Phytophthora Root Rot Resistance and Its Correlation with Fruit Rot Resistance in *Capsicum annuum*

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Abstract. *Phytophthora capsici* causes root and fruit rot and foliar blight of pepper. Multiple sources of resistance to *Phytophthora* root rot have previously been identified, but most display only partial resistance. One source, CM334, has broad spectrum root rot resistance to multiple pathogen isolates but has only low to moderate fruit rot resistance. This study evaluated previously identified pepper lines for resistance to two *P. capsici* isolates, OP97 and 12889, and compared root rot resistance to fruit rot resistance and genetic structure. CM334 was confirmed as a broad spectrum resistance genotype, whereas all other sources of resistance evaluated were susceptible to infection by one or both isolates evaluated. Although not completely resistant, PI 566811 displayed moderate resistance to fruit and root rot to both *P. capsici* isolates. Fruit rot resistance had a significant but small to moderate positive correlation ($r = 0.26\text{--}0.63$) with root rot resistance depending on the isolate and length of exposure. Pepper accessions with resistance to *Phytophthora* root and fruit rot belonging to different genetic subpopulations were identified and could serve as candidates for partial-resistance loci to incorporate into pepper breeding programs.

Phytophthora capsici Leonian infects a wide range of crop species including ornamental and vegetable solanaceous crops (Enzenbacher et al., 2015; Foster et al., 2012; Naegele et al., 2014). Without effective disease management strategies, the pathogen can cause up to 90% crop loss with an estimated annual loss of US\$100 million across susceptible crops (Granke et al., 2012b). To minimize the risk associated with producing *P. capsici*-susceptible crops, researchers have focused on pathogen epidemiology, fungicide, and cultural strategies to understand pathogen dispersal and develop effective management practices that include rotations of drip-applied fungicides and cultivars with reduced susceptibility (Gilardi et al., 2013; Granke et al., 2012b; Parada-Rojas and Quesada-Ocampo, 2019).

Host resistance to diverse isolates of *P. capsici* is an important management tactic and a focus for pepper (*Capsicum annuum*) breeding programs globally. In pepper, the pathogen can cause root rot, foliar blight, and

a fruit rot. A significant challenge for breeding resistant peppers is the high genetic and virulence diversity of the pathogen found in the field (Castro-Rocha et al., 2016; Granke et al., 2012a; Lamour and Hausbeck, 2000, 2001). Sexual reproduction of *P. capsici* occurs in the field at local and regional levels across multiple crops (Quesada-Ocampo et al., 2011). When breeding resistance to pathogens with high genetic diversity, breeders have historically relied on stacking quantitative resistance genes to ensure cultivar longevity. In pepper, race-specific resistance, evident by significant line-by-isolate interactions between identified sources of resistance and *P. capsici* isolates, has been observed. This is true for root and fruit rot resistance and foliar blight resistance in pepper (Candole, 2012; Glosier et al., 2008; Naegele et al., 2013, 2014; Sy et al., 2005).

When ≈ 2300 pepper lines were screened, nearly 80 accessions with some level of resistance to *Phytophthora* root rot were identified (Candole et al., 2010). Despite the many studies evaluating root rot resistance in pepper, including multiple landraces evaluated from Mexico, only one landrace has been consistently resistant to *P. capsici*. CM334, widely acknowledged as stable source of *P. capsici* resistance, is used in breeding programs across the globe. Studies have shown that this landrace is resistant to all isolates of *P. capsici* that have been tested to date (Glosier et al., 2008; Oelke et al., 2003; Ortega et al., 1991; Parada-Rojas and

Quesada-Ocampo, 2019; Sy et al., 2008). *Capsicum* sources of resistance have been identified from Mexico, China, and India (Barksdale, 1984; Candole et al., 2010; Lefebvre and Palloix, 1996; Mallard et al., 2013). Mapping populations derived from some of these sources of resistance have determined that although chromosome 5 is the major locus, there is variation in minor resistance loci (Thabuis et al., 2003). In a study by Thabuis et al. (2003), three sources of resistance including CM334 and PI 201234 had the major locus at chromosome 5, but also had unique smaller effect loci on chromosomes 3, 6, 10, and 12. A better understanding of the genetic diversity and structure of pepper, and its association with *Phytophthora*-induced diseases, is key to stacking novel sources of resistance.

Few studies have evaluated fruit rot resistance to *P. capsici* in pepper, and fewer have evaluated the relationship between fruit and root rot resistance (Biles, 1993; Foster and Hausbeck, 2010; Saini and Sharma, 1978; Tahboub et al., 2008). Complete resistance to *Phytophthora* fruit rot in pepper has not been identified in any accession evaluated to date. CM334, although resistant to root rot, exhibited lesions or spotting on fruit when inoculated (Naegele et al., 2013). In CM334, major quantitative trait loci (qtl) for resistance to fruit rot were identified on chromosome 5, as well as a minor qtl on chromosomes 2 and 4 (Naegele et al., 2013). *Phytophthora* fruit rot resistance was characterized across 170 pepper lines, and low differentiation was detected among resistant and susceptible subpopulations (Naegele et al., 2014). However, the relationship between *Phytophthora* fruit and root rot resistance across diverse germplasm is unclear.

The objectives of this project were to evaluate potential sources of resistance to *P. capsici* root rot against two virulent isolates, determine population genetic structure associated with root rot resistance, and evaluate the correlation between root rot and fruit rot resistance in *Capsicum*.

Materials and Methods

Plant material and isolates. Eighty *C. annuum* lines and one *C. frutescens* (PI 593920) representing wild, cultivated, and landrace materials from the U.S. Department of Agriculture Germplasm Resource Information Network or commercial seed sources: Johnny's Select Seeds, Parks Seed, and Seedway (<http://www.ars-grin.gov/>) were evaluated for *Phytophthora* root rot (Table 1). Twenty seeds from each line were sown into 72-cell trays containing a soilless peat mix (Suremix Michigan Grower Products, Inc., Galesburg, MI) in a polyethylene greenhouse at the Michigan State University (MSU) Horticulture Research Farm (East Lansing, MI). The planted 72-cell trays were placed onto heat pads to maintain a soil temperature of $\approx 26.67^\circ\text{C}$, for ≈ 1 month after planting. Seedlings were transferred to 4-inch black

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Table 1. *Capsicum annuum* genotypes evaluated for resistance to Phytophthora root rot resistance.

Identifier	Country	Continent	Cluster ²	12889 ⁹	OP97 ⁸
CM334	Mexico	N. America	5	R	R
Early Jalapeno	Park's Seed	N. America	5	MR	MR
Grif 9094	Greece	Europe	5	S	S
Grif 9105	Soviet	Asia	4	MS	S
Jn566	USA	N. America	3	S	S
Jn571	USA	N. America	4	S	S
Jn574	USA	N. America	4	S	S
PI 135822	Afghanistan	Asia	5	S	S
PI 138558	Iran	Asia	4	S	S
PI 138560	Iran	Asia	4	S	S
PI 138565	Iran	Asia	4	S	S
PI 142832	Iran	Asia	1	S	S
PI 148628	Iran	Asia	1	S	S
PI 159256	USA	N. America	1	S	S
PI 164311	India	Asia	5	S	S
PI 167063	Turkey	Europe	1	S	S
PI 169129	Turkey	Europe	1	S	S
PI 177301	Italy	Europe	6	S	S
PI 181734	Lebanon	Asia	6	S	S
PI 184039	Serbia	Europe	1	S	S
PI 201232	Mexico	N. America	4	MR	MR
PI 201234	Mexico	N. America	4	MR	MR
PI 201237	Mexico	N. America	4	MR	MR
PI 201239	Mexico	N. America	4	MS	MR
PI 203524	Cuba	S. America	1	S	S
PI 206950	Turkey	Europe	1	S	S
PI 207727	India	Asia	— ^w	S	S
PI 213915	Bolivia	S. America	5	S	S
PI 219896			— ^w	S	S
PI 224438	Mexico	N. America	5	MR	S
PI 226633	Iran	Asia	1	S	S
PI 241641	Colombia	S. America	4	S	S
PI 249908	Portugal	Europe	4	S	S
PI 257283	Spain	Europe	5	S	S
PI 263075	Soviet	Asia	4	S	S
PI 263077	Soviet	Asia	4	S	S
PI 263114	Soviet	Asia	4	S	MS
PI 264662	Germany	Europe	4	S	S
PI 273415	Italy	Europe	5	S	S
PI 281390	El Salvador	S. America	—	S	S
PI 281433	USA	N. America	4	S	S
PI 298647	Spain	Europe	1	S	S
PI 302987	Canada	N. America	4	S	S
PI 338993	Turkey	Europe	— ^w	S	S
PI 339079	Turkey	Europe	1	S	S
PI 339132	Turkey	Europe	4	S	S
PI 342949	USA	N. America	4	S	S
PI 357503	Serbia	Europe	4	S	S
PI 368396	Serbia	Europe	4	S	S
PI 369996	India	Asia	1	S	S
PI 410407	Brazil	S. America	2	S	S
PI 427290	USA	N. America	2	S	MS
PI 432802	China	Asia	6	S	S
PI 438633	Mexico	N. America	2	S	S
PI 511879	Mexico	N. America	2	S	MR
PI 511884	Mexico	N. America	2	MR	R
PI 550700	USA	N. America	2	S	S
PI 555649	Sudan	Africa	2	S	MS
PI 566811	Mexico	N. America	2	MR	MR
PI 585246	Ecuador	S. America	2	S	S
PI 593493	Mexico	N. America	2	MR	MS
PI 593511	Mexico	N. America	2	S	MS
PI 593573	Brazil	S. America	6	MR	MR
PI 593920	Ecuador	S. America	2	S	S
PI 600934	USA	N. America	3	S	S
PI 631126	China	Asia	3	S	S
PI 631131	Yemen	Asia	3	S	S
PI 631140	Guatemala	N. America	4	MS	MR
PI 631143	Guatemala	N. America	1	S	S
PI 631147	India	Asia	2	S	S
PI 640448	Taiwan	Asia	1	S	S
PI 640461	China	Asia	2	S	MS
PI 640532	Mexico	N. America	3	MS	MR
PI 640579	Egypt	Africa	1	S	S

(Continued on next page)

Table 1. (Continued) *Capsicum annum* genotypes evaluated for resistance to *Phytophthora* root rot resistance.

Identifier	Country	Continent	Cluster ^z	12889 ^y	OP97 ^x
PI 640581	Nigeria	Africa	2	S	MR
PI 640670	India	Asia	2	S	S
PI 640676	Kenya	Africa	3	S	S
PI 640803	Philippines	Asia	2	MS	R
PI 640833	USA	N. America	3	S	R
PI 645520	Italy	Europe	3	S	S
PI 653650	Bangladesh	Asia	2	S	S

^zGenetic cluster assignment based on STRUCTURE results.

^yDisease rating based on the lowest level of resistance to isolate 12889 across two experiments.

^xDisease rating based on the lowest level of resistance to isolate OP97 across two experiments.

^wIndicates DNA was not available for this accession.

Fig. 1. Root lesion phenotypes observed on plants that survived until the end of the study inoculated with *Phytophthora capsici* isolate 12889 or OP97.

plastic pots containing the same soilless peat mix before inoculations.

Root rot inoculation and evaluations. Two isolates were selected from the long-term collection of Dr. Mary K. Hausbeck (MSU). Isolates were characterized by host, mefenoxam sensitivity (I = insensitive, S = sensitive) and mating type (A1 or A2). Isolate 12889 (pepper, I, A1) and isolate OP97 (cucumber, S, A1) were maintained on unclarified V8 agar (UCV8; 16 g of agar, 3 g of CaCO₃, 160 mL of V8 juice, and 840 mL of distilled water) for the duration of the experiment. Before inoculations, isolates were activated by inoculating and recovering each isolate from a single pepper fruit. Inoculum was prepared by adding sixteen 6-mm plugs of each isolate from an actively growing culture to a 1-L bottle of prepared millet and grown for 2 to 3 weeks at room temperature (21 °C) to allow the pathogen to colonize and distribute throughout the millet as described by Quesada-Ocampo and Hausbeck (2010). Using a falcon tube, 1.5 g of inoculated millet were applied to the root ball of each plant, covered with soil, and carefully watered to promote infection. Control plants were inoculated with 1.5 g of sterile millet. Four replicate plants were used for each isolate, with two replicate control plants. Plants were evaluated in batches because not all pepper accessions were ready at the same time. Two accessions, CM334 and Grif 9094, were included as resistant and susceptible checks, respectively, in each batch. Disease was assessed three times at weekly intervals for 8 weeks according to the following scale: 0 = no wilting, 1 = 1 <25% wilting, 2 = 25 to <50% wilting, 3 = 50 to <75% wilting, 4 = 75% to 95% wilting, 5 = >95% wilting or plant death modified from Foster and Hausbeck (2010). Approximately

Fig. 2. Pepper germplasm grouped into categories based on disease resistance phenotype to (A) isolate OP97, (B) isolate 12889, or (C) continent of origin. Each bar indicates a unique individual and colors represent identified genetic clusters.

10% of plants with a rating of 5 were returned to the laboratory for confirmation of the pathogen. In brief, roots were surface disinfested with 10% bleach (0.62% sodium hypochlorite) and rinsed in sterile ddH₂O. Three small sections were plated onto UCV8 agar amended with 100 ppm ridomil and 100 ppm ampicillin. The pathogen was confirmed from symptomatic tissue using morphological characteristics. At the end of the study, plants that were not completely wilted (disease rating ≤4) were returned to the laboratory for phenotypic evaluations to determine the effect of *P. capsici* infection on plant growth and root health. Plants were measured for final height, total fruit yield, and root health. Roots were thoroughly rinsed and rated using the following scale: 0 = no symptoms, 1 = small lesions on side roots only, 2 = small lesions on side root or small lesions on main root, 3 = moder-

ately sized lesions on main root, 4 = large lesions on main root (Fig. 1). Total number of plants evaluated for plant traits and root health for each accession varied as not all plants for resistant/semiresistant lines survived to the end of the study. The experiment was conducted two times for each line and isolate. **Genetic diversity and population structure.** Using previously published marker SSR data for the 77 of the 81 individuals evaluated in this study, genetic diversity and population structure were evaluated (Naegel et al., 2014). Genetic population indices based on continent were calculated using 23 markers in *Poppr* (Kamvar et al., 2014) within R (v3.5.1) statistical software (R Core Team, 2019) including number of observed and expected MLG (genotypic richness), rarefaction statistics, genotypic abundance of MLG diversity [Shannon-Wiener Index

(H)] (Shannon, 2001), Simpsons index (λ) (Simpson, 1949), Nei's unbiased gene diversity (H_{exp}) (Nei, 1978), and the linkage disequilibrium [index of association (I_A)] (Brown et al., 1980) (Tables 2 and 3). G_{ST} was calculated using *Poppr* (Kamvar et al., 2014).

Using the software GenAlEx 6.05 (Peakall and Smouse, 2012), analyses of molecular variance (AMOVA) among and between populations based on continent (North America, South America, Europe, Africa, and Asia) and root rot resistance (R, MR, MS, and S) frequencies were calculated and significance determined (based on 999 permutations). Because only a single pepper genotype was resistant to isolate 12889, AMOVA was run using categories for resistant/moderately resistant, moderately susceptible, and susceptible individuals, only. For plants with differential reactions between the two experimental runs, the highest disease susceptibility rating was used for analyses. Population differentiation was tested for statistical significance

(based on the mean and 95% confidence interval). Genetic differentiation (F_{ST}) among subpopulations was classified as little, moderate, great, and very great as described by Wright (1978) and implemented by Hartl and Clark (2007).

Population structure was estimated using STRUCTURE v2.3 (Pritchard et al., 2000) and k (number of subpopulations present) was determined by the log likelihood difference as described by Evanno et al. (2005) using STRUCTURE Harvester (Earl and Vanholdt, 2012). In brief, a single population was used to determine lambda for subsequent testing. A total of 15 k -values were tested in STRUCTURE using the admixed model with independent allele frequencies (Falush et al., 2003). Each k value was tested with a burn in of 100,000 Markov Chain Monte Carlo (MCMC) and 500,000 replicate MCMC a lambda of 0.6459, and five independent runs. Individuals were assigned to a cluster based on the estimated proportion ($\geq 60\%$) of membership (Q). Individuals that could not be assigned to a cluster were considered admixed. Clusters were visualized by category (disease resistance, region, and country of origin) using a population sorting script implemented in R (DeLong et al., 2020).

Statistical analyses. Area under the disease progress curve (AUDPC) values were calculated for each line as described by Shaner and Finney (1977). To account for batch differences, disease values were reported as a proportion of the AUDPC for each line compared with the susceptible control (Grif 9094) in the batch. All statistical analyses were performed in SAS v. 9.3 (SAS Institute, Cary, NC). Data were analyzing using analysis of variance implemented within the PROC MIXED function. Line, isolate, and interaction means were separated using least significant differences at $P = 0.05$, when significant. When analyzing unequal sample sizes, degrees of freedom were accounted using the Kenward-Roger option implemented in SAS. Root rot resistance was classified as resistant (0.0 to 0.1), moderately resistant (>0.10 to 0.25), moderately susceptible (>0.25 to 0.50), and susceptible (>0.50) based on the final AUDPC proportion. Previously published fruit rot data on the same population using the same two isolates OP97 and 12889 were compared with root rot results (Naegele et al., 2015). Correlations were

assessed using the Pearson's correlation coefficient (r) with the PROC CORR function.

Results

Root rot evaluations. Significant differences were observed in disease susceptibility among pepper lines when inoculated with isolate OP97 or 12889. Control plants exhibited no wilting symptoms, and no *P. capsici* was isolated from the roots. Across the different batches, CM334 had an AUDPC ranging from 0 to 27.8 and Grif 9094 had an average AUDPC of 155.4 to 239. The pathogen was confirmed from inoculated plants with symptomatic tissue. There were significant differences between the two repetitions of the experiment ($P = 0.0009$), and data from each were analyzed separately. Pepper line was significant in each replicate ($P < 0.0001$). In the first experiment, isolate was not significant ($P = 0.52$), but the interaction between line and isolate was significant ($P = 0.0037$). Most of the lines were either not significantly less resistant compared with the resistant (34) or less susceptible compared with the susceptible control (42) pepper line when inoculated with isolate 12889. Five lines were significantly different with an intermediate rating from both the resistant and susceptible control when inoculated with isolate 12889. However, only CM334 was classified as resistant, not moderately resistant, in both experiments. Other sources of resistance (PI 201232, PI 201234, PI 201237, PI 224438, PI 511884, PI 566811, and PI 593493) were resistant or moderately resistant in both experiments when inoculated with isolate 12889. When inoculated with isolate OP97, 26 and 45 lines were not significantly more resistant or susceptible than the resistant control or susceptible controls, respectively. Ten lines were significantly different with an intermediate rating from both the resistant and susceptible controls when inoculated with isolate OP97.

In the second experiment, isolate 12889 was more virulent than OP97 ($P < 0.0001$), but the interaction between line and isolate was not significant ($P = 0.5562$). Sixty-two lines were not significantly less or more susceptible than the susceptible control Grif 9094 when inoculated with isolate 12889. Twenty lines were not significantly more or less resistant than the resistant control CM334 (isolate 12889). Two lines displayed an intermediate rating that were not significantly different from either the susceptible or resistant control when inoculated with isolate 12889. When inoculated with isolate OP97, most pepper lines were not significantly more or less resistant compared with the resistant (35) control or more or less susceptible (50) than the susceptible control. Three lines had an intermediate rating and were not significantly different from both the resistant and susceptible control (isolate OP97). Only pepper lines CM334, PI 201232, PI 201237, PI 201234, PI 201237, PI 201239, PI 224438, PI 511844, PI 566811, PI 593573, and Early Jalapeno were consistently resistant/moderately

Table 2. SSR-based markers evaluated against a diverse panel of *Capsicum annuum* lines.

Locus	No Alleles ^z	1-D ^y	H_{exp} ^x	Evenness ^w
P1-1	4	0.49	0.49	0.69
P1-19	3	0.57	0.57	0.81
P1-20	3	0.22	0.22	0.49
P1-21	4	0.25	0.25	0.51
P1-26	4	0.59	0.60	0.76
P1-43	2	0.27	0.28	0.67
P1-47	2	0.34	0.34	0.75
P2-8	3	0.49	0.49	0.72
P2-10	3	0.45	0.45	0.68
P2-29	3	0.56	0.56	0.86
P2-42	4	0.23	0.23	0.50
P2-43	5	0.58	0.58	0.62
P2-46	4	0.28	0.28	0.52
P3-1	4	0.17	0.17	0.43
P3-3	3	0.42	0.43	0.65
P3-7	5	0.63	0.63	0.79
P3-11	3	0.34	0.34	0.60
P3-16	2	0.10	0.10	0.49
P3-17	2	0.11	0.12	0.50
P4-11	6	0.69	0.70	0.77
P4-19	4	0.50	0.50	0.76
P4-21	7	0.67	0.67	0.66
P4-43	6	0.74	0.75	0.82
Mean	3.74	0.42	0.42	0.65

^zNumber of alleles distinguished by each marker.

^ySimpson index.

^xNei's unbiased gene diversity (Nei, 1978).

^wDistribution of alleles.

Table 3. Population summary statistics for *Capsicum annuum* lines evaluated for *Phytophthora capsici* resistance grouped by continent of origin.

Population	N Obs ^z	H^y	Lambda ^x	H_{exp} ^w	rbarD ^v
Africa	4	1.39	0.750	0.339	0.8809
Asia	24	3.18	0.958	0.428	0.00224
Europe	16	2.77	0.938	0.379	-0.00705
North America	12	2.48	0.917	0.393	-0.02041
South America	21	3.04	0.952	0.430	0.01581
Total	77	4.34	0.987	0.424	0.00293

^zNo. of observed samples.

^yShannon-Wiener Index of Multi Locus Genotypes diversity.

^xSimpson's index.

^wNei's unbiased gene diversity (Nei, 1978).

^vStandardized index of association.

Table 4. Analysis of molecular variance for pepper germplasm grouped by continent, resistance phenotype to isolate OP97, and STRUCTURE designated cluster.

Grouping	Source ^z	df ^y	SS ^x	MS ^w	Est Var ^v	% ^u
12889	Among pop	2	10.863	5.432	0.033	1
	Among ind	74	338.923	4.580	1.329	40
	Within ind	77	148.000	1.922	1.922	59
	Total	153	497.786		3.285	100
OP97	Among pop	3	45.058	15.019	0.221	4
	Among ind	73	702.786	9.627	4.090	71
	Within ind	77	111.500	1.448	1.448	25
	Total	153	859.344		5.759	100
Continent	Among pop	4	76.449	19.112	0.349	6
	Among ind	72	672.096	9.335	3.943	69
	Within ind	77	111.500	1.448	1.448	25
	Total	153	860.045		5.740	100
Cluster	Among pop	5	133.283	26.657	0.738	13
	Among ind	71	615.210	8.665	3.608	62
	Within ind	77	111.500	1.448	1.448	25
	Total	153	859.994		5.795	100

^zSource of variation among populations (among pop), among individuals (among ind), within individuals (within ind), or the total population (total).

^yDegrees of freedom for each of the sources of variation.

^xSum of squares.

^wExpected mean of squares.

^vEstimated variation.

^uPercentage of the total variation explained.

resistant to *P. capsici* across both isolates and both experiments. Fifteen pepper lines displayed differential responses from moderately resistant/resistant to moderately susceptible/susceptible across experimental replicates to one or both isolates.

Trait evaluation of surviving plants. Only 20 lines consistently (in both experiments) had three to four surviving plants at the end of the study for a given experiment. No consistent differences were detected among control or inoculated plants in plant height in resistant or partially resistant plants that survived until the end of the study for any of the genotypes evaluated. For root rot visual ratings of the surviving plants, significant negative differences between the control and surviving inoculated plants were detected for partially resistant pepper lines: PI 640803, PI 631140, and PI 640461. Each of these genotypes showed differential responses for wilting between experiments for isolates 12889 or OP97. For the remaining partially resistant genotypes, no significant differences in root lesions were detected among inoculated and millet inoculated control plants compared with CM334.

Fruit rot correlation with root rot. When fruit rot and root rot resistance were compared, fruit rot resistance had a significant positive correlation with root rot resistance for both isolates. Root rot resistance to isolate 12889 had a small positive correlation with fruit rot resistance to the same isolate at 3 d postinoculation (dpi) ($r = 0.35051$, $P = 0.0017$; $r = 0.26493$, $P = 0.0191$ for round 1 and round 2 of the experiment, respectively). A stronger correlation between root rot and fruit rot resistance at 5 dpi was observed when inoculated with isolate 12889 ($r = 0.49894$, $P < 0.0001$; $r = 0.41823$, $P = 0.0001$ for round 1 and round 2 of the experiment, respectively). When inoculated with isolate OP97, root rot resistance and fruit rot resistance had a stronger positive correlation 5 dpi ($r = 0.63499$, $P < 0.0001$; $r = 0.46392$,

$P < 0.0001$) compared with 3 dpi ($r = 0.46628$, $P < 0.0001$; $r = 0.39461$, $P = 0.0004$) for both round 1 and round 2 of the experiment, respectively). Pepper line PI 566811 was the only accession evaluated with moderate resistance to fruit rot five dpi and root rot to both isolates of *P. capsici* in both experiments.

Genetic diversity and population structure. Of the 23 markers evaluated, genetic diversity (Nei, 1978) ranged from 0.15 to 0.78. Evenness for SSR markers ranged from 0.45 to 0.80. The mean genetic diversity and evenness for the population was 0.42 and 0.65, respectively. The adjusted index of association (r_{barD}) was low for the population, indicating frequent recombination based on the 23 markers (Table 2). Genotype accumulation curves indicated that detected genotypic differences for the population reached saturation with only 13 of the 23 loci evaluated (Supplemental Fig. 1). Global G_{ST} was 0.0804. Using AMOVA most of the variation observed was within populations, and not among populations, when grouped by continent and disease resistance for OP97 (Table 4). Significance for each of the groupings was $P = 0.001$. AMOVA values for disease resistance (resistant/moderately resistant, moderately susceptible, and susceptible) to isolate 12889 were not significant ($P = 0.052$). The percentage of molecular variance among populations was 6%, 1%, and 4% when grouped by continent, resistance to isolate 12889, and resistance to isolate OP97, respectively.

For STRUCTURE analysis, a k value of 5 was selected, and individuals were assigned to their respective clusters. Low admixture was detected because most individuals were able to be assigned to a single cluster. When grouped by cluster, 13% of the variation was among clusters based on AMOVA, $P = 0.001$ (Table 4). Cluster 3 (light blue) had the fewest individuals (8), and cluster 4 (dark

blue) had the most (23). Cluster 1 (dark purple), cluster 2 (yellow), and cluster 5 (light purple) and 15, 18, and 9 individuals, respectively. Only 4 individuals could not be placed into a cluster and were considered admixed, designated as cluster 6 (Table 1).

Clustering individuals by resistance level to OP97 or 12889 indicated that no clusters were solely present in the resistant/moderately resistant clusters and not the moderately susceptible/susceptible categories. The exception to this was individuals with 60% membership in the dark purple cluster, which was present only in the susceptible category. The light blue and light purple clusters were present in a higher proportion in the resistant/moderately resistant categories compared in the moderately susceptible/susceptible categories. However, individuals with a majority membership from the yellow and dark blue clusters were also present in the resistant/moderately resistant categories.

When grouped by continent, Europe had no representation of the yellow cluster (2) and a greater than expected representation of the dark purple cluster (1). Light purple and light blue, which were present in higher proportions in the resistant/moderately resistant categories, were found primarily samples from Asia and North America.

Discussion

In this study, we evaluated diverse pepper lines for resistance to *Phytophthora* root rot and compared their resistance to previously published fruit rot using the same two isolates (Naegele et al., 2014). CM334 was consistently resistant to both isolates across both experiments consistent with previous studies identifying it as a broad spectrum source of root rot resistance to *P. capsici* (Glosier et al., 2008; Mallard et al., 2013). Most, though not all, of the material we evaluated had previously been tested for root rot resistance using a mixed set of six isolates (Candole et al.,

2010). While most of the accessions behaved as expected, eight (PIs 201239, 263077, 369996, 427290, 511879, 555649, 631147, and 640532) that had previously been reported as moderately resistant (e.g., survived to fruit set) were susceptible to one or both of the isolates we evaluated in one or both of the experiments.

Many pepper lines were consistently susceptible or R/MR to *P. capsici* across both experiments, however some lines showed variability suggesting an environmental, as well as an isolate-specific, component to resistance. This was evidenced by pepper lines PI 640803, PI 631140, and PI 640461 that exhibited differential disease susceptibility responses. These lines had high variability in disease response between the first and second experiment for isolate 12889, but not OP97, and at the end of the experiment exhibited large root lesions. Lines PI 640803 in the study by Candole et al. (2010) exhibited high susceptibility to *P. capsici*; however PI 640461 had >70% survival with an average root rot severity of 1 to 2.

Isolate-specific interactions are well known, and our results highlight the importance of confirming host resistance using local *P. capsici* populations. Candole et al. (2010) used a mixed set of isolates from Georgia when evaluating for resistance. Of the 75 accessions that we evaluated in common, eight displayed a differential resistance response between the two isolates. Although these accessions may not be useful sources of broad spectrum resistance, they could harbor unique isolate-specific alleles that could bolster the major resistance locus found in CM334 on chromosome 5. One potential accession, PI 566811, a local cultivar from Mexico, had moderate consistent resistance to root and fruit rot to both isolates evaluated.

Fruit rot resistance, although not important in all pepper production systems, should still be considered when breeding resistant peppers. When fruit rot resistance was compared with root rot resistance, there was a moderate positive correlation between the two for both of the isolates evaluated, particularly 5 d postinoculation, across the population. Although correlated, fruit rot resistance and root rot resistance were not strong predictors of each other, and both should still be evaluated when possible. Pepper accession CM334 has broad spectrum root rot resistance to *P. capsici* but had greater fruit rot than accession PI 566811 that only had moderate root rot resistance. This is consistent with previous work that found a major qtl on chromosome 5 was associated with fruit rot resistance but also identified qtl on chromosomes 2, 3, 4, and 6 associated with fruit rot in a small biparental mapping population (Naegele et al., 2013). The significant correlation between fruit rot and root rot is likely the result of a shared major qtl on chromosome 5, with smaller effect unique qtl on chromosomes 2 and 4 possibly being fruit rot resistance specific. Fruit rot and root rot resistance correlations also were affected by isolate, with isolate

12889, previously reported as highly virulent, having a weaker correlation than isolate OP97.

Studies evaluating the population structure of pepper have found regional and global structure (Aguilar-Melendez et al., 2009; Hernández-Verdugo et al., 2001; Hill et al., 2013; Oyama et al., 2006; Pereira-Dias et al., 2019). In our study, five clusters (k) were determined to be the most likely, with low levels of admixture, which differed from the four clusters that were identified when all 157 accessions were included (Naegele et al., 2015). This is consistent with results from Pereira-Dias et al. (2019), who identified five clusters when evaluating a collection of 122 *C. annuum* using more than 4000 single nucleotide polymorphisms, and with results by Hill et al. (2013), who identified six clusters with low levels of admixture.

Numerous studies have identified or mapped resistance to *P. capsici*-incited rot in peppers (Mallard et al., 2013; Naegele et al., 2013; Thabuis et al., 2003). While chromosome 5 has a major qtl associated with *P. capsici* resistance, two other pepper genotypes examined had minor qtl on chromosomes 3, 6, 10, and 12 (Mallard et al., 2013). Using pepper germplasm with isolate-specific or partial resistance could help to identify additional unique loci or alleles for bolstering the resistance on chromosome 5. When assigned to genetic clusters, resistant/moderately resistant individuals were identified in the yellow, light blue, light purple, and dark blue clusters. These clusters were distributed across continents, but the light purple and light blue clusters had a higher frequency in North America (specifically Mexico) and Asia. This was further supported by the AMOVA, which showed a significant separation among continents, clusters, and disease-resistance phenotypes for isolate OP97. These individuals with moderately resistant or resistant phenotypes to both isolates and representing different genetic clusters could be useful targets for identifying divergent resistance loci for breeding *Phytophthora*-resistant peppers. Further studies to evaluate the inheritance and position of resistance loci are needed to confirm the usefulness of these materials for enhancing disease resistance.

Phytophthora capsici-incited rot of pepper is a significant production challenge, and breeding new cultivars with durable resistance is paramount for developing a sustainable management program. Although many studies have identified sources of resistance to *P. capsici* in pepper, our results highlight the importance of using diverse isolates with known virulence variability to evaluate host resistance and identify targets for breeding with unique resistance loci.

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Supplemental Fig. 1. Genotype accumulation curve for pepper germplasm evaluated using SSR markers.