Unravelling the Mysteries of Resurrection Plants using R

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Senescence
Abiotic/biotic stress
Nutrient redistribution

How is this natural process, supposedly hardwired, switched off in resurrection plants?
We know a lot about how it is able to survive desiccation, but *how does X.schlechteri prevent expression of genes related to programmed cell death?*
Research Questions:
1. When during drying are senescence processes initiated?
2. How is senescence regulated in the apex of leaves?
3. How is non-senescent tissue (NST) preventing triggering of this response?
Genes

Expressed genes (transcripts)

Gene Products
(Proteins)

Enzymes

Signals

Regulators

Transcripts and proteins can be sequenced, quantified and compared between conditions.
Experimental design

Withhold water and sample for RNA and water content – store tissue at -80°C

Dissect tissue from sampling points of interest

Grind in liquid Nitrogen

Extract in Trizol

Centrifuge to phase separate

Aqueous phase: RNA
Interphase: DNA
Organic phase: protein, lipids
K-means Clustering
What are the patterns in gene expression between tissue types?

**DEGs of interest:** dissecting the senescence response from the desiccation response – comparing to FT NST is not particularly useful or meaningful because one cannot differentiate drought responsive genes from cellular death genes.
Senescome: extracting DEGs differentially accumulated in AD ST

“Greatest hits of death”: Protein degradation, DNA cleavage, cell death (ACD11, YLS), hormone binding and metabolism (ethylene and salicylic acid), transport (N, C, K, P)

Metabolism overdrive: electron transport, Aox, beta-oxidation, fermentation

https://astridite.shinyapps.io/XeroSenescome
So we have the genes responsible for cellular death, which are only expressed in the most extreme conditions, but how are they regulated?
How is expression of these senescence genes activated?

Are the senescence genes under the control of the same promoters?
Co-expression Network Analysis
Which genes are highly correlated in terms of their expression and can we use this to find promoters and transcription factors?

https://github.com/astridite/transcriptome_coexpression_network
Co-expression Network Analysis
Which genes are highly correlated in terms of their expression and can we use this to find promoters and transcription factors?

https://github.com/astridite/transcriptome_coexpression_network
Promoter Analysis
How is expression of senescence genes regulated?

DNA Sequences:
- GTCAAHS & CRCGTW
- GTCAAHS
Transcription Factor Identification
Which TFs are controlling the network?

**Tomtom**
Motif Comparison Tool
Version 5.1.0

Tomtom compares one or more motifs against a database of known motifs (e.g., JASPAR). Tomtom will rank the motifs in the database and produce an alignment for each significant match (example output for motif and JASPAR CORE 2014 database). See this Manual for more information.
Transcription Factor Identification
Which TFs are controlling the network?

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Predicted Cell Death Regulation Model

TF

Activation of targets in A.thaliana literature:
- Smekalová et al. 2014
- Kim et al. 2018
- Chai et al. 2014
- Liu & Zhang, 2004

Proposed interaction in X. schlechtneri:
- Activates/Produces
- Incomplete regulatory pathway
- Putative transcriptional regulator
Predicted Cell Death Regulation Model

Opens up the opportunity for further experimentation – How are NST blocking this response to prevent cellular death?

If we discover a senescence prevention mechanism unique to resurrection plants, can we use it to improve the response of crops to stress?
Thank You!

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Keren Cooper
Dr Amelia Hilgart
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Arno Duvenage
Halford Dace
Arash Iranzadeh
Dr Maria Cecilia Dias Costa
Plant Stress Lab peeps
Prof Sagadevan Mundree
Dr Brett Williams
Electron Microscopy Unit, UCT –
Miranda Waldron and Mohamed Jafer
John Burroughs – Buffelskloof nature reserve

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