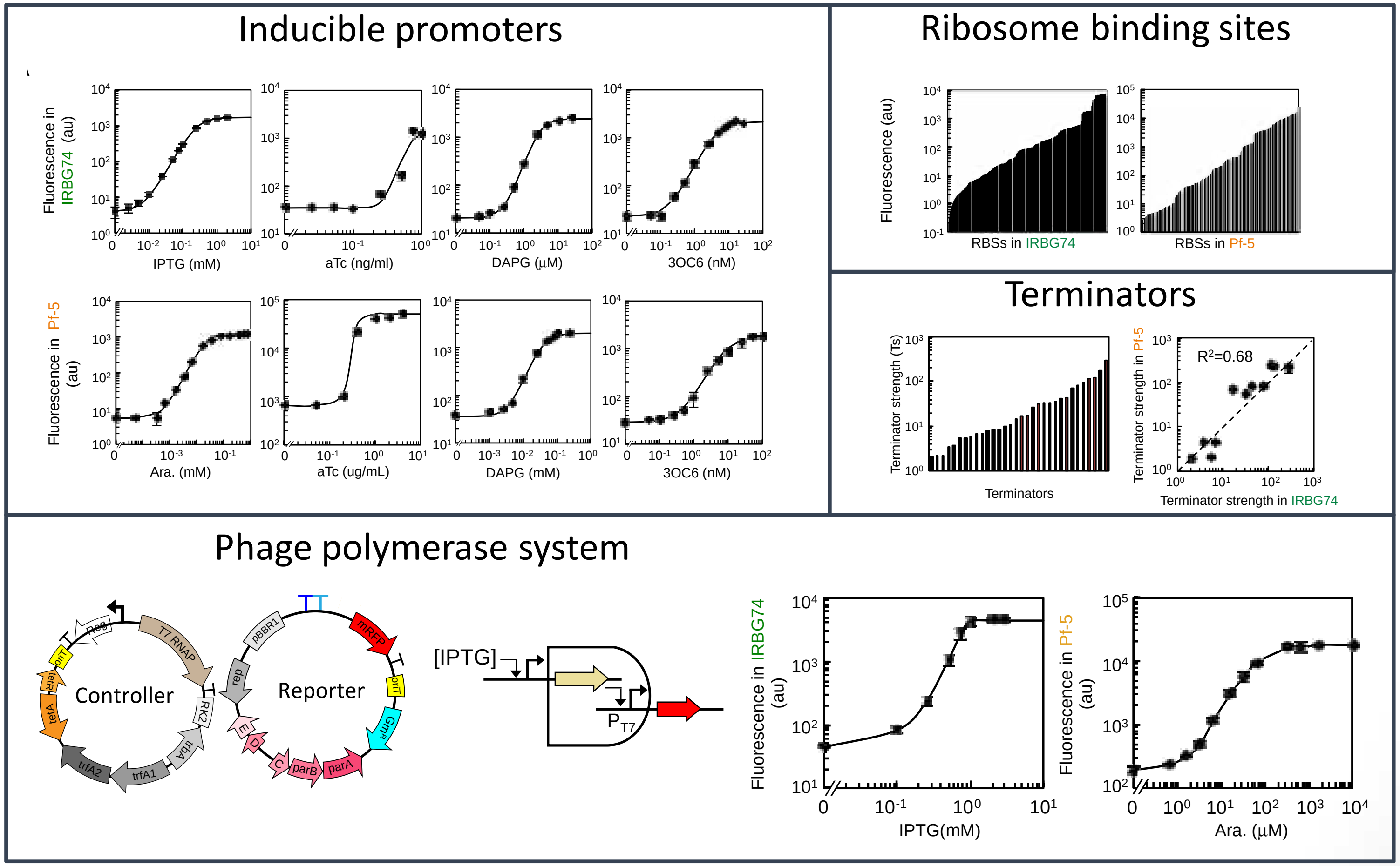

Endophyte / Epiphyte
Plant
1.
LCOs
LCOs
2.
Rhizopine
2.
Rhizopine
ATP
N2
NH4+
3.
5.
Amino-
acids
O2
4.
6.
NH4+

3. Creation of large part libraries for microsymbionts



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Engineering nitrogen fixation clusters for microsymbionts

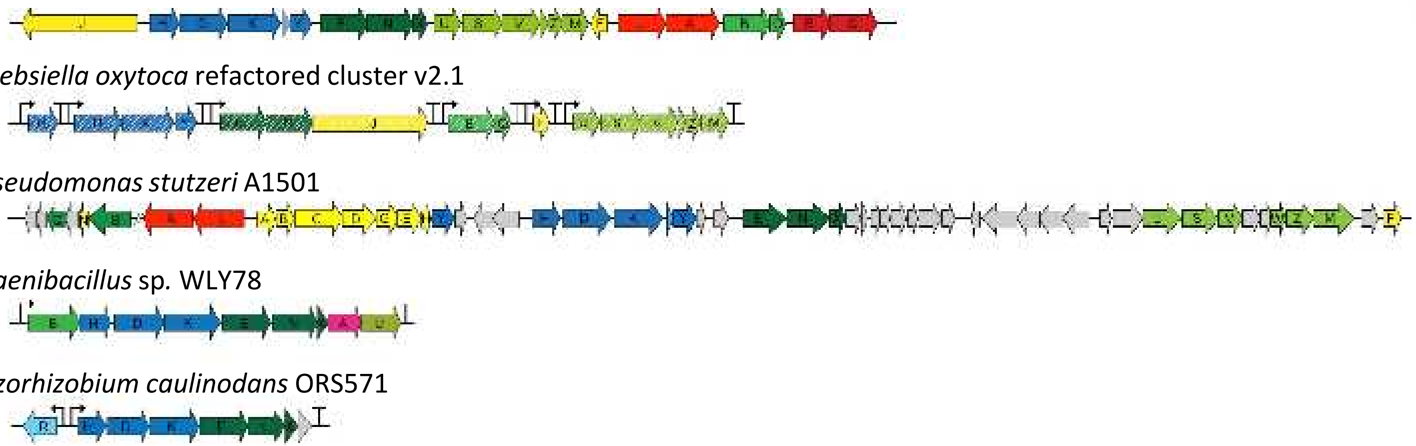
Klebsiella oxytoca M5a1 (+NtrBC)

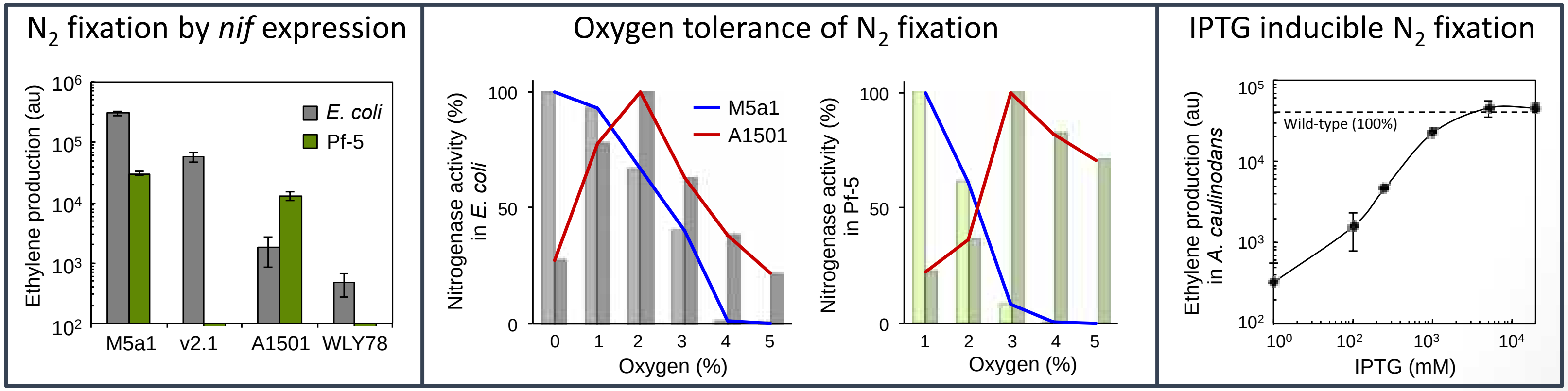
Klebsiella oxytoca refactored cluster v2.1

Pseudomonas stutzeri A1501

Paenibacillus sp. WLY78

Azorhizobium caulinodans ORS571





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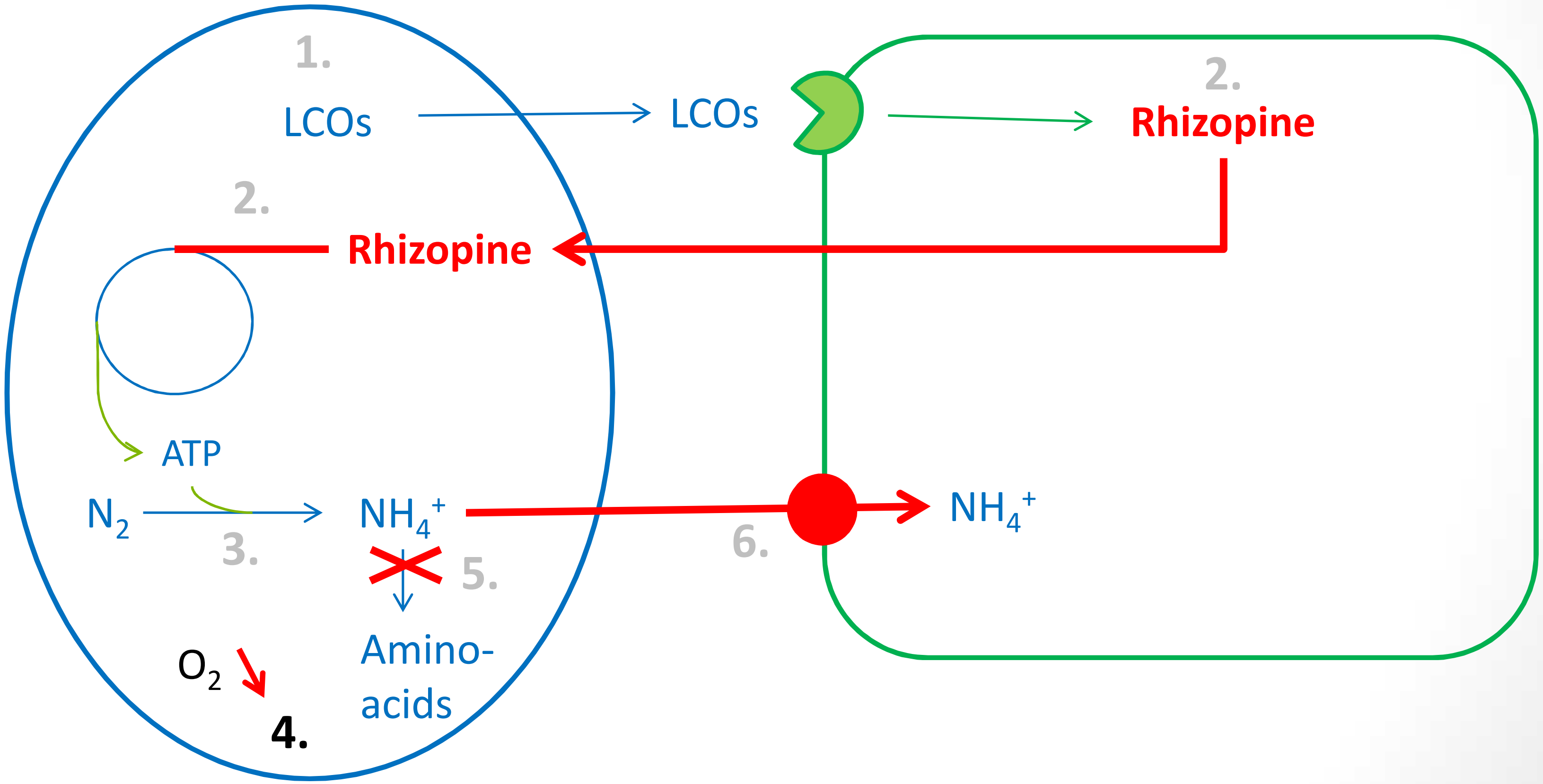
Endophyte / Epiphyte

Plant



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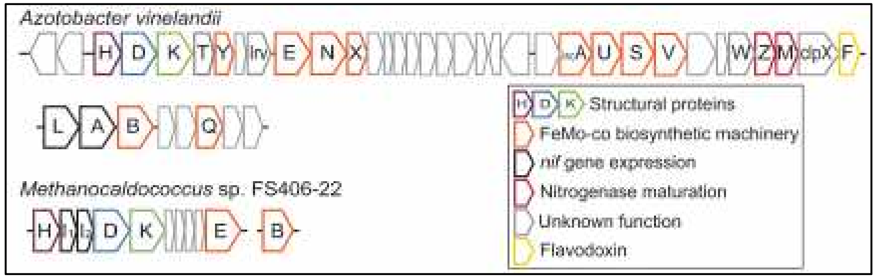
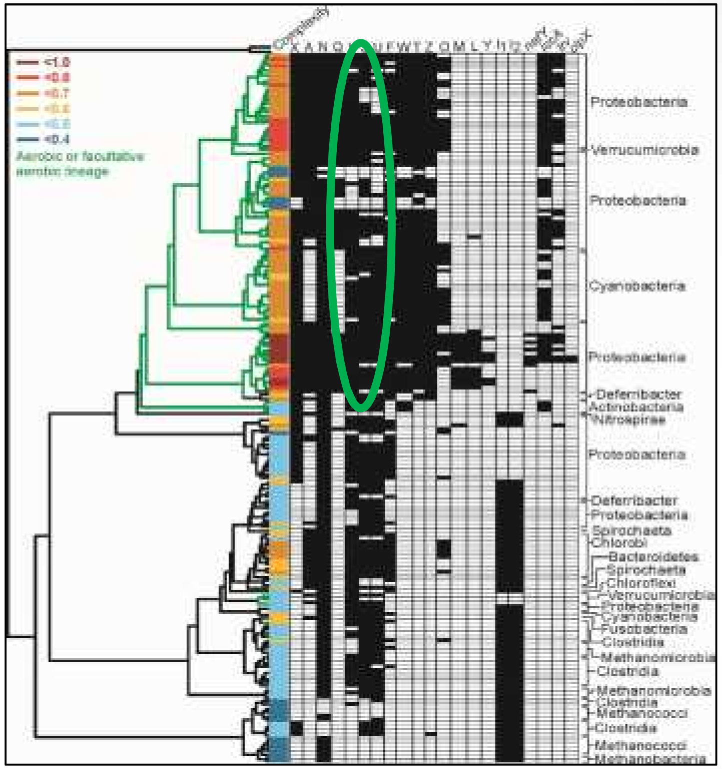
How do *nif* complements differ in aerobes and anaerobes?

***nif* operons differ in the number of *nif* genes they contain**



Work published in J. Bacteriol.



1) Many more nif genes in aerobes than anaerobes
2) Most extensive suite if nif genes are associated with one of our targets (Psuedomonads)
3) Nif genes not associated with specific modes of oxygen protection
4) Anaerobes energetically challenged and likely synthesize and turnover active nitrogenase slowly (postranslational regulation)
5) Aerobes turnover nitrogenase more rapidly (transcriptionally regulated) and have adapted more nif genes to a) improve kinetics of synthesis b) improve fidelity of synthesis and c) perhaps protect and repair oxygen sensitive elements

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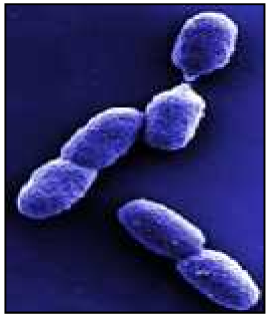
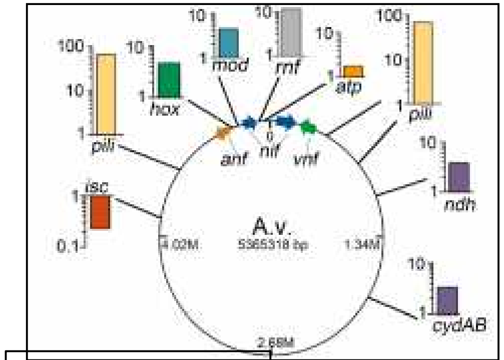
Forging the blueprint for engineering nitrogen fixation in aerobes

Defining patterns of gene expression associated with diazotrophy in aerobes: What genes are involved/critical under diverse carbon and oxygen conditions?



Use *Azotobacter vinelandii* as model organism:

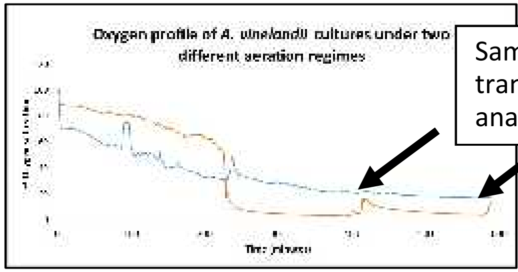
1. Well studied AEROBIC diazotroph
2. Genome sequenced, ease of genetic manipulation



Hamilton *et al*,J Bact 2011

We used transcriptomics to study differential gene expression during diazotrophic growth – in addition to *nif* encoded genes other important genes that support nitrogen fixation have been identified (*rnf, fix,* and genes encoding respiratory complexes important

We have developed capability to culture *A. vinelandii* in bioreactors under various oxygen tensions and have submitted RNA from these cultures for RNAseq



Samples taken for transcriptomic analyses

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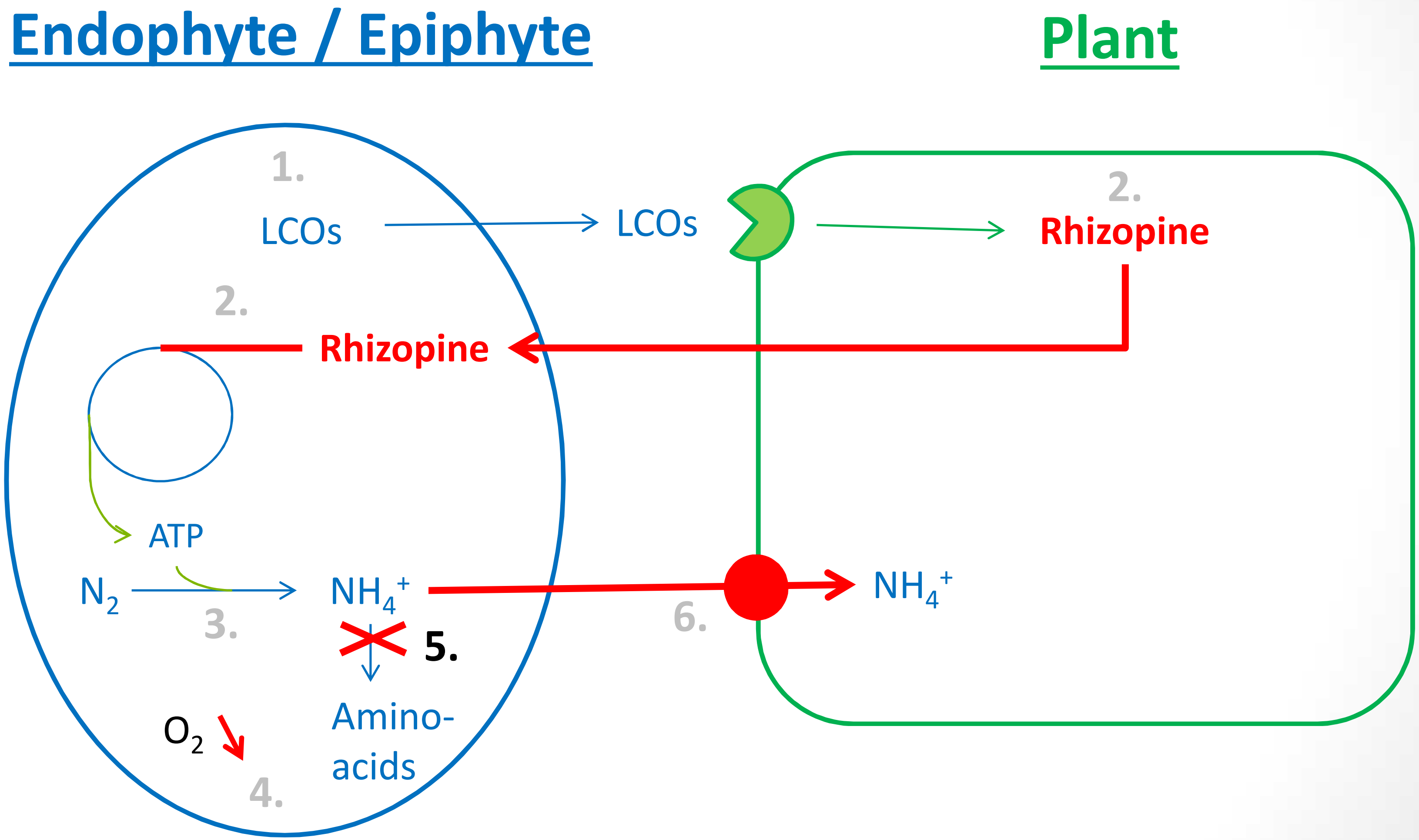

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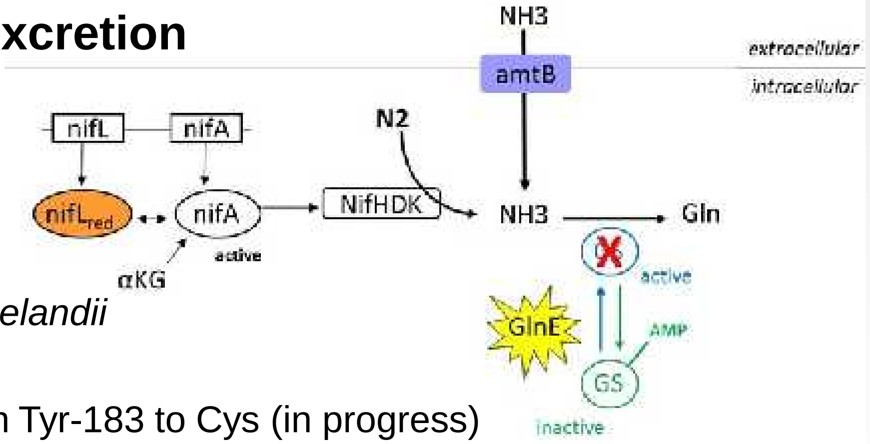
Forging the blueprint for engineering nitrogen fixation in aerobes

Strategies for engineering ammonia excretion



❶ ***glnA* deletion:** *glnA* deletion is lethal for *A. vinelandii* (confirmed experimentally)

❷ **targeted mutagenesis on *glnA*:** point mutation Tyr-183 to Cys (in progress)

Healy *et al.*, 2003 : Tyr-183 to Cys mutation in GS of *A. variabilis* leads to lower GS activity and excretion of ammonium (0.5 mM)

residues involved in formation of negatively charged ammonium-binding pocket

```
A. vinelandii    -GKMFDGSSIAGWKGIEASDMILMPDDSTAVLDPFTEEPTLIIVCDII//QAAWN--TDADFEGGNKGHRPGVKGGYFPVPP
A. variabilis     GVPFDGSSIRGWKAINESDMTMVLDPNTAWIDPFMEVPTLSIVCSIK//ECAWNSGKECTADKPNLAYKPRFKECYFPVSP
                  *  ****** ***  .*: *** :: * .** :*** * *** ***.*  :.***  .:.  :  * .::* .* *****.*

A. vinelandii    VDHDHEIRTAMCNALEEMGLKVEVHHHEVATAGQ//ALNGFINPSTNSYKRLVPGFEAPVMLAYSARNRSASIRIP------
A. variabilis    TDSFQDIRTEMLLTMAKLGVPIEKHHHEVATGGQ//ALLAIINPSTNSYKRLVPGYEAPVNLAYSQGNRSASIRIP------
                 .*  ::*** *  :: ::*: :* *******.**  ** .:**************:**** ****  *********
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• molecular construct carrying the point mutation Tyr-183 to Cys in GS of *A. vinelandii* has been generated

• selection of low affinity GlnA mutants of *A. vinelandii* using GlnE knockout background is in progress (selection on higher concentrations of ammonia)

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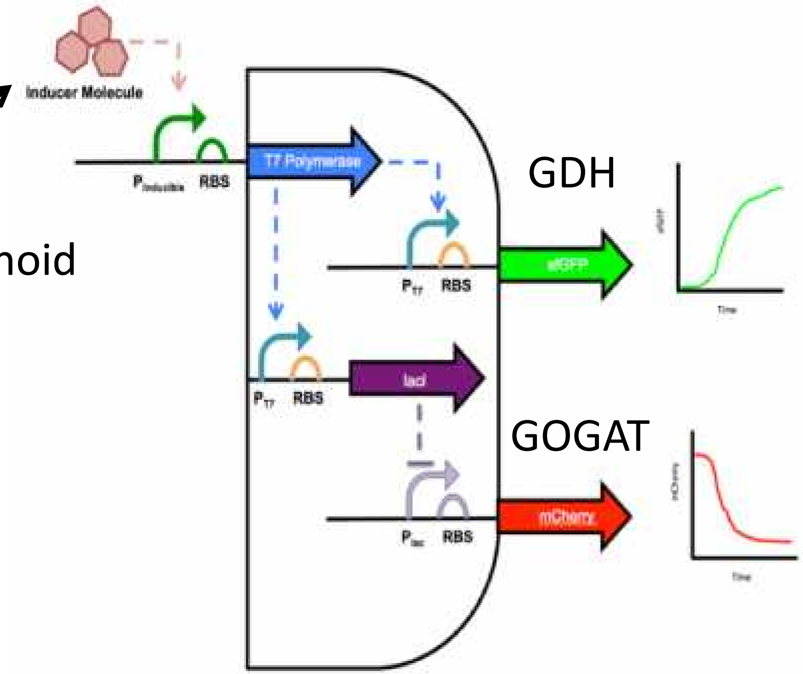
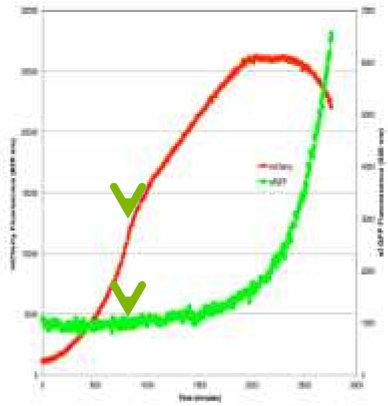
Mutants of *amtB* and *gltB* in IRBG74 and Pf-5

- *amtB* and *gltB* in-frame deletions made in IRBG74
- IRBG74 *gltB* requires glu for growth
- *E. coli gdhA* rescues rhizobial *gltB* growth
- Pf-5 mutants in progress
- Controller (e.g. rhizopine induced) and output modules constructed for repression of *gltB* and induction of *gdh* (tested with Gfp and mCherry)

e.g. SI, flavonoid
IPTG





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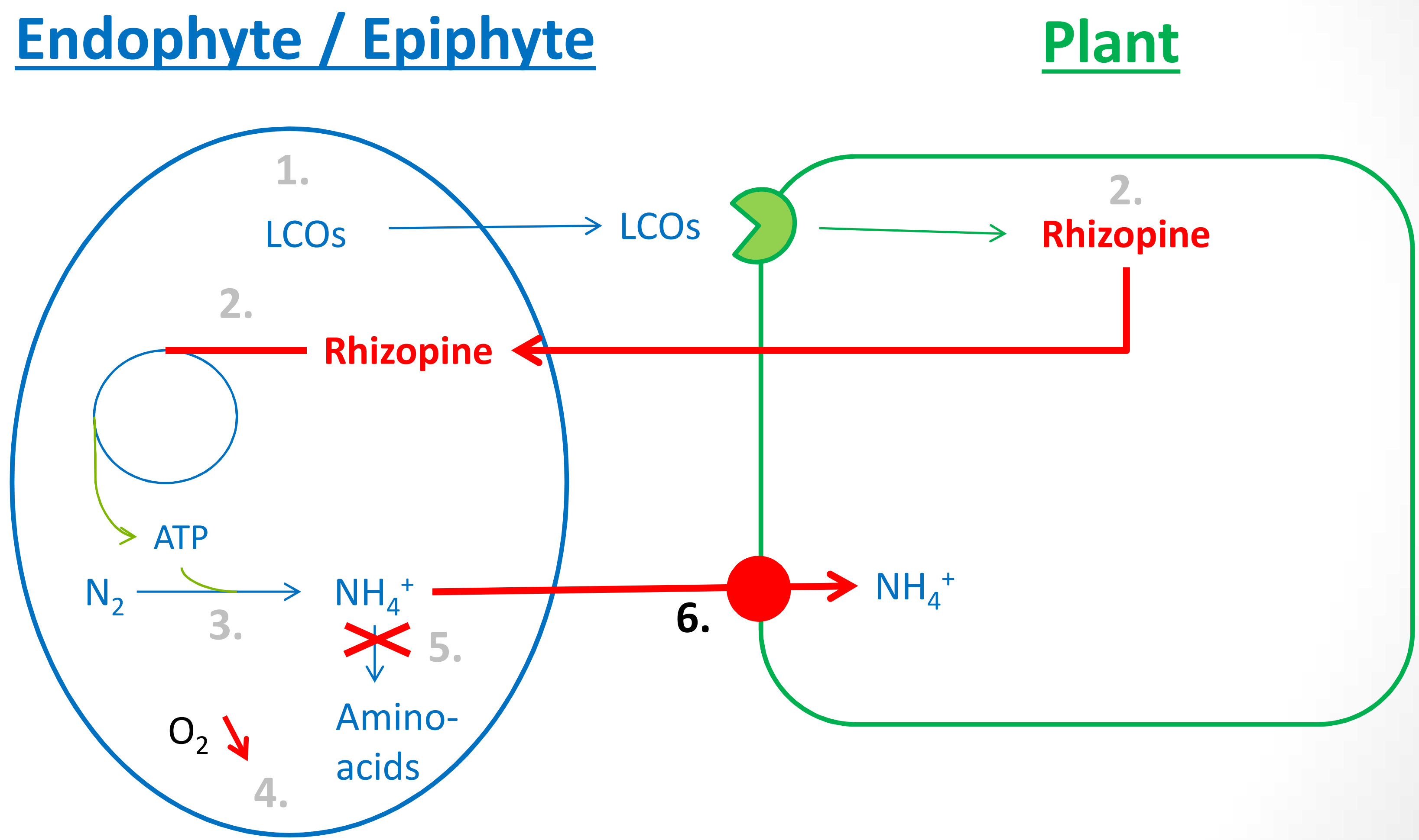

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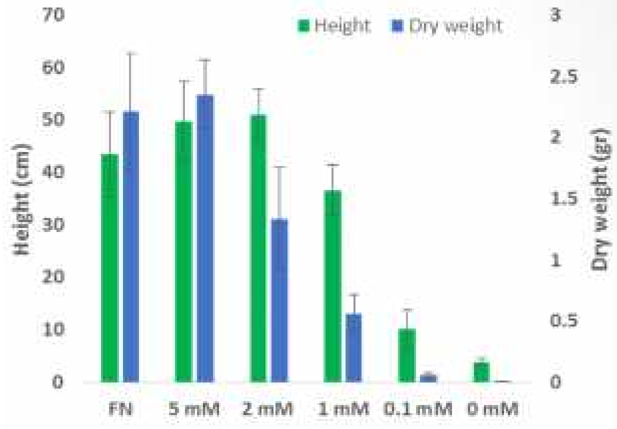
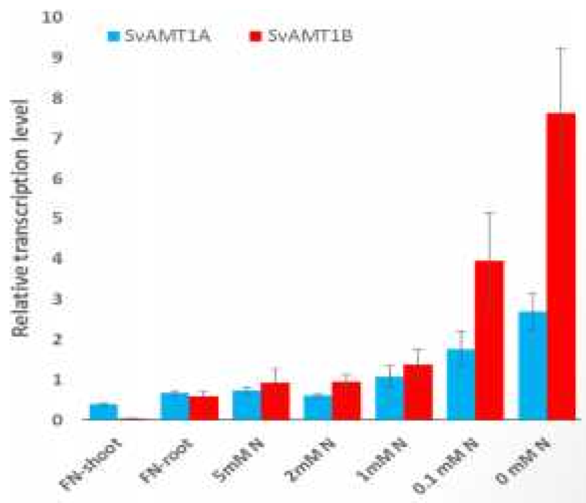
6. Characterize and optimize ammonium uptake

- *Setaria viridis* growth kinetics at different concentrations of ammonium
- Analyze *AMT* gene expression in *S. viridis* roots by quantitative RT-PCR
  - 2 AMT1-type transporters
  - *SvAMT*1A expressed in both shoots and roots
  - *SvAMT*1B expressed mainly in roots Highly expressed under low ammonium concentrations





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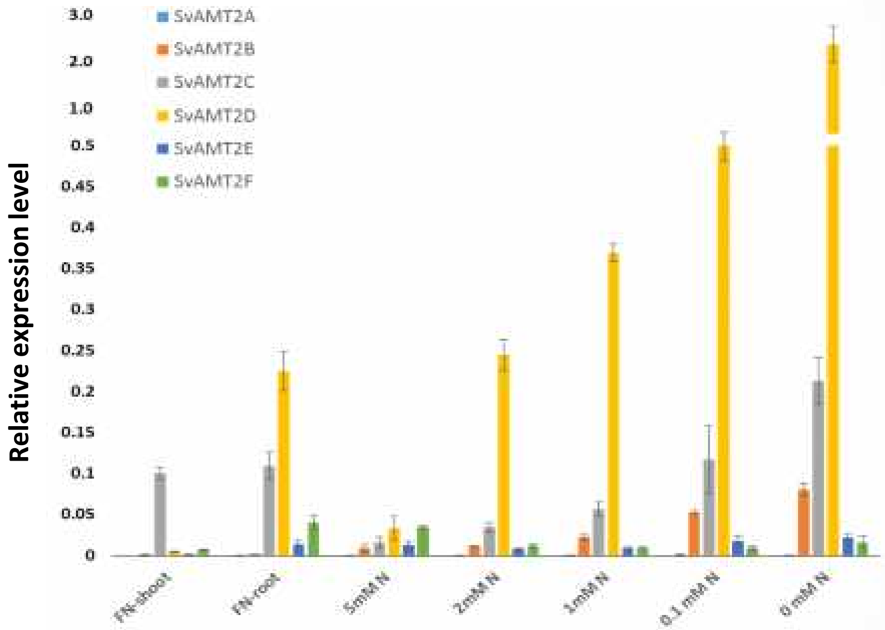
Characterize and optimize ammonium uptake

6 AMT2-type transporters

- *Sv*AMT2C expressed in both shoot and root, under normal N conditions.
- *Sv*AMT2D expression increased substantially at low ammonium concentrations.



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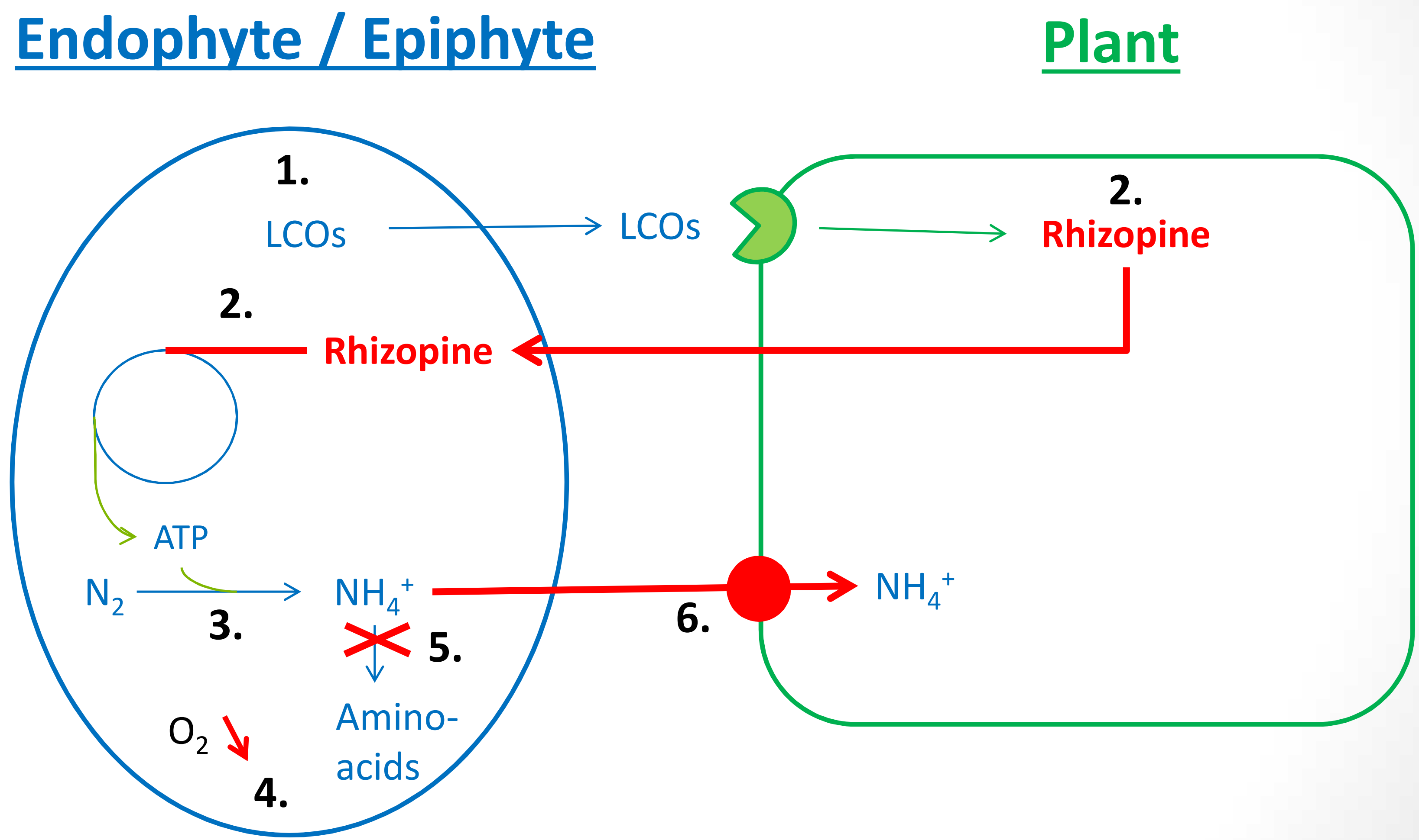

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Acknowledgements

- **Ané Lab**

Kevin Garcia

- **Oldroyd Lab**

Ponraj Paramasivan

- **Peters Lab**

Amaya Garcia Costas
Florence Mus

- **Poole Lab**

Barney Geddes
Ramakrishnan Karunakaran
Alison East

- **Udvardi Lab**

Evangelia Kouri

- **Voigt Lab**

Min-Hyung Ryu

BBSRC bioscience for the future

NSF National Science Foundation WHERE DISCOVERIES BEGIN

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NSF Research Coordination Network

Plant Nitrogen Network (PlaNNet):

Coordinating research on plant nitrogen for sustainable and productive agriculture

PlaNNet aims to coordinate research activities related to the supply, utilization and loss of N by plants with the long-term objective of enhancing the efficiency and sustainability of N-use in agriculture. Research coordination network (RCN) activities will include:

(i) Development of a networking website that includes information about hundreds of researchers within the USA and around the world who are involved in plant N-related research, opportunities for collaboration, and educational resources.

(ii) Annual Workshops-Without-Walls, virtual meetings that will involve hundreds of participants in presentations, discussions, and consensus-building related to plant-N research and development.

(iii) Satellite workshops at major conferences focused on specific aspects of plant N and agriculture.

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Look for us on: http://plannet-rcn.org