Evaluating Quantitative Trait Locus Resistance in Tomato to Multiple *Xanthomonas* spp.

Eduardo Bernal, Debora Liabeuf, and David M. Francis[†]

Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH 44691

Abstract

Bacterial spot of tomato is a foliar disease caused by four *Xanthomonas* species. Identifying genetic resistance in wild tomatoes and subsequent breeding of varieties has been a strategy to reduce the loss from this disease because control using pesticides has been ineffective. Three independent sources of resistance have been identified with quantitative trait loci (QTL) mapping to the centromeric region on chromosome 11. These sources are derived from Hawaii 7998 (QTL-11A), PI 114490 (QTL-11B), and LA2533 (QTL-11C). To determine which QTL introgression from chromosome 11 provides the greatest resistance to multiple species, we developed near-isogenic lines (NILs) using marker-assisted backcrossing. In parallel, we developed an NIL that contains *Rx-4/Xv3*, which provides major gene resistance to *Xanthomonas perforans*.

Additionally, we combined *Rx-4/Xv3* resistance with QTL-11A. These sources of resistance were independently introduced into the susceptible parent, OH88119. During a 3-year period from 2016 to 2018, we evaluated backcross-derived families and NILs from each source in independent field trials inoculated with *X. perforans*, *X. euvesicatoria*, or *X. gardneri*. Our results suggest that both QTL-11C and QTL-11A combined with *Rx-4/Xv3* provide effective genetic resistance against multiple *Xanthomonas* species. In addition, we provide evidence for additive to dominant genetic action for the QTL introgressions.

Keywords: cultivar/resistance, disease management, quantitative resistance, vegetables

Bacterial spot is a foliar disease of tomato (*Solanum lycopersicum* L.) caused by *Xanthomonas euvesicatoria*, *X. perforans*, *X. vesicatoria*, and *X. gardneri*. The disease causes significant yield losses throughout the world, especially in areas with high humidity and rainfall (Potnis et al. 2015). The symptoms include scab-like lesions on the fruit; black and chlorotic spots on the stem, leaves, peduncles; defoliation; and blossom abortion (Yang and Francis 2005). To control the spread of the disease, copper compounds are commonly used, although efficacy is poor under high disease pressure (Louws et al. 2001; Marco and Stall 1983). Additionally, *Xanthomonas* populations tend to be copper insensitive (Liao et al. 2019). Genetic resistance remains a strategy that may reduce losses caused by bacterial spot.

The *Xanthomonas* species causing bacterial spot can be loosely aligned to historical race designations that define specific bacterial effector and resistance gene-mediated responses. *Xanthomonas* isolates with T1 phenotypes are currently considered *X. euvesicatoria* (Scott et al. 1995). Race T1 strains are defined by an *avrRxv*-mediated hypersensitive response (HR) on the tomato accession Hawaii 7998 (Whalen et al. 1993). Hawaii 7998 possesses three loci capable of eliciting an HR when challenged with strains containing the *avrRxv* effector (Wang et al. 1994; Yu et al. 1995). These loci fall on the top and bottom of chromosome 1 (*Rx1* and *Rx2*) and chromosome 5 (*Rx3*), with *Rx3* providing the most effective resistance in the field (Yang and Francis 2005). Races T3 and T4 tend to be *X. perforans*, with T3 strains defined by an *avrXv3*-mediated HR on Hawaii

7981, PI 128216, and several other *Solanum pimpinellifolium* accessions (Scott et al. 2001). The resistance locus *Rx-4/Xv3* maps to chromosome 11 and defines the T3 interaction (Pei et al. 2012; Robbins et al. 2009; Scott et al. 1996; Wang et al. 2011). Race T4 is defined by *xopJ4*-mediated HR on LA0716, with resistance mapping to chromosome 6 (Sharlach et al. 2013). Because race T2 strains are defined by the lack of HR on key differential germplasm, these may be either *X. vesicatoria* or *X. gardneri*.

In addition to major gene resistance, quantitative resistance that seems to be effective against multiple species has been identified. For example, PI 114490 has been described as resistant to T1, T2, and T3 (Scott et al. 2003). Furthermore, genetic studies have defined quantitative trait loci (QTL) that are effective against more than one *Xanthomonas*. For example, PI 114490 (*S. lycopersicum* var. *cerasiforme*) LA2533 (*S. pimpinellifolium*), and Hawaii 7998 all possess QTL in the centromeric region of chromosome 11 (Hutton et al. 2010; Liabeuf 2016; Sim et al. 2015). For Hawaii 7998 and PI 114490, the QTL region on chromosome 11 explained 14.6 to 63.8% of the phenotypic variation for resistance within data derived from T2, T3, and T4 strains (Yang et al. 2005). In a separate study, Hawaii 7998 also displayed QTL associated with resistance on chromosome 11 against T1 and T3 (Sim et al. 2015). For LA2533, the region from 7.8 to 54.8 Mb on chromosome 11 accounted for 38% of the phenotypic variation associated with resistance against *X. gardneri* (Liabeuf 2016). Previous studies have not evaluated all three sources or progeny against the same pathogen strains in common experiments.

To address the relative effectiveness of the QTL on chromosome 11, we implemented a backcross breeding program with marker-assisted selection (MAS) and background genome selection to independently introgress chromosome 11 QTL from different sources to create near-isogenic lines (NILs). Coupling MAS to select marker-linked traits and selecting genomic regions of the recurrent parent using background genome selection (Hospital 2009) allowed us to efficiently develop and evaluate germplasm with QTL of interest in a uniform genetic background. In tomato, >7,720 validated single-nucleotide polymorphisms (SNPs) have been developed using next generation sequencing technology (Sim et al. 2012). We took advantage of these SNPs for selection in three backcross populations. The main objectives were to (i) use MAS and background genome selection to introgress three independent sources of a QTL

[†]Corresponding author: D. M. Francis; francis.77@osu.edu

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found in the same region of chromosome 11, (ii) determine the effectiveness of the resistance from each source against multiple *Xanthomonas* species, and (iii) evaluate homozygous and heterozygous allele genetic effects across QTL introgressions.

Materials and Methods

Plant material. Multiple tomato backcross populations were developed based on three distinct sources of resistance (Hawaii 7998, PI 114490, and LA2533) as the origin of a QTL on chromosome 11 that confers resistance to bacterial spot. Advanced lines from established pedigrees were used as donors of the QTL, because these lines already possessed a greater percentage of elite processing cultivar genetic background. Parent OH087663 is a line derived from a complex population (Sim et al. 2015) and contains a QTL on chromosome 11 inherited from Hawaii 7998 via Fla. 7600 (Hutton et al. 2010; Scott et al. 2003). OH087663 also contains *Rx-4/Xv3* on chromosome 11 inherited from PI 128216 (Robbins et al. 2009; Wang et al. 2011). Parent 01-BR-7087 is a line containing a QTL on chromosome 11 inherited from PI 114490. This line is derived from a cross between PI 114490 and FLA. 7600 (F_1), which was backcrossed twice to OH9242 (Scott et al. 2003). Parent FG12-433E-43 is a line containing a QTL on chromosome 11 inherited from LA2533 derived from two backcrosses to OH2641 (Liabeuf et al. 2015). In all cases, OH88119 (Berry et al. 1995) was used as the susceptible recurrent parent. For ease of naming, each introgression has been designated with a unique name: QTL-11A (Hawaii 7998), QTL-11B (PI 114490), and QTL-11C (LA2533).

NIL development. Development of NILs started in summer 2015 with crosses between each resistant parent containing QTL-11A, QTL-11B, or QTL-11C and our recurrent parent line OH88119 to produce BC₁ populations. Ohio OH88119 was selected because of its susceptibility to all *Xanthomonas* species causing bacterial spot (Yang and Francis 2005). Progeny from BC₁ populations were sown in 288 Square Deep Plug Trays (Hummert International) with PRO-MIX (Hummert International) and covered with a thin layer of

vermiculite in the greenhouse. After 3 weeks, emerged seedlings were processed as described below.

DNA isolation. A small leaflet from seedlings was sampled into 96-well cluster tubes (Corning Inc.). A hexadecyltrimethylammonium bromide (CTAB) method was scaled to 96-well tubes for DNA extraction as described (Sim et al. 2015). Samples were macerated in 150 μ l of extraction buffer (0.35 M sorbitol, 0.1 M Tris buffer, 5 mM EDTA, and 25 mM sodium bisulfite at pH 7.5), 150 μ l of CTAB nuclei lysis buffer (0.2 M Tris, 0.05 M EDTA, 2 M NaCl, and 2% CTAB at pH 7.5), and 60 μ l of 5% Sarkosyl. Two 4-mm metallic beads were added to each tube, and a GenoG-rinder (OPS Diagnostics) was used to grind tissue at 200 strokes per minute for 2 min. Tubes were incubated at 65°C for 20 min; then, they were set at room temperature for 10 min before adding 350 μ l of chloroform isoamyl alcohol (24:1) followed by centrifugation at 5,000 $\times$ g for 10 min. The aqueous phase was transferred to a 96-well round-bottom microtiter plate (Corning Inc.) containing 110 μ l of isopropanol and then centrifuged at 5,000 $\times$ g for 15 min for DNA precipitation. Plates were air dried for 30 min before resuspending the DNA with 100 μ l of Tris EDTA buffer (10 mM Tris and 0.1 mM EDTA).

MAS. Approximately 192 individuals were genotyped from each BC₁ population using two PCR-based DNA markers: Marker Sli_1831 (forward primer: 5'-GGAAGCTTGGATTAAAGGGG-3', reverse primer: 5'-CAGTCGCTTGAACCGAG-3') and Sli_1901 (forward primer: 5'-CGCGTTTCATCTTTTCTC-3', reverse primer: 5'-TCACCTGATAGCAGTGACGTAG-3'). These markers broadly flank the known location of the QTL on chromosome 11 at physical positions 20 and 48 Mb, respectively, based on tomato reference genome (SL 3.0). Additionally, indel marker PCC12 (Pei et al. 2012) at physical position 53 Mb was used to select recombinants with and without *Rx4/Xv3* within the QTL-11A BC₁ population. PCR was conducted in 20- μ l reactions consisting of 2 μ l of buffer A (10 mM Tris-HCl, 50 mM KCl, and 1.5 mM MgCl₂), 0.8 μ l of 1.25 mM dNTP, 0.2 μ l of 10 μ M forward and reverse primers, 12.4 μ l of ddH₂O, 0.4 units of *Taq* DNA polymerase, and 4 μ l of DNA template using the following cycling conditions: (1) 94°C for 3 min, (2) 94°C for 1 min, (3) 54°C for 30 s, (4) 72°C for 1 min, and (5) 72°C for 5 min. Steps 2 to 4 were repeated 35 times before the final annealing step. PCR products were run on a 2% agarose gel at 180 V for 1 h and 30 min and detected using ethidium bromide fluorescence.

Individuals heterozygous for the QTL markers were selected for background genome selection. Markers for background selection were chosen from a set of high-quality validated SNPs based on distribution in the genome and polymorphic information content in germplasm described previously (Blanca et al. 2015; Sim et al. 2012). For each BC₁ population, polymorphic markers spread across the genome, and distinguishing parents were selected (Table 1). SNPs were assayed using the Kompetitive Allele Specific PCR assay (Semagn et al. 2014).

Selected progeny from BC₁ populations developed from QTL-11A, QTL-11B, and QTL-11C were self-pollinated. BC₁S₁ progeny were genotyped and selected for resistant and susceptible alleles at the chromosome 11 QTL introgression. Homozygotes were self-pollinated again (BC₁S₂) and paired as resistant or susceptible members of the same family before transplant in summer 2016 field trials. Family structure was maintained such that background genome effects could be tested based on the original BC₁ selections. At the end of the trials, BC₁S₃ seed from the homozygous resistant progeny was collected from the field for future studies. Beginning in summer 2016, individuals that were homozygous resistant from BC₁S₂ progeny were advanced through two more generations of backcrossing. After the self-pollination step to produce BC₃S₁ seed, selection was imposed for the QTL and/or *Rx4/Xv3*. DNA isolation and selection occurred within BC₃S₁ before transplanting in summer 2017 trials. Individual plants were grouped based on homozygosity and heterozygosity for QTL introgression alleles to test for gene action. BC₃-derived families were evaluated in both summer 2017 and summer 2018 trials.

Table 1. Single-nucleotide polymorphic (SNP) markers used for background genome selection in BC₁ populations

Marker name	Chromosome	Position, Mb (SL3.0)	QTL-11
solcap_snp_sl_14323	1	3.6	B, C
solcap_snp_sl_20440	1	86.64	B, C
solcap_snp_sl_2234	1	94.80	C
solcap_snp_sl_7410	2	8.27	B
solcap_snp_sl_8464	2	39.11	C
solcap_snp_sl_12414	3	2.37	C
solcap_snp_sl_19643	3	4.96	A, B
solcap_snp_sl_7939	3	62.94	B
solcap_snp_sl_9703	3	63.44	A, C
solcap_snp_sl_21385	4	0.85	A, B
solcap_snp_sl_27167	4	3.22	A, B
solcap_snp_sl_47762	4	4.83	A, B
solcap_snp_sl_9856	4	65.79	C
solcap_snp_sl_29473	5	4.83	B, C
solcap_snp_sl_51601	5	20.45	B, C
solcap_snp_sl_231	5	64.96	B
solcap_snp_sl_1337	6	39.77	A, C
solcap_snp_sl_24454	6	44.82	C
solcap_snp_sl_11092	7	3.28	A, B
solcap_snp_sl_37097	7	59.90	B
solcap_snp_sl_5861	7	63.77	A, B, C
solcap_snp_sl_7025	7	65.37	A, B, C
solcap_snp_sl_14530	8	0.71	C
solcap_snp_sl_4374	8	52.58	A, B, C
solcap_snp_sl_7305	8	56.0	A, B
solcap_snp_sl_22830	9	2.45	A, B, C
solcap_snp_sl_30515	10	3.01	A, B, C
solcap_snp_sl_2671	11	55.15	B
solcap_snp_sl_12656	12	1.97	A, B, C
solcap_snp_sl_19345	12	62.81	B, C

Bacterial strains, inoculum preparation, and inoculation. Five *Xanthomonas* strains (*X. euvesicatoria* race T1 [Xcv110C and Xcv767], *X. gardneri* [SM775-12 and SM605-11], and *X. perforans* race T3 [Xcv761]) were used. Strains were grown separately on nutrient yeast broth agar media at 28°C for 48 to 72 h and subsequently resuspended in autoclaved ddH₂O. The optical density of each suspension was measured and standardized to a 10-mm path length at an absorbance of 600 nm = 0.15 (~3.0 × 10⁸ CFU/ml). *Xanthomonas* strains from the same species were then mixed to obtain a uniform concentration. Suspensions were sprayed to runoff with a compressed air sprayer (Preval sprayer) in a greenhouse. Inoculum was prepared and sprayed ~1 week before field transplanting.

Disease evaluation. Disease ratings were conducted on a per plot basis, each having eight plants of the same genotype in 10-foot plots. The severity of bacterial spot was measured using the Horsfall–Barratt scale (Horsfall and Barratt 1945), where 1 = no disease present and 12 = complete defoliation. Susceptible and resistant controls were used to monitor the progression of the disease over the growing season. Two ratings for each field were conducted at time points corresponding to when 80% of the fruits in plots were at the mature green (early) or ripe fruit (late) stage of maturity as described previously (Robbins et al. 2009).

Experimental design. Field trials of germplasm with distinct QTL-11 introgressions were conducted in summer 2016, 2017, and 2018. Three separate fields were used in each year, and each field had backcross-derived families (2016), lines (2017), or NILs (2018); QTL-11 resistant parents; and susceptible control OH88119. Fields were inoculated with *X. perforans*, *X. euvesicatoria*, or *X. gardneri*. In 2016 and 2017, plots were evaluated for disease severity using a randomized complete block design (RCBD) with three blocks and eight-plant plots. In 2016, several BC₁S₂ families were advanced from each QTL-11 introgression, with each family having individuals grouped by resistant and susceptible QTL introgressions. In 2017, BC₁S₄ resistant genotypes and BC₃S₁ families were evaluated in the same locations inoculated with the same pathogens. BC₃S₁ families were selected and grouped based on homozygosity and heterozygosity for markers flanking the QTL introgressions. During 2016 and 2017, fields containing *X. perforans* and *X. gardneri* were located in Wooster, Ohio, and the *X. euvesicatoria* trial was at The Ohio State University's North Central Agricultural Research Station (NCARS) in Fremont, Ohio. In 2018, BC₃S₃ NILs were evaluated for disease severity in an RCBD with four blocks and eight-plant plots in three fields. *X. euvesicatoria* and *X. gardneri* trials were located in Wooster, Ohio, and the *X. perforans* trial was located at NCARS in Fremont, Ohio.

Data analysis. The data for QTL-11 BC₁S₂, BC₁S₄, and BC₃S₁ populations were analyzed independently each year using the fixed effect linear model for analysis of variance (ANOVA):

$$y_{ijk} = \mu + Q_i + F_j + B_k + e_{ijk}$$

where y_{ijk} is the disease rating for the i th introgression within QTL-11 introgression (Q_i) for the j th family within QTL-11 introgression (F_j) in the k th block (B_k). The family term, F_j , was dropped from the model in the analysis of data for BC₃S₃ populations, because individual selections were advanced for each QTL-11 introgression and genetic backgrounds were uniform. Before conducting ANOVA, the disease rating data for individual plots were converted to mean percentages (Bock et al. 2009; Redman et al. 1964). Data for 24 comparisons were then evaluated for normality of the residuals and homogeneity of the variance using the Horsfall–Barratt scale, estimated mean percentages, and estimated mean percentages with an arcsin transformation. Data were tested using the Shapiro–Wilks test and Bartlett test in R using the functions “shapiro.test” and “bartlett.test” (R Core Team 2017). Tukey's honest significant difference (HSD; $\alpha = 0.05$) was used after a significant F test in ANOVA to examine individual introgression effects. Statistical analyses were conducted using the linear model function “lm” in the R Core Package and Tukey's HSD function

“HSD.test” in the agricolae package using R version 3.4.1 (de Mendiburu 2017; R Core Team 2017).

Results

Background genome selection and recovery of recurrent parent. Genome-wide SNP markers were applied to three distinct BC₁ populations with the intent of selecting individuals with a higher percentage of OH88119 background. The average proportion of recurrent parent genome for 241 individuals across the three BC₁ populations was 76.7% (Fig. 1). Selections within each BC₁ population with at least 87.5% of the recurrent parent genome were identified. Eight, seven, and five selections were advanced for QTL-11A, QTL-11B, and QTL-11C BC₁ populations, respectively. Background genome selection allowed us to effectively select BC₁ individuals with a recurrent parent genome equivalent to a BC₂. These individuals were both self-pollinated and backcrossed for evaluation in *Xanthomonas* trials.

Inbred backcross evaluation. Disease pressure across all field trials every year was uniform and severe. Disease severity allowed us to easily discriminate between the resistant and susceptible controls, and data for backcross selections displayed a distribution that approached normal. The mean Horsfall–Barratt scales for our susceptible parent, OH88119, against *X. perforans*, *X. gardneri*, and *X. euvesicatoria* were 7.8, 8.9, and 5.7, corresponding to mean percentages of 75.7, 86.1, and 37.1%, respectively. By comparison, resistant parents rated 3.1, 3.9, and 2.1 for OH087663, corresponding to mean percentages of 5.0, 10.8, and 2.9% when challenged with *X. perforans*, *X. gardneri*, and *X. euvesicatoria*, respectively. Ratings for 01-BR-7087 were 4.0, 5.4, and 3.3, corresponding to 10.8, 28.7, and 6.9%, respectively. Horsfall–Barratt ratings for FG12-433E-43 were 3.0 for *X. perforans*, 3.1 for *X. gardneri*, and 3.9 for *X. euvesicatoria*, corresponding to 4.7, 5.5, and 9.6%, respectively.

The distribution of data and the normality of the residuals were evaluated using Horsfall–Barratt scaled data, Horsfall–Barratt scale converted to mean percentage disease, and the arcsin of the mean percentage disease. Results of this evaluation suggested that either the original Horsfall–Barratt scale or the arcsin of the percentage disease was best suited for additional analysis. For both Horsfall–Barratt scale and the arcsin of the percentage disease, 21 of 24 of the models met the assumptions of normality. In contrast, data converted to the mean percentage violated the assumptions of normality in 10 of 24 models. The general conclusions regarding rank, mean separation, and effectiveness of QTL-11 introgressions did not change significantly despite the data conversion and transformation. We, therefore, present analysis of the original Horsfall–Barratt scaled data, because they better fit the

Fig. 1. The histogram represents the distribution and proportion of OH88119 recurrent parent genome within quantitative trait loci 11 (QTL-11) BC₁ populations. Individuals from each QTL-11 population having a high percentage OH88119 were selected and advanced.

assumptions of the ANOVA and provide a more intuitive interpretation relative to the arcsin transformation of mean percentage disease.

ANOVA was used to determine significant differences between our main effects, QTL introgression, family (independent selections of QTL introgressions), and blocks (within field variation). Across most trials, significant differences were detected owing to QTL introgression at $P \leq 0.0001$ (Table 2). These effects are maintained through the breeding process as material was advanced from BC₁ families to BC₃ selected lines. Family was significant for

Table 2. Analysis of variance of Horsfall–Barratt disease ratings for *Xanthomonas gardneri*, *X. perforans*, and *X. euvesicatoria* for 2016, 2017, and 2018 field trials

Effect	DF ^a	P value	
		Early ^b	Late ^c
<i>X. gardneri</i>			
2016 BC ₁ S ₂			
Introgression (QTL source)	4	0.0202	<0.0001
Family (QTL source)	15	0.0509	<0.0001
Block	2	<0.001	0.5872
2017 BC ₁ S ₄			
Introgression (QTL source)	4	<0.0001	<0.0001
Family (QTL source)	14	<0.001	<0.0001
Block	2	0.9595	0.5964
2017 BC ₃ S ₁			
Introgression (QTL source)	4	0.0217	<0.0001
Family (QTL source)	5	0.5211	0.076
Block	2	0.6853	0.3675
2018 BC ₃ S ₃			
Introgression (QTL source)	4	0.007	<0.0001
Block	3	0.01	0.099
<i>X. perforans</i>			
2016 BC ₁ S ₂			
Introgression (QTL source)	4	<0.0001	<0.0001
Family (QTL source)	14	0.04	0.12
Block	2	0.02	0.02
2017 BC ₁ S ₄			
Introgression (QTL source)	4	<0.0001	<0.0001
Family (QTL source)	14	0.05	0.0034
Block	2	0.74	0.7465
2017 BC ₃ S ₁			
Introgression (QTL source)	4	<0.0001	<0.0001
Family (QTL source)	3	0.8408	0.8408
Block	2	0.5841	0.5841
2018 BC ₃ S ₃			
Introgression (QTL source)	4	<0.0001	<0.0001
Block	3	0.66	0.236
<i>X. euvesicatoria</i>			
2016 BC ₁ S ₂			
Introgression (QTL source)	4	NA ^d	<0.0001
Family (QTL source)	14	NA	0.042
Block	2	NA	0.35
2017 BC ₁ S ₄			
Introgression (QTL source)	4	<0.0001	0.006
Family (QTL source)	14	<0.0001	0.009
Block	2	<0.0001	0.001
2017 BC ₃ S ₁			
Introgression (QTL source)	4	0.201	0.012
Family (QTL source)	5	0.286	0.223
Block	2	0.04	0.0004
2018 BC ₃ S ₃			
Introgression (QTL source)	4	0.002	<0.0001
Block	3	0.6	0.212

^a DF, degrees of freedom.

^b P value for the first disease rating, a time point when 80% of the fruits in plots were at the mature green stage.

^c P value for the first disease rating, a time point when 80% of the fruits in plots were at the ripe fruit stage.

^d NA, not applicable, no early rating.

some BC₁ families (BC₁S₂ and BC₁S₄), but the importance of family differences decreases, becoming insignificant, as material was advanced to BC₃S₁ (Table 2). The block effect was insignificant in most trials, suggesting that fields were uniform and that environmental effects were minimal compared with genetic effects.

QTL-11A. QTL-11A from source OH087663 (tracing to Hawaii 7998) was effective against all three *Xanthomonas* species but displayed a higher level of resistance when combined with Rx4/Xv3 (Fig. 2). In 2016, both QTL-11A and QTL-11A with Rx4/Xv3 displayed strong significant differences compared with OH88119, QTL-11B, and QTL-11C NILs (Fig. 2A, E, and I). In 2017, BC₁S₄ families with QTL-11A or QTL-11A with Rx4/Xv3 were significantly more resistant compared with OH88119 (Fig. 2B, F, and J), but differences were less striking compared with other QTL-11 NILs. In 2017, BC₃S₁ QTL-11A families were significantly more resistant compared with OH88119 in *X. perforans* (Fig. 2G) and *X. euvesicatoria* (Fig. 2K) trials. However, only BC₃S₁ QTL-11A with Rx4/Xv3 displayed a strong level of resistance in the *X. gardneri* trial (Fig. 2C). In 2018 BC₃S₃, the QTL-11A combined with Rx4/Xv3 selection displayed the lowest mean disease rating comparable with QTL-11C (Fig. 2D, H, and L).

QTL-11B. QTL-11B, originating from PI 114490, was effective but provided the least control against multiple *Xanthomonas* species. In 2016, BC₁S₂ families displayed statistically significant differences for disease ratings compared with OH88119 in *X. perforans* and *X. euvesicatoria* trials (Fig. 2E and I), but they were insignificant in the *X. gardneri* trial (Fig. 2A). In 2017, BC₁S₄ families were significantly more resistant than the recurrent parent, OH88119, in the *X. gardneri* trial; however, the mean disease rating was the highest compared with QTL-11A and QTL-11C (Fig. 2B). In 2017, BC₃S₁ families were not different from the recurrent parent control in *X. euvesicatoria* and *X. gardneri* trials (Fig. 2C and K). In 2018, BC₃S₃ selections with QTL-11B introgressions displayed significantly more resistance relative to OH88119 in all trials, and they were comparable with QTL-11A but had a higher mean disease rating than QTL-11C (Fig. 2D, H, and L).

QTL-11C. Among QTL-11 NILs, QTL-11C from source LA2533 was effective across all *Xanthomonas* species and provided the most control in 2018 trials at the BC₃S₃ stage (Fig. 2). In 2016, BC₁S₂ families displayed statistically significant differences for disease ratings compared with OH88119 in *X. perforans* and *X. euvesicatoria* trials (Fig. 2E and I), although differences were insignificant for trials with *X. gardneri* (Fig. 2A). Conversely, in 2017, BC₁S₄ families were significantly different from OH88119 in the *X. gardneri* trial (Fig. 2B) and the *X. perforans* trial (Fig. 2F), but they were not significantly different in *X. euvesicatoria* trials (Fig. 2J). The nonsignificant results reflect variability of introgression performance in different family backgrounds, which decreases during the breeding process (Table 2). In 2017 and 2018, BC₃S₁ and BC₃S₃ selections displayed resistance to all *Xanthomonas* species (Fig. 2C, D, G, H, K, and L). For all trials, QTL-11C displayed a lower disease rating compared with OH88119.

Gene action for QTL introgressions and Rx-4/Xv3. Gene action was evaluated by comparing the mean disease ratings between homozygous introgressions and heterozygous genotypes within BC₃S₁ families and BC₃S₃ selections. We compared sources of the QTL-11 introgression and the additional presence of the major gene Rx4/Xv3 in some combinations. In 2017 and 2018, QTL-11B displayed no statistically significant differences for disease ratings between homozygous and heterozygous genotypes, suggesting dominance (Fig. 3). In 2017, QTL-11C displayed dominant gene action, although statistically significant differences were detected between heterozygous and homozygous resistant genotypes in 2018 trials (Fig. 3B, D, and F), suggesting that they acted with additive to dominant action (Fig. 3). In 2017 and 2018, QTL-11A displayed dominant gene action. In 2017 trials, the combination of QTL-11A and Rx-4/Xv3 also displayed a similar disease rating between homozygous and heterozygous genotypes, suggesting dominance. However, in the 2018 *X. euvesicatoria* trial, QTL-11A in combination with Rx-4/Xv3 displayed additive gene action (Fig. 3F). In 2018,

the introgression of *Rx-4/Xv3* without QTL-11A was also evaluated. In the *X. perforans* trial, lines with only *Rx-4/Xv3* performed better than the QTL-11A introgression (Fig. 3D). This significant reduction of disease severity provides field-based evidence for the effectiveness of *Rx-4/Xv3* against *X. perforans* T3.

Discussion

As sources of resistance are identified and incorporated into breeding programs and varieties, *Xanthomonas* populations have also changed (Jones et al. 1998). When these populations gain or lose effectors that interact with the host, resistance may become ineffective (Abrahamian et al. 2018; Stall et al. 2009). To combat this problem, a strategy to combine major resistance genes and quantitative resistance loci that can target multiple *Xanthomonas* species has been proposed (Sim et al. 2015). Genetic sources Hawaii 7998 (QTL-11A), PI 114490 (QTL-11B), LA2533 (QTL-11C) were previously reported as providing quantitative resistance to control bacterial spot of tomato. In all cases, a major QTL on chromosome 11 was implicated in resistance. It had been unclear which QTL-11 provided the best control against multiple *Xanthomonas* species.

This study developed nearly isogenic resources, determined the best introgression for resistance to several *Xanthomonas* species, and assessed gene action to facilitate the development of advanced breeding material. NILs were developed using a backcross strategy coupled to marker-assisted background genome selection. At the BC₁ generation, we were able to select individuals in each QTL-11 population having a BC₂ equivalent proportion of the recurrent parent genome. We then evaluated NILs in field trials against *X. gardneri*, *X. perforans* (T3), and *X. euvesicatoria*. In general, all QTL-11 selections and introgressions displayed a higher level of resistance compared with the susceptible control OH88119.

In general, we found that QTL-11C and QTL-11A combined with *Rx4/Xv3* provided the best control for all three *Xanthomonas* species

with the most advanced selections (BC₃S₃). For example, QTL-11C reduced the percentages of foliar infection to 11.4, 1.9, and 3.8% relative to OH88119, which were 79.1, 91.0, and 72.9% when challenged with *X. gardneri*, *X. perforans*, and *X. euvesicatoria*, respectively. QTL-11A combined with *Rx4/Xv3* reduced infection to 11.4, 1.2, and 5.3% when challenged with *X. gardneri*, *X. perforans*, and *X. euvesicatoria*, respectively. For QTL-11A and QTL-11B introgressions, statistically improved disease resistance was also noted, although QTL-11B displayed less efficacy against *X. gardneri* and *X. euvesicatoria* in the 2017 BC₃S₃ trials. A surprising finding was the high level of resistance against *X. gardneri* imparted by the combination of the gene *Rx4/Xv3* and the QTL-11A introgression. By itself, *Rx4/Xv3* provides resistance against *X. perforans* race T3 but not against *X. gardneri*. QTL-11A provides moderate resistance against *X. gardneri*. However, the combination of *Rx4/Xv3* and QTL-11A displayed the strongest level of resistance to *X. gardneri*. Additionally, QTL-11A in combination with *Rx4/Xv3* displayed strong resistance against *X. euvesicatoria*. The mechanism of this resistance is unclear, because *X. gardneri* and *X. euvesicatoria* lack an AvrXv3 effector (Timilsina et al. 2016). We prefer not to speculate on the underlying biology of this enhanced resistance given the tendency of tomato resistance genes to be clustered and the large number of genes underlying the coupling phase recombination (Andolfo et al. 2014).

For gene action comparisons, all QTL-11 introgressions displayed additive to dominant effects for resistance. In 2017, gene effects seemed dominant for all three QTL introgressions. In contrast, in 2018, QTL-11A and QTL-11B again displayed dominant gene action, whereas QTL-11C displayed additive effects. It is unclear whether these differences reflect distinct mechanism for each QTL source or variability in the evaluation data. From a breeding standpoint, this information provides knowledge important for the development of hybrid lines.

Fig. 2. Boxplots illustrate the average disease rating and distribution for each of the quantitative trait loci 11 (QTL-11) introgressions. Different letters above each boxplot represent significant differences between genotypes at $P = 0.05$ using the Tukey's honest significant difference test. Data are shown for **A to D**, *Xanthomonas gardneri*, **E to H**, *X. perforans*, and **I to L**, *X. euvesicatoria* at different stages in the development of near-isogenic lines (**A**, **E**, and **I**, BC₁S₂; **B**, **F**, and **J**, BC₁S₄; **C**, **G**, and **K**, BC₃S₁; and **D**, **H**, and **L**, BC₃S₃).

Fig. 3. Boxplots illustrate the average disease rating and distribution for each of the quantitative trait loci 11 (QTL-11) introgressions, heterozygous genotypes, and near-isogenic lines (NILs) lacking QTL-11 introgressions. A only, B, and C refer to NILs that have only a QTL-11 introgression in the homozygous state. A only Het, B Het, and C Het refer to NILs having a QTL-11 introgression in the heterozygous state. A(+)/Xv3 refers to NILs that has both QTL-11A and Xv3 in the homozygous state, and A(+)/Xv3 refers to NILs having both loci in the heterozygous state. Xv3 only refers to an NIL that only contains Xv3 without QTL-11. Different letters above each boxplot represent significant differences between genotypes at $P = 0.05$ using the Tukey's honest significant difference test. Data are shown for **A and B**, *Xanthomonas gardneri*, **C and D**, *X. perforans*, and **E and F**, *X. euvesicatoria* at different stages in the development of NILs (**A, C, and E**, BC₃S₁ and **B, D, and F**, BC₃S₃).

Developing tomato cultivars with genetic resistance to bacterial spot has been an ongoing focus for many years. Breeding for resistance to bacterial spot is difficult owing to the diversity of *Xanthomonas* species, weather conditions that promote disease, and the time that it takes to develop and release new varieties. It is important that new sources of resistance are constantly explored and evaluated against present *Xanthomonas* populations. The nearly isogenic resources described here suggest that some QTL introgressions provide effective resistance against multiple *Xanthomonas* species. These resources can be developed rapidly using MAS and background genome selection, allowing for new loci or combinations to be evaluated rapidly. Furthermore, combinations of specific resistance genes and QTL may enhance resistance.

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Literature Cited

Abrahamian, P., Timilsina, S., Minsavage, G. V., KC, S., Goss, E. M., Jones, J. B., and Vallad, G. E. 2018. The type III effector AvrBsT enhances

Xanthomonas perforans fitness in field-grown tomato. *Phytopathology* 108:1355-1362.

Andolfo, G., Jupe, F., Witek, K., Etherington, G. J., Ercolano, M. R., and Jones, J. D. 2014. Defining the full tomato NB-LRR resistance gene repertoire using genomic and cDNA RenSeq. *BMC Plant Biol.* 14:120.

Berry, S. Z., Aldrich, T. S., Wiese, K. L., and Bash, W. D. 1995. 'Ohio OX38' hybrid processing tomato. *Hortic. Sci.* 30:159.

Blanca, J., Montero-Pau, J., Sauvage, C., Bauchet, G., Illa, E., Díez, M. a. J., Francis, D., Causse, M., van der Knaap, E., and Cañizares, J. n. 2015. Genomic variation in tomato, from wild ancestors to contemporary breeding accessions. *BMC Genomics* 16:257.

Bock, C. H., Gottwald, T. R., Parker, P. E., Cook, A. Z., Ferrandino, F., Parnell, S., and van den Bosch, F. 2009. The Horsfall-Barratt scale and severity estimates of citrus canker. *Eur. J. Plant Pathol.* 125:23-38.

de Mendiburu, F. 2017. *Agricolae: Statistical Procedures for Agricultural Research*. <https://cran.r-project.org/web/packages/agricolae/index.html>

Horsfall, J. G., and Barratt, R. 1945. An improved grading system for measuring plant diseases. *Phytopathology* 35:655.

Hospital, F. 2009. Challenges for effective marker-assisted selection in plants. *Genetica* 136:303-310.

Hutton, S. F., Scott, J. W., Yang, W., Sim, S.-C., Francis, D. M., and Jones, J. B. 2010. Identification of QTL associated with resistance to bacterial spot race T4 in tomato. *Theor. Appl. Genet.* 121:1275-1287.

- Jones, J. B., Bouzar, H., Somodi, G. C., Stall, R. E., Pernezny, K., El-Morsy, G., and Scott, J. W. 1998. Evidence for the preemptive nature of tomato race 3 of *Xanthomonas campestris* pv. *vesicatoria* in Florida. *Phytopathology* 88:33-38.
- Liabeuf, D. 2016. Development of processing tomato lines resistant to *Xanthomonas gardneri*: From screening to breeding. PhD thesis. Ohio State University, Columbus, OH.
- Liabeuf, D., Francis, D. M., and Sim, S. 2015. Screening cultivated and wild tomato germplasm for resistance to *Xanthomonas gardneri*. *Acta Hort.* 1069:65-70.
- Liao, Y. Y., Strayer-Scherer, A. L., White, J., Mukherjee, A., De La Torre-Roche, R., Ritchie, L., Colee, J., Vallad, G. E., Freeman, J. H., Jones, J. B., and Paret, M. L. 2019. Nano-magnesium oxide: A novel bactericide against copper-tolerant *Xanthomonas perforans* causing tomato bacterial spot. *Phytopathology* 109:52-62.
- Louws, F. J., Wilson, M., Campbell, H. L., Cuppels, D. A., Jones, J. B., Shoemaker, P. B., Sahin, F., and Miller, S. A. 2001. Field control of bacterial spot and bacterial speck of tomato using a plant activator. *Plant Dis.* 85:481-488.
- Marco, G., and Stall, R. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. *Plant Dis.* 67:779-781.
- Pei, C., Wang, H., Zhang, J., Wang, Y., Francis, D. M., and Yang, W. 2012. Fine mapping and analysis of a candidate gene in tomato accession PI128216 conferring hypersensitive resistance to bacterial spot race T3. *Theor. Appl. Genet.* 124:533-542.
- Potnis, N., Timilsina, S., Strayer, A., Shanharaj, D., Barak, J. D., Paret, M. L., Vallad, G. E., and Jones, J. B. 2015. Bacterial spot of tomato and pepper: Diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Mol. Plant Pathol.* 16:907-920.
- R Core Team. 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Redman, C. E., King, E. P., Brown, I. F.; Lilly Research Laboratories; and Elanco Products Company. 1964. Tables for Converting Barratt and Horsfall Rating Scores to Estimated Mean Percentages. Eli Lilly and Co., Indianapolis, IN.
- Robbins, M. D., Darrigues, A., Sim, S. C., Masud, M. A., and Francis, D. M. 2009. Characterization of hypersensitive resistance to bacterial spot race T3 (*Xanthomonas perforans*) from tomato accession PI 128216. *Phytopathology* 99:1037-1044.
- Scott, J., Stall, R., Jones, J., and Somodi, G. 1996. A single gene controls the hypersensitive response of Hawaii 7981 to race 3 (T3) of the bacterial spot pathogen. *Tomato Genet. Coop.* 46:23.
- Scott, J. W., Francis, D. M., Miller, S. A., Somodi, G. C., and Jones, J. B. 2003. Tomato bacterial spot resistance derived from PI 114490; inheritance of resistance to race T2 and relationship across three pathogen races. *J. Am. Soc. Hortic. Sci.* 128:698-703.
- Scott, J. W., Jones, J. B., and Somodi, G. C. 2001. Inheritance of resistance in tomato to race T3 of the bacterial spot pathogen. *J. Am. Soc. Hortic. Sci.* 126:436-441.
- Scott, J. W., Jones, J. B., Somodi, G. C., and Stall, R. E. 1995. Screening tomato accessions for resistance to *Xanthomonas campestris* pv. *vesicatoria*, race T3. *HortScience* 30:579-581.
- Semagn, K., Babu, R., Hearne, S., and Olsen, M. 2014. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): Overview of the technology and its application in crop improvement. *Mol. Breed.* 33:1-14.
- Sharlach, M., Dahlbeck, D., Liu, L., Chiu, J., Jiménez-Gómez, J. M., Kimura, S., Koenig, D., Maloof, J. N., Sinha, N., Minsavage, G. V., Jones, J. B., Stall, R. E., and Staskawicz, B. J. 2013. Fine genetic mapping of *RXopJ4*, a bacterial spot disease resistance locus from *Solanum pennellii* LA716. *Theor. Appl. Genet.* 126:601-609.
- Sim, S.-C., Durstewitz, G., Plieske, J., Wieseke, R., Ganai, M. W., Van Deynze, A., Hamilton, J. P., Buell, C. R., Causse, M., and Wijeratne, S. 2012. Development of a large SNP genotyping array and generation of high-density genetic maps in tomato. *PLoS One* 7:e40563.
- Sim, S.-C., Robbins, M. D., Wijeratne, S., Wang, H., Yang, W., and Francis, D. M. 2015. Association analysis for bacterial spot resistance in a directionally selected complex breeding population of tomato. *Phytopathology* 105:1437-1445.
- Stall, R. E., Jones, J. B., and Minsavage, G. V. 2009. Durability of resistance in tomato and pepper to xanthomonads causing bacterial spot. *Phytopathology* 47:265-284.
- Timilsina, S., Abrahamian, P., Potnis, N., Minsavage, G. V., White, F. F., Staskawicz, B. J., Jones, J. B., Vallad, G. E., and Goss, E. M. 2016. Analysis of sequenced genomes of *Xanthomonas perforans* identifies candidate targets for resistance breeding in tomato. *Phytopathology* 106:1097-1104.
- Wang, H., Hutton, S. F., Robbins, M. D., Sim, S. C., Scott, J. W., Yang, W., Jones, J. B., and Francis, D. M. 2011. Molecular mapping of hypersensitive resistance from tomato 'Hawaii 7981' to *Xanthomonas perforans* race T3. *Phytopathology* 101:1217-1223.
- Wang, J. F., Jones, J. B., Scott, J. W., and Stall, R. E. 1994. Several genes in *Lycopersicon esculentum* control hypersensitivity to *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology* 84:702-706.
- Whalen, M. C., Wang, J. F., Carland, F. M., Heiskell, M. E., Dahlbeck, D., Minsavage, G. V., Jones, J. B., Scott, J. W., Stall, R. E., and Staskawicz, B. J. 1993. Avirulence gene *avrRxv* from *Xanthomonas campestris* pv. *vesicatoria* specifies resistance on tomato line Hawaii 7998. *Mol. Plant-Microbe Interact.* 6:616-627.
- Yang, W., and Francis, D. M. 2005. Marker-assisted selection for combining resistance to bacterial spot and bacterial speck in tomato. *J. Am. Soc. Hortic. Sci.* 130:716-721.
- Yang, W., Miller, S., Scott, J., Jones, J., and Francis, D. 2005. Mining tomato genome sequence databases for molecular markers: Application to bacterial resistance and marker assisted selection. *Acta Hort.* 695:241-250.
- Yu, Z. H., Wang, J. F., Stall, R. E., and Vallejos, C. E. 1995. Genomic localization of tomato genes that control a hypersensitive reaction to *Xanthomonas campestris* pv. *vesicatoria* (Doidge) dye. *Genetics* 141:675-682.