Plant and fungal cell-type expression differences in arbuscular mycorrhizal symbiosis using spatial and single-nuclei transcriptomics

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M. truncatula in greenhouse for seed propagation, in cones with sterilized substrate for AMF growth, and in growth chamber for development (left to right)

Background

Arbuscular mycorrhizal (AM) symbiosis is a symbiotic relationship between AM fungi and plant roots that provides mutual nutritional benefit for both partners. This symbiosis occurs along a spatiotemporal gradient, with fungal hyphae penetrating through the plant cell epidermis and forming transitive structures known as arbuscules contained within periarbuscular membranes of cortical cells.

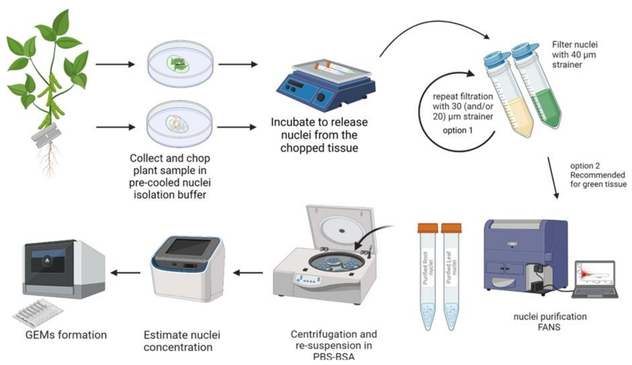
Question

How does arbuscular mycorrhizal symbiosis alter the transcriptional landscape within the plant root at a cell-type specific spatial resolution?

Approach

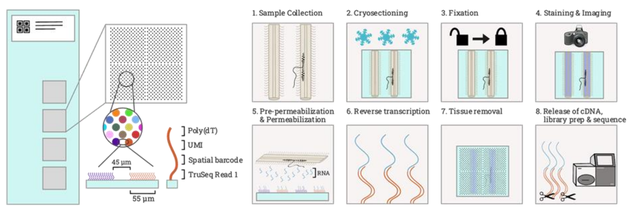
This project aims to map the RNA of model symbiotic legume *Medicago truncatula* plant roots and compare samples with and without AMF *Rhizophagus irregularis* by integrating spatial and single-nuclei transcriptomic datasets from WT plants and colonization mutants.

Single-Nuclei (SN) Methods



Thibivilliers et al., *Methods Mol. Bio.* 2022

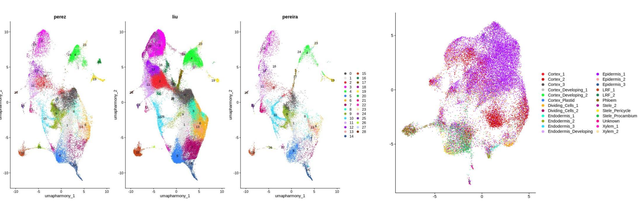
Spatial Transcriptomic (ST) Methods



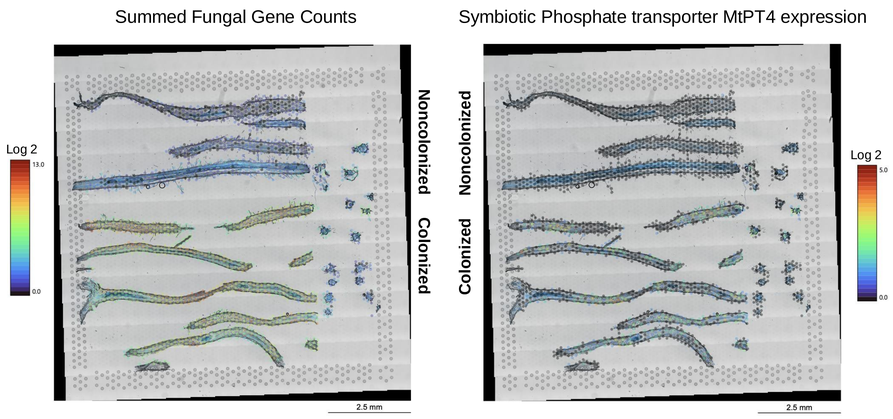
Library Generation and Downstream Analysis

SN and ST libraries were created from *M. truncatula* genotype R108 colonized with *R. irregularis.* 10X Chromium (SN) and Visium (ST) libraries were ran on the Illumina NextSeq 2000 and NovaSeq X. Reads were aligned to the latest version of the *M. truncatula* A17 genome (A17r5.19) and the R. irregularis DAOM197198 genome (Manley et al. 2023). Libraries were preprocessed individually using STARsolo. Ambient RNA contamination was corrected (SoupX) and potential doublets were filtered out (scDoubletFinder). Further QC removed outlier cells with low numbers of gene counts (< 200). Reference SN *Medicago* datasets were used to provide additional evidence for cluster annotation. Secondary analysis was done primarily using Seurat and hdWGCNA.

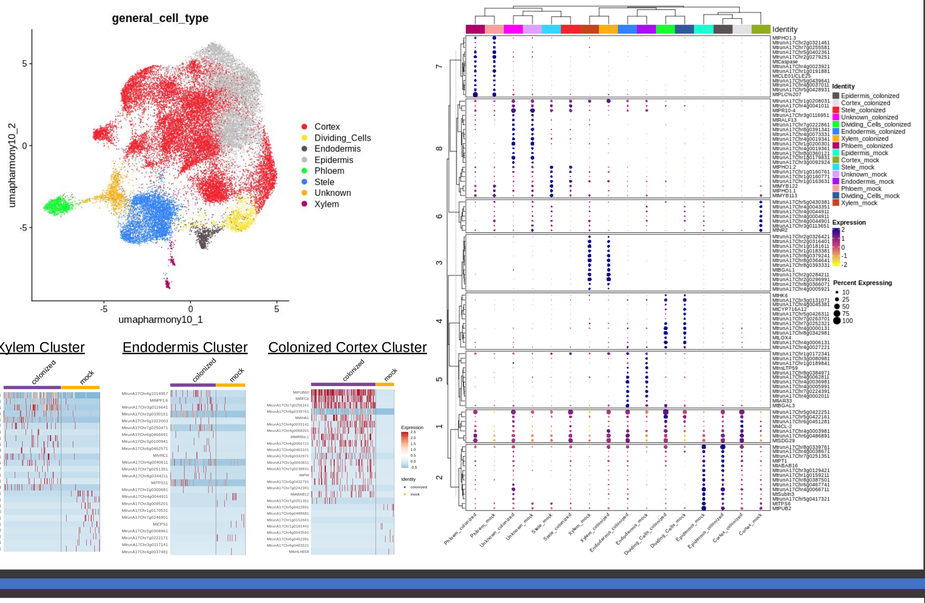
Reference Annotation → predicted cell types



Spatial Transcriptomics: Colonized vs. Noncolonized

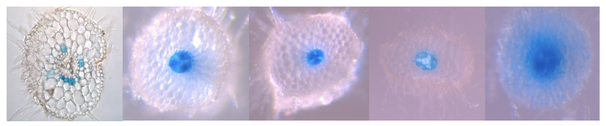


Single Nuclei Transcriptomics: Colonized vs. Noncolonized

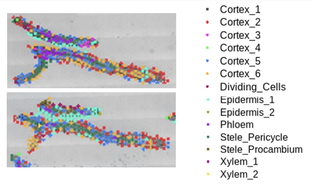


Single-Nuclei Cluster Validation

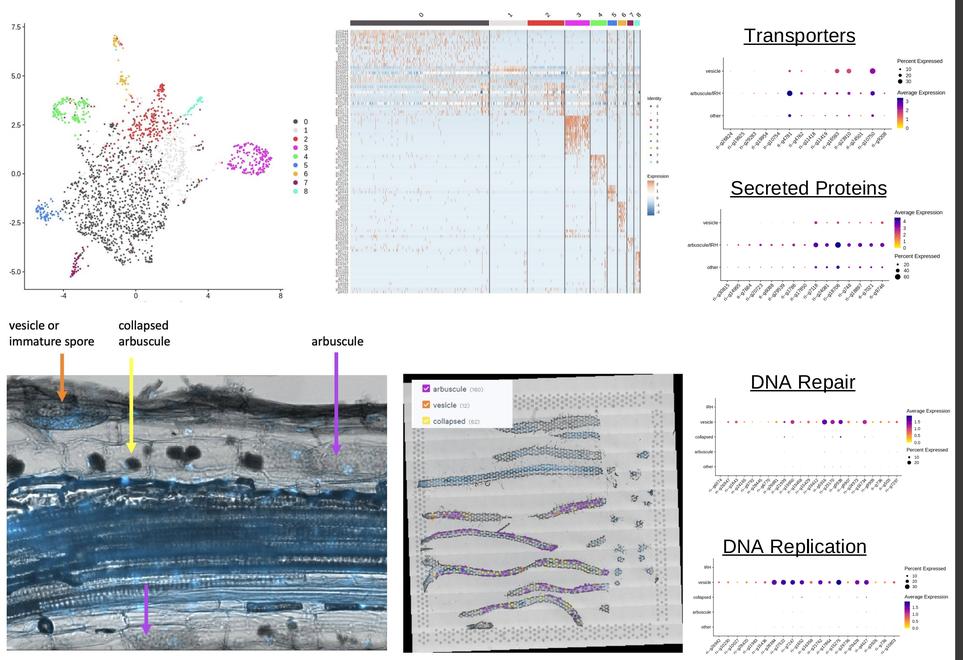
Validation using Promoter-GUS fusion expression



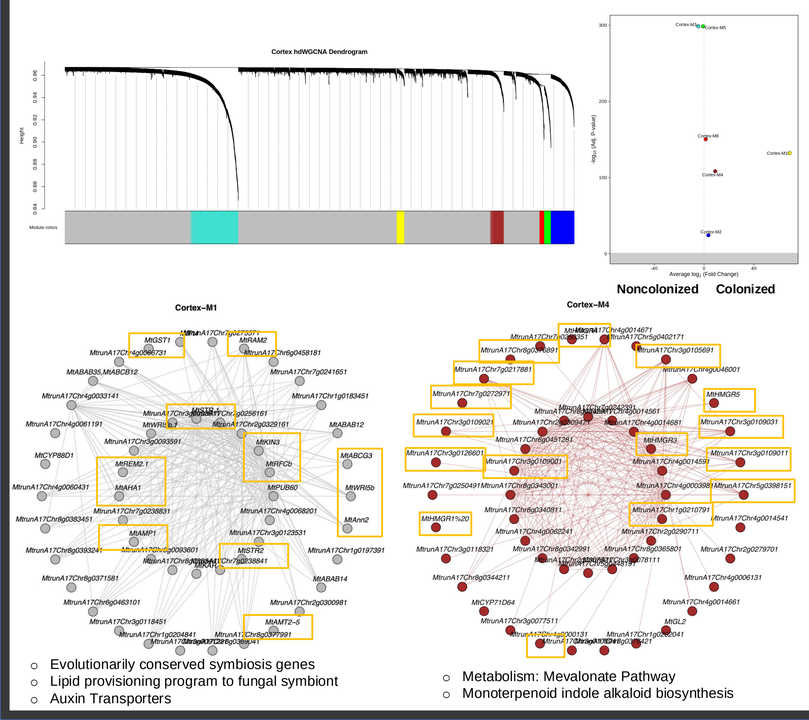
Validation using SN markers to annotate spatial spots



Fungal Single-Nuclei clusters annotate to distinct ST features



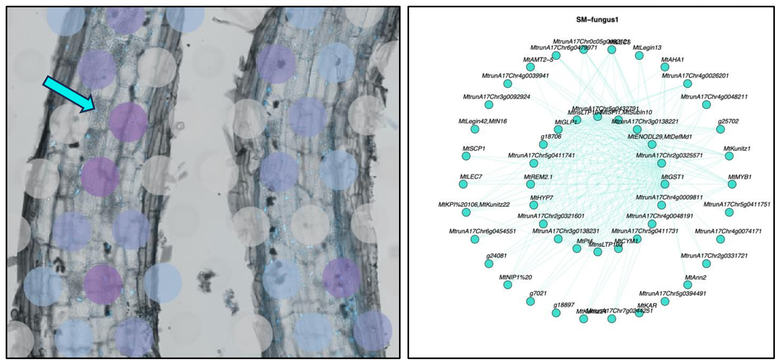
SN WGCNA plant gene expression networks



- Evolutionarily conserved symbiosis genes
- Lipid provisioning program to fungal symbiont
- Auxin Transporters

- Metabolism: Mevalonate Pathway
- Monoterpenoid indole alkaloid biosynthesis

ST WGCNA plant-fungal gene expression networks



Plant genes
Phosphate transport
Plant-microbe recognition
DUFs 538 and 2775
Lipid provisioning program weaker compared to single-cell

Fungal genes
Lipid desaturase
Iron transporters/Iron binding proteins
11β-hydroxysteroid dehydrogenase
Many hypothetical proteins (RNA binding / slicing)

Conclusions & Future Directions

Medicago and *Rhizophagus* reads were able to be captured through both single-nuclei and spatial transcriptomic methods.

Single-nuclei clusters correlated with most *Medicago* cell types using previously described cell markers.

Changes in colonized subset of cortical cells drives root expression differences.

Arbuscules and vesicle structures have strong expression differences. Further work will aim to explore differences in vesicle or spore structures to better elucidate their roles.

Further work will evaluate cell-type expression differences in by comparing this integrated dataset to SN and ST datasets of GRAS transcription factor plant mutants which play a role in AMF colonization (MtRAD1 and MtDella1,MtDella2).

Acknowledgements

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National Institutes of Health

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