Analyzing the effects of AM fungi produced IDA peptides in roots and during arbuscular symbiosis

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BS in Bioinformatics, 2nd year

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Role of AM Symbiosis and AM fungi as Biofertilizer

- Arbuscular mycorrhizal (AM) fungi form mutualistic associations with ~80% of terrestrial plant species.
- They colonize root cortical cells and form arbuscules, where nutrient exchange occurs.
- AM fungi enhance uptake of essential minerals and nutrients
- Promotes plant growth and development

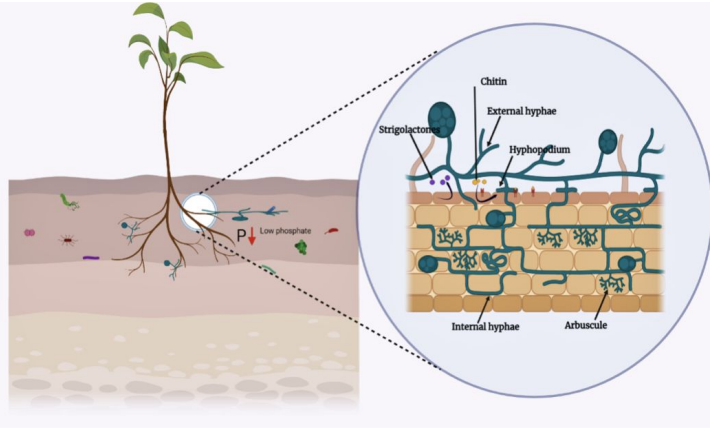
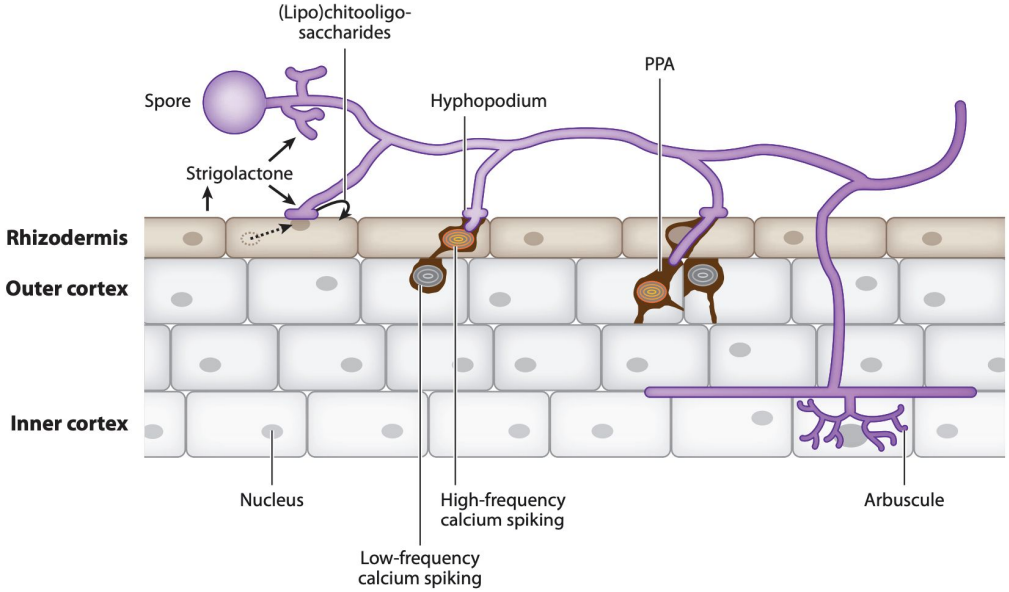


Figure 1. AMF added to Allium genotypes positively influenced plant growth (left: without AMF, right: with AMF, one genotype).

Regulation of AM Symbiosis

- Under low phosphate condition, plants secrete strigolactones
- Strigolactones induce hyphal branching of AM fungi
- Arbuscules are formed inside the root cortical cells.
- Symbiotic maintenance requires the reprogramming of plant signals



The accommodation of AM fungi, taken from Gutjar and Parniske(2013)

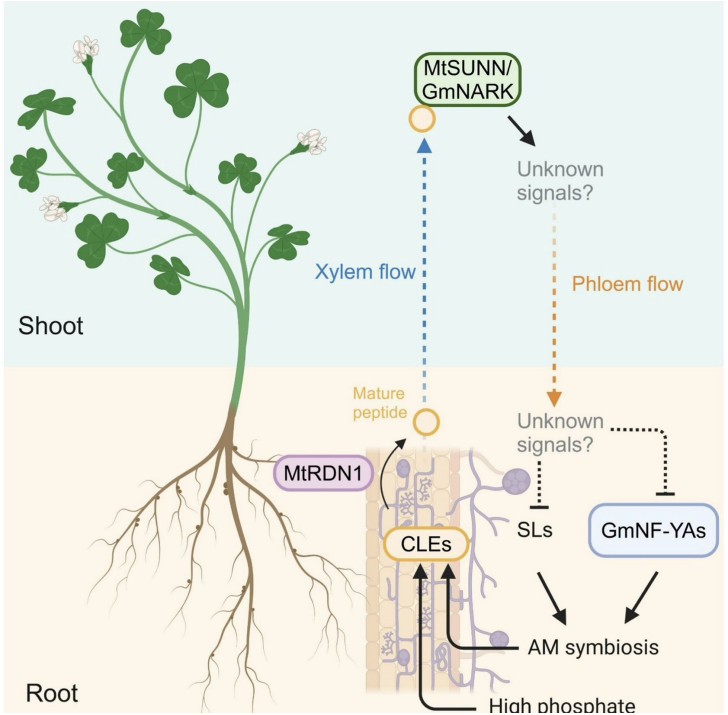
CLE Peptides – Background

- Small, modified peptides (~12–14 aa)

- Functions:
  - -Plant development
  - -Nutrient sensing
  - -Symbiosis regulation (e.g., MtCLE53, MtCLE33)

- CLE peptides are perceived by a family of receptor-like kinases (RLKs)

- Sagars research characterized a so-far-undescribed *M. truncatula* CLE gene, *MtCLE16*, which promotes AM symbiosis, unlike most characterized CLEs which suppress it.

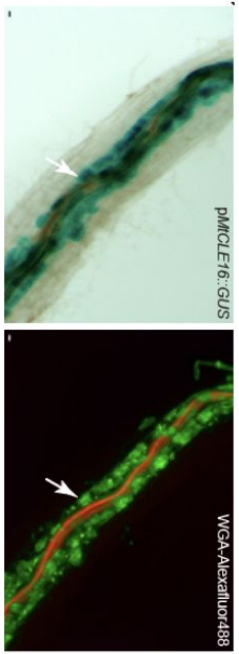


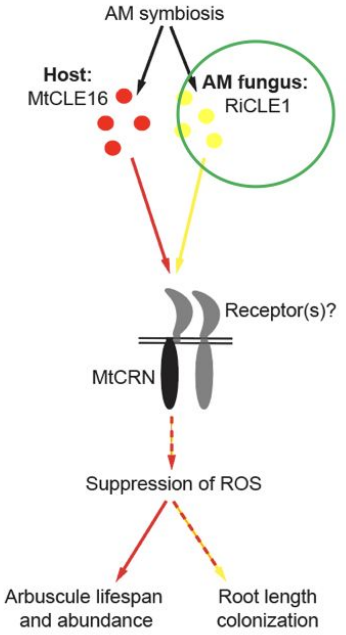
Nakagami et al., 2024

RiCLE1 – A Fungal CLE Mimic

- Secreted by *Rhizophagus irregularis*.
- Positively regulates AM symbiosis
- Mimics MtCLE16: binds to CRN receptor, suppresses reactive oxygen species (ROS), promotes colonization.
- Similarly to RiCLE1, Sagar has recently identified a new class of peptides encoded by AM fungi, called IDA

Medicago truncatula CLE16 as a positive regulator of AM symbiosis. *MtCLE16* is expressed in root cells colonized by AM fungi (AMF)

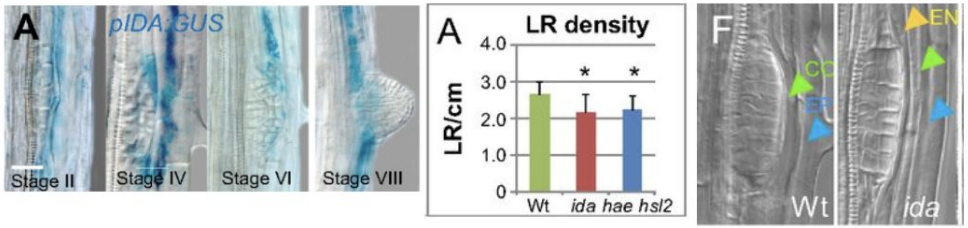




IDA Peptides – Background

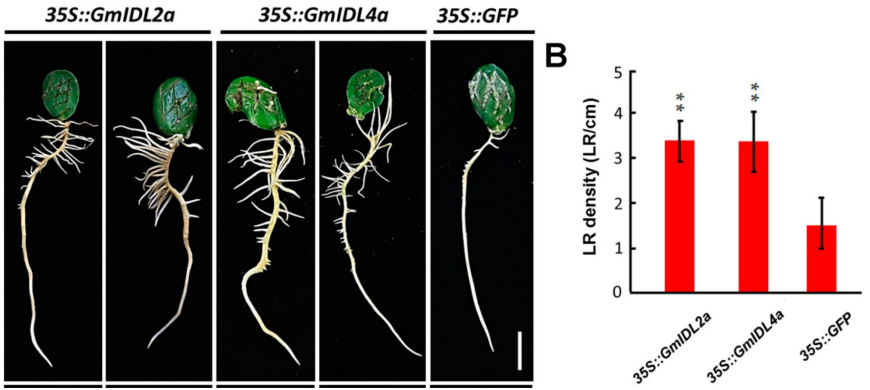
- IDA peptides are a secreted peptide, so it goes to the apoplastic space of the plant cell.
- IDA peptides facilitate lateral root emergence and flower development
- IDA peptide are short secreted peptides ~12-14 amino acids
- Much less studied in AM colonizing plants and less understood than the CLE peptides or other peptide classes such as CEP, PEP, RALF etc.
- IDA peptides are perceived by Leucine-Rich Repeat Receptor-Like Kinases (LRR-RLKs)

Arabidopsis thaliana IDA is required for normal progression of lateral root primordia:



Taken from Kumpf et al., PNAS , 2013

IDA induction in Soybean increases lateral root density



Taken from *Liu et al., Int. J. Mol. Sci. 2018*

RiIDA encodes IDA-Like Peptide

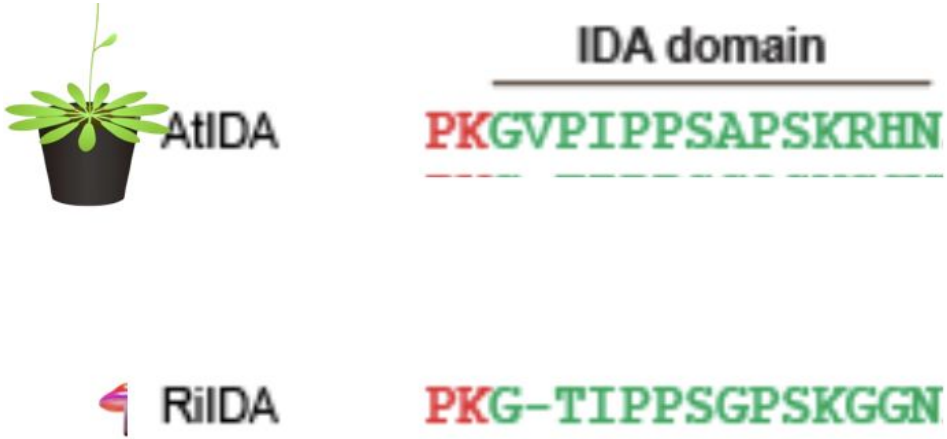
- New peptide class in addition to CLE peptide that has been previously identified
- RiIDA shares conserved residues with AtIDA, including the receptor-binding core (e.g., PKG and TIPP), suggesting functional similarity.
- Only expressed during the AM colonization in the roots but not expressed when fungus is not in the host.

“Hypothesis: IDA Regulates plant root development and AM symbiosis”

- A candidate for novel signaling mechanisms

Shares a very similar sequence to the peptide it mimics:

Further shown through clustering by Sagar: Data not shown



12 amino acids, a functional domain is conserved

Spring quarter (2025): Experimental Aims

"To functionally elucidate the role of fungal IDA peptide during AM symbiosis"

Planned experimental setup:

Analysis to be performed: **Species:**

Root phenotype analysis ---- *Medicago truncatula*, *Arabidopsis thaliana*

- Completed: Measured values including PRL, lateral root count/length, and lateral root primordia using ImageJ (FIJI)

AM phenotypic analysis (microscopy) ---- *Medicago truncatula*

- Completed: roots cleaned and stained with WGA-Alexa Fluor 488. Used the gridline intersect method to estimate colonization rates across different samples.

Genotyping of tnt1 mutant lines ---- Not able to complete due to time constraint

Synthetic peptide application enhances the lateral root length in *M. truncatula*

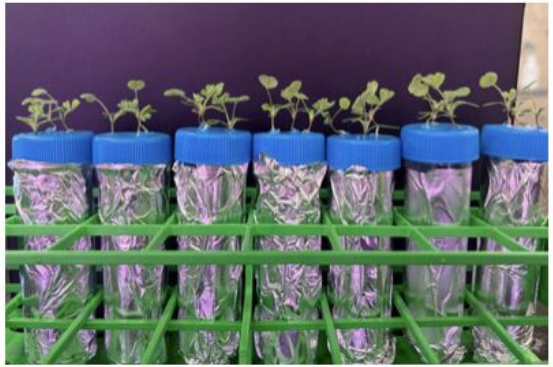
- Plants were grown in falcon tubes and inoculated **with spores**

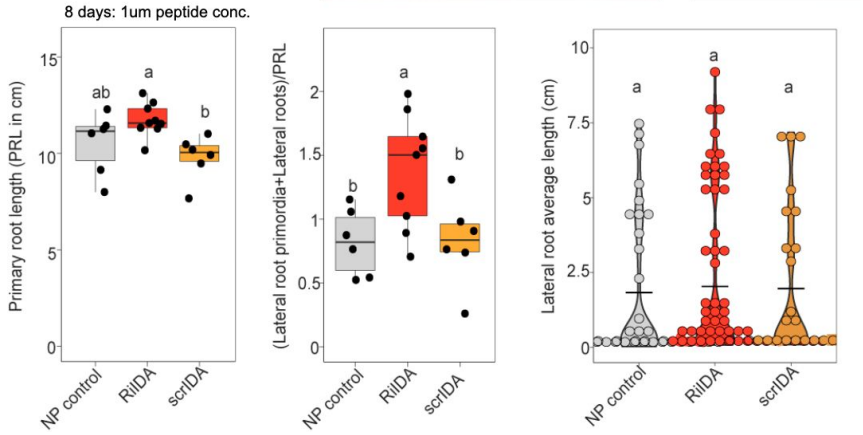
- Treated with **1µM peptide concentration**

- Harvested after **8 days**

-Treated plants showed enhanced primary and lateral root growth

Experimental setup in falcon tubes





NP control

RilDA

ScrIDA (-ve control)

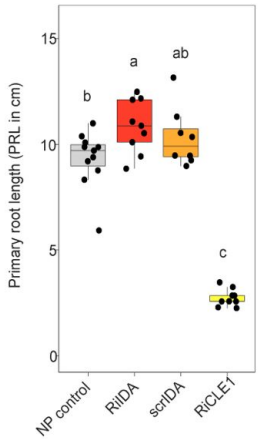
A. thaliana - Synthetic IDA peptide application promotes both primary root growth and lateral root formation

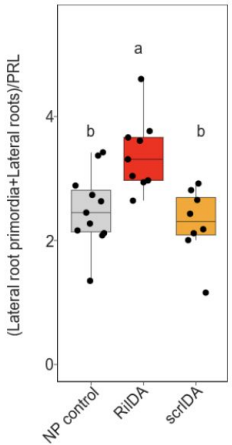
- I quantified the phenotype for plates Sagar was growing

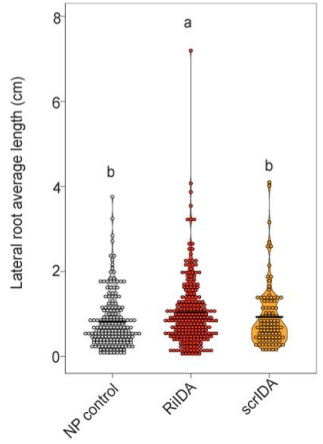
- Treated with **1µM peptide concentration**

- Harvested after **10 days**

- Once again, treated plants showed enhanced primary and lateral root growth







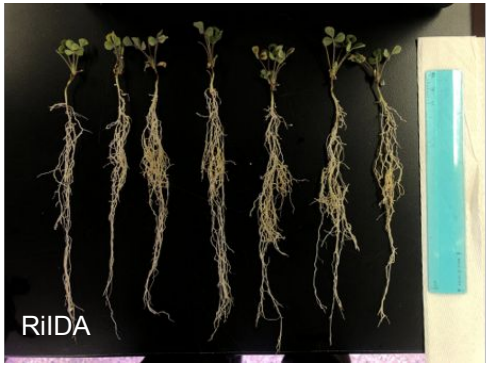
M. truncatula - Synthetic RiIDA & RiCLE1 application promotes AM symbiosis

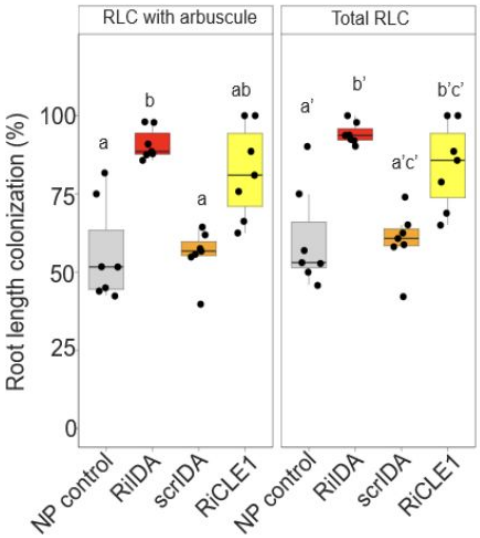
- Plants were grown in cones and inoculated with **200 spores**

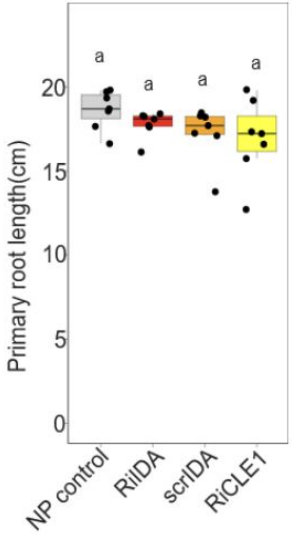
- Treated **biweekly** with **20 mL of 100 nM peptide:hoaglands**

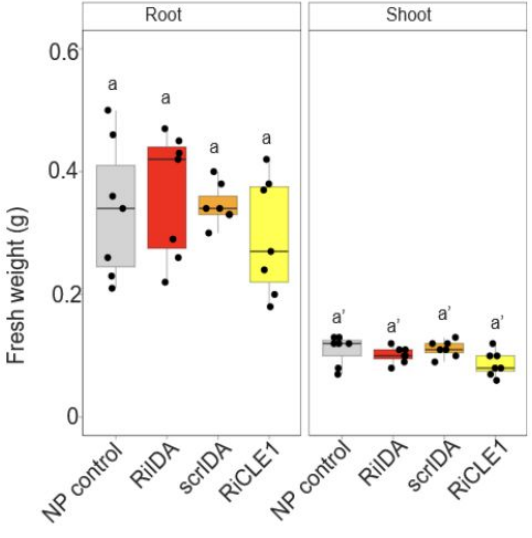
- Harvested after **31 days**

- RiIDA and RiCLE1 greatly enhanced colonization, supporting their role in promoting symbiosis.









Future Work:

- Elucidate **downstream signaling** pathways triggered by RiCLE1 and RiIDA
- Validate **peptide–receptor** interactions
- Explore peptide effects in **additional host species**

Thank you!