# Understanding the Cell Type-Type Specific Response of Medicago Roots to Flood Stress

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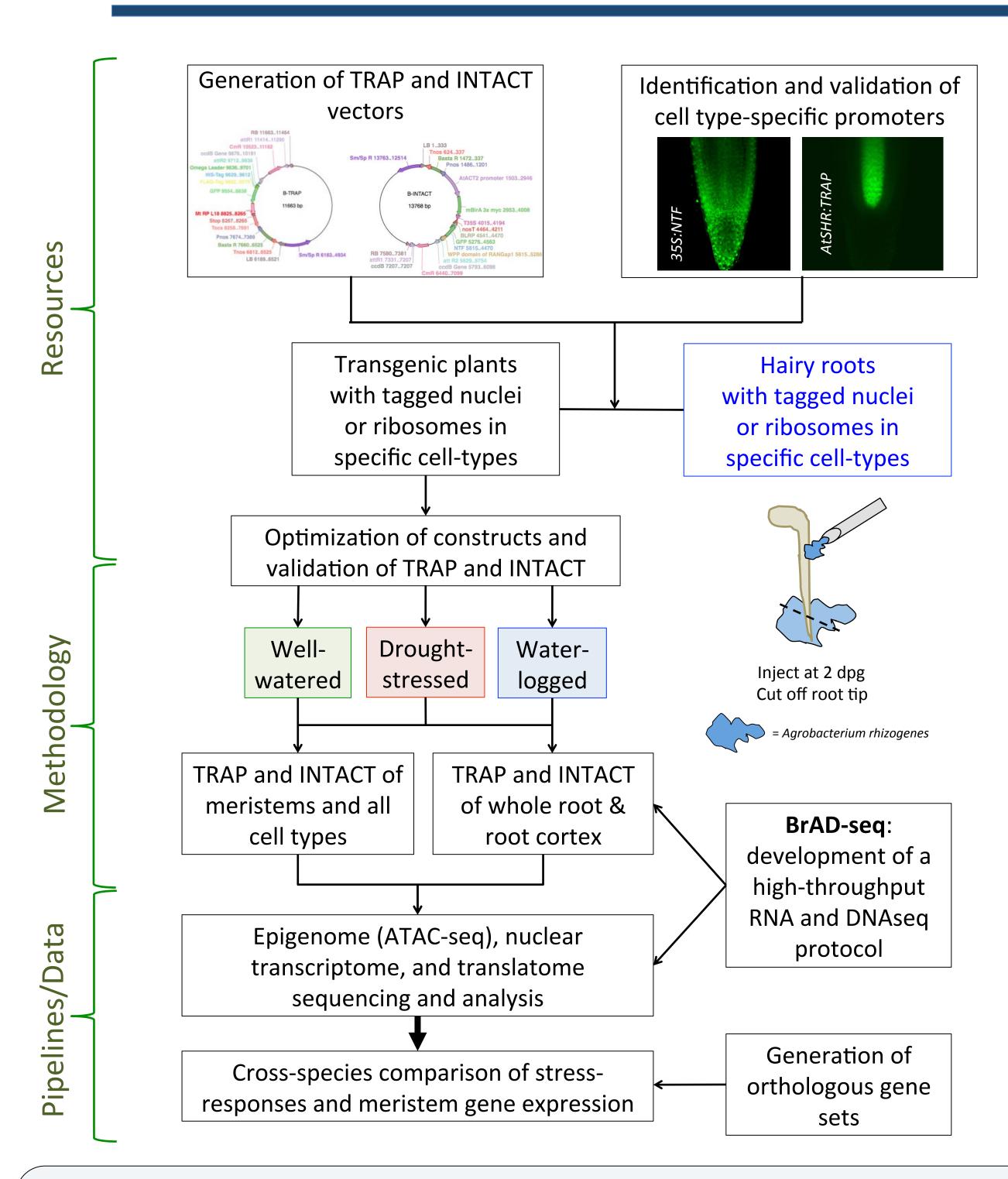
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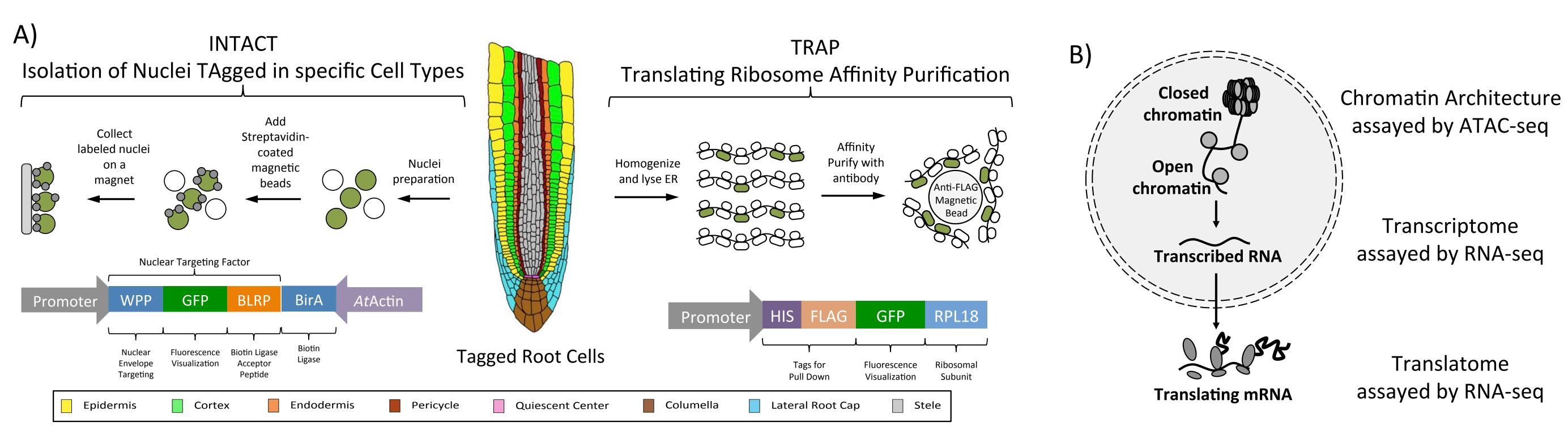
## Abstract

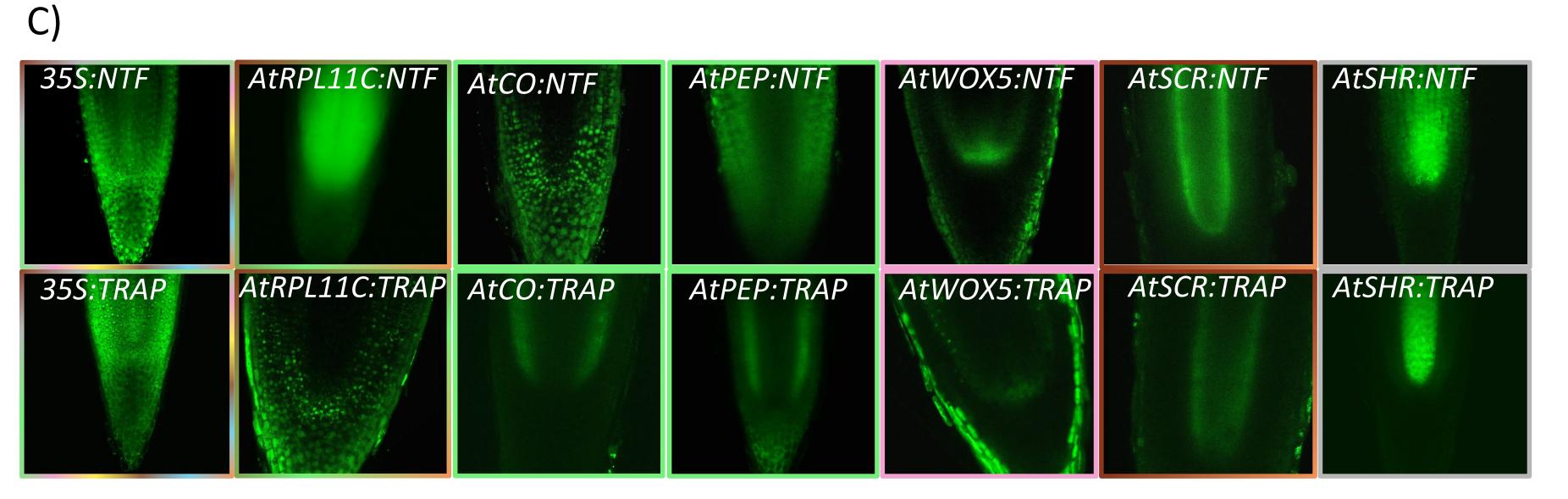
Plants are stationary organisms that must constantly respond to a changing environment in order to survive. Primary detection and response to flood stress occurs in the roots. However, how the specific cell types of the roots respond to these stresses is not completely understood. We have utilized two techniques, INTACT (Isolation of Nuclei TAgged in specific Cell Types) and TRAP (Tagged Ribosome Affinity Purification), to characterize the transcriptional and translational response of Medicago truncatula, alfalfa, roots to submergence stress. The nuclei and translating ribosomes are expressed in specific cell types of the root using Arabidopsis thaliana promoters. Our results show that all of the Arabidopsis promoters, with the exception of AtWOX5, had the same localized expression in *Medicago* truncatula as they do in Arabidopsis. We also show that INTACT and TRAP can be performed using *Medicago* tissue. We are currently characterizing the response of alfalfa root cells to 2 hours of submergence stress. Our results will be compared to parallel experiments performed in tomato and rice in order to identify conserved genes involved in flood stress response in crops. The long-term goal of this research is to establish a comprehensive understanding of drought and flood stress response in crops and to use this information to develop hardier crops.

## **Long Term Project Goals**



## Establishment of INTACT and TRAP lines in M. truncatula for isolation of nuclei and ribosomes from specific cell types





D) 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.

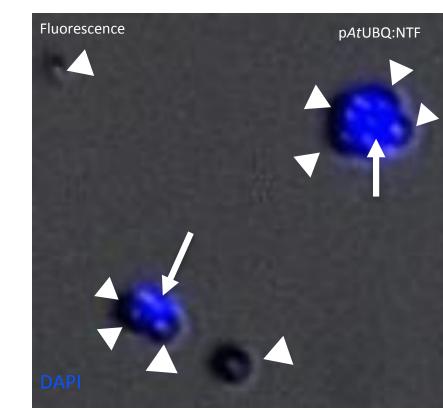
**Target Cell Type** Promoter Cauliflower mosaic virus 35S (35S) **Near Constitutive** Root proliferating cells Ribosomal Protein L11C (RPL11C) SCARECROW (SCR) Root endodermis, quiescent center SHORTROOT (SHR) Root vasculature Cortex specific transcript (CO) Root cortex meristematic zone Root cortex elongation and Endopeptidase (PEP) maturation zone WUSHEL-related homeobox 5 (WOX5) Quiescent center

- 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. Under each cartoon depiction is a schematic depicting the transformed T-DNA that allows for each construct to be expressed in specific cell types.

  B) From chromatin to translation, the molecular response of specific cells to stimuli can be studied in great detail by isolating nuclei and ribosomes using INTACT and TRAP, respectively. We are utilizing ATAC-seq (Assay for Transposase-Accessible Chromatin) to determine chromatin accessibility, and RNA sequencing for
- RNA isolated from nuclei and ribosomes to determine the transcriptional and translational response in the specific cell types.

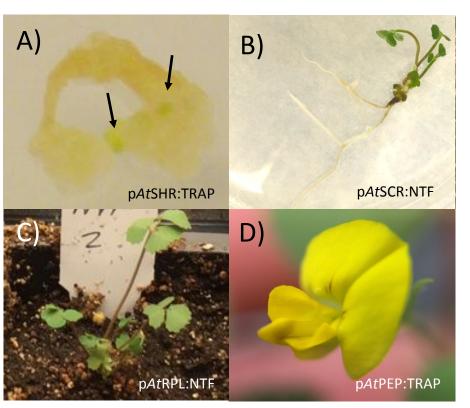
  C) Fluorescence visualization of GFAP was carried out using a fluorescent microscope and/or the Leica SP8 Scanning Laser Confocal Microscope. . Images shown are at different magnifications. All of the A. thaliana promoters show the expected localization and expression in M. truncatula, except for pAtWOX5. The AtWOX5 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. Two lines imaged (pAtRPL11C:TRAP and pAtCO2:NTF) appear to be untransformed roots.

## Figure 1: Nuclei Isolation Using *M. truncatula Root*Tissue Yielded High Purity



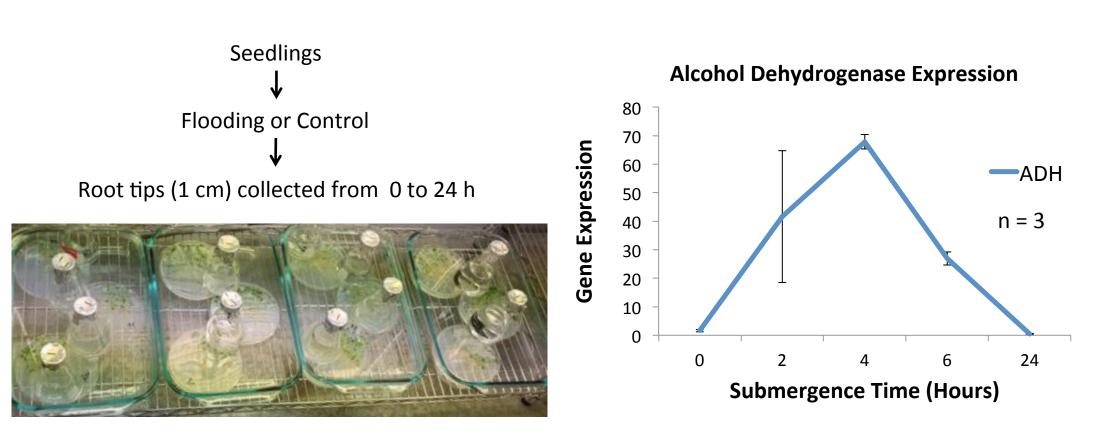
**Figure 1:** Nuclei isolation was performed using *pAt*UBQ:NTF hairy root transformed tissue. a total of 100,000 nuclei were isolated with 9302% purity. Purity was calculated as the ratio of magnetic bead bound nuclei over the total number of observed nuclei. Arrowhead = magnetic bead, arrow = nucleus. DAPI was used to stain the DNA.

## Figure 2: Agrobacterium tumefaciens Transformed Root Explants Have Yielded Adult Plants



**Figure 2: 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.

## Figure 3: Validation of Common Submergence Conditions in M. truncatula



**Figure 3:** One-month-old *M. truncatula* seedlings were submerged under 3 centimeters of water for 0 to 24 hours. At each time point, 1 cm root tips were collected from 3 different plants. Alcohol dehydrogenase (*ADH*) mRNA levels, encoding an enzyme required for anaerobic metabolism, were quantified by qRT-PCR. Values were normalized to Ribosomal Protein Subunit L2 (*RPL2*). **The 2 hour time point was selected for inter-species comparison** of nuclear transcriptome, translatome and epigenetic regulation affected by the stress.

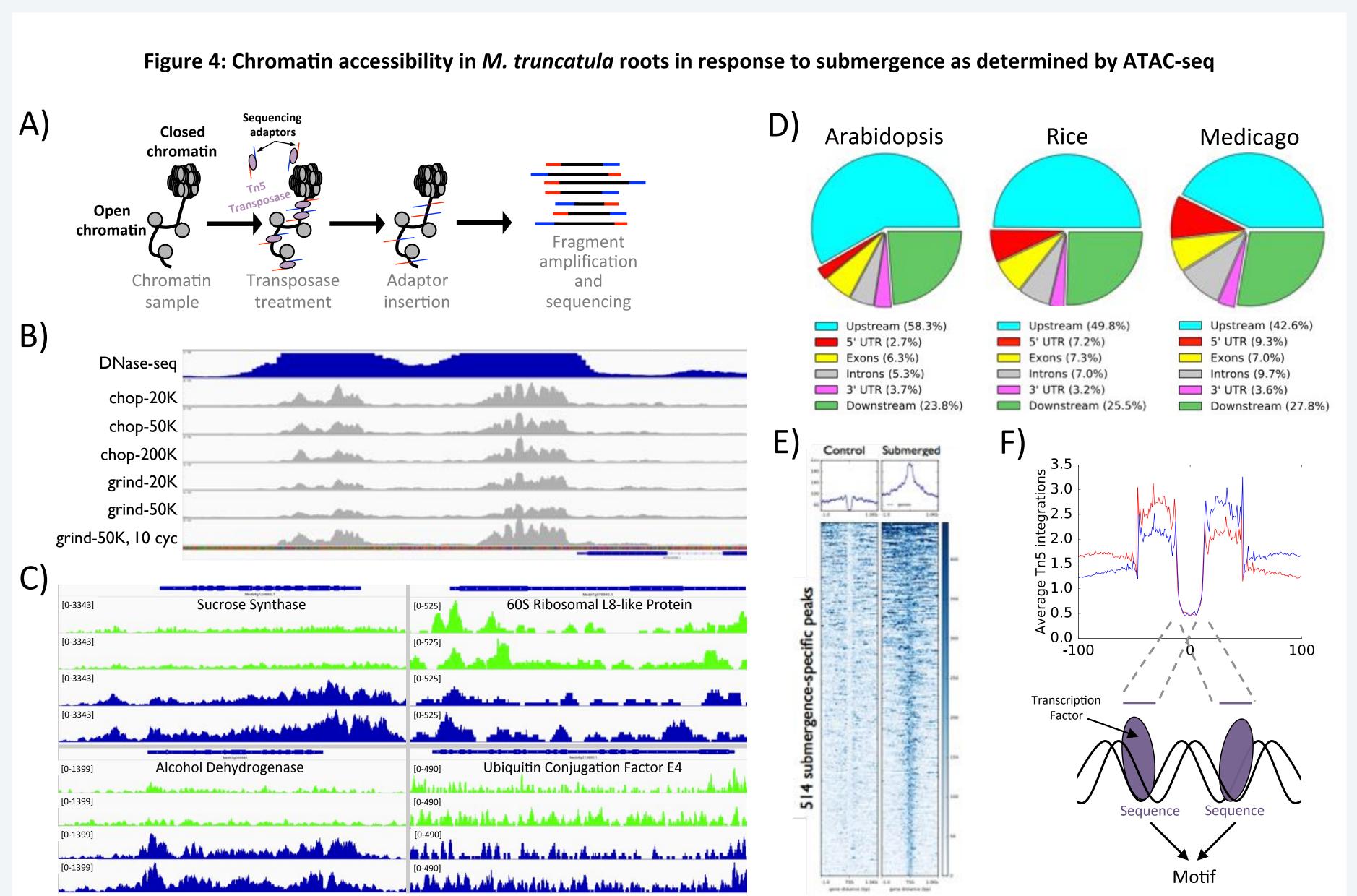


Figure 4: A) The hyperactive Tn5 transposase (violet) loaded with sequencing adapters (blue and red), inserts only in open chromatin regions (between gray nucleosomes) and generates sequencing library fragments that can be PCR-amplified. **B)** In *Arabidopsis* thaliana, 20,000 nuclei isolated from root epidermal cells are sufficient to make sequencing libraries with deep coverage. Our results were similar to previously reported DNasel hypersensitivity sequencing done using nuclei from the same root cell type. Additionally, we saw no difference in sequencing between libraries made from freshly chopped tissue and tissue that was frozen and the ground to a powder. C) Libraries were prepared from 35S:NTF transformed hairy roots by collecting 1 cm root tips from plants that were either submerged for 2 hours (submergence stress) or grown under standard conditions for 2 (control). All four libraries shown had an average of 30 million reads. Chromatin accessibility is shown for four different genes. Open chromatin is expected for genes that are upregulated in response to submergence stress, such as Sucrose Synthase and Alcohol Dehydrogenase). Chromatin accessibility between the two sets of libraries should be the same in genes that do not respond to submergence stress, such as 60S Ribosomal L8-like Protein and Ubiquitin Conjugation Factor E4. Control libraries are shown in green and libraries from submerged samples are shown in blue. D) ATAC-seq peaks were called with HOMER (Hypergeometric Optimization of Motif EnRichment) for the Arabidopsis, Rice, and Medicago. The shown pie charts indicate peak distribution throughout different regions of the genome under control conditions. E) In Medicago, peaks located near or within gene bodies were isolated for analysis. Out of these, 514 peaks that are not found in control conditions are called after 2 hours of submergence. These peaks are found within genes that have Gene Ontology terms associated with signaling and transcription factor activity. F) Within the 35,000 called "peak regions" in Medicago, 60,000 footprints were identified using pyDNase. The shown figure was made using all the Medicago libraries pooled together (~300 million reads). Blue line indicates + strand and

red line indicates - strand. These footprints occur

within open chromatin regions where proteins are

bound to DNA. Future analysis includes identifying

sequence motifs found within these footprints.

## Figure 5: Transcriptional response in Medicago in response to subergence **RNA Yield** Transformation Nuclei Isolated (ng) Injected Selection Efficiency 130,000 52 122,500 79 % 52 57,000 37 290,000 160,000 Closed chromatin Transcribed RNA

Figure 5: A) The submergence experiment was repeated at least 5 times. The injection protocol allows for efficient hairy root transformation that produces lots of transformed hairy roots in *Medicago* in 2-3 weeks. However, around 50% of the injected plants do not survive selection. Out of the survivors, around 80% of their hairy roots are transformed. The transformation efficiency is measured by collecting root tips and measuring how many of the have GFP signal. RNA was quantified using a spectrophotometer. On average, 0.44 picograms of RNA can be isolated from one nucleus. B) Sequencing results were compared between Medicago, Rice, Tomato, and Arabidopsis for genes that show upregulated transcription in response to submergence stress. Around 400 genes are upregulated in Medicago. These genes have Gene Ontology terms associated with metabolism, signaling, and transcription factor activity. C) At each level of gene regulation (chromatin accessibility, gene transcription, mRNA translation) there are three potential responses to submergence stress: Increase, No Change, and Decrease. We are currently analyzing our sequencing results and characterizing the different genes that fall into these different categories.

## Current and Future Work

- 1. Isolate total and translating RNA from 35S:TRAP plants that
- have been stressed with 2 hours of submergence.

  2. Sequence nuclear RNA and total and translating RNA from
- TRAP and compare.Identify the locations and percentage breakdown of chromatin regions that "open" in response to submergence
- stress.

  L. Compare ATAC and RNA-seq data to determine which open
- regions are transcribed, and which of these transcribed regions are translated.
- 5. Compare results to rice and tomato.

## Conclusions

- 1. Except for pAtWOX5, 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. ATAC-seq can be performed with 20,000 nuclei from either freshly chopped or frozen and ground root tissue.
- 5. Fifteen percent of called peaks are unique to submergence stress.