It's a TRAP! Confirming Efficiency of Rice Lines Developed for Cell-type Specific Gene Expression Analysis

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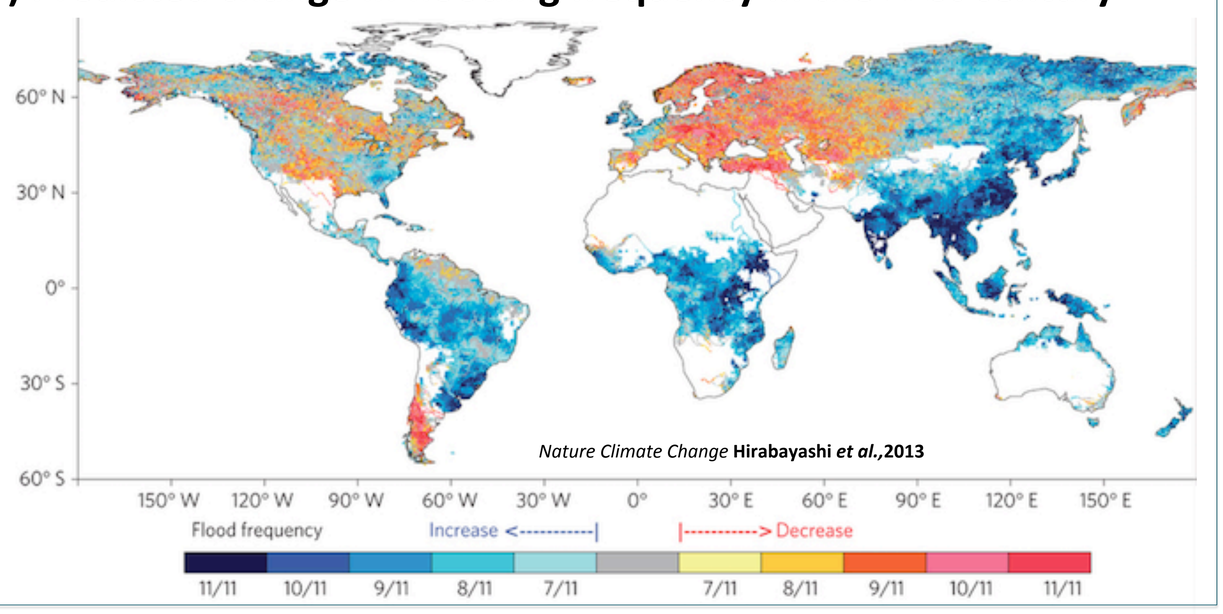
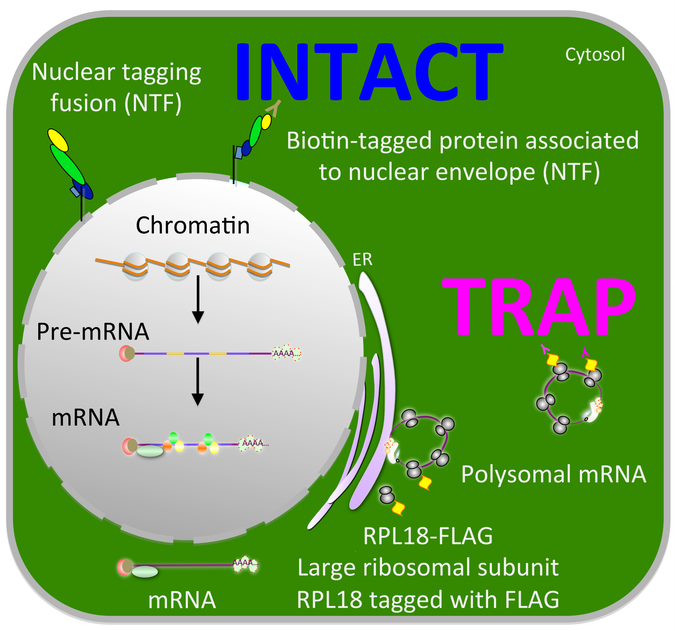
(1) Introduction

- Rice (***Oryza sativa***) is an important global crop species threatened by the unpredictability of future floods and droughts brought on by global **climate change**
- **INTACT** and **TRAP** technologies coupled with bioinformatic analysis tools allow for **cell-type specific gene expression analysis** (Bailey-Serres, 2013)
- Transgenic rice lines developed to express the INTACT and TRAP constructs driven by **cell-type specific promoters** facilitate access to nuclear or polysome associated **mRNA populations** from specific cell-types
- In this pilot study we evaluate the efficiency of TRAP and INTACT rice lines developed for cell-type specific gene expression analysis.
- Here we interrogate a preliminary **NGS** (next generation sequencing) dataset generated from TRAP and INTACT rice plants (3 week-old) in order to evaluate cell-type specific gene expression in response to water stress

(2) Predicted change in flooding frequency in the 21st century



(3) TRAP and INTACT Technologies



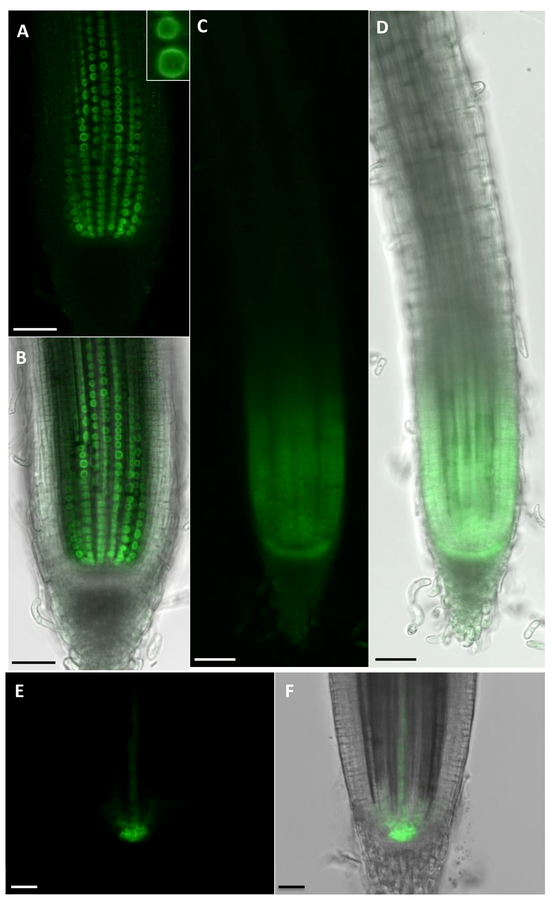
Isolation of Nuclei Tagged in Specific Cell Types (INTACT)

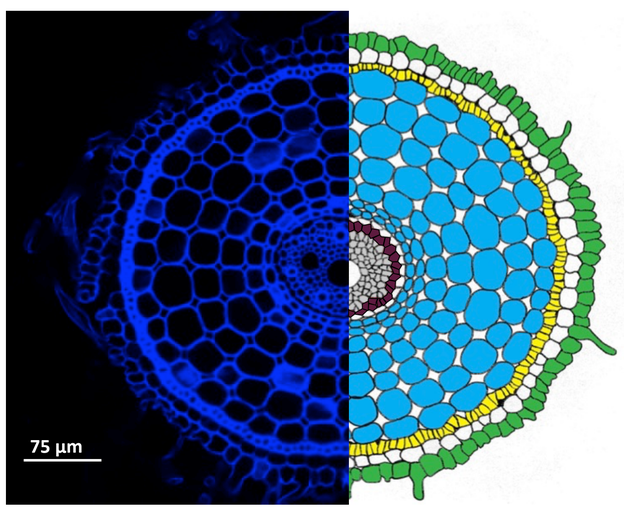
- Access to nuclear chromatin and pre-mRNA
- Isolation of nuclei from specific cell types
- INTACT construct makes this possible

Translating Ribosome Affinity Purification (TRAP)

- Access to polysomal mRNA
- Isolation of translating ribosomes from specific cell types
- TRAP construct makes this possible

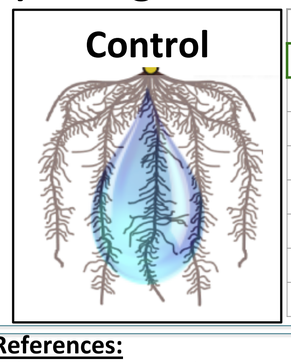
(4) TRAP and INTACT rice lines



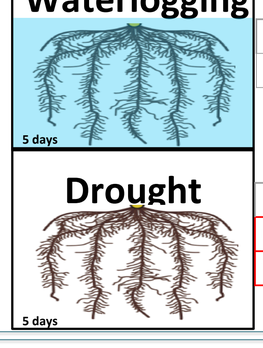


1. TRAP and INTACT transgenic rice line development.
GFP localization in embryonic nodal roots (7 day-old seedlings). **A-B**: Cortex INTACT line. Close up of the root tip (focused on the last cortical cell layer) shows the localization of the NTF (Nuclear Tagging Fusion) to the nuclear envelope. **C-D**: TRAP *RSS1* (*RICE SALT SENSITIVE 1*) promoter to target actively dividing cells. **E-F**: INTACT QHB1 (Quiescent Center Specific Homeobox) promoter to target quiescent center, GFP is localized in the quiescent center: Scale: A-D: 50 µm and E-F: 25 µm

(5)Transgenic rice line treatments



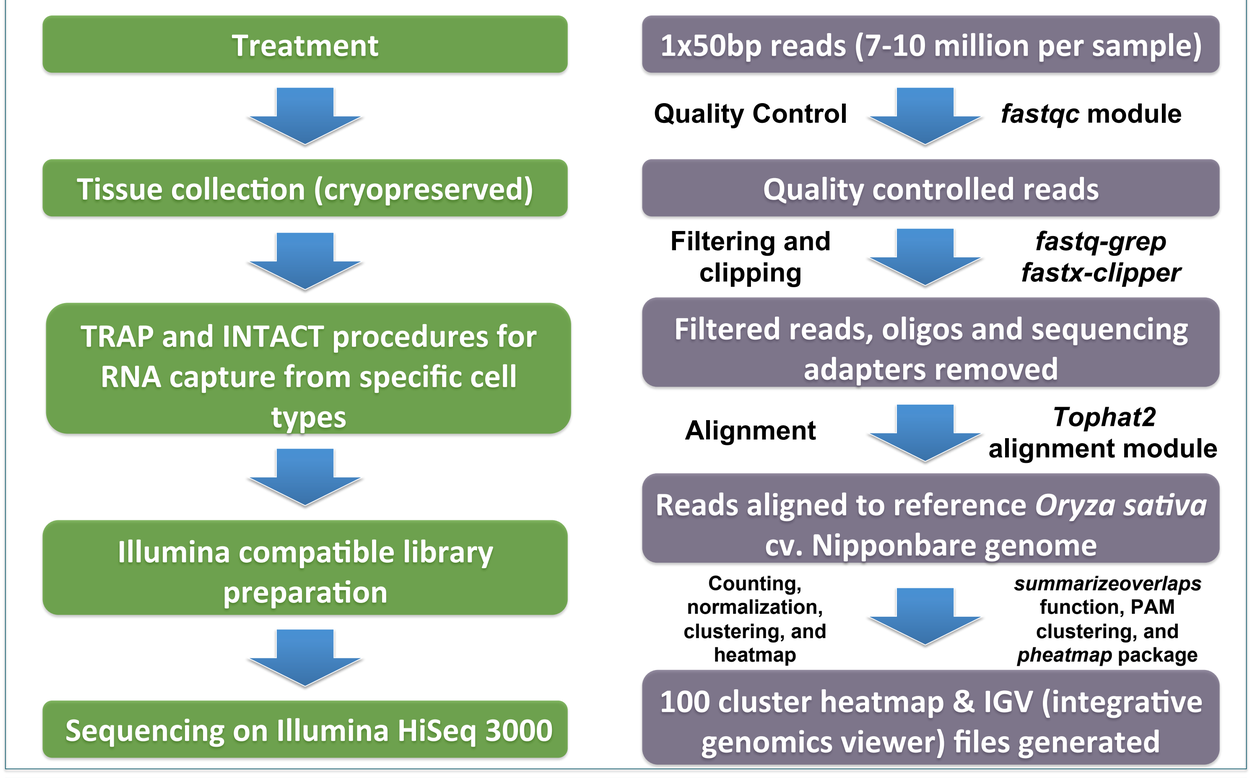
Cell type and technology
Shoot apical meristem (TRAP & INTACT)
Root cortex meristematic zone (TRAP)
Root endodermis meristematic zone (TRAP)
Root dividing cells (TRAP)
Root endodermis differentiated zone (TRAP)
Quiescent center (TRAP)
Endodermis/exodermis differentiated zone (TRAP)
Total mRNA (RNAseq)



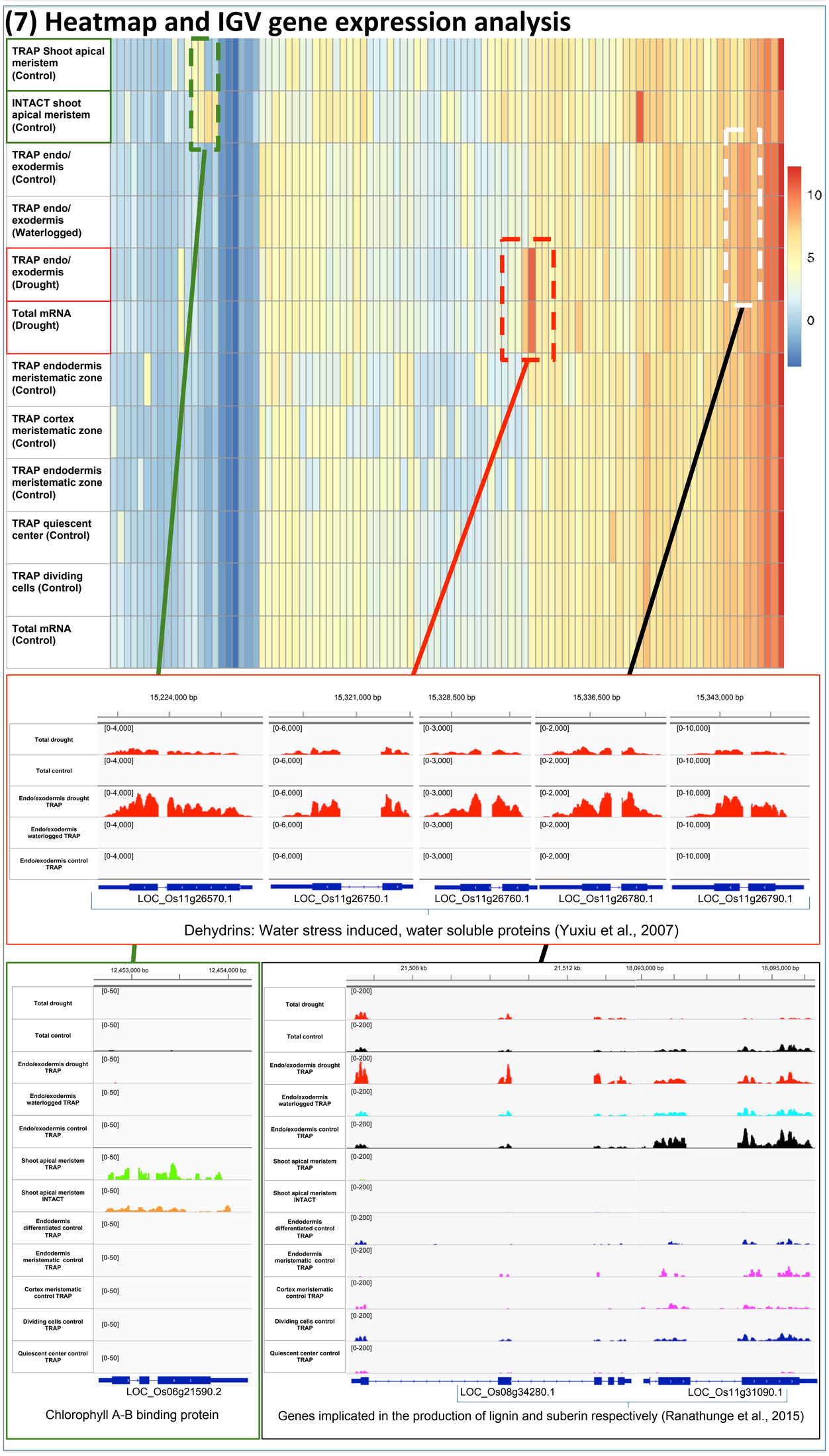
Cell type and technology
Endodermis/exodermis differentiated zone (TRAP)

Cell type and technology
Endodermis/exodermis differentiated zone (TRAP)
Total Drought mRNA (RNAseq)

(6) Data collection and NGS data analysis using bioinformatic tools



(7) Heatmap and IGV gene expression analysis



(8) Conclusions and future directions

- The visualization of the same transcript in its pre-mRNA and ribosome associated mRNA form supports the idea that TRAP and INTACT technologies can be used to isolate different RNA populations within specific cell types
- Water stress induced genes visualized in both total and endodermis/exodermis specific samples help confirm the efficiency of these technologies
- This pilot study confirms the efficiency of TRAP and INTACT technologies within these rice lines which will be used in future, more comprehensive analyses

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