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High resolution RFLP map around the root knot nematode resistance gene (*Mi*) in tomato

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Summary. In the 1940's the root-knot nematode resistance gene (*Mi*) was introgressed into the cultivated tomato from the wild species, *L. peruvianum*, and today it provides the only form of genetic resistance against this pathogen. We report here the construction of a high resolution RFLP map around the *Mi* gene that may aid in the future cloning of this gene via chromosome walking. The map covers the most distal nine map units of chromosome 6 and contains the *Mi* gene, nine RFLP markers, and one isozyme marker (*Aps-1*). Based on the analysis of more than 1,000 F_2 plants from four crosses, we were able to pinpoint the *Mi* gene to the interval between two of these markers – *GP79* and *Aps-1*. In crosses containing the *Mi* gene, this interval is suppressed in recombination and is estimated to be 0.4 c*M* in length. In contrast, for a cross not containing *Mi*, the estimated map distance is approximately 5 times greater (ca. 2 c*M*).

Using RFLP markers around *Mi* as probes, it was possible to classify nematode resistant tomato varieties into three types based on the amount of linked *peruvianum* DNA still present. Two of these types (representing the majority of the varieties tested) were found to still contain more than 5 c*M* of *peruvianum* chromosome – a result that may explain some of the negative effects (e.g. fruit cracking) associated with nematode resistance. The third type (represented by a single variety) is predicted to carry a very small segment of *peruvianum* DNA (<2 c*M*) and may be useful in the identification of additional markers close to *Mi* and in the orientation of clones during a chromosome walk to clone the gene.

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Introduction

Root-knot nematodes (*Meloidogyne* spp) cause serious damage in many crops worldwide (Taylor and Sasser 1978). Their invasion of the root system of host plants results in a shallow, knotted root system, and as soil borne pathogens they can cause repeated crop failures (Wang and Bergeson 1974). While a number of soil fumigants are effective against nematodes, the most economical and environmentally safe method of control is through the use of resistant crop varieties (Fassuliotis 1979).

Tomato is one of the crops in which genetic resistance has been especially effective against root-knot nematodes. Although cultivated tomatoes are naturally susceptible to nematodes, certain accessions of the wild tomato species *L. peruvianum* are highly resistant. During the 1940's, a single dominant gene, *Mi*, which confers high levels of resistance to root-knot nematodes, was transferred from *L. peruvianum* PI 128657 into the domestic tomato (Smith 1944). This gene confers resistance not only to most races of *Meloidogyne incognita* (the most prevalent species in temperate growing areas) but also to *M. javanica* and *M. arenaria* (Barham and Winstead 1957; Roberts and Thomason 1986). Today, all nematode resistant tomato varieties carry the *Mi* gene (Medina-Filho and Stevens 1980).

The *Mi* gene is unique among the nematode resistance genes previously described in other crop plants in that it confers resistance to a wide range of nematode patho-

types, it is relatively stable (i.e., rarely has been overcome by new races), and it has been genetically mapped. The *Mi* locus resides near the centromere on chromosome 6 and is tightly linked to a gene encoding acid phosphatase, *Aps-1* (Gilbert 1958; Rick and Fobes 1974).

If the *Mi* gene could be isolated, it might provide valuable insight into how plants protect themselves against nematodes. Currently, little is known about this process at the molecular level. If cloned, *Mi* could also be transferred, via transformation, into other susceptible crop species in the hope that this gene would also provide protection against nematodes. In eggplant (another solanaceous species), for example, there is no known genetic resistance to root-knot nematodes (Fassuliotis 1979).

The objective of the research reported here was to establish a high resolution RFLP map around the *Mi* gene in tomato. Such a map provides a starting point for a chromosome walk to this gene and also results in markers for detecting the presence of this gene in breeding populations. To achieve this goal, we have analyzed a series of nearly-isogenic lines (NILs) for *Mi* as well as four segregating populations comprising more than 1,000 plants. As a byproduct of this mapping research, we have also generated several new tomato stocks with crossovers near *Mi*. These recombinant stocks may be useful in placing new markers around *Mi* as well as for orienting clones during a chromosomal walk to clone the gene. This research also sheds light on the effectiveness of the breeding process by which *Mi* was originally transferred into the genome of the cultivated tomato and the varying rates of meiotic recombination around *Mi* in different populations.

Materials and methods

Plant material and segregating populations

L. esculentum cv 'Vendor Tm2a' was provided by M. Mutschler, Cornell University, Ithaca, N.Y.; *L. pennellii* LA716, *L. cheesmanii* LA483, and *L. esculentum* cv 'Anahu' (LA655) were provided by C.M. Rick, University of California Davis, Calif.; *L. esculentum* cv 'Moneymaker' was provided by H. Laterrot, INRA, Montfavet, France. All nematode resistant varieties used in this study along with their sources are listed in Table 1.

Four segregating populations were used for genetic mapping around the *Mi* locus. Parents corresponding to each of these populations as well as the markers scored in each are given in Table 2. F_2 populations from each of these crosses were analyzed for RFLP markers as well as for the isozyme marker *Aps-1*. In the crosses 'VFNT Cherry' × *L. cheesmanii* and 'Moneymaker' × 'VFNT Cherry', F_3 seeds from plants recombinant for markers in the vicinity of *Mi* were also tested for resistance to *M. incognita* as described in the section below.

Nematode testing

Nematode testing was performed at the Campbell Institute for Research and Technology using the procedure described by Ammati et al. (1985). Individual plants were scored for the resistance reaction on a scale of 0 (no galls) to 4 (more than 50% galls). Plants were considered resistant only if they received a rating of zero. The genotype at the *Mi* locus of selected F_2 plants was deduced in this way from progeny tests of at least 20 F_3 progeny.

Aps-1 analysis

Crude extracts of young leaves were made for isozyme analysis. Procedures for starch gel preparation and electrophoresis were as described by Shields et al. (1983). The Aps-specific stain was as published by Medina-Filho and Tanksley (1983).

RFLP probes

Two strategies were employed for obtaining RFLP markers closely linked to the *Mi* gene. The first approach employed the

Table 1. Nematode resistant tomato varieties analyzed for markers around the *Mi* gene

Variety	Source	Markers											
		TG297	*CD14*	*Tom25*	*GP79*	*Mi*	*Aps-1*	*CD67*	*TG274A*	*TG97*	*TG178*	*TG231*	*TG232*
VFNT Cherry	UCD	+	+	+	+	+	+	+	+	+	+	+	−
VFN8	UCD	+	+	+	+	+	+	+	+	+	+	+	−
Short Red Ch.	UCD	+	+	+	+	+	+	+	+	+	+	+	−
N1401	Peto	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	−
N1200	Peto	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	−
N1400	Peto	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	−
X2274	CIRT	+	+	+	+	+	−	−	−	−	−	−	−
X1965	CIRT	+	+	+	+	+	−	−	−	−	−	−	−
X2020	CIRT	+	+	+	+	+	−	−	−	−	−	−	−
Camp. 25 Mi	CIRT	+	+	+	+	+	−	−	−	−	−	−	−
Anahu	UCD	+	+	+	+	+	−	−	−	−	−	−	−
Monita	Laterrot	+	+	+	+	+	−	−	−	−	−	−	−
Motabo	Laterrot	+	+	+	+	+	−	−	−	−	−	−	−
Motelle	Laterrot	−	−	−	−	+	−	−	−	−	−	−	−

+, *peruvianum* allele; −, *esculentum* allele; +/−, heterozygous; UCD, University of California, Davis Tomato Genetics Cooperative; Peto, Peto Seed Company, Woodland, CA; CIRT, Campbell Institute for Research and Technology, Davis, CA; Laterrot, see Laterrot (1987)

Table 2. Segregating populations used for construction of the high resolution map around the *Mi* gene in tomato

Parents	Generation analyzed	No. progeny analyzed	Markers assayed
Moneymaker/VFNT Cherry[a]	F2	239	*TG297, CD14, Tom25, GP79, Mi, Aps-1, TG274A, CD67, TG97, TG178, TG231*
VFNT Cherry[a]/*L. cheesmanii* (LA483)	F2	450	*TG297, Tom25, GP79, Mi, Asp-1, TG274A, CD67, TG178, TG231*
Vendor Tm2a/*L. pennellii* (LA716)	F2	400	*TG297, CD14, Tom25, GP79, TG274A, CD67, TG97, TG178, TG231, TG232*
Anahu[a]/VFNT Cherry[a]	F2	289	*Aps-1, TG274A, CD67, TG97, TG178, TG231*

[a] Lines carrying the *Mi* gene

Table 3. Molecular markers used for mapping around the *Mi* gene

Probe	Type	Source	Restriction fragment size[a]							
			EcoRI		EcoRV		XbaI		HindIII	
			E	P	E	P	E	P	E	P
Aps-1	Isozyme									
CD14	cDNA	CNL	–	–	6.0	11.5	–	–	14	9.3 1.6
TG297	PstI genomic	CNL	1.9	2.5	3.8	5.7 1.5	–	–	–	–
Tom25	cDNA	UN	3.7	6.5	–	–	–	–	10.5 3.5	13.5 9.2 3.6 2.9
GP79	PstI genomic	MP	4.3	6.0	8.8	17.0	8.2 4.6	7.6 4.9	6.4	9.0 4.9 3.5
TG274A	PstI genomic	CNL	–	–	4.3	5.7	–	–	8.0	4.7
CD67	cDNA	CNL	–	–	7.4	9.0 4.4	4.1	11.5	5.3	8.7
TG97	Sheared genomic	CNL	7.6 4.5	8.4 7.0	5.2 2.9	1.6	5.4	4.5	–	–
TG178	PstI genomic	CNL	5.3 2.4	4.9 1.8	6.4	7.5 5.5	–	–	15.5	8.6
TG231	PstI genomic	CNL	17.5	13.5	–	–	9.0	13.5 7.0	8.2	16.0

CNL, Cornell University; MP, Max-Planck Institute, Cologne, Germany (Gebhardt et al. 1989); UN, University of Nottinghem (Dr. D. Grierson), Nottingham, England (Kinzer et al. 1990); E, *L. esculentum* (susceptible genotype) DNA; P, *L. peruvianum* (resistant genotype) DNA

[a] All sizes are in kilobases. (–) Denotes that either E and P did not show different bands or in a few cases that the size of the bands was not determined. In all cases only the bands that are different between E and P are listed; bands in common are omitted

use of nearly isogenic lines (NILs) and multi-probing as described by Young et al. (1988). Approximately 750 clones from a PstI genomic library of tomato (1–2 kb inserts) were probed in sets of five to ten clones simultaneously onto DNA from 'Moneymaker' (susceptible) and its resistant counterparts, 'Monita' and 'Motelle'. Clones showing a polymorphism between 'Moneymaker' and either one of the resistant NILs were identified and saved for genetic mapping.

The second approach was to identify clones that had previously been mapped to the region of chromosome 6 where *Mi* is reported to reside. Such clones were identified from a general tomato mapping project ongoing in this laboratory (Bernatzky

and Tanksley 1986b; Ganal et al. in preparation) as well as with mapping data published by Kinzer et al. (1990). One clone GP79 was mapped in potato to the homeologous counterpart of chromosome 6 in tomato (Gebhardt et al. 1989). Table 3 summarizes the types and sources of probes used in this study.

DNA extractions and Southern analysis

Nuclear DNA was isolated according to Bernatzky and Tanksley (1986a). DNA was digested with the appropriate enzyme according to the suppliers (Bethesda Research Laboratories, New England BioLabs), separated in 1.0% agarose gels, and Southern blotted onto GeneScreen Plus (DuPont) or HyBond N^+(Amersham) membranes. The filters were probed with DNA that had been radiolabelled with ^{32}P-dCTP (DuPont) by the random hexamer method (Sambrook et al. 1989).

Construction of linkage maps

Linkage maps from each segregating population were constructed separately using the 'Mapmaker' linkage program of Lander et al. (1987). Markers were ordered along the map only if their orientation was preferred with a LOD score greater than 2–3. In cases where the LOD was less than 3, the map order was verified by examining data manually to account for all crossovers in 3-point linkage tests.

Results and discussion

Genetic map around Mi

An F_2 population of 239 plants from a cross between a susceptible variety ('Moneymaker') and resistant variety ('VFNT Cherry') was used to construct a first map around *Mi*. It was already known that *Mi* is tightly linked to the isozyme locus, *Aps-1*, near the centromere on chromosome 6 (Rick and Fobes 1974; Medina-Filho 1980). Therefore, all RFLP markers previously assigned to this region of chromosome 6 (Bernatzky and Tanksley 1986a, b; Ganal et al. in preparation) as well as probes identified by multi-probing NILs (see Materials and methods) were mapped using this population (Table 2, Fig. 1B). Recombination around the *Mi* gene was very low in this population. Despite this fact, it was possible to assign *Mi* to a 0.4 c*M* interval bounded by *Tom25* and *GP79* on one side and *Aps-1*, *TG274A*, *CD67*, and *TG97* on the other side (Fig. 1B).

F_2 progeny (450 plants) from the cross 'VFNT Cherry' × *L. cheesmanii* LA483 was also examined. This population also revealed very low levels of recombination around *Mi*, but confirmed the order of markers deduced from the first cross and suggested that *Aps-1* is closer to *Mi* than are *TG274A*, *CD67*, and *TG97* (Fig. 1C).

The low levels of recombination observed to this point suggested that crossing-over might be suppressed in the region of chromosome 6 containing the *Mi* gene. With this in mind, we opted to map this region of chromosome 6 in a cross that did not contain the *Mi* gene (and thus *L. peruvianum* DNA). It had previously been shown that F_2 populations from crosses between *L. esculentum* and *L. pennellii* (LA716) yield near normal level of meiotic recombination and yet segregate for most RFLP markers (Bernatzky and Tanksley 1986b). Therefore, an

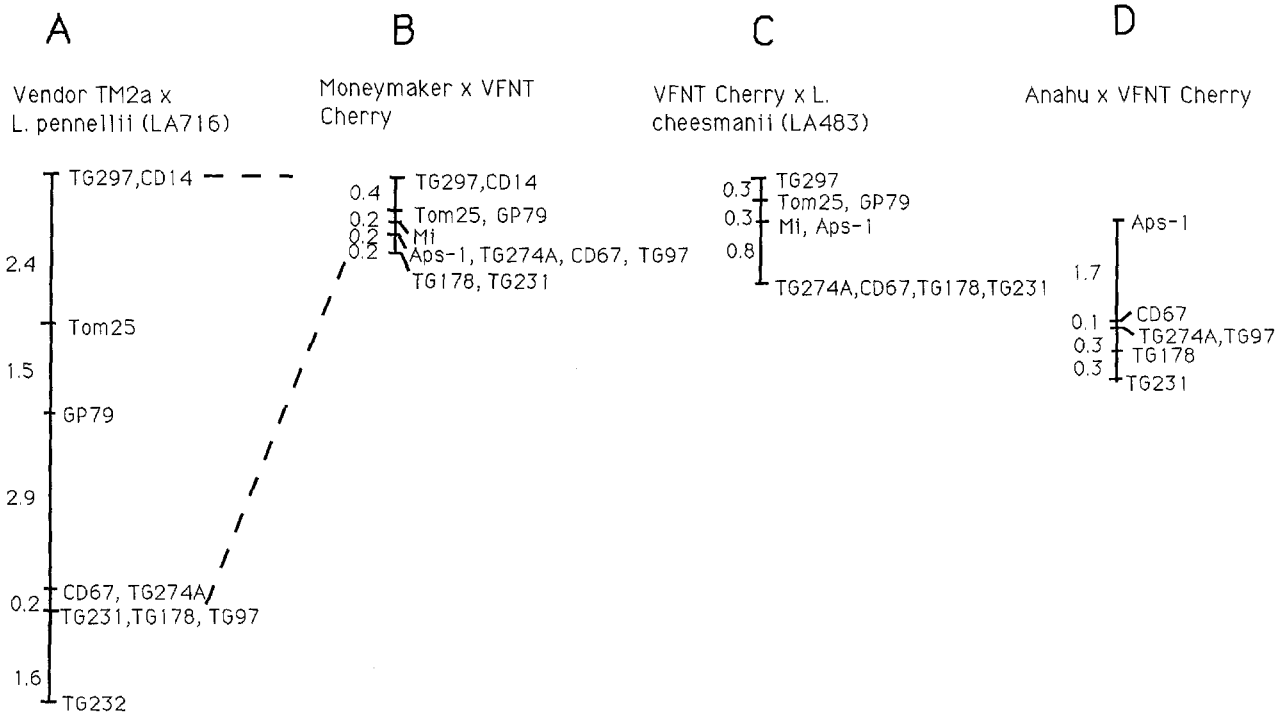

Fig. 1. Linkage maps of the region of chromosome 6 containing the *Mi* gene. Each map (*A*, *B*, *C*, *D*) corresponds to a different cross (see Table 2 for details). Centimorgans are listed on the *left* side of maps. Markers separated by *commas* showed no recombination

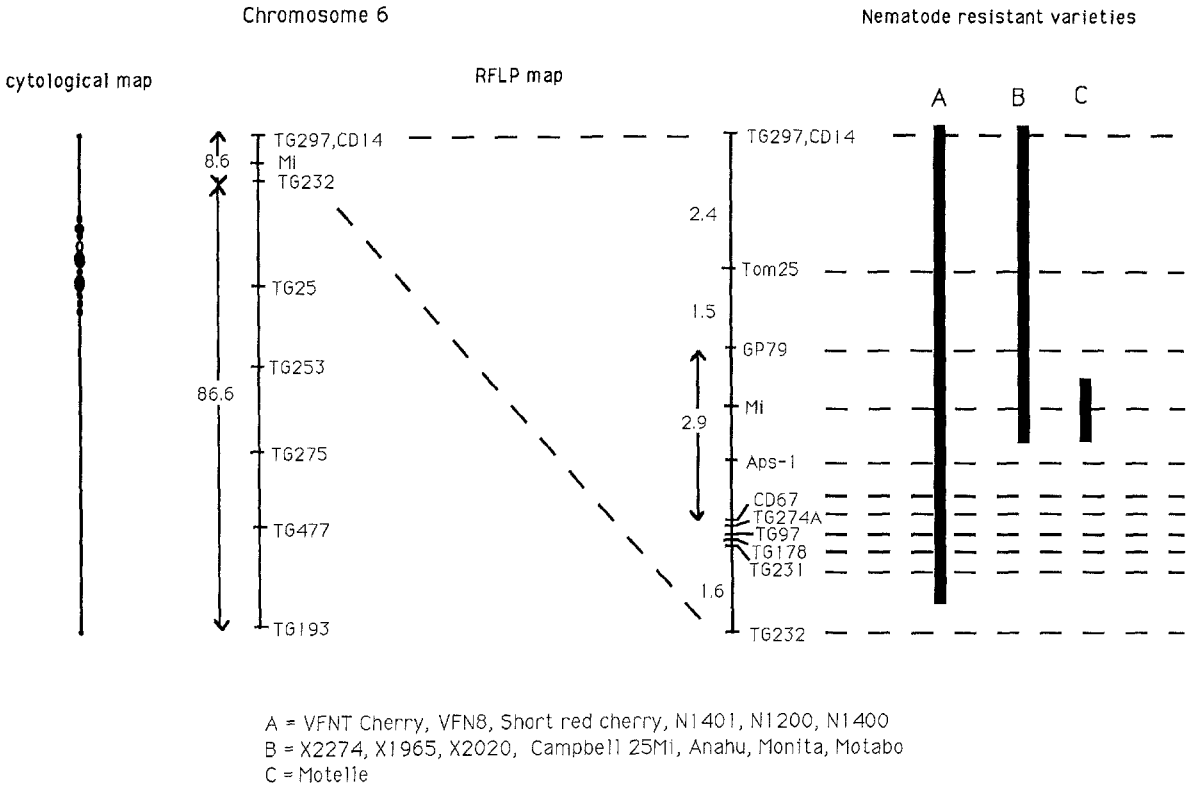

Fig. 2. Consensus linkage map of chromosome 6 with a blowup of the region containing the *Mi* gene. *Black bars* on *right* indicate segments of *peruvianum* DNA contained in different nematode resistant varieties. Resistant varieties can be classified into three groups (*A*, *B*, *C*). Cytological (pachytene) map (*left*) is from Khush and Rick (1968): *black circles* represent heterochromatin; *open circle*, centromere

F_2 population of 400 plants from the cross *L. esculentum* cv 'Vendor Tm2a' by *L. pennellii* (LA716) was probed with clones around *Mi* (Table 1).

More than a five-fold increase in recombination was observed in this region of chromosome 6 in this cross compared with other crosses that did segregate for *Mi* (Fig. 1 A). The increase in recombination cannot be attributed to any specific interval (between RFLP markers) but was manifested in all intervals throughout this region of chromosome 6 (Fig. 1 A). The cause of the reduced recombination in *Mi*-containing crosses (compared with the cross that did not contain *Mi*) is unknown, but may be due to reduced homology between the *esculentum* DNA and the *peruvianum* DNA containing the *Mi* gene. The transfer of the *Mi* gene along with associated (linked) *peruvianum* DNA may somehow be impeding normal meiotic pairing and recombination. Consistent with this notion are reports by Rick (1969) and Paterson et al. (1990) in which it is shown that the frequency of recombination between *esculentum* DNA and wild-species DNA progressively decreases as segments of wild DNA are backcrossed into the cultivated tomato genome.

The overall higher rates of recombination observed in the *L. esculentum* × *L. pennellii* F_2 allowed ordering of markers that had co-segregated in the previous two populations (Fig. 1). By pooling information from the first two maps with the map from this population, it could be deduced that *GP79* is actually closer to *Mi* on one side while the isozyme marker, *Aps-1*, is closest on the other. The closest RFLP markers on the same side of *Mi* as *Aps-1* are *CD67* and *TG274A*.

In the hope of further clarifying the map around *Mi*, a fourth population was employed ('Anahu' × 'VFNT Cherry'). Unlike previous crosses, both parents in this cross carry the *Mi* gene. However, they differ in the amount of linked DNA around the *Mi* gene (see following section, Fig. 2). 'Anahu' has *esculentum* DNA in the region containing *Aps-1*, *CD67*, and *TG274A*, whereas 'VFNT Cherry' still retains *peruvianum* DNA in this region (Fig. 2). The order of markers deduced from 289 F_2 plants is consistent with that deduced from the other populations. In addition, a single plant was obtained that was recombinant between *CD67* and *TG274A*. If this recombinant plant was the result of a single crossover (versus a double crossover), then *CD67* is closer to *Mi* than *TG274A*. It should be pointed out that this single plant is the only evidence that we currently have that *CD67* is closer to *Mi* than *TG274A*, and the order should be tested with additional segregating progeny.

A consensus map of chromosome 6, based on all four populations (and expressed in the map scale from the 'Vendor Tm2a' × *L. pennelli* cross) is presented in Fig. 2. As a point of reference, we also provide a cytological map of chromosome 6 (Fig. 2). From these data we infer than *Mi* is located in an interval bounded by the RFLP markers *GP79* and *Aps-1*. The smallest RFLP-bounded interval for *Mi* is that between *GP79* and *CD67/TG274A*. The size of this interval (in map units) varies with the cross. For those crosses involving the *Mi* gene, the interval is approximately 0.4 c*M*. However, this small map distance is probably the result of suppressed recombination in the region, and a more "normal" map distance is likely be be greater than this (ca. 2.9 c*M*) (Figs. 1, 2).

Finally, while the cytological position of *Mi* is unknown, the fact that this gene maps to the end of the chromosome 6 linkage group suggests that it is either on the short arm or very near the centromere on the long arm of this chromosome (Fig. 2). The exact physical placement of *Mi* awaits conversion of the RFLP map into a physical map and placement of the centromere on the RFLP map of chromosome 6.

Analysis of NILs and other varieties containing Mi

Since its original introgression from *L. peruvianum* more than forty years ago, the *Mi* gene has been transferred (via backcrossing) into many different tomato varieties (Medina-Filho and Stevens 1980). A sample of resistant varieties was analyzed with markers linked to the *Mi* gene (see previous section) to determine the size of the remaining *L. peruvianum* DNA still present around the *Mi* gene. Three of these varieties ('Monita', 'Motabo', and 'Motelle') represent nearly-isogenic lines that can be traced back to a common recurrent parent (Laterrot 1987). A second motivation for conducting a survey of resistant varieties was to hopefully confirm the linkage order deduced from the genetic mapping experiments described in the previous section.

Resistant varieties could be classified into three categories with respect to the amount of linked *peruvianum* DNA retained around the *Mi* gene. Varieties in the first category (Group A, Fig. 2), typified by 'VFN8', have retained *peruvianum* DNA for the entire end of the chromosome 6 linkage group, encompassing nine RFLP markers and one isozyme locus (*Aps-1*). 'N1401', 'N1200', and 'N1400' were all heterozygous at these markers, reflecting the fact that they are hybrid varieties. The simplest explanation for Group A varieties would be the occurrence of a single crossover between *TG231* and *TG232* during the initial transfer of *Mi* from *L. peruvianum* into *L. esculentum*. These varieties would thus contain *peruvianum* alleles for markers extending from *TG231* to *TG297* at the end of the chromosome 6 linkage group. Based on map units, it is estimated that 8% (8/95) of chromosome 6 is comprised of *peruvianum* DNA in these lines.

A second group of varieties (Group B) contains less *peruvianum* DNA than the group A varieties. The length of the segment has been reduced and extends only from *Mi* to the end of the linkage group (approximately 5 c*M* or 5% of chromosome 6). This group does not contain the *L. peruvianum* allele at *Aps-1* or any of the other RFLP markers past this point (moving toward the long arm) (Fig. 2). All group B varieties can be traced back to a Hawaiian variety, 'Anahu' (Medina-Filho and Stevens 1980). 'Anahu' in turn was derived from crosses with an early breeding line that presumably carried the *Aps-1* marker from *peruvianum* (and thus likely belonged to Group A) (Medina-Filho and Stevens 1980). Therefore, the simplest explanation for the derivation of Group B varieties from Group A varieties is the occurrence of a second crossover (this time between *Mi* and *Aps-1*). This would account for the lack of *peruvianum* alleles beyond *Aps-1* in Group B varieties (Fig. 2).

The third category (Group C, Fig. 2) is represented by a single variety, 'Motelle'. 'Motelle' was derived from a cross with a Group B variety ('Monita'); however, while this variety is still nematode resistant, it no longer contains *peruvianum* alleles at any of the markers tested. It seems likely that during the breeding process, a third crossover occurred, removing all *peruvianum* DNA distal from *Mi* (Fig. 2). If this interpretation is correct, then 'Motelle' contains less than 2 c*M* of the *L. peruvianum* chromosome, considerably less than all other tested cultivars.

While it is not known exactly how many generations (mostly backcrosses) have elapsed since the original cross of the cultivated tomato to *L. peruvianum* in the 1940's, the number is expected to be large and probably exceeds 20 generations in most cases (H. P. Medina-Filho personal communication). With such a large number of generations, the size of the introgressed segment should be quite small (< 5 c*M*) (Hanson 1959). While the size of the segment in Group A varieties is greater than expected, the size of the segments in Group B and Group C varieties falls very close to the expectation (Fig. 2). The larger-than-expected size of the introgressed segment in Group A varieties may be due to the reduced level of recombination in the *Mi* region of chromosome 6. A similar situation has been found for the *Tm2a* (tobacco mosaic virus resistance) gene, which has also been introgressed into the cultivated tomato from *L. peruvianum*. Despite many generations of backcrossing, relatively large pieces of linked *peruvianum* DNA still persist in tomato varieties carrying this gene (Young and Tanksley 1989).

It is important to note that results from the survey of nematode resistant varieties supports the order of markers deduced for markers flanking *Mi* based on segregating populations (see previous section). Any order of

markers other than *GP79 – Mi – Aps-1* would require multiple crossovers (e.g., double or triple crossovers) to explain the derivation of Group B from Group A varieties or Group C from Group B varieties.

Use of map and RFLP markers in breeding for nematode resistance

The direct screening of breeding material for resistance requires maintaining live nematodes, which in some instances is not practical. Moreover, since resistance is dominant, using this procedure it is not possible to identify homozygous lines without progeny testing. For this reason, a number of seed companies use the isozyme marker *Aps-1* as an indirect selection for the *Mi* gene. Because of its tight linkage with *Mi*, *Aps-1* provides a high-fidelity indicator of the presence of the resistance gene (Medina-Filho and Stevens 1980). And, because it is codominant, the isozyme marker can be used to identify homozygous, resistant plants. Unfortunately, *Aps-1* selection works only when breeding with Group A varieties, which carry the *peruvianum* allele at this isozyme locus. Group A varieties contain the largest segment of *peruvianum* DNA, and some breeders have reported deleterious effects associated with nematode resistance (e.g., fruit cracking), which may be due to other *peruvianum* genes contained within the introgressed segment (M. Kuehn personal communication). Unfortunately, group B and C varieties (which carry less *peruvianum* DNA) no longer contain the *Aps-1 peruvianum* allele, and thus the isozyme cannot be used for indirect selection of *Mi* (Medina-Filho and Stevens 1980, Fig. 2).

In this paper we report four new codominant RFLP markers (*TG297, CD14, Tom25,* and *GP79*), any one of which can be used for the indirect selection of *Mi* in both group A and group B varieties. The utility of *GP79* for this same purpose has recently been reported by Klein-Lankhorst et al. (1991). Because recombination is suppressed in modern cultivars carrying the *Mi* gene, the rate of recombination between any of these RFLP markers and the *Mi* gene is likely to be less than 1%, which is sufficient for a high fidelity test (Fig. 1). In addition, the new markers (and associated map) around *Mi* should allow breeders to select recombinants from existing progenies in which the introgressed segment contains even less *peruvianum* DNA, thus reducing the possibility of negative effects due to linked DNA.

Potential for cloning Mi via chromosome walking

GP79 and *CD67/TG274A*, which bound the interval containing *Mi*, are estimated to be 0.4 c*M* apart in crosses between resistant and susceptible varieties (Fig. 1). However, as already discussed, this distance is probably an underestimate due to suppressed recombination around *Mi*, and a more realistic estimate is 2.9 c*M*, based on a cross not containing the *Mi* gene (Figs. 1, 2). Given a C-value of 700–900 megabases for tomato (Galbraith et al. 1983) and a map of approximately 1,600 c*M* (Bernatzky and Tanksley 1986b; Ganal et al. in preparation), we estimate that, on average, 1 c*M* equals approximately 500 kb in tomato. An estimated distance between *GP79* and *CD67/TG274A* is thus more than 1.5 Mb. Using pulsed-field gel electrophoresis as described by Ganal and Tanksley (1989) we have checked the distance between *GP79* and *CD67/TG274A*, and preliminary results indicate that these two markers are more than 1 Mb apart.

Yeast artificial chromosomes can be used to clone segments of DNA up to 1 Mb, and whole libaries of clones averaging over 400 kb have been produced for human DNA (Albertsen et al. 1990). With clones this large, it is possible to walk a distance of 1 Mb or greater. *GP79* and *CD67/TG274A* may thus be used as starting points for walking to *Mi*. In addition, as a byproduct of the genetic mapping studies reported here, it has been possible to establish a number of homozygous lines with crossovers in the interval containing *Mi*, which should help both orient clones during a walk and to determine at what point the walk has passed over the target gene (Kerem et al. 1989). The group C variety ('Motelle') which contains the smallest amount of linked *peruvianum* DNA should be useful not only for orientation during a chromosome walk, but also for screening for new markers near the *Mi* gene using random RFLP clones or random PCR markers (Young et al. 1988; Martin et al. 1991).

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