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ORIGINAL PAPER

Identification of QTLs controlling resistance to *Pseudomonas syringae* pv. *tomato* race 1 strains from the wild tomato, *Solanum habrochaites* LA1777

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Abstract
Key message **Screening of wild tomato accessions revealed a source of resistance to *Pseudomonas syringe* pv. *tomato* race 1 from *Solanum habrochaites* and facilitated mapping of QTLs controlling disease resistance.**
Abstract *Pseudomonas syringae* pv. *tomato* (*Pst*) causes bacterial speck of tomato, which is one of the most persistent bacterial diseases in tomato worldwide. Existing *Pst* populations have overcome genetic resistance mediated by the tomato genes *Pto* and *Prf*. The objective of this study was to identify sources of resistance to race 1 strains and map quantitative trait loci (QTLs) controlling resistance in the wild tomato *Solanum habrochaites* LA1777. *Pst* strains A9 and 407 are closely related to current field strains and genome sequencing revealed the lack of the *avrPto* effector as well as select mutations in the *avrPtoB* effector, which are recognized by *Pto* and *Prf*. Strains A9 and 407 were used to screen 278 tomato accessions, identifying five exhibiting resistance: *S. peruvianum* LA3799, *S. peruvianum* var. *dentatum* PI128655, *S. chilense* LA2765, *S. habrochaites* LA2869, and *S. habrochaites* LA1777. An existing set of 93 introgression lines developed from *S. habrochaites* LA1777 was screened for resistance to strain A9 in a replicated greenhouse trial. Four QTLs were identified using composite interval mapping and mapped to different chromosomes. *bsRr1-1* was located on chromosome 1, *bsRr1-2* on chromosome 2, and *bsRr1-12a* and *bsRr1-12b* on chromosome 12. The QTLs detected explained 10.5–12.5 % of the phenotypic variation. Promising lines were also subjected to bacterial growth curves to verify resistance and were analyzed for general horticultural attributes under greenhouse conditions. These findings will provide useful information for future high-resolution mapping of each QTL and integration into marker-assisted breeding programs.

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Introduction

Bacterial speck of tomato is caused by *Pseudomonas syringae* pv. *tomato* (*Pst*), which proliferates mainly on leaves and fruits and can cause yield losses in both field and greenhouse grown tomatoes. Bacterial speck disease develops primarily in cool, moist conditions and is a persistent problem throughout the world on processing tomatoes (Goode and Sasser 1980). *Pst* enters the leaf interior via stomata or wounds where it multiplies and can form necrotic lesions on both fruits and leaves (Yunis et al. 1980). There are two races of *Pst:* 0 and 1 (Bogatsevska et al. 1989; Lawton and MacNeill 1986). Genetic studies have identified dominant genes controlling resistance to bacterial speck, named *Pto-1* through *Pto-4* (Pilowsky MaZ 1982; Pitblado and Kerr 1979; Stockinger and Walling 1994). *Pto-1,* commonly referred to as *Pto*, was identified from *S. pimpinellifolium* and rapidly introgressed into processing tomato cultivars where it provided effective resistance against *Pst* race 0 strains for two decades (Pedley and Martin 2003; Pitblado and Kerr 1979, 1980).

In 1993, the tomato *Pto* gene was cloned which encodes a protein kinase that works in concert with *Prf* (Martin et al. 1993). Prf possesses a central nucleotide binding site and C-terminal leucine-rich repeats, which are found in many plant resistance (R) genes. Pto and Prf specifically recognize two bacterial effectors from *Pst* race 0, called AvrPto and AvrPtoB (Martin et al. 1993; Pedley and Martin 2003). Deleting either *avrPto* or *avrPtoB* from the race 0 strain *Pst* DC3000 results in a subtle decrease in virulence, while deleting both genes significantly reduces bacterial virulence (Lin and Martin 2005; Martin et al. 1993). Furthermore, diverse *avrPtoB* homologs are recognized by *Pto* in tomato, which could significantly impact the host range of different *P. syringae* pathovars (Lin et al. 2006). The recognition of two different bacterial effectors that significantly impact pathogen virulence is the likely explanation for why *Pto*-mediated resistance remained effective over multiple years and locations.

Despite the historical success of *Pto*-mediated resistance, race 1 strains now predominate, rendering *Pto*-mediated resistance ineffective (Cai et al. 2011). *Pst* race 1 was first detected in 1986 in Canada and in 1993 in California, the primary production area for processing tomato cultivars in the US (Arredondo and Davis 2000; Lawton and MacNeill 1986). In California, the vast majority of *Pst* strains isolated from infected field samples between 2005 and 2007 were race 1 (Kunkeaw et al. 2010). Since 2007, all strains isolated from California field samples were race 1 (Kunkeaw et al. 2010) (Coaker, personal communication). Furthermore, the majority of *Pst* race 1 strains represent a common lineage using multi-locus sequence typing and whole-genome derived single-nucleotide polymorphisms (Cai et al. 2011; Kunkeaw et al. 2010). Bacterial speck of tomato can be an important disease in California and New York due to a combination of favorable weather promoting disease development, emergence of race shifting strains, and moderate to high levels of copper resistance. At present, there is a lack of tomato cultivars with resistance to *Pst* race 1 available to tomato growers. Given the close genetic relationship between race 1 strains, identification and deployment of genetic resistance are desirable control strategies.

The identification of sources of resistance to *Pst* race 1 coupled with identification of Quantitative Trait Loci (QTLs) will facilitate breeding for resistance to bacterial speck. Wild tomato species are a rich source of genetic diversity. Resistance to multiple diseases has been identified in wild tomatoes and subsequently introgressed into cultivated tomato (Ji and Chetelat 2007). At least five introgression line (IL) populations have been generated following a cross to a wild tomato species, including ILs derived from: *S. pennellii* LA716 (Eshed and Zamir 1994), S. *habrochaites* LA1777 (Monforte and Tanksley 2000), *S. habrochaites* LA407 (Francis et al. 2001), *S. habrochaites* LYC4 (Finkers et al. 2007) and *S. lycopersicoides* LA2951 (Canady et al. 2005). Populations of ILs that contain only a fragment of the wild donor genome are useful for the discovery of disease resistance QTLs (Li et al. 2011; Smart et al. 2007). These ILs have been used to identify QTLs affecting yield as well as other desirable attributes such as carotenoid production and accumulation of primary metabolites (Fridman et al. 2004; Schauer et al. 2006). *S. pennellii* LA716-derived ILs have been extensively phenotyped and genotyped to identify QTLs controlling various diseases (Astua-Monge et al. 2005), fruit quality (Rousseaux et al. 2005; Smart et al. 2007) and yield (Eshed et al. 1996). Recently, resistance against late blight caused by the oomycete pathogen *Phytophthora infestans* and gray mold caused by fungus *Botrytis cinerea* has been detected in LA1777 ILs (Gabor et al. 2010; Li et al. 2011).

The objectives of this study were to: (1) to screen a wide variety of wild tomato lines to identify those exhibiting resistance against *Pst* race 1 and (2) to identify QTLs controlling resistance to *Pst* race 1 in ILs derived from *S. habrochaites* LA1777. These findings will be useful for breeding programs seeking to incorporate resistance to current strains of *Pst* present in the field.

Materials and methods

Bacterial strains and genome sequencing

Two *P. syringae* pv. *tomato* (Pst) race 1 isolates (A9 and 407) collected from diseased plants from commercial fields in the Central Valley of California were used to assess resistance in wild tomato cultivars (Kunkeaw et al. 2010). Isolate A9 was used for screening of resistance in tomato introgression lines. All bacteria were cultured on nutrient yeast glycerol (NYG) media. For plant inoculations, Pst strains A9, 407, and DC3000 were transformed with the broad host range vector pDSK519 to facilitate selection on media containing the antibiotic kanamycin at a concentration of 25 μg/ml (Keen et al. 1998). For growth curve analyses, the antibiotic concentration was decreased by half.

Genome sequencing was performed using paired 150 bp reads generated on the HiSeq (Illumina) sequencing platform at the Genome Center at the University of California, Davis. Genomes were de novo assembled using SPAdes version 2.5 (Bankevich et al. 2012). *Pst* A9, Pst 407 and *Pst* 838-8 have 136, 134 and 182 contigs with N50 of 152,994, 153,154 and 138,525 bp, respectively. Gene prediction and annotation were carried out using Glimmer3 (Delcher et al. 1999) and rapid annotations using the subsystems technology (RAST) annotation server (Aziz et al. 2008).

Plant materials, bacterial inoculations, and growth curve analyses on Rio Grande

All tomato plants were grown in the greenhouse at 23 °C with a 14 h photoperiod. For all bacterial inoculations, 4- to 5-week-old plants were used. All plant inoculations were performed with at least three biological replicates and repeated twice with similar results.

To investigate the virulence of current *Pst* isolates, *Solanum lycopersicum* cv. Rio-Grande (RG) lines [RG-76R (*Pto/Pto, Prf/Prf*), RG-76R *pto11* (*pto11/pto11, Prf/Prf*)] (Salmeron et al. 1994) were vacuum infiltrated with the following *Pst* strains: DC3000 (race 0), A9 (race 1) and 407 (race 1). Bacterial strains were grown overnight on NYG agar plates and then re-suspended to a concentration of 1×10^4 colony forming units per ml (CFU/ml) in 10 mM $MgCl_2$. Bacterial populations were determined 4 days post-inoculation as previously described (Kunkeaw et al. 2010). Disease symptoms were photographed 4 days post-inoculation. Statistical differences between genotypes were detected using Fisher's least significant difference ($\alpha = 0.05$).

Electrolyte leakage assays

The hypersensitive response (HR) was quantified by electrolyte leakage after bacterial infiltration of leaf tissue. Tomato leaves were infiltrated using a needleless syringe with 1×10^8 CFU/ml of *Pst* A9, 407 and DC3000. After infiltration, plants were placed under a light bank (100 µE/m2/s) for 6 h. Four leaf disks were harvested using a cork borer to generate 1.5 cm^2 of leaf discs, incubated in 4 ml distilled water, and placed under the light bank for 2 h. Conductivity was measured using the Orion 3 Star conductivity meter (Thermo Scientific) 8 h post-inoculation. Statistical differences were detected using Fisher's least significant difference ($\alpha = 0.05$).

Screening of wild tomato accessions

A total of 278 tomato accessions were screened for resistance to *Pst* A9 and 407 (Table S1). These genotypes included 276 wild tomato accessions and two hybrids. The cultivars Bonnie Best and APT 410 were included as susceptible controls. Plants were grown in 23-cm diameter pots in a greenhouse for 4 weeks prior to inoculation. Eleven plants per genotype were inoculated with a mixture *Pst* A9 and 407 strains. Bacterial strains were grown overnight on NYG agar plates and re-suspended to a concentration of 1×10^7 CFU/ml, and mixed together prior to inoculation. A jagged-edged wooden toothpick inserted in the end of a 6 ml syringe was used to generate leaf pricks. The toothpicks were dipped in the bacterial suspension prior to inoculation and five pricks were made on a single leaf. As a negative control, leaves were pricked with a toothpick dipped in distilled water. After inoculation, plants were immediately placed in an enclosed chamber to mist for 2 min for a total of four sprays per day. Plants were held in the mist chamber with 90–100 % humidity for 1 week. Plants were evaluated 7–10 days post-inoculation for lesion development. Evaluation of plants for lesion development was based on a plus/minus reaction scale. Susceptible plants exhibited chlorotic spreading lesions, while resistant plants exhibited no symptoms. Promising genotypes and the susceptible control Bonnie Best and APT410 were re-screened using the same procedure and rated on an additive 0-10 scale, with 0 indicating no symptoms and 10 indicating spreading lesions.

Screening of *S. habrochaites* LA1777 introgression lines and *S. peruvianum* hybrids

Hybrids derived from *S. peruvianum* cv. *dentatum* PI128655 as well as introgression lines (ILs) derived from *S. habrochaites* LA1777 were used in this study. Hybrid plants were derived from a cross between *S. peruvianum* PI128655 × *S. lycopersicum* PI204978 followed by self and open pollination for five generations. Ninety-three introgression lines (ILs) derived from a cross between *S. habrochaites* LA1777 × *S. lycopersicum* E6203 (Monforte and Tanksley 2000) were screened. The LA1777-derived ILs originally consisted of 99 lines, but only 93 lines were currently available (Monforte and Tanksley 2000). Seeds were kindly provided by the Tomato Genetic Resource Center (TGRC, Davis USA). Seeds were germinated in a controlled environmental chamber at 27 °C, 70 % relative humidity for 10–14 days on seedling trays (72 holes, 20 × 50 cm) and then transferred to 10-cm diameter pots in the greenhouse. Plants were grown in a completely randomized design in the greenhouse with three replications per genotype. Parental controls were included in each assay. Four-week-old plants were dip inoculated with *Pst* A9 at a concentration of 1×10^8 CFU/ml as previously described (Kunkeaw et al. 2010). After inoculation, the disease severities of individual genotypes were monitored over time and symptoms were rated 4 days post-inoculation.

ILs and corresponding parental controls were scored based on symptom severity using an additive disease rating scale of 0–4, where 0 = no disease symptoms, 1 = very few specks on the leaves (0–25 % leaf area infected), 2 = some specks on the leaves (25–50 % leaf area infected), 3 = numerous specks on the leaves (50–75 % leaf area infected), 4 = numerous lesions with leaf collapse (75–100 % of leaf area infected).

Pst strain A9 bacterial growth curves were performed on promising ILs, F1 hybrids (cross between *S. peruvianum*

PI128655 × *S. lycopersicum* PI204978), as well as parental controls. Plants were grown and dip inoculated as described above. Plant inoculations were conducted with four biological replications and repeated at least two times.

QTL analyses

Using the phenotypic data obtained from the greenhouse screening, QTL analyses on ILs were conducted with QTL Cartographer using composite interval mapping (Wang et al. 2012). The scan interval was set at 1 cM using the Kosambi mapping function with an automatic selection of peak markers for putative QTLs as a cofactor. The LOD thresholds for declaring the presence of significant QTLs for each trait were determined through 1,000 permutations ($\alpha = 0.05$). The detected QTLs were corroborated by single marker analysis (SMA). The SMA was conducted using one-way analysis of variance with the markers nearest to the QTL peak. Analysis of variance was performed using the PROC GLM procedure of SAS (SAS Institute 2011).

Evaluation of horticultural attributes

Select genotypes exhibiting resistance to *Pst* strain A9 were evaluated for horticultural attributes (Table 3). Flowering, fruit number and size, seed number, and growth habit were analyzed. Plants were grown for 4 months in the greenhouse (23 °C with a 14 h photoperiod) in 25 × 20 cm pots. Two plants per genotype were analyzed.

Results

Characterization of the race structure and effector repertoire of strains A9 and 407

In this study, we used the *P. syringae* pv. *tomato* strains A9 and 407. Both strains were isolated from infected tomato plants in California and phylogenetically cluster with a lineage representing the most common *Pst* strains isolated worldwide (Cai et al. 2011; Kunkeaw et al. 2010). Both strains were inoculated on the tomato near isogenic lines Rio Grande 76R (*Pto/Pto Prf/Prf*) and the Rio Grande 76R *pto* mutant (*pto11/pto11 Prf/Prf*) (Fig. 1). Race 0 strains are recognized by *Pto* in Rio Grande 76R, but can cause disease on Rio Grande 76R *pto11*. Race 1 strains can cause disease on both genotypes. As expected, the race 0 strain *Pst* DC3000 was unable to cause visible disease symptoms on Rio Grande 76R, but was able to cause disease on Rio Grande 76R *pto11* (Fig. 1a, b). In contrast, *Pst* A9 and 407 were able to cause visible disease symptoms and proliferate to high titers on both genotypes (Fig. 1a, b). *Pst* A9 caused more severe disease symptoms and enhanced bacterial growth ($p < 0.05$) compared to either *Pst* DC3000 or *Pst* 407 (Fig. 1a, b). Localized programmed cell death, called the hypersensitive response, is a hallmark of R-gene-mediated resistance. The progression of cell death was quantified by measuring electrolyte leakage using a conductivity meter in the Rio Grande genotypes. *Pst* A9 and 407 exhibited reduced electrolyte leakage compared to *Pst* DC3000 on Rio Grande 76R ($p < 0.05$) and did not exhibit statistically significant differences in electrolyte leakage between Rio Grande 76R and 76R *pto11* ($p < 0.05$) (Fig. 1c). Therefore, both *Pst* A9 and 407 strains are race 1. *Pst* A9 is also hypervirulent compared to *Pst* DC3000 and *Pst* 407 on Rio Grande 76R *pto11*.

To gain insight into the genomic region surrounding the *avrPto* and *avrPtoB* effectors, *Pst* A9, 407 and 838-8 were sequenced and draft genome assemblies were obtained. The genomic context surrounding *avrPto* and *AvrPtoB* is highlighted in Figs. 2 and 3. Both *Pst* A9 and 407 strains lack the *avrPto* effector, which is present in race 0, DC3000 (Fig. 2a). In previous analyses of race 1 strains, we found that approximately 74 % of California strains did not possess the *avrPto* effector, while 26 % still retained *avrPto* based on PCR amplification (Kunkeaw et al. 2010). To gain insight into the genomic context of the *avrPto* cluster in race 1 strains that still retain this effector, we sequenced the genome of *Pst* 838-8 and obtained a draft genome assembly. *AvrPto* is a phage-related gene cluster and it is possible that this region can be actively excised. Consistent with this hypothesis, the *avrPto* cluster is present in different genomic locations in *Pst* 838-8 compared to *Pst* DC3000 (Fig. 2b). $AvrPto_{838\text{-}8}$ possesses five amino acid polymorphisms when compared with the $AvrPto_{DC3000}$ allele, including a key amino acid polymorphism (G95E), which blocks interaction with Pto rendering this effector unable to elicit Pto-mediated resistance (Fig. 2c) (Kunkeaw et al. 2010). In contrast to *avrPto*, *Pst* A9 and 407 strains possess the *avrPtoB* effector and share similar loci surrounding *avrPtoB* with each other and *Pst* DC3000 (Fig. 3a). The *avrPtoB* effector in strains A9 and 407 is identical and highly similar with the well-characterized race 1 allele from *Pst* T1 (99 % amino acid similarity with 1 amino acid polymorphism) (Lin et al. 2006). In contrast, there are several instances of polymorphisms between AvrPtoB from *Pst* DC3000 with the alleles from *Pst* A9 and *Pst* 407 (Fig. 3b). Previously, it has been demonstrated that *avrPtoB* is transcribed, but the protein does not accumulate due to genetic factor(s) present only in race 1 strains (Lin et al. 2006; Kunkeaw et al. 2010). Therefore, *Pst* can use a variety of different mechanisms to overcome *Pto*- and *Prf*-mediated resistance in tomato.

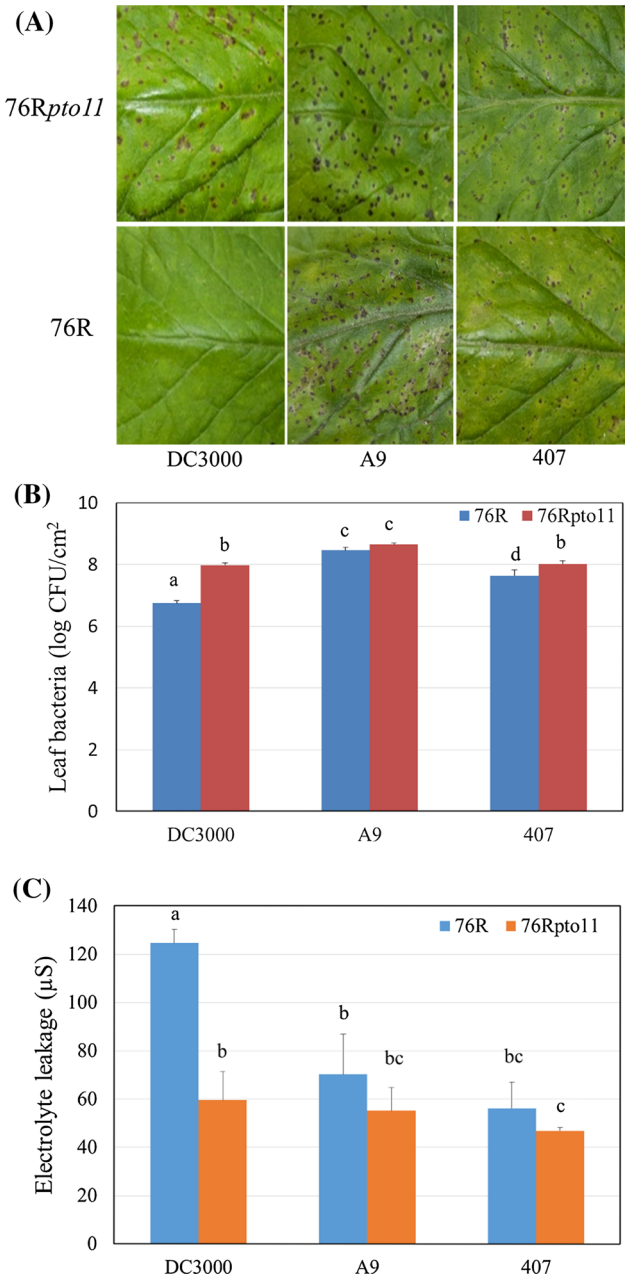

Fig. 1 *Pseudomonas syringae* pv. *tomato* A9 and 407 strains are not recognized by Pto/Prf in tomato and strain A9 exhibits enhanced virulence compared to strain DC3000. **a** Four-week-old Rio Grande 76R and 76R *pto11* tomato plants were dip inoculated with *P. syringae* pv. *tomato* strains DC3000, A9, and 407. Inoculations were performed at a concentration of 1×10^4 CFU/ml after vacuum infiltration. Disease symptoms were photographed 4 days post-inoculation. **b** Tomato genotypes were inoculated as described in (**a**) and bacterial growth curves conducted 4 days post-inoculation. Results are shown as the mean ($n = 3$), ± standard deviation. Statistical differences were detected by Fisher's least significant difference ($\alpha = 0.05$). **c** Quantification of electrolyte leakage 8 h after 4-week-old Rio Grande 76R and 76R *pto11* tomato plants was vacuum infiltrated with 1×10^8 CFU/ml of the indicated strains. Results are shown as the mean ($n = 3$), ± standard deviation. Statistical differences were detected by Fisher's least significant difference ($\alpha = 0.05$)

Screening of tomato accessions for resistance to race 1 *Pst* strains

Presently, there is a lack of tomato varieties exhibiting resistance to *Pst* race 1 although wild tomato species have been reported to carry such resistance (Stockinger and Walling 1994). To identify material exhibiting resistance to representative field isolates, a collection of 278 accessions was screened for reduced symptom severity upon inoculation with a mixture of *Pst* A9 and 407 (Table S1). Among the wild tomato accessions screened, five accessions exhibited reduced symptom severity: *S. peruvianum* LA3799, *S. peruvianum* var. *dentatum* PI128655, *S. habrochaites* LA2869, *S. chilense* LA2765, and *S. habrochaites* 1777 (Table 1). *S. habrochaites* LA1777 and *S. peruvianum* var. *dentatum* PI128655 exhibited lower levels of symptom severity compared to the other accessions (Table 1). Seed obtained from an F1 hybrid *S. peruvianum* cv. *dentatum* PI128655 × *S. lycopersicum* PI204978 followed by self and open pollination for 5 generations also showed decreased symptom severity upon inoculation (Table 1).

The bacterial titer after dip inoculation with *Pst* A9 was also determined for a subset of the accessions listed in Table 1. *S. peruvianum* var. *dentatum* PI128655 and *S. habrochaites* LA1777 were the most resistant and exhibited greater than 100-fold lower bacterial titers 4 days post-inoculation (Fig. 4a, $p < 0.05$). Two additional wild tomato accessions, *S. pimpinellifolium* LA1589 and *S. pennellii* LA0716, with existing inbred backcross and introgression lines were also subjected to bacterial growth assays (Doganlar et al. 2002; Eshed and Zamir 1995) (Fig. 4a). However, LA1589 and LA0716 only exhibited minor levels of resistance to *Pst* A9, with a fivefold reduction in bacterial growth compared to the susceptible control Bonnie Best (Fig. 4a, $p < 0.05$). Plants generated from a cross between PI128655 and PI204978 also exhibited significantly reduced bacterial titers 4 days post-inoculation with *Pst* A9 (Fig. 4b). However, these accessions (PI568258 and PI306812) exhibited poor horticultural attributes and reduced seed germination (data not shown). Therefore, we decided to focus on resistance derived from *S. habrochaites* LA1777.

Screening LA1777 introgression lines and QTL mapping

An existing set of 93 introgression lines developed from *S. habrochaites* LA1777 was screened to identify QTLs controlling resistance to *Pst* strain A9 in a replicated greenhouse trial (Monforte and Tanksley 2000). The recurrent parent, *S. lycopersicum* E6203, was used as a susceptible control for all inoculations. Composite interval mapping (CIM) with QTL Cartographer identified four significant QTLs above the permutation threshold located on chromosomes

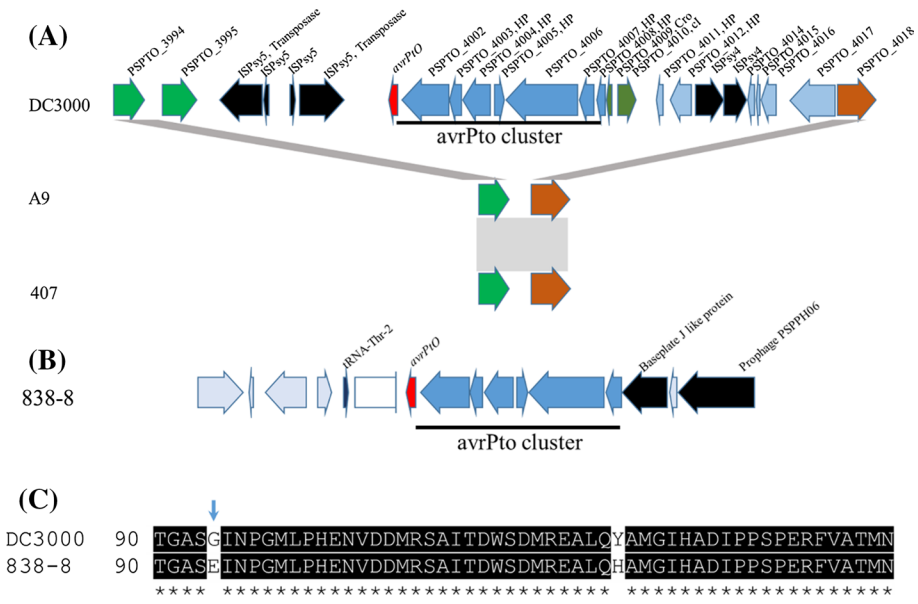

Fig. 2 Genomic context surrounding the *avrPto* cluster in *P. syringae* pv. *tomato* strains. **a** Diagram of the *avrPto* cluster in DC3000 (race 0), A9 (race 1), and 407 (race 1). The *avrPto* effector is colored in *red*, while transposases and insertion elements are colored as *black*. The genomic region surrounding *avrPto* is absent in A9 and 407 strains. **b** Diagram of the *avrPto* cluster in *Pst* strain 838-8 (race1). The cluster is present in this strain but in a different genetic location. **c** The 838-8 *avrPto* allele has a polymorphism in the GINP loop, a region required for the AvrPto–Pto interaction

1, 2 and 12 (Table 2). These QTLs were named *bacterial speck resistance to race 1* (*bsRr1-1, bsRr1-2, bsRr1-12a, and bsRr1-12b*). The percentage of phenotypic variation controlled by each QTL was subsequently determined using marker-trait analyses with the most closely linked markers. The QTL on chromosome 1 is located at 122 cM between markers TG17 and TG27 and named *bsRr1-1* (Fig. 5). *bsRr1-1* accounts for 10.75 % of the phenotypic variance (Table 2). *bsRr1-2* is located at 95 cM between markers CT9 and TG620 (Fig. 5) and accounts for 10.51 % of phenotypic variation. Two QTLs were detected in chromosome 12 (*bsRr1-12a* and *bsRr1-12b*) (Table 2; Fig. 5). *bsRr1-12a* and *bsRr1-12b* are located at 29 cM and 63 cM between markers TG180, CT211 and CT 287A, TG295, respectively (Fig. 5). Permutation analysis resulted in LOD thresholds ranging from 2.29 to 5.65 at $\alpha < 0.05$.

To verify the detected QTLs and examine the bacterial titers in promising *S. habrochaites* ILs, five promising LA1777 ILs were subjected to dip inoculation with *Pst* A9 followed by growth curve analyses at 0 and 4 days post-inoculation (Fig. 6). The parental controls *S. habrochaites* LA1777 and *S. lycopersicum* E6203 were also subjected to growth curve analyses. LA1777 exhibited greater than 1000-fold reduction in bacterial growth compared to E6203. The tested ILs also exhibited reduced bacterial growth and disease symptoms compared to E6203 (10–12-fold lower bacterial titers, $p < 0.05$) (Fig. 6). None of the ILs phenotyped possesses all detected QTLs, which is the most likely explanation for why the decrease in bacterial growth is not as robust as what is seen in LA1777. Only one IL line (LA3968) possessed two QTLs, *bsrR1-12a* and *bsrR1-12b* (Table 2). Four ILs were also identified as having significantly reduced symptom development, but no detected QTLs controlling resistance (Fig. S1). These four ILs (LA3919, LA3929, LA3941, LA3981, and LA3983) also exhibited significantly lower bacterial growth compared to E6203 after dip inoculation with *Pst* A9 (Fig. S1). It is possible that these ILs contain novel QTLs that were not detected due to the low density of markers present in LA1777 IL population. Although all re-tested ILs exhibited reduced symptom development compared to E6203, resistance seemed to be quantitative in nature, rather than qualitative (Fig. 6; Fig. S1). Typically, disease resistance mediated by plant R genes such as *Pto* and *Prf* is described as qualitative and there is a complete lack of disease symptoms after inoculation (Fig. 1a). In contrast, some slight disease symptoms and necrotic specks could still be observed on the ILs as well as very reduced necrotic specks on LA1777 (Fig. 6a). The ILs exhibiting reduced bacterial speck symptoms also support significantly lower bacterial titers (Fig. 6b).

Evaluation of horticultural attributes for select LA1777-derived introgression lines

Morphological traits including flowering, fruit number, fruit size, seed number, and growth habit of selected ILs were surveyed. Two plants of each of the ILs as well as parental controls were grown in the greenhouse for a period of 4 months. The IL LA3941 and *S. habrochaites* LA1777 exhibited similar undesirable horticultural attributes, such as few flowers, a late flowering phenotype, no fruit set, and an indeterminate growth habit. LA3921 and LA3929 were the shortest genotypes and LA1777 and LA3941 were the tallest. LA3968 and LA3969 had larger fruit size compared to E6203 and other genotypes (Table 3; Fig. S2). A variety of different fruit sizes were also detected across ILs (Fig.

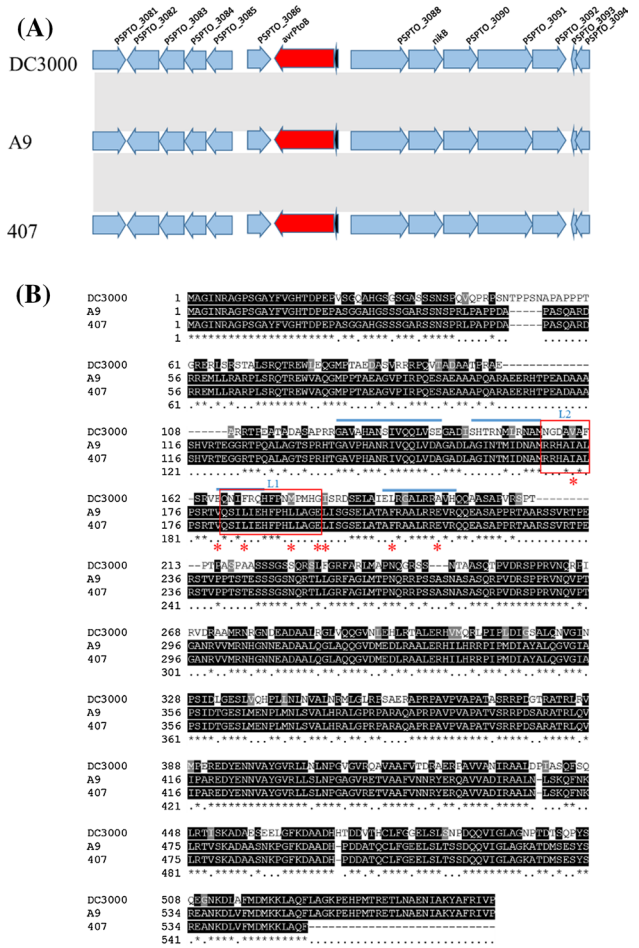

Fig. 3 Genomic context surrounding the *avrPtoB* effector in *P. syringae* pv. *tomato* race 1. **a** The genomic context surrounding *avrPtoB* in *P. syringae* pv. *tomato* DC3000 (race 0), A9 (race 1) and 407 (race 1). All genes are shared across the three strains and in the same genetic location. The *avrPtoB* effector is colored in *red*. **b** ClustalW alignment of AvrPtoB amino acid sequence from *P. syringae* pv. *tomato* DC3000, A9 and 407. *Blue lines* indicate four α-helices within the $AvrPtoB_{121–205}$ domain required for interaction with Pto. *Red blocks* indicate interfaces that contact Pto; *red asterisks* indicate sites that, when mutated, alter the AvrPtoB–Pto interaction

Table 1 Wild tomato accessions exhibiting reduced symptom progression after inoculation with *P. syringae* pv. *tomato* strains A9 and 407 and susceptible controls

Accession	Score	Magnitude of disease
S. habrochaites LA1777	2	Limited spread
S. habrochaites LA2869	4	Limited spread
S. chilense LA2765	5	Moderate spread
S. peruvianum LA3799	6	Limited spread
S. peruvianum PI128655	4	Limited spread
Bonnie best	10	Extreme spread
APT 410	10	Extreme spread

Plants were scored on an additive 0–10 scale, with 0 indicating no symptoms detected

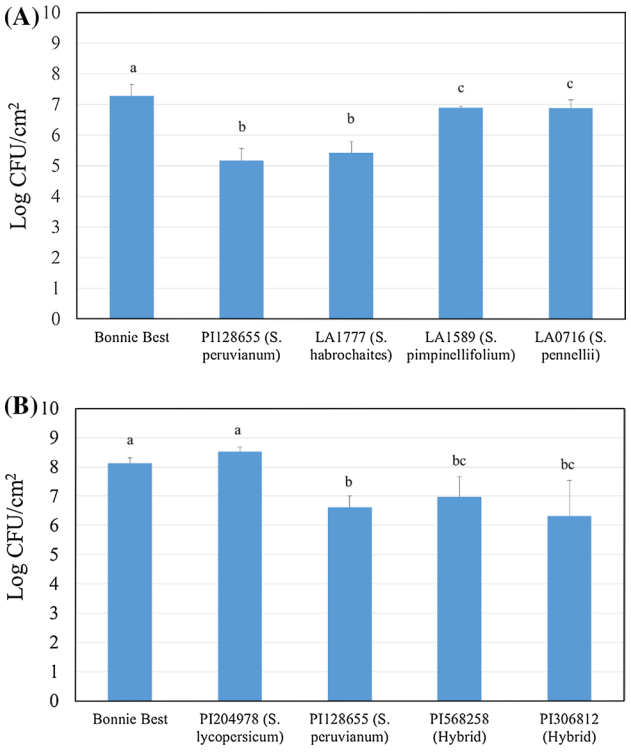

Fig. 4 Growth curve analyses of wild tomato accessions after inoculation with *P. syringae* pv. *tomato* strain A9. **a** Four-week-old wild tomato accessions and the susceptible control *S. lycopersicum* cv. Bonnie Best were dip inoculated with *P. syringae* pv. *tomato* strain A9 at a concentration of 1×10^8 CFU/ml. Bacterial growth curves were conducted 4 days post-inoculation. Results are shown as the mean ($n = 3$) ± standard deviation. Statistical differences were detected by Fisher's least significant difference ($\alpha = 0.05$). **b** Growth curve analyses on parents and hybrids derived from a cross between *S. peruvianum* PI128655 and *S. lycopersicum* PI204978 (PI568258 and PI306812). Bacterial inoculations, growth curves, and statistical analyses were conducted as described in (**a**). Results are shown as the mean ($n = 3$) ± standard deviation

S2). No other obvious differences were observed in the morphological traits in other genotypes and parents under these growth conditions.

Discussion

Pseudomonas syringae pv. *tomato* race 1 strains were first detected in Canada in 1986 and in California in 1996 (Arredondo and Davis 2000; Cai et al. 2011). *Pst* can cause significant losses in processing tomato production due to a combination of suitable environmental conditions and the proliferation of race 1 strains (Kunkeaw et al. 2010). The most common *P. syringae* isolates causing bacterial speck disease in tomato belong to a single, highly related lineage that cluster with other race 1 strains (Cai et al. 2011; Kunkeaw et al. 2010). Because of the close genetic relationship between current

Table 2 QTLs detected for resistance to *P. syringae* pv. *tomato* strain A9 in the *S. habrochaites* LA1777 introgression lines

Chromosome	QTL (ILs)	Position (cM)	Additive effect	LOD	R^2 %
Ch1	*bsRr1-1* (LA3995)	122	0.67	2.29	10.75
Ch2	*bsRr1-2* (LA3921, LA3922)	95	0.67	4.78	10.51
Ch12	*bsRr1-12a* (LA3968)	29	0.26	5.65	12.45
	bsRr1-12b (LA3968, LA3969)	63	0.31	5.53	11.89

LOD logarithm of the odds score for QTLs calculated by composite interval mapping

R^2 % percentage phenotypic variation of the trait explained by individual QTL was estimated by PROC GLM (SAS) ($p < 0.05$)

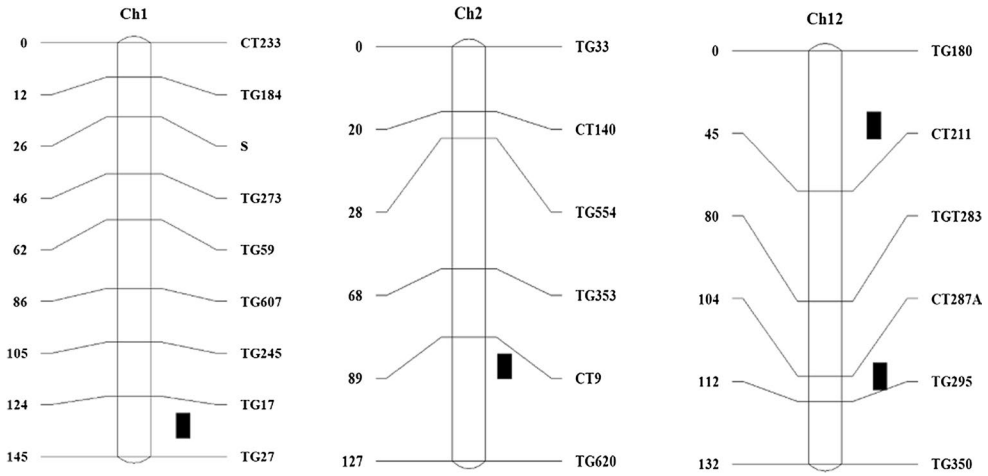

Fig. 5 Linkage maps of tomato chromosomes 1, 2 and 12 highlighting QTLs conferring resistance to *P. syringae* pv. *tomato* strain A9. Molecular markers used to genotype the introgression *lines* are shown to the *right* with distance in centiMorgans to the *left* of each chromosome. QTLs associated with resistance to bacterial speck are highlighted in *black*. QTLs were detected using inclusive composite interval mapping with 1,000 permutations

Pst strains isolated worldwide, it is likely that new sources of quantitative resistance will be effective across a large growing area. In this study, we used two representative *Pst* race 1 strains, A9 and 407, to identify new sources of resistance.

Bacterial strains and sequencing

Next-generation sequencing has made it possible to rapidly analyze pathogen strains from the field for their type III effector repertoires and other virulence components (Baltrus et al. 2011; Bart et al. 2012). The draft genome sequences of *Pst* A9 and 407 provide insight into how these field strains can overcome R-gene-mediated resistance in tomato conferred by Pto and Prf (Pedley and Martin 2003). Both *Pst* A9 and 407 lack the *avrPto* effector (Fig. 2). Other race 1 isolates either lack *avrPto* or have acquired select point mutations within the effector rendering it unable to bind tomato Pto while still retaining some virulence functions (Fig. 2; Kunkeaw et al. 2010). Frequently, bacterial genes encoding traits affecting fitness and virulence, such as antibiotic resistance, heavy metal resistance, and effectors are found in close proximity to mobile genetic elements such as plasmids, transposons, and bacteriophages (Darmon and Leach 2014). *AvrPto* is located in a bacteriophage-related gene cluster and DC3000 appears to be heavily infected with these external elements, while sequenced *Pst* race 1 strains such as T1, A9, and 407 do not exhibit extensive hallmarks of bacteriophage infection (Almeida et al. 2009; Buell et al. 2003; Kim et al. 1998). *AvrPto* is also present in some *Pst* race 1 strains, such as *Pst* 838-8, but in a different genomic location when compared to *Pst* DC3000. Prophage and insertion sequence elements in close proximity to the avrPto cluster may have facilitated its relocation. Race 1 *avrPto* alleles also have five amino acid polymorphisms when compared with $avrPto_{DC3000}$ (Kunkeaw et al. 2010). $AvrPto_{DC3000}$ interacts with tomato Pto at two major interfaces, one of which is polymorphic in race 1 alleles (G95E) (Xing et al. 2007).

AvrPtoB is present in many plant pathogenic *Pseudomonads,* including *Pst* A9 and 407 (Kunkeaw et al. 2010; Lin et al. 2006) (Fig. 3). The *Pst* A9 and 407 *avrPtoB* alleles are identical and only possess a single point mutation compared to *avrPtoB* from *Pst* T1 (Fig. 3). Despite significant polymorphisms between race 0 and 1 *avrPtoB* alleles, *avrPtoB* race 1 alleles can complement *Pst* DC3000 Δ*avrPto/*Δ*avrPtoB* and can be recognized in tomato (Lin et al. 2006). It is interesting to note that *avrPtoB* is transcribed, but the protein is not detectable in race 1 strains, indicating that these strains may possess a protease or small RNA that specifically inhibits AvrPtoB protein level expression to overcome Pto-mediated resistance (Lin et al. 2006; Kunkeaw et al. 2010). Widespread use of processing

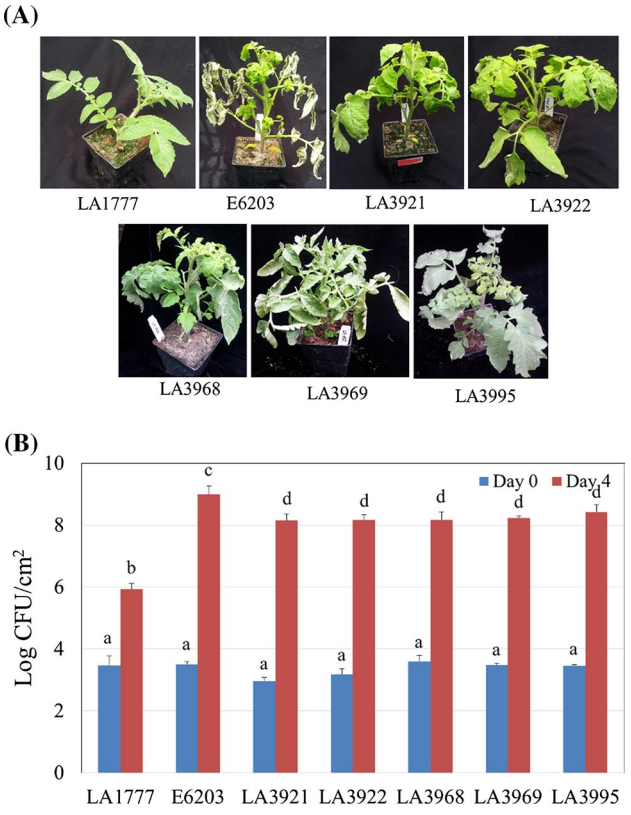

Fig. 6 Bacterial growth curve analyses of promising introgression *lines* exhibiting reduced symptom progression after inoculation with *P. syringae* pv. *tomato* strain A9. Plants included the wild tomato *S. habrochaites* accession LA1777 and the recurrent parent *S. lycopersicum* E6203. Four-week-old tomato plants were dip inoculated with strain A9 at a concentration of 1×10^8 CFU/ml. **a** Disease symptoms were photographed 4 days post-inoculation. **b** Bacterial growth curves were conducted 4 days post-inoculation. Results are shown as the mean ($n = 3$) ± standard deviation. Statistical differences were detected by Fisher's least significant difference ($\alpha = 0.05$)

tomato cultivars likely provided strong selective pressure on *Pst* strains to avoid host recognition.

Germplasm screening

Due to the breakdown of Pto-mediated resistance, there is a need to develop new cultivars that are resistant to current *Pst* field isolates. Genetic resistance based upon a single R gene that recognizes a single pathogen effector (race-specific resistance) has been extensively utilized in breeding programs. However, the disadvantage of this approach is the transient effectiveness of this resistance and continuous evolution of new pathogen races. Some alternative approaches to provide more durable resistance include pyramiding R genes, deploying QTLs to decrease disease severity, and combinations of the two (Castro et al. 2003; Wulff et al. 2011). To identify sources of resistance to *Pst* race 1 in tomato, we screened a large number of wild tomato accessions using seed provided from the Tomato Genetics Resource Center at University of California, Davis and included accessions from each wild tomato core collection. We also received seed from the USDA-ARS Plant Genetic Resources Unit, Geneva, New York. Wild tomato species are rich in genes conferring resistance to wide variety of pathogens. Quantitative resistance to over 40 diseases has been observed in wild relatives and subsequently introgressed into tomato cultivars (Ercolano et al. 2012; Ji and Chetelat 2007; Robertson and Labate 2007). Among 278 accessions screened, we identified five accessions exhibiting resistance to A9 and 407: *S. peruvianum* LA3799, *S. peruvianum* var. *dentatum* PI 128655, *S. habrochaites* LA2869, *S. chilense* LA2765, and *S. habrochaites* LA1777 (Table 1). The presence of resistance to a variety of diseases (single genes and QTLs) in these accessions has been reported in previous

Table 3 Horticultural attributes of selected tomato genotypes exhibiting resistance to *P. syringae* pv. *tomato* strain A9

Genotypes	No. of fruits/plant	Flowers	Fruit size/cm^2	No. of seeds/fruit	Growth habit
LA 1777 (wild accession)	–	Late	–	–	Indeterminate
E6203 (susceptible)	26 ± 4	Yes	2.54	45 ± 3	Determinate
LA3919	12 ± 3	Yes	3.14	36 ± 4	Determinate
LA3921	2 ± 1	Yes	1.13	18 ± 3	Determinate
LA3922	9 ± 2	Yes	2.54	5 ± 2	Determinate
LA3926	7 ± 2	Yes	1.54	48 ± 5	Determinate
LA3929	6 ± 2	Yes	3.14	43 ± 6	Determinate
LA3941	–	Few	–	–	Indeterminate
LA3968	8 ± 3	Yes	7.7	47 ± 2	Determinate
LA3969	20 ± 3	Yes	9.08	46 ± 4	Determinate
LA3979	11 ± 5	Yes	1.77	38 ± 5	Determinate
LA3981	8 ± 2	Yes	2.01	40 ± 2	Determinate
LA3983	8 ± 2	Yes	3.14	5 ± 3	Determinate
LA3989	3 ± 1	Yes	1.13	42 ± 6	Determinate

– = not detected

studies (Foolad and Sharma 2005; Scott and Gardner 2007). *S. habrochaites* LA1777 and *S. peruvianum* var. *dentatum* PI 128655 were the most promising accessions and displayed the highest level of resistance to *Pst* when compared to other wild accessions (Table 1). Growth curve analyses also demonstrated that bacterial populations were significantly lower in these accessions (Fig. 4). *S. habrochaites* LA1777 originates from Peru and is self-incompatible, homozygous, green fruited, and has an indeterminant growth habit (Monforte and Tanksley 2000). LA1777 is well known as a source of begomovirus and insect resistance (Monforte et al. 2001; Muigai et al. 2002; Vidavsky and Czosnek 1998). *S. peruvianum* var. *dentatum* PI 128655 as a source of resistance to different viruses, such as Tomato yellow top virus and Potato leaf roll virus, and bacteria like phytoplasmas has also been documented (Garcion et al. 2014; Hassan and Thomas 1988; Thomas and Boll 1978).

QTL analysis

A set of ILs has been developed in the background of *S. lycopersicum* E6203 (a determinant processing type tomato), covering ~85 % of the LA1777 genome (Monforte and Tanksley 2000). ILs have a number of advantages for QTL mapping, gene identification, and related breeding applications (Zamir 2001; Zamir and Eshed 1998). We focused on identifying QTL from an existing IL population derived from *S. habrochaites* LA1777 to identify specific ILs that could be ultimately deployed more rapidly into a breeding program. QTLs conferring resistance to *Botrytis cinerea* and *Phytophthora infestans* have been detected in the LA1777 × E6203 IL population (Gabor et al. 2010; Li et al. 2011). In this study, four QTLs conferring resistance to *Pst* A9 were detected above the permutation threshold, located on chromosomes 1, 2 and 12 (Table 2; Fig. 5). The percent phenotypic variation (R^2 %) ranged from 10.5 to 12.5 % (Table 2). Subsequent bacterial inoculations coupled with quantification of bacterial titers in tomato leaves revealed that selected ILs also exhibited significantly lower bacterial titers compared to E6203 (Fig. 6b). However, the individual ILs analyzed were still ~100-fold more susceptible than the donor parent LA1777. No single IL possessed all four QTLs. Although there are two QTLs located on chromosome 12, they are 28 cM apart. Thus, pyramiding QTLs into a single line should be feasible. Significant QTLs for some ILs exhibiting resistance were also not detected (Fig. S2). This may be due to the low marker density present in the LA1777 IL population and highlights the need for development of densely saturated markers on this population to facilitate detection and rapid introgression of promising traits while minimizing the contribution of LA1777 donor DNA. The existing set of ILs derived from LA1777 covers ~85 % of the LA1777 genome. Thus, some loci conferring resistance might not be present in the existing set of ILs. This could be the reason why Momotaz and colleagues (Momotaz et al. 2005, 2007a, b) were unable to find resistance to Tomato yellow leaf curl virus (TYLCV) and sweet potato whitefly in the LA1777 ILs, despite reported resistance to TYLCV, Begomovirus and whiteflies in LA1777 (Muigai et al. 2002; Vidavsky and Czosnek 1998).

Wild tomatoes provide a rich source of genetic variation for many different horticultural attributes (Grandillo et al. 1999). The wild accessions *S. pennellii* LA716 (Eshed and Zamir 1994) and *S. pimpinellifolium* LA 1589 (Grandillo et al. 1999; Tanksley et al. 1996) and their corresponding ILs have been well characterized both genetically and phenotypically. The *S. habrochaites* LA1777 ILs have not been extensively exploited but are potentially very useful as a source of diverse traits (Bernacchi et al. 1998; Mathieu et al. 2009; Monforte and Tanksley 2000). Our analysis of the variation in fruit size in different LA1777 ILs uncovered significant variation in fruit size compared to *S. lycopersicum* E6203 (Table S3, Fig. S2). In support of these observations, QTL controlling fruit yield on chromosome 1 has been detected in these ILs (Hanson et al. 2007). One concern with introgression of QTLs from wild accessions is linkage drag, or the simultaneous transfer of DNA segments linked to genes of interest that negatively affect crop performance (Hospital 2005). We did detect several undesirable horticultural attributes in the IL line LA3941 and hybrids derived from a cross between *S. peruvianum* PI128655 × *S. lycopersicum* PI204978. However, our initial evaluation of promising ILs exhibiting resistance to *Pst* race 1 did not reveal obvious instances of linkage drag (Table 3). Future efforts aimed at developing and placing additional markers on the existing LA1777 ILs will facilitate marker-assisted selection in these lines.

In conclusion, we identified wild tomato accessions conferring resistance to *Pst* race 1 strains A9 and 407, which are representative of current strains present worldwide. Analyses of ILs derived from *S. habrochaites* LA1777 resulted in the detection of four major QTLs controlling resistance to *Pst* race 1. These findings will provide useful information to initiate the development of more tightly linked markers for each QTL, facilitating marker-assisted selection for resistance to current *Pst* isolates.

Author Contribution statement SPT and EMM performed the experiments. SPT, RMD and GC conceived the experiments and analyzed the data. SPT and GC wrote the manuscript. We insist that no author be omitted.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that the study complies with the current laws of the country in which they were performed.

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