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Function and Targets of Fusarium oxysporum Effectors

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Summary

A multi-layered immune system protects plants against pathogens. Adapted pathogens can overcome or evade this defense system by secreting small proteins, called effectors. Often host targets of these effectors are encoded by susceptibility (*S*) genes and loss-of-function mutations in these genes sometimes confer resistance. In **chapter 2** we conclude that with increasing numbers of identified effector targets the number of *S* genes that can be used in resistance breeding rises.

Fusarium oxysporum f.sp. *lycopersici* (Fol), a soil-born and host-specific fungus, invades roots and colonizes xylem vessels thereby causing tomato wilt disease. Fourteen effector candidates, called Six proteins for secreted in the xylem proteins, have been identified (Houterman et al., 2007; Schmidt et al., 2013). Besides being involved in pathogenicity, Six1, Six3 and Six4 play a key role in resistance as they correspond to avirulence proteins Avr3, Avr2 and Avr1, respectively. These Avrs are recognized by resistance (*R*) genes *I* or *I-1, I-2* and *I*-3, respectively (Rep et al., 2004; Houterman et al., 2008; Houterman et al., 2009). However, their molecular functions and targets have not been identified yet.

In **chapter 3** we have characterized Six6 and identified homologs in six *formae speciales* of *F. oxysporum* and in two *Colletotrichum* species. A knockout of *SIX6* in Fol significantly increases plant weight, which qualifies Six6 as a genuine effector. Plant growth or development is not affected in *SIX6* expressing transgenic *A. thaliana* plants and also resistance and susceptibility against *F. oxysporum, Verticillium dahliae* and *Pseudomonas syringae* is unaffected. Noticeably, Six6 suppresses the I-2 mediated hypersensitive response in agro-infiltration assays, but not cell death induced by other genes.

Six8, which is encoded by a multi-copy gene family, enhanced cell death mediated by *INF1*, a *Phytophthora infestans* elicitor (**chapter 4**). Pull-down assays in *Nicotiana benthamiana* revealed the co-repressor TOPLESS (TPL) as a potential Six8 target. TPL functions as a negative regulator in various plant developmental, hormonal and stress processes and hence hijacking TPL by Six8 was proposed to lead to manipulation of the plant's molecular processes to mediate susceptibility. In *A. thaliana* genetic evidence indicates that the TPL-Six8 complex is recognized by the *SNC1* gene resulting in a constitutive defense response hallmarked by stunting of the plants and elevated salicylic acid levels. Our model proposes that SNC1 guards TPL and that perturbation of TPL by Six8 triggers immune responses.

To obtain insights in the molecular activities of the Six proteins in tomato, its xylem sap composition has been analyzed following inoculation with wild type Fol or *AVR3, AVR2, SIX2, SIX5* or *SIX6* knockouts (**chapter 5**). Fol inoculations affected the abundance of 217 proteins in the sap and triggered appearance of 16 and disappearance of 63 proteins. Proteins related to stress responses form the main class of proteins whose

accumulation was affected. The abundance of many cell wall modifying proteins was greatly reduced. The different *SIX* knockouts have both a common and a specific effect on the xylem sap composition. The common effect mainly affects proteins involved in stress response signaling while the specific effects are merely involved in cell wall modification. Pathogenicity of *SIX2* knockouts did not clearly differ from the wild type Fol and the xylem sap composition of tomato inoculated with either the *SIX2* knockout or wild type was identical. Therefore, we concluded that a function in pathogenicity correlates with changes in the xylem sap composition. Hence, identification of the xylem sap proteome could be used to define effectors.

In **chapter 6** the experimental pipeline, which was used to characterize Six proteins and determine whether they are true effectors is discussed critically. The possible involvement of reactive oxygen species (ROS) and hormones in susceptibility to Fol are highlighted and their manipulation by Six proteins are shortly reviewed. Lastly, I propose to test whether *TPL* can function as an *S* gene and pinpoint *SNC1* as *bone fide R* gene. Future studies could reveal whether these genes can be used to combat panama disease caused by a *SIX8* carrying *F. oxysporum* f.sp. *cubense* strain.

References

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