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Molecular selection in apple for resistance to scab caused by *Venturia inaequalis*

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Abstract Large-scale marker-assisted selection requires highly reproducible, consistent and simple markers. The use of genetic markers is important in woody plant breeding in general, and in apple in particular, because of the high level of heterozygosity present in *Malus* species. We present here the transformation of two RAPD markers, which we found previously to be linked to the major scab resistance gene *Vf*, into more reliable and reproducible markers that can be applied directly to apple breeding. We give an example of how the use of such markers can speed up selection for the introduction of scab resistance genes into the same plant, reducing labour and avoiding time-consuming test crosses. We discuss the nature and relationship of the scab resistance gene *Vf* to the one present in Nova Easygro, thought to be *Vr*.

Key words *Malus* · Molecular markers · Apple scab · Plant breeding · Marker assisted selection · *Venturia inaequalis*

Introduction

Apple crops are protected by the intensive use of pesticides. Disease-resistant plants must be developed to lessen environmental contamination, to reduce disease control costs and to meet consumer demands in order to avoid pesticide residues on the produce. Despite the obvious advantages, only recently have growers started to introduce disease-resistant varieties into commercial orchards, even though all major apple breeding programmes have included disease resistance as a primary goal for many years (Alston 1989; Kellerhals 1989; Lespinasse 1989).

Scab, caused by the fungal pathogen *Venturia inaequalis* is the most important apple disease with a spread over all apple growing areas. Many sources of resistance to this disease have been described (Williams and Kuç 1969), most of them being under the control of major dominant genes, or R-genes, inherited in a simple Mendelian manner. In the majority of cases the resistance genes derive from wild *Malus* species and are often accompanied by important modifiers, or minor genes, which affect the level of expression of the major gene (Rousselle et al. 1974; Gessler 1989). This results in the production of several phenotypic classes of resistance that cannot be easily distinguished. Nevertheless, tests on several major genes for scab resistance have been made to establish allelic relationships (Dayton and Williams 1968; 1970). Scab resistance is currently available in more than 40 cultivars (Crosby et al. 1994; Goffreda et al. 1994; Merwin et al. 1994; Work et al. 1994).

The use of molecular markers could greatly facilitate apple breeding by speeding up the whole selection process, in searching for rare genetic combinations, and for studying allelic relationships in an F_1 of a cross between apple cultivars.

We reported previously on two RAPD markers ($OpM18_{900}$ and $OpU1_{400}$) linked to the major scab resistance gene *Vf* (introgressed from *Malus floribunda* 821) (Koller et al. 1994). Here we present their transformation into more reliable, consistent and informative markers (RFLP and CAPS: Konieczny and Ausubel 1993; SCAR: Paran and Michelmore 1993) and their use in apple breeding. We also discuss the nature of the resistance gene present in the cultivar Nova Easygro and its relation with *Vf*. Finally, we give an example of how the use of molecular markers allows the recognition of F_1 plants carrying both *Vf* and the resistance gene present in Nova Easygro, so reducing labour and avoiding time-consuming test-crosses.

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Materials and methods

Plant material

The progeny of the crosses Florina×Golden Delicious (F×G) and Florina×Nova Easygro (F×N) were analysed from populations of 100 and 500 individuals respectively. Scab resistance was evaluated after greenhouse tests, where progeny seedlings were sprayed with a conidial suspension of *V. inaequalis* (10^5 conidia/ml). Plants were incubated for 48 h at 18°C and 100% relative humidity, and then transferred to a greenhouse. Disease symptoms were assessed macroscopically after 10–12 days and rated in six classes (Chevalier et al. 1991) from 0 (no scab symptoms) to 5 (high susceptibility and heavy sporulation).

DNA extraction

DNA was extracted following Dellaporta's protocol (Dellaporta et al. 1983) with minor modifications after Koller et al. (1994). DNA was diluted to a final concentration of 1 ng/µl.

Transformation of $OpU1_{400}$ and $OpM18_{900}$ RAPD fragments into more consistent markers

RAPD fragments were excised from agarose gels and re-amplified until only the desired fragment was visible. The amplified fragments were purified from the PCR mix with a PCR purification kit (NucleotraP CR, Macherey-Nagel) and cloned into pUC18 using a Pharmacia SureClone Ligation Kit following the included protocol except that, after the Blunting/Kinasing reaction, samples were heated at 65°C for 10 min in order to inactivate the enzymes and no further column purification was needed. Competent DH5α *E. coli* cells were used for the transformation following a standard protocol (Sambrook et al. 1989). The whole $OpU1_{400}$ fragment and about 150 nucleotides on each side of $OpM18_{900}$ were sequenced using USB Sequenase version 2.0. Specific primers were designed based on sequence data.

PCR amplifications

RAPD amplifications were performed as described previously (Koller et al. 1994). Specific PCR amplification of the $M18_{900}$ fragment was performed in an amplification reaction volume of 15 µl containing 5 ng of genomic DNA, 10 mM Tris-Cl pH 9.0, 0.1 mM of each dNTP (Boehringer Mannheim), 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 µM each of M18-For (TTTATCTCCAAAATCAATAG 20 nt) and M18-Rev (CACACAACTTACAAGAATTT 20 nt) primers, and 0.1 U of *Taq* polymerase (Super*Taq*, Stehelin AG, Basel). Amplifications were performed in a Perkin Elmer Cetus Gene Amp PCR System 9600. The following temperature cycles were used: 2 min 30 s at 94°C then 3 cycles of 30 s at 94°C, 30 s at 48°C, 1 min at 72°C. During these initial cycles the annealing temperature was decreased 1°C per cycle, followed by 35 cycles at the following conditions: 30 s at 94°C, 30 s at 45°C and 1 min at 72°C. After amplification samples were kept at 4°C until used.

The specific $U1_{400}$ fragment was amplified using the same reaction conditions where U1-For (GTAAAGCAAGCACTTCAACG 20 nt) and U1-Rev (GTAAAATAGATGTGTGGGTAGC 22 nt) were used as primers and the $MgCl_2$ concentration was raised to 2.0 mM. The following amplification program was employed: 2 min at 94°C then 35 cycles 30 s at 94°C, 30 s at 58°C and 1 min at 72°C. All amplified products were run on a 1% agarose gel (Bioprobe AG, Basel) with 0.5×TBE (0.045 M Tris-Borate, 0.001 M EDTA) and stained with ethidium bromide.

Cleavage of the specifically amplified $M18_{900}$ fragment was performed at 65°C for 2 h using the following conditions: 15 µl of the PCR amplification, 1 µl of 10× restriction buffer B, and 8 units of restriction enzyme *Taq*I (Boehringer Mannheim) in a final volume of 20 µl.

Southern blot

Approximately 3–5 µg of genomic DNA was digested with 30 units of the selected restriction enzyme, in a final volume of 15 µl. Overnight digestions were usually performed. Then 3 µl of 6× loading dye (Sambrook et al. 1989; gel loading buffer II) were added to the digested DNA and the samples loaded on an 0.7% agarose gel with 1× TBE. The gel was run for 16 h at 60 V and stained with ethidium bromide. DNA was blotted onto Amersham Hybond N^+ nylon membranes using LKB 2016 vacugene vacuum blotting; an alkali blot was performed following the manufacturer's instructions. Then, 25–30 ng of the purified (NucleotraP CR, Macherey-Nagel) colony amplified fragments were labelled using a Pharmacia oligo-labelling kit, following the manufacturer's instructions. No further purification was performed after labelling. Overnight pre-hybridisation was at 65°C in an Appligene hybridisation oven in 20 ml of the following solution: 10% dextransulphate, 1% SDS, 50 mM Tris-Cl pH 7.5, 1 M $NaCl_2$ and 600 ng of sheared and boiled salmon-sperm DNA. Hybridisation was performed by adding the boiled probe to the pre-hybridisation solution and incubating for a further 24 h. Membranes were washed once in 2 × SSC for 10 min at room temperature; twice in 0.5 × SSC+0.1% SDS at 65°C for 20 min; once in 0.2 × SSC+0.1% SDS at 65°C for 20 min. Kodak X-Omat AR autoradiographic films were used and blots were exposed for 2–3 days at –80°C.

Linkage analysis

All linkage-map calculations were made with the software package JoinMap v.1.4 (Stam 1993).

Results

Specific markers

As expected from sequence data, specific PCR amplification of $U1_{400}$ produced, a 320-bp fragment. Such a fragment was amplified from Florina (the resistant parent) and almost all the resistant individuals, while it was absent from Golden Delicious (the susceptible parent) and most of the susceptible progenies (Fig. 1); the new marker was named U1-SCAR (sequence characterised amplified region; Paran and Michelmore 1993).

PCR amplification using primers specific for $M18_{900}$ produced a fragment of about 850 bp that was amplified both from susceptible and resistant individuals. Since the polymorphism revealed by the RAPD primer could no longer be detected when longer primers were used, we decided to look for restriction length polymorphisms within the amplified fragment. We found that, by using *Taq*I, the allele in coupling with *Vf* could be differentiated from the one in repulsion because the former was cleaved at two sites, producing three fragments of about 450, 230 and 170 bp respectively, while the latter remained undigested (Fig. 2). We named this new molecular marker M18-CAPS (cleaved amplified polymorphic sequence: Konieczny and Ausubel 1993).

Using the cloned $U1_{400}$ (Fig. 3) and $M18_{900}$ (data not shown) fragments as RFLP probes it was possible to prove that both sequences are present as single copies in the apple genome. A survey of several apple cultivars revealed a high level of polymorphism in that genomic region. It

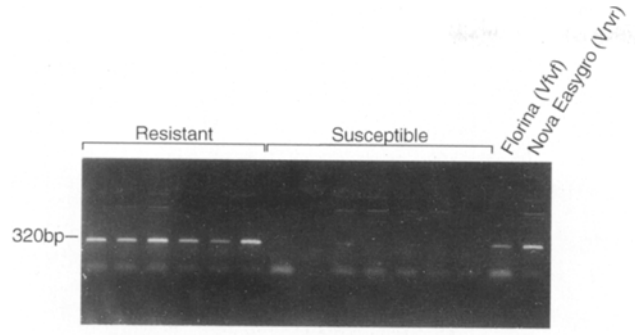

Fig. 1 An ethidium bromide-stained 1% agarose gel containing $U1_{400}$-specific amplification products. A 320-bp band is visible only in the resistant individuals and in the two parental lines of the segregating F×N population

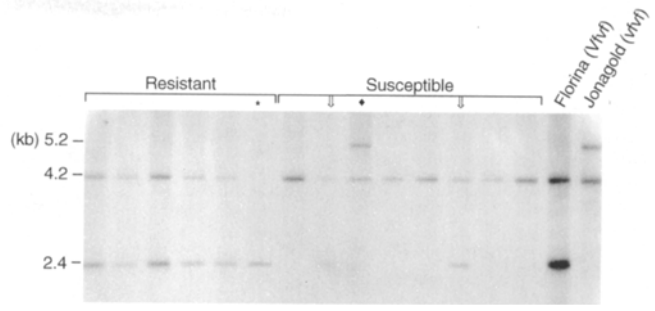

Fig. 3 A Southern blot of some resistant and susceptible individuals of the F×N population, hybridised with a $OpU1_{400}$-specific fragment. The 2.4-kb band is the allele in coupling with *Vf*. * indicates a plant homozygous for the $U1_{400}$ allele in coupling with *Vf*; ⇓ indicates recombinant individuals in which a crossing-over has occurred between $U1_{400}$ and *Vf*; ♦ indicates a contaminant plant obtained by cross pollination (outcrossing)

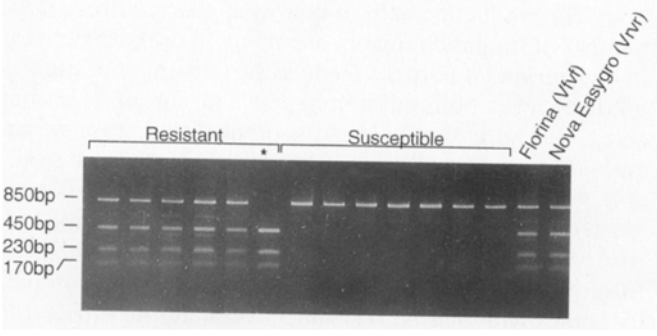

Fig. 2 An agarose gel containing the restriction fragments obtained after cleavage of the amplified 850-bp $M18_{900}$-specific band with the *Taq*I restriction enzyme. The 450-, 230- and 170-bp fragments are present only in resistant individuals and in the two parental lines of the F×N cross. * indicates a plant homozygous for the $M18_{900}$ allele in coupling with *Vf*

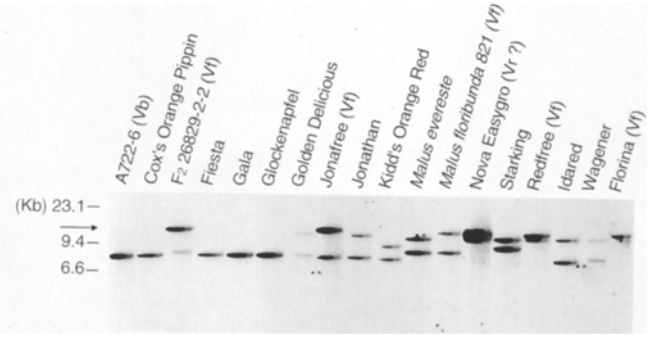

Fig. 4 A Southern blot showing the high level of polymorphism present in the genomic region around *Vf*, as revealed using $M18_{900}$ fragment as a probe. The uppermost band indicated by the *arrow* is the allele in coupling with *Vf*, that is present only in the *Vf*-resistant cultivars, in *M. floribunda* 821 and in Nova Easygro

was in fact possible to distinguish at least four different alleles (Fig. 4).

Analysis of progeny plants

One-hundred FxG progeny plants were analysed for U1-SCAR, M18-CAPS and two RAPD markers ($OpD20_{500}$ and $OpC08_{1100}$). All the markers were linked and it was possible to calculate a genetic linkage map of the genomic region (data not presented). In order to include *Vf* in the map we considered all plants belonging to classes 0 to 3 as resistant and those belonging to classes 4 and 5 as susceptible. This simplification, although artificial, has a biological consistency since 4 and 5 are the only two classes in which clear sporulation occurs. Adopting this classification, a 1:1 segregation was obtained which is in good agreement with the hypothesis that *Vf* is a single dominant gene and that it is heterozygous in the cultivar Florina. Although the version of JoinMap that we employed cannot handle data from outbreeding species, we used the program nevertheless, assuming a backcross type of segregation. This approach was possible because all our dominant markers are in coupling, only two alleles per marker could be recognised, and the expected 1:1 segregation was observed.

The F×N population was analysed with the aim of selecting those resistant plants that inherited the resistant gene derived from Nova Easygro (Fig. 5a), but not *Vf* (present in Florina; Fig. 5b), in order to perform a bulked segregant analysis (Michelmore et al. 1991; Giovannoni et al. 1991) to obtain molecular markers linked to the Nova Easygro resistance gene. In a previous survey we noted that Nova Easygro has all of the *Vf*-linked markers. This information alone was not sufficient to establish whether or not *Vf* and *Vr* are located in the same genomic region. However, the analysis of 491 progeny plants of the F×N population showed that only 7 out of the 355 resistant plants

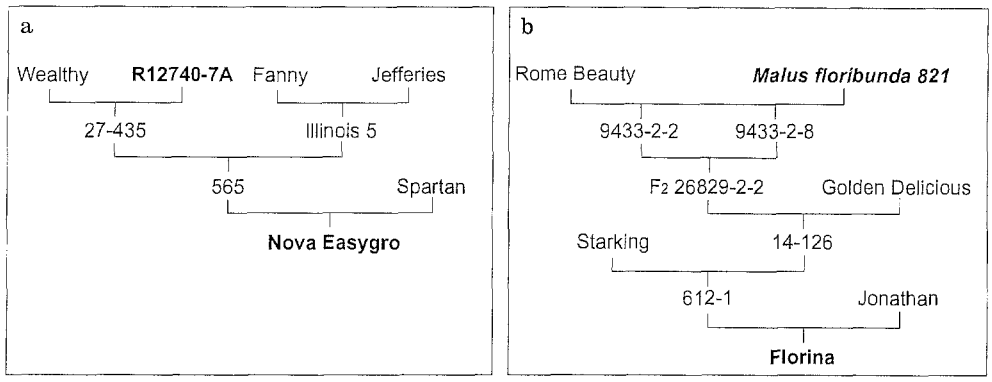

Fig. 5 Pedigrees of Nova Easygro (**a**) and Florina (**b**) cultivars. R12640-7A and *M. floribunda* 821 are respectively the original *Malus* species from which the *Vr* and the *Vf* genes are derived. The Nova Easygro pedigree is probably incorrect (see text)

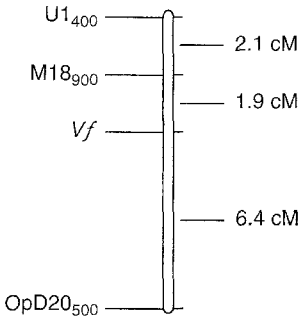

Fig. 6 Linkage map of the *Vf* genomic region as calculated from the segregation data of both F × N and F × G populations

(1.98%) do not carry the M18-CAPS "resistant" allele, suggesting that the two genes are very tightly linked, and may be the same. If we consider the recombination distance from *Vf* to $M18_{900}$ to be 2.0% (SD=1.43), (as estimated in the F×G population), the recombination of 3.0% (SD=0.9) calculated from the F×N progeny is not significantly different. The linkage map of the genomic region was constructed considering *Vf* and *Vr* as alleles of the same locus and using data from both F×G and F×N populations (Fig. 6). JoinMap allows one to construct genomic maps using data obtained from different segregating populations; in our case the markers around the resistance genes are segregating as in a backcross in the F×G population while they segregate as in an F_2 intercross in the F×N population.

We used $M18_{900}$ and $U1_{400}$ as RFLP probes in Southern-blot experiments to establish if the fragments are present in the apple genome as single copies. The presence of only two bands in most of the apple cultivars analysed, and the segregation analysis performed on a sub-sample of the FxN population, revealed that the two bands segregated in perfect agreement with M18-CAPS and the U1-SCAR.

Discussion

Progress in apple breeding is restricted by several factors among which self-incompatibility and a long juvenile period are the most important. The use of molecular markers could ease selection processes, helping breeders to carry out an early selection of the most favourable genetic combinations, thereby reducing time and costs as well as the necessary field space.

Our attention was focused on resistance to scab caused by *V. inaequalis,* certainly the most important apple disease. The production of high-quality apples requires a large number of fungicide treatments, up to 15 or more per year in Switzerland. In order to reduce the amount of pesticides used, to meet both environmental and consumer's concerns, it is necessary to breed good quality disease-resistant varieties. Although some resistant varieties are available, their introduction into commercial orchards is very limited. Most of the resistance genes introgressed into cultivated varieties derive from wild *Malus* species with poor fruit quality (small size, acidic taste etc.), and good-quality apples with disease resistance are therefore difficult to breed. Molecular markers provide a valuable tool to follow the segregation of important genes, and at the same time to reduce the amount of undesired ("wild") DNA linked to the gene of interest (genetic drag). Moreover, the use of molecular markers allows the selection of F_1 plants carrying more than one gene for resistance, so reducing labour and avoiding time-consuming test-crossing. We present here the transformation of two RAPD markers into more reproducible and consistent markers that can be applied directly to apple breeding. These new markers also have an increased information content which could be used in other mapping projects [e.g. QTL mapping, one of the aims of the European Apple Project in which we are involved (King et al. 1992)]. Their use allows one to directly select F_1 seedlings carrying both *Vf* and the resistance gene present in Nova Easygro. It is in fact possible to select plants homozygous for the $M18_{900}$ allele which is in coupling with the resistance gene (either M18-CAPS or M18-RFLP could be used). It is also possible to select plants with a reduced genetic drag by choosing progenies carrying the $M18_{900}$ "resistant" allele and which have lost the other flanking markers.

Furthermore, we proved that the *Vf* resistance gene (derived from *M. floribunda* 821) and the scab resistance gene present in the cultivar Nova Easygro (thought to be *Vr*, derived from *Malus pumila,* Russian seedling R12740-7A) are located in the same genomic region. Our result contrasts with published data (Dayton and Williams 1968;

1970) describing *Vf* and *Vr* as two independent genes. However, a major difference between Dayton and Williams' work and ours is that they used the original *M. pumila* R12740-7A, while we used Nova Easygro, a Canadian selection (Crowe 1975). Theoretically, it is possible that the original Russian seedling contains more than one scab resistance gene (as suggested by Williams and Kuĉ 1969), one of which is tightly linked to *Vf* and was introgressed into Nova Easygro. Genetic markers do not allow us to establish whether *Vf* and the resistance gene present in Nova Easygro are allelic or only tightly linked. However, some evidence strengthens the hypothesis that the two genes are alleles of the same locus (if not the same allele): (1) Their common origin seems to be proved by the observation that all *Vf* molecular markers are also linked to Nova Easygro scab resistance. This is unlikely to be a coincidence since, as described by several authors (Koller et al. 1993; Mulcahy et al. 1993; Dunemann et al. 1994), a very high level of polymorphism is present in apple and our RFLP survey of some apple cultivars confirmed that a high level of polymorphism is also present in the *Vf* genomic region. (2) The observation that a new race of *V. inaequalis* (race 6, Parisi et al. 1993) that overcame *Vf* resistance can also infect Nova Easygro (both *M. floribunda* 821 and the Russian apple R12740-7A are still resistant to race 6; Parisi and Lespinasse 1996. (3) Last, but not least, preliminary data presented by Cheng et al. (1995) lead to the conclusion that the pedigree of Nova Easygro is incorrect and that the resistance in Nova Easygro was not derived from the Russian seedling.

A more detailed analysis of the genomic region concerned will give us more clues about the functions, regulation and relationships among the resistance genes. We know that closer markers are needed for starting a map-based cloning project; nevertheless, useful results seem to come from our European collaboration, and RAPD markers closely linked to *Vf* have been found in other European laboratories (Tartarini 1996). Finally, examples of cloning important plant genes using a map-based approach have been reported (Martin et al. 1993; Wing et al. 1994) and this encourages us to continue our research in order to unravel the mechanisms involved in the plant-pathogen interaction.

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References

Alston FH (1989) Breeding pome fruit with stable resistance to diseases. In: Gessler C, Butt D, Koller B (eds) Integrated control of pome fruit diseases II. OILB-WPRS Bulletin XII/6, pp 90–99

Cheng FS, Brown SK, Weeden NF, Aldwinckle HS (1995) Molecular markers for scab resistance from 'Nova Easygro' apple. Plant Genome III Conference (abstract), San Diego, California. January 1995

Chevalier M, Lespinasse Y, Renaudin S (1991) A microscopic study of the different classes of symptoms coded by the *Vf* gene in apple for resistance to scab (*Venturia inaequalis*). Plant Pathol 40:249–256

Crosby JA, Janick J, Pecknold PC, Korban SS, Ries SM, Goffreda J, Voordeckers A (1994) Seven disease-resistant apple selections released for growers testing. Fruit Var Jour 48:40

Crowe AD (1975) 'Nova Easygro' apple. Fruit Var Jour 29:76

Dayton DF, Williams EB (1968) Independent genes in *Malus* for resistance to *Venturia inaequalis*. Proc Am Soc Hort Sci 93:89–94

Dayton DF, Williams EB (1970) Additional allelic genes in *Malus* for scab resistance of two reaction types. J Am Soc Hort Sci 95:735 -736

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19 -21

Dunemann F, Kahnau R, Schmidt H (1994) Genetic relationships in *Malus* evaluated by RAPD 'fingerprinting' of cultivars and wild species. Plant Breed 113:150–159

Gessler C (1989) Genetic of the interaction *Venturia inaequalis* – *Malus*: the conflict between theory and reality. In: Gessler C, Butt D, Koller B (eds) Integrated control of pome fruit diseases II. OILB-WPRS Bulletin XII/6, pp 168–190

Giovannoni JJ, Wing RA, Ganal MW, Tanksley SD (1991) Isolation of molecular markers from specific chromosomal intervals using DNA pools from an existing mapping population. Nucleic Acids Res 19:6553–6558

Goffreda JC, Polk DF, Rizio GF, Gussman CD, Gianfagna TJ, Crosby JA, Janick J, Pecknold PC, Korban S, Ries SM (1994) Developing scab-resistant apple cultivars with increased quality and ability to store. Fruit Var Jour 48:45

Kellerhals M (1989) Breeding of pome fruit with stable resistance to diseases. In: Gessler C, Butt D, Koller B (eds) Integrated control of pome fruit diseases II. OILB-WPRS Bulletin XII/6, pp. 116–129

King GJ, Alston FH, Battle I, Chevreau E, Gessler C, Janse J, Lindhout P, Manganaris S, Sansavini S, Schimdt H, Tabutt K (1992) The 'European Apple Genome Mapping Project' – developing a strategy for mapping agronomic genes in tree species. Euphytica 56:89–94

Koller B, Lehmann A, McDermott J, Gessler C (1993) Identification of apple cultivars using RAPD markers. Theor Appl Genet 85:901–904

Koller B, Gianfranceschi L, Seglias N, McDermott J, Gessler C (1994) DNA-markers linked to the *Malus floribunda* 821 scab resistance. Plant Mol Biol 26:597–602

Konieczny A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. The Plant Jour 4:403–410

Lespinasse Y (1989) Breeding of pome fruit with stable resistance to diseases: Genes resistance mechanism, present work and prospects. In: Gessler C, Butt D, Koller B (eds) Integrated control of pome fruit diseases II. OILB-WPRS Bulletin XII/6, pp 100–115

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Merwin IA, Rosenberger DA, Engle C (1994) Evaluation of four scab-resistant apple varieties compared with 'Empire' in New York orchards. Fruit Var Jour 48:54–56

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in a specific genomic region by using a segregating population. Proc Natl Acad Sci USA 88:9828–9832

Mulcahy DL, Cresti M, Sansavini S, Douglas GC, Linskens HF, Bergamini Mulcahy G, Vignani R, Pancaldi M (1993) The use of random amplified polymorphic DNA to fingerprint apple genotypes. Scien Hort 54:89–96

Paran I, Michelmore (1993) Development of reliable PCR-based markers linked to the downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Parisi L, Lespinasse Y, Guillaumes J, Krüger J (1993) A new race of *Venturia inaequalis* virulent to apple with resistance due to the *Vf* gene. Phytopathology 83:533–537

Parisi L, Lespinasse Y (1996) Pathogenicity of *Venturia inaequalis* strains of race 6 on apple clones (*Malus* sp.). Plant disease (in press)

Rousselle GL, Williams EB, Hough LF (1974) Modification of the level of resistance to apple scab from the *Vf* gene. In: XIX Int Horti Cong, Warszawa 11–18 September 1974, pp 19–26

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package JoinMap. The Plant Jour 3:739–744

Tartarini S (1995) RAPD markers linked to the *Vf* gene for scab resistance in apple. Theor Appl Genet (in press)

Williams EB, Kuĉ J (1969) Resistance in *Malus* to *Venturia inaequalis*. Annu Rev Phytopathol 7:223–246

Wing RA, Zhang HB, Tanksley SD (1994) Map-based cloning in crop plants. Tomato as a model system. I. Genetic and physical mapping of *jointless*. Mol Gen Genet 242:681–688

Work TM, Bushway RJ, Perkins LB, Schupp JR, Bushway AA (1994) Comparison of sensory, chemical and color attributes of disease-resistant apple cultivars. Fruit Var Jour 48:14–19