# *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] and Laflamme et. al[3] to answer that question:

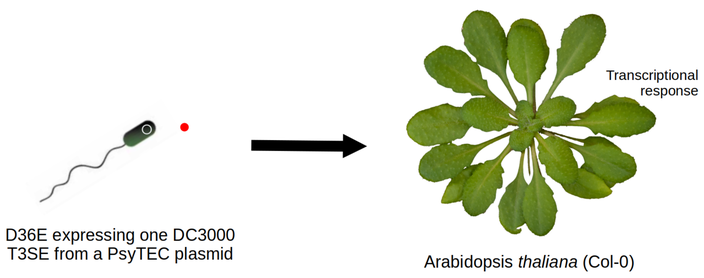


Figure 1: *The model system we used to study individual effectors*

## Methods

### Infection:

- Pressure infiltrate the following well-characterised *P. syringae* effectors, suspended in 10 mM MgSO4 at $OD_{600}$ = 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

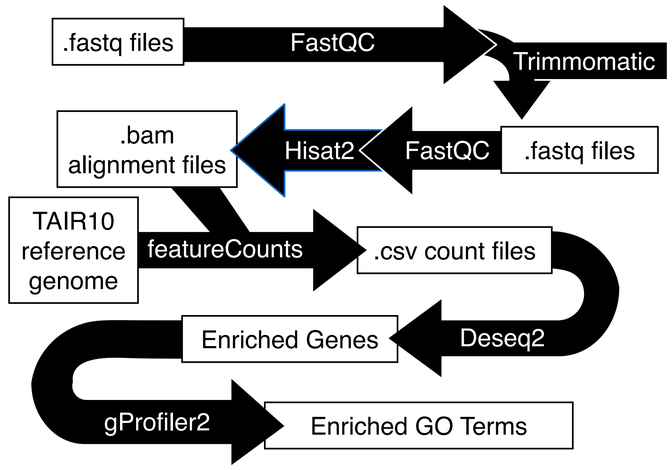


Figure 2: *DEG patterns by treatment 8 hours post-infection*

## Results

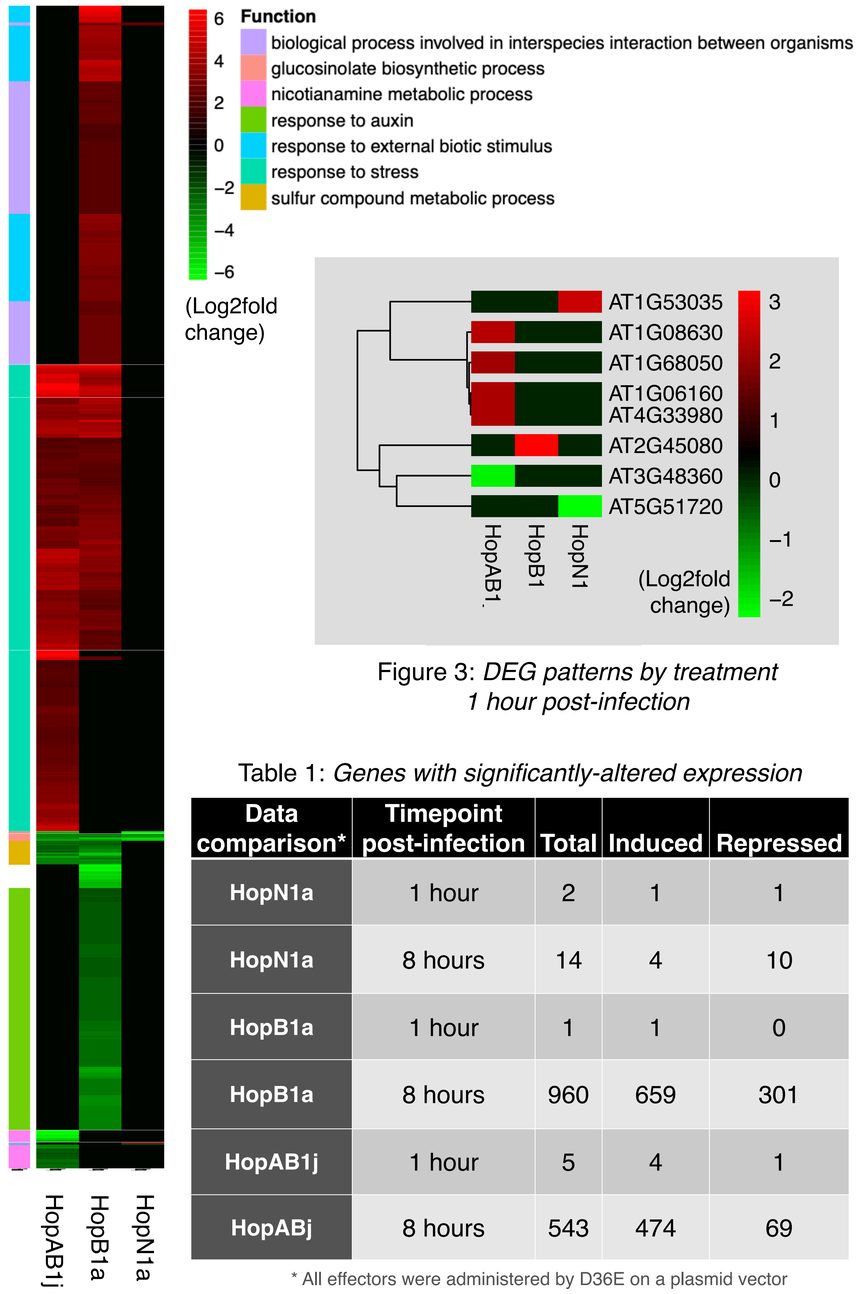


Figure 3: *DEG patterns by treatment 1 hour post-infection*

Table 1: *Genes with significantly-altered expression*

| Data comparison* | Timepoint post-infection | Total | Induced | Repressed |
|---|---|---|---|---|
| HopN1a | 1 hour | 2 | 1 | 1 |
| HopN1a | 8 hours | 14 | 4 | 10 |
| HopB1a | 1 hour | 1 | 1 | 0 |
| HopB1a | 8 hours | 960 | 659 | 301 |
| HopAB1j | 1 hour | 5 | 4 | 1 |
| HopABj | 8 hours | 543 | 474 | 69 |

* All effectors were administered by D36E on a plasmid vector

## Results cont.

Table 2: *DEGs at 1 hour post-infection by effector treatment. Red rows are up-regulated and green genes are down-regulated.*

| 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 |

* All effectors were administered by D36E on a plasmid vector

## 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 "transcriptional 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 Secretion Effector Polymutants Reveal an Interplay between HopAD1 and AvrPtoB". Cell Host & Microbe, 17, 2015, pp. 752-762. 10.1016/j.chom.2015.05.007

3. Laflamme B, Dillon M, Martel A, Almeida R, Desveaux D, Guttman D. "The pan-genome effector-triggered immunity landscape of a host-pathogen interaction". Science, 367(6479), 2020, pp. 763-768. 6/science.aax4079