Development of SCAR Markers Linked to a Phytophthora fragariae Resistance Gene and Their Assessment in European and North American Strawberry Genotypes

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ADDITIONAL INDEX WORDS. Fragaria sp., pedigree-analysis, resistance-gene-mapping, RAPD, SCAR, red stele root rot

ABSTRACT. Two dominant sequence characterized amplified region (SCAR) markers (linked at 3.0 cM, coupling phase) were constructed for the strawberry (Fragaria × ananassa Duch.) gene Rpf1. This gene confers resistance to red stele root rot, caused by the soil-borne fungus Phytophthora fragariae Hickman var. fragariae. The SCAR markers were developed originally from the sequence of RAPD OPO-16C (438) that is linked in repulsion phase to the Rpfl allele. This SCAR primer set produced multiple bands in the resistant test progeny and in some of the susceptible progeny; therefore, new SCARs were developed based on the sequence differences among these bands. These new SCARs were linked in coupling phase to the Rpfl allele and mapped to the same location as the original RAPD OPO-16C (438). The SCAR markers, as well as some additional RAPD markers known to be linked to Rpfl, were shown to be highly conserved in linkage to the gene based on examination of 133 European and North American Fragaria L. sp. cultivars and breeding selections. These flanking RAPD and SCAR-PCR markers can be used in breeding programs for the selection of red stele (Rpfl) resistance.

Many commercial strawberry (Fragaria × ananassa) cultivars are susceptible to red stele root rot caused by the soilborne fungus Phytophthora fragariae var. fragariae (Hickman, 1940). Symptoms of the disease can include dwarfism, wilting of leaves and petioles, reddening of the root stele, and eventual plant death. Soil disinfection with chemical fumigants such as methyl bromide or chloropicrin helps to reduce the inoculum potential of P. fragariae (Bollen, 1972). However, long-term application of such fungicides as metalxy [N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanine methyl ester], and fosetyl-aluminum [aluminum tris (O-ethyl phosphonate)], can result in selection of resistant strains of the fungus (Seemüller and Sun, 1989). Therefore, development of resistant cultivars is highly desirable. The need for naturally resistant strawberry lines is desirable since one of the main chemicals for control of P. fragariae, methyl bromide, is being phased out by the year 2006.

In strawberry, five genes for resistance to thirty races of P. fragariae have been described (Van de Weg, 1997); including Rpfl. Rpfl, a dominant gene (Van de Weg et al., 1997b) that confers resistance to at least 18 races of P. fragariae (Kennedy and Duncan, 1988; Nickerson and Murray, 1993; Schewee, 1994; Van de Weg et al., 1997a). Previously, bulked segregant analysis (BSA) (Michelmore et al., 1991) was used to find eight randomly amplified polymorphic DNA (RAPD) markers linked to Rpfl (Haymes et al., 1997). Mapping this gene provided molecular support to its monogenic inheritance (Van de Weg et al., 1997b).

Molecular markers have many applications in plant breeding programs including marker-assisted selection, allowing breeders to maximize efficiency. One type of molecular marker, RAPD (Williams et al., 1990), has revolutionized genetic analysis and genome characterization for cultivated crops (for reviews see Devos and Gale, 1992; Tingey and Del Tufo, 1993; Waugh and Powell, 1992). More recently, RAPD markers linked to horticultural characteristics have been converted to specific and highly reproducible markers called sequence characterized amplified region (SCAR) markers (Paran and Michelmore, 1993).

In this paper, we report the cloning and sequencing of a RAPD marker linked to Rpfl and its conversion into two highly specific SCAR markers. Moreover, the maintenance of these SCARs and...
some RAPD markers in European and North American strawberry cultivars and breeding selections was examined.

Material and Methods

PLANT MATERIAL. A testcross between two *Fragaria xananassa* cultivars, Md683 (*Rpf1*, resistant) x Senga Sengana (*rpf1*, susceptible), was performed. The resulting progeny of 60 F$_1$ plants segregated in a 1:1 ratio for the *Rpf1* locus (Van de Weg et al., 1997b), and were used for the initial mapping of this gene with RAPD markers (Haymes et al., 1997). The same population was used in the present study to develop the SCAR markers and test their segregation. For determination of marker presence in the present study, strawberry selections from the Centre for Plant Breeding and Reproduction Research, Department of Vegetable and Fruit Crops (CPRO-DLO), Wageningen, The Netherlands, strawberry collection and breeding program or from K. Hummer of the U.S. Department of Agriculture (USDA), National Germplasm Repository, Corvallis, Ore., were used. In total, 68 European and 65 North American cultivars and selections were tested.

RESISTANCE TESTS. Resistance tests were performed at either CPRO-DLO or at the USDA–Agricultural Research Service (ARS) Fruit Laboratory, Beltsville, Md. At CPRO-DLO, *Rpf1* genotypes were identified as such by their resistance to isolate NS2 and susceptibility to one of the isolates A7, A8, NS3, and NS4 (Van de Weg, 1997). Tests were performed according to Van de Weg et al. (1997b). For the F$_1$ mapping population, 29 individuals were shown previously to be resistant and 31 susceptible to virulent race 2.3.4 isolate NS2-25 (Haymes et al. 1997).

At the USDA–ARS Fruit Laboratory, *Rpf1* genotypes were identified by their resistance to isolate A3 or to a mixture of isolates A1, A2, A3, A4, and A6. Occasionally, strawberry selections were also tested with a series of individual inoculations with isolates A1, A2, A4, and A6. Susceptibility to these isolates indicates absence of *Rpf1*. Tests was performed according to Maas et al. (1989) and Scott et al. (1975).

DNA ISOLATION AND PCR AMPLIFICATION OF RAPD MARKERS AND ISOLATION OF THE RAPD MARKER. DNA extraction of the testcross population was done according to Haymes et al. (1997) and that of the European and North American selections, also following procedures of Haymes (1996). Amplifications with RAPD primers were performed according to Haymes et al. (1997). For isolation of the RAPD marker OPO-16C (438), DNA from susceptible plants was amplified with the primer OPO-16: 5′-TCGGCGGTTC-3′ (Operon Tech, Alameda, Calif.), and separated on a 2% agarose gel (1× TBE). The polymerase chain reaction (PCR) fragment corresponding to OPO-16C: 5′-TCGGCGGTTC-3′ (Operon Tech, Alameda, Calif.), and separated on a 2% agarose gel (1× TBE). The polymerase chain reaction (PCR) fragment corresponding to OPO-16C: 5′-TCGGCGGTTC-3′ (Operon Tech, Alameda, Calif.), and separated on a 2% agarose gel (1× TBE). The polymerase chain reaction (PCR) fragment corresponding to OPO-16C: 5′-TCGGCGGTTC-3′ (Operon Tech, Alameda, Calif.), and separated on a 2% agarose gel (1× TBE).

Fig. 1. Sequence homology between the susceptible OPO-16C and the corresponding resistant band fragment of both the upper (U), middle (M), and lower (L) resistant band fragment amplified with the SCAR primers from Table 1 and Fig. 2. Alignment and homology analysis was done using multiple sequence alignment and cluster analysis. Base pair changes are indicated directly below the sequence, deletions are noted by the symbol (–). The symbol (*) indicates two Gs at position 270. The original RAPD primer is underlined.

The DNA was quantified both spectrophotometrically and by running the samples on a 1% TBE agarose gel with DNA standards.
CLONING AND SEQUENCING OF A RAPD MARKER. The purified DNA fragment amplified with RAPD marker OPO-16C (438), was cloned into the *Hinc* II site of plasmid pBluescript SK+ (Stratagene, La Jolla, Calif.), and transformed into *E. coli* DH5α strain according to Sambrook et al. (1989). Recombinant clones were screened for appropriately sized inserts. The selected recombinant clones were sequenced from both sides on an Applied Biosystems 373 Automated Sequencer (Applied Biosystems, Foster City, Calif.) using an *Taq* DyeDexoy Terminator Cycle Sequencing kit [Applied Biosystems (AB)]. Template DNA preparations and sequence reaction mixtures were done according to AB recommended procedures.

PCR FOR SCAR-S PRIMERS. A pair of 24 base pair (bp) primers (SCAR-S) were designed based on the sequence of the cloned OPO-16C (438) fragment (Fig. 1). Each PCR tube contained 50 ng each of the forward and reverse SCAR-S primers, 0.1 mM of each of four dNTP, 20 ng genomic DNA, 2.5 μL 10× reaction buffer (Life Technologies, Gaithersburg, Md.), 0.75 μL 50 mM MgCl₂ (Life Technologies), 0.5 unit of *Taq* polymerase (Life Technologies) and 18 μL of sterilized H₂O, making a total volume of 25 μL. PCR amplification was conducted in either a PE Cetus Thermal Cycler 480 (PE, Foster City, Calif.) or a Hybaid Omnigene Cycler (Hybaid Omnigene, Franklyn, Mass.). PCR conditions for both machines were 94 °C for 3 min followed by 25 cycles of 94 °C for 30 s, 60 °C for 45 s, 72 °C for 1 min, and a 7-min extension at 72 °C. The completed reactions were held at 4 °C for the PE Cycler and 20 °C for the Hybaid Omnigene Cycler.

GEL ANALYSIS AND SEQUENCING OF SCAR PRODUCTS AND DEVELOPMENT OF RESISTANCE-LINKED SCARS. DNA of five susceptible and five resistant plants from the testcross progeny was amplified using the SCAR-S primers. Electrophoresis of the PCR products on a 2% TBE agarose gel. The PCR buffers were optimized for each of the two SCAR-R1 primer sets and differed solely for their pH; SCAR-R1A: 10× buffer (100 mM Tris-HCl pH 8.8; 35 mM MgCl₂, 750 mM KCl) and SCAR-R1B 10× buffer (100 mM Tris-HCl pH 9.2; 35 mM MgCl₂, 750 mM KCl) (Schoettlin et al., 1993).

**Table 1. SCAR primers based on the sequence differences between the OPO-16C RAPD marker for susceptibility and the sequenced markers for resistance from the SCAR-S primers.** Map position, band fragment, and expected band length from which primers were designed are indicated.

| Primer | Sequence | Map position | Band fragment | Expected band length |
|--------|----------|--------------|---------------|---------------------|
| SCAR-S | Forward: 5′-TGC ATC ATT AAT GTA GAA GTC TTT-3′ | 29-52 | (OPO-16C) | 404 |
|        | Reverse: 5′-GTT TTC CCA AAA GAT TAG TAG TTA-3′ | 433-410 | (OPO-16C) |
| SCAR-R1A | Forward: 5′-TGC ATC ATT AAT GTA GAA GTC TTT-3′ | 29-52 | (OPO-16C) | 285 |
|        | Reverse: 5′-TGA TGC GAC ATA CAA AAA TAT TAG-3′ | 320-297 | (Resist M) |
| SCAR-R1B | Forward: 5′-ATG ACC GAA TCA AAA TAT TCT-3′ | 271-298 | (Resist M) | 133 |
|        | Reverse: 5′-ACT AAC ACA GAC AAC CCA CCA -3′ | 410-390 | (Resist M) |

Fig. 2. DNA of three resistant (R) and three susceptible (S) plants amplified with SCAR primer sets. (A) SCAR-S, (B) SCAR-R1A, and (C) SCAR-R1B. Amplified products were analyzed by electrophoresis on a 2% TBE agarose gel. Lanes 1 to 3 are Rpf1 resistant plants and lanes 4 to 6 are rpf1 susceptible plants to *P. fragariae* isolate NS2-25. Molecular weights in (bp) are indicated by M. In A, lane 3, the upper, middle, and the lower band fragments are shown. Primer sequences are listed in Table 1.
ogy, Alameda, Calif.) and one SCAR marker (SCAR-R1A) linked to Rpf1 (Haymes et al., 1997) were used for the conservation analysis. Their relative positions and linkage phase to Rpf1 are presented in Fig. 3.

Table 2. Thirty-four Rpf1-resistant European and North American strawberry cultivars and selections tested with two RAPD markers and one SCAR marker [(+) present, (–) absent] for resistance to Phytophthora fragaria. Marker linkages are illustrated in Fig. 2. Male parents are in bold.

| Genotype | Country of origin | Parental plants | OPO-16C (susceptible) | OPC-8D (resistant) | OPO-8A (resistant) | SCAR-R1A | Rpf1 (resistant) |
|----------|------------------|-----------------|------------------------|-------------------|-------------------|-----------|-----------------|
| Allstar  | USA              | US4419 (Redstar x Surecrop) x [MDUS 1972 x Midland] x MD430] x (NC-1768 x Surecrop) x MDUS 3184 (NC-1768 x Surecrop) | –                 | +             | +             | +        | a               |
| Annapolis| CAN              | Micmac x Raritan x Earliglow | –          | –             | +             | +        | a               |
| Arking   | USA              | Cardinal x ARK-5431 [MDUS 3082 (NC-1768 x Surecrop) x Delite] | –          | –             | +             | +        | b               |
| Auchenincruive-6 | UK    | Frith x Frith | –             | +             | +             | + c      |                 |
| Benton   | USA              | ORUS 2414 x Vale | –             | +             | –             | – d      |                 |
| Cornwallis| CAN            | Earliglow x Kent | +             | +             | +             | + a      |                 |
| CPRO 77191 | NL         | Guardian x Sivetta | +             | +             | –             | + e      |                 |
| CPRO 88218 | NL         | Bogota x Scott | +             | +             | –             | + e      |                 |
| CPRO 88239 | NL         | Bogota x Yalova-4 | –             | –             | +             | + e      |                 |
| CPRO 88246 | NL         | (Redchief x Sivetta) x Bogota x Yalova-4 | –             | –             | +             | + e      |                 |
| CPRO 88275 | NL         | Holiday x (Induka x Sivetta) x Yalova-4 | –             | –             | –             | + e      |                 |
| CPRO 88310 | NL         | (Sivetta x Holiday) x Korona x Scott | +             | +             | –             | – e      |                 |
| CPRO 88312 | NL         | (Sivetta x Holiday) x Korona x Scott | +             | +             | +             | – e      |                 |
| CPRO 89027 | NL         | (Tamella x Redgauntlet) x Md2700 (Pocohantas x Stelemaster) x Allstar | –             | +             | –             | + e      |                 |
| CPRO 90025 | NL         | Allstar x Korona | +             | +             | +             | + e      |                 |
| Darrow   | USA              | Md2713 (Redglow x Surecrop) x MDUS 2787 (Fairland x Midland) x (Midland x MD683) | +             | +             | +             | + a      |                 |
| Delite   | USA              | Albritton x MDUS 2650 (Blakemore x MD683) x Midland x Fairpeake x (Aberdeen x Redheart) | +             | –             | +             | + a      |                 |
| Earliglow| USA              | Md2359 (Fairland x Midland) x Md2713 | +             | +             | +             | + a      |                 |
| Guardian | USA              | NC-1768 [Fairpeake x (Aberdeen x Redheart)] x Tennesse Beauty x Surecrop | +             | +             | +             | + a      |                 |
| Hood     | USA              | ORUS 2315 x Puget Beauty | +             | +             | +             | + a      |                 |
| Linn     | USA              | MDUS 3184 x ORUS 2414 | –             | –             | +             | + a      |                 |
| MD683    | USA              | Scotland BK-46 (Frith selfed) x Fairfax | +             | +             | +             | + a      |                 |
| MDUS 3184| USA              | NC-1768 x Surecrop | +             | +             | +             | + b      |                 |
| Perle de Prague | UK     | Unknown x Unknown | +             | –             | –             | + a      |                 |
| Redchief | USA              | NC-1768 x Surecrop | +             | –             | –             | + a      |                 |
| Scott    | USA              | Sunrise x Tioga | +             | +             | –             | + f      |                 |
| Siltez   | USA              | ORUS 2012 x ORUS 1816 | –             | +             | +             | + a      |                 |
| Stelemaster | USA          | Fairland x MD683 | +             | +             | –             | + a      |                 |
| Sunrise  | USA              | US 4152 (Tennessee Shipper x Maytime) x Stelemaster | +             | +             | –             | + a      |                 |
| Surecrop | USA              | Fairland x MDUS 1972 (Blakemore x MD683) | –             | +             | +             | + a      |                 |
| Tribute  | USA              | EB 18 (NC-1768 x Surecrop) x Cal 65.65-601 x MDUS 4258 (Redglow x Surecrop) x (Midland x Sunrise) | +             | –             | –             | + f      |                 |
| Tristar  | USA              | EB 18 x MDUS 4258 | +             | +             | +             | + a      |                 |
| Yalova-4 | TU                | Cengelköy x Aliso | –             | +             | +             | + e      |                 |
| Yalova-15| TU                | Cengelköy x Tiago | +             | –             | +             | + e      |                 |

Plant material was obtained from CPRO-DLO strawberry collection and breeding program, The Netherlands for all Dutch (NL) selections, MD683, ‘Perle de Prague’, and the two selections from Turkey (TU), and all others came from the USDA National Germplasm Repository, Corvallis, Ore.

Abbreviations: susc = susceptible and resist = resistant.

Results

Development of SCAR primers based on the susceptibility allele. The DNA fragment representing RAPD OPO-16C (438)
Table 3. Fifty-three *Rpf1* susceptible (susc) European and North American strawberry cultivars and selections tested with two RAPD markers and one SCAR marker (+ present; – absent) for resistance (resist) to *Phytophthora fragariae*. The markers are identical to those in Table 2. Male parents are in bold.

| Genotype | Country of origin* | Parental plants | OPO-16C (susc)* | OPC-8D (resist)* | OPO-8A (resist) | SCAR-R1A | Rpf1 (resist)* |
|----------|-------------------|-----------------|----------------|----------------|----------------|-----------|---------------|
| 52 AC 18 | UK | Unknown x Unknown | – | – | – | – | – a |
| 53 Q 13 | UK | Auchincruive 11 x *Fragaria virginiana*-1 | + | + | – | – | – c |
| Aberdeen | UK | Unknown x Unknown | + | – | – | – | – a |
| Avanta | NL | Induka x Sivetta x Karina x Precoce di Romagna | + | – | – | – | d,g |
| Blakemore | USA | Missionary x Howard 17 | + | – | – | – | – a |
| Bogota | NL | Zb.53-11 x Tago | + | – | – | – | – e |
| Brightton | USA | Tufts x Cal 65.65-601 | + | – | – | – | – h |
| Cal 42.8-16 | USA | Sierra x (Blakemore x Nich Ohmer) x (Royal Sovereign x Howard 17) x (Royal Sovereign x Howard 17) | – | – | – | – | – h |
| Cambridge | UK | Etterburgseedling x Avant Tout x Blakemore | + | – | – | – | i |
| Cambridge | UK | US 3378 (Aberdeen x Fairfax) x Early Cambridge | – | + | – | + | – a |
| Cavalier | CAN | Valentine (Howard 17 x Vanguard) x Sparkle | + | – | – | – | g,j |
| Chandler | USA | Douglas x Cal 72.361-105 | – | – | – | – | e |
| Climax | UK | TD-8 (CC-6 O.P) Frith O.P) x Aberdeen | + | – | – | – | a |
| Columbia | USA | WSU 157 x WSU 175 | + | – | – | – | b |
| CPRO 87018 | NL | Elsanta x Cambridge Favourite x (Sivetta x Precoce di Romagna) | + | – | – | – | e |
| CPRO 89028 | NL | (Tamella x Redgauntlet) x Md2700 x Allstar | + | + | – | – | e |
| CPRO 90013 | NL | Rapella x Cambridge Favourite x Elsanta | + | – | – | – | d,e,g,i |
| CPRO 90017 | NL | Rapella x Cambridge Favourite x Gelria | + | + | – | – | d,g,i |
| Del Norte | USA | F. chiloensis (random selection) x F. chiloensis (random selection) | – | – | – | – | – |
| Douglas | USA | Tufts x Cal 64.57-108 | – | – | – | – | a |
| Elvira | NL | Gorella x Vola | + | – | – | – | e |
| Fairfax | USA | Ettersburg 450 x Howard 17 | + | – | – | – | j |
| Florida Belle | USA | Sequoia x Earlibelle | + | – | – | – | b |
| Gorella | NL | Juspa x MDUS 3763 (Suwannee x Midland) | – | – | – | – | e |
| Grenadier | CAN | Valentine x Fairfax | + | – | – | – | g |
| Holiday | USA | Raritan x NY-844 (Redglow x Tennessee Shipper) x Redglow | + | – | – | – | a/g |
| Howard 17 | USA | Crescent x Howard 1 | + | – | – | – | j |
| Jerseybelle | USA | NJ 953 (Lupton x Aberdeen) x Fairfax x NJ 925 (Pathfinder x Fairfax) | + | – | – | – | l |
| Jucunda | ? | Unknown x Unknown | – | – | – | – | h |
| Karola | NL | Gorella x Midway x Karina | + | – | – | – | e |
| Kent | CAN | Frogmore Late Pine x Raritan | + | – | – | – | a |
| Lambada | NL | Sivetta x Holiday x Karina x Primella | + | – | – | – | e |
| Lupton | USA | Joe x Gandy | – | – | – | – | h |
| Lassen | USA | Blakemore x (Marshall x Fendalino) x Nich Ohmer x (Royal Sovereign x Howard 17) x (Marshall x Fendalino) | – | – | – | – | h |
| Macherauch’s Frühernte | DL | Geneva x Deutsch Even | + | – | – | – | h |
| Marmolada | IT | Gorella x Unknown | – | – | – | – | e |
| Marshall | USA | UCM 3585 | Un known x Unknown | + | – | – | – | h |
| Micmac | CAN | Tioga x K61-87 (Guardians S f) | + | – | – | – | a |
| Midland | USA | Howard 17 x Redheart | – | – | – | – | j |
| Midway | USA | Dixieland x Temple | + | – | – | – | f,m |
| Mrak | USA | Cal 69.141-101 (Hecker) x Aiko | + | – | – | – | e |
| Redcoat | CAN | Sparkle x Valentine (Howard 17 x Vanguard) | + | – | – | – | a,g |
was cloned and sequenced (Fig. 1). Using this sequence, a pair of SCAR-S primers was chosen (Table 1) that should have amplified a region of \( \approx 404 \) bp in susceptible individuals. However, this primer set amplified DNA in the susceptible and resistant individuals producing singlet, doublet or triplet band patterns; the multiple bands may have been due to the lack of C/G at the 3′ end. The singlet band was \( \approx 392 \) bp, the doublet was comprised of a 392 bp and a 345 bp fragment, and the triplet was comprised of a 392 bp, 345 bp, and a 330 bp fragment (Fig. 2).

Twenty-four of the resistant genotypes of the test progeny had a doublet while only five had a triplet band. Twenty-four susceptible genotypes were characterized by a single band and seven plants had a doublet band similar to the resistant plants. The sequences of all bands were highly homologous to the OPO-16C sequence (Fig. 1). The sequence from the upper resistant marker band (U), which was present in all progeny, had 98.7% homology to the susceptible allele marker (OPO-16C) (Fig. 1). A 2 bp change in the sequence and one unmatched T were observed as the only

\[ \text{Plant material was obtained from CPRO-DLO strawberry collection and breeding program, The Netherlands for all selections from Germany (DL), Italy (IT), The Netherlands (NL), United Kingdom (UK), except as noted below, Canadian (CAN) selections of 'Kent' and 'Micmac', and the USA selections, 'Blakemore', Del Norte, 'Holiday', 'Mrak', 'Selva', Yaquina A and Yaquina B and 'Jucunda' of unknown country origin. The other selections from the USA and Canada (CAN) came from the USDA National Germplasm Repository, Corvallis, Ore., as did the UK selections 'Cambridge Favourite', 'Cambridge Vigour', and 'Royal Sovereign'.} \]

\[ \text{Abbreviations: susc = susceptible and resist = resistant.} \]

\[ \text{Refer to footnote of Table 2.} \]

![Figure 3](image-url)  
**Fig. 3.** A resistant (R) and susceptible (S) genotype as screened with the RAPD primers OPC-8, OPO-8, OPO-16C, and the SCAR-R1A marker. Arrows indicate the polymorphic markers identified previously as being linked to the \( Rpf1 \) gene. OPO-16C marker is linked in repulsion phase to the \( Rpf1 \) gene. The molecular weight (in bp) is indicated by M.

A linkage map is included to indicate the already published RAPD markers (Haymes et al., 1997) and the SCAR-R1 markers distance from the gene in cM.
differences. The middle fragment (M), present in all resistant progeny and in only a few susceptible progeny, had 95% homology when compared to the OPO-16C sequence (Fig. 1). This fragment contained eight unmatched bases of which seven were part of a deletion region and the extra T (position 212). Besides the eight unmatched bp, a total of 7-bp changes between the OPO-16C and middle fragment sequences were observed (Fig. 1). In a comparison of the upper resistant (U) sequence to the middle resistant (M) sequence, the previously observed 7-bp deletion region (position 285-291) and a 5-bp change were observed. The lowest of the three bands (L), present in only some of the resistant progeny and none of the susceptible, contained a 35 bp deletion, plus the same extra T (position 212) (Fig. 1).

Construction of SCAR primers to the resistant allele of Rpf1. The 7 bp deletion region in the middle band (M) was used for the creation of specific primers linked to the resistant allele. Two SCAR-R1 primer sets were designed to amplify DNA from the resistant plants only. For the SCAR-R1A forward primer, we used the original forward SCAR-S primer. The reverse primer exploited sequence differences at the 3′ end related to the 7 bp deletion region (Table 1). For SCAR-R1A, the expected band size was 285 bp (Table 1 and Fig. 2).

The SCAR-R1A forward primer was based on the deletion region and a 2 bp change in the nucleotide sequence at the 3′ end of the middle fragment (Table 1). The reverse primer for this SCAR started 24 bp upstream from the original RAPD primer and the expected length of the amplified region was 133 bp (Fig. 2). These two SCAR-R1 primer sets were tested on the F1 mapping population of 60 plants and each amplified the expected fragment in resistant plants only, except in the two apparently recombinant plants. The SCAR markers were mapped to the same location as the RAPD OPO-16A/B/C markers at 3 cM from the Rpf1 gene (Fig. 3).

Strawberry genotypes assessed with molecular markers. Genotypes possessing and lacking Rpf1, based on screening tests (Tables 2 and 3), were examined with the following molecular markers: RAPD OPO-8A, RAPD OPC-8D, SCAR-R1A, and SCAR-R1B. The RAPD OPO-8A marker correctly assessed 29 of the 34 resistant genotypes as well as all of the susceptible (Rpf1) individuals (Tables 2 and 3). The divergent resistant genotypes were ‘Perle de Prague’ and four CPRO selections: 88218, 88275, 88310, and 89027.

The SCAR-R1A marker correctly identified 23 of the 34 Rpf1 red stele genotypes (Table 2). Eight of the 11 divergent Rpf1 genotypes are interrelated and their loss of linkage can be explained by a single crossover event during meiosis of Md683. In the resulting cultivar, Stelemaster, Rpf1 is no longer linked to the SCAR but to the alternate allele OPO-16C. Consequently, the SCAR-R1A marker is also absent in descendents of ‘Stelemaster’, ‘Delite’, ‘Scott’, ‘Sunrise’, ‘Tribute’, CPRO 88218, CPRO 88310, and CPRO 88312 while the OPO-16C marker is present in these genotypes. The three other SCAR-R1A divergent genotypes were ‘Benton’, CPRO 77191, and ‘Perle de Prague’. SCAR-R1A produced results identical to those of SCAR-R1A (data not presented). In a screen of 53 rpf1 susceptible genotypes, ‘Cambridge Vigour’ was the only genotype possessing the SCAR-R1A marker (Table 3).

RAPD OPC-8D, the farthest marker from Rpf1, had a total of 16 nonconforming genotypes of the 87 tested. Eleven Rpf1 genotypes did not carry the marker (‘Annapolis’, ‘Arking’, ‘Delite’, ‘Linn’, ‘Tribute’, ‘Perle de Prague’, ‘Yalova-4’, ‘Yalova-15’, CPRO 88239, CPRO 88246, and CPRO 88275) (Table 2). A single crossover event in one of the parental genotypes (‘Cengelköy’) or an earlier ancestor could account for the loss of the marker in the latter five. The absence of the marker in ‘Arking’ may be explained by its absence in the resistant parent ‘Delite’. Out of the 53 susceptible genotypes, five contained the OPC-8D marker, which is explained by crossover events (Table 3).

Additional genotypes. Another six genotypes, whose resistance was unknown, were tested for the presence or absence of Rpf1 markers (Table 4). Based upon the molecular data, five of these genotypes were classified as susceptible while the sixth, ‘Olympus’, could not be determined since it is a recombinant for OPO-8A and SCAR-R1A. If the crossover occurred between Rpf1 and SCAR-R1A, then ‘Olympus’ should possess the gene; however, if the crossover occurred between Rpf1 and OPO-8A then most likely the gene was lost.

An additional 19 cultivars and 21 CPRO selections, whose parents lacked Rpf1 as well as the markers, were assessed with OPO-8A and SCAR-R1A (Table 5). As expected, all lacked the markers.

Discussion

Genetic markers represent a useful tool for plant breeding since the presence of genes can be detected at an early stage of plant development without waiting for the phenotypic expression of the gene in the plants. To overcome the disadvantages associ-
ated with RAPD markers, such as irreproducibility among laboratories, RAPD markers have been converted into highly specific SCAR markers (Paran and Michelmore, 1993). Such SCAR markers have proven useful for fingerprinting, marker-assisted selection, and high-resolution mapping (Kaplan et al., 1996; Paran and Michelmore, 1993; Xu et al., 1995).

In this study, a SCAR marker was constructed for the strawberry gene Rpf1, which represents one of the major sources of genetic resistance to *P. fragariae*. Initially a SCAR-S primer set was constructed based upon a RAPD marker (OPO-16C (438)) linked to the susceptibility allele. Previously, we reported that the OPO-08 markers have a relatively high molecular weight and are difficult to score due to a bright monomorphic band of 1700 bp (Haymes et al., 1997). The OPO-16C marker was chosen over OPO-16A (510) and OPO-16B (450) due to the intensity of the band compared with the other two markers (Haymes et al., 1997). The SCAR primers amplified DNA of different molecular weights in susceptible as well as resistant plants. The bands in the resistant plants allowed creation of two SCAR markers specific for the resistant Rpf1 allele (SCAR-R1 A and SCAR-R1 B), which cosegregated completely with the OPO-16C (438) in a test cross. This was expected based upon the sequence similarity of the resistant bands to the susceptible bands.

**Maintenance of the markers in strawberry selections.**

The linkages of the RAPD and SCAR markers to the Rpf1 gene were maintained in most of the genotypes examined. These genotypes originated from breeding programs in The Netherlands, United States, Canada, Scotland, and Turkey. The linkages

| Genotype     | Country of origin | Parental plants                                                                 |
|--------------|-------------------|---------------------------------------------------------------------------------|
| Blomidon     | CAN               | K72-4 [(Micmac x (Guardian x Tioga)] x Holiday                                  |
| Bounty⁷      | CAN               | Jerseybelle x Senga Sengana                                                     |
| Elsanta⁷     | NL                | Gorella x Holiday                                                              |
| Fairland⁶    | USA               | Aberdeen x Fairfax                                                             |
| Glooscap⁷    | CAN               | Micmac x Bounty                                                                |
| Governor Simcoe⁷ | NL            | Holiday x Guardian                                                            |
| Induka⁷      | NL                | Puget Beauty x Senga Sengana                                                    |
| Korona⁷      | NL                | Tamella x Induka                                                               |
| Polka⁷       | NL                | Induka x Sivetta                                                               |
| Primella⁷    | NL                | Gorella x Macherauch’s Frühernte                                                |
| Raritan⁷     | USA               | Redglow x Jerseybelle                                                          |
| Sivetta⁷     | NL                | Redgauntlet x Gorella                                                          |
| Sparkle⁷     | USA               | Fairfax x Aberdeen                                                            |
| Tago⁷        | NL                | Gorella x Talisman                                                             |
| Tamella⁷     | NL                | Talisman x Gorella                                                             |
| Temple⁷      | USA               | Aberdeen x Fairfax                                                             |
| Tenira⁴      | NL                | Redgauntlet x Gorella                                                          |
| Tioga        | USA               | Lassen x Cal 42.8-16                                                           |
| Valetay⁴     | NL                | Sivetta x Holiday                                                              |
| CPRO 87011   | NL                | Elsanta x Cambridge Favourite x Induka                                          |
| CPRO 87041¹  | NL                | Redchief x Sivetta x Korona                                                    |
| CPRO 88030⁴  | NL                | (Sivetta x Holiday) x Korona x Lambada                                          |
| CPRO 90074⁴  | NL                | (Induka x Sivetta) x Earliglow x (Sivetta x Holiday) x Korona                   |
| CPRO 91008⁴  | NL                | Elsanta x MDUS 3184                                                            |
| CPRO 91012⁴  | NL                | Elsanta x Chandler                                                             |
| CPRO 91020⁴  | NL                | Elsanta x Selva                                                                |
| CPRO 91023⁴  | NL                | Elsanta x Selva                                                                |
| CPRO 91033³  | NL                | Korona x MDUS 3184                                                             |
| CPRO 91046⁷  | NL                | Korona x Chandler                                                              |
| CPRO 91058⁴  | NL                | Korona x Selva                                                                |
| CPRO 91066⁴  | NL                | Korona x Selva                                                                |
| CPRO 91067⁴  | NL                | Korona x Selva                                                                |
| CPRO 91070⁴  | NL                | (Bogota x Sivetta) x Elsanta x MDUS 3184                                        |
| CPRO 91088   | NL                | (Bogota x Sivetta) x Elsanta x Chandler                                        |
| CPRO 91100⁴  | NL                | (Bogota x Sivetta) x Elsanta x Selva                                           |
| CPRO 91113³  | NL                | Holiday x Chandler                                                             |
| CPRO 92033   | NL                | Sivetta x Holiday                                                              |
| CPRO 92041⁴  | NL                | Elsanta x Polka                                                                |
| CPRO 92074   | NL                | Polka x Chandler                                                               |
| CPRO 92075   | NL                | Polka x Selva                                                                  |

⁴Plant material was obtained from CPRO-DLO strawberry collection and breeding program, The Netherlands for all NL selections and the USA selection of ‘Tioga’. The other selections from the USA and Canada (CAN) came from the USDA National Germplasm Repository, Corvallis, Ore. ⁵OPO-16C is present in these selections.
were maintained, irrespective of where these selections were bred. The markers should therefore be applicable for marker-assisted selection in breeding programs, phylogeny studies, and cultivar identification. For example, the data indicate that OPO-8A and the SCAR markers have been maintained in many crosses over succeeding generations. This is illustrated by the pedigree of the Rpf1 selection CPRO 90025 (Fig. 4) in which these markers were maintained through five generations.

The only cultivar in which the presumed presence of Rpf1 could not be established by any of the markers is ‘Perle de Prague’. The origin for this discrepancy could not be determined since its parentage is unknown. The absence of each of the molecular markers may indicate that it carries another gene that has not yet been determined in the strawberry-P. fragariae gene-for-gene model, and which is similar to Rpf1 in respect to its resistance to the relative races (A1-A4, A6-A10, and NS2-NS4) (Van de Weg, 1997) or the present markers may not yet be close enough to detect recombination events in the resistance gene reported.

**Marker-assisted selection.** The RAPD and SCAR markers assessed can be used for marker-assisted selection aimed at the efficient introgression of Rpf1 and the pyramiding of resistance genes into new cultivars. Red stele resistance tests are expensive, laborious, time consuming, affected by environmental factors, and suffer from incomplete resistance and epistatic effects among resistance genes (Van de Weg, 1997b). Additionally, in Europe, *P. fragariae* is a quarantine pathogen, therefore requiring special laboratory facilities. In contrast, RAPD and SCAR markers are relatively inexpensive, reliable, and are easily and quickly screened for, in that the marker is either present or absent, intermediate scores not being possible. The present markers may therefore encourage breeding for red stele resistance since their use is economically and technically more feasible than screening by plant/pathogen tests for the resistant phenotype.

Resistance is best determined by the use of flanking markers since this minimizes detection of false positives. In this respect, OPO-8A and the SCAR markers can be used due to their strong linkage to Rpf1 (Fig 3). In some genotypes, like ‘Stelemaster’ and its descendants, RAPD OPO-16C is linked to Rpf1 instead of the SCAR-R1A. OPO-8A and OPO-16C could then be used as the flanking markers. However, this is only applicable if OPO-16C is absent in the other parent.

Dominant markers such as these SCARs are generally not suitable to distinguish heterozygous from homozygous genotypes. However, the present markers do allow such discernment, since Rpf1 exhibits disomic segregation (Haynes et al., 1997), and since SCAR-R1A and OPO-16C are alternate alleles. In R x R progenies in which both parental genotypes possess SCAR-R1A as well as OPO-16C, progenies lacking OPO-16C should be homozygous for Rpf1. This approach has been followed in a population of 24 progeny of Md683 selfed, of which 7 are predicted to be homozygous resistant, 15 to be heterozygous, and two to be homozygous susceptible for Rpf1 according to the molecular data (data not presented). These numbers fit the expected 1:2:1 segregation ratio at the 95% level ($\chi^2 = 3.9$).

In pyramiding strategies that are considered for development of durable disease resistance, identification of molecular markers for each desired resistance gene is required. Therefore, efforts are underway to map three more Rpf genes. The results should allow application of efficient selection schemes for pyramiding red stele resistance genes in superior cultivars, provided they map to different chromosomal regions.

**Phylogeny.** The occurrence of Rpf1 in most cultivars of eastern North America and in some cultivars (‘Benton’, ‘Linn’, and ‘Hood’) from the west coast of the United States, Scotland, and The Netherlands, was not surprising since they all derived their resistance from the oldest known source of Rpf1, ‘Frith’, generally via Md683, a second generation descendent of ‘Frith’ (Reid, 1952; Scott et al., 1984). Interestingly, Rpf1 is also present in the Oregon cultivar ‘Siletz’ and the Turkish cultivars ‘Yalova-4’ and ‘Yalova-15’, based on the resistance tests and the molecular markers, although to our knowledge ‘Frith’ is not in their

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Fig. 4. Pedigree of CPRO 90025 genotype. Resistant cultivars for Rpf1 are in bold text while susceptible are in normal text. Genotypes that gave positive results in the test for the presence of RAPD OPO-8A and SCAR-R1 markers are indicated by a box whereas genotypes that lacked the markers are underlined. No box and no underline indicate that the individual was not tested.
ancestry. The probability of a similar resistance gene arising independently is highly unlikely, and therefore we believe that this $Rpf$ gene came from a much older common ancestral genotype and that the linkage of the markers was conserved through these earlier generations.

In case of two $Rpf$ resistant parents, the markers can sometimes clarify the parental origin of the gene. For instance, ‘Tribute’ and ‘Tristar’ may have received $Rpf$ from two resistant grand parents, ‘Surecrop’ and ‘Sunrise’ (Table 2). In ‘Surecrop’, $Rpf1$ is linked to the SCAR-RI A1 while in ‘Sunrise’ $Rpf1$ is linked to OPO-16C. Since ‘Tribute’ shows linkage to OPO-16C and not to OPO-16C. Since ‘Tribute’ shows linkage to OPO-16C and not $Rpf1$ grandparents, ‘Surecrop’ and ‘Sunrise’ (Table 2). In ‘Surecrop’, $Rpf1$ is linked to the SCAR-RI A1 while in ‘Sunrise’ $Rpf1$ is linked to OPO-16C. Since ‘Tribute’ shows linkage to OPO-16C and not the SCAR, the $Rpf$ allele should have come from ‘Sunrise’. In contrast, the SCAR-RI A1 marker present in ‘Tristar’ is due to an $Rpf$ allele received from ‘Surecrop’. The simultaneous presence of OPO-16C indicates that ‘Tristar’ also carries an $Rpf$ allele of ‘Sunrise’, thus being homozygous for $Rpf1$, or that one of its susceptible grandparents carries this marker.

**Cultivar Identification.** These markers have proven useful also for cultivar identification. Previously two ‘Aberdeen’ accessions were distinguished by Van de Weg et al. (1997a) based on their resistance to $P. fragariae$. The accession that is most likely to be true to type, namely that of the Scottish Crop Research Institute (SCRI), possesses $Rpf2$ and $Rpf3$, whereas it lacks $Rpf1$ (Van de Weg, 1997). The other accession came from North Carolina State University (NCSU) and had the same resistance as MD683. Our molecular tests confirmed these findings. The Scottish accession lacked the $Rpf1$ flanking markers, while the accession from North Carolina had them.

**Conclusions**

A reliable SCAR marker (SCAR- R1A) that is linked to the $Rpf1$ resistant allele was developed from the sequence of a RAPD marker (OPO-16C), which originally cosegregated with susceptibility in the testcross population. The molecular markers SCAR- R1A and RAPD OPO-8A can be used in marker-assisted selection for resistance to *Phytophthora fragariae* var. *fragariae* since they are highly conserved to the *Rpf1* gene.

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