Role of Erwinia carotovora subsp. atroseptica harpins in the manipulation of host defences

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Introduction

Erwinia carotovora subsp. *atroseptica* (*Eca*) is an important pathogen of potato, causing tuber soft rot and blackleg. Recently, the type three secretion system (T3SS) has been reported in *Eca*. T3SS is used to translocate effector proteins such as DspE across the host membrane into the plant cell, where they appear to interact with host proteins.

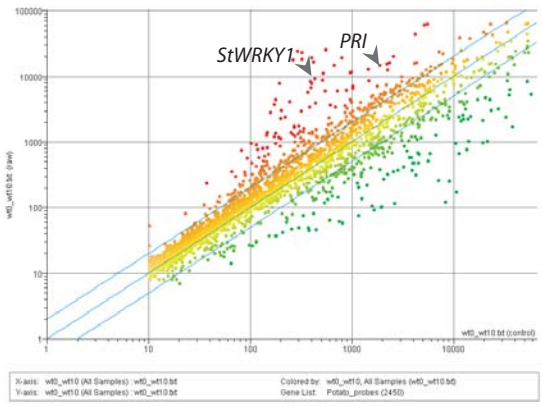
We are investigating the role of harpins in pathogenicity and in manipulation of potato defences using *Eca* mutants and analysing the effect using potato microarrays. This highlighted the *StWRKY* transcription factor that provides resistance to *Eca* and we hope could be useful for breeding *Eca* resistant potatoes.

Aims

- To identify a mutant in *hrpW* and assess its role in pathogenicity
- To identify potato defence pathways modified by effector and helper proteins
- To use this knowledge for enhanced resistance to *Erwinia*

Microarrays

To determine which other genes are up-regulated together with *StWRKY1* transcription factor, cDNA from WT 0.5 vs WT 10 hours post inoculation (hpi) were hybridised to an Agilent microarray. *PRI* gene was also shown to be up-regulated together with the *StWRKY* transcription factor. Both the *StWRKY* transcription factor and *PRI* were also found up-regulated early in response to *hrpW* and *dspE* mutants. Since *PRI* is a marker of a salicylic acid (SA)-dependant pathway, this suggests that both proteins may be involved in suppression of SA-dependant pathways directly or indirectly.



Improved resistance

- The above results suggest that constitutive expression of *StWRKY* transcription factor can increase resistance to *Erwinia*.
- Transgenic lines with increased expression of the *StWRKY* transcription factor were generated.
- Pathogenicity assays on Desiree vs transgenic WRKY lines
- Lesion measured from 2 to 17 days post inoculation (dpi)

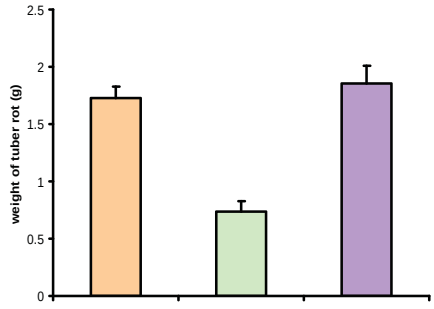
Future Work

- Microarray analysis of plant responses to *hrpW* mutant and SA at 0.5, 3, 7 and 10hpi
- Further analysis of up/down-regulated genes from microarrays to identify pathways targeted by WT vs effector mutants
- Determination of effector localisation *in planta*
- Virus-Induced Gene Silencing (VIGS) of WRKY in Desiree

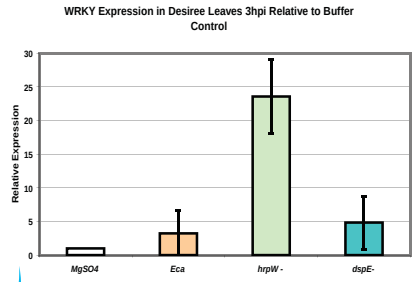
Characterision of *hrpW* mutant and Real time PCR

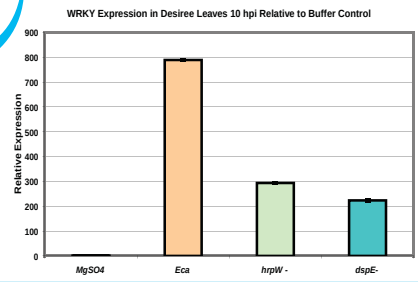
A *hrpW* Tn5 insertion mutant was identified and pathogenicity tests on both potato tubers and stems showed reduced virulence of mutant.

To restore pathogenicity, *hrpW* mutant was complemented with pGEM-T Easy plasmid carrying *hrpW* together with its chaperone. A complemented *hrpW* mutant was as virulent as *Eca* 1043 wild type (WT).



Real time PCR





WT *Eca* 1043 and the *dspE* mutant repress the *Solanum tuberosum* transcription factor *(St)WRKY* expression at 3 hpi. In contrast, *StWRKY* transcription does not seem to be suppressed by *hrpW* mutant.

At 7 hpi, *Eca* WT still suppresses StWRKY expression however, both *dspE* and *hrpW* mutants do not suppress this expression (data not shown).

At 10 hpi, WT *Eca* 1043 no longer suppresses *StWRKY*. Both *hrpW* and *dspE Eca* mutants do not induce *StWRKY* expression as strongly as WT *Eca* 1043.

Could these effector proteins be suppressing a *StWRKY*-dependent potato defence pathway?

Improved resistance

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

Desiree control (17dpi)

Transgenic WRKY plant (17dpi)

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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