Evolution of Races within Fusarium oxysporum f.sp. Lycopersici

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Summary

Fusarium oxysporum f.sp. *lycopersici (Fol)*, the causal agent of Fusarium wilt of tomato, is an asexual, soil-born and highly devastating xylem-colonizing fungal pathogen. Three physiological races of Fol have been reported based on their ability to infect tomato cultivars carrying different monogenic resistance (*R*) genes. Tomato *R* genes that confer race-specific resistance to Fol are known as *Immunity* (*I*) genes. To date, three *I* genes, notably *I* (or *I-1*), *I-2* and *I-3*, have been identified in wild tomatoes and have been introgressed into tomato cultivars. The three *I* genes confer tomato resistant against Fol isolates which contain the avirulence genes corresponding to these *I* genes, notably *AVR1*, *AVR2* and *AVR3,* respectively. *AVR1* is present in all Fol race 1 isolates, but absent in race 2 and 3.. Absence of *AVR1* enables these isolates to infect tomato cultivars carrying *I*. *AVR2* is present in all Fol isolates, albeit that *I-2* breaking (race 3) isolates contain an *AVR2* allele with a point mutation leading to an amino acid change in the protein. So far, three *AVR2* alleles have been described with the following amino acid changes: V41>M, R45>H and R46>P. All three amino acid changes lead to loss of the avirulence function of Avr2, whereas its virulence function remains unaffected. Two *AVR3* alleles have been described, one encoding a protein with a glutamic acid (E) at position 165 and one with a lysine (K) residue at this position (E165<>K165). Both proteins still act as avirulence factor, but differ in the degree of virulence they confer.

Besides its avirulence function, Avr1 (product of *AVR1*) also functions to suppress I-2/Avr2 and I-3/Avr3 triggered immunity. The suppressive ability of Avr1 enables race 1 isolates to infect tomato cultivars containing *I-2* and *I-3* without deleting or mutating the avirulence genes (*AVR2* and *AVR3*) corresponding to *R* genes *I-2* and *I-3* . In Chapter 2, it is shown that not all race 1 isolates infect *I-2* and *I-3* tomato cultivars even though all contain a functional *AVR1* gene. Furthermore, the observed differences in the suppressive ability cannot be explained by one or more mutations in either the Avr1 coding region or the flanking regulatory sequences that would affect the suppressive function but not the avirulence function of Avr1. Based on the results obtained in this study, a model for the suppression of I-2/Avr2 or I-3/Avr3 by Avr1 has been proposed.

In several studies it has been suggested that deletion of *AVR1* from race 1 isolates resulted in the emergence of race 2 (avirulence genotype: –, *AVR2, AVR3*), and that a point mutation in *AVR2* of race 2 brought about the birth of race 3 (–, *avr2, AVR3*). However, the molecular mechanism underlying the loss of *AVR1* has not been determined. In Chapter 3, the molecular mechanism underlying the deletion of *AVR1* in Fol is investigated. To this end, a 100 kb genomic region containing *AVR1* from a race 1 isolate has been sequenced and compared to the reference genome sequence that lacks *AVR1*. The 100 kb genomic region aligned completely to a genomic region in the reference genome with sequence identity over 99.9%, except for a 31 kb genomic region containing *AVR1* in the race 1 isolate and found to be absent in the reference genome. Further analysis suggested that race 2 evolved from race 1 isolate by the deletion of a 31 kb genomic region containing *AVR1*, most probably due to homologous recombination between two Helitron transposable elements. A worldwide collection of Fol isolates was subjected to PCR analysis of the *AVR1* locus, including the two bordering transposable elements. The results obtained showed that race 2 evolved from race 1 by the deletion of either a 31 kb or a 102 kb genomic fragment containing *AVR1* due to the homologous recombination between two Helitrons bordering these fragments. The results suggest that, based on the deletion event that led to the loss of *AVR1*, Fol isolates can be divided into two distinct evolutionary lineages. The results indicate that Helitron transposable elements played a major role in the evolution of races within Fol.

Helitrons are a novel type of DNA transposon that have been and still are being identified in many eukaryotic species. They are distinct from other transposons in their terminal structural features, ability to capture gene fragments and their rolling-circle mode of replication mechanism. In fungi, the structural features of Helitrons have been studied poorly. In Chapter 4, the structural features of Helitrons in *Fusarium oxysporum* (Fo) are identified and described. Six groups of Helitrons were discerned in Fo, called FoHeli1 to FoHeli6. Their terminal features suggest that FoHeli forms a novel group within the Herlitron family. Most importantly, evidence has been obtained for the existence of Helitron in a circular form. Together with this data, a model for the transposition of Helitron has been proposed.

In Chapter 5, the evolution of races was investigated further by extending the number of isolates. The analysis of the *AVR1* locus including its flanking Helitrons in these isolates confirmed the idea that in most cases race 2 evolved from race 1 by a homologous recombination event between two Helitrons. Next to that one race 2 isolate was identified which lost *AVR1* likely due to a homologous recombination event between two *NHT2*-like retro-transposons resulting in a deletion of approximately 457 kb. In two other race 2 isolates no clear indications were obtained as to how the *AVR1* locus was lost. Four point mutations were identified in *Avr2* that caused the emergence of race 3 from race 2. Among these, one is a novel mutation. All these mutations caused the evasion of *I-2* mediated resistance in race 3 isolates. Based on phylogenetic analysis using the sequence of *EF-1 alpha* gene (partial), Fol isolates could be grouped into five evolutionary lineages. Four clonal lineages correlated well with known VCGs of Fol whereas one is a novel clonal lineage associated within an unknown VCG of Fol. All results obtained in this study were used to propose a model to explain the evolution of races within Fol.

In Chapter 6, it is discussed how the knowledge obtained in this thesis contributes to a better understanding the co-evolutionary arms race between a pathogen and its host, particularly in agricultural settings. This research highlights that the introgression of a monogenic resistant gene against a particular pathogen in cultivars and the widespread use of these cultivars in an agrosystem may impose a selection on the pathogen to evolve quickly evasion that particular host resistance. A model for the origin of *formae speciales* of *Fusarium oxysporum* as well as the polyphyletic nature of a *forma specialis* is presented. New research questions are posed and directions for future research aimed to get better understanding of host-pathogen evolution are proposed.