Bacterial wilt disease: host resistance and pathogen virulence mechanisms

Bong-Suk Kim, Elizabeth French, Denise Caldwell, Emily J. Harrington
and Anjali S. Iyer-Pascuzzi

Department of Botany and Plant Pathology, Purdue University, West Lafayette IN, 47906

Email addresses for all authors:

Bong-Suk Kim: kim1@purdue.edu

Elizabeth French: eeckhard@purdue.edu

Denise Caldwell: caldwed@purdue.edu

Emily J. Harrington: ejharrin@purdue.edu

Anjali S. Iyer-Pascuzzi: asi2@purdue.edu

Corresponding author for all stages of publication: Anjali S. Iyer-Pascuzzi, asi2@purdue.edu

Abstract

Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is one of the most destructive bacterial diseases of Solanaceous species worldwide. The species infects plants in more than 200 species and 50 families and was ranked second in a list of the top 10 most scientifically and economically important bacterial plant pathogens(1). The molecular mechanisms underlying resistance and the functions of *R. solanacearum* effectors are beginning to be uncovered, and much remains to be discovered. In this mini-review, we provide a summary of host resistance and *R. solanacearum* virulence mechanisms, with a focus on tomato.

Keywords: Plant disease resistance; tomato; quantitative disease resistance; bacterial pathogen

Introduction

R. solanacearum infects over 200 plant species throughout the world, but is particularly devastating in the *Solanaceae* (2, 3). The pathogen is soil-borne and thrives in hot, humid environments. In Central America and the Southeastern United States, *R. solanacearum* is a severe limiting factor to tomato production, where it regularly reduces crop yields. A newly emerged strain, race 3, biovar 2 is also a severe threat in more temperate regions (4, 5).

Bacteria enter the tomato root either in the elongation zone or through wounds created at the site of lateral root emergence (6). Once in the root, bacteria enter the vasculature, which allows them to move from the root to the shoot. *R. solanacearum* produce several pathogenicity factors, including type III effectors, exopolysaccharide (EPS) (7-11) and cell wall degrading enzymes such as cellulase and pectic lyase (9, 12-15). As bacteria multiply in the xylem, the amount of EPS increases, ultimately preventing water flow to the shoot, and resulting in shoot wilting and eventual plant death.

Bacterial wilt disease management is difficult, and the best means of control has historically been through resistant cultivars. Resistance in tomato is primarily quantitative, the result of many genes contributing a small amount to resistance (16-23). Although several QTL for resistance to *R. solanacearum* have been coarse-mapped (16-23), none have been fine-mapped or cloned.

R. solanacearum is an extremely diverse species. Older classification schemes divided the pathogen into races and biovars (2). The race designation was based on the host range of the pathogen. Within races, *R. solanacearum* was divided into biovars, based in part on the ability of the pathogen to degrade specific carbohydrates (24). However, as the diversity of the species became clear through phylogenetic analyses, the race-biovar designations were changed (25, 26). Currently, *R. solanacearum* is classified according to phylotype and sequevar and is known as the *Ralstonia solanacearum* species complex (RSSC) (25). The RSSC is divided into four phylotypes, which each correspond (approximately) to a different geographic region. Phylotype I is found in Asia, Phylotype II (A and B) in the Americas, Phylotype III in Africa, and Phylotype IV in the Australia-Indonesia region. Phylotypes subsequently are divided into sequevars. Strains in phylotype IIA are found in the southeastern United States and the Caribbean, and typically infect plants in the *Solanaceae*. Strains in Phylotype IIB include the select agent strain of *R. solanacearum* (old designation race 3 biovar 2) that can infect plants at lower temperatures than other strains (4, 5). Phylogenetic analyses have shown little correlation between host range and phylotypes (10, 26).

The genomes of at least 14 different RSSC strains have been completely sequenced (27). These 14 include representatives from all four RSSC phylotypes, including the phylotype II type strain K60 (28), and the phylotype I strain GMI1000 frequently used in laboratory studies (29). Sequencing has been instrumental not only in classification but also in the identification of virulence determinants.

In this mini-review, we provide an overview of host genetic resistance and bacterial virulence mechanisms. For more detailed reviews see (10, 26, 30, 31).

Host resistance mechanisms

Genetic Mechanisms of Resistance

BW resistance in tomato is largely quantitative (polygenic). Although in *Arabidopsis*, resistance to *R. solanacearum* is governed by at least one major gene, *RRS1-R* (32), no major genes for BW resistance have been identified in tomato. Several QTL for BW resistance have

been mapped to regions of the tomato genome (16-23). These are both strain-specific and broad-spectrum QTL and include a large QTL on chromosome 12 to a Taiwanese phylotype I strain (21, 23), weaker QTL on chromosome 6 to both phylotype I and II strains (17-23), and additional small possibly strain-specific QTLs on chromosomes 3, 4 and 8 (22). Most of these QTL were identified using the same recombinant inbred line (RIL) population, Hawaii7996 (HA) x WestVirginia700 (WVa700). HA is unusual in that it has strong resistance in a wide variety of geographic locations throughout the world (3). The published QTL data suggest that HA contains multiple loci contributing to resistance to several *R. solanacearum* strains.

Thus far, the strongest QTL for BW resistance to any *R. solanacearum* strain maps to a 2.8 cM region on chromosome 12. *Bwr-12* is effective against phylotype I (Asian) strains, accounting for approximately 18 – 56% of the resistance (21, 23). This QTL does not appear to be active against phylotype II strains (22).

The other major QTL for BW resistance, *Bwr-6*, localizes to a 15.5 cM region on the short arm of tomato chromosome 6 and is effective against both phylotype I and II strains (17-23). QTL in this region have mapped to slightly different areas of this region in four separate experiments (all in the HA x WVa700 RILs). It is unclear exactly how many QTL are in this region. *Bwr-6* explains approximately 11.5 – 22% of the variation in resistance to *R. solanacearum* (23). This region appears to be a ‘hot-spot’ for both qualitative and quantitative disease resistance genes. Resistance genes that map to this region include *Mi* for resistance to root-knot nematodes and potato aphids (33-35), *Cf2* and *Cf5* for resistance to *Cladosporium fulvum* (36), and the QTL *Ol-1* for resistance to *Oidium lycopersicon* (37), *Ty-1* for resistance to Tomato yellow leaf curl virus (38), and *Am*, for resistance to Alfalfa mosaic virus (39).

In Arabidopsis, resistance to *R. solanacearum* is conferred by the combined action of two NB-LRR genes, *RRS1-R* (resistance to *Ralstonia solanacearum* 1) and *RPS4* (resistance to *Pseudomonas syringae* 4) (40-46). Together these act to confer recognition of the type III effectors (T3Es) PopP2 from *R. solanacearum* and AvrRps4 from *Pseudomonas syringae*. *RRS1-R* is unusual in that it not only encodes the only known recessive NB-LRR *R* gene but the protein also contains a WRKY DNA binding domain (32). The T3E PopP2, an acetyltransferase, directly acetylates a specific lysine within the C-terminal WRKY transcription factor domain of *RRS1-R* (47). This disrupts the connection between *RRS1-R* and DNA, leading to the activation of *RPS4*-induced defenses (45, 46). Thus, *RRS1-R* acts as an ‘integrated decoy’: it uses the WRKY domain as an effector target but can activate defense signaling through a different NBS-LRR protein. Thus, it is able to prevent effector virulence and activate defense genes (45, 46, 48).

Host whole genome transcription profiling

Given the importance of *R. solanacearum*, surprisingly little information is known regarding the transcriptional responses of plants to *R. solanacearum* infection. A time-course of transcriptional changes viewed by microarray analysis of *R. solanacearum* infection in Arabidopsis found that many hormone-related genes were affected (49). At early wilting stages (5 days after inoculation), 471 genes were differentially expressed in the compatible interaction compared to the incompatible. Most (353) were upregulated, and 40% of these genes were involved in the biosynthesis or signaling of the plant hormone abscisic acid (ABA). This is consistent with the idea that during early wilting stages bacteria-produced EPS restricts water flow throughout the plant, and ABA plays a major role in water stress. Many down-regulated genes at the early wilting stage were related to auxin and cytokinin signaling (49).

Because tomato has a different type of resistance to *R. solanacearum* compared to Arabidopsis (poly- vs. monogenic) and pathogenesis of the bacteria within each host appears to be different (49), work directly in tomato is crucial for understanding resistance and disease mechanisms. Examination of tomato transcriptional responses in a resistant and susceptible line with microarrays 24 hours after inoculation showed no change in gene expression in the inoculated susceptible plant relative to the mock-inoculated susceptible plant, but identified 146 up-regulated and 10 down-regulated genes in the resistant plant (50). Many different hormone-related genes were differentially expressed in the resistant cultivar, including those with roles in the jasmonic acid, auxin, and ethylene signaling pathways. PR (pathogenesis-related) genes as well as lignin and hydroxyl cinnamic acid biosynthesis genes were also upregulated, suggesting that gene expression changes leading to alterations in cell wall structure within xylem vessels may be important for resistance (50). In both the transcriptional profiling reports, shoots were sampled for gene expression, but since roots are part of the natural infection process, and are an important part of resistance (51, 52), it is likely that knowledge of root transcriptional reprogramming will provide important information for deciphering resistance mechanisms.

Roots are an important part of resistance

Studies with grafting have demonstrated that roots are key to resistance. Grafting resistant rootstocks to susceptible scions is an effective means of disease control in the Southern United States as well as South Korea and Japan (51, 52). Scions of the susceptible BHN 602 tomato line were grafted onto rootstocks of various tomato lines with resistance to bacterial wilt (51). When disease pressure was low, BW incidence of both grafted and non- or self-grafted was low. When BW disease pressure was high, both fruit yield and BW incidence from grafted hybrids performed much better than non-grafted and self-grafted hybrids (51). Grafting resistant rootstocks had similar effects on resistance in susceptible scions in a field study in North Carolina (52). These studies demonstrate the importance of roots for resistance and tomato yield in the field.

Insights to resistance from histological studies

Several groups have used histological studies to provide insight into how plants are affected by *R. solanacearum*. *R. solanacearum* is thought to enter the root system through secondary roots, root cap or through wounds acquired in the soil (2). In susceptible tomatoes, *R. solanacearum* entry into roots occurs in four stages (6): Bacteria first colonize the root surface either in the elongation zone or at the point of emerging lateral roots. During the second stage of colonization, 2 – 3 days after inoculation, significant numbers of bacteria infect the cortex, an inner layer of the root, with smaller numbers found in the epidermis, an outer root layer. Bacteria enter the vasculature, including the xylem, and begin to degrade cell walls during the third stage of infection, about 3 days after inoculation. In the fourth stage, bacteria multiply and begin to move into the shoot. The increase in EPS in the root and shoot plugs the xylem, preventing water movement, and leading to wilt, the most recognizable disease symptom.

The infection process is less well understood in resistant plants. Multiple reports have shown that resistant cultivars have significant numbers of bacteria within both roots and shoots, although the multiplication of bacteria in resistant plants may be slower (9), and in general, susceptible plants have higher levels of bacteria in the stem. Several reports suggest that bacteria in resistant plants are distributed differently than in susceptible plants, and are limited in their ability to broadly colonize tissues (53). Grimault et al. (53, 54) showed that resistance was

significantly correlated with bacterial colonization in the mid-stem. Consistent with this, QTL controlling *R. solanacearum* race 3 colonization in tomato stems were recently identified (22). There appear to be two types of resistance mechanisms in the stem that both block bacterial movement, but in different ways. Examination of bacteria distribution in stems of resistant and susceptible lines found that resistant lines confined bacteria to the primary xylem, possibly through thickenings of pit membranes in stem xylem tissue (55). In the susceptible line bacteria were found in secondary xylem tissues of the stem or had moved into the pith (55). A second means of preventing bacterial movement may be through the accumulation of tyloses in stem xylem vessels (53). Tyloses are outgrowths of xylem parenchyma cells that balloon into the lumen of adjacent xylem vessels, creating a physical barrier that may prevent bacterial movement.

Experiments in *Medicago truncatula* showed that as in tomato, *R. solanacearum* enters the intercellular spaces of the root cortex and proceeds to the vascular cylinder to allow colonization of the vascular system (56). Bacteria were identified in both resistant and susceptible cultivars but with higher numbers in susceptible plants. As in tomato, once bacteria accessed the central vascular system they were able to rapidly reproduce within the xylem vessel.

The infection process appears to be somewhat similar in *Arabidopsis thaliana* roots grown on agar media. Dignonnet et al. (57) found that six days post inoculation bacteria are found within intercellular spaces of the root cortex. Cell wall degradation allowed the bacteria to enter into the vascular system and multiply, and bacterial pockets to form within xylem vessels (57) of susceptible plants.

Environmental Mechanisms of Resistance

Biocontrol

In recent years, many studies have investigated the use of beneficial microorganisms as biocontrol agents (BCAs) for plant diseases. The most commonly studied microorganisms for the purpose of controlling BW are bacteria (58). Most researchers have focused on avirulent strains of *R. solanacearum*, *Pseudomonas* spp, and *Bacillus* spp., although other species have been used as well. BCAs can suppress disease through multiple mechanisms including competition, production of cell-wall degrading enzymes, antibiotics, or siderophores, and induction of systemic resistance (58). A common strategy for identifying potential BCAs is to isolate them from the rhizospheres of healthy plants (59). However, isolating microorganisms from the rhizospheres of *R. solanacearum*-infected plants to enrich for bacteria with biocontrol capabilities against BW may be a more effective strategy (60).

Hyakumachi et al. (61) showed that soil pretreatment with either a cell culture or cell-free filtrate of *Bacillus thuringiensis* suppresses tomato bacterial wilt and induces expression of defense-related genes. In a follow-up study, they examined transcription patterns in tomato roots pretreated with *B. thuringiensis* cell-free filtrate and subsequently challenged with *R. solanacearum* (62). Salicylic acid (SA)-related defense genes were induced only in main roots, but not in lateral roots, while jasmonic acid (JA)-dependent defense genes were either suppressed or not induced. Ethylene (ET)-responsive genes were induced in both main and lateral roots. Taken together, these results suggest that *B. thuringiensis* mediated disease suppression involves co-activation of SA and ET defense hormone signaling pathways and simultaneous suppression of the JA pathway, which is different than the classic model of induced systemic resistance mediated by activation of JA and ET (63). In an early study on biocontrol of BW, pretreatment

with the oomycete *Pythium oligandrum* transiently induced ET production and expression of ET-related genes and subsequently reduced wilt symptoms (64).

Various rhizosphere-inhabiting fungi have also been found to induce resistance against tomato bacterial wilt. Many studies have focused on mycorrhizal fungi, which develop a symbiotic relationship with plant roots and have also been shown in some cases to induce systemic resistance against plant disease. An early study found that colonization by *Glomus versiformae* induced phenol production in tomato roots and protected them against *R. solanacearum* infection (65). A more recent investigation characterized three different endomycorrhizal fungi for their biocontrol capabilities and found that bacterial wilt disease suppression varied between fungal species (66).

Soil amendments

Several different types of soil amendments have also been shown to provide efficient control for tomato bacterial wilt. Tomatoes grown in soil amended with 20% (v/v) municipal waste biochar, a charcoal-like substance for soil application in agriculture, had a reduced disease incidence compared to tomatoes grown in un-amended soil or soil amended with a wood biochar(67). Addition of bioorganic fertilizer decreased soil *R. solanacearum* populations and reduced disease incidence in tomato field plots compared to plots treated with either an inorganic or organic NPK fertilizer (68). The bioorganic fertilizer, which contains its own populations of bacteria, increased the diversity and richness of the bacterial populations in the amended soil, which was correlated with increased disease resistance. Soil application of systemic resistance elicitors - silicon and chitosan - also reduced bacterial wilt disease incidence and severity and increased expression of defense-related genes, such as chitinases and peroxidases (69). Another study examining silicon-induced resistance to tomato bacterial wilt showed that silicon amendment primed tomato plants for JA-/ET- and reactive oxygen species- dependent defense gene expression, increasing their resistance to disease (70). For a more comprehensive review of biocontrol and soil amendment disease control for bacterial wilt, see Yuliar, et al., (58).

Pathogen Effectors and other Virulence Factors

Non-type III effector Virulence factors

R. solanacearum uses numerous virulence factors during the disease process. The pathogen secretes exopolysaccharide (EPS1), a polymer of N-acetylated sugars and a major virulence factor for infection in tomato (10). EPS1 deficient strains cause significantly less disease in tomato regardless of whether they are inoculated onto unwounded root systems or directly into the xylem(9, 11). In resistant tomato plants, wild type *R. solanacearum* induced greater defense responses than the *eps*⁻ mutant, suggesting that resistant plants can recognize this pathogen trait (11). Coincident with this, *R. solanacearum* growing in roots of infected resistant tomato plants produce less EPS per gram of tissue than those found in susceptible plants(8). The production of EPS1 is regulated by cell density, and is higher when pathogen density is greater than 10⁷ cells/ml (71). In addition to high cell density, EPS1 production requires nitrogen in the form of nitrate. Mutants defective in *nasA*, which encodes a nitrate reductase, do not produce normal amounts of EPS1 (72).

Other virulence factors in both tomato and Arabidopsis include cell wall degrading enzymes (13, 73-75), which are secreted through *R. solanacearum*'s type II protein secretion system (T2SS)(26). Both pectolytic and cellulolytic wall degrading enzymes in *R. solanacearum* have been described. A *R. solanacearum* mutant defective in the T2SS has only weak

virulence(76), and is less virulent than mutants defective only in CWDE, suggesting that in addition to CWDE, other proteins secreted through the T2SS are important for virulence (14). Surprisingly, a triple mutant defective in three pectolytic enzymes was more virulent than either a mutant defective in six CWDE (both pectolytic and cellulolytic)(75) or two cellulolytic enzymes. The authors attribute this to a lack of plant defense stimulation in the triple mutant (75). Similar to infection in tomato, CWDE are important for *R. solanacearum* infection in Arabidopsis. Inoculation with *R. solanacearum* GMI1000 resulted in pectin degradation in the root of wild-type Col-0 Arabidopsis (57).

Another significant aspect of *R. solanacearum* virulence is motility. Nonmotile mutants that either lacked flagella or had defective flagella had reduced virulence when soil-soak inoculated on tomato(77). However, when the nonmotile *fliC* mutant was inoculated directly into the xylem via petiole inoculation, it was able to cause disease (77). These data suggest that flagella are important for locating roots but are dispensable once bacteria have reached the xylem. Twitching motility, which provides a flagella-independent form of surface movement for *R. solanacearum*, also plays a role in virulence (78), as mutants defective in this ability show reduced virulence in tomato (78).

How *R. solanacearum* thrives in the oxygen- and nutrient-limited environment of the xylem has been puzzling. However, recent work has demonstrated that the ability to use nitrate is an important part of *R. solanacearum* virulence in tomato (72, 79, 80). *R. solanacearum* infection reduces already low levels of oxygen in the xylem(80). Tomato xylem sap contains significantly high levels of nitrate. Work by Dalsing et al. 2015 (80) showed that during infection, *R. solanacearum* consumed the available oxygen, and was able to use inorganic N as an energy source with nitrate as a terminal electron acceptor. Consistent with this, the genome of GMI1000, a phylotype I strain of *R. solanacearum*, contains a set of nitrate respiration and denitrification genes (*narG*, *aniA*, *norB*, *nosZ* and *hmpX*)(29, 80). Further, transcriptomic analysis of bacteria growing *in planta* showed that all of these genes were significantly expressed during *R. solanacearum* pathogenesis in tomato (79). Single mutant analysis showed that two mutants with defects in the nitrate detoxification pathway, *aniA* and *hmpX*, caused less wilt disease than wild type *R. solanacearum*(80). Thus, the ability to use inorganic N is an important part of the pathogen's ability to colonize the tomato host and cause disease.

Pathogen Effectors and the T3SS

Like most plant pathogens, *R. solanacearum* uses the type III secretion system (T3SS) to inject type III effectors (T3Es) into the host cell, causing disease in susceptible plants and defense responses in resistant plants (30). *hrp* genes are induced *in planta*, and *R. solanacearum* is thus far the only species in which induction of *hrp* genes has been shown to require physical contact between the bacterium and the plant host cell wall (81-84). In *R. solanacearum*, an outer membrane receptor known as PrhA recognizes a non-diffusible plant cell wall molecule and initiates a transcriptional cascade that results in the activation of the master regulator *hrpG*, followed by *hrpB*. HrpB then directly controls the expression of the T3SS machinery and T3E expression(26). In addition to its role in activating the T3SS through *hrpB*, HrpG also directs the expression of hundreds of *hrpB*-independent genes that are important for pathogen virulence (15, 85). The *hrpB* regulon is expressed *in planta* both at early stages of plant infection and during later wilting stages (79, 86). Both *hrpB* and *hrpG* genes are necessary for tomato root infection, although at different stages of the process (87).

Genomic sequencing has revealed that *R. solanacearum* strains have, on average, between 60 - 75 different T3Es (27, 31), several of which are part of multigene families. About 50% of the predicted T3Es in the RSSC are thought to be specific to this species complex (26), and many are highly conserved within phylotypes. For example, within phylotype II, 54 effectors are conserved among four sequenced strains (26). Thirty-two effectors are conserved across at least 10 of the fully sequenced RSSC genomes (31), although a minimal set of “core” effectors has not yet been identified. Despite the effector conservation within phylotype, there appears to be little correlation between host range and phylotype (26).

Of the predicted 110 T3E gene families in the RSSC (26), only about two dozen have a known function (30). Genetic analyses have demonstrated that several T3Es have roles in *R. solanacearum* virulence. For example, PopS and GALA4 suppress plant defenses in tomato (79, 88), while GALA7 is required for virulence in *Medicago truncatula* (56). In tobacco cells, PopA localizes to the membrane and forms an ion-conducting pore, which may promote translocation of bacterial proteins from the apoplast into the host cell cytoplasm (89). Effectors in the RSSC also have roles in avirulence. AvrA is a major avirulence factor in tobacco (90, 91), while PopP2 interacts with the Arabidopsis resistance protein RRS1-R (32). However, effector function is dependent on host species, as both AvrA and PopP2 contribute to pathogen growth in tomato (AvrA and PopP2) (92) or *Medicago truncatula* (AvrA) (56). Depending on the strain, the *R. solanacearum* genome also contains effectors that are homologs of *Xanthomonas* TAL effectors, known as RipTALs in *R. solanacearum* (27, 93, 94).

In part due to the prevalence of gene families among RSSC effectors, multiple mutations are often necessary to see a reduction in virulence. For example, knocking out all five members of the *AWR* gene family causes reduced virulence in tomato (95). A triple mutant in members of the *HLK* family also shows a reduction in virulence (96). The *GALA* T3E family contains eight genes that encode proteins with F-box motifs (97). Four members of this family interact with Arabidopsis Skp-1 like proteins, suggesting they may be involved in proteasome-mediated degradation of plant host targets (97). Mutants lacking all seven *GALA* genes in GMI1000 have reduced virulence in Arabidopsis, tomato, and eggplant (88). For a more detailed description of T3Es in *R. solanacearum*, the reader is referred to (10, 26, 30, 31).

Understanding the *R. solanacearum* genes used during pathogenesis is helping to shed light on virulence mechanisms *in planta*. For example, using a strain of *R. solanacearum* expressing a luminescent reporter Montiero et al. (86) showed that the gene controlling EPS biosynthesis is activated in bacteria that colonize the xylem. In a different study, examination of gene expression from two strains of *R. solanacearum* *in planta* identified genes that were differentially expressed in tomato during early bacterial wilt disease (79). Surprisingly, 45% of HrpB-regulated genes were upregulated *in planta*, suggesting that T3SS has an important role throughout the disease cycle. Virulence factors such as EPS1 biosynthesis genes and mobility genes (*pilA* and *fliC*) were expressed highly during pathogenesis, as were pathways for sucrose uptake and metabolism. Sucrose regulon genes were upregulated significantly *in planta*. A mutant defective in the *scrA* gene was less virulent than the wild type pathogen when inoculated to unwounded tomato roots, but not when introduced to the xylem through cut petioles. However, calculation of the population size of the wild type and *scrA* mutant in the xylem during wilting showed a reduction in *scrA* growth, suggesting that the *scrA* mutant had a reduction in fitness during pathogenesis (79). Knowledge of the *R. solanacearum* genes active during infection will lead to greater understanding of host resistance and susceptibility.

Conclusions

In conclusion, *R. solanacearum* is a diverse pathogen that uses a battery of virulence mechanisms to cause disease. While there is still much to be learned about host plant resistance, mechanisms of pathogen virulence are becoming clearer. Future studies will continue to investigate pathogen virulence mechanisms and will generate insight into host plant resistance through QTL cloning, examination of the molecular mechanisms of resistance in roots, and through the identification of additional plant proteins targeted by *R. solanacearum* effectors.

Acknowledgements

We thank members of the Iyer-Pascuzzi lab for comments and Purdue University for funding. EF is supported by a NSF graduate research fellowship.

References

1. Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, Ronald P, Dow M, Verdier V, Beer SV, Machado MA, Toth I, Salmond G, Foster GD. Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol Plant Pathol* 2012; **13**(6): 614-629.
2. Hayward AC. Biology and Epidemiology of Bacterial Wilt Caused by *Pseudomonas-Solanacearum*. *Annual Review of Phytopathology* 1991; **29**: 65-87.
3. Scott JW, Wang JF, Hanson PM. Breeding tomatoes for resistance to bacterial wilt, a global view. *Proceedings of the 1st International Symposium on Tomato Diseases* 2005; (695): 161-172.
4. Norman DJ, Zapata M, Gabriel DW, Duan YP, Yuen JM, Mangravita-Novo A, Donahoo RS. Genetic diversity and host range variation of *Ralstonia solanacearum* strains entering North America. *Phytopathology* 2009; **99**(9): 1070-1077.
5. Clarke CR, Studholme DJ, Hayes B, Runde B, Weisberg A, Cai R, Wroblewski T, Daunay MC, Wicker E, Castillo JA, Vinatzer BA. Genome-Enabled Phylogeographic Investigation of the Quarantine Pathogen *Ralstonia solanacearum* Race 3 Biovar 2 and Screening for Sources of Resistance Against Its Core Effectors. *Phytopathology* 2015; **105**(5): 597-607.
6. Vasse J, Frey P, Trigalet A. Microscopic Studies of Intercellular Infection and Protoxylem Invasion of Tomato Roots by *Pseudomonas-Solanacearum*. *Molecular Plant-Microbe Interactions* 1995; **8**(2): 241-251.
7. Denny T, Baek S. Genetic evidence that extracellular polysaccharide is a virulence factor of *Pseudomonas solanacearum*. *Mol Plant Microbe Interactions* 1991; **4**: 198-206.
8. McGarvey JA, Denny TP, Schell MA. Spatial-temporal and quantitative analysis of growth and EPSI production by *Ralstonia solanacearum* in resistant and susceptible tomato cultivars. *Phytopathology* 1999; **89**(12): 1233-1239.

9. Saile E, McGarvey JA, Schell MA, Denny TP. Role of Extracellular Polysaccharide and Endoglucanase in Root Invasion and Colonization of Tomato Plants by *Ralstonia solanacearum*. *Phytopathology* 1997; **87**(12): 1264-1271.
10. Genin S. Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytol* 2010; **187**(4): 920-928.
11. Milling A, Babujee L, Allen C. *Ralstonia solanacearum* extracellular polysaccharide is a specific elicitor of defense responses in wilt-resistant tomato plants. *PLoS One* 2011; **6**(1): e15853.
12. Allen C, Gay J, Simon-Buela L. A regulatory locus, *pehSR*, controls polygalacturonase production and other virulence functions in *Ralstonia solanacearum*. *Mol Plant Microbe Interact* 1997; **10**(9): 1054-1064.
13. Huang Q, Allen C. An exo-poly-alpha-D-galacturonosidase, *PehB*, is required for wild-type virulence of *Ralstonia solanacearum*. *J Bacteriol* 1997; **179**(23): 7369-7378.
14. Liu H, Zhang S, Schell MA, Denny TP. Pyramiding unmarked deletions in *Ralstonia solanacearum* shows that secreted proteins in addition to plant cell-wall-degrading enzymes contribute to virulence. *Mol Plant Microbe Interact* 2005; **18**(12): 1296-1305.
15. Valls M, Genin S, Boucher C. Integrated regulation of the type III secretion system and other virulence determinants in *Ralstonia solanacearum*. *PLoS Pathog* 2006; **2**(8): e82.
16. Young ND, Danesh D. Understanding Bacterial Wilt Resistance in Tomato through the Use of DNA Genetic-Markers. *Bacterial Wilt* 1994: 145-155.
17. Danesh D, Aarons S, McGill GE, Young ND. Genetic Dissection of Oligogenic Resistance to Bacterial Wilt in Tomato. *Mol Plant Microbe Interact* 1994; **7**(4): 464-471.
18. Thoquet P, Olivier J, Sperisen C, Rogowsky P, Laterrot H, Grimsley N. Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii7996. *Molecular Plant-Microbe Interactions* 1996; **9**(9): 826-836.
19. Thoquet P, Olivier J, Sperisen C, Rogowsky P, Prior P, Anais G, Mangin B, Bazin B, Nazer R, Grimsley N. Polygenic resistance of tomato plants to bacterial wilt in the French West Indies. *Molecular Plant-Microbe Interactions* 1996; **9**(9): 837-842.
20. Mangin B, Thoquet P, Olivier J, Grimsley NH. Temporal and multiple quantitative trait loci analyses of resistance to bacterial wilt in tomato permit the resolution of linked loci. *Genetics* 1999; **151**(3): 1165-1172.
21. Wang JF, Olivier J, Thoquet P, Mangin B, Sauviac L, Grimsley NH. Resistance of tomato line Hawaii7996 to *Ralstonia solanacearum* Pss4 in Taiwan is controlled mainly

- by a major strain-specific locus. *Molecular Plant-Microbe Interactions* 2000; **13**(1): 6-13.
22. Carmeille A, Caranta C, Dintinger J, Prior P, Luisetti J, Besse P. Identification of QTLs for *Ralstonia solanacearum* race 3-phylo type II resistance in tomato. *Theor Appl Genet* 2006; **113**(1): 110-121.
 23. Wang J-F, Ho F-I, Truong HTH, Huan S-M, Balatero CH, Dittapongpitch V, Hidayati N. Identification of major QTLs associated with stable resistance of tomato cultivar 'Hawaii 7996' to *Ralstonia solanacearum*. *Euphytica* 2013; **190**: 241-252.
 24. Denny TP, Hayward AC. *Ralstonia*, 3rd edn. APS Press, 2001.
 25. Fegan M, Prior P. How complex is the *Ralstonia solanacearum* species complex? In: C. Allen PP, and A.C. Hayward (ed). *Bacterial Wilt: The Disease and the Ralstonia solanacearum Species Complex*. American Phytopathological Society Press: St. Paul, MN, USA, 2005, pp 449-461.
 26. Genin S, Denny TP. Pathogenomics of the *Ralstonia solanacearum* species complex. *Annu Rev Phytopathol* 2012; **50**: 67-89.
 27. Peeters N, Carrere S, Anisimova M, Plener L, Cazale AC, Genin S. Repertoire, unified nomenclature and evolution of the Type III effector gene set in the *Ralstonia solanacearum* species complex. *BMC genomics* 2013; **14**: 859.
 28. Remenant B, Babujee L, Lajus A, Medigue C, Prior P, Allen C. Sequencing of K60, type strain of the major plant pathogen *Ralstonia solanacearum*. *J Bacteriol* 2012; **194**(10): 2742-2743.
 29. Salanoubat M, Genin S, Artiguenave F, Gouzy J, Mangenot S, Arlat M, Billault A, Brottier P, Camus JC, Cattolico L, Chandler M, Choise N, Claudel-Renard C, Cunnac S, Demange N, Gaspin C, Lavie M, Moisan A, Robert C, Saurin W, Schiex T, Siguier P, Thebault P, Whalen M, Wincker P, Levy M, Weissenbach J, Boucher CA. Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature* 2002; **415**(6871): 497-502.
 30. Coll NS, Valls M. Current knowledge on the *Ralstonia solanacearum* type III secretion system. *Microbial biotechnology* 2013; **6**(6): 614-620.
 31. Deslandes L, Genin S. Opening the *Ralstonia solanacearum* type III effector tool box: insights into host cell subversion mechanisms. *Current opinion in plant biology* 2014; **20**: 110-117.
 32. Deslandes L, Olivier J, Peeters N, Feng DX, Khounlotham M, Boucher C, Somssich I, Genin S, Marco Y. Physical interaction between RRS1-R, a protein conferring resistance

- to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proc Natl Acad Sci U S A* 2003; **100**(13): 8024-8029.
33. Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM. The nematode resistance gene Mi of tomato confers resistance against the potato aphid. *Proc Natl Acad Sci U S A* 1998; **95**(17): 9750-9754.
 34. Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM. The root knot nematode resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *The Plant cell* 1998; **10**(8): 1307-1319.
 35. Vos P, Simons G, Jesse T, Wijbrandi J, Heinen L, Hogers R, Frijters A, Groenendijk J, Diergaarde P, Reijans M, Fierens-Onstenk J, de Both M, Peleman J, Liharska T, Hontelez J, Zabeau M. The tomato Mi-1 gene confers resistance to both root-knot nematodes and potato aphids. *Nature biotechnology* 1998; **16**(13): 1365-1369.
 36. Dickinson MJ, Jones DA, Jones JD. Close linkage between the Cf-2/Cf-5 and Mi resistance loci in tomato. *Mol Plant Microbe Interact* 1993; **6**(3): 341-347.
 37. van der Beek JG, Pet G, Lindhout P. Resistance to powdery mildew (*Oidium lycopersicum*) in *Lycopersicon hirsutum* is controlled by an incompletely-dominant gene Ol-1 on chromosome 6. *Theor Appl Genet* 1994; **89**(4): 467-473.
 38. Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleban T, van-Oss H, Kedar N, Rabinowitch HD, Czosnek H. Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, TY-1. *Theor Appl Genet* 1994; **88**(2): 141-146.
 39. Parrella G, Moretti A, Gognalons P, Lesage ML, Marchoux G, Gebre-Selassie K, Caranta C. The Am Gene Controlling Resistance to Alfalfa mosaic virus in Tomato Is Located in the Cluster of Dominant Resistance Genes on Chromosome 6. *Phytopathology* 2004; **94**(4): 345-350.
 40. Narusaka M, Shirasu K, Noutoshi Y, Kubo Y, Shiraishi T, Iwabuchi M, Narusaka Y. RRS1 and RPS4 provide a dual Resistance-gene system against fungal and bacterial pathogens. *The Plant journal : for cell and molecular biology* 2009; **60**(2): 218-226.
 41. Narusaka M, Kubo Y, Shiraishi T, Iwabuchi M, Narusaka Y. A dual resistance gene system prevents infection by three distinct pathogens. *Plant signaling & behavior* 2009; **4**(10): 954-955.
 42. Narusaka M, Kubo Y, Hatakeyama K, Imamura J, Ezura H, Nanasato Y, Tabei Y, Takano Y, Shirasu K, Narusaka Y. Breaking restricted taxonomic functionality by dual resistance genes. *Plant signaling & behavior* 2013; **8**(6): e24244.

43. Narusaka M, Hatakeyama K, Shirasu K, Narusaka Y. Arabidopsis dual resistance proteins, both RPS4 and RRS1, are required for resistance to bacterial wilt in transgenic Brassica crops. *Plant signaling & behavior* 2014; **9**(7): e29130.
44. Sohn KH, Segonzac C, Rallapalli G, Sarris PF, Woo JY, Williams SJ, Newman TE, Paek KH, Kobe B, Jones JD. The nuclear immune receptor RPS4 is required for RRS1SLH1-dependent constitutive defense activation in Arabidopsis thaliana. *PLoS genetics* 2014; **10**(10): e1004655.
45. Le Roux C, Huet G, Jauneau A, Camborde L, Tremousaygue D, Kraut A, Zhou B, Levaillant M, Adachi H, Yoshioka H, Raffaele S, Berthome R, Coute Y, Parker JE, Deslandes L. A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity. *Cell* 2015; **161**(5): 1074-1088.
46. Sarris PF, Duxbury Z, Huh SU, Ma Y, Segonzac C, Sklenar J, Derbyshire P, Cevik V, Rallapalli G, Saucet SB, Wirthmueller L, Menke FL, Sohn KH, Jones JD. A Plant Immune Receptor Detects Pathogen Effectors that Target WRKY Transcription Factors. *Cell* 2015; **161**(5): 1089-1100.
47. Tasset C, Bernoux M, Jauneau A, Pouzet C, Briere C, Kieffer-Jacquiod S, Rivas S, Marco Y, Deslandes L. Autoacetylation of the Ralstonia solanacearum effector PopP2 targets a lysine residue essential for RRS1-R-mediated immunity in Arabidopsis. *PLoS Pathog* 2010; **6**(11): e1001202.
48. Cesari S, Bernoux M, Moncuquet P, Kroj T, Dodds PN. A novel conserved mechanism for plant NLR protein pairs: the "integrated decoy" hypothesis. *Frontiers in plant science* 2014; **5**: 606.
49. Hu J, Barlet X, Deslandes L, Hirsch J, Feng DX, Somssich I, Marco Y. Transcriptional responses of Arabidopsis thaliana during wilt disease caused by the soil-borne phytopathogenic bacterium, Ralstonia solanacearum. *PLoS One* 2008; **3**(7): e2589.
50. Ishihara T, Mitsuhashi I, Takahashi H, Nakaho K. Transcriptome analysis of quantitative resistance-specific response upon Ralstonia solanacearum infection in tomato. *PLoS One* 2012; **7**(10): e46763.
51. McAvoy T, Freeman J, Rideout S, Olson S, Paret P. Evaluation of Grafting Using Hybrid Rootstocks for Management of Bacterial Wilt in Field Tomato Production. *HortScience* 2012; **47**(5): 621-625.
52. Rivard CL, O'Connell S, Peet MM, Welker RM, Louws FJ. Grafting Tomato to Manage Bacterial Wilt Caused by Ralstonia solanacearum in the Southeastern United States. *Plant Disease* 2012; **96**(7): 973-978.

53. Grimault V, Gelie B, Lemattre M, Prior P, Schmit J. Comparative Histology of Resistant and Susceptible Tomato Cultivars Infected by *Pseudomonas-Solanacearum*. *Physiological and Molecular Plant Pathology* 1994; **44**(2): 105-123.
54. Grimault V, Prior P. Bacterial Wilt Resistance in Tomato Associated with Tolerance of Vascular Tissues to *Pseudomonas-Solanacearum*. *Plant Pathology* 1993; **42**(4): 589-594.
55. Nakaho K, Inoue H, Takayama T, Miyagawa H. Distribution and multiplication of *Ralstonia solanacearum* in tomato plants iwth resistance derived from different origins. *Journal of General Plant Pathology* 2004; **70**(2): 115-119.
56. Turner M, Jauneau A, Genin S, Tavella MJ, Vailleau F, Gentzbittel L, Jardinaud MF. Dissection of bacterial Wilt on *Medicago truncatula* revealed two type III secretion system effectors acting on root infection process and disease development. *Plant Physiol* 2009; **150**(4): 1713-1722.
57. Digonnet C, Martinez Y, Denance N, Chasseray M, Dabos P, Ranocha P, Marco Y, Jauneau A, Goffner D. Deciphering the route of *Ralstonia solanacearum* colonization in *Arabidopsis thaliana* roots during a compatible interaction: focus at the plant cell wall. *Planta* 2012; **236**(5): 1419-1431.
58. Yuliar, Nion YA, Toyota K. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes and environments / JSME* 2015; **30**(1): 1-11.
59. Kurabachew H, Wydra K. Characterization of plant growth promoting rhizobacteria and their potential as bioprotectant against tomato bacterial wilt caused by *Ralstonia solanacearum*. *Biological Control* 2013; (67): 75-83.
60. Huang J, Wei Z, Tan S, Mei X, Yin S, Shen Q, Xu Y. The rhizosphere soil of diseased tomato plants as a source for novel microorganisms to control bacterial wilt. *Applied Soil Ecology* 2013; (72): 79-84.
61. Hyakumachi M, Nishimura M, Arakawa T, Asano S, Yoshida S, Tsushima S, Takahashi H. *Bacillus thuringiensis* supresses bacterial wilt disease caused by *Ralstonia solanacearum* with systemic induction of defense-related gene expression in tomato. . *Microbes and environments / JSME* 2013; (28: 128-134).
62. Takahashi H, Nakaho K, Ishihara T, Ando S, Wada T, Kanayama Y, Asano S, Yoshida S, Tsushima S, Hyakumachi M. Transcriptional profile of tomato roots exhibiting *Bacillus thuringiensis*-induced resistance to *Ralstonia solanacearum*. *Plant cell reports* 2014; **33**(1): 99-110.
63. Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA. Induced systemic resistance by beneficial microbes. *Annu Rev Phytopathol* 2014; **52**: 347-375.

64. Hase S, Shimizu A, Nakaho K, Takenaka S, Takahashi H. Induction of transient ethylene and reduction in severity of tomato bacterial wilt by *Pythium oligandrum*. *Plant Pathology* 2006; **55**(4): 537-543.
65. Zhu HH, Yao Q. Localized and systemic increase of phenols in tomato root induced by *Glomus versiformae* inhibits *Ralstonia solanacearum*. *Journal of Phytopathology* 2004; (152): 537-542.
66. Tahat MM, Siiam K, Othman R. The potential of endomycorrhizal fungi in controlling tomato bacterial wilt *Ralsotnia solanacearum* under glasshouse coditions. . *African Journal of Biotechnology* 2012; (11): 13085-13094.
67. Nerome M, Toyota K, Islam TM, Nishimima T, Matsuoka T, Sato K, Yamaguchi Y. Suppression of bacterial wilt of tomato by incorporation of municipal biowaste charcoal into soil. *Soil Microorganisms* 2005; **59**(1): 9 -14.
68. Liu L, Sun C, Liu S, Chai R, Huang W, Liu X, Tang C, Zhang Y. Bioorganic fertilizer enhances soil suppressive capacity against bacterial wilt of tomato. *PLoS One* 2015; **10**(4): e0121304.
69. Kiirika LM, Stahl F, Wydra K. Phenotypic and molecular characterization of resistance induction by single and combined application of chitosan and silicon in tomato against *Ralstonia solanacearum*. *Physiological and Molecular Plant Pathology* 2013; (81): 1 - 12.
70. Ghareeb H, Bozso Z, Ott PG, Repenning C, Stahl F, Wydra K. Transcriptome of silicon-induced resistance against *Ralstonia solanacearum* in the silicon non-accumulator tomato implicates priming effect. *Physiological and Molecular Plant Pathology* 2011; (75): 83-89.
71. Kang Y, Saile E, Schell MA, Denny TP. Quantitative immunofluorescence of regulated eps gene expression in single cells of *Ralstonia solanacearum*. *Applied and environmental microbiology* 1999; **65**(6): 2356-2362.
72. Dalsing BL, Allen C. Nitrate assimilation contributes to *Ralstonia solanacearum* root attachment, stem colonization, and virulence. *J Bacteriol* 2014; **196**(5): 949-960.
73. Schell MA, Roberts DP, Denny TP. Analysis of the *Pseudomonas solanacearum* polygalacturonase encoded by pglA and its involvement in phytopathogenicity. *J Bacteriol* 1988; **170**(10): 4501-4508.
74. Tans-Kersten J, Guan Y, Allen C. *Ralstonia solanacearum* pectin methylesterase is required for growth on methylated pectin but not for bacterial wilt virulence. *Applied and environmental microbiology* 1998; **64**(12): 4918-4923.

75. Gonzalez ET, Allen C. Characterization of a *Ralstonia solanacearum* operon required for polygalacturonate degradation and uptake of galacturonic acid. *Mol Plant Microbe Interact* 2003; **16**(6): 536-544.
76. Kang Y, Huang J, Mao G, He L, Schell MA. Dramatically reduced virulence of mutants of *Pseudomonas solanacearum* defective in export of extracellular proteins across the outer membrane. *Mol Plant Microbe Interact* 1994; (7): 370-377.
77. Tans-Kersten J, Huang H, Allen C. *Ralstonia solanacearum* needs motility for invasive virulence on tomato. *J Bacteriol* 2001; **183**(12): 3597-3605.
78. Liu H, Kang Y, Genin S, Schell MA, Denny TP. Twitching motility of *Ralstonia solanacearum* requires a type IV pilus system. *Microbiology* 2001; **147**(Pt 12): 3215-3229.
79. Jacobs JM, Milling A, Mitra RM, Hogan CS, Ailloud F, Prior P, Allen C. *Ralstonia solanacearum* requires PopS, an ancient AvrE-family effector, for virulence and To overcome salicylic acid-mediated defenses during tomato pathogenesis. *MBio* 2013; **4**(6): e00875-00813.
80. Dalsing BL, Truchon AN, Gonzalez-Orta ET, Milling AS, Allen C. *Ralstonia solanacearum* uses inorganic nitrogen metabolism for virulence, ATP production, and detoxification in the oxygen-limited host xylem environment. *MBio* 2015; **6**(2): e02471.
81. Marena M, Brito B, Callard D, Genin S, Barberis P, Boucher C, Arlat M. PrhA controls a novel regulatory pathway required for the specific induction of *Ralstonia solanacearum* hrp genes in the presence of plant cells. *Molecular microbiology* 1998; **27**(2): 437-453.
82. Brito B, Marena M, Barberis P, Boucher C, Genin S. prhJ and hrpG, two new components of the plant signal-dependent regulatory cascade controlled by PrhA in *Ralstonia solanacearum*. *Molecular microbiology* 1999; **31**(1): 237-251.
83. Aldon D, Brito B, Boucher C, Genin S. A bacterial sensor of plant cell contact controls the transcriptional induction of *Ralstonia solanacearum* pathogenicity genes. *The EMBO journal* 2000; **19**(10): 2304-2314.
84. Brito B, Aldon D, Barberis P, Boucher C, Genin S. A signal transfer system through three compartments transduces the plant cell contact-dependent signal controlling *Ralstonia solanacearum* hrp genes. *Mol Plant Microbe Interact* 2002; **15**(2): 109-119.
85. Occhialini A, Cunnac S, Reymond N, Genin S, Boucher C. Genome-wide analysis of gene expression in *Ralstonia solanacearum* reveals that the hrpB gene acts as a regulatory switch controlling multiple virulence pathways. *Mol Plant Microbe Interact* 2005; **18**(9): 938-949.

86. Monteiro F, Genin S, van Dijk I, Valls M. A luminescent reporter evidences active expression of *Ralstonia solanacearum* type III secretion system genes throughout plant infection. *Microbiology* 2012; **158**(Pt 8): 2107-2116.
87. Vasse J, Genin S, Frey P, Boucher C, Brito B. The *hrpB* and *hrpG* regulatory genes of *Ralstonia solanacearum* are required for different stages of the tomato root infection process. *Mol Plant Microbe Interact* 2000; **13**(3): 259-267.
88. Remigi P, Anisimova M, Guidot A, Genin S, Peeters N. Functional diversification of the GALA type III effector family contributes to *Ralstonia solanacearum* adaptation on different plant hosts. *New Phytol* 2011; **192**(4): 976-987.
89. Racape J, Belbahri L, Engelhardt S, Lacombe B, Lee J, Lochman J, Marais A, Nicole M, Nurnberger T, Parlange F, Puverel S, Keller H. Ca²⁺-dependent lipid binding and membrane integration of PopA, a harpin-like elicitor of the hypersensitive response in tobacco. *Molecular microbiology* 2005; **58**(5): 1406-1420.
90. Carney BF, Denny TP. A cloned avirulence gene from *Pseudomonas solanacearum* determines incompatibility on *Nicotiana tabacum* at the host species level. *J Bacteriol* 1990; **172**(9): 4836-4843.
91. Robertson AE, Wechter WP, Denny TP, Fortnum BA, Kluepfel DA. Relationship between avirulence gene (*avrA*) diversity in *Ralstonia solanacearum* and bacterial wilt incidence. *Mol Plant Microbe Interact* 2004; **17**(12): 1376-1384.
92. Macho AP, Guidot A, Barberis P, Beuzon CR, Genin S. A competitive index assay identifies several *Ralstonia solanacearum* type III effector mutant strains with reduced fitness in host plants. *Mol Plant Microbe Interact* 2010; **23**(9): 1197-1205.
93. de Lange O, Schreiber T, Schandry N, Radeck J, Braun KH, Koszinowski J, Heuer H, Strauss A, Lahaye T. Breaking the DNA-binding code of *Ralstonia solanacearum* TAL effectors provides new possibilities to generate plant resistance genes against bacterial wilt disease. *New Phytol* 2013; **199**(3): 773-786.
94. Li L, Atef A, Piatek A, Ali Z, Piatek M, Aouida M, Sharakuu A, Mahjoub A, Wang G, Khan S, Fedoroff NV, Zhu JK, Mahfouz MM. Characterization and DNA-binding specificities of *Ralstonia* TAL-like effectors. *Molecular plant* 2013; **6**(4): 1318-1330.
95. Sole M, Popa C, Mith O, Sohn KH, Jones JD, Deslandes L, Valls M. The *awr* gene family encodes a novel class of *Ralstonia solanacearum* type III effectors displaying virulence and avirulence activities. *Mol Plant Microbe Interact* 2012; **25**(7): 941-953.
96. Chen L, Shiota M, Zhang Y, Kiba A, Hikichi Y, Ohnishi K. Involvement of HLK effectors in *Ralstonia solanacearum* disease development in tomato. *J Gen Plant Pathol* 2014; **80**: 79-84.

97. Angot A, Peeters N, Lechner E, Vailleau F, Baud C, Gentzbittel L, Sartorel E, Genschik P, Boucher C, Genin S. *Ralstonia solanacearum* requires F-box-like domain-containing type III effectors to promote disease on several host plants. *Proc Natl Acad Sci U S A* 2006; **103**(39): 14620-14625.