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Genetics and Genomics of Resistance

Rating System for Phytophthora Root Rot Influences Quantitative Trait Locus Detection and Reveals Incomplete Dominance and Duplicative Recessive Epistatic Gene Action in *Capsicum annuum*

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Abstract

Phytophthora capsici is one of the most devastating pathogens facing pepper (*Capsicum annuum*) producers worldwide. Numerous factors, such as the race of the pathogen, the growing environment, and the source of resistance, have resulted in an overall lack of widely applicable molecular markers associated with resistance. Our objective was to determine the effect of the rating system on quantitative trait locus (QTL) detection and understand inheritance patterns of host resistance that can influence selection and molecular marker accuracy. We evaluated an $F_{2:11}$ recombinant inbred line population screened against the highly virulent strain (Pc134) and scored using two widely used methods, developed by Bosland and Lindsey and by Black. The rating system developed by Bosland and Lindsey resulted in slightly higher logarithm of odds for the QTL on chromosome 5, and we detected a QTL on chromosome 12 uniquely using this rating system. A QTL on chromosome 10 was detected using both rating systems, but Black resulted in considerably higher logarithm of odds for this QTL compared with the Bosland and Lindsey system. Molecular markers developed were nominally better at accurately predicting the phenotype than previously published molecular markers but did not completely explain resistance in our validation populations. The inheritance pattern of resistance in one of our F_2 populations did not significantly deviate from a 7:9 segregation ratio, indicating duplicative recessive epistasis. However, these results could be confounded by the presence of incomplete gene action, which was found through the improved selection accuracy when the phenotypes of heterozygous individuals were grouped with those with susceptible alleles.

Keywords: genotyping-by-sequencing, molecular marker, oomycetes, *Phytophthora capsici*, single nucleotide polymorphism

Pepper (*Capsicum annuum*) is an increasingly important vegetable crop and is among the most important spice crops worldwide (Bosland and Votava 2012). Consumer demand for pepper has increased substantially over the past 30 years, especially for hot chili pepper (Barchenger et al. 2018a). It has been estimated that peppers are consumed daily by approximately a quarter of the world's population (Halikowski Smith 2015). Global production of pepper was 41.1 million tonnes on an area of 3.7 million hectares in 2021, and approximately 60% of pepper is produced in Asia (Food and Agriculture Organization of the United Nations 2022). The primary limitations to increased pepper productivity and quality are biotic and abiotic stresses.

Globally, Phytophthora root rot caused by *Phytophthora capsici* is one of the most devastating diseases affecting pepper production worldwide, causing more than $100 million USD in annual losses (Bosland 2008). Current management practices for *P. capsici* include irrigation management, crop rotation, soil solarization, fungicide applications (Granke et al. 2012; Hausbeck and Lamour 2004; Sanogo et al. 2023), and the planting of cultivars that are resistant to local isolates (Barchenger et al. 2018b). *P. capsici* can readily move from field to field and rapidly establish itself in different regions, as surface water used for irrigation is an important means of disseminating the pathogen (Gevens et al. 2007). Fully restricting the movement of *P. capsici* among sites is often impossible. Therefore, the best approach to preventing *P. capsici* infection in vegetable crops is the development of resistant cultivars, because it is less expensive and a sustainable alternative to fungicide applications and other management practices (Hausbeck and Lamour 2004).

Breeding for host resistance to *P. capsici* in pepper is very complex (Barchenger et al. 2018a). Depending on the point of infection, growing environment, and plant maturity, *P. capsici* can cause disease on effectively every part of the pepper plant (Alcantara and Bosland 1994). It has been shown that for each *P. capsici* disease syndrome (root rot, foliar blight, stem blight, and fruit blight), separate and independent resistant systems have evolved in the host (Monroy-Barbosa and Bosland 2010), requiring the presence of independent resistance genes for the control of each disease syndrome (Sy et al. 2005; Walker and Bosland 1999). However, some quantitative trait loci (QTLs) have been consistently identified that show effects against many disease symptoms and pathogen strains (Ogundiwin et al. 2005; Rehrig et al. 2014). Within the Phytophthora root rot and foliar blight disease syndromes, more than 45 physiological races have been identified (Barchenger et al. 2018b; da Costa Ribeiro and Bosland 2012; Glosier et al. 2008; Hwang et al. 1995; Jiang et al. 2015; Lee et al. 2010; Monroy-Barbosa

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and Bosland 2011; Oelke et al. 2003; Sy et al. 2008) with different resistance genes/alleles controlling the resistant phenotype against each physiological race of *P. capsici* within each disease syndrome (Monroy-Barbosa and Bosland 2008).

Efforts have been made to identify loci contributing to host resistance to *P. capsici* both in biparental populations (Lozada et al. 2021; Rehrig et al. 2014; Truong et al. 2012; Wang et al. 2016; Xu et al. 2016) and to a much lesser extent using genomewide association studies (Ro et al. 2022; Siddique et al. 2019). With a limited exception (Chunthawodtiporn et al. 2019), most of these efforts were made using a single race (Lozada et al. 2021; Ro et al. 2022; Wang et al. 2016; Xu et al. 2016) or a few races (Siddique et al. 2019). Molecular markers within these identified loci associated with resistance have been developed (Liu et al. 2014; Siddique et al. 2019; Wang et al. 2016; Xu et al. 2016); however, the efficacy of the markers for different populations inoculated with different isolates using different scoring techniques is low (Ro et al. 2022). It is known that breeding for resistance to *P. capsici* is heavily dependent on the accuracy and precision of the disease screening method used (Chavez and Kabelka 2009). Several disease screens have been developed for *P. capsici*. For root-rot screening, 10,000 zoospores per plant (Bosland and Lindsey 1991) and 100,000 zoospores per plant (Black 1999) have been used. Inoculum concentration and plant age play a major role in the level of resistance displayed in the host (Barchenger 2017; da Costa Ribeiro and Bosland 2012; Mansfeld et al. 2017). To breed effectively for resistance and correctly identify races of *P. capsici*, standardized screening protocols should be developed and followed by scientists worldwide (Barchenger et al. 2018b). Our aim was to determine the efficacy of two different disease scoring methods for quantitative trait locus (QTL) detection to identify the most appropriate method to guide breeding for host resistance for *P. capsici*.

Materials and Methods

For this study, we utilized a recombinant inbred line (RIL) population (designated as CCA175), which consisted of 139 $F_{2:11}$ lines that were developed from a hybridization between PBC 518, a susceptible WorldVeg breeding line, and a selection made from the *P. capsici*-resistant line PI 201234 (Barksdale et al. 1984). Seeds of the RILs, the parental lines, a resistant check ('Criollo de Morelos 334'), and two susceptible checks (NMCA10399 and 'Early Calwonder') were sown into sterilized peat moss media in 72-celled plastic trays. Two seeds per cell were sown and then later thinned to one plant per cell for experimentation. The inoculation experiment was replicated three times, with blocking done by greenhouse bench, with 12 plants in each replication. For validation, we used two segregating populations derived from 1705-5612-1 (resistant; not derived from PI 201234) × AVPP1711 (susceptible) with 216 individuals and PI 201234 (resistant) × PBC 142 (susceptible) with 144 individuals, which were sown and maintained as described above.

All plants were maintained in a controlled-environment greenhouse at 28 ± 3°C with an average relative humidity (RH) of 86.5% for 4 weeks. Regular pest and disease scouting was conducted, and appropriate steps were taken to manage diseases, including pesticide applications until inoculation. Plants were hand irrigated twice daily and the fertilizer Nitrophoska (20-19-19) (EuroChem, Switzerland) was applied 2 weeks after sowing. Seedlings were inoculated 5 weeks after sowing, at the 4 to 6 true leaf stage.

The *P. capsici* race 3 isolate Pc134, a highly virulent diploid isolate of the A1 mating type (Barchenger et al. 2017), was used for inoculation. Before inoculation, the pathogen was cultured on V-8 juice agar medium at 28°C with continuous lighting for 4 days. After 4 days, each culture was cut into four equal pieces and each piece was moved into an empty Petri dish to be cut into ~0.5-cm^2 pieces. Sterile water was added to cover the agar pieces at room temperature for 1 h. Later, the water was decanted and replaced with 18 ml of sterile water to recover the agar pieces for 24 h at 28°C with continuous lighting to induce stress on the pathogen in order to produce sporangia. The plates were then transferred to 4°C for 1 h to cause zoospore release. The zoospore suspension was decanted, filtered, and collected into a beaker. The inoculum concentration was adjusted to 10^5 zoospores/ml with a hemocytometer. For each plant, the medium was saturated with 5 ml zoospore suspension added to the surface. There was a total of 5.0×10^5 zoospores inoculum per plant, and the inoculated plants were watered twice a day to maintain high soil moisture.

Disease severity was evaluated 3 weeks after inoculation, using two different rating systems. A rating system developed by Black (1999) utilized a standardized 0 to 4 scale was used to score each plant, where 0 = no symptoms, 1 = 1 to 10% stem necrosis below the cotyledons, 2 = 11 to 50% necrosis below the cotyledons, 3 = 51 to 100% necrosis below the cotyledons, 4 = necrosis above the cotyledons or plant dead. If a plant was scored 0, it was considered resistant, whereas a score of 1 to 4 was susceptible. The rating system developed by Bosland and Lindsey (1991) utilized a standardized 10-point interaction scale to score each plant, where 0 = no symptoms, healthy, white and vigorous roots; 1 = slightly brown roots, healthy and vigorous; 3 = brown roots, necrosis might be at the base of plants; 5 = brown and weak roots, necrosis on the stem, slightly stunting; 7 = seriously brown and weak roots, wilting, stunting with more than 10% necrosis below the cotyledons; 9 = plant dead and almost no roots. Even numbers were used for intermediate symptoms. Plants with scores of 0 to 2 were considered resistant, whereas plants with scores of 3 to 9 were susceptible.

Prior to inoculation, young actively growing leaves were collected from three individual plants of each RIL. DNA was isolated from the leaves using the FavorPrep Plant Genomic DNA extraction Mini kit following the instructions provided by the manufacturer. GBS libraries (*ApeKI*) were prepared according to the GBS protocol per Elshire et al. (2011) except that a centrifugal evaporator of PCR product was used for enrichment, followed by separation on a 6% acrylamide gel. Fragments of between 300 and 450 bp were cut out, and the DNA was recovered from the gel following Sun et al. (2012) and purified using the MinElute PCR fragment purification kit. The recovered DNA was requantified (Qubit) and sent to the High Throughput Genomics Core Facility, Biodiversity Research Center, Academia Sinica, Taipei, Taiwan, for quality control on an Agilent DNF-474 HS NGS fragment analyzer and sequencing using the Illumina HiSeq2500 platform as 151-bp single-end reads.

For SNP calling, FastQ files were developed using Tassel 5.0 (Glaubitz et al. 2014) and sequence reads were mapped to the UC Davis *Capsicum* reference genome (Hulse-Kemp et al. 2018) using "bwa aln" from Burrows–Wheeler Alignment (BWA) v0.7.17. SNPs were first filtered for minimum sequencing depth of 3, maximum missing data of 50%, minimum allele frequency of 0.05, and maximum heterozygosity of 0.3. Before the second step, samples with missing data or heterozygosity rate three standard deviations larger or smaller than the samples' average were removed from the analysis. Next, the filters were set at maximum missing data rate of 50%, minimum allele frequency of 0.1, and maximum heterozygosity of 0.4. Minor SNP states and sites with indels were removed. Preprocessing of output genotypes consisted of correction and imputation at Sliding window size = 11 and postprocessing (debug and binning) using the Genotype-Corrector (GC) (Miao et al. 2018) to generate a genotype file for linkage mapping.

After SNP calling, 2,910 SNPs from the CCA175 RIL population were used to build the genetic map based on JoinMap version 4.0 using Kosambi's mapping function. To obtain high-quality genetic mapping, the genotype files from GC were used for filtering out individuals with missing >30%, SNP markers missing >20%, and loci with significant segregation ratios ($P < 0.001$). Finally, 70 RILs and 1,198 SNPs from the CCA175 RIL population were used to build a genetic map based on JoinMap version 4.0 using Kosambi's mapping function.

Composite interval mapping (Zeng 1994) using QGene (Joehanes and Nelson 2008) was done for QTL identification. The QTL analysis of the resistance to *P. capsici* based on two inoculation methods was conducted individually according to average results of the three replications, each with 12 plants of the RIL population, and intervals having the logarithm of odds (LOD) score above 3 were identified as significant QTLs. Additionally, for validation of candidate SNPs, the following criteria were used for filtering individuals and SNPs to acquire standby SNPs: minimum sequencing depth of 2, maximum missing data of 50%, minimum allele frequency of 0.05, and maximum heterozygosity of 0.6. The visualization of linkage maps integrated with QTL results was done using MapChart 2.32 (Voorrips 2002).

Either cleaved amplified polymorphic sequence (CAPS) or derived-CAPS (dCAPS) molecular markers were designed in the flanking regions of polymorphisms within the significant QTLs identified (Table 1). These molecular markers were used to validate the identified QTLs in the two segregating F_2 populations. For validation, DNA was isolated from the segregating populations using a modified CTAB methodology. The molecular markers from each individual F_2 plant were amplified using the BIO-RAD PTC-0200 DNA Engine Cycler (Bio-Rad, United States), which included an initial incubation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 45 s, with a final extension step for 5 min at 72°C. Amplicons were visualized on a 6% polyacrylamide gel stained with FluoroStain DNA Fluorescent Staining Dye (Green, 10,000×) (Smobio, Taiwan) using the MICROTEK Bio-1000F (Microtek, Taiwan) visualizer. The amplicons were digested, following the manufacturer's instruction, with the respective restriction enzymes listed in Table 1. The digested amplicons were visualized as above and manually scored as being either the susceptible, resistant, or heterozygous allele, based on the paternal lines. Mismatch percentage for each molecular marker was calculated by determining the proportion of accurate identification of the phenotype based on the genotype for each molecular marker, with heterozygotes assumed to predict both a phenotype of resistance and susceptibility. A modified version of the previously published molecular marker Phyto5NBS1, which has been found to have a high level of selection accuracy (Liu et al. 2014), was used for comparison of selection accuracy in our populations. Selection accuracy was determined by calculating the percentage of individuals across the validation populations with a match in the phenotype and the induvial and combination of molecular marker using Fisher's exact test in R.

Results

Phenotypic screening

The parental lines (PBC 518 and PI 201234-1) as well as the resistant (Criollo de Morelos 334) and susceptible (NMCA10399 and Early Calwonder) checks performed as expected, with average scores of 4.0 and 9.0 for PBC 518, NMCA10399, and

TABLE 1. List of cleaved amplified polymorphic sequence (CAPS) or derived-CAPS (dCAPS) molecular markers developed in the quantitative trait loci used for validation in segregating populations[a]

Marker type	Name	Chrom./ contig	Position	Forward primer	Reverse primer	SNP (S/R)	PCR (bp)	Restriction enzyme
dCAPS	CaPC2082.1	2,082	410822	GTAAATAACAGCAACTTA TGATCTAGCACAGAT	CTTTTTAGGTTCTGATTG TCTTGGTAGT	A/C	135	*Dpn*I/*Mbo*I
dCAPS	CaPC5.1	5	14943541	CATATTAGGTGAAAAGGA TCCGTGCAA	CCTTCTTAAACTTCACTC GTGGGATT	G/T	122	*Bsr*DI
CAPS	CaPC5.2	5	18011286	GGGATCACCGTTTA TTCCAA	CAATTGCAGAATTG CTCCTT	G/T	228	*Sty*I/*Bsa*JI
CAPS	CaPC5.3	5	22044718	CCAGGAGGTCCAAT GACAGT	TGGAACTTGGGTGT TTTTCAG	T/C	205	*Bce*AI
CAPS	CaPC5.3b	5	22044774	CCAGGAGGTCCAAT GACAGT	TTTCTGCTGCTACA CCTCCAC	A/G	324	*Cac*8I
CAPS	CaPC5.4	5	21236262	TTGTTTGCTTGCAT CCCTTT	CAAGCTGCATACCA CTGCAT	A/T	299	*Afl*II, *Mse*I
dCAPS	CaPC5.5	5	200868258	TTGTCATGTTTGTT GTACTTCGGACCAATT	GCCTACAAATACAA CAGCTATTCAACC	C/G	147	*Mfe*I
CAPS	CaPC5.5b	5	200868258	GCTGAAATGAGTTG CATACATTG	GCCTACAAATACAA CAGCTATTCAACC	C/G	225	*Hpy*188III/*Taq*I
CAPS	CaPC5.6	5	14776234/ 14776380	GCAAGATCGAAGCA TCACAA	TTGCCCTAATGCAC TCAGC	TT/CC	228	*Bst*UI
dCAPS	CaPC5.7	5	21997642	TGTGGTCCTACCAA CAAGTCATGTAGA	TATTAGAGCTTCTC GATCCGTTTC	T/G	250	*Acc*I
CAPS	CaPC10.1	10	219923293	TGCCGTTGGATAGT TGTTCA	CCTGGCATCACACA CATTTC	T/G	159	*Nsp*I
CAPS	CaPC10.2	10	220984405	CCGAGGGAAGCCTT AATGTC	GTTATTTGAAGGGG GCCTTG	T/C	305	*Bcc*I
CAPS	CaPC10.3	10	218711926/ 218711930	TCGGTCACGAGATG TGAAAA	AATGCACCACTTCT GACACG	AT/GC	164	*Hpy*188I
CAPS	CaPC10.4	10	219102077	CAGCCTCACGGAAG AAAAGA	GCTGCATTTCTCAA CCGACT	A/C	129	*Bts*I/*Bts*IMutI
CAPS	CaPC10.5	10	220797590	CACCCAAAGCATCA CCAGTA	GCAACACTTGGGAT TTTCGT	A/C	133	*Mbo*II
CAPS	CaPC10.6	10	220928080	GCGACATCGACACT TTTGAA	TGCATTAACCAATG TGAAGGA	G/A	127	*Hpy*CH4IV
dCAPS	CaPC12.1	12	21484714	CAGCTGACAGATCG ACTAAT	AGAAGTGAATATTT AGAGACTGA	T/C	114	*Dde*I
CAPS	CaPC12.2	12	21486779/ 21486784	AGGCTGCTCCTCAA GTGAAC	GGATATCCGCACAC CTCACT	AA/CG	170	*Bcc*I/*Tsp*DTI
CAPS	CaPC12.3	12	18307572	TACCCTATGCTCCC CTGATG	TTCCATCTTTCGTC CAGAAAC	T/G	205	*Nla*IV
CAPS	CaPC12.4	12	18311133	TCCGATATGGTGGA GATGGT	TACAACATGCACTG CAGCAA	T/C	199	*Cac*8I

[a] SNP, single nucleotide polymorphism.

Early Calwonder, for the Black (1999) and Bosland and Lindsey (1991) rating systems, respectively, and scores of 0.0 for both rating systems for PI 201234-1 and Criollo de Morelos 334. The disease response to *P. capsici* was not normally distributed among the RIL population using either the rating system established by Black (1999) or Bosland and Lindsey (1991) (Figs. 1 and 2). Both rating systems resulted in bimodal distribution with skewness towards extremes in resistance and susceptibility, with fewer intermediate lines. The average score among the RILs was 1.4 using the Black (1999) rating system and 3.9 using the Bosland and Lindsey (1991) system. Using the rating system established by Black (1999), we found that 57 RILs were highly resistant (average score <1) (Fig. 1), while 33 were resistant (average score <2), using the Bosland and Lindsey (1991) rating system (Fig. 2). For both rating systems, seven RILs were highly susceptible with average scores of 4 (Black 1999) or 9 (Bosland and Lindsey 1991) (Figs. 1 and 2). The most resistant RILs were CCA175-044 and CCA175-053, which had average scores of 0 and 0 using the Black (1999) system and 0.22 and 0.25, respectively, using the Bosland and Lindsey (1991) rating system. The most susceptible RILs, CCA175-037, CCA175-048, and CCA175-068, had average scores of 4.0 and 9.0 for the Black (1999) and Bosland and Lindsey (1991) rating systems, respectively, with all plants in all replications being completely dead.

GBS-derived SNP markers

We identified 7,579 SNPs distributed across the twelve chromosomes of *Capsicum annuum* among the RILs, and an additional 1,469 SNPs were mapped to 588 scaffolds. Chromosome 3 had the greatest number of SNPs, at 1,003, whereas chromosome 4 had the fewest, at 401. On the average, 632 SNPs per chromosome and 2.5 SNPs per unmapped scaffold were identified among the RIL population. The most common point mutation observed in our population was cytosine with thymine with 2,648 C to T or T to C SNPs, followed by adenine with guanine with 2,571, A to G or G to A SNPs. With 678, point mutations for guanine and cytosine were the least common in our population, while the average substitution rate among the other nucleotides was 1,040.

QTL associated with *P. capsici* resistance

For both rating systems, we identified major QTLs associated with resistance to *P. capsici* race Pc134. In the region from 18 to 22 Mb on chromosome 5, a QTL with LODs of 14.2 and 16.4 was found, for the rating systems established by Black (1999) and Bosland and Lindsey (1991), respectively. Another QTL detected in both methods was located at the distal end of chromosome 10 (219 to 220 Mb), LOD = 3.4 (Bosland and Lindsey) and 9.4 (Black). On the other hand, two QTLs were identified exclusively by the Bosland-and-Lindsey rating system in the regions on chromosome 10 (214 to 215 Mb) and 12 (21.4 to 21.5 Mb), suggesting that both rating systems could provide robust results for QTL analysis and the detection of novel QTL (Fig. 3).

Inheritance and selection accuracy of *P. capsici* resistance

Generally, inheritance of resistance to *P. capsici* in our F_2 populations deviated significantly from expected gene models, with the exception of the population derived from PI 201234-1 and PBC 142, which did not deviate significantly from a 7:9 resistant-to-susceptible ratio, indicating duplicative recessive epistatic gene control (Table 2).

The published molecular marker Phyto5NBS1 (Liu et al. 2014) had a selection accuracy of 61.2 and 56.9% for the Bosland and Lindsey (1991) and the Black (1999) screening methods, respectively, when the heterozygote allele was grouped with the resistance phenotypes. The molecular markers CaPC5.3 and CaPC5.7, developed in this study, had slightly improved selection accuracy for the Bosland and Lindsey (1991) (63.8 and 62.8%, respectively) and for the Black (1999) (58.7 and 57.8%, respectively) (Table 3). However, the improvement in selection accuracy for the QTL on chromosome 5 was not substantially greater than that for the nearly decade-old Phyto5NBS1. The molecular markers evaluated for the QTLs on chromosomes 10 and 12 did not have sufficient selection accuracy, with chromosome 10 molecular markers having less than 60% selection accuracy (Table 3). The Fisher's exact test confirmed that the combination of molecular markers on chromosomes 5 and 10 was not associated with the resistant or susceptible phenotype using the Black (1999) scoring method ($P < 0.001$) and the combination of molecular markers on chromosomes 5 and 12 was not associated with the resistant or susceptible phenotype using the Bosland and Lindsey (1991) method ($P < 0.001$).

Interestingly, when we grouped the heterozygous allele with the susceptible phenotypes, the selection accuracy almost always im-

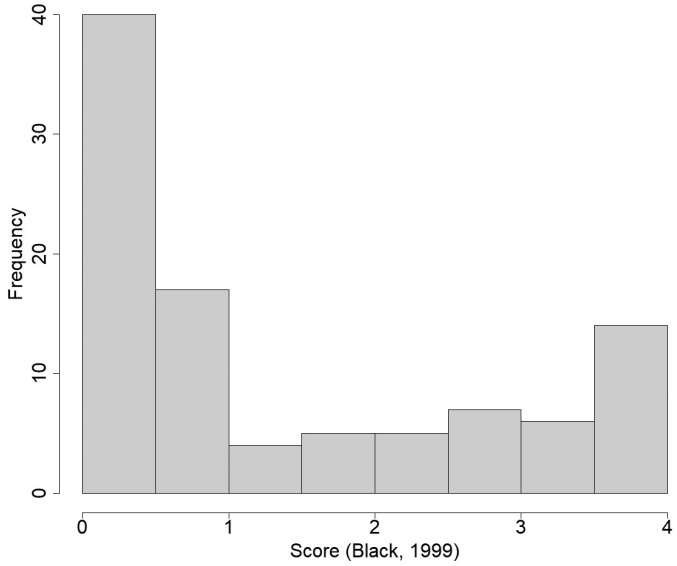

Fig. 1. Distribution of phenotypes in the $F_{2:11}$ recombinant inbred line pepper (*Capsicum annuum*) population derived from PBC 518 and PI 201234 using the Black (1999) scoring methodology screened against the Pc134 strain of *Phytophthora capsici*.

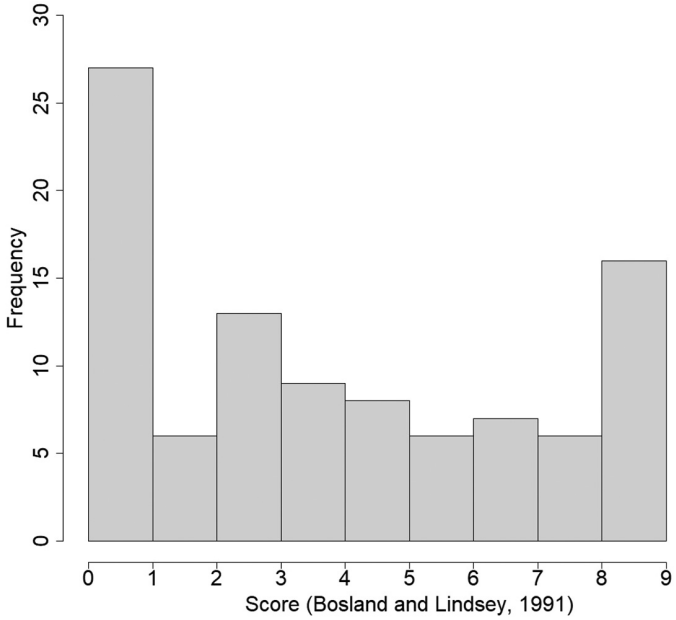

Fig. 2. Distribution of phenotype in the $F_{2:11}$ recombinant inbred line pepper (*Capsicum annuum*) population derived from PBC 518 and PI 201234 using the Bosland and Lindsey (1991) scoring methodology screened against the Pc134 strain of *Phytophthora capsici*.

proved for the molecular markers located on chromosomes 5 and 10, although not for chromosome 12 (Table 3). This was especially true for CaPC5.4, which had around 72% selection accuracy using both methods when heterozygotes were considered to be susceptible. The individuals with heterozygosity for the molecular markers typically had intermediate performance and not as high a score as the homozygous recessive individuals, indicating incomplete or partial dominance gene action.

Discussion

Extensive work has been previously carried out to study inheritance patterns of resistance to *P. capsici*, identify QTLs associated with resistance, and develop molecular markers within the associated QTLs for marker-assisted selection (Lee et al. 2012; Lefebvre and Palloix 1996; Liu et al. 2014; Naegele et al. 2014; Reifschneider et al. 1992; Sy et al. 2005; Thabuis et al. 2004; Walker and Bosland 1999; Wang et al. 2016; Xu et al. 2016). Host resistance to *P. capsici* is highly complex, and varies by race used for screening, method of screening, source of resistance, and other factors (summarized in Barchenger et al. 2018b). We evaluated the effect of the rating system on QTL detection for host resistance to *P. capsici* in pepper. Siddique et al. (2019) detected QTLs associated with multiple races of the pathogen in multiple environments, using both QTL mapping and GWAS, some of which were common, while others were specific. Our work builds upon this study and highlights the importance of selecting the most appropriate rating system for QTL detection and molecular marker development, in addition to race, host, and growing environment.

Disease screening method influences QTL identification for *P. capsici* resistance

The rating system developed by Bosland and Lindsey (1991) resulted in slightly higher LOD for the QTL on chromosome 5, and we detected a QTL on chromosome 12 uniquely using this rating system. A QTL on chromosome 10 was detected using both rating systems, but Black (1999) resulted in a very high LOD for this QTL compared with the Bosland and Lindsey (1991) system. Similarly to our work, Poland and Nelson (2011) reported detecting different QTLs utilizing an ordinal rating system, a percentage estimate, or a combination of both systems for northern leaf blight (caused by *Exserohilum turcicum*) resistance in maize (*Zea mays*), although there were common QTLs detected across systems.

Chromosome 5 has long been known to have QTLs associated with resistance to *P. capsici* across different environments and using different strains or races of the pathogen (Liu et al. 2014; Lozada et al. 2021; Mallard et al. 2013; Rehrig et al. 2014; Siddique et al. 2019). However, the location of the QTLs reported on chromosome 5 varies, which is likely because the chromosome is saturated with nucleotide-binding site leucine-rich repeat (NBS-LRR) genes with high levels of duplication resulting from transposable elements

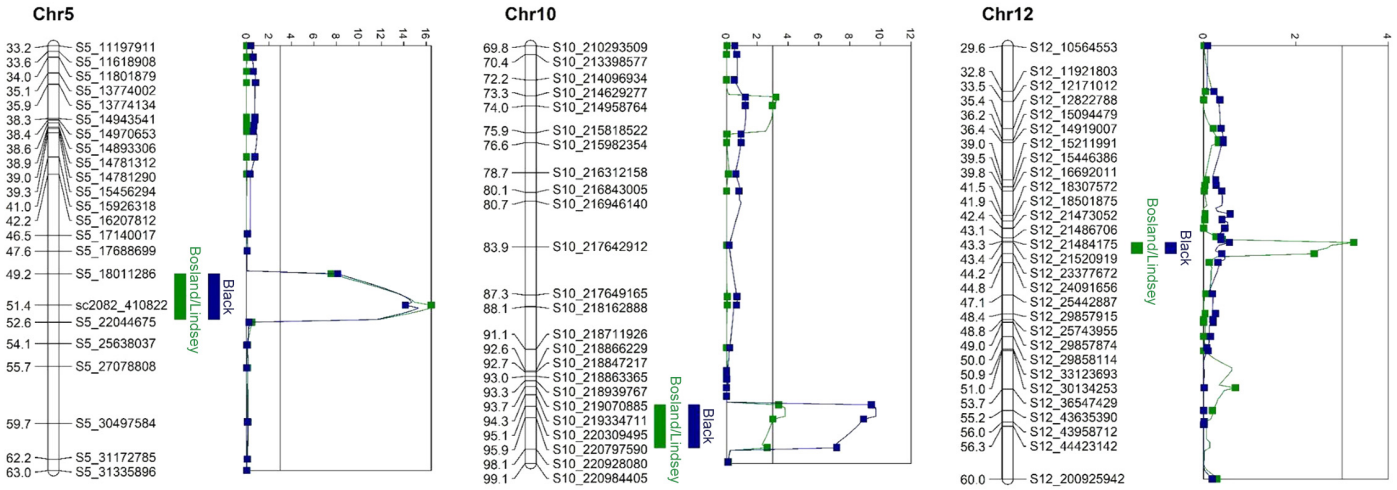

Fig. 3. Significant quantitative trait loci associated with resistance to *Phytophthora capsici* in pepper (*Capsicum annuum*) using the Black (1999) in blue and the Bosland and Lindsey (1991) in green scoring methods. The linkage maps of chromosomes 5, 10, and 12 are zoomed in to show major quantitative trait loci related to *P. capsici* resistance. In each zoomed-in linkage map, the genetic position in centimorgans (cM) and the physical position in base pairs (bp) are indicated on the left and right of the chromosome bar, respectively. Logarithm of odds for each marker is on the top axis of the chart.

TABLE 2. χ^2 goodness of fit test for the two populations developed to determine the inheritance patterns of resistance and for validation of developed molecular markers

Entry	Expected ratio	Black resistant	Black susceptible	χ^2	*P* value	Bosland and Lindsay resistant	Bosland and Lindsay susceptible	χ^2	*P* value
1705-5612-1	1:0	36	0	–	–	36	0	–	–
AVPP1711	0:1	0	36	–	–	0	36	–	–
F_1	1:0	36	0	–	–	36	0	–	–
F_2 (216)	3:1	75	141	186.9	<0.001	76	140	182.6	<0.001
	9:7			40.7	<0.001			38.9	<0.001
	7:9			7.2	0.007			6.4	0.012
PI 201234	1:0	36	0	–	–	36	0	–	–
PBC 142	0:1	0	36	–	–	0	36	–	–
F_1	1:0	36	0	–	–	36	0	–	–
F_2 (144)	3:1	56	88	100.2	<0.001	54	90	108.0	<0.001
	9:7			17.6	<0.001			20.6	<0.001
	7:9			1.4	0.239			2.3	0.131

(Kim et al. 2017; Seo et al. 2016). For example, the chromosome 5 QTL reported here was located in the pericentromeric region, which is similar to that reported by Liu et al. (2014) and Rehrig et al. (2014); however, Siddique et al. (2019) found two major QTLs on the distal and proximal ends of the chromosome. The QTL on chromosome 5 (49.2 to 52.6 cM) identified here was upstream of the major QTL indented by Liu et al. (2014), which was associated with the Phyto5NBS molecular marker and downstream of the major *Pc5.1* QTL at 8.35 and 38.13 cM (Du et al. 2021; Mallard et al. 2013; Rehrig et al. 2014) and that reported by Siddique et al. (2019) between 27.0 and 29.5 cM and Lozada et al. (2021) at 81.2 cM. In addition to chromosome 5, we also found significant QTLs on chromosomes 10 and 12, which have also been reported previously (Siddique et al. 2019; Truong et al. 2012; Xu et al. 2016). The previously reported QTLs detected on chromosome 10, at 14,299 Kb (Siddique et al. 2019; Truong et al. 2012) and at 196 Mb (Siddique et al. 2019; Xu et al. 2016), were both upstream of the chromosome 10 QTL detected here, which was located at the distal end of the chromosome from 219 to 220 Mb. The chromosome 12 QTL detected here was upstream of the previously reported QTLs (Siddique et al. 2019; Truong et al. 2012).

Selection accuracy for molecular markers shows potential for marker-assisted breeding for *P. capsici* resistance in pepper

Generally, but not always, the selection accuracy using the Black (1999) method was slightly higher than that when the Bosland and Lindsey (1991) method was used. The selection accuracy for the molecular markers developed on chromosome 5 was nominally better than that for the widely used Phyto5NBS1 molecular marker (Liu et al. 2014). None of the molecular markers we developed could select completely for host resistance in our validation populations. The highest rate of selection accuracy achieved in this study was 72% for the Ca5.4 marker using the Black (1999) method and 71.6% for the Bosland and Lindsey (1991) rating system. Generally, the molecular markers developed in the QTL on chromosome 10 did not predict the phenotype accurately approximately 50% of the time, which would indicate random chance. The molecular marker developed in the QTL on chromosome 12 had a negative association, with selection accuracy of around 38.5%. While we used two rating systems based on numerical scores associated with disease severity, the actual phenotype of *P. capsici* is more qualitative. It has been found that plants with a score of >2 will eventually die before setting seeds, and therefore, are considered susceptible (Bosland and Lindsey 1991). The χ^2 goodness-of-fit test was based on the phenotypes resistant and susceptible, and did not consider intermediate performance; despite this, we found non-Mendelian inheritance patterns.

TABLE 3. Percentage selection accuracy of the molecular markers developed in this study for a resistant phenotype[a]

Marker	Heterozygous allele grouping	Bosland and Lindsey (1991)	Black (1999)
Phyto5NBS1	R	61.2	56.9
	S	69.3	68.8
CaPC2083	R	61.5	56.4
	S	69.3	70.2
CaPC5.3	R	63.8	58.7
	S	71.6	70.2
CaPC5.4	R	60.1	56.9
	S	71.6	72.0
Ca5.7	R	62.8	57.8
	S	71.6	70.2
CaPC10.2	R	53.2	49.1
	S	54.6	56.0
CaPC10.3	R	51.8	49.1
	S	56.1	57.3
CaPC10.5	R	54.2	49.1
	S	54.6	55.5
CaPC12.1	R	38.0	39.0
	S	38.0	39.0

[a] Selection accuracy represents the percentage of individuals across the validation populations with a match in the phenotype and the molecular marker. The heterozygous alleles were grouped with both the resistant and the susceptible alleles for marker selection accuracy.

In addition to the association of individual molecular markers, we also evaluated the association of combinations of molecular markers with the three significant QTLs. We did not find significant association between a combination of molecular markers that was greater than for the individual markers on chromosome 5. While selection accuracy greater than 70% could be considered applicable for a breeding program, it highlights that other factors are involved in host resistance and were not captured in this study. However, we achieved the highest selection accuracy when we considered heterozygous alleles to be susceptible. Previous studies found that resistance to *P. capsici* in pepper was generally dominant in nature (Lee et al. 2012; Reeves et al. 2013; Sy et al. 2005; Walker and Bosland 1999; Wang et al. 2016; Xu et al. 2016), and heterozygous alleles could be considered to confer a phenotype similar to that for resistant individuals. Contrary to this, we found that the individuals with heterozygous alleles for the chromosome 5 markers had intermediate performance. This intermediate performance indicates potentially incomplete or partially dominant gene action for host resistance to *P. capsici*, which supports the work of Bonnet et al. (2007), who found F_1 individuals to have intermediate levels of resistance compared with the resistant and susceptible parental lines used to develop the hybrid.

Inheritance pattern demonstrates the genetic complexity of *P. capsici* resistance

Duplicative recessive epistasis occurs when the homozygous recessive allele of one gene masks the effect of either the homozygous dominant or the heterozygous allele of another gene, and performs the same as homozygous recessive. The inheritance pattern of resistance in one of our F_2 populations, derived from PI 201234 and PBC 142 and used for molecular marker validation, did not significantly deviate from a 7:9 segregation ratio, indicating duplicative recessive epistasis. However, these results could be confounded by the presence of incomplete gene action. Epistatic interactions of QTLs and inheritance models for *P. capsici* resistance has been previously reported (Lefebvre and Palloix 1996; Naegele et al. 2014; Reifschneider et al. 1992; Siddique et al. 2019; Thabuis et al. 2004). However, there are also extensive reports of resistance following typical Mendelian inheritance models (Lee et al. 2012; Sy et al. 2005; Walker and Bosland 1999; Wang et al. 2016; Xu et al. 2016). There are also reports of resistance following additive gene action (Bonnet et al. 2007; Lefebvre and Palloix 1996). It was reported more than 50 years ago that two resistance loci exist, and either one could confer resistance to *P. capsici* (Smith et al. 1967). Overall, the genetic inheritance for *P. capsici* resistance observed in the current study and in previous reports show its complex genetic architecture imposing challenges in breeding for disease resistant cultivars.

Conclusions

We found that resistance to *P. capsici* in pepper is more complex than widely accepted. Here we report evidence for incomplete dominance and potentially duplicative recessive epistasis, although the presence of one might be resulting in the identification of the other. We also found significant QTLs associated with host resistance that largely support previous studies. In addition, the use of a screening method plays a role in QTL detection and could contribute to the overall lack of widely applicable molecular markers. The molecular markers developed in this study nominally improve selection accuracy relative to the widely used Phyto5NBS1 molecular marker. Overall, this work sheds light on some of the most challenging aspects of breeding for host resistance to *P. capsici* and provides a basis for further research in this important area.

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