

Using overexpression of predicted hub genes to test models of gene regulatory networks mediating defence against *Botrytis cinerea* in *Arabidopsis thaliana*

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1. Identification of genes that are differentially expressed following infection has identified potential key regulatory genes (hubs). Testing overexpressor lines of predicted hub genes for disease resistance, and determining potential downstream target genes of confirmed regulatory genes was conducted using expression profiling.

2. Plants are subject to a wide variety of biotrophic and necrotrophic fungal pathogens, which pose a huge economical and agricultural threat. *Botrytis cinerea*, a type of grey mould, infects a wide variety of soft fruits, such as strawberries and grapes causing significant pre- and post-harvest losses.

3. Real-time PCR was conducted for *ANAC059* overexpressor line against Col-0 wt with Actin primers as reference to ensure the line was overexpressed. The Crossing point (Cp) of *ANAC059* occurred before Col-0 wt, confirmed this line is overexpressed.

Sample name	Target	Reference	Mean Cp
TP5	TP5	Actin	31.80
Col-0 wt	TP5	Actin	36.01

Table 1 - Mean crossing points of samples

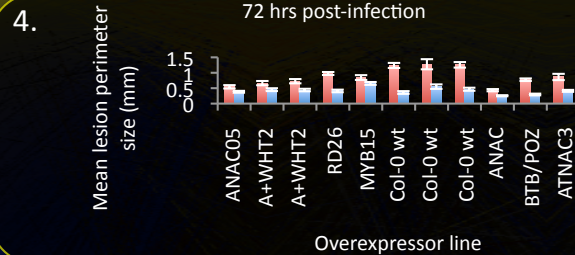
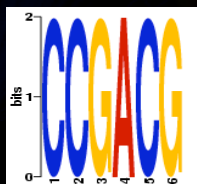


Figure 1 – mean lesion size of each overexpressor line with standard error bars. Red bars represent the first infection profile. Blue bars represent the second infection profile.

Two-week old *Botrytis* was isolated and used to infect leaves from each overexpressor line. Photos were taken and the lesion perimeter size was analysed in ImageJ.

The lowest mean lesion perimeter was observed for overexpressor line *ANAC092*, suggesting resistance, a phenotype not seen before for this gene. *BTB/POZ* also conferred good resistance to *Botrytis* at 72 hours post-infection.

5. *ANAC059* and *BTB/POZ* aRNA samples were labelled with Cy3 and Cy5 dyes, and hybridised to CATMAv4 slides against Col-0 wt.



A MEME search was conducted for the upregulated *ANAC059* genes, returning this motif, which is currently unknown.

Figure 2 – MEME output

6. BiNGO was used to visualise clusters of overrepresented genes from the microarray. *BTB/POZ* overexpressor line showed overrepresented genes involved in plant resistance.

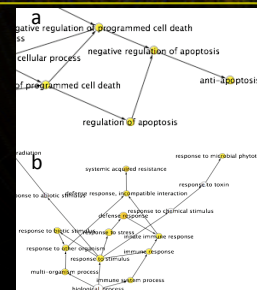


Figure 3 – overrepresented genes found in the *BTB/POZ* upregulated gene list; a) shows genes involved in apoptosis; b) shows genes involved in immune response

7. Overexpression of *BTB/POZ* conferred resistance to *Botrytis*. *ANAC092*, previously only known for drought resistance properties, showed *Botrytis* resistant properties when overexpressed. Microarray analysis revealed possible targets for future investigation.