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Summary

Fusarium oxysporum f.sp. *lycopersici* (*Fol*), the causal agent of Fusarium wilt of tomato, is a soil-born and highly devastating xylem-colonizing fungal pathogen. Upon colonization *Fol* secretes over 42 proteins in the xylem sap. Among these secreted proteins 11 small proteins, so called SIX (Secreted In Xylem) proteins, have been identified. Some of the SIX proteins represent Avirulence (Avr) proteins. Avirulence proteins can trigger resistance when recognised by a matching Resistance (R) protein of the host. Three race specific resistance (*R*) genes against *Fol* have been introgressed from wild tomato relatives into commercial tomato cultivars, these genes are called *I* or *I-1*, *I-2* and *I-3*. Hence, the interaction between an R protein and its cognate effector (avirulence) protein plays a key role in the *R*-gene mediated resistance. To get insights into how *Fol* effector proteins contribute to pathogenicity and *R*-gene mediated resistance, I here describe the molecular characterisation of two effector proteins: SIX3 and SIX5. In **chapter 2** the molecular basis of effector recognition by R proteins that contain an NB domain and an LRR domain (NB-LRR) is reviewed. We focus on the structural features of the subdomains of NB-LRRs, their interdomain contacts and their interactions with chaperones and the 26S proteasome. Together these interactions appear to keep NB-LRRs in a signalling-competent, yet auto-inhibited state. We also describe how the NB-LRRs building blocks assemble into signalling competent proteins and how the accumulation of these proteins in the cell is controlled. In addition different effector recognition models by NB-LRRs and the subsequent induction of defence signalling is discussed.

Two *Fol* avirulence genes (*AVR1* and *AVR3*) and one *R* gene *I-2* were cloned at the start of the project. However, these genes do no constitute a complete *R-AVR* pair. In **chapter 3** I describe the identification and characterization of effector Avr2, which completes the first gene-pair of a xylem colonizing fungus and its host. The Six3 protein was identified as Avr2 since a *SIX3* knockout became pathogenic on *I-2* tomato plants. Avr2 actually displays two distinct activities, besides triggering resistance in *I-2* tomato plants*,* it was found to be required for full virulence on susceptible plants. Specific point mutations in Avr2, causing single amino acid changes, were found in *I-2*-breaking *Fol* strains. These point mutations were shown to prevent recognition by *I-2,* both in tomato and upon co-expression of these genes in leaves of *Nicotiana benthamiana*. *Fol* strains carrying the *I-2*-breaking Avr2 variants are fully virulent, showing that virulence and avirulence functions can be uncoupled. Although Avr2 is secreted into xylem sap when *Fol* colonizes tomato, the Avr2 protein can be recognized intracellularly by I-2, implying its uptake by host cells. In **chapter 4** I show that expression of *AVR2* is strongly induced in root- and xylem-colonizing hyphae three days post *Fol* inoculation. Local accumulation of Avr2, in discrete structures of unknown identity, was observed alongside fungal hyphae growing between the cortical cells of tomato roots. Heterologous expression of mis-localised Avr2 chimeras revealed that Avr2 requires a nuclear localization to trigger an I-2-specific cell death response. Subsequent structure-function analyses of Avr2 demonstrated that, except for the N-terminal 17 amino

acids, the entire protein is required for *I-2*-mediated recognition and that Avr2 forms homodimers *in planta*. Dimerization alone, however, was found to be insufficient to activate I-2 signalling.

Although Avr2 is essential for *I-2*-mediated resistance, a *SIX5* knockout was surprisingly also found to be able to break *I-2* resistance in tomato plants. In **chapter 5**, I provide a detailed molecular characterisation of the Six5-Avr2 effector pair. Expression of both genes is controlled by a shared promoter region. Knockout of either gene compromises *I-2*-mediated resistance and partially impairs virulence on a susceptible plant. Whereas the effector pair is required for *I-2*-mediated resistance, Avr2 alone is sufficient to trigger *I-2*-mediated cell death in heterologous systems suggesting that cell death and resistance can be uncoupled. Both effectors interacted in the yeast two-hybrid system and *in planta* accumulation of Six5 might depend on the presence of Avr2. In **chapter 6** the application of agroinfiltration in molecular plant pathology, and especially for expression of plant *R* and fungal *AVR* (effector) genes in leaves of *Nicotiana benthamiana,* is described. The matching gene pair *I-2* and *AVR2* is used as a typical example. Co-expression of the *I-2* gene with the *AVR2* gene triggers host defence responses that culminate in a hypersensitive response (HR). This HR is visible as a necrotic sector in the infiltrated leaf area expressing both genes. Staining of the infiltrated leaves with trypan blue allows visual scoring of the HR. Fusion of a fluorescent tag to the recombinant protein Avr2 facilitates determination of its subcellular localization by confocal microscopy.

Avr2 not only acts as an avirulence factor to activate I-2 triggering a resistance response, it also functions as a virulence factor of *Fol*. To examine the virulence activity of Avr2 *in planta,* the generation of stable transgenic Arabidopsis and tomato plants that constitutively express the *AVR2* gene is described in **chapter 7**. Heterologous expression of *AVR2* in *Arabidopsis thaliana* causes enhanced susceptibility towards *Verticillium dahliae,* a xylem-colonizing fungal pathogen. In tomato, *AVR2* expression results in plants with elongated internodes and upward pointing leaves. F1 progeny derived from a cross between *AVR2*-expressing tomato and *I-2* plant is viable but the plants have necrotic sectors in their leaves and are elongated.

In **chapter 8** I discuss how the knowledge obtained in my thesis contributes to a better understanding of the role of effector protein Avr2 in resistance and pathogenicity. A tentative model for the function of the Avr2/Six5 effector pair in *I-2*-mediated resistance and susceptibility in tomato is presented. Our model proposes that Avr2 and Six5 act as a pair to trigger *I-2*-mediated resistance, but act separately as independent virulence factors; each of them manipulating distinct plant targets. New research questions are posed together with directions for future research aimed to get further insights into how Avr2 induces *I-2*-mediated resistance and how Avr2 contributes to *Fol* pathogenicity.