Theor Appl Genet (2005) 111: 128–135
DOI 10.1007/s00122-005-2002-z

ORIGINAL PAPER

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Identification of a major QTL together with several minor additive or epistatic QTLs for resistance to fire blight in apple in two related progenies

Received: 17 December 2004 / Accepted: 10 March 2005 / Published online: 26 April 2005

Abstract Although fire blight, caused by the bacterium *Erwinia amylovora*, is one of the most destructive diseases of apple (*Malus* × *domestica*) worldwide, no major, qualitative gene for resistance to this disease has been identified to date in apple. We conducted a quantitative trait locus (QTL) analysis in two F_1 progenies derived from crosses between the cultivars Fiesta and either Discovery or Prima. Both progenies were inoculated in the greenhouse with the same strain of *E. amylovora*, and the length of necrosis was scored 7 days and 14 days after inoculation. Additive QTLs were identified using the MAPQTL software, and digenic epistatic interactions, which are an indication of putative epistatic QTLs, were detected by two-way analyses of variance. A major QTL explaining 34.3–46.6% of the phenotypic variation was identified on linkage group (LG) 7 of Fiesta in both progenies at the same genetic position. Four minor QTLs were also identified on LGs 3, 12 and 13. In addition, several significant digenic interactions were identified in both progenies. These results confirm the complex polygenic nature of resistance to fire blight in the progenies studied and also reveal the existence of a major QTL on LG7 that is stable in two distinct genetic backgrounds. This QTL could be a valuable target in marker-assisted selection to obtain new, fire blight-resistant apple cultivars and forms a starting point for discovering the function of the genes underlying such QTLs involved in fire blight control.

Communicated by H. Nybom

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Introduction

Fire blight, caused by the necrogenic gram-negative bacterium *Erwinia amylovora*, is one of the most destructive diseases of apple (*Malus* × *domestica*) and pear (*Pyrus communis*) worldwide. The number of countries in which this disease has been reported has increased continually since it first appeared in 1873 in New York state, USA (Vanneste 2000). It now occurs in at least 40 countries spread across North America, Europe, the Middle East and New Zealand (Bonn and Van der Zwet 2000). Pathogen infection occurs primarily via natural openings in the flowers (nectarthodes) or through wounds on aerial vegetative parts. When established, bacteria multiply and progress into intercellular spaces between the parenchyma cells, leading to a rapid necrosis of infected tissues and to ooze production, the two most typical symptoms of the disease (Thomson 2000). On highly susceptible genotypes, necroses may extend to the entire tree within only one growing season. While antibiotics, such as streptomycin and some copper-derived chemical compounds, may partly control the disease, in many countries, such compounds are not registered for use. Moreover, streptomycin-resistant populations of *E. amylovora* have already been isolated in several orchards throughout the world (Jones and Schnabel 2000). Consequently, the use of genetically resistant cultivars would be a valuable alternative to chemical control.

Apple cultivars display a great variability for resistance to fire blight (Le Lezec et al. 1985, 1990), but the

genetic basis of resistance is poorly understood. A few studies have identified a quantitative, most probably polygenic, determinism in several apple cultivars (Korban et al. 1988; for a review see Lespinasse and Aldwinckle 2000). There is no known ''gene-for-gene '', R-Avr relationship for the interaction *E. amylovora*/*M.* × *domestica*, and specific interactions between the pathogen genotypes and the apple cultivars have rarely been reported (Lespinasse and Aldwinckle 2000), which may be partly attributable to the quantitative nature of resistance.

Molecular markers can help identify genes underlying quantitative disease resistance in apple. Several apple genetic linkage maps have already been produced from different segregating F_1 progenies (Hemmat et al. 1994; Conner et al. 1997; Maliepaard et al. 1998; Liebhard et al. 2003a), and apple reference linkage maps have been constructed successively from progenies derived from the crosses Prima × Fiesta (Maliepaard et al. 1998) and later from Fiesta × Discovery (Liebhard et al. 2003a). Major genes and quantitative trait loci (QTLs) for resistance to scab, caused by *Venturia inaequalis*, and powdery mildew, caused by *Podosphaera leucotricha*, have been identified, and some have been mapped in apple (Alston et al. 2000; Hemmat and Brown 2002; Durel et al. 2003; Liebhard et al. 2003b; Calenge et al. 2004a, b), but no gene for resistance to fire blight has been identified to date.

The objective of this study was to explore the genetic determinism underlying resistance to fire blight in two related apple progenies through a QTL analysis. We found that the resistance to fire blight in these progenies is polygenic, but mainly explained by a major QTL derived from Fiesta, which was identified in both progenies. To our knowledge, this study is the first one to identify loci for resistance to fire blight in apple.

Materials and methods

Plant material

An F_1 progeny of 144 individuals was derived from a cross between apple (*Malus* × *domestica*) cultivars Prima (female) and Fiesta (male) (P × F) during the EAGMAP project (European Apple Genome Mapping Project) (King et al. 1991). Genetic linkage maps of both parents of this progeny have been previously constructed using microsatellite, isozyme, random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) markers (Maliepaard et al. 1998) and recently completed with additional microsatellite, RFLP and AFLP markers (Van de Weg, unpublished results). A second progeny of 188 individuals was derived from a Fiesta (female) × Discovery (male) (F × D) cross created in 1996 at Plant Research International (Wageningen, The Netherlands). The genetic linkage maps of both parents of this progeny were constructed within the frame of the DARE project (Durable Apple Resistance in Europe; Lespinasse et al. 2000) using microsatellite, AFLP and isozyme markers (Van de Weg, unpublished results). The seedlings of this progeny are different from those of the Fiesta × Discovery progeny described by Liebhard et al. 2003a). Duplicates of the original P × F and F × D progenies at Plant Research International were planted in 1997 and 2000 in two local orchards in Angers, France. In early 2002 (or 2003), graftwood of these duplicated progenies, their parental cultivars, two susceptible control cultivars (Idared and Golden Delicious) and a resistant control cultivar (Evereste) was collected and grafted onto the apple rootstock MM106, giving several tree scions (replicates) per genotype.

Inoculation and disease resistance assessment

The bacterial strain CFBP 1430 of *Erwinia amylovora* was used for inoculating both progenies. Inocula were prepared from an overnight culture in sterile, distilled water to reach a concentration of 10^7 CFU/ml. Bacterial suspensions were kept at 4°C before inoculation. Inoculations were performed by cutting the two youngest well-expanded leaves of each shoot with scissors dipped in the bacterial suspension. Two distinct disease resistance experiments were performed in the greenhouse: the P × F progeny was tested in 2002 with six replicates per genotype, and the F × D progeny was tested in 2003 with seven replicates per genotype. Inoculations were made on shoots at least 15 cm long in order to keep physiological conditions as homogeneous as possible. All of the trees in each experiment could not be inoculated at the same time due to different growth rates and, consequently, several inoculations were needed. Eight and ten inoculation series per experiment were carried out within a 10-week period to inoculate every individual tree from the P × F and F × D progenies. Shoots that never reached 15 cm were not inoculated. On each shoot, the length of necrosis was measured 7 days and 14 days post-inoculation (dpi) using the following scale: 0.5, necroses affecting only veins of the inoculated leaves;1.0, necroses reaching the petioles of the inoculated leaves; necroses reaching the stem were measured in centimeters and 1.0 cm was added to the value obtained.

Statistical analyses

Statistical analyses of the phenotypic data were conducted using SAS software (SAS Institute, Raleigh, N.C.). Analyses of variance (ANOVA) were performed using the GLM procedure to test the significance of ''genotypes'' and ''date of inoculation'' effects. Data were adjusted according to the ''date of inoculation'' effects using the MIXED procedure, with ''genotypes'' and ''date of inoculation'' considered as random and fixed effects, respectively. Broad-sense heritability of

genotypic means within each progeny was calculated using the following formula $h^2 = \sigma^2_g/(\sigma^2_g + (\sigma^2_e/n))$, where n is the mean number of replicates per genotype, σ^2_g is the genetic variance (i.e. inter-genotype variance) and σ^2_e is the residual error variance.

Additive QTLs were detected with the RMQM (restricted multiple QTL mapping) procedure of the MAPQTL software (Van Ooijen and Maliepaard 1996) using the markers nearest to the QTL peaks as cofactors (one marker per peak). QTLs with a maximum LOD (logarithm of odds ratio) score greater or equal to 3.0 were declared significant. For each significant QTL, confidence intervals corresponding to a LOD score drop-off of 1 or 2 on either side of the likelihood peak were calculated.

To detect putative epistatic QTLs, we identified those markers involved in digenic interactions by testing the simple effects and interaction effects of all pairwise combinations of molecular markers of each parental map in two-way ANOVA using SAS software with the formula $Y_{ijk} = \mu + M^1_I + M^2_j + (M^1 * M^2)_{ij} + E_{ijk}$, where Y_{ijk} = the phenotypic value of the genotype k having markers M^1 and M^2 in the Ith and jth marker classes (i.e. alleles), μ = the phenotypic mean of the progeny, M^1_I and M^2_j are the proper effects of marker classes I and j for markers M^1 and M^2, $(M^1 * M^2)_{ij}$ is the interaction effect between markers M^1 and M^2 for the combination of marker classes I and j and E_{ijk} is the residual effect. Interaction effects detected with a probability under 10^{-4} (p-value) were declared significant.

Results

Disease assessments

For both experiments, the susceptible controls Idared and Golden Delicious displayed necroses along the full lengths of the shoots at 14 dpi, while the parents of the first progeny, Prima and Fiesta, showed intermediate phenotypes, with Prima tending to be more susceptible than Fiesta (Table 1, Fig. 1). Conversely, Discovery exhibited a mean length of necrosis similar to that of Golden Delicious and could be considered to be as a susceptible parent in the second cross (Table 1). Conditions seemed more favourable to the disease in 2002, since all cultivars were more diseased in that year than in 2003, with the exception of Evereste, which was fully resistant in 2002 but displayed slight necroses on petioles on a few shoots in 2003. Nevertheless, the P × F progeny as a whole (tested in 2002) was less diseased than the F × D progeny (tested in 2003) (Table 1). Therefore, we concluded that Prima had a better contribution to the resistance of its progeny than Discovery, a result consistent with its higher fire blight resistance score. The average values of the P × F and F × D progenies were generally in between the two values of their respective parents, except for P × F at 14 dpi. This may indicate a rather additive genetic determinism. With respect to the distribution of both progenies (Fig. 1), transgressive individuals were observed in both cases, either as more resistant or more susceptible individuals than their respective parents. This was consistent with a putative polygenic determinism of fire blight resistance in these progenies with resistance factors inherited from both parents.

Table 1 Mean length of necrosis of parental cultivars of the Prima × Fiesta (P × F) and Fiesta × Discovery (F × D) progenies and of the control cultivars. Average values of the whole set of P × F and F × D progenies are also indicated

Cultivar	2002		2003	
	7 dpi	14 dpi	7 dpi	14 dpi
Prima	5.4	10.9	1.8	3.8
Fiesta	1.9	7.5	1.3	4.6
Discovery	10.1	15.4	5.3	12.3
Evereste	0.0	0.0	0.35	0.64
Golden	4.7	14.0	5.4	12.3
Idared	11.3	18.7	6.7	14.9
P × F	2.6	5.3	–	–
F × D	–	–	3.8	6.6

Statistical analyses and QTL detection

Strong "genotype" and "date of inoculation" effects (p-value = 0.001) were observed for both tests by ANOVA on raw data. In the P × F progeny, the broad-sense

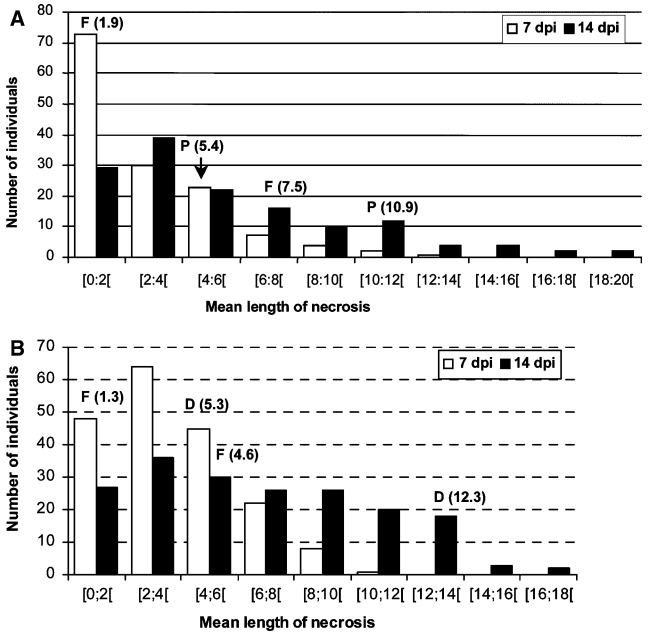

Fig. 1 Distribution of the genotypes of the Prima (P) × Fiesta (F) (**a**) and Fiesta × Discovery (D) (**b**) progenies according to their mean length of necrosis at 7 and 14 days post-inoculation (dpi) (data adjusted). Mean values of the parents at both dates are also indicated

heritability of the length of necrosis was 0.86 at 7 dpi and 0.85 at 14 dpi. In the F × D progeny, it was 0.88 and 0.86, respectively. In both progenies, the correlation coefficient between data collected at 7 dpi and 14 dpi was very high: 0.92 in P × F, and 0.85 in F × D.

In the P × F progeny, two additive QTLs were detected with MAPQTL at both 7 dpi and 14 dpi on linkage groups (LGs) 3 in Prima (P3) and 7 in Fiesta (F7) (Table 2, Fig. 2). The QTL on F7 explained a large part of the phenotypic variation (R^2) in the progeny at both 7 dpi and 14 dpi (43.2–46.6%, Table 2). The QTL on LG P3 was detected with a LOD score just below the LOD threshold of three at 7 dpi but showed a clearly significant effect at 14 dpi.

In the F × D progeny, four additive QTLs were detected with MAPQTL on four LGs: F3, F7, D12 and D13 (Table 2, Fig. 2). Two QTLs derived from Fiesta were detected at both 7 dpi and 14 dpi on LGs 3 and 7. The QTL on F7 was again detected with a high R^2 (42.6%) at 7 dpi, though it was weaker than in the P × F progeny at 14 dpi (34.3%). The QTL on F3 was detected with a LOD score under the LOD threshold of 3 at 14 dpi but was nevertheless declared significant since it was consistent with the QTL effect identified at 7 dpi. In addition, two minor effect additive QTLs from Discovery were detected only at 7 dpi on D12 and D13. In both progenies, the maximum likelihood position (QTL peak) of the QTL on F7 was close to the common RAPD marker GE80-19-0550 (about 1 cM in P × F, 4–6 cM in F × D). The QTLs on P3 and F3 were not detected at the same genetic position and had likelihood peaks very far from each other (about 40 cM).

In the P × F progeny, seven significant digenic interactions, indicating putative epistatic QTLs, were detected with probabilities ranging from 8.3×10^{-5} to 3.2×10^{-6} (Table 3), whereas five significant interactions were detected in the F × D progeny with probabilities from 5.9×10^{-5} to 3.5×10^{-5}. Interactions with the lowest *p*-values occurred in the P × F progeny. The P3-F7 interaction was identified between markers belonging to the confidence intervals of the identified additive QTLs on P3 and F7. This might indicate an interaction component between these QTLs. Two interactions, F5-F9 and F1-D10, were identified at both 7 dpi and 14 dpi.

Discussion

We detected several QTLs in two progenies sharing one common parent (Fiesta) that had been crossed with two cultivars displaying contrasting fire blight resistance levels (Prima and Discovery). The QTL associated with the strongest effect was derived from the common parent Fiesta and was detected in both progenies, i.e. in two contrasting genetic backgrounds, with a very similar impact on the respective phenotypic variations.

The broad-sense heritability of the genotypic means was high in both progenies, thus allowing a reliable QTL detection. A polygenic determinism was identified for the major QTL on LG 7 of Fiesta (F7) and several minor QTLs, either additive or epistatic, from Discovery, Fiesta or Prima. The identification of one major QTL together with several minor QTLs is frequent in plant resistance QTL analyses (Young 1996). Although

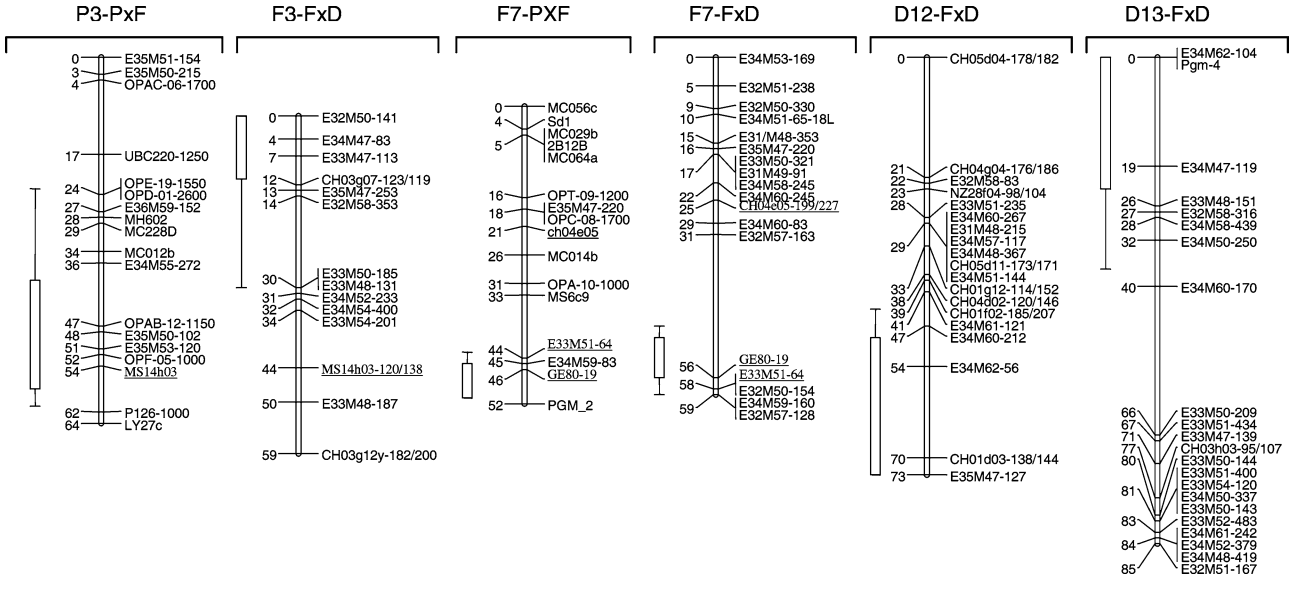

Fig. 2 QTLs identified for resistance to fire blight. LGs belonging to the Prima × Fiesta (*P* × F) and Fiesta × Discovery (*F* × D) genetic linkage maps on which QTLs for fire blight resistance were identified are shown. Homologous LGs from different linkage maps are represented *side by side*. QTLs are represented by *white boxes extended by lines*, which stretch over the LOD-1 and LOD-2 confidence intervals, respectively. Markers mapped in both progenies and consequently enabling some LGs to be aligned are *underlined*. Only QTLs identified with data collected at 14 dpi in the P × F progeny and at 7 dpi in the F × D progeny are represented. These data are those that maximize the QTL likelihood peaks

Table 2 Parameters associated with the QTLs detected with the MAPQTL software in the Prima × Fiesta (P × F) and Fiesta × Discovery (F × D) progenies based on the mean length of necrosis. Values are indicated in italics when the LOD score of the QTL is lower than 3

	LG	LOD[a]	Distance[b] (cM)	R^{2c} (%)	Confidence interval (cM)	ac[d]	ad[d]	bc[d]	bd[d]	A_f[e]	A_m[e]	Cofactors[f]
2002 P × F												
7 dpi	*P3*	*2.88*	*53.8*	*5.1*	*60.3*	*5.49*	*5.49*	*4.33*	*4.33*	*0.58*	–	*MS14h03*
	F7	18.43	46.7	43.2	13.0	5.55	2.12	5.55	2.12	–	1.72	GE80-19-0550
14 dpi	P3	4.09	53.8	7.5	37.7	9.65	9.65	7.31	7.31	1.17	–	MS14h03
	F7	19.14	46.7	46.6	5.8	9.72	3.86	9.72	3.86	–	2.93	GE80-19-0550
2003 F × D												
7 dpi	F3	3.57	0.0	4.4	30.1	6.71	6.71	5.80	5.80	0.46	–	E32M50-141-12
	F7	26.82	52.7	42.6	12.2	6.72	6.72	3.73	3.73	1.49	–	GE80-19-0550
	D12	3.53	62.3	5.4	29.3	6.77	5.76	6.77	5.76	–	0.51	CH01d03
	D13	4.87	10.2	7.9	36.5	6.87	5.65	6.87	5.65	–	0.61	E34M62-104-15
14 dpi	*F3*	*2.66*	*0.0*	*4.9*	*10.5*	*9.53*	*9.53*	*7.73*	*7.73*	*0.90*	–	E32M50-141-12
	F7	13.39	50.7	34.3	18.1	9.92	9.92	5.14	5.14	2.39	–	GE80-19-0550

[a]Maximum LOD (logarithm of odds ratio) score (QTL peak)
[b]Position of the QTL peak on the corresponding LG
[c]Proportion of phenotypic variation explained by each QTL
[d]Mean length of necrosis associated with each of the four possible genotypic classes, ac, ad, bc and bd, in the P × F and F × D progenies coded as ab × cd
[e]Female (A_f) and male (A_m) additive effects computed as [(ac + ad)−(bc + bd)]/4 and [(ac + bc)−(ad + bd)]/4, respectively
[f]Markers close to the QTL peaks and used as cofactors for QTL detection

Prima is generally considered to be quite resistant (Le Lezec et al. 1985, 1990) and did show a higher resistance than Discovery in our analysis, we were able to identify only one, minor additive QTL in this cultivar. This may be explained by the putative existence of other homozygous and, therefore, unidentifiable resistance QTLs in Prima. Additional putative explanations may include: (1) the limited size of the P × F progeny, which might prevent the detection of minor QTLs; (2) the occurrence of favourable dominant interactions in Prima that could have been broken after the crossing with Fiesta and could not be detected in the resulting progeny; (3) complex epistatic interactions that were undetectable in this cross. Prima is also possibly more susceptible to fire blight under the conditions of the present experiment—i.e. at an early growing stage in the geenhouse with inoculations performed on actively growing shoots—than under field conditions at an older growing stage, such as that investigated by Le Lezec et al. (1985, 1990). Interestingly, Golden Delicious, which is a grandparent of Prima, also had a reduced resistance level in our experiment compared to that observed in previous

Table 3 Interactions for resistance to fire blight identified between molecular marker pairs from the parental maps of the Prima × Fiesta (P × F) and Fiesta × Discovery (F × D) progenies. For each genomic region detected, only marker pairs detected with the strongest probability (lowest *p*-value) are indicated

Progeny	Markers	LG[a]	Number of individuals[b]				Distension[c]	P[d]
			ac	ad	bc	bd		
P × F	7 dpi							
	OPAF-12-2000/E35M52-133	P1-F1	29	31	36	32		6.71×10^{-5}
	UBC220-1800/MC221a	P2-P16	29	31	36	32		8.34×10^{-5}
	E34M59-104/OPAE-01-1190	F5-F9	23	23	28	24		4.87×10^{-6}
	14 dpi							
	E34M59-265/OPE-04-1400	P2-F6	26	27	41	22	$P < 5 \times 10^{-2}$	3.22×10^{-6}
	MC228D/GE80-19-0550	P3-F7	26	40	37	28	$P < 5 \times 10^{-2}$	6.14×10^{-6}
	E32M52-242/pB610	P9-F1	22	25	34	32	$P < 5 \times 10^{-2}$	5.40×10^{-5}
	E34M59-104/E36M51-480	F5-F9	19	28	29	24		6.29×10^{-6}
F × D	7 dpi							
	E31M48-362-20/E34M47-100-25	F1-D10	43	21	52	20	$P < 5 \times 10^{-5}$	5.25×10^{-5}
	E36M51-348-2/E32M50-237-7	F14-F16	10	13	18	14		3.46×10^{-5}
	CH04d02/CH05a04	D12-D16	19	21	8	11	$P < 5 \times 10^{-2}$	4.83×10^{-5}
	14 dpi							
	E31M48-362-20/E34M47-100-25	F1-D10	43	21	56	20	$P < 1 \times 10^{-5}$	5.92×10^{-5}
	E33M51-265-4/E33M52-219-6	F17-D5	30	38	35	31	$P < 5 \times 10^{-2}$	5.44×10^{-5}

[a]LGs to which the interacting molecular markers belong
[b]Number of individuals with phenotypic and molecular data available for each of the four possible genotypic classes, ac, ad, bc and bd, for two molecular markers coded with alleles a/b and c/d
[c]Segregation distortion of the different genotypic classes compared to the 1:1:1:1 expected segregation, tested with a chi-square test (*P*: probability)
[d]Probability associated with the interaction detected

analyses. Both genotypes could share one or several resistance loci that were either overcome by the *E. amylovora* strain tested or were less efficient under the conditions of our experiment than in the field. In both progenies, a QTL effect was identified at both dates (7 dpi and 14 dpi) on LG 3 either on P3 (P × F) or F3 (F × D). The genes underlying these QTL effects on P3 and F3 are most probably different, since the likelihood peaks of the QTLs were very far from each other. In addition to these additive QTLs that were stable over the two assessment dates, two additional QTLs were identified at 7 dpi in F × D on D12 and D13, which were not detected at 14 dpi. These QTLs possibly lose their efficiency with increased disease levels. This lack of efficiency might be related to the very weak resistance level of Discovery, the parent donating these QTLs.

The QTLs for resistance to fire blight identified in the present analysis can be compared to those of a previous study conducted in pear (Dondini et al. 2004). The use of common microsatellite markers and the remarkable synteny between apple and pear (Yamamoto et al. 2004) enables the apple and pear maps to be aligned. In pear, four QTLs have been identified on LGs 2, 4 and 9 (Dondini et al. 2004), two of which have been mapped at positions similar to those of epistatic QTLs identified in apple. The pear QTL on LG 9 is close to the microsatellite CH05a03, which was mapped at less than 2 cM from CH01h01 in an apple genetic map (European project DARE, unpublished results), whereas in our apple study two epistatic QTLs of Prima and Fiesta also mapped very close to CH01h01 on LG 9. Similarly, the same region on the top of LG 2 was involved in epistatic interactions in apple (in Prima) and identified in pear. These two pear QTLs on LGs 2 and 9 might also be involved in epistatic interactions, which were not tested by Dondini et al. (2004).

Lespinasse and Aldwinckle (2000) reported that some apple cultivars with the major gene *Vf*, conferring resistance to *V. inaequalis*, also displayed a higher resistance to fire blight. If this were the case, the presence of a QTL near *Vf*, which is carried by Prima, would be expected. This was not observed, possibly due to the absence of an actual common genetic basis between fire blight and scab resistance in the *Vf*-cultivars, as already proposed by Korban et al. (1988). We were also unable to detect a minor QTL, or the link between the *Vf* genomic region and fire blight might be more complex involving, for instance, epistatic interactions between two or more loci.

In other pathosystems, epistatic loci have been shown to account for a significant part of phenotypic variation in progenies segregating for a quantitative resistance (see, for example, Manzanares-Dauleux et al. 2000; Ahmadi et al. 2001). Nevertheless, these loci are rarely considered, partly because many current QTL mapping softwares (including MAPQTL) do not yet include routines to identify them. To try to detect such epistatic QTLs, we examined the occurrences of significant inter-loci interactions for all possible two-way combinations of markers. The same genomic region was often detected two or more times for its involvement in distinct digenic interactions, on homologous LGs (P9-F9; F1-P1; F16-D16-P16) or homeologous segments (F5-D10; P9-F17; F6-F14). Such recurrent involvements are additional indications in favour of the presence of significant epistatic QTLs in these genomic regions. The microsatellite markers used in constructing both genetic maps made it possible to align homologous LGs among the three parents of the two crosses (data not shown). Moreover, additional RFLP markers tend to demonstrate that the apple genome contains many homeologous segments (Maliepaard et al. 1998; Van de Weg, unpublished results). Homeologous segments are genomic regions presumed to derive from the same ancestor chromosome, in accordance with the probable allopolyploid origin of the *Maloideae* subfamily within the *Rosaceae* (Phipps et al. 1991). The detection of homeologous regions involved in digenic interactions is again a consistent indication of the putative presence of significant epistatic QTLs in those regions. Nevertheless, it was not possible to evaluate the confidence intervals of all of these epistatic QTLs, the intervals of which are most probably very large. Therefore, the results have to be seen as convergent indications of the probable involvement of epistasis in fire blight resistance in apple. Interestingly, the region identified three times on LG16 also co-localizes with a gene cluster for pathogenesis-related proteins of the PR10 family (Gao et al. 2005). Such genes can be considered to be candidate genes putatively involved in the control of fire blight. These epistatic interactions have first to be confirmed in enlarged or additional progenies in future experiments, since it can not be excluded that some of them could be statistical artefacts due to the high number of interactions tested, the small sizes of both progenies tested or the small sample sizes of some of the genotypic combinations.

The validation of QTLs in several highly different genetic backgrounds is crucial in plant breeding, especially for heterozygous allogamous plants with complex pedigrees, such as apple. The F7 QTL effects were found at very similar positions in both progenies, close to the RAPD marker GE-80-19-0550 and to the AFLP marker E33M51-64. It is highly probable that both QTLs correspond to the same locus. The F7 QTL was efficient in both a moderately resistant (Prima) and a susceptible (Discovery) background. Ideally, its existence should be validated in other genetic backgrounds by studying other crosses between Fiesta and various cultivars. This requires the development of flanking, co-dominant markers that could be used to validate its expression by simple variance analyses. The RAPD marker GE80-19-0550, which has already been converted into a sequence-characterised amplified region (SCAR; data not shown), might be used for this purpose. Such markers could also be used to mine for putative orthologous alleles at the F7 QTL that are associated with stronger phenotypic effects in related or unrelated apple cultivars. Conserved genomic regions conferring resistance towards the same pathogen in unrelated cultivars of the same species have

already been reported (McMullen and Simcox 1995; Miklas et al. 2000; Gebhardt and Valkonen 2001). Ideally, the F7 QTL should also be validated with other strains of *E. amylovora*, since differential virulences have been reported in the *M.* × *domestica*/*E. amylovora* pathosystem (Norelli et al. 1984). We are currently testing several strains of *E. amylovora* on individuals carrying this QTL to determine the putative existence of differential effects (C.-E. Durel et al., in preparation).

It would be interesting to determine if the gene(s) underlying this QTL is (are) similar to major resistance genes previously cloned in many other plant species (see for review McDowell and Woffenden 2003). Some nucleotide-binding site/leucine-rich repeat (NBS/LRR)-type genes have been shown to confer a partial resistance (Ori et al. 1997; Wang et al. 1998). Calenge et al. (2005) mapped 53 resistance gene analogues (RGAs) belonging to the NBS/LRR class of resistance genes (Hammond-Kosack and Jones 1997) in apple. Baldi et al. (2004) also located 18 RGAs belonging to different classes, including the NBS/LRR class. Nevertheless, none of the RGAs from both of these studies mapped close to the QTL on F7. Although it is certain that Calenge et al. (2005) and Baldi et al. (2004) did not map all possible RGA apple sequences, their results could be an indication that the QTL on F7 does not correspond to a NBS-type resistance gene. Candidate genes involved in the transduction cascade leading from pathogen recognition to the resistance phenotype or in the defence response should also be mapped to look for genetic co-localizations. Such candidates have already been identified by Venisse et al. (2002), and the genetic mapping of some of these has been initiated (data not shown). The characterization of the F7 QTL could also be achieved by looking for genes expressed differentially between individuals carrying either the favourable or the unfavourable allele at this QTL by means of a differential display approach (for example, cDNA-AFLP performed on genotype bulks).

Acknowledgements We thank Roland Chartier and Lysiane Leclout for their excellent technical work in the greenhouse. This research was based on unpublished results from the European project FAIR5 CT97-3898 (DARE: Durable Apple Resistance in Europe). It was partly supported by a grant from INRA/Région Pays de la Loire, France, allocated to the first author.

References

Ahmadi N, Albar L, Pressoir G, Pinel A, Fargette D, Ghesquiere A (2001) Genetic basis and mapping of the resistance to rice yellow mottle virus. III. Analysis of QTL efficiency in introgressed progenies confirmed the hypothesis of complementary epistasis between two resistance QTL. Theor Appl Genet 103:1084–1092

Alston FH, Phillips KL, Evans KM (2000) A *Malus* gene list. Acta Hortic 538:561–570

Baldi P, Patocchi A, Zini E, Toller C, Velasco R, Komjanc M (2004) Cloning and mapping of resistance gene homologues in apple. Theor Appl Genet 109:231–239

Bonn WG, Van der Zwet T (2000) Distribution and economic importance of fire blight. In: Vanneste J-L (ed) Fire blight: the disease and its causative agent: *Erwinia amylovora*. CAB Int, Wallingford, pp 37–53

Calenge F, Faure A, Drouet D, Parisi L, Brisset M-N, Paulin J-P, Van der Linden CG, Van de Weg WE, Schouten H, Lespinasse Y, Durel C-E (2004a) Genomic organization of resistance factors against scab (*Venturia inaqualis*), powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*) in apple. In: Tikhonovitch I, Lugtenberg B, Provorov N (eds) Proc 11th Int Congr Mol Plant-Microbe Interact. Biol Plant Microbe Interact 4:35–39

Calenge F, Faure A, Goerre M, Gebhardt C, Van de Weg WE, Parisi L, Durel C-E (2004b) A QTL analysis reveals both broad-spectrum and isolate-specific QTL for scab resistance in an apple progeny challenged with eight isolates of *Venturia inaequalis*. Phytopathology 94:370–379

Calenge F, Van der Linden CG, Van de Weg WE, Schouten HJ, Van Arkel G, Denancé C, Durel C-E (2005) Resistance gene analogues identified through the NBS-profiling method map close to major genes and QTL for disease resistance in apple. Theor Appl Genet 110:660–668

Conner PJ, Brown SK, Weeden NF (1997) Randomly amplified polymorphic DNA-based genetic linkage maps of three apple cultivars. J Am Soc Hortic Sci 3:350–359

Dondini L, Pierantoni L, Gaiotti F, Chiodini R, Tartarini S, Bazzi C, Sansavini S (2004) Identifying QTLs for fire blight resistance via a European pear (*Pyrus communis* L.) genetic linkage map. Mol Breed 14:407–418

Durel C-E, Parisi L, Laurens F, Van de Weg E, Liebhard R, Koller B, Jourjon MF (2003) Genetic dissection of partial resistance against two monoconidial strains of the new race 6 of *Venturia inaequalis* in apple. Genome 46:224–234

Gao ZS, Van de Weg WE, Schaart JG, Schouten HJ, Tran DH, Kodde L, Van der Meer IM, Van der Geest AHM, Kodde J, Breiteneder H, Hoffmann-Sommergruber K, Bosch D, Gilissen LJWJ (2005) Genomic cloning and linkage mapping of the Mal d 1 (PR-10) gene family in apple (*Malus* × *domestica*) Theor Appl Genet (in press)

Gebhardt C, Valkonen JPT (2001) Organization of genes controlling disease resistance in the potato genome. Annu Rev Phytopathol 39:79–102

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Physiol Plant Mol Biol 48:575–607

Hemmat M, Brown SK (2002) Tagging and mapping scab resistance genes from R12740-7A Apple. J Am Soc Hortic Sci 127:365–370

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular linkage map for apple. J Hered 85:4–11

Jones AL, Schnabel E (2000) The development of streptomycin-resistant strains of *Erwinia amylovora*. In: Vanneste J-L (ed) Fire blight: the disease and its causative agent: *Erwinia amylovora*. CAB Int, Wallingford, pp 335–367

King GJ, Alston FH, Batlle I, Chevreau E, Gessler C, Janse J, Lindhout P, Manganaris AG, Sansavini S, Schmidt H, Tobutt KR (1991) "The European Apple Genome mapping Project"—developing a strategy for mapping genes coding for agronomic characters in tree species. Euphytica 56:89–94

Korban SS, Janick J, Williams EB, Emerson FH (1988) "Dayton" apple. HortScience 23:927–928

Le Lezec M, Thibault B, Balavoine P, Paulin J-P (1985) Sensibilité variétale du pommier et du poirier. Phytoma Février:37–44

Le Lezec M, Babin J, Lecomte P (1990) Le feu bactérien: sensibilité de nouvelles variétés de pommes, premiers essais. L'arboriculture Fruitière 434:24–32

Liebhard R, Koller B, Gianfranceschi L, Gessler C (2003a) Creating a saturated reference map for the apple (*Malus* × *domestica* Borkh.) genome. Theor Appl Genet 106:1497–1508

Liebhard R, Koller B, Kellerhalls M, Pfammatter W, Jermini M, Gessler C (2003b) Mapping quantitative field resistance in apple against scab. Phythopathology 93:493–501

Lespinasse Y, Aldwinckle H (2000) Breeding for resistance to fire blight. In: Vanneste J-L (ed) Fire blight: the disease and its causative agent: *Erwinia amylovora*. CAB Int, Wallingford, pp 253–273

Lespinasse Y, Durel C-E, Laurens F, Parisi L, Chevalier M, Pinet C (2000) A European project: DARE—durable apple resistance in Europe (FAIR5 CT97-3898) durable resistance of apple to scab and powdery-mildew: one step more towards an environmental friendly orchard. Acta Hortic 538:197–200

Maliepaard C, Alston F, Van Arkel G, Brown L, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, Van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, Den Nijs APM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-Van Ginkel M, King GJ (1998) Aligning male and female linkage maps of apple (*Malus pumila*) using multi-allelic markers. Theor Appl Genet 97:60–73

Manzanares-Dauleux MJ, Delourme R, Baron F, Thomas G (2000) Mapping of one major gene and of QTLs involved in resistance to clubroot in *Brassica napus*. Theor Appl Genet 101:885–891

McDowell JM, Woffenden BJ (2003) Plant disease resistance genes: recent insights and potential applications. Trends Biotechnol 21:178–183

McMullen M, Simcox KD (1995) Genomic organization of disease and insect resistance genes in Maize. Mol Plant Microbe Interact 8:811–815

Miklas PN, Delorme R, Stone V, Daly MJ, Stavely JR, Steadman JR, Bassett MJ, Beaver JS (2000) Bacterial, fungal, and viral disease resistance loci mapped in a recombinant inbred common bean population (''Dorado''/XAN 176). J Am Soc Hortic Sci 125:476–481

Norelli JL, Aldwinckle HS, Beer SV (1984) Differential host × pathogen interactions among cultivars of apple and strains of *Erwinia amylovora*. Phytopathology 74:136–139

Ori N, Eshed Y, Paran I, Presting G, Aviv D, Tanksley S, Zamir D, Fluhr R (1997) The *I2C* family from the wilt disease resistance locus *I2* belongs to the nucleotide binding, leucine rich repeat superfamily of plant resistance genes. Plant Cell 9:521–532

Phipps JB, Robertson KR, Rohrer JR (1991) Origins and evolution of subfamily Maloideae (Rosaceae). Syst Bot 16:303–332

Thomson S (2000) Epidemiology of fire blight. In: Vanneste J-L (ed) Fire blight: the disease and its causative agent: *Erwinia amylovora*. CAB Int, Wallingford, pp 370–382

Vanneste J-L (2000) What is fire blight? Who is *Erwinia amylovora*? How to control it? In: Vanneste J-L (ed) Fire blight: the disease and its causative agent: *Erwinia amylovora*. CAB Int, Wallingford, pp 1–6

Van Ooijen JW, Maliepaard C (1996) MAPQTL version 3.0: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen

Venisse JS, Malnoy M, Faize M, Paulin JP, Brisset MN (2002) Modulation of defence responses of Malus spp during compatible and incompatible interactions with Erwinia amylovora. Mol Plant Microbe Interact 15:1204–1212

Wang GL, Ruan DL, Song WY, Sideris S, Chen LL, Pi LY, Zhang S, Zhang Z, Fauquet C, Gaut B, Ronald P (1998) *Xa21D* encodes a receptor-like molecule with a leucine-rich-repeat domains that determine race specific recognition and is subject to adaptative evolution. Plant Cell 10:765–779

Yamamoto T, Kimura T, Saito T, Kotobuki K, Matsuta N, Liebhard R, Gessler C, Van de Weg WE, Hayashi T (2004) Genetic linkage maps of Japanese and European pears aligned to the apple consensus map. Acta Hortic 663:51–56

Young N (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479–501