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Mapping the *Sw-5* locus for tomato spotted wilt virus resistance in tomatoes using RAPD and RFLP analyses

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Abstract The *Sw-5* locus confers dominant resistance to tomato spotted wilt virus (TSWV). To map the location and facilitate the identification of markers linked to *Sw-5* we developed a pair of near-isogenic lines (NILs) and an F_2 *Lycopersicon esculentum* × *L. pennellii* population segregating for resistance to TSWV. DNA from the NILs was analyzed using 748 random 10-mer oligonucleotides to discern linked molecular markers using a random amplified polymorphic DNA (RAPD) approach. One random primer (GAGCACGGGA) was found to produce a RAPD band of about 2200 bp that demonstrates linkage to *Sw-5*. Data from co-segregation of resistance and restriction fragment length polymorphisms (RFLPs) in a F_2 interspecific population position *Sw-5* between the markers CT71 and CT220 near the telomere of the long arm of chromosome 9.

Key words *Lycopersicon esculentum* · *Lycopersicon peruvianum* · RAPD · RFLP · Tomato spotted wilt virus (TSWV)

Introduction

Tospoviruses similar or identical to tomato spotted wilt virus (TSWV) are recognized as infecting over 550 monocot and dicot species worldwide (Wijkamp and Peters 1993). Severe pandemics caused by TSWV have been reported in many economically important crops (Miller 1989; O'Malley and Hartmann 1989; Ullman et al. 1993).

TSWV infections in the cultivated tomato (*Lycopersicon esculentum* Mill.) can severely reduce fruit yield and quality. In addition to plant stunting and yield reduction, the fruit is blemished by necrotic or chlorotic ringspots that usually appear after the development of color, rendering infected fruit unmarketable. Although TSWV incidence is somewhat sporadic, losses as high as 38% have been observed in commercial tomato fields in southeastern Arkansas (Paterson et al. 1989).

The tomato cultivar Stevens has been reported to express TSWV resistance originating from *L. Peruvianum* (van Zijl et al. 1986). This cultivar contains a single dominant gene (*Sw-5*) that confers resistance to TSWV (Stevens et al. 1992; Boiteux et al. 1993).

The international, cross commodity, destructive nature of TSWV warrants detailed studies of the *Sw-5* resistance gene. Identifying the position of this gene within the tomato genome will facilitate cultivar development incorporating TSWV resistance. The map location of *Sw-5* would be the foundation for positional cloning of this gene using yeast artificial chromosomes (YACs) (Martin et al. 1993a, c). Cloning of *Sw-5* would allow characterization of the gene product(s) responsible for resistance and allow direct introduction of the gene into other hosts.

Tomato is considered to be a model crop for molecular studies and an ideal species for disease resistance gene localization (Rick 1988; Hille et al. 1989). Gene mapping in tomato has been dramatically facilitated by the development of a restriction fragment length polymorphism (RFLP) linkage map of over 1 200 DNA molecular markers spanning all 12 chromosomes (Tanksley, et al. 1992). Additional molecular markers linked to a trait can be generated using Random Amplified Polymorphic DNAs (RAPDs). Martin et al. (1991) used RAPDs on near-isogenic lines (NILs) to find molecular markers linked to the *Pto* gene; responsible for resistance to *Pseudomonas syringae* pv *tomato*.

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A number of resistance genes have been localized in tomato using molecular techniques. Molecular markers have been located approximately 0.4 centiMorgans (cM) from the tobacco mosaic virus resistance gene *Tm-2a* (Young et al. 1988; Young and Tanksley 1989), < 2 cM from the *Mi* gene responsible for nematode resistance (Messeguer et al. 1991), < 10 cM from the *Il* gene responsible for resistance to race 1 of *Fusarium oxysporum* f. sp. *lycopersici* (Sarfatti et al. 1991), and 0.4 cM from *Pto* (Martin et al. 1993c). Molecular markers were used to isolate a YAC containing the *Pto* gene, (Martin et al. 1993b). The cloned *Pto* gene was found to resemble a protein kinase gene (Martin et al. 1993a).

The objective of the present study was to use RAPDs and previously mapped molecular probes to identify molecular markers linked to the *Sw-5* gene.

Materials and methods

Origin, maintenance, inoculation and scoring of TSWV

Isolate maintenance, inoculation and scoring were done as previously described (Stevens et al. 1992). Virus isolate 85-9 was used to screen all tomato plants because of the severe disease symptoms it induces. This virus was originally isolated from infected tomatoes in southeastern Arkansas in 1985. The disease symptoms caused by 85-9 in tomato are similar to the "tip blight" symptoms described by Norris (1946). This isolate was serologically identified as a common isolate of TSWV (formerly TSWV-L; German et al. 1992) using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Agdia Inc. Mishawaka, Ind).

Genetic material

An inbred Arkansas TSWV susceptible line, 89S, was used to create a pair of *L. esculentum* NILs, 89S and 89R. The 89R line derives its *Sw-5* resistance allele from *L. peruvianum*; however, it has been thoroughly introgressed into *L. esculentum* (possibly by as many as 20 generations; SA is the source of *Sw-5*, Stevens et al. 1992). The resistant 89R line has undergone five backcrosses to the susceptible 89S parent followed by two selfing generations. TSWV-resistant plants from the first selfed generation were selected for self pollination. Progeny from 48 plants originating from each resistant parent plant were tested for resistance. Plants whose progeny did not segregate for resistance were considered homozygous for *Sw-5*. One second-generation population with complete resistance was the source for bulked DNAs in RAPD analysis.

Isozyme and RFLP marker analyses were performed on 53 plants from a segregating F_2 population from an interspecific cross of SA to *L. pennellii* (LA 716). This interspecific cross [*L. esculentum* × *L. pennellii* (LA 716)] is noted for the generation of polymorphisms (Tanksley et al. 1992) which are generally sparse in *L. esculentum* × *L. esculentum* crosses (Miller and Tanksley 1990; van der Beek et al. 1992). The interspecific (SA × *L. pennellii*) F_2 plants were rigorously evaluated for TSWV resistance. Five cuttings from each plant were inoculated with TSWV isolate 85-9 and evaluated by ELISA for detectable TSWV in plant extracts (Stevens et al. 1992) after 1 month. One cutting was also evaluated 4 months post-inoculation. As a result of this evaluation protocol, 31 plants could confidently be defined as resistant (TSWV not detectable) or susceptible (TSWV detected and disease symptoms). All 31 plants (17 resistant, 14 susceptible) were examined in the RFLP and isozyme analyses.

Additional F_2 populations were developed that segregated known single recessive genes in addition to *Sw-5*.

Isozyme analyses

The preparation of proteins for electrophoresis was according to Suurs et al. (1989) with minor modifications. Samples were electrophoresed in a discontinuous (Laemmli 1970) 7 × 8-cm, 7.5% polyacrylamide gel at a constant 150 V for approximately 1.5 h and then immediately tested for the specific isozyme (Vallejos 1983). The isozymes examined included glutamate oxaloacetate transaminase (*GOT*), 6-phosphogluconate dehydrogenase (*6-PGDH*), phosphoglucomutase (*PGM*), and shikimic acid dehydrogenase (*SKDH*).

DNA analyses

Plant DNA isolation was performed according to the method reported by Bernatzky and Tanksley (1986). Bulked DNA samples (Michelmore et al. 1991) contained equal amounts of DNA from 21 young plants of the 89R NIL or pooled DNA from five 89S NIL plants.

Seven-hundred 10-mer oligonucleotide primers were obtained from John Hobbs (University of British Columbia, Vancouver, BC), and 20 were set X from Operon Technologies Inc. (Alameda, Calif). Custom-synthesized primers included 16 from Bio-Synthesis Inc. (Lewisville, Tex.), and 12 from Integrated DNA Technologies Inc. (Coralville, Iowa). Screening for primer amplification (10 µl) was in 50 mM Tris-HCl (pH 8.3), 0.25 µg BSA, 1 mM $MgCl_2$, 1 mM tartrazine, 0.5% ficoll, dATP, dCTP, dGTP, and dTTP (each at 0.2 mM), 0.4 µM primer, 50 ng of genomic DNA, and 0.38 units of *Taq* DNA polymerase (Promega Corp., Madison, Wis.). Reactions were performed in an Idaho Technologies thermal cycler programmed for 1 cycle of 30 s at 94 °C followed by 45 cycles of 10 s at 94 °C, 20 s at 35 °C, heat ramp of '1', and 2 min at 72 °C, followed by a final incubation of 5 min at 72 °C. Reaction products were resolved on 1.75% agarose gels. Resolution of occasional larger products utilized 0.7% agarose gels. Some RAPD products (see results) were labelled by incorporation of α-^{35}S-dATP (DuPont NEN, Boston, Mass.) and resolved on 40-cm 6% denaturing polyacrylamide gels to verify suspected polymorphisms.

For RFLP analyses, purified DNA was restricted to completion according to the supplier's (Gibco BRL, Gaithersburg, M.) recommendations, separated in 0.7% agarose gels and blotted onto MagnaGraph nylon membranes (Micron Separations Inc., Westborough, Mass.). Restriction enzymes included *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, *Pst*I, *Sst*RI, and *Xba*I. DNA probes were obtained from Steven D. Tanksley (Cornell University, Ithaca, N.Y.). Probes were radiolabelled with α-^{32} P-dATP (DuPont NEN, Boston, Mass.) either by random priming or by nick translation (Sambrook et al. 1989), and hybridized to the filters (Church and Gilbert 1984).

Results and discussion

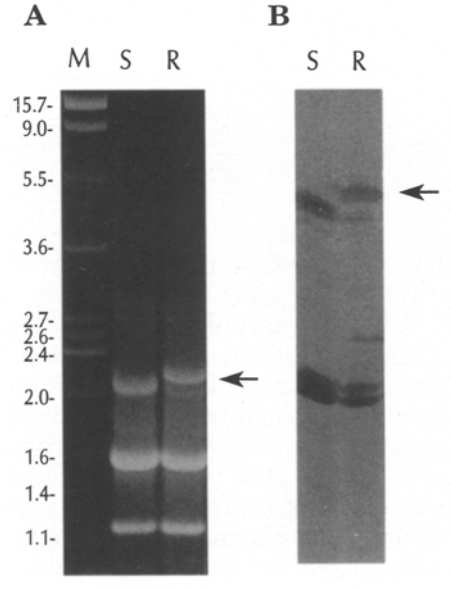
RAPD PCR results

Of the 748 primers, 265 (35.4%) failed to amplify distinct DNA fragments, 226 (30.2%) produced one to four bands, 188 (25.1%) produced five to nine bands, and 69 (9.2%) produced more than nine fragments. More than 2 600 DNA fragments were amplified using these primers. No clear polymorphisms were identified between the NIL RAPD products. Therefore we re-examined several minor polymorphisms. Thirteen oligonucleotides were selected for RAPD reactions containing ^{35}S-dATP and the products were resolved on denaturing polyacrylamide gels to distinguish bands of similar molecular weights and to minimize artifacts from secondary structure. Autoradiographic results revealed that primer #72 (5'-GAGCACGGGA-3') detected a

polymorphism (Fig. 1 Panel B). This polymorphism was initially observed as a more intense band produced in the 89R amplifications when compared to the 89S products. The primer #72 RAPD reaction products were then separated on a 15-cm, 0.7% agarose gel revealing an approximately 2200-bp fragment unique to the products from resistant NILs (Fig. 1 Panel A) and the resistant parent plants (data not shown). Unfortunately, the production of this polymorphic band has at, times, been inconsistent in RAPD reactions. The denaturing gel analysis of ^{35}S-labelled products demonstrates that this band is not a gel or secondary-structure artifact. Based on our results, a commercial seed company has evaluated co-segregation of the approximately 2 200-bp RAPD polymorphism and *Sw-5* resistance in a separate backcross population. In several hundred plants no crossovers (segregants) between these markers were detected (anonymous, personal communication). These results not only confirm the close association of the approximately 2 200-bp RAPD band and *Sw-5*, but also extend our observations to an additional breeding line. Therefore this marker should prove invaluable in marker-assisted selection for *Sw-5*.

There are approximately 1 276 map units and 950 megabase pairs (Mbp) in the tomato genome (Tanksley et al. 1992). Thus, 1 cM is approximately equal to 750 kilobase pairs (kbp). We examined over 2 600 bands using the 748 primers, consistent with a coverage of about 0.5 cM. Therefore, the detection of only one polymorphic band out of the 2 600 bands from each NIL DNA suggests that the 2.2-kbp RAPD band is most likely within 0.5 cM of *Sw-5*. Further, the infrequency of RAPD polymorphisms implies that the 89R line has little remaining *L. peruvianum* DNA and that the introgressed region containing the *Sw-5* locus is relatively small.

Fig. 1A, B Detection of the 2 200-bp polymorphic band using primer #72 in RAPD reactions. RAPD reaction products were for DNA from either the resistant 89R NIL (*lane R*) or the susceptible 89S NIL (*lane S*). Panel **A** Separation of RAPD products in a 0.7% agarose gel. Sizes in kbp for molecular-weight marker bands (*M*) are indicated to the left. Panel **B** Autoradiographic results from separation of reactions incorporating ^{35}S-dATP on denaturing acrylamide gels



RFLP results

Our initial RFLP data suggested linkage between *Sw-5* and the chromosome-5 telomeric markers TG623 and CT101 (Stevens et al. 1993). Examination for linkage to additional markers on chromosome 5 (*tf*, CT201, and TG185, see Tanksley et al. 1992) revealed no significant linkage to *Sw-5*. Therefore, *Sw-5* is apparently not located on chromosome 5.

Linkage analyses for markers from all chromosomes except 7 and 11 gave P values ≤ 0.05 (Table 1). However, the *L. esculentum* × *L. pennellii* cross has been shown to have numerous chromosomal regions demonstrating unequal segregation (Zamir and Tadmor 1986). Careful examination of our data (Table 2) reveals that the telomeric molecular probe CT71 on the long arm of chromosome 9 demonstrates the highest frequency of co-segregation with *Sw-5*. The chi-square for co-segregation with CT71 is the highest of any marker tested (Table 1).

Consistent with a marker tightly linked to *Sw-5*, only one susceptible plant (plant #14) was heterozygous for CT71 while the remaining 13 susceptible plants were homozygous for the RFLP marker from the susceptible *L. pennellii* parent (Table 2). All 17 of the resistant plants had at least one copy of the NIL *L. esculentum* marker (Table 2, EE or EP).

According to Tanksley et al. (1992), TG328, GP41, TG591A co-segregate at 1.6 cM centromeric to CT71 and CT220 is 2.7 cM telomeric to CT71 (Fig. 2). Susceptible plant #14 (PP for *Sw-5*) was found to be heterozygous for markers TG328 and TG591A (EP in Table 2) yet homozygous for the *L. pennellii* allele (PP) at CT220. These data are most consistent with *Sw-5* being positioned between CT71 and CT220. Tanksley et al. (1992) reported a map order of TG424, TG591A, CT71, and CT220 (Fig. 2) with which our data are in agreement. However, the map distances we determined do differ. For the 31 F_2 plants we detected five crossovers in the TG424–TG591A interval deriving a map distance of approximately 8 cM. There were three crossovers in the interval between TG591A and CT71 for a map distance of approximately 4.8 cM. The interval between CT71 and CT220 had 11 crossovers suggesting a distance of about 17.7 cM. Although RFLP analyses for CT220 using four different restriction enzymes were sometimes difficult to interpret, composite data reveal that 5 of the 14 susceptible plants (PP for *Sw-5*) were heterozygous (EP) for CT220 while only 2 of the 17 resistant plants were homozygous for the *L. pennellii* marker. These results are most consistent with a tighter linkage of *Sw-5* to CT71 than to CT220. *Sw-5* is dominant and therefore

Table 1 Linkage analysis of isozyme and molecular markers with TSWV resistance. Chi-square values are for an expected 1:2:1 ratio co-dominant molecular markers in progeny plants. An F_2 population from TSWV-resistant *L. esculentum* × susceptible *L. pennellii* was scored for segregation of *Sw-5* resistance and subsequently scored for co-segregation of RFLP or isozyme markers

Chromosome	Marker	Chi-square	*P* value	Chromosome	Marker	Chi-square	*P* value
1	*SKDH-1*	6.51	0.04	7	CT195	2.62	0.27
	TG389	7.71	0.02		*GOT-2*	3.27	0.19
	TG27	4.51	0.11		TG61	4.37	0.11
2	TG480	4.65	0.10	8	TG176	6.04	0.05
	CD35	17.30	< 0.01		TG330	3.14	0.21
3	TG479	1.79	0.51		TG294	3.23	0.20
	TG366	5.71	0.06	9	TG18	5.36	0.07
	TG533	16.07	< 0.01		TG390	8.59	0.01
4	*6-PGDH-1*	7.15	0.03		TG424	26.62	< 0.01
	PGM-2	8.25	0.02		TG328	33.42	< 0.01
	TG163	1.86	0.40		TG591A	33.42	< 0.01
5	TG623	13.93	< 0.01		CT71	40.25	< 0.01
	CT101	20.29	< 0.01		CT220	8.20	0.02
	CT201	1.29	0.53	10	TG313	10.47	0.01
	TG185	2.39	0.30		TG386	5.89	0.05
6	TG178	3.95	0.14		TG233	2.41	0.30
	TG153	2.96	0.23	11	TG393	2.88	0.24
	TG444	10.79	< 0.01	12	TG565	8.45	0.02
	TG548	6.45	0.04				
	TG220	5.69	0.06				

Table 2 Segregation patterns for *Sw-5* resistance and RFLP markers. Each of the molecular probes displayed a *P* value of ≤ 0.01 (Table 1). RFLP genotypes for resistant or susceptible plants were assessed as: EE- two *L. esculentum* alleles, PP- two *L. pennellii* alleles, or EP- one allele from each parent. The *L. peruvianum Sw-5* gene was introgressed into a *L. esculentum* background (Stevens et al. 1992); therefore, linkage results in co-segregation with the E RFLP marker

Chromosome	Probe	Restriction endonuclease	RFLP genotype of segregants					
			Resistant progeny			Susceptible progeny		
			EE	EP	PP	EE	EP	PP
2	CD35	*Eco*RV	0	8	9	2	12	0
3	TG533	*Pst*I	10	3	4	6	3	5
5	TG623[a]	*Xba*I	7	7	2	0	13	1
	CT101	*Eco*RI	7	10	0	0	14	0
6	TG444	*Eco*RV	6	4	7	1	6	7
9	TG390	*Xba*I	5	6	6	0	7	7
	TG424	*Eco*RI	2	13	2	0	3	11
	TG328	*Eco*RV	6	11	0	0	2	12
	TG591A	*Eco*RV	6	11	0	0	2	12
	CT71	*Xba*I	5	12	0	0	1	13
	CT220	*Eco*RV	5	9	3	1	5	8
10	TG313	*Eco*RI	1	10	6	0	7	7

[a] The genotype of one resitant plant could not be determined from the blot

resistant interspecific F_2 plants may be either heterozygous or homozygous for *Sw-5*. Thus, three of the five crossovers we detected in the CT71-CT220 interval for the resistant plants cannot be positioned relative to *Sw-5* while the remaining two crossovers occured between *Sw-5* and CT220. In the susceptible plants we ascertained a single crossover between CT71 and *Sw-5*, while there were five crossovers between *Sw-5* and CT220. Therefore, we estimate that *Sw-5* is between 1.6 and 4.8 cM centromere-distal to CT71 and between 11.3 and 16.1 cM proximal to CT220.

Tanksley et al. (1992) reported a map distance of 2.7 cM between CT71 and CT220; however, data from our cross indicate a distance about 16–20 cM. The probable reason for this discrepancy is that *Sw-5* originated from *L. peruvianum*. Thus, in our F_2 interspecific cross, meiotic recombinations near *Sw-5* involve DNA from *L. peruvianum* and *L. pennellii*. In contrast, map distances of Tanksley et al. (1992) were based on DNA from *L. esculentum* and *L. pennellii* for this region. There is evidence for suppression of crossover events between *L. esculentum* chromosomal regions and regions introg-

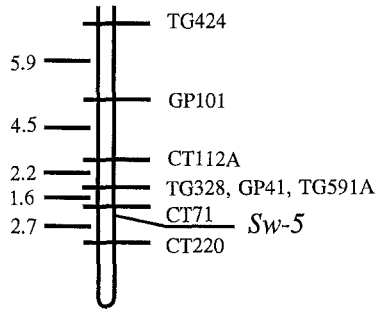


Fig. 2 Molecular linkage map of the telomeric region of the long arm on chromosome 9 from tomato. The approximate location of *Sw-5* is indicated (see text). Markers separated by commas have never been observed to segregate. *Numbers* to the left are map units in centiMorgans according to Tanksley et al. (1992). Map based on Tanksley et al. (1992)

ressed from *L. peruvianum* (Messeguer et al. 1991). Thus, crossover frequencies in regions surrounding *Sw-5* would be expected to vary according to the genomic origin of the paired homologs. The data presented here suggests that the region between CT71 and CT220 in our 89R line is either physically larger than in the standard *L. esculentum* map (Tanksley et al. 1992), or that in our 89R × *L. pennellii* F_1 population this area is highly active for crossover events. The altered crossover frequencies in this region are consistent with the presence of heterologous chromosomal material, further supporting this region as the site of the *Sw-5* introgression.

Potential use of molecular probe CT71

Linkage of CT71 and *Sw-5* will help define the specific map location of the polymorphic fragment produced by primer #72. These linked markers could then be used to monitor this resistance gene in a breeding program (i.e., marker-assisted selection). With more than 200 *L. peruvianum* accessions available in the USDA germplasm collection, additional genes for TSWV resistance may be identified. The identification of the probable linkage of CT71 and *Sw-5* will facilitate discrimination from other TSWV-resistance genes. Molecular markers will allow sequential addition of distinct TSWV-resistance genes to the same genome, either through transformation with molecular clones or conventional breeding. This would generate quantitative resistance which Fraser (1988, 1992) has suggested would provide a sizable barrier to virus infection. The eventual cloning of virus-resistance genes and the transformation of other TSWV host species with these genes would be an important step in TSWV control. Cloning and characterization of the actual gene products responsible for resistance would greatly enhance our understanding of the biochemical steps involved in host-virus interactions.

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References

Bernatzky R, Tanksley SD (1986) Genetics of actin-related sequences in tomato. Theor Appl Genet 72:314–321

Beek JG van der, Verkerk R, Zabel P, Lindhout P (1992) Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: *Cf9* (resistance to *Cladosporium fulvum*) on chromosome 1. Theor Appl Genet 84:106–112

Boiteux LS, Nagata T, Giordano LdeB (1993) Field resistance of tomato *Lycopersicon esculentum* lines to tomato spotted wilt disease. Rep Tomato Genet Coop 43:7–9

Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81, 1991–1995

Fraser RSS (1988) Virus recognition and pathogenicity: implications for resistance mechanisms and breeding. Pestic Sci 23:267–275

Fraser RSS (1992) The genetics of plant-virus interactions: implications for plant breeding. Euphytica 63:175–185

German TL, Ullman DE, Moyer JW (1992) *Tospoviruses*: diagnosis, molecular biology, phylogeny, and vector relationships. Annu Rev Phytopathol 30:315–348

Hille J, Koornneef M, Ramanna MS, Zabel P (1989) Tomato: a crop species amenable to improvement by cellular and molecular methods. Euphytica 42:1–23

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

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

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

Martin GB, de Vicente C, Ganal M, Miller L, Tanksley S (1993b) Towards positional cloning of the *Pto* bacterial resistance locus from tomato. In: Nester EW, Verma DPS (eds) Advances in molecular genetics of plant-microbe interactions. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 451–455

Martin GB, de Vicente MC, Tanksley SD (1993c) High-resolution linkage physical characterization of the *Pto* bacterial resistance locus in tomato. Mol Plant-Microbe Interact 6:26–34

Messeguer R, Ganal M, 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

Miller JC, Tanksley SD (1990) RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor Appl Genet 80:437–448

Miller R (1989) Identifying the most serious problem we face today – TSWV. Grower Talks July:100–106

Norris DO (1946) The strain complex and symptom variability of tomato spotted wilt virus. Aust Coun Sci Ind Res Bull 202

O'Malley PJ, Hartmann RW (1989) Resistance to tomato spotted wilt virus in lettuce. HortScience 24:360–362

Paterson RG, Scott SJ Gergerich RC (1989) Resistance in two *Lycopersicon* species to an Arkansas isolate of tomato spotted wilt virus. Euphytica 43:173–178

Rick CM (1988) Molecular markers as aids for germplasm management and use in *Lycopersicon*. HortScience 23:55–57

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

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

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, Rhoads DD, Lamb EM, Gergerich RC, Morelock TE (1993) Use of PCR and RFLP techniques to map the *Sw-5* locus conferring resistance to tomato spotted wilt virus (TSWV) in tomatoes. HortScience 28:583

Suurs LCJM, Jongedijk E, Tan MMC (1989) Polyacrylamide gradient-gel electrophoresis: a routine method for high-resolution isozyme electrophoresis of *Solanum* and *Lycopersicon* species. Euphytica 40:181–186

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, 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

Ullman DE, German TL, Sherwood JL, Westcot DM, Cantone FA (1993) *Tospovirus* replication in insect vector cells: immunocytochemical evidence that the nonstructural protein encoded by the S RNA of tomato spotted wilt tospovirus is present in thrips vector cells. Phytopathology 83:456–463

Vallejos CE (1983) Enzyme activity staining. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding. Elsevier Science Publishers B. V., Amsterdam, The Netherlands (Part A, pp 469–516)

Wijkamp I, Peters D (1993) Determination of the median latent period of two tospoviruses in *Frankliniella occidentalis*, using a novel leaf disk assay. Phytopathology 83:986–991

Young ND, Tanksley SD (1989) RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. Theor Appl Genet 77:353–359

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

Zamir D, Tadmor Y (1986) Unequal segregation of nuclear genes in plants. Bot Gaz 147:355–358

Zijl JJB van, Bosch SE, Coetzee CPJ (1986) Breeding tomatoes for processing in South Africa. Acta Hort 194:69–75

Note added in proof Recent analysis of other tomato breeding lines by RAPD with primer #72 has revealed that many lines produce a band equivalent to the 2200-bp polymorphic band from 89R. Therefore, this band likely drives from *L. esculentum* DNA tightly linked with *Sw-5* and complicating use of the band for marker-assisted selection in these other lines.