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Restriction Fragment Length Polymorphism Mapping of the *Stemphylium* Resistance Gene in Tomato

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The resistance of tomato (*Lycopersicon esculentum*) to the gray leaf spot disease caused by four *Stemphylium* species is conferred by a single incompletely dominant gene, *Sm*. The resistance gene was introgressed into cultivars from the wild species *L. pimpinellifolium* and was found to be linked to a Fusarium race 1 resistance gene on chromosome 11. To place *Sm* on the restriction fragment length polymorphism (RFLP) map, we analyzed by means of progeny tests the genotypes of 124 F2 plants segregating for the resistance. The results were compared to the RFLP genotypes of the plants with respect to eight DNA markers that map to chromosome 11. *Sm* was located between *T10* and *TG110*. The linkage between *T10* and *Sm* was not broken in eight independently bred resistant lines that showed the same polymorphism as the donor *L. pimpinellifolium* accession. The results indicate the usefulness of RFLP markers for screening of plants for *Stemphylium* resistance and as potential starting points in a chromosome walk aimed at cloning *Sm*.

Additional keywords: breeding, disease resistance.

The gray leaf spot disease in tomato is caused by four different known species of *Stemphylium*: *S. solani* Weber, *S. floridanum* Hannon and Weber, *S. botryosum* Wallr., and *S. vesicarum* (Wallr.) Simmons (Bashi *et al.* 1973; Blancard and Laterrot 1986). The symptoms of the disease are gray lesions on the foliage, followed in severe attacks by complete defoliation. Resistance to the disease was identified (Andrus *et al.* 1942) in the red-fruited species *L. pimpinellifolium*, and this led to the breeding of resistant tomato cultivars. Hendrix and Frazier (1949) determined that the resistance is due to a single gene with incomplete dominance, *Sm*, which confers resistance to all four species of *Stemphylium* (Bashi *et al.* 1973; Blancard and Laterrot 1986). This resistance has not been overcome by new virulent races of the pathogen since its introgression nearly 50 years ago. The new cases of disease in presumably resistant plants were due to impurity of the lines being tested (Laterrot and Blancard 1983). This host-pathogen system provides a good example of a resistance gene that has been widely used in an extensive crop for a long period without losing its total immunity.

Dennett (1950) reported a crossover value approximating 36% between *Sm* and *I* genes, the latter conferring resistance against a wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* race 1. Both genes were introgressed from the same accession of *L. pimpinellifolium* (PI 79532). The gene *I* was assigned to chromosome 11 (Paddock 1950), although its exact position on the map is still not known. On the basis of these data, DNA restriction fragment length polymorphism (RFLP) markers that map to chromosome 11 were used in this study to place *Sm* on the map.

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MATERIALS AND METHODS

Plant material. The tomato cultivars Moneymaker (Sm^+/Sm^+, susceptible) and Motelle (Sm/Sm, resistant) were crossed, and an F1 plant was selfed to create a segregating F2 population of 142 plants. DNA was extracted from each plant, and F3 seed were collected from 124 F2 plants for use in progeny tests for resistance to the gray leaf spot disease.

The following tomato varieties and breeding lines were analyzed for RFLP variation: *Stemphylium* resistant Motelle, Ideucenzi, IC 2-8, Vendor, Mobox, Romitel, Peto 95-43, M82-1-8, and 86LB410-10; *Stemphylium* susceptible Moneymaker, Rossol, Marmande, and 86LB410-11.

***Stemphylium* inoculations.** A *S. vesicarum* isolate originating from Beja, Tunisia, was used for inoculation tests. The pathogen was grown on a solid medium consisting of 200 ml of tomato and vegetable juice, V8, 2 g of $CaCO_3$, 18 g of gelose, and 800 ml of distilled water (Laterrot and Blancard 1983). The inoculation test was performed on 20 F3 seedlings for each F2 plant, as described by Blancard and Laterrot (1986). The cultivars Motelle and Moneymaker and their F1 population were used as controls. Seeds were sown in compost disinfected by vapor and placed in a glasshouse. Three weeks after germination the leaves were sprayed to runoff with *S. vesicarum* conidial suspension (10^4/ml), and the plants were then transferred to a moist chamber at 24° C and sprayed twice a day with water. The plants were covered by a plastic sheet for the

first 4 days after inoculation and illuminated for 12 hr a day with dim fluorescent light (4,000 lx). The first symptoms of the disease, small gray spots on the leaves, were observed 4–5 days after inoculation. Ten days later, the plants were visually assessed for the severity of symptoms on a scale of 1–5: 1 = coalescence of lesions, 2 = numerous lesions, 3 = few lesions, 4 = rare lesions, 5 = no symptoms. A weighted mean disease rating was calculated for each genotype on the basis of its progeny test.

RFLP analysis. To identify RFLPs suitable for the mapping analysis, DNA was extracted from Motelle and Moneymaker plants and digested with the following 22 restriction enzymes: *Pvu*II, *Pst*I *Hin*dIII, *Bam*HI, *Ava*I, *Bgl*II, *Eco*RI, *Xba*I, *Xho*I, *Cfo*I, *Bcl*I, *Bst*EII, *Hpa*II, *Msp*I, *Eco*RV, *Rsa*I, *Hin*cII, *Alu*I, *Hin*fI, *Taq*I, *Dra*I, and *Hae*III. The digested DNA was gel electrophoresed, Southern blotted, and hybridized to the following eight radiolabeled DNA markers that map to chromosome 11: *TG7*, *TG107*, *TG108*, *TG110*, *TG36*, *TG105*, *TG26* (Zamir and Tanksley 1988), and *T10* (a cDNA clone encoding the chloroplastic superoxide dismutase; Perl-Treves *et al.* 1990). Restriction enzymes revealing polymorphism were used to digest the DNA of the F2 population. DNA isolation, restriction digests, electrophoresis on agarose gels, Southern blots, hybridizations, and autoradiography were as described by Bernatzky and Tanksley (1986) except that the filters were probed with random hexamer-labeled plasmids (Feinberg and Vogelstein 1983).

Statistical analysis. The Mapmaker program (Lander *et al.* 1987) was used for mapping analysis by the Kosambi function. Statistical analysis was performed using the Data Desk computer program for the MacIntosh (Velleman and Pratt 1989).

RESULTS

Monogenic inheritance of *Stemphylium* resistance. Inoculation results and disease ratings of the parental lines Motelle and Moneymaker and their F1 hybrid are shown in Table 1. The weighted mean disease rating value of the F1 population indicates the partial dominance of the resistance. The frequency distribution of the disease rating of an F2 population of 124 individuals was determined after a progeny test of 20 F3 seedlings from each plant (Fig. 1). Based on the results of the inoculation of the parents, the F1, and the trimodal shape of the distribution, we defined three genotypic groups in the F2. First, Moneymaker had a mean disease rating of 1.86 and is of the genotype Sm^+/Sm^+ (homozygous for the susceptibility allele). The eleven F2 plants that were assigned as Sm^+/Sm^+ had a mean disease rating of 2.16, with a minimum of 1.71 and a maximum of 2.60. Second, sixty-three F2 plants were assigned the genotype Sm/Sm^+; the mean disease rating for this group was 4.16, with a minimum of 3.10 and a maximum of 4.70. Third, Motelle (Sm/Sm) had a mean disease rating of 4.95. Fifty F2 plants were assigned the genotype Sm/Sm; their mean disease rating was 4.97, with a minimum of 4.90 and a maximum of 5.00. The results of the F2 population indicate that *Sm* deviated significantly from the expected 1:2:1 Mendelian ratio (Table 2).

Linkage analysis. RFLPs for the single copy markers *TG7*, *TG107*, *TG108*, *T10* (Perl-Treves *et al.* 1990), *TG36* and *TG26*, and for the duplicate markers *TG105* and *TG110*, were observed between the parental lines Motelle and Moneymaker (Fig. 2). Unequal segregation was detected for all the RFLP markers that map to chromosome 11; in all cases, including *Sm*, there was a deficiency of plants homozygous to the Moneymaker alleles and an excess of homozygotes for Motelle alleles (Table 2). Mapping analysis placed *Sm* between *T10* and *TG110* (Fig 3). The LOD score ($\log_{10}$ of the odds ratio) for the placement of *Sm* in that position was 0.0 compared to a LOD score of −14.9 for the position between *TG110* and *TG107*, a LOD score of −17.1 for the position between *T10* and *TG36*, and a LOD score of −47.2 for the placement of *Sm* at infinity. The most likely order of genes on the map is always indicated with a relative log-likelihood of zero, while others will have negative relative log-likelihoods, indicating as a power of 10 the degree to which they provide less likely explanations of the data. These results clearly show that *Sm* strongly prefers to be between *T10* and *TG110*.

Screening of nine *Stemphylium* resistant and four susceptible tomato lines with *T10* indicated that all the resistant genotypes including the original *L. pimpinellifolium* accession (PI 79532), which was the source of the resistance, showed the polymorphism of Motelle, whereas the susceptible lines had the polymorphism of Moneymaker.

Table 1. Results of inoculations of cultivars Motelle, Moneymaker, and their F1 hybrid with *Stemphylium vesicarum*

Accession	Genotype	Visual rating[a] (number of plants) 1	2	3	4	5	Total	Mean disease rating
Motelle	Sm/Sm	0	0	0	3	61	64	4.95
F1	Sm/Sm^+	0	0	21	43	6	70	3.79
Moneymaker	Sm^+/Sm^+	2	58	5	0	0	65	1.86

[a]Disease rating: 1 = severe symptoms, coalescence of lesions; 5 = no symptoms.

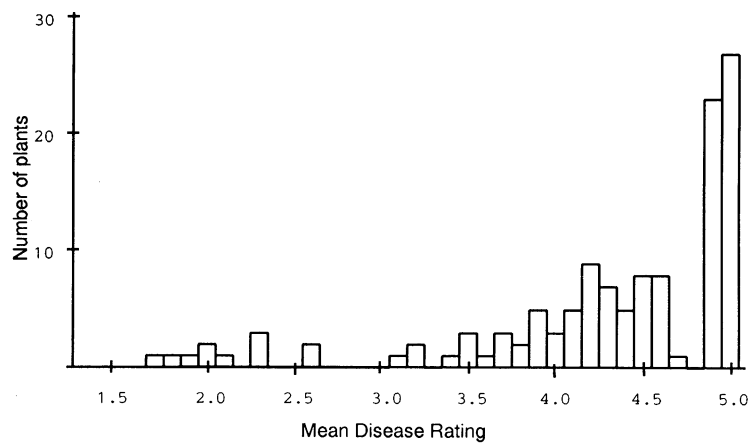

Fig. 1. Frequency distribution of *Stemphylium* disease rating in an F2 population of 124 plants resulting from selfing the hybrid between cultivars Motelle and Moneymaker.

DISCUSSION

Identification of genetic markers closely linked to disease resistance genes has long been an objective of plant breeders, because the markers can be used to screen genotypes in a breeding program without resorting to inoculation with the pathogen. In tomato, a recessive seedling morphological marker *ah* (anthocyaninless) was found to be linked to *Tm-2*, a gene for resistance to tobacco mosaic virus (Robinson *et al.* 1970). The codominant isozyme marker *Aps-1* is linked to *Mi* (*Meloidogyne incognita*), the nematode resistance gene (Rick and Fobes 1974), and *Got-2* is linked to *I3*, which confers resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 3 (Bournival *et al.* 1989). The development of an RFLP map covering the entire tomato genome made it possible to follow in a single population the segregation of hundreds of DNA markers and the gene of interest. By using the RFLP system, markers were found that are closely linked to *Tm-2* (Young *et al.* 1988), *Tm-1* (Levesque *et al.* 1990), *I1* and *I2* (*F. o.* f. sp. *lycopersici* races 1 and 2 resistance genes; Sarfatti *et al.* in press; Sarfatti *et al.* 1989), *Mi* (Klein-Lankhorst *et al.* 1991; Messeguer *et al.* in press), and *Pto* (*Pseudomonas syringae* pv. *tomato*; Martin *et al.* 1991). In addition to their breeding applications, RFLP markers tightly linked to disease resistance genes can be used as starting points for physical mapping and chromosome walking aimed at cloning of the genes (Michelmore *et al.* 1987; Tanksley *et al.* 1989). Both the breeding and the molecular applications require precise mapping of the factors responsible for resistance.

Resistance of plants to *Stemphylium* is a quantitative trait determined by the activity of a single gene. The mapping of *Sm* requires transformation of the quantitative disease rating into Mendelian genotypes. The degree and severity of the *Stemphylium* symptoms are influenced by environmental conditions (Hendrix and Frazier 1949), and variations in disease response can therefore be observed for individuals with identical resistance genotypes. To assign Mendelian genotypes in the F2 progeny test populations, we defined the "cut-off" points within the disease rating distribution between the genotypes Sm^+/Sm^+ and Sm/Sm^+, and between Sm/Sm^+ and Sm/Sm.

The present study demonstrates how the RFLP markers flanking *Sm* provide a way to confirm the genotypic assignments. The segregation pattern of *Sm* deviated significantly from the expected Mendelian ratios. The results demonstrate that *T10* and *TG110*, which are linked to *Sm*, deviated in the same direction, indicating that aberrant segregations of this chromosome segment are chiefly responsible for

Table 2. Monogenic segregations of chromosome 11 markers in an F2 generation resulting from selfing of a hybrid between cultivars Motelle and Moneymaker

	Genotype[a]			x^2
Locus	1	2	3	1:2:1
TG7	28	49	46	10.3[b]
TG110	13	67	61	33.0[c]
TG107	12	64	51	24.0[c]
TG108	12	(—— 129 ——)		22.3[d]
Sm	11	63	50	24.6[c]
T10	14	70	51	20.5[c]
TG36	23	64	55	15.8[e]
TG105	23	62	55	16.5[e]
TG26	25	62	55	15.0[e]

[a]1, Homozygous for the Moneymaker allele; 2, heterozygous; 3, homozygous for the Motelle allele.
[b]Significant at the 5% level.
[c]Significant at the 0.1% level.
[d]Significant at the 0.1% level for 1:3 ratio.
[e]Significant at the 1% level.

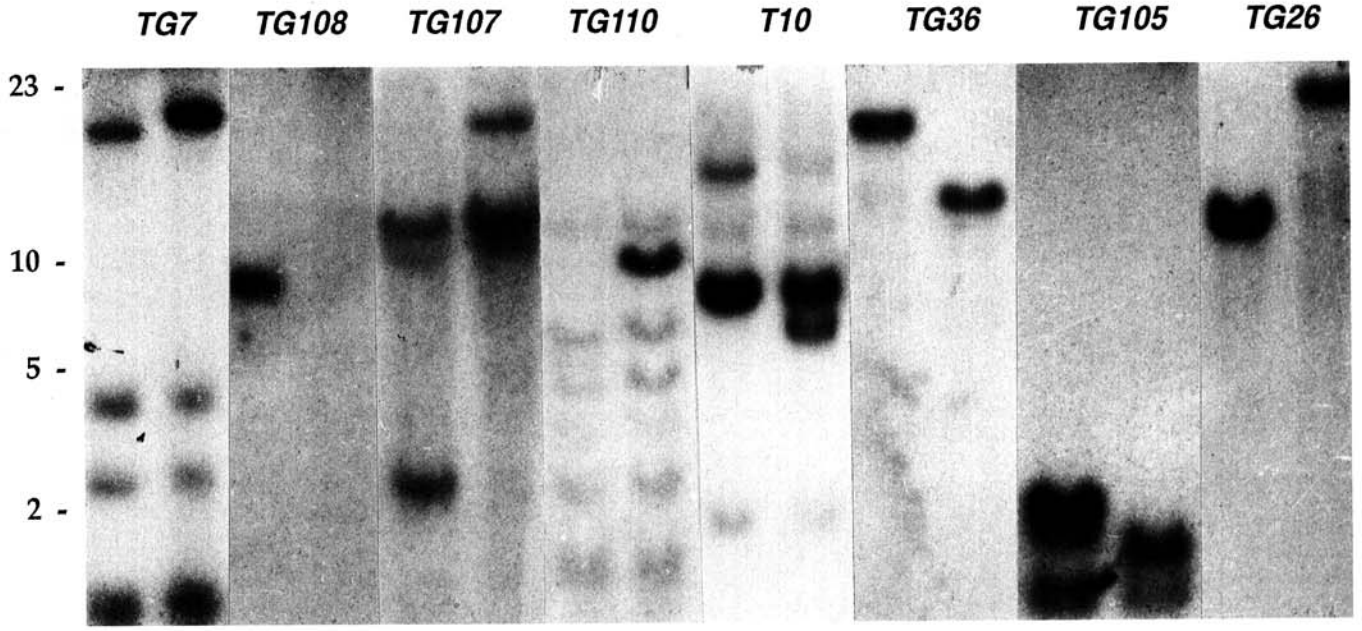

Fig. 2. Restriction fragment length polymorphism between cultivars Motelle (left lane) and Moneymaker (right lane) for the markers *TG7* (polymorphism detected with *Cfo*I), *TG108* (*Hpa*II), *TG107* (*Hin*dIII), *TG110* (*Hae*III), *T10* (*Bgl*II), *TG36* (*Hpa*II), *TG105* (*Taq*I), and *TG26* (*Bgl*II). Lefthand margin indicates molecular weights in kilobases.

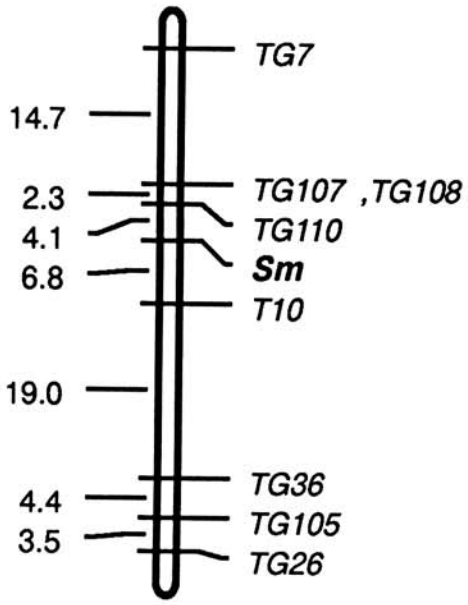

Fig. 3. The molecular map of chromosome 11. CentiMorgans are listed on the left side of the map. Markers separated by comma showed no recombination.

the results. Nonrandom segregations due to preferential fertilizations were previously reported for the gene *I*, which is also linked to *Sm* (Kedar *et al.* 1967; Zamir and Tadmor 1986). Further confirmation of the genotype assignments came from the mapping analysis: the three-point additive distance, *TG110-Sm-T10*, was 10.9 cM, similar to the distance between *TG110* and *T10*, which was calculated independently of *Sm* (10.4 cM). Any misclassification of the *Stemphylium* genotype would have resulted in large discrepancies between the distances obtained in the two-point and three-point tests.

The linkage between *T10* and *Sm* (6.8 cM) is confirmed by the lack of recombinants between the RFLP marker and the resistance gene in eight independently bred lines. *T10* can therefore be used as a marker in screening of resistant plants in a breeding program.

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