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.

Characterisation 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 and 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

- 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

References

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Improved resistance

Transgenic lines are significantly resistant to Erwinia compared to control plants

Desiree control (17dpi)

Transgenic WRKY plant (17dpi)