

Delineation of a Scab Resistance Gene Cluster on Linkage Group 2 of Apple

V. Bus¹, W.E. van de Weg², C.E. Durel³, C. Gessler⁴, F. Calenge³, L. Parisi³,
E. Rikkerink⁵, S. Gardiner⁶, A. Patocchi⁴, M. Meulenbroek², H. Schouten² and
F. Laurens³

¹HortResearch, Hawkes Bay Research Centre, Private Bag 1401, Havelock North, New Zealand

²Plant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands

³Institut National de la Recherche Agronomique, Centre de Recherches d'Angers, BP 57, 49071 Beaucoz , France

⁴Swiss Federal Institute of Technology, Institute of Plant Science/Phytopathology, Universit tstrasse 2, 8092 Zurich, Switzerland

⁵HortResearch, Mt Albert Research Centre, Private Bag 92 169, Auckland, New Zealand

⁶HortResearch, Palmerston North Research Centre, Private Bag 11 030, Palmerston North, New Zealand

Keywords: *Venturia inaequalis*, genetic markers, breeding strategy

Abstract

With the advent of genetic maps for apple that carry common transferable markers, it is possible to investigate genomic relationships between genes present in different accessions. Co-dominant markers, such as microsatellites, are particularly useful for this purpose. In recent years, genetic markers have been developed for a number of resistance genes for apple scab (*Venturia inaequalis*). In this paper, we present the discovery of a new scab resistance gene (*Vh8*) that maps to linkage group 2 (LG2). We then bring together the findings from different research groups on other scab resistance genes that also map to LG2 in an attempt to delineate their arrangement. These other genes comprise major genes (*Vh2*, *Vr*, *Vbj* and *Vr2*), as well as several race specific quantitative trait loci (QTLs) from 'Discovery' and 'TN10-8'. Results indicate that *Vh2* and *Vr* are probably identical, and that *Vh2/Vr*, *Vh8*, *Vbj* and at least one of the QTLs are closely linked to each other on the lower half of LG2. A putative map of this gene cluster is presented. The fourth major gene, *Vr2*, maps at a significant distance from this gene cluster at the top end of LG2. We discuss the consequences of resistance gene cluster arrangements on breeding strategies for durable resistance to apple scab and the use of marker-assisted selection.

INTRODUCTION

Breeding for durable resistance to apple scab (*Venturia inaequalis*) goes hand in hand with the development of marker-assisted selection for scab resistance genes. In recent years, different research groups around the world have identified markers for many of these genes. With the advent of genetic maps for apple that have transferable markers, such as microsatellite markers, in common, it is possible to investigate the genomic relationships between different sources of scab resistance.

In this paper, we describe the discovery of a new scab resistance gene (*Vh8*) with the aid of a new race of *V. inaequalis* (race 8). This race enabled us to distinguish this new gene from the *Vh2* gene, whereas no definite distinction could be made based on either resistance symptoms, or the genetic marker studies. The marker studies suggested the presence of a resistance gene cluster on LG2. Recent research by different research teams around the world has shown that other scab resistance genes also map to this linkage group. We attempt to delineate the resistance genes on LG2 by integrating the localised genetic maps that have been developed for the individual genes to date. We also discuss the consequences of the resistance gene cluster arrangements on breeding strategies for durable resistance to apple scab.

Vh8, A NEW SCAB RESISTANCE GENE

HortResearch has a large germplasm collection, which includes *Malus sieversii* (Ledeb.) Roem. accessions collected in Kazakhstan and Kyrgystan (Luby et al., 2001). A modified backcross programme was started with accession *M. sieversii* W193B, which was selected for its resistance to apple scab in New Zealand. A 'Royal Gala' x W193B progeny was screened by artificial inoculation in the glasshouse with conidia from a mix of isolates. Half of the progeny from this cross showed both resistance (in the form of stellate necrosis) and susceptible (R + S) symptoms on the same leaf, while the other half of the progeny only showed susceptible symptoms (S). The segregation ratio was (R + S) : S = 77 : 75, which is not significantly different from an expected R : S = 1 : 1 ratio for a single major gene ($P(\chi^2 > 0.03) \sim 0.88$). The stellate necrotic symptoms (SN) caused by this gene were very similar to those conditioned by the *Vr* and *Vh2* genes from Russian apple R12740-7A. However, (R + S) symptoms were never observed in progenies segregating for either of these two genes inoculated with the same conidia mix, which suggests that these scab resistance genes are different from the one in *M. sieversii* W193B. Monospore cultures of virulent strains isolated from (R + S) leaves were able to infect accession W193B, but not accession TST34T15 (scab differential host 2) carrying the *Vh2* gene. These findings confirmed that a new gene was segregating, and that it was overcome by a new race of *V. inaequalis*, which we have designated race 8 (Bus et al., 2003). However, genetic marker studies revealed that the OPL19SCAR marker linked to the *Vh2* gene, was also linked to the new resistance gene in the accession W193B. The polymerase chain reaction (PCR) products of this SCAR marker were cloned and sequenced for both hosts. They were completely homologous. This high level of homology provided further evidence that the linked SCAR markers are allelic and therefore that the new *M. sieversii* gene, which we have named *Vh8*, and the *Vh2* gene are clustered or allelic.

CLUSTER DELINEATION

Both the *Vh2* and *Vh8* genes share markers with other scab resistance genes for which localised genetic maps have been developed, providing further support for the presence of a gene cluster on LG2. Here we attempt to delineate the scab resistance gene cluster based on the information available to date. Delineation was aided by the recent development of transferable marker systems, such as simple sequence repeat markers (SSRs), which are capable of integrating genetic maps from diverse genetic backgrounds. We note here that map distances between the same loci can sometimes vary significantly in different genetic backgrounds. This potential variation makes the inferred relative positions of the genes with respect to each other less certain, hence in this paper we focus mainly on the order of the genes on the chromosome.

The SSR marker map developed for 'Discovery' from a 'Fiesta' x 'Discovery' (FxD) family (Liebhard et al., 2002) was used as the base, and three SSRs (CH02c06, CH05e03, and CH03d01) on the lower half of LG2 provided the anchor points (Fig. 1A). This excludes the *Vr2* gene, which is located near the top of LG2 very close to CH02c02aSSR (Patocchi et al., 2004). The anchor points of the different genes that mapped to the cluster on LG2 are presented in Table 1 in the order that the genes were delineated. We started delineation with the *Vbj* gene, since all three SSRs mentioned above were used to map this gene (Gygax et al., 2004). It mapped very close to CH05e03SSR. The Z13SCAR marker provided the anchor point for the *Vh2* gene, since the 900 bp allele, which was linked to the susceptibility allele of the *Vbj* gene (Gygax et al., 2004), was found to be linked to the *Vh2* allele for resistance. Theoretically, this places the *Vh2* and *Vbj* alleles in repulsion phase in the cluster if they had been in the same genetic background. Linkage of the CH02b10SSR to the *Vh2* gene and its position above CH02c06SSR on the FxD map (Liebhard et al., 2002) determined the orientation of the map of the *Vh2* gene and placed this gene below the *Vbj* gene. The CH02b10SSR marker is also linked to the *Vr* gene (Hemmat et al., 2002), at about the same distance as it was from the *Vh2* gene. Although it is possible that both resistance factors are different alleles of the

same gene, it is more likely that they are actually the same, since they both originate from Russian apple R12740-7A and condition the same stellate necrotic resistance reactions. Hence we have putatively assigned them to the same position in the cluster. The *Vh8* gene has not been mapped with any SSR marker to date (Table 1). Anchor points for the positioning of this gene were provided by OPL19SCAR, which is linked to *Vh2* (Bus et al., 2000), and the 628 bp allele of the OPB18SCAR marker, which is linked to *Vr* (Hemmat et al., 2002). While the 628 bp allele of OPB18SCAR is in repulsion phase with the *Vh8* gene in the 'Royal Gala' x W193B family, a second allele of 799 bp is in coupling phase to the gene (Fig. 1A). Finally, a number of quantitative trait loci (QTLs) identified in a 'Discovery' x TN10-8 (DxT) family by different isolates, mapped to the same region of LG2 (Fig.1B) (Calenge et al., 2004). They are mapped here based on their 'QTL peaks', which place them all above the major genes (Fig. 1A). These QTLs came from both parents in the DxT cross, hence alleles for resistance will be present on both homologous chromosomes. The confidence interval (Fig. 1B) for the positions of some of these QTLs covers a large region of chromosome 2. Since these QTL regions at least partially co-localise with known major qualitative resistance genes and the QTL peak for one of these QTLs is reasonably close to the inferred position of *Vbj*, it is possible that both are encoded by similar genes, possibly from the same cluster. The fact that these QTLs appear specific in their host-pathogen interactions (Calenge et al., 2004), adds further weight to the argument that some QTLs may be specific partial resistances fitting the gene-for-gene model, rather than general resistance factors that are non-differential. However, the gene(s) underlying the QTLs might be at any position within their confidence interval (Fig. 1B), and the mapping of the DxT QTLs to this cluster may therefore be merely coincidence.

BREEDING STRATEGY

The delineated map presented in Fig. 1A is only one of the possible configurations of these scab resistance genes on LG2. Based on the data to date, we cannot exclude the possibility that some or all of the genes are allelic, which will allow for the pyramiding of a maximum of only two resistance alleles. However, from experience in other crops, it is more probable that we are dealing with a gene cluster, or perhaps a group of genes that are merely linked. In any case, the fact that resistance genes are linked, has consequences for the breeding strategy when pyramiding these genes. In a typical modified backcross situation, where the resistant parent is crossed with a susceptible parent with high fruit quality, genes linked in the coupling phase will remain together. Half of the progeny will still carry the same resistance gene combination (Fig. 2A), except for those in which the genes have separated due to recombination events, which will be rare if the genes are closely linked. When two resistance genes are present in the repulsion phase, i.e. the genes came from different genetic backgrounds and were combined into a single genotype through the pairing of the two homologous chromosomes, one from each of the parents, they will dissociate again in the next generation (Fig. 2B). In this case, one can either continue back-crossing the resistance genes in separate breeding lines and bring them together again in the final cross for cultivar development, or select for the few recombinants carrying the genes in the coupling phase and continue back-crossing the genes in the same breeding line (Fig. 2A). In either case, the success of a breeding strategy aimed at pyramiding two or more (see example in Fig. 2C) linked genes hinges on the availability of highly informative genetic markers.

CONCLUSION

In this paper, we have presented the discovery of a new scab resistance gene *Vh8*, which forms part of a gene cluster on LG2 of apple, including the *Vh2/Vr*, and *Vbj* genes as well as several scab resistance QTLs. We have attempted to delineate the cluster based on the information available to date, but further research, including the testing of a series of well-chosen progenies to a series of monospore isolates, is required to dissect the precise arrangement of scab resistance genes on LG2 as well as other linkage groups.

Literature Cited

- Bus, V., Laurens, F., Gardiner, S., Plummer, K. and Rikkerink, E. 2003. Molecular detection reveals a new gene-for-gene combination in the *Malus*-apple scab interaction. 8th Intl. Congress of Plant Pathology, 2-7 February 2003, Christchurch, New Zealand. Abstract book Vol. 2 - Offered papers. p.217.
- Bus, V., Ranatunga, C., Gardiner, S., Bassett, H. and Rikkerink, E. 2000. Marker assisted selection for pest and disease resistance in the New Zealand apple breeding programme. *Acta Hort.* 538:541-547.
- Calenge, F., Faure, A., Goerre, M., Gebhardt, C., Van de Weg, W.E., Parisi, L. and Durel, C.-E. 2004. Quantitative trait loci (QTL) analysis reveals both broad-spectrum and isolate-specific QTL for scab resistance in an apple progeny challenged with eight isolates of *Venturia inaequalis*. *Phytopathol.* 94:370-379.
- Gygax, M., Gianfranceschi, L., Liebhard, R., Kellerhals, M., Gessler, C. and Patocchi, A. 2004. Molecular markers linked to the apple scab resistance gene *Vbj* derived from *Malus baccata jackii*. *Theor. Appl. Genet.* 109:1702-1709.
- Hemmat, M., Brown, S.K. and Weeden, N.F. 2002. Tagging and mapping scab resistance genes from R12740-7A apple. *J. Amer. Soc. Hort. Sci.* 127(3):365-370.
- Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C.D., Tarchini, R., Van de Weg, E. and Gessler, C. 2002. Development and characterisation of 140 new microsatellites in apple (*Malus x domestica* Borkh.). *Molecular Breeding* 10:217-241.
- Luby, J., Forsline, P., Aldwinckle, H., Bus, V. and Geibel, M. 2001. Silk Road apples – collection, evaluation, and utilization of *Malus sieversii* from Central Asia. *HortScience* 36(2):225-231.
- Patocchi, A., Bigler, B., Koller, B., Kellerhals, M. and Gessler, C. 2004. Vr2: a new apple scab resistance gene. *Theor. Appl. Genet.* 109:1087-1092.

Tables

Table 1. The major scab resistance genes that were mapped to a gene cluster on the lower half of linkage group 2 in the order that they were delineated for Fig. 1. The genetic markers are listed in the order from top to bottom in which they were mapped to LG2. The anchor points presented in the table are the marker alleles that were linked to the respective genes.

Marker	Scab resistance gene (in order of delineation)			
	1 <i>Vbj</i>	2 <i>Vh2</i>	3 <i>Vr</i>	4 <i>Vh8</i>
CH02b10 SSR		121bp ^z	122bp ^z	
CH02c06 SSR	248bp			
Z13 SCAR	773bp	900bp		
CH05e03 SSR	150bp			
OPB18 SCAR			628bp	799bp
OPL19 SCAR		433bp		433bp
CH03d01 SSR	115bp			

^z The measurement of the size of these alleles was determined in two different ways: on agarose (for *Vr*) and on a DNA sequencer (for *Vh2*). As the accuracy measurement of at least one of these methods is likely to be greater than the 1 bp difference, it also is likely that they are actually the same allele and therefore have the same size.

Figures

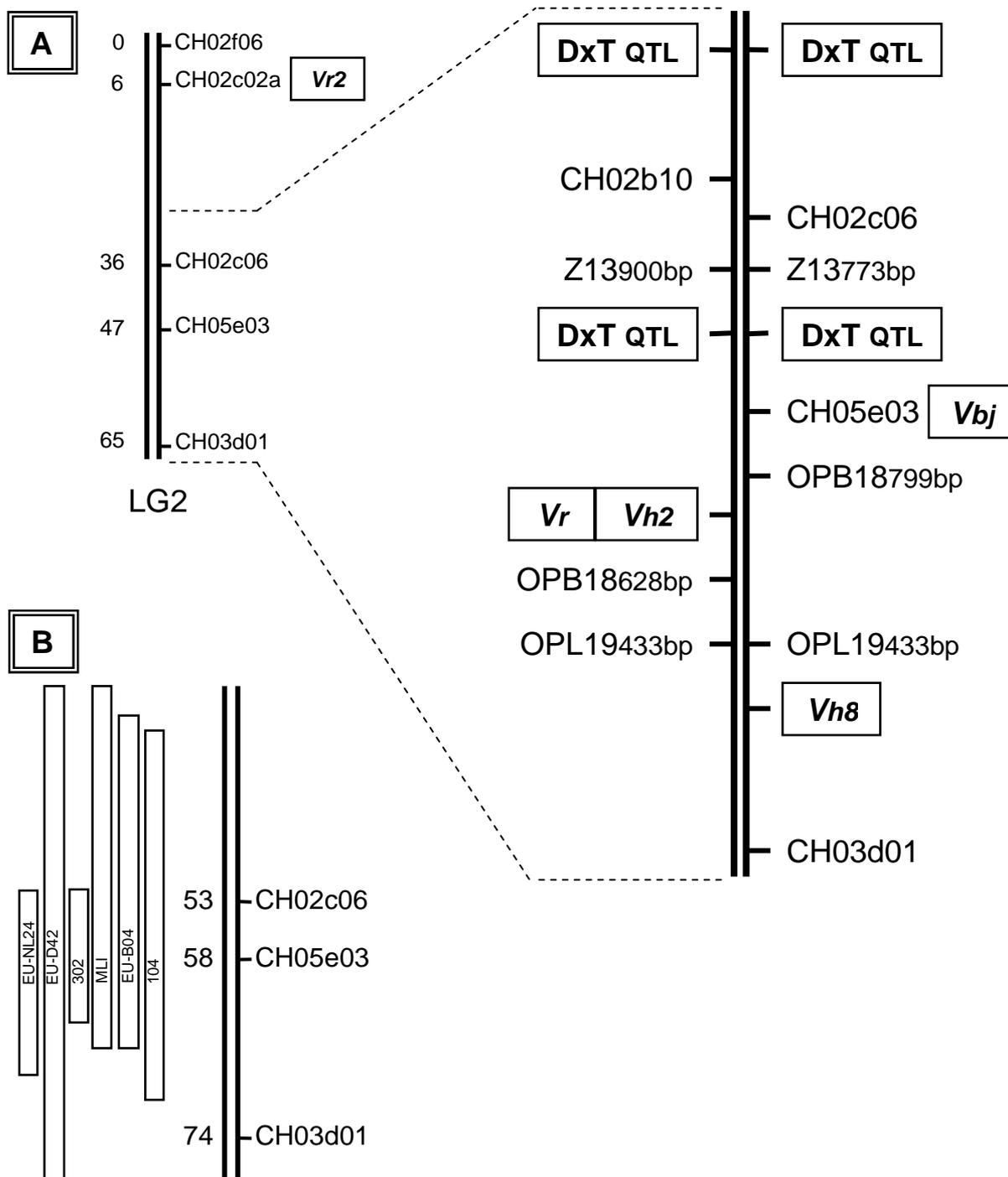


Fig. 1. Delineation of a scab resistance gene cluster on linkage group 2 (LG2) of apple based on the genetic maps for the individual major genes (A). The chromosome regions of LG2 identified as carrying QTLs for scab resistance in a 'Discovery' x TN10-8 (DxD) family (B), have been mapped according to their QTL peaks.

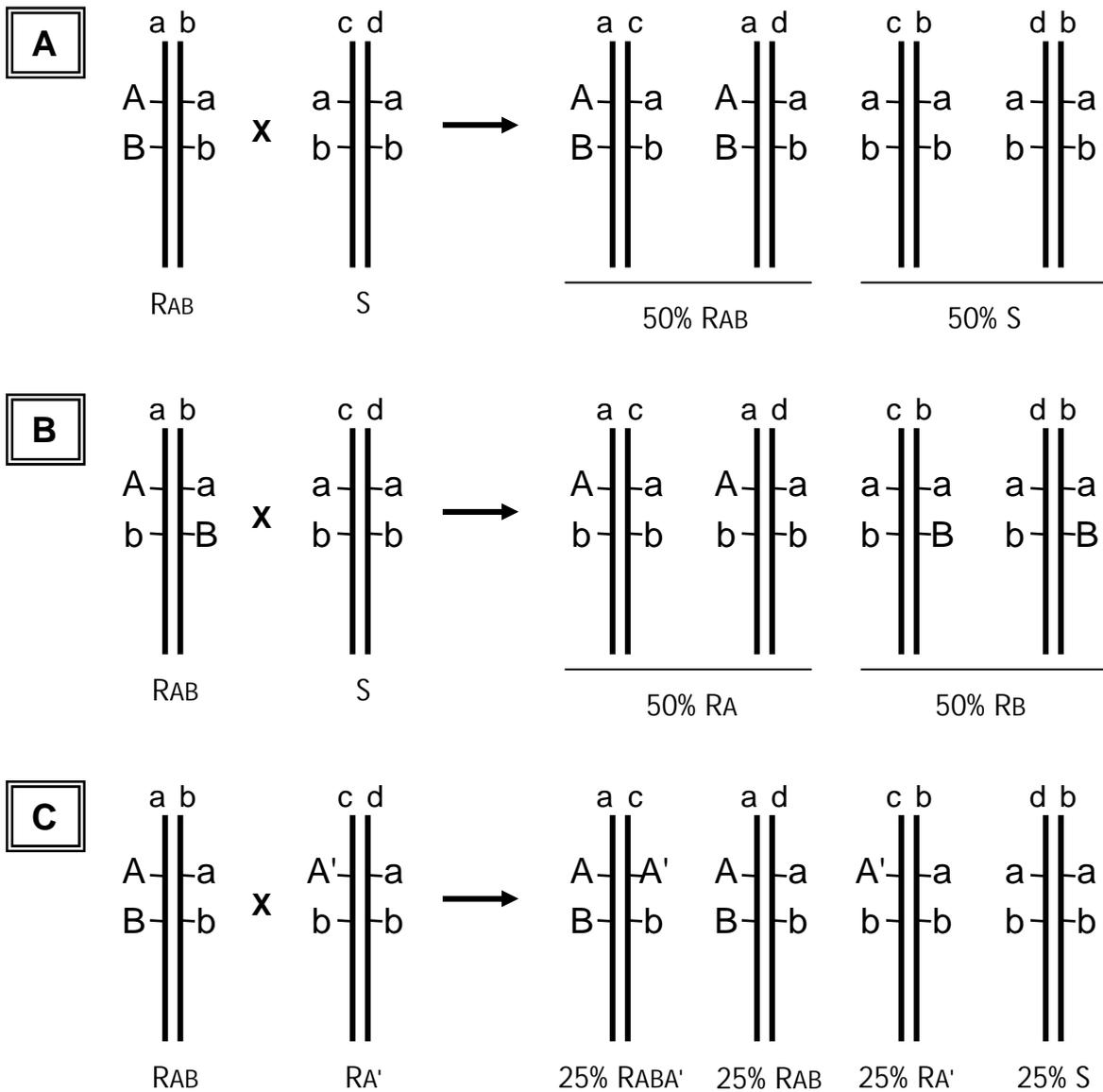


Fig. 2. Possible segregations of the progeny from a susceptible parent crossed with a resistant parent carrying two closely linked resistance genes with a low frequency of cross-over between the genes in coupling (A) or in repulsion (B) phase, and between a parent carrying two resistance genes in coupling phase and a parent carrying an allelic resistance to one of the resistance genes (C).