Inheritance studies of apple scab resistance and identification of *Rvi14*, a new major gene that acts together with other broad-spectrum QTL

V. Soufflet-Freslon, L. Gianfranceschi, A. Patocchi, and C.-E. Durel

Abstract: Scab, caused by the fungal pathogen *Venturia inaequalis*, is the most common disease of cultivated apple (*Malus ×domestica*). The fungal races 6 and 7 have now overcome the major resistance gene *Vf*, which is widely used in apple breeding programmes. New breeding strategies to achieve durable resistance are thus necessary. The aim of this study was to determine the genetic basis of quantitative resistance of the apple cultivar 'Dülmener Rosenapfel', known to be scab resistant under different environmental conditions. An F_1 progeny derived from the cross between the susceptible cultivar 'Gala' and 'Dülmener Rosenapfel' was tested in a greenhouse with a multi-isolate inoculum of *V. inaequalis*. *Rvi14*, a new major gene that conditions a chlorotic-type reaction, was mapped on linkage group (LG) 6 in a genomic region not known to be involved in disease resistance. A further three quantitative trait loci (QTL) for resistance were identified. One co-localized with *Rvi14* on LG6, whereas the remaining two were detected on LG11 and LG17, in genomic regions already reported to carry broad-spectrum QTL in other genetic backgrounds. Since a selective genotyping approach was used to detect QTL, an expectation-maximization (EM) computation was used to estimate the corrected QTL contributions to phenotypic variation and was validated by entire progeny genotyping.

Key words: Malus ×domestica, *Venturia inaequalis*, major gene, quantitative resistance, genetic mapping.

Résumé : La tavelure, causée par le champignon pathogène *Venturia inaequalis*, est la maladie du pommier cultivé (*Malus ×domestica*) la plus répandue. Les races 6 et 7 du champignon ont maintenant contourné le gène majeur de résistance *Vf*, largement utilisé dans les programmes d'amélioration du pommier. De nouvelles stratégies d'amélioration sont alors nécessaires en vue d'une résistance durable. Le but de cette étude était d'étudier le déterminisme génétique de la résistance quantitative de la variété de pommier 'Dülmener Rosenapfel', connue pour être résistante à la tavelure dans différentes conditions environnementales. Une descendance F_1 issue du croisement entre la variété sensible 'Gala' et 'Dülmener Rosenapfel' a été testée en serre avec un inoculum multi-souches de *V. inaequalis*. Un nouveau gène majeur, *Rvi14*, déterminant une réaction de type chlorotique, a été localisé sur le groupe de liaison (GL) 6 dans une région génomique pas encore connue pour être impliquée dans la résistance aux maladies. Trois QTL (« quantitative trait loci ») de résistance ont été identifiés. Un a co-localisé avec *Rvi14* sur le GL6 alors que les deux autres ont été détectés sur le GL11 et le GL17, dans des régions génomiques déjà connues pour porter des QTL à large spectre dans d'autres fonds génétiques. Les QTL ayant été détectés par génotypage sélectif, leur contribution à la variation phénotypique a été corrigée par maximum de vraisemblance; cette méthode corrective a ensuite été validée par le génotypage de la population entière.

Mots-clés : Malus ×domestica, *Venturia inaequalis*, gène majeur, résistance quantitative, cartographie génétique.

Introduction

Apple scab, caused by the fungal pathogen *Venturia inaequalis* (Cooke) G. Wint., is one of the most prevalent diseases in apple-growing areas with high spring and summer rainfall. Most commercial apple cultivars (*Malus ×domestica*) are susceptible to this disease and its control requires up to 12–15 fungicide treatments per season. It is now crucial to reduce such intensive use of pesticides in apple orchards for environmental and consumer health reasons and because of the appearance of fungicide resistance. Growing genetically resistant or tolerant apple cultivars should provide an alternative to chemical control of the disease, provided that this resistance is durable.

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Scab resistance studies have been focused on identifying major resistance genes, mainly in small-fruited Asiatic *Malus* species. These include *Vf* and *Vfh* from *Malus floribunda* 821 (Williams and Kuc 1969; Bénaouf and Parisi 2000); *Vr-DW*, *Vh2* (also called *Vr* or *Vr-A*), and *Vh4* (also called *Vx* or *Vr1*) from *Malus pumila* R12740-7A (Dayton and Williams 1968; Aldwinckle et al. 1976; Hemmat et al. 2002; Bus et al. 2005*a*; Boudichevskaia et al. 2006); *Vh8* from *Malus sieversii* (Bus et al. 2005*b*); *Vbj* from *Malus baccata jackii* (Williams and Kuc 1969); *Vb* from 'Hansen's baccata #2' (Williams and Kuc 1969); *Va* from PI172623 (Lespinasse 1989); *Vm* from *Malus micromalus* 245-38 or *Malus atrosanguinea* 804 (Williams and Kuc 1969); *Vg* from 'Golden Delicious' (Bénaouf and Parisi 1997); *Vr2* from GMAL 2473 (Patocchi et al. 2004); and *Vd* from 'Durello di Forli' (Tartarini et al. 2004). Most of these resistance genes have been mapped in the apple genome (for a review, see Gessler et al. 2006), but only the *Vf* gene has been widely introgressed into the apple selections released over the last 50–60 years (Laurens 1999). However, in the late 1980s, *Vf* was overcome by two new *V. inaequalis* races (race 6, Parisi et al. 1993; race 7, Bénaouf and Parisi 2000). The identification of different fungal races with the ability to overcome specific apple scab resistance genes triggered a need to develop new breeding strategies to promote durable resistance.

Pyramiding of several resistance genes within the same genetic background has been proposed to develop apple cultivars with durable scab resistance (MacHardy et al. 2001; Gessler et al. 2006). However, pyramiding of major resistance genes has proven to be risky in pathosystems such as lettuce–*Bremia lactucae* (Lebeda 1992). Conversely, polygenic quantitative resistance is considered to be more durable (Burdon 1993; Parlevliet 1993, 2002; Lindhout 2002), and combining major and minor resistance genes could offer more stable resistance than combining only major genes. A research programme is thus underway at INRA, Angers, France, to detect quantitative trait loci (QTL) for scab resistance. QTL were identified in the cultivars 'Fiesta', 'Discovery', and 'TN10-8' after inoculation of two segregating progenies with several monoconidial isolates in controlled conditions (Durel et al. 2003; Calenge et al. 2004). Some of these QTL were also identified in 'Fiesta' × 'Discovery' progeny grown in unsprayed fields (Liebhard et al. 2003). 'Discovery' showed a high level of resistance against a wide range of pathogen isolates under different environmental conditions (Laurens et al. 2003) and is a promising candidate for deciphering some of the genetic factors underlying scab resistance. Calenge et al. (2004) showed that 'Discovery' carries a combination of a major gene (*Vg* on LG12) and specific or broad-spectrum QTL on LG2, LG5, LG13, LG15, and LG17.

In the present study, we explored the genetic basis of quantitative resistance against scab of another apple cultivar, 'Dülmener Rosenapfel', which is known to be resistant in unsprayed field conditions (A. Peil, personal communication 2007). During the European DARE (Durable Apple Resistance in Europe) project (Lespinasse et al. 2000), this cultivar showed resistance to a dozen inocula of *V. inaequalis* under different experimental conditions (Laurens et al. 2003), making it a prime candidate to study new and existing gene combinations for durable scab resistance.

Material and methods

Plant material and *Venturia inaequalis* isolates

A population of 275 F_1 individuals (GaxDR) was derived from a cross between the susceptible cultivar 'Gala' (female parent) and the German resistant cultivar 'Dülmener Rosenapfel' (male parent). Twenty-five susceptible control individuals were derived from a cross between 'Golden Delicious' and 'Granny Smith'. In spring 2005, seedlings were grown in the greenhouse up to the 6–8 leaf stage before inoculation. After a first inoculation, the plants continued to grow for approximately 2 months before pruning and then were transferred for 2.5 months to a cold chamber (1 °C) during the summer to mimic winter vernalization. After vernalization and when plant growth resumed, leaves were exposed to a second inoculation in autumn 2005. The parents could not be evaluated because they were not in the growth stage when their progeny and susceptible control individuals were inoculated, but they had been previously evaluated for their disease response in the same greenhouse by Laurens et al. (2003).

Six isolates of *Venturia inaequalis* were used for inoculation. Their origin and characteristics are described in Table 1. The monoconidial isolates 301 and 1066 are reference isolates from the INRA collection (Bénaouf and Parisi 2000). The monoconidial isolates EU-B04, EU-NL19, EU-D42, and EU-NL24 were from the European *V. inaequalis* collection of the European DARE project (Parisi et al. 2004).

Pathological tests and phenotyping

Two inoculation techniques were used, i.e., spray inoculation and droplet inoculation. In a first step, spray inoculation was used to identify qualitative and quantitative resistance factors in the GaxDR population. In a second step, droplet inoculation was used to characterize the interaction pattern of a new major gene previously identified by spray inoculation with *V. inaequalis*.

The GaxDR population (275 individuals) and susceptible control seedlings were spray-inoculated in spring 2005 (first screen). Spray inoculation was repeated on a subset of 155 GaxDR individuals and on susceptible control seedlings in autumn 2005 (second screen). For both screens, individuals were spray-inoculated in the greenhouse with a mixture of the same 5 isolates according to the conditions described by Chevalier et al. (1991). The 5 isolates (EU-B04, EU-NL19, EU-D42, EU-NL24, and 1066, Table 1) were prepared separately, as described by Parisi and Lespinasse (1996), and mixed in equal proportions. The final concentration of each isolate was about 8×10^4 conidia/mL. The rate of germinated conidia ranged from 56% to 93% after 24 h on an agar plate. After inoculation, the seedlings were incubated under transparent plastic sheets. Humidity was maintained at 90% by misting the greenhouse (with a fogging system) and the air temperature was maintained at 18 °C for 48 h to allow conidia germination. The seedlings were then taken out of the sheets, and the humidity was reduced to 70% and the temperature to 17 °C until scoring.

A subset of 120 GaxDR individuals was droplet-inoculated in autumn 2005. Six isolates were used for droplet inoculation, including the 5 isolates used for spray inoculation and another one (301, Table 1). Seedlings were inoculated

Table 1. Characteristics of the *Venturia inaequalis* monoconidial isolates tested on 'Gala' × 'Dülmener Rosenapfel' progeny.

Isolate	Host cultivar or hybrid[a]	Country	Race	Inoculation technique[c]
301	'Prima' × 'KI 40'	Germany	1	Droplet
EU-B04	'Golden Delicious'	Belgium	1	Spray and droplet
EU-NL19	'Golden Delicious'	The Netherlands	1	Spray and droplet
EU-D42	'Prima'	Germany	6	Spray and droplet
1066	*Malus floribunda* 821	France	7	Spray and droplet
EU-NL24	'Prima'	The Netherlands	6+7[b]	Spray and droplet

[a]Host cultivars or hybrids from which isolates were collected.

[b]Isolate combining the virulences of races 6 and 7.

[c]Isolates were included in the mixture used for spray inoculation and (or) were tested separately for droplet inoculation.

with each isolate separately, the final conidia concentration was about 4×10^4 conidia/mL, and the rate of germinated conidia ranged from 53% to 92%. The 120 individuals were distributed into three groups of 40. Each group was tested with two isolates (group 1: isolates 301 and 1066; group 2: isolates EU-NL19 and EU-NL24; group 3: isolates EU-B04 and EU-D42) and every plant of a group was simultaneously, but separately, inoculated with the two isolates. The droplet inoculation technique was described by Bus et al. (2005*b*). The seedlings were inoculated by placing 150 µL of inoculum into small inoculation chambers, which were clipped onto the youngest expanded leaf of actively growing shoots. The seedlings were incubated under transparent plastic sheets for 48 h, and the inoculation chambers were then removed. The environmental conditions in the greenhouse were the same as for the spray inoculation technique.

Chlorotic-type symptoms and sporulation intensity were scored on leaves. The presence or absence of chlorotic-type symptoms was scored at 7 days (spray) or at 11 and 19 days (droplet) after inoculation. For spray inoculation, the sporulation intensity was scored at 14, 21, and 28 days after inoculation following an ordinal scale corresponding to the percentage of leaf surface showing sporulation (described by Croxall et al. 1952 and modified by Parisi et al. 1993). The ordinal scale was as follows: 0, no sporulation; 1, $0 <$ sporulation $\leq 1\%$; 2, $1\% <$ sporulation $\leq 5\%$; 3, $5\% <$ sporulation $\leq 10\%$; 4, $10\% <$ sporulation $\leq 25\%$; 5, $25\% <$ sporulation $\leq 50\%$; 6, $50\% <$ sporulation $\leq 75\%$; and 7, $75\% <$ sporulation $\leq 100\%$. AUDPC (area under the disease progress curve) was computed and used to measure scab susceptibility using the ordinal data from the three dates.

Statistical analyses

The χ^2 test was used to compare the chlorotic-type reaction segregation in the GaxDR progeny with 1:1 segregation. The Mann–Whitney–Wilcoxon test was used to compare AUDPC means of two samples, while Spearman's rank correlation coefficient was implemented to measure correlation between AUDPC computed for both screens.

DNA extraction and specific PCR amplification

DNA from the progeny and parental genotypes was isolated from leaves of seedlings and grafted plants, respectively, using the QIAGEN DNeasy 96 Plant Kit (QIAGEN, Hilden, Germany).

Eighty-three (83) simple sequence repeat (SSR) markers, previously described by Liebhard et al. (2002), Vinatzer et al. (2004), and Silfverberg-Dilworth et al. (2006), were used to screen the GaxDR progeny. An additional SSR marker, HB09-SSR (G.A.L. Broggini et al., unpublished data), was derived from a bacterial artificial chromosome (BAC) clone (Vinatzer et al. 2001) that was isolated using a probe derived from the *HcrVf2* scab resistance gene (homologous to the *Cladosporium fulvum* resistance genes of the *Vf* region). The set of 84 SSR markers was selected on the basis of polymorphism between 'Dülmener Rosenapfel' and 'Gala', and the distribution spanning the apple genome as assessed in the reference genetic map of Silfverberg-Dilworth et al. (2006). The estimated SSR coverage was about 65% of the genome based on the 'Discovery' reference map (Silfverberg-Dilworth et al. 2006).

PCR amplification of SSR from genomic DNA was performed as described by Gianfranceschi et al. (1998) with minor modifications, including 0.33 µmol/L of both forward and reverse primers, and REDTaq DNA Polymerase (Sigma-Aldrich Corporation, St. Louis, Missouri, USA). Uniplex SSR amplification was done in a Peltier Thermal Cycler (MJ Research, Waltham, Massachusetts, USA) as described by Gianfranceschi et al. (1998) with minor modifications: 1 cycle of 2 min 30 s at 94 °C; 5 cycles of 30 s at 94 °C, 1 min annealing at 60 °C (dropping 1 °C/cycle), 1 min at 72 °C; 30 cycles of 30 s at 94 °C, 1 min at 55 °C, 1 min at 72 °C; and a final extension cycle of 10 min at 72 °C. A half volume of denaturing gel loading buffer (80% deionized formamide, 10 mmol/L EDTA pH 8.0, 1 mg/mL xylene cyanol, 1 mg/mL bromophenol blue) was added to the PCR product and heated at 95 °C for 5 min. Products were separated on 7 mol/L urea denaturing 5% polyacrylamide sequencing gels by electrophoresis at 60 W for 2 h. PAGE gels were stained using silver nitrate as described by Creste et al. (2001).

Multiplex SSR amplification was performed with the QIAGEN Multiplex PCR Kit. The PCR amplification solution consisted of an 11 µL reaction volume containing 5 ng of apple genomic DNA, 1× QIAGEN Multiplex PCR Master Mix, and 0.2 µmol/L of each primer. SSR amplifications were done in a Peltier Thermal Cycler with the following programme: 1 cycle of 15 min at 94 °C; 30 cycles of 30 s at 94 °C, 1 min 30 s at 55 °C, 1 min at 72 °C; 1 cycle of 15 min at 55 °C; and a final extension cycle of 15 min at 72 °C. PCR products were loaded onto an ABI PRISM 3130

sequencer (Applied Biosystems, Foster City, California) with an internal 50–400 bp size standard (GeneScan 400HD ROX, Applied Biosystems). Data were collected with ABI PRISM Data Collection software V3.0 and analysed with ABI PRISM GeneMapper software V3.7 (Applied Biosystems).

Selective genotyping

All progeny at the extremes of the first-screen AUDPC scores (beyond one standard deviation from the mean) and 34% of the remaining progeny (within one standard deviation from the mean) were genotyped at every marker to obtain a representative population of 166 individuals (Fig. 1). These 166 individuals accounted for 60% of the entire progeny.

To demonstrate the efficiency of a maximum-likelihood approach for correcting the overestimation of QTL contributions to phenotypic variation, the entire GaxDR progeny of 275 individuals was genotyped for markers closest to the likelihood peaks of the detected QTL (see below).

Genetic map construction

The integrated female + male linkage map of GaxDR was constructed using JoinMap software V3.0 (Van Ooijen and Voorrips 2001; PRI, Wageningen, the Netherlands). The initial map construction was done using a critical LOD (logarithm of odds ratio) score of 6 to associate loci and generate linkage groups. Markers linked to their neighbors with LOD ≤ 3 were assigned to linkage groups according to their respective positions on the apple reference linkage map (Silfverberg-Dilworth et al. 2006). Linkage analysis to refine the marker order within linkage groups was carried out using the standard default settings in JoinMap for LOD and REC thresholds (1.0 and 0.4, respectively). The final locus order was determined by extensive manual analysis of data points generating double crossovers between flanking loci. Recombination frequencies were converted to map distances using Kosambi's mapping function (Kosambi 1944), and segregation distortions of parental alleles were examined by the χ^2 test with a 95% confidence level. Linkage groups were assigned according to the position of common markers and numbering described by Maliepaard et al. (1998).

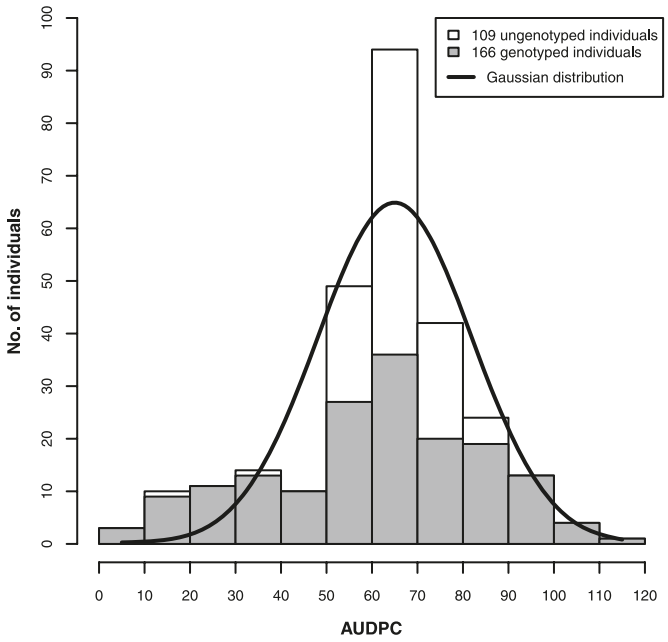
QTL detection

Two sets of 166 and 135 jointly phenotyped and genotyped individuals were available for QTL analyses for the first and second screens, respectively. Twenty individuals among the 155 inoculated for the second screen were stunted and not scored, leaving only 135 individuals for QTL analysis.

QTL detection was performed using AUDPC data with MapQTL software V4.0 (Van Ooijen et al. 2002; PRI, Wageningen, the Netherlands). MapQTL software is able to deal with a full-sib family derived from heterozygous parents of a cross-pollinating species (e.g., apple). The integrated linkage map was used for detection of both additive and dominance effects.

The AUDPC data scored in the first screen (i.e., with a normal distribution for the entire progeny) were used for interval mapping. A LOD threshold at which a QTL was declared significant (p = 0.05) was determined using 1000 permutations (Churchill and Doerge 1994). Each significant QTL was characterized by its LOD score and its confidence interval, corresponding to a LOD score drop of 1 or 2 on either side of the likelihood peak with a probability over 90% and 95%, respectively (Van Ooijen 1992). A multiple analysis of variance (ANOVA), including molecular markers closest to the QTL peaks and their first-order interaction, was performed to test the effects of parental alleles and epistatic effects among QTL (SAS Institute Inc. 2000). QTL detection using AUDPC data in the second screen (non-normal distribution) was achieved by performing a Kruskal–Wallis test at each marker.

Fig. 1. AUDPC distributions for the entire (275 individuals) 'Gala' × 'Dülmener Rosenapfel' progeny and for the subset selected for genetic studies (166 individuals), scored at the first screen. The Gaussian curve was plotted with data on the 275 individuals.



QTL contributions to phenotypic variation

Selective genotyping is known to lead to overestimation of QTL effects (Lander and Botstein 1989). Different solutions have been proposed to account for such overestimations (e.g., Muranty and Goffinet 1997; Xu and Vogl 2000), but they have been rarely implemented in QTL mapping software. MapQTL is able to take selective genotyping into account in single-interval mapping but not in multiple-QTL mapping (MQM function). Thus we developed the following approach to compute unbiased QTL contributions to phenotypic variation when there were several QTL. To validate our approach, we compared such unbiased QTL contributions with QTL contributions computed with complete genotyping data (i.e., with genotyping data from the entire GaxDR progeny of 275 individuals for markers closest to the QTL peaks).

1. QTL detection: QTL were detected using the single-QTL model of MapQTL (Interval Mapping function) using phenotypic and genotypic data from the 166 individuals.
2. Overestimated effects: ANOVA, using data from the 166 individuals and including markers closest to the

QTL peaks, allowed joint analysis of the effects of QTL-associated markers and computation of the total percentage of explained phenotypic variation (R^2). The contribution of each QTL-associated marker to phenotypic variation was calculated according to Scherrer (1984).

3. EM correction: an expectation-maximization (EM) computation implemented within NORM software (Schafer 1999) was used to estimate the corrected simple linear correlation coefficients between phenotype and QTL-associated markers or among QTL-associated markers. The EM-corrected simple linear correlation coefficients were then used for unbiased computation of the contribution of each QTL-associated marker and of the total R^2 according to Scherrer (1984).
4. Validation: complete genotyping of QTL-associated markers was analyzed on the entire unselected progeny and the total R^2 computed by ANOVA. The contribution of each QTL-associated marker was re-estimated (Scherrer 1984). These values (total R^2 and QTL contributions) were compared with the respective EM-corrected values computed in step 3.

Results

Phenotyping

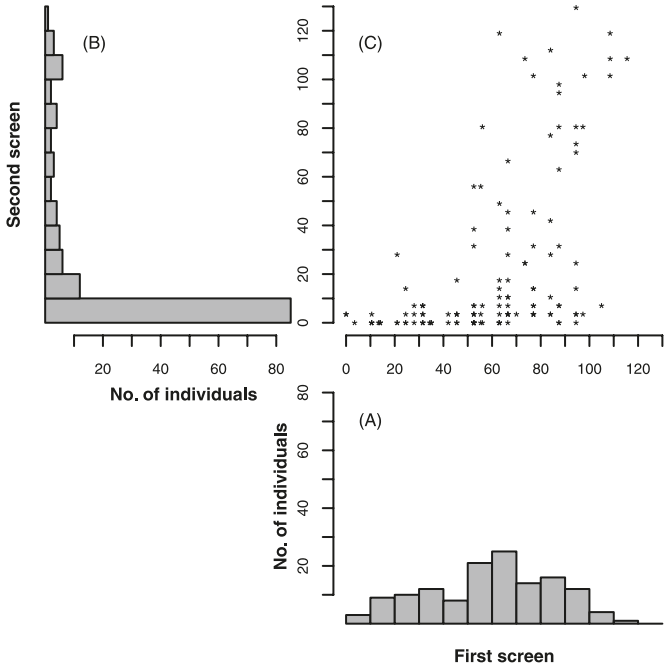
Susceptible control individuals displayed a high level of sporulation on leaves for both screens. AUDPC in the first screen (83.7 ± 15.3) was significantly lower ($p < 0.02$) than AUDPC in the second screen (101.2 ± 39.0). AUDPC in the GaxDR progeny exhibited different distributions between the first and second screens (Fig. 2). The distribution was close to normal in the first screen, whereas most individuals were either completely resistant or slightly susceptible, thus giving a highly skewed distribution, in the second screen. Average AUDPC was significantly higher ($p < 0.0001$) in the first screen (59.0 ± 27.1) than in the second screen (22.0 ± 34.1). The rank correlation coefficient between the two screens was 0.58 ($p < 0.0001$). Note that only individuals tested in both screens were included in the above analyses (susceptible controls and GaxDR progeny).

A total of 129 out of 275 individuals of the GaxDR progeny displayed a chlorotic-type reaction scored on leaves 7 days after inoculation with the mixture of 5 isolates of *Venturia inaequalis* (symptoms scored in the first screen and checked in the second screen on spray-inoculated seedlings; Table 2). This segregation did not differ significantly from 1:1 segregation ($P(\chi^2 \geq 0.33) = 0.57$), which confirmed the assumed segregation of a single heterozygous gene in one of the parents.

Genetic mapping of a new major gene in apple

The chlorotic-type reaction was mapped as a qualitative trait at the distal end of LG6 in the apple genome. The gene associated with this reaction was found to derive from 'Dülmener Rosenapfel'. The gene was assigned *Rvi14* (previously called *Vdr1*, Freslon et al. 2006) according to the new nomenclature of scab races proposed by Bus et al. (2008). On the integrated linkage map, the genetic distance between *Rvi14* and the nearest marker, HB09-SSR, was 4.0 cM (LOD = 52.0, 253 individuals). The size of the HB09-SSR allele coupling with *Rvi14* was 210 bp. The linkage of *Rvi14* to CH03d07 and NZ23g04 was also significant at an LOD of 21.65 and 5.22, respectively (Fig. 3).

Fig. 2. AUDPC for 'Gala' × 'Dülmener Rosenapfel' progeny after co-inoculation with five *Venturia inaequalis* isolates: (A) AUDPC distribution for the first screen; (B) AUDPC distribution for the second screen; (C) individuals plotted for both screens.



Characterization of the interaction pattern of *Rvi14* with *Venturia inaequalis*

Six independent monoconidial isolate inoculations were performed on three subsets of the segregating population. The chlorotic-type reaction was observed only with the two isolates EU-NL24 and EU-NL19 in individuals that had already shown this reaction in the first screen (apart from 1 to 3 escapes). Nevertheless, both isolates sporulated on leaves of some individuals carrying *Rvi14*, with EU-NL19 sporulating more frequently than EU-NL24 (47% and 25%, respectively). The other 4 isolates did not elicit the chlorotic-type reaction on individuals identified as carrying *Rvi14* during the first screen. Isolates EU-D42 and EU-B04 caused sporulation on most of the individuals, whereas isolates 301 and 1066 sporulated on about one third of the individuals.

QTL analyses

For AUDPC data on 166 individuals in the first screen (selective genotyping), three QTL located on different linkage groups of the GaxDR integrated linkage map were significantly associated with scab resistance (Table 3). Two QTL were mapped to distal regions, i.e., one on LG6 (LOD = 7.42, Fig. 3) and one on LG11 (LOD = 13.69, Fig. 3). The third QTL was mapped to the proximal region of LG17 (LOD = 11.32, Fig. 3). The QTL on LG6 was detected in 'Dülmener Rosenapfel' (male effect), while the QTL on LG11 was detected in 'Gala' (female effect) (Table 3). The QTL on LG17 was detected in both parents (Table 3). ANOVA including molecular markers closest to

Table 2. Segregation of a chlorotic-type reaction in 'Gala' × 'Dülmener Rosenapfel' progeny after co-inoculation with five *Venturia inaequalis* isolates in both screens.

		Chlorotic-type reaction				
	No. of individuals	Present	Absent	d.t.s.*	χ^2 value	*p* value
1st screen	275	113	126	36	0.71	0.40
2nd screen	155	61	60	34	0.01	0.93
1st screen + 2nd screen	275	129	120	26	0.33	0.57

*Difficult to score.

the QTL peaks and their first-order interaction showed slight epistatic interactions ($0.01 < p < 0.05$) between LG11 (CH04h02) and LG6 (HB09-SSR) or LG17 (CH01h01). ANOVA was thus restricted to variation factors with a highly significant effect ($p < 0.01$), i.e., the male marker for the QTL on LG6, the female marker for the QTL on LG11, and both parents' markers for the QTL on LG17. The QTL on LG6 was located close to *Rvi14* and the peak of the QTL co-localized with the marker HB09-SSR (Fig. 3). The quantitative resistance allele was involved in coupling with the favourable *Rvi14* allele.

The three scab resistance QTL detected in the first screen were confirmed in the second screen in 135 individuals, i.e., QTL on LG6 (marker HB09-SSR, $p < 0.001$), LG11 (marker CH04h02, $p < 0.0001$), and LG17 (marker CH01h01, $p < 0.0001$).

QTL contributions to phenotypic variation

R^2 were estimated from the first-screen phenotypic data. The total R^2 computed with the complete genotyping data for the 275 individuals was 42.7%, whereas it was 54.8% for the selected data set for 166 individuals (Table 3). Selective genotyping resulted in overestimation of the total R^2 of about 12%. On the other hand, the EM-corrected R^2 was 44.9%, which is close to the R^2 computed with complete genotyping data. Similar patterns were observed for each QTL contribution to phenotypic variation (Table 3).

Discussion

A new genomic region involved in apple scab resistance

In addition to genomic regions already known to be involved in the control of scab resistance (major genes and QTL) in several apple cultivars, a new region was identified in the German cultivar 'Dülmener Rosenapfel' at the distal end of LG6. No other resistance genes, for scab or any other apple diseases, have been identified in this region of the apple genome. Despite some sporulation on seedlings exhibiting a chlorotic-type reaction, the *Rvi14* gene appears to confer resistance to isolates EU-NL24 (virulences of races 6 and 7) and EU-NL19 (race 1), which would carry a corresponding avirulence gene. A similar situation has often been described for the *Vf* gene, which was found to confer complete resistance or only allow some sporulation in favourable controlled conditions, despite the fact that it has a substantial effect against *Venturia inaequalis* in the orchard when not overcome by races 6 and 7 (Chevalier et al. 1991; Gardiner et al. 1996; Gessler et al. 2006).

The *Rvi14* gene determines a chlorotic-type reaction similar to the *Vf* gene phenotype (e.g., in 'Florina'). Moreover, the marker HB09-SSR was derived from a BAC clone that had been identified after hybridization with the *HcrVf2* gene (Vinatzer et al. 2001), which is the most probable candidate for the *Vf* resistance gene (Xu and Korban 2002; Belfanti et al. 2004). Allo- or auto-polyploidy has been implicated in the origin of the apple genome (Darlington and Moffett 1930; Sax 1933; Challice 1981) and several duplicated regions have been identified in the apple genome (Chevreau et al. 1985; Chevreau and Laurens 1987; Maliepaard et al. 1998). The LG6 genomic region carrying the *Rvi14* gene thus might be homeologous to the LG1 region carrying the *Vf* gene. These two chlorotic-type resistance genes (or gene clusters) might derive from the same ancestor and might have duplicated and diverged over evolutionary time.

Co-localization of resistance QTL with major resistance genes has been observed in apple (Durel et al. 2003; Bus et al. 2004) and in numerous other plant species such as rapeseed (Manzanares-Dauleux et al. 2000), common bean (Kelly and Vallejo 2004), and poplar (Jorge et al. 2005). Most of the resistance genes characterized at the molecular level belong to allelic series or to families of tightly linked genes (Hulbert et al. 2001). Therefore, major genes and co-localizing QTL could be members of the same allelic series, as suggested by Robertson (1985), or members of a multigene family. Co-localization of the QTL detected on LG6 with *Rvi14* suggests that both resistance factors actually belong to the same locus, or that they are part of the same gene family. In the first case, the *Rvi14* gene would control both the chlorotic-type reaction for isolates carrying a corresponding avirulence factor (EU-NL19 and EU-NL24) and some residual resistance to the virulent isolates (all of the other four). The mixed inoculum we used did not enable us to clarify whether *Rvi14* had a general effect towards all the isolates tested or a differential effect dependent on the isolates present in the mixture and their relative proportions. Physical mapping and sequencing of BAC clones should reveal whether a single gene for *Rvi14* or a cluster of genes at the *Rvi14* locus provides differential resistance to apple scab.

Two confirmed genomic regions involved in partial resistance to scab in apple

Two QTL were detected on LG11 and LG17 of the GaxDR integrated linkage map with the mixture of isolates of *Venturia inaequalis*. These QTL have already been reported by Durel et al. (2003) in grafted progeny of 'Prima' × 'Fiesta' inoculated in a controlled climatic chamber with two monoconidial isolates of race 6. The QTL on LG17 was detected only in 'Fiesta', whereas the QTL on LG11 was detected in both 'Prima' and 'Fiesta'. QTL on LG11 and LG17 were also identified by Liebhard et al. (2003) in 'Fi-

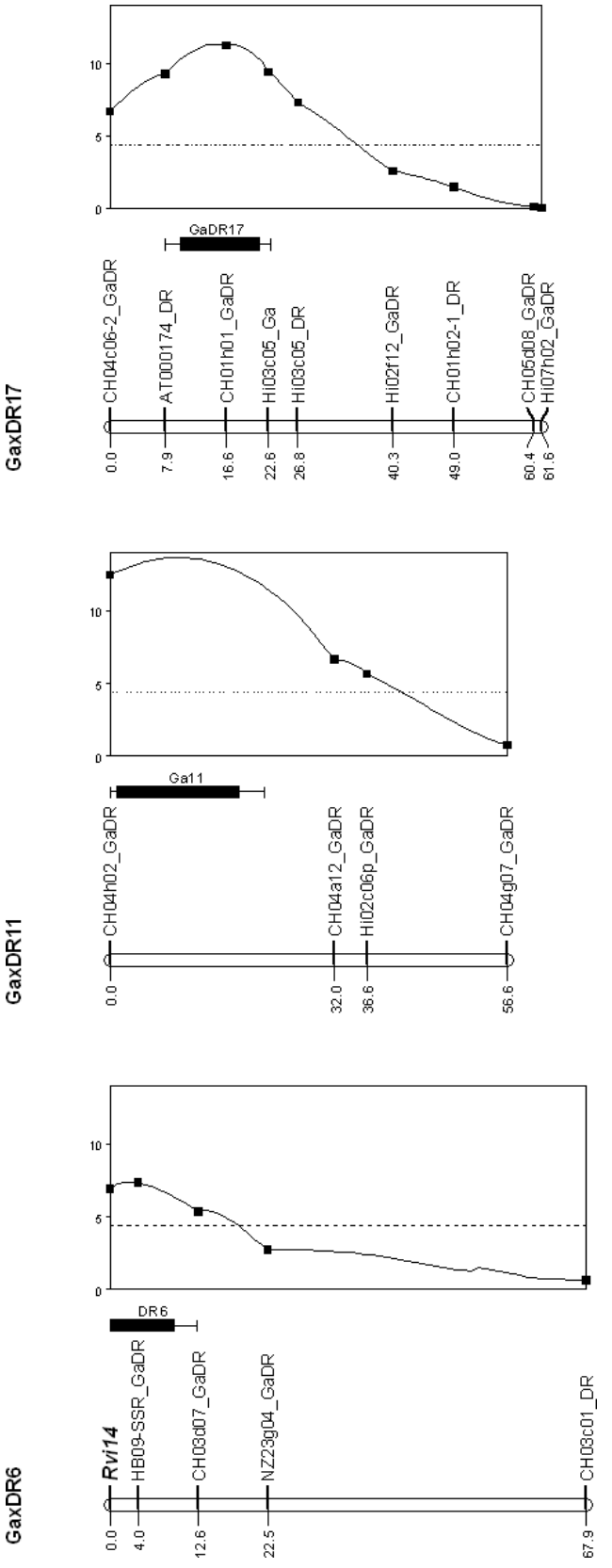


Fig. 3. Genomic localization of QTL for AUDPC and LOD plots for linkage groups 6, 11, and 17 of the 'Gala' × 'Dülmener Rosenapfel' integrated linkage map (interval mapping detection), after co-inoculation with five *Venturia inaequalis* isolates. Markers with a "_Ga" or a "_DR" extension are polymorphic only for 'Gala' or 'Dülmener Rosenapfel', respectively. Markers "_GaDR" are polymorphic for both parents. Distances are in centimorgans. QTL are represented by boxes, in which length represents the LOD – 1 confidence interval and extended lines represent that of LOD – 2. In the LOD plots, dotted lines represent the genome-wide LOD threshold.

esta' × 'Discovery' progeny grown in field conditions with a Swiss local inoculum. The QTL on LG11 was detected only in 'Discovery', whereas the QTL on LG17 was detected in both parents. Likewise, Calenge et al. (2004) showed a significant effect of the QTL on LG17 in seedlings and grafted plants derived from a 'Discovery' × 'TN10-8' cross, which were inoculated in greenhouse conditions with isolates of races 1, 6, 7, and '6 + 7' and with two local inocula from France and Switzerland. This QTL was detected in 'Discovery'. The previous results and ours thus demonstrate the stability of these two genomic regions in the control of partial scab resistance. Indeed, these QTL are expressed in different genetic backgrounds, physiological stages, and environmental conditions. Based on previous studies, it could be postulated that these are broad-spectrum QTL, as supported by our study involving an inoculum consisting of various *V. inaequalis* isolates. Each isolate of our inoculum would need to be separately tested to confirm the broad spectrum of the QTL detected in 'Dülmener Rosenapfel' and 'Gala'.

QTL contributions to phenotypic variation

Selective genotyping resulted in overestimation of both the total R^2 and the QTL contributions to phenotypic variation. The correction by maximum likelihood (EM correction as proposed by Xu and Vogl 2000) provided a reasonable R^2 estimation; the same was true for each QTL contribution. Here we validated this EM correction with experimental data, which has seldom been achieved to date. Another approach to correct the overestimation of QTL contributions was proposed by Lagarrigue et al. (2006) for QTL detected in chicken. It is based on the hypothesis of strict truncations of the normal distribution, i.e., a situation that was not adapted to our experimental conditions. In the present study, the slight overestimation observed in the EM correction when compared with complete genotyping might have been due to some deviation from the Gaussian distribution for AUDPC (Fig. 1).

The contributions of the three detected QTL ranged from 4.4% to 18.9% (Table 3). In a previous study (Durel et al. 2003), the QTL detected on LG11 explained 16%–23% of phenotypic variation, according to *V. inaequalis* isolates used in the greenhouse. The contribution of this QTL in field conditions was estimated at 11% (Liebhard et al. 2003). The contribution reported in our study (18.9%) is in agreement with these previous results. On the other hand, the QTL detected on LG17 explained 10%–13% of phenotypic variation (Durel et al. 2003). In a second greenhouse study (Calenge et al. 2004), the contribution of this QTL ranged from 5% to 19%, but was higher than 10% with most tested isolates. The contribution of this QTL was about 23% in field conditions (Liebhard et al. 2003). In our study,

Table 3. Individual and combined QTL contributions to scab disease variation in 'Gala' × 'Dülmener Rosenapfel' progeny after co-inoculation with five *Venturia inaequalis* isolates in the first screen.

LG	Marker[a]	Favourable allele size[b]	Selective genotyping[c] (166 individuals)	EM correction (275 individuals)	Complete genotyping (275 individuals)
LG6	HB09-SSR_DR	210 bp	12.3	10.1	10.0
LG11	CH04h02_Ga	185 bp	22.4	18.3	18.9
LG17	CH01h01_Ga	136 bp	14.4	11.8	9.4
	CH01h01_DR	132 bp	5.7	4.7	4.4
Total R^2			54.8	44.9	42.7

Note: EM, expectation-maximization.

[a]SSR closest to the likelihood peak of each QTL.

[b]Favourable parental alleles associated with scab resistance.

[c]Overestimated values.

the QTL on LG17 accounted for a maximum contribution to phenotypic variation of 9.4%, which is lower than noted in previous studies. Comparing QTL contributions is meaningful only if genetic variations are similar among different studies, since these contributions are assessed in relative terms (%). However, data obtained in the above studies did not enable us to check this condition, so the above comparisons have to be considered cautiously.

Genetic determinants of resistance

The small number of genomic regions apparently involved in the quantitative scab resistance of 'Dülmener Rosenapfel' contrasts with the higher number of such regions detected for 'Discovery' scab resistance by Calenge et al. (2004). Different factors could explain this discrepancy: (*i*) phenotypes of each individual were less accurately estimated with single-copy seedlings, as in the present study, than with multiple-copy grafted plants, as tested in the 'Discovery' genetic analysis, and small QTL effects were thus less easily detected in our study; (*ii*) only one mixture of *Venturia inaequalis* isolates was used here, whereas 8 successive inocula with either monoconidial isolates or local isolates were used on the 'Discovery' progeny, so specific QTL may be missing here; (*iii*) some regions of the apple genome were not covered by markers in the present GaxDR linkage map, so a complete genome scan could not be performed; (*iv*) there may be more "homozygous" or epistatic undetected genetic factors in 'Dülmener Rosenapfel' than in 'Discovery'.

Note that AUDPC showed a normal distribution in the GaxDR progeny in the first screen, whereas it became highly skewed towards resistance in the second screen. This variation between the two screens has also been observed in other progenies derived from 'Dülmener Rosenapfel' (unpublished results). In the first screen, inoculated plants were very young seedlings (6–8 leaves after seed germination), whereas they were physiologically older in the second screen. The differences in data distribution between the first and the second screen may have been due to the differential expression of resistance at different physiological stages. The expression of seedling and adult plant resistances can be controlled by different genes, which is particularly common in several plant species (e.g., resistance to powdery mildew (Wang et al. 2005) or to stripe rust (Bariana et al. 2006) in wheat, resistance to leaf rust in barley (Qi et al. 1998), and resistance to stem canker in rapeseed (Delourme et al. 2004)). In other situations, some resistance genes are effective at both seedling and adult stages (Camargo et al. 1995), whereas others are effective only in adult plants (Zhang and Knott 1993). Such developmentally regulated resistance genes could also be present in apple.

Another interesting point was the presence of two QTL detected in 'Gala'. This cultivar is commonly regarded as a reference apple genotype for scab susceptibility. Indeed, so far 'Gala' has been considered susceptible, regardless of the *V. inaequalis* isolate. For instance, Laurens et al. (2003) tested the same isolates as those used in our experiment, except for isolate 301, and showed that 'Gala' was highly susceptible to all the tested isolates. Nevertheless, macroscopic and microscopic studies have shown that its level of susceptibility differs according to the fungal isolate (M. Chevalier, personal communication 2007). In the greenhouse, 'Gala' can thus be considered a "moderately" susceptible cultivar. Our study showed that 'Gala' carries at least two QTL whose effects alone are not sufficient to confer a high level of resistance. Many authors have pointed out the contribution of susceptible genotypes to disease resistance, and this effect was weak (Schön et al. 1993; Lefebvre and Palloix 1996) or moderate (Pilet et al. 1998; Paillard et al. 2004).

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