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ORIGINAL PAPER

Identification of Novel Strain-Specific and Environment-Dependent Minor QTLs Linked to Fire Blight Resistance in Apples

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Abstract
Since its first report almost 200 years ago, fire blight, caused by the gram-negative bacterium *Erwinia amylovora*, has threatened apple and pear production globally. Identifying novel genes and their functional alleles is a prerequisite to developing apple cultivars with enhanced fire blight resistance. Here, we report 13 strain-specific and environment-dependent minor QTLs linked to fire blight resistance from a segregating *Malus sieversii* × *Malus* × *domestica* mapping population. Interval mapping at 95% confidence and Kruskal–Wallis analysis at P value = 0.005 were used to identify QTLs for three strains of *E. amylovora* differing in virulence and pathogenicity. The QTLs identified explain a small to moderate part of resistance variability, and a majority was not common between years or *E. amylovora* strains. These QTLs are distributed in eight linkage groups of apples and comparison of their map position to previously identified fire blight resistance QTLs indicates that most are novel loci. Interaction between experimental conditions in the greenhouse and field, and between years, and differences in virulence levels of strains might be responsible for strain- and year-specific QTLs. The QTLs identified on LG10 for strain Ea273 in 2011 and strain LP101 in 2011, and on LG15 for strain LP101 could be the same QTLs identified previously with strain CFBP1430 in cultivar "Florina" and "Co-op16 × Co-op17" mapping population, respectively. We discuss the potential impact of newly identified minor fire blight QTLs and major gene-based resistance on the rate of mutation in pathogen populations to overcome resistance and durability of resistance.

Keywords Fire blight · *Erwinia amylovora* · *Malus domestica* · Host resistance · QTL mapping · Durable resistance

Introduction

New apple cultivars are constantly needed for optimal performance under changing biotic and abiotic stresses and to meet market demands. Identification of novel genes and their functional alleles is essential to breed cultivars with enhanced disease resistance. A wide variety of bacterial diseases cause significant economic loss to fruit growers throughout the world (Harshman et al. 2017); of these, fire blight, caused by the gram-negative bacterium *Erwinia amylovora*, is a particularly devastating disease of pome fruits, mainly apples and pears (Norelli et al. 2003a). In the USA alone, the economic damage of fire blight to the apple industry was estimated at over $100 million annually through blossom, shoot, or rootstock blight. The actual cost varies yearly and is difficult to estimate due to the recurring cost of sprays and pruning of infected parts, and the multi-year impact of tree and orchard loss. Since its first report in the Hudson Valley, New York almost 200 years ago, fire blight has spread from North America to Europe, western Asia, northern Africa, and New Zealand (van der Zwet et al. 2012). Some preventive measures, including pruning of infected plant parts, restricted movement between infected orchards, and use of antibiotics in combination with fire blight prediction models, and

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resistant rootstock and scion can slow the spread and reduce the impact of the damage (Norelli et al. 2003a).

Varying levels of resistance to fire blight both in cultivated apples and pears and their wild relatives have been reported (Luby et al. 2002; Aldwinckle et al. 1999). Selection based on visual phenotyping of natural infections is possible, but expensive, and has some intrinsic limitations. For example, due to high heterozygosity in pome fruits, it is difficult to discern heterozygous from homozygous effects, and unfavorable weather for fire blight development will result in a lack of symptoms in susceptible individuals even though bacteria are present (Khan et al. 2012a; Kellerhals et al. 2008). Quantitative trait loci (QTLs) have been identified in different *Malus* genetic backgrounds with small to major effects on fire blight resistance/susceptibility (Khan et al. 2012a; Malnoy et al. 2012). QTL donor accessions for major fire blight loci on LG3, LG10, and two on LG12 are wild apples *Malus robusta* 5, *M. fusca*, and *M.* × *arnoldiana*, and "Evereste" and *M. floribunda* 821, respectively; the QTL on LG7 was identified from *Malus* × *domestica* cultivar "Fiesta" (Emeriewen et al. 2014, 2017; Fahrentrapp et al. 2013; Gardiner et al. 2012; Parravicini et al. 2011; Khan et al. 2007). Incorporating these QTLs into susceptible but commercially important and consumer-favored backgrounds is a challenge when using traditional plant-breeding techniques, as the poor fruit quality of most of these donors is inherited and can take up to five generations to select out. The aggressiveness of *E. amylovora* strains also varies, resulting in different levels of fire blight infection (Malnoy et al. 2012; Lee et al. 2010; Norelli et al. 2003b; Norelli et al. 1986). The majority of the major QTLs identified so far are strain-specific and not valid for multiple pathogen strains, as are often present in orchards, and can break down when used extensively (Malnoy et al. 2012; Khan et al. 2012a). For example, highly aggressive *E. amylovora* strains (E2002a, LA637) can overcome FB_MR5 resistance (Peil et al. 2011). High selection pressure from resistance alleles on the pathogen, along with its capacity to rapidly mutate, can lead to this breakdown of resistance. Exposing mapping populations and germplasm to multiple strains can not only demonstrate the stability and effectiveness of QTLs to a large number of bacterial strains, but will lead to the identification of novel QTLs. Resistance genes with minor effects, both strain-specific and non-specific, can provide a wider spectrum of resistance and may induce mutations more slowly within the pathogen population (Wöhner et al. 2014; Khan et al. 2012a; Norelli et al. 2003b).

Identification of new sources of both major strain-specific and non-specific minor resistance genes is important for ideal broad-spectrum resistance. Durable resistance barriers can be created by combining multiple monogenic and polygenic resistances with complementary action toward different strains of the same pathogen to provide broad-spectrum resistance for several diseases in pre-breeding germplasm stocks. Monogenic and polygenic resistance could be pyramided through marker-assisted pseudo-backcrossing, cis-genically, or via genome-editing (Bortesi and Fischer 2015; Khan and Korban 2012; Khan et al. 2007; Baumgartner et al. 2015; Broggini et al. 2014; Khan et al. 2012a). Regardless of the strategy to improve resistance, characterization of sources of resistance and development of DNA tests to readily identify novel functional genes and their allelic variants is required as current resistances lose effectiveness. We evaluated a mapping population (GMAL 4593) derived by crossing *M.* × *domestica* cultivar "Royal Gala" and an accession of *M. sieversii* (PI 613981), using three strains of *E. amylovora*, in field and greenhouse to identify QTLs linked to fire blight resistance.

Materials and Methods

Plant Material

A total of 169 progeny plants from GMAL 4593 mapping population, together with their parents, were used for fire blight resistance phenotyping and QTL identification. The GMAL 4593 population was derived from a cross between *M.* × *domestica* "Royal Gala" as the female parent and an elite accession of *M. sieversii* "KAZ 95 18-07" (PI 613981) as male parent, previously described by Norelli et al. (2017). The progeny and parents were bud grafted onto seedling rootstocks with at least three replications of each genotype. Initially, grafted plants were planted in a peat-perlite-vermiculite mixture in 13-cm diameter pots, grown in a greenhouse (ca. 20 °C (14–25 °C)) in Geneva, NY and trained to a single shoot (Aldwinckle and Preczewski 1976). Subsequently, 3–4 replicates of each genotype of the same potted plants were planted in the field in Kearneysville, WV in a randomized block design.

Bacterial Strains and Inoculum

E. amylovora (Ea) strains used in this study are listed in Table 1. Inoculum was prepared by growing Ea strains in liquid Kado 523 (Kado and Heskett 1970) broth at 28 °C, 250 rpm for 14–18 h. Concentration of inoculum was adjusted to approx. 1×10^{10} cfu/ml based on optical absorbance at 620 nm using a standard curve and subsequent dilution with sterile 0.05 M potassium phosphate buffer, pH 6.5. Inoculum was maintained on ice until used for inoculation.

Inoculation and Evaluation of Disease Resistance

Greenhouse plants were inoculated and evaluated for fire blight resistance in 2008. Greenhouse-grown plants were then shipped to Kearneysville, WV, planted in the field autumn 2008, and evaluated for their fire blight resistance in spring

Table 1 *Erwinia amylovora* strains used in this study

Strain	Origin		Isolator	Characteristics	Trial year used
	Location	Host			
AFRS273	Unknown	Unknown	J.L. Norelli	Highly aggressive strain	2010, 2011
Ea273	New York	*Malus* × *domestica* "R.I. Greening"	S.V. Beer	Standard NY strain previously used for evaluation of cultivars	2008, 2011
LP101	Washington	Malling 26 apple rootstock	P.L. Pusey	Used to screen fire blight resistance in Washington State Univ. apple breeding program	2011

2010 and 2011. Shoots of both greenhouse- and field-grown plants were inoculated by transversally bisecting the 2–4 youngest leaves of actively growing shoot-tip leaf whorls with scissors dipped in a suspension of a single Ea strain (approx. 1×10^9 cfu/ml) (Norelli et al. 2003b). The unit of replication in the greenhouse trial was individual, single-shoot-potted plants, whereas in the field trials, the unit of replication was individual shoots on plants with multiple shoots. On field-grown plants, shoots were selected on separate scaffold branches to limit systemic spread between inoculated shoots, and inoculated shoots were labeled to facilitate multiple observations and/or inoculation with different Ea strains. Evaluation of resistance/susceptibility was done as described in Norelli et al. (2003b) and Harshman et al. (2017). Total shoot length and fire blight lesion length from the point of inoculation to the furthest point of visible necrosis were measured 6–10 weeks after inoculation when fire blight lesions had ceased extension and a distinct margin was visible between necrotic and healthy stem tissue. Percentage of the current season's shoot necrosis (PSN) was calculated by dividing the lesion length (cm) by the shoot length (cm) and multiplying the quotient by 100.

Statistical Analysis

Datasets for each year and *E. amylovora* strain were treated separately for analysis. Phenotypic data collected for entire mapping population was checked for outliers, normality, and frequency distribution. Analysis of variance (ANOVA) was performed for percentage of shoot necrosis (PSN) to test genotypic differences and to estimate variance components (Khan et al. 2006). Best linear unbiased predictions (BLUPs) were also estimated to summarize the different replicates per genotypes, using the LME4 package in R software (R Core Team 2014). The model used for BLUP estimates was as follows: Model=lme4 [Trait~1| Genotype) + (1|Rep)]. The normality was checked using the Shapiro test in R software (R Core Team 2014). The datasets corresponding to the inoculation in 2008 and 2011 with Ea273 present a normal distribution. Mathematical transformations (sqrt, arsin sqrt, log, exp) were used in the datasets corresponding to the inoculation in 2010 and 2011 with AFRS273 strain but none of these transformations resulted in a normal distribution. Frequency distributions of the PSN were plotted using R software (R Core Team 2014). The statistical differences between the years and strains were tested with the Wilcoxon-paired test and the correlations between PSN, shoot length, and datasets from years and strains were assessed with Pearson's correlation using R software (R Core Team 2014).

Genetic Linkage Map

The apple genetic map previously developed by Norelli et al. (2017) was used in the present study. This genetic map is composed of 578 SSR, SNPlex, HRM, and GBS markers, constructed with JoinMap 4.1 software (van Ooijen 2006).

QTL Analysis and Heritability Estimates

BLUP estimates for PSN for strains and years were used for QTL mapping analysis in MapQTL 5 software (van Ooijen 2009). Kruskal–Wallis test, a single marker analysis, interval, and multiple QTL mapping were used to identify QTLs. Interval mapping was performed for the dataset following a normal distribution (2008 and 2011 with Ea273) and Kruskal–Wallis test was performed for the other dataset (2010 and 2011 with AFRS273 and 2011 with LP101). The logarithm of odds (LOD) genome-wide threshold was calculated by permutation analysis to estimate the position of significant QTLs with 95% confidence. The map location of the QTLs was visualized on MapChart 2.30 (Voorrips 2002). Variance components were used to estimate heritability. Heritability was estimated as the ratio between the genotypic and the phenotypic variances: $H^2 = \sigma^2_g/\sigma^2_p$, where $\sigma^2_p = (\sigma^2_g + \sigma^2_e/n)$, σ^2_g is the genotypic variance, σ^2_e is the environmental variance, and n is the mean number of replicate per genotype. We estimated phenotypic variation explained by the QTLs, markers flanking the QTLs, and the magnitude of the effect of the presence and absence of the marker alleles. Genetic map positions of identified QTLs were also compared with QTLs reported in the literature to confirm if they are the same or novel.

Results

Genotype, Strain, and Year Interaction

The progeny of the GMAL 4593 mapping population showed varying responses to three *E. amylovora* strains (Fig. 1). Percentage of shoot necrosis (PSN) of fire blight for the population inoculated with strain Ea273 was similar between 2008 and 2011 (Wilcoxon paired test *P* value = 0.4). However, for inoculation with AFRS273, PSN differed between 2010 and 2011 (Wilcoxon paired test *P* value < 0.001), with more symptoms in 2011. The environmental conditions in the orchards were different between experiments conducted in 2010 and 2011. No significant difference was found for temperatures in 2010 and 2011 but humidity was significantly higher (Student's *t* test *P* value < 0.05) in 2011 than in 2010 (Online Resource 1). In 2011, simultaneous infection with the three strains under similar environmental conditions showed a higher PSN in the population challenged with AFRS273 than with Ea273, which showed a higher PSN than with LP101 (Wilcoxon paired test *P* value < 0.001). AFRS273 produced greater PSN than the two other strains when comparing all genotypes common between the three strains in 2011 inoculations. Only 69% of these genotypes showed higher PSN with Ea273 than LP101 (Fig. 2).

Percentage of shoot necrosis (PSN) of fire blight for the Ea273 strain from 2008, 2010, and 2011 showed a normal distribution. The progeny plants inoculated with strain AFRS273 in 2010 had the highest variation at 79% of variation between the minimal and the maximal PSN. Genotypes inoculated with strain AFRS273 in 2011 skewed toward high PSN, indicating severe fire blight infection while LP101 infection had more genotypes below 40% necrosis, indicating low to mild severity of fire blight infection compared to other datasets (Fig. 3). There is no significant difference between the mean PSN between 2008 and 2011 for strain Ea273 (Table 2), but there are differences for individual genotypes (Online Resource 4). The genotypes with high PSN differ from year to year. In general, there was no significant correlation between the BLUPs of PSN and the corresponding shoot length. There was moderate positive correlation (0.25–0.5) between strain and year datasets, except for LP101-2011 and Ea273-2008, where it was weak positive correlation. There is positive significant correlation (0.51) between PSN from Ea273 inoculations in 2011 and LP101 inoculations in 2011 (Online Resource 2). The broad-sense heritability of necrosis (%) varied between 0.54 and 0.75, depending on the strain and the year. Broad-sense heritabilities for fire blight resistance from strain Ea273 inoculation were 0.70 and 0.74 in 2008 greenhouse and 2011 field experiments, respectively. However, for strain AFRS273, there were large differences in average fire blight necrosis (67.5% for 2010 and 89.56% for 2011) and heritability estimates in 2010 (0.72) and 2011 (0.53). Only Ea273 had both greenhouse and field experiments, other strains had only field evaluations (Table 2).

Identification of QTLs Linked to Fire Blight Resistance

A total of 13 marker-trait associations were significant in eight linkage groups for three *E. amylovora* strains for 3 years. Three of these QTLs were significant at 95% in interval mapping analysis, while the remaining ten QTLs were significant (*P* value = 0.005 or above) in Kruskal–Wallis analysis. Four QTLs were detected for the LP101 strain, one on LG5, two on LG10, and one on LG15. For Ea273 strain, two QTLs were identified in 2011 on LG9 and 10, and one QTL was identified in 2008 on LG8. Six QTLs were detected for AFRS273 strain,

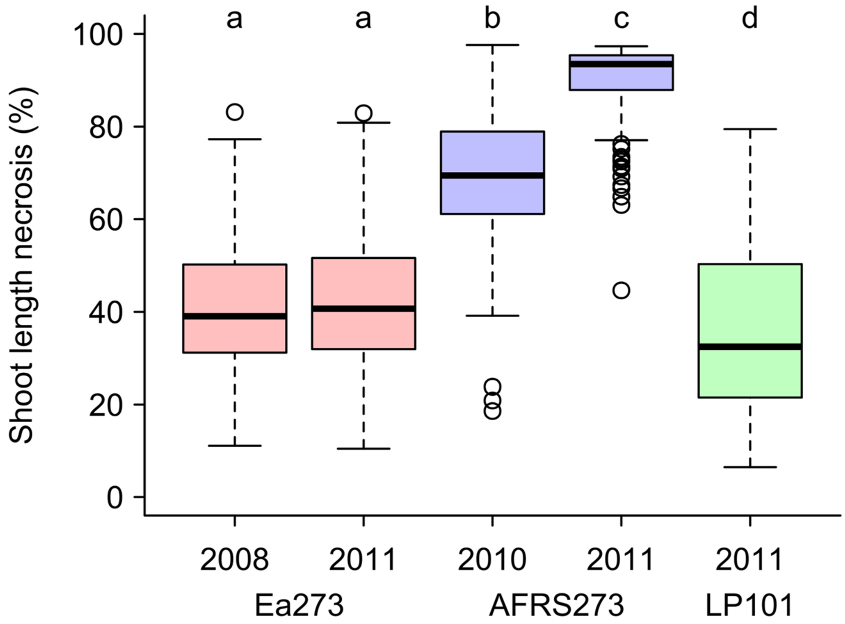

Fig. 1 Best linear unbiased predictors (BLUPs) for mean shoot length necrosis (%) of fire blight for the GMAL 4593 apple mapping population, depending on *Erwinia amylovora* strain (Ea273, AFRS273, LP101) in 3 years (2008, 2010, 2011). Letters correspond to the significant (*P* value < 0.05) difference with Wilcoxon-paired test

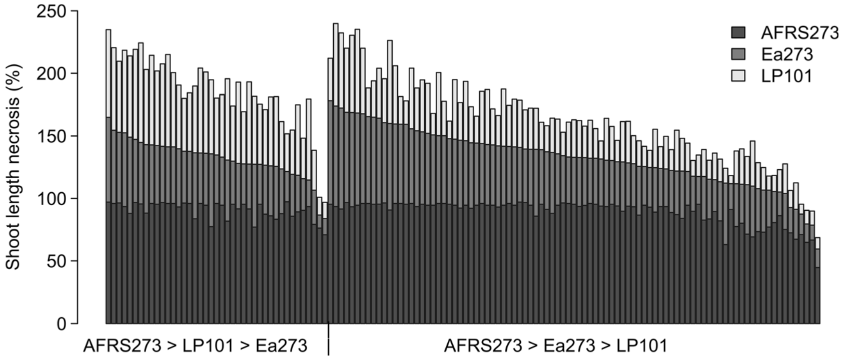

Fig. 2 Best linear unbiased predictors (BLUPs) of shoot length necrosis (%) of fire blight for the apple mapping population (GMAL 4593) inoculated in the orchard with three *E. amylovora* strains, LP101 (light gray), Ea273 (gray) and AFRS273 (dark gray) in 2011. Each bar corresponds to a unique genotype. In the first group, genotypes present a higher shoot length necrosis (%) when infected by AFRS273, then LP101 and then Ea273. The second group includes genotypes that show higher shoot length necrosis (%) when infected by AFRS273, followed by Ea273, and then LP101

three were in 2010, and three in 2011 (Table 3 and Fig. 4). Among the QTLs detected with the three strains in each of the 3 years, only the QTLs detected on LG8 with strain AFRS273 in 2011 and with strain Ea273 in 2008 are physically close and could correspond to the same resistance loci. QTL detected on LG5 with LP101 in 2011 and on LG8 with strain Ea273 in 2008 were physically close to QTLs detected for shoot length (Online Resource 3).

Discussion

Novel Fire Blight QTLs

A total of 13 novel marker-trait associations linked to fire blight resistance were identified from a *Malus sieversii* × *Malus* × *domestica* mapping population through interval mapping at 95% confidence and Kruskal–Wallis analysis at P value = 0.005. These QTLs were distributed in eight linkage groups. Although several QTLs were identified on the same linkage groups as previously identified QTLs, the majority are far enough from previously identified fire blight resistance QTLs to assume that they are new loci representing novel resistance mechanisms. Fire blight QTLs identified on LG10 at 15.9 cM with strain Ea273 in 2011 could be the same as the QTL identified with strain CFBP1430 for lesion length after first and second weeks in cultivar "Florina" (Le Roux et al. 2010). This QTL had approximately 15% phenotypic variation explained (PVE) in both studies. QTL identified by Le Roux et al. (2010) was between common SSR markers CH02b07 and Hi03f06 but closer to CH02b07, as in this study. However, the precise location varies between studies, possibly

Fig. 3 Frequency distribution of the best linear unbiased predictors (BLUPs) of percentage of shoot length necrosis (PSN) of fire blight for the apple mapping population GMAL 4593. Three *Erwinia amylovora* strains (Ea273, AFRS273, LP101) were used to inoculate the population in three years (2008, 2010, 2011). Black arrows indicate PSN for parents of the respective progeny

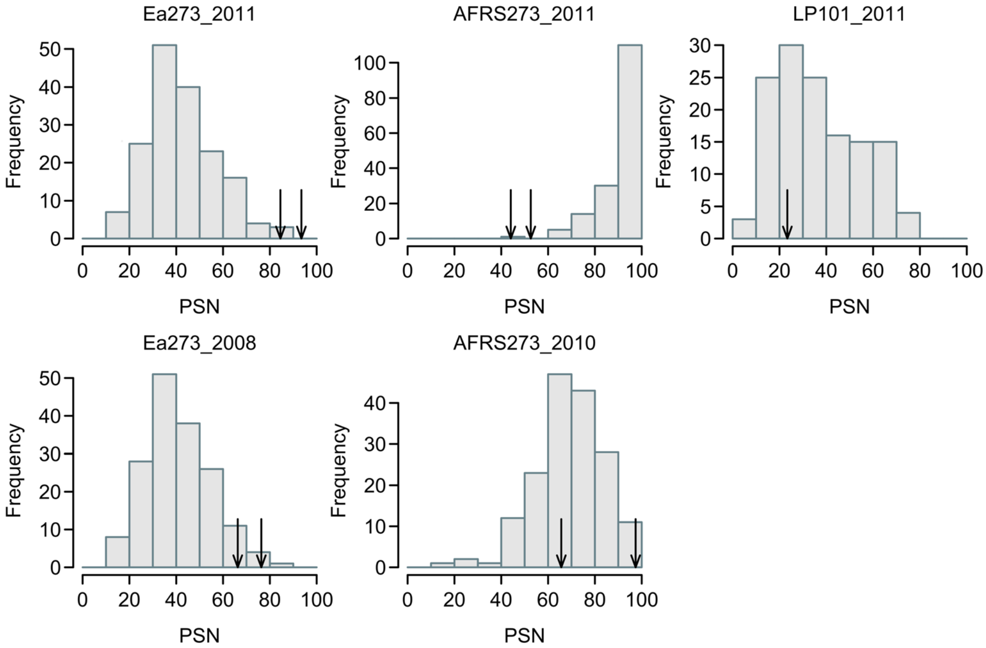

Table 2 Number of genotypes, mean percentage of the shoot necrosis (PSN) of fire blight, genotypic variance (σ2g), environmental variance (σ2e), phenotypic variance (σ2p), and broad-sense heritability (H2) for 3 years and three *Erwinia amylovora* strains for the apple mapping population GMAL 4593

Strain	Year	Genotypes	PSN	σ^2_g	σ^2_e	$\sigma^2_p = (\sigma^2_g + \sigma^2_e/n)$	H^2
Ea273	2008	167	38.24	0.028	0.036	0.063	0.701
AFRS273	2010	168	67.5	0.026	0.077	0.103	0.725
Ea273	2011	169	39.82	0.028	0.047	0.075	0.749
AFRS273	2011	160	89.56	0.016	0.047	0.064	0.536
LP101	2011	133	31.52	0.049	0.058	0.108	0.665

due to the effect of population size. The QTLs identified for strain LP101 in 2011 using KW test at 73.8 and 87.9 cM seem to be novel fire blight QTLs on LG10. The QTL identified using KW test on LG15 with LP101 is also in a similar region (12.7 cM) as one reported by Khan et al. (2013) in "Co-op16 × Co-op17" mapping population for lesion lengths in the first and second weeks after inoculation, between 4.5 and 25.3 cM peak at MdSNPui07111 and NZ02b14.06 markers, respectively. However, there are no common markers across both studies for accurate comparison of mapping positions. This QTL was identified using Ea strain CBFP1430 and explained 17.4 and 15.4% PVE. Although no QTL was identified consistently over 2 years, two QTLs on LG8 in close proximity, with markers S8_29532144 and GD_SNP01764 close to the QTL peak, identified for strains AFRS273 and EA273 in 2011 and 2008, respectively, could be the same. Although there was a significant positive correlation (0.51) between PSN data from Ea273 and LP101 in 2011, no overlapping QTL was detected. This QTL explained 12.5% phenotypic variation in IM analysis. QTLs on LG10, LG15, and LG8 could be promising for further validation. We cannot rule out the effect of population size and marker density on identification of small-effect QTLs, accurate map position, and consistency over years and strains (Khan et al. 2012b; Beavis 1998). This can be confirmed by adding more recombinant individuals and fine mapping the QTL regions. More than 30 major and minor fire blight resistance loci conferring strain-specific and broad-spectrum resistance have been identified in the literature so far; some may not be distinct loci (Khan et al. 2012a; Malnoy et al. 2012). Overlapping mapping positions could indicate that some loci identified in different studies may be the same. However, R genes tend to cluster, and loci in close proximity may indicate R genes in close proximity, thus distinct loci. The stability of some of the identified loci has to be tested over multiple years, and they may need to be validated in different genetic backgrounds before potential application.

Effect Size of QTLs and G × E × Strain

The efficacy of most of the QTLs found in this study appeared to be affected by the environment and *E. amylovora* strain. These QTLs explain a small to moderate part of the variability observed for resistance/susceptibility to infection by *E. amylovora*, and a majority was not common between years and *E. amylovora* strains. The broad-sense heritability for fire blight resistance estimated here was somewhat lower than reported previously by Le Roux et al. (2010), Khan et al. (2006), and Calenge et al. (2005), potentially due to different experimental conditions. Broad-sense heritability values for strain Ea273 inoculation in 2008 greenhouse and 2011 field experiments were similar. The major difference between mean fire blight necrosis and heritability estimates for strain AFRS273 between 2010 and 2011 in the field experiment might be due to differences in temperature and humidity. The heritability values indicate that the majority of phenotypic variation within this population could be attributed to genetic variability. However, as the total variation in fire blight resistance explained by the QTLs is less than the broad-sense heritability, some additional small-effect QTLs may not have been identified. Although the size of the population used is satisfactory to map strong or moderate-effect QTLs, it may not be sufficient to precisely map the position and estimate the contribution of small-effect QTLs, as population size is one major factor influencing QTL detection (Beavis 1998). Increasing the number of progeny and improvement of the map coverage might lead to the identification of additional, small-effect QTLs in this population (Le Roux et al. 2010). Previous studies have noted that QTLs with minor effects detected in one population can have a major effect in another population or with a different strain. For example, the major QTL detected on LG3 by Peil et al. (2007) was also detected as a minor QTL by Calenge et al. (2005) and the major QTL detected on LG12 (Calenge et al. 2005) was also detected as a minor QTL by Durel et al. (2009). Minor QTLs exhibit strong interaction with the environment and are difficult to deploy in commercial backgrounds through breeding. Interaction between experimental conditions in the greenhouse and field, between years, and differences in virulence levels of strains might be responsible for strain- and year-specific QTLs. Although there was no significant difference between the PSN of fire blight between 2008 and 2011 for strain Ea273, the individual genotypes differ significantly from year to year.

Table 3 QTLs identified for fire blight resistance in GMAL 4593 apple mapping population for 3 years and three *Erwinia amylovora* strains

Year	Strain	Analysis type	LG	Position (cM)	Nearest marker	LOD	K	Sig.	mu_ac	mu_ad	mu_bc	mu_bd	ee	ef	eg	fg	ll	lm	nn	np	PVE
2008	Ea273	IM	8	10.5	GD_SNP01764	4.64		**	0.40	0.48	0.34	0.43									12.5
2011	Ea273	IM	9	65.9	S9_31836168	5.39		**	0.55	0.39	0.43	0.38									15.2
			10	15.9	S10_4183672	5.76		***	0.42	0.51	0.38	0.36									15.4
2010	AFRS273	KW	9	8.4	S9_1692625		10.2	****									0.66	0.74			
			9	47.8	GD_SNP00803HRMa		15.0	****					0.76	0.68	0.68	0.65					
			12	0.4	S12_633846		7.9	****									0.66	0.72			
2011	AFRS273	KW	3	95	S3_37067287		11.5	*****									0.92	0.88			
			8	7.8	S8_29532144		10.9	*****											0.88	0.92	
			11	32.4	S11_11316802		10.5	****									0.92	0.89			
2011	LP101	KW	5	61.8	S5_21449608		8.2	****									0.41	0.32			
			10	73.8	S10_29121625		8.0	****									0.40	0.32			
			10	87.9	CH02b03b_71		9.9	****									0.43	0.32			
			15	12.7	S15_4422339		11.9	*****									0.42	0.30			

Best linear unbiased predictors (BLUPs) of percentage of shoot necrosis (PSN) were used for QTLs for interval mapping (IM) and Kruskal–Wallis (KW) analyses. LOD threshold for a significant QTL was set as 4.5 (95%). K is the value for KW test. LG represents linkage group while Sig. represent significance as ** = 0.05, *** = 0.01, **** = 0.005, and *****= 0.001; PVE is the phenotypic variation explained in IM analysis. mu_ac, mu_ad, mu_bc, and mu_bd are mean BLUP estimates for genotypic classes for ab × cd marker types; ee, ef, eg, and fg are mean BLUP estimates for ef × eg marker class; ll and lm are mean BLUP estimates for marker class ll × lm; and nn and np for marker class nn × np

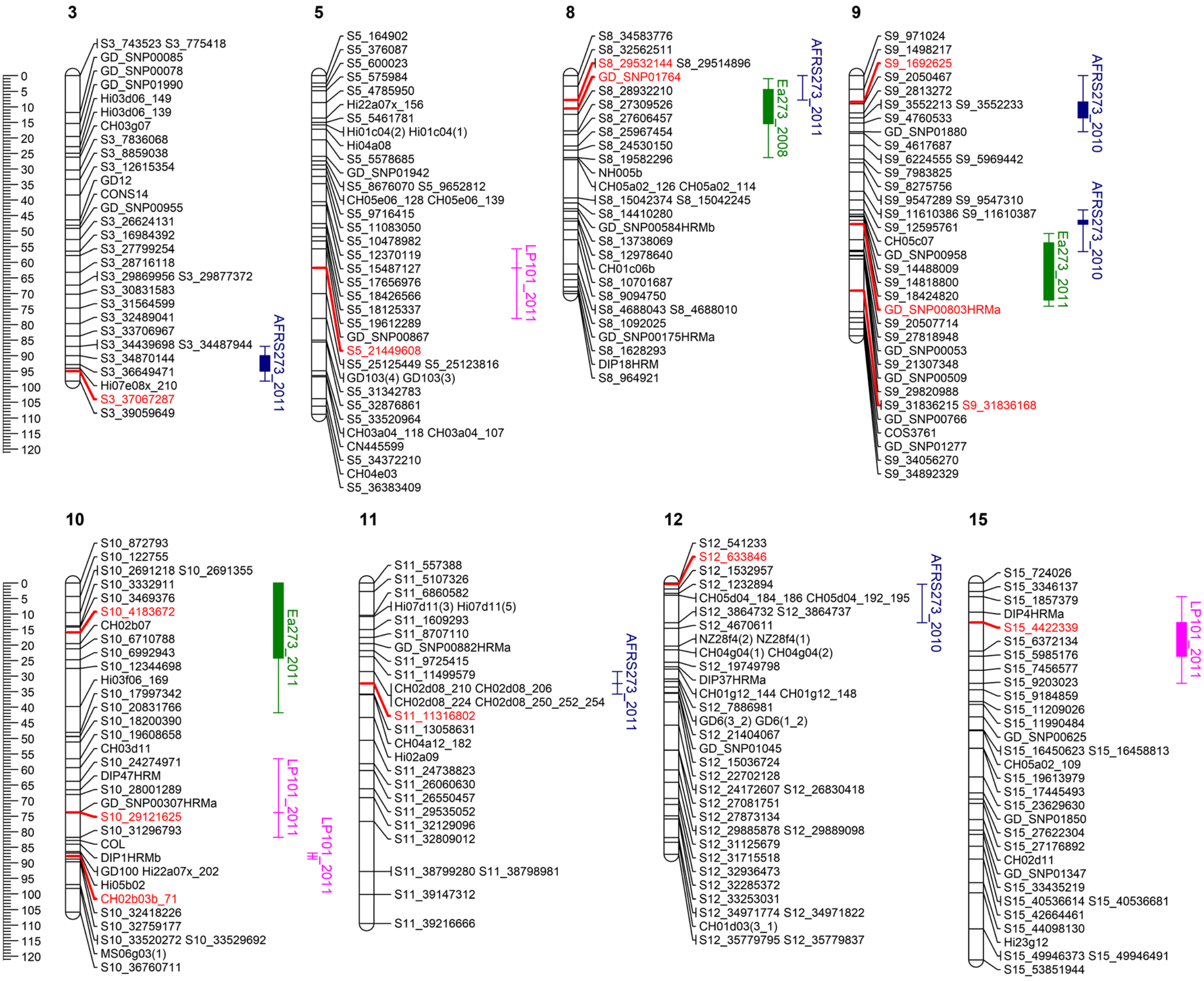

Fig. 4 Fire blight resistance QTLs identified in GMAL 4593 apple mapping population for 3 years and three *Erwinia amylovora* strains. Best linear unbiased predictors (BLUPs) of percentage of the shoot necrosis (PSN) of fire blight were used for QTLs for interval mapping (IM) and Kruskal–Wallis (KW) analyses. LOD threshold to declare a significant QTL in IM was set as 4.5 (95%) and KW was **** 0.005. Note: The QTLs detected with the *E. amylovora* strain Ea273 using IM are represented in green: the lines correspond to the 1-LOD and 2-LOD confidence intervals. The QTLs detected with AFRS273 and LP101 using KW are represented in blue and pink, respectively, the boxes correspond to the markers significant at least at $P = 0.005$ and the confidence interval lines for the significant QTL are up to $P = 0.1$

These differences in fire blight necrosis could be due to environmental differences. In 2008, the experimentation took place in the greenhouse, and in 2011, the experiment was carried out in orchards, which could have triggered different resistance mechanisms. Additionally, the trees would be a year older in the orchard and probably have more shoots, impacting the vigor and thus the infection rate. Significant (P value < 0.05) difference in humidity between 2011 and 2010 (Online Resource 1) indicates that moisture might be an important factor for differences in the infection rate of *E. amylovora* and specificity of QTLs to the years. Usually QTLs conferred by major R-genes are robust and have less effect from the environment in the same genetic mapping population. *E. amylovora* strains have varying levels of pathogenicity and virulence, resulting in different levels of fire blight infection due to different resistance mechanisms (Smits et al. 2014; Malnoy et al. 2012; Norelli et al. 1984). *E. amylovora* strain-specific QTLs have been reported in previous fire blight resistance QTL mapping studies. For example, fire blight QTLs on linkage group (LG)3, LG7, LG10, and two on LG12 have major effects and showed strain specificity (Emeriewen et al. 2014; Fahrentrapp et al. 2013; Parravicini et al. 2011; Khan et al. 2007). R genes have been identified for the QTLs FB_MR5 from *Malus* × *robusta* 5 on LG3 and FB_E from the crab apple “Evereste” on LG12 (Fahrentrapp et al. 2013; Parravicini et al. 2011). A CC-NBS-LRR gene was found responsible for the major QTL FB_MR5. Transformation of the CC-NBS-LRR gene into the fire blight-susceptible cultivar "Gala" showed significantly lower infection upon artificial inoculation with *E. amylovora* (Broggini et al. 2014; Fahrentrapp et al. 2013; Peil et al. 2007). Some *E. amylovora* strains can overcome the major resistance of *M.* × *robusta* R5 (Wöhner et al. 2014). In the long run, small effects may slow down the rate of mutation to overcome resistance in pathogen populations and thus can be more durable than resistance based on major genes (Laloi et al. 2017). Therefore, we speculate that strain-specific minor effect alleles may provide a wider spectrum of resistance to fire blight and induce slower mutation rates within *E. amylovora* population.

Conclusions

An effective and sustainable management strategy for fire blight requires an integrated control strategy aimed at reducing primary inoculum, interfering with infection through the application of antibiotics, biological agents, or chemicals, as well as enhancing host resistance. Apple cultivars with improved fire blight resistance, either to specific *Erwinia* strains, or wide resistance across strains, can reduce losses, increase orchard profitability, and limit the impact and cost of antibiotic use, for positive economic effect on apple production globally. Pyramiding multiple strain-specific and non-specific resistances could lead to the development of ideal broad-spectrum and durable resistance to the strains present in production areas through marker-assisted pseudo-backcrossing, cis-genically, or via genome-editing (Nishitani et al. 2016; Malnoy et al. 2016; Broggini et al. 2014; Kellerhals et al. 2008).

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