

An Overview of the Position and Robustness of Scab Resistance QTLs and Major Genes by Aligning Genetic Maps of Five Apple Progenies

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Keywords: *Malus x domestica*, *Venturia inaequalis*, quantitative resistance, quantitative trait loci, genetic map, molecular markers

Abstract

In the frame of the D.A.R.E. project, five mapping populations have been studied for partial scab resistance against several races of *Venturia inaequalis*. A main objective was to identify QTLs (quantitative traits loci) with broad spectrum of resistance towards a wide range of strains of the fungus. Genetic markers (mainly SSR and AFLP) were tested on each population and genetic maps were constructed for both parents of each population. Meanwhile, pathological tests with several isolates of different races of *V. inaequalis* were performed. Four major genomic regions appear to be involved in scab resistance: they are located on linkage groups (LG) LG-1, LG-2, LG-11, and LG-17. Some other linkage groups carry either QTLs or major resistance genes that are isolate specific: a QTL on LG-5, *Vd* on LG-10, and *Vg* on LG-12. The QTL region located on LG-17 clearly exhibited the widest spectrum of resistance.

INTRODUCTION

Scab, caused by the fungus *Venturia inaequalis* (Cooke) G. Wint., is one of the most important diseases of apple (*Malus x domestica*) worldwide. Its control requires 12 to 20 fungicide sprays per year in a commercial orchard. Most of the apple cultivars grown in Europe are susceptible to scab. The use of genetically scab resistant cultivars is an alternative to the chemical control of the disease. The major resistance gene *Vf* has been widely used in many apple breeding programmes (Laurens, 1999). To date, two races able to overcome this resistance gene have been identified and characterised (Bénaouf and Parisi, 2000). Durability of scab resistance in new apple cultivars is a major objective for the future. The European project D.A.R.E. was dealing with this aspect (Lespinasse et al., 2000). Partial scab resistance, frequently called “polygenic” resistance, received special attention in this project which focused on the identification of QTLs (quantitative resistance loci) with broad spectrum resistance towards this genetically diverse pathogen. Quantitative polygenic resistance should be more durable than monogenic resistance (Parlevliet, 2002). Here, we present a synthetic overview of the genomic organisation of scab resistance in apple, focusing on the specificity or broad spectrum action of the detected resistance genes to a range of *V. inaequalis* isolates. Part of the results has already been published (Durel et al., 2003; Calenge et al., 2004). To our knowledge, this is the first in-depth study of the genetic dissection of partial scab resistance in apple while taking into account the variability of *V. inaequalis*.

MATERIALS AND METHODS

Five F1 progenies derived from crosses between three partial resistant cultivars or hybrids (‘Discovery’, TN10-8, and ‘Durello di Forli’), one moderately susceptible

cultivar ('Fiesta'), and one cultivar carrying the *Vf* and *Vg* major resistance genes ('Prima'), were studied (Table 1). Each progeny consisted of about 150 individuals.

Pathological tests were performed on the same progenies replicated at different locations either in a glasshouse with controlled isolates, or in the field with natural infection (Table 1). At INRA, the five progenies were also tested with 3 reference isolates corresponding to 3 races of *V. inaequalis* (race 1, race 6, and race 7), with the latter two capable of overcoming the *Vf* gene (Bénaouf and Parisi, 2000). Four more isolates were inoculated onto progeny C1 to further explore the genetic control of scab resistance in this progeny (Parisi et al., 2004; Calenge et al., 2004). For most of the pathological tests performed in the glasshouse, scions were grafted on MM106 in 3 replicates. The inoculum concentration was close to 300 000 conidia/ml for each isolate. Two traits were assessed 14 days and 21 days after inoculation: sporulation severity and symptom class as described by Calenge et al. (2004).

Molecular markers were developed to construct genetic maps for the five progenies (Table 1). The genetic map of progeny J was already published by Maliepaard et al. (1998) and completed during the DARE project (Van de Weg, pers. commun.). For the four other progenies, many AFLP primer combinations (Vos et al., 1995) and SSR markers (Gianfranceschi et al., 1998; Liebhard et al., 2002) were used. Forty common SSR markers well-distributed over the genome were selected to allow alignment of the different genetic maps. Genetic maps were constructed independently for the two parents of each progeny. A QTL analysis was then performed to detect QTLs. The software used were JoinMap (Stam and Van Ooijen, 1995) and MapQTL (Van Ooijen and Maliepaard, 1996).

RESULTS

Genetic maps were first constructed progeny by progeny. In all cases it was possible to design the 17 expected linkage groups of each parent. Only those LGs containing resistance factors are depicted in Fig. 1.

First, several major resistance genes, which had been mapped previously were positioned on the map: *Vf*, *Va*, and *Vb* on LG-1 (Maliepaard et al., 1998; Hemmat et al., 2003), *Vr2*, *Vr*, *Vh8* and *Vbj* on LG-2 (Patocchi et al., 2003; Bus et al., 2004), *Vd* on LG-10 (Tartarini et al., 2004), and *Vg* on LG-12 (Calenge et al., 2004).

New QTLs with rather strong specificities towards the tested isolates were identified on LG-3, LG-5, LG-10, and LG-15 in four different progenies (J, C1, C2, C4).

On LG-1 and on LG-2, two other new QTLs or QTL clusters were identified, which are involved in resistance to several tested isolates. In progenies C1 and J, a QTL effect was detected for several isolates at a similar position on LG-1. These QTLs are located in the same genomic region to which the major resistance genes *Vf*, *Va*, and *Vb*, have been mapped. *Vf* is not segregating in the progeny C1, but thanks to the SSR marker 'CH-Vf1' developed by Vinatzer et al. (2004), the position of these QTLs could be aligned with the location of *Vf*. CH-Vf1 is very tightly linked to *Vf*, and was easily mapped in progeny C1 to determine the position of the *Vf* locus (Calenge et al., 2004). All the QTL effects detected in progeny C1 were associated with the presence of a favourable allele in the parent TN10-8, which is derived from a polygenic 'Antonovka' (P.I. 172632). The favourable effect was shown to originate from this Antonovka with the SSR. On LG-2, different QTL effects were detected in progeny C1 in both 'Discovery' and TN10-8. This QTL or QTL cluster has been shown to co-localise with 3 major resistance genes (Bus et al., 2004). No QTL effect was identified on either LG-1 or LG-2 that was effective against all the tested isolates, i.e. the QTL or QTL clusters exhibited at least partial specificities towards the different *V. inaequalis* isolates.

A QTL effect on the top of LG-11 of 'Fiesta' was identified in progeny J for two isolates of race 6 (Durel et al., 2003). In progeny C2, the same region was carrying a QTL (or a QTL cluster) for resistance to all three isolates tested in the glasshouse with the same favourable allele from 'Fiesta'. A broad spectrum QTL may thus be present at this position in this cultivar.

Finally, QTL effects were detected very frequently on LG-17 of both 'Discovery' and 'Fiesta' in all the progenies with nearly all the isolates tested. Every time the same QTL region in the cultivar 'Discovery' was detected: in progeny C1 for all the isolates tested except one; in progeny C2 for all isolates (greenhouse and field) tested; and in progeny C3 for an isolate of race 1. This region was also detected in 'Fiesta' in progeny C4, either in the field or in greenhouse test with Italian isolates. This QTL can be considered as a robust broad spectrum resistance QTL. Nevertheless, in some rare cases, no QTL effect was observed for D17 or F17: the presence of a major gene or a too high inoculum pressure may be reasons for such negative observations. A preliminary study revealed that an integrated analysis of the 3 progenies involving 'Discovery' revealed more significantly this QTL than when each progeny was analysed separately. This was to be expected, since integration of progenies increases the number of genotypes and thereby the power of statistical analysis (higher LOD score). The consecutive reduction of confidence interval of the QTL has to be further explored.

DISCUSSION

We now have a rather good overview of the genomic organisation of scab resistance factors in apple taking into account the fungus variability. Several regions with quite high isolate specificities were detected. Two regions, on LG-1 and LG-2, exhibited an intermediate spectrum of resistance. Interestingly, major resistance genes are co-localising in these two regions hence one can hypothesize that the detected QTL may belong to the same corresponding resistance gene families. In four cases, QTL might be considered as major R genes with only partial effect. In that way, they may be involved in pathogen recognition. A similar situation may be present on LG-10 involving both a major gene and QTL. The co-localisation of the QTL from TN10-8, which is derived from 'Antonovka' P.I. 172632, and the major gene *Va* from 'Antonovka' P.I. 172633 mapped by Hemmat et al. (2003) on LG-1 is another argument in favour of major genes and QTLs being different alleles of a single locus or different paralogs of a resistance gene family. Finally, two regions with broad spectrum of resistance were identified on LG-11 and LG-17. Especially on LG-17, most of the isolates tested indicated a QTL effect in this region, with always the same favourable allele in 'Discovery' and probably also in 'Fiesta' (data not checked). These two regions are not known to carry any major R genes for scab resistance hence these two regions may be rather involved in either signalling pathways or defence mechanisms. These broad spectrum resistance loci (or clusters of loci) should be good candidates for durable resistance.

ACKNOWLEDGEMENTS

This project was carried out with the financial support from the Commission of the European Communities (contract N° FAIR5-CT97-3898). This publication does not necessarily reflect the Commission's views and in no way anticipates its future policy in this area. Its content is the sole responsibility of the publishers.

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Tables

Table 1. Pathological tests performed on the five analysed progenies and distribution of the work for genetic map construction.

Progeny	Pathological tests ⁽¹⁾							Origin map
	Race 1	Race 5	Race 6	Race 7	Race NL	Field isolate	Nat. inoc.	
C1 Discovery x TN10-8	X	X	X	X	X	X	-	INRA
C2 Fiesta x Discovery	X	-	X	X	-	-	X	PRI + ETHZ
C3 Discovery x Prima	X	-	X	X	-	-	-	IOZ
C4 Durello di Forli x Fiesta	X	-	X	X	-	X	X	DCA-BO
J Prima x Fiesta	X	-	X	X	-	-	-	PRI + ETHZ

⁽¹⁾ Most of the different isolates are described in Bénaouf and Parisi (2000) or in Parisi et al. (2004): Race 1 = n° 104 and EU-B-04; Race 5 = n° 163; Race 6 = n° 302 and EU-D-42; Race 7 = n° 1066; Race NL = EU-NL-24; Field isolates derives from French (C1) or Italian (C4) orchards.

Figures

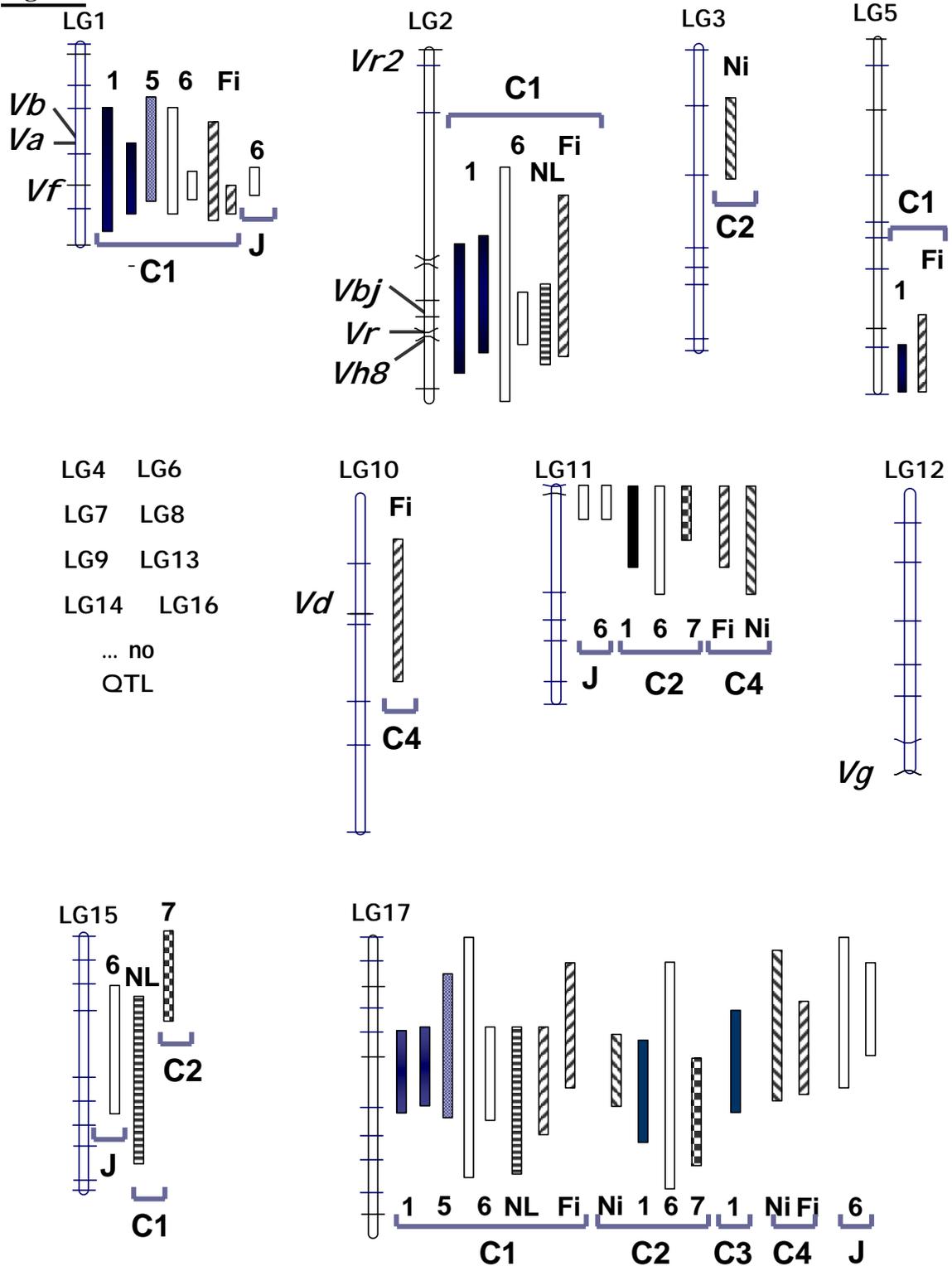


Fig. 1. Genomic organisation of scab resistance factors in apple. Vertical bars represent the confidence intervals of the position of the QTL (2-LOD interval support). Isolates tested: race 1 (black), race 5 (grey), race 6 (white), race 7 (draughtboard), Dutch isolate (horizontal stripe), field isolate (ascending line), natural inoculation (descending line). Each bar presents the result of a single isolate. Since in some cases more than one isolate of a race was tested, various bars can be present for a single race.