

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

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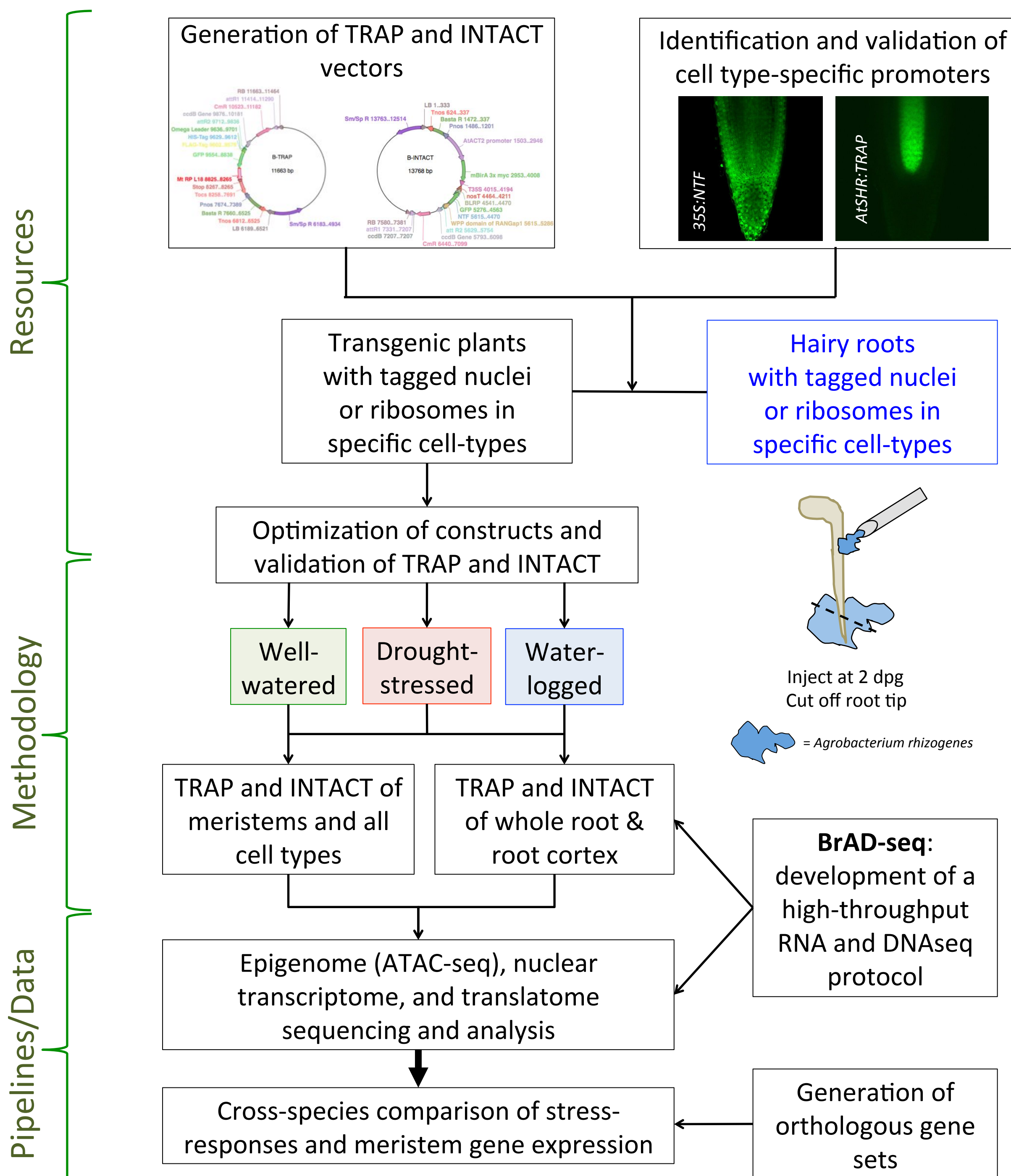


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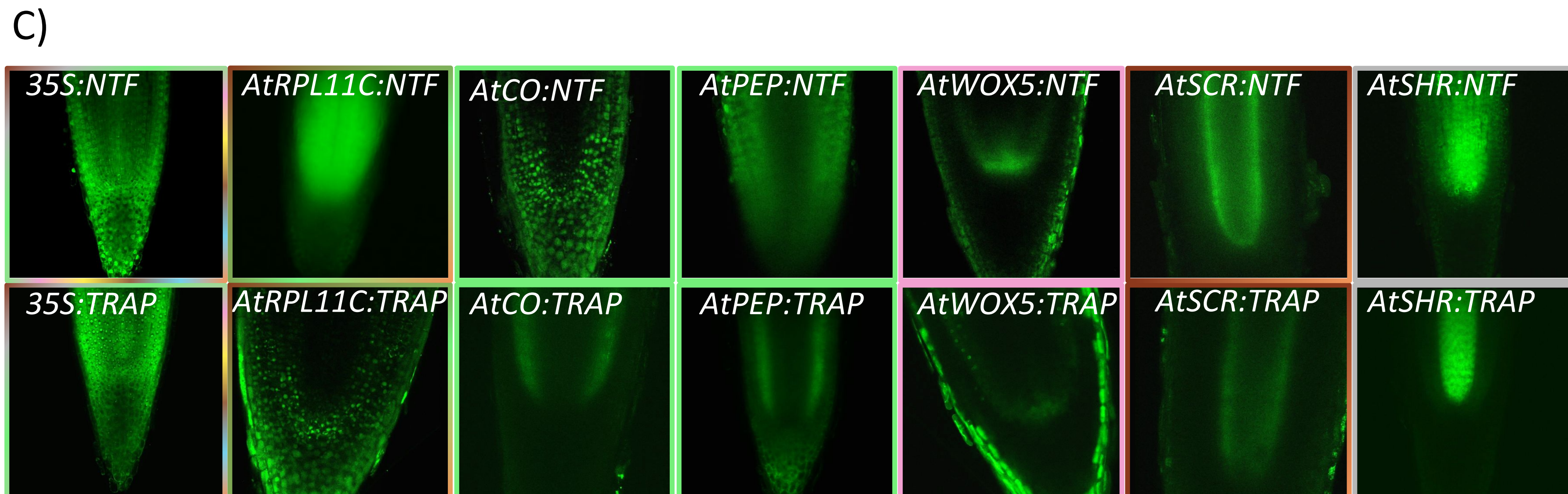
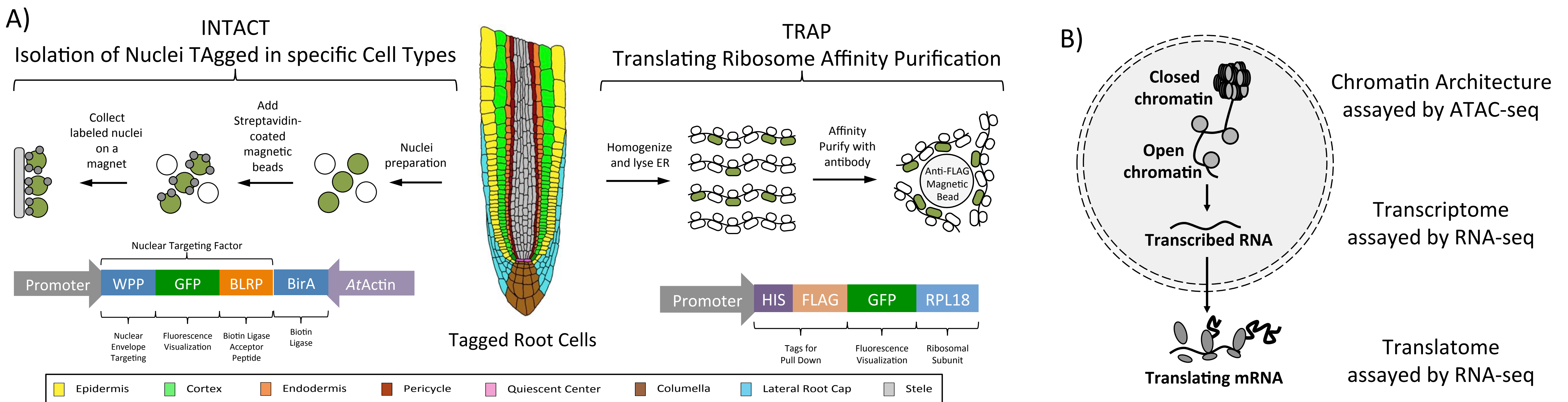
Abstract

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



C) 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 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 GFP 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.
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.

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

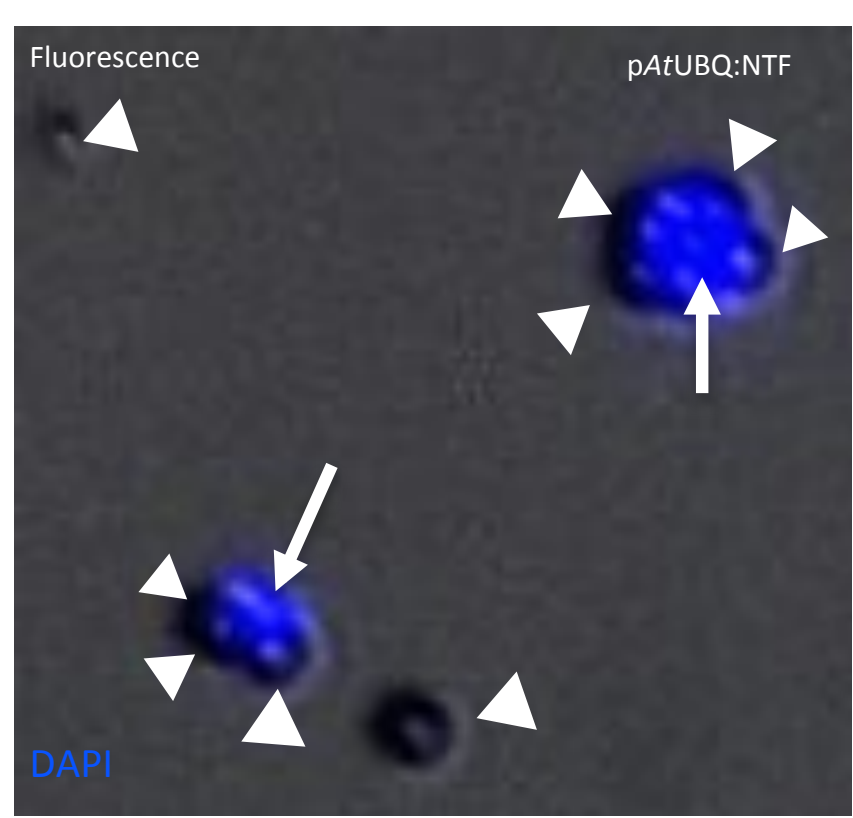


Figure 1: Nuclei isolation was performed using pAtUBQ:NTF hairy root transformed tissue. A total of 100,000 nuclei were isolated with 9302% purity. Purity was calculated as the ratio of magnetic bead bound nuclei over the total number of observed nuclei. Arrowhead = magnetic bead, arrow = nucleus. DAPI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

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

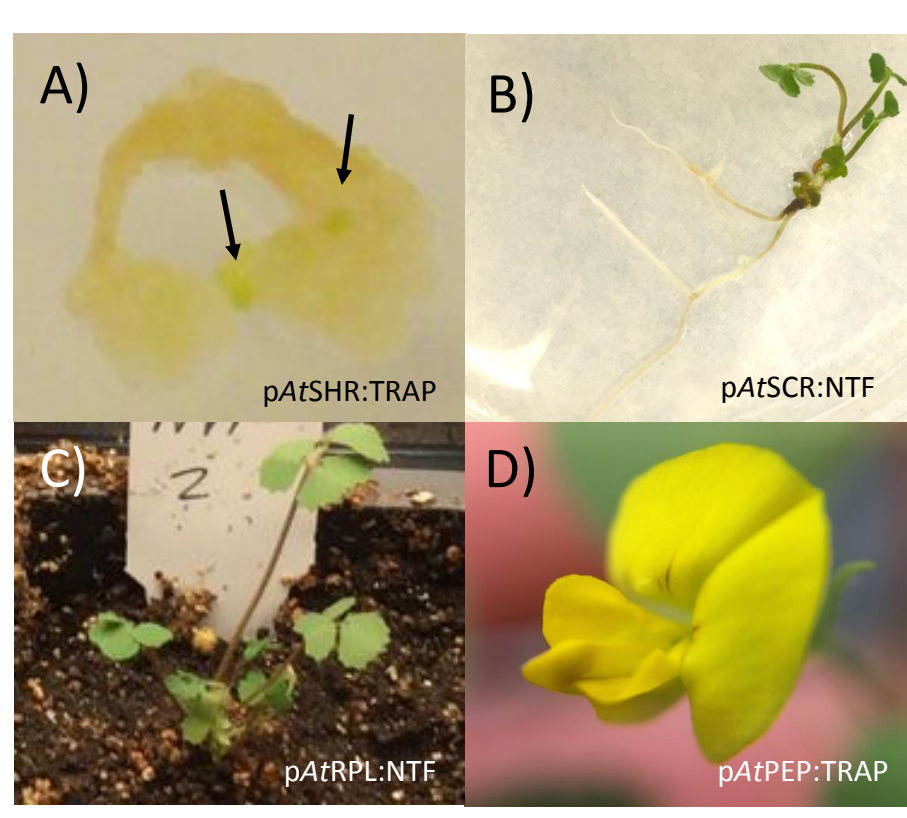


Figure 2: A) Callus from *A. tumefaciens* transformed root explants. Arrows show green spots that will develop into seedlings. **B)** A seedling that has differentiated from a callus, ready to be planted. **C)** A planted seedling adjusting to soil. **D)** A flowering adult ready to form pods and produce 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*

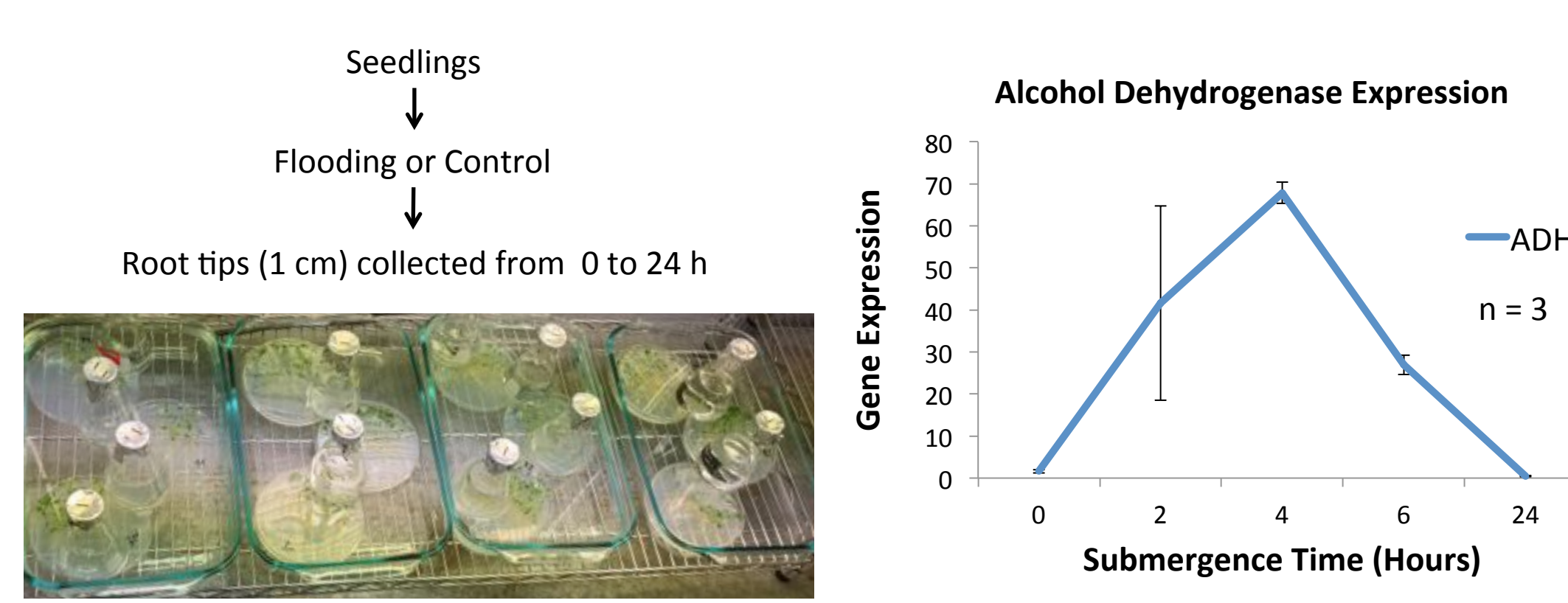
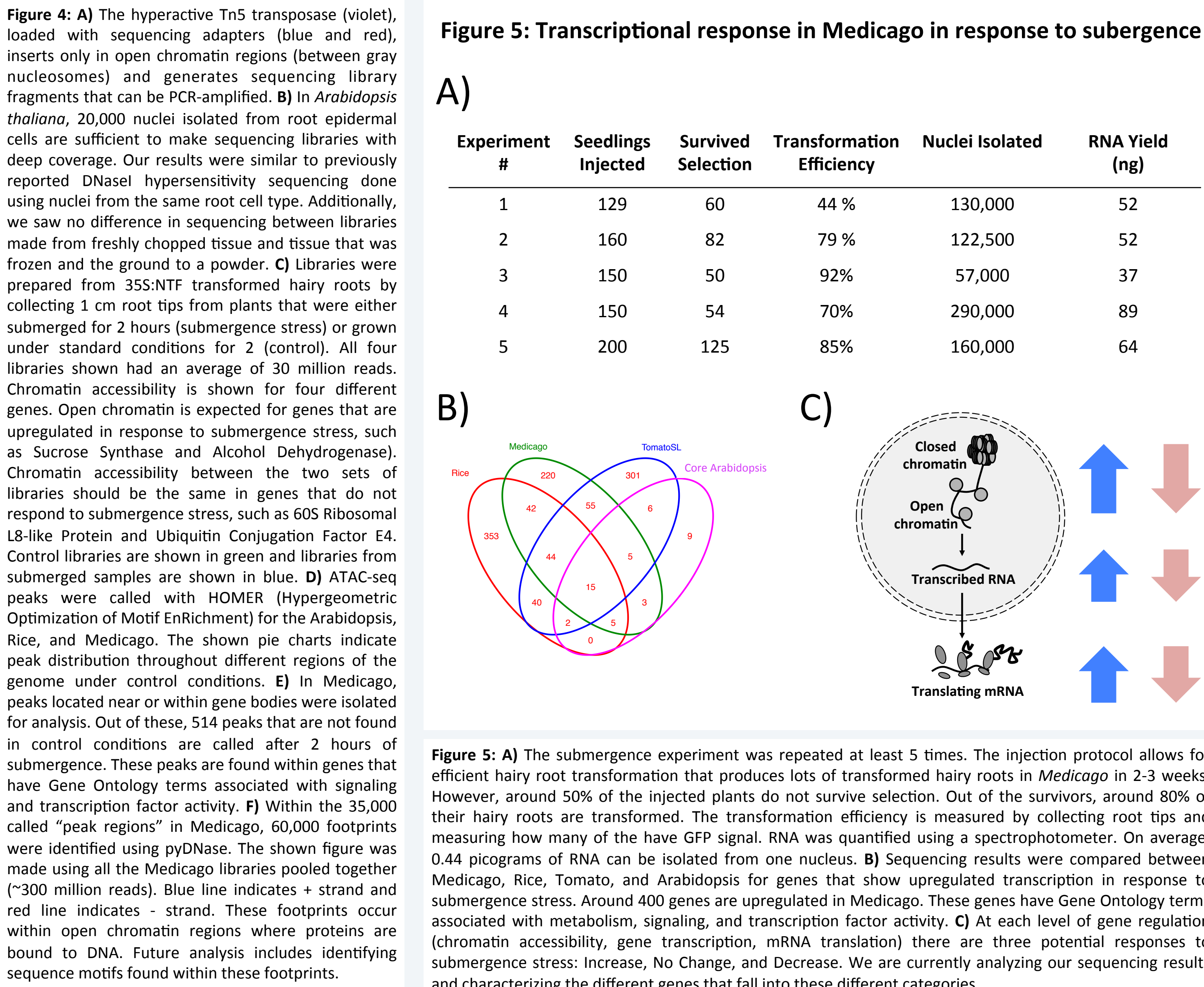
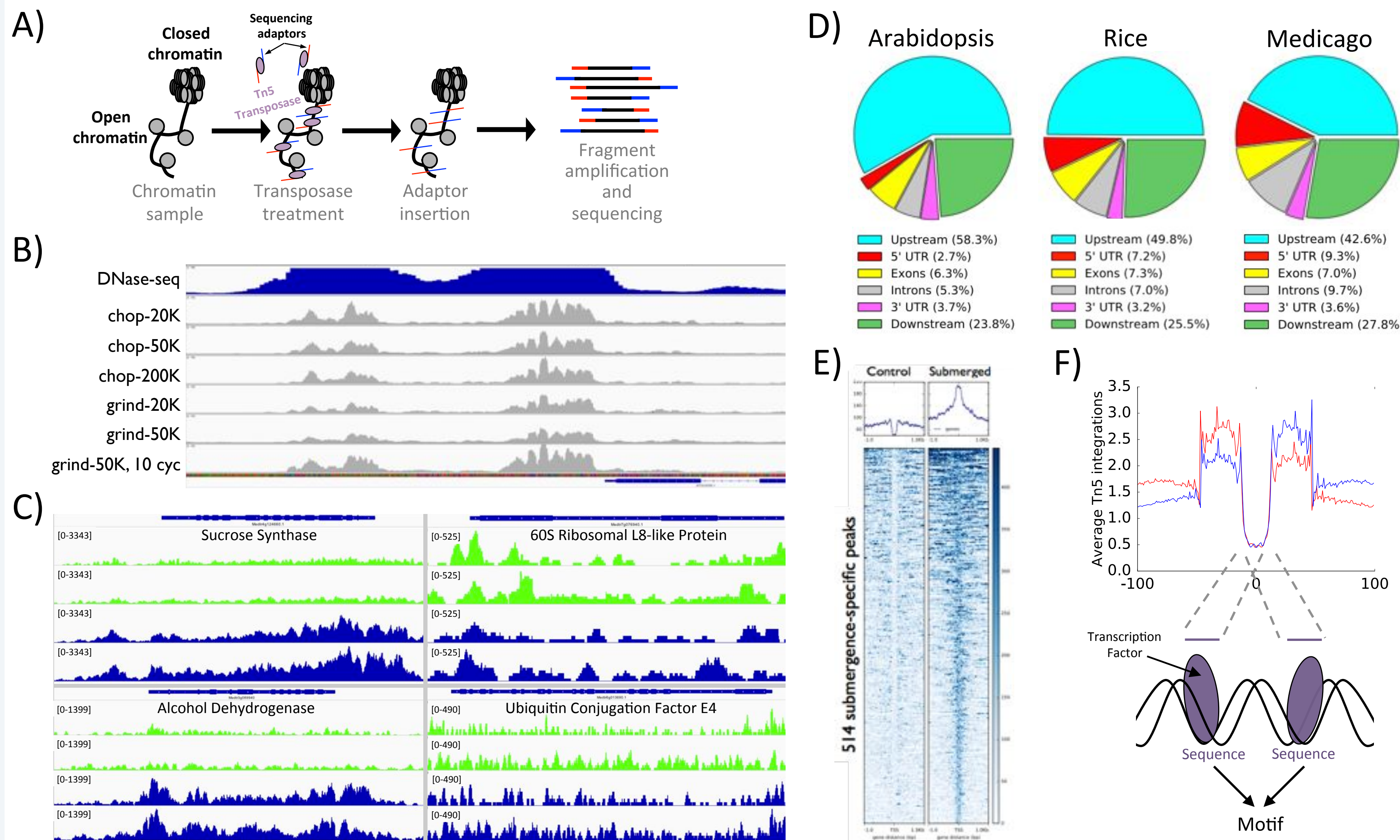


Figure 3: One-month-old *M. truncatula* seedlings were submerged under 3 centimeters of water for 0 to 24 hours. At each time point, 1 cm root tips were collected from 3 different plants. Alcohol dehydrogenase (ADH) mRNA levels, encoding an enzyme required for anaerobic metabolism, were quantified by 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: Chromatin accessibility in *M. truncatula* roots in response to submergence as determined by ATAC-seq



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.
3. Identify the locations and percentage breakdown of chromatin regions that "open" in response to submergence stress.
4. 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.