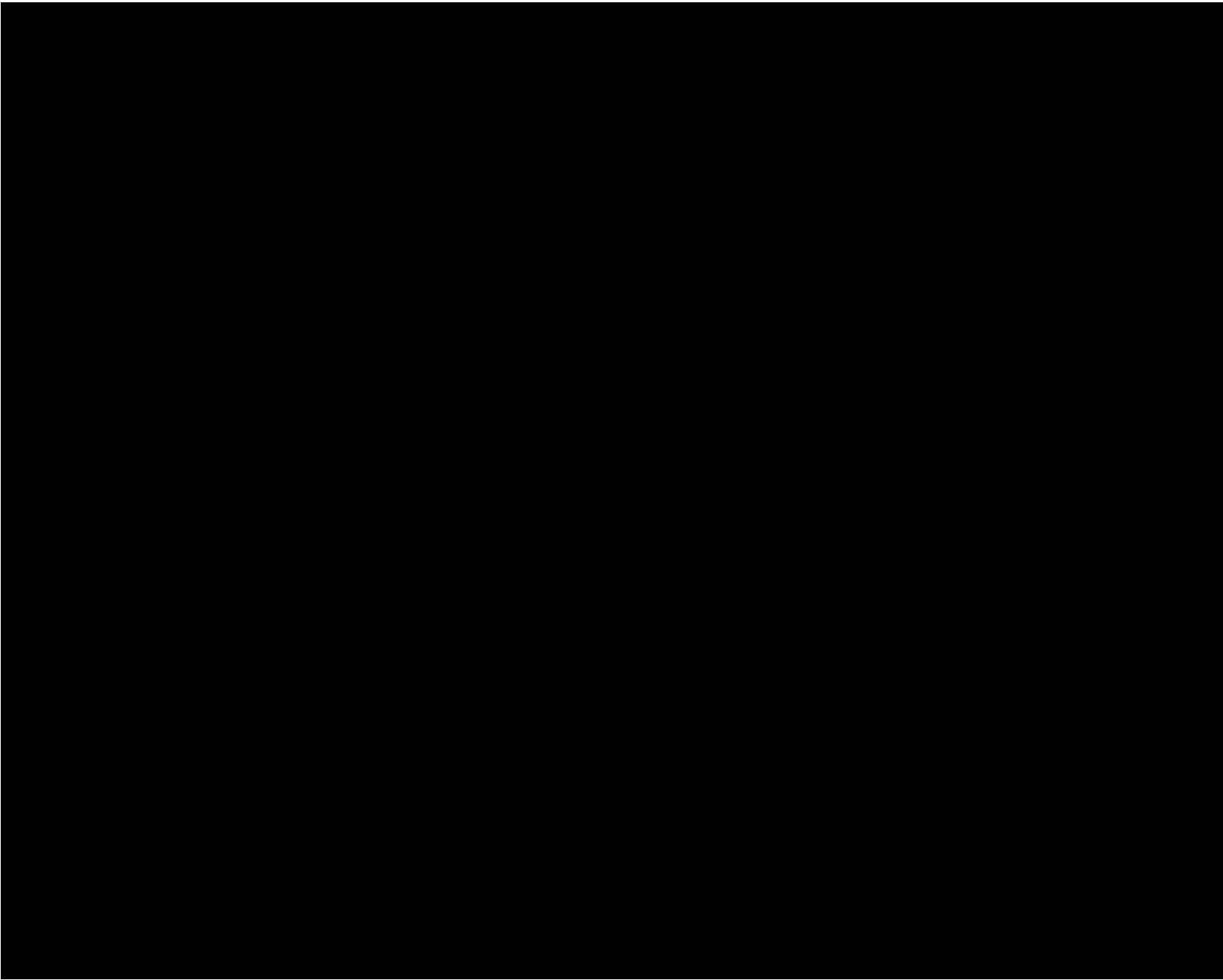


# Unravelling the Mysteries of Resurrection Plants using R



Dr Astrid Radermacher  
Plant Stress Lab  
Molecular and Cell Biology  
University of Cape Town



<https://www.youtube.com/watch?v=33GN8HRNG2E>





Aging

Abiotic/biotic stress

# Senescence

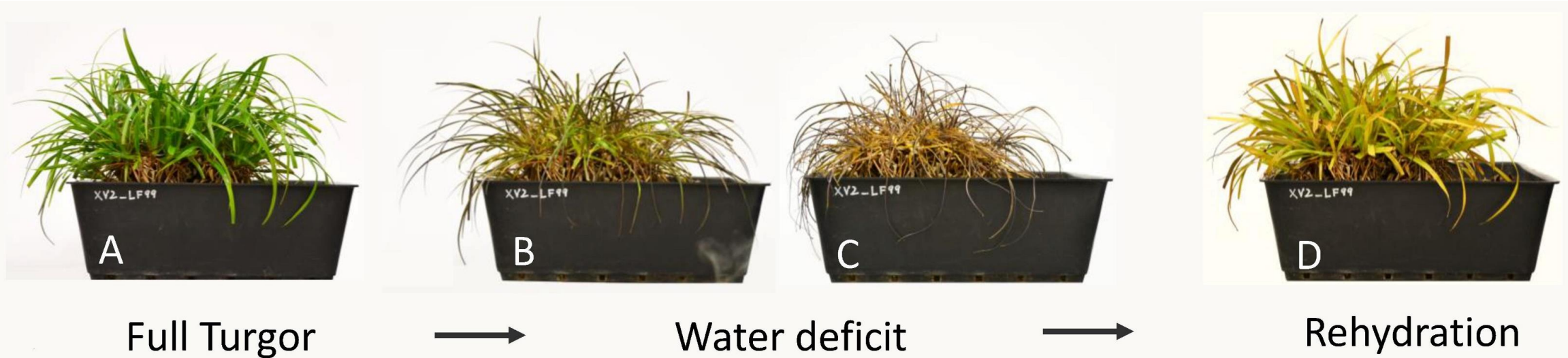
Nutrient redistribution

How is this natural process, supposedly hardwired, switched off in resurrection plants?





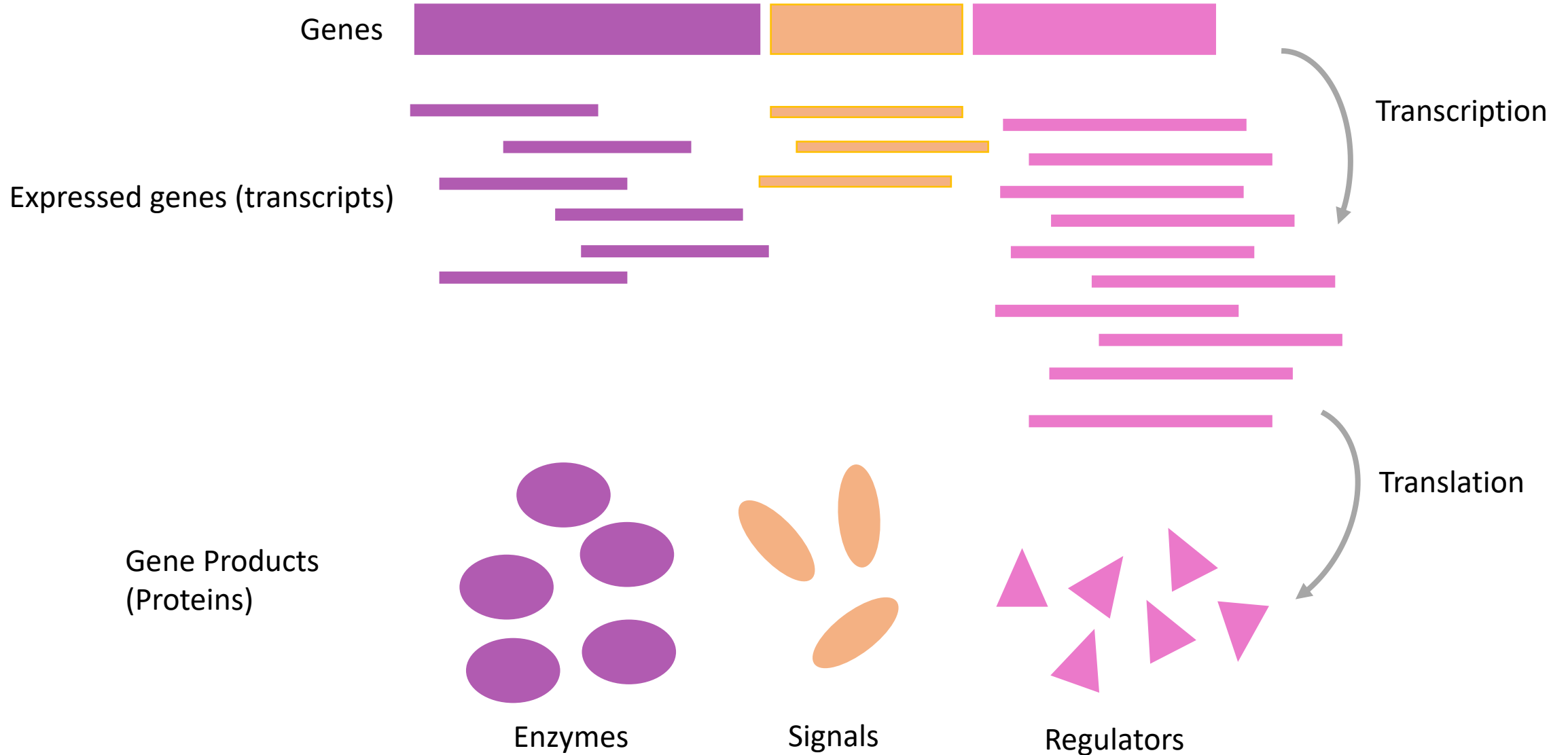
***Xerophyta schlechteri*: a monocotyledonous resurrection plant and model for understanding improved drought tolerance in cereals.**



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:

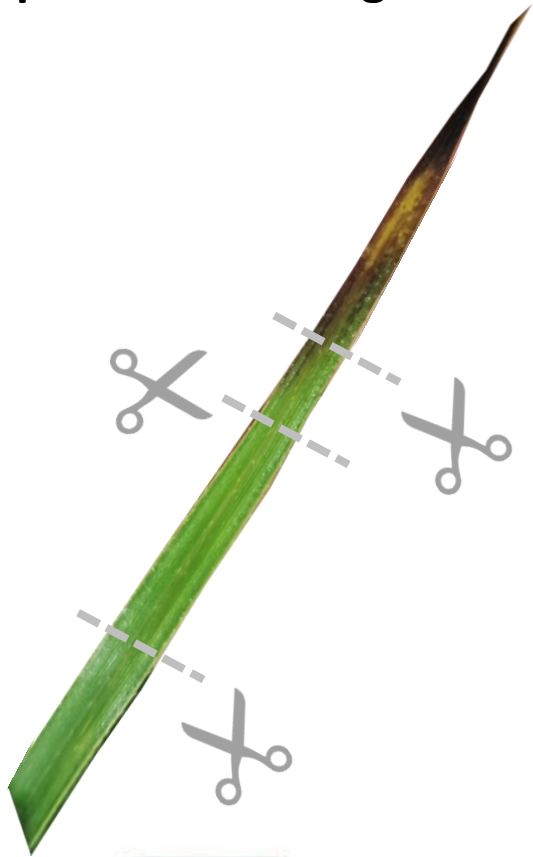
1. When during drying are senescence processes initiated?
2. How is senescence regulated in the apex of leaves?
3. How is non-senescent tissue (NST) preventing triggering of this response?



Transcripts and proteins can be sequenced, quantified and compared between conditions

## Experimental design

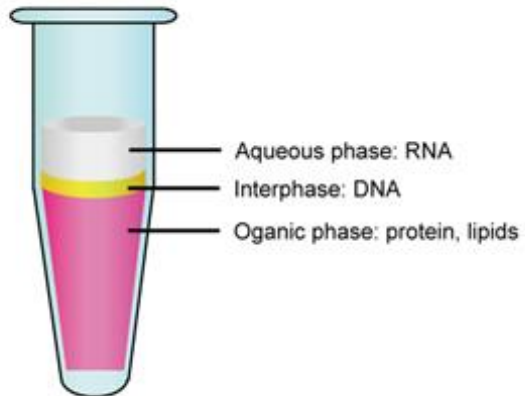
Withhold water and sample for RNA and water content – store tissue at  $-80^{\circ}\text{C}$



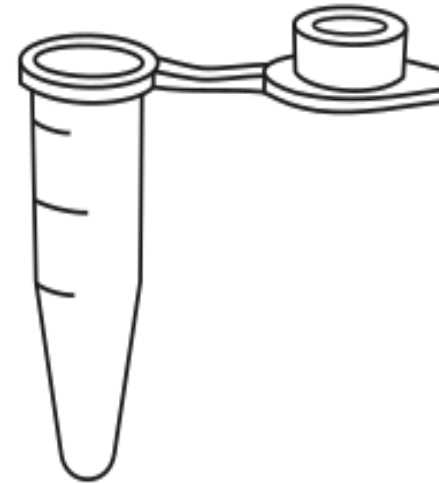
Dissect tissue from sampling points of interest



Centrifuge to phase separate



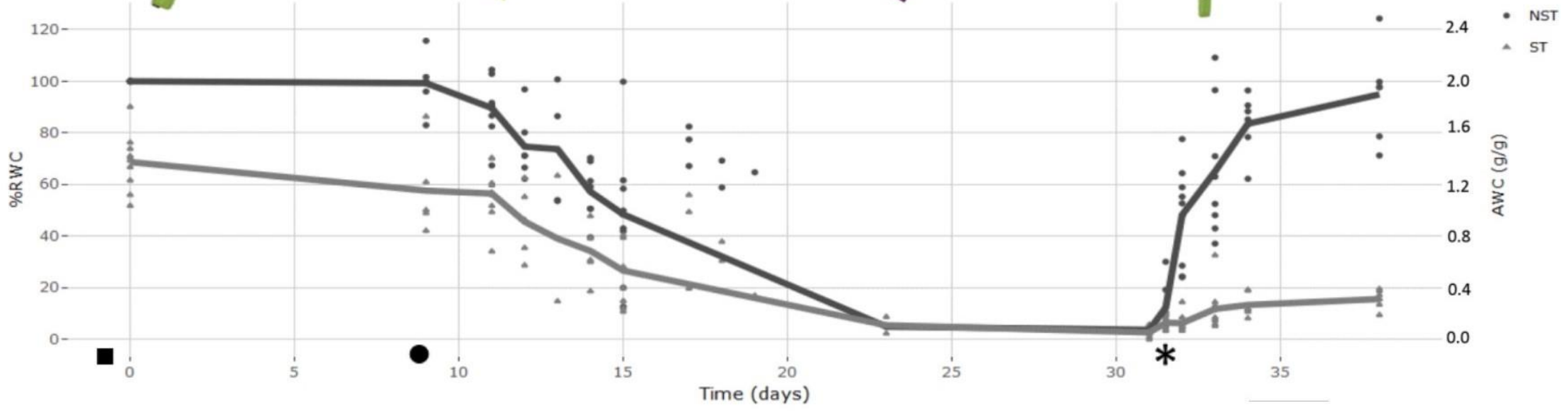
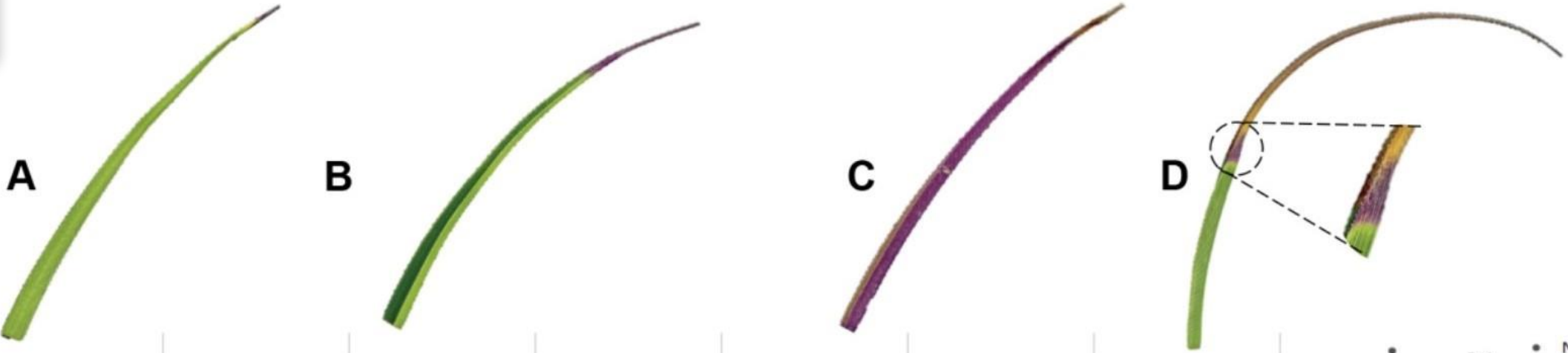
Aqueous phase: RNA  
Interphase: DNA  
Organic phase: protein, lipids



Extract in Trizol

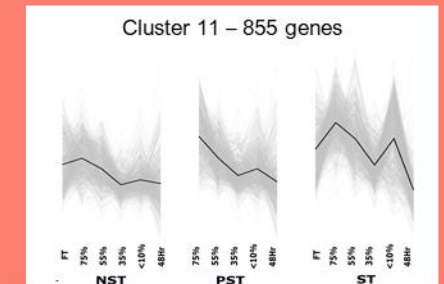
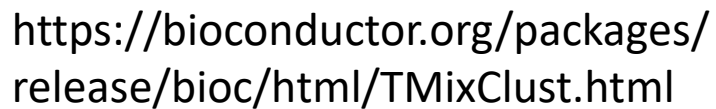


Grind in liquid Nitrogen

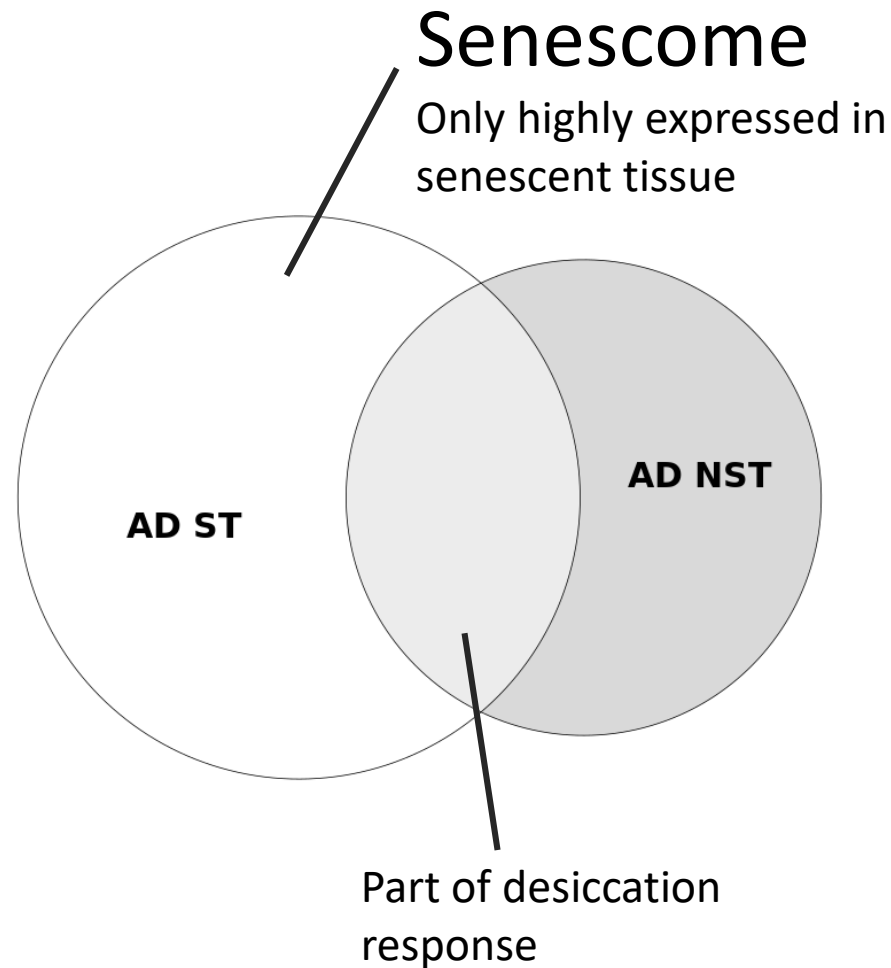
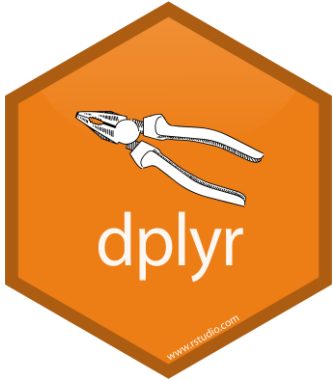




## What are the patterns in gene expression between tissue types?



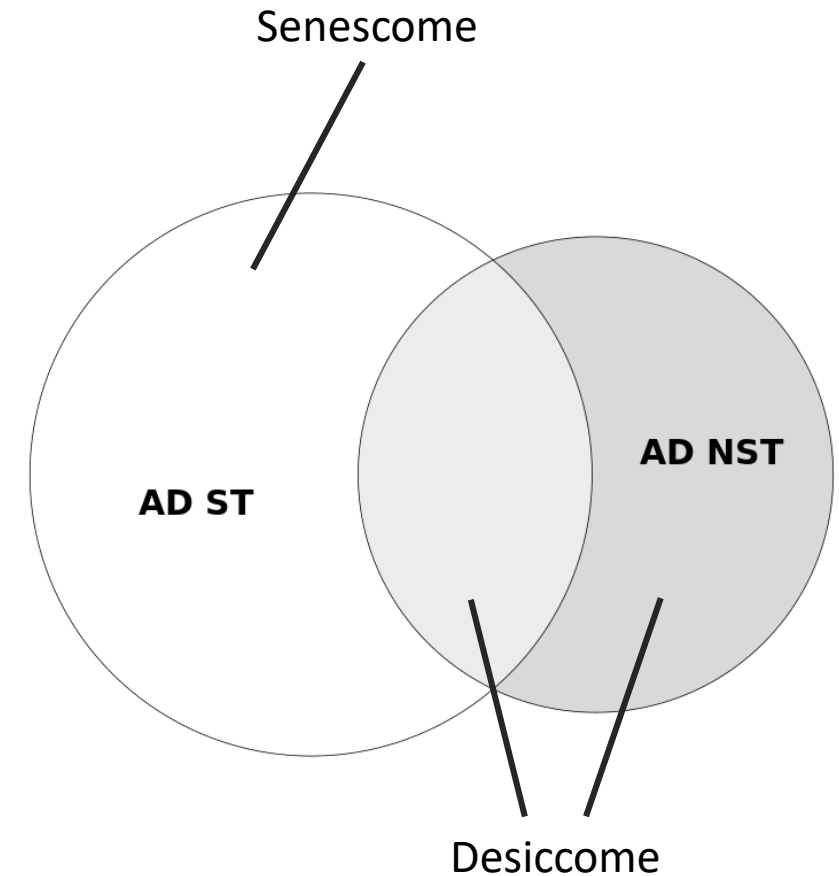
**DEGs of interest:** dissecting the senescence response from the desiccation response – comparing to FT NST is not particularly useful or meaningful because one cannot differentiate drought responsive genes from cellular death genes



## 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)

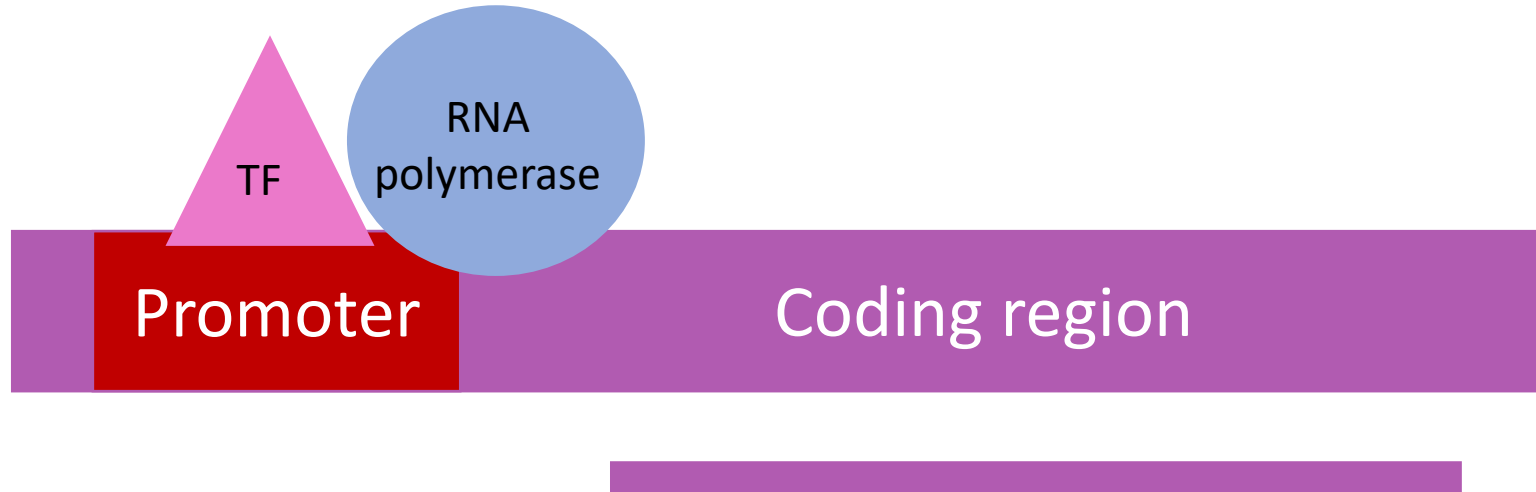
Metabolism overdrive:  
electron transport, Aox,  
beta-oxidation,  
fermentation





So we have the genes responsible for cellular death, which are only expressed in the most extreme conditions, but how are they regulated?

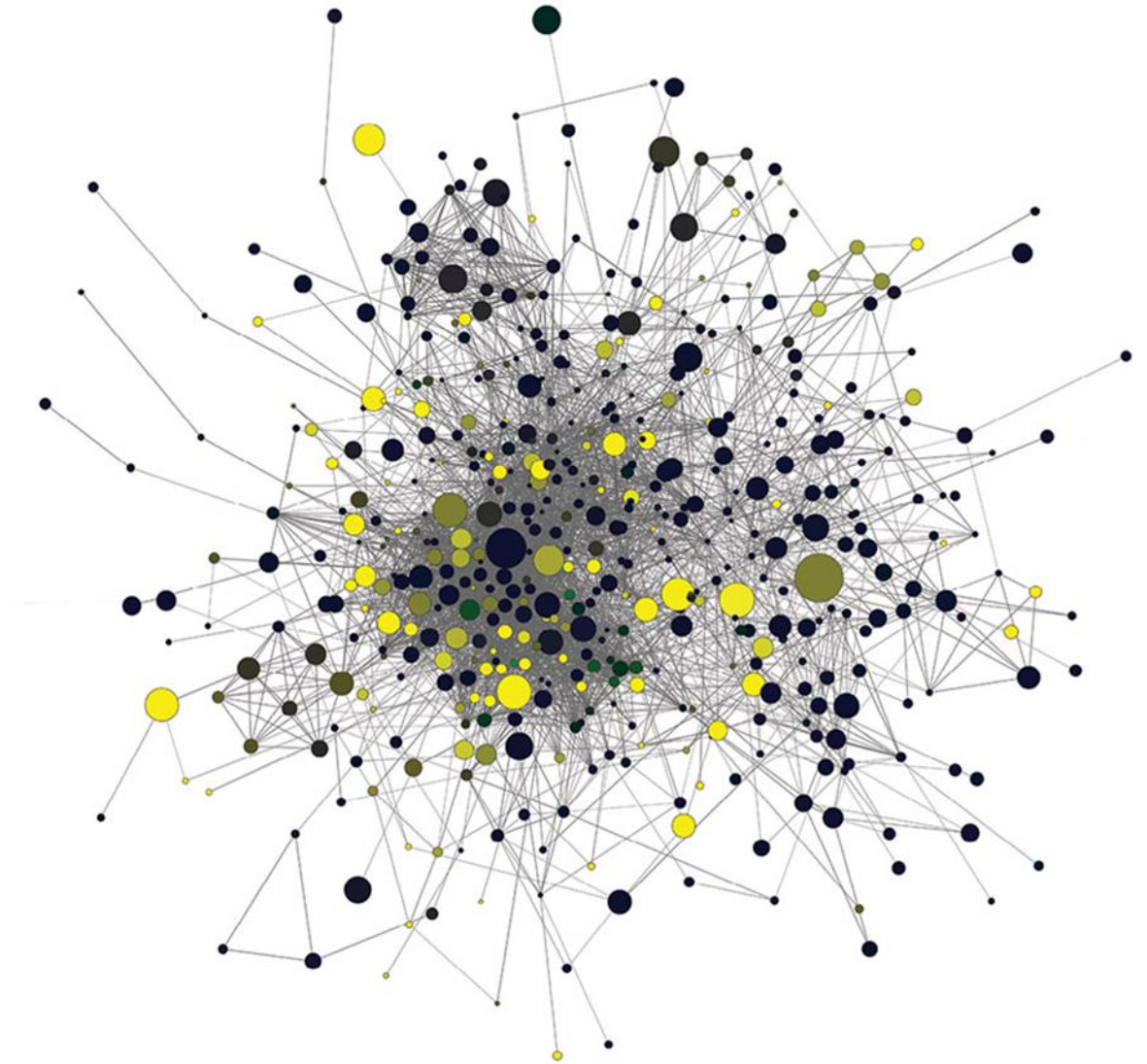
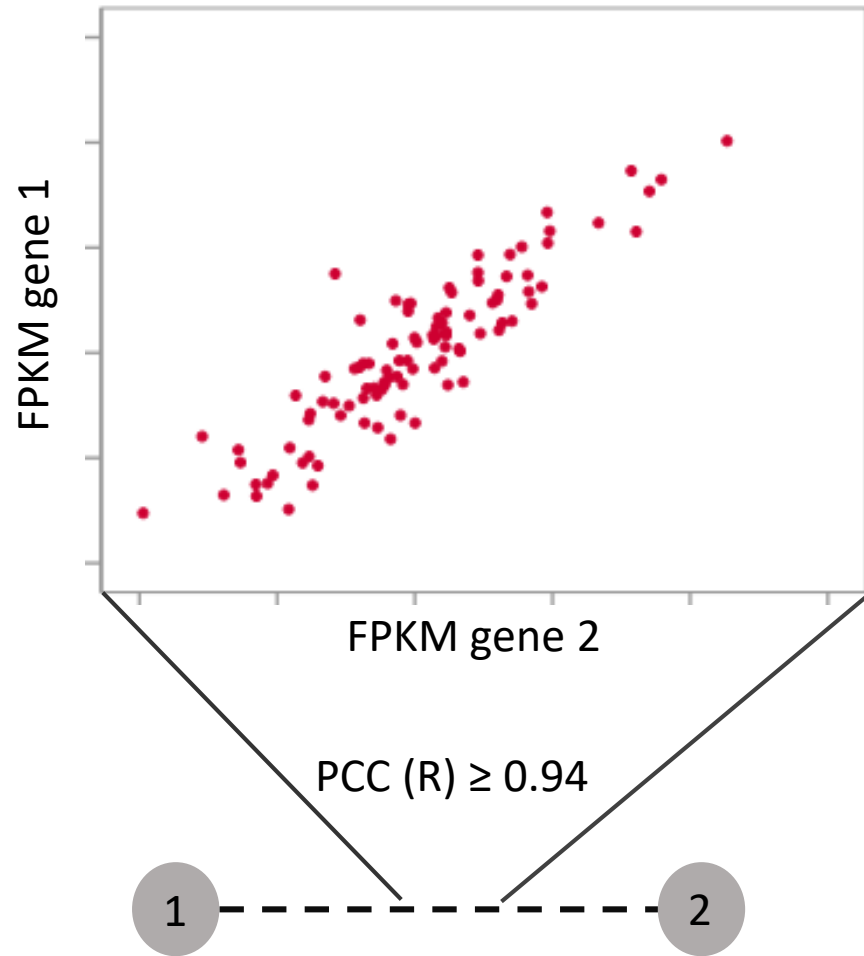
How is expression of these senescence genes activated?



Are the senescence genes under the control of the same promoters?

## Co-expression Network Analysis

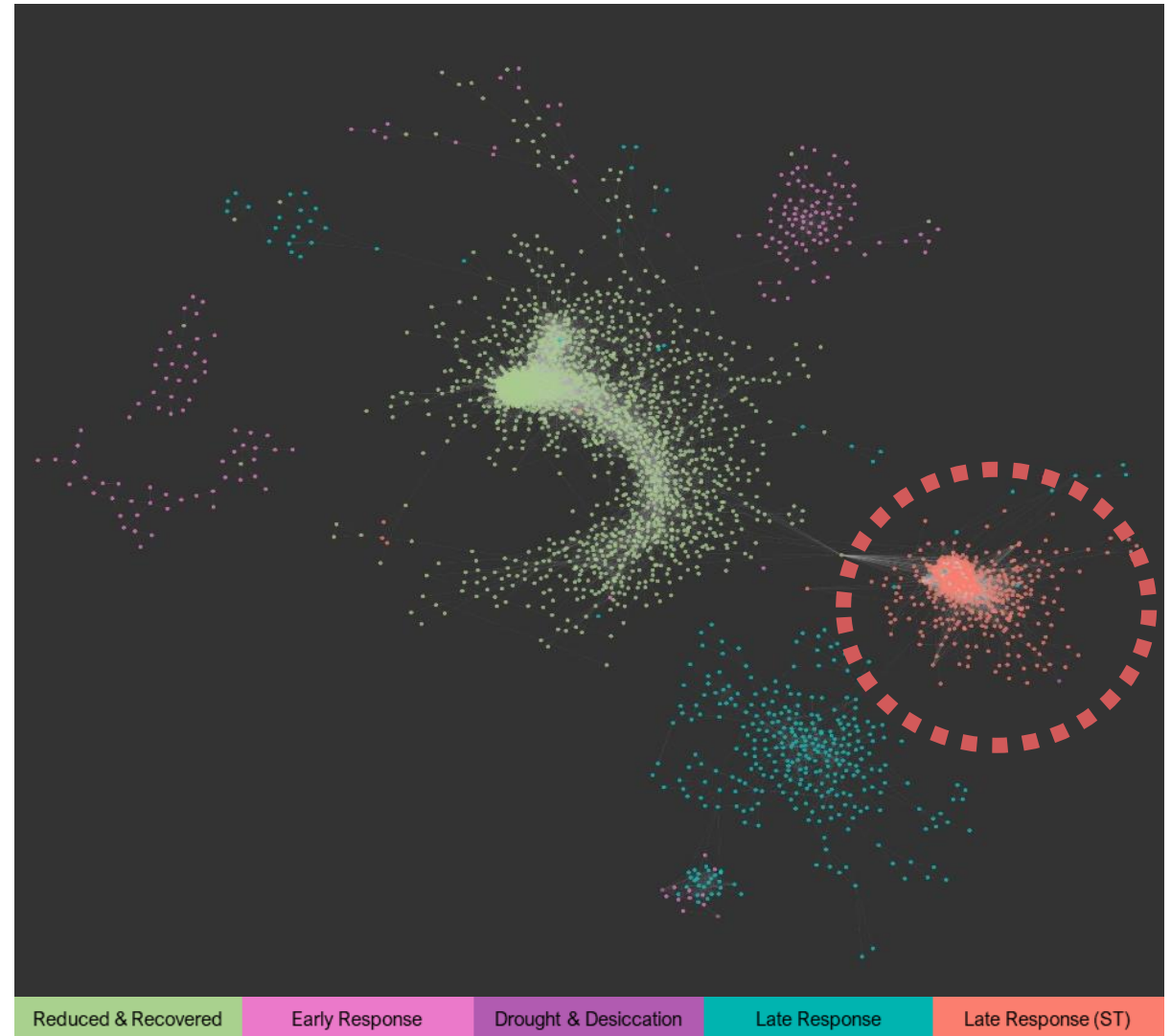
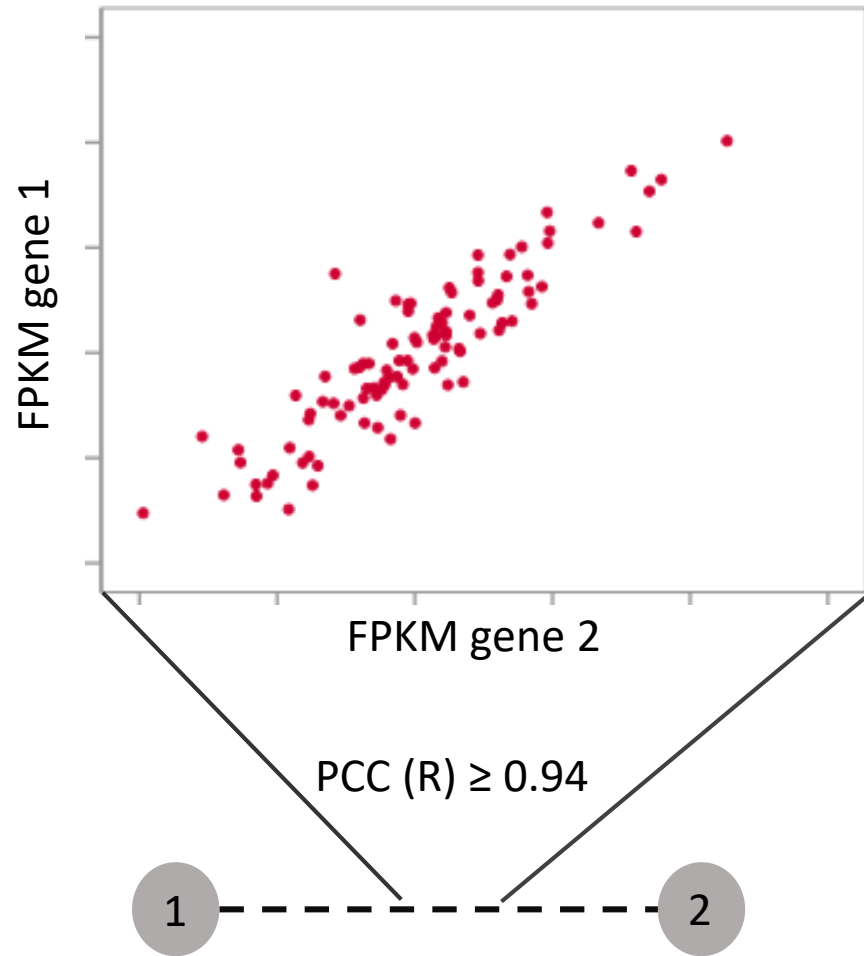
Which genes are highly correlated in terms of their expression and can we use this to find promoters and transcription factors?





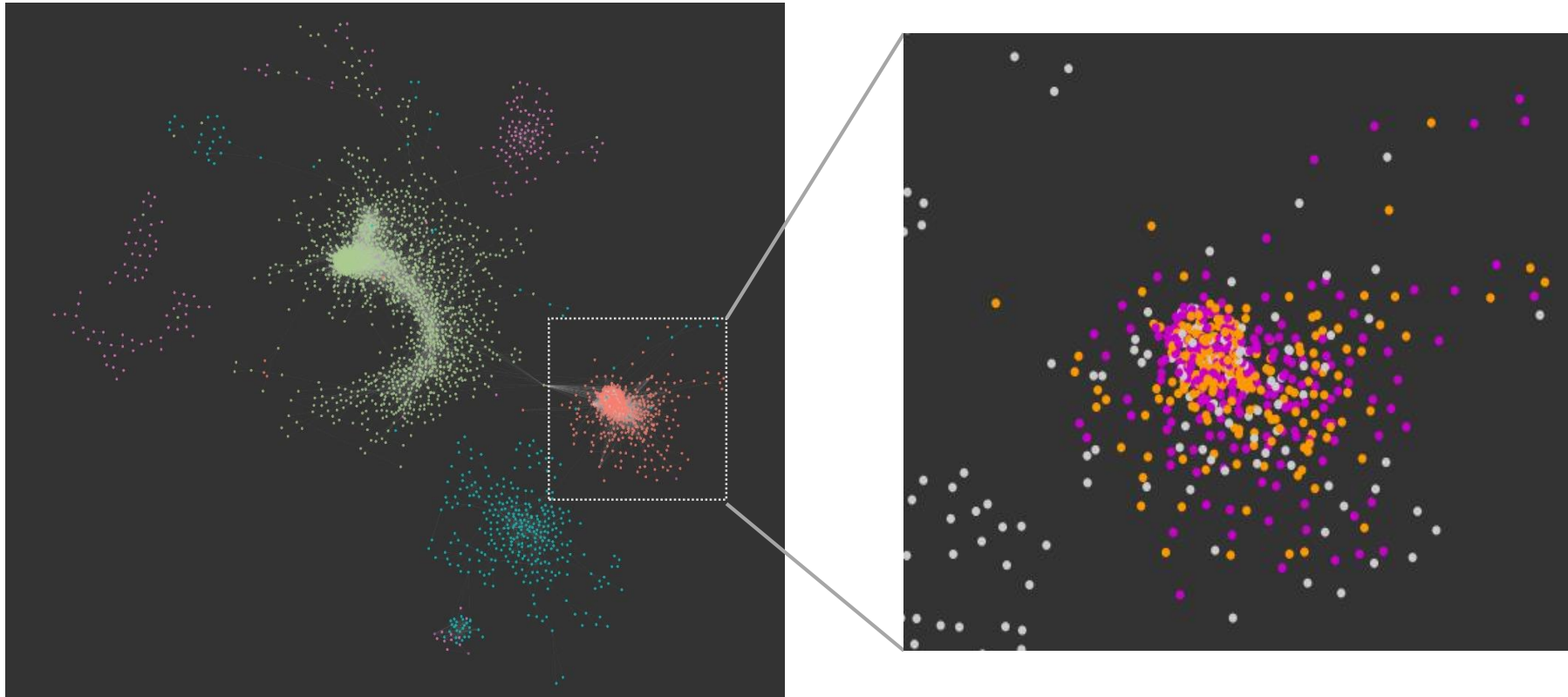
# Co-expression Network Analysis

Which genes are highly correlated in terms of their expression and can we use this to find promoters and transcription factors?



# Promoter Analysis

How is expression of senescence genes regulated?



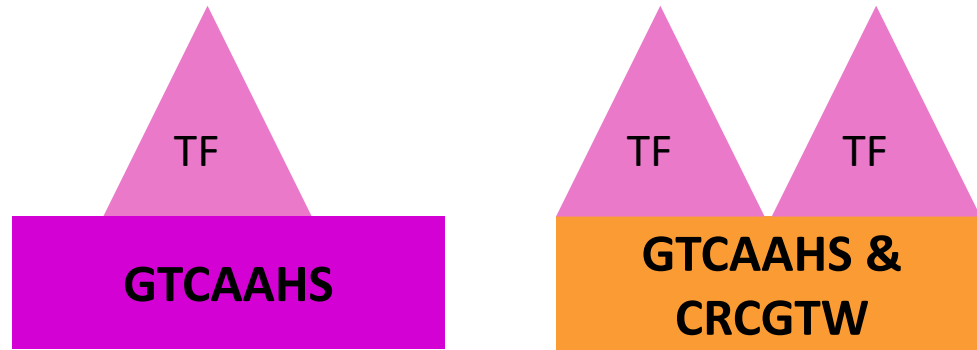
Promoter

DNA Sequences:

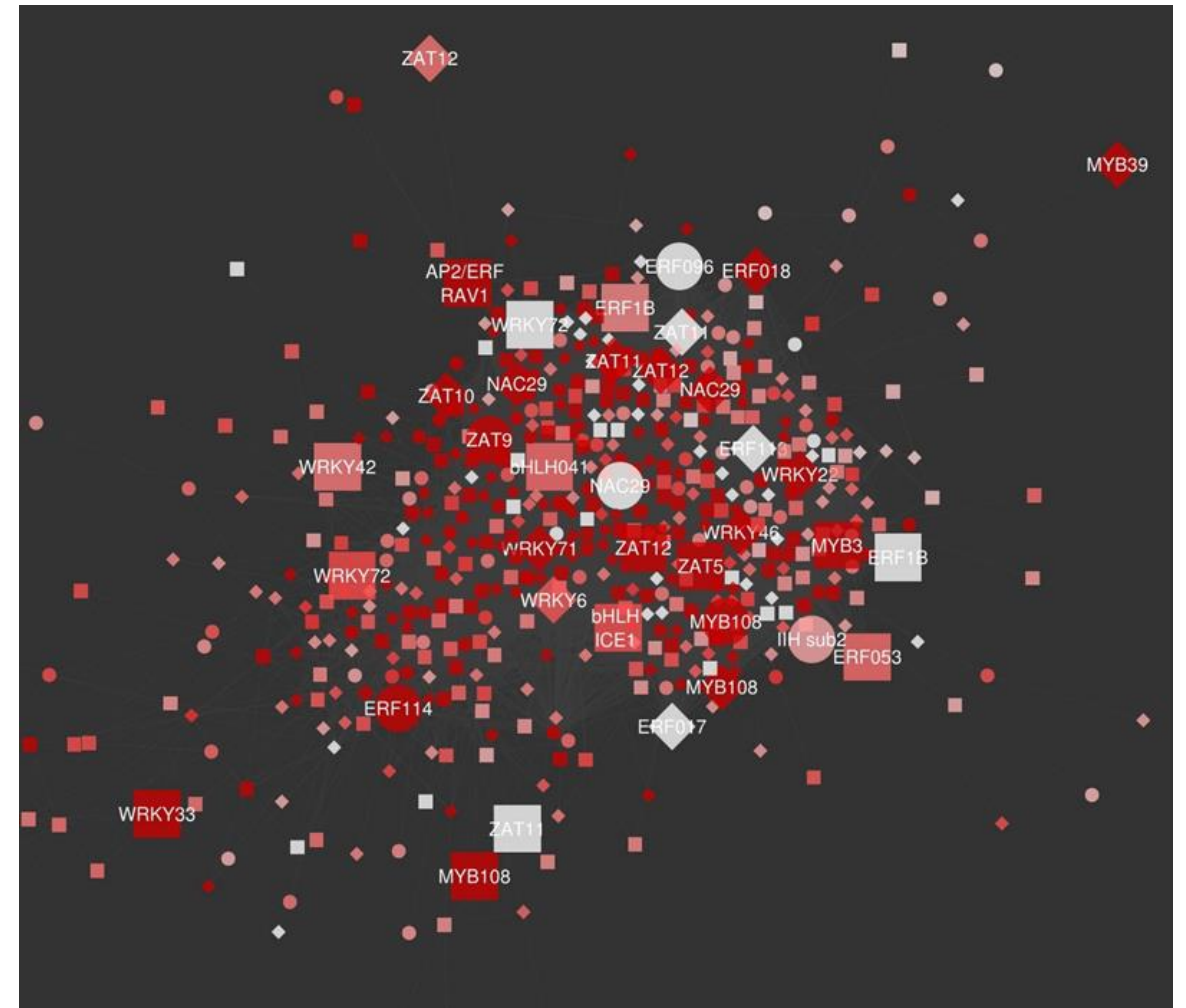
**GTCAAHS &  
CRCGTW**

**GTCAAHS**

## Which TFs are controlling the network?



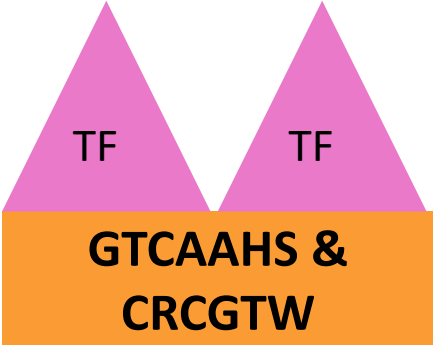
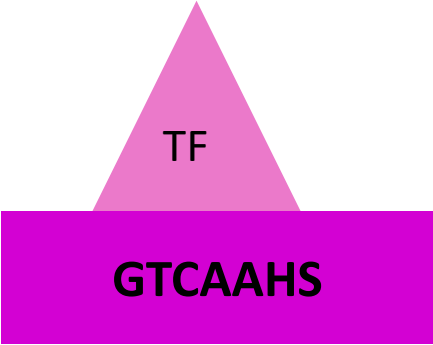
Tomtom compares one or more motifs against a database of known motifs (e.g., JASPAR). Tomtom will rank the motifs in the database and produce an alignment for each significant match (sample output for motif and JASPAR CORE 2014 database). See this [Manual](#) for more information.





# Transcription Factor Identification

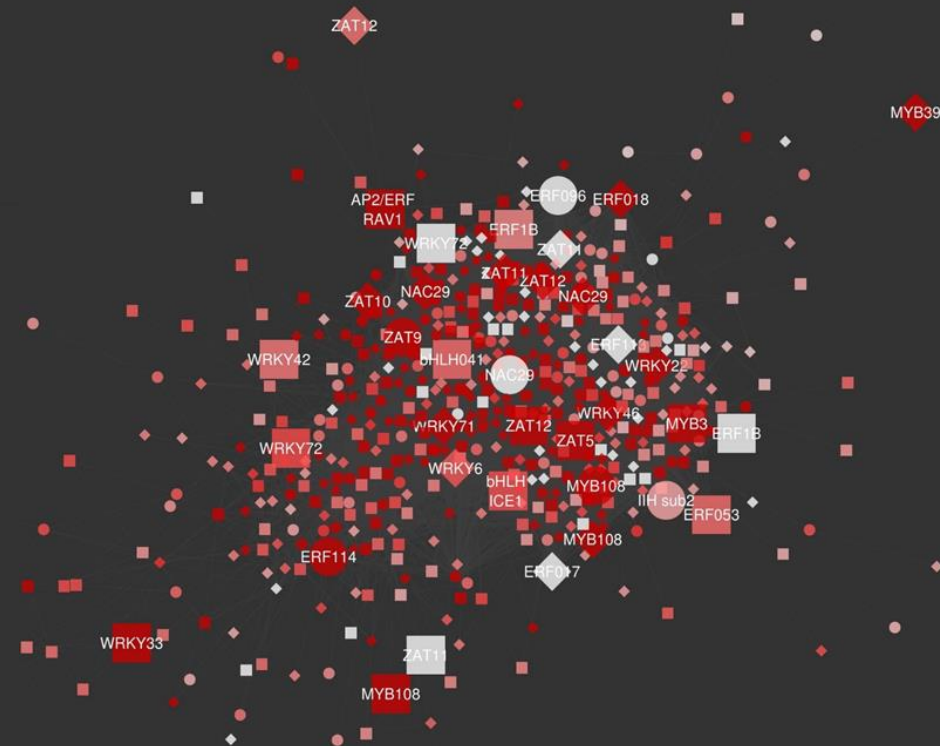
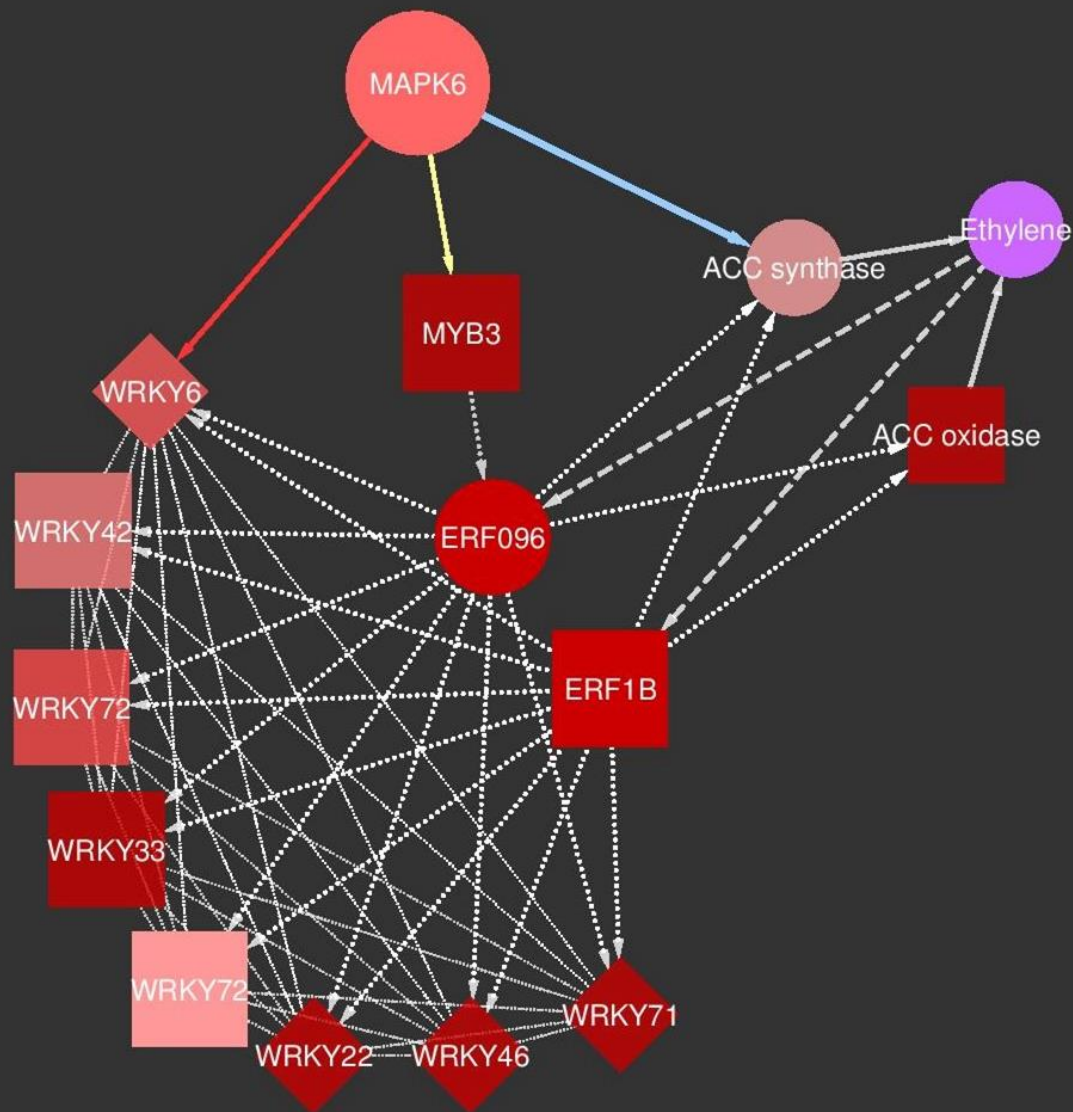
## Which TFs are controlling the network?



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 (sample output for motif and JASPAR CORE 2014 database). See this Manual for more information.

TomTom ID	Gene ID	X. schlechteri ID	Optimal offset	p-value	E-value	Overlap	Query consensus	Target consensus	Orientation
MA1165.1	AT1G49560		0	0.01	7.01	7	GTCAAAG	ATCAAAGATTC	+
MA1390.1	AT1G68670		2	0.02	9.56	7	GTCAAAG	GAATCAAAGATTC	+
MA1306.1	WRKY11	Xvis03_201752; Xvis03_213823; Xvis03_205846; Xvis03_205928; Xvis03_214066	5	0.00	0.98	7	GTCAAAG	AAAAAGTCAACGCT	-
MA1075.1	WRKY12		1	0.00	0.30	7	GTCAAAG	GGTCAACG	-
MA1314.1	WRKY14		4	0.00	1.04	7	GTCAAAG	AAAAGTCAACGAT	+
MA1076.1	WRKY15		2	0.00	0.13	7	GTCAAAG	AGGTCAACGC	+
MA1299.1	WRKY17		5	0.00	1.42	7	GTCAAAG	AAAAAGTCAACGCC	+
MA1077.1	WRKY18		3	0.00	1.20	7	GTCAAAG	ATGGTCAACG	+
MA1078.1	WRKY2	Xvis03_212849	2	0.00	1.54	6	GTCAAAG	CGGTCAAC	+
MA1295.1	WRKY20	Xvis03_200083; Xvis03_218672	4	0.00	0.43	7	GTCAAAG	AATAGTCAACGTT	-
MA1079.1	WRKY21		3	0.00	0.33	7	GTCAAAG	AAGGTCAACG	+
MA1303.1	WRKY22		4	0.00	1.49	7	GTCAAAG	AAAAGTCAACGAT	+
MA1080.1	WRKY23	Xvis03_221572; Xvis03_203616; Xvis03_203836; Xvis03_211583	1	0.00	0.17	7	GTCAAAG	AGTCAACG	+
MA1315.1	WRKY24		6	0.00	1.19	7	GTCAAAG	AAAAAGTCAACGA	-
MA1081.1	WRKY25		2	0.01	2.57	6	GTCAAAG	CGGTCAAC	+
MA1297.1	WRKY26	Xvis03_209784	4	0.00	0.95	7	GTCAAAG	AAAAGTCAACGGT	+
MA1318.1	WRKY27		4	0.00	1.62	7	GTCAAAG	AAAAGTCAACGAT	-
MA1311.1	WRKY28	Xvis03_211038	4	0.00	0.64	7	GTCAAAG	AAAAGTCAACGAT	-
MA1298.1	WRKY29		4	0.00	1.06	7	GTCAAAG	AAAAGTCAACG	+
MA1309.1	WRKY3	Xvis03_200369; Xvis03_208183; Xvis03_215320; Xvis03_206017; Xvis03_219578	4	0.00	0.34	7	GTCAAAG	AAAAGTCAACG	+
MA1083.1	WRKY30		1	0.00	0.37	7	GTCAAAG	GGTCAACGCT	+
MA1307.1	WRKY31		12	0.00	2.31	7	GTCAAAG	GGATAAAAAAAGTCAACG	+
MA1301.1	WRKY33	Xvis03_210468; Xvis03_221299	4	0.00	0.34	7	GTCAAAG	AAAAGTCAACG	+
MA1084.1	WRKY38		1	0.00	0.36	7	GTCAAAG	GGTCAACG	-
MA1085.2	WRKY40	Xvis03_201790; Xvis03_219584; Xvis03_214127; Xvis03_205822 Xvis03_223134	3	0.01	2.89	7	GTCAAAG	AAAGTCAAAA	+
MA1310.1	WRKY42		4	0.01	2.91	7	GTCAAAG	AAAAGTCAACGCTAATTAATA	-
MA1086.1	WRKY43		2	0.00	1.01	7	GTCAAAG	AAGTCAACAC	+
MA1087.1	WRKY45		1	0.00	0.27	7	GTCAAAG	GGTCAACG	-
MA1296.1	WRKY46	Xvis03_201984; Xvis03_209794	4	0.00	0.38	7	GTCAAAG	CAAAGTCAACG	-
MA1312.1	WRKY47		2	0.00	0.67	7	GTCAAAG	AAGTCAACGCCGGT	-
MA1088.1	WRKY48		3	0.00	0.24	7	GTCAAAG	GAGGTCAACG	+
MA1317.1	WRKY50	Xvis03_214509; Xvis03_220179	6	0.00	0.53	7	GTCAAAG	AAAAAGTCAAAAG	-
MA1305.1	WRKY55		3	0.00	0.89	7	GTCAAAG	AAAGTCAACGCT	-
MA1089.1	WRKY57	Xvis03_200381	3	0.00	0.22	7	GTCAAAG	AAAGTCAACG	+
MA1304.1	WRKY59		5	0.00	0.07	7	GTCAAAG	AAAAAGTCAAAAG	+
MA1300.1	WRKY6	Xvis03_215220; Xvis03_201108; Xvis03_209315	12	0.00	2.11	7	GTCAAAG	ATGTTAAAAAAGTCAACG	-

# Predicted Cell Death Regulation Model

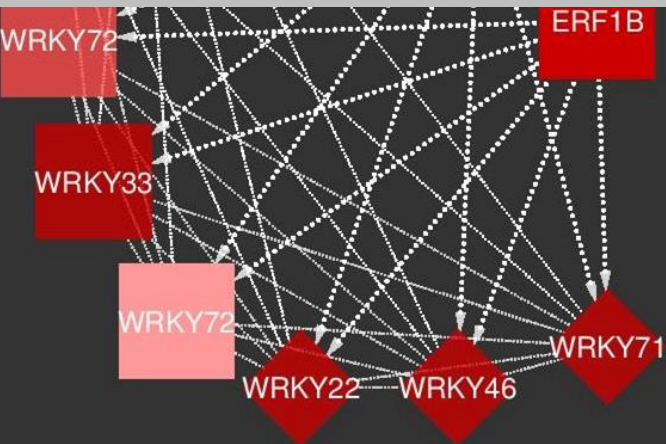
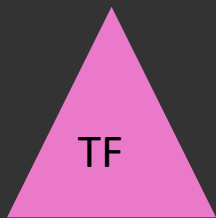


Activation of targets in <i>A.thaliana</i> literature:	Proposed interaction in <i>X. schlechteri</i> :
Smékalová et al. 2014	Activates/Produces
Kim, et al. 2018	Incomplete regulatory pathway
Chai, et al. 2014	Putative transcriptional regulator
Liu & Zhang, 2004	

# 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?



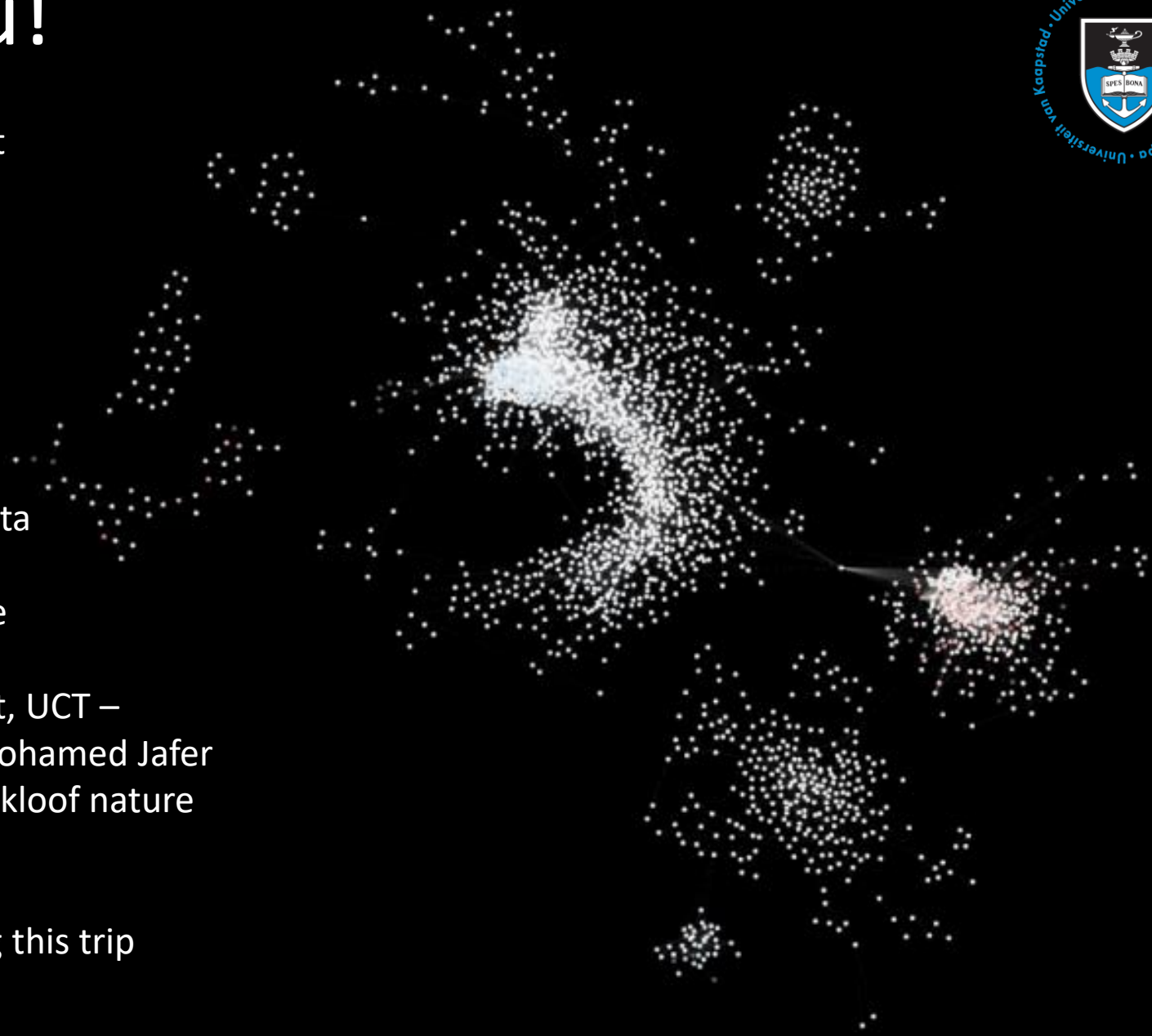
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# Thank You!

Prof Jill Farrant  
Assoc. Prof Henk Hilhorst  
Dr Suhail Rafudeen  
Keren Cooper  
Dr Amelia Hilgart  
Pei-Yin Liebrich  
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

R consortium for funding this trip



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