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How to Conquer a Tomato Plant? Fusarium Oxysporum Effector Targets

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Summary

With a continuously growing global population, expected to reach almost ten billion in the year 2050 (as predicted by the UN), food requirements are ever higher. With pathogen infestation leading to great annual losses and with the use of pesticides becoming more restricted due to their negative effect on the environment and human health, there is a need for other forms of crop protection. Understanding the molecular basis of plant-pathogen interactions can help identify new sources of resistance and/or new strategies to improve disease resistance in crops.

To be able to infect, pathogens secrete proteins that create a 'pathogen-friendly' environment, by suppressing immunity, providing protection or releasing nutrients. Plants on the other hand, contain a multi-layered immune system to defend themselves. In **Chapter 1** we present an overview of the fungal and plant components involved in vascular wilt diseases. We describe the proteins secreted by vascular wilt fungi during host colonization and discuss their role in pathogenicity. Amongst others, we discuss effectors: small, usually cysteine-rich proteins that are required for full virulence of the pathogen. Some of these effectors can be recognized by Resistance (R) proteins, which are part of the second layer of plant immunity, initiating defense responses to protect the plant.

One of the vascular wilt pathogens described in Chapter 1 is *Fusarium oxysporum* (*Fo*). In the remainder of the thesis we focus on the interaction between *Fo* f. sp. *lycopersici* (*Fol*) and its host plant: tomato (*Solanum lycopersicum)*. We try to identify plant targets of effectors to better understand how *Fol* shapes the host environment to facilitate infection. In **Chapter 2** we describe the interaction between putative effector Six8 and the plant protein Topless (TPL). We initially identify the interaction by performing Affinity Purification (AP) with Six8 as bait, followed by Mass-Spectrometry (MS) analysis. We then confirm it by using yeast two-hybrid (y2h) assays and Bimolecular Fluorescence Complementation (BiFC). Topless can interact with plant proteins containing an EAR-motif. We identify an EAR-like motif in Six8 and show that it is required for the interaction between TPL and Six8. The BiFC experiment shows that the Six8-TPL association primarily takes place in the plant nucleus. Finally, we show that TPL is a susceptibility target by silencing *TPL* in tomato plants and performing a *Fol* disease assay.

In **Chapter 3** we focus on the role of *Fol* Six1 in virulence. We generate transgenic Arabidopsis (*Arabidopsis thaliana*) plants expressing different forms of *SIX1*. None of them showed a phenotype giving us no clues on effector function. We continue to perform AP-MS and y2h experiments to identify Six1 plant targets. We find multiple putative host targets, with small heat shock proteins being identified by both methods.

In **Chapter 4** we search for targets of Six4 in an AP-MS experiment and find an association with plant GlutAmate Decarboxylases (GADs). This enzyme produces γ-Amino Butyric Acid (GABA) from glutamate. We show that GABA levels decrease during a susceptible interaction, but this effect is not dependent on the presence of Six4. Also, transgenic expression of *Fol SIX4* in tomato plants does not alter GABA levels. We already knew that Six4 is able to suppress the Hypersensitive Response (HR) initiated by the recognition of Avr2/Six3 by the tomato R protein Immunity-2 (I-2) in a transient expression system *in planta*. Here, we show that a C-terminally truncated GAD can phenocopy this effect.

In **Chapter 5** we describe the generation of stable transgenic tomato plants, without R genes against *Fol*, expressing *Fol SIX1* or *Fol SIX4*. The transgenic plant lines that we obtained did not show a phenotype. *Fol* Six4 can trigger immune responses when it is recognized by the tomato R protein I. A cross between one tomato line expressing an intronless variant of full-length *SIX4* and an *I*-containing plant resulted in plants with a semi-dominant 'constitutive resistance' phenotype that persisted in the F2 generation. As none of the tomato lines producing mature (intracellular) Six4 showed a phenotype when crossed to an *I*-containing plant, it is likely that Six4 is recognized outside the plant cell.

Chapter 6 provides a general discussion. I suggest that the hemi-biotrophic lifestyle of *Fol* might influence timing of effector secretion and discuss the current knowledge on effector uptake into the plant cell. I also discuss methodology to identify effector targets. As effector targets are potential Susceptibility (S) factors – plant proteins required for pathogen infection – I discuss their potential role in plant breeding. Finally, I reflect on the resistance response against *Fol* mediated by R proteins.

Knowledge about the molecular interactions between plants and pathogens contributes to our understanding of plant disease. In this thesis multiple putative effector targets are identified and for one of them we have shown it is a S factor. Future research with tomato plants producing both Six4 and I could give us more insight in *R*-gene mediated resistance against Fusarium wilt disease. *R* and *S* genes can both contribute to resistance against pathogens and will continue to play a crucial role in crop protection.