Dissecting Genetic Resistance to Fire Blight in Three Pear Populations

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Accepted for publication 11 March 2020.

ABSTRACT

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is a persistent problem for pear (*Pyrus* spp.) growers in the United States. Growing resistant cultivars is one of the best options for managing fire blight. The cultivars Potomac and Old Home and the selection NJA2R59T69 display resistance to fire blight. As such, three mapping populations (El Dorado × Potomac, Old Home × Bartlett, and NJA2R59T69 × Bartlett) were developed to identify genomic regions associated with resistance to fire blight. Progeny were phenotyped during 2017 and 2018 by inoculating multiple actively growing shoots of field-grown seedling trees with *E. amylovora* isolate E153n via the cut-leaf method. Genotyping was conducted using the recently developed Axiom Pear 70 K Genotyping Array and chromosomal linkage groups were

created for each population. An integrated two-way pseudo-testcross approach was used to map quantitative trait loci (QTLs). Resistance QTLs were identified on chromosome 2 for each population. The QTLs identified in the El Dorado × Potomac and Old Home × Bartlett populations are in the same region as QTLs that were previously identified in Harrow Sweet and Moonglow. The QTL in NJA2R59T69 mapped proximally to the previously identified QTLs and originated from an unknown Asian or occidental source. Future research will focus on further characterizing the resistance regions and developing tools for DNA-informed breeding.

Keywords: genetics and resistance

Fire blight, caused by *Erwinia amylovora*, is the most important bacterial disease of pear (*Pyrus* spp.). The pathogen originated in North America and can now be found throughout North America and Europe, parts of Asia, and northern Africa (Bell 2019; van der Zwet 2002). The pathogen overwinters in cankers and, as temperatures warm, the bacteria emerge in a polysaccharide ooze (Malnoy et al. 2012). The ooze serves to protect the bacteria from abiotic stresses and to attract insects for dissemination (Griffith et al. 2003). In addition to insects, *E. amylovora* can be spread via wind and rain. Infection of pear can occur on blossoms, where it enters through floral nectaries, or on shoots through wounds (Malnoy et al. 2012). Because wounding is required for shoot blight, hail damage can be a major factor in the development of epidemics.

In conducive climates, the disease can be very difficult to control. Management of the disease currently relies on the removal of infected plants and tissue and the application of antibiotics and copper-based compounds (Duffy et al. 2005; McManus et al. 2002; Norelli et al. 2003b; Psallidas and Tsiantos 2000). In some regions of the world, resistance to the antibiotics streptomycin and oxolinic acid has been detected in *E. amylovora* populations (Laforest et al. 2019; Manulis et al. 2003; McManus et al. 2002; Rezzonico et al. 2009; Sundin and Wang 2018). Concerns have been raised

regarding the impacts of off-target applications of antibiotics on the environment and microbiomes and on the possibility of horizontal gene transfer of antibiotic resistance between plant and human pathogenic microorganisms (Chiou and Jones 1995; McManus 2014; Sundin et al. 1995). The use of genetic resistance is an attractive option for disease management and can help reduce the perceived risks associated with off-target antibiotic applications. Unfortunately, the most commonly grown pear cultivars in the United States are susceptible to fire blight (Bell 2019). A number of fire blight-resistant scions have been developed globally but are not commercially cultivated widely. Therefore, there is a great need to develop new resistant pear cultivars that meet the demands of growers, processors, and consumers.

Very little work has been done to identify genomic regions associated with fire blight resistance in pear. To date, four studies have been conducted using three different populations. Three of these studies had relatively low-density maps, using a mix of amplified fragment length polymorphism and simple sequence repeat markers (Bokszczanin et al. 2009; Dondini et al. 2004; Le Roux et al. 2012). Dondini et al. (2004) and Le Roux et al. (2012) evaluated the same Passe Crassane × Harrow Sweet population and identified the same quantitative trait loci (QTLs) on Harrow Sweet linkage groups (LGs) 2 and 4. The Dondini et al. (2004) study also identified a minor effect QTL on Harrow Sweet LG 9 that was not identified by Le Roux et al. (2012). Bokszczanin et al. (2009) identified two QTLs in an interspecific cross between Doyenné du Comice and a *Pyrus ussuriensis* selection known as “no. 18”. The first QTL was on Doyenné du Comice LG 4 in a similar region to the one identified in Harrow Sweet (Le Roux et al. 2012). The second QTL was on *P. ussuriensis* no. 18 LG 11 (Bokszczanin et al. 2009). The most recent QTL study to be conducted for fire blight resistance was accomplished using another interspecific population, PEAR3 (PremP003, *P. ×bretschneideri* × *P. communis*) × Moonglow (*P. communis*), and an Illumina single-nucleotide polymorphism (SNP) array (Montanari et al. 2016). In this study, the same population was evaluated in two locations over multiple years. Six QTLs were identified, five on PEAR3 LGs 7, 9, 10, 12,

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Funding: This research was funded through the USDA National Institute of Food and Agriculture–Specialty Crop Research Initiative project, “RosBREED: Combining Disease Resistance and Horticultural Quality in New Rosaceous Cultivars” (2014-51181-22378), the California Pear Advisory Board, and USDA-ARS CRIS numbers 2072-21000-049-00D and 8080-21000-029-00D.

*The e-Xtra logo stands for “electronic extra” and indicates that supplementary materials are published online.

The author(s) declare no conflict of interest.

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and 15 and one on Moonglow LG 2 (Montanari et al. 2016). The PEAR3 LG 9 QTL was in a similar region to the Harrow Sweet LG 9 QTL (Dondini et al. 2004; Montanari et al. 2016; Yamamoto et al. 2013). The Moonglow LG 2 QTL was also in a similar region as the Harrow Sweet LG 2 QTL (Montanari et al. 2016). The Montanari et al. (2016) study was able to achieve narrower confidence intervals for the LG 2 QTL due to a much higher marker density. This marker density was not consistent across other LGs, possibly due to the mix of both *Malus* and *Pyrus* SNPs on the array used and the interspecific nature of the mapping population (Montanari et al. 2013).

Advancements in pear genomic resources can spur the identification of new fire blight resistance QTLs. Recently, a high-quality genome assembly was completed for a double-haploid offspring of Bartlett (Linsmith et al. 2019). A 70 K SNP Axiom array consisting only of *Pyrus* SNPs was also developed (Montanari et al. 2019). This array is a significant improvement over the 9 K (8 K apple + 1 K pear) Infinium array that was previously available. When used in conjunction, these tools can provide new insights into the genomic regions associated with fire blight resistance. As such, a new QTL mapping study was conducted on three pear populations to characterize the resistance present in the *P. communis* cultivars Old Home and Potomac and the hybrid selection NJA2R59T69.

MATERIALS AND METHODS

Germplasm. Three populations were developed. The first was a cross of the resistant Old Home with susceptible Bartlett (OH×B; US200502; 100 individuals). The second was the result of a cross between resistant Potomac and susceptible El Dorado (P×ED; US97211; 81 individuals). Finally, the third was created by crossing the resistant hybrid selection NJA2R59T69 with Bartlett (NJ×B; US200536/37; 82 individuals). Each population was maintained in a separate orchard as adult plants at the United States Department of Agriculture–Agricultural Research Service Appalachian Fruit Research Laboratory in Kearneysville, WV.

Phenotypic evaluation. Plant material was phenotyped for its resistance to fire blight under orchard conditions during the 2017 and 2018 growing seasons. Inoculum was prepared by culturing *E. amylovora* isolate E153n overnight in Kado523 broth, as previously described (Norelli et al. 2003a). In 2017 and 2018, inoculum concentration was adjusted to 1 and 5 × 10⁹ CFU ml⁻¹, respectively, in a 10 mM dibasic phosphate buffer (pH 7.0). Isolate E153n is a spontaneous nalidixic-acid-resistant derivative of isolate Ea153 originating from eastern Oregon (Johnson et al. 1993; Stockwell et al. 1998).

For each plant, 10 actively growing shoots on independent branches that had at least 10 cm of new growth were inoculated. Inoculations were conducted by bisecting at least three immature leaves immediately below the shoot tip meristem of actively growing shoots with scissors dipped into the inoculum suspension (Norelli et al. 1988). The shoot length was measured from the first bud scale (previous season's) to the first cut leaf. Four to six weeks after inoculation, lesion length was measured. Severity for each shoot was calculated by dividing the lesion length by the shoot length and multiplying by 100. Mean severity was calculated for each tree and used for future analysis.

Linkage mapping. Young, recently expanded leaf tissue was collected from each plant for the parents and offspring of the three populations in Kearneysville, WV and shipped overnight to the University of California, Davis, CA. DNA was extracted using the E-Z 96 Plant DNA extraction kit (Omega BioTek, Norcross, GA, U.S.A.) using a protocol described by Gilmore et al. (2011). The extracted genomic DNA was quantified using the Quant-iT Picogreen Assay (Invitrogen, Eugene, OR, U.S.A.) and a Tecan Infinite M Plex multimode plate reader (Tecan Group Ltd., Zürich, Switzerland). DNA for each sample was adjusted to between 15 and 50 ng/μl and submitted to Thermo Fisher Scientific, Inc.

(Waltham, MA, U.S.A.) for genotyping using the Axiom Pear 70 K genotyping array (Montanari et al. 2019). The genotypic data for each population was scored using the Axiom Analysis Suite (v 4.0; Thermo Fisher Scientific, Inc.) following the best practices described in the software documentation. The R package SNPish v 1.5.2 was used to assign the markers into one of six quality classes according to their clustering performance. Markers that were assigned into the “Poly High Resolution” (three defined clusters) and “No Minor Homozygote” (two defined clusters) classifications were used for mapping in each population.

Linkage mapping for the OH×B population was conducted as part of the work of Linsmith et al. (2019) and the previously created map was used for this study. The P×ED and NJ×B populations were mapped using the same strategy as the OH×B population. Markers were first filtered to have <5% missing data. An individual that had allele calls not expected from their respective cross at a rate of ≥5% was considered to have originated from an open pollination event and was removed from analysis. Mapping was conducted with JoinMap 5 using the maximum-likelihood algorithm and Kosambi's mapping function (Kosambi 1943; van Ooijen 2011). An initial map was created for each population by using markers that fit 1:1 or 1:2:1 segregation ratios ($\alpha = 0.01$). An iterative process followed using both JoinMap 5 and graphical genotyping to fix genotyping and marker ordering errors. After a high-quality framework map was constructed, markers that fit segregation ratios of 2:1 or 2:3:1 ($\alpha = 0.01$) were added to improve low-density regions caused by segregation distortion.

QTL analysis. A spatial autocorrelation analysis was conducted on the phenotypic data for each population to determine whether the plant location within the orchard influenced disease development and whether plant location should be used as a cofactor in QTL analysis. This analysis was conducted by calculating the inverse of Euclidian distance between each tree based on their row and position within each row for each population. The resulting distance matrix was used to calculate Moran's I using the R v 3.6.1 (R Core Team 2019) package ‘ape’ v 5.3 (Paradis et al. 2004). Moran's I is analogous to a correlation coefficient where a value of 0 indicates a random pattern and positive or negative 1 indicates a strong positive or negative spatial autocorrelation, respectively. Phenotypic data normality were also visually assessed for each population. Data that were not approximately normally distributed were transformed to meet data assumptions associated with interval mapping algorithms (Lander and Botstein 1989; Zurn et al. 2018).

QTL analysis was conducted using MapQTL 6 using a two-way pseudo test cross approach with the multiple QTL mapping algorithm (van Ooijen 2009). Significant QTLs ($\alpha = 0.05$) were identified using a resampling analysis of 1,000 permutations (Churchill and Doerge 1994). For significant QTLs, the 95% confidence intervals were estimated using the 2-LOD drop method (Lander and Botstein 1989).

RESULTS

Phenotypic evaluation. Phenotypic data for each of the populations were skewed toward resistance (Fig. 1). This trend was observed during each growing season. The 2018 growing season had higher disease pressure than the 2017 growing season. This is reflected in the distributions of the data because the 2018 trials tended to have a greater number of higher mean severity ratings (Fig. 1). For each trial, missing data were observed. During the 2017 trials, 15 (15.0%), 6 (7.4%), and 9 (11.0%) individuals did not have data for the OH×B, P×ED, and NJ×B populations, respectively. In 2018, the OH×B, P×ED, and NJ×B populations had 13 (13%), 5 (6.2%), and 5 (6.1%) individuals with missing data, respectively. Missing data were caused by the death of the plant or the loss of tags marking the inoculated shoot over the course of the growing season.

Linkage mapping. The OH×B map described by Linsmith et al. (2019) consists of 29,703 markers distributed across 17 chromosomal LGs (Supplementary Table S1). The total length of the OH×B map is 1,147.22 centimorgans (cM). The map has an average density of 1.59 unique marker bins/cM. Severe segregation distortion was observed on the long arm of chromosome 17 for markers that were heterozygous in Bartlett. This region is known to contain the gametic incompatibility S-locus (De Franceschi et al. 2011)

For the P×ED population, 70,726 markers had <5% missing data. Of those markers, 27,052 were identified that were classified as

either “Poly High Resolution” or “No Minor Homozygote” markers and were segregating in the P×ED population. One individual in the P×ED population was found to be the result of an open pollination event and was removed from analysis. In total, 22,039 markers were included in the final P×ED map (Supplementary Table S2). The P×ED map has 17 LGs that each correspond to one of the 17 *P. communis* chromosomes. The total length of the P×ED map is 1,034.97 cM with an average density of 1.22 unique marker bins/cM.

There were 70,424 markers with <5% missing data for the NJ×B population. Of these markers, 38,793 had <5% missing data, were

Fig. 1. Phenotypic distributions for each population during the 2017 and 2018 growing season. Populations consisted of crosses of Old Home with Bartlett (OH×B), Potomac with El Dorado (P×ED), and NJA2R59T69 with Bartlett (NJ×B).

segregating in the NJxB population, and were classified as either “Poly High Resolution” or “No Minor Homozygote” markers. All individuals were identified to be the result of the expected cross. The final NJxB map consists of 37,182 markers distributed across 17 chromosomal LGs (Supplementary Table S3). The NJxB map is 1,080.50 cM in total length and has an average density of 1.70 unique marker bins/cM. Like the OHxB map, severe segregation distortion was observed on the long arm of chromosome 17 for markers that were heterozygous in Bartlett.

QTL analysis. A spatial autocorrelation analysis of each trial for each population revealed that plant location within an orchard did not influence disease development (Table 1). As such, field location was not used as a cofactor in any of the QTL analyses. Data were not normally distributed during any of the trials and were transformed to approach normality and meet QTL mapping assumptions (Fig. 1). Transformations were chosen via visual assessment of Quantile-Quantile plots of the posttransformed data. A square root transformation was applied to the OHxB data, cubic root transformation was applied to the PxED data, and a quartic root transformation was applied to the NJxB data.

One significant ($\alpha = 0.05$) QTL was identified on the short arm of chromosome 2 for the OHxB and PxED populations during the 2017 and 2018 growing season (Fig. 2; Table 2). These QTLs were inherited from Old Home and Potomac, respectively. There was some ambiguity as to the location of the chromosome 2 QTL in the OHxB population. During the 2017 growing season, the QTL was detected in a slightly different location than during the 2018 growing season, likely due to environmental variation. Physically, these QTLs on chromosome 2 for the OHxB and PxED populations are in the same location when considering the 2018 OHxB trial (Table 2). A significant ($\alpha = 0.05$) QTL was identified in a different position on the short arm of chromosome 2 for the NJxB population during the 2017 and 2018 growing seasons (Fig. 2; Table 2). A significant ($\alpha = 0.05$) QTL was also identified for the NJxB population on the short arm of chromosome 13 during only the 2017 growing season. The chromosome 2 and 13 QTLs were both inherited from NJA2R59T69.

The physical regions of the QTLs were identified in the double-haploid Bartlett genome assembly v2 (Linsmith et al. 2019) and were evaluated for resistance associated genes. The physical region describing the chromosome 2 QTLs in the OHxB and PxED populations is 1.86 Mb in size. There are 205 reported genes in this region. Twelve of the genes have functions associated with disease resistance, with eight being nucleotide-binding leucine-rich repeats (NB-LRRs) and four being serine/threonine-protein kinases. The physical region describing the chromosome 2 QTL in the NJxB population is 1.71 Mb in size and there are 204 reported genes in this region. Resistance-associated genes in this region consist of 15 NB-LRRs and three serine/threonine-protein kinases.

DISCUSSION

QTLs in three regions were identified during the present study. Similar to previous studies, QTLs were identified on chromosome 2 (Fig. 2; Table 2) (Dondini et al. 2004; Le Roux et al. 2012; Montanari et al. 2016). The chromosome 2 QTL identified in the

OHxB mapped to two locations between the 2017 and 2018 disease trials. The 2017 trial had lower disease pressure (Fig. 1), which could have led to the slightly different mapping position. When considering the physical position of the 2018 OHxB chromosome 2 QTL, the OHxB and PxED chromosome 2 QTLs map to the same physical location (Table 2). The QTL identified from Potomac in the PxED population is likely the same as the one identified in Moonglow, because Moonglow is a parent of Potomac (Montanari et al. 2016). Old Home is a grandparent of Harrow Sweet, in which Le Roux et al. (2012) identified a QTL on chromosome 2. The markers delimiting the Harrow Sweet chromosome 2 QTL, TsuENH017 and NH033b, were evaluated in Old Home and none of the fire blight resistance-associated alleles were identified (Le Roux et al. 2012). Le Roux et al. (2012) subsequently hypothesized that the resistance present in Harrow Sweet was inherited from the pollen grandparent Early Sweet. TsuENH017 was also associated with the Moonglow chromosome 2 QTL inherited from Roi Charles de Wurtemberg (Montanari et al. 2016). The same Moonglow fire blight resistance haplotype was identified in Old Home and the two cultivars were hypothesized to have the same resistance QTLs despite no known shared pedigree (Montanari et al. 2016). The chromosome 2 QTL identified in the OHxB population inherited from Old Home confirms this hypothesis. The overlap of the physical regions between the chromosome 2 QTLs identified in the OHxB and PxED populations adds further support to this hypothesis. Because the pedigree of Old Home remains a mystery, it may not be possible to understand how Old Home and Moonglow are related.

The chromosome 2 QTL identified in the NJxB population mapped proximal to the QTLs detected in the OHxB and PxED populations and appears to be unique (Table 2). During the 2018 growing season, the NJxB chromosome 2 QTL explained 51.2% of the phenotypic variation (Table 2). Genotypic profiling of NJA2R59T69 suggests that it is a hybrid of an unknown Asian pear species and *P. communis* (S. Montanari, personal communication). It is unknown whether the resistance is of Asian or occidental origin. To date, no fire blight resistance QTLs have been reported for chromosome 2 that have originated from an Asian source. The only study conducted on an Asian pear species was by Boksaczanin et al. (2009). In that study, only one QTL was found that originated from the *P. ussuriensis* selection no. 18 on chromosome 11 (Boksaczanin et al. 2009). A second significant QTL was also identified on chromosome 13 during the 2017 growing season in the NJxB population. This QTL was not observed during the 2018 growing season, where disease pressure was higher (Fig. 1). As such, the chromosome 13 QTL may be due to environmental rather than genetic factors.

Disease resistance genes were identified in the two chromosome 2 QTL regions. Most of the genes identified are NB-LRR genes that are typically involved in effector-triggered immunity (ETI) through the recognition of effectors or effector–host protein interactions. The remaining genes are serine/threonine-protein kinase genes. Resistance in either of the chromosome 2 QTL regions may be governed by a molecular gene-for-gene model, where an NB-LRR protein directly interacts with an avirulence protein. In apple, the FB_MR5 protein is thought to directly interact with the *E. amylovora* AvrPrt2_{EA} protein to induce an ETI response (Broggini et al. 2014). Unlike the FB_MR5–AvrPrt2_{EA} interaction, an immune reaction was not observed for the three populations. Despite the absence of such an immune reaction, it is not uncommon for NB-LRR and other receptor-like genes to be major factors in pathogen recognition in quantitative resistance pathways (Corwin and Kliebenstein 2017). Often, these receptor genes will have major effects on phenotypic variability, and polymorphisms in downstream genes within the resistance pathway will have smaller impacts on the trait. To effectively identify minor effect resistance QTLs, large populations consisting of thousands of individuals may be necessary (Corwin and Kliebenstein 2017). This often is not

TABLE 1. Moran's I for spatial autocorrelation for each population during each growing year^a

Population ^b	2017	2018
OHxB	0.04 (0.13)	−0.02 (0.72)
PxED	0.03 (0.19)	−0.01 (0.99)
NJxB	0.05 (0.10)	−0.02 (0.88)

^a P values for the null hypothesis that there is no spatial autocorrelation are reported in parentheses.

^b Crosses consisted of Old Home with Bartlett (OHxB), Potomac with El Dorado (PxED), and NJA2R59T69 with Bartlett (NJxB).

feasible in tree plants such as pear. A serine/threonine-protein kinase gene in the OHxB and PxED chromosome 2 QTL region is of particular note. This gene (*pycom02g07320*) has homology to a receptor-like protein kinase known as RPM1-interacting protein kinase (*RIPK*). *RIPK* has been studied in *Arabidopsis* and soybean (*Glycine max*) and is involved in host defense responses to the bacterial pathogens *Pseudomonas syringae* and *Xanthomonas campestris* pv. *campestris* (Guy et al. 2013; Liu et al. 2011; Russell et al.

2015). *RIPK* was shown to be involved as part of an ETI response that fits the guard hypothesis (Jones and Dangl 2006; Liu et al. 2011; Russell et al. 2015). In the model, the *P. syringae* effector AvrB recruits *RIPK* to phosphorylate RIN4 (Liu et al. 2011). The phosphorylation of RIN4 negatively regulates pathogen-associated molecular pattern triggered immunity (PTI). The NB-LRR protein RPM1 recognizes *RIPK* phosphorylation of RIN4 and triggers a host defense response. As part of the molecular arms race between

Fig. 2. Comparison of the chromosome 2 integrated linkage maps for the three populations: Old Home with Bartlett (OHxB), Potomac with El Dorado (PxED), and NJA2R59T69 with Bartlett (NJxB). Quantitative trait locus (QTL) regions are described as the 95% confidence intervals for the QTL based on the 2-LOD drop method (Lander and Botstein 1989). Figure was created using MapChart v2.3 (Voorrips 2002).

TABLE 2. Quantitative trait loci (QTLs) detected in each population^a

Trial, year ^b	Chr	Map position (cM)	Physical position	LOD (<i>R</i> ²)
OHxB 2017	2	AX-171807929 (1.01)–AX-172155651 (3.80)	177,428–3,010,397	3.42 (16.9)
OHxB 2018	2	AX-171887756 (10.91)–AX-171778348 (13.20)	3,601,869–5,093,429	3.79 (18.2)
PxED 2017	2	AX-171771958 (6.33)–AX-171963799 (8.54)	3,355,998–5,212,116	3.44 (15.0)
PxED 2018	2	AX-171771958 (6.33)–AX-171802715 (7.60)	3,355,998–3,675,804	5.13 (26.7)
NJxB 2017	2	AX-171864058 (16.22)–AX-171810219 (19.15)	5,869,948–6,531,759	4.23 (18.4)
NJxB 2017	13	AX-172009310 (11.74)–AX-172032675 (15.29)	6,048,936–7,519,604	3.46 (14.7)
NJxB 2018	2	AX-171780642 (13.60)–AX-171974482 (16.22)	4,817,406–5,953,553	11.99 (51.2)

^a Chr = chromosome and cM = centimorgans. Map positions reported are the markers and their map position in parentheses delimiting the 95% confidence interval identified using the 2-LOD drop method (Lander and Botstein 1989). Physical positions correspond to the nucleotide position of the markers on the *P. communis* Bartlett DH v2.0 genome assembly (Linsmith et al. 2019). LOD values and the phenotypic variance explained by the QTL (*R*²) are reported.

^b Crosses consisted of Old Home with Bartlett (OHxB), Potomac with El Dorado (PxED), and NJA2R59T69 with Bartlett (NJxB).

hosts and pathogens, *X. campestris* pv. *campestris* and *P. syringae* have also evolved the effectors AvrAC and AvrPphB, respectively, that interact with RIPK and prevent an *RPM1*-mediated defense response by not allowing RIN4 phosphorylation (Guy et al. 2013; Russell et al. 2015). AvrAC and AvrPphB prevent the RIN4 phosphorylation through uridylylation and cleavage of RIPK, respectively. A mutation in *pycom02g07320* that would prevent the resulting protein from phosphorylating its interacting host protein (RIN4 homolog) would allow the PTI response to remain active. Conversely, a mutation in *pycom02g07320* could prevent an *E. amylovora* effector (AvrAC- or AvrPphB-like effectors) from modifying the *pycom02g07320* protein, allowing for phosphorylation of its interacting host protein (RIN4 homolog) and the subsequent triggering of an *RPM1*-like host resistance response. Cloning *pycom02g07320*, the NB-LRR genes, and any interacting genes followed by protein–protein interaction studies would be needed to provide support to any of the proposed models.

It may be some time before a more definite answer is available for questions surrounding the host defense responses mediated by the identified chromosome 2 QTLs. Recent genomic and molecular tools and resources developed for pear will help accelerate future research of this interesting pathosystem. Future work will focus on cloning the genes mediating resistance in the chromosome 2 QTL regions and developing molecular tools to facilitate DNA informed breeding for these QTLs.

ACKNOWLEDGMENTS

We thank R. Lewis of the USDA-ARS, Kearneysville, WV for his technical support.

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