DISCOVER NEW GENES REQUIRED FOR PLANT SURVIVAL STRATEGIES TO WATER EXTREMES

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Abstract

Rice (*Oryza sativa* L.) is the primary source of carbohydrates and calories for more than half of humanity. With climate change, one of the main areas of research on rice is its ability to combat abiotic stresses such as extremes in water availability. Indeed, floods and droughts are increasingly experienced in agricultural systems worldwide. These extremes in water availability diminish the production of rice grain. Improving yields under these conditions is important.

Drought and flooding invoke responses at the organ-, tissue- and cell-specific levels that enable metabolic to developmental responses relevant to survival (Voesenek and Bailey-Serres, 2014). As an example, several layers of the root cortex differentiate into lysigenous cortical aerenchyma. These large lacunae serve as gas conduits from aerial tissues to the roots and alleviate oxygen deprivation in waterlogged and compact soils.

Our project goal is to assess gene regulation in specific cell-types in response to extremes in water availability. To achieve this goal we have established technologies that allow access to the chromatin (epigenome) and nuclear transcriptome with INTACT, and ribosome-loaded transcripts (translatome) with TRAP.

Background

Promoters

Promoters are regulatory regions of DNA located near transcription start sites that initiate transcription. The specific DNA sequence of the promoter determines which transcription factors and regulatory elements will bind to the promoter. Thus, this also controls transcription of the downstream gene. In our project, the promoters that target our tissue of interest, such as cortex or endodermis, need to be identified.

TRAP (Translational Ribosome Affinity Purification)

This method is based on the expression of an epitope-tagged ribosomal protein (His_6-FLAG-RPL18) by a cell type-specific promoter. This protein is incorporated in ribosome complexes. TRAP allows for the purification of these ribosomes (Mustroph *et al*., 2009). Starting from a total ribosome preparation, the tagged ribosome complexes are separated from the non-tagged ones using beads coated with an antibody specific to the epitope used to tag the ribosomal subunit (Zanetti *et al.*, 2005). The ribosome-associated mRNAs (Translatome) can be sequenced or amplified to study what is being translated in specific cell types.

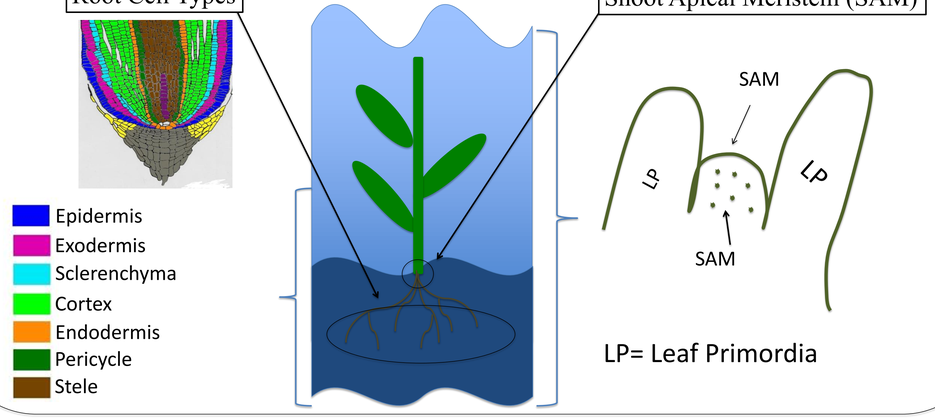
INTACT (Isolation of Nuclei TAgged in Specific Cell Types)

This technology uses the strong interaction between biotin and streptavidin to isolate nuclei from specific cell types of a tissue. This method transgenically tags nuclei by the expression of a nuclear targeting fusion protein (NTF) composed of a WPP domain, a green fluorescent protein (GFP) and biotin ligase recognition peptide (BLRP). The WPP domain anchors NTF to the nuclear envelope and the ubiquitously expressed Biotin ligase (BirA) biotinylates the BLRP of NTF (Deal and Henikoff, 2010). Nuclei that have biotinylated nuclear envelopes can be isolated from tissue using streptavidin-coated magnetic beads and a magnet. This technique allow access to the DNA (Epigenome) and nuclear RNA (Transcriptome).

***Agrobacterium tumefaciens* mediated rice genetic transformation**

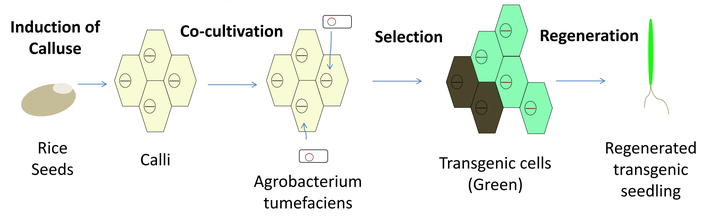
To perform both TRAP and INTACT or to identify cell type-specific promoters, transgenic lines that expressed the transgenes NTF, His_6-FLAG-RPL18 or reporter gene (GFP/GUS) were generated. The method uses the soil bacterium *Agrobacterium tumefaciens* to transfer DNA segments. The advantages are high efficiency, integration of a small numbers of copies of transfer DNA (T-DNA) and a transfer of large segments (Yukoh Hiei & Toshihiko Komari, 2008). T-DNA is inserted in rice genome using calli cells induced from mature seeds. Cells that have incorporated the T-DNA will generate transgenic plants through different steps of selection (using antibiotics) and regeneration (using phytohormones).

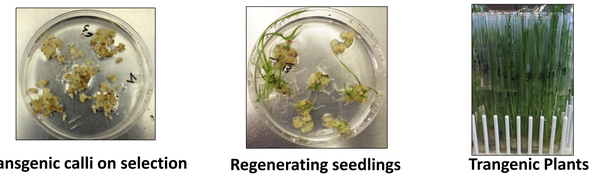
Under Waterlogging Conditions



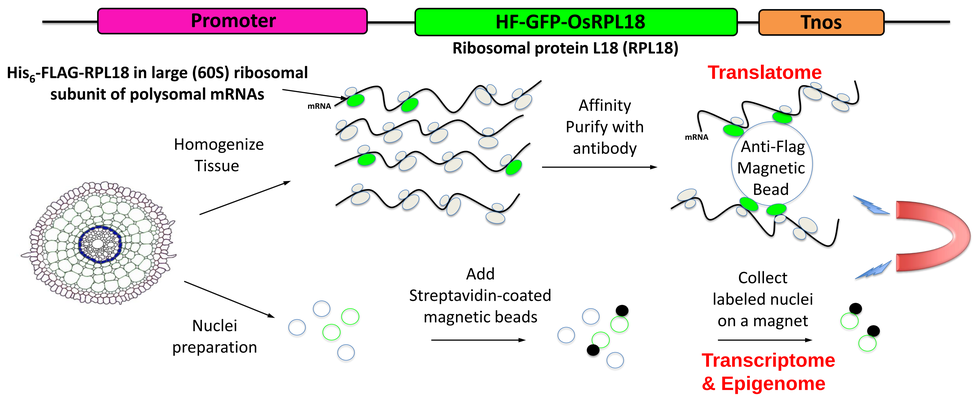
Under Complete Submergence

Agrobacterium tumefaciens mediated rice genetic transformation

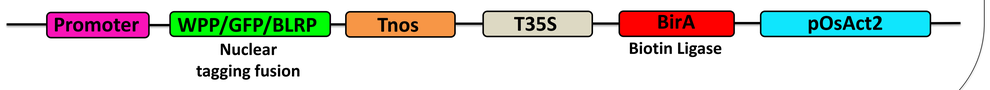




T-DNA used for Translating Ribosome Affinity Purification (TRAP) Lines

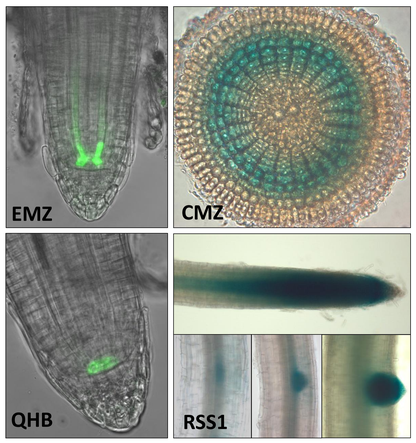


T-DNA used for Isolation of Nuclei TAgged in specific Cell Types (INTACT) Lines

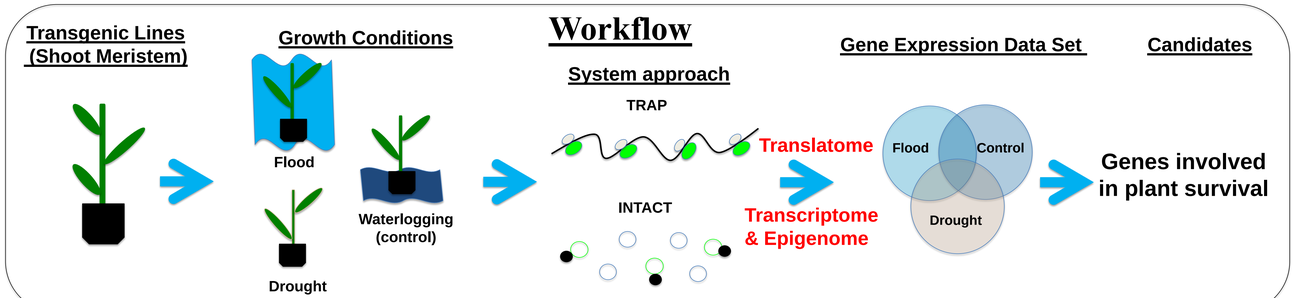


Reporter Lines to test Promoters

Target cell Type	Promoter
Near Constitutive	*Cauliflower mosaic virus 35S*
Root proliferating cells	*Rice salt sensitive 1* (*RSS1*)
Quiescent center	*Quiescent center specific homeobox* (*QHB*)
Endodermis	*Endodermis meristematic zone* (*EMZ*)
Cortex	*Cortical meristematic zone* (*CMZ*)
Exodermis/ Endodermis	*Low silicon 1* (*LSI1*)
Shoot apical meristem	*Oryza sativa homeobox1* (*OSH1*)



Workflow



References & Acknowledgments

1) Mustroph *et al* (2009). *Proc Natl Acad Sci* 209, 1–6
2) Zanetti *et al* (2005). *Plant Phys* 138, 624
3) Deal and Henikoff (2010). *Nature Prot* 6, 56.
4) Yukoh Hiei and Toshihiko Komari (2008). *Nature Prot* 3, 824
5) Voesenek and Bailey-Serres (2014). *New Phytol* 206(1):57-73

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