CHARACTERISATION OF EFFECTOR PROTEINS SECRETED BY *Erwinia carotovora* ssp. *atroseptica* AND THEIR ROLE ON HOST RESISTANCE

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Introduction

The type three secretion system (TTSS) is used to translocate effector proteins (DspE, HrpW and HrpN) across the host membrane into plant cells, where they appear to interact with the host.

Mutations in HrpN and DspE are reduced in pathogenicity (Holeva et al., 2004). The role of HrpW and 8 unique coding sequences within the TTSS cluster are being investigated. We believe that the above proteins alter plant defense pathways.

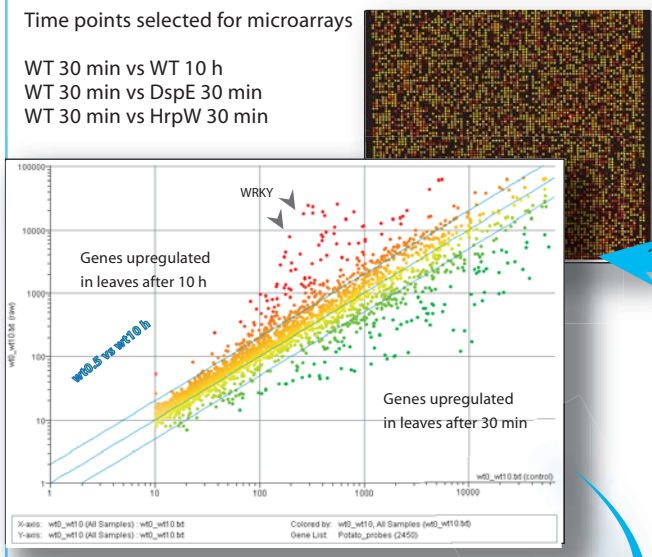
Aims

- To identify mutants in HrpW and the 8 unique cds and assess their role in pathogenicity
- To identify potato defense pathways modified by the effector proteins
- To use this knowledge for enhanced resistance to *Erwinia* (Eca)

Microarrays

Time points selected for microarrays

WT 30 min vs WT 10 h
WT 30 min vs DspE 30 min
WT 30 min vs HrpW 30 min

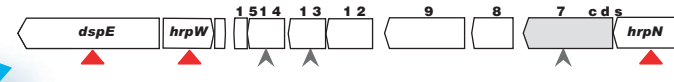


Improved resistance

- Up regulation of WRKY after 10h confirmed by microarrays
- Transgenic lines were produced that showed an increase in WRKY gene expression
- Pathogenicity assays on Desiree vs transgenic WRKY lines
- Lesion measured from 2 to 17 days post inoculation

Mutant identification and Real time PCR

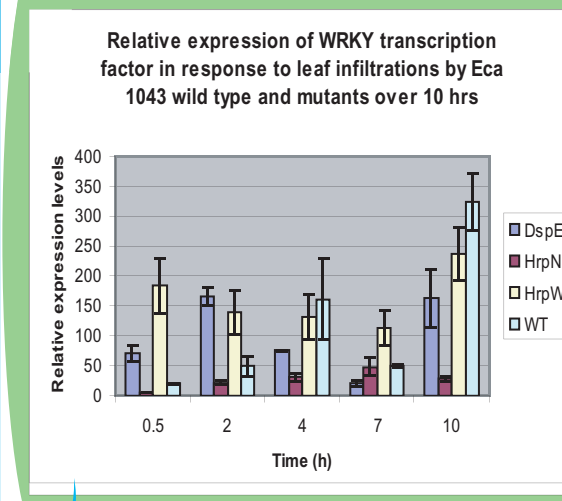
Mutant identification



Mutations with pathogenicity assays underway

Mutations with reduced pathogenicity

Real time PCR



WRKY is highly induced at 10 h in response to Eca 1043 WT.

In contrast, WRKY seems to be induced as early as 30 mins in response to HrpW and DspE mutants.

Could effector proteins block WRKY in WT interactions?

Improved resistance

Transgenic lines are significantly resistant to *Erwinia* compared to control plants

Desiree control (17dpi)

Transgenic WRKY plant (17dpi)

Future Work

- Biological repeats for both Taqman and microarray analysis to confirm results
- Further analysis of up/down regulated genes from microarrays to identify pathways targeted by WT vs effector mutants
- Identify specific host proteins that interact with effector proteins using yeast-2-hybrids

References

Holeva, M.C., K.S. Bell, L.J., Hyman, A.O. Avrova, S.C. Whisson, P.R.J. Birch, and I.K. Toth. 2004. Use of a Pooled Transposon Mutation Grid to Demonstrate Roles in Disease Development for Erwinia carotovora subsp artroseptica Putative Type III secreted effector (DspE/A) and Helper (HrpN) Proteins. MPMI 17:943-950

Acknowledgements

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