UNDERSTANDING THE CELL TYPE-SPECIFIC RESPONSE OF ALFALFA ROOTS TO FLOOD STRESSES

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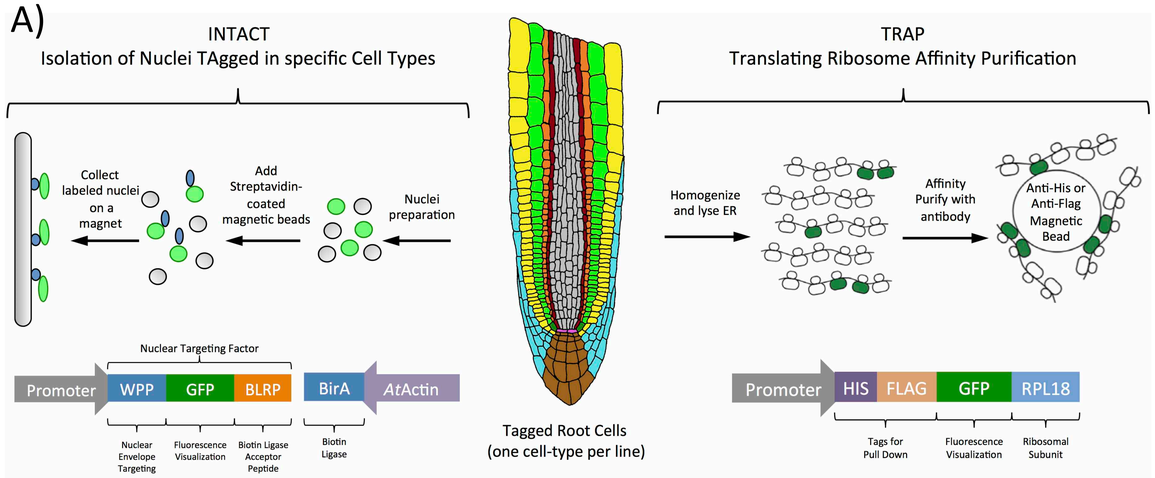
Abstract

Plants are stationary organisms that must constantly respond to a changing environment in order to survive. Primary detection and response to drought and flooding occurs in the roots. However, how the specific cell types of the roots respond to these stresses is not completely understood. Our lab utilizes two techniques, INTACT (Isolation of Nuclei TAgged in specific Cell Types) to isolate DNA and RNA present in nuclei, and TRAP (Tagged Ribosome Affinity Purification) to isolate translating ribosome from the cytoplasm, in order to study, in detail, how plants responds to environmental cues. For this work, we used *Arabidopsis thaliana* promoters to drive expression of NTF (Nuclear Targeting Factor) or TRAP in specific cell types of *Medicago truncatula*, alfalfa, roots in order to study how alfalfa responds to flood stress. Our results show that all of the *A. thaliana* promoters, with the exception of *At*WOX5, had the same time and place expression in *M. truncatula* as in *A. thaliana*. We also show that INTACT and TRAP can be performed using *M. truncatula* tissues, which has not been shown before. We are currently characterizing the gene response of alfalfa root cells to 2 hours of flooding stress. The transcriptional and translational response to flood stress in alfalfa will be compared to tomato and rice. The long-term goal of this research is to establish a comprehensive understanding of how crops respond to a variety of growth conditions so that this information can be used to develop hardier crops.

Project Goals

1. Target specific cell types of the alfalfa root using known promoters.
2. Isolate nuclei from specific cell types.
3. Isolate translating mRNA from specific cell types.
4. Develop a simple, robust model for hypoxic stress.
5. Isolate nuclei and translating mRNA from hypoxic roots.
6. Establish stable lines for cell type-specific expression of NTF or TRAP.

APPROACH: Cell Type-Specific Isolation of Nuclei and Ribosomes



B)

Target Cell Type	Promoter
Near Constitutive	Cauliflower mosaic virus 35S (p35S) Ubiquitin (p*At*UBQ)
Root proliferating cells	Ribosomal protein L11C (p*AtPL11C*)
Root endodermis, quiescent center	SCARECROW (p*At*SCR)
Root vasculature	SHORTROOT (p*At*SHR)
Root cortex meristematic zone	Cortex specific transcript (p*At*CO2)
Root cortex elongation and maturation zone	Endopeptidase (p*At*PEP)
Quiescent center	WUSHEL-related homeobox 5 (p*At*WOX5)

C)

Bacteria	Strain	Purpose
Agrobacterium rhizogenes	K599	Hairy root transformation
Agrobacterium tumefaciens	EHA105	Root explant transformation

APPROACH

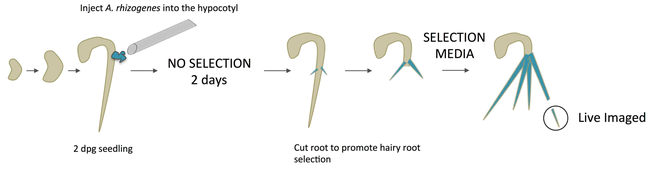
A) The different cell types of the root are shown in the root diagram and labeled in the box below. The cell types that make up the root that are being targeted in this project are: Epidermis (yellow), Cortex (green), Endodermis (orange), Pericycle (red), Quiescent Center (pink), Columella (brown), Lateral Root Cap (light blue), and Stele (grey). A cartoon depicting the protocol for performing INTACT is shown on the left side of the root diagram and a cartoon depicting the TRAP protocol is shown on the right side of the root diagram. Under each cartoon depiction is a schematic depicting the T-DNA that allows for each construct to be expressed in specific cell types.

B) The *Arabidopsis thaliana* promoters listed were used to target expression of NTF or TRAP in the target cell types. The promoters listed were either already isolated or were amplified using primers reported in prior publications.

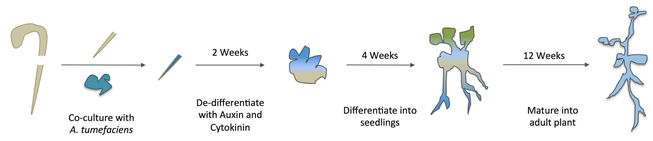
C) Medicago truncatula can be transformed by two different species of *Agrobacterium*. *A. rhizogenes* is used to transform the hairy roots. *A. tumefaciens* is used to transform leaf or root explants and establish stable lines through tissue culture.

METHODS

A) *A. rhizogenes* Transformation of Alfalfa Hairy Roots



B) *A. tumefaciens* Transformation of Alfalfa Root Explants



METHODS

A) Hairy Root Transformation of *M. truncatula* was done using *Agrobacterium rhizogenes* strain K599. Two day old *M. truncatula* seedlings were injected with a liquid culture of *A. rhizogenes* (OD_{600} = 1.0) that was suspended in water. The bacteria were allowed to culture in the roots for 2 days on Fahraeus Media (FM). The seedlings were then moved to selection FM media for 7 days. After the 7 days, the injected radicle was cut. Transformed hairy roots could be collected after 1-2 weeks. Live imaging was done using cut root tips. Dpg = days post germination.

B) Root Explant Transformation of *M. truncatula* was done using *Agrobacterium tumefaciens* strain EHA105. Two week old *M. truncatula* roots were cut into 1 cm sections and incubated with a liquid culture of *A. tumefiens* (OD_{600} = 0.5) for 30 minutes. The roots were incubated on nutrient plates, in the dark, for another 2 days. Transformed roots were de-differentiated, under selection, using the plant hormones Auxin and Cytokinin for two weeks. The formed calli were removed from Auxin and Cytokinin containing media and were allowed to differentiate into seedlings. Transgenic seedlings were planted into soil and grown to mature adults. T1 seeds were collected and propagated after genotyping.

RESULTS

Figure 1: *A. thaliana* Promoters Show Similar Localization in *M. truncatula*

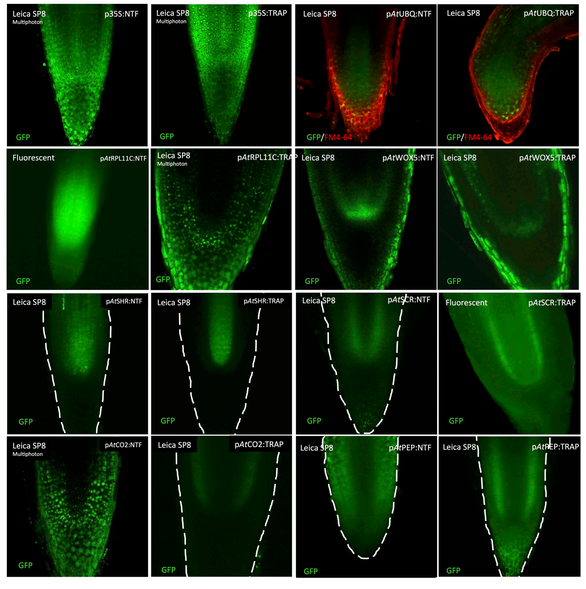


Figure 1
Fluorescence visualization of GFP was carried out using a fluorescent microscope and/or the Leica SP8 Scanning Laser Confocal Microscope. Images shown are at different magnifications. Some of the cut root tips were stained with 1g/L FM4-64 for 1 minute before being visualized with Leica SP8. FM4-64 is a cell membrane staining dye that fluoresces red. All of the *A. thaliana* promoters show the expected localization and expression in *M. truncatula*, except for p*At*WOX5. The *At*WOX5 promoter should only express in the 2 cells of the Quiescent Center. However, in *M. truncatula* this promoter has ectopic expression throughout the Endodermis, Cortex, and Stele.

Figure 2: Nuclei Isolation Using *M. truncatula Root* Tissue Yielded High Purity

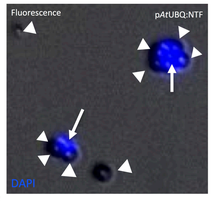


Figure 2
Nuclei isolation using INTACT had never been done in *M. truncatula*. We were able to isolate nuclei from p*At*UBQ:NTF and p*At*SCR:NTF transformed *Medicago truncatula* root tissues; 100,000 nuclei (93.02% purity) and 85,000 nuclei (91.89% purity), respectively. Purity was calculated as the ratio of magnetic bead bound nuclei over the total number of observed nuclei. Arrowhead = magnetic bead, arrow = nucleus. DAPI was used to stain the DNA.

Figure 3: Submerged Roots Experience Sufficient Flood Stress by 2 Hours

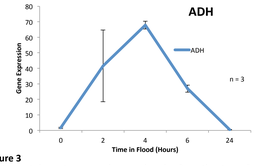


Figure 3
M. truncatula roots were submerged under 3 centimeters of water for the indicated time points. At each time point, 1 cm of root was cut from the tip. Each time point had 3 different samples. Reverse transcribed cDNA was quantified using qPCR. Ribosomal Protein subunit L2 (RPL2) was used as the endogenous reference. Alcohol dehydrogenase, which becomes upregulated under hypoxic conditions, was elevated as early as 2 hours. This time point will be used in future flood stress experiments. ADH = Alcohol dehydrogenase.

FUTURE DIRECTION

Figure 4: *Agrobacterium tumefaciens* Transformed Root Explants Have Yielded Adult Plants

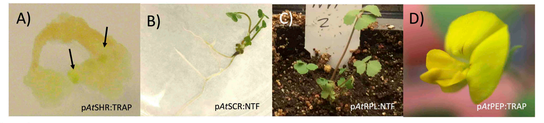


Figure 4
A) Callus obtained from *A. tumefaciens* transformed root explants. Arrows show green spots that will develop into seedlings. B) A seedling that has differentiated from a callus, ready to be planted. C) A planted seedling adjusting to soil. D) A flowering adult ready to form pods and produce seeds. T1 seeds have been collected from 6 lines. We are still working to establish the 10 remaining lines.

Current Projects

1. We are constructing a *At*UBQ:3XmCherry-SYP122 construct to visualize root cell membranes for imaging.
2. We are confirming ADH upregulation in 2 hour submerged plants, transformed with either *At*35S:NTF or *At*35S:TRAP.

Conclusions

1. Except for p*At*WOX5, all the other *Arabidopsis thaliana* promoters showed similar expression in *Medicago truncatula* roots, compared to *Arabidopsis thaliana* roots.
2. Nuclei isolation using INTACT was successfully performed in *M. truncatula*.
3. Two hours of flood stress is sufficient to elicit a stress response in *M. truncatula* roots.
4. *Agrobacterium tumefaciens* transformed calli are successfully developing into mature adults

References

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