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A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato

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Abstract. A PCR-based codominant marker has been developed which is tightly linked to *Mi*, a dominant genetic locus in tomato that confers resistance to several species of root-knot nematode. DNA from tomato lines differing in nematode resistance was screened for random amplified polymorphic DNA markers linked to *Mi* using decamer primers. Several markers were identified. One amplified product, REX-1, obtained using a pair of decamer primers, was present as a dominant marker in all nematode-resistant tomato lines tested. REX-1 was cloned and the DNA sequences of its ends were determined and used to develop 20-mer primers. PCR amplification with the 20-mer primers produced a single amplified band in both susceptible and resistant tomato lines. The amplified bands from susceptible and resistant lines were distinguishable after cleavage with the restriction enzyme *Taq*I. The linkage of REX-1 to *Mi* was verified in an F_2 population. This marker is more tightly linked to *Mi* than is *Aps-1*, the currently-used isozyme marker, and allows screening of germplasm where the linkage between *Mi* and *Aps-1* has been lost. Homozygous and heterozygous individuals can be distinguished and the procedure can be used for rapid, routine screening. The strategy used to obtain REX-1 is applicable to obtaining tightly-linked markers to other genetic loci. Such markers would allow rapid, concurrent screening for the segregation of several loci of interest.

Key words: *Lycopersicon esculentum* – RAPD – PCR – *Meloidogyne* – Root knot nematode

Introduction

Resistance to root-knot nematodes in tomato (*Lycopersicon esculentum*) is conferred by the presence of a single, dominant gene *Mi* (Gilbert and McGuire 1956; Braham and Winstead 1957). Because of the severe damage that these pests can cause to tomato, and due to increased interest in nonchemical agricultural management, incorporating nematode resistance is a component of many modern tomato breeding programs. Direct screening for nematode resistance is tedious and progeny testing is required to distinguish heterozygotes. The isozyme marker *Aps-1*, encoding acid phosphatase-1, is tightly linked to *Mi* and is a classic example of the use of an easily-scored marker to follow the segregation of a linked agronomically-important trait (Rick and Fobes 1974; Medina-Filho and Stevens 1980; Bolkan et al. 1987). *Aps-1*^{1}, encoding an electrophoretic variant of *Aps-1*, was introgressed from the wild tomato species *L. peruvianum* along with *Mi*. Recent studies have shown that there is repressed recombination in the region of the genome carrying the *Aps-1*^{1} allele in crosses segregating for *Mi* (Messeguer et al. 1991; Ho et al. 1992). This repressed recombination has resulted in the retention of a large block of *L. peruvianum* DNA, possibly carrying detrimental traits, in many nematode-resistant lines. Germplasm that carries *Mi* and the *L. esculentum Aps-1* allele, *Aps-1*$^+$, presumably due to recombination between these two genes, is available, and the introgressed region of chromosome 6 in these lines is much smaller than in plants

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with the *Aps-1*^{1} allele (Messeguer et al. 1991; Ho et al. 1992). However, this germplasm has not been fully utilized due to the lack of available linked markers.

RFLPs have been used extensively as genetic markers in tomato and have been especially useful for mapping genes in introgressed regions of tomato (van der Beek et al. 1992). Polymorphisms linked to *Mi* have been identified (Klein-Lankhorst et al. 1991a; Messeguer et al. 1991; Ho et al. 1992). However, the necessity to carry out Southern-blot hybridization makes RFLPs impractical for routine analysis. PCR amplification of genomic DNA primed by arbitrary oligomers has been used to generate random amplified polymorphic DNA (RAPD) markers for mapping (Williams et al. 1990). RAPD markers linked to disease resistance genes have been identified in tomato by screening nearly-isogenic lines differing in the presence of a single resistance gene (Klein-Lankhorst et al. 1991b; Martin et al. 1991). These markers have the advantage that they are easy to generate and do not require the use of isotopes. However, because of the non-stringent hybridization conditions used to generate RAPD markers, the amplification pattern varies between DNA preparations and with assay conditions. The method is thus not suitable for routine analysis. RAPD markers can be converted into stable and reliable markers by cloning the amplified bands, sequencing their ends, and then using this sequence to generate extended oligomer primers. This strategy has been used successfully to generate markers for lettuce (Paran and Michelmore 1993). These extended oligomers when annealed under stringent hybridization conditions reproducibly prime the amplification of single bands, corresponding to genetically-defined loci, called sequence-characterized amplified regions (SCARs; Paran and Michelmore 1993). SCARs resemble the sequence-tagged sites (STSs) that have been a major tool in the alignment of clones in the human genome project (Olson et al. 1989; Chumakov et al. 1992). Here we apply this technique to the production of a marker that is very tightly linked to *Mi* and that is suitable for large-scale screening in a breeding program.

Materials and methods

Plant material

The tomato lines used in this work and their sources are listed in Table 1. These lines differ in *Aps-1* alleles, nematode resistance, and the extent of introgressed DNA from *L. peruvianum* (Ho et al. 1992). Sun6082 and CastlerockII are nearly-isogenic lines that differ in nematode resistance. Plants with the prefix "VL" are individual F_2 progeny from a cross between LA55 (*mi/mi Aps-1*$^+$/*Aps-1*$^+$) and VFNT cherry (*Mi/Mi Aps-1*^{1}/*Aps-1*^{1}) (see Ho et al. 1992). Monita, Monalbo, Motelle, and Mopérou are from a series of nearly-isogenic tomato lines in Moneymaker type (Laterrot 1987).

DNA extraction

Plant DNA used for RAPD analysis was extracted as described previously (Williamson and Colwell 1991). For some amplifications with 20-mer REX-1 primers, a quick DNA extraction protocol designed for PCR analysis was used (Edwards et al. 1991).

RAPD amplification conditions

RAPD amplifications were carried out basically as described by Williams et al. (1990) with a modified reaction mix (10 mM Tris, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; 0.01% gelatin; 0.1 mM each of dATP, dCTP, dGTP and dTTP; 0.1% Triton X-100 (v/v); 0.2 µM primer, 20 ng template DNA and 1.0 U *Taq* polymerase) on a Perkin-Elmer Cetus DNA Thermal Cycler 480. The decamer primers for RAPD markers were obtained from Operon Technologies (Alameda, Calif.; Operon kits A, B, G, K, M, P, V, and X). PCR conditions were: 95 °C for 5 min followed by 45 cycles of 1 min at 94 °C, 1 min at 35 °C and 2 min at 72 °C, followed by 7 min at 72 °C.

Cloning of RAPD fragment REX-1

Amplification products were resolved by agarose-gel electrophoresis and the band of interest purified with a "GeneClean" kit (Bio101, Inc., La Jolla, Calif., USA) as recommended by the manufacturer. The purified product was cloned using a TA cloning kit (Invitrogen Corp., San Diego, Calif., USA). Dideoxy DNA sequencing was carried out using a "Sequenase" kit (US-Biochemical Corp., Cleveland, Ohio, USA).

Analysis of tomato DNA for the REX-1 allele

Amplification of marker REX-1 as a SCAR was carried out in 25 µl of the same reaction mix as for RAPD amplifications except that 0.5 µM each of primers REX-F1 (5′-TCGGAGCCTTGGTCTGAATT-3′) and REX-R2 (5′-GCCAGAGATGATTCGTGAGA-3′) were used. Samples were placed at 94 °C for 3 min, then amplification was carried out under the following conditions: 30 cycles of 94 °C for 1 min; 55 °C, 2 min; and 72 °C, 2 min. Following amplification, 7 µl of the reaction mix were transferred to a new tube and digested with 5 units of *Taq*I (Promega, Madison, WIS., USA) as recommended by the manufacturer in a total volume of 10 µl. Amplification products were resolved on a 1.5% agarose gel.

Results

Screening for RAPDs linked to Mi

PCR amplification of DNA from a set of six tomato lines (lines 1–6, Table 1) that differed in their *Mi* and *Aps-1* alleles was carried out using 160 different decamers as primers. Upon agarose-gel electrophoresis, on average 7–8 distinguishable bands were observed after amplification with each primer, though there was considerable variability among primers in the number of bands amplified. Twenty-six primers resulted in amplification of at least one polymorphic fragment that correlated with the *Aps-1* allele present: 18 primers amplified an additional fragment in *Aps-1*^{1} lines, five amplified an extra fragment in *Aps-1*$^+$ lines, and three primers produced a codominant marker, revealing size

Table 1. Tomato lines and markers linked to *Mi*

Tomato line	Source[a]	*Aps-1*	*Mi*[b]	OPV07ba[c]	OPV07b[d]	REX-1[e]
1. Castlerock II	1	1^+	mi	0	e	e
2. Sun 6082	1	1^1	Mi	p	0	p
3. VFNT cherry	5	1^1	Mi	p	0	p
4. LA655 (Anahu)	5	1^+	Mi	0	0	p
5. Rossol	3	1^+	Mi	0	0	p
6. VL642-40	9	1^+	mi	0	e	e
7. 83M-S	2	1^3	mi	0	e	e
8. 83M-R	2	1^1	Mi	p	e	p
9. Monalbo	3	1^+	mi	0	e	e
10. Monita	3	1^+	Mi	0	0	p
11. N118	4	1^1	mi	p	e	e
12. Moneymaker-Cf2	6	1^3	mi	0	e	e
13. Roma	3	1^+	mi	0	e	e
14. VL607-54	10	$1^1/1^+$	–	p	e	p/e
15. VL627-4	10	1^1	Mi	p	0	p
16. Motelle	3	1^+	Mi	0	e	p
17. Hawaii	2	$1^+/1^+$	Mi	0	0	p
18. Small Fry	3	$1^1/1^+$	Mi/mi	–	–	p/e
19. H8892	9	$1^1/1^+$	Mi/mi	–	–	p/e
20. HM201	7	1^+	Mi	–	–	p
21. Fame	8	1^+	Mi/mi	–	–	p/e
22. Sierra	8	1^+	Mi/mi	–	–	p/e
23. Pacheco	8	1^+	Mi/mi	–	–	p/e
24. Centurion	8	1^+	Mi/mi	–	–	p/e
25. C8202	1	1^+	Mi	–	–	p
26. UC82B	1	1^+	mi	–	–	e
27. LA55	5	1^+	mi	–	–	e
28. Mopérou	3	1^+	mi	–	–	e

[a] Source 1 is Sunseeds, Hollister Calif., USA; 2 is DeRuyter Seeds, Bleiswijk, The Netherlands; 3 is Petoseed, Woodland, Calif., USA; 4 is G. Dickenson, University of California, Davis Calif., USA; 5 is C. Rick, Tomato Genetics Stock Center, University of California, Davis; 6 is P. Lindhout, Center for Plant Research, Wageningen, The Netherlands; 7 is Harris Moran Seed Co., Davis, Calif., USA; 8 is Asgrow Seed Co., San Juan Bautista, Calif., USA; 9 is Heinz U.S.A, Stockton, Calif.; 10 is our laboratory

[b] 'Mi' indicates homozygous for the nematode resistant allele, 'mi' is homozygous for the susceptible allele, and 'Mi/mi' indicates heterozygous at *Mi*

[c] 'p' indicates the presence of the dominant *L. peruvianum*-specific RAPD marker and '0' indicates the absence of this marker

[d] 'e' indicates the presence of the dominant *L. esculentum*-specific RAPD marker and '0' indicates the absence of this marker

[e] PCR amplification products using REX-1 primers F1 and R2 were cleaved with *Taq*I to differentiate 'p' and 'e' alleles

polymorphisms corresponding to each of the two *Aps-1* alleles. Polymorphic bands that did not correlate with the *Aps-1* or *Mi* alleles were frequently observed among the lines but most polymorphisms between the isogenic pair CastlerockII and Sun6082 appeared to be correlated with the *Aps-1* allele present. Eleven of the decamers (OPA06, OPA09, OPB01, OPB07, OPB12, OPM08, OPM11, OPM13, OPK10, OPK20, and OPV07) that primed amplification of a polymorphic band correlating with an *Aps-1* allele were used to amplify bands from eight additional tomato lines (lines 7–14, Table 1) that differed in the *Aps-1* and *Mi* alleles present and in the length of the remaining *L. peruvianum* DNA linked to *Mi* (Ho et al. 1992). In all but one case, the polymorphic band correlated with the *Aps-1* allele, even for lines carrying a crossover between *Mi* and *Aps-1*. The exception was primer OPV07 which resulted in the amplification of two sets of polymorphic markers (Fig. 1a). One band, OPV07a, was present only in lines carrying the *Aps-1*^{1} allele. The second polymorphic band, OPV07b, was present in many lines carrying the susceptible allele of *Mi*. Comparison of the pattern of polymorphisms in tomato lines 1–17 (Table 1), for which the length of the remaining introgressed region carrying *Mi* had been previously determined (Ho et al. 1992), indicated that the OPV07b marker was amplified from DNA of *L. esculentum*, but not *L. peruvianum*, origin.

Some decamers primed amplification of only a few bands, or in a few cases no band, from tomato genomic DNA. When these decamers were used in pairs to prime the amplification of tomato DNA several bands (on the average seven) were obtained, allowing us to generate additional markers from available decamers. Fifty-five such pairs were used to amplify DNA from tomato lines CastlerockII, Sun6082 and Motelle.

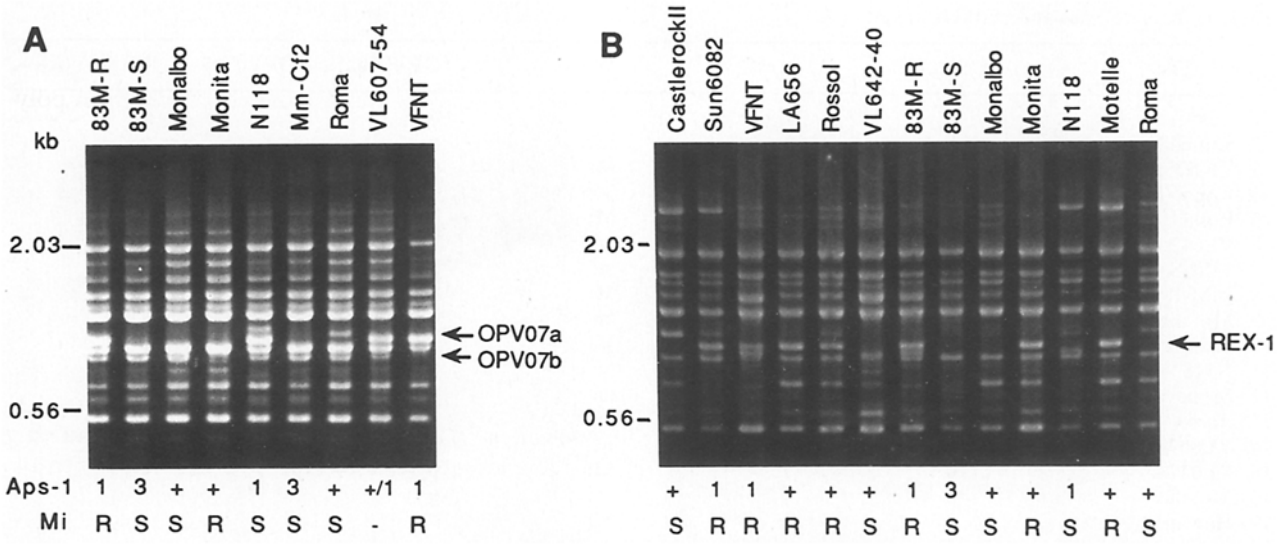

Fig. 1A, B. RAPD amplification products showing polymorphisms linked to *Mi*. Genomic DNA from the tomato lines indicated above each lane was amplified using primer OPV07 (**A**) or the primer pair OPG01 and OPX17 (**B**). Moneymaker-Cf2 is abbreviated *Mm-Cf2*. The *Aps-1* and *Mi* (*R* for resistant and *S* for susceptible) phenotypes are indicated at the bottom of each lane. The positions of the two polymorphic bands, OPV07a and OPV07b, amplified by OPV07 are indicated and the *Mi*-specific polymorphic band amplified by OPG01 and OPX17 is designated *REX-1*

Motelle was chosen for this screening as it is thought to contain the shortest remaining introgressed region of the available nematode-resistant tomato lines (Messeguer et al. 1991; Ho et al. 1992). Pairs of primers revealing a polymorphism between the susceptible line CastlerockII and the two resistant lines, Sun6082 and Motelle, were used to amplify DNA from additional lines in Table 1 to further test for linkage to *Mi*. Among the primers thus tested, one pair, OPG01 and OPX17, yielded a dominant band that was present in all lines tested that carried *Mi* and was not present in any susceptible lines (Fig. 1b). This marker was designated REX-1.

Characterization of REX-1

The fragment corresponding to the dominant RAPD marker REX-1 was cloned and the DNA sequences of the ends of the cloned insert were determined. One hundred and seventy seven nucleotides of sequence beginning with the sequence of decamer OPG01 were obtained from one end of the insert, and 169 nucleotides of sequence starting from the OPX17 sequence were obtained from the other end. A pair of 20-mer primers, REX-F1 and REX-R2 (see Materials and methods) were designed from the sequence data. The oligonucleotide sequences of the primers were chosen to be 50% GC and to lack secondary structure by visual examination. The primers were located 49 and 146 bases from the ends of the cloned RAPD marker and thus were expected to prime amplification of a DNA fragment 195 base pairs shorter than the original fragment from resistant tomato. Upon PCR amplification, using an annealing temperature of 55 °C, a single band of the expected size, 750 bp, was amplified from both resistant and susceptible tomato DNA (Fig. 2).

Amplified DNA from resistant and susceptible tomato was cleaved with several restriction enzymes (*Alu*I, *Dra*I, *Hin*dIII, *Msp*I, *Sau*3A, *Ssp*I, and *Taq*I). Only one enzyme (*Taq*I) revealed a polymorphism (Fig. 2). Amplified DNA from resistant plants was cleaved into two bands of approximately 570 and 160 bp whereas the amplified DNA from susceptible plants did not contain a *Taq*I cleavage site. Heterozygotes displayed all three bands, thus generating a codominant marker. In heterozygotes, the 750-bp band was somewhat more intense than the 570-bp band suggesting that amplification of the *L. peruvianum* allele was more efficient than that of the *L. esculentum* allele (see Fig. 2). The reason for this slight difference in amplification is unknown. DNA extracts from 69 individuals in an F_2 population segregating for nematode resistance were amplified with the REX-1 primer set, then cleaved with *Taq*I (Fig. 3). Individuals in this population had previously been characterized for *Mi* and *Aps-1* alleles and for RFLP markers linked to *Mi* (Ho et al. 1992). In all cases the amplified fragment derived from plants with the resistant allele of *Mi* was cleaved by *Taq*I whereas the fragment from susceptible plants was not cleaved. Heterozygotes were easily distinguished.

The REX-1 phenotypes of tomato lines from diverse breeding programs were determined (Table 1). In

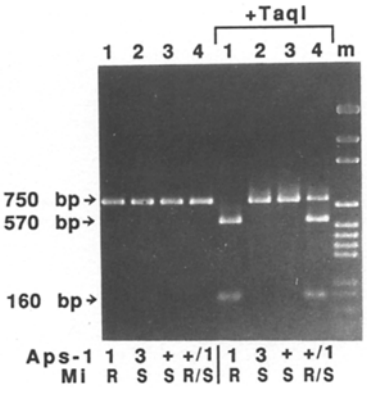

Fig. 2. REX-1 amplification products obtained using 20-mer primers REX-F1 and REX-R2. A band of approximately 750 bp is amplified from both resistant (*R*) and susceptible (*S*) tomato lines: Sun6082, *lane 1*; Moneymaker-Cf2, *lane 2*; CastlerockII, *lane 3*, and Fame, *lane 4*. After digestion with *Taq*I two bands are seen in the *R* line and three are seen in a line heterozygous (*R/S*) at the *Mi* locus. Lane '*m*' contains size markers

all cases nematode-resistant varieties carried the *Taq*I-cleaved allele of REX-1 and no susceptible varieties carried this allele. Tomato lines that carry a third *Aps-1* allele, *Aps-1*^{3}, which was introduced along with the linked *Cladosporium fulvum* resistance gene *Cf2* from *L. pimpinellifolium* (Ho et al. 1992), were included in the analysis. These lines result in the amplification of a 750-bp band that was not cleaved by *Taq*I, and was thus not distinguishable from the *L. esculentum* allele (Fig. 2; Table 1). Three recombinants have been obtained between the *Mi*-flanking markers GP79 and *Aps-1* from a cross of VFNT cherry × LA55 among 339 F_2 progeny, placing these markers about 0.9 cM apart (Ho et al. 1992; Ho and Williamson, unpublished data). Two of these recombinants were tested for nematode resistance and REX-1 alleles were determined. In both cases *Mi* and REX-1 cosegregated, with one recombination site mapping to each side of REX-1. The third plant died and could not be tested for resistance.

Discussion

Because of the *L. peruvianum* origin of *Mi*, this region of the tomato genome was expected to reveal DNA polymorphisms when compared to susceptible lines (Miller and Tanksley 1990). We examined approximately 1120 RAPD loci (160 primers and about seven bands per primer) and found 26 polymorphic bands (2.3%) between *Aps-1*^{1} and *Aps-1*$^+$-containing lines. All but one of these polymorphisms, OPV07b, is lacking in resistant lines carrying the *L. esculentum Aps-1* allele, *Aps-1*$^+$. The high frequency of *Aps-1*^{1}-associated polymorphisms is consistent with findings for RFLP markers and strongly suggests that the amount of *L. peruvianum* DNA is much larger in *Aps-1*^{1}-containing resistant tomato lines than in resistant lines with the *Aps-1*$^+$ allele (Ho et al. 1992; Messeguer et al. 1992). A detailed RFLP map of the *Mi* region of chromosome 6 has been deduced by comparison of *Mi*-linked polymorphisms in available lines and in recombinants in an F_2 population segregating for nematode resistance (Fig. 4). Using characterized lines we can position most of the RAPD markers identified here in tight linkage to *Aps-1* (Fig. 4). Comparison of the *L. esculentum-L. peruvianum* junction positions and the OPV07b polymorphism, places this marker near RFLP markers CD14 and H5G4. The *L. peruvianum* REX-1 allele is inseparable from *Mi* in all lines tested and, thus, along with LC379 represents the only described diagnostic marker present in Motelle which appears to retain the smallest introgressed region of the available resistant lines (Ho et al. 1992). F_2 segregation analysis has so far not resolved *Mi* and REX-1 and places these loci between GP79 and *Aps-1* which are separated by approximately 0.9 cM.

Nematode-resistant lines with the *L. esculentum* allele *Aps-1*$^+$ have not been utilized in some breeding programs due to the lack of available linked markers and the difficulty of direct screening for nematode resistance. REX-1 provides a tightly-linked and easily-scored marker that distinguishes all resistant and sus-

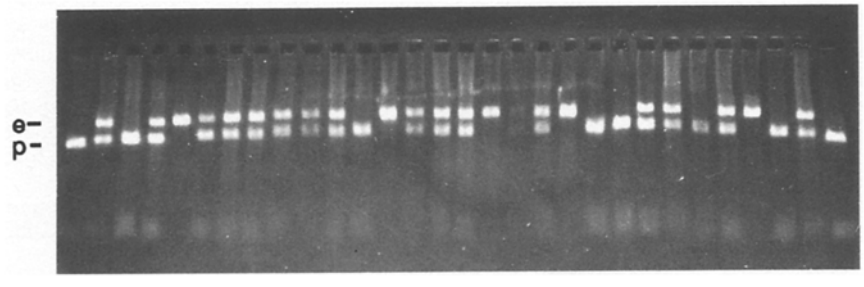

Fig. 3. F_2 segregation pattern of REX-1. DNA from 30 F_2 plants from a cross of VFNT cherry (*Aps-1*^{1} *Mi yv*) and LA55 (*Aps-1*$^+$ *mi yv*) was amplified with 20-mer primers REX-F1 and REX-R2 and cleaved with *Taq*I. The *Aps-1* alleles, nematode resistance, and alleles of surrounding RFLP markers of the F_2 plants have been previously determined (Ho et al. 1992) and segregate as expected for the REX-1 resistant (*p*) and susceptible (*e*) alleles. Thirty-nine additional F_2 were analyzed (data not shown)

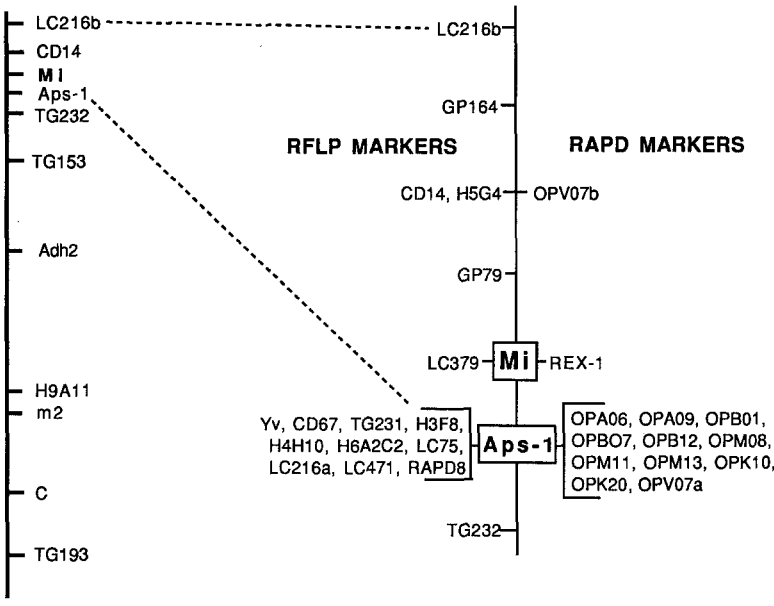

Fig. 4. Genetic map of the *Mi* region. A map of chromosome 6 is shown at the left and the relative positions of RFLP markers tightly linked to *Mi* are indicated (Ho et al. 1992). The position of RAPD markers deduced from an analysis of chracterized tomato lines is indicated at the right

ceptible lines tested, including Motelle which has the smallest introgressed region of the so-far characterized nematode-resistant lines (Messeguer et al. 1991; Ho et al. 1992). Because the REX-1 marker is codominant, heterozygous individuals can be easily identified. DNA extracted by a very simple procedure (Edwards et al. 1991) that requires less than 1 cm^2 of leaf tissue and is easy enough that hundreds of extractions can be carried out in 1 day was found to give consistent amplification with the REX-1 20-mer primers. Thus the marker is suitable for routine screening in a breeding program. It should be noted that failure of the enzyme *Taq*I to cleave the amplified fragment from the *L. peruvianum*-derived allele of REX-1 would result in incorrectly scoring a resistant plant as susceptible. The activity of the enzyme should be monitored by including a known resistant control in each experiment. In practice we have found cleavage by *Taq*I to be very reliable.

The strategy used here to generate a SCAR (or STS) from a RAPD marker tightly linked to *Mi* should be applicable to obtaining similar markers for other loci encoding agronomically-useful traits. In lettuce six of nine RAPD markers converted to SCARs were amplified in both parents and were useful markers either due to length polymorphism or restriction site polymorphisms (Paran and Michelmore 1993). In addition to markers originating from RAPDs, many of the more than 1000 RFLP markers that have already been mapped in tomato (Tanksley et al. 1992) could be converted to STS markers by sequencing their ends. Several markers of this type could be screened by PCR from the same DNA extract, allowing rapid, concurrent screening for several traits.

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References

Beek JG van der, Verkerk R, Zabel P, Lindhout P (1992) Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: Cf9 (resistance to *Cladosporium fulvum*) on chromosome 1. Theor Appl Genet 84:106–112

Bolkan HA, Williamson VM, Waters, CM (1987) Use of cellulose-acetate electrophoresis as an alternative to starch-gel electrophoresis for detecting root-knot nematode resistance in tomato. Plant Dis 71:1001–1003

Braham WS, Winstead NN (1957) Inheritance of resistance to root-knot nematodes in tomatoes. Proc Am Soc Hort Sci 69:372–377

Chumakov I, Rigault P, Guillou S, Ougen P, Billaut A, Guasconi G, Gervy P, LeGall I, Soularue P, Grinas L, Bougueleret L, Bellanne-Chantelot C, Lacroix B, Barillot E, Gesnouin P, Pook S, Vaysseix G, Frelat G, Schmitz A, Sambucy JL, Bosch A, Estivill X, Weissenbach J, Vignal A, Riethman H, Cox D, Patterson D, Gardiner K, Hattori M, Sakaki Y, Ichikawa H, Ohki M, Le Paslier D, Heilig R, Antonariakis S, Cohen D (1992) Continuum of overlapping clones spanning the entire human chromosome 21 q. Nature 359:380–387

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res 19:1349

Gilbert JC, McGuire DC (1956) Inheritance of resistance to severe root-knot from *Meloidogyne incognita* in commercial-type tomatoes. Proc Am Soc Hort Sci 63:437–442

Ho J-Y, Weide R, Ma HM, van Wordragen MF, Lambert KN, Koorneef M, Zabel P, Williamson VM (1992) The root-knot

nematode resistance gene (*Mi*) in tomato: construction of a molecular linkage map and identification of dominant cDNA markers in resistant genotypes. The Plant J 2: 971–982

Klein-Lankhotrs RM, Rietveid P, Machiels B, Verkert R, Weide R, Gebhardt C, Koornneef M, Zabel P (1991a) RFLP markers linked to the root knot nematode resistance gene *Mi* in tomato. Theor Appl Genet 81:661–667

Klein-Lankhorts RM, Vermunt A, Weide R, Liharska T, Zabel P (1991b) Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD) Theor Appl Genet 83:108–114

Laterrot H (1987) Neat-isogenic tomato lines in Moneymaker type with different genes for disease resistances. Tomato Genet Coop Rep Rep 37:91

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

Medina-Filho HP, Stevens MA (1980) Tomato breeding for nematode resistance: survey of resistant varieties for horticultural characteristics and genotype of acid phosphatases. Acta Hortic 100:383–391

Messeguer R, Ganal M, de Vicente MC, Young ND, Bolkan H, Tanksley SD (1991) High-resolution RFLP map around the root knot nematode resistant gene (*Mi*) in tomato. Theor Appl Genet 82:529–536

Miller JC, Tanksley SD (1990) RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor Appl Genet 80:437–448

Olson M, Hood L, Cantor C, Botstein D (1989) A common language for physical mapping of the human genome. Science 245:1434–1435

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Rick CM, Fobes J (1974) Association of an allozyme with nematode resistance. Tomato Genet Coop Rep 24:25

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High-density molecular-linkage maps of the tomato and potato genomes. Genetics 132:1141–1161

Williams JGK, Kubelik AR, Livak KJ, Rafalsky A, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Williamson VM, Colwell G (1991) Acid phosphatase-1 from nematode resistant tomato: isolation and characterization of its gene. Plant Physiol 97:139–146

Note added in proof

We have found that substitution of primer REX-R3 (5′-ATGCC-AGAGATGATTCGTGA-3′) for primer REX-R2 results in equal amplification of resistant and susceptible alleles of REX-1.