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

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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.

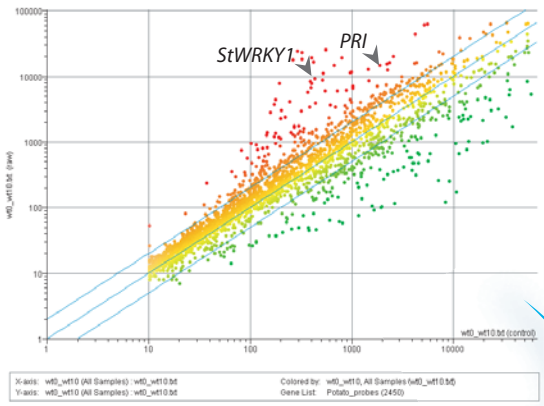
Harpins/helpers (HrpN and HrpW) are believed to assist in the translocation of true effectors. In *Eca*, mutants in HrpN and DspE were found to be reduced in pathogenicity. However, the role of HrpW in pathogenicity is yet to be characterised.

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 *StWRKY1* transcription factor. Both the *StWRKY1* 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.



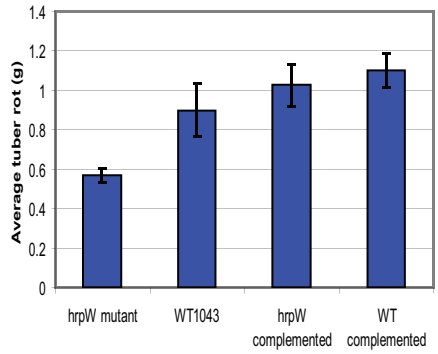
Improved resistance

- The above results suggest that constitutive expression of *StWRKY1* transcription factor can increase resistance to *Erwinia*.
- Transgenic lines with increased expression of the *StWRKY1* transcription factor were generated.
- Pathogenicity assays on Desiree vs transgenic WRKY lines
- Lesion measured from 2 to 17 days post inoculation (dpi)

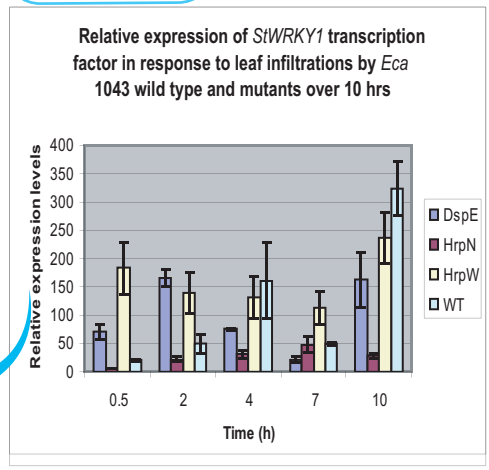
Characterision of *hrpW* mutant and Real time PCR

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



Eca 1043 represses *Solanum tuberosum (St)WRKY1* transcription during early hours of infection.

In contrast, *StWRKY1* transcription factor seems to be induced as early as 30 mins in response to HrpW and DspE mutants.

Could these effector proteins block *StWRKY1* transcription during WT interactions?

Improved resistance

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

Desiree control (17dpi)

Transgenic WRKY plant (17dpi)

Future Work

- Microarray analysis of plant response to *hrpW* mutant 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*
- Identification of 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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