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High-Density Linkage Mapping and Identification of Quantitative Trait Loci Associated with Powdery Mildew Resistance in Flowering Dogwood (*Cornus florida*)

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Abstract: Flowering dogwood (*Cornus florida* L.) is a popular, spring-blooming ornamental tree native to the eastern United States. The species is in general very susceptible to powdery mildew caused by *Erysiphe pulchra*, which disfigures leaves, decreases growth, and negatively affects flowering. Breeding for resistance has been recognized as an ideal strategy for controlling the disease in *C. florida*, but efforts have been hindered by the rarity of PM resistance in available germplasm and knowledge of its genetic control. In this study, we mapped quantitative trait loci (QTL) associated with PM resistance/tolerance in two full sibling populations segregating for PM response: Rutgers H4AR15P25 (P25) × Rutgers H4AR15R28 (P28) (n = 195) and Rutgers H4AR15R25 × Rutgers H4AR15P35 (P35) (n = 83). High-density genetic linkage maps were constructed for the mapping populations using double digest restriction-site associated DNA sequencing-derived single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs). The P25 × P28 map consisted of 2265 markers, spanning 1520 cM and 11 linkage groups (LGs) with an average marker spacing of 0.69 cM. The P25 × P35 map was constructed with 1788 markers, spanning 1256 cM and 11 LGs, with an average marker spacing of 0.72 cM. The maps had 604 markers in common and exhibited excellent collinearity. Through multiple QTL model mapping, one major QTL (LOD = 11.36 and R^2 = 58.9%) was identified in P25 × P35. Furthermore, a minor QTL (LOD = 3.30 and R^2 = 7.8%) was detected in P25 × P28. Due to their proximity onLG3, these QTL may be designating the same locus or tightly linked loci. The negative additive effects of both QTL signify that the PM susceptible male parents were contributing susceptibility alleles to the progeny. This is the first report of QTL associated with PM response on LG3 in *C. florida* and lays the groundwork for the development of marker-assisted selection for PM resistance in *C. florida* breeding programs.

Keywords: *Cornus florida*; flowering dogwood; ddRadseq; linkage mapping; QTL; powdery mildew; disease resistance

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1. Introduction

Flowering dogwood (*Cornus florida* L.) is a small ornamental tree native to the eastern United States. Often described as a quintessential four-season plant, *C. florida* is valued for its spring blooms with showy bracts, red fruit, reddish-purple fall color, and graceful architecture for summer and winter interest. Culturally and economically significant, it has been honored as the state flower or tree of North Carolina, Virginia, and Missouri. Dogwood tree sales, of which *C. florida* makes up a significant portion, are worth 31 million dollars per year in the United States [1]. This is third in value only to crape myrtle and flowering cherry for deciduous flowering trees.

Despite its popularity, flowering dogwood is susceptible to several pests, environmental stresses, and diseases, with one of the most serious being dogwood powdery mildew (PM) (*Erysiphe pulchra* Cook and Peck). PM-infected leaves can exhibit unattractive white fungal growth, increased red pigmentation, curling, and stunting. Untreated PM in the

nursery can reduce stem caliper by 80% and tree height by 50% in a single growing season [2]. For mature trees, the disease can decrease flowering, and repeated severe infections can stunt growth and reduce landscape appeal [3,4]. Dogwood PM is believed to be a non-native disease imported from Asia based on genetic evidence and the fact that it was unobserved on *C. florida* in the U.S. until 1994, when it became an annual problem [5,6]. Fungicide control with biweekly applications is effective but expensive, with management costs of dogwood nurseries increasing more than 16-fold after 1994 [7]. In addition, there are concerns surrounding the environmental side effects of conventional fungicides and the potential development of fungicide resistance with repeated chemical applications.

Currently, the use of disease-resistant cultivars is considered an ideal strategy for controlling PM in *C. florida*. However, there are few resistant cultivars available, especially of the desirable pink-bracted var. *rubra* [8–11]. Developing PM-resistant *C. florida* cultivars is challenging because of self-incompatibility, long generation time (3–5 years), and limited knowledge of PM resistance genes and their inheritance [12,13]. In addition, resistance is very rare in natural populations, estimated to be 0.1% [14]. Despite these challenges, the costs of developing PM-resistant dogwoods in the context of a breeding program would be returned with a premium charged for the final product. One study found consumers were willing to pay an average of $13.35 more for a PM-resistant *C. florida* [15]. Although this number represents the total premium that would be shared among all stages of production from breeder to retailer, nurseries producing PM-resistant trees could also save an estimated $1465/hectare ($0.148/tree) in PM fungicide costs [16].

Marker-assisted selection (MAS) could help make breeding PM-resistant cultivars more efficient through early selection and elimination of seedlings lacking desirable marker combinations. This would allow for the maximization of greenhouse and field space and screening of more progeny to achieve desirable combinations of ornamental traits and disease resistance. Mapping of economically important quantitative trait loci (QTL) for use in MAS in *C. florida* has been limited due to a lack of genetic resources. In 2009, Wang et al. constructed the first flowering dogwood genetic linkage map [17]. This map consisted of 255 simple sequence repeat (SSR) markers spanning 1175 cM and 11 linkage groups (LGs), corresponding to the haploid chromosome number of *C. florida* [18]. The linkage map has been used by studies mapping QTL related to red foliage [19] and PM resistance [20]. The PM QTL mapping study found four QTL explaining between 9.51–13.21% of the phenotypic variance. The two mapping populations in the study shared a susceptible parent and two of the QTL (QTLPM-3 and QTLPM-4) may be designating the same locus based on their logarithm of odds (LOD) scores, R^2 values, and proximity on the same LG. However, the study was limited by low SSR marker coverage: 35 and 29 markers for the two mapping populations. One LG had no marker coverage for either population, allowing potential QTL on the LG to go undetected. The low coverage was due to loci in the linkage maps not segregating in the mapping populations and the laborious and time-intensive nature of SSR genotyping. These difficulties can be overcome with reduced representation sequencing methods, such as double-digestion restriction-site associated DNA sequencing (ddRadseq). The ddRadseq technique is reproducible, straightforward, and economical and can simultaneously discover and genotype thousands of single nucleotide polymorphism (SNP) and insertion–deletion (indel) markers in hundreds to thousands of samples. [21,22]. Recently, ddRadseq and other reduced representation sequencing methods have been used for high-density linkage map construction and QTL analysis in chickpea [23], hazelnut [24], hydrangea [25], lettuce [26], peanut [27], pecan [28], and sesame [29].

One of the Rutgers University dogwood breeding program's breeding selections, H4AR15P25, exhibits excellent resistance to PM. This resistance has been stable over multiple years and locations with high disease pressure in New Jersey and Tennessee [11]; however, little is known about the nature of this resistance. The objectives of the current study were to (1) build high-density genetic linkage maps of *C. florida* using ddRadseq and (2) investigate QTL associated with PM resistance in the H4AR15P25 breeding selection.

The goal is to better understand the nature of the PM resistance as a step toward developing marker-assisted selection tools for use in dogwood breeding programs.

2. Materials and Methods

2.1. Assembling the Mapping Populations

Mapping population development using open-pollinated seeds and SSR markers was detailed in Pfarr et al. [30]. Briefly, to expedite mapping population development, open-pollinated (OP) seeds were harvested in 2017 from a tree of white-bracted PM resistant H4AR15P25 grown in an isolated crossing block. The crossing block contained 16 different PM-susceptible and PM-tolerant *C. florida* breeding selections and cultivars spaced in various locations from the original seed tree. Six hundred and thirty-six OP seedlings of H4AR15P25 were genotyped with 8 SSR markers to determine pollen parents. Using CERVUS 3.0.7, 202 seedlings were identified as progeny of H4AR15P25 × H4AR15P28 (closest tree to seed parent) and 196 were selected for the development of the QTL mapping population. Further, 101 seedlings were identified as progeny of H4AR15P25 × H4AR15P35 and 84 were selected for the second QTL mapping population. For brevity in the current paper, H4AR15P25 × H4AR15P28 and H4AR15P25 × H4AR15P35 will be referred to as P25 × P28 and P25 × P35, respectively. P28 is a blush pink-bracted tree with moderate PM tolerance, and P35 is white-bracted and exhibits PM resistance intermediate between P25 and P28.

2.2. Phenotyping

In summer 2019, the 196 full-sibling population from P25 × P28 was grown in the research greenhouse at Rutgers Horticultural Research Farm 1 (New Brunswick, NJ, USA) with 24/18 °C (day/night) with 16-h daylengths. Plants were grown in 3.7 L containers with Frey's NX-6 Mix (Frey's Group, Quarryville, PA, USA), top-dressed with 5 g of six-month time-release fertilizer (Osmocote Plus 15N-9P2O5-12K2O with micronutrients; The Scotts Co., Marysville, OH, USA), and watered as needed. The population was arranged in a completely randomized design. Five visibly PM-diseased seedling trees originating from other populations held in the Rutgers breeding program collection were placed around the population and used as a source of inoculum that spreads naturally under greenhouse conditions. Disease ratings were taken once a month in June, July, and August 2019 using a 0–100% PM categorical severity scale (0, 1, 5, 10, 20, 30, … 100%). In fall 2021, both populations, P25 × P28 and P25 × P35, were field planted at Rutgers Horticultural Research Farm III (East Brunswick, NJ, USA). The P25 × P35 population was phenotyped in 2020 and 2021 using the same 0–100% PM categorical severity scale, as previously described. Mean disease ratings for each genotype per season were used in the QTL analysis. Histograms of phenotypic data were visualized in R with the 'ggplot2' and 'gridExtra' packages [31–33].

2.3. ddRadseq Library Preparation and Sequencing

Young leaves were collected from progeny and parents, flash-frozen in liquid nitrogen, and ground using a Tissuelyser II (Qiagen, Hilden, Germany). DNA was extracted using the Qiagen DNeasy Plant kit following manufacturer's instructions. The ddRadseq libraries were constructed for parents and progeny using a protocol adapted from Poland et al. [34]. Briefly, 200 ng DNA/sample was digested with the rare cutter PstI-HF and common cutter MspI (NEB, Ipswich, MA, USA) at 37 °C for 2 h. For each sample, a uniquely barcoded forward PstI adapter and universal reverse MspI Y-adapter were ligated to the digested DNA in a master mix containing 2 µL of 10× NEBuffer 4, 200 U T4 DNA ligase, and 4 µL of 10 mM ATP per sample. The ligation was carried out at 22 °C for 2 h, with a subsequent incubation of 65 °C for 20 min to inactivate the ligase. Samples were cleaned using 0.5 v/v magnetic AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA), with a 70% ethanol washing step to remove DNA fragments <300 bp. Individual cleaned library samples were subsequently PCR amplified with the following cycling parameters: initial denaturation at 95 °C for 30 s; followed by 16 cycles of 95 °C for 30 s, 62 °C for 20 s, 68 °C for 15 s; with

a final extension of 68 °C for 5 min. The PCR primers included sequences for Illumina (San Diego, CA, USA) flow cell binding. DNA libraries were quantified with the Qubit 3.0 (Thermo Fisher Scientific, Waltham, MA, USA) normalized to 9.0 ng/µL, and pooled into 48-plex sequencing libraries. A final cleanup step was performed with magnetic beads and ethanol wash as described previously. The pooled libraries were paired-end sequenced (2 × 150) on an Illumina HiSeq with 30% PhiX spike-in by GENEWIZ (South Plainfield, NJ, USA). The parental samples were sequenced at 8–10× the coverage of the progeny.

2.4. Calling SNP Markers

Stacks v2.53 was used to assemble reads and call SNP markers [35,36]. The integrated alignment strategy of Paris et al. [37] was followed, as this work demonstrated that building loci de novo and then aligning loci to a reference genome resulted in the identification of more SNP markers than initially aligning raw reads directly to a reference genome. Briefly, raw sequencing reads were cleaned, quality filtered, and demultiplexed using Stacks process_radtags. Parameter values M and *n* were optimized for population P25 × P28 using the r80 method [37]. For both mapping populations, denovo_map was run with M = 4 and *n* = 6 to construct loci and call SNP markers. Consensus sequences for assembled loci in the catalog.ga.gz file were aligned to the *Cornus florida* reference genome [38] using Burrow-Wheeler Alignment tool [39]. The script stacks–integrate–alignments [37] was used to integrate the alignment position for each locus back into the Stacks output files. The populations program was rerun to write SNP markers and loci SNP haplotypes that were present in both parents, >90% of the progeny, and suitable for a CP mapping population in a JoinMap output file. This strategy identified SNP marker classes with the following segregation patterns: 1:1 (<lm × ll> or <nn × np>), 1:1:1:1 (<ab × cd> or <ef × eg>), or 1:2:1 (<hk × hk>).

2.5. Linkage Map Construction

Markers from the two mapping populations were combined, then sorted by reference genome contig and reference genome base pair position. Markers were renamed according to their order in the reference genome: 1st marker on contig 1 = 1, 2nd marker on contig 1 = 2, and so forth. Markers present in both mapping populations (originating from the same locus in the reference genome) were assigned the same number allowing for comparison of the two populations' linkage maps (Table S1). LG1 is equivalent to contig 1 in the reference genome, LG2 is equivalent to contig 2, etc., except LG11, which is equivalent to contig 0. This change was made to avoid designating an LG0, following with conventional naming of LGs. Six and eight polymorphic SSR loci were added to the P25 × P28 and P25 × P35 datasets, respectively. These loci were previously used to genotype the progeny and segregated in the two populations [30].

JoinMap 4.1 was used to construct the linkage maps [40,41]. For both populations, markers were tested for segregation distortion from expected frequencies with chi-square tests, and loci with segregation distortion significance of 0.005 or greater were excluded from the analysis. For determining linkage group assignment of markers, the independence LOD score was set from 1 to 10 and a final independence LOD cutoff of 7 was used. Linkage groups were constructed using Maximum Likelihood (ML) mapping with default parameters. In JoinMap 4.1, linkage map construction for cross-pollinated (CP) species follows the pseudo-testcross method of Grattapaglia and Sederoff [42], first building separate maternal and paternal maps, then integration into a final consensus map using markers segregating in both parents.

2.6. QTL Analysis

Due to the memory constraints of performing QTL mapping on combined CP population linkage maps, the separate maternal and paternal maps were used for QTL analysis. Genotypes were re-coded in Backcross (BC1) notation according to phase information supplied by JoinMap as shown in Table S2. Loci segregating in both parents (<ab × cb>,

ef × eg>, and <hk × hk>) were duplicated for separate conversion for the paternal parent and ".2" was added to the duplicated paternal locus name to ensure unique locus names for QTL analysis. Heterozygote hk alleles were recoded as missing data because heterozygote progeny could not be accurately assigned an allele from either parent.

QTL analysis was conducted with MapQTL 6 [43]. For each population, the 95% genome-wide significance LOD threshold was determined by permutation test with 10,000 permutations. An initial round of regression Interval Mapping was conducted. Linkage groups with peaks near or above the significance threshold were chosen for rounds of automatic cofactor selection (ACS) as described in the MapQTL 6 manual. Cofactors found through ACS were added individually to rounds of regression multiple-QTL model (MQM) mapping. For each round of MQM, only cofactors within a significant LOD peak were retained, and when need be, were iteratively shifted to adjacent markers with higher LOD until the significant QTL location stayed constant. The integrated linkage maps and QTL regions were visualized and compared with MapChart 2.32 [44]. For markers associated with significant QTL, phenotypic data for each genotype class was visualized in R with 'ggplot2' and 'ggpubr' packages [31,32,45]. In order to compare QTL from the previous PM QTL study [20], at least 5 SSR primers from each previous LG were blasted against the genome to determine which LGs correspond to the reference genome contigs and this study's LGs.

3. Results

3.1. Phenotyping

For population P25 × P28 summer 2019 ratings, disease severity increased steadily over the course of the season: June mean severity rating was 7.4%, July mean was 17.6%, while August mean disease severity ratings peaked at 24.1%. The overall season mean disease severity rating was 16.6%, and the standard deviation was 10.4% (Figure 1). Unfortunately, a phytophthora outbreak in the field during winter 2019/2020 killed many trees, leaving 29 remaining trees for P25 × P28 and 64 trees for P25 × P35; therefore, 2020 and 2021 phenotypic data from the P25 × P28 population was not used for QTL analysis because of the small population size.

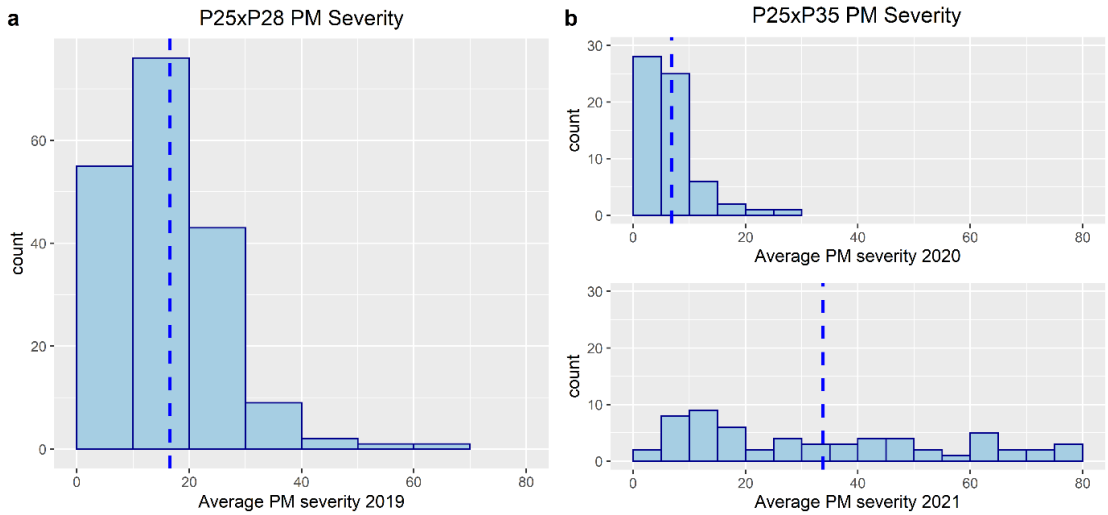

Figure 1. Histograms for mean powdery mildew (PM) severity ratings per season for trees in two full sibling QTL mapping populations: (**a**) P25 × P28 average PM severity in 2019 and (**b**) P25 × P35 average PM severity in 2020 and 202. Population mean PM severity for each year is denoted by a dashed blue line.

For the population P25 × P35 2020 ratings under field conditions, the July mean severity rating was 7.0%, August was 6.3%, and early October 7.1%. The season average was 6.8%. and the standard deviation was 5.2%. Disease pressure increased the following

year. For 2021, the July mean severity rating was 27.7%, August was 33.3%, and September was 40.5%. The season average was 33.8% and the standard deviation was 23.6%.

3.2. Sequencing Statistics and SNP Marker Discovery

The six HiSeq sequencing run mean Q values ranged from 35.1–36.7 with the overall run mean of 36.0. The average percentage of bases with Q scores $\geq$ Q30 was 83.4% with individual runs ranging from 80.1% to 86.0%. The combined sequencing runs for all samples yielded a total of 479.9 Gb of data in the raw fastq files. After the initial sequencing, additional parental samples were sequenced to increase the parental depth of coverage relative to the progeny samples. The mean Q score for the additional run was 36.14 with 82.3% of bases with Q scores $\geq$ Q30.

After the process_radtags step of stacks demultiplexed and discarded low-quality reads, the P25 $\times$ P28 population had an average of 6.6 million paired-end reads per sample, with a maximum of 12.8 million, a minimum of 4.4 million, and a standard deviation of 0.82 million. The P25 $\times$ P35 population had an average of 7.0 million paired-end reads per sample, with a high of 11.1 million, a low of 3.6 million, and a standard deviation of 1.63 million. The parents had an average of about 4.6–6.7 times more sequencing reads than the progeny with P25 = 44.0 million, P28 = 37.9 million, and P35 = 32.3 million paired-end reads. This increased read depth is important for the Stacks pipeline to accurately call SNP markers in a mapping population.

For P25 $\times$ P28, the initial run of the Stacks denovo pipeline returned 181,070 raw SNP markers with an effective per sample read coverage mean of 83.9. After the alignment of loci to the reference genome and an additional filter for loci present in both parents and more than 80% of the progeny, 13,178 polymorphic loci remained. After a final filter with populations to return loci present in greater than 90% of the progeny and suitable for a cross-pollinated mapping population, 2410 SNP markers and haplotypes were returned. In JoinMap, an additional 151 markers were removed from the dataset before the final linkage grouping due to large fit and stress (1) or severe segregation distortion (150) ($p \leq 0.005$).

For P25 $\times$ P35, the initial run of the Stacks denovo pipeline returned 45,902 raw SNP markers with an effective per sample coverage mean of 84.7. After the same two filtering steps described above, 9835 and then 1891 SNP markers and haplotypes remained. 109 markers were removed in JoinMap due to severe segregation distortion or large fit and stress.

Before the final analysis, two progeny were eliminated from the study. For P25 $\times$ P28, progeny F-545 was excluded because of a large number of missing loci and consistently high (10–30) expected recombination count per LG indicating a high level of genotyping error. For P25 $\times$ P35, F-290 was excluded when it became apparent that it was not a progeny of P35 due to the presence of non-parental alleles. F-290 was originally assigned to P35 with only 80% confidence by Cervus parentage analysis using eight SSR markers [30].

For P25 $\times$ P28, of the 2265 SNP haplotypes and SSR markers used for final linkage map construction, 1804 segregated 1:1 (<lm $\times$ ll> or <nn $\times$ np>) in the progeny, 255 segregated 1:1:1:1 (<ab $\times$ cb> or ef $\times$ eg>), and 205 segregated 1:2:1 (<hk $\times$ hk>). For the 1788 SNP haplotypes and SSR markers used in the final linkage map construction of P25 $\times$ P35, 1377 segregated 1:1, 207 segregated 1:1:1:1, and 204 segregated 1:2:1.

3.3. Linkage Maps

The P25 $\times$ P28 and P25 $\times$ P35 populations had 11 clear linkage groups at LOD 7.0. The P25 $\times$ P28 map was constructed with 2265 markers, spanning 1520 cM, with an average marker spacing of 0.69 cM (Figure 2). The P25 $\times$ P35 map consisted of 1788 markers, spanning 1256 cM, with an average marker spacing of 0.72 cM. Summary statistics for the linkage maps of the two populations are presented in Table 1. Detailed maps for both populations with loci names and positions can be found in Figures S1 and S2. The two linkage populations had 604 markers in common and exhibited excellent synteny as shown in Figures 3 and 4a. The slight difference in size between the two populations' maps may

be due to less recombination in the smaller population (195 vs. 83 individuals). The linkage map of P25 × P28 also displayed good synteny with the reference genome of *C. florida* 'Appalachian Spring' (Figure 4b).

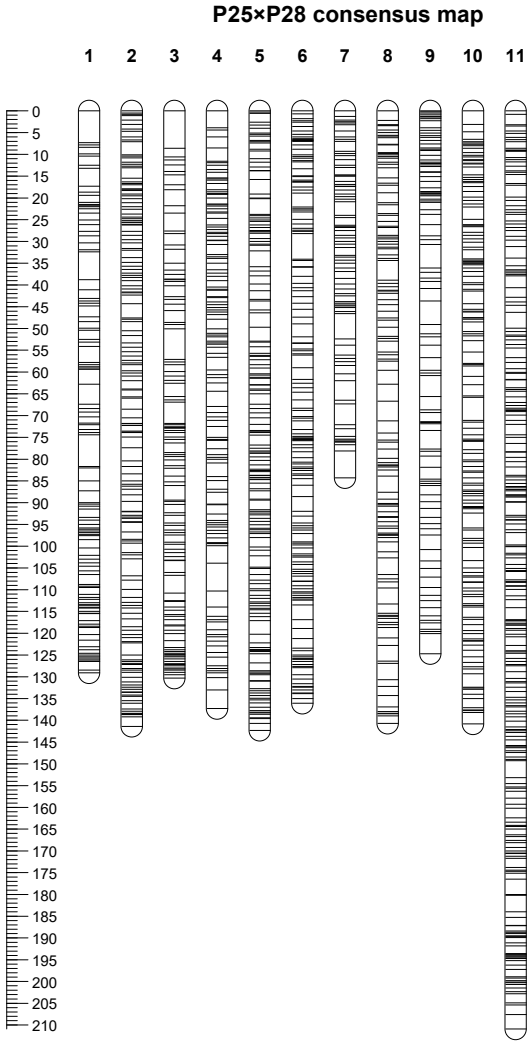

Figure 2. Linkage map of *Cornus florida* P25 × P28 constructed with a 195 individual full sibling QTL mapping population. Size in cM is shown on the left axis.

Table 1. Summary statistics for linkage maps constructed from two *Cornus florida* full-sibling cross-pollinated mapping populations: P25 × P28 and P25 × P35. Statistics from the previously published SSR map are from Wang et al. [17].

Linkage Group	Mapping Population	Number of Markers	Total Distance (cM)	Average Distance (cM/marker)	Maximum Gap (cM)
LG1	P25 × P28	174	130.2	0.75	7.3
	P25 × P35	154	110.9	0.72	5.8
LG2	P25 × P28	239	141.4	0.59	3.9
	P25 × P35	159	97.2	0.61	7.1
LG3	P25 × P28	155	130.3	0.84	8.6
	P25 × P35	132	83.7	0.63	6.8
LG4	P25 × P28	176	137.3	0.78	6.4
	P25 × P35	135	127.9	0.95	5.1

Table 1. *Cont.*

Linkage Group	Mapping Population	Number of Markers	Total Distance (cM)	Average Distance (cM/marker)	Maximum Gap (cM)
LG5	P25 × P28	258	142.3	0.55	3.7
	P25 × P35	216	109.9	0.51	4.2
LG6	P25 × P28	231	136.1	0.59	5.9
	P25 × P35	166	111.3	0.67	7.7
LG7	P25 × P28	118	84.3	0.71	6.3
	P25 × P35	128	111.6	0.87	4.8
LG8	P25 × P28	191	141.3	0.74	4.6
	P25 × P35	140	134.8	0.96	14.8
LG9	P25 × P28	147	124.7	0.85	5.4
	P25 × P35	114	81.2	0.71	8
LG10	P25 × P28	221	140.8	0.64	5.3
	P25 × P35	181	101.4	0.56	8.8
LG11	P25 × P28	355	210.9	0.59	3.8
	P25 × P35	263	186.1	0.71	9.7
Total	P25 × P28	2265	1520	0.69	
	P25 × P35	1788	1256	0.72	
Previously published map		255	1175	4.6	

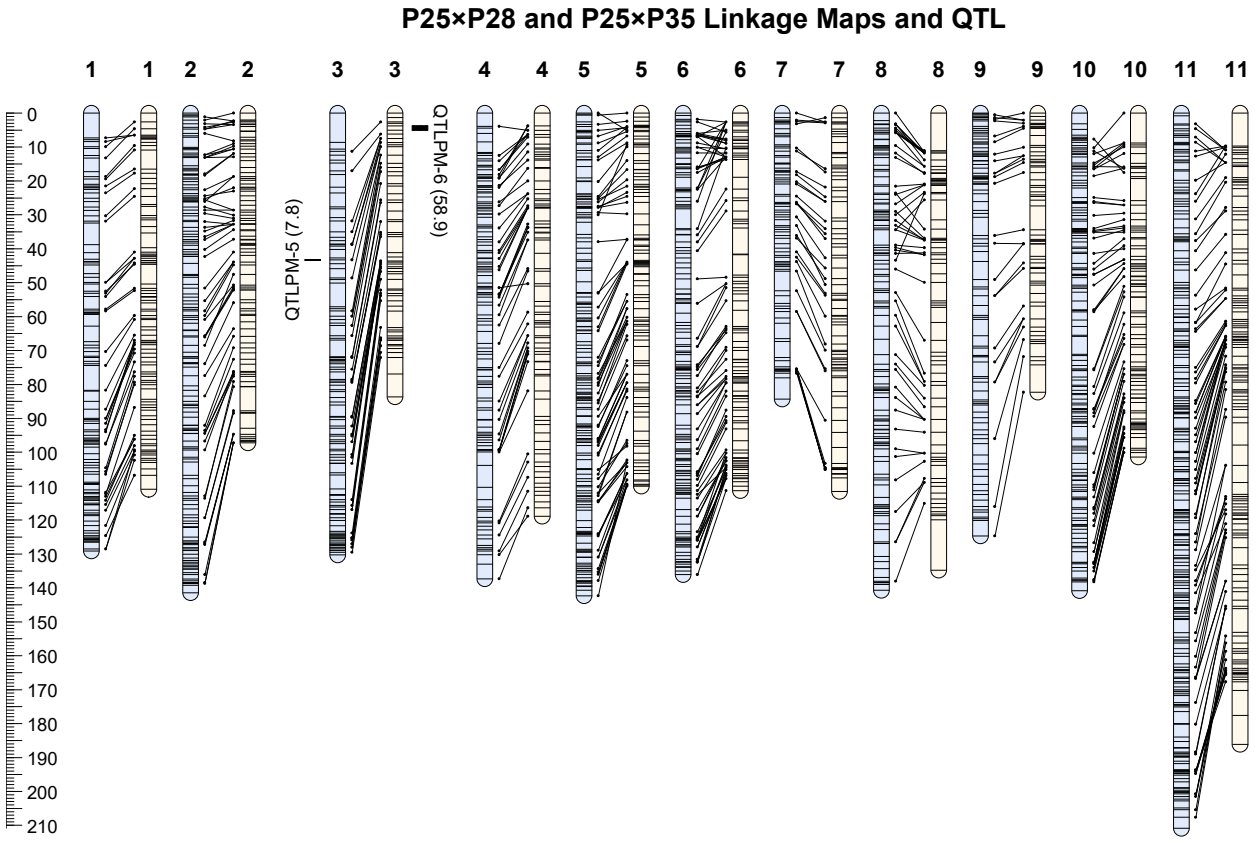

Figure 3. Comparisons of linkage maps from the P25 × P28 and P25 × P35 populations with QTL. P25 × P28 LGs are light blue and P25 × P28 LGs are tan. Markers in common between the two maps are connected by a black line. Size in cM is shown on the left axis. Significant QTL from this study are shown with their respective linkage groups. R^2 values are in parentheses.

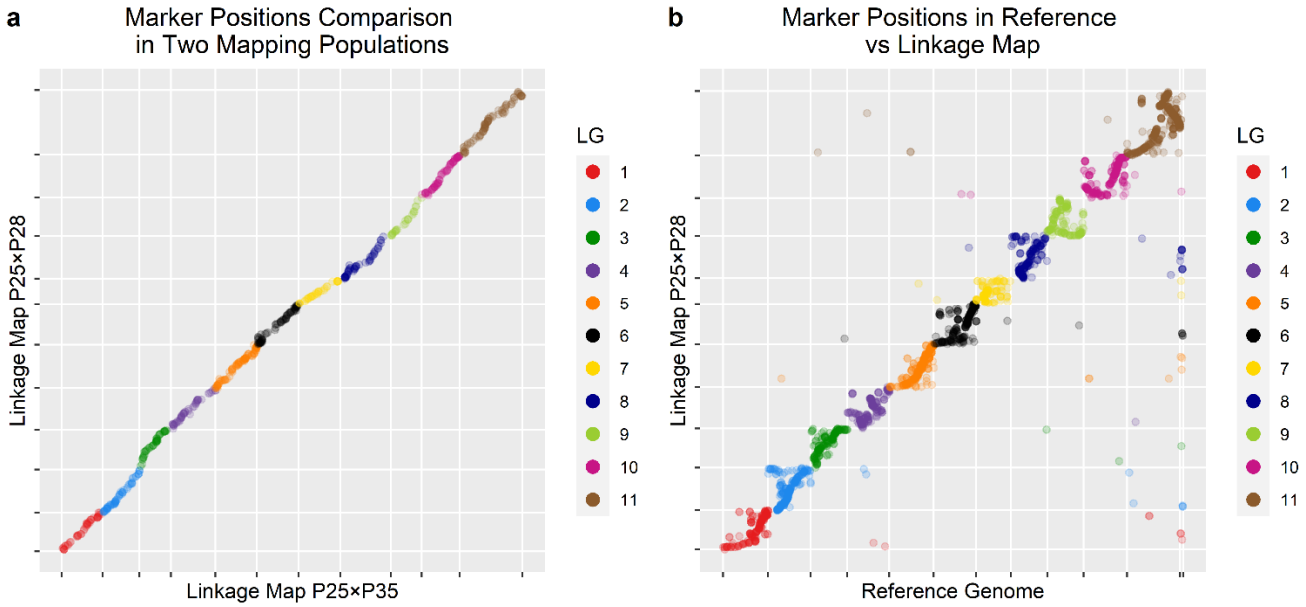

Figure 4. (**a**) Comparisons of marker positions (cM) between common markers in P25 × P28 and P25 × P35 mapping population's linkage maps. (**b**) Comparisons of marker positions in P25 × P28 linkage map with their physical positions in the reference genome.

To compare QTL and linkage maps from previously published work [17,20], SSR primers from the previous linkage maps were blasted to the reference genome to anchor the maps to the reference genome. For clarity in the results and discussion, LGs from previous work are referred to by their corresponding contigs and LGs numbers in our study. This was done to be consistent with the reference genome for future research. Name conversions between the two LMs are listed in Table 2.

Table 2. Comparison of Contig numbers in *C. florida* reference genome, LGs in this study, and LGs from Wang et al. [17].

Contig in Reference Genome	Linkage Group in This Study	Linkage Group in Wang et al. [17]
1	LG1	LG9
2	LG2	LG10
3	LG3	LG5
4	LG4	LG2
5	LG5	LG8
6	LG6	LG6
7	LG7	LG1
8	LG8	LG11
9	LG9	LG4
10	LG10	LG3
0	LG11	LG7

3.4. QTL Mapping

For the P25 × P28 population with 2019 data, the 95% LOD threshold was 3.2. The analysis returned one significant QTL on LG3 associated with marker 799 (designated QTLPM-5). QTLPM-5 had a LOD score of 3.3 and explained 7.8% of the phenotypic variance. The additive effect was −5.82.

For the P25 × P35 mapping population's 2020 data, the LOD threshold was 2.8. There were no significant QTL with the 2020 phenotypic data. For the P25 × P35 mapping population's 2021 data, the LOD threshold was 3.4. There was one significant QTL, QTLPM-6, associated with markers 702 and 686 on LG 3. It had a peak LOD score of 11.36 and

explained 58.9% of the phenotypic variance. The additive effect of QTLPM-6 was −62.86. QTL statistics are summarized in Table 3.

Table 3. Information for two QTL found in two *C. florida* populations mapping populations segregating for PM resistance.

QTL	Population	LG	Position (cM)	LOD	R^2	Marker(s)	Additive Effect
QTLPM-5	P25 × P28	3	43.3	3.30	7.8	799	−5.82
QTLPM-6	P25 × P35	3	3.78–5.01	11.36	58.9	702 and 686	−62.86

4. Discussion

The obligate outcrossing nature and high inbreeding depression of *C. florida* prevent the development of a classical F_2 mapping population; therefore, F_1 mapping populations were used for the present study. In this case, F_1 progeny can be considered as "pseudo F_2" because they segregate for alleles from their highly heterozygous parents. However, this complicates linkage map construction as there are several different marker classes with two to four alleles segregating, and linkage phases are initially unknown. In JoinMap, this can be overcome through the pseudo-testcross analysis, where the two parental meiosis maps are constructed separately and then combined into one map with maximum likelihood using bridging markers segregating in both parents (<hk × hk>, <ef × eg, or <ab × cd>) [40]. This strategy is commonly used in other outcrossing crops with recent examples in hazelnut [24], hydrangea [25], passionfruit [46], and walnut [47]. In the present study, the integration of the consensus maps was facilitated using Stacks-determined SNP loci haplotypes and a small number of SSRs, with 460 bridging markers for P25 × P28 and 411 bridging markers for P5 × P35. The consensus linkage maps constructed in this study are the densest to date for *C. florida*, with the average distance per marker decreasing from 4.6 cM to 0.69 and 0.72 cM. These high-density genetic maps will be valuable tools for future genetic studies and will be used to improve the reference genome assembly.

The two QTL found in this study may be referring to the same locus or tightly linked loci based on their similar locations. QTLPM-5 and QTLPM-6 are located near the same distal ends of LG3 on P25 × P28 and P25 × P35, respectively. Marker 799 associated with QTLPM-5 is present in both populations. In population P25 × P35, marker 799 is situated at 9.88 cM, only 4.87 cM away from QTLPM-6. However, QTLPM-5 was a relatively small effect QTL explaining 7.8% of the phenotypic variation for P25 × P28 in 2019, while QTLPM-6 was a large effect QTL explaining 58.9% of the phenotypic variation for P25 × P35 in 2021. It is possible that the higher PM severity present in the field in 2021 allowed for a greater distinction between resistant vs susceptible plants. Another explanation is the causal locus may have diminished effects in the genetic background of blush pink-bracted P28, which groups with PM-susceptible var. rubra cultivars in a diversity study analysis [48]. Whether or not these QTL are the same locus, LG3-associated PM resistance in these populations is distinct from previously discovered QTL on LG1, LG5, and LG9 [20].

For both QTL, the susceptible parents were likely contributing susceptibility alleles that are associated with increased PM severity in the progeny. The QTL were segregating in the male parents (P28 and P35), not the resistant female parent (P25). However, the negative additive effects of both QTL signify that heterozygotes ("np", P28 and P35 genotype) in the mapping populations had higher PM severity ratings than homozygotes ("nn", P25 genotype) (Figure 5). Additionally, the first $\frac{1}{4}$ to $\frac{1}{2}$ of LG3 in both populations is made exclusively of markers segregating in the male parents (<nn × np>), suggesting that this region of the genome is homozygous in the female parent (Figure 6). For the male parents, the <nn × np> markers in this region are solely in phase (−0), meaning that all the unique p alleles are linked on the same chromosome in the male parent. Because of this, the actual casual loci in this LG3 region should also theoretically segregate for heterozygotes to have a higher PM severity rating. It is not possible to distinguish between an additive or dominance mode of action with this data because the markers only segregated into

two genotype classes, "nn" or "np," without homozygotes for the p allele available for observation in the progeny.

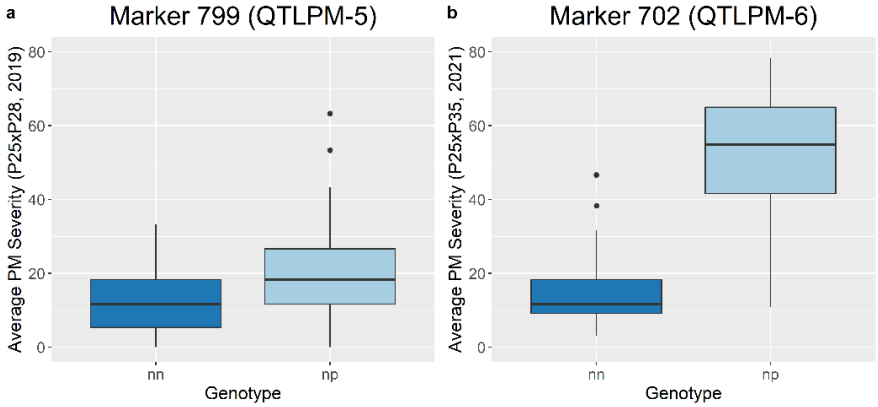

Figure 5. Average PM severity by genotype for markers associated with significant QTL: (**a**) Marker 799 (QTLPM-5) and (**b**) Marker 702 (QTLPM-6).

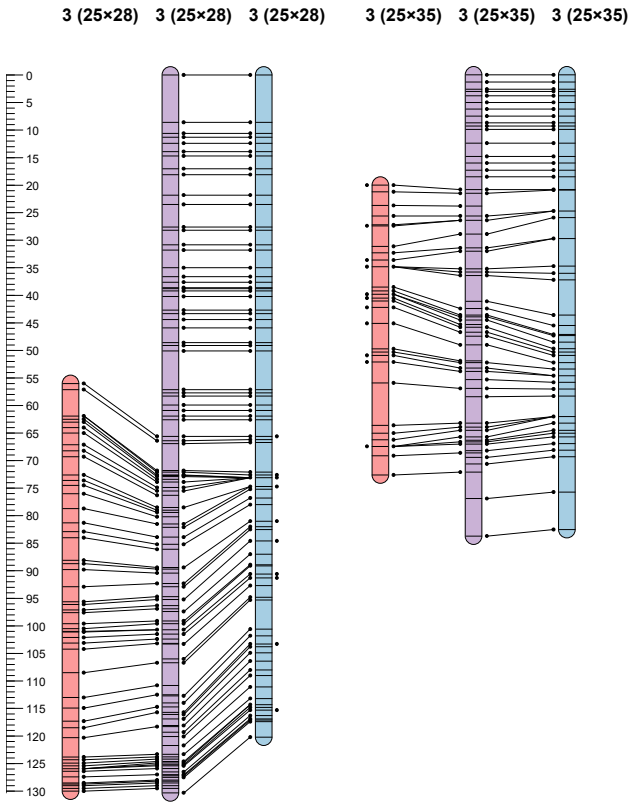

Figure 6. LG3 maps for P25 × P28 and P25 × P35 QTL mapping populations. Red LGs are maternal, blue are paternal, and purple are consensus maps. Markers in the maternal and paternal maps are connected to their positions in the consensus map by black lines.

Additional QTL may have been missed because the power of this study to detect minor loci associated with quantitative PM resistance may have been limited. There was no genotypic replication within years due to the difficulty and expense of asexually propagating woody *C. florida*. Replication over years was cut short for P25 × P28 when most of the population succumbed to phytophthora in winter 2020. It is possible that additional resistance in P25 is highly quantitative, and its allelic contribution was diminished when crossed with the more PM susceptible trees. QTLPM-5 only explained 7.8% of the pheno-

typic variation for P25 × P28 in 2019 so the contributions of minor QTL in this population may have been too small to detect under our experimental design.

In general, there was a weak correlation in PM severity ratings between years and environments for both populations, except between the 2020 and 2021 field data for the surviving P25 × P28 trees (Pearson's correlation coefficient = 0.61). These results demonstrate the challenges of working with the PM pathosystem, which is complex and highly environmentally influenced. However, PM-resistant parent P25 exhibited no or very little PM over more than a decade under high disease pressure and replication in New Jersey, Tennessee, and Oregon. P25′s replicated resistance combined with our study's findings of QTL in similar regions of LG3 in two P25-derived populations provide confidence in the presence of genetic resistance.

5. Conclusions

The *C. florida* genetic linkage maps constructed for this project are the densest to date and will be valuable for improving the reference genome assembly. This study expands our understanding of PM QTL in *C. florida* with the discovery of LG3-associated PM resistance. Marker 702 may be a good candidate for use in MAS as it is associated with a major QTL. However, more studies need to be conducted with different breeding populations over multiple years to further verify QTL from this study and identify other QTL that may be specific to diverse germplasm and could be stacked with additive effects. In addition, fine mapping of causal genes will be important to develop tightly linked, effective markers for use in marker-assisted selection.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8050405/s1, Figure S1: P25 × P28 full linkage map; Figure S2: P25 × P35 full linkage map; Table S1: Marker name conversion from STACKS assigned names to new names based on reference genome order. Table S2: Marker conversion from Cross Pollinated (CP) notation to Backcross 1 (BC1) notation.

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