Theor Appl Genet (1996) 92:1045–1051

© Springer-Verlag 1996

V. Chagué · J. C. Mercier · M. Guénard
A. de Courcel · F. Vedel

Identification and mapping on chromosome 9 of RAPD markers linked to *Sw-5* in tomato by bulked segregant analysis

Received: 3 July 1995 / Accepted: 8 October 1995

Abstract Bulked segregant analysis was used to identify random amplified polymorphic DNA (RAPD) markers linked to the *Sw-5* gene for resistance to tomato spotted wilt virus (TSWV) in tomato. Using two pools of phenotyped individuals from one segregating population, we identified four RAPD markers linked to the gene of interest. Two of these appeared tightly linked to *Sw-5*, whereas another, linked in repulsion phase, enabled the identification of heterozygous and susceptible plants. After linkage analysis of an F_2 population, the RAPD markers were shown to be linked to *Sw-5* within a distance of 10.5 cM. One of the RAPD markers close to *Sw-5* was used to develop a SCAR (sequence characterized amplified region) marker. Another RAPD marker was stabilized into a pseudo-SCAR marker by enhancing the specificity of its primer sequence without cloning and sequencing. RAPD markers were mapped to chromosome 9 on the RFLP tomato map developed by Tanksley et al. (1992). The analysis of 13 F_3 families and eight BC_2 populations segregating for resistance to TSWV confirmed the linkage of the RAPD markers found. These markers are presently being used in marker-assisted plant breeding.

Key words Tomato · RAPD markers · Bulked segregant analysis · TSWV resistance gene · Marker-assisted selection

Communicated by M. Koornneef

V. Chagué (✉) · F. Vedel
Institut de Biotechnologie des Plantes, Bâtiment 630, URA CNRS 1128, Université Paris-Sud, 91405 Orsay cedex, France

J. C. Mercier
Clause Semences, Station de Recherche de Saint Rémy, Le Mas St Pierre, 13210 St Rémy de Provence, France

M. Guénard · A. de Courcel
Clause Semences, 1 Avenue Lucien Clause, 91220 Brétigny sur Orge, France

Introduction

The cultivated tomato (*L esculentum*) is seriously affected by tomato spotted wilt virus (TSWV); in addition to a reduction in yield, fruits from plants affected by TSWV may develop chlorotic and necrotic ringspots, and are thus unmarketable. Virus attacks are increasing in all of the Mediterranean countries. Natural vectors of TSWV are thrips; in particular *Frankliniella occidentalis* which has recently been introduced in Europe. As they transmit the virus before insecticides incapacitate them, plant resistance appears to be the best way to control the disease.

One of the highest levels of resistance to TSWV has been found in *L. peruvianum* (for review, Stevens et al. 1992) and transferred to the fresh market cultivar 'Stevens' (Stevens 1964). This cultivar carries resistance gene *Sw-5*, considered to be incompletely dominant; i.e. heterozygous plants are less resistant than homozygous plants, and the resistance does not seem to be isolate-specific (Stevens et al. 1992). The introduction of this resistance into new cultivars is needed to satisfy growers. Phenotypic screening of resistance requires time, plants at the right stage, inoculum and restricted areas in which to control virus spread. The development of molecular markers linked to the resistance gene would facilitate its introgression, the breeding process should be accelerated and analyses would be easier as the presence of the gene could be detected without disease testing.

Stevens et al. (1995) recently mapped *Sw-5* between restriction fragment length polymorphism (RFLP) markers CT 71 and CT220 near a telomeric area of chromosome 9. They also identified a random amplified polymorphic DNA (RAPD) marker linked to *Sw-5* within an average of 0.5 cM with a pair of near-isogenic lines by incorporating [^{35}S]-dATP in polymerase chain reactions (PCR) and separating amplified products on denaturing acrylamide gels. However, they reported the presence of a band equivalent to their RAPD marker in many tomato lines, thereby complicating the use of the

band for marker-assisted selection. It would therefore be useful for introgression efficiency to increase the number of known markers surrounding *Sw-5*.

Random amplified polymorphic DNA (Williams et al. 1990; Welsh and McClelland 1990) analysis provides a good tool to identify molecular markers linked to disease resistance loci (Martin et al. 1991; Paran et al. 1991; Michelmore et al. 1991). In most cases, the identification of molecular markers linked to important traits or genes depends on the availability of near-isogenic lines (NILs) for the gene or the trait of interest; however, the development of these lines is costly, time-consuming, and often unnecessary in breeding programs. Michelmore et al. (1991), described an alternative method, called bulked segregant analysis (BSA), to screen efficiently for RAPD and RFLP markers linked to a specific region of the genome. This strategy has the advantage of reducing identification of markers unlinked to the target region, in contrast with NILs where the introgressed fragment that carries the gene can be larger. BSA has been successfully applied to target resistance genes in different crop species: in lettuce, *Dm 5* and *Dm 8* resistance genes to downy mildew (Michelmore et al. 1991); in onion, resistance to downy mildew (de Vries et al. 1992); in common bean, *Up2*, a resistance gene to *Uromyces phaseoli* (Miklas et al. 1993), and a resistance gene block against *U. phaseoli* (Haley et al. 1993); in tomato, *Ol-1*, a resistance gene to *Oidium lycopersicum* (van der Beek et al. 1994).

In tomato, the development of a saturated RFLP map (Tanksley et al. 1992) has facilitated the mapping of several disease resistance genes through the use of molecular markers. These genes have been located throughout the tomato genome on different chromosomes: chromosome 1 *Cf 9* (resistance to *Cladosporium fulvum*, van der Beek et al. 1992; Jones et al. 1993); chromosome 2, *Tm-1* (resistance to tobacco mosaic virus, Levesque et al. 1990); chromosome 3, *Asc* (resistance to *Alternaria alternata*, Witsenboer et al. 1989; van der Biezen et al. 1995); chromosome 5, *Pto* (resistance to *Pseudomonas syringae*, Martin et al. 1991); chromosome 6, *Mi* (resistance to root knot nematode, Messeguer et al. 1991), *Cf2* (resistance to *Cladosporium fulvum*, Jones et al. 1993; Dickinson et al. 1993), *Cf5* (resistance to *C. fulvum*, Dickinson et al. 1993), *Ty-1* (tolerance to tomato yellow leaf curl virus, Zamir et al. 1994), and *Ol-1* (resistance to *Oidium lycopersicum*, van der Beek et al. 1994); chromosome 7, *I-1* (resistance to *Fusarium oxysporum* race 1, Sarfatti et al. 1991); chromosome 9, *Tm-2a* (resistance to tobacco mosaic virus, Young et al. 1988), *Ve* (resistance to *Verticillium*, Zamir et al. 1993); chromosome 11, *I-2* (resistance to *F. oxysporum* race 2, Sarfatti et al. 1989 and *Sm* (resistance to *Stemphylium*, Behare et al. 1991).

We report here the identification of RAPD markers linked to *Sw-5* which confers resistance to tomato spotted wilt virus in tomato. Molecular markers were identified by BSA on an F_2 population segregating for *Sw-5*. One of the RAPD markers, R2, close to *Sw-5* was used to develop a SCAR (sequence characterized amplified region) marker. Another RAPD marker, S1, was stabilized into a pseudo-SCAR marker. RAPD markers were integrated to chromosome 9 on the RFLP tomato map developed by Tanksley et al. (1992).

Materials and methods

Plant material

RAPD analysis was performed on an F_2 population obtained from a cross between two *L. esculentum* varieties, 'Stevens' (resistant) and 'Trend' (susceptible). 'Stevens' carries the *Sw-5* gene on an introgressed *L. peruvianum* fragment. This F_2 population consisted of 100 individuals that segregated for *Sw-5*. Thirteen F_3 families (80 plants in total) were derived by selfing of the F_2 plants.

RFLP analysis was performed on an F_2 population consisting of 168 individuals that was obtained from a cross between 'Venise' (*L. esculentum* cultivar) and *L. hirsutum*. One hundred and thirty RFLP markers covering the entire genome, with an average of 1 marker each 9–10 cM, had previously been mapped in this population (unpublished results, markers from Tanksley et al. 1992).

Inoculation

Young tomato plants (1 month old, 3–4 leaf stage) were inoculated with TSWV isolate LYE 51 (from INRA Montfavet). Inoculum was prepared by homogenizing 10-day-old infected leaves in an ice-cold mortar and pestle with chilled inoculation buffer (30 m*M* Na_2HPO_4 buffer, pH 8, containing 0.2% Na-diethylthiocarbamate). To inoculate the virus, 75 mg/ml charcoal and 75 mg/ml carborundum were added to freshly prepared inoculum. After inoculation of the third leaf and rinsing with distilled water, plants were placed in a growth chamber (24°C, 14 h light). Infection was visually scored 3 weeks after inoculation (i.e. when the tomato plants were 6–7 weeks old), when the control plants (24 susceptible *L esculentum* 'Trèsor' plants treated in the same way as the test plants that were regularly spaced between the test F_2 plants to control inoculation homogeneity) showed viral symptoms: susceptible individuals showed chlorotic and necrotic spots with a general browning of the leaves.

DNA Extraction

For RAPD analysis, DNA was extracted from each plant of the segregating population and the parent cultivars, before inoculation, as described by Dellaporta et al. (1983), for small quantities of material. A leaf disc (approximately 40 mg fresh weight) was placed in an Eppendorf tube containing 750 µl EB buffer (100 m*M* Tris-HCl, 50 m*M* EDTA, 500 m*M* NaCl, 10 m*M* β-mercaptoethanol, pH 8) and 1.4% SDS, and crushed with a teflon pestle. After incubation at 65 °C for 10 min, 250 µl 5 *M* K-acetate was added, and the tube placed on ice for 20 min. After centrifugation at 4 °C for 20 min at 13 000 *g*, 600 µl of supernatant was removed and added to 300 µl isopropanol in a fresh sterile Eppendorf tube. After centrifugation at 4°C for 10 min at 13 000 *g*, the pellet was dried and redissolved with either 100 µl sterile distilled water or TE (10 m*M* Tris, 1 m*M* EDTA, pH 8). DNA was kept at −20 °C.

For RFLP analysis, DNA was extracted from desiccated leaves of each individual as described by Saghai-Maroof et al. (1984).

Bulk composition

Although *Sw-5* has been considered to be incompletely dominant (Stevens et al. 1992), we saw no differences in symptoms between homozygous and heterozygous resistance plants. Two bulks were prepared : a resistant bulk of DNA from 67 F_2 individuals (heterozygous and homozygous resistant plants) and a susceptible bulk

consisting of DNA from 33 F_2 individuals (homozygous susceptible plants).

RAPD analysis

Bulked genomic DNA was used as a template for PCR amplification. A single 10-mer oligonucleotide primer (Operon Technologies) was used in each PCR amplification. Approximately 20 ng of DNA (2–5 μl) was used as template in 25 μl containing 1 × buffer (10 m*M* Tris-HCl pH 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.1% Triton × 100, 0.2 mg/ml gelatin), 200 μ*M* of each dNTP (Promega) 0.2 μ*M* primer and 1 unit *Taq* polymerase (Appligene). The reaction volume was overlaid with 50 μl of mineral oil (Perkin Elmer). Amplification was performed in a Braun Bio-med Thermocycler 60 for 45 cycles. After initial denaturation for 3 min at 92 °C, each cycle consisted of 30 s at 92 °C, 60 s at 42 °C, and 30 s at 72 °C. The 45 cycles were followed by a 8-min final extension at 72 °C. Amplification products were resolved by electrophoresis in 1.4% agarose gel with TBE buffer (Sambrook et al. 1989) for 3 h at 150 V and revealed by ethidium bromide staining.

SCAR analysis

A band of amplified DNA corresponding to a RAPD marker was excised from a gel and eluted by microcentrifugation through a 1.2-μm nylon membrance (Pall Biodyne) presoaked in 50 m*M* Tris HCl pH 8, 0.1% SDS. DNA was reamplified and cloned as described by Adam-Blondon et al. (1994). Double-stranded sequencing (T7 Sequencing TM kit, Pharmacia) was performed according to the dideoxychain-termination method using the T3 and T7 primers. The sequence was used to design two specific oligonucleotides for each end of the marker, that contained the 10 original bases of the RAPD primer followed by the next 10 internal bases. Primers were synthesized by Appligene. The amplification procedure consisted of 30 cycles of 30 s at 94 °C and 1 min at 65 °C, after an initial denaturation for 1 min at 94 °C. The 30 cycles were followed by an 8-min final extension at 65 °C.

Pseudo SCAR

In order to improve the efficiency of the RAPD assay we developed a new approach to marker enhancement, which we called pseudo-SCAR, by adding a genome-specific base to the primer without sequencing of the marker. After the primer which generated a polymorphic marker had been identified a set of four primers, called primers-1, was synthesized by adding one of the four bases, A, T, G, C, to the original primer. Ten PCR reactions were made with different combinations of primers-1, and the pair of primers-1 generating the desired marker was then selected. From this pair, eight novel primers, called primers-2, were synthesized that consisted of the two primers-1 plus one of the four bases. Sixteen PCR reactions were performed, and only one of the primer-2 combinations produced the desired marker, which appeared as a darker band than found in classic RAPD assays. Amplifications were performed as described in RAPD analysis, except that the annealing temperature of the PCR reaction was increased to 44 °C with the selected primers-2.

RFLP analysis

Seven micrograms DNA per individual was digested with 35 units of either *Eco*RI, *Bam*HI or *Hin*dIII restriction enzymes according to the manufacturer's instructions (Boehringer). DNA fragments were separated on 0.8% (w/v) agarose gels in TAE buffer (Sambrook et al. 1989) and Southern-blotted on Hybond N membranes according to the manufacturer's instructions (Amersham). RFLP probes were random single and low-copy-number sequences provided by Dr. S. D. Tanksley. Cloned inserts were amplified by PCR (Bernatzky and Tanksley 1986) before radiolabeling and hybridization (Radprime DNA labeling system, Gibco BRL).

Linkage analysis

Marker order and map distances were estimated using MAPMAKER version 2.0 (Lander et al. 1987). RAPD markers were first ordered with a LOD score > 3. Recombination fractions were transformed by the Kosambi map function to estimate the map distance (Kosambi 1944).

Results

RAPD markers

Parents and bulks were screened with 382 random primers. Approximately 80% of these allowed DNA amplification in both parents and bulks. Screening revealed that 117 primers gave polymorphic DNA fragments between the parents, 6 of which generated polymorphic DNA fragments that were observed in the two parents and the two bulks (Table 1). Four of these primers, r1, r2, r3 and r4, generated markers R1, R2, R3, and R4 (2000, 400, 1200 and 1800 bp in size, respectively) linked to the resistance gene: these markers are present in 'Stevens', the resistant parent, and in the resistant bulk. Amplification patterns of these primers are shown in Fig. 1. The two other primers, s1 and s2, generated markers S1 and S2 (1700 and 2000 bp, respectively) linked to the susceptible allele, in repulsion phase: they are present in 'Trend', the susceptible parent, and in the susceptible bulk. Amplification patterns are shown in Fig. 2.

Use of RAPD markers

In order to confirm the genetic association of polymorphic DNA fragments with TSWV resistance, several progenies were tested for segregation to TSWV resistance with the RAPD markers found. Thirteen F_3 families (80 plants in total) were tested with RAPD markers R1, R2, R3 and S1. We observed that R1 and R2 segregated together, while R3 was often undetectable. Marker S1 was used to detect the heterozygotes among the resistant plants and to confirm the susceptibility of plants which showed no markers in coupling phase. On the basis of these tests, some plants were chosen, and their F_4 progeny was submitted to a pathological test for TSWV resistance. Genetic analysis was confirmed by

Table 1 Sequence of 10-mer oligonucleotide primers for each RAPD marker linked to the *Sw-5* gene

Locus	Primer	Sequence
R1	r1	CTTCCCCAAG
R2	r2	CTGGGTGAGT
R3	r3	GGTGCACGTT
R4	r4	GAACGGACTC
S1	s1	CCACAGCAGT
S2	s2	TGCTGCAGGT

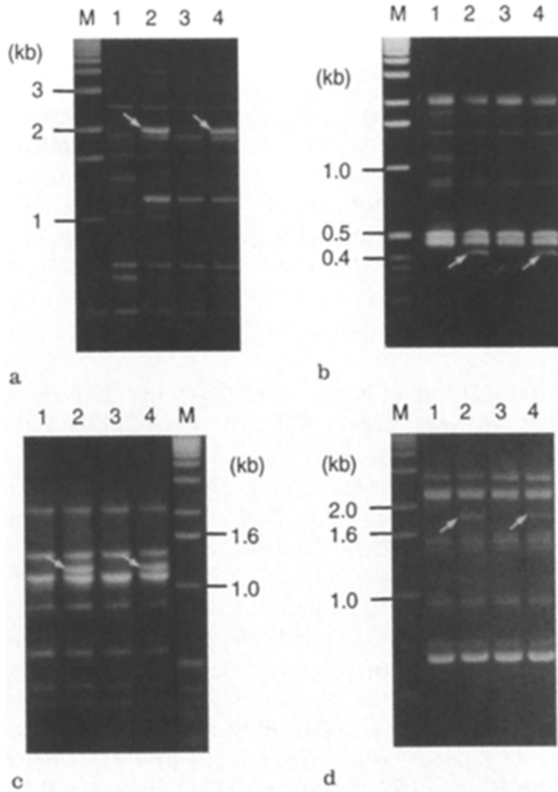


Fig. 1a–d Amplification patterns using RAPD primers to identify the R1 (**a**), R2 (**b**), R3 (**c**) and R4 (**d**) markers (*arrows*) linked to the TSWV resistance gene. *Lane M* 1-kb molecular-weight ladder, *1* susceptible parent 'Trend', *2* resistant parent 'Stevens', *3*: susceptible F_2 bulk, *4* resistant F_2 bulk

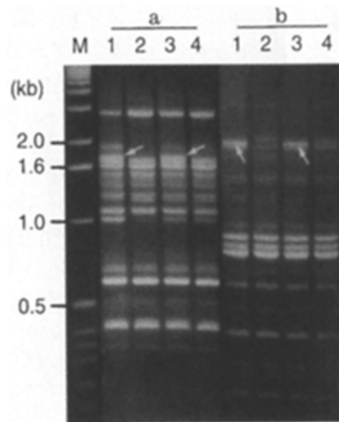


Fig. 2a, b Amplification pattern using RAPD primers to identify the S1 (**a**) and S2 (**b**) markers linked to the TSWV susceptible allele (S1 and S2 are indicated by *arrows*). *Lane M* 1-kb molecular-weight ladder, *1* susceptible parent, *2* resistant parent, *3* susceptible F_2 bulk, *4* resistant F_2 bulk

disease test results: R1 and R2 markers appeared to be in coupling phase and to determine TSWV resistance, whereas S1 was in repulsion phase. Eight BC_2 families (42 plants in total) were also screened with the four RAPD markers, and their analyses confirmed the results obtained earlier, i.e. a tight linkage between R1, R2 and TSWV resistance.

Marker improvement

R2 was used to produce a SCAR marker. The polymorphic band R2 was cloned and its extremities sequenced to generate a pair of 20-mer oligonucleotide primers (Table 2). When used as a probe on the RAPD profile, this cloned product revealed a 400-bp fragment present in the resistant parent and individuals only that corresponded to RAPD marker R2. Amplification with SCr2-a and SCr2-b primers resulted in a single band that was the same size as the progenitor fragment present in resistant parent 'Stevens' and in resistant bulk and resistant individuals (Fig. 3). Both RAPD and SCAR analyses of F_2 and BC progenies led to identical conclusions: observed polymorphisms detected with these techniques correspond to the same locus.

The stability of S1 was particularly sensitive to small variations in $MgCl_2$ concentration and to the quality of the primer solution, *Taq* polymerase and DNA samples. Minor variations in these components could especially occur after several freeze-thaw cycles. While the enhancement of marker S1 appeared a good way of overcoming these problems, we developed another alternative, which we called pseudo-SCAR. This type of marker enhancement is particularly interesting, especially with PCR products which appear difficult to clone, such as S1. When two specific bases were added to primer s1 (Table 3), marker S1 appeared as a stronger band than

Table 2 Sequence of the two 20-mer oligonucleotide primers derived from marker R2

Locus	Primer	Sequence[a]
SC-R2	SCr2-a	CTGGGTGAGTCTTGACATTT
	SCr2-b	CTGGGTGAGTACATCAGATT

[a] The underlined sequence represents the initial RAPD primer

Fig. 3a, b Parents, bulks and representative individuals of the F_2 progeny (*R* for resistant and *S* for susceptible) were scored for the RAPD marker R2 (**a**) and for the corresponding SCAR marker SC-R2 (**b**). The RAPD and SCAR markers are indicated by *arrows*. *Lane M* 1-kb molecular-weight ladder, *1* susceptible parent, *2* resistant parent, *3* susceptible F_2 bulk, *4* resistant F_2 bulk

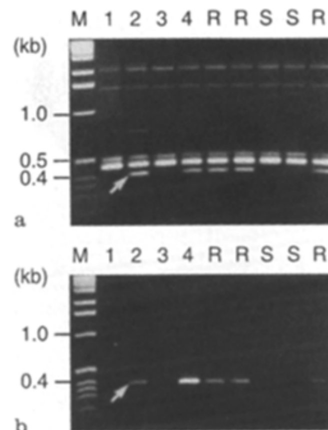


Table 3 Sequence of the two 12-mer oligonucleotide primers-2 derived from marker S1

Locus	Primer	Sequence[a]
pS-S1	pSs1-a	CCACAGCAGTCC
	pSs1-b	CCACAGCAGTCA

[a]The underlined sequence represents the initial RAPD primer

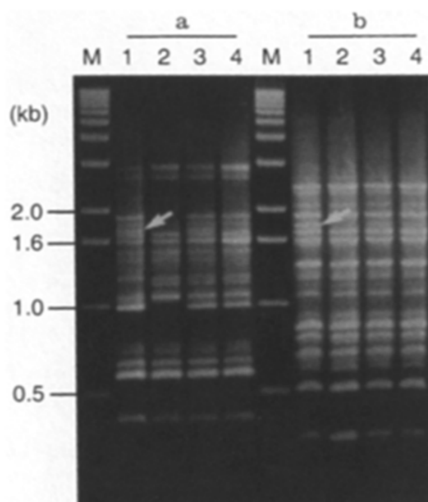
what was usually found in a RAPD assay, enhanced by an annealing temperature of 44 °C (Fig. 4). Pseudo-SCAR patterns showed new bands which could seem unexpected because of the extra bases and the higher annealing temperature. This has been observed in other instances (unpublished results) where a single extra nucleotide in 3' thoroughly changes the RAPD pattern even when the marker band is conserved. When used as a probe on the pseudo-SCAR pS-S1 profile, the S1 marker revealed a 1700-bp fragment in the susceptible parent, bulk and individuals, and in the heterozygous resistant individuals, as was expected based on the pS-S1 data. Data obtained with RAPD and pseudo-SCAR analysis led to identical conclusions, and both polymorphisms were linked to the same locus.

Linkage analysis

The six primers (r1, r2, r3, r4, s1, s2) were used to amplify DNA from F_2 individuals ('Stevens' x 'Trend') to confirm linkage with *Sw-5*. Primers r1, r2, r3 and s1 generated the same markers as the parents and the bulks, but the patterns of primers r4 and s2 were not reproducible.

Linkage of the four RAPD markers to the *Sw-5* locus was analyzed in the 'Stevens' × 'Trend' F_2 population

Fig. 4a, b Comparison of amplification patterns using RAPD primer s1 (**a**) to those using pseudo-SCAR primers pSs1-a and pSs1-b (**b**). The RAPD pseudo-SCAR markers are indicated by *arrows*. *Lane M* 1-kb molecular-weight ladder, *1* susceptible parent, *2* resistant parent, *3* susceptible F_2 bulk, *4* resistant F_2 bulk

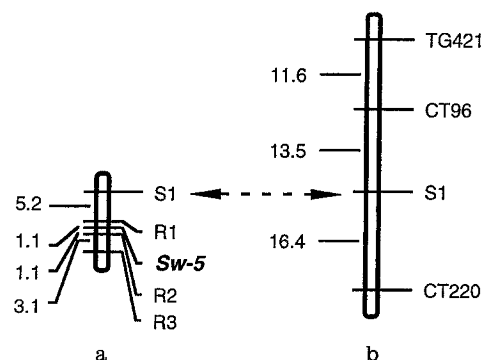


(Fig. 5). All markers were mapped to the same linkage group near *Sw-5*, within a 10.5 cM fragment. Markers R1 and R2 were located on each side of *Sw-5* and were the most tightly linked. In order to include these RAPD markers in a genetic map of tomato, segregations of RAPD and RFLP markers were analyzed in the 'Venise' × *L. hirsutum* F_2 population. R3 and S1 were the only *Sw-5* markers detected in the population. It is likely that R1 and R2 are specific to the *L. peruvianum* genome from which *Sw-5* was introgressed into 'Stevens'. R3 showed strong segregation distorsion and was not used for mapping. The segregation data suggested that S1 is situated on chromosome 9 between CT96 (LOD value for CT96-S1 was 8.8) and CT220 (LOD value for CT220-S1 was 8.76) within a distance of 13.5 and 16.5 cM, respectively.

Discussion

Our goal was to find RAPD markers linked to the *Sw-5* gene in order to use them in marker-assisted breeding programs. BSA allowed us to rapidly find markers linked to *Sw-5*. Screening with 382 random primers showed that only three of the RAPD markers generated were linked to the resistant locus and only one was linked to the susceptible allele. Thus, BSA allowed us to directly target the gene, as demonstrated by Michelmore et al. (1991). If two of the three markers, R1 and R2, are tightly linked to the gene and can be used to determine the resistance of plants to TSWV, the third, R3, may be useful to eliminate part of the linkage drag. The high recombination frequency of R3 allowed us to detect breakage of this introgressed fragment and confirmed the better linkage of markers R1 and R2 with *Sw-5*. Marker S1 is useful for distinguishing homozygous resistant plants from heterozygous plants or for confirming the absence of resistance markers. However, stability of the S1 marker can be altered by the presence of polymorphisms within *L. esculentum* cultivars,

Fig. 5a, b The *Sw-5* linkage group and chromosome 9 integrated map. Map distances, on the *left*, are centiMorgans. They have been estimated using the Kosambi map function. A linkage group obtained by RAPD analysis of 'Trend' × 'Stevens' F_2 progeny. **B** chromosome 9 linkage group obtained by RFLP and RAPD analysis of 'Venise' × *L. hirsutum* F_2 progeny



particularly since there is no selection pressure for this trait.

The pooling of large numbers of individuals increases the probability that the two pools will not differ for alleles other than those adjacent to the target trait. Giovanni et al. (1991) suggested that increasing the pool size could also reduce the effect of false positives due to non-specific annealing. In this paper we chose a large population since our previous experience (unpublished results) showed it minimized «one-off» individual effects. Resistant individuals in the bulk were either homozygous or heterozygous for *Sw-5*: the expected proportion of homozygous individuals was 33% of the bulk. Increasing the size of the pool could also help match the observed and expected number of homo- zygous resistant individuals. Heterozygous plants present in the resistant bulk did not interfere with the search for markers linked to the gene. This bulk was approximately composed of 33% susceptible allele and 66% resistant allele and, as demonstrated by Michelmore et al. (1991), it proved sufficient to detect most of the markers in the bulks. The S1 marker (Fig. 2) could be detected in the resistant bulk as a minor band. It is possible that other susceptible markers were not taken into account if they were very visible in the resistant bulk. A third bulk made of homozygous resistant plants from the same origin would be useful for detecting markers linked in repulsion phase. In addition, comparison between *L. peruvianum* and 'Stevens' showed that the three RAPD resistance markers are *L. peruvianum*-specific and located on the introgressed *L. peruvianum* fragment in 'Stevens'.

It has been shown that RAPD markers can vary according to experimental conditions – $MgCl_2$ concentration (Wolf et al. 1993), presence of glycerol in the reaction buffer (Hai Lu and Nègre 1993), *Taq* polymerase quantity and quality (Schierwater and Ender 1993) and type of thermal cycler (Wolf et al. 1993). These problems can be partly solved by converting RAPD markers into SCARs (sequence characterized amplified regions; Paran and Michelmore 1993). Pseudo-SCARs could be an alternative when marker enhancement is necessary but cloning appears difficult. These techniques were applied to R2 and S1, thereby generating markers SC-R2 and pS-S1 with improved experimental stability and reproducibility. Primer specificity improvement by addition of two bases proved sufficient to overcome S1 instability, although SCARs may be more effective in general.

These markers are now being used in breeding programs for *Sw-5*. They were successfully transferred from the research laboratory to the breeding laboratory, despite the fact different thermocyclers were used (Perkin Elmer and MJ Research PTC-100), thus allowing a reduction in the number of pathological tests. In addition, identification of resistant plants will take less time: DNA extraction (with the method described by Edwards et al. 1991) and RAPD analysis can be performed within a day, without the complications and risks of pathological tests.

Integration of *Sw-5* markers to the tomato RFLP map (Tanksley et al. 1992) mapped S1 at the end of chromosome 9 between CT96 and CT220. Our map distances differ from the map established by Tanksley et al. (1992). These authors reported a map distance of 6.5 cM between CT96 and CT220, while we found a distance of 29.9 cM. Stevens et al. (1995) also reported a discrepancy for their map distances obtained with cross SA (*L. esculentum* carrying a *L. peruvianum* introgressed fragment) × *L. pennellii* LA 716. As they suggested, mapping based on different species from the original cross mapped by Tanksley et al. (1992) (i.e. *L. esculentum* cv 'VF-36' *Tm2a* × *L. pennellii* LA 716) could alter crossover frequencies. The use of *L. hirsutum* in our cross could explain some variation in crossover frequencies, especially in regions where crossover events showed unequal recombination rates. Our segregation data agree with Stevens et al. (1995) who described this telomeric area as being "highly active for crossover events".

In addition, it was assumed that the level of polymorphism detected with RAPDs is higher than with RFLPs, which could be limiting when targeting a particular region of the genome (Williams et al. 1990; Welsh and McClelland 1990). RAPD markers map on the whole genome, regardless of the presence of restriction sites and number of gene copy. The four RAPD markers found could help fill the gaps in this region, and our information could be useful to adding to the knowledge of this telomeric area. In addition, it is necessary to saturate a region before chromosome walking, and SC-R2 could be a starting point in the molecular characterization of *Sw-5* locus to help understand the molecular mechanisms of virus and, more generally, pathogen resistance in plants.

Acknowledgements We are grateful to Chantal Mathieu, Philippe Boudec (Rhône-Poulenc Agrochimie) and Anne Françoise Adam-Blondon, respectively, for their help in the sequencing, RFLP and Mapmaker analyses. We also wish to thank Roland Boyer for the photographs.

References

Adam-Blondon AF, Sévignac M, Bannerot H, Dron M (1994) SCAR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean. Theor Appl Genet 88:865–870

Beek JG van der, Verkerk R, Zabel P, Linhout P (1992) Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: *Cf-9* (resistance to *Cladosporium fulvum*) on chromosome 1. Theor Appl Genet 84:106–112

Beek JG van der, Pet F, Lindhout P (1994) Resistance to powdery mildew (*Oidium lycopersicum) in Lycopersicon hirsutum* is controlled by an incompletely dominant gene *Ol-1* on chromosome 6. Theor Appl Genet 89:467–473

Behare J, Laterrot H, Safartii M, Zamir D (1991) RFLP mapping of the Stemphylium resistance gene in tomato. Mol Plant Microbe Interact 4:489–492

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Biezen EA van der, Glagotskaya T, Overduin B, Nijkamp HJH, Hille J (1995) Inheritance and genetic mapping of resistance to *Alternaria alternata* f. sp. *lycopersici* in *Lycopersicon pennellii*. Mol Gen Genet 247:453–461

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

Dickinson MJ, Jones DA, Jones JD (1993) Close linkage between the *Cf-2*/*Cf-5* and *Mi* resistance loci in tomato. Mol Plant Microbe Interact 6: 341–347

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res 19:1349

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

Hai Lu Y, Négre S (1993) Use of glycerol for enhanced efficiency and specificity of PCR amplification. Trends Genet 9:297

Haley SD, Miklas PN, Stavely JR, Byrum J, Kelly JD (1993) Identification of RAPD markers linked to a major rust resistance gene block in common bean. Theor Appl Genet 86:505–512

Jones DA, Dickinson MJ, Balint-Kurti PJ, Dixon MS (1993) Two complex resistance loci revealed in tomato by classical and RFLP mapping of the *Cf-2*, *Cf-4*, *Cf-5* and *Cf9* genes for resistance to *Cladosporium fulvum*. Mol Plant Microbe Interact 6:348–355

Kosambi DD (1994) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural population. Genomics 1:174–181

Levesque H, Vedel F, Mathieu C, de Courcel AGL (1990) Identification of a short DNA spacer sequence highly specific to a tomato line containing the *Tm-1* gene introgressed from *Lycopersicon hirsutum*. Theor Appl Genet 80:602–608

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

Messeguer R, Ganal MW, de Vicente MC, Young ND, Bolkan H, Tanksley SD (1991) High resolution RFLP map around the root knot nematode resistance gene (*Mi*) in tomato. Theor Appl Genet 82:529–536

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

Miklas PN, Stavely JR, Kelly JD (1993) Identification and potential use of molecular marker for rust resistance in common bean. Theor Appl Genet 85:745–749

Paran I, Kesseli R, Michelmore R (1991) Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome 34:1021–1027

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

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Sambrook L, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Sarfatti M, Katan J, Fluhr R, Zamir D (1989) An RFLP marker in tomato linked to the *Fusarium oxysporum* resistance gene *I-2*. Theor Appl Genet 78:755–759

Sarfatti M, Abu-Abied M, Katan J, Zamir D (1991) RFLP mapping of *I1*, a new locus in tomato conferring resistance against *Fusarium oxysporum* f. sp. *lycopersici* race 1. Theor Appl Genet 82:22–26

Schierwater B, Ender A (1993) Different thermostable DNA polymerases may amplify different RAPD products. Nucleic Acids Res 21:4647–4648

Stevens JM (1964) Tomato breeding. Project report W-Vv1, Dept Agric Tech Serv.

Stevens MR, Scott SJ, Gergerich RC, (1992) Inheritance of a gene for resistance to tomato spotted wilt virus (TSWV) from *Lycopersicon peruvianum* Mill. Euphytica 59:9–17

Stevens MR, Lamb EM, Rhoad DD (1995) Mapping the *Sw-5* locus for tomato spotted wilt virus resistance in tomatoes using RAPD and RFLP analyses. Theor Appl Genet 90:451–456

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovanni JJ, Grandillo S, Martin GB, Messeguer R, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132: 1141–1160

Vries J, de Jongerius R, Sandbrink H, Lindhout P (1992) RAPD markers assist in resistance breeding. Prophyta 2:50–51

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primer. Nucleic Acids Res 24:7213–7218

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic makers. Nucleic Acids Res 18:6531–6535

Witsenboer HMA, van de Griend EG, Tiersma JB, Nijkamp HJJ, Hille J (1989) Tomato resistance to *Alternaria* stem canker: localisation in host genotypes and functionnal expression compared to non-host resistance. Theor Appl Genet 78:457–462

Wolf K, Schoen ED, Peters-Van Rijn J (1993) Optimizing the generation of random amplified polymorphic DNAs in chrysanthemum. Theor Appl Genet 86:1033–1037

Young ND, Zamir D, Ganal MW, Tanksley SD (1988) Use of isogenic lines and simulataneous probing to identify DNA markers tightly linked to the *Tm-2a* gene in tomato. Genetics 120:579–585

Zamir D, Bolkan H, Juvik JA, Watterson JC, Tanksley SD (1993) New evidence for placement of *Ve* the gene for resistance to Verticillium race 1. Tomato Genet Coop Rep 43: 51–52

Zamir D, Ekstein-Michelson J, Zakay Y, Navot N, Zeidan M, Safartii M, Eshed Y, Harez E, Pleban T, van Oss H, Kedar N, Rabinowitch HD, Czosnek H (1994) Mapping and introgression of a yellow leaf curl virus tolerance gene, *Ty-1*. Theor Appl Genet 88:141–146