The *Ptr1* Locus of *Solanum lycopersicoides* Confers Resistance to Race 1 Strains of *Pseudomonas syringae* pv. *tomato* and to *Ralstonia pseudosolanacearum* by Recognizing the Type III Effectors AvrRpt2 and RipBN

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Accepted 17 February 2019.

Race 1 strains of *Pseudomonas syringae* pv. *tomato*, which cause bacterial speck disease of tomato, are becoming increasingly common and no simply inherited genetic resistance to such strains is known. We discovered that a locus in *Solanum lycopersicoides*, termed *Pseudomonas tomato* race 1 (*Ptr1*), confers resistance to race 1 *P. syringae* pv. *tomato* strains by detecting the activity of type III effector AvrRpt2. In *Arabidopsis*, AvrRpt2 degrades the RIN4 protein, thereby activating RPS2-mediated immunity. Using site-directed mutagenesis of AvrRpt2, we found that, like RPS2, activation of *Ptr1* requires AvrRpt2 proteolytic activity. *Ptr1* also detected the activity of AvrRpt2 homologs from diverse bacteria, including one in *Ralstonia pseudosolanacearum*. The genome sequence of *S. lycopersicoides* revealed no RPS2 homolog in the *Ptr1* region. *Ptr1* could play an important role in controlling bacterial speck disease and its future cloning may shed light on an example of convergent evolution for recognition of a widespread type III effector.

Keywords: AvrRpt2, disease resistance, Mr5, NLR-triggered immunity, RIN4, RPS2, *Solanum lycopersicum*, *Solanum lycopersicoides*

Bacterial speck disease of tomato, caused by *Pseudomonas syringae* pv. *tomato*, occurs in cool, wet environments that

favor bacterial spreading and leaf colonization through stomata (Pedley and Martin 2003). Two races of *P. syringae* pv. *tomato* are currently defined, which differ in their ability to cause disease on tomato varieties expressing the resistance gene *Pto*. Race 0 strains express the type III effectors AvrPto or AvrPtoB, are recognized by *Pto*, and, consequently, are unable to cause disease on *Pto*-expressing tomato varieties. Race 1 strains do not have the *avrPto* or *avrPtoB* genes or do not express these effector proteins and are therefore not recognized by *Pto* (Kunkeaw et al. 2010; Lin et al. 2006). Recently, strains with virulence attributes intermediate between race 0 and race 1 strains have been discovered (Kraus et al. 2017). These strains express AvrPto but nevertheless multiply to levels intermediate between race 0 and race 1 strains in tomato plants that express *Pto*.

In order to combat pathogens, plants have evolved a two-layered immune system. In an initial defense response, plants use extracellular pattern recognition receptors to detect the presence of microbe-associated molecular patterns (Dangl et al. 2013). In the second immune response, plants use intracellular proteins (resistance [R] proteins or nucleotide-binding oligomerization domain-like receptors [NLRs]) to detect pathogen effector proteins translocated inside the host cell during the infection process. NLR-triggered immunity (NTI) is typically associated with programmed cell death and significant inhibition of pathogen multiplication (Büttner 2016; Jones and Dangl 2006).

After translocation of AvrPto or AvrPtoB into the plant cell, *Pto*, which is a serine and threonine protein kinase, physically interacts with either one of these effectors and acts in concert with Prf, an NLR, to activate NTI (Pedley and Martin 2003; Salmeron et al. 1996). *Pto* was originally identified in a wild relative of tomato, *Solanum pimpinellifolium*, and it has been introgressed into many processing-type tomato varieties. For over 30 years, the *Pto* gene has provided effective control of speck disease caused by *P. syringae* pv. *tomato* race 0 strains (Pitblado and Kerr 1980). However, the increasing prevalence of race 1 *P. syringae* pv. *tomato* strains able to overcome *Pto*/Prf-mediated NTI has led to the search for sources of resistance to these virulent strains. Since no race 1 resistance has

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Funding: This work was supported by National Science Foundation (NSF) grants IOS-1546625 (to G. B. Martin) and IOS-0923312 (to J. J. Giovannoni), NSF Research Experience for Undergraduates DBI-1358843 (to M. Vachev), Laboratoire d'Excellence (LABEX), TULIP (ANR-10-LABX-41 to N. Peeters and F. Maviane-Macia), and Colciencias Departamento Administrativo de Ciencia, Tecnología e Innovación grant 673 (to C. Mazo-Molina).

*The e-Xtra logo stands for “electronic extra” and indicates that eight supplementary figures and six supplementary tables are published online.

The author(s) declare no conflict of interest.

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been identified in cultivated tomato, wild relatives of tomato are likely to be the best potential source for this trait (Peralta et al. 2008).

Wild relatives of tomato have been screened previously to identify resistance against race 1 *P. syringae* pv. *tomato* strains. One study reported a screen of introgression lines (ILs) derived from *S. habrochaites* LA1777, using the race 1 strain A9 from California (Thapa et al. 2015). The detection of four quantitative trait loci (QTLs), on chromosomes 1, 2, and 12 (two loci), explained the moderate resistance to this strain; however, overall they accounted for a small percentage of the variability observed (10.5 to 12.5% of the phenotypic variation) (Thapa et al. 2015). A second study identified two QTLs, on chromosomes 2 and 8, in *S. habrochaites* LA2109, that contributed to resistance to race 1 strain T1, which accounted for 24 and 26% of the phenotypic variability, respectively (Bao et al. 2015). Recently, another study reported a screen of 96 wild accessions and identified two accessions that display resistance toward race 1 strain T1, *S. neorickii* LA1329 and *S. habrochaites* LA1253. Resistance in LA1253 appears to be a complex genetic trait and its inheritance remains unclear (Hassan et al. 2017). Although together these QTLs might contribute to the breeding of enhanced race 1 *P. syringae* pv. *tomato* resistance in tomato, their quantitative nature and relatively weak race 1 resistance limits their usefulness.

Here, we report the identification of race 1 resistance in a distantly related relative of tomato, *Solanum lycopersicoides*. The resistance is likely due to a single locus, which we refer to as *Pseudomonas tomato* race 1 (*Ptr1*). *Ptr1* confers resistance to several race 1 *P. syringae* pv. *tomato* strains but not to the race 0 strain DC3000. A test of type III effectors that are present

in race 1 strains but lacking in DC3000 identified AvrRpt2 as the effector recognized by *Ptr1*. AvrRpt2 is a cysteine protease that has been intensively studied because it cleaves RIN4, leading to activation of the *Arabidopsis* NLR protein RPS2 (Axtell et al. 2003; Mackey et al. 2003). By using site-directed mutagenesis of AvrRpt2, we discovered that, like RPS2, *Ptr1* detects the proteolytic activities of this effector. *Ptr1* also detects the activity of AvrRpt2 variants expressed by diverse bacterial phytopathogens, and this recognition correlates with ability of the variants to cleave the *Arabidopsis* RIN4 protein. Notably, *Ptr1* conferred strong resistance to a *Ralstonia pseudosolanacearum* strain that expresses an AvrRpt2 homolog (RipBN); no resistance gene that gives protection against this important pathogen, causative agent of bacterial wilt disease, has been reported previously.

RESULTS

A locus on chromosome 4 from *S. lycopersicoides* LA2951 confers resistance to *P. syringae* pv. *tomato* race 1 strains.

During the summer of 2015 in upstate New York, a research plot of 110 *S. lycopersicum* VF36 × *S. lycopersicoides* LA2951 ILs (Canady et al. 2005) became naturally infected by *P. syringae* pv. *tomato*, resulting in severe symptoms of bacterial speck disease. However, two ILs, LA4245 and LA4277, remained essentially free of disease. LA4245 and LA4277 have large overlapping introgressed segments from chromosome 4 of *S. lycopersicoides* (Canady et al. 2005). The introgression in LA4245 is smaller, so we focused on that line for further characterization. In order to determine the race and other characteristics of the *P. syringae* pv. *tomato* strain involved in the outbreak, isolates were collected from the field and were analyzed. The presence of both *avrPto* and *avrPtoB* genes, immunoblot detection of the AvrPto protein, and subsequent inoculation of tomato plants with and without the *Pto* gene indicated the field isolates were race 0 *P. syringae* pv. *tomato* strains (Kraus et al. 2017). One *P. syringae* pv. *tomato* strain, referred to as NY15125, was chosen for further analysis.

The chromosome 4 introgression segment in LA4245 is maintained in a heterozygous condition because homozygotes are very rarely obtained, as noted previously (Canady et al. 2005). We named the putative LA4245 resistance locus *Ptr1* and used a nomenclature in which its presence or absence is denoted as LA4245-R (*Ptr1*/*ptr1*) or LA4245-S (*ptr1*/*ptr1*), respectively. To follow up the field observations, LA4245 plants were inoculated with *P. syringae* pv. *tomato* strains DC3000, NY15125, and T1 in the greenhouse. Pathogen assays showed that DC3000 caused severe symptoms on LA4245-R plants, whereas NY15125 and T1 caused the appearance of few or no specks on LA4245-R, respectively (Fig. 1A). All the *P. syringae* pv. *tomato* strains caused more disease on LA4245-S plants. Consistent with these observations, bacterial population assays showed that T1 attained levels approximately 65-fold lower than DC3000 on LA4245-R plants (Fig. 1B). NY15125 grew to a level intermediate between T1 and DC3000 on LA4245-R. Such intermediate growth was observed previously for NY15125 on *Pto*-expressing plants (Kraus et al. 2017). The three *P. syringae* pv. *tomato* strains all attained similar population levels on LA4245-S plants (Fig. 1B). Therefore, the putative *Ptr1* locus from *S. lycopersicoides* LA2951 confers resistance to *P. syringae* pv. *tomato* strains NY15125 and, notably, T1, which is a race 1 strain.

P. syringae pv. *tomato* resistance in LA4245-R plants is due to recognition of the bacterial effector AvrRpt2.

A comparison of the type III effector genes in DC3000 and T1 identified eight that are present exclusively in T1 (*avrA1*,

Fig. 1. LA4245-R confers resistance to certain strains of *Pseudomonas syringae* pv. *tomato*. **A**, Disease symptoms of LA4245-R and LA4245-S leaves vacuum-infiltrated with strains DC3000, NY15125, and T1 at 3×10^4 CFU ml⁻¹ and photographed 5 days later. **B**, Bacterial populations measured 2 days after inoculation with the *P. syringae* pv. *tomato* strains indicated. Significance was determined by a pair-wise *t* test. An asterisk (*) indicates significant at $P < 0.05$ and n.s. means not significant at $P > 0.05$. Bars indicate the mean of three independent experiments using three plants per strain. Error bars represent $\pm$ standard error of the mean.

avrRpt2, *hopAE1*, *hopAG1*, *hopAII*, *hopAS1*, *hopS1*, and *hopW1*) (Jones et al. 2015) (Supplementary Table S4). To determine whether LA4245-R resistance involves the recognition of any of these effectors, T1-specific effectors were individually cloned into the expression vector pCPP5372, the plasmids were introduced, via electroporation, into DC3000 Δ *avrPto* Δ *avrPtoB*, and the strains were inoculated onto LA4245-R plants. All the strains except the one expressing AvrRpt2 caused disease on LA4245-R plants. Subsequent experiments showed that the DC3000 strain expressing AvrRpt2 reached a population size 80-fold less in leaves of LA4245-R plants compared with LA4245-S plants; a DC3000 strain carrying an empty vector grew to the same level in the two plant lines (Fig. 2A).

Next, we asked whether *P. syringae* pv. *tomato* isolate NY15125, which was collected from the naturally infected tomato field, has the *avrRpt2* gene. Genome sequencing and PCR analysis confirmed the presence of *avrRpt2* in this isolate (Supplementary Fig. S1). A comparison of AvrRpt2 protein sequences from NY15125 and T1 showed they are 100% identical. To investigate the activity of *avrRpt2* in NY15125, we generated a NY15125 Δ *avrRpt2* deletion mutant and tested whether the strain was altered in its ability to grow on LA4245-R or LA4245-S plants. LA4245-R plants inoculated with the NY15125 wild-type strain supported an approximately 60-fold lower bacterial population compared with LA4245-S plants (Fig. 2B). In contrast, this difference was not observed when LA4245-R and LA4245-S plants were inoculated with NY15125 Δ *avrRpt2*. Importantly, complementation of the mutant strain with *avrRpt2* restored the wild-type growth difference in LA4245-R plants (Fig. 2B).

AvrRpt2 was originally identified in *P. syringae* pv. *tomato* JL1065 (Whalen et al. 1991). A comparison between the AvrRpt2 protein sequences from JL1065 and NY15125 showed that the proteins have just two divergent amino acids; proline-24 and alanine-152 in AvrRpt2_{JL1065} are replaced by threonine and glycine, respectively in AvrRpt2_{NY15125}. We therefore investigated if there was any difference in effector recognition on LA4245-R plants when infected with JL1065 wild-type and JL1065 Δ *avrRpt2* strains (Lim and Kunkel 2005). Bacterial population assays indicated that AvrRpt2_{JL1065} is also recognized by LA4245-R (Supplementary Fig. S2). Finally, we tested three additional race 1 *P. syringae* pv. *tomato* strains that all carry the *avrRpt2* gene (NYT1, CA-A9, and CA-407) and found that LA4245-R is resistant to all of them, whereas LA4245-S was susceptible to these strains (Supplementary Fig. S3). Thus, *Ptr1* confers AvrRpt2-mediated resistance to multiple *P. syringae* pv. *tomato* race 1 strains.

The *Ptr1* locus and *RPS2* detect the activity of the same variants of AvrRpt2.

To gain insight into the mechanism of *Ptr1* recognition of AvrRpt2, we performed site-directed mutagenesis to alter amino acids in the effector that have been reported to be essential for its recognition by RPS2 in *Arabidopsis* (Chisholm et al. 2005; Jin et al. 2003; Lim and Kunkel 2004a and b). Ten AvrRpt2 variants were generated, of which eight have been reported to abolish recognition by RPS2 (Axtell et al. 2001, 2003; Jin et al. 2003; Lim and Kunkel 2004b); two variants, F70R, which disrupts the AvrRpt2 autocleavage site, and E150S, were still recognized by RPS2 (Chisholm et al. 2005; Jin et al. 2003). Each AvrRpt2 variant was introduced into DC3000 on a plasmid and shown to be expressed by immunoblotting (Supplementary Fig. S4). The strains were then vacuum-infiltrated into LA4245-R and LA4245-S plants, bacterial populations in leaves were measured, and plants were scored for disease symptoms. We observed that DC3000 expressing AvrRpt2 variants with the substitutions C122A, C122Y, G131D, G141D, G194R, or H208A grew to high

levels and caused severe disease on LA4245-R plants, indicating a loss of *Ptr1* recognition (Fig. 3A and B). In contrast, similar to wild-type AvrRpt2, strains with variants F70R, E150S, Y191C, or D216E reached population levels, on average, approximately 60-fold lower than the empty vector control strain and did not cause any disease symptoms on LA4245-R plants (Fig. 3A and B). All the DC3000 strains caused disease and reached similar population levels in LA4245-S plants (Supplementary Fig. S5).

Interestingly, AvrRpt2 variants with the substitutions Y191C and D216E were recognized by *Ptr1* but have been reported earlier not to be recognized by RPS2 and to be unable to induce *Arabidopsis* RIN4 degradation (Lim and Kunkel 2004a). However, recently it was shown that *Arabidopsis* RIN4 is cleaved by variants AvrRpt2(Y191C) and AvrRpt2(D216E) (Eschen-Lippold et al. 2016). To investigate this discrepancy, *Arabidopsis* Col-0 *RPS2* plants were inoculated with DC3000 expressing AvrRpt2 and several of the variants, including Y191C and D216E, and bacterial populations were measured 3 days later. We observed a significant reduction in bacterial growth and an absence of disease symptoms in Col-0 *RPS2*

Fig. 2. *Pseudomonas syringae* pv. *tomato* race 1 resistance in LA4245-R plants is due to the recognition of the bacterial effector AvrRpt2. **A**, LA4245-R and LA4245-S plants were vacuum-infiltrated with DC3000 carrying *avrRpt2*_{NY15125} or an empty vector (EV) at 5×10^4 CFU ml⁻¹ and bacterial populations were measured 2 days later. **B**, LA4245-R and LA4245-S plants inoculated with NY15125 wild-type, NY15125 Δ *avrRpt2*, a complemented strain, or an empty vector (EV) at 5×10^4 CFU ml⁻¹. Bacterial populations were measured 2 days after inoculation. Significance was determined by a pair-wise *t* test. An asterisk (*) indicates significant at $P < 0.05$ and n.s. means not significant at $P > 0.05$. Bars in both A and B indicate the mean of three independent experiments using three plants per strain. Error bars represent $\pm$ standard error of the mean.

plants inoculated with DC3000 carrying AvrRpt2 and the variants E150S, Y191C, and D216E; the F70R variant appeared to be weakly detected by RPS2 (Supplementary Fig. S6). Thus, *Ptr1* and *RPS2* detect the activity of the same variants of AvrRpt2.

***Ptr1* recognition of the AvrRpt2 variants correlates with the effector's ability to cleave tomato Rin4 proteins.**

In *Arabidopsis*, AvrRpt2-mediated degradation of RIN4 leads to the activation of RPS2 (Mackey et al. 2003). Accordingly, we hypothesized that AvrRpt2 variants that are recognized by *Ptr1* will also be capable of degrading Rin4 in tomato. Tomato has three genes with similarity to *AtRIN4* that are expressed in leaves and two of these are induced during *Pto*-mediated NTI (*SlRin4-1*, *SlRin4-2*, and *SlRin4-3* [Supplementary Table S5]). To avoid the *Ptr1*-mediated defense response, we vacuum-infiltrated LA4245-S plants with the DC3000 strains expressing each AvrRpt2 variant and wild-type AvrRpt2 and detected *SlRin4* abundance by immunoblotting 6 h later.

Wild-type AvrRpt2 and the variants F70R, E150S, Y191S, and D216A each induced a reduction in tomato Rin4 abundance that correlates with *Ptr1* recognition of these proteins in LA4245-R plants (Fig. 4A). DC3000 strains with AvrRpt2 variants C122A, G131D, G141D, G194R, H208A, or an empty vector, all of which caused disease on LA4245-R plants, failed to induce tomato Rin4 elimination (Fig. 4A). Similar experiments in *Arabidopsis* supported our earlier observations in that both AvrRpt2(Y191C) and AvrRpt2(D216E) were able to degrade RIN4 in *rps2* plants (Fig. 4B). Therefore, there is a perfect correlation between the recognition of each AvrRpt2 variant by *Ptr1* and the effector's ability to induce tomato Rin4 disappearance.

AvrRpt2 proteins from different bacterial species are recognized by *Ptr1*.

Homologs of AvrRpt2 are found in diverse bacterial species including the plant pathogens *Erwinia amylovora*,

Fig. 3. *Ptr1* and *RPS2* detect the same variants of AvrRpt2. **A**, Symptoms of LA4245-R plants vacuum-infiltrated with *Pseudomonas syringae* pv. *tomato* DC3000 expressing *avrRpt2* wild type (WT), *avrRpt2* variants, or an empty vector (EV) at 5×10^4 CFU ml⁻¹. Photographs were taken 5 days after inoculation. **B**, Bacterial populations on LA4245-R plants inoculated with DC3000 expressing *avrRpt2* WT, *avrRpt2* variants, and empty vector (EV) were measured 2 days after inoculation. Significance was determined using analysis of variance with a Tukey's post hoc multiple comparison test; different letters indicate significant differences between treatments ($P < 0.001$). Bars indicate the mean of three independent experiments using three plants per strain. Error bars represent $\pm$ standard error of the mean.

R. pseudosolanacearum, *Acidovorax citrulli*, and *Acidovorax avenae*, the soil bacterium *Burkholderia pyrrocinia*, the fungal parasite *Collimonas fungivorans*, and the symbiotic bacteria *Mesorhizobium huakuii* and *Sinorhizobium medicae* (Eschen-Lippold et al. 2016; Zhao et al. 2006). Some of these AvrRpt2 proteins have very divergent amino acid sequences or are truncated in comparison with *P. syringae* pv. *tomato* AvrRpt2 and we wished to determine whether *Ptr1* would recognize them. *Agrobacterium*-mediated expression (agroinfiltration) was used to express each AvrRpt2 protein or a yellow fluorescent protein control in leaves of LA4245-R and LA4245-S. The AvrRpt2 homologs from five of the eight bacterial species induced cell death in LA4245-R leaves but not in LA4245-S leaves, indicating their activity is recognized by *Ptr1* (Fig. 5). Detection of protein expression of each AvrRpt2 homolog was done via agroinfiltration in LA4245-S leaves (Supplementary Fig. S7).

An amino acid substitution in AvrRpt2 that abolishes its recognition by the apple Mr5 NLR resistance protein does not affect recognition by *Ptr1*.

Mr5 is an NLR fire blight resistance protein in apple that recognizes strains of *E. amylovora* that express AvrRpt2 (Fahrenttrapp et al. 2012; Vogt et al. 2013). A single amino acid substitution in AvrRpt2 at position 156 (cysteine to serine) abolishes recognition of the effector by Mr5. In AvrRpt2^{NY15125}, the comparable residue is tyrosine 191. Since a Y191C substitution in AvrRpt2 was shown previously to be recognized by *Ptr1* (Fig. 3), we asked whether a Y191S substitution in AvrRpt2

would abolish *Ptr1* recognition as it does for Mr5 recognition. Inoculation of LA4245-R plants with a DC3000 strain expressing AvrRpt2(Y191S) revealed this variant is recognized by *Ptr1*, as it induced disease resistance and reduced bacterial growth compared with an empty vector DC3000 control strain (Fig. 6A and B). Mr5 and *Ptr1*, therefore, appear to use different mechanisms to detect AvrRpt2 activity.

Ptr1 confers resistance to bacterial wilt disease caused by *R. pseudosolanacearum*.

Agroinfiltration experiments indicated that RipBN, the AvrRpt2 homolog from *R. pseudosolanacearum*, is recognized by *Ptr1* (Fig. 5). The *ripBN* gene is present in a strain of *R. pseudosolanacearum*, CMR15, that was collected from tomato in Cameroon in 2009 (Mahbou Somo Toukam et al. 2009) and was later sequenced (Remenant et al. 2010). Bacterial wilt, caused by *R. pseudosolanacearum*, is a devastating disease for which no NLR-mediated resistance in tomato has been reported (Huet 2014). We therefore soil drench-inoculated LA4245-R and LA4245-S plants with CMR15 and scored the number of plants showing symptoms of bacterial wilt over a 13-day time period. Beginning at 6 days after inoculation, LA4245-S plants started to wilt and, 13 days after inoculation, approximately 70% of the plants were dead (Fig. 7A and B). Remarkably, at this timepoint, there was 100% survival of the LA4245-R plants. *Ptr1*-mediated resistance can therefore be effective against bacterial wilt and might be useful for controlling bacterial wilt disease in tomato-growing areas that have *R. pseudosolanacearum* strains expressing RipBN.

Fig. 4. AvrRpt2 variants F70R, E150S, Y191C, D216E, and AvrRpt2 wild-type, which are recognized by *Ptr1*, are able to degrade tomato Rin4 proteins. **A**, Degradation of the endogenous tomato Rin4 proteins after vacuum infiltration of LA4245-S leaves with *Pseudomonas syringae* pv. *tomato* DC3000 strains carrying *avrRpt2* wild type (WT), *avrRpt2* variants, or an empty vector (EV). Plant tissue was harvested 0 and 6 h postinfiltration (hpi). Total protein extracted from infiltrated plants was subjected to immunoblotting using a α -RIN4 antibody. Locations of tomato Rin4-1, Rin4-2, and Rin4-3 are shown. Asterisks indicate an unknown cross-reacting protein. **B**, *Arabidopsis* Col-0 *rps2* leaves were syringe-infiltrated with DC3000 carrying *avrRpt2* wild type (WT), *avrRpt2* variants, or an EV. Leaf tissue was harvested 4 hpi. Total protein extracted from infiltrated leaves was subjected to immunoblotting using a α -RIN4 antibody. Ponceau staining shows the amount of protein loaded in each lane.

No RPS2, Mr5, or RIN4 orthologs are present in the *S. lycopersicoides* chromosome 4 introgression segments of LA4245-R.

To initiate the map-based cloning of *Ptr1*, we generated sequence data, at 14x coverage of the Heinz 1706 reference genome from genomic DNA of LA4245-R, using an Illumina HiSeq2000. The reads were mapped to the *S. lycopersicum* Heinz 1706 genome sequence to identify areas of high sequence polymorphism. This analysis revealed that LA4245-R contains two *S. lycopersicoides* introgression segments on chromosome 4 in the background of the tomato parent VF36. One small segment lies within coordinates 1 to 260,000 bp (260 kb) and the other lies between coordinates 4,480,000 and 62,030,000 bp (approximately 57.5 Mb) (Supplementary Fig. S8A). A high-quality genome sequence of *S. lycopersicoides* LA2951, a parent of the ILs, has been generated recently (discussed below). Synteny between Heinz 1706 and LA2951 was determined, and gene models predicted to be in the introgressed regions of LA4245-R were identified in the *S. lycopersicoides* annotation. *Ptr1* recognition of AvrRpt2 suggests it likely encodes an NLR protein or possibly a guard gene such as Rin4. No NLR-encoding genes are annotated within the small introgression segment and just 14 NLR genes are

annotated within the large *S. lycopersicoides* introgression segment. Interestingly, none of these 14 genes encode a predicted protein with similarity to RPS2 or Mr5. In fact, the *S. lycopersicoides* genes with predicted proteins having the highest similarity to RPS2 and Mr5 are all located on other chromosomes (Supplementary Table S6). None of the four *Rin4* genes in tomato are located on chromosome 4.

DISCUSSION

From a serendipitous observation during a natural occurrence of bacterial speck disease, we identified and further characterized two *S. lycopersicoides* ILs that have strong resistance to multiple race 1 strains of *P. syringae* pv. *tomato*. Such resistance is important, since race 1 *P. syringae* pv. *tomato* strains are becoming increasingly common throughout the world and yet no simply inherited genetic resistance to these strains is known. The two ILs contain a large overlapping introgression segment from *S. lycopersicoides* chromosome 4, which carries a putative locus that we refer to as *Ptr1*, which recognizes the effector AvrRpt2. This effector is present in *P. syringae* pv. *tomato* strains collected from diverse tomato-growing regions including all race 1 *P. syringae* pv. *tomato* strains for which a

Fig. 5. AvrRpt2 homologs from diverse bacteria are recognized by *Ptr1*. Agroinfiltration of each *avrRpt2* homolog (Aa, *Acidovorax avenae*; Ac, *Acidovorax citrulli*; Bp, *Burkholderia pyrrocinia*; Cf, *Collimonas fungivorans*; Ea, *Erwinia amylovora*; Mh, *Mezorhizobium huakuii*; Rs, *Ralstonia pseudosolanacearum*; Sm, *Sinorhizobium medicae*; Pst, *Pseudomonas syringae* pv. *tomato*) and a control (yellow fluorescent protein [YFP]) into LA4245-R and LA4245-S leaves. Detached leaves were cleared with ethanol to better visualize cell death associated with AvrRpt2 recognition. Photographs were taken 4 days after infiltration. Shown are the number of times cell death was observed over the total number of agroinfiltrations performed.

genome sequence is available (T1, K40, NY-T1, CA-A9, CA-407) and some race 0 *P. syringae* pv. *tomato* strains (JL1065, NY15125). The *Ptr1* locus, if combined with *Pto* in the same tomato variety, has the potential to become an important component of bacterial speck disease control. Here, we compare the activity of *Ptr1* to *Arabidopsis* *RPS2* and apple *Mr5*, two genes that also detect AvrRpt2, discuss the potential utility of *Ptr1* and approaches to identifying the *Ptr1* gene, and we propose the definition of additional *P. syringae* pv. *tomato* races to account for the fact that two NTI loci are now known to confer resistance to bacterial speck disease.

Cleavage of Rin4 by AvrRpt2 in *Arabidopsis* leads to activation of RPS2 and resistance to *P. syringae* pv. *tomato*, although the specific mechanism by which Rin4 degradation activates RPS2 is unknown (Day et al. 2005; Toruño et al. 2019). Our analysis of AvrRpt2 variants revealed a perfect correlation between the ability of a variant to degrade tomato

Rin4 proteins and its recognition by *Ptr1* and *RPS2*; AvrRpt2 variants that do not degrade Rin4 are not recognized by *Ptr1* or *RPS2*. It is possible, therefore, that the *Ptr1* and *RPS2* proteins are activated via the same mechanism subsequent to Rin4 degradation, although the possibility that a mechanism specific to tomato or *Arabidopsis* exists cannot currently be ruled out. The apple *Mr5* protein detects activity of AvrRpt2 from *E. amylovora*. The AvrRpt2_{Ea} effector does not induce *Arabidopsis* Rin4 degradation when the proteins are transiently co-expressed in *N. benthamiana*, although it is possible that this method masks moderate degradation; whether Rin4 degradation in apple is correlated with *Mr5* activation has not been reported (Vogt et al. 2013). Interestingly, an AvrRpt2_{Ea} variant that is not detected by *Mr5* is detected by *Ptr1* and is able to degrade *Arabidopsis* RIN4, suggesting that the recognition mechanism of these two proteins is different.

Using an anti-AtRin4 antibody, we observed that all three tomato Rin4-like proteins that are expressed in leaves were degraded in the presence of AvrRpt2. Soybean also has four Rin4 proteins, and virus-induced gene silencing experiments showed that just two of them, GmRIN4a and GmRIN4b, play a role in the hypersensitive response induced by AvrB and the NLR protein Rpg1b (Selote and Kachroo 2010). A subsequent study showed that overexpression of any one of the four soybean RIN4s with Rpg1b/AvrB in leaves of *Nicotiana glutinosa* caused a hypersensitive response; the authors concluded that the expression level differences in these two studies might account for these different observations (Kessens et al. 2014).

Fig. 6. *Pseudomonas syringae* pv. *tomato* AvrRpt2(Y191S) variant, analog of the *Erwinia amylovora* AvrRpt2(C156S) virulent variant is recognized by *Ptr1*. **A**, Disease symptoms of LA4245-R and LA4245-S plants 4 days after inoculation with DC3000 expressing *avrRpt2*(Y191S), *avrRpt2* wild type (WT), or an empty vector (EV) at 5×10^4 CFU ml⁻¹. **B**, Bacterial populations were measured in LA4245-R plants 2 days after inoculation. Significance was determined using analysis of variance with a Tukey's post hoc multiple comparison test, and different letters indicate significant differences between treatments ($P < 0.001$). Bars indicate the mean of three plants and error bars represent $\pm$ standard deviation. Results shown are representative of three independent experiments.

Fig. 7. *Ptr1* confers resistance to *Ralstonia pseudosolanacearum* CMR15 expressing AvrRpt2 homolog RipBN. **A**, LA4245-R plants ($n = 25$) and LA4245-S plants ($n = 30$) were soil drench-inoculated with 50 ml of 10^8 CFU ml⁻¹ *Ralstonia pseudosolanacearum* CMR15. The percentage of plants surviving (showing no wilt symptoms) or dying (showing severe wilting) 1 to 13 days postinoculation (DPI) is shown. The two survival curves are significantly different at $P < 0.0001$, using the Mantel-Cox test. **B**, Photographs taken 13 days after inoculation show extreme wilting of a LA4245-S plant (right) and no wilting of a LA4245-R plant (left). Results shown are representative of three independent experiments.

Apple contains two Rin4-like genes although, as noted above, any specific roles they might have in activation of Mr5 have not yet been reported (Fahrenttrapp et al. 2012; Vogt et al. 2013). In the future, we will investigate the requirement of the three tomato Rin4 proteins for activation of Ptr1, using both CRISPR-generated mutations in the corresponding genes and, once the *Ptr1* gene is identified, transient coexpression in *N. benthamiana*.

We found that *Ptr1* is able to recognize AvrRpt2 homologs from a diverse array of different bacteria, including several plant pathogens. Each of the AvrRpt2 proteins recognized by *Ptr1* had been previously shown to also induce AtRin4 degradation in *Arabidopsis*, although their ability to activate RPS2-mediated resistance was not reported (Eschen-Lippold et al. 2016). If *avrRpt2* homologs are widespread in field isolates of *Acidovorax citrulli* and *E. amylovora*, then our results suggest that *Ptr1* might be useful if expressed transgenically in cucurbits to control bacterial fruit blotch caused by *Acidovorax citrulli* and in apple or pear to confer resistance to fire blight caused by *E. amylovora*. Despite extensive screening of tomato germplasm, no single *R* gene has been identified that confers resistance to bacterial wilt disease caused by *R. pseudosolanacearum* (Huet 2014). We found that *Ptr1* was remarkably effective in preventing symptoms of bacterial wilt, in a growth chamber assay, using strain CMR15. Unfortunately, CMR15 seems to be a rare example of a *R. pseudosolanacearum* strain that expresses an AvrRpt2 homolog (RipBN) (Peeters et al. 2013) (LIPM Ralso T3E database) and *Ptr1* will, therefore, likely not be of broad utility for controlling bacterial wilt disease. Nevertheless, further study of interaction between CMR15 and LA4245-R might provide some useful insights into the molecular basis of the NTI response against *R. pseudosolanacearum* that is induced by *Ptr1*.

Considering that *Ptr1* plays a role in detecting the type III effector AvrRpt2, it seems most likely that it encodes an NLR, although it could encode a decoy or guardee protein that is monitored by an NLR. In either case, one of these genes must be located in the introgression segments, although the other one may or may not lie in the introgressions. Examples of each possibility are known; the *Pto* (decoy) and *Prf* (NLR) genes are located in a 20-kb region of chromosome 5, whereas the *RIN4* (guardee) and *RPS2* (NLR) genes are located on a different chromosome in *Arabidopsis*. In the Heinz 1706 tomato reference genome sequence, chromosome 4 has 56 NLR-encoding genes, the most of any chromosome; 43 of these genes are clustered tightly within a 100-kb region, with the other 13 located throughout the rest of the chromosome. *S. lycopersicoides* has 66 NLR-encoding genes on chromosome 4 with a similar distribution as seen in Heinz 1706—58 are tightly clustered at one end and eight are distributed along the chromosome. One boundary of the large introgression segment occurs in the middle of the large NLR cluster, which potentially eliminates as *Ptr1* candidates 51 NLR-encoding genes. Each of the remaining 14 NLR genes is a candidate for *Ptr1*. None of these candidates encode

proteins with obvious similarity to RPS2 or Mr5, and, if one of these is *Ptr1*, it will be just the third known example of convergent evolution in different plant species for recognition of the same effector (Ashfield et al. 2004; Carter et al. 2019). The obvious decoy/guardee proteins that might be involved with AvrRpt2 recognition in tomato are the three Rin4-like proteins expressed in leaves. None of these genes are located on chromosome 4 of tomato or *S. lycopersicoides*. Another, perhaps less likely, scenario that should be considered is that a gene in the introgression segment encodes a novel host protein that acts with Rin4 to activate an NLR located on another chromosome in VF36. All these possibilities will be investigated in the future as we seek to identify the genes involved in AvrRpt2 recognition and understand the associated molecular mechanisms.

Although LA4245-R plants are morphologically very similar to VF36, the IL line has two introgression segments on chromosome 4, including one large (about 57.5 Mb) segment encompassing the majority of the 66.56-Mb chromosome; however, this IL line cannot be maintained in a homozygous condition. If *Ptr1* is to be useful for control of speck disease, it will need to be introgressed into other tomato breeding lines and it will be necessary to reduce the size of the introgressed segment in order to reduce ‘linkage drag’ (i.e., deleterious alleles). However, recombination is often severely suppressed in plants carrying chromosomal regions from a wild relative of tomato, and *S. lycopersicoides* is particularly distant from cultivated tomato (Grandillo et al. 2011). One approach that might be used in this case is to cross LA4245-R with a species that is phylogenetically intermediate between tomato and *S. lycopersicoides*. A good candidate for such a ‘bridge’ species is *S. pennellii* which has been shown previously to be useful in increasing recombination frequency in *S. lycopersicoides* introgression regions (Canady et al. 2006). Importantly, *Ptr1* and *Pto* are located on different chromosomes (4 and 5, respectively), which will facilitate introgressing them both into advanced breeding lines.

The identification of a second *R* gene that confers resistance to bacterial speck disease presents the opportunity to extend the currently defined race structure of *P. syringae* pv. *tomato*. The two current races were defined based on the *Pto* gene in which race 0 strains express either or both of the type III effectors AvrPto and AvrPtoB, and race 1 strains evade *Pto* detection by either losing or mutating these effector genes or, in the case of AvrPtoB, suppressing its protein accumulation (Kunkeaw et al. 2010; Lin et al. 2006; Pedley and Martin 2003). Based on the discovery of *Ptr1* and focusing on *P. syringae* pv. *tomato* strains related to T1, we propose that race 0 strains might be considered the original state of the pathogen and refer to those strains that express *avrPto* or *avrPtoB* along with *avrRpt2* (Table 1). Race 1 can then refer to strains that express *avrRpt2* but lack *avrPto* or *avrPtoB*. A newly defined race 2 would refer to strains that have *avrPto* or *avrPtoB* but lack *avrRpt2*. Finally, a hypothetical strain that

Table 1. Proposed races for *Pseudomonas syringae* pv. *tomato* based on the *Pto* and *Ptr1* genes^a

Race	Example	References	Relevant effectors	R gene present		
				<i>Pto</i>	<i>Ptr1</i>	None
0	JL1065 NY15125	Whalen et al. 1991; Kraus et al. 2017	Either AvrPto, AvrPtoB or both, AvrRpt2	R	R	S
1	T1, NYT1, CA-A9, CA-407 ^b	Almeida et al. 2009; Jones et al. 2015; Kunkeaw et al. 2010	AvrRpt2	S	R	S
2	Max13	Yan et al. 2008	Either AvrPto, AvrPtoB or both	R	S	S
3	Unknown		No AvrPto, AvrPtoB, AvrRpt2	S	S	S

^a Resistance and susceptibility are denoted as R and S, respectively.

^b Race 1 strains T1, NYT1, CA-A9, and CA-407 are positive for the presence of the *avrPtoB* gene, but AvrPtoB protein does not accumulate for unknown reasons.

lacks all three of these effectors would be defined as race 3. Examples of race 0, race 1, and race 2 *P. syringae* pv. *tomato* strains along with the ability of one or both *Ptr1* and *Pto* to recognize them are provided in Table 1.

In summary, *Ptr1* has the potential to become an important component (along with *Pto*) for the control of bacterial speck disease in tomato. In addition, the eventual cloning and characterization of the gene will allow its molecular characterization and, in particular, might shed light on whether it uses the same or a different mechanism than used by RPS2 and Mr5 to respond to AvrRpt2-mediated degradation of Rin4.

MATERIALS AND METHODS

Bacterial strains and plasmids.

Pseudomonas syringae pv. *tomato* strains DC3000 (Buell et al. 2003), NY15125 (Kraus et al. 2017), T1 (Almeida et al. 2009), JL1065 (Whalen et al. 1991), NYT1 (Jones et al. 2015), CA-A9, and CA-407 (Kunkeaw et al. 2010) were grown on King's B (KB) (King et al. 1954) semiselective media at 30°C (Supplementary Table S1). *Ralstonia pseudosolanacearum* CMR15 (Remenant et al. 2010) was grown on rich B medium (27.7 mM glucose, 10 g of bacto-peptone per liter, 1 g of yeast extract per liter, 1 g of casamino acids per liter). pCPP5372 plasmids (Oh et al. 2007) carrying wild-type *avrRpt2*_{NY15125}, *avrRpt2* variants, or the empty vector were introduced into DC3000 by electroporation (Supplementary Tables S2 and S3). All *P. syringae* pv. *tomato* strains were stored in 20% glycerol + 60 mM sucrose at -80°C. *Escherichia coli* TOP10 was used for plasmid maintenance and were grown in Luria-Bertani (LB) medium at 37°C.

Plant material.

Solanum lycopersicoides IL seeds of LA4245 and LA4277 were obtained from the Tomato Genetics Resource Center. The genotype of LA4245 can be heterozygous for the presence of *Ptr1* (*Ptr1/ptr1*) (LA4245-R) or homozygous for the lack of the gene (*ptr1/ptr1*) (LA4245-S). *S. lycopersicoides* ILs were grown in a greenhouse at 24°C during daylight and 22°C at night. *Nicotiana benthamiana* Nb-1 (Bombarely et al. 2012) was maintained in a growth chamber with 16 h of light at 24°C and 8 h of dark at 20°C and 50% humidity. Tomatoes and *N. benthamiana* plants were grown in Cornell Osmocote Mix soil (0.16 m³ peat moss, 0.34 m³ vermiculite, 2.27 kg of lime, 2.27 kg of Osmocote Plus15-9-12, and 0.54 kg of Uni-Mix 11-5-11 [Everris, Israeli Chemicals Ltd.]). After pathogen inoculation, plants were moved to a growth chamber with 25°C, 50% humidity, and 16 h of light. *Arabidopsis thaliana* accession Columbia (Col-0) seeds were ethanol-sterilized, were suspended in 1 ml of water, and were cold stratified for 2 days at 4°C. *A. thaliana* was grown in Fafard Mix (Sungro Horticulture) in a growth chamber under fluorescent lighting (100 μmol m⁻² s⁻¹) with a 12-h light and 12-h dark cycle at 21°C and 40% humidity.

Genome sequencing, assembly, and type III effector annotation of NY15125.

The NY15125 genome was sequenced to 163× coverage with long reads from the PacBio RSII platform. A Canu assembly was performed with a stringent error rate (corrected error rate = 0.035) (Koren et al. 2017). Illumina sequencing was also done to generate paired-end reads for a coverage of 114×. Adapter clipping and quality filtering of the Illumina reads was done with Trimmomatic (Bolger et al. 2014). Concordant read-mapping of the Illumina paired-end reads was used to evaluate the quality of the assembly. Two rounds of base-level error corrections were done with PacBio reads using Arrow

(available online), followed by two rounds of error correction with Illumina reads using Pilon (Walker et al. 2014). The polished assembly includes a 6.2 Mb chromosome and three putative plasmids (88, 116, and 122 kb). It was annotated with Prokka (Seemann 2014), using proteins from the T1 genome (Almeida et al. 2009) as supporting evidence. The NY15125 chromosome was compared with the DC3000 and T1 genomes using BRIGG (Alikhan et al. 2011). Pseudomolecules were constructed from the deposited contigs for *P. syringae* pv. *tomato* NY15125 and were annotated using MG-RAST (Meyer et al. 2008). Effector genes were identified from the MG-RAST annotation, by alignment with other *P. syringae* pv. *tomato* sequences, and based on proximity to HrpL binding sites, predicted using the methods described previously (Saha and Lindeberg 2013). The NY15125 genome sequence and plasmid sequences are available from GenBank (accession numbers CP034558 to CP034561).

Development of a *P. syringae* pv. *tomato* NY15125Δ*avrRpt2* strain.

A 1,024-bp promoter fragment and an 841-bp fragment downstream of the *avrRpt2* gene sequence were PCR-amplified and *EcoRI* or *XmaI* restriction sites were added, respectively. Fusion of both DNA fragments was cloned into the suicide vector pK18mobsacB and was transformed into *Escherichia coli* S17-1. Deletion of *avrRpt2* in NY15125 was performed by biparental mating, as described previously (Kraus et al. 2017) with modifications (Kvitko and Collmer 2011).

P. syringae pv. *tomato* inoculation and population assays in tomato.

P. syringae pv. *tomato* strains were grown on KB plates for 2 days at 30°C. Strains were diluted in 10 mM MgCl₂ + 0.002% Silwet L-77 at a final concentration of 5 × 10⁴ CFU ml⁻¹. Four-week-old plants were vacuum-infiltrated, and three leaf disk samples (7 mm in diameter) were collected at 2 h (day 0) and 2 days postinoculation (dpi) to quantify bacterial populations. The experiments were repeated three times. Results shown are the mean of three independent experiments using three biological replicates per strain, including standard error of the mean. Photographs for each technical replicate were taken 7 dpi. Statistical analyses were performed using Prism 6.0 (GraphPad software).

P. syringae pv. *tomato* inoculation and population assays in *A. thaliana*.

Five-week-old plants were dip-inoculated for 20 s in a bacterial suspension (1 × 10⁸ CFU ml⁻¹ of *P. syringae* pv. *tomato*) containing 10 mM MgCl₂ + 0.02% Silwet L-77. Bacterial populations were measured 3 dpi by submerging the aerial plant tissue in 10 mM MgCl₂ + 0.2% Silwet L-77 for 2 h at 28°C. The bathing solution was serially diluted and plated (Tornero and Dangl 2002). Experiments were repeated three times. Results shown are the mean of three independent experiments using three biological replicates per strain, including standard error of the mean. Statistical analyses were performed using Prism 6.0 (GraphPad software).

Immunodetection of AvrRpt2 proteins in *P. syringae* pv. *tomato*.

Strains of *P. syringae* pv. *tomato* grown on KB plates for 2 days at 30°C were resuspended in *hrp*-inducing liquid minimal media [50 mM KH₂PO₄, 7.6 mM (NH₄)₂SO₄, 1.7 mM NaCl, 1.7 mM MgCl₂, 10 mM fructose, pH 5.7] or KB liquid media containing the appropriate antibiotics at an optical density at 600 nm (OD₆₀₀) of 0.4 and 0.1, respectively. Bacterial cultures were grown at 28°C for 16 h of shaking at

220 rpm, and the OD₆₀₀ was adjusted to a final concentration of 0.5. Bacterial culture (1 ml of each) was washed with water and was centrifuged. Bacterial pellets were resuspended in 100 µl of Laemmli buffer (20 mM Tris, 1% sodium dodecyl sulfate [SDS], 0.05% bromophenol blue, and 10% glycerol, pH 6.8), were boiled for 5 min, and 5 µl of each was used for immunoblot analysis. To detect AvrRpt2 proteins, membranes were probed with α -hemagglutinin antibody (Roche) conjugated with horseradish peroxidase (HRP).

***Agrobacterium*-mediated transient protein expression in leaves.**

Agrobacterium tumefaciens 1D1249 (Wroblewski et al. 2005) harboring the various expression vectors was grown on LB media with the appropriate antibiotics for 2 days at 30°C. Bacteria were scraped from the plate, were resuspended in infiltration buffer (10 mM MgCl₂, 10 mM morpholineethane sulfonic acid [pH 5.6], and 200 mM acetosyringone), and were maintained for 4 h in the dark at room temperature on a nutator rocker. Bacterial cultures were then washed and centrifuged, and the pellet was resuspended in fresh infiltration buffer before diluting cultures at a final OD₆₀₀ of 0.15. Tomato or *N. benthamiana* leaves were infiltrated using a needle-less syringe and were placed in a growth chamber (24°C day and 22°C night). Leaf samples for protein expression were taken 32 h later.

Immunoblot detection of plant-expressed proteins.

Protein samples were analyzed by grinding three leaf disks (9 mm in diameter) in protein sample buffer (50 mM Tris HCl [pH 7.5], 10% glycerol, 2% SDS, 2 mM EDTA, 1 mM dithiothreitol, and 1% protease inhibitor [Sigma-Aldrich]). Samples were separated by SDS-polyacrylamide gel electrophoresis on 4 to 20% gradient polyacrylamide gels and were transferred to Immobilon-P polyvinylidene difluoride membranes (Millipore), according to standard procedures (Taylor 2015). To detect AvrRpt2 proteins, membranes were probed with α -c-Myc (GeneScript) antibody conjugated with HRP. For tomato Rin4 detection, AtRin4 polyclonal antiserum (gift from G. Coaker, University of California Davis) was used at a concentration of 1:2,000. Secondary goat antirabbit immunoglobulin G conjugated with HRP was used at a dilution of 1:10,000 (Promega).

***R. pseudosolanacearum* disease assays.**

For survival assays with *R. pseudosolanacearum* CMR15, 4-week-old tomato plants (grown in peat pots) were transferred in potting mixture in 3-liter pots to the Toulouse Plant-Microbe Phenotyping facility (28°C, 16 h of light). Each tomato plant was soil drench-inoculated with 50 ml of 10⁸ CFU per milliliter. Disease scoring was performed daily, using a visual index in which the numbers 1, 2, 3, and 4 corresponded to 25, 50, 75, and 100% wilted leaves, respectively. Disease scores were transformed into binary data for the purpose of statistical comparison between disease curves (Remigi et al. 2011).

Tomato genome sequencing.

Genomic DNA was extracted from a single LA4245-R plant using a DNeasy plant mini kit (Qiagen). DNA was mechanically sheared, using the Covaris S2 adaptative focused acoustic disruptor (Covaris, Inc.), to an average size of 500 to 600 bp and was used to prepare a library. Single-end 100-bp DNA reads were sequenced using the Illumina HiSeq 2000 platform. The reads from LA4245-R were mapped to the *S. lycopersicum* Heinz 1706 genome sequence SL2.50 (Tomato Genome Consortium 2012) using hisat2 version 2.1.0 (Kim et al. 2015), and

single nucleotide polymorphisms (SNPs) were called using GATK version 4.0. (McKenna et al. 2010). SNPs were plotted in 10-kb bins using R. Synteny between SL2.50 and LA2951 version 0.6 was determined using SynMap at CoGe. Protein similarity for RPS2 and MR5 were calculated with Geneious R1. The genome sequence of LA4245 is available at the National Center for Biotechnology Information Sequence Read Archive under BioProject ID PRJNA516877. The genome sequence of LA2951 is available in the Sol Genomics Network database.

ACKNOWLEDGMENTS

We thank R. Roberts and A. Collmer for helpful comments on the manuscript, J. Lee for the AvrRpt2 homologs, B. Kunkel for JL1065 strains, G. Coaker for anti-RIN4 antibodies, D. Klessig for *A. thaliana* *rps2* seeds, K. Munkvold and F. Meng for generating DC3000 strains with T1 effectors, and R. Chetelat and the Tomato Genetics Resource Center for seeds of LA4245. The *Ralstonia* assays were performed at the Toulouse Plant-Microbe Phenotyping facility of the LIPM-UMR (INRA441/CNRS2594).

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AUTHOR-RECOMMENDED INTERNET RESOURCES

CoGe database: <https://genomevolution.org/coge/SynMap.pl>
 Geneious R11: <https://www.geneious.com>
 LIPM Ralsto T3E database:
<https://iant.toulouse.inra.fr/bacteria/annotation/site/prj/T3Ev3>
 PacBio Arrow software: <https://github.com/PacificBiosciences/pbbioconda>
 Sol Genomics Network:
https://solgenomics.net/organism/Solanum_lycopersicoides/genome
 Tomato Genetics Resource Center:
https://tgrc.ucdavis.edu/lycopersicoides_ils.aspx