Close Linkage Between the *Cf-2/Cf-5* and *Mi* Resistance Loci in Tomato

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Analysis of restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers in tomato plants segregating for resistance to the fungus *Cladosporium fulvum* was used to localize the resistance genes *Cf-2* and *Cf-5* to the same region of chromosome 6. This region, between GP79 and *Aps-1,* is the same as that reported for the *Mi* gene, which confers resistance to root-knot nematodes (*Meloidogyne* spp.). Recombination values based on F_2 populations from crosses between near-isogenic lines of *L. esculentum* 'Moneymaker' carrying *Cf-2* or *Cf-5* and *Lycopersicon pennellii,* indicate that this region occupies 4–5 centiMorgans (cM). However, in F_2 populations from crosses between the *L. esculentum* stock LA1190 carrying *yv* and these lines, this value is 1–2 cM. The *Cf-2* gene, introduced into *L. esculentum* from *L. pimpinellifolium,* is on an introgressed segment that extends from a point distal to GP79 to a point between TG232 and H2D1. The origin of *Cf-5* was found to be *L. esculentum* var. *cerasiforme* rather than *L. pimpinellifolium* as previously reported. No RFLP markers and only one RAPD marker showed a polymorphism between Moneymaker and the near-isogenic line carrying *Cf-5.*

Additional keyword: tomato leaf mold.

Leaf mold of tomato (*Lycopersicon esculentum* Miller), caused by the fungus *Cladosporium fulvum* Cooke, can cause major crop losses in greenhouses if adequate precautions are not taken to control it. At least 11 dominant genes, which confer resistance to different races of the fungus, have been postulated (Stevens and Rick 1988). The majority of these have been identified in wild relatives of tomato, and bred into *L. esculentum*. One of these genes, *Cf-2,* was identified in *L. pimpinellifolium* in 1934. This gene was assigned to chromosome 6 (Langford 1937) and was given a map location 43 on the long arm by Kerr *et al.* (1980). In this same study, *Mi,* which confers resistance to the root-knot nematode (*Meloidogyne* spp.), was assigned to position 35, and a number of recombinants were reported between *Cf-2* and *Mi*. More recently, Koornneef and Zabel (1990) have revised the linkage map of chromosome 6 in this region. Although *Cf-2* was not used in this study, *Mi* was given the location 44, *Cf-2* the location 52, and the leaf color marker *yv* (yellow virescent) and the isozyme marker *Aps-1* (acid phosphatase-1) were assigned to position 43. In the accompanying paper (Jones *et al.* 1993), we report *Cf-2* to be 1–2 centiMorgans (cM) proximal to *yv,* and thus near position 41 or 42.

During 1970, *Cf-5* was identified in PI 187002, reported to be an *L. pimpinellifolium* accession, and was introgressed into *L. esculentum* (Kerr *et al.* 1971). This gene was provisionally mapped to chromosome 4 (Kanwar *et al.* 1980). We have shown that this gene is in fact located on chromosome 6, linked to *yv*, and allelic or very closely linked to *Cf-2* (Jones *et al.* 1993).

To date, no gene products for plant genes conferring resistance to biotrophic fungal pathogens have been identified. One approach for cloning the *Cf* genes would be to identify closely linked flanking markers, as starting points for chromosome walking. Such approaches have been used successfully to clone human genes such as that responsible for cystic fibrosis (Rommens *et al.* 1989). This technique requires the isolation and ordering of probes tightly linked to the resistance genes. Both restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs) have been used to develop detailed molecular maps. With RFLPs, high resolution maps have been constructed for chromosome 6 around the *Mi* gene (Messeguer *et al.* 1991; Klein-Lankhorst *et al.* 1991). These maps have positioned *Mi* between the markers GP79 and *Aps-1,* and the distance between these markers has been estimated as between 0.4 and 2.0 cM, depending on the nature of the parents used in the cross. The advent of RAPDs (Williams *et al.* 1990) has provided an efficient means of generating additional markers to refine these maps further. Near-isogenic lines (NILs) of tomato differing with respect to resistance genes carried on introgressed regions of DNA are ideal for the use of this technique to identify closely linked markers.

In this study, RFLP mapping has been performed on F_2 populations from crosses between near-isogenic lines of *L. esculentum* 'Moneymaker' carrying *Cf-2* or *Cf-5* and *Lycopersicon pennellii.* Since *Cf-2* and possibly *Cf-5* were introgressed into *L. esculentum* from related polymorphic species, NILs of the cultivar Moneymaker with *Cf-2* or *Cf-5* present or absent were used to screen for RAPDs linked to the resistance genes, and these polymorphisms were similarly mapped on the segregating populations. The second part of this study was to generate recombinants

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between the visible marker *yv*, and the *Cf-2* and *Cf-5* genes. As well as confirming the position of these genes on the RFLP map, these recombinants are of value in ordering new probes, and in deciding when to initiate chromosome walking experiments to locate and clone the resistance genes.

RESULTS

RFLP mapping around *Cf-2* and *Cf-5*.

Figure 1 shows the order of the RFLP markers used in this study based on a compilation of published maps of chromosome 6 (Messeguer *et al.* 1991; Klein-Lankhorst *et al.* 1991). Initial testcross data indicated that *Cf-2* and *Cf-5* were linked to the RFLP marker TG232 and to the visible marker *yv* (Jones *et al.* 1993). Two hundred sixty-eight *Cf2* × *L. pennellii* F_2 plants and 159 *Cf5* × *L. pennellii* F_2 plants were screened with the fungus for resistance/susceptibility (Table 1). The susceptible plants were then screened with the RFLP markers listed in Table 2; the results are summarized in Tables 3 and 4.

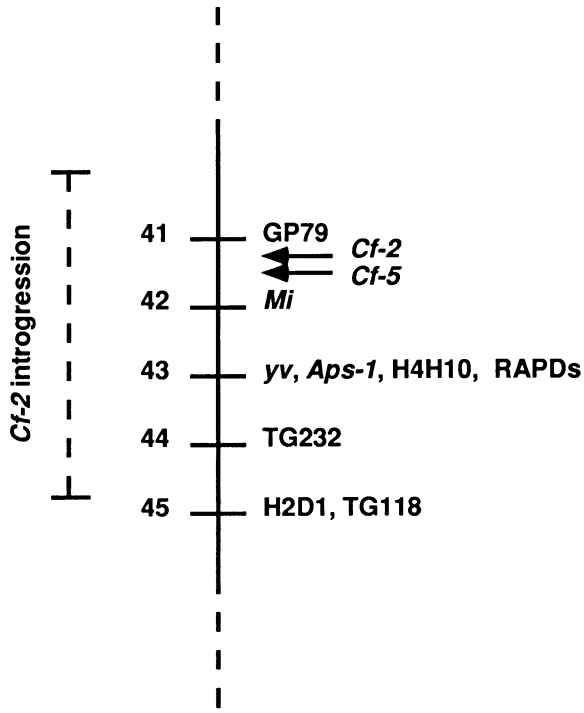

Fig. 1. Integrated linkage map of chromosome 6 in the region of the *Cf-2* and *Cf-5* resistance genes. Because of the high variation in recombination distances obtained from different crosses, the map location numbers shown are only a guide for positioning this region on the classical map of tomato. The cluster of RAPDs at position 43 comprised $OPB12_{0510}$, $OPL2_{0400}$, $OPN2_{0400}$, and $OPN20_{1200}$.

Most of the plants were homozygous for *L. pennellii* RFLPs throughout the region, as would be expected for susceptible plants. Those plants that were heterozygous were then used to position the resistance genes. In the cross with *Cf-2*, four of the 45 susceptible plants tested with *Aps-1* were heterozygous. All of these and two additional plants were also heterozygous for TG232. These plants and an additional three were heterozygous for H2D1, and a further two plants were heterozygous for TG118. None of the 45 plants tested was heterozygous for GP79. This indicates that *Cf-2* is most tightly linked to GP79, although it is not possible to conclude from these data which side of this marker the resistance gene lies.

In the cross with *Cf-5*, two of the 29 susceptible plants tested were heterozygous for *Aps-1*. These were also heterozygous with H4H10 and TG232, and an additional plant was heterozygous with TG118. One individual out of the 29 was heterozygous for GP79, and this plant was homozygous for all the other markers. Similarly, all of the plants that were heterozygous for *Aps-1* were homozygous for GP79. This indicates that *Cf-5* is located between the markers *Aps-1* and GP79.

The recombination frequency between *Cf-2* and *Aps-1* was 4.4 ± 2.2%, and there was no recombination between *Cf-2* and GP79, giving a recombination value r = 0% with an upper limit of 6.3% at $P = 0.05$. By contrast the data for *Cf-5* gave recombination values of 3.4 ± 2.4% between *Cf5* and both TG232 and *Aps-1*, and a recombination value of 1.7 ± 1.7% between *Cf-5* and GP79.

RAPD markers linked to *Cf-2* and *Cf-5*.

More than 300 random 10-mer primers were screened on the Cf0, Cf2, and Cf5 NILs. From these, three polymorphisms were found linked to *Cf-2*, with the primers B12, L2, and N2. These linked polymorphic loci were designated $OPB12_{0510}$, $OPL2_{0400}$, and $OPN2_{0400}$, respectively (the subscripts indicate the band sizes in base pairs). A fourth primer, D16, amplified a Cf2-specific locus designated $OPD16_{0750}$ that was unlinked to *Cf-2*. When primers B12, L2, and N2 were used on resistant progeny from a testcross

Table 1. Segregating F_2 populations used for mapping *Cf-2* and *Cf-5*

	Phenotype[a]						
Parents	**R**	**S**	**+R**	**+S**	**yv R**	**yv S**	**Markers analyzed**
Cf2/ *Lycopersicon pennellii*	209	59					RFLPs TG118, H2D1, TG232, *Aps-1*, and GP79
							RAPDs $OPB12_{0510}$, $OPL2_{0400}$, and $OPN2_{0400}$
Cf5/ *L. pennellii*	125	34					RFLPs TG118, TG232, H4H10, *Aps-1*, and GP79
							RAPD $OPN20_{1200}$
Cf2/LA1190			744	5	11	210	RFLPs *TG232, Aps-1*, and GP79
							RAPDs $OPB12_{0510}$, $OPL2_{0400}$ and $OPN2_{0400}$
Cf5/LA1190			170	6	2	56	RAPD $OPN20_{1200}$

[a] R = resistant; S = susceptible; + = wild type; yv = yellow virescent.

of Cf2, the specific bands were found in all of them (data not shown). The results using these primers on susceptible progeny from F_2 crosses are listed in Table 3. These data indicate that all the plants that were heterozygous for *Aps-1* contained the RAPD bands, whereas all the individuals which were homozygous for the *L. pennellii* allele of *Aps-1* lacked the RAPD band. This indicates that the three RAPD bands are all on the *Aps-1* side of the recombination sites. For Cf5, one RAPD band was found linked to *Cf-5* using the primer N20. The linked polymorphic locus was designated $OPN20_{1200}$. The segregation of this band is shown in Table 4. As with the Cf2 RAPD bands, this band segregated on the *Aps-1* side of the recombination sites.

Extent of introgression.

As well as detecting polymorphisms between *L. pennellii* and *L. esculentum,* some of the probes used also detected polymorphisms between the NILs. These results are shown in Table 2. For Cf0 and Cf2 there were detectable polymorphisms with GP79, *Aps-1,* H4H10, and TG232, but no polymorphisms with H2D1 and TG118. This suggests that the introgressed region in Cf2 extends from a point distal to GP79 to a point between TG232 and H2D1. The three Cf2-specific RAPD bands were also present in *L. pimpinellifolium* LA100 (data not shown), confirming the *L. pimpinellifolium* origin of the introgressed segment carrying *Cf-2.* No RFLPs were found between Cf0 and Cf5 with any of the probes, and the only detectable RAPD polymorphism between these lines was with RAPD primer N20. However, this RAPD band was not detected in the *L. pimpinellifolium* line LA100 (data not shown), but was present in the *L. esculentum* var. *cerasiforme ms-32* line LA359 (results not shown), indicating that the origin of the *Cf-5* gene may have been *L. esculentum* var. *cerasiforme.* This origin was confirmed by obtaining the line PI 187002, which was found to be listed as an *L. esculentum* var. *cerasiforme* accession, from the USDA-ARS Plant Genetic Resources Unit, Cornell University, Ithaca, NY.

Table 2. Restriction fragment length polymorphism markers used for mapping *Cf-2* and *Cf-5*

		Restriction enzymes used to reveal RFLPs between:			
Probe	**Source[a]**	**LA716[b]/Cf2**	**LA716/Cf5**	**Cf0/Cf2**	**Cf0/Cf5**
GP79	C.G.	*Dra*I	*Eco*RV	*Eco*RV	None[c]
Aps-1	V.W.	*Dra*I	*Dra*I	*Eco*RI	None
H4H10	P.Z.	*Eco*RV	*Eco*RV	*Dra*I	None
TG232	S.T.	*Eco*RV	*Eco*RV	*Bst*NI	None
H2D1	P.Z.	*Eco*RI	*Eco*RI	None	None
TG118	S.T.	*Eco*RV	*Eco*RV	None	None

[a] C.G. = Christina Gebhardt, Max Planck Institute, Cologne; V.W. = Valerie Williamson, University of California, Davis; P.Z. = Pim Zabel, Agricultural University, Wageningen; S.T. = Steve Tanksley, Cornell University.
[b] LA716 = *Lycopersicon pennellii.*
[c] None = none of the enzymes tested, *Dra*I, *Eco*RV, *Eco*RI, *Bst*NI, and *Hin*dIII, revealed a polymorphism.

Table 3. Analysis of restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers in susceptible F_2 plants from crosses between Cf2 and *Lycopersicon pennellii* LA716

RFLP[a]/RAPD[b] marker phenotype						No. of
TG118	**H2D1**	**TG232**	***Aps-1***	***OPB12$_{0510}$***[c]	**GP79**	**plants**
P	P	P	P	−	P	34
H	P	P	P	−	P	2
H	H^{pi}	P	P	−	P	3
H	H^{pi}	H^{pi}	P	−	P	2
H	H^{pi}	H^{pi}	H^{pi}	−	P	0
H	H^{pi}	H^{pi}	H^{pi}	+	P	4
P	P	P	P	−	H^{pi}	0

[a] P = homozygous for *L. pennellii* RFLPs; H = heterozygous for *L. esculentum* and *L. pennellii* RFLPs; H^{pi} = heterozygous for *L. pimpinellifolium* and *L. pennellii* RFLPs.
[b] − = RAPD band absent; + = RAPD band present.
[c] The RAPD markers $OPL2_{0400}$ and $OPN2_{0400}$ gave the same result as $OPB12_{0510}$.

Recombination with *yv*.

Three-point linkage data from the F_2 of crosses between Cf2/Cf5 and LA1178 (*yv-coa-c*) had shown that both *Cf-2* and *Cf-5* were located about 2% recombination proximal to *yv* (Jones *et al.* 1993). To generate more recombinants within this region, 970 Cf2 × LA1190 and 234 Cf5 × LA1190 F_2 plants were screened for resistance/susceptibility to the fungus. Recombination between *Cf-2*/*Cf-5* and *yv* would result in F_2 plants which were either green and susceptible or yellow virescent and resistant. The results are shown in Table 1. A total of five green/susceptible recombinants were obtained in the *Cf-2* cross, and 11 yellow virescent/resistant plants. For *Cf-5,* six green susceptible plants and two yellow virescent/resistant recombinants were obtained.

The *Cf-2*/*yv* recombinants were screened with RAPD primers B12, L2, and N2, and with the probes GP79, *Aps-1,* and TG232, which showed polymorphisms between Cf0 and Cf2. All five green susceptible plants gave identical results with the probes and similarly all the 11 *yv*-resistant plants gave identical results. The results for representative plants are shown in Figures 2 and 3A. The green susceptible plants show the Cf2-specific RAPD bands and have both the Cf0 and Cf2 forms of TG232 and *Aps-1.* However, these plants only have the Cf0 form of GP79. Conversely, the *yv*-resistant recombinants do not have the RAPD bands, and have only the Cf0 form of TG232 and *Aps-1,* but show both the Cf0 and Cf2 forms of GP79. This indicates that all the recombination events have occurred

Table 4. Analysis of restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers in susceptible F_2 plants from crosses between Cf5 and *Lycopersicon pennellii* LA716

RFLP[a]/RAPD[b] marker phenotype						No. of
TG118	**TG232**	**H4H10**	***Aps-1***	***OPN20$_{1200}$***	**GP79**	**plants**
P	P	P	P	−	P	25
H	P	P	P	−	P	1
H	H	P	P	−	P	0
H	H	H	P	−	P	0
H	H	H	H	−	P	0
H	H	H	H	+	P	2
P	P	P	P	−	H	1

[a] P = homozygous for *L. pennellii* RFLPs; H = heterozygous for *L. esculentum* and *L. pennellii* RFLPs.
[b] − = RAPD band absent; + = RAPD band present.

between GP79 and the other markers, suggesting that *Cf-2* is between GP79 and the *Aps-1, yv,* RAPD cluster of markers.

To confirm this location, probe GP79 and the Cf2-specific RAPD primers were used on Ontario 7620 which is a *Cf-2-Mi* recombinant line (Kerr *et al.* 1980). The presence of *Cf-2* in this line was confirmed by separate inoculations with races 0, 2, and 5 of the fungus. This line had the *L. esculentum* form of GP79 after *Eco*RV digestion, and lacked the Cf2 RAPD bands (results not shown), indicating that the remaining *L. pimpinellifolium* introgressed DNA does not extend to GP79 or the *Aps-1, yv,* RAPD cluster of markers, and confirming that the *Cf-2* gene lies between GP79 and *Aps-1*. The parental *Mi* line for Ontario 7620 was Anahu, which carries the *Mi* gene on a large segment of *L. peruvianum* DNA that does not extend as far as the *Aps-1* locus (Messeguer *et al.* 1991). At least two recombination events must have occurred to produce the Ontario 7620 *Mi-Cf-2* chromosome with *L. esculentum* alleles at the GP79 and *Aps-1* loci. These data do not orient the *Cf-2* and *Mi* loci relative to these two RFLP markers. Further analysis of this line, and linkage analysis of *Cf-2* and *Mi* with additional RFLP and visible markers such as *tl* and *yv* (Jones *et al.* 1993) will be needed to address this question.

For the Cf5/*yv* recombinants, only the RAPD$OPN20_{1200}$ could be mapped relative to *Cf-5* (Fig. 3B). As with the Cf2-specific RAPD bands, $OPN20_{1200}$ mapped on the *yv* side of all the recombinants, indicating that this marker is probably also in the *Aps-1, yv,* RAPD cluster of markers.

DISCUSSION

As part of a map-based strategy for cloning the *Cf-2* and *Cf-5* resistance genes from tomato, closely linked flanking markers have been identified as possible starting points for chromosome walking, and recombinants have been generated between the resistance genes and a linked visible marker. From this analysis, further evidence has been obtained that a cluster of resistance genes exists on chromosome 6.

Cf-2 was previously reported to be at a position 8 cM from *Mi* by Kerr *et al.* (1980). While *Mi* has subsequently been repositioned relative to *yv* and other chromosome 6 markers (Koornneef and Zabel 1990), *Cf-2* has not been remapped and has been assumed to be 8 cM from *Mi*. Our initial mapping, reported in the accompanying paper, showed that *Cf-2* and *Cf-5,* like *Mi* were approximately 1–2 cM from *yv,* and the three-point data obtained located them proximal to *yv* (Jones *et al.* 1993). By combining these data together with our RFLP mapping data and previously published mapping data for *Mi* (Messeguer *et al.* 1991; Klein-Lankhorst *et al.* 1991), an integrated map of the *Cf-2, Cf-5, Mi* region of chromosome 6 has been constructed (Fig. 1).

The order of probes on this map is consistent with the previously published data. As with the data presented by Messeguer *et al.* (1991), the recombination distances depended on the parents used in the cross. In their study, the distance between TG232 and GP79 was 4.7% in the F_2 from a cross between Vendor and *L. pennellii.* From

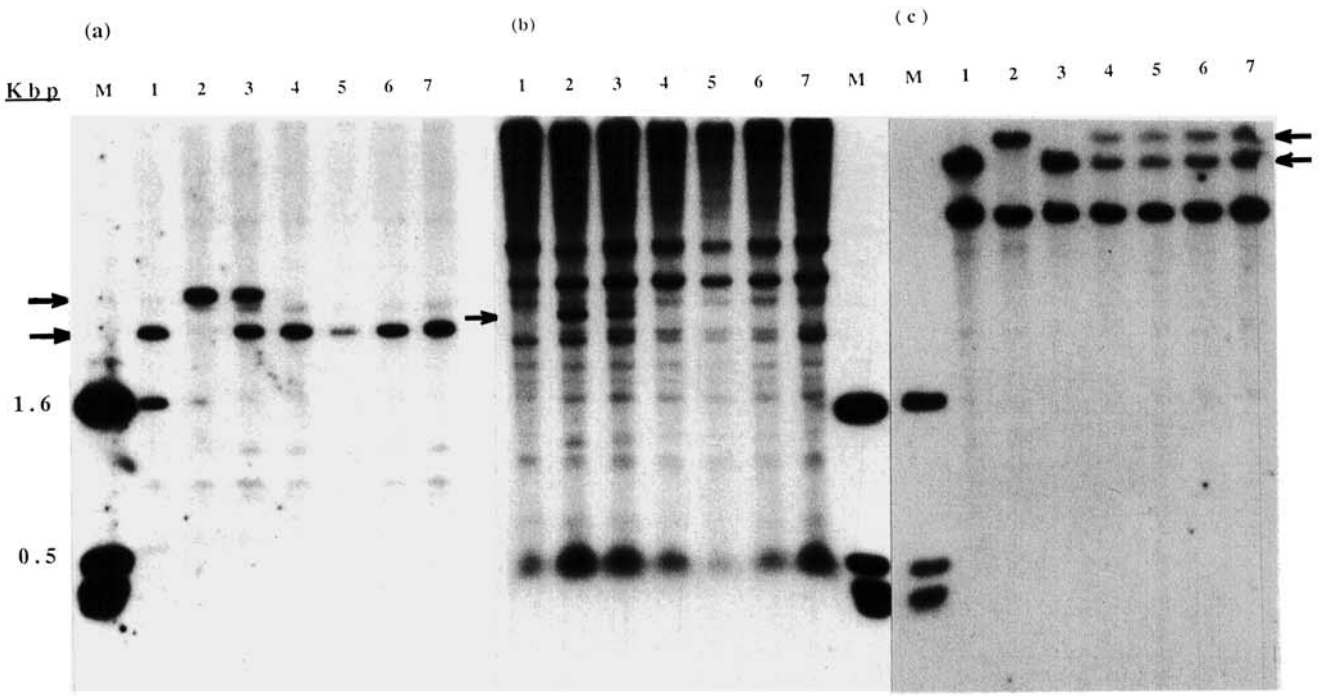

Fig. 2. Southern blots of recombinant F_2 progeny from the Cf2 × LA1190 *yv* cross. Lane 1, Cf0; lane 2, Cf2; lane 3, green susceptible recombinant; lanes 4–7, yellow virescent resistant recombinants. **A,** DNA cut with *Bst*NI and probed with TG232; **B,** cut with *Eco*RI and probed with *Aps-1*; **C,** DNA cut with *Eco*RV and probed with GP79. The positions of the Cf0 and Cf2 specific bands for TG232 and GP79 are arrowed. For the *Aps-1* polymorphism there appears to be no Cf0 specific band corresponding to the Cf2 specific band.

our data, this value is 5.6% in the F_2 progeny from the cross between Cf2 and *L. pennellii,* and 5.1% in the F_2 progeny from the cross between Cf5 and *L. pennellii.* However, in the F_2 populations from their crosses of VFNT cherry × *L. cheesmanii* and VFNT cherry × Moneymaker the distance from GP79 to *Aps-1* was reduced to 0.3–0.4%. Similarly, our F_2 data for the *yv* crosses gave values of 1–2%, recombination between *yv* and *Cf-2/Cf-5.*

The data for RFLP and RAPD differences between the NILs give insight into the origin of the *Cf* genes and the size of the introgressed DNA. In Cf2, there are RFLP differences extending from a point between H2D1 and TG232 to a point between GP79 and the tip of the short arm, consistent with the *L. pimpinellifolium* origin of *Cf-2* and defining the extent of introgression. The extent of this region in physical terms is unknown, particularly in view of the different recombination values in different crosses. In the Ontario 7620 line, the *L. pimpinellifolium* DNA does not extend to GP79 or the *Aps-1* cluster of markers, suggesting that the amount of introgressed DNA carrying the *Cf-2* gene in this line is reduced. In the case of Cf5, only one RAPD primer showed a polymorphism,

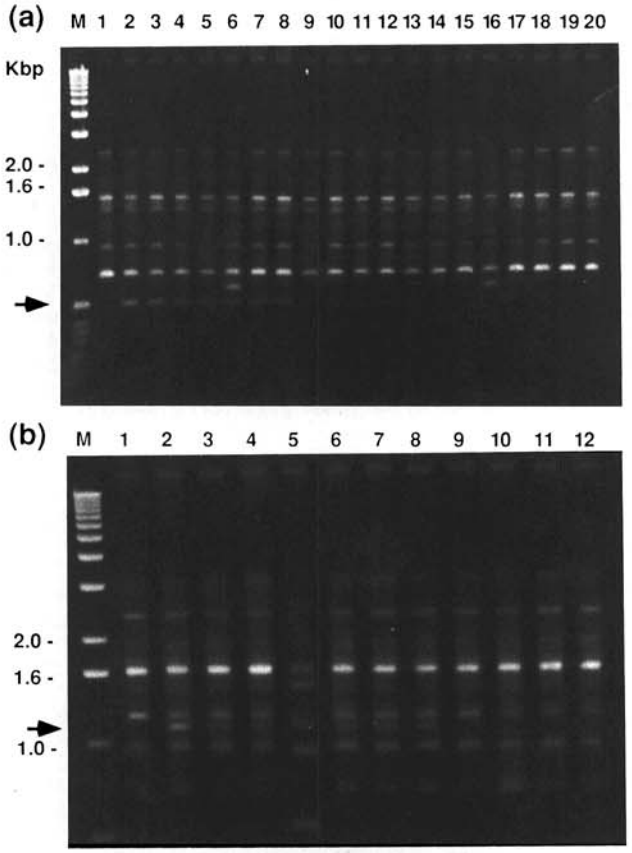

Fig. 3. A, Segregation of the Cf2-specific RAPD marker $OPB12_{0510}$ in recombinant F_2 progeny from the Cf2 × LA1190 *yv* cross. Lane 1, Cf0; lane 2, Cf2; lanes 3–8, green susceptible recombinants; lanes 9–20, yellow virescent resistant recombinants. The position of $OPB12_{0510}$ is arrowed. **B,** Segregation of the Cf5-specific RAPD marker $OPN20_{1200}$ in recombinant F_2 progeny from the Cf5 × LA1190 *yv* cross. Lane 1, Cf0; lane 2, Cf5; lanes 3–8, green susceptible recombinants; lanes 9-12, yellow virescent resistant recombinants. The position of $OPN20_{1200}$ is arrowed.

and this polymorphic band was not present in *L. pimpinellifolium* LA100, but was present in *L. e.* var. *cerasiforme* LA359. *Cf-5* was reported to originate from *L. pimpinellifolium* accession PI 187002 (Kerr *et al.* 1971), but in fact the USDA-ARS Plant Genetic Resources Centre record PI 187002 as an *L. e.* var. *cerasiforme* accession from Guatemala, confirming that the origin of *Cf-5* is *L. esculentum.*

Evidence presented in the accompanying paper (Jones *et al.* 1993) suggests that *Cf-2* and *Cf-5* may be allelic. It has also been reported that a *Cf* gene from *L. cheesmanii* is allelic to *Cf-5* (Stamova and Yordanov 1978). The occurrence of multiple allelic series of resistance genes to fungi occurs in many plant species (reviewed by Pryor 1987). In flax rust, the *K, L, M, N,* and *P* loci contain 1, 13, 7, 3, and 5 alleles respectively. In barley, 12 alleles are known at the *Ml-a* locus and in maize there are 14 known alleles at the *Rp1* locus. The clustering of resistance genes to more than one pathogen has also been reported. In maize, resistance to *Puccinia polysora (Rpp9)* lies 1.6 map units from the *P. sorghi Rp1* locus (Pryor 1987). RFLP mapping of the tomato *I-1* gene (resistance to *Fusarium oxysporum*) and the potato *Gro1* gene (resistance to the nematode *Globodera rostochiensis*) also places these loci at nearly identical positions (Barone *et al.* 1990; Sarfatti *et al.* 1991).

The data presented in this paper place *Cf-2* and *Cf-5* in the same region as *Mi.* The reasons for clustering of resistance genes are unknown. It has been postulated that resistance loci comprise complex regions within which rearrangements or recombination events (e.g., unequal crossing over) lead to the production of novel resistance specificities (Pryor 1987). It is understandable that different specificities for resistance to the same pathogen might evolve in this way. It would be remarkable if resistances to such different pathogens as a leaf-invading fungus and a root-invading nematode had arisen in this manner. If the *Cf-2/5* and *Mi* genes were derived from the same ancestral gene, then this might suggest functional similarity either in the ways the corresponding avirulence genes interact with the host or, more plausibly, a similarity in the mechanism by which such recognition activates host defenses. The latter interpretation would suggest that these resistance gene products might be bifunctional, with a variable domain that accomplishes recognition, and a conserved domain required for activation of plant defenses. Alternatively, similar locations may not reflect a functional relationship between *Cf-2/5* and *Mi,* but a localized mechanism for creating genetic variation in specificity.

The true relationship between these resistance genes will only be revealed with their isolation. The identification of closely linked visible markers to facilitate the recovery of progeny recombinant for *Cf-2/Cf-5,* together with the generation and ordering of additional molecular markers within this region, should make it possible to walk to and clone these resistance genes.

MATERIALS AND METHODS

Plant material and segregating populations.

Near-isogenic lines of the susceptible *L. esculentum* cultivar Moneymaker carrying *Cf-2* or *Cf-5* (designated Cf2

and Cf5, respectively), were obtained from R. Oliver (University of East Anglia, Norwich) and their authenticity confirmed as described in the accompanying paper (Jones *et al.* 1993). These are true breeding NILs developed at the Centre of Genetic Resources (CPRO, the Netherlands) by backcrossing (×5) the donor parents to Moneymaker (designated Cf0) as the recurrent parent and selfing for five generations (Tigchelaar 1984). *L. pennellii* LA716, *L. esculentum* LA1190 (*yv*), *L. e.* var. *cerasiforme* LA359 (*ms-32*) and *L. pimpinellifolium* LA100 were provided by C. Rick (Tomato Genetic Resource Centre, Davis, CA). Ontario 7620, a recombinant line carrying both *Mi* and *Cf-2* (Kerr *et al.* 1980), was provided by H. Laterrot (INRA, Montfavet, France).

Four segregating populations were used for mapping the *Cf-2* and *Cf-5* loci. The parents for each of these populations along with the segregation data and RFLP and RAPD markers used are listed in Table 1. Linkage analysis was carried out as described in the accompanying paper (Jones *et al.* 1993).

Testing resistance by inoculation with *C. fulvum*.

Pure cultures of *C. fulvum* races 0, 2, and 5 were obtained from R. Oliver (University of East Anglia, Norwich) and their authenticity confirmed as described in the accompanying paper (Jones *et al.* 1993). Fungal cultures were grown on one quarter-strength potato-dextrose agar (Oxoid Ltd., Basingstoke, Hants.) for 2 wk at 28° C. Spores were washed off, and heavy suspensions were used for inoculation. Plants for inoculation were treated with the plant-growth regulator, paclobutrazol (ICI Agrochemicals, Bracknell, Berks.). This prevents etiolation of the seedlings under the conditions of high humidity required for fungal development. The paclobutrazol was applied to the compost in one treatment of 50 ml 10^{-5} M solution/500 ml of compost at the cotyledon stage. Plants were inoculated by dipping in the spore suspension at the four-leaf stage. The plants were maintained at 100% humidity in propagators for 3 days, after which the humidity was lowered to about 80% for 11 days. Symptoms of mycelial growth and profuse sporulation or resistant flecks were clearly scorable 14 days after inoculation.

DNA extraction/RFLP analysis.

Nuclear DNA was extracted essentially as described by Bernatzky and Tanksley (1986), except that the 2 g of frozen leaf material was ground to a fine powder in liquid nitrogen using a mortar and pestle before transfer to DNA extraction buffer. DNA was digested with the appropriate restriction endonuclease according to the suppliers (Northumbria Biologicals Limited, Cramlington, Northumberland; Bethesda Research Laboratories, Gaithersburg, MD) directions, separated in 0.8% agarose gels and Southern blotted onto GeneScreen Plus membranes (Du Pont Co., Wilmington, DE). Filters were probed with DNA labeled with ^{32}P-dCTP (Du Pont) by the random hexamer method (Sambrook *et al.* 1989) using a multiprime kit (Amersham, Aylesbury, Bucks.) and washed according to the manufacturer's instructions. Final washes were at 65° C in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 sodium citrate), 0.1% sodium dodecyl sulfate. The sources of the probes used in this study are listed in Table 2. Inserts for radiolabeling were prepared by digestion of the plasmid DNA and electroelution from agarose gels.

RAPD analysis.

DNA from the NILs were used as templates for polymerase chain reaction amplification using single oligonucleotide primers. The 10-mer oligonucleotides used were from the commercially available RAPD primer kits A to P (Operon Technologies, Alameda, CA). The reaction conditions were as described by Williams *et al.* (1990) for amplification of RAPDs. Approximately 20 ng of DNA was used as template in a 25-μl reaction volume containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.9 mM $MgCl_2$, 0.01% gelatin, 100 μM each dNTP, 0.2 μM primer, and 1 unit of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT). Amplification was performed using a Perkin Elmer Cetus thermocycler for 35 cycles. Each cycle consisted of 30 sec at 94° C, 30 sec at 31° C, 15 sec at 45° C, and 1 min at 72° C. The 35 cycles were followed by a period of 10 min at 72° C, and the reactions were held at 4° C until assayed. Linkage of polymorphic bands to *Cf-2* or *Cf-5* was determined by using the primers on populations segregating for resistance to the fungus. The sequences of the primers that gave products that distinguished the NILs are: B12, CCTTGACGCA; D16, AGGGCGTAAG; L2, TGGGCGTCAA; N2, ACCAGGGGCA; N20, GGTGCTCCGT.

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