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RFLP markers linked to the root knot nematode resistance gene *Mi* in tomato

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Summary. The *Mi* gene originating from the wild tomato species *Lycopersicon peruvianum* confers resistance to all major root knot nematodes (*Meloidogyne* spp.). This single dominant gene is located on chromosome 6 and is very closely linked to the acid phosphatase-1 (*Aps-1*) locus. Resistance to nematodes has been introgressed into various cultivars of the cultivated tomato (*L. esculentum*), in many cultivars along with the linked *L. peruvianum Aps-1[1]* allele. By using a pair of nearly isogenic lines differing in a small chromosomal region containing the *Mi* and *Aps-1* loci, we have identified two RFLP markers, GP79 and H6A2c2, which are located in the introgressed *L. peruvianum* region. Analysis of a test panel of 51 *L. esculentum* genotypes of various origins indicated that GP79 is very tightly linked to the *Mi* gene and allows both homozygous and heterozygous nematode-resistant genotypes to be distinguished from susceptible genotypes, irrespective of their *Aps-1* alleles. Marker H6A2c2 is linked to the *Aps-1* locus and is capable of discriminating between the *L. peruvianum Aps-1[1]* allele and the *L. esculentum Aps-1[3]* and *Aps-1[+]* alleles. In combination, these RFLP markers may provide a powerful tool in breeding tomatoes for nematode resistance.

Key words: Tomato – *Lycopersicon esculentum* – Root knot nematode resistance – *Meloidogyne* – RFLP markers

Introduction

Infection with root knot nematodes (*Meloidogyne* spp.) is an important cause of tomato (*Lycopersicon esculentum*) crop losses, especially in areas between latitudes of 35 °S and 35 °N (Baker et al. 1976; Mac Farlane et al. 1946). Infested plants show an aberrant development of the root system characterized by swellings (referred to as galls or knots), which inhibit the uptake of water and nutrients and interfere with the translocation of minerals and photosynthates (Wang and Bergeson 1974; Berlinger 1986; Hussey 1985). As a result, typical deficiency symptoms appear, such as wilting, stunted growth, and early senescence, which lead to severe yield decreases.

Although chemical control of nematodes is feasible, the adverse effects of using nematicides have made breeding for nematode-resistant tomato cultivars the method of choice in dealing with the nematode problem (Medina-Filho and Tanksley 1983). Resistance to root knot nematodes was found to be present in the wild species *L. peruvianum* (Bailey 1941), and resistant *L. esculentum* cultivars were obtained by introgressing the *L. peruvianum* resistance gene, designated *Mi*, into *L. esculentum* (Smith 1944; Watts 1947; Frazier and Dennett 1949). From the initial *L. esculentum* × *L. peruvianum* cross, one single F_1 plant was used for further breeding by repeated backcrossing. Nowadays, all known nematode-resistant *L. esculentum* cultivars are derived from this single F_1 plant.

The *Mi* gene, which confers resistance to all major *Meloidogyne* spp. known to attack tomato, is located at position 44 on chromosome 6 (Gilbert 1958; Koornneef and Zabel 1990) and is very closely linked to the leaf color marker *yv* (yellow virescent) and to the acid phosphatase-1 (*Aps-1*) locus (Gilbert 1958; Medina-Filho 1980). The fortuitous linkage of the *Mi* locus with the *Aps-1* locus has been exploited in breeding tomatoes for nematode resistance, as the *L. peruvianum Aps-1* allele (*Aps-1[1]*), which was carried along with the *Mi* gene in the original cross, is easily distinguishable from the *L. escu-*

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lentum Aps-1 alleles (*Aps-1*$^+$ and *Aps-1*^{3}) by isozyme staining (Fobes and Rick 1976). Thus far, the presence of the *L. peruvianum Aps-1*^{1} allele in tomatoes has always been indicative of resistance to nematode infection. However, all nematode-resistant cultivars and breeding lines developed at the Hawaii Experimental Station (HES) from backcross material obtained by Watts (1947) possess the wild-type (*Aps-1*$^+$) allele. Apparently, a crossover between the *Aps-1* and *Mi* loci had occurred in the early generations of the HES breeding program (Medina-Filho and Stevens 1980). Therefore, selection for resistance in material derived from these HES lines depends on a nematode infection assay that is cumbersome and often less reliable.

The nature of the plant defence reaction against nematodes has remained elusive so far. Obviously, cloning of the *Mi* gene will be a prerequisite for gaining more insight into the mechanism of this plant-pathogen interaction. As for most plant genes conferring resistance to pathogens, no gene product of the *Mi* gene is known and, consequently, none of the standard molecular cloning techniques can be applied. A conceivable approach to clone the *Mi* gene would be to identify closely linked, flanking molecular markers and to subsequently clone all the sequences contained between them, using "reverse genetics" strategies (Herrmann et al. 1990; Orkin 1986; Rommens et al. 1989). This approach seems particularly attractive in plants in view of the relative ease with which restriction fragment length polymorphism (RFLP) markers closely linked to a target gene can be obtained by exploiting pairs of nearly isogenic lines (NILs), differing only in a small chromosomal segment containing the target gene (Young et al. 1988). In this paper we describe the application of this strategy to the isolation of two RFLP markers that are very tightly linked to the *Mi* locus and the *Aps-1* locus, respectively.

Materials and methods

Plant materials

L. esculentum lines 83M7133 and 83M7138 form a pair of nearly isogenic lines differing only in the chromosome 6 region containing the *Aps-1* and *Mi* loci. These lines are derived from 83M (De Ruiter, The Netherlands), which is a nematode-resistant *L. esculentum* line containing the *L. peruvianum Aps-1*^{1} and *Mi* alleles. The backcross program involved in breeding this line consisted of at least 20 backcrosses of nematode-resistant cultivars obtained from the USA in the late 1950s with various breeding lines adapted to Dutch greenhouse conditions. The 83M line that segregated for *Mi*/*Aps-1* was subsequently selfed for 11 generations under selection of heterozygous plants. Two homozygous plants, identified in a segregating F_{10} line on the basis of their isozyme patterns, were selfed, resulting in the two NILs. The nematode-resistant line 83M7138 contains the *L. peruvianum* introgressed region derived from line 83M with the *Aps-1*^{1} and *Mi* alleles. The nematode-sensitive line 83M7133 possesses a novel *L. esculentum Aps-1* allele (*Aps-1*^{3}) and the *L. esculentum Mi* allele.

L. esculentum LA 1641 is a chromosome 6 substitution line in which the *L. esculentum* chromosome 6 has been replaced by chromosome 6 from *L. pennellii* LA716, except for the very distal end of the long arm encompassing RFLP marker TG193. This line has been developed from a *L. esculentum* × *L. pennellii* cross by Rick (1969). A detailed molecular and genetic characterization of this substitution line will be published elsewhere (R. Weide, R. Klein-Lankhorst, M. Koornneef and P. Zabel, in preparation).

L. pennellii LA 716, *L. esculentum* LA 1641, and the *L. esculentum* cultivars VFN8, Anahu (LA 655), and LA 656 (a genotype related to Anahu) were obtained from Dr. C. M. Rick, University of California, Davis/CA. Both resistant and sensitive *L. esculentum* cultivars and breeding lines were a gift from the Dutch seed companies Royal Sluis (Enkhuizen), De Ruiter Seeds (Bleiswijk), Nunhems Zaden (Haelen), and Rijk Zwaan (De Lier), as specified in Table 1. The varieties represent both F_1 hybrids and open-pollinated cultivars.

DNA methodology

All standard DNA methodologies were carried out according to Maniatis et al. (1982). Plant DNA was isolated from leaves as described by Murray and Thompson (1980). Labeling of DNA with α^{32}P-dATP was done using the "random primer" method according to Feinberg and Vogelstein (1984).

Hybridization of Southern blots containing tomato genomic DNA (3 µg/lane) with single- or low-copy clones from the genomic library was carried out at 65 °C in 15 ml 1 *M* NaCl, 1% SDS, 10% dextran sulfate, 50 m*M* TRIS-HCl, 0.1 mg/ml denatured salmon sperm DNA (final pH 7.5), using a roller-bottle hybridization oven (Highbaid, Teddington, UK). After hybridization, blots were rinsed in 0.1 × SSC at room temperature for 30 s and washed in 0.5 × SSC, 1%SDS at 65 °C, 2 × 30 min. Hybridization of tomato DNA with labeled potato RFLP markers was carried out at 55 °C under the same conditions as described above.

Construction and screening of the L. esculentum genomic library

A genomic *L. esculentum* DNA library was constructed by ligating HindIII-digested total DNA (4 µg) from the nematode-resistant line 83M7138 into the dephosphorylated HindIII site of pUC 18 (1 µg). Clones were screened for the presence of repetitive sequences by hybridizing dot blots prepared from the library [using a Minifold apparatus (Schleicher and Schuell Inc., Dassel, Germany)] with labeled total DNA from *L. esculentum* 83M7138. Strongly hybridizing clones were omitted from further screening and the remaining clones were subjected to a second round of screening for repeats by hybridizing Southern blots of plasmid DNA. Insert DNA from clones showing no hybridization signal after overnight exposure was considered to represent either a single- or low-copy *L. esculentum* sequence. Inserts were isolated and used in pools of four to probe Southern blots containing DNA from *L. esculentum* 83M7138, 83M7133, LA1641, and *L. pennelli* LA716 digested with HindIII, EcoRI, EcoRV, HaeIII, BglII, DraI, TaqI, or XbaI. When a pool of inserts exhibited a chromosome 6-specific RFLP, as shown by a *L. pennellii*-type hybridization pattern in the digest of the chromosome 6 substitution line, individual inserts were rehybridized separately on Southern blots to identify the cloned *L. esculentum* fragment responsible for the polymorphism.

Acid phosphatase isozyme analysis

Leaf segments (ca. 1 × 2 cm) were frozen in liquid nitrogen, grounded using a Teflon pestle, and homogenized in 0.5 ml of ice-cold, freshly prepared extraction buffer [100 m*M* TRIS-HAc, 100 m*M* KAc, 10% (v/v) glycerol, 2 m*M* EDTA, 0.1 m*M*

PMSF, 0.25 *M* sodium ascorbate, 5 m*M* DTT, 125 mg/ml Polyclar AT (final pH 8.0)]. After centrifugation (13,000 *g*, 15 min), the supernatant was transferred to a new tube and stored at −80 °C.

Forty microliters of the leaf extract was loaded on a nondenaturing polyacrylamide gel [7.5% (w/v) stacking gel, 12.5% (w/v) separating gel]. Electrophoresis was performed for 18 h at 70 V and 4 °C in 50 m*M* TRIS, 380 m*M* glycine. The gel was subsequently equilibrated for 2 × 15 min with staining buffer (50 m*M* NaAc, 10 m*M* $MgCl_2$, final pH 5.5) and stained overnight for acid phosphatase activity at 30 °C under gentle agitation in staining buffer containing 0.03% (w/v) β-naphtyl acid phosphate and 1 mg/ml Fast Black K salt (Medina-Filho and Tanksley 1983).

Results

Identification of RFLP markers for the Aps-1/Mi region

To identify RFLP markers for the *Mi/Aps-1* region on chromosome 6, Southern blots of a pair of NILs differing for a small introgressed region around the *Aps-1* and *Mi* loci were hybridized with single and low-copy genomic clones from tomato and potato. Clones mapping in this region were expected to exhibit a restriction fragment length polymorphism between the NILs, due to the difference in source of the target DNA. To confirm the chromosome 6 location of the putative *Aps-1/Mi* region-specific RFLP markers, DNA from a *L. esculentum* chromosomal 6 substitution line and from *L. pennellii* were simultaneously probed. If a clone showing a polymorphism between the NILs maps in the *Aps-1/Mi* region, then it should exhibit a *L. pennellii*-specific hybridization pattern in the *L. esculentum* substitution line carrying chromosome 6 from *L. pennellii*.

Of the 250 tomato genomic clones thus tested, 21 could be assigned to chromosome 6. Only one clone, H6A2c2, also exhibited an RFLP between the pair of NILs (Fig. 1, left panel), apparently due to its location on the introgressed *L. peruvianum* chromosome 6 segment that carries the *Aps-1* and *Mi* loci. Assuming a random distribution of the genomic sequences among the library, this result suggests that the introgressed segment comprises only a very small proportion of the genome of resistant line 83M7138.

In addition to the tomato genomic clones, potato clones derived from chromosome 8 (Gebhardt et al. 1989; C. Gebhardt, E. Ritter, A. Barone, T. Debener, B. Walkemeier, V. Schachtschabel, H. Kaufmann, R. D. Thompson, M. Ganal, S. Tanksley and F. Salamini, in preparation), which is homeologous to tomato chromosome 6 (Bonierbale et al. 1988), were similarly tested for polymorphism between the pair of NILs and the chromosome 6 substitution line. Of the seven clones tested, six (CP12, GP79, GP89, GP136, GP164, GP202) revealed a *L. pennellii*-specific hybridization pattern for the chromosome 6 substitution line, indicating that these potato RFLP markers indeed map on chromosome 6 in tomato (results not shown). Among these potato clones, GP79 appeared to be of particular interest by virtue of exhibiting a polymorphism between the pair of NILs (Fig. 1, right panel).

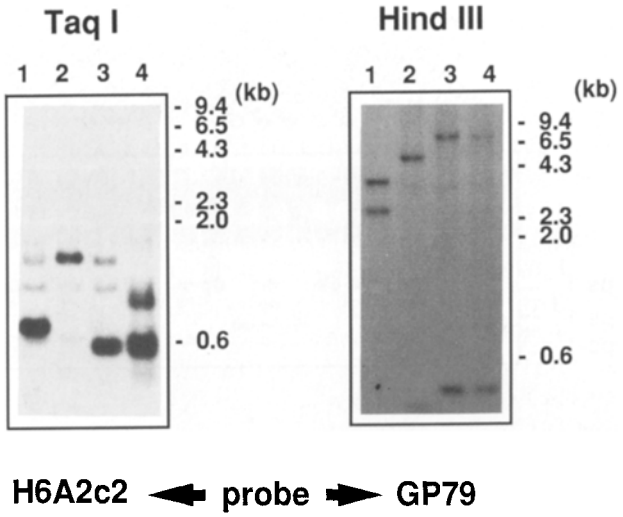

Fig. 1. Identification of RFLP markers in the *Aps-1/Mi* region. Total DNA (3 µg) from the nematode-resistant line *L. esculentum* 83M7138 (*lanes 1*), the sensitive line 83M7133 (*lanes 2*), the chromosome 6-substitution line LA 1641 (*lanes 3*), and from *L. pennellii* LA716 (*lanes 4*) was digested with TaqI (*left panel*) and HindIII (*right panel*), separated on 1% agarose gels, transferred to Gene Screen Plus membranes, and hybridized with the RFLP markers H6A2c2 (*left*) and GP79 (*right*)

Linkage of H6A2c2 with the Aps-1 locus

Although the use of the NILs had thus provided two RFLP markers that are located in the introgressed *L. peruvianum* segment carrying the *Mi* locus and the tightly linked *Aps-1* locus, the mapping data have not provided as yet any information on their relative position and distance to these loci. For that purpose, a wide variety of nematode-resistant and -sensitive cultivars, including those in which the linkage between the *Mi* locus and the *L. peruvianum* $Aps\text{-}1^1$ allele is broken (so-called Hawaii-type cultivars, derived from the HES lines), was subjected to acid phosphatase staining and Southern blot analysis. The *APS-1* isozyme patterns of a representative set of 22 genotypes are shown in Fig. 2. Among the 51 genotypes tested, three *Aps-1* alleles were distinguished: the *L. peruvianum*-specific $Aps\text{-}1^1$ allele that is only present in nematode-resistant tomato lines and two alleles encoding allozymes with a higher mobility on polyacrylamide gels. One of these ($Aps\text{-}1^+$) represents the standard *L. esculentum* (Marglobe) allele that is known from the literature (Fobes and Rick 1976). The other allele, which to our knowledge has not been described before, encodes an allozyme with a mobility slightly lower than $Aps\text{-}1^+$ and is here referred to as $Aps\text{-}1^3$. [The designation $Aps\text{-}1^2$ has already been used to describe an *Aps-1* allele present in

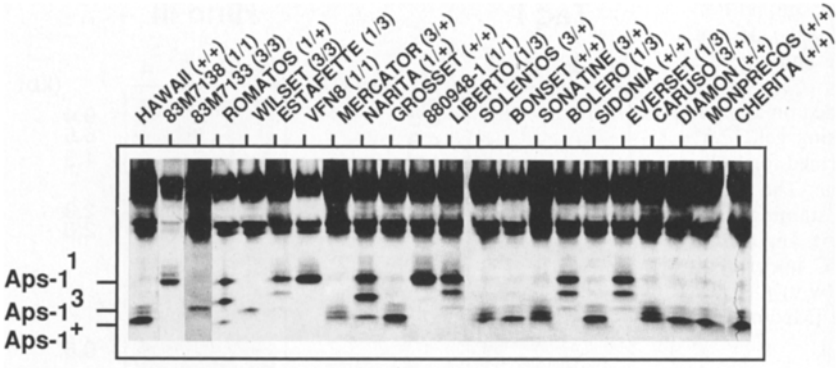

Fig. 2. *APS-1* isozyme patterns of various nematode-resistant and -sensitive *L. esculentum* genotypes. Leaf extracts of various *L. esculentum* genotypes from the test panel (see Table 1) were prepared and analyzed on 12.5% acrylamide gels for acid phosphatase activity as described in "Materials and methods." The resulting *APS-1* isozyme patterns of a representative set of 22 of the 51 tested genotypes are shown. The *Aps-1* alleles of individual genotypes are indicated in parentheses: $+ = Aps\text{-}1^+$, $1 = Aps\text{-}1^1$, $3 = Aps\text{-}1^3$. The genotype designated "Hawaii," *first lane*, corresponds to line 870482 (see Table 1)

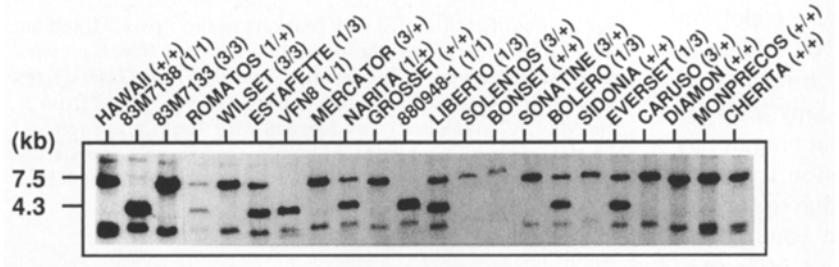

Fig. 3. RFLP marker H6A2c2 discriminates the *L. peruvianum Aps-1^1* allele from the *L. esculentum Aps-1^+* and *Aps-1^3* alleles. Southern blots of EcoRI-digested DNA from 51 *L. esculentum* genotypes were hybridized to RFLP marker H6A2c2. The hybridization patterns of the same set of 22 genotypes used in Fig. 2 are shown. The hybridizing fragments (4.3 kb and 7.5 kb) diagnostic for the different *Aps-1* alleles are indicated. *Aps-1* alleles of the individual genotypes are shown in parentheses: $+ = Aps\text{-}1^+$, $1 = Aps\text{-}1^1$, $3 = Aps\text{-}1^3$

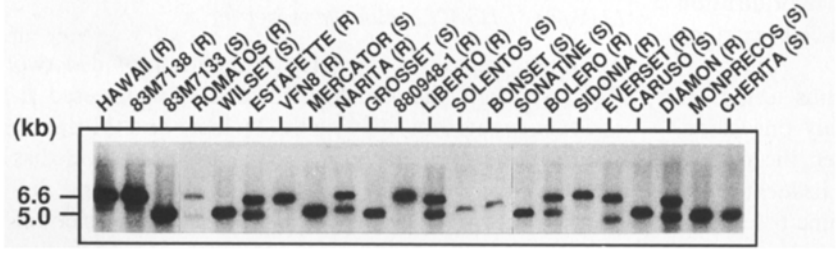

Fig. 4. RFLP marker GP79 discriminates nematode-resistant *L. esculentum* cultivars from sensitive cultivars. Southern blots of EcoRI-digested DNA from 51 *L. esculentum* genotypes were hybridized to RFLP marker GP79. The hybridization patterns of the same set of 22 genotypes used in Fig. 2 are shown. The response to nematode infection of the different genotypes is shown in parentheses: R = resistant, S = sensitive. (The migration of restriction fragments from cultivars Romatos, Solentos, and Bonset was slightly disturbed due to impurities in the DNA preparations)

the wild tomato species *L. chmielewskii* (Fobes and Rick 1976)]. Since tomatoes homozygous for this new allele are all sensitive to nematode infection, *Aps-1^3* is most likely to represent an *L. esculentum* allele.

According to their *APS-1* isozyme patterns and their response to nematodes, the 51 tomato cultivars were divided into eight classes (see Table 1): Nematode-sensitive cultivars, being either *Aps-1^+/Aps-1^+*, *Aps-1^3/Aps-1^3*, or *Aps-1^3/Aps-1^+*; resistant cultivars homozygous or heterozygous for the *L. peruvianum Aps-1* allele (*Aps-1^1/Aps-1^1*, *Aps-1^1/Aps-1^+*, or *Aps-1^1/Aps-1^3*); and resistant cultivars of the Hawaii-type (*Aps-1^+/Aps-1^+* or *Aps-1^3/Aps-1^+*), in which the linkage between the *L. peruvianum Aps-1* gene and the *Mi* gene has been lost by recombination.

To establish a possible correlation between the *Mi* gene, the different *Aps-1* alleles, and the RFLPs detected by clone H6A2c2 and GP79, EcoRI-digested DNA from each of the 51 *L. esculentum* genotypes was subjected to Southern blot analysis. Hybridization with clone H6A2c2 produced restriction patterns as shown in Fig. 3. Among the three major restriction fragments observed, two fragments of 4.3 and 7.5 kb, respectively, were diagnostic for the *Aps-1* alleles. All genotypes homozygous for the *L. peruvianum Aps-1^1* allele exhibited the 4.3-kb EcoRI fragment. On the other hand, genotypes homozygous for the *L. esculentum Aps-1* alleles, *Aps-1^+* or *Aps-1^3*, only contained the 7.5-kb fragment, whereas heterozygous genotypes (*Aps-1^1/Aps-1^+* and *Aps-1^1/Aps-1^3*) showed both the 4.3 and 7.5 kb fragment (Table 1). These

Table 1. *Aps-1* alleles and restriction fragments detected by RFLP markers H6A2c2 and GP79 in 51 *L. esculentum* genotypes. A test panel consisting of 51 different nematode-resistant and -sensitive *L. esculentum* genotypes was surveyed by *APS-1* isozyme staining and hybridization with the RFLP markers H6A2c2 and GP79. The *Aps-1* alleles and the size (kb) of the hybridizing EcoRI fragments of the individual genotypes are given. The genotypes are grouped according to their reaction upon nematode infection and their *Aps-1* alleles.

Nematode-sensitive *L. esculentum* genotypes

Name	*Aps-1* alleles	H6A2c2 (EcoRI)	GP79 (EcoRI)	Source
Marglobe	+/+	7.5	5.0	5
Robin	+/+	7.5	5.0	1
Crisolita	+/+	7.5	5.0	1
Bornia	+/+	7.5	5.0	1
Grosset	+/+	7.5	5.0	1
Bonset	+/+	7.5	5.0	1
Monprecos	+/+	7.5	5.0	1
Cherita	+/+	7.5	5.0	2
Incas	+/+	7.5	5.0	3
CastleRock	+/+	7.5	5.0	3
Azteca	+/+	7.5	5.0	3
83M7133	3 /3	7.5	5.0	2
Wilset	3 /3	7.5	5.0	1
880948-2	3 /3	7.5	5.0	6
RZ43	3 /3	7.5	5.0	4
Concreto	3 /+	7.5	5.0	2
Mercator	3 /+	7.5	5.0	2
Solentos	3 /+	7.5	5.0	2
Sonatine	3 /+	7.5	5.0	2
Caruso	3 /+	7.5	5.0	2

Nematode-resistant *L. esculentum* genotypes

Name	*Aps-1* alleles	H6A2c2 (EcoRI)	GP79 (EcoRI)	Source
83M7138	1/1	4.3	6.6	2
880948-1	1/1	4.3	6.6	6
VFN8	1/1	4.3	6.6	5
RZ1793	1/1	4.3	6.6	4
RZ1795	1/1	4.3	6.6	4
Parana	1/+	4.3/7.5	6.6/5.0	1
Romatos	1/+	4.3/7.5	6.6/4.5	2
Narita	1/+	4.3/7.5	6.6/5.0	1
Monix	1/+	4.3/7.5	6.6/5.0	3
Solido	1/+	4.3/7.5	6.6/5.0	3
Aratino	1/3	4.3/7.5	6.6/4.5	2
W1182	1/3	4.3/7.5	6.6/4.5	2
Credito	1/3	4.3/7.5	6.6/5.0	2
Romada	1/3	4.3/7.5	6.6/5.0	2
Restino	1/3	4.3/7.5	6.6/4.5	2
Belcanto	1/3	4.3/7.5	6.6/4.5	2
Estafette	1/3	4.3/7.5	6.6/5.0	2
Liberto	1/3	4.3/7.5	6.6/5.0	2
Bolero	1/3	4.3/7.5	6.6/5.0	2
Everest	1/3	4.3/7.5	6.6/4.5	1

Nematode-resistant *L. esculentum* genotypes, Hawaii-type

Name	*Aps-1* alleles	H6A2c2 (EcoRI)	GP79 (EcoRI)	Source
870482 (Hawaii)	+/+	7.5	6.6	2
Rakata	+/+	7.5	6.6/5.0	2
Roma	+/+	7.5	6.6	1
Royesta	+/+	7.5	6.6/5.0	1
Sidonia	+/+	7.5	6.6	1
Diamon	+/+	7.5	6.6/5.0	1
Anahu	+/+	7.5	6.6	5
LA656	+/+	7.5	6.6	5
Ranger	+/+	7.5	6.6/5.0	3
Floriset	3 /+	7.5	6.6/5.0	1
Multiset	3 /+	7.5	6.6/5.0	1

1 = Royal Sluis, 2 = De Ruiter Seeds, 3 = Nunhems Zaden, 4 = Rijk Zwaan, 5 = C. Rick, 6 = own breeding program

results demonstrate that clone H6A2c2 is very tightly linked to the *Aps-1* locus and is capable of distinguishing the *L. peruvianum* $Aps\text{-}1^1$ allele from the *L. esculentum* $Aps\text{-}1^+$ and $Aps\text{-}1^3$ alleles.

Linkage of GP79 with the Mi locus

A similar survey was conducted with the RFLP marker GP79 (Fig. 4 and Table 1). In this case, a strict correlation was found between the presence of a 6.6-kb EcoRI fragment and resistance to nematodes. All the nematode-resistant genotypes surveyed, including Hawaii-type lines ($Aps\text{-}1^+$ or $Aps\text{-}1^3$), were found to contain a diagnostic 6.6-kb EcoRI fragment. In old tomato cultivars such as VFN8 and Roma VFN, which are homozygous for *Mi*, this was the only fragment detected by GP79. Modern resistant cultivars, which are mostly F_1 hybrids, contained in addition to the 6.6-kb fragment either a 5.0-kb or 4.5-kb EcoRI fragment. Nematode-sensitive cultivars only exhibited a single 5.0-kb EcoRI fragment. In conclusion, these results demonstrate that clone GP79 is highly discriminatory and is capable of identifying nematode-resistant genotypes, irrespective of their *Aps-1* phenotype.

To determine the genetic distance between H6A2c2, GP79, *Aps-1*, and the leaf color marker *yv* (yellow virescent), which is tightly linked to *Aps-1*, segregation for these four markers was analyzed in a population of 50 F_2 plants from a *L. esculentum* LA802 ($Aps\text{-}1^+/Aps\text{-}1^+$) × *L. esculentum* 83M7138 ($Aps\text{-}1^1/Aps\text{-}1^1$) cross. LA802 is a nematode-sensitive line carrying the chromosome 6 markers *yv*, *m2* (mottled), and *c* (potato leaf). The F_2 plants were analyzed by *APS-1* isozyme staining and Southern blot analysis, using H6A2c2 and GP79 as probes (results not shown). No recombinants were found between the RFLP markers, *yv* and *Aps-1*. On the assumption that the chance of finding a recombinant in a

population of a certain size is determined by a Poisson distribution, the percentage of recombination between these markers was estimated to be less than 3%.

Discussion

In principle, RFLP markers closely associated with various economically important traits in tomato are easily identifiable with the aid of pairs of nearly isogenic lines, which differ only in a small introgressed chromosomal segment containing the gene of interest (Young et al. 1988; Sarfatti et al. 1989). With respect to the nematode resistance region on chromosome 6, we have thus been able to identify two RFLP markers that are located in the introgressed *L. peruvianum* segment carrying the nematode resistance gene *Mi*.

The HindIII genomic tomato library employed here provided a useful and apparently representative source of potential RFLP markers. Among the 250 clones tested, 21 (8.4%) were found to map on chromosome 6, which represents in size an "average" tomato chromosome among the 12 chromosomes constituting the haploid genome. In principle, the source of potential RFLP markers for linkage analysis in tomato is not limited to tomato itself. Clones derived from other members of the nightshade family (Solanaceae) are feasible as well. Using tomato clones as RFLP markers in pepper (Tanksley et al. 1988) and potato (Bonierbale et al. 1988), a high degree of linkage conservation was detected. Conversely, mapped potato clones (Gebhardt et al. 1989) should be supplementary to tomato markers and, in some cases, highly useful when located at or near a region that has been shown to carry an important trait in tomato. The potato clone GP79, which had been mapped already in a linkage group homeologous to chromosome 6 of tomato (C. Gebhardt, E. Ritter, A. Barone, T. Debener, B. Walkemeier, V. Schachtschabel, H. Kaufmann, R. Thompson, M. Ganal, S. Tanksley and F. Salamini, in preparation) but is not yet known to be linked to a nematode resistance locus in potato, provides a striking example in this respect. Its tight linkage to the nematode resistance locus in tomato, as demonstrated in this paper, may in turn provide a first clue as to the location of a root knot nematode resistance gene in wild *Solanum* species (see also Mendoza and Jatale 1985).

In tomato, GP79 is very tightly linked to the *Mi* gene and is capable of identifying both the *Aps-1*^{1}-type as well as the Hawaii-type (*Aps-1*$^+$ or *Aps-1*^{3}) nematode-resistant cultivars. Apparently, this RFLP marker is located at the *Mi* side of the *Aps-1*/*Mi* crossing-over point in the HES lines. In breeding programs involving cultivars derived from these lines, the *Aps-1* locus is not a useful marker and selection for nematode resistance depends on a bioassay that is time-consuming and not always foolproof. Obviously, GP79 provides a powerful molecular marker allowing segregating populations to be screened for the nematode resistance trait at both the homozygous and heterozygous level. The RFLP marker H6A2c2 is not useful for that purpose, as it is located at the *Aps-1* side of the *Aps-1*/*Mi* crossing-over point in the HES lines.

The APS-1 isozyme staining of the test panel of 51 *L. esculentum* cultivars showed the existence of a second, hitherto unrecorded, *L. esculentum Aps-1* allele, which we refer to as *Aps-1*^{3}. This *Aps-1* allele seems to be present mainly in tomato varieties used for greenhouse culture, such as Wilset, Sonatine, and Estafette (see Table 1).

Genetic linkage analysis revealed a close linkage between both RFLP markers, the *Aps-1* locus and the *yv* locus. As no recombinants were recovered between any of these markers among 50 F_2 plants analyzed, no gene order could be established. The maximum map distance between any of these markers was estimated to be 3 cMorgans. It remains to be tested whether this close genetic linkage corresponds to a physical distance of approximately 1.5 Mbp that can be calculated for tomato, assuming a constant ratio between genetic distance and physical distance. The mere location of the *Aps-1*/*Mi* region near the centromere may significantly contribute to suppression of recombination and, consequently, to an underestimation of the physical distance (Loh et al. 1987). As recombination is also known to be suppressed in genotypes heterozygous for a chromosomal segment originating from a different species (Rick 1969), the chromosomal region habituated by *Aps-1, Mi,* GP79, and H6A2c2 in the nematode-resistant line 83M7138 is likely to be larger in terms of DNA base pairs than suggested by estimates based on genetic linkage data. Southern blot analysis of high-molecular-weight DNA separated by pulsed field gel electrophoresis (Van Daelen et al. 1989) using GP79 and H6A2c2 as probes did not show any common bands hybridizing in the 100–1,000 kb region (results not shown).

Studies are now in progress which aim at the construction of a long-range physical map of the *Mi* region using pulsed field gel electrophoresis and a tomato YAC library (R.A.I. van Daelen, in preparation).

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Note added in proof. Recently, molecular and genetic evidence has been obtained indicating that the Aps-1^3 allele was introgressed into tomato from *L. pimpinellifolium* along with the Cf2 gene conferring resistance to the pingus *Cladosporium fulvum*.

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