Dissection of race 1 anthracnose resistance in a watermelon (Citrullus lanatus var. lanatus) biparental mapping population

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Abstract Anthracnose, caused by the fungal pathogen Colletotrichum orbiculare (Berk. & Mont.) Arx syn. lagenaria, is one of the most important diseases of watermelon in the United States and worldwide. The study was conducted to identify C. orbiculare race 1 resistance quantitative trait loci (QTL) in a ‘Charleston Gray’, resistant parent, and ‘New Hampshire Midget’, susceptible parent, biparental mapping population. The mapping population consisted of 228 F$_2$ and the validation population consisted of 60 individuals each in BC$_1$P$_1$ and BC$_1$P$_2$. The disease severity was rated using a disease index comprising a rating scale of 0–100%. IciMapping was used to draw the linkage map and R/qtl non-parametric method (’model = np’) was used to identity QTL. We identified a major disease resistance QTL, Qar1-8, on chromosome 8. The significant SNP marker S8_5149002, part of a putative coiled-coil (CC)–nucleotide-binding site (NBS)–leucine-rich repeat (LRR) (CC-NBS-LRR or CNL; CICG08G002410), had a LOD of 14.06. The significant marker was validated on mapping populations using R package functions ‘chisq.test’, ‘wilkox.test’, ‘kruskal.test’, and ‘dunn.test’. The significant marker S8_5149002 was also tested for its ability to differentiate race 1 anthracnose resistance on 61 watermelon germplasm including 41 plant introduction (PI) lines. Hence, the diagnostic SNP marker S8_5149002 could be used for marker assisted selection (MAS) for race 1 anthracnose resistance in watermelon breeding programs.

Keywords Watermelon · Colletotrichum orbiculare · Race 1 anthracnose · QTL · PACE SNP marker · Qar1-8

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Introduction

Watermelon (Citrullus lanatus var. lanatus) occupies 7% of global vegetable production acreage and is among the top five most consumed fresh fruits in the world (Yong and Guo 2017). In 2020, watermelon was grown on 100,000 acres and worth $574 million in the U.S. (USDA-NASS 2021). The major watermelon-growing states in the U.S. are Florida, Texas, Georgia, and California. Anthracnose is one of the major diseases of watermelon and other cucurbits and is caused by the fungal pathogen Colletotrichum orbiculare (Berk. & Mont.) Arx syn. lagenaria. The fungus is a hemibiotroph ascomycete that occurs intracellularly in the plant hosts (Perfect et al. 1999; Dickman 2000; Xuei et al. 1988). Seven races of C. orbiculare have been described based on differential host reaction (Goode 1958; Dutta et al. 1960; Jenkins 1964). Wasilwa et al. (1993) grouped a total of 92 isolates of C. orbiculare into ten vegetative compatibility groups (VCGs), of which three (VCG 1, 2, and 3) were pathogenic in cucurbit differentials. Two distinct virulent phenotypes were observed and isolates in VCG 1/VCG 3 had disease reactions similar to previously described race 2 (Wasilwa et al. 1993). The three races of the fungus (races 1, 2, and 3) belong to the pathogenic VCGs and have received particular attention in watermelon (Boyhan et al. 1994). A large number of watermelon germplasm are resistant to Colletotrichum orbiculare race 1 and 3, while others are susceptible (Wasilwa et al. 1993; Maynard and Hopkins 1999). The disease affects all above ground parts and symptoms include angular, brown to black leaf spots; tan, oval-shaped lesions in stems; sunken, and water-soaked spots on fruits (Elwakil et al. 2013; Dutta 1958; Layton 1937). Wet weather conditions such as rain and high humidity provide a favorable environment for dispersion and germination of conidia, and subsequent infection in plant (Maynard and Hopkins 1999).

Several accounts of anthracnose as a major disease in cucurbits can be traced back to the late nineteenth century and early twentieth century (Gardner 1918; Parris 1949). The most severe reports of this disease was mainly in south, southeast, northeast, and midwest regions of the U.S. (Wasilwa et al. 1993), with up to 30% yield loss reported in watermelon (Parris 1949) and 60% yield loss reported in other cucurbits (Thompson and Jenkins 1985). A significant negative impact on plants due to anthracnose is on fruit quality, as this disease influences grading standards of watermelon outlined by the United States Department of Agriculture (USDA-AMS 2021). Research on anthracnose disease management in watermelon has been prioritized in the past (King and Davis 2007), and is still considered a major research priority (Kousik et al. 2016).

Several efforts focused on breeding watermelon varieties for anthracnose resistance have been reported (Huh et al. 2010a, b; Crall et al. 1994; Norton et al. 1993; Crall 1990). Resistance to race 1 anthracnose in watermelons has been shown to be governed by a single dominant locus, Ar-1, and resistance was dominant to susceptibility (Layton 1937; Wehner 2012). Utilizing molecular markers closely associated with underlying genes can increase efficiency of the breeding programs (Xu and Crouch 2008). Single nucleotide polymorphism (SNP) markers are the latest of the molecular markers, succeeding restriction fragment length polymorphisms (RFLP) markers (Beckmann and Soller 1986), random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990), simple sequence repeats (SSRs) or microsatellite markers (Litt and Luty 1989; Akkaya et al. 1992), and amplified fragment length polymorphisms (AFLP) markers (Vos et al. 1995). The popularity of SNP markers stems from the fact that they are commonly occurring DNA sequence variations, the basis of most genetic variation (Ganal and Röder 2007; Chagné et al. 2008), high density, cost effective and efficient compared to previous types of markers (Xu and Crouch 2008), and may affect protein function if present in the coding sequences (Yuan et al. 2006). The quantitative trait loci (QTL) discovery in watermelon facilitates marker-assisted selection (MAS) and breeding for several traits such as Fusarium wilt resistance (Lambel et al. 2014; Branham et al. 2018, 2020; Meru and McGregor 2016), gummy stem blight resistance (Lee et al. 2021; Gimode et al. 2021), and fruit/flesh quality traits (Fall et al. 2019; Ren et al. 2018; Yang et al. 2021).

In the current study, we identified a major QTL for C. orbiculare race 1 resistance from ‘Charles Gray’ in a F2 population and validated on BC1 populations. We further delineated a putative race 1 anthracnose resistant gene in the QTL region and used a previously reported SNP marker (Jang et al. 2021).
to differentiate race 1 anthracnose resistant and susceptible individuals from the mapping population, as well as the broader watermelon germplasm pool.

Materials and methods

Developing biparental mapping populations

The watermelon mapping populations were developed at North Carolina State University. Two parental lines, ‘Charleston Gray’ (resistant, female parent, P₁) developed by C. F. Andrus in 1954 (Andrus 1955), and ‘New Hampshire Midget’ (susceptible, male parent, P₂) were used to generate F₁, F₂, BC₁P₁, and BC₁P₂ mapping populations. The mapping populations consisted of 228 F₂ individuals as well as 60 individuals each in BC₁P₁ and BC₁P₂.

Inoculum preparation and pathogen inoculation

Colletotrichum orbiculare race 1, collected in North Carolina in 1998, was used to inoculate seedlings. The inoculum preparation and inoculation were conducted as described by Patel (2019). In brief, the fungus was grown on green bean agar (GBA) media for three-weeks. Spores were harvested by adding 10–15 mL distilled water to each agar plate, rubbing the surface of the agar with a sterile metal spreader, pouring the spore suspension into a sterile conical flask, and passing it through four layers of cheesecloth. Concentration of the inoculum was measured using a hemocytometer and adjusted to 100,000 spores mL⁻¹ prior to inoculation. One drop of Tween-20 was added to every 500 mL of the spore inoculum. The three-week-old watermelon seedlings grown in the greenhouse were inoculated with the spore inoculum. After inoculation, seedlings were kept in a humidity chamber, in the greenhouse, for 48 h in darkness at 80–100% relative humidity, and at a temperature of 22–24 °C. Then, seedlings were moved to the natural light, and rated at 8, 11, and 14 days post inoculation (dpi).

Disease rating

The disease index was rated on a scale of 0 to 100%, with an interval of 5%, with weightage on different parts of the plants—true leaves (50% total: yellowing-5%, complete necrotic leaf-40%, petiole-10%), meristem (25% total: necrosis spots-10%, mostly necrotic-20%, dead-25%), hypocotyl (20% total: 1–2 brown patches-5%, many brown patches-15%, completely brown-20%), cotyledons (5% total: little to complete necrosis: 5%). Individuals were designated as resistant and susceptible when the overall rating score was ≤ 40, and ≥ 41%, respectively (Patel 2019).

DNA isolation, ddRADseq library construction, and genotyping by sequencing

A total of 360 watermelon leaf samples (three P₁, three P₂, six F₁, 60 BC₁P₁, 60 BC₁P₂, and 228 F₂ individuals) were collected from three-week-old seedlings. Samples were freeze-dried immediately, and genomic DNA was extracted from lyophilized samples using E.Z.N.A. Plant DNA Kit (Omega BioTek, GA, USA) following manufacturer’s protocol. The DNA were quantified using Quant-iT-PicoGreen (Invitrogen, Thermo Fisher Scientific, USA) following the manufacturer’s instructions. Due to some samples yielding low amounts of DNA, a total of 188 watermelon samples (three P₁, two P₂, six F₁, 48 BC₁P₁ and 129 F₂ individuals) were sent to Texas A&M AgriLife Genomics and Bioinformatics Service, College Station, TX (https://www.txgen.tamu.edu/) for double digest restriction-site associated DNA sequencing (ddRADseq) as described previously (Yang et al. 2020) with the following changes. The restriction enzymes EcoRI and NlaIII were used for library prep and inserts from 400 to 600 bp were selected on a Pippin prep (Sage Science, Boston, MA, USA). The ddRADseq libraries were sequenced using 40% of a NovaSeq S4 X lane (2 × 150 bp paired-end run; Illumina, Inc., San Diego, CA, USA).

Raw sequences were demultiplexed using Illumina bcl2fastq, allowing for 1 base error in the barcode sequences. Sequences were first quality-filtered using the program FASTX-Toolkit (http://hannonlab.cshl.edu/fastx-toolkit). Raw sequencing reads were first trimmed to remove low quality bases with quality score less than 20 on the ends of reads and reads with 30% or more bases showing low quality score (Q < 15) were removed. The reference genome for watermelon was downloaded from NCBI website (GCA_000238415.2). Bowtie2 [http://bowtie-bio.sourceforge.net/bowtie2/index.shtml] was used...
to align quality-filtered reads to the reference with the default parameters. Aligned reads were then processed with SAMtools v1.19 to generate coordinate sorted binary SAM files (BAM). Reads with mapping quality (MQ) less than 5 were removed. The local re-alignment tool in the Genome Analysis Toolkit (GATK, https://software.broadinstitute.org/gatk/) was used to perform re-alignment in Insertion/Deletion regions as previously described. Finally, the processed alignment files were fed to the tool Haplotype-Caller, which is part of the GATK, to call variations and perform genotyping for each sample. Once the SNP calling process was completed, individual SNPs with more than 20% missing data and Minor Allele Frequency (MAF) less than 0.05 in each population group were removed.

QTL mapping

Genotypic data were assigned to A (P1 type, homozygous resistant), B (P2 type, homozygous susceptible), H (heterozygous), and X (missing) types. Since the phenotypic disease rating data for F2 population were found to be in a non-normal distribution, QTL analysis was done on ‘qtl’ package (Broman et al. 2003) with a non-parametric method (‘model = np’) on R software (R Core Team 2014; version 3.6.2) with RStudio GUI (RStudio-Team 2021). The logarithm of odds (LOD) threshold of 4.11 for QTL detection was estimated with 1000 permutations. As genotyping-by-sequencing (GBS) genotypic data had higher missing values, QTL analysis was also performed after imputing missing genotypic data using a multiple imputation method (‘method = imp’) (Sen and Churchill 2001) on R ‘qtl’. An additional 3 to 9 cycles of final denaturation and annealing/extension was done to improve the amplification, as well as to obtain tight and well separated clusters. Several non-parametric analysis—Chi-Square, Mann–Whitney-Wilcoxon test (Wilcoxon 1945; Mann and Whitney 1947), Kruskal–Wallis test (Kruskal and Wallis 1952), and Dunn’s test (Dunn 1964) were conducted on data on R software (R Core Team 2014; version 3.6.2) with RStudio GUI (RStudio-Team 2021) using functions ‘chisq.test’, ‘wilcox.test’, ‘kruskal.test’, and ‘dunn.test’, respectively.

Results

Disease response of the mapping population

Phenotypic disease response of race 1 anthracnose inoculated mapping populations (N=360) resulted in Mendelian ratios for a single gene (Supplementary Table S2). All 60 BC1P1 individuals showed no
segregation and had 100% resistance phenotype. The 60 \text{BC1P2} individuals (\chi^2_{1:1} = 0.26, P = 0.60) failed to reject the null hypothesis of 1:1::resistant:susceptible segregation ratio. The 228 \text{F2} individuals (\chi^2_{3:1} = 2.33, P = 0.12) also failed to reject the null hypothesis of 3:1::resistant:susceptible segregation ratio. The histograms of the \text{BC1P1}, \text{BC1P2}, and \text{F2} population (Fig. 1) indicated a non-normal phenotypic distribution.

Analysis of ddRADseq data

Generated DNA fragments (400–600 bp inserts) were selected on the Pippin Prep platform. After construction of ddRADseq libraries, they were sequenced using 40% of a NovaSeq S4 X lane (2 \times 150 bp paired-end run), and an average of 4.79 million (M) reads/sample or 1.44 giga base (Gb) per sample were generated. A 4X genome coverage (depth) was obtained on average. Approximately 50% of reads were chloroplast or mitochondria based on the basic local alignment search tool (BLAST). However, upon manually checking several reads to the reference genome (https://www.ncbi.nlm.nih.gov/assembly/GCA_000238415.2), samples aligned from 89.06 to 99.45% to the reference genome. This attests to a recent finding that there is exchange of genetic material between nuclear and organelle genome, and the mitochondrial and chloroplast genomes in watermelon share about 33% and 47% homology, respectively with the nuclear genome (Cui et al. 2021). The reference genome obtained from the National Center for Biotechnology Information (NCBI) website (GenBank assembly accession: GCA_000238415.2) corresponds to the watermelon cultivar ‘97103’ v2 Genome in the Cucurbit Genetics Database (http://cucurbitgenomics.org/ftp/genome/watermelon/97103/v2/). At median 3 and mean 11 coverage depth, a total of 147,600 raw, unfiltered single nucleotide polymorphisms (SNPs) were obtained. After removing SNPs with depth > 20, a total of 134,136 SNPs were remaining. After filtering SNPs with minor allele frequency (MAF) < 0.05 and more than 20% missing data, a total of 653 SNP markers were left.

QTL mapping, genetic linkage map and resistant gene

After aligning the SNP regions between ‘97103’ and ‘Charleston Gray’ genomes, the physical coordinates of markers were updated to represent ‘Charleston Gray’ and used in the linkage map construction for Chromosome 8 (Fig. 2). The genetic linkage maps were also drawn for remaining ten chromosomes using physical coordinates of ‘97103’ watermelon genome (Supplementary Fig. S2). The rank based non-parametric QTL analysis was done on R ‘qtl’ and a significant SNP marker S8_5149002 was observed in the major QTL region (LOD = 14.06) (Table 1 and Fig. 2). The effect plot for the marker showed that the disease index was low and similar for the homozygous resistant (\text{Ar-1Ar-1}) and heterozygous individuals (\text{Ar-1ar-1}) as compared to the homozygous susceptible (\text{ar-1ar-1}) (Fig. 3). Since GBS genotypic data resulted in higher missing value, QTL analysis was re-analyzed after multiple imputation in R/qtl. The LOD score for the significant marker increased from 14.06 to 44.42 after imputation. The QTL was validated on \text{BC1P1} and \text{BC1P2} populations, where only the latter population showed a significant QTL with S8_5149002 being the significant marker (LOD = 8.32; Supplementary Fig. S3). The physical coordinate of S8_5149002 marker did not align with the physical positions of adjacent markers on \text{F2} population probably due to inversion or crossover in this genome segment on the mapping population or due to the small population size of the mapping population. Such discrepancy in the order of marker locations was also observed earlier in the same region of Chromosome 8 in watermelon (Shang et al. 2016; Jang et al. 2019). The results from this study showed that a significant QTL, \text{Qar1-8}, from ‘Charleston Gray’ contributed to race 1 anthracnose resistance.

PACE based SNP genotyping and significant marker validation

Out of 34 PACE markers designed, only three markers (S8_4483489, S8_4714069, and S8_5149002) were found to be polymorphic and clustered populations into three distinct groups—homozygous resistant, heterozygous, and homozygous susceptible. The proportion of individuals into different groups based on the PACE SNP genotyping and their Chi-square values are presented in Supplementary Table S3. Non-parametric tests (Chi-Square, Mann–Whitney, and Kruskal–Wallis) using PACE-based genotypic data of three markers (S8_4483489, S8_4714069 and S8_5149002) showed varying results (Table 2).
Fig. 1 Histogram showing disease response on race 1 anthracnose inoculated ‘Charleston Gray’ × ‘New Hampshire Midget’ populations: a BC₁P₁ population (N = 60); b BC₁P₂ population (N = 60); c F₂ population (N = 228). The arrows mark the average disease rating (%) for the resistant and susceptible parents in the F₂ population.
For Marker S8_4483489 and S8_4714069, the observed segregation ratios significantly deviated from expected Mendelian ratios ($P<0.05$) in the backcross population [BC$_1$P$_2$, expected 1(Ar-1Ar-1):1(ar-1ar-1)]. However, the Mann–Whitney test failed to reject the null hypothesis ($P=0.12$) for 1:1 ratio. The Chi-Square test for markers S8_4483489 and S8_4714069 on the F$_2$ population failed to reject ($P=0.06$) and rejected ($P<0.001$), respectively, the null hypothesis for expected ratios—1(Ar-1Ar-1):2(Ar-1ar-1):1(ar-1ar-1). Furthermore, the Kruskal–Wallis test in F$_2$ population resulted in a significant difference ($P<0.001$) among Ar-1Ar-1 (homozygous resistant), ar-1ar-1 (homozygous susceptible), and Ar-1ar-1 (heterozygous) groups. Since the Kruskal–Wallis test in F$_2$ population was significant for the marker, a post-hoc analysis using Dunn’s test was done to compare how the three groups (Ar-1Ar-1, ar-1ar-1, and Ar-1ar-1) differed from each other. There was significant difference between...
all groups: (Ar-1Ar-1 vs ar-1ar-1), (Ar-1Ar-1 vs Ar-1ar-1), and (ar-1ar-1 vs Ar-1ar-1) (P<0.001), indicating that markers categorized homozygous dominant (Ar-1Ar-1), heterozygous (Ar-1ar-1), and homozygous recessive (ar-1ar-1) into three separate groups based on phenotype. Results indicated that markers failed to correctly assign individuals into resistant and susceptible groups based on the single dominant gene. Thus, markers S8_4483489 and S8_4714069 were not diagnostic markers for Qar1-8.

Contrastingly, the marker S8_5149002 did not show deviation between the observed and expected Mendelian ratios for both the Chi-Square and Mann–Whitney test (P=0.09). The Chi-Square test in the F2 population showed that there was no deviation (P=0.69) from expected Mendelian ratios – 1(Ar-1Ar-1):2(Ar-1ar-1):1(ar-1ar-1). The Kruskal–Wallis test in F2 population showed that there was significant difference (P<0.05) among Ar-1Ar-1, ar-1ar-1, and Ar-1ar-1 groups. The post-hoc

Table 2 Results of non-parametric tests (Chi-Square, Mann–Whitney = MW, Kruskal–Wallis = KW, Dunn’s Test = DT) in BC1P2 and F2 population using information obtained from PACE-based genotyping

| Marker | BC1P2 | | | F2 | | | | | |
|--------|-------|---|---|---|---|---|---|---|---|
|        | χ²    | P  | MW (P) | χ² | P  | KW: χ² | P  | DT: z | P  |
| S8_4483489 | 5.5  | 0.02 | 0.12 | 5.6  | 0.06 | 78.2 | <0.001 | -8.69 | <0.001 |
| S8_4714069 | 3.9  | 0.04 | 0.12 | 11.0 | <0.001 | 67.7 | <0.001 | -8.21 | <0.001 |
| S8_5149002 | 0.2  | 0.69 | 0.09 | 3.4  | 0.18 | 124.3 | <0.001 | -9.46 | <0.001 |

P<0.05 indicates there is significant difference between observed and expected Mendelian ratios or between groups Ar-1Ar-1, ar-1ar-1, and Ar-1ar-1; Ar-1Ar-1=homozygous resistant parent alleles, ar-1ar-1=homozygous susceptible parent alleles, and Ar-1ar-1=heterozygous
Dunn’s test showed significant difference between only the two groups (Ar-1Ar-1 vs ar-1ar-1) and (ar-1ar-1 vs Ar-1ar-1) \( (P<0.001) \) but not for the (Ar-1Ar-1 vs Ar-1ar-1) group \( (P=0.15) \). Results indicated that the marker, S8_5149002, was able to distinguish phenotypes, resistant (Ar-1Ar-1 and Ar-1ar-1) versus susceptible (ar-1ar-1) and agrees with earlier genetic studies that a single dominant gene controls race 1 anthracnose resistance. Results showed that S8_5149002 marker is the diagnostic marker located in the Qar1-8 region.

The SNP marker S8_5149002 was used to discriminate the watermelon germplasm \( (N=61) \) for Colletotrichum orbiculare race 1 resistance. There were 19, 12, and 30 germplasm showing homozygous resistant, heterozygous, and homozygous susceptible alleles, respectively (Supplementary Table S4).

Discussion

Phenotypic and genotypic ratios

Colletotrichum orbiculare race 1 affects watermelon and cucumber in which a single dominant resistance gene was reported (Layton 1936; Hall et al. 1960; Barnes and Epps 1952). In this study, the Chi-square analysis showed a goodness of fit for a single dominant gene controlling race 1 anthracnose resistance both phenotypically and genotypically. Similar Mendelian phenotypic segregation ratios were reported and suggested that a single dominant gene was involved for race 1 anthracnose resistance in watermelon—‘Africa 8’ (Layton 1937), and ‘Charleston Gray’, ‘Congo’ and ‘Fairfax’ (Hall et al. 1960) (Table 3). Resistance to anthracnose in beans was also found to be dominant in crosses of resistant × tolerant and resistant × susceptible varieties (Andrus and Wade 1942). A single dominant gene for anthracnose resistance was also reported in cucumber (Barnes and Epps 1952). Robinson et al. (1976) assigned Ar gene symbol for the anthracnose resistance gene in watermelon and cucumber. Winstead et al. (1959) also reported that race 1 anthracnose resistance gene also conferred race 3 anthracnose resistance in watermelon by superimposing race 3 inoculum on race 1 inoculated plants and vice-versa. The pedigree of ‘Charleston Gray’ had ‘Africa 8’, whereas the pedigree of ‘Congo’ and ‘Fairfax’ had ‘African’ (Table 3).

It is most likely that Ar-J in ‘Charleston Gray’ might had been inherited from ‘Africa 8’, a race 1 anthracnose resistance founder parent.

Resistance QTL and putative genes

In the study, the preliminary analysis using GBS markers identified race 1 anthracnose resistance QTL on chromosome 8 (in between coordinates 4,847,957 and 6,294,791). The QTL on chromosome 8 was on the similar region to the previous study (Jang et al. 2019). One of the genes, CC-NBS-LRR (CNL; Cla001017 or ClCG08G002410), in the QTL region was reported for race 1 anthracnose resistance on breeding line ‘DrHS7250’ (Jang et al. 2019). We converted the high-resolution melting (HRM) SNP marker, CL14-27-9, for ClCG08G002410 onto the PACE marker and designated it as S8_5149002. We reanalyzed F2 mapping population data by including genotypic data for marker S8_5149002. The result showed that S8_5149002 was the significant marker

| Genotype        | Pedigree                                                                 | Race 1 Anthracnose response | References                                      |
|-----------------|--------------------------------------------------------------------------|----------------------------|------------------------------------------------|
| Charleston Gray | \[((\text{Africa } 8 \times \text{Iowa Belle}) \times \text{Garrison}) \times \text{Garrison}] \times [(\text{Hawkesbury} \times \text{Leesburg}) \times \text{Garrison}] | R                          | (Hall et al. 1960; Levi et al. 2001b)          |
| Congo           | (\text{African} \times \text{Iowa Belle}) \times \text{Garrison}        | R                          | (Hall et al. 1960; Levi et al. 2001b)          |
| Fairfax         | [\text{Garrison} \times (\text{African} \times \text{Iowa Belle})] \times [(\text{Leesburg} \times \text{Hawkesbury})] | R                          | (Hall et al. 1960; Levi et al. 2001b)          |
| New Hampshire Midget | (\text{Favorite Honey} \times \text{Dakota Sweet})             | S                          | (Yeager 1950; Rhodes et al. 1992)              |
with LOD 14.6 and up to 44 (with imputation) and could indicate that \textit{ClCG08G002410} could also be the race 1 anthracnose resistance gene in ‘Charleston Gray’.

Variable genotype of watermelon germplasm

The significant SNP marker S8\_5149002 clearly differentiated the disease response of the individuals of the mapping population as well as the germplasm present in the watermelon breeding program. Genotype of several watermelon germplasm and hybrids showed homozygous resistance to race 1 anthracnose (Supplementary Table S4). These include ‘Crimson Sweet’, ‘TASTIGOLD’, ‘AU-Sweet Scarlet’, ‘AU-Golden Producer’, ‘Perola’, ‘Crimson Diamond’, ‘Graybelle’, ‘Verona’, ‘SUNSHADE’, ‘Sugarlee’, ‘Dixielee’, ‘Jubilee’, ‘Big Stripe’, ‘Pronto’, ‘Pathfinder F1’, and ‘Fascination’. For most of them the source of race 1 anthracnose resistance might have inherited from the founder parent—‘Africa 8’. It is intriguing that the \textit{Ar-1} gene is exhibiting resistance for more than 50 years. Interestingly, the germplasm PI 189225 which is resistant to race 2 anthracnose (Levi et al. 2001a), showed susceptible genotype (\textit{arl-1}) for race 1 anthracnose suggesting race-specific resistance provided by the \textit{R}-genes.

Conclusion

The study delineates a major QTL region on chromosome 8 governing race 1 anthracnose resistance and putative CC-NBS-LRR (CNL; \textit{ClCG08G002410}) could be a potential resistance gene in Charleston Gray. Further study is needed to validate that the CNL is the \textit{Ar-1} gene. The S8\_5149002 is a diagnostic marker for race 1 anthracnose resistance and could be used in MAS in watermelon breeding programs.

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Declarations

Conflict of interest  The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Consent to participate  Not applicable.

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References

3crBioscience (2018) PACE genotyping master mix user guide. https://3crbio.com/wp-content/uploads/2019/01/PACE-IR-User-Guide-v1.5.pdf, Accessed on 18 July 2021

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132:1131–1139

Andrus CF (1955) New watermelon varieties: bring new life to that industry. Seed World 4:36–40

Andrus CF, Wade BL (1942) The factorial interpretation of anthracnose resistance in beans. U.S. Department of Agriculture, Washington DC, pp 1–29

Barnes W, Epps W (1952) Two types of anthracnose resistance in cucumbers. Plant Dis Rptr 36:479–480

Beckmann J, Soller M (1986) Restriction fragment length polymorphisms in plant genetic improvement. Oxf Surv Plant Mol Cell Biol 3:196–250

Boyhan G, Norton J, Abrahams B, Wen H (1994) A new source of resistance to anthracnose (Race 2) in watermelon. HortScience 29:111–112

Branham SE, Patrick Wechter W, Lambel S, Massey L, Ma M, Fauve J, Farnham MW, Levi A (2018) QTL-seq and marker development for resistance to Fusarium oxysporum f. sp. niveum race 1 in cultivated watermelon. Mol Breed 38:1–9

Branham SE, Patrick Wechter W, Ling K-S, Chanda B, Massey L, Zhao G, Guner N, Bello M, Kabelka E, Fei Z (2020) QTL mapping of resistance to Fusarium oxysporum f. sp. niveum race 2 and Papaya ringspot virus in Citrullus amarus. Theor Appl Genet 133:677–687

Broman KW, Wu H, Sen Š, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890

Chagné D, Gasic K, Crowhurst RN, Han Y, Bassett HC, Bowatte DR, Lawrence TJ, Rikkerink EH, Gardiner SE,
Korban SS (2008) Development of a set of SNP markers present in expressed genes of the apple. Genomics 92:353–358

Crall JM (1990) “Charlee” watermelon. HortScience 25:812–813

Crall JM, Elmstrom GW, McCuistion FT (1994) SSDL: a high-quality icebox watermelon breeding line resistant to fusarium wilt and anthracnose. HortScience 29:707–708

Cui H, Ding Z, Zhu Q, Wu Y, Qiu B, Gao P (2021) Comparative analysis of nuclear, chloroplast, and mitochondriad genomes of watermelon and melon provides evidence of gene transfer. Sci Rep 11:1–9

Dickman MB (2000) Colletotrichum. In: Kronstad JW (ed) Fungal pathol. Springer, Dordrecht, pp 127–147

Dunn OJ (1964) Multiple comparisons using rank sums. Technometrics 6:241–252

Dutta SK, Hall C, Heyne E (1960) Observations on the physiological races of Colletotrichum lagenarium. Bot Gaz 121:163–166

Dutta SK (1958) Inheritance of resistance to anthracnose in watermelon. Kansas State College of Agriculture and Applied Science. https://krex.k-state.edu/dspace/bitstream/2097/25212/LD2668T41958D89.pdf?sequence=1

Elwakil WM, Dufault NS, Freeman JH, Mossler MA (2013) Florida crop/pest management profile: watermelon: CIR1236. Electronic data information source (EDIS). https://edis.ifas.ufl.edu/pdf/PI/PI031/PI031-D9ymdph1t3.pdf. Accessed 22 Mar 2022

Fall LA, Perkins-Veazie P, Ma G, McGregor C (2019) QTLs associated with flesh quality traits in an elite elite watermelon population. Euphytica 215:1–14

Ganal MW, Röder MS (2007) Microsatellite and SNP markers in wheat breeding. In: Varshney RK, Tuberosa R (eds) Genomics-assisted crop improvement, 1st edn. Springer, Dordrecht, pp 127–147

Gardner MW (1918) Anthracnose of cucurbits. U.S. Department of Agriculture, Washington, DC

Gimode W, Bao K, Fei Z, McGregor C (2021) QTL associated with gummy stem blight resistance in watermelon. Theor Appl Genet 134:573–584

Goode MJ (1958) Physiological specialization in Colletotrichum lagenarium. Phytopathology 48:79–83

Hall CV, Dutta SK, Kalia HR, Rogerson CT (1960) Inheritance of resistance to the fungus Colletotrichum lagenarium (Pass.) Ell. and Halst. in watermelons. Proc Am Soc Hort Sci 75:638–643

Huh Y-C, Hong K-H, Ko H-C, Park K-S, Park D-K, Lee J-S, Cho M-C, Lee S-Y, Ko K-D, Lee W-M (2010a) Breeding of a Mid-Late Maturing Watermelon Cultivar, ‘Hanbit’ with Resistant to Anthracnose Race 3. Korean J Breed Sci 42

Huh Y-C, Hong K-H, Ko H-C, Park K-S, Park D-K, Lee J-S, Cho M-C, Lee S-Y, Ko K-D, Lee W-M (2010b) Breeding of a Mid Maturing Watermelon Cultivar, ‘Hangyeco’ with Resistance to Anthracnose Race 3. Korean J Breed Sci 42

Jang YJ, Seo M, Hersh CP, Rhee S-J, Kim Y, Lee GP (2019) An evolutionarily conserved non-synonymous SNP in a leucine-rich repeat domain determines anthracnose resistance in watermelon. Theor Appl Genet 132:473–488

Jenkins SF (1964) Pathogenic comparisons of three new and four previously described races of Glomerella cingulata var. orbiculare.

King S, Davis A (2007) Hot topics for watermelon research: a survey of the industry. HortScience 42:454–454

Kousik CS, Brusca J, Turechek WW (2016) Diseases and disease management strategies take top research priority in the watermelon research and development group members survey (2014 to 2015). Plant Health Prog 17:53–58

Kruskal WH, Wallis WA (1952) Use of ranks in one-criterion variance analysis. J Am Stat Assoc 47:583–621

Lambel S, Lanini B, Vivoda E, Fauve J, Patrick Wechter W, Harris-Shultz KR, Massey L, Levi A (2014) A major QTL associated with fusarium oxysporum race 1 resistance identified in genetic populations derived from closely related watermelon lines using selective genotyping and genotyping-by-sequencing for SNP discovery. Theor Appl Genet 127:2105–2115

Layton DV (1936) The parasitism of Colletotrichum lagenarium (Pass.) Ell. and Halst. Iowa State College. https://dr.lib.iastate.edu/server/api/bitstreams/58dc1b3f-a3a3-49c7-b2d4-44eacafeb6ba/content

Layton DV (1937) The parasitism of Colletotrichum lagenarium (Pass.) Ell. and Halst. Res Bull, Agric Exp St, Iowa State College of Agric and Mech Arts 223:37–67

Lee ES, Kim D-S, Kim SG, Huh Y-C, Back C-G, Lee Y-R, Siddique MI, Han K, Lee H-E, Lee J (2021) QTL mapping for gummy stem blight resistance in watermelon (Citrullus spp.). Plants 10:500

Levi A, Thomas CE, Keinath AP, Wehner TC (2001a) Genetic diversity among watermelon (Citrullus lanatus and Citrullus colocynthis) accessions. Gen Resour Crop Evol 48:559–566

Levi A, Thomas CE, Wehner TC, Zhang X (2001b) Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. HortScience 36:1096–1101

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397

Mann HB, Whitney DR (1947) On a test of whether one of two random variables is stochastically larger than the other. Ann Math Stat 18:50–60

Maynard DN, Hopkins DL (1999) Watermelon fruit disorders. HortTechnology 9:155–161

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop Journal 3(3):269–283

Meru G, McGregor C (2016) Genotyping by sequencing for SNP discovery and genetic mapping of resistance to race 1 of Fusarium oxysporum in watermelon. Sci Hortic 209:31–40

Norton J, Boyhan GE, Smith DA, Abravams BR (1993) AU-golden producer: a high quality, disease resistant watermelon for the South. Alabama Agricultural Experiment Station. Auburn University, Auburn, Alabama

Parisi G (1949) Watermelon breeding. Econ Bot 3:193–212

Patel TK (2019) Studies on anthracnose resistance in watermelon germplasm. PhD Dissertation, North Carolina State University, Raleigh, North Carolina
Perfect SE, Hughes HB, O’Connell RJ, Green JR (1999) Colletotrichum: a model genus for studies on pathology and fungal–plant interactions. Fungal Genet Biol 27:186–198

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/

Ren Y, Guo S, Zhang J, He H, Sun H, Tian S, Gong G, Zhang H, Levi A, Tademor Y (2018) A tonoplast sugar transporter underlies a sugar accumulation QTL in watermelon. Plant Physiol 176:836–850

Rhodes B, Zhang X, Dean RA, Frick J, Nong Zhang J (1992) Use of a detached leaf assay for race 1 and race 2 anthracnose resistance in a diallel cross with Citrullus. Cucurbit Genet Coop

Robinson RW, Munger HM, Bohn GW (1976) Genes of the cucurbitaceae. HortScience 11:554–568

RStudio-Team (2021) RStudio: integrated development environment for R. RStudio, PBC. http://www.rstudio.com/

Sen Ś, Churchill GA (2001) A statistical framework for quantitative trait mapping. Genetics 159:371–387

Shang J, Li N, Li N, Xu Y, Ma S, Wang J (2016) Construction of a high-density genetic map for watermelon (Citrullus lanatus L.) based on large-scale SNP discovery by specific length amplified fragment sequencing (SLAF-seq). Sci Hort 203:38–46

Thompson D, Jenkins S (1985) Influence of cultivar resistance, initial disease, environment, and fungicide concentration and timing on anthracnose development and yield loss in pickling cucumbers. Phytopathology 75:1422–1427

USDA-AMS (2021) United States standards for grades of watermelons. United States Department of Agriculture (Agricultural Marketing Service, Specialty Crops Program, Specialty Crops Inspection Division), Washington, D.C. https://www.ams.usda.gov/sites/default/files/media/WatermelonStandards.pdf, Accessed on 23 Mar 2022

USDA-NASS (2021) Vegetables 2020 summary. United States Department of Agriculture (National Agricultural Statistics Service). https://downloads.usda.library.cornell.edu/usda-esmis/files/02870v86p/j6731x86f/9306t664/vegea

Voorrips R (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Vos P, Hogers R, Bleeker M, Reijans M, Tvd L, Hornes M, Friters A, Pot J, Paleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wasilwa L, Correll J, Morelock T, McNew R (1993) Reexamination of races of the cucurbit anthracnose pathogen Colletotrichum orbiculare. Phytopathology 83:1190–1198

Wehner TC (2012) Gene List for Watermelon; https://cucurbit.info/2012/07/2012-gene-list-for-watermelon/, Accessed on 23 Mar 2022

Wilcoxon F (1945) Individual comparisons by ranking methods. Biom Bull 1:80–83

Williams JG, Kebeler AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Winstead N, Goode M, Barham W (1959) Resistance in watermelon to Colletotrichum lagenarium races 1, 2, and 3. Plant Dis Rptr 43:570–577

Xu Y, Crouch JH (2008) Marker-assisted selection in plant breeding: from publications to practice. Crop Sci 48:391–407. https://doi.org/10.2135/cropsci2007.04.0191

Xuei X, Järflors U, Kuć J (1988) Ultrastructural changes associated with induced systemic resistance of cucumber to disease: host response and development of Colletotrichum lagenarium in systemically protected leaves. Can J Bot 66:1028–1038

Yang T, Amanullah S, Pan J, Chen G, Liu S, Ma S, Wang J, Gao P, Wang X (2021) Identification of putative genetic regions for watermelon rind hardness and related traits by BSA-seq and QTL mapping. Euphytica 217:1–18

Yang Y, Dhakal S, Chu C, Wang S, Xue Q, Rudd JC, Ibrahim AMH, Jessup K, Baker J, Fuentebalba MP, Devkota R, Baker S, Johnson CD, Metz R, Liu S (2020) Genome-wide identification of QTL associated with yield and yield components in two popular wheat cultivars TAM 111 and TAM 112. PLoS One 15:e0237293

Yeager AF (1950) Breeding improved horticultural plants. I. Vegetables. The University of New Hampshire. https://scholars.unh.edu/cgi/viewcontent.cgi?article=1341&context=agbulletin, Accessed on 23 Mar 2022

Yong X, Guo S (2017) The watermelon genome. In: R. Grumet NK, Garcia-Mas J (ed) Genetics and genomics of cucurbits. Springer, pp 199–210

Yuan H-Y, Chiou J-J, Tseng W-H, Liu C-H, Liu C-K, Lin Y-J, Yang T, Amanullah S, Pan J, Chen G, Liu S, Ma S, Wang J, Wang H-H, Yao A, Chen Y-T, Hsu C-N (2006) FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. Nucleic Acids Res 34:W635–W641

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