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A Randomly Amplified Polymorphic DNA (RAPD) Marker Tightly Linked to the Scab-resistance Gene V_f in Apple

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Abstract. Almost 200 random sequence decamer primers were used to screen a pair of bulked samples of apple (*Malus ×domestica* Borkh.) DNA and that of the donor parent *Malus floribunda* Sieb. clone 821 for molecular markers linked to the V_f gene conferring resistance to apple scab [*Venturia inaequalis* (Cke.) Wint.]. Identified was a single primer that generated a polymerase chain-reaction (PCR) fragment, OPAR4/1400, from the donor parent *M. floribunda* clone 821 and the scab-resistant selections/cultivars bulk, but not from the scab-susceptible recurrent-parent bulk. Cosegregation analysis using a segregating apple progeny and polymorphism analysis of individual scab-resistant selections/cultivars confirmed that this marker was linked to the scab-resistance gene V_f. OPAR4/1400 was then cloned and sequenced. Sequence-specific primers of 25 oligonucleotides based on the marker were developed and used to screen further *M. floribunda* clone 821, scab-susceptible apple cultivars, scab-resistant apple cultivars, and scab-resistant Purdue, Rutgers, and Univ. of Illinois apple breeding program selections. The sequence-specific primers identified polymorphisms of OPAR4/1400 based on the presence or absence of a single band. This molecular marker is at a distance of about 3.6 cM from the V_f gene.

Apple is the most important temperate tree fruit crop in the world. Apple orchards require frequent treatments with various fungicides, insecticides, and herbicides, which increase production costs and reduce profit. More importantly, applying these chemicals has received widespread criticism and increased restrictions due to the public's concern over environmental and health risks. Apple scab, caused by *Venturia inaequalis*, is one of the most widespread fungal diseases of apples and accounts for much of the pesticide use in apple orchards. All currently grown commercial apple cultivars, including 'Jonathan', 'Gala', 'Golden Delicious', 'Delicious', and 'Granny Smith', among others, are known to be susceptible to this fungal disease. Fungicides for controlling this disease are available; however, recent registration restrictions for most key broad-spectrum fungicides, including the ethylene-bis-dithiocarbamates (EBDCs), captan, and the benzimidazoles, have severely limited chemical control options in the apple industry (National Research Council, 1987). Moreover, resistance of *V. inaequalis* to the narrow-spectrum sterol biosynthesis-inhibiting fungicides and to some broad-spectrum fungicides, including dodine, benomyl, and thiophanate-methyl, has been reported in various orchards (Fiaccadori et al., 1987; Hildebrand et al., 1989), thereby rendering these chemicals ineffective for controlling scab in these areas.

Although management programs for apple scab have evolved, including integrated pest management (IPM) practices, the most promising option so far has been developing scab-resistant apple cultivars (Merwin et al., 1994). The Purdue, Rutgers, and Univ. of Illinois (PRI) apple breeding program has had a long history of breeding apples for scab resistance. Currently, there are 11 named scab-resistant apple cultivars that have been released, and hundreds of advanced selections are under testing from this program (Goffreda et al., 1995). Most of these cultivars carry a single major gene for scab resistance, V_f, which has been introgressed into commercial scab-susceptible apples from the small-fruited crabapple species, *M. floribunda* clone 821, by sexual hybridization. While the interest of apple growers in these new cultivars has increased, most commercial plantings are still devoted to susceptible cultivars such as 'Golden Delicious', 'Delicious', and 'Gala'. Therefore, there is a need to combine resistance to apple scab with high-quality fruit traits and other traits necessary for successful apple cultivars. Transferring the V_f gene into commercial apple cultivars via genetic engineering techniques is a viable approach to achieving this goal.

For map-based cloning and isolation of the V_f gene, closely linked molecular markers are needed as starting points for chromosome walking. Previously, isozyme markers (Manganaris et al., 1994) and RAPD markers (Durham and Korban 1994; Gardiner et al., 1996; Koller et al., 1994; Yang and Kruger, 1994) have been reported that are associated with the V_f gene. These markers have been identified by screening numerous decamer random primers and using the bulked-segregant analysis of Michelmore et al. (1991). In this paper, we describe a RAPD marker that is more closely linked to the V_f gene than any of the above mentioned markers and demonstrate the significance of developing sequence-specific primers.

Materials and Methods

Plant material. Commercial apple cultivars, including 'Golden Delicious', 'Jonathan', 'Jonagold', 'McIntosh', 'Delicious', 'Gala', 'Empire', 'Cox Orange Pippin', 'Idared', and 'Rome Beauty',

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were pooled as a scab-susceptible bulk. For the scab-resistance bulk, 10 selections released from the PRI breeding program, including Co-ops 3, 4, 5, 6, 7, 8, 10, 11, 14, and 17, were used. In addition to the resistant and susceptible bulks, *M. floribunda* 821, the original source of the V_f scab-resistance gene, was used in the electrophoretic analysis to compare DNA profiles of the three groups of samples. Leaf tissue was collected from mature trees of all Co-op selections, cultivars, and *M. floribunda* 821 growing in the field of the Pomology Research Center at the Univ. of Illinois, Urbana. Leaves were used immediately or stored frozen at –70 °C until needed.

The progeny '93/22', derived from a cross between a scab-resistant selection '81/19-35' (carrying the V_f gene) and a scab-susceptible selection '87/7-10', was used for segregation analysis. Seedlings of this progeny were inoculated in the greenhouse with *V. inaequalis* and scored for resistance/susceptibility to scab. One-hundred thirty-eight plants, including 48 susceptible and 90 resistant seedlings, were used for mapping putative DNA markers.

DNA EXTRACTION AND RAPD ANALYSIS. Total genomic DNA was extracted from fresh leaf material following the procedure described by Colosi and Schaal (1993) and by using liquid nitrogen and ball bearings. For bulked samples, an equal amount of leaf tissue from each individual was pooled before DNA isolation.

The polymerase chain reaction (PCR) conditions described by Williams et al. (1990) were used. The reaction was conducted in 25 µL containing 2 µL of genomic DNA template, 5 pmol of a single decamer-primer (Operon Technologies, Alameda, Calif.), 0.5 U of *Taq* polymerase, 10 M tris-HCl (pH 8.3), 50 mM KCl_2, 2 mM $MgCl_2$, 0.001% gelatin, and 100 µM each of dATP, dTTP, dGTP, and dCTP. The mixture was overlaid with 25 µL of mineral oil. Amplification was conducted on a programmable thermal cycler (model PTC-100; MJ Research Inc., Watertown, Mass). Template DNA was initially denatured at 94 °C for 5 min, followed by 40 cycles of PCR amplification using the following parameters: 30 s denaturation at 92 °C, 1 min primer annealing at 35 °C, and 2 min primer extension at 72 °C. A final 7-min incubation at 72 °C was allowed to complete primer extension. Amplified products were electrophoretically resolved on 1.3% agarose gels with 1× TAE (40 mM tris-acetate and 1 mM EDTA) and containing 0.1 $\mu g \cdot mL^{-1}$ ethidium bromide.

DETECTION OF DNA FRAGMENTS UNIQUE TO *M. FLORIBUNDA* 821 IN THE RESISTANT BULK. Analyses were performed using bulked samples following the method of Michelmore et al. (1991), with some modification. Instead of using a segregating progeny, we used two bulked samples consisting of resistant selections/cultivars and susceptible cultivars, respectively, and one nonbulked sample of *M. floribunda* 821. The three samples were used for screening individual decamer primers for DNA polymorphism. Primers that gave rise to amplified fragments in the resistance bulked sample that were also unique to the *M. floribunda* 821 sample were considered to be putative markers and were selected for cosegregation analysis.

Since polymorphisms were detected by only a few primers in an initial analysis, we decided to use a two-step strategy in which only the two bulks, instead of all three samples, were analyzed in the first step. Then, primers that amplified a DNA fragment that was exclusively present in the resistant bulk were further analyzed with all three samples in the second step. This approach allowed us to analyze for a large number of primers and reduce the overall number of reactions needed.

COSEGREGATION ANALYSIS OF PUTATIVE MARKERS. To check for potential cosegregation of DNA fragments and scab-resistant phenotypes, primers that generated unique fragments in the resistant bulk and *M. floribunda* 821 were analyzed against individuals of known scab-resistant/susceptible phenotypes from the segregating progeny '93/22', 38 Co-op selections/cultivars, and 10 scab-susceptible apple cultivars, including 'Golden Delicious', 'Jonathan', 'Jonagold', McIntosh', 'Delicious', 'Gala', 'Empire', 'Cox Orange Pippin', 'Idared', and 'Rome Beauty'.

CLONING AND SEQUENCING OF RAPD FRAGMENTS OF OPAR4/1400. The vector pBluescript M13 (Stratagene, La Jolla, Calif.) was used to clone RAPD fragments. The procedure was previously described by Yang and Korban (1996). The polymorphic RAPD band was excised from the gel with a surgical blade, and TE buffer was added to the gel slice. The agarose gel piece containing the DNA fragment was minced using a pipette-tip, and the crushed agarose was discarded following 2 min of centrifugation. The prepared DNA was reamplified using the appropriate primers. An undiluted PCR reaction, containing the desired RAPD fragment, was directly used for blunt-end ligation, which inserted the fragment into the Sma I site of the pBluescript M13 vector. The ligation reaction followed the procedures described by Yang and Korban (1996). Recombinant clones were screened for the appropriate size of insert by PCR using vector-specific primers (M13 forward and reverse sequencing primers) and restriction digestion before sequencing. Plasmid DNA containing RAPD inserts were prepared for sequencing following the protocol of Goode and Feinstein (1992). Both ends of each DNA insert were sequenced using an automated sequencer (model 373A; Applied Biosystem, Perkin Elmer, Foster, Calif.).

SYNTHESIS OF PRIMERS AND PCR ANALYSIS USING MARKER SEQUENCE-SPECIFIC PRIMERS. Based on the sequence data of the cloned RAPD fragments, primers were synthesized at the Genetic Engineering Facility of the Univ. of Illinois. The primers synthesized were 25-er, with the first 10 bases corresponding to the sequence of the original random primer that produced the respective RAPD fragment. PCR was conducted using a pair of 25-er primers as described by Yang and Krüger (1994) with two modifications. The annealing temperature was increased from 35 to 70 °C and the reaction was reduced from 40 to 30 cycles of 30 s denaturation of 94 °C, 1 min annealing at 70, and 3 min of primer extension at 72 °C.

Results

IDENTIFICATION OF OPAR4/1400. Initial screening of 79 operon decamer-primers, which generated about 360 bands, against the scab-resistant bulk, scab-susceptible bulk, and *M. floribunda* 821 resulted in the identification of seven primers that generated DNA

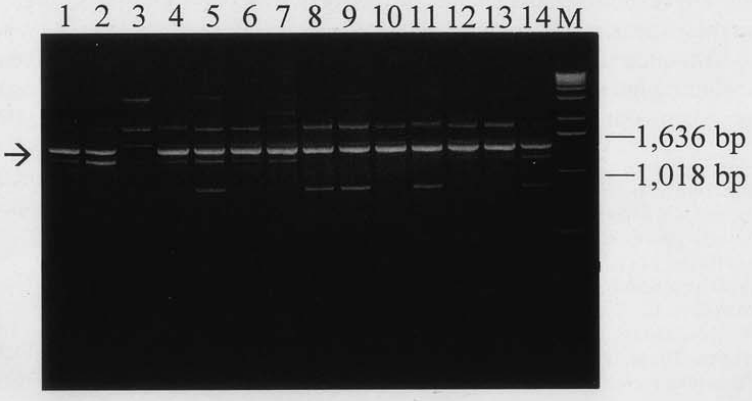

Fig. 1. Ethidium bromide-stained electrophoretic pattern of RAPD fragments generated by OPAR4. Arrow corresponds to the fragment of 1400 bp cosegregating with scab resistance. Lanes 1–3 are *M. floribunda* 821, scab-resistant bulk, and scab-susceptible bulk, respectively; lanes 4–15 are scab-resistant individuals Co-ops 27, 29-38; M is 1-kb DNA ladder.

Table 1. Cosegregation of scab resistance and the RAPD marker OPAR4/1400 in the apple progeny '93/22'.[z]

Phenotypic greenhouse reaction of seedlings to scab inoculation	Plants (no.)	Segregation of seedlings for OPAR4/1400		Recombination ratio (%) ±SD
		Present	Absent	
Susceptible	48	2	46	3.6 ± 0.4
Resistant	90	87	3	

[z]The progeny '93/22' was derived from a cross of '81/19-35' x '87/7-10'. Seedlings were inoculated in the greenhouse with *Venturia inaequalis* and then rated for presence or absence of scab resistance 2 weeks following inoculation.

fragments that were present in *M. floribunda* 821 and the resistant bulk, but absent in the susceptible bulk. Six of these primers, OPAQ6, OPAQ14, OPAS2, OPAS10, OPAT2, and OPAT18, were subsequently shown to generate a DNA fragment that did not cosegregate with scab resistance when analyzed using individual seedlings of the segregating progeny '93/22' (data not shown). Although these DNA fragments were not found to be linked to the V_f gene, they probably corresponded to *M. floribunda* 821 chromosomal segments unrelated to the scab-resistance gene. One primer, OPAR4, generated a polymorphic fragment that was found to be linked to the V_f gene. RAPD fragments amplified by this primer are presented in Fig. 1. This fragment of about 1400 bp, cosegregating with scab resistance, has been designated as OPAR4/1400. All but 2 of the 48 scab-susceptible seedlings did not contain the OPAR4/1400 fragment, while all but 3 of 90 scab-resistant seedlings were OPAR4/1400-positive (Table 1).

SCREENING DIFFERENT APPLE GENOTYPES WITH OPAR4/1400. Scoring across a diverse collection of apple genotypes, including scab-resistant Co-op selections/cultivars and scab-susceptible apple cultivars, showed a close linkage between the RAPD marker OPAR4/1400 and scab resistance. The presence/absence of OPAR4/1400 fragment in the genotypes tested have been summarized in Table 2. For all scab-susceptible cultivars tested, including 'Gala', 'Golden Delicious', 'Jonathan', 'Jonagold', 'McIntosh', 'Delicious', and 'Wijcik', no amplification of OPAR4/1400 was detected. Out of 38 scab-resistant Co-op selections/cultivars, 36 showed the OPAR4/1400. This corresponded to an approximate 95% frequency of occurrence. This provided strong evidence that the RAPD fragment OPAR/1400 was linked to the V_f gene for scab resistance derived from the wild species *M. floribunda* 821.

CLONING AND SEQUENCING THE RAPD FRAGMENT OF OPAR4/1400. The reamplified PCR products of OPAR4/1400 were cloned into pBluescript M13. The identities of the cloned products were verified by PCR using vector sequence-specific primers and double-restriction digestion with restriction endonucleases Xba I and Pst I. One of the clones having the correct size of 1400 bp was used for DNA sequencing. The entire RAPD fragment of OPAR4/1400 was sequenced using M13/pUC forward and reverse sequencing primers. The sequencing results showed that the OPAR4/1400 RAPD fragment had a size of 1411 bp (Fig. 2)

Based on sequencing results, oligonucleotides of 25 bases were synthesized and used as sequence-specific primers for DNA amplification (Fig. 2). The first two oligonucleotides, OPAR4/1400 I and OPAR4/1400 II, were derived from both ends of the RAPD fragment of OPAR4/1400 and contained the original 10 bases of the RAPD primer OPAR4 plus the next 15 internal bases from the 3' end.

When the two 25-er primers were used to screen individual apple cultivars, Co-op selections, and *M. floribunda* 821 at an annealing temperature of 70 °C, two major fragments were amplified in the scab-resistant genotypes. One of these fragments corresponded to OPAR4/1400 and another fragment had a small size of about 390 bp. Interestingly, both seemed to be linked to each other since they always appeared together (Fig. 3). This 390-bp fragment was not amplified from the same locus, OPAR4/1400, since no sequence repeats of the primers OPAR4/1400 I and II have been found within the DNA fragment OPAR4/1400.

To amplify a single fragment of OPAR4/1400, a third primer, OPAR4/1400 IIa, was designed, which was 25 bp in length and included the nucleotides from 868 to 900 from the 5' end of the OPAR4/1400 (Fig. 2). OPAR4/1400 IIa along with OPAR4/1400 I gave rise to a PCR product of 900 bp in all resistant genotypes tested including *M. floribunda* 821, while this PCR product could not be amplified in scab-susceptible genotypes (Fig. 4). Since the 900-bp PCR product matched the distance between the primers OPAR4/1400 I and OPAR4/1400 IIa, it is likely that this fragment was derived from part of the molecular marker OPAR4/1400. In addition to the 900-bp fragment, several weak background fragments were also produced. In resistant and susceptible genotypes, a PCR fragment of about 2100 bp was amplified (Fig. 4). A similar result was also obtained with a fourth primer, OPAR4/1400 IIb, which had a 25-bp fragment and included the nucleotides 808 to 833 from the 5' end (Fig. 2).

Table 2. Survey for OPAR4/1400 in Co-op selections/cultivars developed from the scab-resistant apple breeding program.[z]

Genotype	Cultivars and selections with (+) or without (–) OPAR4/1400	
	+	–
Resistant selections	Co-ops 1, 3, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 25, 27, 24, 26, 29, 31, 33, 34, 35, 36, 37	Co-op 28
Resistant cultivars	Enterprise, Dayton, GoldRush, Jonafree, Prima, Priscilla, Pristine, Sir Prize, Williams' Pride	Redfree
Both groups	36	2

[z]All 10 scab-susceptible cultivars tested did not contain the OPAR/1400 fragment, while *M. floribunda* 821, the original source of the V_f gene, did.

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      5'        10          20          30          40          50
 1    CCAGGAGAAG  TCATATTACC  TGTGTATCCA  ACGCTCCCCA  GGGAAGATGT   50
          (OPAR4/1400 I) →
 51   GAGTTCCTTA  AATGGAAAAG  TGTCCTCATC  GAATAGGACA  TGGCGAGACA  100
101   AATAGATTCG  TCTCGTGGAC  AAATCTAAGC  ATTTGTACCC  TTGATGATTC  150
151   AGTGAGTAAC  CTAAAAACAC  ACATGGTTTG  GACTTTGGTT  GGAGTTTATT  200
201   TTGTGCGTAA  GGCACTAACC  AGGGATAACA  TTGACATCCA  AACACTTTAA  250
251   GAGTTTCATA  TTTTGGCACT  TTGGCATACA  GTTGTTCATA  GGGAGACTTG  300
301   TGAGACAAGG  CACGACTTGG  CAACCTATTA  ATTAAATAAG  TAGCTGTTAA  350
351   ACAAGCATCC  ACCCAATATT  TATTTGGAAT  ATGTGCATGA  GAAAGCATGA  400
401   CGATACTAGT  CTCAATAATG  TGACGATGTT  TTCTTTCGGC  AATGCCATTT  450
451   TGTTCAGGAT  GTTTTGGACA  CGAAGATCTT  TGGGAAATAC  CATGAGTGGC  500
501   AAGGTAGGTT  TGAAATACAT  GACTTTTATA  CTCACCACCT  TCATCACATT  550
551   GAAGAGTTTT  GATAGACCTG  TTAAACATAT  TTTCAACCTG  TGCTTTAAAC  600
601   TTCACAAAGA  TTTCAAAGAC  CTCACATTTA  TTTTTCATTG  GAAAAACCCA  650
651   AGAATACCGA  GAATAATCAT  CCACAAATAA  CACATAATAG  CGAAAACCTT  700
701   CAACTGAATT  AACGGCGTGA  GAGGTCCTCT  TGCTCGCCAC  GACCGAGAGC  750
751   ACAGACGGCG  TGAGCGATCC  ATTAATCCAG  CTGAGTATCA  TTTGATCGTA  800
801   CTGCACCCAT  GTTGTGTACG  CAGGATTGGT  AGTTGTACTG  CCAGAAAGTT  850
                ← (OPAR4/1400 IIb)
851   GTTTGGGAGG  ACAAACAACC  GTCCTATCAA  CGTAACCCAT  CAGGTCCCGA  900
                              ← (OPAR4/1400 IIa)
901   CTTTTGAGAA  CAGGTAAGAT  CTGAGCCAAC  CATAAAGGAT  AATTGGTCCG  950
951   ATCCAGCTTA  ATATTCACCA  CTGTGGTCAA  CGACGAGAAG  GGGTTCATGG 1000
1001  TGGAATTGGC  GGGGTTGGAG  GTTTGGGTTG  CAGCACCGTT  GCTAGAATCG 1050
1051  GCCATGGAAG  AGAAAAAAAA  ACTGATATGT  GTCTTTAGAC  TGCTAGGGCT 1100
1101  CTGATACCAT  GTAAAACTTA  CAACACTCAC  AGTTTCACTT  CAATTGCATT 1150
1151  ACCCAATCAG  TGATGGGCTG  CATGTATTTA  TAATGTATTT  CAGATTACAT 1200
1201  CAGGGAACTG  AAATCCCTAT  TTACAAGGAT  ATTTGAAAAA  CAAAGATACA 1250
1251  ACAGAAAGAT  CATACTGAGA  GTTACACGGA  AAGGAATATT  TGAAAGAGTC 1300
                ← (OPAR4/1400 IIc)
1301  TTAACCTAAA  CCAACTCTTG  TTGGATAACA  ATTGGACTTG  ATCACGAACT 1350
1351  CTTGAAACCG  CACGCGGACT  ATGACTTGGT  CTAACACCTT  CAACCCTCAA 1400
1401  CCTTCTCCTG  G  ← (OPAR4/1400 II)                          1450
          10          20          30          40          50
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Fig. 2. DNA sequence of the RAPD marker OPAR4/1400 of apple and sequences of the 25-er primers designed and used for amplifying apple genomic DNA.

A fifth primer, OPAR4/1400 IIc, covering the nucleotides 1256 to 1280 from the 5' end (Fig. 2), used together with OPAR4/1400 I, produced exclusively a single fragment. Thus, polymorphisms of the RAPD marker OPAR4/1400 were identified by the presence or absence of a single band (Fig. 5). All background DNA fragments, which could be otherwise amplified by the decamer primer OPAR4, were no longer detected on the gel. All OPAR4/1400-positive genotypes including *M. floribunda* 821 showed a single fragment of 1280 bp, while all OPAR4/1400-negative genotypes failed to generate any fragments (Fig. 6).

Discussion

Map-based cloning of a gene of interest is possible since techniques for cloning and manipulating large fragments of genomic DNA have been developed (Michelmore et al., 1992). The main approach in map-based cloning of a gene involves identifying overlapping genomic clones by chromosome walking. One of the prerequisites for chromosome walking is knowledge of the location of a gene on a genetic linkage map. DNA markers tightly linked to or flanking a target gene can provide a starting point for chromosome walking (Martin et al., 1991; Reiter et al., 1992). In this study, we identified a RAPD marker, OPAR4/1400, that is linked to the V_f gene conferring resistance to apple scab. With a frequency of recombination of 3.6%, this marker showed a closer linkage to the V_f gene than a previously reported marker, OPD20/600 (Yang and Krüger, 1994). The presence of the OPAR4/1400 fragment in 95% of Co-op selections/cultivars tested strongly indicates that this DNA fragment is closely linked to the V_f gene and at a genetic mapping distance of 3.6 cM.

Based on sequence analysis, no sequence repeats of the primers OPAR4/1400 I and II have been found within the DNA fragment OPAR4/1400. Therefore, it is apparent that the 390-bp fragment amplified by OPAR4/1400 I and II is not derived from OPAR4/1400. It must have been amplified from genomic DNA sequences that are closely linked to OPAR4/1400. Thus, the 390-bp fragment provides another DNA marker for scab resistance.

It is known that RAPD techniques are sensitive to changes in reaction conditions, such as Mg^{2+} concentration in the buffer and temperature conditions. Often, these changes do not allow accurate comparisons among results obtained in different laboratories. In addition, production of background fragments in the agarose gel sometimes interferes with the identification of bands of interest. The use of 25-er marker-sequence-specific primers allows amplification of a single DNA fragment. This improves the reliability of the RAPD fragment as a genetic marker (Paran et al., 1991; Paran and Michelmore, 1993), and facilitates its application in marker-aided selection (Tanksley, 1983). Therefore, OPAR4/1400 provides a useful genetic marker for the V_f gene for scab resistance.

Although several scab-resistance genes have been identified in different *Malus* species (Dayton and Williams,

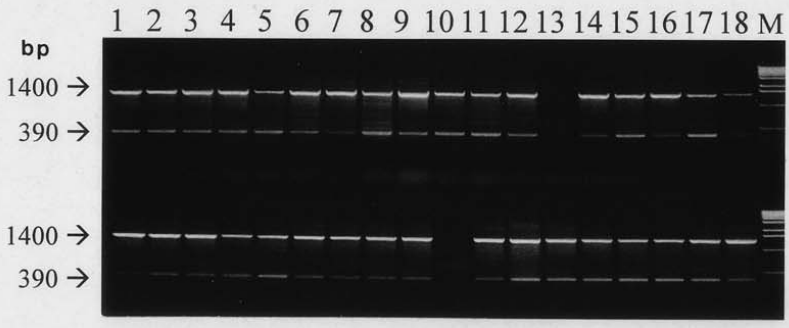

Fig. 3. Amplification of genomic DNA using the pair of 25-er primers OPAR4/1400 I and II. (**top gel**) Lanes 1 to 18 are Co-ops 1–18. (**bottom gel**) Lanes 1 to 18 are Co-ops 19–36. M is 1-kb DNA ladder.

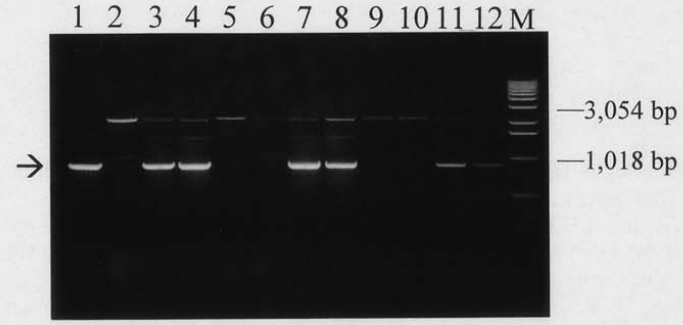

Fig. 4. PCR fragments amplified using OPAR4/1400 I and IIa. From left to right: lanes 1–8 are *M. floribunda*, scab-susceptible bulk, 'Prima', 'Dayton', 'Redfree', Co-op 28, Co-op 4, and Co-op 5; lanes 9–10 are two OPAR4/1400 negative seedlings of '93/22'; lanes 11–12 are two OPAR4/1400-positive seedlings of '93/22'; M is 1-kb DNA ladder.

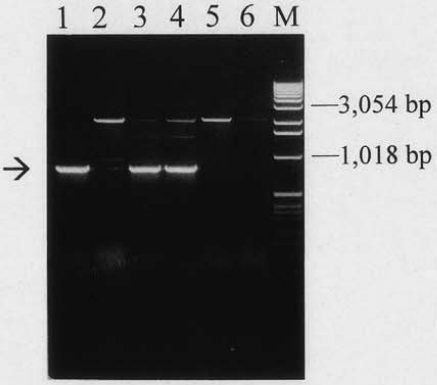

Fig. 5. Amplification of genomic DNA using OPAR4/1400 I and IIb. (**left to right**) *M. floribunda*, scab-susceptible bulk, Co-op 1, 'Prima', 'Redfree', Co-op 28, and 1-kb DNA ladder. Arrow corresponds to an 883-bp fragment that co-segregates with the V_f gene.

1968, 1970), the V_f gene has been most widely used in breeding programs because it is believed to be the most stable (Hough, 1944). Cultivars and selections carrying the V_f gene have been field-immune to apple scab for all five known races of *V. inaequalis* for more than 50 years in countries where they have been grown. Since 1988, scab symptoms have been observed on 'Prima' and a few V_f selections including Co-ops 7, 9, and 10 in Ahrensburg, Germany (Krüger, 1988). Greenhouse inoculations with the German inoculum in France resulted in infection of some, but not all, cultivars and selections carrying the V_f gene (Parisi et al., 1993), indicating that the V_f resistance may have been overcome by a new race of *V. inaequalis*, designated as race 6. These events raise the urgency of diversifying the sources of resistance to *V. inaequalis* in the scab-resistant breeding material. Thus, additional efforts must be made to combine the independent sources of scab resistance genes (Dayton and Williams, 1968, 1970) in a new breeding strategy (Lespinasse, 1989). Although scab resistance is a qualitative trait, screening a progeny for the presence of different resistance genes based on phenotypic response requires time-consuming testing procedures. Moreover, expression of the resistance genes can be influenced by the environment and their reactions are difficult to resolve (Lamb and Hamilton, 1969). DNA markers provide valuable tools for screening progenies for the presence of several nonallelic scab-resistance genes and should be used to map these genes.

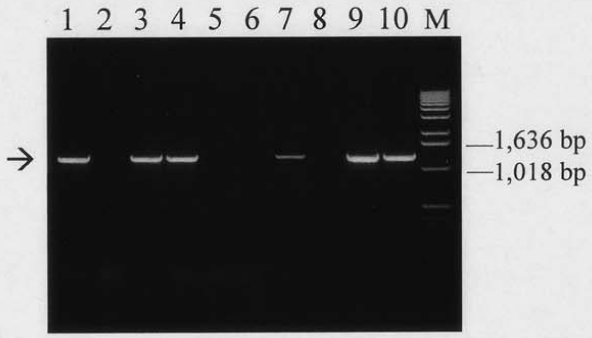

Fig. 6. Amplification of genomic DNA using OPAR4/1400 I and IIc. (**left to right**) *M. floribunda,* scab-susceptible bulk, 'Prima', 'Dayton', 'Redfree', Co-op 28, an OPAR4/1400-positive seedling of the progeny '93/22', an OPAR4/1400-negative seedling of the progeny '93/22', 'Priscilla', and 'Sir Prize'; M is 1-kb DNA ladder.

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