Molecular Markers for the Scab Resistance (V_f) Region in Apple

Minou Hemmat,¹ Norman F. Weeden,¹ Herb S. Aldwinckle,² and Susan K. Brown¹
Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456

ADDITIONAL INDEX WORDS. *Malus*, markers, STS, multiplexing

ABSTRACT. Bulk segregant analysis was used to identify RAPD markers that display tight linkage to the V_f gene in apple (*Malus* sp.) that confers resistance to five races of apple scab [*Venturia inaequalis* (Cke.) Wint.]. We identified several new RAPD markers linked to V_f . The most tightly linked marker in the test population, S5₂₅₀₀, was cloned and sequenced. A linkage map of the V_f region was developed using these markers, RAPD markers previously described by other laboratories, and the isozyme locus *Pgm-1*. An assay was developed for V_f by multiplexing the two markers closely flanking the V_f locus. This assay has a theoretical 'escape' value (discarding a resistant plant) of 3% and an error rate (selection of a susceptible plant) of 0.02%.

Apple scab caused by *Venturia inaequalis* (Cke.) Wint. is one of the most damaging diseases of apple. Multiple application of fungicides is currently the most effective control method in commercial orchards. One alternative to chemical control is the use of scab-resistant apple cultivars. In most breeding programs, resistance to scab has been introduced through crosses derived from *Malus floribunda* 821 (Hough et al., 1953; Lamb and Hamilton, 1969). This resistance is believed to be conferred by a single dominant gene, designated V_f , and is distinct from five other scab resistance genes V_r , V_b , V_{b1} , V_m , and V_a (Williams and Kuc, 1969).

Resistant plants must be identified and selected as early as possible in a breeding program to minimize costs associated with plant maintenance. Currently, the standard method of selecting for resistant plants in segregating progenies is a laborious direct inoculation with the pathogen. The method has practical limitations such as availability of appropriate testing facilities and inconsistent expression of phenotypes under these conditions. An isozyme marker, *Pgm-1*, was reported to be useful in screening progenies segregating for V_f (Manganaris et al., 1994). However, the recombination rate between *Pgm-1* and V_f is significant ($\approx 8\%$) and the marker locus does not always segregate in progenies segregating for V_f . Therefore, identification of molecular marker(s) linked to the resistance gene is important to effectively screen for resistant plants.

DNA markers such as RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers have been used in apple to study genetic variation (Nybom et al., 1990), to construct genetic linkage maps (Conner et al., 1997; Hemmat et al., 1994), and to identify rootstocks (Koller et al., 1993; Landry et al., 1994). RAPD markers also have been used to verify gene introgression into cultivated apple (Durham and Korban, 1994; Yang et al., 1997b), as well as tagging an aphid resistance gene (*Sd1*) (Roche et al., 1997) and a number of morphological traits in apple (Cheng et al., 1996; Hemmat et al., 1997; Lawson et al., 1995).

Bulked segregant analysis (BSA) together with the RAPD technique is a powerful approach for identification of closely linked markers for disease resistance and economically important traits (Michelmore et al., 1991; Pineda et al., 1993). Recently,

maps of the chromosome region containing the V_f gene have been constructed (Gardiner, 1996; Gianfranceschi et al., 1996; Tartarini, 1996). Although several RAPD markers on both sides of V_f have been identified (Koller et al., 1994; Tartarini, 1996; Yang and Krüger, 1994; Yang et al., 1997a, 1997b), a highly reliable ($<1\%$ error) and tightly linked assay has yet to be described. Markers within 1 cM of the gene of interest are occasionally difficult to find. An alternative way to increase efficiency of a marker-assisted selection program is to bracket the gene with a marker on each side and select for nonrecombinants between the markers (Tanksley, 1983). Two markers flanking the gene by 10 cM on each side will incorrectly identify a susceptible plant as resistant 1% of the time, although $\approx 20\%$ of the resistant plants will be discarded. Markers flanking the gene by 1 cM would mistakenly identify a susceptible plant as resistant only one time in 10,000, and only 1% of the resistant individuals tested would escape selection.

The objectives of this study were to identify tightly flanking markers and develop a highly reliable screening procedure for the V_f gene.

Materials and Methods

PLANT MATERIAL. Progenies of three crosses were studied. A population of 38 apple plants was produced from 'Prima' x 'Spartan' (cross 1). 'Prima' was the source of V_f resistance. This population was used initially to identify markers that cosegregated or displayed close linkage with isozyme marker *Pgm-1*. A second population of 73 plants was produced from 'Golden Delicious' x 'Prima' (cross 2). Cross 2 was the primary population for mapping DNA markers. A third population of 78 plants from 'Idared' x NY81204-42 (cross 3) was screened for PGM-1 and segregating DNA markers. This population was chosen because a large quantity of seed was available for this study. NY81204-42 was derived from *M. floribunda* 821 but not through 'Prima'. This population was of particular interest because the scab resistant reaction of NY81204-42 was more consistent than Prima and the fruit quality was superior. Therefore NY81204-42 represented a more appropriate parent for the breeding program.

SCAB INOCULATION. Spores from five races of *V. inaequalis* were used for inoculation as described by Manganaris et al. (1994). Frozen inoculum was thawed and sprayed on all the leaf surface when the seedlings were at the two-leaf stage. For 48 h the seedlings were kept at 15 to 20 °C with $>95\%$ relative humidity. The seedlings were then transferred to the greenhouse where the symptoms were expressed after ≈ 2 weeks. Plants which did not

Received for publication 29 Jan. 1998. Accepted for publication 20 July 1998. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Department of Horticultural Sciences.

²Department of Plant Pathology.

show symptoms were reinoculated and scored after 2 weeks.

ISOZYME ANALYSIS. Assays for phosphoglucosyltransferase (PGM) were performed on all three progenies. Tissue extraction and horizontal starch gel electrophoresis were performed as described by Weeden and Lamb (1985). Assay conditions were as described by Manganaris et al. (1994) and Weeden and Lamb (1987). Isozyme analyses were performed three times on all populations.

DNA ISOLATION AND RAPD AMPLIFICATION. DNA was isolated from the cotyledons or young leaf tissues using the method of Doyle and Doyle (1990). Bulk segregant analysis (BSA) (Michmore et al., 1991) was performed on pooled DNA samples from five plants with the faster migrating, and five with the slower migrating PGM-1 allozyme. About 400 random decamer oligonucleotides were used for screening the bulked samples. Primers that generated a fragment displaying polymorphism between bulk samples were tested on the entire population individually. PCR reaction conditions were carried out as described by Hemmat et al. (1994). DNA was amplified in a PTC100 thermocycler (MJ Research, Inc., Watertown, Mass.) with the following program: one cycle of 1 min at 94 °C, 40 cycles of 1 min at 94 °C, 2 min at 35 °C, 2 min at 72 °C, and a final extension cycle of 8 min at 72 °C. For the S5 primer, the annealing temperature was raised to 40 °C. RAPD products were separated on 2% gels: 1% agarose (International Biotechnologies, Inc., New Haven, Conn.) and 1% NuSieve GTG agarose (FMC Biproducts, Rockland, Maine). Amplifications were replicated on separate dates to assure the reproducibility of the results. Primers were synthesized at Cornell University, New York State Center for Advanced Technologies, or purchased from the University of British Columbia (Vancouver B.C., Canada) and Operon Technologies (Alameda, Calif.). Gels were stained with ethidium bromide and visualized and photographed under UV light.

Data from the PGM-1 assay, scab inoculation and RAPD analysis were used for linkage analysis and mapping around the *V_f* region in cross 2. RAPD markers included five newly identified—S5₂₅₀₀, B505₁₇₀₀, S29₁₁₅₀, P198₇₅₀, and B398₄₈₀—and nine previously reported markers—OPA15₉₀₀, OPM18₉₀₀, OPU01₄₀₀, OPD20₆₀₀, OPAL07₅₈₀, OPC08₁₁₀₀, OPC09₉₀₀, OPAM19₂₂₀₀, and OPAR04₁₄₀₀—and OPK16₁₃₀₀ (Durham and Korban, 1994; Koller et al., 1994; Yang and Kruger, 1994; Tartarini, 1996; Yang et al., 1997a, 1997b). All RAPD markers were scored as present or absent. MAPMAKER (Lander et al., 1987) was used to confirm locus order for each RAPD marker and to determine multipoint recombination frequency among loci.

CLONING AND SEQUENCING THE RAPD FRAGMENT S5₂₅₀₀. To clone DNA marker S5₂₅₀₀, the identified band was cut from the agarose gel and soaked in 50 µL type I water for 4 h at room temperature. One microliter of this solution was used as template DNA to reamplify the RAPD fragment at a 50 °C annealing temperature. The subsequent PCR product was directly cloned into pCR II (TA Cloning Kit, Invitrogen, San Diego, Calif.). The cloned fragment carried an internal *Eco*RI site which gave two fragments of ≈1000 and 1500 bp when cut with this enzyme. To obtain the full sequence of the S5₂₅₀₀ fragment, the plasmid containing the 2500-bp fragment was digested with restriction enzyme *Eco*RI and the two subsequent fragments were each ligated in pUC118 vector, following the methods described by Promega (Protocols and Application Guide). Ligated products were then transformed into DH5α competent cells (GIBCO BRL, Life Technologies, Inc., Gaithersburg, MD.), and transformed colonies were screened for plasmids containing appropriate length inserts. Identified plasmids were isolated and purified according to the ABI protocol (Applied Biosystems Inc., Foster City, Calif.). M13MUT forward and M13

reverse primers were used for end sequencing the inserts. Sequencing was performed at Cornell University, New York State Center for Advanced Technologies.

PRIMER DESIGN AND ANALYSIS. Based on sequence data from the cloned RAPD fragment, we identified and designed a pair of primers for the region in the sequence that was responsible for polymorphism in the S5₂₅₀₀ marker. S5₁₄₅₀ sequence data was used to develop primers that amplified a specific fragment in resistant plants but did not amplify a fragment in susceptible plants. S5₁₄₅₀ was amplified using the same PCR reactions and conditions as described for RAPD amplification except that the annealing temperature was increased to 65 °C.

DEVELOPING A MULTIPLEXED ASSAY FOR *V_f*. An assay was developed to simultaneously amplify two sequence tagged sites (STSs) of different length in one reaction. The PCR reaction conditions were as described by Hemmat et al. (1994), but two sets of primers, U1₄₀₀ forward and reverse, developed from the OPU1₄₀₀ marker (Gianfranceschi et al. 1996), and S5₁₄₅₀ forward and reverse were used in one reaction mix. Touchdown amplification steps were applied to amplify the bands (Mellersh and Sampson, 1993). A profile was optimized to amplify both STSs. The profile consisted of 2 min at 94 °C, 2 min at 69 °C, 2 min at 72 °C. The cycle was repeated each time 1 min at 94 °C with a reduction of 1 °C for annealing temperature per cycle. The cycle was repeated 30 times when annealing temperature reached 62 °C. A final extension cycle of 8 min at 72 °C was applied.

Results

IDENTIFYING DNA MARKERS USING 'PRIMA' x 'SPARTAN' (CROSS 1). PGM-1 segregated 1:1 in population 1 (Table 1) and linkage to *Pgm-1* was confirmed using this population. Although *V_f* was not scored in this population, a preliminary linkage map could be constructed using just the markers. Bulk segregant analysis identified five new RAPD markers (S5₂₅₀₀, B505₁₇₀₀, S29₁₁₅₀, P198₇₅₀, and B398₄₈₀). In addition, previously reported RAPD markers OPA15₉₀₀, OPK16₁₃₀₀, OPM18₉₀₀, OPD20₆₀₀, OPU01₄₀₀, OPAL07₅₈₀, and OPC08₁₁₀₀ segregated in this population (Table 2). A linkage map gave the following order of markers in relation to *Pgm-1*: OPK16₁₃₀₀, B398₄₈₀, B505₁₇₀₀, *Pgm-1*, OPM18₉₀₀, OPU01₄₀₀, OPAL07₅₈₀, S5₂₅₀₀, OPA15₉₀₀, OPC08₁₁₀₀, OPD20₆₀₀, P198₇₅₀, and S29₁₁₅₀.

PGM-1, SCAB RESISTANCE, AND DNA MARKER SEGREGATION RESULTS IN CROSSES 2 AND 3. Progenies from crosses 2 and 3 segregated for PGM-1, and this segregation ratio did not deviate significantly from 1:1 (Table 1). The segregation ratio for scab resistance in cross 2 was also near 1:1, although six plants gave ambiguous phenotypes; four were 'Asp' (chlorotic wrinkled phenotype with some sporulation upon second inoculation) and two were type 3' (few restricted sporulating lesions) (Aldwinckle et al., 1976).

Table 1. Segregation for PGM-1 in 'Prima' x 'Spartan', 'Golden Delicious' x 'Prima', and 'Idared' x NY81204-42.

Cross	No. with designated phenotype ^a		χ^2 (1:1)
	Fast	Slow	
'Prima' x 'Spartan'	14	16	0.32
'Golden Delicious' x 'Prima'	28	30	0.068
'Idared' x NY81204-42	44	34	1.28

^aData not available for 8 plants from 'Prima' x 'Spartan' cross or for 15 plants from 'Golden Delicious' x 'Prima'.

Table 2. List of the newly identified and previously reported primers that were used in the linkage map of the V_f region.

RAPD primer	Sequence	Reference
B398	5'-CAGTGCTCTT-3'	F. Cheng, Hemmat et al., 1998
B505	5'-CCCTTTACAC-3'	Hemmat et al., 1998
OPAL07	5'-CCGTCCATCC-3'	Tartarini 1996
OPAR04	5'-CCAGGAGAAG-3'	Yang et al. 1996
OPK16	5'-GAGCGTCGAA-3'	Hemmat et al., 1998
OPA15	5'-TTCCGAACCC-3'	Durham and Korban 1994
OPAM19	5'-CCAGGTCTTC-3'	Tartarini 1996
OPC08	5'-TGGACCGGTG-3'	Tartarini 1996
OPC09	5'-CTCACCGTCC-3'	Tartarini 1996
OPD20	5'-ACCCGGTCAC-3'	Yang and Kruger 1994
OPM18	5'-CCAGGTCTTC-3'	Koller et al. 1994
OPU01	5'-ACGGACGTCA-3'	Koller et al. 1994
P198	5'-GTGTTTGCGG-3'	Hemmat et al., 1998
S29	5'-CCAGACAAGC-3'	Hemmat et al., 1998
S5	5'-CCGGCTCTTG-3'	Hemmat et al., 1998
S5 ₁₄₅₀ forward	5'-ACA GGT CGA TTA CTT GCG	Hemmat et al., 1998
S5 ₁₄₅₀ reverse	5'-GGC TCT TGC AGT TGG GAA	Hemmat et al., 1998
U1 ₄₀₀ forward	5'-GTA AAG CAA GCA CTT CAA CG	Gianfranceschi et al., 1996
U1 ₄₀₀ reverse	5'-GTA AAA TAG ATG TGT GGG TAGC	Gianfranceschi et al., 1996

Plants with an ambiguous phenotype were not placed in either the resistant or susceptible categories. However, after the map was constructed the genotype of the flanking markers in each of these plants was used to determine the V_f genotype. Our data indicate that the four seedlings scored as 'Asp' possessed the markers adjacent to V_f and presumably had the resistance gene. In contrast, both plants with a class 3 susceptible reaction lacked all V_f markers. All new RAPD markers (S5₂₅₀₀, B505₁₇₀₀, S29₁₁₅₀, P198₇₅₀, and B398₄₈₀) and most of the previously reported RAPD markers (OPA15₉₀₀, OPK16₁₃₀₀, OPM18₉₀₀, OPU01₄₀₀, OPD20₆₀₀, OPAL07₅₈₀, and OPC08₁₁₀₀) segregated in population 2 for a total of 14 markers. Three previously reported RAPD markers—OPC09₉₀₀, OPAM19₂₂₀₀, and OPAR04₁₄₀₀—did not segregate in this population. Gardiner et al. (1996), reported a number of RAPD markers around 900 bp when they used OPC09 in the population they examined. In our analysis, very few RAPD markers were observed between 600 and 1100 bp and none cosegregated with V_f . In the case of OPAR04₁₄₀₀, neither the decamer OPAR04 nor the specific primers designed for a SCAR produced any segregating band around 1400 bp. A band at 2200 bp was observed for OPAM19, but this band did not segregate in our populations.

Cross 3 segregated for some RAPD markers, primarily those close to *Pgm-1* (including OPM18₉₀₀, U1₄₀₀, and OPAL07₅₈₀). RAPD fragments corresponding to S5₂₅₀₀, OPA15₉₀₀, OPD20₆₀₀, and P198₇₅₀ were not observed in this population. No data were obtained for OPC08₁₁₀₀ and S29 RAPD markers. These results suggest that a recombination has occurred between S5₂₅₀₀ and V_f in the pedigree of NY81204-42, thus eliminating the region from *M. floribunda* that contains S5₂₅₀₀, OPA15₉₀₀, OPD20₆₀₀, and P198₇₅₀. Markers OPU01₄₀₀ and OPAL07₅₈₀ are on the same side of the ancestral recombination event, and based on the data from cross 2 appear to be on the opposite side of V_f from this recombination.

DEVELOPING A LINKAGE MAP. Using 'Golden Delicious' x 'Prima' population (cross 2) data, a linkage map was constructed. The markers covered ≈ 45 cM in the region containing the V_f gene. Markers OPM18₉₀₀, OPU01₄₀₀ and OPAL07₅₈₀ mapped between V_f and *Pgm-1*. The S5₂₅₀₀ marker was placed immediately adjacent to V_f on the side opposite to U01₄₀₀ (Fig. 1). Log likelihood of the order gave an LOD of 2.4 for the order shown in Fig. 1 relative to the next most likely arrangement of the markers. Only one of the

four alternative orders within an LOD of 3.0 of that shown in Fig. 1 had a marker other than S5₂₅₀₀ as the closest marker to V_f .

DEVELOPING AN STS FROM THE S5₂₅₀₀ RAPD MARKER. Based on sequence data from the S5₂₅₀₀ fragment (Fig. 2), we identified a pair of 18-bp primers (long primers in bold and underlined). One primer started with the original 10-mer of the RAPD primer (S5) on the 3' end of the priming site. The second primer started 1100 bp from the 5' end of the sequence. These specific primers

amplified the region of polymorphism in the S5₂₅₀₀ RAPD marker. A BLAST search of GenBank revealed no significant homology between the S5₂₅₀₀ sequence and anything reported in the data base.

MULTIPLYED ASSAY FOR V_f . The multiplexed assay amplified two STSs simultaneously (Fig. 3). The 1450-bp fragment is amplified by the S5 forward and reverse primers and the 400-bp fragment by the U1 primers. Plants possessing both fragments are considered resistant. Absence of both

Fig. 1. Genetic map of the V_f region constructed from progeny of 'Golden Delicious' x 'Prima' using previously published and newly identified RAPD markers. The arrow shows the point of recombination event in the line leading to NY81204-42.

CCGGCTCTTGGATATTCGGACAAGTTAACCACCCCTTTTATGGGTTTGTCAACCAGTCTTCTTCTTCTTCTTATGACCTTCTCTTTTCCCTTCTCCG 100
 CCTAGGCCATACATGATCAGATTATATAGGGGCTCTGAGAAGGGATCCGTATGTTTATCCGTACCACAGCTTCTTCTAGTCGATTGGTTTTAAATCCCT 200
 CTTCTCCCTATCATCTTCTCCCCAAGGTGAGGTAAAGATATAGGAATAGGTGGTCTTAGAACTGGATCATCTTCTTTCTGGAGCCTGCAGAGGCGTTC 300
 TCCAGTCTTTGTAGCATGTGCAACAAAGCAGTTTGCAAATCTTCTGATCCCTTTGACGTACTTCTTTGATCAAAAGTCCCACTGGGCGTGCCAAAATTA 400
 TGTTCGTGGCAGAAATTCGTGCGGGATGCTTTCTGGCGACGAGCACACAGCTCTCGAGAGGTGACGCCCCGTGATGCTTCTGTGCGGGCTTCTGC 500
 TTTACTGGCGGTTTGTGAGGCAAGCCTGTGCGGTAAGGCTTGTAGGGCAAGGCTGAAAGAGAAGGACAGAATTAACCTTTGAAAGGTGCCCTTTGTGGGG 600
 CCTTAGGTGTAGGCCTTAAGGCTCACAATCAAGATTAACTTTTAAAGTGTCTAGGCGCTTGCCACTGATATTTTCAACATGGCAGATGTAACACGGATGTTG 700
 AGTTTAAATATATATATTCAGAGACAAGTTAGTTATTTAGAGAGTGCCTTTGTGGGGCTTTAGGTGTAGCCTTGAAGGTTCTGTTAATAACTAATTA 800
 NTACTCCAATACATAAGGTCAATTTCTTGGTTATATGAGTCTTATAACCAATTATACCTTTTGATTACCTGAGCCCCGAGAGCAGAATGAATCCAAATAGGT 900
 GCCTTTGTAGGGCCTTAGATAGGCCTTGAGGCTTCTATCAGAACTCCTTGTATACTCAATGTTCTAGCCCCGAGAAACAGGATGAATCCAAATAGGTGCC 1000
 TTTGTAGGGCCTTGAATAGGCGTTGAGGTTCCCATCAGAATTCATTCCGTACTCGAACGTTCTATGGTAATCATAATATAATAGAAATAAACTAAGTAA 1100
CAGGTGGAATTAAGTTCGGCTCAAGTAACCTTCGGACTTCTTGTATCAAGCTTGTGCTGGATGGTTAAGTCCACATCAGGTTCTTTTTTATGCCTCGAG 1200
 GCCAAGGACTTTTGTAGGTGATCANATCTTATATGCCCAGGGCTTGAACCTTAGATTGACTTGAAGTGTGAAGACATATTCAAATCGGATCAAGCGTT 1300
 GAGAGAGTCTTTTAATAACCATATTACTCGACGGAGCTTCAGAAATCGAAGAGGCGCTGCTCAGAAATCNAAGAGAGCGTTTGTCTTCTCAAAGCTGG 1400
 GCTGCTCAGAGATCTCGAGGTCGATCTCAGAAATCGAAGAGGCGCTGCTCTTCTCAAAGCTGGGCTGCTCAAAGACACGAAGGCGGATCTCACAAA 1500
 TCGATGAGCGCTTGTCTTCTCAAAGTTGGGCTGCTTAGAGACGACGAAGGCGGATCTCACAANTCGAAGAGGCGACCTACTTTTCCAGCCTTCTCAGCA 1600
 CCTGTACACGCACACTCAGCTTTGCGGAAATATGGGCATCTGTCTNAANACTCTGGTGAAGTAGAAAGCGCATGAATCTTACTGTTCAATCNGCCAC 1700
 TTCCACACGCAATAGTACTCATGGGTATCAGATAAAATTTGCCAAAAGTTTTCGCCAAAAGTGAGCACGTTGAAGCTTGCAAACCTTCCCAATACTTNGTTT 1800
 CGACCAAGACGGGTAAAGAATAGCAAAGAAACAGCATTAACAAAGTTTAGAACACATAAAATTTGGAAGGTTTAGCTAACCATATTATTACCCACAAGGG 1900
 TAAAGGAACATACACTGCTGGATAAATTGGAAGTCCCTGTGTCTCAAACCTTCTGTGCTTCTGTCGCAAGGTAGATTAGCAACATGCCCAACCTTTAAIT 2000
 GACATTCGAGAAACACTCCCAACAGATTGCTTGTCTCAAAATCGAAAAGCAGCAGTCTCCGAATCTCGAGAGCCAGACTCCCAACATGATAACTTTT 2100
 CTCAAAAATCGAAGAGAGGTTAAAGGAACAGTACCATTGCTGGATAAATTGGAAGTCCCTGTGTCTCAACCTCTGTGCTTCTGTTGGCAAGGTAGACTAGC 2200
 AAACATGCCCAACCTTTACTTACATTCGAGAAAACACTCCCAACAAGATTGCTTGTCTCAAATCGAAGAGGACCGCCCTCCGAATCTCGAGAGCCAGA 2300
 CTCCAACATGATTACTTTCTCAAAAATCGAAGAGACACCGCTCTCCGAATCTCGAGAGCCAGACCCCGCATGATTGCTTTCTCAAAAATCGAAGAGG 2400
 CATCGTTCTCCGAATCTCGAGAGCCAGATCCCGATAGGATTGCTTGTTCAAAACCGAAGAGGCAACACT**TTCCCAACTGCAAGAGCC** 2489

Fig. 2. Sequence of the S5₂₅₀₀ RAPD fragment. S5 primer is shown in bold. Forward primer for S5₁₄₅₀ marker is shown in bold and underlined. Sequence homologous to the S5₁₄₅₀ reverse primer is shown in italics and underlined.

fragments indicates a susceptible individual. Individuals possessing only one of the fragments ($\approx 2\%$ of the population) are recombinant between the markers and their V_f genotype can not be determined.

Discussion

Marker-assisted selection has become popular in crop improvement and disease resistance breeding (Pineda et al., 1993; Michelmore et al., 1991). For such an approach to be successful, the marker must be highly reliable and easily screened. The assay developed in this study for V_f involves two sequence tagged sites (STSs) that appear to be close to but on opposite sides of V_f . The two sets of primers are compatible with each other, permitting simultaneous screening (multiplexing) of both in a single amplification reaction. This assay has a theoretical escape value (discarding a resistant plant) of $\approx 2.6\%$ and an error rate (selection of a susceptible plant) of 0.017%.

The screened cross 'Golden Delicious' $\times$ 'Prima' (cross 2) was not available at the start of this study. Therefore we used an unscreened population 'Prima' $\times$ 'Spartan' (cross 1) and bulked based on *Pgm-1* genotype. This approach proved successful for identifying other markers that were linked to the V_f locus. Once the scoring for scab resistance had been performed on population of cross 2, we were able to place the markers in a linear arrangement with respect to V_f as well as to determine the probable V_f genotype for the six plants displaying ambiguous responses to scab inoculation. All 'Asp' plants possessed the markers and thus presumably the V_f gene while the class 3 susceptible plants both lacked markers. As 'Prima' also has been reported to generate an 'Asp' reaction when inoculated with *V. inaequalis*, we concluded that this reaction reflected the presence of V_f . One or more additional 'modifier' genes may be present in the original *M. floribunda* source of resistance because 'Asp' phenotype is not observed when this clone is inoculated (Roussele, et al., 1974).

Our results have resolved some of the ambiguities in the marker

order around V_f . The absence of OPC08₁₁₀₀, OPA15₉₀₀, OPD20₆₀₀, and P198₇₅₀ in the 'Idared' $\times$ NY81204-42 population suggests a recombination has occurred between V_f and these markers in the pedigree leading to NY81204-42. A separate population derived from a sib of NY81204-42 also segregated for V_f but not for the above markers, confirming that the recombination probably occurred in an earlier generations (M. Hemmat, unpublished). As S5₁₄₅₀ is lacking in this population, it must be placed on the OPA15₉₀₀ side of V_f . Although there is a slight possibility that OPAL07₅₈₀ is located between V_f and S5₁₄₅₀, the log likelihood for this order is 2.75 less than that for the order presented in Fig. 2. The possibility that U1₄₀₀ and S5₁₄₅₀ is on the same side of V_f is over five orders of magnitude less than our preferred order. These results indicate that U1₄₀₀ and S5₁₄₅₀ should be an excellent set of flanking markers for marker assisted selection. Tartarini (1996) placed OPAM19₂₂₀₀ and OPAL₇₈₀ on the same side of V_f . Others have indicated that OAL07₅₈₀ is on the S5 side of V_f (C. Maliepaard, personal communication). We did not observe a 2200-bp fragment generated by the OPAM19₂₂₀₀ primer segregating in any of the populations we examined, and our data on OPAL07₅₈₀ suggest that this marker is more likely to be on the other side of V_f . Lack of resolution of some markers in our mapping population (cross 2) is probably due to differences in the PCR systems used to generate RAPD phenotypes rather than to the plant materials involved. The PCR system used by Gardiner is known to generate slightly different RAPD phenotypes than ours (Cheng et al., 1998), therefore

Fig. 3. Single assay test using two STS primers (S5₁₄₀₀ and U1₄₀₀) simultaneously. (left to right) Lanes 1-100 bp, DNA ladder; lanes 2, 3, and 7, resistant individuals possessing 1400- and 400-bp markers; lanes 4 and 5, susceptible plants (lacking bands); lane 6, an individual with only one band showing recombination between the two markers.

the same problems may have occurred with other primers.

Gardiner et al. (1996) investigated three populations segregating for *Pgm-I*, OU1₄₀₀, OPM18₉₀₀ and developed two different orders for the *V_f*, OU1₄₀₀, OM18₉₀₀, and *V_f*. In both maps they placed OU1₄₀₀ closer to *V_f*. However, Gianfranceschi et al. (1996) used OU1₄₀₀ and OPM18₉₀₀ as RFLP markers and placed OPM18₉₀₀ closer to *V_f* and both markers on the opposite side of the *V_f* from OPD20₆₀₀. Our results could not determine which marker was closer to *V_f* but clearly placed both on the PGM-1 side of the *V_f*. Our map places most of the commonly used markers in relatively same distance as in the maps of Gardiner et al. (1996), Gianfranceschi et al. (1996), and Tartarini (1996). We have placed OP08₁₁₀₀ about the same distance as reported by Tartarini (1996). The distance of OA15₉₀₀ to *V_f* in our map is very close to that reported by Gardiner et al., 1996. OU1₄₀₀, and OM18₉₀₀ in our map have the same distance to *V_f* as the combined distance of these two markers in the map reported by Gianfranceschi et al. (1996). The recombination frequency reported for OK161300 (Yang et al., 1997b) is 4.3% but this marker mapped ≈16 cM from *V_f* in our linkage map.

Although some of the new markers are specific only to select population, knowledge of diversity of markers available will be important in breeding. Breeders are not using the same parents repeatedly for resistant breeding, but are using a wide array of parents that have been derived from *M. floribunda* 821.

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