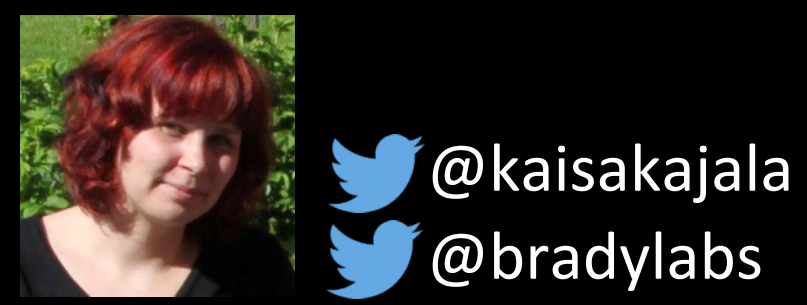


MAPPING THE TOMATO ROOT GENE EXPRESSION IN DROUGHT AND WATERLOGGING




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1. TOMATO AS A CROP MODEL

CULTIVATED  Tomato has

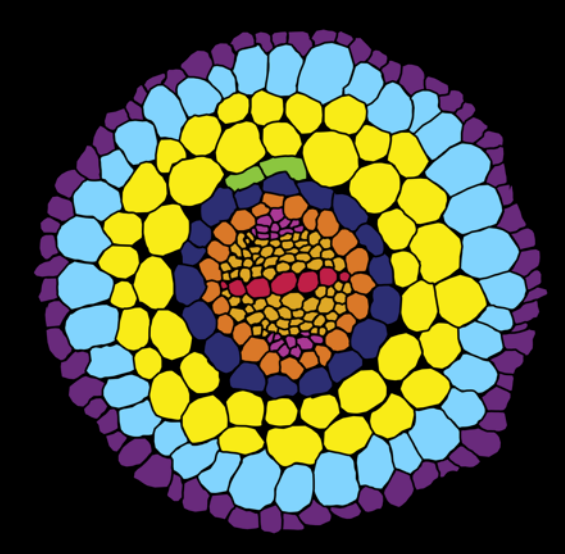
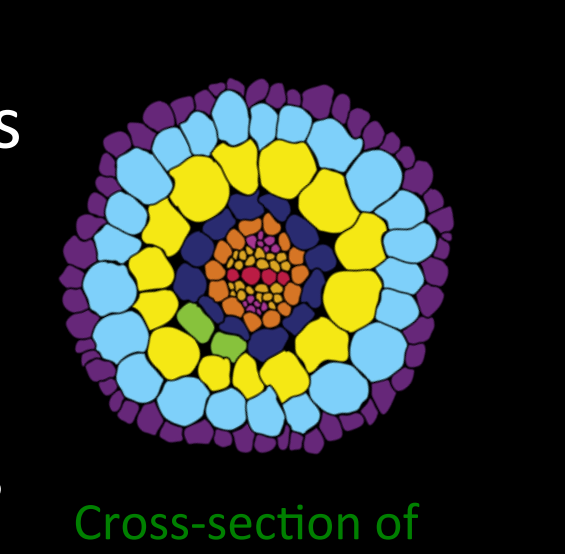
- a sequenced genome
- drought-tolerant inter-crossable species with developmental differences

DROUGHT-TOLERANT!  *Solanum pennellii* "PENN"

We are investigating the responses to drought and flooding in

- Specific cell types
- Different regulatory levels of gene expression
- Roots and shoots
- Multiple different species

Solanum lycopersicum var. M82

Legend for cell types: Epidermis, Exodermis, Cortex, Middle Cortex, Endodermis, Pericycle, Phloem, Procambium, Xylem.

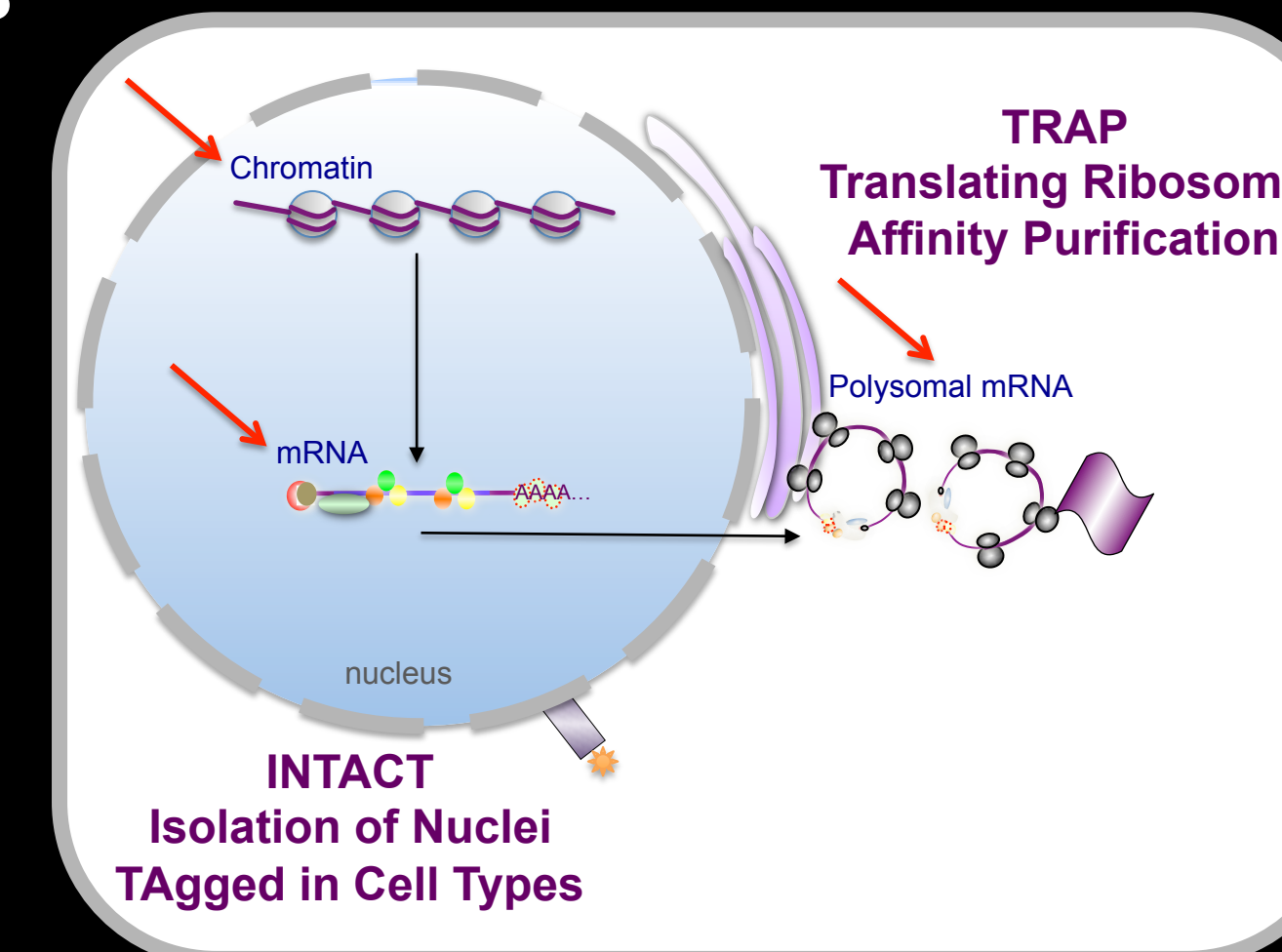
2. MULTILEVEL, CELL TYPE-SPECIFIC PROFILING OF GENE EXPRESSION

INTACT: ISOLATION OF NUCLEI TAGGED IN CELL TYPES

- Use a promoter to express a nuclear biotin tag.
- Pull down the nuclei with streptavidin beads.
- Subsequent analyses:

ATAC-seq (Assay for Transposon Accessible Chromatin) comparable to DNase-seq but easier! (Buenrostro et al., 2013)

RNA-seq – we established a pipeline to sequence nuclear mRNA. Nuclei have low amounts of polyA mRNA, so total nuclear RNA is subjected to rRNA degradation.



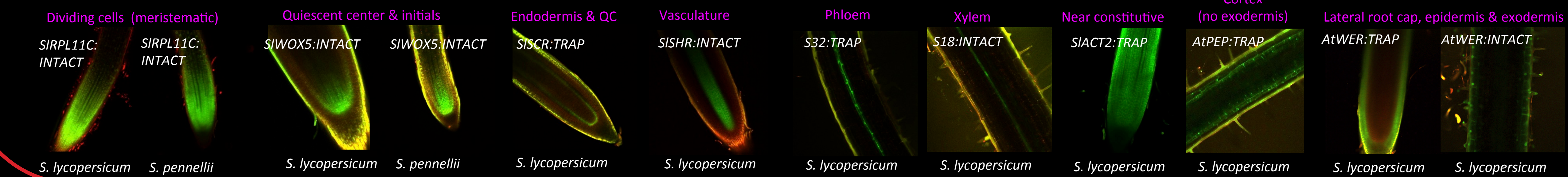
TRAP: TRANSLATING RIBOSOME AFFINITY PURIFICATION

- Use a promoter to express a ribosome flag tag.
- Pull down the ribosomes with α -flag beads.
- Subsequent analyses:

RNA-seq – rapid random-primed libraries (Townsend et al., 2015)

are generated from polysomal mRNA to query which transcripts are associated with polysomes

Ribo-seq – RNA immunoprecipitation allows identification of exact ribosome locations on mRNA.



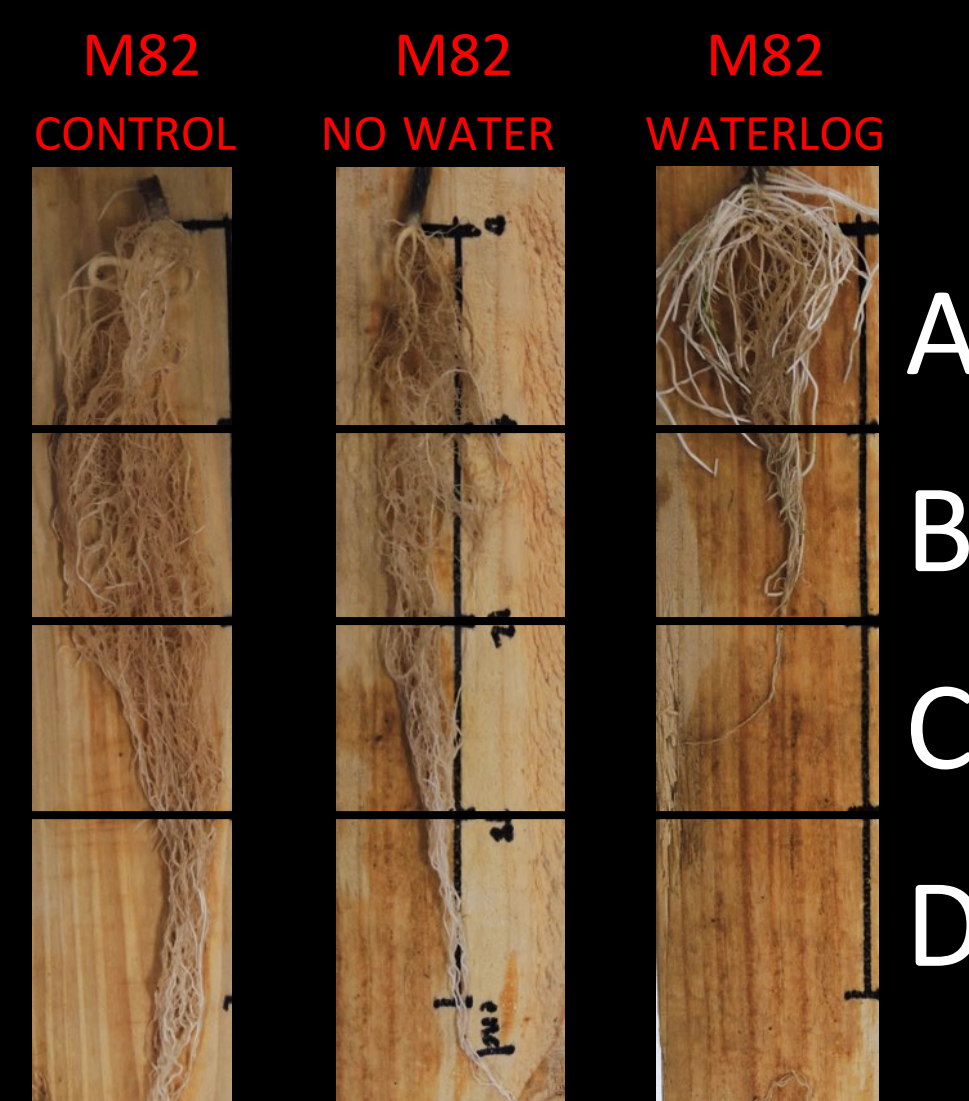
3. SPECIFIC CELL TYPES RESPOND TO LONG-TERM WATER STRESSES

We tested the effect of long-term drought and waterlogging on roots.

Plants grown on clay media. 21 days well watered + 12 days treatment

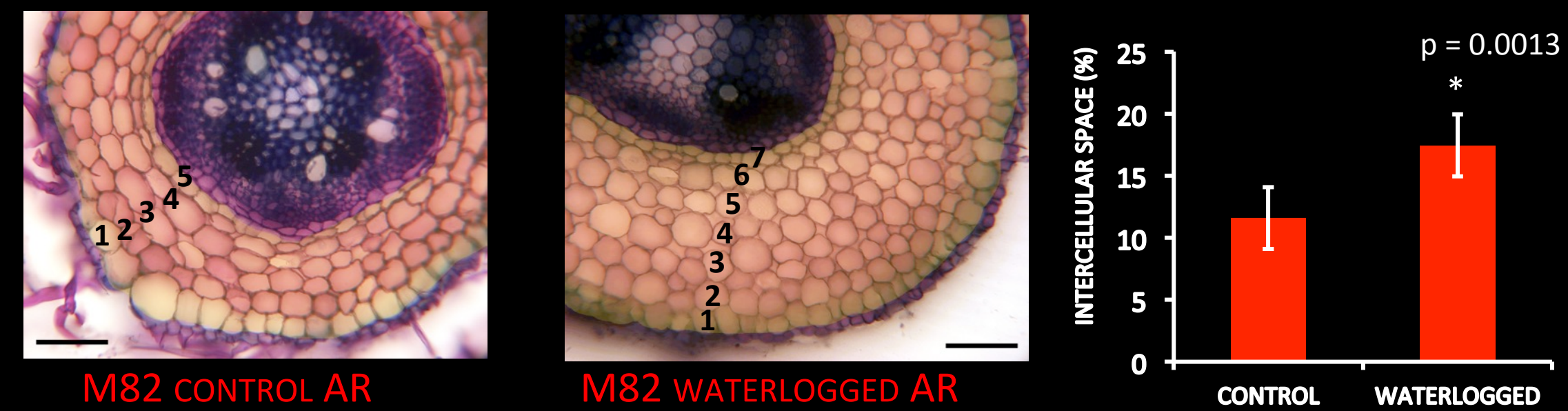
- control (well watered)
- no water
- waterlogging (rootball submerged)

Samples taken in 6 cm segments.

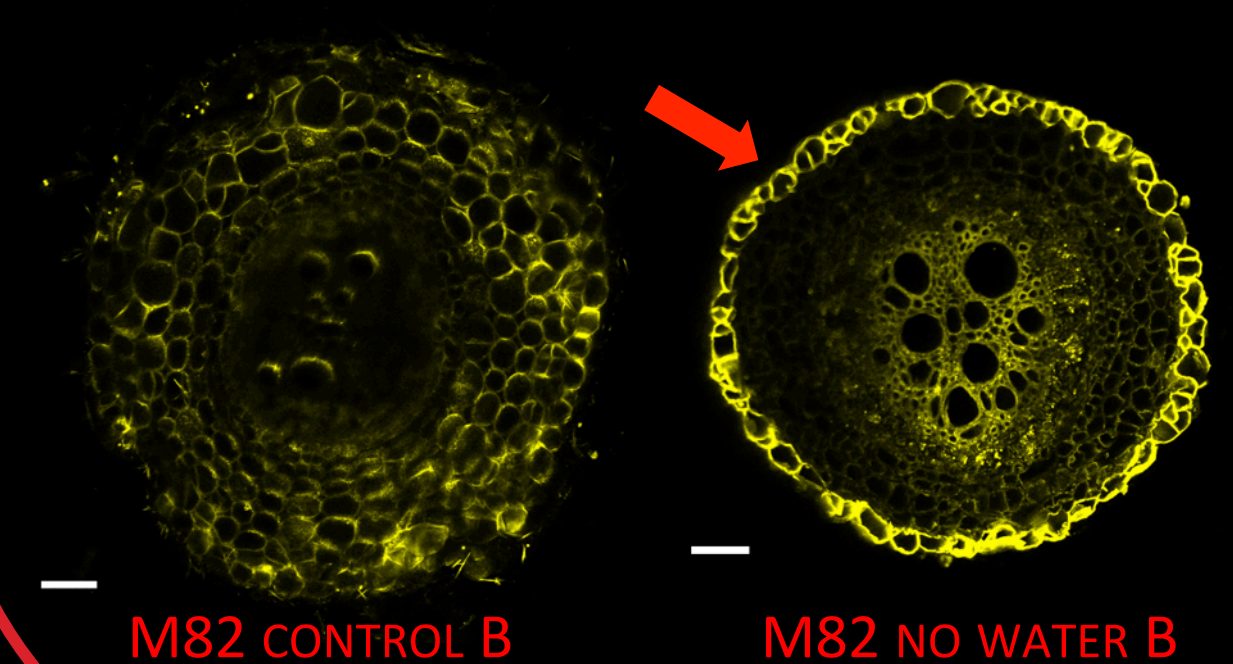


HYCOTYL-DERIVED ADVENTITIOUS ROOTS (AR) INCREASE IN WATERLOGGING.

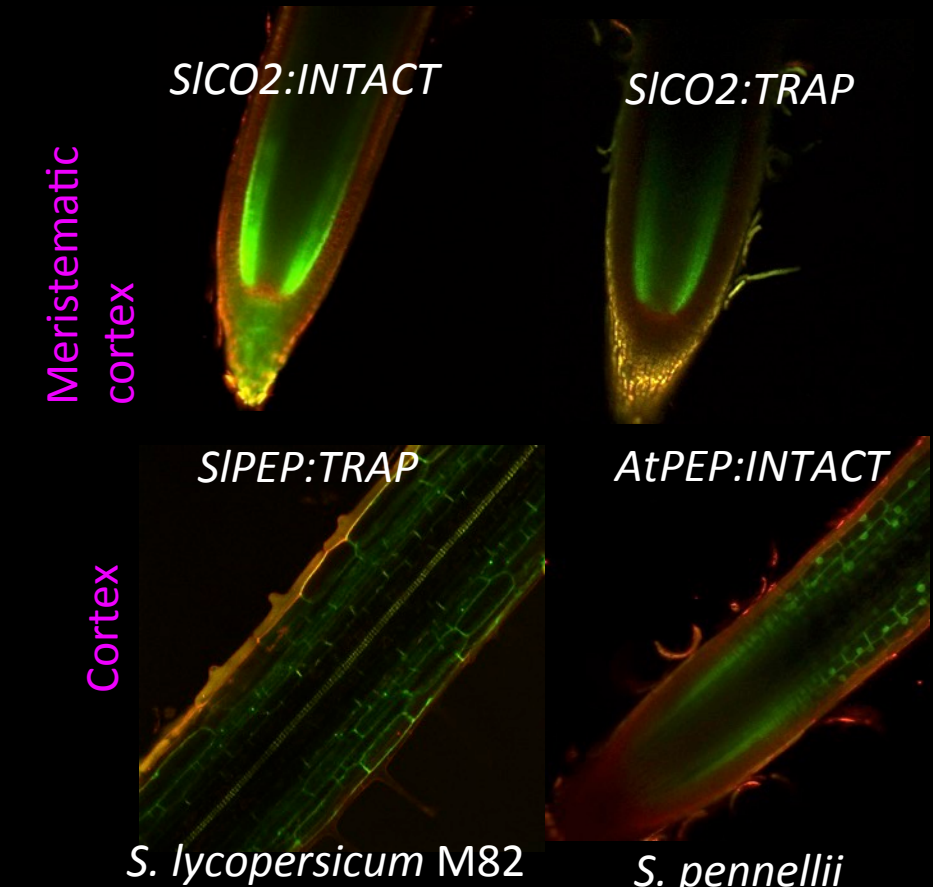
ARS HAVE MORE CORTEX LAYERS WITH MORE EXTRACELLULAR SPACE AFTER WATERLOGGING.



SUBERIN STAINING WITH FLUOROL YELLOW SHOWS SUBERIN ACCUMULATION IN EXODERMIS AFTER NO WATER TREATMENT IN M82, WHILE EXODERMAL SUBERIN IS CONSTITUTIVE IN *S. PENNELLII*.

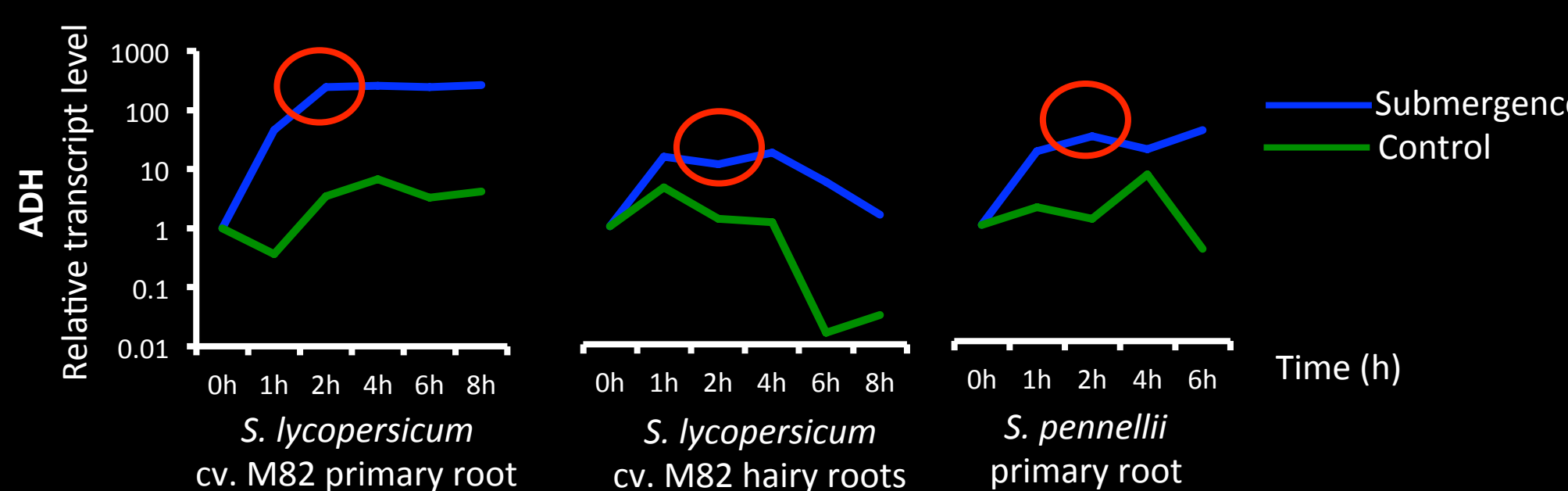
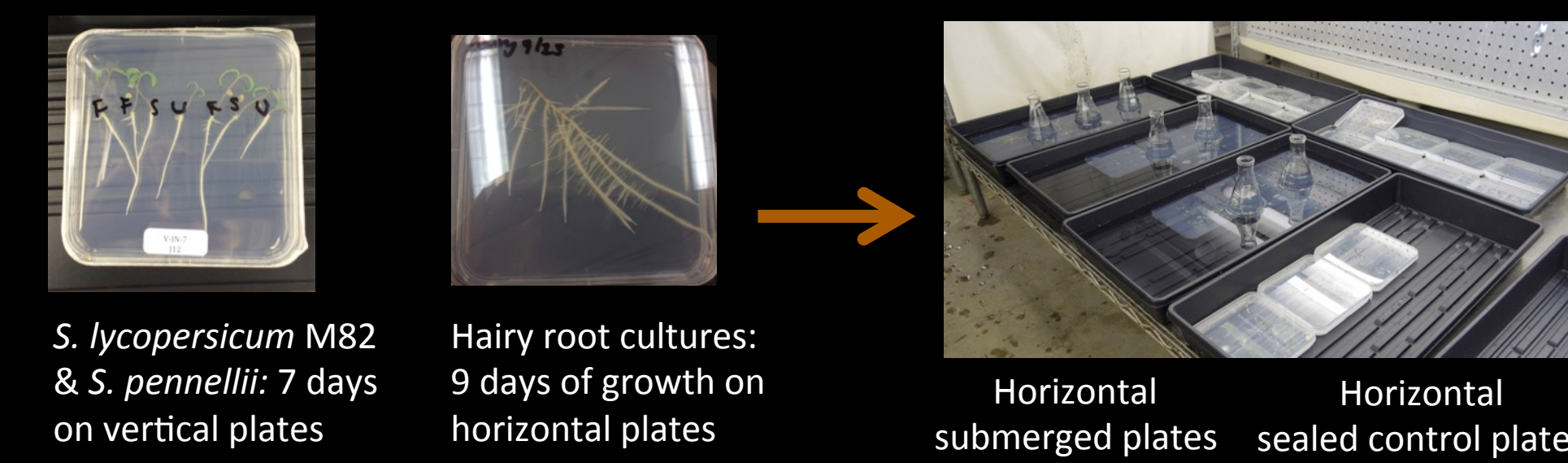


TRANSGENIC MARKER LINES TO STUDY UNDERLYING GENE EXPRESSION



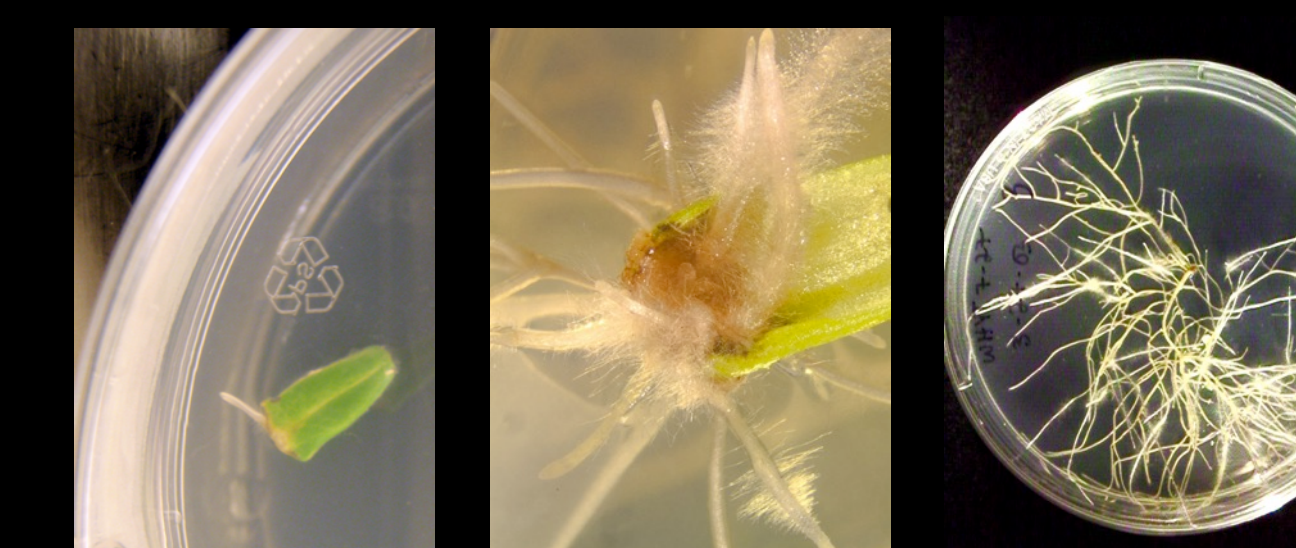
4. ROOT SUBMERGENCE RESPONSE IN HAIRY ROOTS IS PERTURBED (VERY FRESH PRELIMINARY DATA AHEAD)

EXPERIMENTAL SET-UP & TIMECOURSE OF RESPONSE

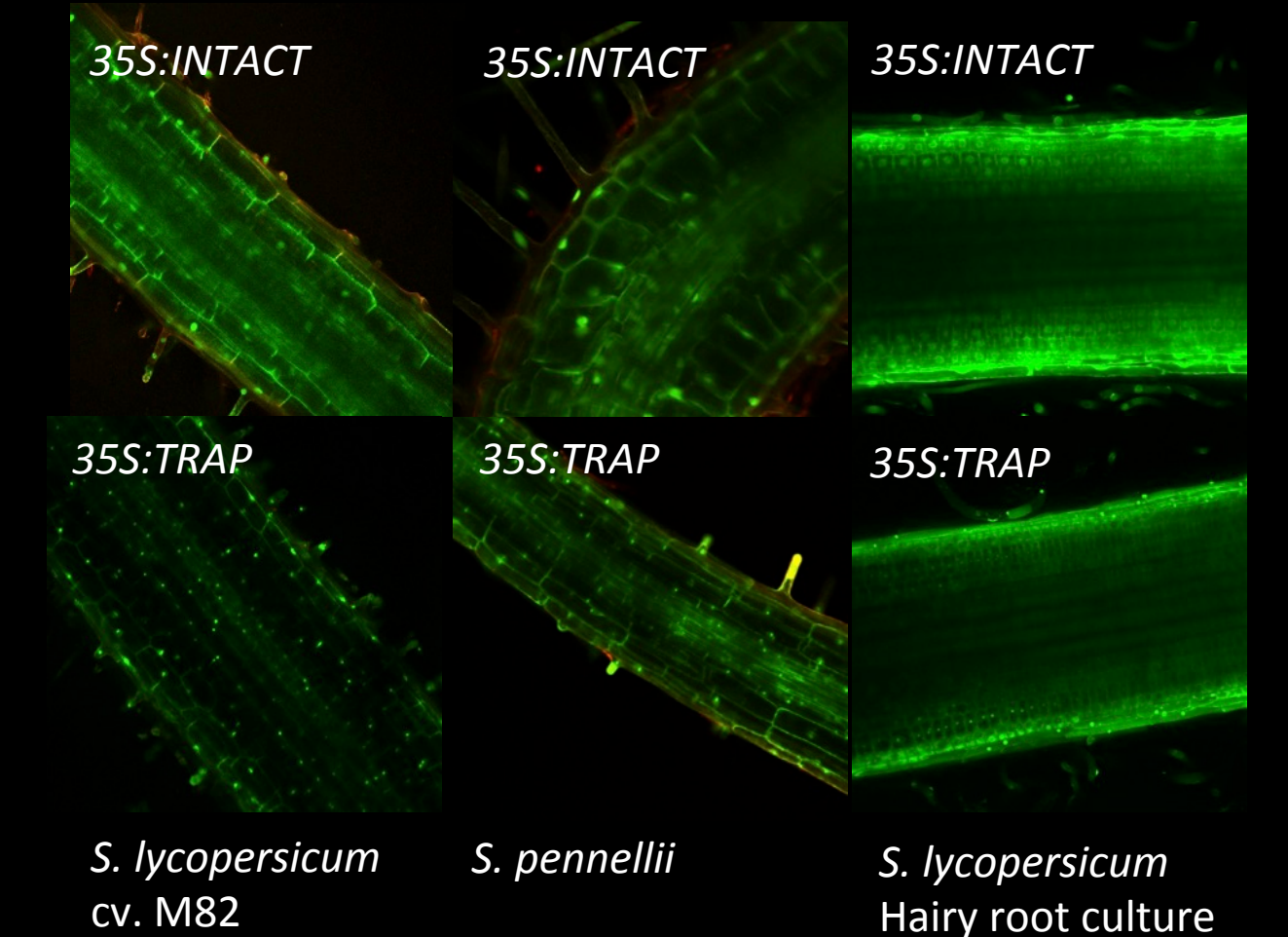


HAIRY ROOT CULTURES

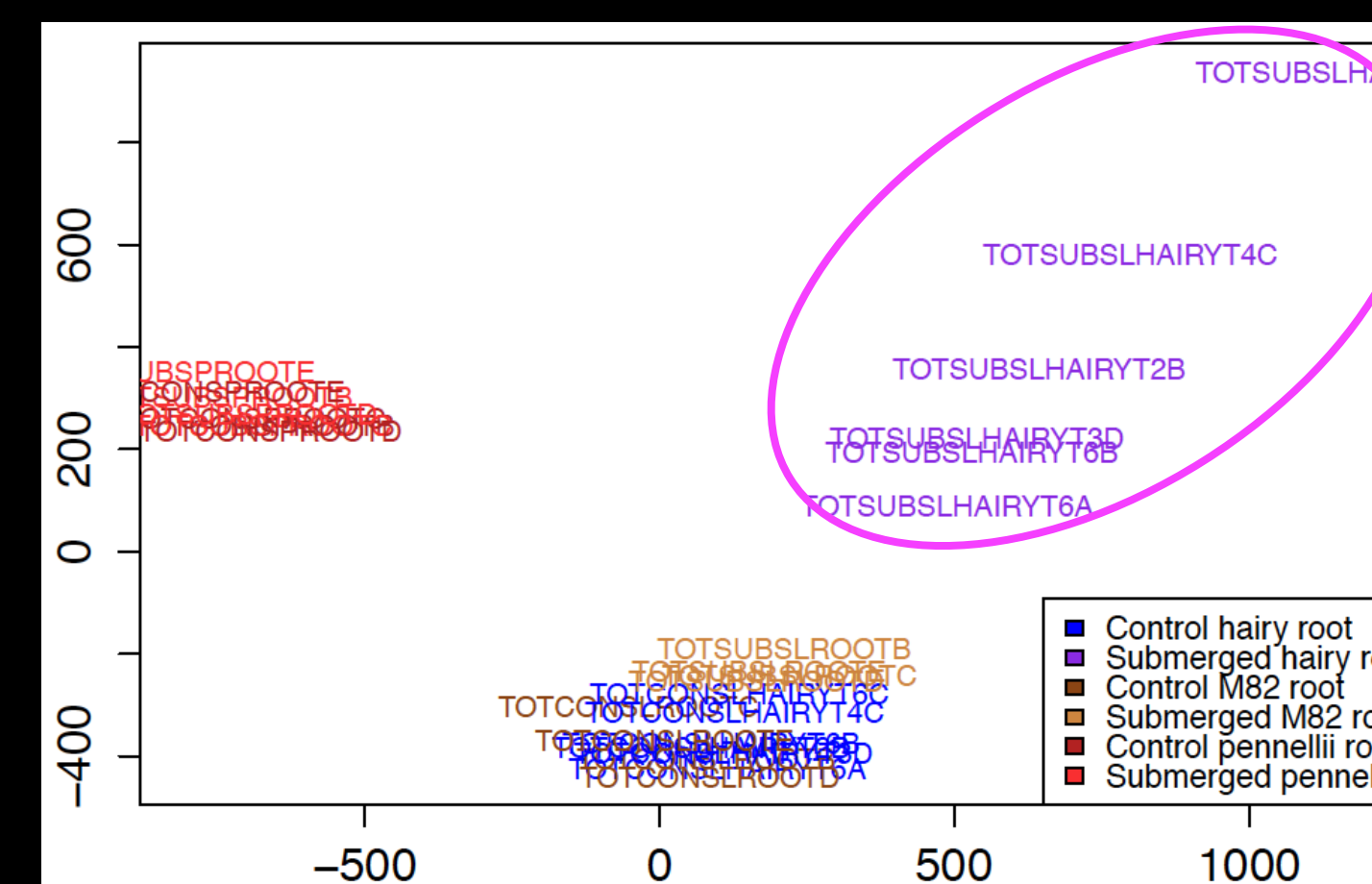
- induced by *Agrobacterium rhizogenes*
- a useful tool for generating rapid transgenic tomato roots to study root development (Ron et al., 2014)



TRANSGENIC MARKER LINES USED IN PROFILING MULTIPLE LEVELS OF GENE EXPRESSION



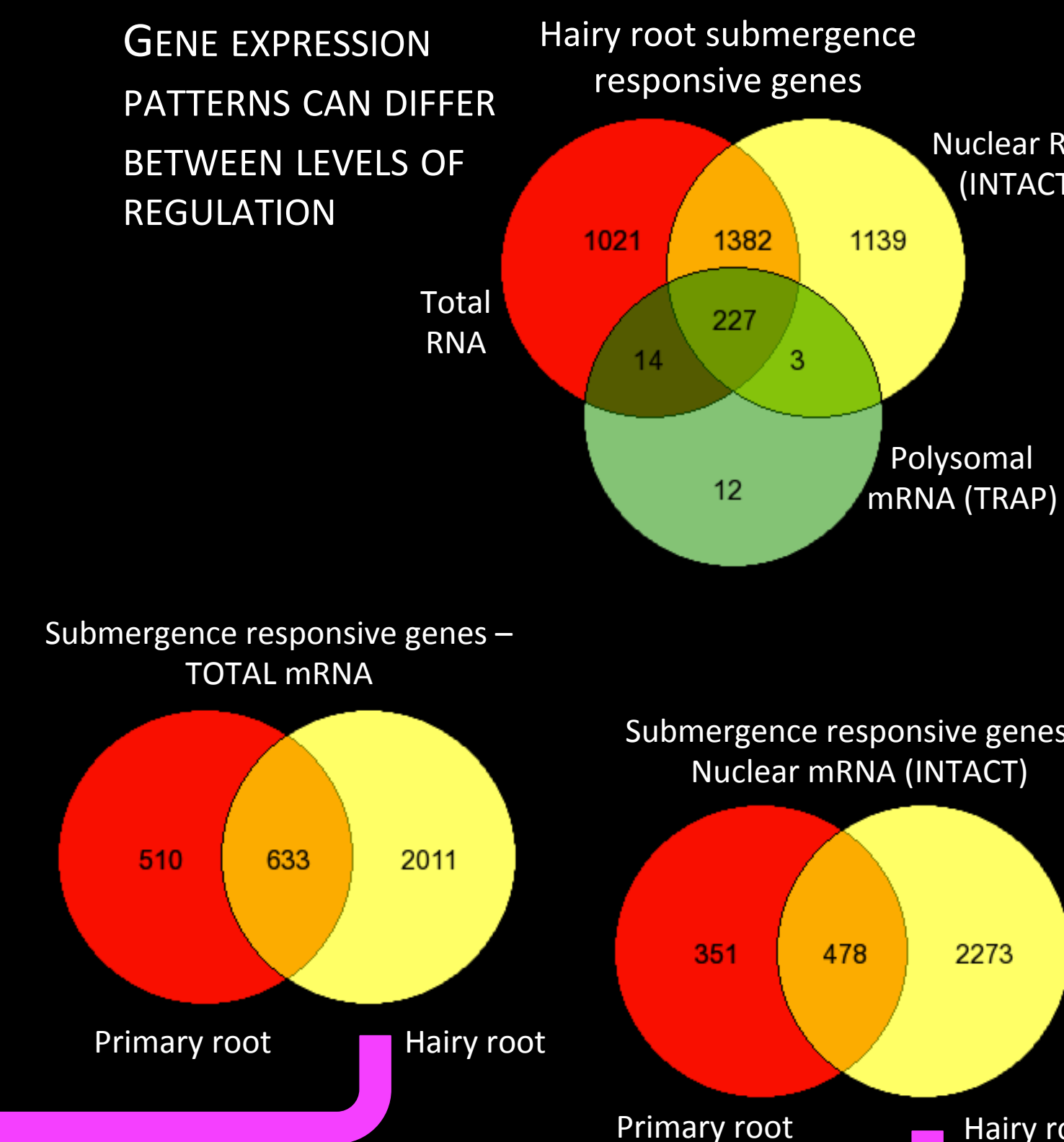
HAIRY ROOT SUBMERGENCE RESPONSE IS DISTINCT FROM PRIMARY ROOT RESPONSE



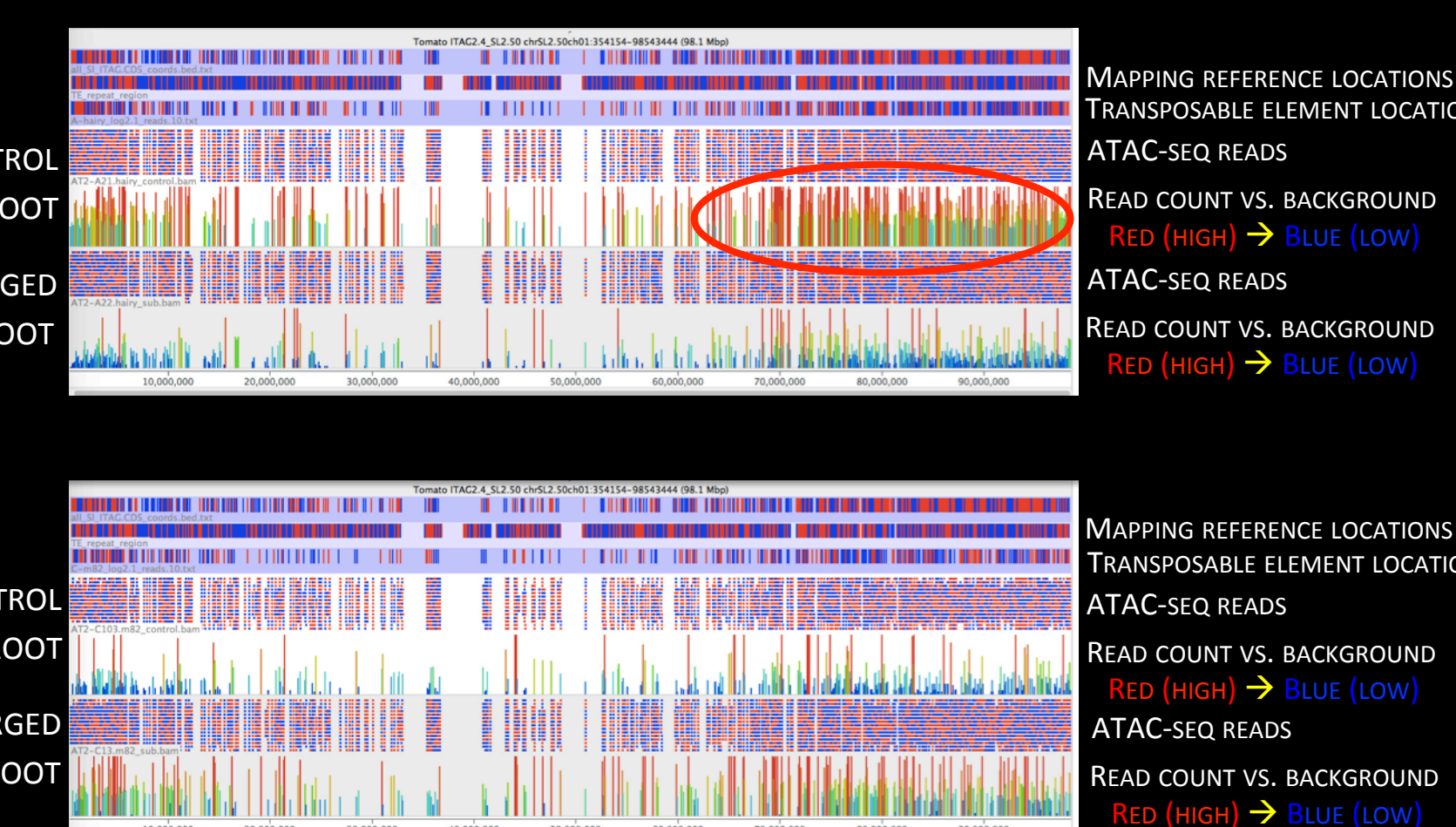
UNLIKE PRIMARY ROOTS, HAIRY ROOT SUBMERGENCE-RESPONSIVE GENES ARE ENRICHED IN

- transcription factors
- lateral root initiation related genes

GENE EXPRESSION PATTERNS CAN DIFFER BETWEEN LEVELS OF REGULATION



CHROMATIN ACCESSIBILITY OF TRANSPOSABLE ELEMENTS IS GLOBALLY HIGH IN CONTROL HAIRY ROOTS, BUT IS REDUCED TO PRIMARY ROOT LEVEL IN SUBMERGENCE



NEXT UP:

- DIFFERENTIALLY ACCESSIBLE CHROMATIN REGIONS!
- COMPARISON OF DIFFERENT REGULATORY LEVELS!
- COMPARISONS ACROSS TOMATOES, MEDICAGO & RICE!