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ORIGINAL PAPER

M Gygax · L Gianfranceschi · R Liebhard ·
M Kellerhals · C Gessler · A Patocchi

Molecular markers linked to the apple scab resistance gene *Vbj* derived from *Malus baccata jackii*

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Abstract Breeding for scab-resistant apple cultivars by pyramiding several resistance genes in the same genetic background is a promising way to control apple scab caused by the fungus *Venturia inaequalis*. To achieve this goal, DNA markers linked to the genes of interest are required in order to select seedlings with the desired resistance allele combinations. For several apple scab resistance genes, molecular markers are already available; but until now, none existed for the apple scab resistance gene *Vbj* originating from the crab apple *Malus baccata jackii*. Using bulk segregant analysis, three RAPD markers linked to *Vbj* were first identified. These markers were transformed into more reliable sequence-characterised amplified region (SCAR) markers that proved to be co-dominant. In addition, three SSR markers and one SCAR were identified by comparing homologous linkage groups of existing genetic maps. Discarding plants showing genotype–phenotype incongruence (GPI plants) plants, a linkage map was calculated. *Vbj* mapped between the markers CH05e03 (SSR) and T6-SCAR, at 0.6 cM from CH05e03 and at 3.9 cM from T6-SCAR. Without the removal of the GPI plants, *Vbj* was placed 15 cM away from the closest markers. Problems and pitfalls due to GPI plants and the consequences for mapping the resistance gene accurately are discussed. Finally, the usefulness of co-dominant markers for pedigree analysis is also demonstrated.

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M. Gygax · L. Gianfranceschi · R. Liebhard · C. Gessler ·
A. Patocchi (✉)
Plant Pathology, Institute of Plant Sciences, Swiss Federal Institute of Technology,
8092 Zurich, Switzerland
e-mail: andrea.patocchi@ipw.agrl.ethz.ch
Tel.: +41-1-6325789
Fax: +41-1-6321572

M. Kellerhals
Agroscope FAW Wädenswil,
8820 Wädenswil, Switzerland

Present address:
L. Gianfranceschi
Department of Biomolecular Sciences and Biotechnology,
University of Milano,
20133 Milan, Italy

Introduction

Apple scab caused by the pathogen *Venturia inaequalis* (Cooke) G. Wint. is the most important apple disease in temperate regions throughout the world. A high number of fungicide treatments, usually between 15 and 20 per growing season, are necessary to control this disease. Environmental considerations and consumer health concerns call for a reduction of the intensive use of pesticides in apple orchards. Breeding for disease-resistant or less susceptible apple plants offer a way of achieving this goal.

Apple breeders have tried for many years to improve the scab resistance of their apple selections by introducing major resistance genes derived from wild apples (MacHardy 1996). The most successful breeding programs for scab resistance have been achieved using the resistance gene *Vf* (Janick et al. 1996). *Vf* is a major dominant resistance gene originating from the crab apple *Malus floribunda* 821. Until recently, *Vf* had not been overcome by the pathogen, and therefore, for about 50 years, this type of scab resistance was thought to be durable. Parisi et al. (1993), however, demonstrated that a new race of the pathogen (race 6) was able to develop scab lesions on apple cultivars or selections carrying *Vf*. Furthermore, Bénaouf and Parisi (2000) identified race 7 of *V. inaequalis*, a race that showed virulence towards *M. floribunda* 821. These observations clearly show that the resistance encoded by *Vf* can be overcome by the pathogen and can lead to boom and bust cycles, a phenomenon well-known, for example, in the barley powdery mildew pathosystem (Wolfe 1993).

The intensive use of *Vf* in breeding programs has led to a small genetic basis of scab resistance in apple selections released over the last 50–60 years. Since the *Vf* resistance can no longer be considered durable, there is an urgent

need to diversify the sources of scab resistance in apple breeding programs (Parisi et al. 1993). Consequently, new resistance breeding strategies have to be set up. A promising way is the incorporation of two or more resistance genes in the same cultivar or selection, the so-called pyramiding of resistance genes (MacHardy et al. 2001). This strategy should delay, or even prevent, the breakdown of the resistance genes. However, breeding for more than one resistance gene can hardly be achieved without the use of molecular markers, which greatly facilitate the identification of favourable genetic combinations during the selection process (Gianfranceschi et al. 1996).

Pyramiding resistance genes in an apple cultivar is not an easy task. First, resistance genes have to be available, preferably in advanced selections, and second, molecular markers linked to those genes have to be developed. Until now, six major scab resistance genes (*Vf*, *Vr*, *Vb*, *Va*, *Vm* and *Vbj*), all originating from crab apples have been identified. They have been singularly incorporated into commercial apples or selections, which makes them available for breeding purposes (MacHardy 1996). For all of them except *Vbj*, molecular markers have been developed: *Vf* (between others: Koller et al. 1994 Vinatzer et al. 2004); *Vr* (Hemmat et al. 2002); *Vb* and *Va* (Hemmat et al. 2003); and *Vm* (Cheng et al. 1998). Two other apple scab resistance genes, *Vx* and *Vr2*, and molecular markers associated to them have recently been identified (Hemmat et al. 2002; Patocchi et al. 2004). Among these genes, however, only *Vf* has been intensively studied. A positional cloning project led to the identification in the *Vf* region of a cluster of genes homologous to the *Cf* resistance genes of tomato and consequently called *HcrVfs* (homologues to the *Cladosporium fulvum* resistance genes of the *Vf* region, Patocchi et al. 1999a, b; Vinatzer et al. 2001). One of these genes, *HcrVf2*, when introduced into the susceptible cultivar Gala, conferred apple scab resistance to the transgenic plants, proving the identification of the first apple scab resistance gene (Barbieri et al. 2003; Belfanti et al. 2004).

In this paper, we present the first molecular markers associated to the apple scab resistance gene *Vbj* originating from the crab apple *M. baccata jackii*. Furthermore, the problems encountered during the construction of the genetic map of the *Vbj* region are discussed.

Materials and methods

Plant material

A population of 173 individuals from the cross selection A722-7 × cv. Golden Delicious (A×GD) was analysed. The apple selection A722-7 carries the scab resistance gene *Vbj* derived from the wild species *M. baccata jackii*, whereas Golden Delicious is a susceptible cultivar. Scab resistance was evaluated under glasshouse conditions at Agroscope FAW Wädenswil. The progeny seedlings were inoculated with a suspension of *V. inaequalis* field conidia (4×10^5 conidia/ml) as described by Gianfranceschi et al. (1996). Assessment of susceptibility and resistance was performed macroscopically after 10–12 days and rated in six classes (Chevalier et al. 1991) from 0 (no scab) to 4 (high susceptibility and heavy sporulation).

For pedigree analysis, apple cultivars or selections and other *Malus* species were available from Agroscope FAW Wädenswil (*M. baccata* Hansen's #2-CH, *M. baccata jackii* CH, A722-7, Starking, Golden Delicious and Worcester Pearmain), from the National Germplasm Repository for Apple and Grape, NYS Agricultural Experiment Station, Geneva, N.Y., USA (*M. baccata* Hansen's #2-US and *M. baccata jackii*-US), from the Purdue University, USA (selection OR18T26-US) or from INRA research station in Angers, France (selection OR18T26-Fr).

DNA extraction, bulked segregant analysis and RAPD amplification

DNA was extracted as described by Dellaporta's protocol (Dellaporta et al. 1983), with minor modifications following Koller et al. (1994).

Bulked segregant analysis (BSA) was performed as described by Michelmore et al. (1991). The diluted DNA samples (1 ng/µl) of ten resistant (class 0–2) and ten susceptible (class 4) progeny individuals were pooled into two resistant and two susceptible bulks. A total of 506 random ten-base Operon-primers (Operon Technologies) were evaluated via PCR under the following conditions: amplification reaction volume was 15 µl containing 10 mM Tris-HCl (pH 9.0); 50 mM KCl; 1.5 mM $MgCl_2$; 100 µM each of dATP, dCTP, dGTP, dTTP (Roche, Switzerland); 0.3 µM primer; 5 ng genomic DNA; and 0.85 U *Taq* DNA Polymerase (Pharmacia). Amplification was performed in a PerkinElmer GeneAmp PCR System 9600 following Koller et al. (1994). Amplicons were separated on 1% agarose gels with 0.5× TBE and stained with ethidium bromide.

Transformation of RAPD fragments into sequence-characterised amplified region

The RAPD fragments associated with the resistance were transformed into more consistent sequence-characterised amplified region (SCAR) markers as previously described by Gianfranceschi et al. (1996). Plasmids were purified (Wizard Minipreps, Promega, Madison, Wis., USA), and the inserted fragments were sequenced on an Applied Biosystems 373 automated sequencer, using the ABI PRISM dye terminator cycle sequencing kit. Specific primers were designed using the program Primer, version 0.5 (Whitehead Institute for Biomedical Research, Cambridge, Mass., USA). Primers were synthesised by MWG-Biotech (Ebersburg, Germany).

Table 1 Primer sequences, product length in base pairs and annealing temperature of the molecular markers used in the study

Marker	Primer sequence (5′→3′)	Product length (bp)	Annealing temperature (°C)
RAPD			
OPB08	GTC CAC ACG G	710	36–45
OPK08	GAA CAC TGG G	848	36–45
OPT06	CAA GGG CAG A	801	36–45
OPZ13	GAC TAA GCC C	869	36–45
SCAR[a]			
K08-SCAR for	GAA CAC TGG GCA AAG GAA AC	743 (900)[b]	67
K08-SCAR rev	TAA AAG CCA CGT TCT CTC GC		
T06-SCAR for	CGT TCA ACT CAT AAG TGG TCC C	410 (790)[b]	60
T06-SCAR rev	AAG GGC AGA ATC ATA AAA GCC		
Z13-SCAR for	CCC TAG CAT GCC ATA AAA CC	773 (750, 870, 900)[b]	66
Z13-SCAR rev	CCC AGT GGA ATA TTT CGA GG		
SSR[c]			
CH02c06-for	TGA CGA AAT CCA CTA CTA ATG CA	248 (230, 236, 240)[b]	60
CH02c06-rev	GAT TGC GCG CTT TTT AAC AT		
CH03d01-for	CGC ACC ACA AAT CCA ACT C	115 (109, 113)[b]	60
CH03d01-rev	AGA GTC AGA AGC ACA GCC TC		
CH05e03-for	CGA ATA TTT TCA CTC TGA CTG GG	150 (176, 182, 190)[b]	60
CH05e03-rev	CAA GTT GTT GTA CTG CTC CGA C		

[a] *SCAR* Sequence-characterised amplified region
[b] In *brackets* are the products also amplified by the specific primers, but not linked to the resistance gene *Vb*
[c] Gianfranceschi et al. (1998) and Liebhard et al. (2002)

Specific PCR amplification

Specific PCR amplification of the SCAR markers was performed in an amplification reaction volume of 20 µl containing 5 ng of genomic DNA, 10 mM Tris-HCl (pH 9.0); 50 mM KCl; 1.5 mM $MgCl_2$; 100 µM each of dATP, dCTP, dGTP, dTTP (Roche, Switzerland); 0.2 µM each of the corresponding SCAR specific primers (Table 1) and 1.4 U *Taq* DNA Polymerase (Pharmacia Biotech). Amplifications were performed in a PerkinElmer GeneAmp PCR System 9600 at the following conditions: 2 min 30 s at 94°C; then 35 cycles of 30 s at 94°C; 30 s at the corresponding annealing temperature for the specific primers used (Table 1); 1 min at 72°C with the last cycle, followed by extension at 72°C for 10 min. After amplification samples were kept at 4°C until used. Amplification products were electrophoresed as described above.

Comparative mapping

Additional markers linked to the resistance gene *Vbj* were identified by comparative mapping. Z13-SCAR was tested on a cv. Iduna × selection A679-2 (I×A) progeny population from which an RAPD and SSR linkage map has been generated by Seglias (1997). Segregation analysis and mapping was performed with 48 individuals from the I×A progeny population. Once the map position of Z13-SCAR was identified in that second cross, molecular markers belonging to the homologous I×A linkage group, closely linked to Z13-SCAR and therefore potentially linked to *Vbj*, were then tested on the A×GD population. Finally, comparative mapping was also performed with a cv. Fiesta × cv. Discovery map generated by Liebhard et al. (2002). Linkage analysis with genetic markers originating from homologous linkage group of this third population allowed the detection of new SSRs (simple sequence repeat) markers potentially linked to the *Vbj*. These SSRs were tested on all individuals of the cross A×GD following Gianfranceschi et al. (1998).

Mapping

All linkage map calculations were performed with the software JoinMap, version 2.0 (Stam and van Ooijen 1995), in connection with JMDesk, version 3.6 (http://www.ecogenics.ch).

In order to construct the maternal linkage map (A722-7), co-dominant SCAR and SSR markers were used as dominant markers; only the allele in coupling with the resistance was taken into consideration. To define the linkage groups, a LOD score of 5.5 was used. Map distances were calculated according to the Kosambi mapping function (Kosambi 1944).

Results

Resistance screening

A 1:1 segregation ratio was observed between resistant and susceptible plants. Considering all individuals of the classes 0 to 3b as resistant, 92 individuals were classified

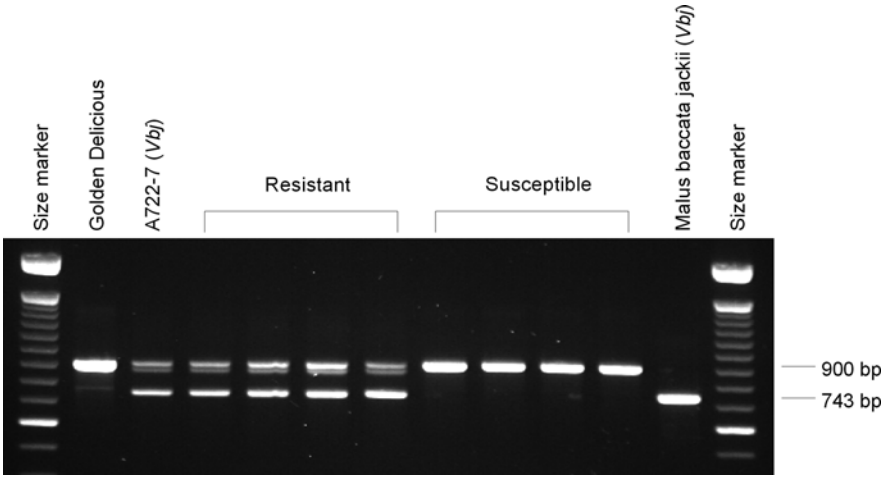

Fig. 1 Amplification profile of the marker K08-SCAR from Golden Delicious, A722-7, four resistant and four susceptible seedlings and *Malus baccata jackii*. The fragment of 743 bp is linked to the resistance gene *Vbj*. This fragment is clearly also amplified from *M. baccata jackii*, the donor of the resistance gene

as resistant (eight of class 0, 29 of class 2, 20 of class 3a and 35 of class 3b) and 81 as susceptible (class 4). This segregation pattern corresponds to a 1:1 ratio (χ^2=0.58) and confirms the hypothesis of *Vbj* being a dominant resistance gene present heterozygously in the apple selection A722-7.

Identification of RAPD markers by means of bulked segregant analysis

Three out of 506 random decamer primers produced polymorphic PCR products between the resistant and the susceptible bulks. These three RAPD primers, named OPB08, OPK08 and OPZ13 (Table 1), amplified PCR fragments of 710, 848 and 869 bp respectively, which were present in the two resistant bulks and the female parent A722-7 (*Vbj*), but absent in the two susceptible bulks and the male parent Golden Delicious. Analysis of the A×GD progeny (173 plants) showed that the three RAPD markers were linked to the scab resistance gene with recombination frequencies of 16.2–22.0%.

SCAR markers derived from RAPD markers

The two RAPD markers OPK08 (848 bp) and OPZ13 (869 bp) were transformed into SCAR markers. The specific primers pairs K08-SCAR for/rev and Z13-SCAR for/rev (Table 1) produced the expected amplicons, 743 bp and 773 bp, respectively. Each SCAR marker co-segregated with the RAPD fragment from which it was derived.

The specific primers K08-SCAR for/rev produced a second fragment of 900 bp which was amplified from both parents and from all progeny individuals (Fig. 1). Also, Z13-SCAR yielded extra bands. Z13-SCAR in A722-7 amplified two extra fragments of 900 bp and 750 bp, while in Golden Delicious it amplified two fragments of 750 bp and 870 bp (Fig. 2). According to these amplifications, we concluded that the specific Z13-SCAR and K08-SCAR each amplify two loci.

Identification of three SSR markers and one SCAR from existing maps

The Z13-SCAR segregated in the I×A progeny population showing a 1:1 segregation (data not shown). According to linkage analysis, Z13-SCAR mapped on linkage group 4 of A679-2 (Seglias 1997), which corresponds to linkage

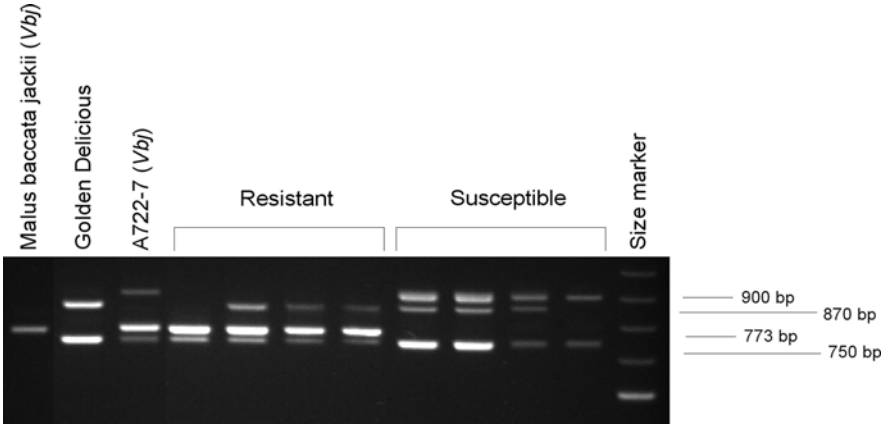

Fig. 2 Amplification profile of the marker Z13-SCAR from Golden Delicious, A722-7, four resistant and four susceptible seedlings and *M. baccata jackii*. The fragment of 773 bp is linked to the resistance gene *Vbj*. This fragment is also clearly amplified from *M. baccata jackii*, the donor of the resistance gene

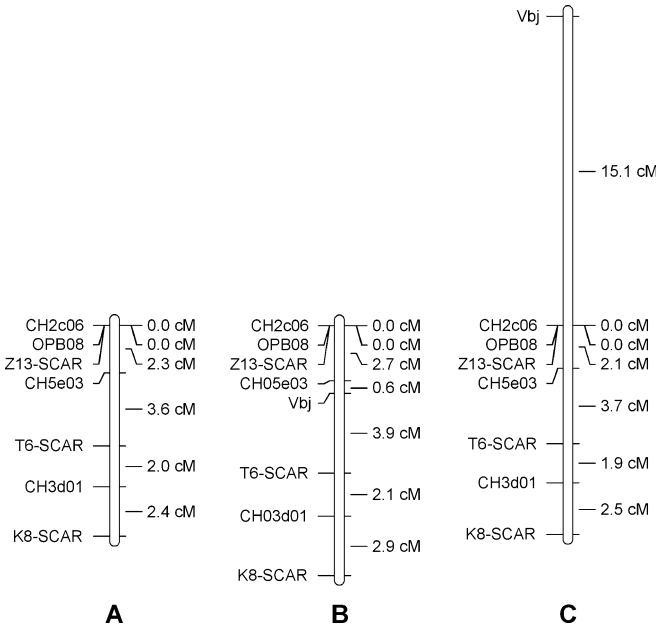

Fig. 3 **a** Linkage map of the *Vbj* genomic region calculated solely from the segregation data of the molecular markers of the complete A722-7 × Golden Delicious progeny population (173 plants). **b** Linkage map including *Vbj*, calculated by excluding from the dataset the 25 plants showing genotype–phenotype incongruence (GPI) plants. **c** Linkage map calculated from the segregation data including the 25 GPI plants

group 2 according to Liebhard et al. (2002). Two markers from the former linkage group, the RAPD marker OPT06 and SSR CH02c06 (Table 1), were tested on the parental plants A722-7 and Golden Delicious, and on a sample of four resistant and four susceptible progeny. Since they segregated as expected, the RAPD marker OPT06 was transformed into a SCAR, and linkage analysis was performed on the whole A×GD population. T06-SCAR primers (Table 1) produced the expected 790-bp fragment, which was amplified from both parents and all progeny. A second unexpected DNA product of 410 bp was also amplified, but only from A722-7 and the resistant progeny.

The SSR marker CH02c06 (Gianfranceschi et al. 1998) amplified two fragments of 230 bp and 248 bp, the latter (248 bp) was in coupling with the scab resistance.

Since CH02c06 also mapped on linkage group 2 of the cultivar Fiesta (Liebhard et al. 2002), two further microsatellites from the same region of linkage group two, SSRs CH05e03 and CH03d01 (Table 1), were tested. Their two alleles in coupling with the resistance gene are 150 bp and 115 bp long, respectively.

Construction of the linkage map of the *Vbj* region

Using the segregation data of the alleles in coupling with the resistance of all seven markers, OPB08, K08-SCAR, T06-SCAR, Z13-SCAR, CH02c06, CH05e03 and CH03d01, a map has been calculated. All seven markers were linked in a single linkage group with a total length 10.3 cM (Fig. 3a).

Before calculating the map with the segregating data of *Vbj*, the seedlings were carefully checked. Thirteen resistant plants (classes 2 to 3b) were identified that did not have any of the seven marker alleles in coupling with *Vbj*, while 12 susceptible plants (class 4) amplified all marker alleles in coupling with *Vbj*. These 25 plants showing genotype–phenotype incongruence (GPI plants) plants were discarded for the map-construction. The resistance gene *Vbj* was then mapped between the markers CH05e03 and T06-SCAR, with map distances of 0.6 cM and 3.9 cM, respectively. No change of the order of the markers and only a small increase (1.9 cM) of the total length of the linkage group has been observed (Fig. 3b). Without the removal of the 25 GPI plants, *Vbj* was mapped 15.1 cM from CH2c06 and the linkage group reached 25.3 cM (Fig. 3c).

Pedigree analysis of A722-7 and origin of the resistant gene

At the start of this investigation, according to the breeder's information, A722-7 was said to carry the resistance allele *Vb* originating from *M. baccata* Hansen's #2. The test of this accession, with the molecular markers identified in this study, raised some doubt about the correctness of the pedigree of A722-7. Although Z13-SCAR, K08-SCAR and CH02c06 amplified the alleles associated to the apple scab resistance gene identified in A722-7 from *M. baccata* Hansen's #2, all other molecular markers did not. Moreover, none of the SCAR markers or any SSRs alleles

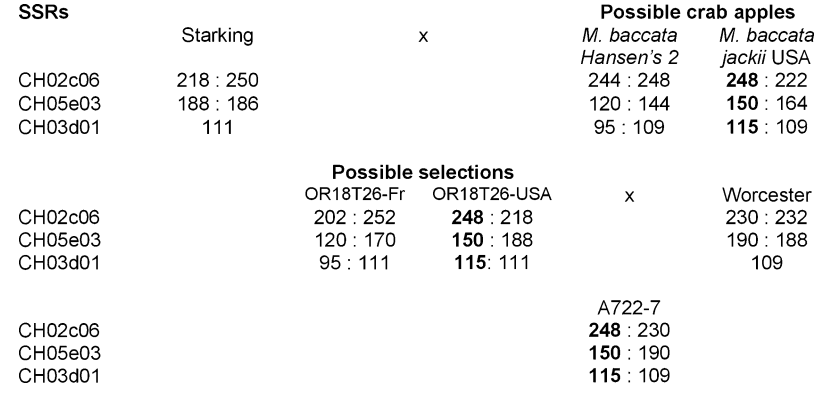

Fig. 4 The SSR analysis of the pedigree of A722-7. In the initial crosses, the two possible scab-resistant crab apples are shown. Also the two putative OR18T26, i.e. one from INRA Angers France and one from the USA, were tested. *Roman numbers* indicate the size of the fragments amplified with the corresponding combination of SSR and cultivar/selection. *Numbers in boldface* indicate the alleles in coupling with the resistance gene *Vbj*

linked to the resistance gene were present in OR18T26-Fr, the apple selection from INRA Angers (France), which should be the female parent of A722-7 and the donor of the resistance gene (Fig. 4).

According to Vincent Bus from The Horticulture and Food Research Institute of New Zealand and Jules Janick from Purdue University, USA (V. Bus and J. Janic, personal communications), the original cross that led to A722-7 was actually cv. Starking × *M. baccata jackii*. This hypothesis was then tested by analysing new plant material from the United States. All SCAR and SSR markers alleles found in A722-7 were amplified from *M. baccata jackii*-USA and OR18T26-USA. These plants are therefore the ancestors of A722-7, and the resistance gene present in A722-7 is *Vbj* (Fig. 4). Furthermore, we concluded that OR18T26-Fr (from Angers, France) was not identical to the OR18T26-USA, although theoretically the former should be a clone of the latter.

Discussion

Resistance screening and identification of RAPD markers by means of BSA

Resistance phenotyping is one of the first tasks in a project with the aim of mapping a resistance gene. This allows the segregation ratio of resistant to susceptible individuals from a controlled cross to be determined.

Since a 1:1 segregation ratio between resistance and susceptible plants was observed, it is concluded that the selection A722-7 carries the dominant resistance gene *Vbj* in a heterozygous state. This result confirms the findings of Dayton and Williams (1968), who first demonstrated that *Vbj* is a single dominant gene originating from the wild species *M. baccata jackii*.

A high number of arbitrary primers were screened for the detection of a few molecular markers: out of 506 primers, only three produced RAPD markers linked to the resistance gene *Vbj*. The success rate of 0.6% can be considered quite low; however, this result does not differ greatly from previous studies which identified RAPD markers linked to the dominant resistant gene *Vf* (Koller et al. 1994; Yang and Krüger 1994; Tartarini 1996; Gardiner et al. 1996). This can be explained by the use of heterozygous resistant bulks, which reduce the probability of detecting polymorphism by 50% (Melchinger 1990).

Identification of additional markers by comparison with existing maps

Besides the three markers found by BSA, four molecular markers were identified by comparative mapping, i.e. molecular markers developed for the gene of interest are mapped in other populations allowing the identification of homologous linkage groups, then the molecular markers identified in these linkage groups are tested for their linkage to the gene of interest. In this way, additional genetic markers closely linked to *Vbj* were identified: T06-SCAR and SSR CH02c06 previously mapped on linkage group 4 of the I×A population (Seglias 1997) and the two SSRs CH05e03 and CH03d01 from linkage group 2 of a Fiesta × Discovery population (Liebhard et al. 2002). This clearly demonstrated that multi-allelic markers can be used as an allelic bridge to identify potential new markers, by identification of homologous linkage groups. The advantage of such a method is obvious: new markers can be rapidly identified, eliminating all the routine work associated with BSA.

Construction of the linkage map of the *Vbj* region

Three linkage maps of the *Vbj* region were constructed. The first map was calculated using only the segregation data of the markers and the complete population (173 individuals, Fig. 3a). The second map composed of the seven markers, and the apple scab resistance phenotype was calculated by eliminating from the dataset the 25 GPI plants (13 resistant plants without any of the seven marker alleles in coupling with *Vbj* and 12 susceptible plants with all marker alleles in coupling with *Vbj*, Fig. 3b). Without the removal of the 25 GPI plants, *Vbj* was mapped outside the group of markers at 15.1 cM from CH02c06 (Fig. 3c).

The precise mapping of the resistance gene relies on the accuracy of the biological assay, i.e. on phenotyping the segregating progeny, but sometimes this is not sufficient to correctly map a resistance gene. In fact, the presence/absence of *Vbj* in the plants can still be falsely inferred from their phenotypes without a mistake in the scoring. This is the case, for instance, when the resistance of a progeny plant is due not to the presence of the major gene but to a positive assembly of resistance QTLs or when the susceptibility of a plant is due to a negative assembly of modifiers of the major gene in its presence.

All these phenomena have already been reported in the apple breeding literature. During the fine mapping of the *Vf* gene, Patocchi et al. (1999a) found about 9% of resistant individuals that did not carry the alleles in coupling with *Vf*. To construct the genetic map of the *Vf* region that was used as starting point for the positional cloning of the first apple scab resistance gene, the authors discarded those dubious plants. In addition, Hemmat et al. (1994) had to eliminate the 'double recombinants' to construct the linkage map of the two apple cultivars White Angel and Rome Beauty.

A further factor that can influence the host reaction, and therefore the classification of plant, is the concentration of the inoculum used (MacHardy 1996). Too high a concentration of inoculum may shift some plants into a higher class. Since the inoculum used for phenotyping the A×GD population had a concentration of 4×10^5 conidia/ml, which is rather high compared to usual tests where the concentration does not exceed 1.5×10^5 conidia/ml, the symptoms expressed in the plants were probably more severe. Consequently, this could have moved some plants carrying *Vbj* into the susceptible class 4, leading to GPI.

A further source of GPI could be due to the action of modifiers. As Rousselle et al. (1974) pointed out, the degree of resistance conferred by a major resistance gene can be modified by the action of minor or modifier genes inherited from both parents. In addition, Gessler (1989), studying the resistance gene *Vf*, postulated that the differences in the degree of resistance among the plants carrying the *Vf* gene could be due to two modifier genes. Depending on the combination of alleles present in the parents of a cross and the combination inherited, plants carrying the *Vf* gene may also show a susceptible phenotype. Therefore the 12 susceptible GPI plants carrying the *Vbj* markers could be plants carrying the *Vbj* gene and a combination of modifier alleles that shifted the plant into the susceptible class.

For the various reasons mentioned, we are convinced that it is correct to remove the 25 GPI plants from the dataset, and that the correct map position of *Vbj* is that shown in Fig. 3b.

Origin of the resistance gene

In a full-sib family microsatellites can potentially identify all four parental alleles at a given locus and are therefore a very powerful tool for pedigree analysis. According to the three SSR markers associated with *Vbj*, the original pedigree of the apple selection A722-7 was incorrect, showing that *M. baccata* Hansen's #2 could not be the original donor of the resistance gene (Fig. 4). The data clearly indicate that the resistance gene present in A722-7 originates from *M. baccata jackii*. It is not easy to find an explanation for this mistake, but since apple breeding is a very long process, breeders are forced to use mass-selection methods, exchange plant material or keep interesting selections for many years, sometimes between 30 and 60 years. Often, the precise source of the pollen donor of the resistance gene could be lost, simply because breeders do not remember exactly where it originates. As the trait (phenotype) was and still is relevant, the exact origin of the gene is of secondary importance to breeders. This seems likely to have occurred in our case as the original cross was done more than 25 years ago. Such errors seem not to be an exception in apple breeding. Cheng et al. (1995) already concluded that the pedigree of cv. Nova Easygro was incorrect and Gianfranceschi et al. (1996) demonstrated that the scab resistance gene present in Nova Easygro was not *Vr*, but *Vf*. Patocchi et al. (2004) proved that the accession GMAL 2473 cannot be cv. Russian Seedling, as previously thought. These few examples show that with the use of molecular markers and the availability of complete apple genetic maps, the pedigree of some apple varieties will probably have to be corrected in the near future.

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