Pseudomonas syringae Type-III Secreted Effectors Elicit Unique Transcriptional Responses in *Arabidopsis thaliana*

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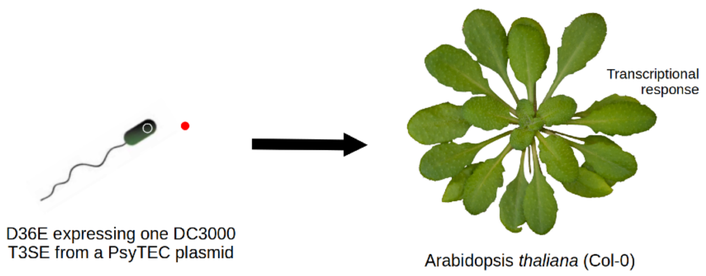
Introduction

The bacterium *Pseudomonas syringae* destroys millions of dollars of crops as a hemibiotrophic phytopathogen.

P. syringae enters the stomata and injects its type-III secreted effector proteins[1] into the symplast to disable host immunity. The plant responds with a transcriptional counter-attack (PAMP-triggered immunity, or *PTI*).

But does each effector induce a *unique* transcriptional response in the plant?

We've adapted a system developed by Wei et. al[2] to answer that question:



Isolated effectors > overexpression

Methods

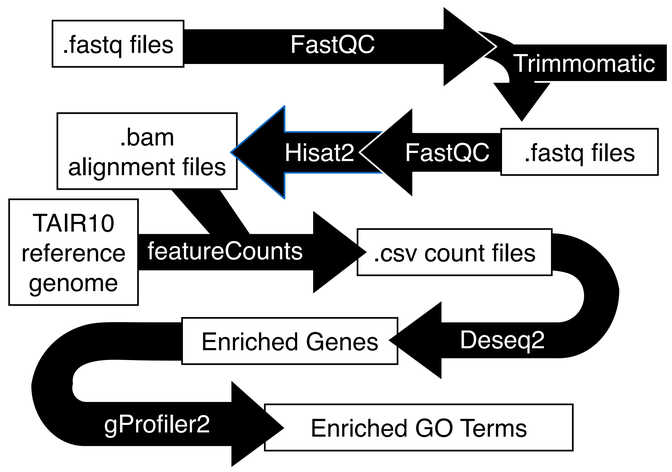
Infection:

- Pressure infiltrate the following well-characterised *P. syringae* effectors, suspended in 10 mM MgSO4 at OD = 0.0002, in into *A. Thaliana* in biological triplicate:
  - D36E::**HopN1**a
    - Cleaves PsbQ in chloroplast's photosystem II to suppress SA signalling
  - D36E::**HopB1**a
    - Cleaves BAK1 at the membrane to suppresses PTI
  - D36E::**HopAB1**j
    - Ligates ubiquitin to FLS2 at membrane to suppress PTI
- With D36E::EV and 10 mM MgSO4 as controls, leaves were frozen 1h and 8h post-infiltration.

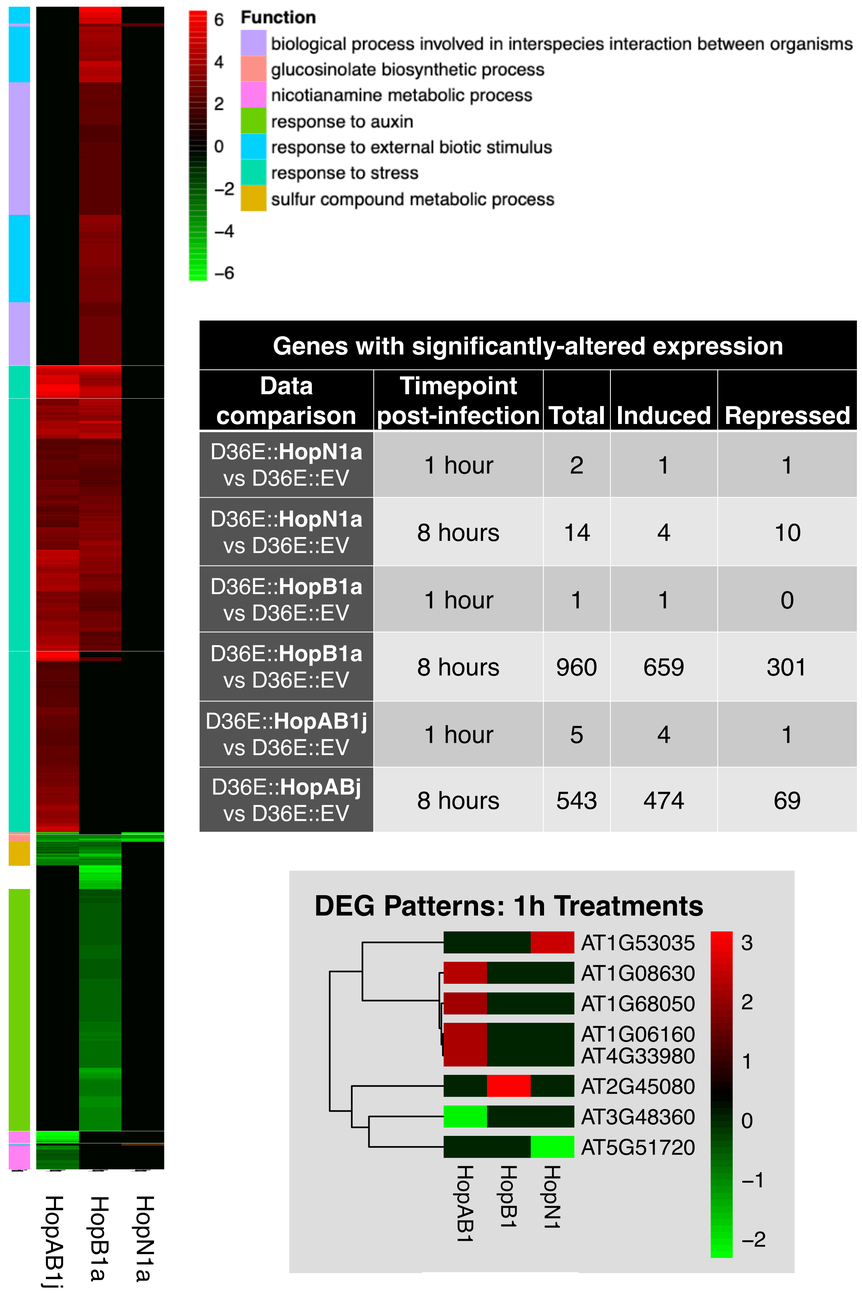
RNA Extraction & Sequencing:

- Frozen leaves were ground in LN2 via mortar & pestle, then suspended in TRIzol.
- Centrifuged then supernatant was mixed with chloroform
- Centrifuged again and span down through RNEasy spin column kit
- Samples were stored at -80°C
- Samples were sequenced on an Illumina NextSeq 2000

Computational Pipeline



Results



Genes with significantly-altered expression				
Data comparison	Timepoint post-infection	Total	Induced	Repressed
D36E::**HopN1a** vs D36E::EV	1 hour	2	1	1
D36E::**HopN1a** vs D36E::EV	8 hours	14	4	10
D36E::**HopB1a** vs D36E::EV	1 hour	1	1	0
D36E::**HopB1a** vs D36E::EV	8 hours	960	659	301
D36E::**HopAB1j** vs D36E::EV	1 hour	5	4	1
D36E::**HopABj** vs D36E::EV	8 hours	543	474	69

Results cont.

DEGs at 1 hour post-infection by effector treatment				
Effector	Locus	Locale	Regu-lation	Product
HopN1a	AT1G53035	Chloroplast	Up	Transmembrane protein
HopN1a	AT5G51720	Chloroplast	Down	**NEET**, involved in ROS homeostasis
HopB1a	AT2G45080	Cytoplasm	Up	Cyclin P3, enables protein kinase binding
HopAB1j	AT1G08630	Cytosol	Up	THA1, degrades Thr → Gly
HopAB1j	AT1G68050	Cytosol & nucleus	Up	Part of **SCF uqituitin ligase** complex
HopAB1j	AT1G06160	Nucleus	Up	**ORA59**, master regulator of JA pathway
HopAB1j	AT4G33980	Nucleus	Up	COR28
HopAB1j	AT3G48360	Nucleus	Down	BT2, part of TAC1-mediated telomerase pathway

Discussion

- **1 hour post-infection**
  - HopN1 demonstrates locale specificity, HopAB1 & HopB1 less so
  - HopN1 NEET and HopAB1j ORA59, SCF demonstrate functional specificity, HopB1 less so
- **8 hour post-infection**
  - Unique expression patterns visible across all treatments
  - Stress response activation and auxin

Conclusion

D36E::HopAB1j, D36E::HopB1a, and D36E::HopN1a induce unique transcriptional responses in A. Thaliana.

Their "transcriptonal fingerprints" are partially capable of characterising localisation and/or functional outcomes.

References

1.Xin X, Kvitko B, He SY. "*Pseudomonas syringae*: what it takes to be a pathogen". Nat Rev Microbiol, 16, 5, 2018, pp. 316-318. 10.1038/nrmicro.2018.17

2. Wei H, Chakravarthy S, Mathieu J, Swingle B, Martin G, Collmer A. "*Pseudomonas syringae* pv. *tomato* DC3000 Type III Secretio Effector Polymutants Reveal an Interplay between HopAD1 and AvrPtoB". Cell Host & Microbe, 17, 2015, pp. 752-762. 10.1016/j.chom.2015.05.007