Review

Molecular interactions between tomato and its wilt pathogen *Fusarium oxysporum* f. sp. *Lycopersici*

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Abstract

Fusarium oxysporum f.sp. *lycopersici* Snyd. et Hans.(*Fol*) is a soil-borne plant pathogen that causes wilt in tomato plants and threatens tomato industry worldwide. Successful plant infection and tissue colonization by *Fol* is an active process that involves a variety of cell wall-degrading enzymes (CWDE), regulation of nutrient metabolism, and secretion of effectors to suppress and/or overcome the physical basal defense in tomato plants. The effector-encoding avirulence genes have been identified and their combinations in the genome of *Fol* determine the 3 races of the pathogen. Avirulence genes and other pathogenicity factors are assembled in a lineage-specific genomic region, including 4 entire chromosomes that *Fol* acquired probably by horizontal gene transfer from other closely related species. In the course of co-evolution with *Fol*, tomato evolved 3 resistance genes to counteract pathogen effector-triggered disease. The interactions between tomato and *Fol* has become a model system for the study of the molecular basis of disease resistance and susceptibility in plants.

Keywords: *Fusarium oxysporum* f.sp. *lycopersici*, tomato, avirulence genes, resistance genes

Résumé

Fusarium oxysporum f.sp. *Lycopersici* Snyd. et Hans. (*Fol*) est un champignon phytopathogène tellurique qui cause le flétrissement vasculaire chez, et menace l'industrie de, la tomate au niveau mondial. Le processus d'infection et de colonisation des tissues par ce pathogène impliquent une variété d'enzymes de dégradation des parois cellulaires végétales, la régulation du métabolisme des nutriments, et la sécrétion d'effecteurs pour inhiber et/ou vaincre le système basal de défense chez les plantes. Trois gènes d'avirulence, codant des effecteurs, ont été identifiés et leur combinaisons dans le génome du *Fol* déterminent les 3 races connues du pathogène. Les gènes d'avirulence et d'autres facteurs de pathogénicité sont assemblés dans une région génomique de lignée spécifique qui englobe 4 chromosomes entiers que *Fol* aurait probablement acquis via transfert horizontal de gènes provenant d'autres espèces génétiquement proches. Au cours de la coévolution avec *Fol*, la tomate a évolué 3 gènes de résistance pour contrecarrer les effets pathogéniques des effecteurs du *Fol*. Les interactions entre la tomate et *Fol* est devenue un modèle systémique pour étudier les bases moléculaires de susceptibilité et résistance aux maladies chez les plantes.

Mots clés: *Fusarium oxysporum* f.sp. *lycopersici*, tomate, gènes d'avirulence, gènes de résistance

INTRODUCTION

Plants, during their life cycle, are exposed to a wide range of pathogens that have the potential to affect their fitness and threaten their survival. Microbial plant pathogens produce proteins (effectors) to interfere with plant defense and colonize the host tissue. As a response, plants have evolved different resistance mechanisms going from simple and general reactions to more complex and specific ones. It is now known that plants have two lines of defense. The first confers basal defense against all potential pathogens and is based on the recognition of microbial shared features termed pathogen-associated molecular patterns (PAMPS) by so-called PAMP-recognition receptors (PRRs) that activate PAMP-triggered immunity (PTI) and prevent further colonization of the host (reviewed in Ioannis and de Wit, 2009). Once pathogens outweigh plant basal defensive system, plants deploy more specialized detection machinery involving resistance gene-encoded effectors that initiate effector-triggered immunity leading to an acute defense response in plants, the hallmark of which is the hypersensitive cell-death response.

Extracellular fungal pathogens are limited to intracellular spaces and do not enter host cells by the means of feeding structures. Most of the effectors released by this group of pathogens have been extracted from the apoplastic fluid or xylem sap of diseased tissues and many of the effector-encoding genes have been cloned. The effectors of extracellular fungal pathogens are small and generally cysteine-rich and display a high stabil-

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ity in protease-rich environment. Their main role is the suppression or avoidance of host defense (Catanzarity and Jones, 2010).

The genus *Fusarium* (teleomorph *Gibberella*) comprises a large number of species that are cosmopolitan, widely distributed in the soil as saprophytes, and exist commonly in association with below ground and aerial plant parts as both endophyte and exophyte symbionts (Booth, 1971; Burgess, 1981; Bacon and Yates, 2006; and Moretti, 2009). The economic importance of many *Fusarium* species as plant pathogens has been demonstrated in a wide range of agricultural, horticultural and silvicultural crops grown worldwide. Among the species of this genus, *Fusarium oxysporum* is notable for its capacity to cause disease on various plant species (with grasses as an outstanding exception). Pathogenic strains of *F. oxysporum* can invade plant roots and colonize the xylem, thereby causing wilt diseases. *F. oxysporum* has many host-specific special forms, called *formae speciales* (f. sp.), which suggests a gene-for-gene relationship between this pathogen and its wide host range.

Fusarium oxysporum f. sp. *Lycopersici* (*Fol*) is a devastating pathogen that causes wilt in tomato (*Lycopersicon esculentum* Mill.) and threatens the industry of this economically important crop in many parts of the globe. The pathogen was first reported in Europe (Italy) in 1933 (reviewed in Huang and Lindhout, 1997). As a soilborne pathogen, *Fol* enters tomato through the roots, and can infect tomato plants at all growth stages. The fungus colonizes the vascular bundle and clog water flow and nutrient movement, leading to wilt, and ultimately causes plant death. In several cases, losses in tomato production can reach 80% (reviewed in Huang and Lindhout, 1997). Many techniques are used for the control of this pathogen in tomato fields, including soil steaming, fumigation, solarization or combinations of these. However, all of these management techniques are costly and mainly restricted to greenhouses. In field the only effective and convenient control method is the use of resistant cultivars.

As a consequence of host-pathogen interactions over a long period of time, many "fingerprints" are left in their genomes, reflecting the rapid evolution of genes that encode proteins directly involved in these interactions. Examples of such rapidly evolving genes are those that encode effectors in the pathogen and resistance proteins from the host. This review sheds light on the pathogen profile of *Fol* and its interactions with tomato. We focus mainly on the state of art of research in this field and provide useful insight into the process of infection and new developments that involve the discovery and molecular analysis of xylem sap proteomics, avirulence/effector proteins, transcriptomes, and novel pathogenicity genes and genome organization. Finally, we construct one possible scenario of coevlutionary interactions between *Fol* and tomato that led to the emergence of the three races of the pathogen and the evolution of resistance genes in tomato based on gene for gene theory (Flor, 1941) and recent advances in *Fol*-tomato pathosystem.

THE PROCESS OF TOMATO INFECTION BY *FOL*

Light fluorescence and electron microscopy have been used to study plant infection by *F. oxysporum*. The process consists of several steps, including root surface attachment and colonization, penetration and colonization of the root cortex and, in the case of wilt inducing *formae speciales*, mycelia proliferation inside the xylem vessels (Fig. 1; reviewed in Di Pietro *et al*., 2003). Working with *Fol*, Di Pietro *et al*. (2001) observed conidial germination on roots, growth in the tomato root cortex and colonization of the xylem by the pathogen. They also showed that the Fmk1 mutant (fmk1), a dispensable gene for vegetative growth and conidiation, could germinate on roots but was unable to develop any further. Chlamydospores (thick-walled, survival spores) of green fluorescent protein-labeled *Fol* were also observed on and in tomato roots 7 and 22 days post-inoculation, respectively. However, neither conidiophores nor microconidia were detected in xylem vessels (van der Does *et al*. 2008). The role of microconidia in xylem colonization has been shown to be trivial in a study of Ren1, a putative transcription factor essential for micro-and macroconidia formation. A mutant disrupted in this gene produces only chlamydospores and abnormal rod-shaped, conidium-like cells, but is not affected in pathogenesis, suggesting that microconidia and macroconidia are not important for pathogenicity (Ohara *et al*. 2004).

Following penetration, Czymmek *et al*. (2007) observed that fungal growth was initially intercellular but, ultimately, became intracellular, and the collapse of plant cells was observed at sites of fungal penetration, presumably as a consequence of a loss of turgor pressure. Plant cells that were not in direct contact with mycelium were also subjected to changes such as the loss of auto-fluorescent vacuole content and changes in the appearance of the endoplasmic reticulum.

ROLE OF CELL WALL-DEGRADING ENZYMES (CWDE) AND NUTRIENT METABOLISM IN PATHOGENICITY

The contribution part of CWDE in the process of infection is not completely elucidated. To penetrate and colonize plant tissues, pathogenic *F. oxysporum*, like most fungi, secrete an arsenal of CWDE, such as polygalacturonases, pectatelyases, xylanases and proteases. However, shutting down individual CWDE or protease-encoding genes did not show any impact on virulence (reviewed in Di Pietro *et al*., 2003). The reason for this might be functional redundancy of these genes.

Carbon metabolism can have an impact on pathogenesis through its effects on the expression of CWDE-encoding genes as it was shown in the analysis of *Fusarium oxysporum* f. sp. *lycopersici* Frp1 disruptant (frp1). Frp1 is a gene absolutely required for pathogenicity and frp1 is impaired in root colonization capacity (Duyvesteinj *et al*., 2005). Jonkers *et al*. (2009) have shown that mutation in the Frp1 results in reduced assimilation of organic acids,

amino acids and/or polysaccharides, which explains the poor root colonization of the frp1 mutant. External root colonization, but not virulence (as a result of an inability to penetrate the roots), was restored by the addition of glucose or proline. Surprisingly, Icl1 (isocytrate lyase) disruptant, produced in the same study, conserved its ability to penetrate the roots and be virulent, despite the similar reduced growth pattern it shares with Frp1 disruptant on several carbon resources. In addition to impaired carbohydrates uptake ability, fpr1 has defects in the expression of CWDE genes, suggesting that collective secretion of several enzymes is very likely to be required for superficial colonization (through the release of cell wall- components for nutrients) as well as root penetration (through weakening of cell walls).

Nitrogen regulation has also been demonstrated to play an important role in the process of infection. Utilization of several secondary nitrogen resources has been reduced by shutting down the global nitrogen regulator Fnr1, which abolished the expression of nutrition genes normally upregulated in the early phase of infection, and resulted in reduced pathogenicity towards tomato (Divon *et al*., 2006).

Overall, although direct proof of causal relationship between extracellular enzymes production and pathogenicity has not been elucidated, available results indicate that the collective secretion of CWDE, carbon metabolism, and nitrogen uptake regulation are important for the infection process in *Fol*-tomato pathosystem.

TOMATO RESPONSES TO INFECTION BY *FOL*

Recent development in the fields of plant genomics and transcriptomics driven by advances in computational methods has expanded our understanding of plant-microbe interactions and their outcomes at the molecular level. Investigation of plant-expressed molecules following pathogen infection provides valuable insights into mechanisms that underlie plant defense. Such mechanisms involve the regulation of gene expression, cascade signaling activation, hormone balancing and synthesis of defensive metabolites (Mithofer and Boland, 2012).

Substantial body of work has addressed tomato-*Fol* interactions and provided accumulating evidence of specific responses of tomato plants to *Fol* attack. In a recent study (Andolfo *et al*., 2014) genome-wide transcriptional analysis evidenced the overexpression of 2392 genes (about 64% of the differentially expressed genes during infection) in resistant tomato plants infected by *Fol*, indicating considerable gene activation upon inoculation. The upregulated genes are associated to maintenance of cellular structures and cellular homeostasis. These are very important metabolic activities required by plants to survive fungus-inflicted stresses. For example, the master gene of inflammation was one of the up-regulated genes in tomato-*Fol* interactions. This gene is a key player in anti-apoptotic (anti programmed cell death) signaling and is able to prevent apoptotic signaling pathway by inhibiting map-kinases (Paul *et al*., 2011). Since *Fusarium oxysporum* is a necrotrophic fungus (Trusov *et al*., 2006) that kills host cells prior to infection, through the predicted deployment of cell death inducing toxins and enzymes, the overexpression of the anti-apoptosis gene could confer resistance to *Fol*.

The expression of plant resistance genes leads also to the chemical modification of plant cell wall. In tomato plants, extract of *Fol* induces an increase in cell wall strengthening via the deposition of lignin, and an increased concentration of phenolic compounds, such as ferulic acid, 4-hydrobenzoinc acid and 4-coumaric acid (Mandal and mitra, 2007). Such reactions build strong physical barriers at the infection sites and pose major hurdle for the pathogen to overcome for successful infection.

Another way of plant defense strategies against pathogen attack is the release of anti-microbial compounds to counteract pathogen ingression upon infection. For instance, *Fol*-infected tomato plants secrete the steroidal glycoalkaloid saponin α-tomatine that forms complexes

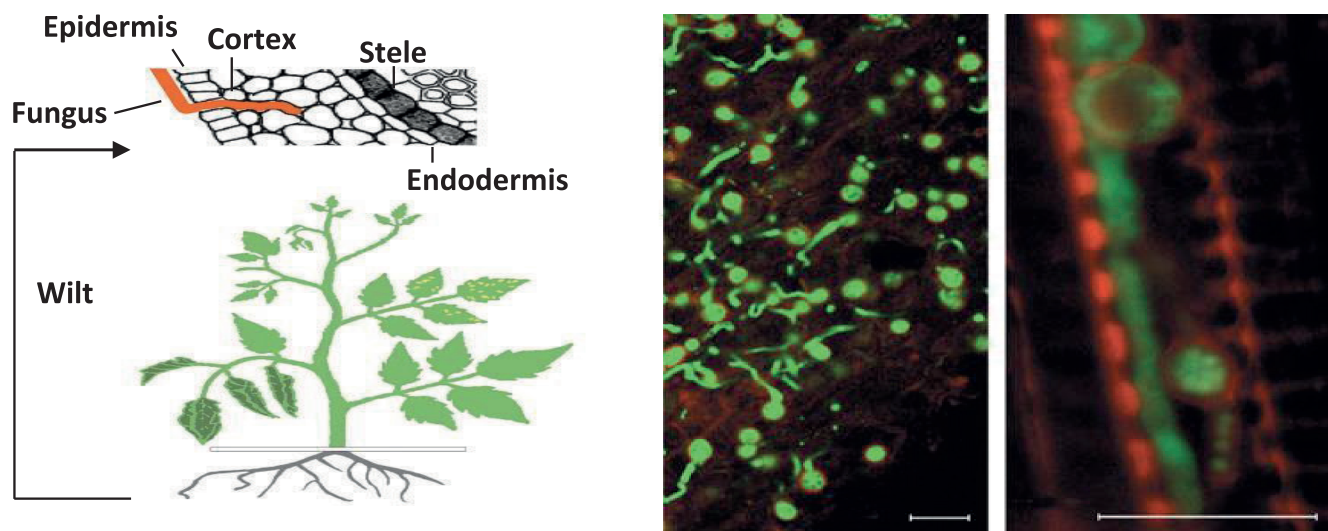

Figure 1: Infection by Fusarium oxysporum f. sp. lycopersici. (Left) penetration through the root cortex (Catanzariti and Jones, 2010). (Middle) confocal image of chlamydospores of green fluorescent protein-labeled pathogen outside of a tomato root. (Right) image of chlamydospores in a xylem vessel (Michielse and Rep, 2009)

with sterol in the pathogen fungal membrane. These structures affect membrane plasticity and cause pores in fungal cell wall that lead to leakage of cellular contents (Rodick, 1977), contributing thereby to resistance.

SUPPRESSION OF PLANT RESPONSES

Like the majority of plant pathogens, *Fusarium oxysporum* has to overcome a variety of plant defense mechanisms for successful invasion and colonization, including physical barriers and antifungal compounds. For instance, when pathogenic *Fusarium oxysporum*, including tomato pathogen, sense the presence of cell wall strengthening compounds in the hosts they increase the production of mycotoxins and the activity of hydrolytic enzymes, such as pectinase, cellulose and amylase (Wu *et al*., 2008). Additionally, *Fol* can release a tomatine degrading enzyme, called tomatinase, to counteract α-tomatine antifungal effect in diseased tomato plants (Lairini *et al*., 1996). The genome of *Fusarium oxysporum* contains five putative tomatinase genes (Pareja-Jaime *et al*., 2008). The role of one of these genes, Tom1, in degradation of tomatinase was studied. Its constitutive overexpression yielded high level of tomatinase activity regardless of the presence of α-tomatine. Disease symptoms development was slower in Tom1-overexpressing strain- infected tomato plants, whereas disruption of Tom1 resulted in 25% decrease in tomatinase activity and a delay in disease symptoms apparition (Pareja-Jaime *et al*., 2008). These findings prove that tomatine inactivation takes part in the virulence of *Fusarium oxysporum* f. sp. *lycopersici*.

Fungal plant pathogens can also adapt their cell wall during pathogenicity to resist damages inflicted by plant enzymes or compounds and/or to reduce release of cell wall-derived defense elicitors. Madrid *et al*. (2003) showed that chitin synthase V (ChsV), an enzyme catalyzing chitin biosynthesis, is absolutely required for pathogenicity in *Fusarium oxysporum* vascular wilts. Peroxisomal function has also been shown to play a role in the modification of fungal cell wall as a mechanism of resistance to plant defense machinery. Peroxisomes are single-membrane-bound organelles which, in filamentous fungi, are involved in the β-oxydation of fatty acids, peroxide detoxification and occlusion of septal pores (Jedd and Chua, 2000). Peroxisomal function and fatty acid metabolism have been shown to be required for virulence of *Fusarium oxysporum* where four different Pex genes, Pex1, Pex10, Pex12, and Pex26 were identified as potential pathogenicity genes (Michielse *et al*., 2009). The requirement of peroxisomal function for pathogenicity could be explained by their possible role in host nutrients utilization and/or plugging septale pores to prevent cytoplasmic leakage during invasive growth.

In summary, degradation of plant-derived chemicals through the upregulation of genes involved in hydrolytic enzymes biosynthesis and adaptation of cell wall composition to prevent damages caused by plant-derived antifungal compounds are key strategies for *Fusarium oxysporum* species, particularly *Fol*, to be successful pathogens.

ROLE OF TRANSCRIPTION FACTORS IN PATHOGENICITY

Substantial advance has been made in the identification of transcription factors closely related to pathogenicity in different *Fusarium oxysporum formae speciales*, such as in *Fol*. In *Fol*, disruptant of Sge1 lost its pathogenicity on tomato plants (Imazaki *et al*., 2009) although it showed no deficiency in vegetative growth or the utilization of different carbon sources. However, Sge1 mutant displayed reduced conidiation even though the conidia generated were similar in germination rate and morphology to the wild type. Fluorescence microscopy was used to observe its behavior during infection. Sge1 disruptants were not impaired in root attachment and superficial root colonization, but affected in invasion ability and/or in planta growth (Imazaki *et al*., 2009). These results imply that Sge1 is a master switch of a set of genes in the pathogenicity pathway in *Fol*.

Transcriptional factors may also serve as a means of sectoring pathogenicity gene expression to assure functional redundancy and prevent shutdown of virulence machinery. The XlnR gene encoding transcription factor XlnR, a regulator of many xylanolytic and cellulolytic genes, was impeded in *Fusarium oxysporum* f. sp. *lycopersici* to unravel the role of xylanases in pathogenicity. Knock-out of this gene negatively influenced the expression of two but not all xylanase genes,Xyl3 and Xyl4, and did not suppress xylanase activity totally. However, virulence was not affected (Calero-Nieto *et al*., 2007). The incomplete loss of xylanase activity in the xlnR disruptant and its ensuing residual activity indicates that XlnR governs only a subset of genes involved in pathogenicity.

GENE FOR GENE RELATIONSHIPS BETWEEN AVIRULENCE GENES IN *FOL* AND RESISTANCE GENES IN TOMATO

The gene for gene concept states that every gene for resistance (R) in the host is matched by a corresponding dominant avirulence gene(Avr) for virulence in the pathogen (Flor, 1941), and the interaction between the products of these genes (R and Avr) induces a cascade of signal transduction and gene upregulation that leads to the activation of host defense response. In tomato plants, 7 proteins, termed secreted in xylem (Six proteins), have been isolated from xylem sap during infection by *Fol*- (Catanzariti and Jones, 2010). Most Six proteins are unique to *F. oxysporum*. However, a homologue of Six6 is also present in two *Colletotrichum spp* (Gawehns *et al*., 2014). Among these proteins, Avr1 (Six4), Avr2 (Six3) and Avr3 (Six1) turned out to have avirulence activities. In addition, Six6 has recently been reported to have effector property in *Fol* and its expression was found to require living host cells (Gawehns *et al*, 2014). Compatible/incompatible (susceptibility/resistance) interactions in tomato-*Fol* pathosystem are controlled by avirulence genes; Avr1, Avr2, and Avr3; and their cognate resistance genes; I, I2, and I3; in tomato, respectively. 3 known races of *Fol* carrying the 3 avirulence genes (Avr1-3) in different

combinations have been identified. The outcomes of the interactions between the 3 *Fol* races and all possible combinations of resistance genes in tomato cultivars are summarized in Table 1.

Avr1 (Six4) is processed after secretion and is part of the xylem sap in the form of a 184 amino acids protein (Houterman *et al*., 2008). *Fol* strains (race 1) having Avr1 gene induce resistance in tomato cultivars harboring either the I resistance genes, but this avirulence factor is not required for virulence on plants lacking the cognate I genes (Houterman *et al*., 2008). Moreover, inhibition of effector-triggered immunity induced by Avr2 and Avr3 is another function of Avr1. Houterman *et al*. (2008) demonstrated that when strains of *Fol* that are avirulent on I2 and/or I3 are transformed with Avr1 they gained virulence on these lines, indicating that, by some an as yet unknown mechanism, Avr1 suppresses I2 and I3-mediated resistance. Avr1 gene is absent in races 1 and 2 that are avirulent on I2 and I3 cultivars, and no variation in the sequence of this gene has been found among different *Fol* isolates (Houterman *et al*., 2008).

Avr2 (Six3) effector consists of 144 amino acids and contains just two cysteine residues. Its corresponding I2 resistance gene, currently the only gene to be cloned, encodes and intracellular nucleotide-binding leucine rich repeat (NB-LRR) protein with an N-terminal coiled coil (CC) domain (reviewed in Catanzariti, 2010). Houterman *et al*. (2009) proved that recognition of Avr2 occurs inside the host plant, consistent with the intracellular localization of I2, although it is not known whether these two proteins directly interact. In addition, recognition inside host cell implies that Avr2 is transported from the xylem into the intracellular space, a property that is common to effectors from oomycetes (Schornack *et al*., 2010). Avr2 is required for full virulence and no *Fol* strains lacking this effector have been found. The most frequent mechanism underlying virulence on I2 cultivars is inhibition of I2-mediated resistance by Avr1 (Houterman *et al*., 2008). Nonetheless, variants with single amino acid changes that circumvent I2- mediated resistance without fitness cost have also been reported (Houterman *et al*., 2009). In addition, as Six6 requires living tissue for expression, this effector has been shown to suppress I2-mediated programmed cell death (I2CD) in tomato and contribute to pathogenicity. However, I2CD suppressing activity of Six6 does not allow the fungus to overcome I2 resistance in tomato, suggesting that I2-mediated resistance is independent from cell death (Gawehns *et al*., 2014).

Avr3 (Six1) is a small cysteine-rich protein of approximately 32 KDa. After secretion, this protein is

Table 1: Gene for gene Interactions between avirulence genes in *Fusarium oxysporum* f. sp. *lycopersici (Fol)* races and cognate resistance genes in tomato

***Fol* race (avirulence genes)**	**Possible combinations of resistance genes in tomato cultivars**								**Explanation of host-pathogen interaction outcomes**
	i i2 i3	***i i2 I3***	***i I2 i3***	***i I2I3***	***I I2 I3***	***I I2 i3***	***I i2 I3***	***I i2 i3***	
1 (*Avr1 Avr2 Avr3*)	S	S	S	S	R	R	R	R	Resistance gene *I* in the host recognizes avirulence gene *Avr1* in the pathogen and triggers resistance. *I* mutants (*i*) don't recognize *Avr1* in the pathogen which induces disease either as a result of compatible interaction between *Avr1 and i* or as a consequence of the suppression by *Avr1* of *I2*- and/ or *I3*- mediated resistance.
2 (-*Avr2 Avr3*)	S	R	R	R	R	R	R	S	Transposan-insertion into, or deletion of, *Avr1* impedes the capacity of *I* to recognize race 2. Resistance genes *I2* and/or *I3* in the hosts recognize avirulence genes *Avr2* and or/ *Avr3*, respectively, and suppress disease.
3 (- *avr2 Avr3*)	S	R	S	R	R	S	R	S	*Avr2* mutant (*avr2*) prevents genotypes with *I2* resistance gene from detecting race 3 of the pathogen. Only genotypes with *I3* are capable of detecting race 3 and initiate resistance mechanisms. Host mutants defective in *I3* (*i3*) cannot recognize race 3 of the pathogen and, thus, are susceptible.

S, susceptibility leading to the occurrence of disease (compatible interaction)
R, resistance leading to the suppression of disease (incompatible interaction)

cleaved at both the N- and C-terminus into a 22 KDa (189 amino acids) protein and a 12 KDa derivative, the former was proven to be the active form of the protein (Rep *et al*., 2004). The presence of living plant tissue in the vicinity of the pathogen is not only required for Avr3 expression during infection and colonization of tomato plants, but might also trigger a switch from saprophytic to pathogenic style. This expression is neither cultivar-specific nor depends on morphological features of the roots (Van Der Does *et al*., 2008). Like Avr2, Avr3 is also required for full virulence on tomato plants and relies on inhibition by Avr1 of I3-driven plant defense response.

GENOMIC ORGANIZATION OF PATHOGENICITY IN *FOL*

Genome sequencing of fungal phytopathogens has revolutionized the study of plant pathogenisis. Whole genome sequence data for individual fungal genomes accelerated classical forward and reverse genetic for biological function attribution to diverse genes including pathogenicity ones. To understand the molecular underpinnings of pathogenicity in *Fol*, a study compared the genome of this pathogen to two other fungi of the same genus, *Fusarium verticilloides* Sacc. Nirenberg (*Fv*) and *Fusarium graminearum* Schwabe (*Fg*) (Ma *et al*., 2010). The genome of *Fol* (60 megabases) is about 44% and 65% larger than those of *Fv* (42 Mb) and *Fg* (36 Mb) respectively, implying a greater number of protein-encoding genes in *Fol*.

Fusarium genomes consist of a core region with approximately 9000 genes considered to be orthologuous, and each species contains thousands of genes that are specific to each genome (Martinj and kistler, 2010). The genetic patrimonies of *Fol*, *Fv* and *Fg* are assembled in 15, 11 and 4 chromosomes, respectively (Ma *et al*., 2010). Comparison among the three species genomes attributed the increased genomic territory in *Fol* to additional unique sequences carried by extra chromosomes. *Fol*-specific sequences are a substantial part (40%) of the *Fol* assembly, designated as *Fol* lineage-specific (*Fol* LS), to distinguish them from the conserved core genome (Ma *et al*., 2010).

Fol LS regions include four entire chromosomes and contain more than 74% of transposable elements in *Fol* ge-

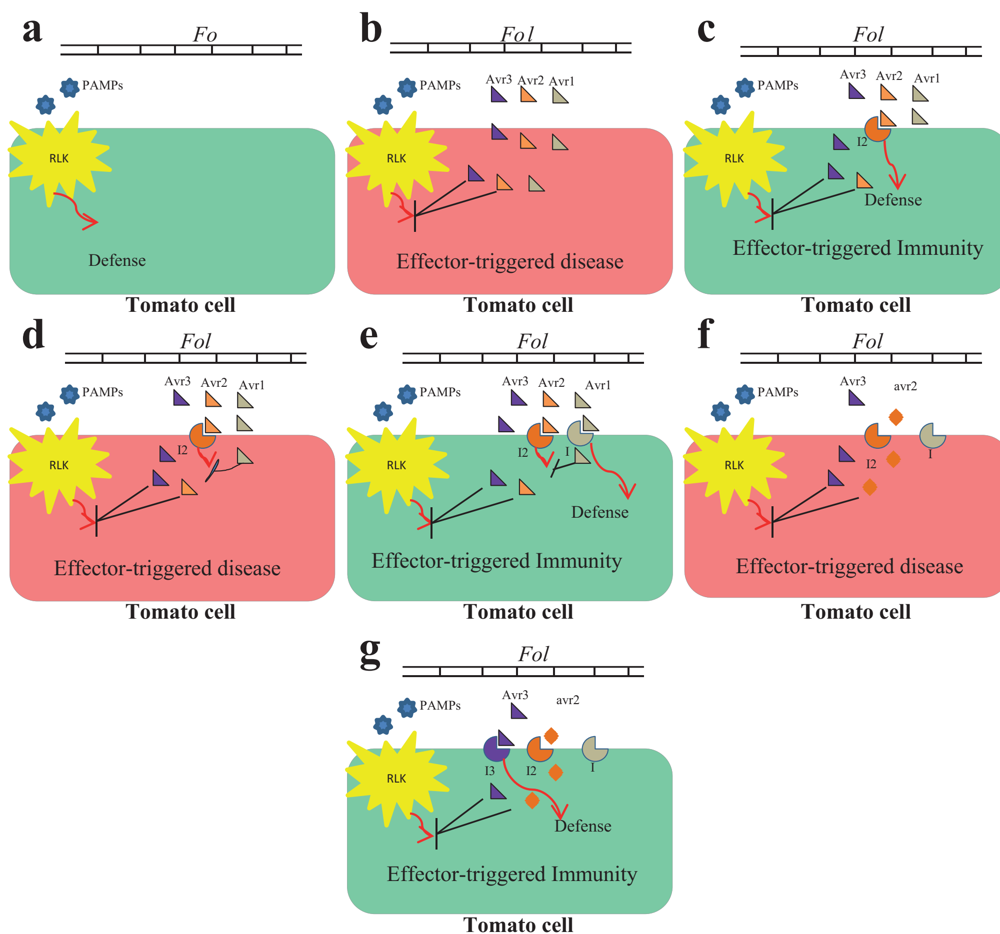

Figure 2: Conceptual model depicting coevolution and molecular arms race between tomato and Fusarium oxysporum f. sp. lycopersici (Fol). Arrows signifies activation. Lines ending in cross bars signifies suppression. (a) Non-pathogenic Fo strains trigger the induction of basal defense preventing disease through recognition of pathogen-associated molecular pattern by receptor-like kinases (RLKs). (b) Effectors, such as Avr2 and Avr3, suppress the PAMP-triggered Immunity (PTI) response, allowing pathogenic Fol strains to cause disease. (c) Perception of Avr2 by I2 activates host defense. (d) Avr1 suppresses I2-mediated defense, resulting in disease development. (e) Avr1 is recognized by I resulting in the activation of host defenses. (f) Transposan-insertion into (not shown), or deletion of Avr1 (shown), plus point mutation in Avr2, resulting in avr2, allow the pathogen to elude I and I2- mediated plant defense response and cause disease. (g) Tomato evolved I3 that recognizes Avr3 and initiates defense reaction

nome, including 95% of all DNA transposons. 20% of the entire *Fol* genome was identified as repetitive sequences, including many retro elements, short interspersed nuclear elements and several large segmental duplications (Ma *et al*., 2010).

Only 20% of the predicted genes in the *Fol* LS have been functionally classified based on homology to known proteins. These genes are enriched for the functional categories "secreted effectors and virulence factors", "transcription factors", and "proteins involved in signal transduction", but are deficient in genes for house-keeping functions (Ma *et al*., 2010). Genes with predicted functions related to pathogenicity that have been identified are effectors as well as necrosis and ethylene-inducing peptides and a variety of fungal and plant cell- wall- degrading or modifying enzymes that are mostly expressed during early stages of tomato root infection. *Fol* LS regions were also found to be rich in genes for lipid metabolism and lipid-derived secondary messenger, suggesting an important role for lipid signaling in fungal pathogenicity (Ma *et al*., 2010).

Fol LS genes have no clear orthologues in the other *Fusarium* species nor do they have paralogues in the core region of *Fol*. Basic Local Alignment Search Tool (BLAST) indicated that 93% of the of the 1285 LS-encoded proteins having their homologs in the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) protein database belong to ascomycetes fungi, indicating that *Fol* LS regions are of fungal origin. These results along with other phylogenetic analysis involving seven selected ascomycetes and four sequenced *Fusarium* genomes suggest that the *Fol* LS regions were acquired via horizontal transfer from other *Fusarium* species (Ma *et al*., 2010, Inami *et al*., 2014).

CO-EVOLUTION BETWEEN *FOL* AND TOMATO

A conceptual model of co-evolution between *F. oxysporum* (*Fo*) and tomato, based on gene-for-gene interactions, that led to the emergence of the 3 known races of *Fol* is presented in Fig. 2. Briefly, non-pathogenic *Fo* strains colonize the roots but are restricted to the surface by the basal defense system of the plant (Fig. 2a). Extracellular receptor-like kinases (RLKs) are probably involved in the detection of pathogen-associated molecular patterns (PAMPs) associated with these non-pathogenic strains and trigger PAMP-triggered immunity (Boller and Felix, 2009). Virulence on tomato evolved by the acquisition of effectors (Avr1, Avr2, and Avr3) and pathogenicity factors, probably via horizontal gene transfer as demonstrated by Ma *et al*. (2010) and specific adaptation to tomato, that enabled *Fo* to overcome plant defense machinery giving birth to *Fol* race 1. At least Avr2 and Avr3 are involved in the development of disease in tomato (Figure 2b). Tomato responded by evolving I2 to detect Avr2 and turn on defense machinery (Fig. 2c). To circumvent the newly developed plant defense mechanism, the pathogen evolved Avr1 to inhibit I2-mediated resistance via an as yet unknown mechanism (Fig. 2d). The evolvement of Avr1 by the pathogen selected for tomato plants that carry I resistant gene, capable of recognizing Avr1 and initiating defense cascades against the pathogen (Fig. 2e). As a way of escaping I-triggered defense mechanism, the pathogen underwent two successive molecular events. The first one is transposon-insertion into (Inami *et al*., 2012), or complete deletion of Avr1 (Houterman *et al*., 2009; Fig. 2f) to avoid recognition by I resistance gene. This led to the emergence of race 2 (Avr2 and Avr3) of the pathogen, which is virulent on tomato plants lacking both I2 and I3 resistance genes. The second molecular event is the emergence of single point mutation Avr2 variants (Inami *et al*., 2012; Houterman *et al*., 2009; Fig. 2f). These mutations prevent recognition by I2 but do not impair virulence. The plant bounced back to assault and evolved I3 that senses the presence of Avr3 and activates plant defense mechanisms, providing thereby protection against the pathogen (Fig. 2g).

CONCLUSIONS AND PROSPECTS

Significant advances have been made in the past decade towards the understanding of molecular bases underpinning interactions in the *Fol*-tomato pathosystem. Highlights are the identification of 3 efferctor-coding avirulence genes; Avr1, Avr2, and Avr3, in *Fol* and 3 cognate resistance genes in tomato. Of these resistance genes; I, I2, and I3; have been introgressed into tomato commercial varieties as a strategy for effective management of tomato wilt disease in agricultural settings. However, the ongoing co-evolution between *Fo* land tomato is likely to lead to the emergence of new races of the pathogen capable of breaking down resistance. Comparative genomics among populations of the pathogen from different geographic locations will be useful in the identification of new genes that code the synthesis of effector proteins and possibly the prediction of their virulence functions on the currently resistant cultivars. A function in virulence can be investigated experimentally by gene disruption, gene knock-down, or overexpression assays. In anticipation of eventual epidemic outbreaks of *Fol* races carrying such genes, large explorations among natural populations of wild tomato will help identify cognate resistance genes to be used in resistance breeding for a durable control of the disease.

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