New Tools for Evaluation of Cell-Specific Environmental Plasticity in Rice, Tomato and Medicago

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Abstract

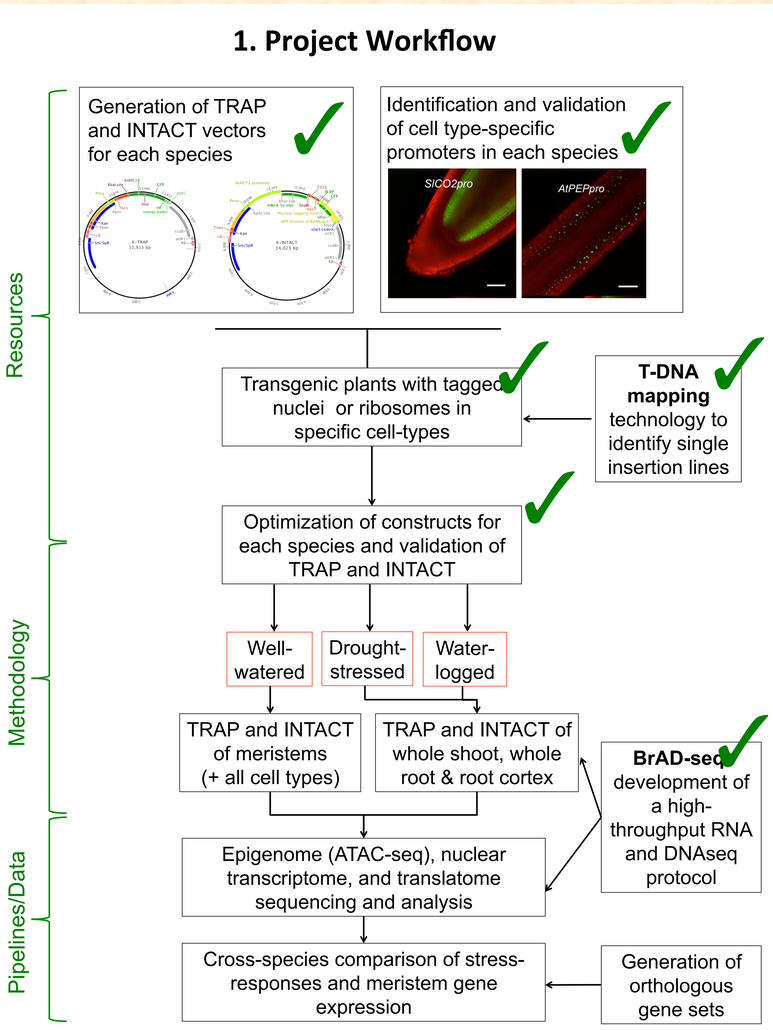
From seed germination to ovule fertilization, plant development is exquisitely orchestrated by genetic processes that are fine-tuned by environmental cues. This plasticity entails the precise regulation of networks of genes in individual cells. Of all the stresses experienced by crops, extremes in water are particularly damaging to crop yield stability. We are asking: **How does gene activity in stem cells (meristems) of roots and shoots differ across species? How do flooding and drought stresses influence the development of specialized cell types in the root?**

To address these questions, we have refined the **INTACT** (Isolation of nuclei tagged in specific cell types) and **TRAP** (Tagged ribosome affinity purification) technologies that enable examination of the epigenome, transcriptome, and translatome of specific cell types.

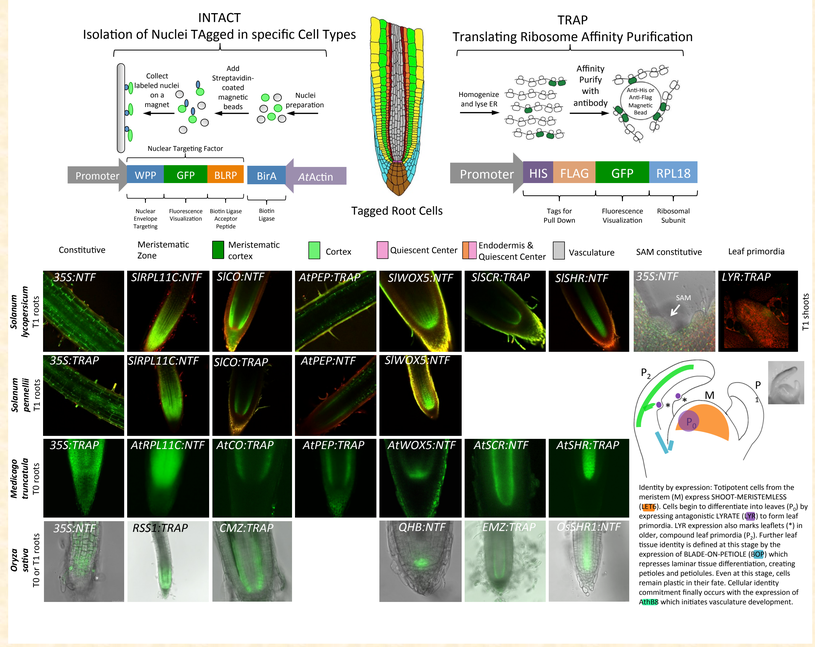
Our challenges and successes have been:

- To translate these systems from Arabidopsis to other dicots
- To establish *Agrobacterium rhizhogenes*-promoted hairy roots in tomato and Medicago
- To identify meristem and root cell-specific promoters
- To optimize INTACT in a monocot
- To integrate INTACT with "tagmentation" (ATAC-seq)
- To advance nuclear RNA, mRNA and ribosome footprint library construction with limited rRNA contamination
- To establish pipelines for data analysis

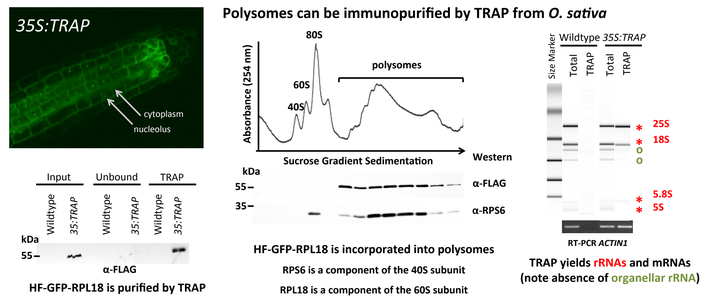
1. Project Workflow



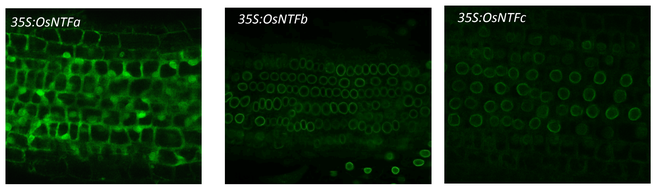
2. Establishment of INTACT and TRAP lines in four species



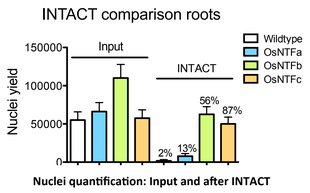
3. Purification of nuclei by INTACT and polysomes by TRAP as illustrated in rice



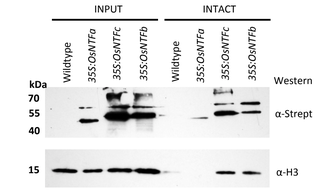
INTACT isolation of nuclei - optimized for rice



Expression of different Nuclear Tagging Fusion (NTF) proteins to capture nuclei

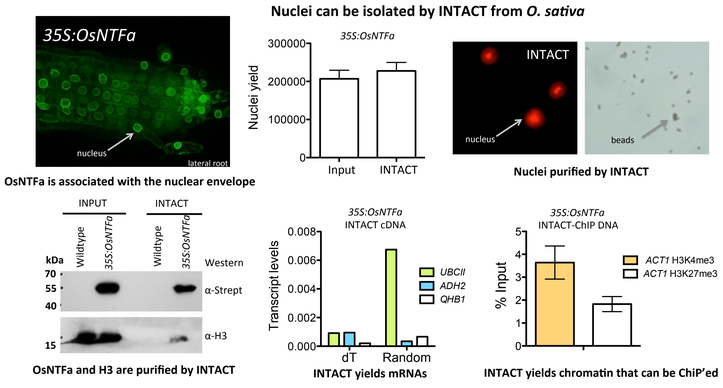


Nuclei quantification: Input and after INTACT



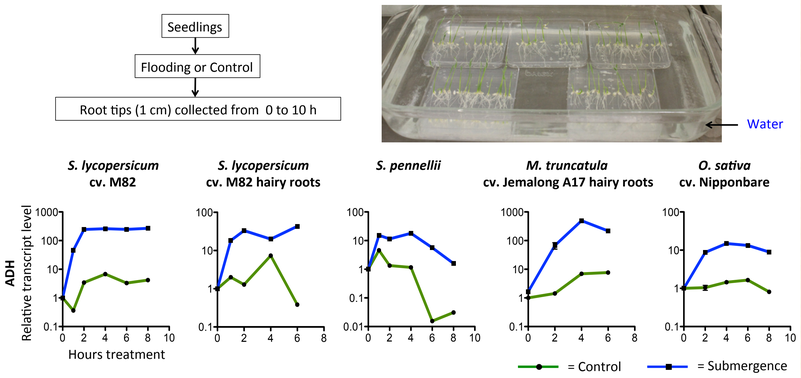
Histone H3 is present in captured nuclei

Nuclei can be isolated by INTACT from *O. sativa*



INTACT and TRAP was also established by our project team for tomato (Ron et al., Plant Phys 2014) and Medicago (Reynoso et al., 2012 and unpublished data by Bajic et al., Emory U)

4. Validation of common submergence conditions in seedlings for three crop species

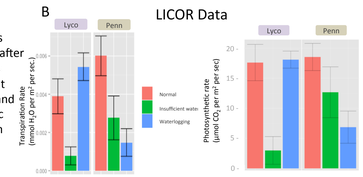


Alcohol dehydrogenase (*ADH*) mRNA levels, encoding an enzyme required for anaerobic metabolism, were quantified by qRT-PCR. Values were normalized to *ACT2* mRNA levels for *S. lycopersicum* and *S. penelli*, *RPL2* for *M. truncatula*, and *UBCII* for *O. sativa*. **The 2 hour time point was selected for inter-species comparison** of nuclear transcriptome, translatome and epigenetic regulation affected by the stress.

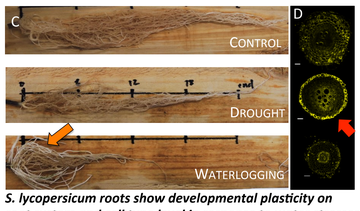
5. Plasticity of development in response to drought and waterlogging as illustrated in tomato and rice

Shoot responses under stresses

A. Tomato shoots experience obvious turgor loss and chlorosis as well as premature loss of leaves.



B. LICOR measurements taken 12 days after onset of stress reveal different transpiration and photosynthetic levels between species and stresses.

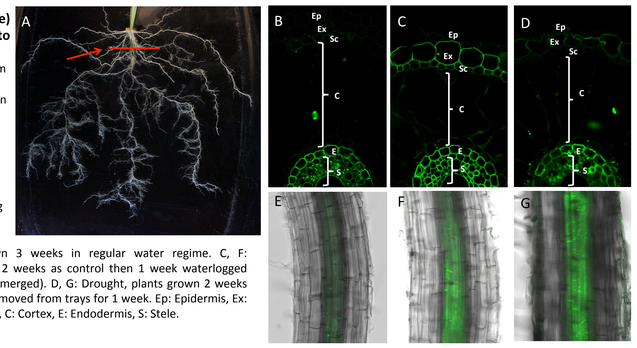


***S. lycopersicum* roots show developmental plasticity on root system and cell type level in response to water stresses**

C. Total root biomass is not affected by 12-day stress, but biomass shifts to the surface due to growth of hypocotyl-derived roots in response to waterlogging (orange arrow).

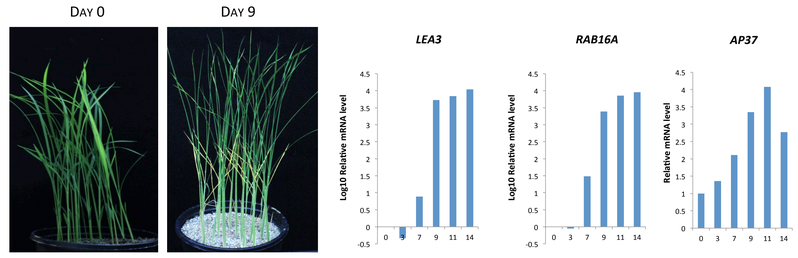
D. Staining root sections for suberin with fluorol yellow shows that in drought, suberin accumulates in cell type-specific fashion in exodermis (red arrow), waterproofing the root.

***O. sativa* (cv. Nipponbare) root system response to water extreme**



A: 3 week-old rice root system
B, C, D: Primary roots
Exodermis suberization shown by Fluorol Yellow stained transection (2 cm from the root / shoot junction (red arrow)) (false color green).
E, F, G: Lateral root stained with Yellow Fluorol show enhanced suberization during drought and waterlogging.

B, E: Control, plants grown 3 weeks in regular water regime. C, F: Waterlogging, plants grown 2 weeks as control then 1 week waterlogged (root system completely submerged). D, G: Drought, plants grown 2 weeks as control then water was removed from trays for 1 week. Ep: Epidermis, Ex: Exodermis, Sc: Sclerenchyma, C: Cortex, E: Endodermis, S: Stele.



Shoot drought time course experiment

Plants were grown in pots for 2 weeks and watered every day until Day 0 when water was removed from trays. Rolled up leaves were observed after 8 days without water. qRT-PCR analysis of three drought molecular markers in rice shoots at different time points. *LEA3* and *RAB16A* are highly induced after 7 days without water.

6. Future Work