Resistance to Crown and Root Rot Caused by *Phytophthora capsici* in a Tomato Advanced Backcross of *Solanum habrochaites* and *Solanum lycopersicum*

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Abstract

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Phytophthora capsici causes devastating disease on many vegetable crops, including tomato and other solanaceous species. *Solanum habrochaites* accession LA407, a wild relative of cultivated tomato, has shown complete resistance to four *P. capsici* isolates from Michigan cucurbitaceous and solanaceous crops in a previous study. Greenhouse experiments were conducted to evaluate 62 lines of a tomato inbred backcross population between LA407 and the cultivated tomato 'Hunt 100' and 'Peto 95-43' for resistance to two highly virulent *P. capsici* isolates. Roots of 6-week-old seedlings were inoculated with each of two *P. capsici* isolates and maintained in the greenhouse. Plants were evaluated for wilting and plant

death three times per week for 5 weeks. Significant differences were observed in disease response among the inbred tomato lines. Most lines evaluated were susceptible to *P. capsici* isolate 12889 but resistant to isolate OP97; 24 tomato lines were resistant to both isolates. Heritability of *Phytophthora* root rot resistance was high in this population. Polymorphic molecular markers located in genes related to resistance and defense responses were identified and added to a genetic map previously generated for the population. Resistant lines and polymorphic markers identified in this study are a valuable resource for development of tomato varieties resistant to *P. capsici*.

Phytophthora capsici Leonian is a destructive, soilborne pathogen that has a wide host range within the Solanaceae, including tomato, eggplant, and pepper (Granke et al. 2012b). In tomato (*Solanum lycopersicum* L.), a major vegetable crop grown in every country of the world, *P. capsici* causes root, crown, and fruit rot (Granke et al. 2012b; Quesada-Ocampo et al. 2011b). *P. capsici*-induced disease in tomato has been reported in several regions of the United States, including Michigan (Grube et al. 2000), California (Bolkan 1985; Critopoulos 1955; Satour and Butler 1967), and Colorado (Kreutzer et al. 1940). In recent years, the occurrence and severity of *P. capsici* infections in different crops in the United States has increased, in part, due to the use of *P. capsici*-infested water for irrigation (Hausbeck and Lamour 2004; Quesada-Ocampo et al. 2011a).

Commonly used approaches to manage *P. capsici* have included crop rotation and fungicide applications. The success of crop rotation is limited by the long-term survival of oospores in the soil and an increasing list of susceptible hosts (Geuens et al. 2008; Kousik et al. 2015; Quesada-Ocampo et al. 2009). Similarly, applications of the commonly used fungicide mefenoxam may not provide adequate control due to resistance in *P. capsici* populations throughout the United States (Quesada-Ocampo et al. 2011b). In the United States, tomato fruit are grown for the fresh-market and processing industry. Fresh-market tomato are grown using raised beds, plastic mulch, and

trellising that reduce the risk of *P. capsici* root and crown rot due to poor water drainage and the risk of fruit rot due to splashing or direct contact with the soil. In contrast, the production system of processing tomato, harvested mechanically, uses lower beds where water drainage may not be optimal and no mulch, so that fruit are in direct contact with the soil. Although disease caused by *P. capsici* is not a significant challenge in fresh-market tomato production, processing tomato growers have experienced severe losses when inoculum is available and weather conditions have been warm and wet. With the lack of affordable and dependable chemical control measures and limitations of cultural control strategies, developing resistant varieties is desirable. However, little is known about resistance to *P. capsici* in cultivated tomato (Quesada-Ocampo and Hausbeck 2010).

The limited genetic diversity following domestication in cultivated tomato has led researchers to use wild tomato species as sources of disease resistance (Rick and Yoder 1988). One example is *S. habrochaites*, a wild tomato that has been used to produce F₁ interspecific hybrids, backcrosses (BC₁), and advanced backcrosses (BC₂) used in tomato breeding programs (Kabelka et al. 2002). Developing disease-resistant tomato cultivars by introgression of resistance genes from wild relatives has provided effective disease control for some tomato diseases (Finkers et al. 2007). In recent studies, *S. habrochaites* accession LA407 showed resistance to four *P. capsici* isolates from cucurbitaceous and solanaceous crops (Quesada-Ocampo and Hausbeck 2010). Nonetheless, segregating tomato populations derived from wild relatives have not been evaluated for resistance to *P. capsici* to assess their potential for tomato improvement. In addition, available populations that have been evaluated for disease resistance to other tomato pathogens have limited molecular markers available to establish marker-trait associations (Kabelka et al. 2002). Such associations can be used for marker-assisted selection, allowing plant breeders to more quickly identify varieties with desirable agricultural traits (Robert et al. 2001). In this study, we aimed to evaluate a tomato breeding population as a genetic resource for crown and root rot resistance caused by *P. capsici*. The population includes 62 lines of a tomato inbred advanced backcross (IBC) population (IBC₂S₄) developed by crossing *S. habrochaites* accession LA407 to *S. lycopersicum* 'Hunt100' and 'Peto 95-43', two cultivated accessions with characteristics of contemporary processing varieties (Kabelka et al. 2002). A genetic map has

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also been generated for this tomato population that could facilitate breeding for traits such as disease resistance (Kabelka et al. 2002). Specifically, we sought to (i) determine which lines of the backcross are resistant to *P. capsici* and the heritability of the trait, (ii) add markers related to disease resistance and defense responses to the genetic map of the backcross population, and (iii) investigate associations between introgressions from the wild parent and resistance to *P. capsici*.

Materials and Methods

Plant material. A segregating population consisting of 62 homozygous lines was used in this study (Table 1). The LA407 IBC population consists of 62 BC₂S₄ lines developed by crossing *S. habrochaites* LA407 to *S. lycopersicum* Hunt100 (LA3144) followed by a backcross to Hunt100 (Francis et al. 2001). Individual BC₁ lines were then crossed to Peto 95-43 (LA3528); BC₂ lines were then advanced by self-fertilization and single-seed descent for four generations (Francis et al. 2001). Each line in the LA407 IBC population has a theoretical *S. lycopersicum* contribution of 87.5% and random introgressions from LA407 accounting for 12.5% (Kabelka et al. 2002).

Seed of the 62 inbred lines and the parents LA407 and LA3144 were treated with 2.7% sodium hypochlorite for 30 min, rinsed with sterile water, and immediately sown on 72-square cell plastic flats (Hummert International, Earth City, MO) containing soilless medium (Baccto Professional Planting Mix; Michigan Peat Company, Houston) and a top layer of medium-grade vermiculite (Hummert International). Seedlings were grown for 4 weeks in a greenhouse under approximately 14 h/day illumination and transplanted into 2.5-liter square plastic pots (Hummert International) containing the same soilless medium described above. Plants were allowed to recover from transplant stress for 2 weeks prior to being inoculated with millet seed infested with one of two different *P. capsici* isolates and a control inoculum consisting of uninfested millet seed. For the duration of the experiment, plants were fertilized weekly with Peter's 20-20-20 water-soluble fertilizer (The Scotts Company, Marysville, OH) and irrigated as needed.

Isolate selection and maintenance. *P. capsici* isolates that were obtained from infected cucurbit and solanaceous crops in Michigan were selected for evaluations. Isolate OP97 (A1 mating type [MT]), from pickling cucumber, is sensitive to mefenoxam, whereas isolate 12889 (A1 MT) from pepper, is insensitive. Isolates were phenotypically characterized for MT, sensitivity to mefenoxam (Quesada-Ocampo et al. 2009), morphology (Granke et al. 2011), virulence (Granke et al. 2012a), and genetic identity (Quesada-Ocampo et al. 2011b) in previous studies.

Actively growing, single-spore cultures were propagated on unclarified V8 agar (UCV8A; 3 g of CaCO₃, 15 g of agar, 160 ml of unfiltered V8 juice, and 840 ml of distilled water) following long-term storage as stock cultures and activated by reinoculation and re-isolation from cucumber fruit to maintain virulence, as previously described (Quesada-Ocampo et al. 2009).

Inoculum preparation and root inoculation. *P. capsici*-infested millet seed (100 g of millet seed, 0.08 g of asparagine, and 72 ml of water) was prepared as previously described (Quesada-Ocampo and Hausbeck 2010). Plants were inoculated by mixing 1 g of millet seed infested with one of the two *P. capsici* isolates (OP97 or 12889) with approximately 10 g of soilless medium that was removed from the area adjacent to each plant stem. For each isolate, 10 replicate plants were used. Ten additional control plants were inoculated with millet seed containing sterile UCV8 plugs. The entire experiment was replicated three times.

Resistance screening. Disease assessment was conducted three times per week for 5 weeks and was initiated 2 days following inoculation on each line and parents. Disease progression was recorded for each plant as percent wilting, as previously described (Quesada-Ocampo and Hausbeck 2010). The area under the disease progress curve (AUDPC) was calculated using percent wilting values and data followed assumptions for statistical analyses. The experiment was designed as a split-plot, with whole plots arranged in a randomized complete block design. Because no differences were detected among the three experiments, combined data were subjected to analysis of

variance using the PROC MIXED and PROC GLIMMIX procedures of SAS (SAS Institute Inc., Cary, NC), and multiple comparisons among the means were conducted using Tukey's test when effects were found to be statistically significant at $P < 0.05$. Plants were considered resistant to an isolate of *P. capsici* when their AUDPC value was lower than or not significantly different from the value of the resistant parent LA407.

Plant DNA extraction and purification. DNA was extracted from three young healthy leaves of each line and parent using the DNeasy plant mini kit, following the manufacturer's instructions (Qiagen, Valencia, CA). The DNA concentration for each sample was determined using a NanoDrop 100 spectrophotometer and the NanoDrop software (ver. 2.4.7c; Nano-Drop Technologies Inc., Wilmington, DE). A genomic DNA concentration of 15 ng/μl was used for amplification of simple sequence repeats (SSR) and resistance gene analogs for single-nucleotide polymorphism (SNP) detection, while 100 ng/μl was used to obtain amplified fragment length polymorphisms (AFLP).

Molecular markers. To map additional markers to a previously published linkage map of the tomato population (Kabelka et al. 2002), four different types of markers were tested for polymorphism: 70 AFLP, 63 restriction fragment length polymorphism (RFLP) (Francis et al. 2001), 64 SNP (Supplementary Table S1), and 106 SSR (Supplementary Table S2). All molecular markers were first tested in the parents to confirm polymorphism between them. After parent evaluations, all AFLP, SNP, and SSR without polymorphism were discarded for progeny evaluations. The RFLP analysis for the tomato population was previously published (Kabelka et al. 2002) and the data were used to generate a framework map to add the markers genotyped in this study.

AFLP analysis. Approximately 500 ng of DNA was used with the AFLP kit for regular plant genomes (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. After preselective amplification, *MseI* CAC-*EcoRI* ACA, *MseI* CAG-*EcoRI* AAG, *MseI* CAC-*EcoRI* AAG, *MseI* CAA-*EcoRI* AAC, *MseI* CAA-*EcoRI* AAG, *MseI* CAC-*EcoRI* AAC, and *MseI* CAA-*EcoRI* ACA primer combinations were chosen for selective polymerase chain reaction (PCR). Labeled products, with a size equal to or larger than 70 bp and with a fluorescence signal equal to or higher than 300 relative fluorescence units in at least one parent, were scored using the Peak Scanner software (ver. v1.0; Applied Biosystems).

SNP analysis. Transcripts shared by *S. habrochaites* (LA1777) and *S. lycopersicum* (TA496), annotated as resistance or defense genes and containing SNP, were identified in release 161a of the Solanaceae Genomics Resource (<http://solanaceae.plantbiology.msu.edu/>) and selected for primer design using Primer 3 v.0.4.0 (Rozen and Skaletsky 2000). In total, 64 pairs of primers were designed to amplify a product with an SNP located approximately in the middle of the fragment. Transcripts were amplified and sequenced in LA407 and Hunt100 to confirm SNP. Restriction enzymes that selectively digested at the SNP site in one parent but not the other were identified with CLC DNA workbench software (v5.7.1; CLC Bio, Cambridge, MA). Briefly, each PCR was performed in a 15-μl volume consisting of 10 μM each dNTP, 2.5 mM MgCl₂, 0.5 U of Taq DNA polymerase (Promega Corp., Madison, WI), 3 μl of 10× buffer, 0.4 μM each primer, and 15 ng of genomic DNA that was used as a template. The enzyme digestion was performed in a 15-μl volume with 4.5 μl of PCR product, 1× enzyme buffer, 1× bovine serum albumen, and 1 U of restriction enzyme (New England Biolabs, Ipswich, MA). The PCR program was 5 min at 94°C for an initial denaturation; 35 cycles of 30 s at 94°C, 45 s at 50 to 57°C, and 45 s at 72°C; followed by a final extension at 72°C by 5 min. Digestions were performed for 1 h at 37°C and 5 min at 10°C. Digested products were evaluated in high-resolution 2% metaphor agarose gels (Lonza, Rockland, ME) and scored in the parents and progeny as present or absent.

SSR analysis. *S. habrochaites* and *S. lycopersicum* transcripts annotated as resistance or defense genes and containing SSR were identified in release 161a of the Solanaceae Genomics Resource and selected for primer design using Primer 3. Nine SSR derived from expressed sequence tags of *Capsicum annuum* and obtained at the

Table 1. Mean area under the disease progress curve (AUDPC) values, percent death, disease reaction, and LA407 introgression percentage of each IBC₂S₄ line evaluated for resistance to *Phytophthora capsici*

ID ^z	Mean AUDPC ^x		Death (%)		DR ^y		LA407 (%)
	12889	OP97	12889	OP97	12889	OP97	
LA407	13.6 wxyzABCD	8.8 DE	10.5	6.8	R	R	...
HUNT100	95.7 ab	71.2 hijkl	73.6	54.7	S	S	...
2304	32.2 pq	12.9 yzABCD	24.7	10.0	S	R	14.1
2305	31.5 pqr	12.5 yzABCD	24.2	9.6	S	R	11.2
2306	15.8 vwxyzABCD	13.0 xyzABCD	20.0	12.2	R	R	14.6
2307	30.8 pqrs	21.0 qrstuvwxyzABC	23.7	16.2	S	S	28.4
2309	20.3 rstuvwxyzABCD	0.0 E	15.6	0.0	R	R	22.8
2310	83.6 cdefg	13.2 wxyzABCD	64.3	10.1	S	R	18.6
2311	13.4 wxyzABCD	12.1 ABCD	10.3	9.3	R	R	26.6
2313	60.6 lmn	0.0 E	46.6	0.0	S	R	1.4
2314	62.1 klmn	0.0 E	47.7	0.0	S	R	6.7
2315	79.9 cdefgh	0.0 E	61.5	0.0	S	R	13.5
2316	83.3 cdefg	0.0 E	64.1	0.0	S	R	5.7
2317	99.4 a	56.2 mno	76.5	43.2	S	S	8.2
2318	0.0 E	0.0 E	0.0	0.0	R	R	14.2
2319	0.0 E	0.0 E	0.0	0.0	R	R	14.5
2321	91.5 abc	20.8 qrstuvwxyzABC	70.4	16.0	S	S	9.7
2322	60.3 lmn	14.9 vwxyzABCD	46.4	11.5	S	R	18.3
2323	26.6 pqrstuv	0.0 E	20.4	0.0	S	R	23.7
2325	74.3 fghij	0.0 E	57.1	0.0	S	R	6.9
2326	33.4 p	0.0 E	25.7	0.0	S	R	15.7
2327	86.7 bcde	24.8 pqrstuvw	66.7	79.1	S	S	14.1
2328	11.8 ABCDE	0.0 E	9.1	0.0	R	R	12.9
2330	85.4 bcdef	0.0 E	65.7	0.0	S	R	17.4
2331	23.2 pqrstuvwxyza	0.0 E	17.9	0.0	R	R	11.3
2332	14.1 ABCD	10.9 BCDE	12.3	8.4	R	R	5.9
2333	14.6 wxyzABCD	0.0 E	11.2	0.0	R	R	16.6
2334	19.9 rstuvwxyzABCD	17.1 uvwxyzABCD	15.3	13.1	R	R	13.4
2335	13.9 wxyzABCD	21.3 qrstuvwxyzABC	10.7	16.4	R	S	8.6
2336	30.7 pqrst	0.0 E	23.6	0.0	S	R	10.5
2337	62.9 jklmn	24.0 pqrstuvwxyz	48.4	18.5	S	S	3.9
2338	12.4 yzABCD	0.0 E	9.6	0.0	R	R	22.5
2339	13.7 wxyzABCD	14.9 vwxyzABCD	10.6	11.5	R	R	12.2
2340	82.6 cdefgh	19.5 stuvwxyzABCD	63.5	15.0	S	R	15.1
2341	12.3 yzABCD	0.0 E	9.5	0.0	R	R	14.1
2342	22.2 pqrstuvwxyzaB	0.0 E	17.1	0.0	R	R	15.2
2343	70.8 hijkl	12.5 yzABCD	54.4	9.6	S	R	22.4
2344	89.4 abcd	9.9 CDE	68.8	7.6	S	R	15.6
2345	52.0 no	0.0 E	40.0	0.0	S	R	9.2
2346	90.8 abcd	72.4 ghijk	69.8	55.7	S	S	8.3
2347	0.0 E	0.0 E	0.0	0.0	R	R	16.1
2348	89.3 abcd	79.3 defgh	68.7	61.0	S	S	11.9
2349	73.1 ghijk	17.9 uvwxyzABCD	56.2	13.8	S	R	8.1
2350	21.8 pqrstuvwxyzaB	0.0 E	16.8	0.0	R	R	10.7
2351	17.7 uvwxyzABCD	0.0 E	13.6	0.0	R	R	12.5
2352	88.9 abcd	13.7 wxyzABCD	68.4	10.5	S	R	8.4
2353	0.0 E	0.0 E	0.0	0.0	R	R	10.3
2354	75.3 efghi	10.8 BCDE	57.9	8.3	S	R	5.6
2355	18.9 zABCD	12.3 tuvxyzABCD	14.6	9.4	R	R	5.7
2356	16.4 vwxyzABCD	0.0 E	12.6	0.0	R	R	23.4
2357	24.1 pqrstuvwxy	0.0 E	18.5	0.0	R	R	9.6
2358	46.9 o	23.6 pqrstuvwxyza	36.1	18.1	S	S	12.0
2359	32.3 pq	20.5 qrstuvwxyzABCD	24.8	15.8	S	R	11.1
2360	48.3 o	0.0 E	37.2	0.0	S	R	13.4
2361	0.0 E	0.0 E	0.0	0.0	R	R	14.4
2362	21.2 qrstuvwxyzABC	24.8 pqrstuvw	16.3	19.1	R	S	12.2
2363	0.0 E	0.0 E	0.0	0.0	R	R	13.6
2364	55.9 mno	17.7 uvwxyzABCD	43.0	13.6	S	R	18.3
2365	71.2 hijkl	13.7 wxyzABCD	54.8	10.6	S	R	32.3
2366	16.8 uvwxyzABCD	21.2 qrstuvwxyzABC	12.9	16.3	R	S	11.1
2367	28.3 pqrstu	21.2 qrstuvwxyzABC	21.8	16.3	S	S	10.1
2368	66.7 ijklm	16.3 vwxyzABCD	51.3	12.5	S	R	22.1
2369	81.9 cdefgh	0.0 E	63.0	0.0	S	R	14.6
2370	13.2 E	0.0 wxyzABCD	10.1	0.0	R	R	25.9

^x Mean AUDPC values for each isolate followed by the same letter are not significantly different.

^y Disease reaction (DR): lines with no significant differences in mean AUDPC values with LA407 or with lower mean AUDPC values were considered resistant (R) and all other lines were considered susceptible (S).

^z Genotype identification number.

Sol Genomics Network website (<https://solgenomics.net/>) were also included for primer design using Primer 3. PCR assays were performed as described above. Products were evaluated in high-resolution 3% metaphor agarose gels (Lonza) and scored in the parents and progeny as present or absent.

Heritability analysis and genetic map construction. Heritability for resistance to each isolate was estimated using the mean squares implemented within a formula previously described (Fehr 1987). For disease data, the experimental repetitions were treated as a single location with multiple dates. Confidence intervals (CI) were calculated as previously described (Knapp et al. 1985). Linkage groups were generated using 152 polymorphic markers (63 RFLP, 48 AFLP, 20 SSR, and 21 SNP), a linkage criterion of $P = 0.05$, and the Kosambi mapping function with the software Map Manager QTXb20.1 (Manly et al. 2001). Local permutations of the order of markers within a chromosome (ripple function) were performed to find the best distance between loci. The informative loci, genotype frequencies, number of crossovers, map distance, confidence limit (low and high according with the 95% CI for map distance), log of the odds (LOD) for linkage, and coverage in centimorgans were determined for each locus within a chromosome. Quantitative trait loci (QTL) analyses were not performed due to the limited size of the tomato population.

Introgression analysis. The program STRUCTURE (version 2.3.4) (Pritchard et al. 2000) was used for backcross introgression analysis using all available markers for the population. The analysis was performed using linked loci correlations on each chromosome, with the ancestry linkage model and correlated allele frequencies (Falush et al. 2003). This option uses the admixture model and takes into account the linkage disequilibrium between chromosomes. Several analyses were performed with different combinations of burn-in values (50,000 to 200,000), Markov chain Monte Carlo (MCMC) chains (50,000 to 100,000), values of K from 1 to 5, and $\lambda = 1$ using the 62 tomato lines and parents ($n = 64$), with all loci genotyped ($l = 152$) and grouped by each of the 12 tomato chromosomes. After preliminary analyses, the burn-in value was set to 100,000, the MCMC chains to 100,000, the λ to 1, and the K value with the highest likelihood was determined to be $K = 2$. Using the linkage model, the LA407 or *S. lycopersicum* genetic cluster content per chromosome in each tomato line and the parents was determined. The clustering quality as a function of number of markers per chromosome was also established. The parameters used for this analysis were the same as for the previous analysis. When the fixation index (F_{st}) value had a symmetrical distribution at a specific K , the assumption was that the data did not have structure and, consequently, did not provide information about the introgressions per chromosome.

Results

An advanced backcross (BC_2S_4) population ($n = 62$) was used to determine the resistance to *P. capsici* for each line (Table 1). As observed in previous studies (Quesada-Ocampo and Hausbeck 2010), disease symptoms in susceptible lines were observed 4 to 5 days postinoculation

whereas resistant lines did not present any disease symptoms or symptoms were significantly delayed compared with susceptible ones. Symptoms included ascending wilt and chlorosis, root and crown rot, cankers at the crown that progressed upward along the stem, and stunting. When the crown rot and cankers were severe in the most susceptible lines, the base of the plant collapsed, resulting in rapid wilting of the canopy and death of the plant. Some tolerant lines were able to rapidly generate secondary roots above rotted areas of the crown, which allowed them to maintain healthy canopies and recover from disease.

AUDPC values were significantly different among tomato lines and between isolates ($P < 0.01$). In all, 24 lines were found to be resistant to both *P. capsici* isolates OP97 and 12889, 27 lines were resistant to isolate 12889, and 50 lines were resistant to isolate OP97 (Table 1). Lines 2335, 2362, and 2366 were resistant to *P. capsici* isolate 12889 but susceptible to isolate OP97 (Table 1). Additionally, AUDPC values of lines 2318, 2319, 2347, 2353, 2361, 2363, and 2370 showed significant differences from LA407 in disease response after inoculation with 12889, due to a lower AUDPC value (more resistant) than LA407. No lines resistant to isolate OP97 were significantly different from LA407 but 29 of the resistant lines had lower AUDPC values. Interestingly, AUDPC values of resistant lines 2318, 2319, 2347, 2353, 2361, and 2363 were lower than LA407 when inoculated with each of the two isolates (Table 1). Additionally, 12 lines resistant to OP97 showed lower AUDPC values than LA407 but had higher AUDPC values and were significantly more susceptible than LA407 when challenged with isolate 12889. The lines that were susceptible to *P. capsici* 12889 but resistant to OP97 were 2313, 2314, 2315, 2316, 2323, 2325, 2326, 2330, 2336, 2345, 2360, and 2369 (Table 1). Lines 2307, 2317, 2321, 2327, 2337, 2346, 2348, 2358, and 2367 were susceptible to both *P. capsici* isolates (Table 1). In susceptible lines for one or both of the *P. capsici* isolates, mild wilting was evident 4 days after inoculation and symptoms progressed as previously described (Quesada-Ocampo and Hausbeck 2010). Symptoms caused by *P. capsici* isolate 12889 were more severe and progressed faster than in plants inoculated with OP97. The resistant parent LA407 and other resistant lines showed a very mild level of wilting 10 or more days after inoculation and subsequently recovered.

Broad sense heritability (H) in this population was high for Phytophthora root rot resistance to isolates 12889 ($H = 0.95$, $CI = 0.92$ to 0.96) and OP97 ($H = 0.98$, $CI = 0.97$ to 0.98). The 62 lines from the IBC_2S_4 population were used to generate a genetic map and locate additional markers that could be used in future breeding efforts. After evaluating 301 molecular markers, approximately half of the markers (152 markers) had polymorphism between the parents and showed segregation in the progeny. Polymorphic and segregating markers included 63 framework RFLP markers, 48 AFLP (three primer combinations: *MseI* CAC-*EcoRI* ACA, *MseI* CAG-*EcoRI* AAG, and *MseI* CAC-*EcoRI* AAG), 20 SSR, and 21 SNP. The genetic map covered 1,099 centimorgans (cM) and included 12 linkage groups that corresponded to the 12 tomato chromosomes using a recombination fraction

Table 2. Tomato linkage groups for IBC_2S_4 LA407 $\times$ Hunt100^z

Linkage group	Length (cM)	Number of markers	RFLP	AFLP	SSR	SNP	Average spacing (cM)	Largest distance (cM)	LOD	Recombination fraction (Q)
Chr1	127.2	22	7	12	1	2	5.5	17	3.23–18.06	0.02–0.17
Chr2	120.4	13	6	1	4	2	9.3	20.8	3–18.66	0.02–0.26
Chr3	132.7	23	7	5	7	4	6.0	13.5	3.29–18.36	0.02–0.27
Chr4	149	29	6	19	0	4	5.1	10.9	3.29–18.36	0.02–0.29
Chr5	68.8	10	4	1	4	1	6.9	11.5	3.91–18.66	0.02–0.29
Chr6	73.1	9	5	0	2	2	8.1	15.2	3.29–18.36	0.02–0.26
Chr7	83.3	8	5	0	1	2	10.4	17.9	3.54–11.68	0.02–0.24
Chr8	75.2	6	6	0	0	0	12.5	27	6.32–18.36	0.01–0.17
Chr9	41.1	3	3	0	0	0	13.7	25.8	3.12–15.56	0.02–0.26
Chr10	80.4	13	5	3	1	4	6.2	11.9	4.42–18.66	0.016–0.211
Chr11	67.4	11	4	7	0	0	6.1	11.7	3.77–14.25	0.033–0.24
Chr12	80.4	5	5	0	0	0	16.1	28	4.25–13.40	0.02–0.23
Total	1,099	152	63	48	20	21	...	...	...	...

^z Abbreviations: cM = centimorgans, RFLP = restriction fragment length polymorphism, AFLP = amplified fragment length polymorphism, SSR = simple sequence repeats, SNP = single-nucleotide polymorphisms, LOD = log of the odds, and Chr = chromosome.

from 0.016 to 0.29 and LOD of 3.0 or higher (Table 2). In total, 89 markers were added to the original linkage map previously published (Kabelka et al. 2002); of those markers, 48 constituted nonannotated markers (AFLP) but the remaining 41 markers (SSR and SNP) were located in genes with annotations related to disease resistance and defense responses. The map coverage achieved corresponds approximately to 81.4% of the total genome for an advanced backcross population (Bernacchi et al. 1998). Chromosomes 8, 9, and 12 had lower marker coverage than the remaining chromosomes, and no new markers mapped to these groups. Markers located in genes with annotations related to disease resistance and defense responses were mapped to all chromosomes except for 8, 9, 11, and 12. Markers with no annotation (AFLP) were mapped to all chromosomes except for 6, 7, 8, 9, and 12.

The results obtained from the STRUCTURE analysis for each line (Fig. 1) indicated that the average percentage of the *S. habrochaites* LA407 genome in the IBC₂S₄ progeny was 13.7%, which was confirmed by performing the same analyses on individual chromosomes (data not shown). Chromosomes with low marker coverage such as 8, 9, and 12, had symmetrical *F_{st}* values in all K evaluations performed; nonetheless, the population structure with the data of all chromosomes combined accurately established the LA407 content. Results were consistent with the theoretical expectation of 12.5% LA407 content per line (Kabelka et al. 2002) for this advanced backcross. The percentage of the LA407 genome varied among lines. Lower values were found in the lines 2313, 2337, 2354, 2316, 2355, 2332, 2314, and 2325 with an LA407 content between 1.5 and 7% (Table 1). Higher values from 22 to 32% were observed in lines 2365, 2307, 2311, 2370, 2323, 2356, 2309, 2338, 2343, and 2368 (Table 1). No significant correlation was observed between percentage of LA407 content in the lines and resistance to either *P. capsici* isolate.

Discussion

In this study, resistance to *P. capsici* in an IBC₂S₄ tomato population was investigated using two *P. capsici* isolates with different virulences; isolate 12889 produced a susceptible reaction on 55% of the lines, while 17% were susceptible to OP97. The phenotypic variation in resistance found in each line had a consistent relationship with the virulence of the isolate used; that is, fewer lines were resistant to the more virulent isolate *P. capsici* 12889. Our results provide evidence for the existence of physiological races in *P. capsici* infecting tomato similar to those in pepper and eggplant (Foster et al. 2013; Naegele et al. 2014; Sy et al. 2005). Partial resistance to *P. capsici* physiological races has been previously reported on tomato cultivars and wild relatives, and related species (Naegele et al. 2014; Quesada-Ocampo and Hausbeck 2010). The quantitative and isolate-specific nature of the response of tomato lines to *P. capsici* infection suggests that resistance to this pathogen in tomato is polygenic, similar to resistance in pepper. Most commercial pepper varieties currently available do not show high levels of resistance to *P. capsici* due to the complex inheritance of the trait and difficulty with reproducible phenotypic screens (Naegele et al. 2014; Oelke et al. 2003; Thabuis et al. 2004). QTL for resistance to *P. capsici* have been identified in pepper using segregating populations (Lefebvre and Palloix 1996; Naegele et al. 2014; Oelke et al. 2003; Pflieger et al. 2001; Thabuis et al. 2003, 2004) but not in tomato, due to the lack of knowledge of lines with high resistance and derived segregating populations of a sufficiently large size for this type of analysis. Although, in this study, we were unable to reliably detect QTL conferring resistance to *P. capsici* in tomato due to the small size of the backcross population, we did identify several progeny that show resistance to one or both highly virulent *P. capsici* isolates and that have desirable horticultural characteristics due to their significant Hunt100 genetic background. The high heritability of *Phytophthora* root rot resistance further suggests that integrating resistance into commercial tomato will be possible.

Annotated markers such as SNP and SSR derived from resistance or defense-related genes can provide information to link phenotype to genotype because they represent coding regions of the genome (López et al. 2007). In this study, we developed and mapped annotated markers in a previously published tomato linkage map to

evaluate their usefulness as markers for future breeding efforts. Although we did not find any annotated markers strongly associated with *P. capsici* resistance, markers representing defense-related genes segregated within the population. Evaluation of the segregation of these markers in a larger population may lead to insight into the *P. capsici*–tomato interaction.

Fig. 1. LA407 and Hunt100 shared genome percentage on each line. Dark-gray zone represents genotype in common with Hunt100 (susceptible to disease caused by *Phytophthora capsici*) and light-gray zone represents genotype in common with LA407, the resistant parent.

The donor *S. habrochaites* has been reported to be resistant to *P. infestans* in other studies suggesting it may have broad resistance to several *Phytophthora* spp. Interspecific mapping using *S. habrochaites* as a donor of resistance to several pathogens has identified various QTL conferring resistance to *Botrytis cinerea*, bacterial canker, and early blight on the same chromosome regions as the QTL found for *P. infestans* resistance (Brouwer et al. 2004; Finkers et al. 2007; Francis et al. 2001; Zhang et al. 2002, 2003). Several *P. capsici* resistance QTL have been identified on chromosomes 1, 2, and 3 using different pepper crosses (Grube et al. 2000; Lefebvre and Palloix 1996; Naegele et al. 2014; Pflieger et al. 2001; Sy et al. 2005; Thabuis et al. 2003), suggesting the possible clustering of resistance to *Phytophthora* spp. within the family Solanaceae.

In tomato, advanced backcross populations have been an effective method to simultaneously incorporate quality and disease resistance traits into a cultivated background while also identifying regions of the genome underlying the desired traits (Francis et al. 2001; Foolad et al. 2002; Kabelka et al. 2002; Kreutzer et al. 1994; Zhang et al. 2003). *S. habrochaites* is an important source of disease resistance for several tomato pathogens and, in the IBC population, the donor parent *S. habrochaites* LA407 contributed favorable alleles that were backcrossed and fixed in the progeny. This introgression varied widely among the lines, with as little as 1.5% and as much as 32% of the genome estimated to be from LA407. No correlation was observed between LA407 content and resistance to *P. capsici*, indicating that specific genomic regions have to be introgressed into a line to achieve resistance.

Lines 2311, 2318, 2319, and 2341 were resistant to both *P. capsici* isolates. These lines only have one to four fragments of chromosome 3 introgressed. Also, some lines with resistance to OP97 and 12889 had specific segments introgressed on chromosomes 1 or 2. For example, line 2338 had a specific fragment at 30 cM on chromosome 2, line 2347 at 60 cM on chromosome 1, and line 2353 at 80 cM on chromosome 2. Lines 2322 and 2310 had a high percentage of introgression on chromosome 1 but were both susceptible to *P. capsici* isolates. Lines 2340, 2344, 2352, and 2354 were susceptible to both *P. capsici* isolates and shared specific segments on chromosome 2 located at 38 cM. Additional experiments with larger mapping populations are needed to verify whether these introgressions have a direct role in resistance or susceptibility to *P. capsici*.

This study is the first evaluation of the genetic control of *Phytophthora* root rot resistance in tomato. LA407, the donor parent of the IBC population, is a valuable source of economically important traits, including resistance to diverse pathogens for the improvement of cultivated tomato crops (Finkers et al. 2007; Eshed and Zamir 1995; Zhang et al. 2002, 2003). After analysis of the *S. habrochaites* × *S. lycopersicon* IBC₂S₄ population, we found candidate lines with resistance to *P. capsici* and low levels of introgression of additional LA407 alleles. Molecular markers related to resistance and defense responses were also located in the genetic map of this tomato population, and will provide valuable tools for breeders incorporating resistance. These findings will be useful for disease management strategies that reduce fungicide use while decreasing losses due to disease.

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