

1. Introduction

Food security is becoming a crucial issue, with crop yields being decreased by plant pathogens

One of the major pathogens is the grey mould, *Botrytis cinerea*

The key to developing resistant plants is to gain a better understanding of the molecular mechanisms that take place when a plant is infected

The PRESTA (Plant Responses to Environmental Stress in Arabidopsis) project seeks to determine the gene networks in Arabidopsis that mediate the responses to abiotic and biotic stresses, using high definition time series microarrays

Several genes with multiple up- and down-stream interactions ("hub" genes) were identified in network reconstruction modelling, and in this project, three were selected for further investigation

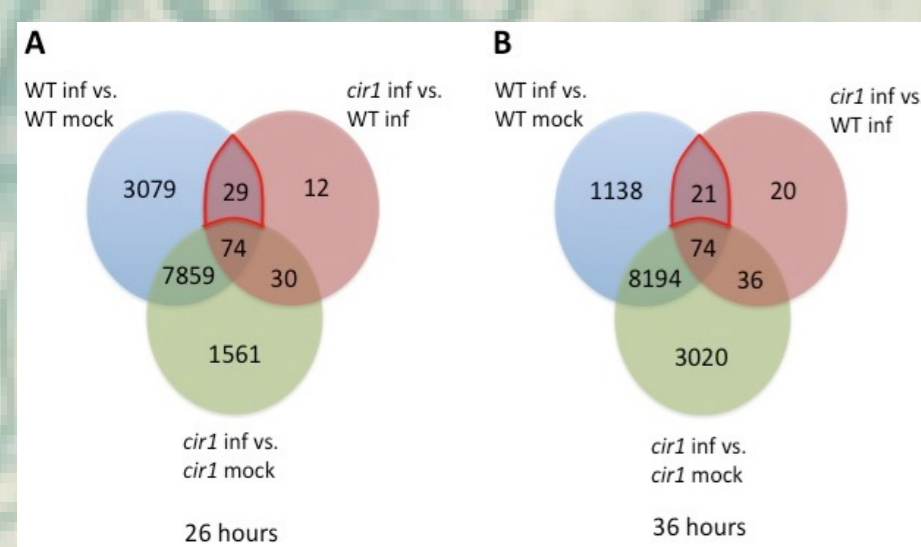
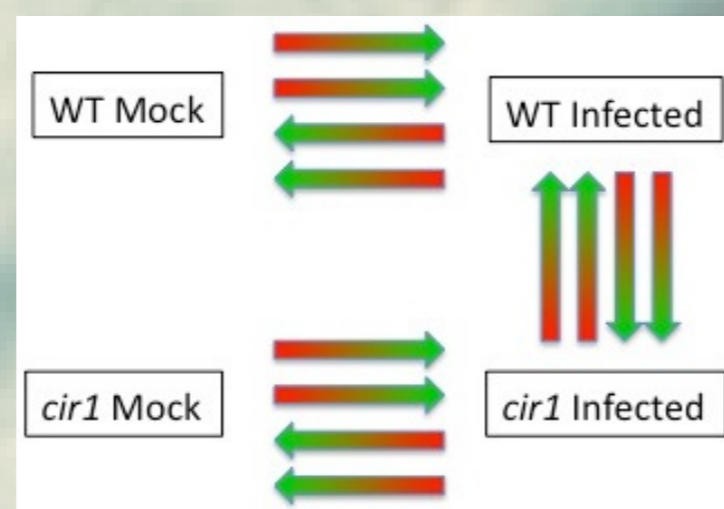
2. Downstream targets of CIR1

Performed **microarrays**

- CATMA v4 arrays
- 2 timepoints - 26 and 36 hpi
- 4 pooled biological replicates
- 4 technical replicates
- Slide hybridisations shown on right
- Same hybridisations at each timepoint

Data analysis using limma - linear models
FDR corrected p-value cutoff of 0.01

CIR1 (CIRCADIAN 1, AT5G37260.1)
MYB family TF
Controls auxin levels
Indirect circadian control
CIR1 knockout more susceptible to *B. cinerea*



The number of common genes between the different datasets are shown on the left

Of most interest were those circled in red

These genes are potentially downstream of CIR1, as well as being induced in response to *B. cinerea* infection

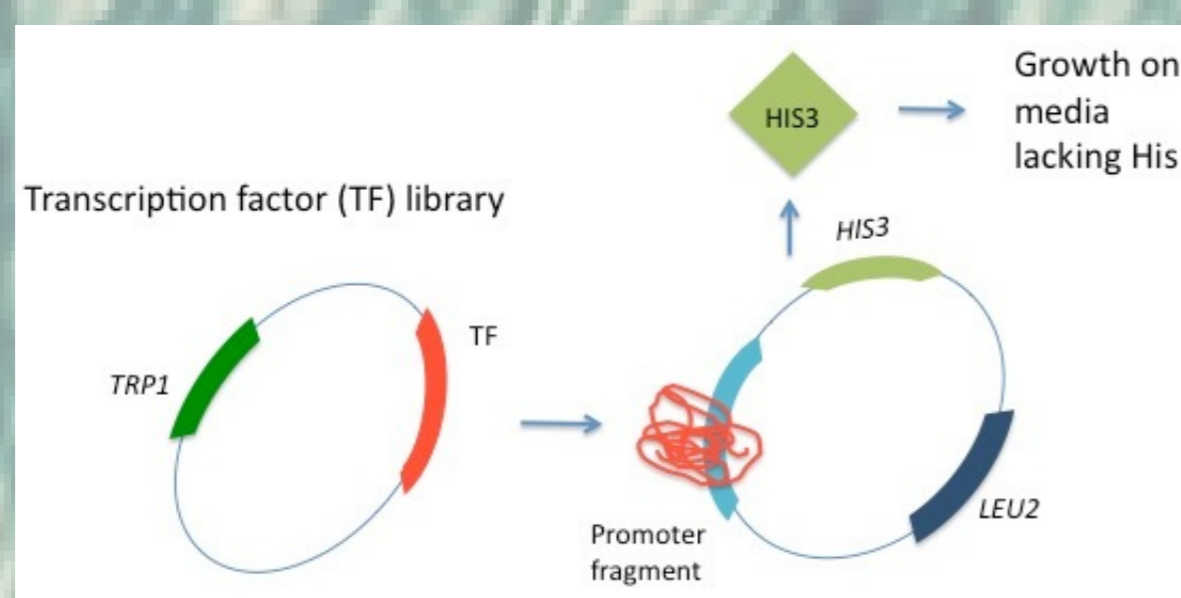
Interesting genes (from 36 hour data) included:

- § *AT3G22235* - similar to a gene that is circadian and pathogen controlled
- § *EBF2* - an ethylene regulator

3. Yeast one-hybrid

Yeast one-hybrid works by allowing a transcription factor (TF) from a library, to interact with a promoter of interest.

If a positive interaction occurs, the production of the *HIS3* reporter gene is induced, allowing the yeast cells to grow on media lacking histidine.



4. Upstream targets of TGA3

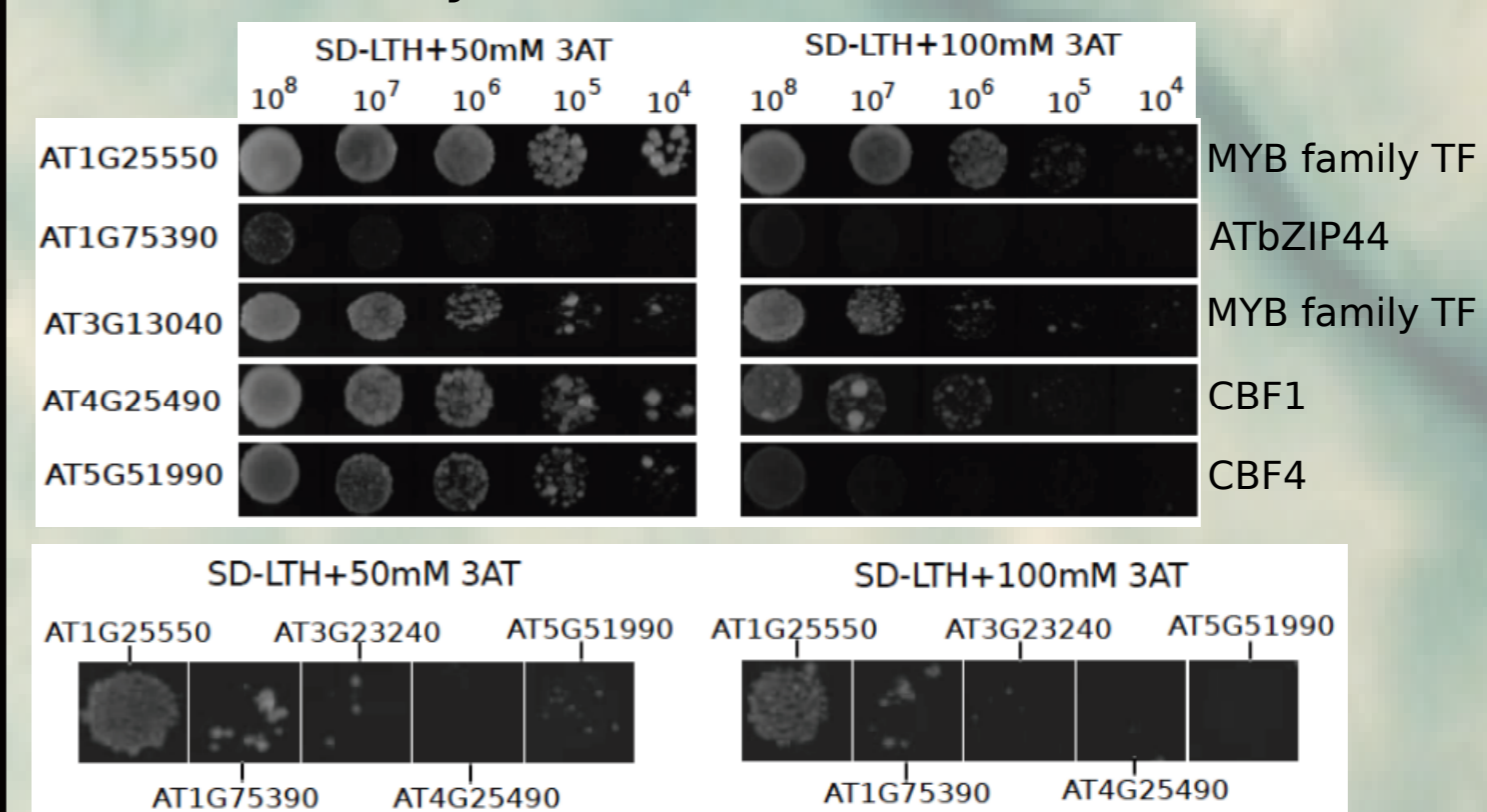
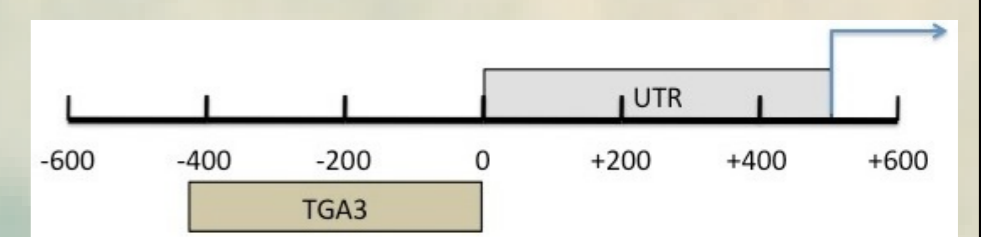
Performed **yeast one-hybrid**

- Promoter fragment shown on right

Interesting TFs that bound:

- § MYB family TFs
- § ATbZIPs
- § CBF family

TGA3 (AT1G22070.1)
Binds to NPR1, inducing expression of *PR-1*
TGA3 knockout more susceptible to *B. cinerea*



Interactions confirmed by co-transformation (left top) and mating (left bottom)

5. Upstream targets of ARF2

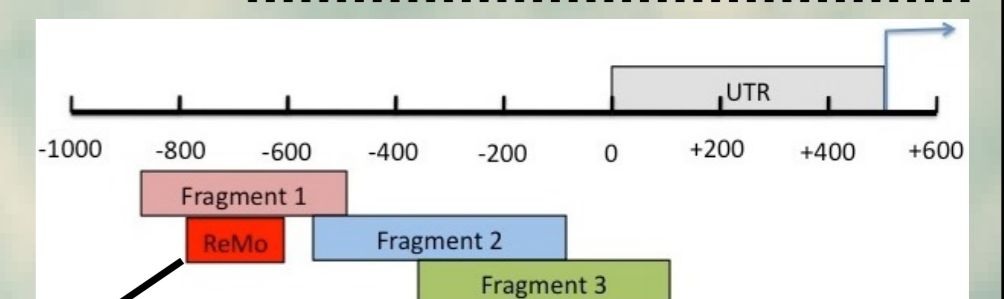
Performed **yeast one-hybrid**

- Three overlapping promoter fragments screened
- Shown on right

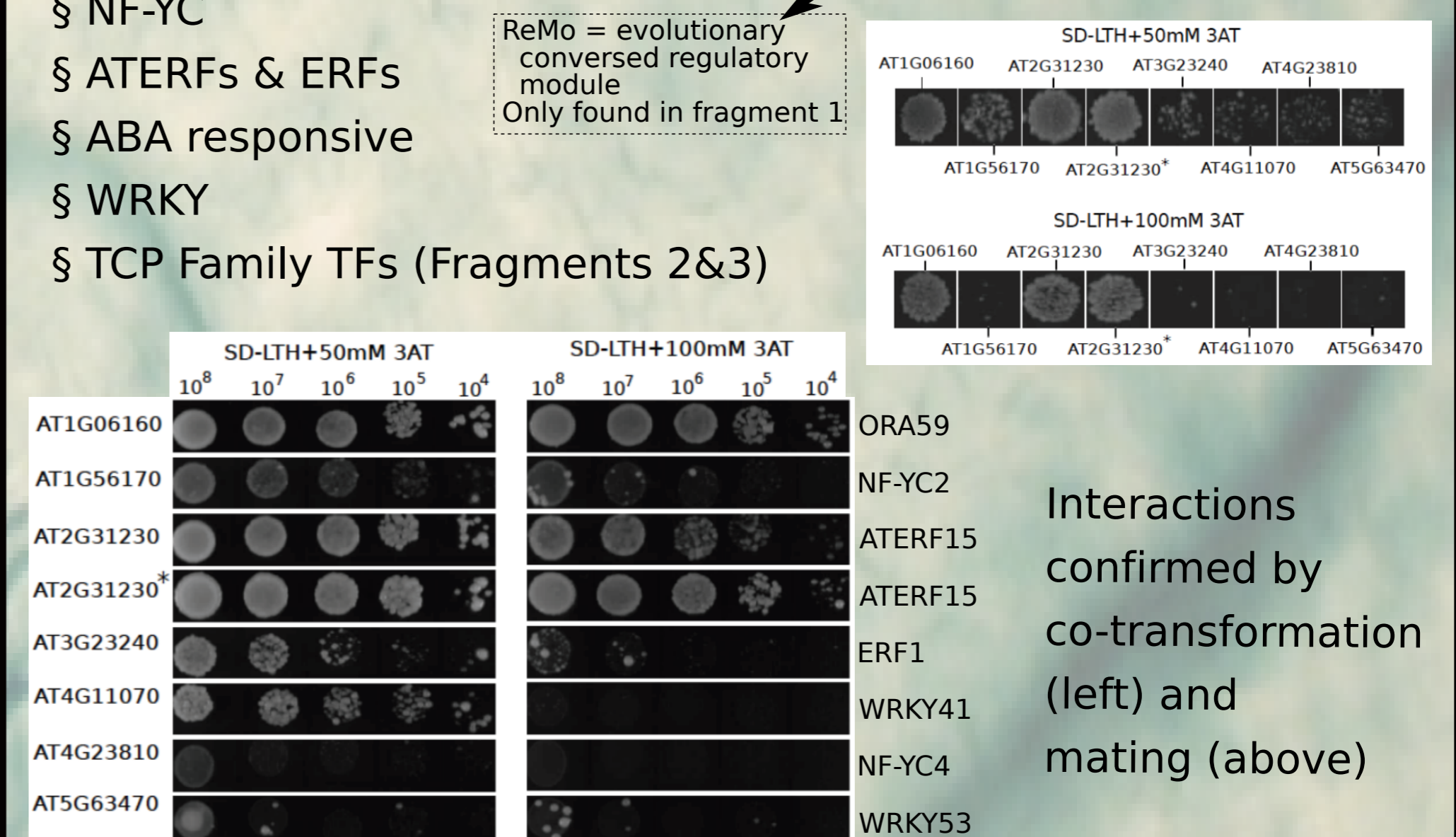
Interesting TFs that bound:

- § ORA59
- § NF-YC
- § ATERFs & ERFs
- § ABA responsive
- § WRKY
- § TCP Family TFs (Fragments 2&3)

ARF2 (AUXIN RESPONSE FACTOR 2, AT5G62000.1)
Binds and represses genes involved in auxin signalling
ARF2 knockouts exhibit delayed senescence and more resistant to *B. cinerea*



ReMo = evolutionary conserved regulatory module
Only found in fragment 1



Interactions confirmed by co-transformation (left) and mating (above)

6. Conclusion

Extended local networks of three hub genes

- Can use this information in further modellings

Further work:

- § Mutagenise portions of the promoter
 - Which TFs no longer bind?
 - Identify sections that are the most important
- § Test other members of the same TF family
 - Specificity of the interaction

