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ORIGINAL ARTICLE

Identification and marker development of a moderate-effect fire blight resistance QTL in *M. sieversii*, the primary progenitor of domesticated apples

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Abstract

Fire blight, a bacterial disease caused by *Erwinia amylovora*, is the most devastating disease of apples and a major threat to apple production. Most commercial apple cultivars are susceptible to fire blight, driving the need to develop fire-blight-resistant cultivars. Although several major fire blight resistance QTLs have been identified from wild species of *Malus*, the challenges of breeding apples due to long juvenile phase and heterozygosity greatly limit their use. *M. sieversii*, the primary progenitor of domesticated apples, is one of the wild *Malus* species that is sexually compatible with *M. domestica* and has some favorable fruit quality traits. In this study, we performed QTL analysis on two F1 apple populations of *M. domestica* cv. "Royal Gala" × *M. sieversii* (GMAL4591 and GMAL4592) to identify fire blight resistance QTL. Parental linkage maps were constructed for each family using marker sets of approximately 20K GBS-SNPs. Phenotype data was collected from parents and progeny through controlled fire blight inoculations in the greenhouse for two subsequent years. A significant ($P < 0.0001$) moderate-effect fire blight resistance QTL on linkage group 7 of GMAL4591 was identified from the paternal parent *M. sieversii* "KAZ 95 17-14" (*Msv_FB7*). *Msv_FB7* explains about 48–53% of the phenotyping variance across multiple years and time points. Additionally, a significant ($P < 0.001$) minor effect QTL explaining 18% of the phenotypic variance was identified in population GMAL4592 on LG10 from "Royal Gala." We developed diagnostic SSR markers flanking the *Msv_FB7* QTL to use in apple breeding. These findings have the potential to accelerate the development of fire-blight-resistant cultivars.

Keywords Genetic disease resistance · Quantitative trait loci · Erwinia amylovora · Plant domestication

Introduction

Apples are a highly valued tree fruit crop globally. The production of apples generates an estimated $73 billion worldwide (FAOSTAT 2020). Most commercial apple cultivars are moderately to highly susceptible to several fungal and bacterial diseases including fire blight, apple scab, and powdery mildew (Yoder 2000; Kostick et al. 2019; Papp et al. 2020). Fire blight, a bacterial disease caused by *Erwinia amylovora*, is a major threat to apple production(Winslow et al. 1920; Emeriewen et al. 2019). This pathogen can rapidly spread across large orchards and kill productive trees in a single season (Norelli et al. 2003). The distribution of *E. amylovora* has become a worldwide issue, threatening apple-growing regions in 46 countries across 4 continents (Peil et al. 2009; van der Zwet et al. 2016). In addition, pathogen resistance to the widely used antibiotic, streptomycin, has also become prevalent in the USA (Loper 1991; Mcmanus et al. 2002; Dougherty et al. 2021; Wallis et al. 2021). Many European countries have strict and limited use of antibiotics for fire blight management (Peil et al. 2009). The use of host genetic resistance to manage fire blight is a sustainable option and a high priority for apple breeding programs (Peil et al. 2021). The identification of genetic loci linked to fire blight resistance is an important component of the

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development of resistant cultivars through breeding (Peil et al. 2021; Khan and Korban 2022).

Several quantitative trait loci (QTL) linked to fire blight resistance have been previously identified across both wild *Malus* species and domesticated apples (*M. domestica*). The sources of putative fire blight resistance alleles from wild *Malus* species include *M.* × *robusta* 5, *M. fusca*, *M. floribunda* 821, *M.* × *arnoldiana*, and *M.* "*Evereste*" (Peil et al. 2007; Durel et al. 2009; Emeriewen et al. 2014, 2017, 2021). These are all major effect loci with a range of 60–80% variance explained on linkage groups (LG) 3, 10, and 12 of apples (Peil et al. 2007; Durel et al. 2009; Emeriewen et al. 2014, 2017, 2021). *M. sieversii* accession KAZ 95 18-07 was found to possess 13 minor-effect loci that were strain- and environment-dependent (Desnoues et al. 2018a). Several other minor-effect loci have been identified from *M. domestica* on LGs 2, 5, 6, 10, and 15 (Le Roux et al. 2010a; Khan et al. 2013). The only moderate-effect QTL from an elite domesticated background has been identified in "Fiesta" on LG07 from the cross "Fiesta" × "Discovery" with 38% variance explained by the QTL (Calenge et al. 2005; Khan et al. 2006a). This QTL has also been found across a range of commercially viable cultivars including "Kidd's Orange Red" and "Enterprise" (Khan et al. 2007a; van de Weg et al. 2018a). While this QTL alone may not provide a high level of resistance against fire blight, it can still be utilized effectively when pyramided with other resistance loci, resulting in better control of the disease. Moreover, this QTL would be useful for marker-assisted selection to breed fire-blight-resistant apple cultivars as it originates from an elite genetic background (Luo et al. 2020).

Wild *Malus* species are a valuable source of disease-resistant QTL; however, the biological challenges of breeding apples greatly limit their use. Apples are a long-cycle perennial crop with a 4- to 6-year juvenile period, high heterozygosity, and gametophytic self-incompatibility (Hanke et al. 2007; Peil et al. 2009; Brown 2012). Introducing resistance QTL from wild relatives *via* conventional crossing would take nearly 25 years to effectively break the linkage drag of unfavorable fruit quality alleles (Peil et al. 2021; Khan and Korban 2022). Additionally, making wide crosses with distantly related apple genotypes can result in low fertilization efficiency or poor seed viability (Korban 1986). This was seen in breeding programs such as PRI (Purdue; Rutgers; Illinois) which have taken over 30 years to achieve apple scab-resistant cultivars with acceptable fruit quality *via* conventional crossing (Crosby et al. 1992; Janick 2006; Peil et al. 2009). The robust resistance loci derived from wild species are primarily utilized in commercial production through rootstocks (Peil et al. 2021). The fire blight resistance gene FB_MR5 was efficiently bred into the Geneva© series rootstocks, given selecting fruit quality is not a focus (Fazio et al. 2015; Fazio 2021). Although both scion and rootstock breeding programs utilize these resistance QTL, breeding fire-blight-resistant scion cultivars with good fruit quality using wild *Malus* species is especially challenging (Khan and Korban 2022).

The obstacles of breeding for disease-resistant apple cultivars with good fruit quality can be overcome by leveraging closely related wild progenitor species of domesticated apples rather than distant wild species. *M. domestica* is a result of hybridization events between the primary (*M. sieversii*), secondary (*M. sylvestris*), and minorly contributing (*M. orientalis; M. bacatta*) progenitor species (Cornille et al. 2012, 2014; Duan et al. 2017; Sun et al. 2020; Volk et al. 2022). *M. sieversii*, the primary progenitor to domesticated apples, is one of the only wild *Malus* species that are both sexually compatible with *M. domestica* and have moderate fruit size (Cornille et al. 2012). *M. sieversii* is originally from the geographically diverse Caucus region in western Asia and is an important resource of beneficial alleles for biotic stress resistance and abiotic stress tolerance (Luby et al. 2001; Forsline et al. 2008; Harshman et al. 2017). Several QTL for drought tolerance, as well as resistance to blue mold, fire blight, and apple scab, have been identified (Bus et al. 2005; Norelli et al. 2014; Desnoues et al. 2018a; Yang et al. 2019). *M. sieversii* accessions with strong fire blight resistance have been identified through controlled inoculations over multiple years and environments (Harshman et al. 2017). Germplasm screenings in the U.S. Department of Agriculture (USDA) *Malus* collection in Geneva, NY, have also revealed *M. sieversii* accessions that have a robust level of resistance to fire blight from recurrent natural infections (Forsline and Aldwinckle 2004; Forsline et al. 2008; Dougherty et al. 2021). Since *M. sieversii* already has moderate fruit size, it is highly advantageous for breeding because fewer pseudo-backcross generations would be necessary (Harshman et al. 2017).

In this study, we performed QTL analysis on two F1 interspecific populations of *M. domestica* cv. "Royal Gala" crossed with two different *M. sieversii* accessions. Parental linkage maps for all 17 linkage groups were constructed using marker sets of approximately 20K GBS-SNPs for each family. Parents and progeny were phenotyped for fire blight resistance in the greenhouse for two subsequent years. Through QTL mapping, a moderate effect and stable fire blight resistance QTL "*Msv_FB7*" on LG07 was identified, and markers were developed to use in apple breeding.

Methods

Plant material

The populations used for QTL analysis are the two interspecific crosses with *M. domestica* cv. "Royal Gala" (PI651008)

as the common maternal parent. The paternal parent of population GMAL4591 KAZ 95 17-14 (PI613959). The paternal parent of population GMAL4592 is KAZ 95 10-04F (PI613978). The accessions used as the paternal parents were collected in 1995 from Kazakhstan during U.S. Department of Agriculture (USDA) led expeditions from 1989 to 1996 (Hokanson et al. 1997; Luby et al. 1999). These F1 populations and their parents were developed and maintained in the orchards at the Plant Genetic Resources Unit (PGRU) USDA-ARS *Malus* collection in Geneva, NY. Dormant budwood of the F1 progeny was collected from the PGRU orchard for approximately 120 F1 progeny from each population and grafted using the whip and tongue technique to M.7 rootstocks in 2018. Five clonal replicates of each genotype were potted in D40H deepots (Stuewe and Sons, Tangent, OR) using Cornell Potting mix (50:50 peat moss and vermiculite, 6.2 kgm^{-3} lime, 1.25 kgm^{-3} superphosphate, 0.62 kgm^{-3} calcium nitrate). Both parental genotypes and controls ("Gala" PI392303; "Robusta5" PI588825) were included in this experiment with 10 clonal replicates each. The trees were allowed to acclimatize to the greenhouse and maintained under consistent conditions of 24–25 °C, 60% humidity, and 14-hour day lengths. These populations were watered and fertilized weekly with Peters Excel CAL-MAG special (SKU# E99140; N-P-K:15-5-15; ~250 ppm). The pots were randomly arranged in the greenhouse as a randomized complete block design separated by replicate. Trees were placed in a climate-controlled cold cellar with a minimum of 4.4 °C for the winter months (December–February) each year of use and were brought back to the greenhouse in the spring (April–May).

Fire blight inoculations

Plants taken out of chilling were acclimatized and grown in the greenhouse for approximately one month before inoculation. The population was ready for infection when 90% of the shoots were measured on a scale above 10 cm. Artificial inoculations with *E. amylovora* were conducted in the greenhouse. *E. amylovora* cultures were plated 24 hours before inoculation on LB media at 28 °C. The inoculum was prepared as a liquid bacterial suspension of phosphate buffer saline (PBS) at a concentration of 1×10^9 CFU/ml. For all years, inoculations were performed with the *Erwinia amylovora* strain Ea2002A. One-year-old shoots were inoculated by cutting along the midrib of the youngest leaf with scissors dipped in the liquid bacterial inoculum. The greenhouse environment was adjusted during the infection period to a temperature of 27 °C during the day and 25 °C at night. Hoses with misting nozzles spraying water were placed under the greenhouse benches for the first 3 days to maintain a humidity of approximately 85% during the early infection period.

Fire blight assessment and data analysis

We measured leaf length (LL) with a scale (cm) from the cut edge of the leaf to where the blade meets the petiole. Shoot length (SL) was measured with a scale (cm) from the base of the fire blight inoculated shoot to the apical meristem. Leaf (LN) and shoot necrosis (SN) are measured in cm as the length of necrotic tissue from the inoculation site at 7 dai, 10 dai, and 15 dai. PSLL (percent shoot lesion length) is calculated by dividing SN by SL and multiplying by 100 to represent the percentage of the young shoot that is visibly necrotic. For 2019, SL, SN, LL, and LN were taken only at 7 days after infection (dai). In 2020, SL, SN, LL, and LN were taken at three time points (7 dai, 10 dai, and 15 dai). The area under the disease progress curve (AUDPC) was calculated for the 2020 PSLL data using the agricolae package in R with the following formula in Eq. (1) (Mendiburu and Yaseen 2020).

$$A_k = \sum_{i=1}^{N_i-1} \frac{y_i + y_{i+1}}{2} \left(t_{i+1} - t_i\right) \tag{1}$$

The data was cleaned of impossibilities (e.g., value >100%) and outliers due to typing errors. The data were filtered by removing extremely large leaves (leaf lengths greater than 5 cm) and extremely short shoots (keeping samples with shoot lengths greater than 9 cm), as described by Khan et al. (2007a). Genotypes with less than 3 replicates that meet the filtering criteria were removed. The final population sizes for GMAL4591 were 85 progeny in 2019 and 120 progeny in 2020. For GMAL4592, the population size included 90 progeny in 2019 and 123 progeny in 2020. The data for PSLL was averaged across replicates and transformed using the Box-Cox transformation, using an optimal lambda value of 0.6, which is most similar to a square root transformation (Osborne 2010). The effects on the normality of residuals using a combination of a Shapiro-Wilks test, QQ-plot, and histograms of the model residual values (Supplementary Fig. 4). SL was used as a co-factor in the models used to calculate the optimal lambda. Broad-sense heritability was calculated by dividing the total genetic variance by the phenotypic variance. The phenotypic variance was estimated by adding the genetic variance with the environmental variance divided by the mean number of replicates (Calenge et al. 2004).

Genotyping by sequencing and SNP calling

The DNeasy 96 Plant Kits (Qiagen, Valencia, CA, USA) were used for DNA extraction of parents and progeny of GMAL4591 and GMAL4592. Genotyping-by-sequencing (GBS) library preparations were performed separately for

each population, as performed by Elshire et al. (2011), and processed with two different restriction enzymes (ApeKI, PstI-EcoT22I). Illumina Hi-Seq 2000 (96 samples per lane) was used to sequence the samples at Cornell University (Ithaca, New York, USA) across 42 lanes, generating 100-bp single-end reads. Read filtering and SNP calling procedures were done in accordance with Migicovsky et al. (2021). SNPs were called to the *M. domestica* GDDH13 v1.1 reference genome (Daccord et al. 2017a). The marker name includes information about the linkage group and the physical position (bp) in the GDDH13 v1.1 genome.

GBS filtering

The filtering process for genotypic data was conducted with a combination of PLINKv1.9 and TASSELv5 (Bradbury et al. 2007; Purcell et al. 2007a). The filtered genotypic data contained 19,295 SNPs for GMAL4591 and 16,440 SNPs for GMAL4592. Filtering was performed with PLINKv1.9 with parameters set to minor allele frequency of 0.05 (--maf), missing SNPs per marker site <0.1 (--geno), missing SNPs per individual <0.1 (--mind), and max heterozygosity of 0.7 (Purcell et al. 2007b). The vcf file was converted with a custom R script to the nnxnp, hkxhk, and llxlm formatting requirements of JoinMap5 (Lee et al. 2019). In the later analysis, only pseudo-test cross markers nnxnp and llxlm were used for mapping. Markers showing heterozygosity in both parents were removed. Markers segregating for the alleles of a single parent remained. Multidimensional scaling (MDS) analysis was performed on the two families separately to identify genetic outliers and possible pollen contamination. Using a 3D plot of the first three MDS dimensions, any progeny that deviated from the familial cluster was considered a possible pollen contaminant.

Linkage map construction

The GBS SNP data for the two populations were inputted into the JoinMap5 software (Lee et al. 2019) and checked for genotyping code errors. Each locus was filtered with a chi-square test for segregation distortion, allowing a *p*-value value down to 0.001. The percent similarity between all loci was calculated, and identical loci were removed. The loci were grouped by the independent LOD values to form linkage groups with a minimum LOD score of 10. Distinct linkage groups were identified and checked for suspect linkages by removing loci that have recombination fractions greater than 0.5. The markers in each linkage group were ordered initially *via* a maximum likelihood (ML) algorithm with default settings and 10K permutations. The ordered output of each linkage group from the ML algorithm was then used as the fixed order in a regression-based model. The regression-based marker ordering was performed with recombination fractions smaller than 0.350, LOD larger than 10, a jump threshold of 5, and a ripple of 1. The Kosambi mapping function was used for each linkage group. The final ordering for each linkage group was plotted with the physical position of the markers according to the *M. domestica* GDDH13 v1.1 reference genome (Daccord et al. 2017b) to evaluate ordering on the correlation between cM and base pair distance. This was also used to detect any possible structural variations. The final proposed map from this analysis was manually scanned for minor improvements by removing double recombinations/singletons and any chi-square nearest neighbor fit over 40 cM.

QTL mapping

R/QTL and MapQTL5 were used to calculate LOD scores and run multiple models to identify QTL (Broman et al. 2003; Van Ooijen 2004). In R/QTL, the data was converted to the backcross format where heterozygous states are B and homozygous states are A. The genotypic data was imputed prior to QTL analysis using the method of Sen and Churchill (2001). Given the data was slightly skewed from the normality of residuals assumption, every analysis was run with both the "Harley Knott" parametric regression and "np" non-parametric methods. Interval and composite interval mapping were performed separately for each year and trait. The LOD thresholds were calculated using a permutation test with 10K iterations and two stringent alpha values of $P < 0.001$ and $P < 0.0001$. A region of interest for a QTL is defined by the cM length of the linkage group where the LOD curve is above and intersects with the significance threshold. The percent variance explained was calculated using Eq. (2), where n is the population size and LOD is the logarithm of the odds value for an SNP marker (Broman et al. 2003).

$$PVE = 1 - \left(10^{\left(-\frac{2}{n}\right)*LOD}\right) \quad (2)$$

SSR marker development for Msv_FB7 fire blight QTL

Three SSR markers were designed spanning the QTL region of interest. This includes one marker at the peak of the 1.5-LOD QTL support interval on LG07 and two markers flanking upstream and downstream of the QTL. The physical position of the SNP in the Golden Delicious Double Haploid Genome (GDDH13v1.1) (Daccord et al. 2017b) was used to find di- and tri-short tandem repeats (STR) and their flanking sequences. The Primer3 software (Untergasser et al. 2012) was used to design forward and reverse primers flanking the STRs (Supplementary Table 2). An M13 tail (5'-TGTAAA ACGACGGCCAGT-3') was added to the 5'-end of each

forward primer. The forward M13 primer labeled with the 6-FAM fluorescent dye was ordered from Eurofins (Fleuri, Luxembourg). To check if these primer sequences were unique, the sequences were aligned using BLAST (Camacho et al. 2009) to the "Gala" and *M. sieversii* phased diploid reference genomes (Sun et al. 2020). The resulting alignments were filtered to retain only unique primer sequences conserved in all three genomes.

To test if the SSR markers were polymorphic, a subset of highly resistant and highly susceptible progeny and parents of the GMAL4591 population were genotyped. DNA was extracted from young leaf tissue using the Qiagen DNeasy kit (Germantown, Maryland). The SSR markers were run in separate PCR reactions with the EmeraldAmp® GT PCR Master Mix (Takara Bio) according to the manufacturer's instructions. The thermocycler protocol was set for (1) 95 °C for 3 min, (2) 95 °C for 1 min, (3) 55 °C for 1 min, (4) 72 °C for 30 s, 30X repeat of steps 2–4, and (5) 72 °C for 30 min. During the PCR reaction, the forward M13 primer labeled with the 6-FAM fluorescent dye was added to the mix at a rate of 1 ul of a 5-uM stock per 10-ul reaction. The PCR amplicons were sized using the Applied Biosystems 3730xl instrument with the LIZ500 sizing standard. The fragment size data were scored using the GeneMarker genotyping software (Hulce et al. 2011). Fragment sizes of the SSR markers were compared across parents and progeny to determine the inheritance patterns. The significance of the effect size of the fire blight resistance allele was determined with a Wilcox rank sum test. Moreover, accessions with known fire blight resistance QTL were screened using these markers to compare fragment sizes with the QTL donor.

Results

Phenotypic distributions and heritability

The average shoot lengths for GMAL 4591 and GMAL 4592 were respectively 11.01 ± 1.76 cm and 13.54 ± 3.61 cm in 2019 and 13.72 ± 2.67 cm and 15.48 ± 2.42 cm in 2020. High variability in the fire blight percent shoot lesion length (PSLL) values was observed, ranging from 0 to 81% in 2019 and 0 to 100% in 2020 across all populations (Fig. 1). The mean PSLL for the 2019 at 7 dai was 16.56 ± 21.36% for GMAL4591 and 26.31 ± 22.04% for GMAL4592 (Fig. 1). In 2020, the mean PSLL at time point 7 dai, 10 dai, and 15 dai were 13.36 ± 15%, 26.15 ± 23.63%, and 37.7 ± 29.7% for GMAL4591 and 39.09 ± 17.86%, 52.08 ± 19.19%, and 75.31 ± 19.57% for GMAL4592 (Fig. 1). The average AUDPC PSLL 2020 values for GMAL4591 and GMAL4592 were 214.69 ± 186.89 and 455.24 ± 140.02, respectively (Fig. 1). The parents were on the extreme ends of the distributions for all years and populations (Fig. 1). The exception was GMAL4592 in 2019 due to an unsuccessful inoculation of "Royal Gala" (Fig. 1). Transgressive segregation for both populations was observed only in the 2020 evaluations. Based on the AUDPC PSLL 2020, the percent of progeny more resistant than the *M. sieversii* parents for GMAL4591

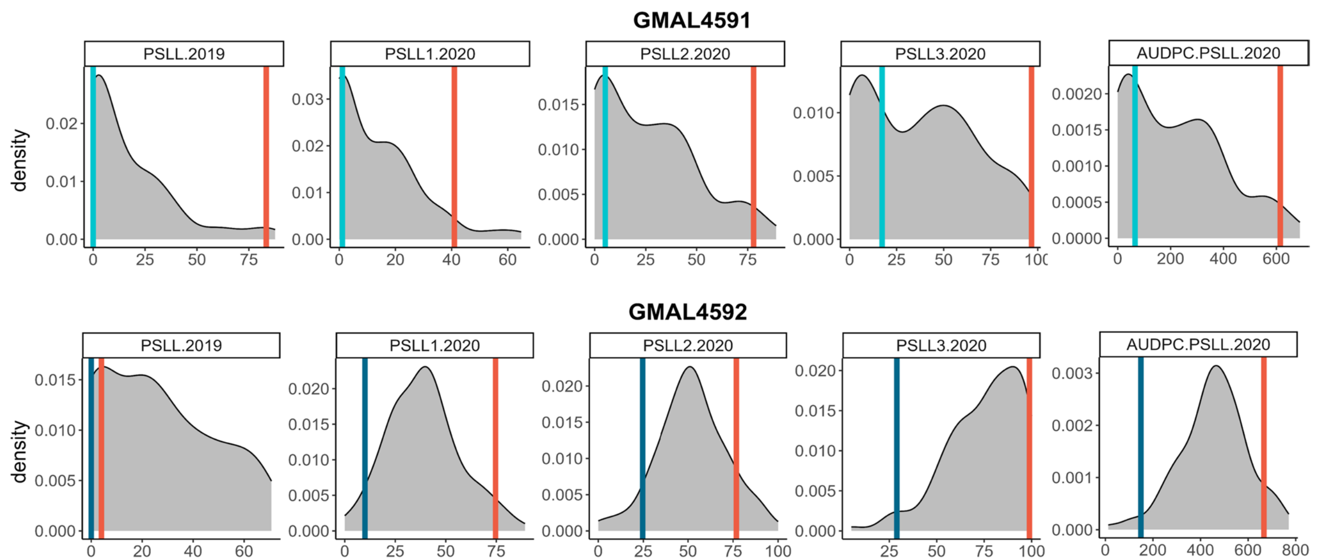

Fig. 1 Density plots of the fire blight percent shoot lesion length (PSLL) and area under the disease progress curve of percent shoot lesion length (AUDPC PSLL) used for QTL analysis across two years (2019–2020) and two F1 mapping populations (GMAL4591-top; GMAL4592-bottom). The blue bars represent the phenotypic value of the *M. sieversii* paternal parents (PI613959-turquoise; PI613978-light blue), and the red bar represents the phenotypic value of the maternal parent ("Royal Gala"). For 2020 data, PSLL1, PSLL2, and PSLL3 represent the phenotypic value taken on 7 dai, 10 dai, and 15 dai, respectively

and GMAL4592 were 31.2% and 6.5%, respectively (Fig. 1). For GMAL4591, the broad-sense heritability was high for both years ranging from 0.86 to 0.92 (Supplementary Table 1). The broad-sense heritability for GMAL4592 in both years for all traits ranged from 0.61 to 0.87 (Supplementary Table 1).

Linkage map construction

Four parental linkage maps were constructed for GMAL4591 and GMAL4592 using the pseudo-test cross markers from the GBS SNP data set (Supplementary Fig. 1). The filtered GBS SNP data sets were grouped into 17 distinct linkage groups after grouping loci with an independence test LOD score above 10. For GMAL4591, the final linkage maps consisted of 590 paternally segregating markers and 824 maternally segregating markers. The GMAL4592 linkage maps consisted of 559 paternally segregating markers and 864 maternally segregating markers. The centiMorgan distance for a single linkage group ranged from 40 to 110 cM with an average of 42 SNPs per linkage group. The total length of the paternal and maternal linkage maps for GMAL4591 was 1571 cM and 1425 cM, respectively. For GMAL4592, the average length of the paternal and maternal linkage maps was 1552 cM and 1518 cM, respectively. The average marker density for all linkage maps ranges from 1.8 to 3 cM. The maximum gap between markers of the paternal and maternal linkage maps for GMAL4591 was 21 cM and 26 cM, respectively. The maximum gap between markers of the paternal and maternal linkage maps for GMAL4592 was 26 cM and 17 cM, respectively. Larger gaps in the linkage maps were found spanning centromeric and telomeric regions due to low recombination rates (Antanaviciute et al. 2012) (Supplementary Fig. 1). The correlations of the physical positions from the GDDH13v1.1 reference genome (Daccord et al. 2017b) to the cM distances between SNPs were high for all linkage maps. The correlations were 0.91 and 0.88 for the maternal and paternal linkage maps of GMAL4591. For GMAL4592, the correlations were 0.89 and 0.92 for the maternal and paternal linkage maps, respectively (Supplementary Fig. 1). No observable structural variations were identified in any of the linkage maps constructed.

QTL mapping

A moderate-effect QTL from the paternal parent of GMAL4591 (KAZ 95 17-14; PI613959) was identified on LG07 with stringent LOD thresholds of 5.51 ($P < 0.001$) and 5.86 LOD ($P < 0.0001$) in 2019 and 2020, respectively (Khan et al. 2006b, 2007b) (Supplementary Fig. 2). Based on the CIM results, the LG07 QTL was observed to explain 48% of the phenotypic variance of PSLL 2019 (7 dai), 48% of PSLL 2020 (7 dai), 52% of PSLL 2020 (10 dai), 51% of PSLL 2020 (15 dai), and 53% of AUDPC PSLL 2020. The regions of interest of the QTL from the IM results are approximately 17 cM and 8 cM for CIM in 2019 and 2020, respectively. The 1.5-LOD QTL support interval was 5 cM with the maximum of each peak being the SNP marker, SLG7_PHT_25489931. The QTL region of interest spans the markers SLG7_PHT_22699693 and SLG7_PHT_27725770. The QTL was detected on an *M. sieversii* accession KAZ 95 17-14 (PI613959) linkage group, and therefore, this QTL was named as “Msv_FB7.” There was a significant ($P < 0.0001$) mean difference in all disease-related traits depending on the inheritance of the paternal allele from *M. sieversii* “KAZ 95 17-14” allele B on LG07 (Fig. 2B). At the SNP site SLG7_PHT_25489931, the mean PSLL 2019 was 20.2 ± 18.04% if the paternal allele A was inherited and 2.3 ± 5.54% if the paternal allele B was inherited (Fig. 2B). The mean AUDPC PSLL 2020 value was 298.1 ± 108.32 if the paternal allele A was inherited and 77.1 ± 78.57 if the paternal allele B was inherited (Fig. 2B). For progeny that shows no evidence of recombination across the 1.5-LOD QTL support interval, the mean AUDPC PSLL 2020 was 339.9 ± 134.49 for individuals with the paternal haplotype A and 104.8 ± 96.86 with the paternal haplotype B (Fig. 3).

A minor-effect QTL was identified on the proximal end of LG10 from the maternal parent of GMAL4592, *M. domestica* cv. “Royal Gala” (PI651008). This QTL was identified at the 4.56 LOD ($P < 0.001$) threshold for only the trait PSLL2 2020. The peak LOD value for this QTL was found at the SNP marker SLG10_PHT_118207, explaining 18% of the phenotypic variance. The 1.5-LOD QTL support interval was 2 cM. There was a significant ($P < 0.001$) mean PSLL2 2020 difference observed at this marker site with an average of 47.8% ± 14.54 when the maternal allele A was inherited and 58.9% ± 19.53 when the maternal allele B was inherited.

Marker development and QTL validation

Three SSR markers linked to *Msv_FB7* have been developed to use for marker-assisted selection in apple breeding programs. The fragment analysis of the upstream flanking marker of the QTL peak, Msv_FB7_5, showed a 342 bp fragment for “Royal Gala” and 312/316 bp for KAZ 95 17-14. There was a significant mean difference ($P < 0.05$, $n = 33$ progeny) for AUDPC PSLL 2020 with values of 291.4 for the 312 bp (−) allele and 138.4 for the 316 (+) allele. The marker at the QTL peak, Msv_FB7_1, showed a 131 bp fragment for “Royal Gala” and 120/147 bp for KAZ 95 17-14. There was a significant mean difference ($P < 0.0001$, $n = 55$ progeny) for AUDPC PSLL 2020 with values of 278.9 for the 120 bp (-) allele and 107.5 for the 147 (+) allele. The downstream flanking marker of the QTL peak, Msv_FB7_6, showed a 147 bp fragment for “Royal Gala” and 131/135 bp

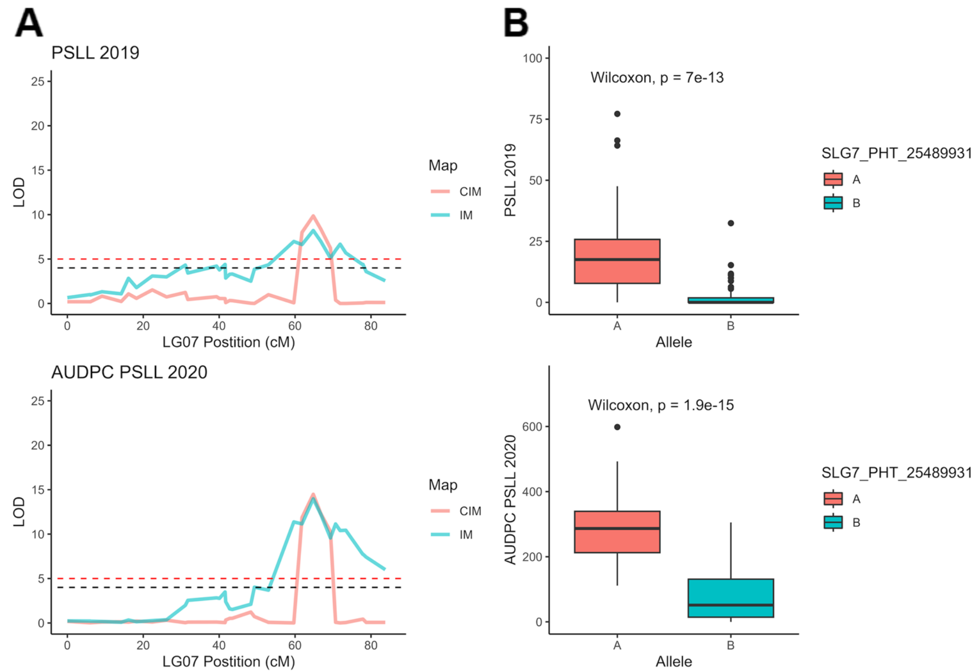

Fig. 2 (**A**) Logarithm of the odds (LOD) plot from the QTL analysis for two years of fire blight phenotype data from the F1 population, GMAL4591. The percent shoot lesion length (PSLL 2019, top) and area under the disease progress curve of the percent shoot lesion length (AUDPC PSLL 2020, bottom) show a significant ($P < 0.0001$) QTL for fire blight resistance on the linkage group (LG) 7. Interval mapping results are in blue, and composite interval mapping results are in red. (**B**) The fire blight infection severity by genotype boxplot for the SNP marker at the QTL peak on LG07 (SLG7_PHT_25489931) across two years (top: 2019; bottom: 2020). Genotypes marked A are homozygous and B are heterozygous. The marker name includes information about the linkage group and the physical position (bp) in the GDDH13v1.1 genome (Daccord et al. 2017b)

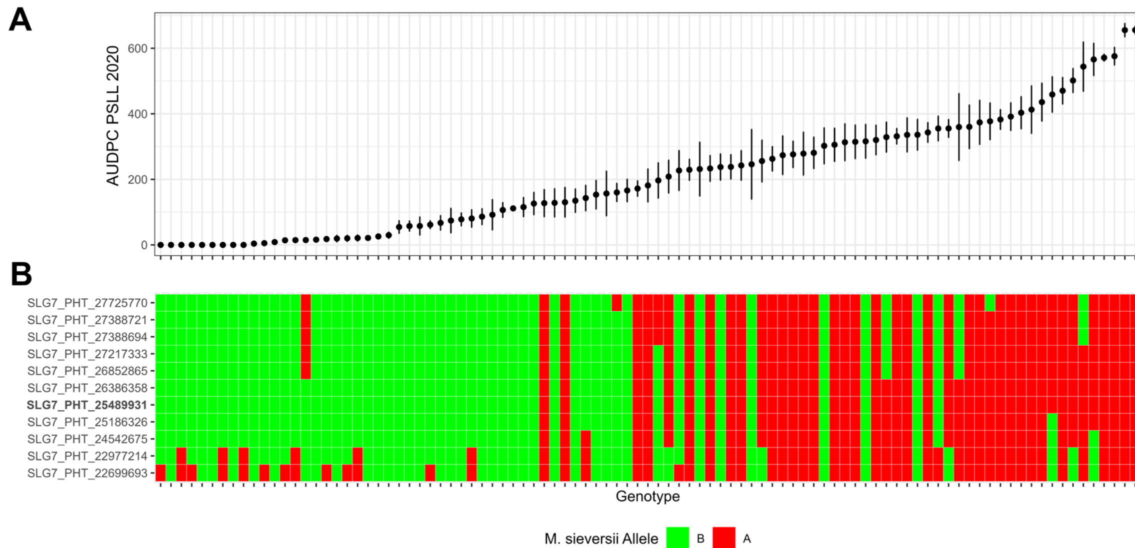

Fig. 3 (**A**) Line graph where the *y*-axis contains the sorted mean fire blight infection severity values measured by the area under the disease progress curve of the percent shoot lesion length (AUDPC PSLL 2020) in the F1 population, GMAL4591. Each dot represents F1 progeny or parent of GMAL4591 at each tick on the shared *x*-axis. (**B**) Heat map showing a graphical representation of the introgression of the **A** (red) and **B** (green) alleles in GMAL4591 from the *M. sieversii* paternal parent (PI613959) in the 1.5-LOD QTL support interval. The SNP names in the *y*-axis contain the linkage group and the physical position in the GDDH13v1.1 reference genome. The SNP with the highest LOD (SLG7_PHT_25489931) is bolded

for KAZ 95 17-14. There was a significant mean difference ($P < 0.01$, $n = 25$ progeny) for AUDPC PSLL 2020 with values of 294.9 for the 135 bp (−) allele and 86.3 for the 131 bp (+) allele.

Combining Msv_FB7_5 and Msv_FB7_1 had a significant mean difference ($P < 0.01$, $n = 25$ progeny) for AUDPC PSLL 2020 with a value of 216.8. Combining Msv_FB7_1 and Msv_FB7_6 had a significant mean difference ($P < 0.01$, $n = 18$ progeny) for AUDPC PSLL 2020 with a value of 273.2. Combining Msv_FB7_5 and Msv_FB7_6 had a significant mean difference ($P < 0.01$, $n = 11$ progeny) with a value of 343.5. Msv_FB7_5 and Msv_FB7_6 showed no evidence of recombination or outliers when combined.

Discussion

A moderate-effect fire blight resistance QTL "*Msv_FB7*" explaining 48–53% of the phenotypic variance was identified at the distal end of linkage group 7 from the *M. sieversii* parent KAZ 95 17-14. The identification of this QTL was based on stringent significance thresholds ($P < 0.0001$) across multiple time points and two years of data, indicating the reliability of the results. The LOD scores of *Msv_FB7* for each time point in the 2020 disease evaluations increased over time, similar to previously identified fire blight QTL on LG7 (Khan et al. 2006b). The broad-sense heritability values for all traits were moderate to high (0.61–0.92), suggesting that experimental/environmental factors had minimal influence on these traits. These estimates were comparable to previous greenhouse screenings of fire blight QTL mapping populations that ranged from 0.71 to 0.96 (Calenge et al. 2005; Khan et al. 2006b; Durel et al. 2009; Desnoues et al. 2018b). Since the *Msv_FB7* QTL explains only around half of the observed phenotypic variation, it is possible that the residual variation in the defense response is attributable to undetected minor-effect QTL (Desnoues et al. 2018b). These undetected minor QTLs may explain the occasional fire blight susceptibility responses of some GMAL 4591 progeny that inherit the resistance haplotype (Fig. 3). Minor-effect QTL is often not detected when moderate-sized populations are used. Population sizes near 100 F1 individuals can contribute to an overestimation of the genetic effect of *Msv_FB7* or a lack of power to detect smaller effect QTL due to the Beavis Effect (Xu 2003). Additionally, the phenotypic evaluations were performed with a single strain, opening the possibility of strain-specific effects of *Msv_FB7*. For example, the *Msv_FB7* donor "KAZ 95 17-14" demonstrated resistance to the Canadian *E. amylovora* strain Ea2002A in our experiment; however, it did not exhibit resistance against the Swiss strain, FAW610 (Baumgartner et al. 2011). Similar strain-specific effects have been observed for the fire blight resistance QTL *MR5* and *Mfu10* (Peil et al. 2011; Emeriewen et al. 2015). Therefore, further investigation is needed to better understand the stability and strain effects on *Msv_FB7*.

Additionally, *Msv_FB7* is colocalized with the putative moderate-effect fire blight resistance QTL identified in *M. domestica*, *FB_F7* based on marker positions in the GDDH13 v1.1 genome (Khan et al. 2006b, 2007b; Daccord et al. 2017b, van de Weg et al. 2018b). Given the close genetic relationship and admixture events between *M. sieversii* and *M. domestica*, it opens the question of whether these QTL are the same or different alleles (Khan et al. 2021; sieversii et al. 2022). If these are different QTLs, colocalization may pose challenges in pyramiding as they would be in tight coupling or repulsion phase linkage. Similar observations have been made with a major effect fire blight resistance QTL, *FB_Mar12*, on the distal end of LG12 colocalized in *M. floribunda, M. "Evereste,"* and *M.* × *arnoldiana* (Durel et al. 2009; Emeriewen et al. 2017, 2021). These two species have evidence of hybridization, but it is currently still not known if the fire blight resistance QTL is the same locus or independent loci (Emeriewen et al. 2021). To better understand the inheritance of *Msv_FB7*, several genotypes that possess *FB_F7* were genotyped with the SSR markers developed in the study (Supplementary Table 3). We did not find any matching fragment sizes of SSR markers linked to the *Msv_FB7* resistance allele and the genotypes known to possess *FB_F7* (Supplementary Table 3).

Three SSR markers have been developed to perform a marker-assisted selection for *Msv_FB7* in apple breeding and future fine-mapping studies. Consistently across all markers, significant ($P < 0.05$) mean differences in the AUDPC PSLL 2020 were observed depending on the paternal allele inherited (Fig. 4). The results of the SSR genotyping matched the effect of the SNP markers from the QTL analysis with a near 1:1 ratio of A and B alleles inherited (Fig. 4). Furthermore, the combination of multiple markers reduced the likelihood of a recombination event between the marker and the actual position of the QTL (Fig. 4). This is seen with the reduction of outliers in groups that are both positive and negative for the paternal resistance allele (Fig. 4). Combining the two flanking markers (Msv_FB7_5 and Msv_FB7_6) resulted in no evidence of recombination in the QTL region, as expected (Fig. 4). Additionally, when comparing the results obtained with the SNP and SSR haplotypes that had no recombination in the QTL region of interest, the effect on the AUDPC PSLL 2020 values was consistent (Fig. 3). Confirmation of *Msv_FB7 via* SSR genotyping provides evidence against this being the result of SNP genotyping errors (Wallin et al. 2021). However, validating this QTL across different genetic backgrounds is needed to better understand the true QTL effects (Khan et al. 2007b). These findings suggest that these markers can be reliably utilized for marker-assisted selection (MAS) of *Msv_FB7* in an apple breeding

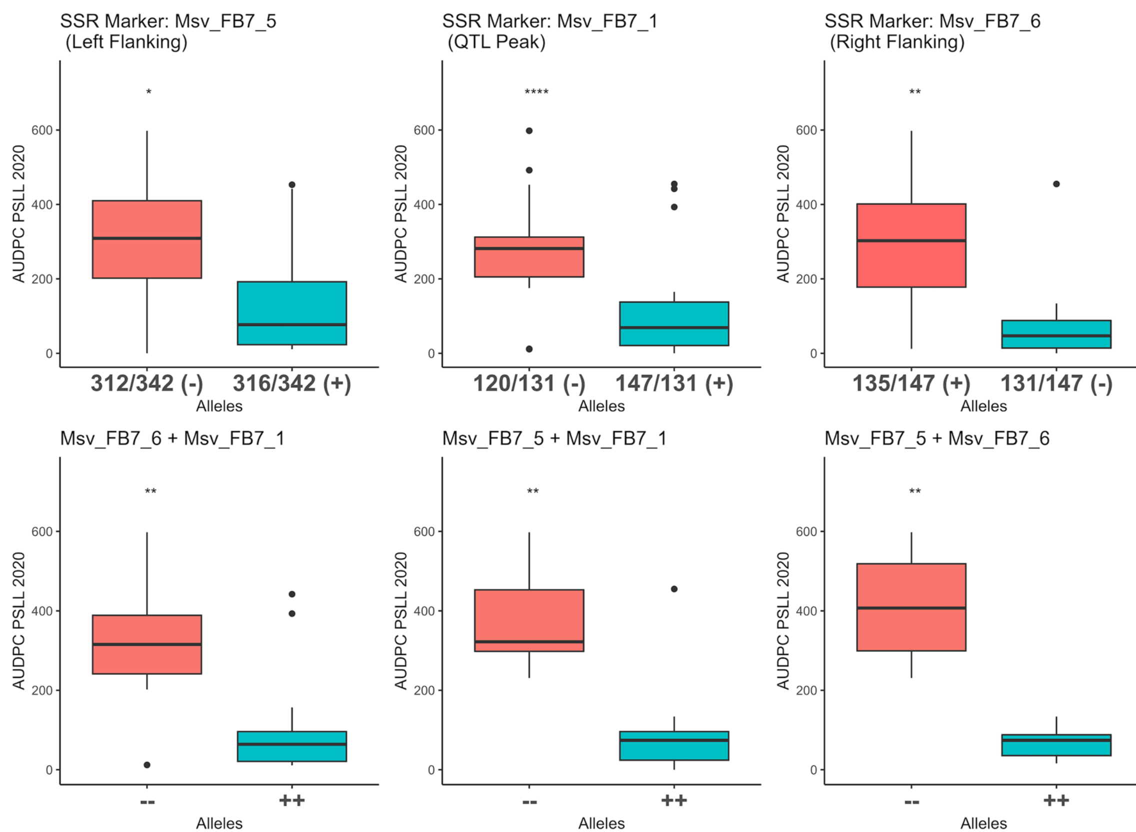

Fig. 4 Boxplots showing the effect of the GMAL4591 F1 progeny allele size from three SSR markers (*x*-axis) on the fire blight infection severity measured by the area under the disease progress curve of the percent shoot lesion length from 2020 (AUDPC PSLL 2020; *y*-axis). The allele sizes indicate the fragment length in bp, and in parentheses is the status of whether *Msv_FB7* was inherited. The first row shows the results of single markers, and the second row shows varying combinations of when two markers were both positive or negative for the QTL. The stars above the boxes indicate varying levels of statistical significance (* = 0.05; ** = 0.01; ***=0.001; **** = 0.0001)

program. This QTL can be pyramided with other fire blight resistance QTL as well as for other important apple diseases and pests such as powdery mildew, apple scab, and wooly apple aphid (Bus et al. 2000; Patocchi et al. 2009). This is more attainable using *M. sieversii*, given it is among the only wild *Malus* species with moderate fruit size (Cornille et al. 2012; Harshman et al. 2017). Additionally, several important apple production traits were found to be highly similar when comparing across *M. sieversii* accessions and *M. domestica* cultivars, including soluble solids (°Brix), precocity date (years to bloom), firmness (kg/cm^2), and the percent change in acidity and firmness in storage (Davies et al. 2022). This alleviates the bottleneck to develop fire-blight-resistant cultivars by requiring fewer generations to break the linkage drag of unfavorable fruit quality alleles (Russo et al. 2007; Harshman et al. 2017).

We also detected a minor-effect QTL on linkage group 10 in the maternal parent “Royal Gala” of GMAL4592, which explains 18% of the phenotypic variance and is specific to the PSLL2 2020 time point and year (Supplementary Fig. 2). The instability and time-point specificity could be due to sample sizes and relatively low number of replicates limiting the power to reliably detect minor-effect QTL (Xu 2003). Additionally, the moderate broad-sense heritability value for the 2019 evaluation in GMAL4592 could indicate experimental noise that further limits QTL detection from that data (Supplementary Table 1). It is suspected that the resistance from the paternal parent “KAZ 95 10-04F” is comprised of many additive minor-effect loci. This genetic architecture is proposed, given the near normal distribution of fire blight resistance in the progeny, low level of transgressive segregants, and higher disease severity ratings across GMAL4592 compared to GMAL4591 (Le Roux et al. 2010b; Brachi et al. 2011) (Fig. 1). This QTL also colocalizes with a QTL previously identified in the “Florina” × “Nova Easygro” segregating mapping population, where a minor-effect QTL was mapped to LG10, explaining 15.3–17.9% of the phenotypic variance (Le Roux et al. 2010b). Interestingly, in both studies, the minor-effect QTL was derived from the susceptible parent. Identifying QTL from the susceptible parent has been observed across multiple studies mapping fire blight and apple scab resistance

(Liebhard et al. 2003; Calenge et al. 2004; Soufflet-Freslon et al. 2008; Kostick et al. 2021). The effect of the QTL could be recessive in the parental genotype, making it detectable when it segregates across a biparental population (Liebhard et al. 2003). The effect of this QTL could also be due to newly formed epistatic interactions with alleles from the parent KAZ 10-04F, explaining why it is not observed in GMAL4591 (Lorang et al. 2007).

Conclusion

We identified the moderate-effect QTL on LG07, *Msv_FB7*, from the *M. sieversii* male parent (PI613959; "KAZ 95 17-14"). Additionally, a minor-effect QTL was identified from GMAL4592 on LG10 from the maternal parent *M. domestica* cv. "Royal Gala." *Msv_FB7* was identified across 2 years and multiple time points of data with high broad-sense heritability. Three diagnostic SSR markers were successfully developed to be used for the marker-assisted selection of *Msv_FB7*. Therefore, *Msv_FB7* shows potential to be a useful source of fire blight resistance to develop fire-blight-resistant cultivars with less generation time than other wild species of apple.

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Author contributions A.K. conceptualized, designed, and managed the project. R.T. and D.C-S. performed the experiments. R.T. performed all analysis and interpretation as well as drafted the manuscript. R.T., D.C-S., G-Y.Z., and A.K. revised and finalized the manuscript. All authors read and approved the final version.

Data availability The genotype by sequencing SNP data sets for GMAL4591 and GMAL4592 to construct genetic maps are made available on the Dryad database (10.5061/dryad.15dv41p3c).

Declarations

Competing interests The authors declare no competing interests.

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