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Identifying QTLs for fire-blight resistance via a European pear (*Pyrus communis* L.) genetic linkage map

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Abstract

The existence of different levels of susceptibility to fire blight (*Erwinia amylovora*) in European pear (*Pyrus communis* L.) cultivars suggests that it is possible to identify QTLs related to resistance in pear germplasm. Given the polygenic nature of this trait, we designed two genetic maps of the parental lines ‘Passe Crassane’ (susceptible) and ‘Harrow Sweet’ (resistant) using SSRs, MFLPs, AFLPs, RGAs and AFLP-RGAs markers. RGA-related markers should theoretically map in chromosome regions coding for resistance genes. The ‘Passe Crassane’ map includes 155 *loci*, for a total length of 912 cM organised in 18 linkage groups, and the ‘Harrow Sweet’ map 156 *loci*, for a total length of 930 cM divided in 19 linkage groups; both maps have a good genome coverage when compared to the more detailed apple maps. Four putative QTLs related to fire blight resistance were identified in the map. A suite of molecular markers, including two AFLP-RGAs, capable of defining resistant and susceptible haplotypes in the analysed population was developed.

Abbreviations: AFLP – amplified fragment length polymorphism; cv – cultivar; HS – Harrow Sweet; ISV – index of varietal susceptibility; LG – linkage group; LOD – log of odds; MAS – marker assisted selection; MFLP – microsatellite-anchored fragment length polymorphism; PC – Passe Crassane; PCR – polymerase chain reaction; QTL – quantitative trait locus; RAPD – random amplified polymorphic DNA; RGA – resistance gene analog; SSCP – single strand conformational polymorphism; SSR – simple sequence repeat

Introduction

Few plant diseases are as devastating to cash crops as fire blight (*Erwinia amylovora*) is to *Maloideae* (apple, pear and other rosaceous species). Indeed, despite quarantine measures, the disease continues to spread throughout western, central and southern Europe (Jock et al. 2002). The lack of completely effective control strategies has accentuated the importance of breeding as one of the most promising tools of an integrated disease-management programme. While most European pear (*Pyrus communis* L.) cultivars are highly susceptible to infection, it is also known that different sources of resistance with a wide range of responses to the pathogen occur in pear germplasm (van der Zwet and Bell 1984; Thibault et al. 1989; Lespinasse and Aldwinckle 2000). Fire-blight resistance in pear is known as a quantitative trait (Le Lézec et al. 1985; Dondini et al. 2002), so that the identification of resistance QTLs can be pursued by constructing a genomic map on a specific segregating F1 population and evaluating the resistance of each

seedling. Most of the reported pear maps (Weeden et al. 1994; Iketani et al. 2001) are based on dominant RAPD markers, so that their usefulness is usually limited to the F1 population in which they were developed. While it is also known that SSR markers offer several advantages over other molecular markers for genetic mapping because of their codominant inheritance, the large number of alleles per *locus* and their abundance in any genome, only a few pear-specific SSR markers have so far been isolated (Yamamoto et al. 2002). It has been demonstrated that some SSR-based apple genetic maps (Maaliepaard et al. 1998, Liebhard et al. 2002) can theoretically facilitate the construction of a pear map because of the good degree of synteny between the two species (Yamamoto et al. 2001), and a first pear map enriched with codominant markers was built using this approach (Yamamoto et al. 2002). The need to denominate or orientate several linkage groups and to enhance the map in codominant SSR-like markers led us to develop and map, MFLP markers (a PCR technique in which an AFLP primer is used in combination with a primer anchored to SSR motifs). This technique is described by Yang et al. (2001) and was previously applied only in fingerprinting.

Several disease-resistance genes have been isolated in recent years from a wide range of plant species (from Whitham et al. 1994 until Cooley et al. 2000). Cloned gene structure shows a remarkable degree of similarity and specific conserved domains play a role in protein-protein interaction and signal transduction (Staskawicz et al. 1995). Based on the assumed structure of their protein products, these genes have been grouped into several classes, the most prevalent encoding proteins with a predicted nucleotide-binding site (NBS) attached to a C-terminal leucine-rich repeat (LRR) of variable length. The conservative structure of these genes makes it possible to design primers, whether degenerate or not, to amplify RGAs (resistance gene analogs) by PCR. This approach has been successfully applied to isolated NBS-LRR genes from several monocot and dicot species (Leister et al. 1996; Kanazin et al. 1996). Michelmore and Meyers (1998) reported that RGAs are commonly organised in clusters and genes encoded within a single cluster may determine resistance to different pathogens and races; thus, on the basis of their clustered distribution and by inference from other cell-cell recognition systems, they postulated RGAs as encoding functionally and evolutionarily related members of plant/pathogen recognition systems.

A potential approach to identifying QTLs related to fire blight resistance is to integrate the map with RGA markers. Dondini et al. (2002) identified pear RGAs from the fire-blight resistant cvs ‘Old Home’, ‘US 309’ and ‘Harrow Sweet’ using heterologous primers to amplify the DNA fragments between the *p-loop* and *GLPL* motifs. An AFLP-RGA technique (a modified-AFLP in which one of the two AFLP primers is substituted by a primer designed on highly conserved resistance sequences: Hayes et al. 2000) has also been used to identify putative disease-resistance polymorphic markers.

Here we report two SSR-enriched European pear maps in which the main QTLs related to fire-blight resistance were identified. The analysis of phenotypic and molecular data made it possible to define the resistant and susceptible haplotypes in the analysed population (‘Passe Crassane’ x ‘Harrow Sweet’) and, hence, a marker-assisted approach (MAS) can now be developed for easier selection of fire-blight resistant genotypes in breeding programs as well as to obviate the difficulties and costs of resistance evaluation and the limitations due to the fact that *E. amylovora* is a quarantine organism in several countries.

Materials and methods

Plant material and inoculation with E. amylovora

A segregating F1 population derived from ‘Passe Crassane’ x ‘Harrow Sweet’ (99 seedlings) was used to investigate resistance-segregation pattern and for molecular analyses. Each seedling and the parental lines were grafted (two buds each) on three potted quince rootstocks and inoculated by bisecting the two youngest leaves with scissors dipped in 7×10^8 cfu ml^{-1} suspension of the local *E. amylovora* strain OMP-BO 1077.7/94. Three independent greenhouse-resistance assessments (summer 1999, spring 2000 and spring 2001) were carried out on one plant per seedling for an average total of 10 analysed shoots/seedling; symptom progression was monitored for about one month after inoculation. The non-growing shoots were excluded from analysis since shoot growth is essential for a better evaluation of resistance (Le Lézec et al. 1985; Bell et al. 1990). Indexes of frequency and severity of infection were determined and their weighted mean was used to calculate a susceptibility index (ISV) after Le Lézec et al. (1997):

Table 1. List of the heterologous PCR primers used to amplify conserved regions of Resistance Gene Analogs (RGA).

Name	Primer Sequence	Designed on R-gene sequence of
P1	ATGGGAAGCAAGTATTCAAGGC	*Pto* gene of tomato
P2	TTGGCACAAAATTCTCATCAAGC	
T1	GGGGGAGTCGGTAAAACAACA	*N* gene of tobacco
T2	TTGAGGGCTAAAGGAAGGC	
B1	GGGTGGAATAGGCAAGAC	*L6* gene of flax
B2	CTTCAGAGTCAATGGAAGTCC	
B3	GGGTTCAACGCATCATAACT	
INO1	IGGIGGIATIGGIAAIAC	Leister et al. (1996)
INO2	ITTIAGIGTIAAIGGIAGICC	

$$\frac{(10\times Ns1)+(30\times Ns2)+(50\times Ns3)+(70\times Ns4)+(90\times Ns5)}{\text{N}^\circ\text{ of shoots for each genotype}},$$

where N represents the number of infected shoots in each severity class (S1 to S5); phenotypic data were used for QTL analyses.

DNA extraction and marker analyses

For each genotype, 4g of fresh young leaves were ground in liquid nitrogen, and DNA extraction was performed following a modified CTAB protocol (Maguire et al. 1994). DNA quality was checked in 1% agarose gel and the amount was estimated by image analysis (Molecular Analyst 1.4.1 software; Bio-Rad, USA). SSRs, RGAs, AFLPs, AFLP-RGAs and MFLPs markers were used for map construction.

A total number of 112 apple SSR markers, all reviewed by Liebhard et al. (2002) except VFC9 (Vinatzer pers. comm.) were screened. PCR amplifications were performed in a MJ PTC-200 Peltier Thermal Cycler under the following conditions: 50 ng genomic DNA, 1 μM each primer, 100 μM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.7 U (5U/μl) *Taq* DNA polymerase (Amersham, UK). A first cycle of denaturation at 94 °C for 2.5 min was followed by 32 cycles of annealing (60 °C for 45 s), extension (72 °C for 1 min) and denaturation (94 °C for 30 s), with a final extension of 10 min at 72 °C.

Specific primer combinations were designed on the most divergent regions of RGA clones (Dondini et al. 2002) identified by ClustalW analysis. A segregating polymorphism for the RGA marker called US60BT (Forward: CGTTGATGAACCACTACCTGT; Reverse: GATGACTGATCAAGTGCTGAG) was obtained by SSCP after Slabaugh et al. (1999) and by performing the PCR reaction (annealing at 55 °C) after Dondini et al. (2002). Additional RGA markers were identified using heterologous primers, whether degenerate (INO 1 and INO 2, Table 1) or not (B1 and T2, Table 1), as reported in Dondini et al. (2002).

For AFLP, an aliquot of 500 ng genomic DNA was digested with restriction enzymes *Mse*I and *Eco*RI or *Pst*I (Vos et al. 1995; Vuylsteke et al. 1999, respectively). Restriction fragments were ligated with double-stranded adapters, and a pre-amplification was done using a primer with one added selective nucleotide (*Mse*I + C and *Eco*RI + A or *Pst*I + A). The reaction mix was diluted 1/20, and an aliquot of 5 μl was used for the final amplification with two primers each having three selective nucleotides. Sixty-seven primer combinations were preliminarily screened in the F1 population derived from 'Passe Crassane' and 'Harrow Sweet' to analyse the most polymorphic.

The peculiar modified-AFLP technique called AFLP-RGA was performed after Hayes et al. (2000). It consists of a standard AFLP protocol up to pre-amplification with *Eco*RI + 1 and *Mse*I + 1 selective primers. An aliquot of these reaction products (equivalent to 25 ng of DNA) was then used in a second round of amplification employing the *Eco*RI + 3 or *Mse*I + 3 primers and a heterologous primer specific for RGA amplification; these RGA heterologous primers are reported in Table 1. Seven primer combinations were analysed in the F1 mapping population (P1-M55, P2-M51, B3-M51, B3-M55, T1-E32, T2-E32, B2-M51).

Another modified-AFLP technique, called MFLP, was performed to identify markers containing microsatellite domains (Yang et al. 2001). A standard AFLP is performed to pre-amplification (with *Eco*RI + 1 and *Mse*I + 1 selective primers) and is followed by a final amplification carried out by coupling an AFLP primer (*Ec*oRI + 3 or MseI + 3) and a primer anchored to an SSR sequence with five additional bases

Table 2. List of the primers designed on the MFLP-derived sequences. R represent the microsatellite-anchored primer.

Name	Excised bands	Primer sequence
C	E32R-3	GATTGCGTCTGAGACTG
H	M62R-2	CACCCTATACCTACTGC
I	E36R-2	GGGCATTGTGAGTCATG
L	E32R-4	GAGTATACCTGTTGGAAG
N	E36R-1	CAGCCATGAAATTCGAC

(called RAMP primer: AGCTGCTCTCTCTCTC). As suggested by Yang et al. (2001) to obtain more specific amplification products, polymorphic bands were excised from poly-acrylamide gels and cloned in pGEM vector (Promega, USA). A new primer was designed on each sequence (Table 2) and used in combination with the above RAMP primer. The PCR amplification protocol was the same as that used for the SSRs but at lower annealing temperature (51 °C).

Electrophoresis conditions and gel staining

DNA fragments identified with the different PCR techniques (except SSCP) were separated in 5% poly-acrylamide gels (65W constant). For the SSCPs, a 14% poly-acrylamide concentration (as suggested by Hauser et al. 1998) was used and the gels were run at 6W overnight. PCR-products were visualized by silver staining as per silver sequencing kit (Promega, USA).

Map construction

JoinMap 3.0 (van Ooijen and Voorrips, 2002), including the Kosambi mapping function, was used for the construction of genetic linkage maps of ‘Passe Crassane’ and ‘Harrow Sweet’. A LOD score of 6.0 was used to group markers. Given that the population was an F1 of an outbreeding plant species, the SSR markers that segregated as <abxab> (presence of the same SSR alleles in both parents of the F1 population) or <aaxbb> (presence of the same homozygous allele in a parent of the cross and of a second homozygous allele in the second parent), as well as the AFLP markers that segregate as <a0xa0>, were not scored. A single *locus* analysis was performed to identify markers showing a distortion from the expected segregation ratio. Skewed markers (significance 0.01 after χ^2 analysis) were discarded only if they introduced relevant discrepancies during map construction. During the scoring of each polymorphism, all the parental genotypes were evaluated three times. The denomination of the pear linkage group was decided by the distribution of the apple SSR on analogy with the apple maps available (Liebhard et al. 2002). The linkage groups in which both parents lacked the apple SSR markers were named with letters.

QTL analysis

Interval Mapping analysis using MapQTL 4.0 (Van Ooijen et al. 2002) was performed for QTL identification. Three-year pooled data of fire-blight resistance as severity, incidence and ISV per seedling were used. The ‘permutation test’ was used to determine the significance threshold of the LOD score after Interval Mapping analysis: the frequency distribution of the maximum LOD score is determined over a set of iterations and in each iteration the quantitative trait data are permuted (1000 permutations were used) over the individuals while the marker data remain fixed. A LOD threshold of 1.3 (corresponding to a probability of over 95%) was used for declaring a putative QTL as significant. The percentage of the variation explained and the average of phenotypic effect by the significant QTL were estimated for each inoculation assay.

Results and discussion

SSRs

A large proportion (78.8%) of the 112 apple SSR markers screened produced reproducible amplifications; another 24 SSRs tested (21.2%) did not show any amplification product or the patterns were not clear or polymorphic (data not shown). Some of the SSRs showing a useful segregation in our mapping of the F1 population (41, 37.2%; Table 3) evinced a dominant segregation and therefore were mapped only in one parent (ten *loci* in ‘Passe Crassane’ and seven in ‘Harrow Sweet’); twenty-eight *loci* showed a codominant segregation pattern and map in both parents. The SSR CH04C06 marked two *loci,* as in apple (Liebhard et al. 2002). Two SSRs, i.e., CH03H03 and CH01F02, mapped in the analysed population in more than one *locus* (three and two *loci*, respectively) while in apple they are reported as single *locus*. By contrast, in the pear F1 population used, the multilocus apple SSRs CH01D03,

Table 3. Segregation data and map position of the apple SSR markers that segregate in the pear F1 population 'Passe Crassane' x 'Harrow Sweet' (PC x HS). Null alleles are indicated as '0'.

SSR	Segregation	χ^2	LG	SSR	Segregation	χ^2	LG
CH01C06	abxaa	1.5	8	CH04C06-1	abxcd;	0.5;	10
CH01D03	abxac	3.4	4	CH04C06-2	abxaa	0.8	17
CH01D08	abxac	0.7	15	CH04C07	a0xbc	0.1	14
CH01D09	abxac	0.1	12	CH04C10	abxaa	0.1	17
CH01F02-1	aaxab	0.8	4	CH04D02	abxcd	0.3	14
CH01F02-2	abxcc	0.8	12	CH04E05	abxcd	0.0	7
CH01F07	aaxbc	2.9	10	CH04F06	abxaa	0.0	14
CH01G05	abxaa	0.0	14	CH04G04	abxaa	1.0	12
CH02B07	abxcd	1.7	10	CH04G09	aaxbc	2.1	10
CH02C02	abxcd	3.2	4	CH04H02	aaxab	0.8	11
CH02C11	abxac	4.5	10	CH05A02	abxcc	0.8	8
CH02F06	abxac	0.1	2	CH05A03	abxc0	0.0	9
CH02H11a	abxaa	2.7	4	CH05C06	abxac	1.5	16
CH03D02	abxcd	0.3	11	CH05C07	abxac	0.0	9
CH03D10	abxcd	0.5	2	CH05D03	abxcd	0.3	14
CH03D11	abxcd	2.7	10	CH05D04	abxac	0.0	12
CH03D12	aaxab	0.2	6	CH05D11	abxcd	0.0	12
CH03G06	abxcd	0.5	14	CH05E06	abxcd	1.7	5
CH03G07	abxac	0.4	3	CH05F04	abxac	0.8	13
CH03H03-1	abxcd	0.2	2	CH05F06	abxc0	2.2	5
CH03H03-2	aaxab	0.3	13	CH05G01	a0xbc	0.4	11
CH03H03-3	aaxab	0.4	14	CH05G11	abxcc	0.0	14
				VFC9	abxac	0.1	1

CH04G09 and CH05A02 amplify only a single locus. In sum, 37 and 35 apple SSRs mapped in 'Passe Crassane' and 'Harrow Sweet', respectively.

Pear RGAs

Three primer combinations (INO1/2, B1/T2 and US60BT) produced 7 segregating fragments in the F1 population derived from 'Passe Crassane' and 'Harrow Sweet'– 4 mapped in 'Passe Crassane' and 3 in 'Harrow Sweet'. PCR using the primer combinations INO1/2 and B1/T2 produced 6 polymorphisms (3 each) smaller than 500bp and directly detectable in 5% poly-acrylamide gels. Most of the RGAs (except BT350 and BT445) mapped in the LG 5 (Figure1a and b), indicating that in pear these genes may also be organised in clusters (Michelmore and Meyer 1998).

AFLPs and AFLP-RGA

The number of polymorphic bands per primer combination ranged from 0 (E32/M54, E32/M58, E32/M59, E33/M50, E33/M55, E34/M54, E34/M56, E35/M55, E39/M59, E43/M50) to 16 (E35/M48), with an average number of 5.0. The most polymorphic primer combinations produced 223 reproducible polymorphisms. Approximately 90% (199/223) of the scored AFLP were mapped, i.e., 98 in 'Passe Crassane' and 101 in 'Harrow Sweet'; about 10% of the polymorphisms collected (24/223) did not find a position on the map. The number of polymorphic AFLP-RGA fragments per primer combination ranged from 1 (B2/M51) to 6 (P2/M51), the average being 3.8. Of the 29 polymorphisms scored in the whole population, 10 mapped in 'Passe Crassane' and 14 in 'Harrow Sweet'.

MFLP

After the first experimental step (an AFLP protocol) a number of stutter bands for each primer combination were found, although the amplification patterns were unclear and difficult to use for mapping. According to Yang at al. (2001) the sequencing of excised bands made it possible to design more specific primers and amplify very clear polymorphisms. Five primer combinations identify six different *loci*; in fact, the marker '*I*' is multilocus. Three MFLP markers are codominant ('*H*' '*I1*' and '*N*') and the other three ('*C*','*I2*' and '*L*') are dominant.

Table 4. Total number of scored markers, number and percentage of skewed markers (χ^2 threshold 0.01) and their presence/absence in the map of 'Passe Crassane' x 'Harrow Sweet' (PC x HS).

Markers	Total	Skewed markers		Mapped		Unmapped	
	number	number	%	PC	HS	PC	HS
SSR	45	4	8.9	3	1	–	–
MFLP	6	2	33.3	1	1	–	–
AFLP	223	30	13.4	9	8	3	10
AFLP-RGA	29	8	27.6	2	1	4	1
RGA	7	2	28.6	1	–	–	1
Total	310	46	14.8	16	11	7	12

Skewed markers

A small amount of skewed segregation was found for all types of markers (Table 4). The markers with the highest percentage distortion were found using MFLPs (32%, 2/6), AFLP-RGAs (28%, 8/29) and RGAs (27%, 2/7); the AFLPs and SSRs evinced the lowest percentages (about 10%). All the skewed SSR and MFLP markers were mapped and about 50% of the other skewed markers (AFLP, AFLP-RGA and RGA) were unmapped. Thus, of the 15% skewed markers (on a dataset of more than 300), only 6% went unmapped.

Map construction

The 'Passe Crassane' map included 155 *loci* (98 AFLPs, 37 SSRs, 6 MFLPs, 4 RGAs, 10 AFLPs-RGA) for a total length of 912 cM. This map is organised in 18 LGs ranging from 7.0 to 92.9 cM with an average size of 50.6 cM and an average distance between *loci* of 5.8 cM (Figure 1a and b). The 'Harrow Sweet' map included a total of 156 *loci* (101 AFLPs, 35 SSRs, 3 MFLPs, 3 RGAs and 14 AFLPs-RGA) distributed in 930 cM of length. This map is organised in 19 LGs ranging from 12.8 to 79.7 cM with an average size of 49.3 and an average distance of 6.0 cM between loci (Figure 1a and b). The sizes of these maps are comparable with the results reported for apple by Maliepaard et al. (1998) and by Yamamoto et al. (2002) in pear.

Only the codominant markers were used to align the two parental maps. The AFLP markers that segregate as < a0xa0 > were not considered because, as suggested by Maliepaard et al. (1997), they could be used as allelic bridges for combining the parental maps but they provide little linkage information. Thus, in aligning two apple maps, Maliepaard et al. (1998) assigned these markers to linkage groups but not used in map construction because the recombination frequency estimates were typically inaccurate (Maliepaard et al. 1997).

It proved possible to align a total 14 LGs of 'Passe Crassane' and 'Harrow Sweet' using common SSR *loci*. Of these, the LGs 2, 4, 5, 7, 9, 10, 11, 12, 14 are well consolidated and oriented by the presence of at least two codominant SSR anchor *loci* (Figure 1a and b), whereas the LGs 1, 3, 13, 15, 16 were coupled by only one codominant SSR *loci*. A codominant MFLP marker made it possible to denominate the LG HS8, coupling it with the correspondent LG of 'Passe Crassane'. Linkage group 6 of 'Passe Crassane' and 17 of 'Harrow Sweet' were not identified because the apple-SSRs in these LGs are not segregating in one parent. However, they should be represented in the maps because a further 5 LGs (2 in 'Passe Crassane' and 3 in 'Harrow Sweet'), defined only by AFLP markers and indicated by letters (Figure 1a and b), are available. This is also because the number of identified LGs is slightly larger than the seventeen expected from the chromosome number.

The 'Harrow Sweet' LG 2 (HS2) is still divided into two parts (they join at LOD 3.0 with a gap of 32 cM) but the presence of the SSRs CH02F06 and CH03D10 enables alignment with the homolog LG of 'Passe Crassane' (Figure 1a). PC4 and HS4 are denominated by the presence of the codominant SSR CH01D03 (Figure 1a), even if it is reported that this marker in apple is a multilocus marker and maps in LG 4 and 12 (Liebhard et al. 2002). The presence of the first locus of CH01F02 in HS4, which in apple maps in LG 12 (Liebhard et al. 2002), was not considered ambiguous because this marker is multilocus in pear; moreover, the second locus of CH01F02 maps in PC12. Finally, the PC12 and HS12 are un-

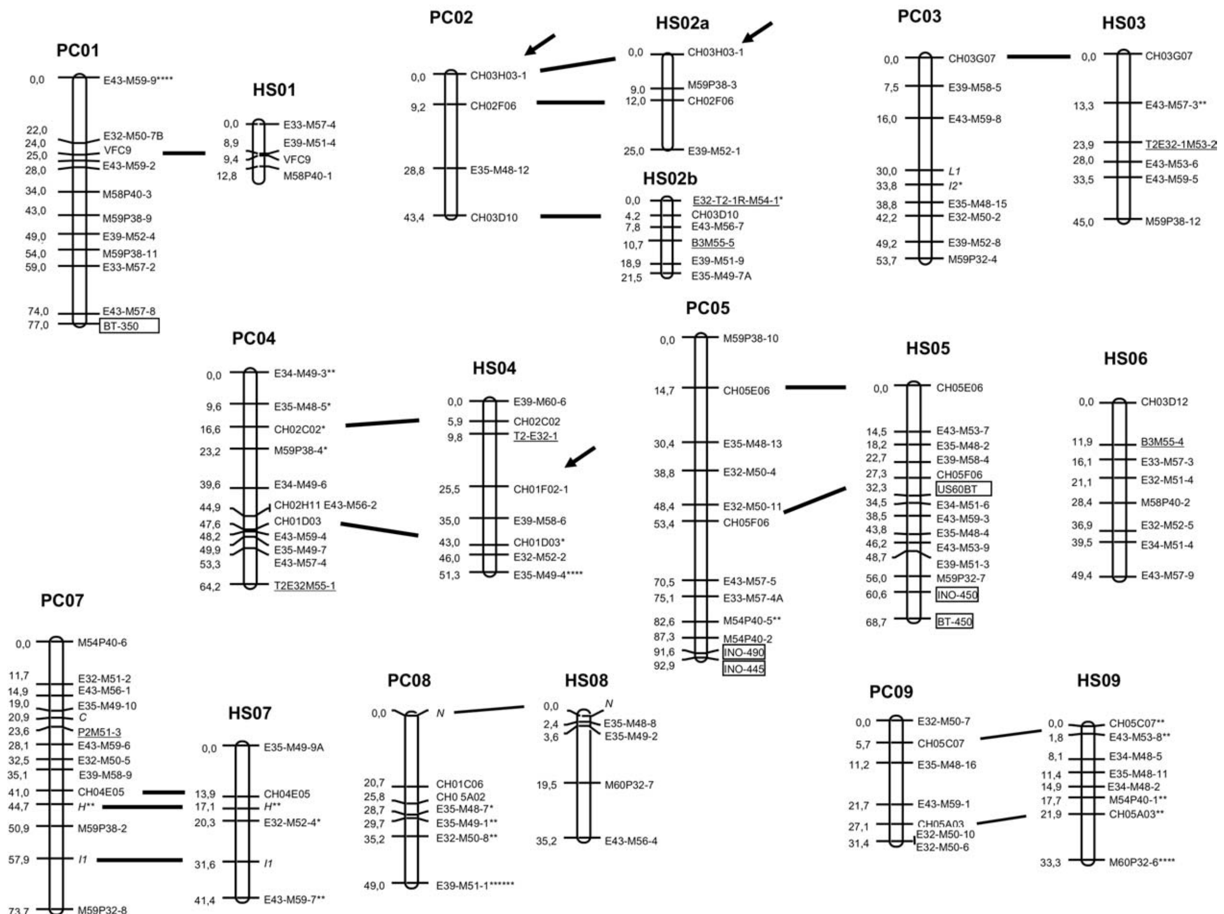

Figure 1a and b. Genetic linkage map of the pear varieties 'Passe Crassane' (PC) and 'Harrow Sweet' (HS). Multilocus SSR markers are indicated by arrows, RGAs by boxes, AFLP-RGAs are underlined and MFLPs are in *Italics*.* indicates the distorted segregation (significance after χ^2 analysis: *P 0.05;**P 0.01; ***P 0.005;****P 0.001; *****P 0.0005; ******P 0.0001).

ambiguously denominated by the presence of many codominant SSRs.

PC17 is denominated by the presence of two SSR loci with dominant segregation pattern (CH04C10 and CH04C06-2), although in future confirmation may be provided through mapping the *S-locus* alleles, which in apple are located in this LG. HS13 is still indicated in two sub-groups (HS13a and HS13b). The presence of CH05F04 in HS13a and PC13 clearly identify the two homologs. HS13b includes one of the CH03H03 loci of pear while in apple it is reported only in LG 13 (Liebhard et al., 2002). Consequently, it is possible that this latter LG represents part of a different chromosome (Figure 1b).

Only two SSRs, i.e., CH02F06 and CH01F02, reported by Yamamoto et al. (2002) are found in our work. The former makes it possible to align LG 11 of 'Bartlett' and 'Hosui' with LG 2 of 'Passe Crassane' and 'Harrow Sweet'. While the latter identified LG 1 of 'Bartlett' and 'Hosui', aligned with LG 17 in apple, in the present paper is a multilocus marker that maps in LG 12 and LG 4 of 'Passe Crassane' and 'Harrow Sweet', respectively. This discrepancy between maps cannot be easily explained since 'Harrow Sweet' derives directly from 'Bartlett' (the pedigree is 'Bartlett' x 'Purduet').

Fire-blight resistance QTLs

Disease incidence, severity and ISV from three years of experimental inoculations with *E. amylovora* were pooled and the distribution in classes (Table 5) confirmed the polygenic nature of the trait (Le Lézec et al. 1985). As reported (Le Lézec et al. 1985; Thibault

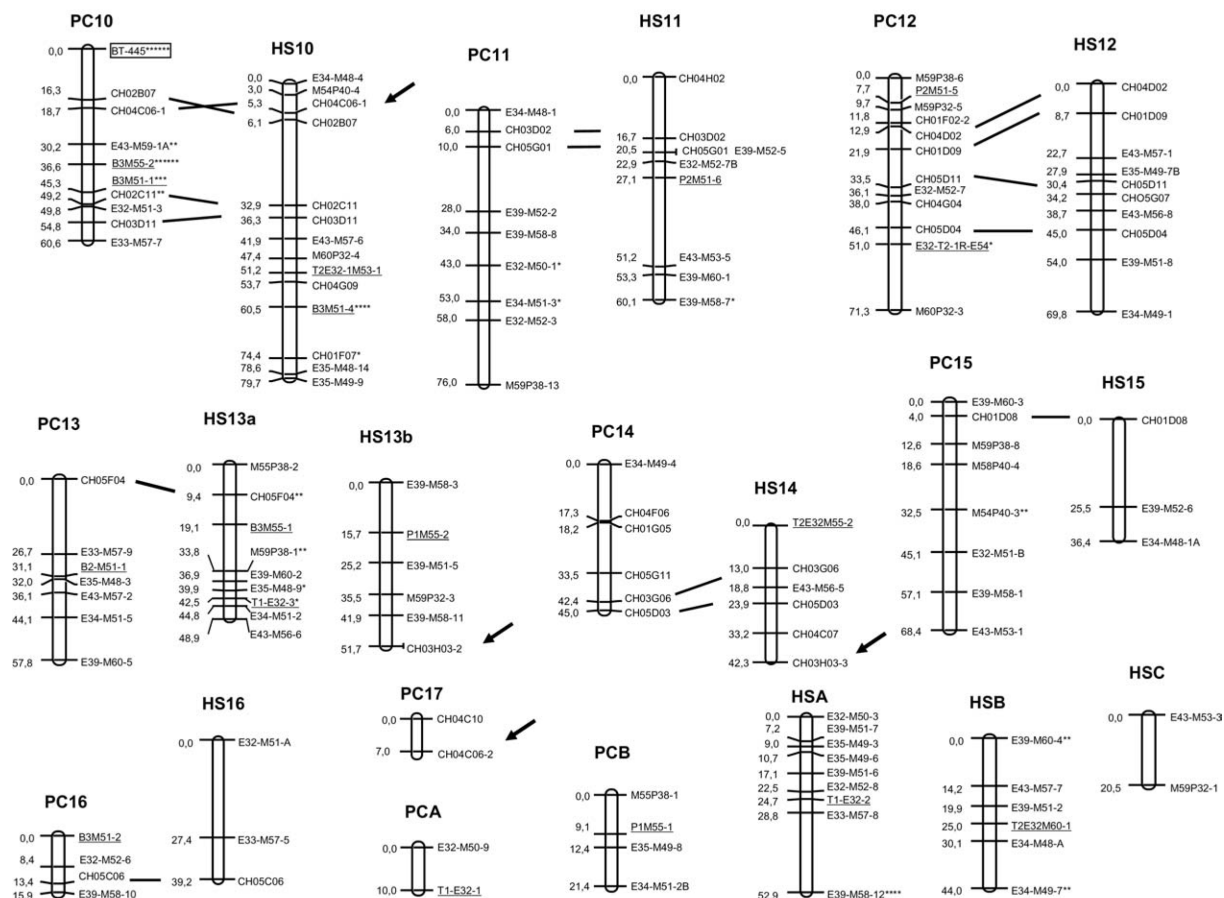

Figure 1a and b. Continued

Table 5. Evaluation of the degree of resistance to fireblight of the individuals derived from the F1 population 'Passe Crassane' x 'Harrow Sweet': disease incidence, severity and ISV data collected during three years of analyses and used to group all the seedlings in five classes. * indicates the class positions of the resistant cultivar 'Harrow Sweet' and + that of the susceptible 'Passe Crassane'.

Class	Range	% of individuals Incidence	Severity	ISV
1	0–20	6.2*	15.7	10.4
2	20.1–40	11.5	20.8*	23.9*
3	40.1–60	13.5	13.5	18.8
4	60.1–80	11.5	24.0	27.1
5	80.1–100	57.3+	26.0+	19.8+

et al. 1989; Le Lézec et al. 1997) a certain degree of discrepancy among the data collected in the three years of assessments was observed. It is known that each pear genotype may develop different degrees of symptom intensity even within the same assessment. Indeed, such well-known 'resistant' genotypes as 'Harrow Sweet' can show damaged shoots. The different shoot growth rate in any given plant can determine different levels of infection and the non-actively growing shoots appear resistant even in susceptible seedlings (Bell et al. 1990). To decrease this variability, the ISV index was developed more than ten years ago to take into account simultaneously frequency and severity data (Le Lézec et al. 1985; Le Lézec et al. 1997). QTL analysis was performed using both the indices calculated for each seedling and the data ranked in classes of resistance/susceptibility.

QTL analysis (by Interval Mapping) identifies, for all the phenotypic parameters used, four regions of 'Harrow Sweet', the resistant parent, significantly associated with fire-blight resistance (LG HS2a and b, HS4 and HS9; Figure 2, Table 4); no QTLs related to resistance were found in susceptible 'Passe Crassane'. The most significant association is between the SSR markers CH03H03-1 and the AFLP M59P38-3 in

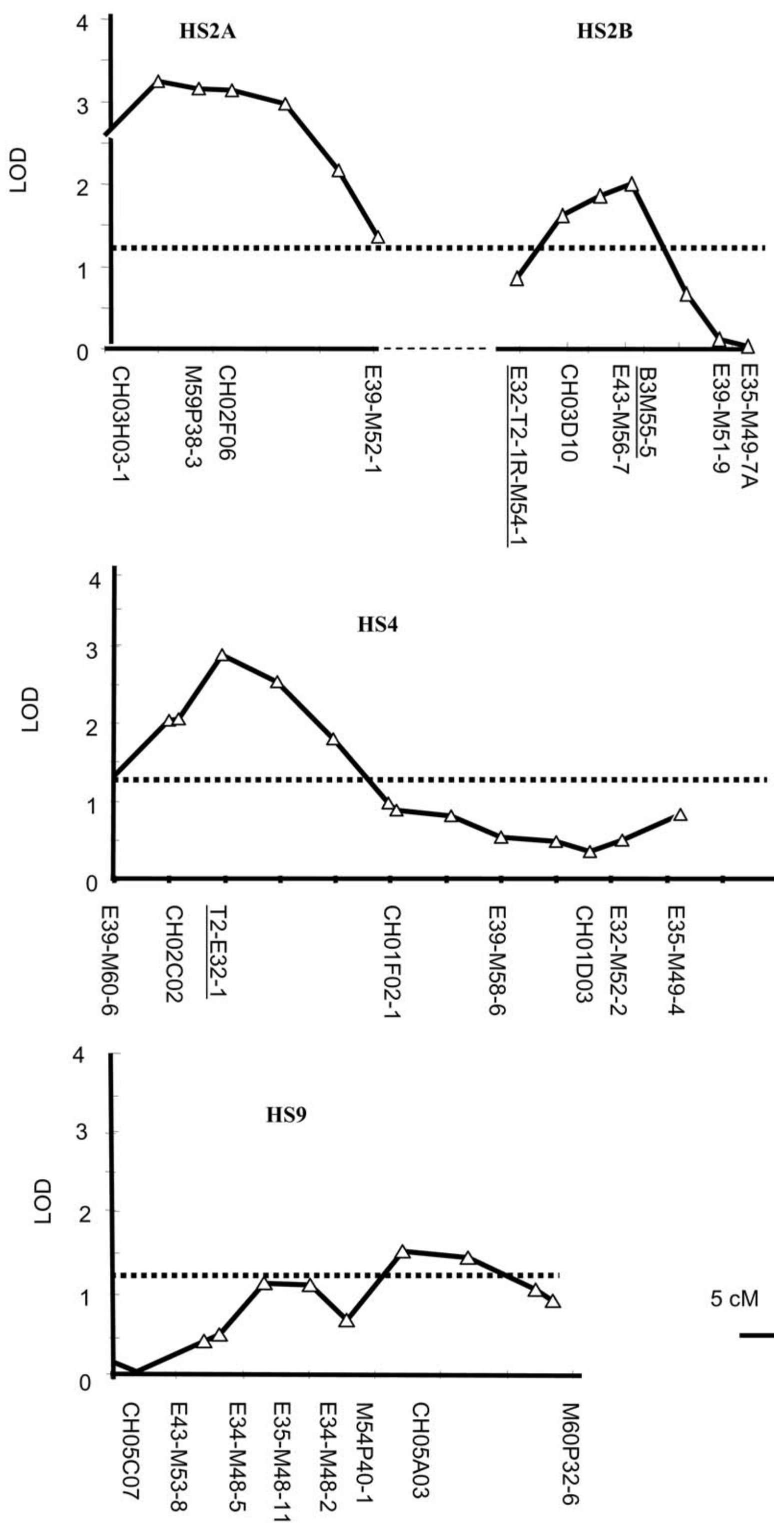

Figure 2. QTLs (for ISV trait) of fire blight resistance identified on the linkage groups of the fire blight-tolerant cultivar 'Harrow Sweet'. AFLP-RGA markers are underlined. The probability of the association of the markers to the 'resistance trait' (after Interval Mapping analysis) is indicated as LOD score. A black line indicates the LOD threshold of 1.3 (corresponding to 95% of probability) determined after 'permutation test' by using MapQTL 4.0.

Table 6. QTLs of fire-blight resistance identified in the linkage groups of the fire blight-resistant cultivar ‘Harrow Sweet’. The probability of the association of the markers to the ‘resistance trait’ (after Interval Mapping analysis) is indicated as LOD score. Markers reported correspond to the maximum LOD score observed. The percentage of the explained variance by the QTLs (% exp.) is also reported; the columns + and – indicate the average of phenotypic effects for the ‘plus QTL’ and ‘minus QTL’ alleles. The allele responsible for the phenotypic effects is indicated in brackets.

Marker	LG	Incidence				Severity				ISV			
		LOD	% exp	–	+	LOD	% exp	–	+	LOD	% exp	–	+
M59P38-3 <aaxab>	2a	4.83	24.6	1.33(–a)	1.76(–b)	3.31	16.6	1.33(–a)	1.75(–b)	3.25	16.4	0.92(–a)	1.29(–b)
B3M55-5 <aaxab>	2b	2.60	11.8	1.30(–a)	2.25(–b)	2.13	9.9	1.30(–a)	2.24(–b)	2.06	9.6	0.95(–a)	1.60(–b)
T2E32-1 <aaxab>	4	1.97	9.5	1.69(–a)	1.77(–b)	2.45	8.7	1.60(–a)	1.80(–b)	2.64	12.0	1.19(–a)	1.39(–b)
CH05A03 <abxc0>	9	1.50	6.9	1.37(–c)	1.81(–0)	1.83	8.4	1.37(–c)	1.81(–0)	1.85	8.5	1.01(–c)	1.25(–0)

HS2a; other significant associations are the AFLP-RGA B3M55-5, which is closely associated with SSR CH03D10) in HS2b, AFLP-RGA called T2E32-1, which maps next to CH01F02) in HS4, and SSR CH05A03 in HS9 (Figure 2). For all three parameters considered, even the use of the indices per genotype instead of the classes did not affect the position and the LOD scores of the QTLs. The percentages of the explained variance for the different traits ranged between 6.9 and 21.9, thereby providing further support for the polygenic nature of fire-blight resistance (Table 6). An indicative value of about 50% of the total variance explained by all the QTLs also indicates that some markers may be positioned close to the real QTL peak. The QTL positioned in LG HS2a appears to be more evident when associated with the data for the incidence of the disease, whereas the reverse was observed in LG HS9 (Table 6). The ISV and severity QTLs showed a very similar trend. The presence of two AFLP-RGAs among the markers identifying the QTLs may indicate the presence of a resistance gene, which may be involved in the expression of the resistance trait itself.

Another indication of the polygenic nature of fire-blight resistance can be found by analysing the average phenotypic effects associated with the ‘plus QTL alleles’ for the different traits (incidence, severity and ISV) used for the statistics (Table 6). These data also confirm that each QTL made a small contribution to resistance expression. In any case, the genotypes sharing all the ‘plus QTL alleles’ (10 seedlings) or the ‘minus QTL alleles’ (5 seedlings) show a very different response to pathogen inoculation, thereby confirming that the marker associations are significant. If we consider the 5 markers that most closely indicate the position of the putative QTLs (CH03H03-1, M59P38-3, B3M55-5, T2E32-1 and CH05A03, Figure 3), 80% of the genotypes that share all the ‘plus alleles’ are grouped in the first two ISV classes and 100% of the genotypes that share all the ‘minus alleles’ are grouped in class 5 (the most susceptible). Two genotypes sharing all the plus alleles in the ISV classes 3 and 4, respectively, were also observed (Table 5). This discrepancy might depend on the variability of the phenotypic data collected after experimental inoculation with *E. amylovora*, a phenomenon also reported by Le Lézec et al. (1985); alternatively recombinants may occur between certain markers and the real position of a QTL. It also suggests that other QTLs might be identified in the resistant cv. ‘Harrow Sweet’.

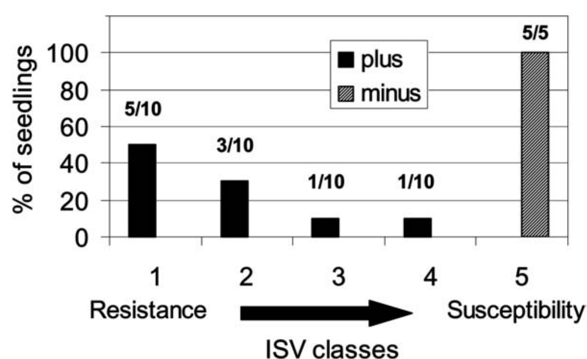

Figure 3. Distribution of the genotypes sharing all the ‘plus’ (black bars) and ‘minus’ (white and black bars) QTL alleles in the five ISV classes (numbers from 1 to 5 indicate the progressive increase of genotype susceptibility). The number of the individuals sharing the ‘plus’ or the ‘minus’ alleles is indicated over the top of the bars.

The present study identifies four putative QTLs for fire-blight resistance and defines the resistant (in most cases) and susceptible haplotypes (Figure 3). It thus represents a first step in developing a MAS approach in pear breeding programmes designed to select new fire-blight-resistant genotypes. The presence in each

putative QTL of SSR markers makes it possible to transfer map information to different pear cross populations and verify the relationships with other sources of fire-blight resistance in pear. The fact that these maps have many SSR loci also makes them very informative, because this feature enables information to be transferred to any pear progeny, and is a solid tool for pear genetic research.

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