Enhanced Resolution of Plasticity to Environmental Stimuli in Meristems and Individual Root Cell Types of Rice

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Abstract

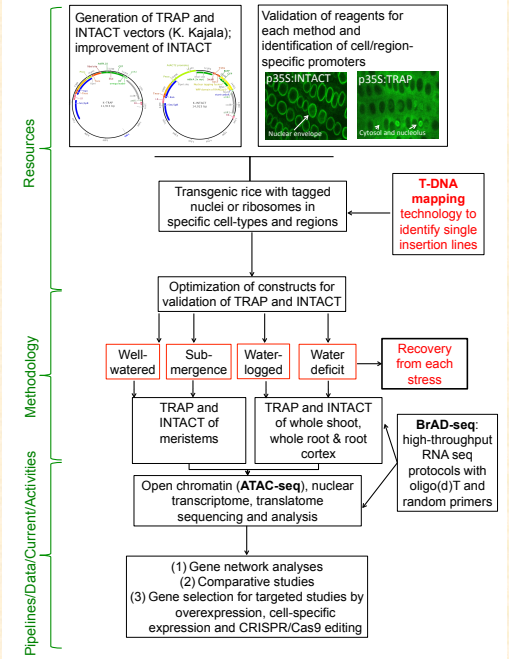
Too little and too much water due to climatic events is a significant cause of global food insecurity. Crops are less productive under water-limited conditions and all major crops, with the exception of rice (*Oryza sativa*), die within a few days of flooding. To complement our analyses of the key submergence-tolerance gene SUB1A, an ERF-VII transcription factor, we retooled **INTACT** (Isolation of Nuclei TAgged in specific Cell Types) and **TRAP** (Translating Ribosome Affinity Purification) for rice. **ATAC**-seq (Assay for Transposase Accessible Chromatin) was coupled with INTACT to monitor chromatin. We used these technologies to monitor responses to submergence, waterlogging and water deficit, and the return to homeostasis. The data uncover multiple layers of reversible chromatin and mRNA regulation including nuclear retention, turnover, alternative splicing, translation and *trans*-regulation by non-coding RNAs.

We are asking: **How does gene activity in stem cells (meristems) of roots and shoots differ? How do flooding and water-deficit stress influence plasticity of specialized cell types in the root?**

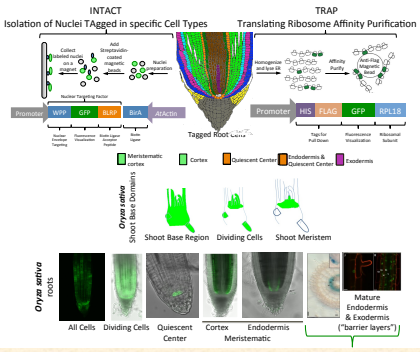
Our challenges and successes have been:

- To optimize TRAP and INTACT in rice
- To identify cell-specific promoter for certain root cell types
- To integrate INTACT with "tagmentation" (ATAC-seq)
- To limit rRNA and organelle contamination in nuclear RNA and ribosome footprint libraries.
- To establish pipelines for multi-scale data analysis

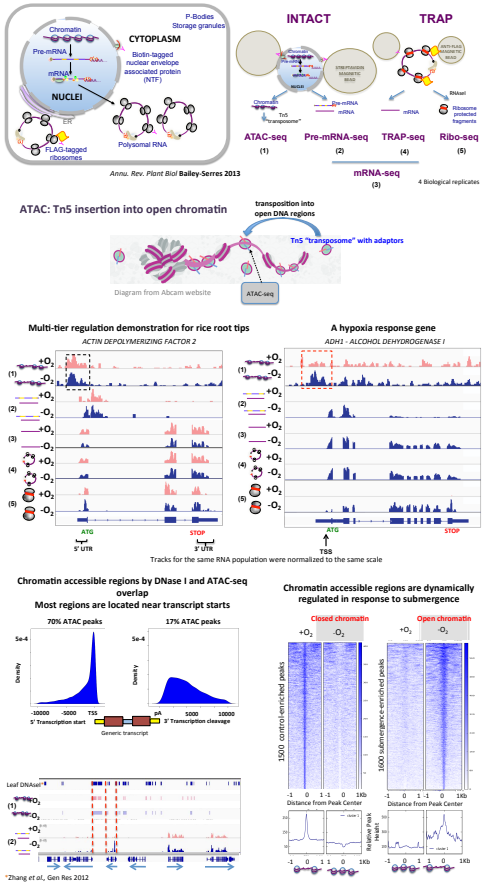
1. Project Workflow



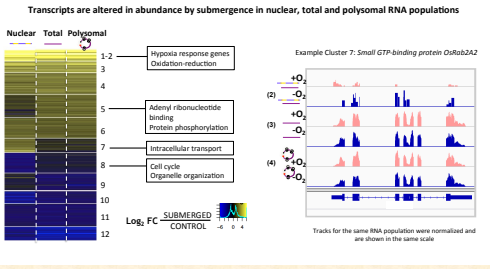
2. Establishment of INTACT and TRAP lines in rice



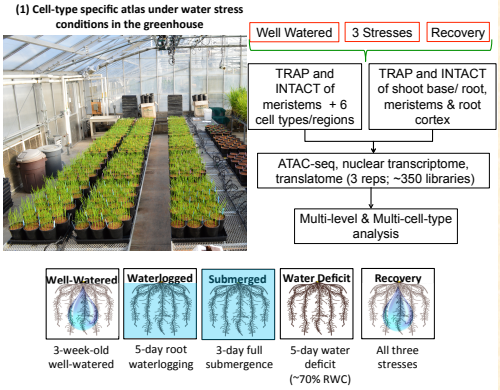
3. Open chromatin, nuclear RNA and polysomal mRNA analyses in root meristematic regions under submergence



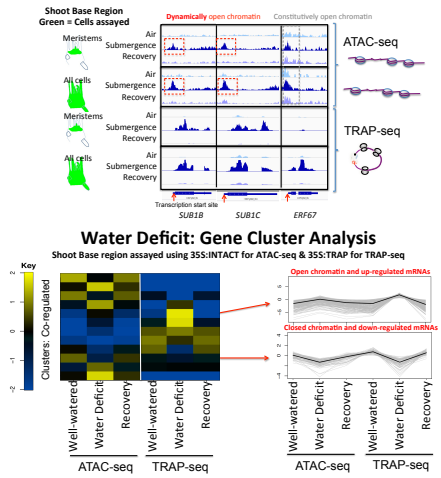
4. Comparison of nuclear RNA, total and polysomal mRNAs demonstrates nuclear retention of some RNAs



5. Plasticity to water extremes in plants grown in Profile®



6. Examples: Chromatin Accessibility versus TRAP'ed mRNA Submergence: *SUB1A*-related ERF-VIIs



Water Deficit: Gene Cluster Analysis