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Acibenzolar-*S*-Methyl and Resistance Quantitative Trait Loci Complement Each Other to Control Apple Scab and Fire Blight

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Abstract

Diversifying disease control methods is a key strategy to sustainably reduce pesticides. Plant genetic resistance has long been used to create resistant varieties. Plant resistance inducers (PRI) are also considered to promote crop health, but their effectiveness is partial and can vary according to the environment and the plant genotype. We investigated the putative interaction between intrinsic (genetic) and PRI-induced resistance in apple when affected by scab and fire blight diseases. A large F1 mapping population was challenged by each disease after a pre-treatment with acibenzolar-*S*-methyl (ASM) and compared with the water control. Apple scab and fire blight resistance quantitative trait loci (QTLs) were detected in both conditions and compared. ASM exhibited a strong effectiveness in reducing both diseases. When combined, QTL-controlled and ASM-induced resistance acted complementarily to reduce the symptoms from 85 to 100%, depending on the disease. In our conditions, resistance QTLs were only slightly or rarely affected by ASM treatment, despite their probable implication in various stages of the resistance buildup. Implications of these results are discussed considering already known results, the underlying mechanisms, cross protection of both types of resistance against pathogen adaptation, and practical application in orchard conditions.

Keywords: *Erwinia amylovora*, induced defense, intrinsic resistance, *Malus domestica*, *Venturia inaequalis*

Plant diseases threaten global agricultural production, leading to the extensive use of pesticides. Plant genetic resistance is considered as a major lever, and breeding for new resistant varieties is very active in most crop species. However, resistance genes exert selection pressures on pathogens, triggering their evolution (McDonald and Linde 2002). Diversifying and pyramiding resistance genes, especially when controlling both qualitative and quantitative resistance, is considered as a promising approach for achieving durable resistance (Pilet-Nayel et al. 2017). Such diversification is indeed expected to promote conflicting selection pressures on pathogen populations, which should constrain them to an evolutionary compromise, limiting their development.

Reduced reliance on conventional pesticides can be also achieved by biocontrol (Burketova et al. 2015; Pal and McSpadden Gardener 2006). Plant resistance inducers (PRIs, also called elicitors or plant defense activators) are part of biocontrol methods. They include a range of chemical (Bektas and Eulgem 2015) or biological (Wiesel et al. 2014) stimulators that are able to activate plant defenses, without direct toxicity against pathogens (Oliveira et al. 2016; Oostendorp et al. 2001). PRIs most often provide only partial resistance, and their performance is greatly influenced by abiotic and biotic factors that include the type of pathogen and the plant genotype (Walters et al. 2013). However, mechanisms underlying these numerous interactions are not clearly understood. Regarding the plant, comprehensive knowledge on genotype-PRI interactions could reorient plant breeding programs toward responsive genotypes or assist the choice of varieties to be deployed in practice, especially if PRIs are intended to be used in pest management programs. A combination of genetic resistance and the use of PRIs is expected to multiply selection pressures on pathogen populations and thus limit their evolution, similarly to a combination of various genetic resistance factors.

Apple (*Malus domestica*) is a major fruit tree species that requires a tremendous amount of pesticide in temperate climate conditions (MacHardy 1996). Apple genotypes display a great variability of responses to apple scab (caused by the ascomycete fungus *Venturia inaequalis*) and fire blight (caused by the bacterium *Erwinia amylovora*), two major diseases impacting orchards. Genetic mapping studies revealed numerous resistant (R) genes and QTLs involved in resistance against apple scab (summarized in Khajuria et al. 2018) and fire blight (reviewed in Emeriewen et al. 2019; Malnoy et al. 2012), conferring partial to complete resistance to the diseases. Some of these loci are known to be strain-specific, with breakdown of resistance already demonstrated (Caffier et al. 2010, 2015; Parisi 1993; Patocchi et al. 2020; Peil et al. 2020; Wöhner et al. 2014). The *Rvi6* gene, also called *Vf* (from *Malus floribunda*), was one of the genes most widely used in apple breeding programs but was overcome as early as 1981 by *V. inaequalis* race 6 (Parisi 1993). In apple, as in most plant species, quantitative resistance is considered more durable than qualitative resistance because of the multiple loci controlling selection pressure on pathogen populations (Parlevliet 2002; Pilet-Nayel et al. 2017). However, a slow erosion of some QTLs has already been demonstrated (Caffier et al. 2014, 2016).

Acibenzolar-*S*-methyl (ASM), a functional salicylic acid (SA) analog, is one of the most promising PRIs in several plant species (Gozzo and Faoro 2013). It promotes systemic acquired resistance, and consequently, the induction of various defense responses, including release of pathogenesis-related proteins, leading to the protection of many species against a broad spectrum of pathogens (Assis et al. 2015; Ishiga et al. 2020; Matsuo et al. 2019; Romero et al. 2001; Youssef et al. 2019). In apple, several studies reported significant control of apple scab (Bengtsson et al. 2006, 2009; Marolleau et al. 2017) and fire blight (Abo-Elyousr et al. 2010; Aćimović et al. 2015; Brisset et al. 2000; Dugé de Bernonville et al. 2014; Hassan and Buchenauer 2007; Johnson et al. 2016; Marolleau et al. 2017; Maxson-Stein et al. 2002; Shahini Sough et al. 2010), as well as the triggering of a molecular defense response (Brisset et al. 2000; Dugé de Bernonville et al. 2014; Maxson-Stein et al. 2002; Warneys

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et al. 2018; Ziadi et al. 2001). Although significant, performance of ASM exhibits variability that remains to be understood for its practical use in the orchard. Among others, the genetic factor is still poorly investigated.

In this article, we explored the added value of combining intrinsic (genetic) resistance against apple scab and fire blight with induced resistance conferred by ASM using a genetic-mapping approach. Composition of QTLs in a segregating population was compared between ASM-treated and -untreated plants to highlight genetic determinants that could explain, or potentially interact with, the performance of ASM.

Materials and Methods

Plant material. All experiments were performed with a F1 population referred as the "TxF progeny," which was described in Laloi et al. (2016). The 267 individuals were derived from a controlled cross between TN10-8 and Fiesta, two genotypes partially resistant to apple scab. Fiesta is also partially resistant to fire blight. For each experiment, up to 10 replicates per individual were grafted and grown in greenhouse under semi-controlled growing conditions (23°C day/20°C night, humidity 40 to 80%, and artificial light to complement natural light). Graftwood was collected from a conservatory orchard located at the Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE, Angers, France) and grafted on MM106 apple rootstock. The two parents and two susceptible control cultivars (Golden Delicious and Gala) were also included in each experiment in 10 replicates.

Experimental design, treatment application, and phenotypic data collection. For each experiment, out of the 10 replicates, 4 replicates with active growing shoots per individual and per treatment condition were chosen and distributed in each treatment group in randomized blocks in the greenhouse. Two days before inoculation, plants were sprayed with a solution of Syngenta BION 50 WG (50% ASM, called here "ASM treatment") or with reverse osmosis water as control (called the "water control"). Water dilutions of 0.2 g.liter^{-1} for *V. inaequalis*-inoculated plants, and 0.4 g.liter^{-1} for *E. amylovora*-inoculated plants, were applied. The commercial treatment advice (0.4 g.liter^{-1}) was reduced for scab to maintain enough phenotypic variability across TxF progeny.

Two *V. inaequalis* isolates were used: the reference isolate 'EU-B04' (Origin: Belgium, host: Golden Delicious) previously described in Caffier et al. (2015) and Le Cam et al. (2019) and the isolate '09BCZ014' (Origin: France, host: TN10-8 × Prima progeny [individual E063]), referred to as isolate '2557' in Laloi et al. (2016). Monoconidial suspensions were prepared from diseased dry leaves at a concentration of 2.5×10^5 conidia.ml^{-1} and sprayed on grafted trees, incubated thereafter 2 days at 17°C under a plastic sheet to maintain a high humidity, according to the conditions described by Caffier et al. (2010). The percentage of leaf surface exhibiting sporulating lesions was scored at 14, 21, and 28 days post-inoculation using the ordinal scale (0 to 7) described in Calenge et al. (2004). Two experiments were performed with isolate 'EU-B04' (coded "Vi-B04_1 et Vi-B04_2"), and one with '09BCZ014' (coded "Vi-Z14").

The reference strain CFPB1430 of *E. amylovora* from the French collection of phytopathogenic bacteria (Paulin and Samson 1973) was used for inoculation. The bacterial suspension was prepared as described in Dugé de Bernonville et al. (2014) at 10^8 CFU.ml^{-1}. Growing shoots (>10 cm) were inoculated by cutting the two youngest unrolled leaves with scissors previously soaked in the bacterial suspension. The length of necrosis developing on stem was measured at 7, 14, and 21 days post-inoculation. The ratio between necrosis length and total shoot length was used as a severity score. Two experiments were performed (coded "Ea-1430_1" and "Ea-1430_2").

Data analysis of phenotypic data. Phenotypic data were analyzed separately for ASM treatment or the water control. The area under the disease-progress curve (AUDPC) was calculated as a quantitative summary of disease severity over time:

$$\text{AUDPC} = \sum_{i=0}^{2} \frac{y_i + y_{i+1}}{2} x(t_{i+1} - t_i),$$

where y_i is the disease score at the ith day of observation, and t_i is the number of days post-inoculation at the ith observation.

All statistical analyses were performed using the software suite R (Dalgaard 2010). AUDPCs were fitted for environmental trend effects using a SpATS package (Rodríguez-Álvarez et al. 2018) that estimates a Best Linear Unbiased Prediction (BLUP) for each individual. Broad-sense heritability of each trait for each treatment was also estimated with the function "getHeritability" of the same package.

Genotyping data and genetic maps construction. DNA was extracted from fresh young leaf samples using an LGC Genomics oKtopure robot and quantified with a Hoechst protocol. The TxF progeny was genotyped with the Illumina Infinium 20K single nucleotide polymorphisms (SNPs) array (Bianco et al. 2014). Genotyping data were analyzed using Illumina GenomeStudio Genotyping Module software (V2.0) with manual correction whenever necessary to improve dataset quality. Markers were filtered according to their parental segregation profile (ABxAA for the female map and AAxAB for the male map; markers in ABxAB were not considered). Then a linkage map was constructed for each parent with JoinMap 4.1 software using the Kosambi mapping function (van Ooijen 2006). Map quality was checked by comparison with the apple reference genetic map of Di Pierro et al. (2016) and an updated version of the map (Howard et al. 2020). The software Biomercator V4.2 (Arcade et al. 2004) was used to curate manually erroneous marker positions. Seventeen microsatellite (simple sequence repeat) markers located in genomic regions where scab resistance QTLs were expected according to previous publications were added after genotyping of the TxF progeny (Supplementary Table S1).

QTL mapping. To compare the contribution of QTLs in resistance and in the interaction between genotypes and ASM, a mapping of QTLs was performed independently in water-control and ASM-treated populations. QTL analyses were conducted using the R/qtl package (Broman et al. 2003). Simple interval mapping and composite interval mapping were estimated using a multiple imputation method and a normal distribution model. Co-factors for composite interval mapping were determined from the best prediction model simulated with the "stepwise" function. The logarithm of the odds (LOD) score threshold was determined using 1,000 permutations to identify the statistically significant QTLs ($\alpha = 0.05$ genome-wide). LOD thresholds were approximately 5 for both scab and fire blight experiments. Suggestive QTLs with LOD scores between 3 and 5 were also considered. The LOD score, the 2-LOD support confidence interval (CI), and the contribution of each QTL to the overall phenotypic variance (individual R^2) were extracted from R/qtl analyses, together with the global QTL contribution (global R^2). Individual and global R^2 were calculated with the "fitqtl" function (used for fitting a defined multiple-QTL model). Interactions between QTLs were studied by variance analysis using the genotyping data of each SNP closest to the peak of each QTL, and were detailed by the "effectplot" function. These results were used to define the model for the calculation of the global R^2 with the fitqtl function. To adequately compare QTL effects between treatments by considering the overall phenotypic variation in each treatment, the relative QTL effect (r_{Effect}) was calculated for each QTL as follows:

$$r_{\text{Effect}} = \frac{1}{\sigma_B} \times \frac{|\text{BLUP(AA)} - \text{BLUP(AB)}|}{2},$$

where AA and AB are the allelic versions at the SNP closest to the QTL peak, and σ_B is the standard deviation of BLUP (adjusted for all significative QTLs effects, other than just the one for the QTL being considered) for a given treatment condition. The CIs at 5% of r_{Effects} were simulated by bootstrapping with 5,000 replicates using the "boot" function: when comparing r_{Effects} of a given QTL between the water control and ASM treatment, the overlap of the CIs was interpreted as if the effects were not significantly different. For

simplicity, QTL alleles were then considered equivalent to SNP alleles at QTL peaks. A QTL detected at almost the same genomic position in various experiments was also considered, and named as the same QTL.

In Vi-B04 experiments, a clear bimodal distribution was observed (see "Results" below). This distribution fitted with the strong effect of the major QTL (simply called "qT1" here) detected on LG1 of TN10-8 after inoculation with this isolate (Calenge et al. 2004) or with a mixture of isolates including 'EU-B04' (Lê Van et al. 2012). For that reason, the TxF progeny was subdivided into two subsets of individuals according to the presence/absence of the resistance allele at qT1 predicted by SNP data at the QTL peak. QTL analyses were then conducted on both subpopulations (further called "qT1+ and qT1− *subpopulations*"). Conversely, the isolate '09BCZ014' (also numbered '2257') was shown to partially overcome the QTL qT1 by Laloi et al. (2016). QTL analysis was thus performed on the whole TxF progeny with the latter isolate.

Results

Phenotypic variability. Only experiments involving the most replicates per individual, Vi-B04_1, Vi-Z14, and Ea-1430_1 are presented here (the two additional experiments Vi-B04_2 and Ea-1430_2 are available as Supplementary Data).

A significant reduction of AUDPC was observed for both scab and fire blight after ASM treatment compared with the water control (Table 1). For the TxF progeny, AUDPC was reduced by 54% for Vi-B04_1, 73% for Vi-Z14 and 50% for Ea-1430_1. Similar reductions were observed for the two parents and susceptible controls (Fig. 1). A large variation was observed among individuals regardless of the conditions, with AUDPC values ranging from 0 to 150 for Vi-B04_1, from 0 to 75 for Vi-Z14, and from 1 to 11 for Ea-1430_1 (Fig. 1). A higher coefficient of variation was observed for scab (*CV* = 0.75 to 1.27) than for fire blight (*CV* = 0.27 to 0.34; Table 1). The distributions were generally unimodal except for the water control in Vi-B04_1, where a clear bimodal distribution was observed (Fig. 1A), fitting with the expected effect of QTL qT1 (see "Materials and Methods"). Broad-sense heritabilities (h^2) were generally high whatever the conditions, ranging from 0.63 for fire blight after ASM treatment to 0.94 for scab after the water control (Table 1). Adjusting the data for spatial effects resulted in a limited increase of heritability from 0.01 to 0.08 units compared with no adjustment (data not shown). Correlation coefficients (Cor) between the water control and ASM treatment were intermediate to high (0.6 to 0.9) according to the experiments (Fig. 1). The range of AUDPC values observed in ASM-treated individuals increased as AUDPC values of the corresponding water-treated individuals increased, suggesting interactions between genotypes and treatments especially for the less resistant individuals (Fig. 1). AUDPC mean values were lower in Vi-B04_2 and Ea-1430_2 experiments, but the variation of AUDPC was similar (Supplementary Table S2; Supplementary Fig. S1). The Cor values between both Vi-B04 experiments were 0.74 for the water control and 0.73 for

Table 1. Mean values (± standard deviation) of the area under the disease-progress curve, relative effectiveness of acibenzolar-*S*-methyl (ASM), coefficient of variation (CV) of individual Best Linear Unbiased Predictions, broad-sense heritability (h^2), and phenotypic variation explained by all detected resistance quantitative trait loci (global R^2, variance) against scab (*Venturia inaequalis* isolates 'EU-B04' and '09BCZ14'; experiments Vi-B04_1 and Vi-Z14, respectively) and fire blight (*Erwinia amylovora* isolate 'CFPB1430'; experiment Ea-1430_1) in the TxF progeny[a]

Experiment	Treatment	Mean	Effectiveness ASM (%)	CV	h^2	Global R^2
Vi-B04_1	Water	41.2 ± 36.8		0.84 $[0.29]_{qT1-}$	0.94	81.9 $[65.1]_{qT1-}$
	ASM	18.9 ± 26.4	54	1.27 $[0.48]_{qT1-}$	0.91	70.2 $[53.8]_{qT1-}$
Vi-Z14	Water	24.1 ± 21.4		0.75	0.84	47.5
	ASM	6.6 ± 8.4	73	0.88	0.71	15.0
Ea-1430_1	Water	6.2 ± 2.4		0.27	0.70	35.0
	ASM	3.1 ± 1.8	50	0.34	0.63	33.1

[a] Values for the subpopulation qT1− are indicated within brackets. Effectiveness of acibenzolar-*S*-methyl is computed as: (*Mean-water – Mean-plant resistance inducers*)/*Mean-water*.

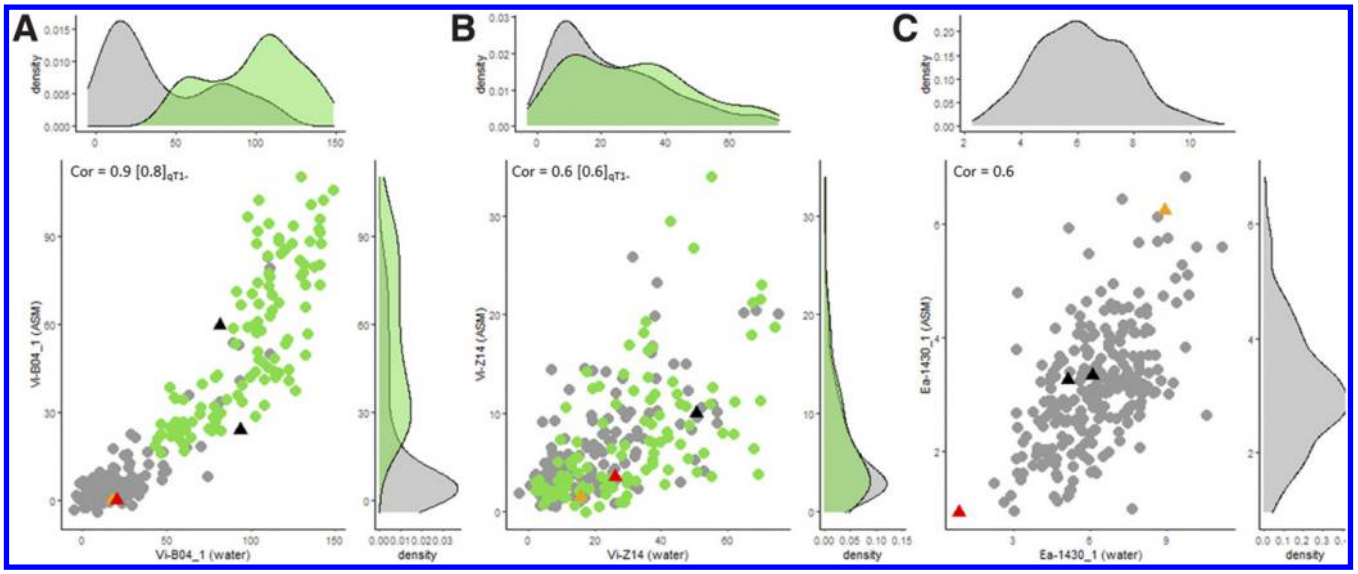

Fig. 1. Relationship between disease severity (Best Linear Unbiased Prediction estimates; BLUPs) for water- and acibenzolar-*S*-methyl (ASM)–treated individuals of the TxF progeny in **A,** Vi-B04_1, **B,** Vi-Z14, and **C,** Ea-1430 experiments. Distributions of BLUPs are shown in the upper (water control) and right (ASM treatment) part of each graph where the complete population is represented in gray and the subpopulation qT1− (i.e., individuals selected as not carrying the resistance allele of the major quantitative trait loci qT1) in green. The same color code is used in the graph where the green dots are superimposed on the gray dots. Control and parental genotypes are shown with triangles in orange (TN10-8), red (Fiesta), and black (Gala and Golden Delicious). Values for the subpopulation qT1− are indicated within brackets. Cor, Pearson coefficient of correlation between water and ASM BLUPs.

ASM treatment, while they were only 0.19 and 0.16 between both Ea_1430 experiments, respectively.

QTL detection for *V. inaequalis* isolate 'EU-B04'. A total of 6,245 SNPs was found to be polymorphic in the TxF progeny and one or both parents. After discarding 4,433 SNPs that were polymorphic in both parents and redundant, two parental genetic maps of 17 linkage groups were constructed with 853 and 959 SNPs for TN10-8 and Fiesta, respectively. Both parental maps were merged in a single file for further detections of QTLs (Supplementary Table S3).

For the water-control condition, a major QTL effect (LOD approximately 77; R^2 = 69.9%) was detected on LG1, together with three other significant QTLs on LG T13, F11, and F17, and with one suggestive on LG F12, when considering the whole TxF progeny (Supplementary Table S4; Fig. 2). The presence of the former QTL (corresponding to qT1) was consistent with the bimodal distribution observed above. In the qT1− subpopulation, four significant QTLs and one suggestive QTL were detected on linkage groups T13, F3, F11, F17, and F12, respectively (Table 2; Fig. 2). They explained from 3.7 to 29.0% of phenotypic variation; their relative effect (r_{Effect}) varied from 0.32 (QTL qF12) to 0.66 (QTL qF17), compared with 0.85 for QTL qT1 in the whole TxF progeny (Fig. 2). A significant interaction was found between QTLs qF11 and qF17 (Table 3). No AUDPC difference was detectable between both alleles of qF11 when the "unfavorable" (i.e., susceptible) allele of qF17 (AA) was present (genotypic classes AA:AA and AB:AA with high AUDPC values at 149.6 and 146.2, respectively). With the "favorable" (i.e., resistant) allele of qF17, the "favorable" allele of qF11 was, on the contrary, very efficient with AUDPC values decreasing from 131.3 to 99.5 for the combination of QTL alleles AA:AB, indicating a complementary relationship between "favorable" alleles of both QTLs. Altogether, QTLs on LG T1, T13, F11, F12, and F17 explained 81.9% of phenotypic variation in the whole progeny, whereas the five QTLs including the QTL interaction explained 65.1% of phenotypic variation in the qT1− subpopulation (Table 1). For the ASM-treatment condition, QTL qT1 still exhibited a strong effect (LOD approximately 46; R^2 = 60%) in the whole TxF progeny (Supplementary Table S4; Fig. 2) together with QTLs qF17 and qF11 as a suggestive QTL (Supplementary Table S4). All three explained 70.2% of the phenotypic variation. In the qT1− subpopulation, only three out of the five QTLs detected in the water-control condition were detected (Table 2). QTLs qT13 and qF3 completely disappeared after ASM treatment with a LOD score <0.2 at the SNP near to the peak of the same QTL detected in the water control and a non-significant effect for qF3 (Fig. 2). The three other QTLs were detected at roughly the same positions on linkage groups as with the water control, and the significant interaction between qF11 and qF17 was also found with the same pattern as above (Table 3). Their relative effects were not significantly different from the water control and were thus not significantly modulated by ASM treatment, despite much stronger LOD score and R^2 for qF17 (21.2 and 39.9% with ASM treatment compared with 12.4 and 29.0% with the water control) and lower LOD score and R^2 for qF11 (7.6 and 10.7% compared with 10.7 and 18.1%, respectively; Table 2; Fig. 2).

For experiment Vi-B04_2, the same QTLs were detected despite being at lower LOD scores than in the first experimentation (Supplementary Table S5). In the whole TxF progeny, qT1 was still detected as the strongest QTL for both treatment conditions (LOD approximately 35; R^2 approximately 40 to 48%) together with qF11 and qF12 as suggestive QTL (water control) and qF17 (both conditions) and still a significant interaction between qF11 and qF17 (Supplementary Table S6, water control). In the qT1− subpopulation, qF17 was the single significant QTL detected, while qT13, qF3, qF11, and qF12 were detected as suggestive QTLs with mostly similar R^2 values compared with the Vi-B04_1 experiment. Three significant interactions between QTLs were detected, two of them involving qT13 with qF11 and qF17, respectively (Supplementary Table S6). In this experiment, ASM treatment did not significantly modulate the QTL effect on any of the QTLs detected (Supplementary Fig. S2).

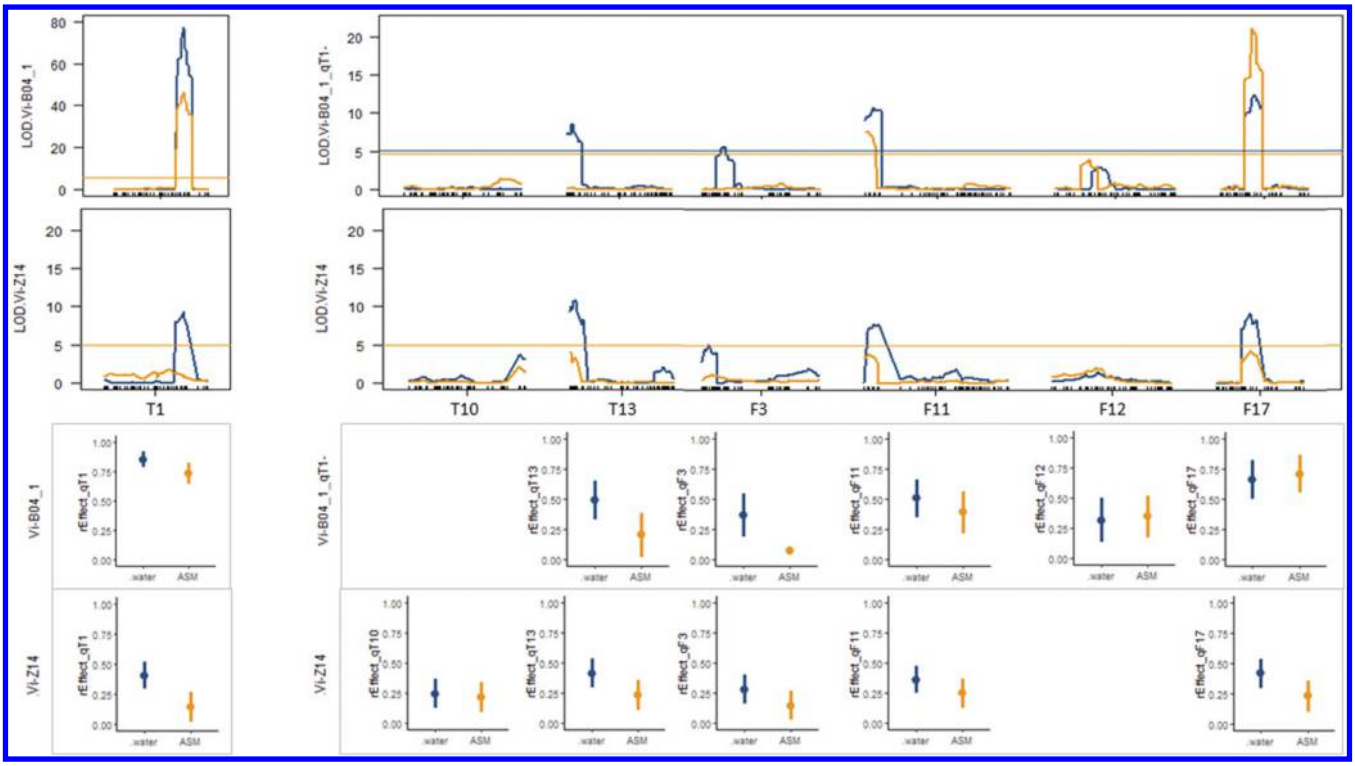

Fig. 2. Logarithm-of-the-odds (LOD) score curves of quantitative trait loci involved in resistance against scab (Vi-B04_1 and Vi-Z14) for each treatment identified in the TxF progeny (upper part) and relative quantitative trait loci effect (r_{Effect}) of these quantitative trait loci (lower part). Blue and orange represent, respectively, the water control and the acibenzolar-*S*-methyl (ASM) treatment. LOD score thresholds are drawn as horizontal lines with the respective colors. In each r_{Effect} plot, mean and standard deviation of each r_{Effect}, calculated with bootstrapping, are represented by a point and a vertical bar, respectively, with the respective blue and orange colors for water (control) or ASM (treatment).

QTL detection for *V. inaequalis* isolate '09BCZ14'. For the water-control condition, all the QTLs detected in Vi-B04_1 were also detected in Vi-Z14, except on LG F12, and a new suggestive QTL was detected on LG T10. The phenotypic variation explained by these QTLs ranged from 3.5 to 10.7% and the relative effects from 0.25 (QTL qT10) to 0.42 (QTLs qT13 and qF17; Table 2; Fig. 2). Interaction between QTLs qF11 and qF17 was still significant whereas new interactions were found to be significant between qT1 and qF3, between qT13 and qF3, and between qT10 and qF11 (Table 3). Altogether, these QTLs and interactions explained 47.5% of the phenotypic variation (Table 1). For the ASM-treatment condition, three out of the six QTLs identified in the water control were still detected but only as suggestive QTLs (qT13, qF11, and qF17; Table 2). Altogether, they explained only 15.0% of the phenotypic variation without any interactions. The relative effects of these QTLs were not significantly different when compared with the water control (Fig. 2). Conversely, the relative effect of qT1 was significantly lower for ASM treatment than for the water control (Fig. 2).

QTL mapping for fire blight resistance. For the water-control condition, four QTLs (two significant and two suggestive) were detected on LG T3, T5, F7, and F15, explaining from 3.1 to 20.1% of total phenotypic variation (Table 2; Fig. 3). Altogether, these QTLs explained 35.0% of the phenotypic variation. The same QTLs except LG F15 were found for the ASM-treatment condition, together with a new significant QTL on LG F5 (Table 2). These QTLs explained from 4.6 to 13.2% of the phenotypic variation (33.1% altogether). On LG T5, the peak of the QTLs was offset by approximately 20 cM between the water control and ASM treatment, which suggests two different QTLs. The relative effects of these QTLs were not significantly different

Table 2. Parameters associated with the quantitative trait loci (QTLs) identified for disease resistance against scab (experiments Vi-B04_1, subpopulation qT1–, and Vi-Z14) and fire blight (experiment Ea-1430_1) in the TxF progeny after water or acibenzolar-S-methyl (ASM) treatment

Trait	Treatment	Linkage group	Position	LOD[a]	R^2	CI[b]
Vi-B04_1$_{qT1-}$	Water	**T13**	**3.58**	**8.6**	**9.3**	**0.00 – 6.80**
		F3	**13.36**	**5.7**	**5.5**	**8.25 – 18.54**
		F11	**5.00**	**10.7**	**18.1**	**0.00 – 20.98**
		F12	*25.99*	*3.0*	*3.7*	*20.46 – 31.56*
		F17	**18.92**	**12.4**	**29.0**	**16.52 – 27.35**
	ASM	T13	3.58	0.3	–	–
		F3	44.80	0.8	–	–
		F11	**0.00**	**7.6**	**10.7**	**0.00 – 7.91**
		F12	*19.28*	*4.0*	*5.1*	*10.83 – 24.43*
		F17	**18.00**	**21.2**	**39.9**	**16.52 – 20.90**
Vi-Z14	Water	**T1**	**45.03**	**9.4**	**10.7**	**39.01 – 52.89**
		T10	*62.09*	*3.6*	*3.5*	54.88 – 65.69
		T13	**3.18**	**10.9**	**10.1**	**0.00 – 6.80**
		F3	**3.92**	**5.1**	**5.3**	**0.00 – 9.03**
		F11	**5.53**	**7.8**	**7.9**	**0.00 – 20.98**
		F17	**18.92**	**9.1**	**10.7**	**14.18 – 27.35**
	ASM	T1	36.61	1.7	–	–
		T10	62.09	2.2	–	–
		T13	*0.00*	*4.1*	*6.3*	*0.00 – 6.80*
		F3	5.50	1.1	–	–
		F11	*3.17*	*3.9*	*5.0*	*0.00 – 8.69*
		F17	*18.92*	*4.2*	*5.0*	*13.79 – 27.35*
Ea-1430_1	Water	*T3*	*23.57*	*4.1*	*3.1*	*11.81 – 26.76*
		T5	**54.33**	**5.8**	**5.2**	**43.24 – 62.05**
		F5	39.73	2.5	–	–
		F7	**69.46**	**18.4**	**20.1**	**67.10 – 71.42**
		F15	*76.05*	*3.7*	*3.9*	*70.94 – 81.15*
	ASM	*T3*	*20.38*	*3.7*	*4.6*	*17.13 – 29.18*
		T5	**35.22**	**4.9**	**4.6**	**28.37 – 40.84**
		F5	**38.56**	**6.3**	**8.4**	**32.18 – 42.08**
		F7	**69.07**	**12.2**	**13.2**	**67.10 – 71.42**
		F15	86.70	2.9	–	–

[a] Logarithm of the odds (LOD) thresholds were obtained after permutations (n = 1,000): 5.1 (Vi-B04$_{qT1-}$_water), 4.6 (Vi-B04$_{qT1-}$_acibenzolar-*S*-methyl [ASM]), 5.0 (Vi-Z14_water and Vi-Z14_ASM), 4.7 (Ea-1430_water), and 4.6 (Ea-1430_ASM). Significant QTLs are shown in bold. Suggestive QTLs with LOD score between 3.0 and the LOD threshold are shown in italics. QTLs being insignificant in one condition but significant in another comprise the remainder of the numbers. R^2, variance; CI, confidence interval.

[b] Position of 2-LOD support QTL CI borders.

Table 3. Interactions between significant and suggestive quantitative trait loci (QTLs) identified in Vi-B04_1$_{qT1-}$ and Vi-Z14 experiments after water or acibenzolar-S-methyl (ASM) treatment[a]

		Water					ASM				
Trait	QTLs combination	AA:AA	AB:AA	AA:AB	AB:AB	*F*-value	AA:AA	AB:AA	AA:AB	AB:AB	*F*-value
Vi-B04_1$_{qT1-}$ Vi-Z14	qF11:qF17	149.6	146.2	99.5	131.3	12.1***	94.7	93.2	49.0	70.0	8.9**
	qT1:qF3	34.2	19.2	24.7	16.6	6.6*	–	–	–	–	–
	qT13:qF3	19.9	35.5	15.8	25.4	6.6*	–	–	–	–	–
	qT10:qF11	15.7	24.3	23.1	31.2	4.9*	–	–	–	–	–
	qF11:qF17	31.3	32.3	12.8	24.9	4.2*	–	–	–	–	–

[a] *F*-value of the variance analysis Fisher test, where asterisks represent the significance of the test at the risk of 0.05 (*), 0.01 (**), or 0.005 (***).

when compared with the water control (Fig. 3). No significant interaction could be identified between the QTLs in both conditions.

In the Ea-1430_2 experiment for the water-control condition, three QTLs previously identified were maintained (qT3, qT5, and qF15), two new QTLs were detected (qT15 and qF5), and one was no longer detected (qF7; Supplementary Table S5). Surprisingly, qF7 was the QTL with the strongest effect in the first experiment; only a weak peak was detected here with an LOD of 1.8. For the ASM treatment condition, three new QTLs (qT1, qT7, and qF1) were identified while three others disappeared (qT3, qT15, and qF15). The QTL interactions in the two conditions are totally different (Supplementary Table S6). Among the nine QTLs identified, the relative effects of qT3 and qF15 were significantly modulated by ASM (Supplementary Fig. S3).

Discussion

A first result of our study has been to confirm already known scab and fire blight resistance QTLs and to detect new ones under the water-control condition. For scab resistance, QTLs located on LG1 of TN10-8 (qT1) and on LG11 and LG17 of Fiesta (qF11 and qF17, respectively) were previously detected and confirmed in various studies (Calenge et al. 2004; Durel et al. 2003; Lê Van et al. 2012; Liebhard et al. 2003; Soufflet-Freslon et al. 2008). The added value of this study is the refinement of their genetic position with reduced CIs compared with previous studies. As stated above, qT1 is precisely co-localized with the *Rvi6* (*Vf*) gene at position 43.03 cM of the genetic map corresponding to the CH-Vf1 simple sequence repeat marker, tightly associated to that R-gene (Vinatzer et al. 2004). qT1 is thus a potential allele or paralog of *Rvi6* and can thus be postulated as an extracellular leucine-rich repeat receptor-like gene (Belfanti et al. 2004; Calenge et al. 2004). Another key aspect is the confirmation of the strong complementary (synergistic) interaction between qF11 and qF17 as already highlighted by Caffier et al. (2014). Such an interaction can be interpreted as the complementary action of two genes successively involved in a metabolic pathway (McMullen et al. 1998). The dependency of the qF11 allelic contrast upon the unfavorable or favorable allele of qF17 could be tentatively interpreted as an upstream position of the gene underlying qF17 and a downstream position of the gene underlying qF11. Thus, qF11 and qF17 could instead be involved in a signaling or defense pathway, unlike qT1, which is more probably involved in pathogen effector or pathogen-associated molecular patterns (PAMPs) recognition. Although reduced, the CIs of these QTLs were still too wide to search for sufficiently plausible candidate genes. An attempt was made for qF17 and yielded >160 positional candidates (data not shown). New scab resistance QTLs were detected on LGs 10 and 13 of TN10-8 and LGs 3 and 12 of Fiesta. The most significant were qT13 and qF3 detected at genomic positions not yet identified in other scab resistance studies. They will therefore be considered as a priority for further marker-assisted breeding.

For fire blight resistance, the QTL qF7 was consistently detected on LG7 of Fiesta in the first experiment as already published (Calenge et al. 2005; Khan et al. 2007). The refinement of its genetic position was allowed with reduced CIs compared with previous studies. This QTL was shown to interact with two other ones on LG8 and LG13 (van de Weg et al. 2018), but these genomic regions were not detected in our study. Implication in a metabolic pathway could nevertheless be postulated for qF7 because of such interactions. We also identified resistance QTLs on LGs 3, 5, and 15, which may coincide with those published by Calenge et al. (2005), Durel et al. (2009) and Le Roux et al. (2010), respectively, despite that their genomic position was not very accurate. In our second experiment, qF7 was no longer detected; this calls into question the relevance of this experiment, especially in view of the low AUDPC and heritability values obtained and the weak correlation between both Ea-1430 experiments. This second experiment was performed very early in the season (early February) at an unusual time for fire blight experiment, which could explain this surprising result.

In this study, we propose for the first time a QTL mapping approach to search for genetic factors influenced by a PRI. A few studies highlighted the influence of genotypes on the effectiveness of PRIs, but without any link to intrinsic genetic resistance of varieties (Maisonneuve et al. 2013; Sharma et al. 2010; Vallad and Goodman 2004). Pawlowski et al. (2016) underlined the specific interaction between the genetic and PRI-induced resistance but did not characterize the genetic resistance factors. A mapping approach has the advantage of exploring in detail the putative modulation of genetic resistance factors by PRIs. In our study, the results show that genetic resistance controlled by QTLs is only slightly or rarely affected by ASM treatment. Out of the seven scab resistance QTLs detected, only two QTLs (qF3 for Vi-B04_1, and qT1 for Vi-Z14) almost disappeared after treatment with ASM. We also detected two fire blight resistance QTLs (qT3 and qF15), the effects of which significantly decreased after ASM treatment in the second Ea-1430 experiment, although the experimental conditions were questionable. Despite a large

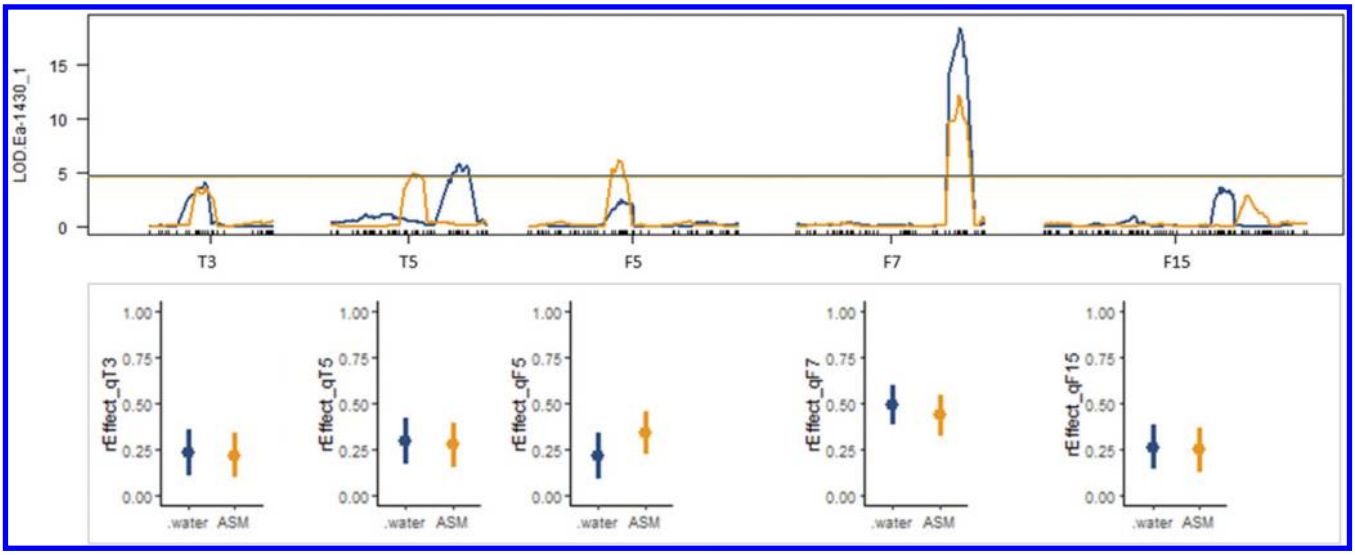

Fig. 3. Logarithm of the odds (LOD) score curves of quantitative trait loci involved in resistance against fire blight (Ea-1430_1) for each treatment identified in the TxF progeny (upper part) and relative quantitative trait loci effect (r_{Effect}) of these quantitative trait loci (lower part). Blue and orange represent, respectively, the water control and acibenzolar-*S*-methyl (ASM) treatment. LOD score thresholds are drawn as horizontal lines with the respective colors. In each r_{Effect} plot, mean and standard deviation of each r_{Effect}, calculated with bootstrapping, are represented by a point and a vertical bar, respectively, with the respective blue and orange colors for water (control) or ASM (treatment).

mapping population, the CIs surrounding relative effects of QTLs were large and therefore precluded the detection of low modulation of QTL effects by ASM. More individuals and replicates per individual would have been necessary to detect such modulations. Furthermore, no QTL specific to the ASM treatment condition was detected: all QTLs detected with ASM were already identified as resistance QTLs under the water control. The general trend was either a maintenance of, or a reduction in, the relative effect of QTLs. Some of them fully disappeared (significantly), but this observation was hard to repeat for both diseases. To some extent, this QTL effect disappearance could also be explained by a scale effect. Indeed, the application of ASM strongly reduces the global variability in disease severity, and the weakest QTLs under the water control can become insignificant under ASM treatment from the consequent lack of statistical power. However, a general trend of maintenance or small reduction of most QTLs is surprising: considering the overall effectiveness of ASM on reducing disease symptoms and the interactions observed between less resistant genotypes and treatment, it would have been expected to find more interactions between the pathways that lead to this resistance.

To go further, according to the diverse nature of the QTLs present in our population, we expected to observe some QTLs responding to ASM, with others remaining unchanged. ASM is known to act on the SA signaling pathway. In tobacco, it is perceived by salicylic acid-binding protein2 (SABP2), which transforms it into acibenzolar, which then interacts with nonexpressor of pathogenesis-related protein1 (NPR1) to activate the transcription of a set of genes in the SAR pathway (de Jong et al. 2019; Ishiga et al. 2020; Li et al. 2020; Tripathi et al. 2010; Warneys et al. 2018). If we consider the three stages of resistance buildup, namely recognition, signaling, and defense, we could postulate that QTLs involved in signaling and defense could be more affected by ASM than QTLs involved in effectors or PAMP recognition. However, the qT1 previously described as most probably involved in pathogen recognition disappeared with 09BCZ014 isolate but not with EU-B04, whereas the QTLs qF11, qF17, and qF7, potentially involved in signaling or defense pathways were not significantly affected by ASM. Various hypotheses can be formulated to interpret our results. In Arabidopsis, Tsuda et al. (2009) showed that each of the SA, jasmonic acid, and ethylene signaling sectors can positively contribute to immunity against both biotrophic and necrotrophic pathogens. The authors schematized various situations of compensation or synergy between different sectors of a complex signaling network where the effector or PAMP recognition (input) can generate an equivalent restriction of pathogen growth (output) while mobilizing very different sectors interacting or not. In our situation, ASM treatment would reinforce one of the signaling sectors with possible consequences on other sectors given SA versus jasmonic acid/ethylene compensation, while resistance QTLs positioned at various locations of the network would impact the flow along some of these pathways. Some of the detected QTLs could therefore be located on metabolic pathways independent of the SA pathway while others could be implied in the SA pathway and thus be affected by the strong initial supply of ASM. In-depth exploration of the metabolic mechanisms involved in genotypes, and whether or not they carry resistance QTL alleles, either with or without ASM treatment, is needed to precisely determine the interactions between pathways.

Our study revealed that the intrinsic resistance controlled by QTLs and the resistance induced by ASM have a complementary effect for the control of apple scab and fire blight. In our study, the average effectiveness of ASM in reducing the disease ranged from 50% for Ea-1430_1 to 78% for V1-B04_2. Under the water control, the individuals cumulating in favorable alleles at all detected QTLs exhibited a disease reduction of 91% for Vi-B04_1 and 98% for Vi-Z14 compared with individuals carrying only unfavorable QTL alleles for scab, and 78% for fire blight. When intrinsic and ASM-induced resistance was combined, the disease reduction was close to 100% for scab and 85% for fire blight. These results confirm that there is no incompatibility between intrinsic and ASM-induced resistance for apple as reported for cucumber (da Rocha and Hammerschmidt 2005). These results were obtained under controlled conditions, but arguments suggest that the same trends could be observed in orchards. On the one hand, Caffier et al. (2014, 2016) showed that quantitative resistance related to QTLs qT1, qF11, and qF17 significantly reduced scab severity in orchards. On the other hand, Marolleau et al. (2017) confirmed that ASM could be integrated into orchard protection practices to control apple scab. Combining both types of resistance in the orchard should allow a better control of apple scab and similarly for fire blight.

In addition to improving protection effectiveness, the interest in combining intrinsic and PRI-induced resistance could rely on a cross protection of both types of resistance through a diversification of selection pressures on pathogen populations to reduce or slow down pathogen adaptation (Caffier et al. 2014; Lê Van et al. 2013). The use of ASM in combination with genotypes cumulating in several QTLs involved in different defense pathways could be a promising sustainable protection for the control of apple scab and fire blight. A thorough analysis of the mechanisms activated by this resistance will confirm these hypotheses, and all of these results must be confirmed in the orchards for possible integration into the protection practices.

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Author-Recommended Internet Resources

SpATS software package, CRAN:
https://www.google.com/search?client=firefox-b-1-d&q=SpATS+package
Illumina GenomeStudio Genotyping Module software, V2.0:
https://www.illumina.com/techniques/microarrays/array-data-analysis-experimental-design/genomestudio.html
JoinMap 4.1 software, Kyazma:
https://www.kyazma.nl/index.php/JoinMap/
Biomercator V4.2 software:
https://urgi.versailles.inra.fr/Tools/BioMercator-V4
R/qtl software package: https://rqtl.org/

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