Two Complex Resistance Loci Revealed in Tomato by Classical and RFLP Mapping of the *Cf-2, Cf-4, Cf-5,* and *Cf-9* Genes for Resistance to *Cladosporium fulvum*

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Received 28 October 1992. Accepted 9 March 1993.

To develop effective strategies for cloning tomato genes for resistance to *Cladosporium fulvum,* we have tested the reported genetic locations of four such *Cf* genes. *Cf-2* has been reported to be on chromosome 6, *Cf-4* on chromosome 1, *Cf-5* on chromosome 4, and *Cf-9* on chromosome 10. Our mapping studies confirm that *Cf-2* is on chromosome 6. *Cf-5* is not on chromosome 4, but on chromosome 6 at the same location as *Cf-2.* Classical mapping places both *Cf-2* and *Cf-5* approximately 2 centiMorgans (cM) proximal to *yv* on the long arm of chromosome 6, and less than 1 cM from *tl* on the short arm. Restriction fragment length polymorphism (RFLP) mapping places *Cf-2* close to the chromosome 6 marker TG232. *Cf-4* is on the short arm of chromosome 1, loosely linked and distal to the visible marker *au. Cf-9* is not on chromosome 10, but on chromosome 1 at the same location as *Cf-4.* Classical mapping places *Cf-9* approximately 14 cM distal to *au* and approximately 19 cM from *ses,* loosely linked to the chromosome 1 markers *irr, Lpg,* and *com,* but unlinked to *ms-32, imb,* and *br.* RFLP mapping places *Cf-9* between the chromosome 1 markers TG236 and TG301. Tests for allelism show that *Cf-2* is allelic or very closely linked to *Cf-5* and that *Cf-4* is allelic or very closely linked to *Cf-9.*

Additional keywords: Lycopersicon esculentum, tomato leaf mold.

Plant genes for resistance to biotrophic fungal pathogens have been known for more than 80 years (Biffen 1905), but we remain ignorant of their functions. Numerous attempts have been made to elucidate resistance gene function using biochemical approaches, but these have invariably failed. Recent approaches to this problem have turned to the cloning of resistance genes in the hope that molecular characterization will lead to an understanding of how they work. Unfortunately, the choice of strategies for cloning resistance genes is also limited by a lack of biochemical knowledge about their functions. Almost all that is known about resistance genes are their genetic properties, so it is largely these that determine the cloning strategy. We are attempting resistance gene isolation using both map-based cloning and insertional mutagenesis by transposition of the maize transposable elements *Ac* (*Activator*) or *Ds* (*Dissociation*) from linked sites. Both of these strategies require accurate knowledge of the location of the target resistance genes in the plant genome.

In tomato (*Lycopersicon esculentum* Miller), our target genes are the *Cf-2, Cf-4, Cf-5,* and *Cf-9* genes for resistance to the fungus *Cladosporium fulvum* Cooke (syn. *Fulvia fulva*), the causal agent of leaf mold. *Cf-2* was first mapped to chromosome 6 by Langford (1937), who found loose but significant linkage (43.0 ± 1.9%) with *c* (*cut* or potato leaf) in a coupling-phase F_2 population of 1,316 plants. Kerr *et al.* (1980) found no significant linkage between *Cf-2* and *c* in four testcross populations totaling 222 plants, but did find significant linkage (32.3 ± 3.7%) between *Cf-2* and another chromosome 6 marker *m-2* (mottled) in two testcross populations totaling 158 plants. They also found close linkage (8.3 ± 1.5%) between *Cf-2* and another resistance gene on chromosome 6 *Mi* (resistance to the nematode *Meloidogyne incognita*) in seven testcross populations totaling 351 plants. From the two populations of 158 plants segregating all four markers, they postulated the gene orders and linkage distances *Mi*–8 centiMorgans (cM)–*Cf-2*–33 cM–*m-2*–27 cM–*c*.

Kerr and Bailey (1964) found an indication of loose linkage between *Cf-4* and the linked chromosome 1 markers *br* (*brachytic*) and *y* (fruit epidermis lacking *yellow* pigment) with *Cf-4* probably distal to *y* relative to *br.* Previously, Langford (1937) had mapped *Cf-1* to a similar position with significant linkages (30.9 ± 3.6% and 40.1 ± 3.6%) between *Cf-1* and *y* in a coupling-phase F_2 population of 261 plants and a testcross population of 187 plants, respectively. He found no significant linkage (50.5 ± 3.7%) between *Cf-1* and *br* in a testcross population of 186 plants, indicating that *Cf-1* was distal to *y* relative to *br.* Kerr and Bailey tested the possibility that *Cf-1* and *Cf-4* were at the same location by producing a repulsion-phase F_2 population of 293 plants that they inoculated with a race of *Cladosporium* avirulent to both *Cf-1* and *Cf-4.* Only one susceptible plant was recovered, which they suspected was due to seed admixture rather than recombination, indicating that *Cf-1* and *Cf-4* were either allelic or very closely linked.

Lenhardt and Kerr (1972) stated that *Cf-5* was located at the same position on chromosome 1 as *Cf-1* and *Cf-4*

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MPMI, Vol. 6, No. 3, pp. 348-357

but presented no supporting data. Kanwar *et al.* (1980a, 1980b) examined the linkage relationships of many *Cf* genes including *Cf-2, Cf-4, Cf-5,* and *Cf-9.* They confirmed the location of *Cf-2* on chromosome 6 and *Cf-4* on chromosome 1 and presented data indicating that *Cf-5* was between *ful* (*fulgens*) and *e* (*entire*) on chromosome 4 and *Cf-9* distal to *hy* (*homogeneous yellow*) on chromosome 10. However, these data were later cast into doubt by Kerr (1982), and a number of their proposed locations were subsequently contradicted by other observations (Gerlagh *et al.* 1989; Laterrot and Moretti 1989; Laterrot and Moretti 1991; van der Beek *et al.* 1992). We have tested the authenticity of the reported locations of *Cf-2, Cf-4, Cf-5,* and *Cf-9* by a combination of classical and restriction fragment length polymorphism (RFLP) mapping. We have refined the positions of those genes whose chromosomal locations we were able to confirm and remapped those whose locations we were unable to confirm. Based on these new locations, we tested for allelism between *Cf* genes that mapped to similar positions.

RESULTS

Testing the reported locations of *Cf-2, Cf-4, Cf-5,* and *Cf-9.*

The results of mapping experiments designed to check the reported locations of *Cf-2, Cf-4, Cf-5,* and *Cf-9* are shown in Tables 1 and 2. The location of *Cf-2* on chromosome 6 was confirmed by close linkage to *yv* (*yellow virescent*) (Table 1) and to TG232 (Table 2). The location of *Cf-4* on chromosome 1 was confirmed by linkage to *au* (*aurea*) (Table 1). We could not detect linkage of *Cf-5* to markers on chromosome 1 or chromosome 4, or linkage of *Cf-9* to markers on chromosome 10, by either classical or RFLP mapping.

Table 1. Linkage of *Cf* genes to visible markers in chromosome tester crosses to test the reported locations of *Cf-2, Cf-4, Cf-5,* and *Cf-9*[a]

					Phenotype[b]					Recombination[d]
Cf gene	Tester	Chromosome	Cross	Marker	+R	+S	mR	mS	χ^2 Assoc.[c]	(%)
Cf-2	LA1190[e]	6	F_2	*yv*	75	1	0	24	94.7**	1.0 ± 1.0
Cf-4	LA1186	1	F_2	*au*^{tl}	102	17	13	33	51.8**	18.7 ± 3.4
				scf	92	32	23	18	4.78*	39.1 ± 5.1
				inv	88	33	27	17	1.97	–
				dgt	90	32	25	18	3.68	–
Cf-5	LA917	4	F_2	*clau*	59	25	21	8	0.11	–
				ful	52	21	23	12	0.34	–
				ra	53	23	21	11	0.18	–
				e	55	25	20	8	0.07	–
				di	54	26	21	7	0.55	–
Cf-9	LA1002	10	Test	*ag*	8	8	8	9	0.03	–
				h	10	10	6	7	0.05	–
				l-2	7	13	9	4	3.70	–
			F_2	*ag*	39	11	15	6	0.35	–
				h	45	11	9	6	2.69	–

[a] Resistance gene segregation was scored by inoculation with race 0 for *Cf-2*, race 5 for *Cf-4* and race 4 for *Cf-5*, and by injection with race 0 apoplastic fluid for *Cf-9*

[b] + = Wild type for the visible marker; m = mutant for the visible marker; R = resistant; S = susceptible.

[c] χ^2 test for association with 1 df; * = significant at $P = 0.05$; ** = significant at $P = 0.001$.

[d] ± Standard error; – = unlinked.

[e] The LA1190 line segregated for *pds*, and in this instance the F_1 did not carry *pds*.

Table 2. Linkage of *Cf* genes to restriction fragment length polymorphism (RFLP) markers in *Lycopersicon esculentum* Cf0 × (*L. esculentum* Cf2, Cf5, and Cf9 × *L. pennellii* LA716) testcrosses to test the reported locations of *Cf-2, Cf-5,* and *Cf-9*[a]

				Phenotype[b]					Recombination
Cf gene	Chromosome	Probe	Digest	ER	ES	HR	HS	χ^2 Assoc.	(%)
Cf-2	6	TG232	*Bst*NI	35	–	0	–	35.0**	0[c]
		TG118	*Eco*RV	32	–	3	–	24.0**	8.6 ± 4.7
		TG73	*Dra*I	30	–	5	–	17.9**	14.3 ± 5.9
		TG99	*Eco*RV	20	–	15	–	0.71	–
		TG115	*Dra*I	13	–	22	–	2.31	–
Cf-5	1	TG71	*Eco*RV	7	10	7	9	0.03	–
		TG83	*Eco*RV	4	8	7	10	0.03	–
	4	TG62	*Pst*I	6	8	4	7	0.04	–
		TG22	*Eco*RI	5	8	9	13	0.03	–
Cf-9	10	TG122	*Xba*I	9	4	5	3	0.10	–
		TG43	*Eco*RV	5	8	6	10	0.003	–
		TG63	*Eco*RV	4	5	6	10	0.12	–

[a] Resistance gene segregation was scored by injection with race 0 apoplastic fluid. Only the resistant progeny from a 35 resistant:39 susceptible segregation of *Cf-2* were used for RFLP mapping of *Cf-2*.

[b] E = homozygous for *L. esculentum* RFLP except for TG232 which was homozygous for the introgressed *L. pimpinellifolium* RFLP; H = heterozygous for *L. esculentum* and *L. pennellii* RFLPs; R = resistant; S = susceptible.

[c] Upper limit of recombination = 8.2% at $P = 0.05$.

Remapping *Cf-5* and *Cf-9*.

The results of a wide range of classical mapping experiments designed to locate *Cf-5* and *Cf-9* are shown in Tables 3 and 4, respectively. From these experiments *Cf-5* was found to be on chromosome 6 and, like *Cf-2*, closely linked to *yv*, raising the possibility that *Cf-2* and *Cf-5* may be closely linked or even allelic. These experiments did not locate *Cf-9* but did exclude *Cf-9* from approximately 75% of the classical map. This provided a guide for RFLP mapping experiments which focused on regions of the genome not covered by the classical mapping experiments. The results of these RFLP mapping experiments are shown in Table 5. From these experiments *Cf-9* was found to be on the short arm of chromosome 1 cosegregating with TG24. This location was later confirmed by linkage of *Cf-9* to *au* in a 2-655A (au^{ll}) × Cf9 F_2 population of 102 progeny which gave 20.6 ± 4.6% recombination between *Cf-9* and *au*. This distance of *Cf-9* from *au* was very similar to that for *Cf-4*, raising the possibility that *Cf-4* and *Cf-9* may also be closely linked to each other or even

Table 3. Linkage of *Cf-5* to visible markers in chromosome tester crosses[a]

Tester	Chromosome	Cross	Marker	Phenotype[b] +R	+S	mR	mS	χ^2 Assoc.	Recombination (%)
LA1444	2	F_2	*wv*	27	12	8	1	1.43	–
			d	28	10	7	3	0.05	–
LA1430	3	F_2	*sy*	36	9	6	3	0.77	–
			bls	31	9	11	3	0.01	–
			sf	34	10	8	2	0.04	–
LA1444	5	F_2	*af*	23	8	12	5	0.07	–
			tf	29	9	6	4	1.07	–
LA1190[b]	6	F_2	*pds*	27	7	7	4	1.12	–
			yv	33	1	1	10	35.0**	4.6 ± 3.2
LA1103	7	Test	*var*	12	7	7	10	1.74	–
			not	8	8	11	9	0.09	–
LA1666	8	Test	*l*	8	13	16	7	4.38*	–
			bu	8	13	16	7	4.38*	–
			dl	8	13	16	7	4.38*	–
			ae	11	11	13	9	0.37	–
LA1002	10	F_2	*ag*	23	9	5	1	0.34	–
			h	23	9	5	1	0.34	–
			l-2	19	7	9	3	0.02	–
LA881	11	Test	*neg*	8	7	1	4	1.68	–
			l	6	6	3	5	0.30	–
			a	5	5	4	6	0.20	–
LA1111	12	Test	*alb*	7	16	6	11	0.11	–
LA1171	12	Test	*aud*	8	17	6	15	0.06	–

[a] Resistance gene segregation was scored by inoculation with race 0 except for the LA1666 testcross, which was scored by injection with race 0 apoplastic fluid.

[b] The LA1190 line segregated for *pds*, and in this instance the F_1 carried *pds*.

Table 4. Linkage of *Cf-9* to visible markers in chromosome tester crosses[a]

Tester	Chromosome	Cross	Marker	Phenotype +R	+S	mR	mS	χ^2 Assoc.
LA1444	2	F_2	*wv*	23	9	13	4	0.12
			d	26	9	10	4	0.04
LA1430	3	Test	*sy*	6	4	4	4	0.22
			bls	6	5	4	3	0.00
			sf	6	5	4	3	0.00
LA917	4	F_2	*ful*	35	14	5	0	1.93
			e	34	14	6	0	2.36
			di	32	12	8	2	0.22
LA1444	5	F_2	*af*	25	9	11	4	0.0002
			tf	29	12	7	1	0.97
LA1190	6	F_2	*yv*	26	8	10	3	0.001
LA651	6	F_2	*m-2*	35	8	14	2	0.31
			c	36	6	13	4	0.73
LA1103	7	Test	*var*	10	19	11	9	2.03
			not	7	13	14	15	0.85
LA1100	9	F_2	*marm*	35	12	11	3	0.10
LA881	11	F_2	*neg*	34	8	12	6	1.44
			hl	36	10	10	4	0.28
			a	30	9	16	5	0.004
LA1111	12	Test	*alb*	13	7	2	3	1.04
LA1171	12	Test	*aud*	6	13	11	17	0.29

[a] Resistance gene segregation was scored by injection with race 0 apoplastic fluid.

allelic.

Refining the locations of *Cf-2* and *Cf-5*.

In the F_2 of a cross between *Cf-2* and the visible markers *yv* and *pds* (*phosphorus deficiency syndrome*), *yv* was found to be linked to *Cf-2,* but both *yv* and *Cf-2* were unlinked to *pds* (Table 6). The unexpected lack of linkage between *yv* and *pds* is inconsistent with the present map of chromosome 6 (Tanksley and Mutschler 1990) showing them 27 cM apart and suggests that *pds* may not be on chromosome 6 as previously suggested by Rick *et al.* (1970). In the F_2 of a cross between *Cf-2* and the visible markers *yv* and *coa* (*corrotundata*), *Cf-2* was found to be about 2 cM proximal to *yv* (Table 6) rather than distal, as shown on the current classical map of tomato (Tanksley and Mutschler 1990). Almost identical results for the same cross have been obtained by M. Koornneef (personal communication). This places *Cf-2* very close to the centromere, since *yv* has been localized cytologically to a short segment of interstitial euchromatin near the centromeric heterochromatin on the long arm of chromosome 6 (Khush and Rick 1968). It also places *Cf-2* near to *tl* (*thiamineless*) which has been localized cytologically to euchromatin on the short arm of chromosome 6 (Khush and Rick 1968). The F_2 of a cross between *Cf-2* and *tl* revealed *Cf-2* to be more tightly linked to *tl* than to *yv* (Table 6), suggesting that *Cf-2,* like *tl,* may be on the short arm of chromosome 6 rather than the long arm. Experiments are in progress to determine whether *Cf-2* is proximal or distal to *tl.* Similarly, *Cf-5* was also found to be about 2 cM proximal to *yv* and much closer to *tl* (Table 6), strengthening the possibility of allelism with *Cf-2.* Experiments refining the locations of *Cf-2* and *Cf-5* on the RFLP map of chromosome 6 are reported in the accompanying paper (Dickinson *et al.* 1993) and remain in progress.

Table 5. Linkage of *Cf-9* to restriction fragment length polymorphism (RFLP) markers in a *Lycopersicon esculentum* Cf0 × (*L. esculentum* Cf9 × *L. pennellii* LA716) testcross to determine the location of *Cf-9*[a]

			Phenotype				
Chromosome	Probe	Digest	ER	ES	HR	HS	χ^2 Assoc.
1	TG24	*Hin*dIII	16	0	0	7	23.0**
	TG83	*Dra*I	8	6	12	4	1.07
2	TG31	*Xba*I	10	4	7	4	0.17
3	TG130	*Hae*III	11	4	6	2	0.01
4	TG62	*Dra*I	10	5	9	5	0.02
8	TG45	*Xba*I	12	6	3	1	0.10
11	TG30	XbaI	8	3	6	3	0.01
12	TG68	*Xba*I	4	2	6	2	0.12
	TG28	*Dra*I	7	4	10	2	1.15

[a] Resistance gene segregation was scored by injection with race 0 apoplastic fluid. A segregation of 22 resistant to 13 susceptible testcross progeny was obtained but in no case was it possible to score a complete RFLP segregation for all 35 progeny.

Table 6. Linkage of *Cf-2* and *Cf-5* to visible markers in F_2 progeny of crosses to refine the loctions of *Cf-2* and *Cf-5* on chromosome 6[a]

			Phenotype					
Cf gene	Tester	Marker	+R	+S	mR	mS	χ^2 Assoc.	Recombination (%)
Cf-2	LA1190[b]	*yv*	530	13	10	146	573.5**	3.5 ± 0.7%
	LA1178	*yv*	378	8	4	143	475.3**	2.2 ± 0.6%
		coa	320	69	62	82	79.6**	27.0 ± 2.3%
		c	297	98	85	53	9.31*	41.2 ± 2.9%
	GCR472	*tl*	346	1	1	95	431.3**	0.47 ± 0.33%
Cf-5	LA1178	*yv*	198	2	3	62	238.6**	1.9 ± 0.9%
		coa	175	41	26	23	17.0**	32.8 ± 3.6%
		c	164	44	37	20	4.74*	40.3 ± 4.1%
	GCR472	*tl*	340	1	1	109	440.2**	0.45 ± 0.32%

[a] Resistance gene segregation was scored by inoculation with either race 4 or race 5 for *Cf-2* and race 0 or race 4 for *Cf-5*. The data generated by this use of alternative races to screen different batches of each segregating population were homogeneous and so were pooled.

[b] LA1190 segregated for *pds*, and in this instance the F_1 carried *pds*. The F_2 segregated 543 wild type, 156 yv, 135 pds and 47 pds yv, but because *pds* was so debilitating and assorted independently of *yv* (χ^2 assoc. = 1.21, $P > 0.05$), resistance gene segregation was scored for the non pds progeny only.

Table 7. Linkage of *Cf-9* to visible markers in progeny of tester crosses to refine the location of *Cf-9* on chromosome 1[a]

			Phenotype					
Tester	Cross	Marker	+R	+S	mR	mS	χ^2 Assoc.	Recombination (%)
LA1185	F_2	*au*[ll]	265	32	24	83	172.4**	14.4 ± 1.9%
		scf	220	76	66	37	3.95*	43.2 ± 3.5%
GCR376	F_2	*irr*	117	26	31	21	10.3*	35.2 ± 4.4%
LA664	F_2	*com*	188	53	53	34	9.58*	38.7 ± 3.6%
LA359	F_2	*ms-32*	81	27	18	9	0.77	–
GCR362	F_2	*imb*	120	32	32	8	0.02	–
LA826	F_2	*ses*	717	90	87	154	289.1**	19.3 ± 1.4%
GCR705	Test	*au*	43	9	6	34	41.6**	16.3 ± 3.9%
		Lpg	32	18	17	25	5.07*	38.0 ± 5.1%
LA2069	F_2	*br*	35	15	17	5	0.40	–

[a] Resistance gene segregation was scored by injection with race 5 apoplastic fluid. Some batches of the LA826 × Cf9 F_2 population were screened with race 4 apoplastic fluid. The data generated were homogeneous with those generated using race 5 apoplastic fluid and so were pooled.

Refining the locations of *Cf-4* and *Cf-9*.

In the F_2 of a cross between *Cf-4* and the visible markers *au* and *scf* (*scurfy*) (Table 1), barely significant linkage was observed between *scf* and *Cf-4*, and this was slightly less than that between *au* and *scf*, initially suggesting the possibility that *Cf-4* was proximal to *au*. In a similar cross (Table 7), *Cf-9* also showed barely significant linkage to *scf*, but this was slightly greater than that between *au* and *scf*, suggesting the possibility that *Cf-9* was distal to *au*. Linkage distances are usually approximately additive, but in both these crosses the linkage distances were extremely nonadditive, i.e., *au*–18.7%–*Cf-4* + *Cf-4*–39.1%–*scf* ≠ *au*–42.5%–*scf* and *Cf-9*–14.4%–*au* + *au*–42.1%–*scf* ≠ *Cf-9*–43.2% *scf*. The *au–scf* and *Cf-4/9–scf* distances did not differ significantly from one another in either cross. They also did not differ significantly from the *au–inv* (44.2 ± 5.5%), *au–dgt* (41.7 ± 5.3%), *Cf-4–inv* (42.6 ± 5.3%), and *Cf-4–dgt* (40.3 ± 5.2%) distances in the *Cf-4* cross. Since *inv* (*invalida*) and *dgt* (*diageotropica*) are known to be well distal on the long arm of chromosome 1 and unlinked to *au* (Tanksley and Mutschler 1990), it would appear that a recombination distance of 40–45% reflects lack of linkage in both crosses. This is inconsistent with *Cf-4/9* locations between *au* and *scf* because linkage much less than 40% would have been expected with *scf*. These data are therefore only consistent with *Cf-4/9* locations distal to *au* on the short arm of chromosome 1 rather than between *au* and *scf*. Consistent with this conclusion, *irr* (*irregularis*) and *com* (*complicata*), which lie in the region between *au* and *scf* on the current genetic map (Tanksley and Mutschler 1990) were only loosely linked to *Cf-9* (Table 7), and *ms-32* (male sterile) and *imb* (*imbecilla*), which also lie in this region, assorted independently from *Cf-9* (Table 7). However, inconsistent with this conclusion, *ses* (*semisterilis*) and *Lpg* (*Lapageria*) which are, respectively, 7 and 16 cM distal to *au* on the current genetic map, were further from *Cf-9* than *au* (Table 7). In fact *Lpg* was 23.9 ± 4.4% distal to *au* relative to *Cf-9*. Also inconsistent with this conclusion, *br*, which is 32 cM distal to *au* on the current genetic map, assorted independently from *Cf-9* (Table 7).

RFLP mapping (Table 8) showed *Cf-9* to be located between TG236 and TG301 on the short arm of chromosome 1, possibly closer to TG301 than TG236. Linkage of *Cf-9* to TG301 has been demonstrated independently by van der Beek *et al.* (1992), but they did not determine the location of *Cf-9* relative to TG301 on the RFLP map. Experiments with *Cf-4*, although less conclusive, suggested a very similar RFLP location (data not shown), strengthening the possibility of allelism with *Cf-9*. RFLP mapping of *au* and *ms-32* (Table 8) show *au* to cosegregate with TG236, and *ms-32* to be proximal to TG51, consistent with distal locations for *Cf-4* and *Cf-9*. Experiments to refine the RFLP map locations of *Cf-4*, *Cf-9*, and visible

Table 8. Restriction fragment length polymorphism (RFLP) mapping of *Cf-9*, *au*, and *ms-32* in *L. esculentum* Cf9, 2-655A (*au*^{tl}) and LA359 (*ms-32*) × *Lycopersicon pennellii* LA716 crosses to determine their relative locations[a]

Gene	Cross	Phenotype	RFLP genotype[b] with probe[c]						Number of progeny
			721A[d]	CT233	TG301	TG236	TG24	TG51	
Cf-9	F_2	Susceptible	P	–	P	P	P	P (or -)	12 (+2)
			P	–	P	P	P	H	1
			P	–	P	H	H	H	2
			H	–	H^{pi}	P	P	P	1
			H	–	P	P	P	P	1
Fraction of F_1 gametes recombinant			2/38	–	1/38	2/38	2/38	3/34	
au^{tl}	Test	Aurea	–	E	E	E	–	E	14
			–	E	E	E	–	H	1
			–	H	H	E	–	E	2
			–	H	E	E	–	E	2
		Wild type	–	H	H	H	–	H	8
			–	H	H	H	–	E	1
			–	E	E	H	–	H	1
Fraction of F_1 gametes recombinant			–	5/29	3/29	0/29	–	2/29	
ms-32	Test	Male sterile	–	E (or -)	–	E	–	E	16 (+2)
			–	H	–	E	–	E	4
			–	H	–	H	–	E	3
			–	H	–	H	–	H	2
		Male fertile	–	H	–	H	–	H	13
			–	E	–	H	–	H	3
			–	E	–	E	–	H	1
			–	E	–	E	–	E	2
Fraction of F_1 gametes recombinant			–	15/44	–	8/46	–	4/46	

[a] Resistance gene segregation was scored by inoculation with race 0. Only the susceptible progeny from a 54 resistant:20 susceptible F_2 segregation were used for RFLP mapping of *Cf-9*. A nonrandom subset of testcross progeny was used for RFLP maping of *au*, but a complete set of testcross progeny was used for RFLP mapping *ms-32*.

[b] E = homozygous for *L. esculentum* RFLP; H = heterozygous for *L. esculentum* and *L. pennellii* RFLPs; H^{pi} = heterozygous for *L. pimpinellifolium* and *L. pennellii* RFLPs; P = homozygous for *L. pennellii* RFLP; – = not scorable.

[c] Probes are listed in order of their location on the short arm of chromosome 1 with the most distal probe on the left and the most proximal on the right. RFLPs were revealed by digestion with *Eco*RV for 721A, *Eco*RI for TG301 in the *au* mapping, and *Hin*dIII for CT233, TG301 in the *Cf-9* mapping, TG236, TG24 and TG51.

[d] A chromosome 1 probe generated in this laboratory (Thomas *et al.* submitted).

markers on the short arm of chromosome 1 are in progress and will be reported elsewhere.

Testing for allelism between *Cf-2* and *Cf-5* and between *Cf-4* and *Cf-9*.

To test for allelism between *Cf-2* and *Cf-5* and between *Cf-4* and *Cf-9*, an F_1 plant heterozygous for *Cf-2* and *Cf-5* and another heterozygous for *Cf-4* and *Cf-9* were testcrossed to Cf0. If the two genes being tested were allelic then either one or the other would have been inherited by the testcross progeny. However, if they were nonallelic then recombinant progeny carrying neither or both would also have been recovered. The testcross progeny were inoculated with a *Cladosporium* race that would detect both resistance genes, so that if the resistance genes were allelic no susceptible plants would have been recovered (assuming no intraallelic recombination), but if they were nonallelic, then susceptible recombinants would have been recovered (assuming they were not closely linked). No susceptible plants were recovered among 484 progeny from the *Cf-2/5* testcross inoculated with either race 0 or 4, nor among 486 progeny from the *Cf-4/9* testcross inoculated with either race 0 or 5. This indicates that *Cf-2* is allelic or very closely linked to *Cf-5* and that *Cf-4* is allelic or very closely linked to *Cf-9*, with a 0.6% upper limit of recombination at $P = 0.05$ in both cases. Experiments designed to examine the allelism or close linkage of these genes more rigorously are in progress and will be reported elsewhere.

DISCUSSION

These mapping experiments, summarized in Figure 1, reveal that the *Cf-2, Cf-4, Cf-5,* and *Cf-9* genes have all been mapped inaccurately to various extents. The complete mislocation of *Cf-5* and *Cf-9* on the wrong chromosomes is inexplicable, but the poor positioning of *Cf-2* and *Cf-4* on their respective chromosomes is probably due to the fact that, in common with many other genes on the classical map, their positions are based on heterogeneous data, often from two-point crosses, compiled from different sources. It is therefore perhaps not surprising that these mapping experiments have also generated results inconsistent with the locations of other genes on the classical map, including the possible mislocation of *ses, Lpg,* and *br* distal rather than proximal to *au* on the short arm of chromosome 1, and the possible misplacement of *pds* on chromosome 6 (Fig. 1).

The position of *ses* is based on a two-point cross, which gave 7.5% recombination with *au*, and a three-point cross, which gave 40.5 and 46% recombination with *scf* and *inv*, respectively (Reeves *et al.* 1968). The position of *Lpg* is based on four separate two-point crosses which resulted in 15, 16.5, 33.5, and 38.5% recombination with *y, au, scf,* and *inv,* respectively (Rick 1964; Rick and Boynton 1966). Although consistent with locations of *ses* and *Lpg* distal to *scf* relative to *inv,* these data allow either proximal or distal locations relative to *au.* However, only locations proximal to *au* appear consistent with our data.

Three-point data were used to orient *br* relative to *y* and *Cf-1* or *Cf-4* (Langford 1937; Kerr and Bailey 1964), but there are no data to orient this group on the map of chromosome 1, which has largely been built up relative to *y*. It is entirely possible that this group of genes was placed in the wrong orientation, so allowing either a proximal or distal location of *br* relative to *au.* However, only a location proximal to *au* appears consistent with our data.

The position of *pds* is based on three-point data from two replicate crosses that place it on the short arm of chromosome 6 loosely linked to *yv* but unlinked to *c* on the long arm (Rick *et al.* 1970). In both these crosses there was significant segregation distortion for *yv* and in one cross there was also significant segregation distortion for *pds.* Such distortions tend to invalidate the linkage analysis, making the case for a location on chromosome 6 much weaker. Our data suggest that *pds* is unlinked to *yv.* Ho *et al.* (1992) have also found *pds* to assort independently of *yv.* If *pds* is not located on chromosome 6, then perhaps it is located on chromosome 9, since Rick *et al.* (1970) also reported "significant indications of linkage" with *marm* (*marmorata*), which do not appear to have been followed up.

One unfortunate consequence of these probable mislocations of markers on the classical map has been to remove

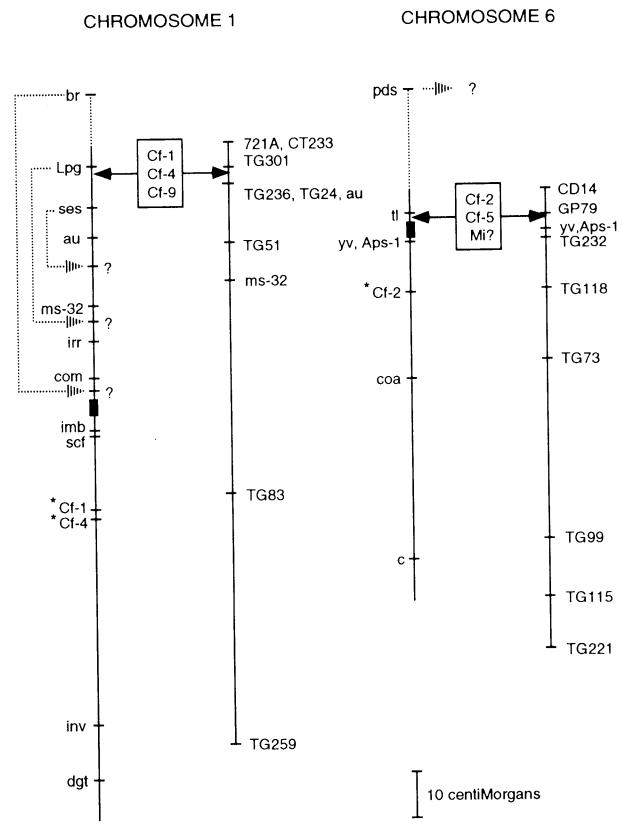

Fig. 1. Summary of the *Cf-2, Cf-4,* and *Cf-5,* and *Cf-9* mapping data represented on simplified classical (left) and RFLP (right) maps of chromosomes one and six of tomato. These maps are based on the most recent classical (Tanksley and Mutschler 1990) and RFLP (Tanksley *et al.* 1992) maps available. New locations of the *Cf* genes are shown boxed, previously supposed locations are indicated by asterisks. Dashed lines on the classical map represent regions whose existence is cast into doubt by the possible relocation of genes as indicated by the dashed arrows. Filled in boxes on the classical map represent the approximate locations of centromeres.

almost all the visible markers distal or just proximal to *Cf-9* on the short arm of chromosome 1 that could have been used to select progeny recombinant in the vicinity of *Cf-9* for RFLP fine mapping. *Brown seed* (*bs*) remains the only potentially useful marker in this region, and its location relative to *Cf-9* is currently being examined. The usefulness of *yv* for selecting progeny recombinant in the vicinity of *Cf-2* and *Cf-5* for RFLP fine mapping is well demonstrated in the accompanying paper (Dickinson *et al.* 1993). *Thiamineless* (*tl*) is closer to *Cf-2* and *Cf-5* and should be more useful because recombinants, though less frequent, should be more informative. If *tl* proves to be distal to *Cf-2* and *Cf-5* on the short arm of chromosome 6, then it would be even more useful because it could be used in conjunction with *yv* to select for recombination events either side of these resistance genes.

These mapping experiments have also revealed two complex resistance loci in tomato, one on chromosome 6, of which *Cf-2, Cf-5,* and possibly *Mi* (Dickinson *et al.* 1993) are members, and another on chromosome 1, of which *Cf-4, Cf-9,* and probably *Cf-1* are members (Fig. 1). The existence of these complex loci reveals the genetic control of resistance to leaf mold in tomato to be similar to the genetic control of resistance to biotrophic fungal pathogens in other plants, many of which also possess complex resistance loci. Examples include resistance to rust in maize (Saxena and Hooker 1968), to powdery mildew in barley (Wise and Ellingboe 1985), to rust in flax (Shepherd and Mayo 1972), and to downy mildew in lettuce (Hulbert and Michelmore 1985). These complex loci may comprise a series of multiple alleles, such as the *L* locus for resistance to rust in flax (Flor 1965; Shepherd and Mayo 1972; Islam *et al.* 1989; Mayo and Shepherd 1980) or possibly the *Ml-a* locus for resistance to powdery mildew in barley (Wise and Ellingboe 1985). Alternatively, they may comprise closely linked but separate genes, such as the *M* and *N* loci for resistance to rust in flax (Flor 1965; Shepherd and Mayo 1972; Islam *et al.* 1989; Mayo and Shepherd 1980) or the *Rp1* locus for resistance to rust in maize (Saxena and Hooker 1968; Hulbert and Bennetzen 1991). For allelic resistance genes it is not possible to obtain recombinants that express more than one resistance specificity in *cis* arrangement unless they are determined by separate functional domains within a single gene. For example, recombination between resistance alleles at the *L* locus of flax results in the loss or modification of both specificities or the gain of a new specificity, and in some cases one of the original specificities or a new specificity can be recovered by further recombination (Flor 1965; Shepherd and Mayo 1972; Islam *et al.* 1989; Islam *et al.* 1991; Islam and Shepherd 1991). For closely linked resistance genes, such as those at the *M* and *N* loci in flax, it is possible to obtain recombinants that express more than one resistance specificity in the *cis* arrangement (Flor 1965; Shepherd and Mayo 1972; Mayo and Shepherd 1980). Such intra- or intergenic recombinants, if they could be identified for the *Cf-2/5* or *Cf-1/4/9* loci, would be extremely powerful tools for the analysis and cloning of resistance genes. For example, if a chromosome walk were to be initiated from a molecular marker linked to the *Cf-2/5* locus, advantage could be taken of the RFLPs between the introgressed segment of *L. pimpinellifolium* DNA bearing *Cf-2* and the homologous segment of *L. esculentum* var. *cerasiforme* DNA bearing *Cf-5* (Dickinson *et al.* 1993). A recombinant with *Cf-2* and *Cf-5* in *cis* or losing both specificities would show a switch in RFLPs from the *pimpinellifolium* form to the *esculentum* form or even a novel recombinant RFLP for probes at or between the genes. This would solve the problem in a walking strategy of knowing when one had arrived at the destination. Similar possibilities exist for the *Cf-1/4/9* locus with respect to RFLPs between the *L. esculentum* DNA bearing *Cf-1,* the introgressed *L. hirsutum* DNA bearing *Cf-4* and the introgressed *L. pimpinellifolium* DNA bearing *Cf-9* (Stevens and Rick 1988).

For *Cf-2* and *Cf-5,* the flanking markers *tl* and *yv* could be used to enrich for intragenic or intralocus recombinants between the resistance genes by selection for progeny recombinant between the visible markers. For example, an F_1 between a *yv Cf-2* recombinant and a *tl Cf-5* recombinant (and vice versa) could be crossed to a *tl yv* Cf0 plant and wild-type or *tl yv* recombinants recovered and screened for loss or gain of both resistances. Experiments of this nature are under way.

Linked visible markers are not only useful in map-based cloning strategies, but also for transposon-tagging strategies. Studies examining the patterns of *Ac* or *Ds* transposition in maize (Greenblatt 1984; Dooner and Belachew 1989), tobacco (Jones *et al.* 1990), and tomato (Osborne *et al.* 1991) suggest that the prospects for gene tagging by linked transposition of *Ac* or *Ds* elements are greater the tighter the linkage between the transposon and the target gene. Linked visible markers could be used either to identify transposon insertions near to the target *Cf* gene, or as targets themselves, from which subsequent linked tagging experiments could be initiated. The wild-type allele of *yv* has proven an unsuitable target near the *Cf-2/5* locus because of its instability both documented in the literature (Hagemann 1962) and revealed in attempted tagging experiments using *Ac* (G. Bishop, unpublished). The wild-type allele of *tl* may prove a better target not only because it is closer to the *Cf-2/5* locus, but because it is likely to be more stable and because the mutant allele confers a conditional phenotype that can be restored to normal by provision of exogenous thiamine. Either or both markers could be used to select closely linked transposon insertions.

The allelism or close linkage of *Cf-2* to *Cf-5* and *Cf-4* to *Cf-9* has reduced the number of targets for transposon tagging or map-based cloning to two defined regions, so that if one gene from each region is isolated it may be possible to obtain the other gene(s) from the same region either by homology or a short walk. The identification of closely linked visible markers provides powerful tools to assist in targeting the *Cf-2/5* locus and potentially the *Cf-1/4/9* locus. The isolation of *Cf* genes by either linked tagging or map-based cloning is now a realistic objective.

MATERIALS AND METHODS

Plant material.

Four *Cladosporium*-resistant near-isogenic lines of the *Cladosporium*-susceptible cultivar Moneymaker, homozy-

gous for *Cf-2, Cf-4, Cf-5,* and *Cf-9,* respectively (Tigchelaar 1984), were obtained from R. Oliver (University of East Anglia, Norwich) and used in these mapping experiments. Moneymaker is here designated Cf0 (because of its lack of detectable resistance genes) and the four near-isogenic lines Cf2, Cf4, Cf5, and Cf9 (consistent with the resistance genes they carry), respectively. These near-isogenic lines interacted differentially with *Cladosporium* as described by de Wit *et al.* (1987) (Fig. 2), confirming their authenticity. A number of *Cladosporium*-susceptible chromosome-tester lines, homozygous for various combinations of recessive genes marking individual chromosomes and conferring visible early-seedling phenotypes, were obtained from C. M. Rick (University of California, Davis) and used in the classical mapping experiments (see Results). These lines are denoted by their LA or 2- prefix. In theory, these lines contained sufficient markers in total to cover almost the entire classical genetic map of tomato. In practice, we found some of the reported markers to have been lost or too difficult to score reliably, so that our coverage was somewhat reduced. Some additional lines homozygous for single recessive genes, were obtained from J. Maxon-Smith (HRI, Littlehampton) and used in the classical mapping experiments (see Results). These lines are denoted by their GCR prefix. The self-compatible *Cladosporium*-susceptible *Lycopersicon pennellii* accession LA716 was also obtained from C. M. Rick and used in the RFLP mapping experiments.

Crossing strategy.

To detect linkage by classical mapping, Cf2, Cf4, Cf5, and Cf9 plants were crossed as males to the chromosome testers and the resultant F_1 plants were also crossed as males to the chromosome testers to generate testcross seed. To detect linkage by RFLP mapping, Cf2, Cf4, Cf5, and Cf9 plants were crossed as females to LA716 and the F_1 plants were crossed as males to Cf0 plants to generate testcross seed. The F_1 plants were testcrossed as males rather than allowed to self-pollinate because reduced recombination in male versus female gametes (de Vicente and Tanksley 1991) was expected to enhance the detection of linkage. For mapping experiments to refine linkages, or if the production of sufficient testcross seed for the detection of linkage was problematic, the above F_1 plants were allowed to self-pollinate to generate coupling phase F_2 seed.

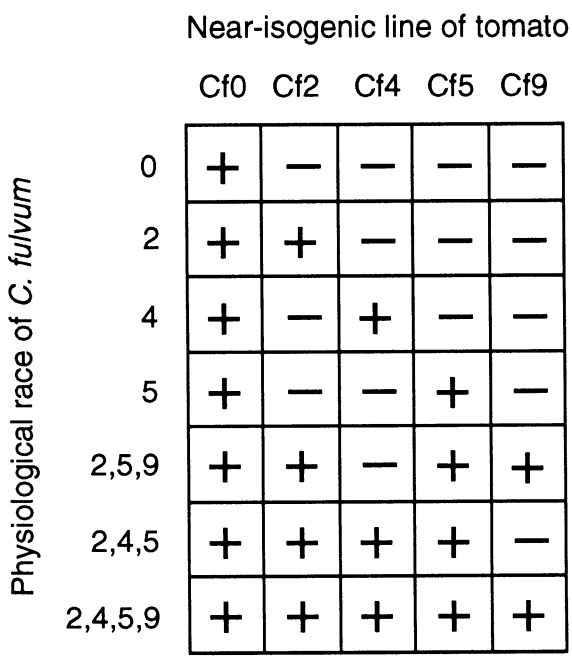

Fig. 2. Differential interactions between the Cf2, Cf4, Cf5, and Cf9 near-isogenic lines of tomato cv. Moneymaker (Cf0) carrying the *Cf-2, Cf-4, Cf-5,* and *Cf-9* gene for resistance to *Cladosporium fulvum,* respectively, and physiological races of *C. fulvum.* + = Compatible interaction, − = incompatible interaction.

Scoring segregation of resistance genes by inoculation with *C. fulvum.*

Race 0, named according to its lack of virulence for any known resistance specificity, and races 4 and 5 of *C. fulvum,* named according to the resistance specificities for which they are virulent (Day 1956), were obtained from R. Oliver (University of East Anglia, Norwich) and used to inoculate plants segregating for resistance. These races interacted differentially with Cf0, Cf2, Cf4, Cf5, and Cf9 plants as described by de Wit *et al.* (1987) (Fig. 2), confirming their authenticity. Aqueous suspensions of approximately 10^5 to 10^6 spores per milliliter were prepared from cultures of *Cladosporium* grown on 10 g of potato-dextrose agar (Oxoid Ltd., Basingstoke, Hants.) per liter for 2 wk at 28° C with supplementary lighting. Following scoring of visible markers, but prior to scoring of RFLP markers, seedlings at the three- to four-leaf stage were inoculated by dipping them in the spore suspension. Immediately prior to inoculation, seedlings were watered with 10^{-5} M paclobutrazol (ICI Agrochemicals, Bracknell, Berks.), an inhibitor of gibberellin biosynthesis, at an approximate dosage of 100 ml per liter of compost. Paclobutrazol treatment prevented the etiolation of seedlings that would have otherwise occurred under the incubation conditions. Following inoculation the plants were kept in the greenhouse under plant propagation frames at 100% humidity without supplementary lighting for 3 days and then at approximately 80% humidity with supplementary lighting when necessary for 11 days and then scored for disease symptoms.

Scoring segregation of resistance genes by injection with apoplastic fluids containing avirulence peptides.

The production of avirulence gene-encoded peptides in the apoplastic fluid of susceptible tomato plants supporting the growth of *C. fulvum,* and their use in the induction of necrotic or chlorotic responses following injection into resistant tomato plants, has been well documented (De Wit and Spikman 1982; Scholtens-Toma and De Wit 1988). Apoplastic fluids were isolated as described by de Wit and Spikman (1982) from Cf0 plants 14 days after inoculation with race 0, race 4, or race 5. Apoplastic fluids were injected via the underside into the leaves of plants to be scored for resistance, using a 1-ml disposable syringe without a needle. The plants were kept in the greenhouse, under supplementary lighting when necessary, until symptoms (necrosis for *Cf-9* or chlorosis for the other *Cf* genes)

were observed (usually 1 or 2 days after injection for *Cf-9* or 4 or 5 days or later for the other *Cf* genes).

Scoring RFLP segregation.

Nuclear DNA was extracted essentially as described by Bernatzky and Tanksley (1986), except that the frozen leaf material was ground to a fine powder in liquid nitrogen using a mortar and pestle before addition of extraction buffer. DNA was digested to completion with appropriate restriction endonucleases, and the resulting fragments were electrophoretically separated on 0.8% agarose gels (5–10 μg of DNA per lane) then vacuum or capillary blotted and UV crosslinked onto GeneScreen Plus membranes (Du Pont Co., Wilmington, DE). Previously mapped RFLP probes (Tanksley *et al.* 1992) comprising tomato genomic (TG) or cDNA (CT) clones were provided by S. Tanksley (Cornell University). The cloned tomato DNA fragments were released by restriction endonuclease digestion, electrophoretically separated from the vector on agarose gels, electroeluted, and random hexamer labeled with ^{32}P-dCTP (Feinberg and Vogelstein 1983). Probes were hybridized to the blots and the membranes washed according to the manufacturer's directions. Final washes were at 65° C in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate. X-ray films were exposed to the membranes for 1–10 days depending on the specific activity of the probe and the extent of probe hybridization.

Linkage analysis.

To detect linkage between *Cf* genes and visible or RFLP markers, joint segregations were tested pairwise for departures from independent assortment by carrying out χ^2 tests for association on 2×2 contingency tables. For testcross data, recombination values (r) were measured directly and the standard error calculated assuming a binomial distribution of r. For F_2 data recombination values and standard errors were estimated using the maximum likelihood method (Mather 1951). For recombination values of zero the upper limit of recombination at $P = 0.05$ for n F_1 gametes contributing to n testcross or $n/2$ F_2 progeny was calculated according to the formula $1 - p^{1/n}$ (see Table VIII, Fisher and Yates 1963).

ACKNOWLEDGMENTS

We thank C. Rick and J. Maxon-Smith for provision of visible marker stocks of tomato, R. Oliver for provision of *Cladosporium fulvum* cultures and *Cladosporium*-resistant and -susceptible NILs of tomato, S. Tanksley for provision of RFLP probes, K. Hammond-Kosack for guidance in work with apoplastic fluid extracts, M. Koornneef and P. Zabel for valuable discussion, R. Chetelat for valuable criticism of the manuscript, ICI Agrochemicals for the gift of the gibberellin biosynthesis inhibitor paclobutrazol, and L. and S. Perkins for plant care. This work was supported by the Gatsby Foundation and AFRC Plant Molecular Biology Program grant PMB/523.

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