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RAPD markers linked to the *Vf* gene for scab resistance in apple

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Abstract Scab (*Venturia inaequalis*) is one of the most harmful diseases of apple, significantly affecting world apple production. The identification and early selection of resistant genotypes by molecular markers would greatly improve breeding strategies. Bulked segregant analysis was chosen for the identification of RAPD markers linked to the *Vf* scab resistant gene. Five different RAPD markers, derived from the wild species *Malus floribunda*. 821, were identified, and their genetic distance from *Vf* gene was estimated. The markers $OPAM19_{2200}$ and $OPAL07_{580}$ were found to be very closely linked to the *Vf* gene. This result was indirectly confirmed by the analysis of resistant genotypes collected from various breeding programmes. Except for cv 'Murray', which carries the *Vm* gene, all these resistant genotypes showed the markers $OPAM19_{2200}$ and $OPAL07_{580}$.

Key words Apple · Scab resistance · *Vf* gene · RAPD · Linkage

Introduction

Apple scab, caused by the fungus *Venturia inaequalis* (Cke.) Wint., is a major disease in apple. In many parts of the world, at least ten fungicide sprayings are needed for effective crop protection. Genetic control of the disease is one way to reduce the risk of apple scab in the orchard and would effectively reduce fungicide input as requested by producers and consumers.

Eight different major resistance genes have been identified in apple germplasm from *Malus floribunda* 821 (*Vf*), *Malus micromalus* (*Vm*), Hansen's baccata no. 2 (*Vb*), *Malus baccata* Jackii (*Vbj*), *Malus pumila* R12740-7A (*Vr*), Antonovka Plant Introduction 172612 (or 172623?) (*Va*), Jonsib crab and Cathay crab (Williams et al. 1966; Dayton and Williams 1968; Williams and Kuc 1969; Hough et al. 1970). Ten allelic genes at the *Vf* locus have been identified in different *Malus* species (Williams et al. 1966; Williams and Dayton 1968), and a common origin can be postulated since most of them derive from the Arnold Arboretum (Williams and Kuc 1969). The *Vf* gene from *Malus floribunda* 821 is the main genetic source of scab resistance and has been widely used in breeding programmes since 1914 (Crandall 1926).

Scab symptoms under greenhouse conditions are assigned to five or six reaction classes (Hough et al. 1953; Chevalier et al. 1991). Usually, only the extreme class with clear and abundant sporulation is considered to be susceptible. Hough (1944) was the first to note that the "*floribunda*" type of resistance must be mainly determined either by a single factor or by a closely linked factor complex since backcross progeny segregation approximated a 1:1 ratio. Yet the change in reaction type through the backcross generation (class 1 for *Malus floribunda* 821; class 2 for $F_2$26829-2-2 and $F_2$26830-2; class 2–3 for the next modified backcross) suggested that "the original level of resistance was due either to a group of rather closely linked quantitative genes or to a class 3 reaction qualitative gene closely linked to one or more quantitative genes" (Williams and Kuc 1969). A low level of resistance (class 3) for the *Vf* gene is also supported by the finding that the same or an allelic gene, inducing a class 3 reaction type in seedlings, is present in *Malus*×*atrosanguinea* 804 and in *Malus micromalus* 245-38 but masked by the *Vm* "pit type" gene (Williams and Dayton 1968; Rousselle et al. 1974).

The variation in the level of resistance found in segregating progenies after being inoculated in a controlled atmosphere, seems to be due to the presence of minor genes modifying the expression of resistance that are independent of the major gene responsible for the "field immunity" (Lamb and Hamilton 1969). The presence of minor resistance genes (modifiers) which are inherited in a cumulative manner from both resistant and susceptible parents has also been postulated by others (Rousselle et al.

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1974; Gessler et al. 1993). Rousselle et al. (1974) observed changes in the distribution of resistant reaction classes in crosses between a resistant variety (or tester) with different susceptible cultivars. Also, a higher level of resistance was found in testcrosses with scab resistant selection from the early part of the breeding programme than from later generations. Some susceptible apple cultivars possess a differential resistance against scab, and a particular fungus isolate carries virulence which can overcome a particular host resistance (Gessler et al. 1993). Therefore, a clear specificity between susceptible apple cultivars and pathogen isolates can be postulated.

The selection of resistant seedlings in breeding programmes has been carried out by inoculating apple seedlings with a scab conidia suspension, but results can vary slightly depending on the inoculum used or the environmental conditions (Lamb and Hamilton 1969). Rousselle et al. (1974), in order to explain the excess of resistant seedlings in some progenies, further postulated that the presence of several modifiers in the absence of the *Vf* gene may be sufficient for the expression of a class 3 or M resistance.

The identification of molecular markers linked to scab resistance could represent a starting point to enhance backcross-breeding programmes and to perform an early selection of resistant seedlings, thereby avoiding the effects of the environment and differences in the evaluation criteria observed with the scab inoculum. Furthermore, the availability of markers tightly linked to physiologically different scab resistance genes will allow their combination in the same genotype so as to achieve a more durable resistance. This strategy is even more important after the identification in Germany of a new race of scab, named 6, that is able to overcome the resistance of advanced selections but not that of the original *Malus floribunda* 821 (Parisi et al. 1993). More recently, another scab isolate (FL1) capable of rendering the resistance of *Malus floribunda* 821 ineffective but not that of some later selections has been isolated in England (Roberts and Crute 1994).

The detection of random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) linked to major genes in specific genomic regions can be performed in segregating populations by 'bulked segregant analysis'. This technique has been used for the identification of RAPD markers linked to the downy mildew resistance gene in lettuce (Michelmore et al. 1991), to sex in *Silene latifolia* (Mulcahy et al. 1992) and to rust resistance in common bean (Haley et al. 1993).

In apple, the isozyme marker PGM-1 is reported to be linked at about 8 cM to the *Vf* scab resistance gene (Manganaris et al. 1994) and more recently, three RAPD markers linked to the *Vf* gene have been identified by bulked segregant analysis: $OPM18_{900}$, $OPU01_{400}$ (Koller et al. 1994) and $OPD20_{600}$ (Yang and Krüger 1994). The present article reports the identification of other RAPD markers linked to the *Vf* gene in apple and their consequent usefulness in breeding programmes.

Materials and methods

Plant materials and assessment of scab infection

A progeny of 'Prima' × 'Golden Delicious' (40 seedlings) was used for bulked segregant analysis; segregation of the identified markers was also performed on 'Prima' × 'Summerred' (27) and 'Prima' × 'Jerseymac' (42) progenies. Crosses were done in 1981 and, after 3 years of growth in nursery, the seedlings were transplanted to the field in 1985. Figure 1 shows the pedigree of the seedling progenies.

Triennal data on scab resistance were collected in full field on unsprayed seedlings. Only trees evincing clear sporulating lesions were considered susceptible. The usefulness of identified molecular markers was tested on a sample of resistant genotypes from various breeding programmes (USA, France, Canada, Germany) and on some tolerant and susceptible cultivars.

DNA extraction and bulk composition

Apple DNA was extracted from 1 g of freeze-dried leaves using a CTAB protocol (Doyle Doyle 1990; modified by G. J. King personal communication). RNA was removed from the DNA preparation by adding 2 µl of RNase A (10 mg/ml) and then incubating at 37°C for 2 h. The DNA was extracted with an equal volume of dichloromethane-isoamyl alcohol (24:1) and precipitated with 2 volumes of cold ethanol; it was then dissolved in sterile water. Sample DNA concentration was estimated by DNA fluorometry (Hoefer TK100, Hoefer Scientific Instrument, San Francisco, Calif.).

Two DNA samples were bulked by mixing 1 µg of DNA from each of 10 susceptible (bulk S) or 10 resistant (bulk R) seedlings of the 'Prima' × 'Golden Delicious' progeny. One hundred and eighty different 10-mer primers were used for screening DNA bulks (kits AA, AB, AC, AD, A, C, AL, AM and AN from Operon Technology, Alameda, Calif.).

RAPD analysis

The amplification reactions were performed in 25-µl volumes containing 10 m*M* Tris-HCl pH 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 200 µ*M* each dNTPs, 400 n*M* primer, 0.6 U *Taq* polymerase (Boehringer Mannheim, Germany) and 50 ng of genomic DNA. Amplification was performed in an Omnigene Thermal Cycler (Hybaid Limited, UK) programmed as follows (slightly modified after Koller et al. 1993): 1 cycle of 150 s at 94°C, 30 s at 36°C, 120 s at 72°C; 21 cycles of 20 s at 94°C, 15 s at 36°C, 15 s at 45°C, 90 s at 72°C; 19 cycles of 20 s at 94°C (increased 1 s/cycle), 15 s at 36°C, 15 s at 45°C, 120 s at 72°C (increased 3 s/cycle); 1 cycle of 600 s at 72°C.

Amplification products were size-fractionated by electrophoresis at about 3.5 V/cm through a 2% Seakem LE agarose gel (FMC, Rockland, Me.) in 1× TAE buffer. DNA was then stained in a 0.5 µg/ml ethidium bromide solution for 30 min (Sambrook et al. 1989).

Linkage analysis

Chi-square analysis for goodness of fit to the backcross type of segregation (single or two point) was performed for both the phenotypic and genotypic data. The Yates chi-square correction for continuity was used for the single-point test involving only one degree of freedom (Zar 1984). Heterogeneity chi-square analysis was performed to test the opportunity of pooling the data set from the three seedling progenies (Zar 1984).

Recombination frequencies and corresponding LOD values were calculated using the software programme JOINMAP (Stam 1993). A minimum LOD score of 3.0 was used to establish the degree of linkage. Map distances (in cM) were calculated using the Kosambi mapping function.

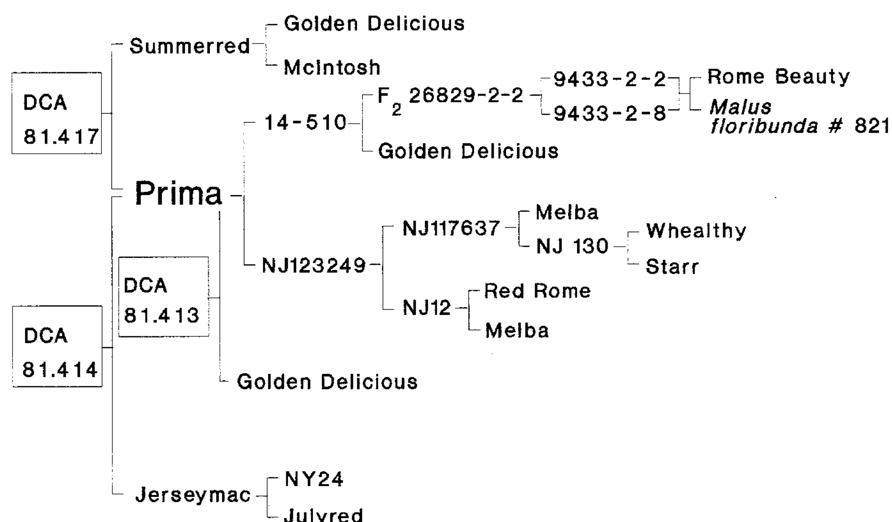

Fig. 1 Seedling progenies pedigree: 'Prima' × 'Golden Delicious' (DCA 81.413), 'Prima' × 'Jerseymac' (DCA 81.414) and 'Prima' × 'Summerred' (DCA 81.417)

Results

Scab infection

Differences were found in the intensity of scab lesions among the three populations derived from the same resistant cultivar tester ('Prima'). Scab lesions were abundant in leaves of susceptible seedlings of the 'Prima' × 'Golden Delicious' and 'Prima' × 'Summerred' progenies, where a moderate to heavy defoliation was recorded during the summer. In the 'Prima' × 'Jerseymac' population the scab symptoms were less pronounced and little defoliation occurred.

The triennal scab data on the three seedling progenies were in good agreement. Two exceptions (possible infection escapes), both observed in the 'Prima' × 'Jerseymac' progeny, have to be mentioned: trees nos. 11 and 40, classified as resistant in 1992 and 1993, exhibited scab lesions in 1994. Chi-square analysis of the *Vf* segregation showed a good fit to a 1:1 ratio for the 'Prima' × 'Jerseymac' and 'Prima' × 'Summerred' progenies; an unexpected deviation from the 1:1 backcross type of segregation was observed in the 'Prima' × 'Golden Delicious' progeny when fewer resistant seedlings were obtained (Table 1). The latter controlled cross was repeated in 1994 to check this anomalous segregation pattern. About a quarter of the seedlings died within a month after sowing, which was as expected since both parentals were heterozygous for the pale green lethal gene (Alston 1976; Way et al. 1976), although preliminary data on scab susceptibility collected after a greenhouse inoculation did not confirm this deviation (data not reported). This finding suggested pooling the data of the three seedling populations even if the heterogenity chi-square was significant ($P<0.05$), a result that was mainly due to the anomalous segregation in the 'Prima' × 'Golden Delicious' progeny (Table 1).

Bulked segregant analysis

Amplification patterns for the two DNA bulks (R and S) were identical for most of the random primers. More than 1,000 RAPD bands and as many loci were examined. Some primers (5%) did not amplify apple DNA at all.

Table 1 Segregation of the *Vf* gene for scab resistance in different apple seedling progenies. 'Prima' is the heterozygous resistant parent (*Vfvf*)

Seedling progeny (number of seedlings)	Phenotype[a]		Expected ratio	χ^{2b}	df	*P*
	Vfvf	*vfvf*				
'Prima' × 'Golden Delicious' (40)	11	29	1:1	7.23	1	**
'Prima' × 'Summerred' (27)	12	15	1:1	0.15	1	$0.5<P<0.75$
'Prima' × 'Jerseymac' (42)	23	19	1:1	0.21	1	$0.5<P<0.75$
			Total chi-square[c]	8.81	3	
			Pooled chi-square[c]	2.65	1	
			Heterogeneity chi-square	6.16	2	*

* Significant at 0.05; ** Significant at 0.01
[a] Number of seedlings; *Vfvf*=scab resistant; *vfvf*=scab susceptible
[b] Calculated with Yates chi-square correction for continuity
[c] Calculated without Yates chi-square correction for continuity

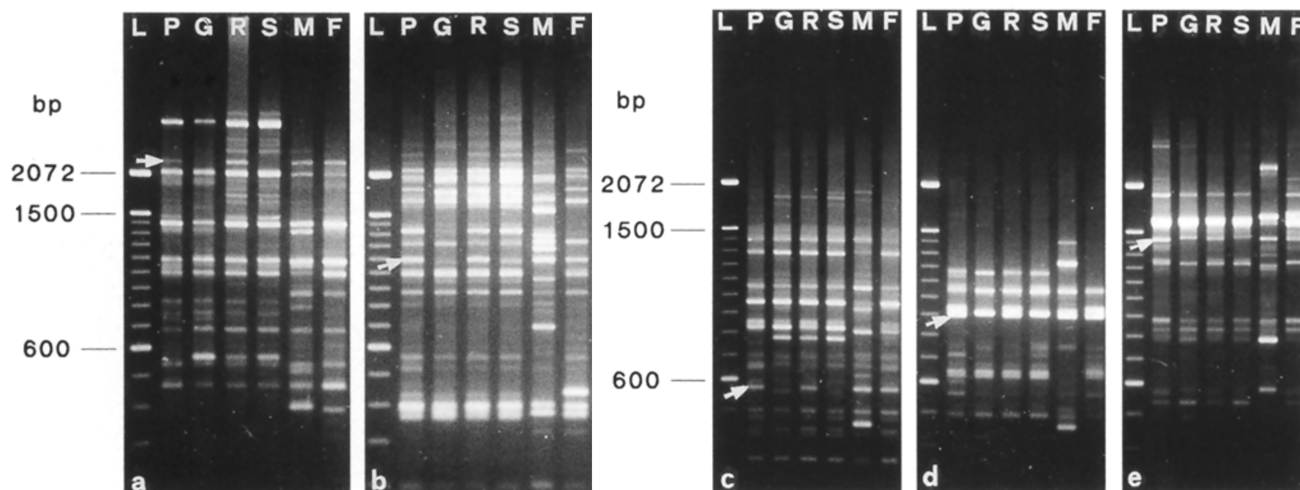

Fig. 2 RAPD amplification pattern using primers OPAM19 (**a**), OPC08 (**b**) OPAL07 (**c**), OPC09 (**d**) and OPAB19 (**e**) in the 'Prima' × 'Golden Delicious' apple seedling progeny. The polymorphisms linked to the *Vf* gene for scab resistance are marked by *arrows*. *P* 'Prima' scab-resistant parent, *G* 'Golden Delicious' scab-susceptible parent, *R* scab-resistant bulk, *S* scab-susceptible bulk, *M Malus floribunda* 821, *F* $F_2$26829-2-2, and *L* 100-bp ladder molecular-weight marker (Gibco BRL; size of bands indicated in bp)

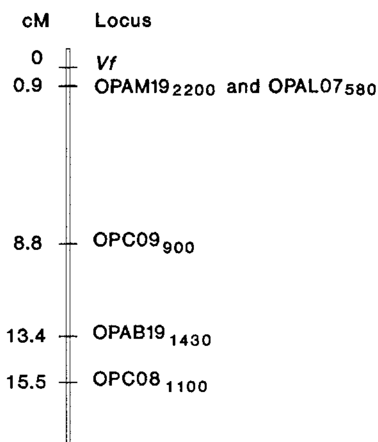

Fig. 3 Genetic map of the chromosome region containing the *Vf* gene for scab resistance

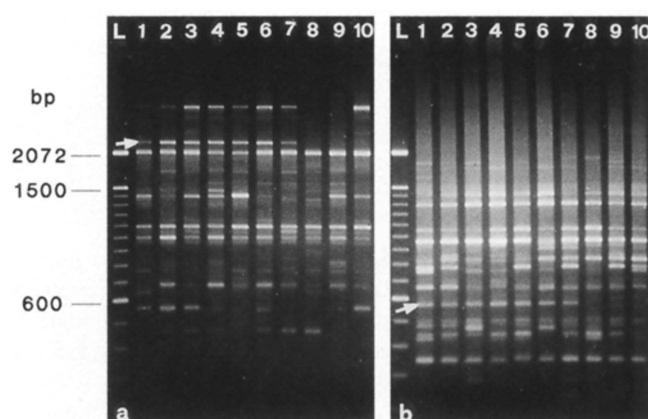

Fig. 4 RAPD amplification pattern using primers OPAM19 (**a**) and OPAL07 (**b**) in some advanced scab-resistant selections and cultivars. The polymorphisms linked to the *Vf* gene for scab resistance are marked by *arrows*. *1* 'Jonafree', *2* 'GoldRush', *3* 'Enterprise', *4* 'Freedom', *5* 'Relinda', *6* 'Florina', *7* 'Britegold', *8* 'Murray', *9* 'Royal Gala', *10* 'Jonagold', *L* 100-bp ladder molecular-weight marker (Gibco BRL; size of bands indicated in bp)

Five clear polymorphisms were identified: $OPAM19_{2200}$, $OPAL07_{580}$, $OPC09_{900}$, $OPC08_{1100}$ and $OPAB19_{1430}$. These fragments were amplified both in the resistant DNA bulk and in the resistant parent 'Prima'. They were also clearly derived from the wild species *Malus floribunda* 821, from which the *Vf* gene was introgressed (Fig. 2). A second polymorphism at 940 bp was also observed using the primer C09; this marker co-segregated with $OPC09_{900}$, but is derived from $F_2$26829-2-2 (and probably from its parent 'Rome Beauty') and not from *Malus floribunda* 821 (Fig. 2d). A longer electrophoresis period is required for a better resolution of the OPC09 amplification products since there are at least three fragments between 900 bp and 940 bp.

Chi-square analysis showed a deviation from the expected 1:1 ratio for all the markers in the 'Prima' × 'Golden Delicious' progeny, as was also observed for the *Vf* gene. Markers identified in the 'Prima' × 'Golden Delicious' progeny were useful both in the 'Prima' × 'Summerred' and in the 'Prima' × 'Jerseymac' progenies, where no deviation from the expected 1:1 ratio was found for RAPD markers (Table 2).

Co-segregation analysis with pooled data from the three seedling progenies confirmed the linkage between scab resistance and the five RAPD markers (Table 3). Only one seedling showed recombination between *Vf* and the two co-segregating markers, $OPAM19_{2200}$ and $OPAL07_{580}$, indicating that these two markers are quite close to *Vf*. The pooled recombination frequency estimated for $OPAM19_{2200}$ and $OPAL07_{580}$ was 0.9±0.9 (Table 3). Ten recombinant seedlings between the *Vf* gene and $OPC09_{900}$ were identified, and the pooled recombination frequency was estimated to be 9.2±2.8%. The other two markers

Table 2 Segregation of RAPD markers in different apple seedling progenies. 'Prima' is the heterozygous parent (Aa)

Seedling progeny (number of seedlings)	RAPD markers	Phenotype[a]		χ^{2b}	P
		Aa	aa		
'Prima' × 'Golden Delicious' (40)	$OPAM19_{2200}$	11	29	7.23	**
	$OPAL07_{580}$	11	29	7.23	**
	$OPC09_{900}$	13	27	4.23	*
	$OPC08_{1100}$	12	28	5.63	*
	$OPAB19_{1430}$	11	29	7.23	**
'Prima' × 'Summerred' (27)	$OPAM19_{2200}$	12	15	0.15	$0.50<P<0.75$
	$OPAL07_{580}$	12	15	0.15	$0.50<P<0.75$
	$OPC09_{900}$	14	13	0.0	$0.95<P<0.99$
	$OPC08_{1100}$	14	13	0.0	$0.95<P<0.99$
	$OPAB19_{1430}$	14	13	0.0	$0.95<P<0.99$
'Prima' × 'Jerseymac' (42)	$OPAM19_{2200}$	22	20	0.02	$0.75<P<0.90$
	$OPAL07_{580}$	22	20	0.02	$0.75<P<0.90$
	$OPC09_{900}$	23	19	0.21	$0.75<P<0.90$
	$OPC08_{1100}$	22	20	0.02	$0.75<P<0.90$
	$OPAB19_{1430}$	23	19	0.21	$0.50<P<0.75$

* Significant at 0.05; ** Significant at 0.01
[a] Number of seedlings; Aa=presence of RAPD marker; aa=absence of RAPD marker; expected ratio 1:1
[b] Calculated with Yates chi-square correction for continuity

Table 3 Loci, phenotypic frequency, χ^2 values for goodness of fit to backcross type of segregation (single point: χ^2A and χ^2B; two-point: χ^2AB), recombination frequency between loci and relative LOD score[a]

Locus A	Locus B	Phenotype[b] AB	Ab	aB	ab	χ^2A[c]	χ^2B[c]	χ^2AB[d]	Recombination frequency ±SE	LOD score
Vf	$OPAM19_{2200}$	45	1	0	63	2.35	2.97	111	0.9±0.9	30.30
Vf	$OPAL07_{580}$	45	1	0	63	2.35	2.97	111	0.9±0.9	30.30
Vf	$OPC09_{900}$	43	3	7	56	2.35	0.59	76.06	9.2±2.8	18.30
Vf	$OPC08_{1100}$	39	7	9	54	2.35	1.32	58.60	14.7±3.4	13.10
Vf	$OPAB19_{1430}$	40	6	8	55	2.35	1.32	64.39	12.8±3.2	14.70
$OPAM19_{2200}$	$OPAL07_{580}$	45	0	0	64	2.97	2.97	115.62	0.0	32.80
$OPAM19_{2200}$	$OPC09_{900}$	43	2	7	57	2.97	0.59	80.03	8.3±2.6	19.30
$OPAM19_{2200}$	$OPC08_{1100}$	39	6	9	55	2.97	1.32	62.12	13.7±3.3	13.80
$OPAM19_{2200}$	$OPAB19_{1430}$	40	5	8	56	2.97	1.32	68.06	11.9±3.1	15.50
$OPAL07_{580}$	$OPC09_{900}$	43	2	7	57	2.97	0.59	80.03	8.3±2.6	19.30
$OPAL07_{580}$	$OPC08_{1100}$	39	6	9	55	2.97	1.32	62.12	13.7±3.3	13.80
$OPAL07_{580}$	$OPAB19_{1430}$	40	5	8	56	2.97	1.32	68.06	11.9±3.1	15.50
$OPC09_{900}$	$OPC08_{1100}$	46	4	2	57	0.59	1.32	88.61	5.5±2.2	22.70
$OPC09_{900}$	$OPAB19_{1430}$	45	5	3	56	0.59	1.32	81.64	7.3±2.5	20.40
$OPC08_{1100}$	$OPAB19_{1430}$	46	2	2	59	1.32	1.32	96.69	3.7±1.8	25.40

[a] Data from the three seedling progenies were pooled for both chi-square and JOINMAP analysis
[b] Number of seedlings; A and/or B=scab resistant or presence of RAPD marker; a and/or b=scab susceptible or absence of RAPD marker
[c] Calculated with Yates chi-square correction for continuity (critical $\chi^2_{1df;\,P=0.05}$=3.841)
[d] Independence chi-square (critical value $\chi^2_{3df;\,P=0.01}$=11.345)

($OPC08_{1100}$, $OPAB19_{1430}$) are also linked to the *Vf* gene, though with a higher recombination frequency (Table 3).

Map distances in centiMorgans between the *Vf* gene and RAPD markers are shown in Fig. 3. The average chi-square for the goodness of fit of the map calculated by JOINMAP was in agreement with the normal fluctuation.

The $AM19_{2200}$ and $AL07_{580}$ markers were detected in all of the resistant genotype selections derived from *Malus floribunda* 821 (Fig. 4), although a few genotypes lacked the more distant markers (Table 4). *Vf*-linked RAPD markers were absent not only in all of the susceptible cultivars tested ('Fiesta', 'Fuji', 'Golden Delicious', 'Granny Smith', 'Jerseymac', 'Jonagold', 'Jonathan', 'Law Red Rome Beauty', 'Red Chief', 'Royal Gala', 'Staymared', 'Summerred') but also in the two genotypes tolerant to scab, 'Delbard Jubile' (particularly tolerant on fruit) and 'Antonovka' (clone of unknown origin planted at the DCA's Bologna station). While no *Vf* markers were present in cv 'Murray', which carries the *Vm* gene, all the markers were found in the cvs 'Nova Easygro' and 'Reglindis', which are reported to carry the *Vr* gene and *VA* polygenic resistance, respectively.

Table 4 Survey of RAPD markers in some advanced scab-resistant selections and cultivars from various breeding programmes

Origin	Genotype	Scab resistance gene[a]	Molecular marker[b]			
			$OPAL07_{580}$	$OPAM19_{2200}$	$OPC09_{900}$	$OPC08_{1100}$
USA	Dayton	*Vf*	+	+	+	+
	Enterprise	*Vf*	+	+	+	+
	Freedom	*Vf*	+	+	–	–
	GoldRush	*Vf*	+	+	–	–
	Jonafree	*Vf*	+	+	+	–
	Liberty	*Vf*	+	+	+	+
	Primiera	*Vf*	+	+	–	–
	Priscilla	*Vf*	+	+	–	–
	Redfree	*Vf*	+	+	+	+
	Sel. PRI COOP 11	*Vf*	+	+	–	–
	Sel. PRI COOP 16	*Vf*	+	+	+	+
	Sel. PRI COOP 27	*Vf*	+	+	–	–
	Sel. PRI COOP 31	*Vf*	+	+	+	+
	Sel. PRI COOP 34	*Vf*	+	+	+	+
	Sel. PRI COOP 36	*Vf*	+	+	+	+
	Sir Prize	*Vf*	+	+	–	–
	Williams' Pride	*Vf*	+	+	+	+
France	Baujade	*Vf*	+	+	–	–
	Florina	*Vf*	+	+	+	+
	Priam	*Vf*	+	+	+	+
	Sel. INRAX 3189	*Vf*	+	+	+	+
Germany	Reanda	*Vf*	+	+	+	+
	Relinda	*Vf*	+	+	+	+
	Remo	*Vf*	+	+	+	+
	Rewena	*Vf*	+	+	+	+
	Reglindis	*VA*	+	+	+	+
Poland	Sava	*Vf*	+	+	+	+
	Witos	*Vf*	+	+	+	+
Canada	Britegold	*Vf*	+	+	+	–
	Moira	*Vf*	+	+	+	+
	Richelieu	*Vf*	+	+	+	+
	Trent	*Vf*	+	+	+	+
	Murray	*Vm*	–	–	–	–
	Nova Easygro	*Vr*	+	+	+	+

[a] *Vf*=scab resistance from *Malus floribunda* 821; *Vm*=scab resistance from *Malus*×*atrosanguinea* 804; *Vr*=scab resistance from Russian seedling R12740-7A; *VA*=polygenic scab resistance from 'Antonovka'
[b] +, presence of RAPD marker; –, absence of RAPD marker

Discussion

The variation in the extent of susceptibility among the three progenies suggests that cv 'Jerseymac' possesses more minor genes that strengthens scab resistance conferred by *Vf* than cvs 'Golden Delicious' and 'Summerred'. The presence of minor genes for resistance in susceptible varieties is also reported by Rousselle et al. (1974).

The molecular results confirm the usefulness of bulked segregant analysis for the identification of markers in specific chromosomal regions. The deviation observed in the segregation of *Vf* and RAPD markers in the original 'Prima' × 'Golden Delicious' progeny cannot be easily explained, and the data do not seem to be reproducible. $OPAM19_{2200}$ and $OPAL07_{580}$ seem to be the two closest molecular markers to the *Vf* gene identified so far. The limited number of seedlings analysed could have affected the calculation of the genetic map distance between markers and the *Vf* gene, although the findings that these markers were found in all of the resistant genotypes analysed (derived from different breeding programmes) should be taken as an indirect confirmation of close linkage.

The markers $OPAM19_{2200}$ and $OPAL07_{580}$ can be used for early screening of resistant seedlings even if their transformation in more reproducible SCAR (sequence characterized amplified regions) or CAPS (cleaved amplified polymorphic sequences) markers (Paran and Michelmore 1993; Konieczny and Ausubel 1993), now in progress, will enable easier scoring. Furthermore, the conversion of dominant RAPDs to codominant SCAR or CAPS markers will be very useful even for high-resolution genetic mapping of the *Vf* region.

Despite the fact that the *Vf* RAPD markers identified in the present study seem to map on the same side of the resistant gene, they can still be used for the construction of a graphical genotype (Young and Tanksley 1989) limited to the region containing the resistant gene. This enables se-

lection of the parents exhibiting the smallest possible chromosomal fragment from *Malus floribunda* 821 in future breeding programmes, since such a fragment may contain genes with unwanted effects on the phenotype. In this connection, cvs 'Freedom', 'Baujade', 'GoldRush', 'Priscilla', 'Sir Prize', 'Sel. PRI 2750/1' ('Primiera'), 'Sel. PRI COOP 11' and 'Sel. PRI COOP 27' seem to be very promising since they did not exhibit either the $OPC09_{900}$ or the $OPC08_{1100}$ marker.

Vf-linked molecular markers are also useful for studying the relationships among different scab resistance genes. The absence of all of the identified markers in cv 'Murray' (*Vm* gene) can be taken as an indirect confirmation of the diversity of the two resistance genes both for the different reaction types (Williams and Kuc 1969) and the absence of homology between the flanking chromosomal regions tagged by these RAPD markers. The presence of *Vf* markers in cvs 'Nova Easygro' and 'Reglindis', which carry, respectively, the *Vr* gene from the Russian seedling R12740-7A (Crowe 1975) and a polygenic resistance from 'Antonovka' (Fischer 1994), needs further investigation, first by checking the correspondence of our trees to the true type. If these results would be confirmed, they would point to a homology between the chromosomal regions flanking these resistance genes. Tests with race six may even prove the identity of the resistance gene carried by 'Nova Easygro' and 'Reglindis'. Furthermore, the 'Antonovka' clone at the DCA station should differ from the 'Antonovka' clone used in the 'Reglindis' pedigree.

The availability of *Vf*-linked molecular markers is a first step in combining different resistance genes for a more durable resistance in future breeding programmes without the need for very time-consuming progeny tests. This aspect is becoming more and more important following the identification of different races of *Venturia inaequalis* that can overcome specific types of resistance (Shay and Williams 1956; Williams and Brown 1968; Parisi et al. 1993; Roberts and Crute 1994). Also, upon adoptation of the strategies of cloning large DNA fragments in yeast and chromosome walking, these markers could be used both for linking the genetic and physical maps and as the starting point for identification of the *Vf* resistance gene.

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