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FULL RESEARCH PAPER

Identification of a CAPS marker tightly linked to the Tomato yellow leaf curl disease resistance gene *Ty-1* in tomato

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Abstract During the process of breeding programmes, several resistance genes have been introgressed into tomato (*Solanum lycopersicum*) cultivars from different wild tomato relatives. A number of these resistance genes have been mapped to chromosome 6. Among them, *Ty-1* and *Mi*, which confer resistance to Tomato yellow leaf curl disease and to *Meloidogyne* spp., respectively, are in most cases incorporated in commercial hybrids. Several molecular markers tightly linked to *Mi* have been identified. This study was conducted in order to find an informative molecular marker linked to *Ty-1*. Six markers mapped in the same region as *Ty-1* were analysed in plant material carrying different combinations of *Ty-1* and *Mi* alleles. Three of the six markers revealed polymorphism among the assayed accessions. One allele of JB-1 marker showed association with *Ty-1*. Furthermore, the presence of *Mi* did not interfere with the results. The analysis of several accessions of wild tomato relatives with the three polymorphic markers allowed the establishment of the origin of the alleles found in cultivated plant material, showing that introgressions from *S. lycopersicum, S. pimpinellifolium* and *S. habrochaites* will not interfere with the results of this marker which tags *Ty-1*. Furthermore this analysis enabled the location of CT21, the RFLP marker from which JB-1 was designed.

Keywords Marker-assisted selection · *Mi* gene · *Solanum lycopersicum* · TYLCD

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Introduction

Tomato yellow leaf curl disease (TYLCD) causes important yield losses in tomato (*Solanum lycopersicum*) crops all over the world (Picó, Díez, & Nuez, 1996; Pilowsky & Cohen, 2000). This disease is caused by different viral species, all members of the genus *Begomovirus* (family Geminiviridae). Nine species from different geographical areas have been described and five more are considered tentative species (Fauquet & Stanley, 2005). Resistance to this disease has been identified in some wild tomato relatives such as *Solanum pimpinellifolium*, *Solanum habrochaites*, *Solanum peruvianum* and *Solanum chilense* (reviewed in Laterrot, 1992; Picó, Díez, & Nuez, 1996; Picó et al., 1999; Pilowsky & Cohen, 2000). The genetic basis of the resistance, which depends on the species, ranges from a single incompletely-dominant gene to a polygenic recessive pattern (Lapidot & Friedmann, 2000).

The resistance derived from accession LA1969 of *S. chilense*, has been thoroughly studied by Zamir et al. (1994); a major incompletely dominant gene, *Ty-1*, and two or more modifier genes control the resistance to TYLCD in this accession. The major gene, *Ty-1*, maps to chromosome 6, concretely to the region around markers TG297 and TG97. The resistance found in LA1969 has been introgressed into the cultivated species and several lines partially resistant to TYLCD have been developed (Laterrot, 1995; Michelson, Zamir, & Czosnek, 1994; Zamir et al., 1994). Most of the commercial cultivars resistant to TYLCD carry *Ty-1* gene. The availability of molecular markers tightly linked to this gene would allow the screening of putative resistant genotypes without inoculation with the pathogen, thereby shortening the length of breeding programmes. To date it has not been reported a concrete marker always linked to the presence of *Ty-1* gene. Several resistance genes have been mapped in tomato chromosome 6, apart from *Ty-1*, (reviewed in Zhang, Khan, Niño-Liu, & Foolad, 2002). The region around TG297 and TG97 markers holds some of these genes, i.e., *Mi*, *Cf-2* and *Cf-5* and *Ty-1*. This region has been studied in detail, initially with the purpose of cloning the *Mi* gene (Kaloshian et al., 1998; Liharska, Hontelez, van Kammen, Zabel, & Koornneef, 1997; Messeguer et al., 1991; van Daelen et al., 1993; van Wordragen et al., 1994).

Mi is a single dominant gene which confers resistance to the main species of the genus *Meloidogyne* (Gilbert, 1958). During the 1940s, this gene was transferred to cultivated tomato from *S. peruvianum* PI128657 (Bailey, 1941). Just one F_1 plant, derived from accession PI128657, was obtained (Smith, 1944). Breeding programmes were thus continued with this single plant. However, these programmes were developed from different backcrosses of the F_1 plant to *S. lycopersicum* at the Hawaiian Experimental Station (HES) and also at the University of California (Davis). The result was the release of the first resistant cultivars: VFN8 in California and Anahu and other HES lines in Hawaii. So, commercially available resistant plant materials are derived from one of these two original cultivars (Medina-Filho & Tanksley, 1983).

In *S. lycopersicum* plant material with introgressions for the region containing *Mi*, recombination is severely suppressed (Ho et al., 1992; Liharska, Koornneef, van Wordragen, van Kammen, & Zabel, 1996; Messeguer et al., 1991). As a consequence, tomato plants containing *Mi* retain a large introgression of *S. peruvianum*, although variable in length among cultivars (Messeguer et al., 1991). In order to facilitate breeding programmes, several molecular markers tightly linked to *Mi* have been identified, such as the acid phosphatase-1 (*Aps-1*) gene (Rick & Fobes, 1974) as well as RFLP (Ho et al., 1992; Klein-Lankhorst et al., 1991a; Messeguer et al., 1991) and RAPD (Klein-Lankhorst, Vermunt, Weide, Liharska, & Zabel, 1991b) markers. Even more specific PCR-based markers have also been developed (Kaloshian et al., 1998; Williamson, Ho, Wu, Miller, & Kaloshian, 1994), which are more suitable for routine analysis.

The purpose of the research reported here was to develop a molecular marker tightly linked to the resistance gene *Ty-1*. As has already been stated, the region containing *Ty-1* and *Mi* is genetically very short. It is very feasible that repression of recombination occurs also for introgressions from *S. chilense*, so it is likely that large amounts of *S. chilense* DNA are kept in resistant plant material derived from LA1969. Therefore, the molecular markers linked to *Mi* could be useful as markers for *Ty-1*, if alleles from *S. peruvianum* and *S. chilense* differed for these markers. On the other hand, if the alleles from these two species were the same for these markers, their use could lead to false positive results.

We have assayed different plant material for some of the markers that are tightly linked to *Mi* and, in addition, some other markers previously described for this region of chromosome 6. We describe a marker with one allele tightly associated with *Ty-1*.

Materials and methods

Plant material

Plant materials employed in this study along with their sources and main characteristics are listed in

Table 1 Plant material analysed

Species	Accession	*Mi*[a]	*Ty-1*[b]	Aps-1[c]	REX-1[d]	JB-1[e]	Source[f]
S. lycopersicum	UPV21183	*mi/mi*	*ty-1/ty-1*	1	1	1	1
	UPV21745	*mi/mi*	*ty-1/ty-1*	1	1	1	1
	FC	*mi/mi*	*ty-1/ty-1*	1	1	1	1
	Gévora	*Mi/Mi*	*ty-1/ty-1*	1	2	1	2
	H1124	*Mi/Mi*	*ty-1/ty-1*	2	2	2	2
	Fitó 1	*Mi/Mi*	*ty-1/ty-1*	1	2	1	3
	Fitó 2	*Mi/Mi*	*ty-1/ty-1*	2	2	2	3
	Fitó 3	*mi/mi*	*Ty-1/Ty-1*	2	1	3	3
	Fitó 4	*Mi/Mi*	*Ty-1/Ty-1*	2	2	3	3
	SC	*mi/mi*	*Ty-1/Ty-1*	2	2	3	4
	Boludo	*Mi/mi*	*Ty-1/ty-1*	1/2[g]	1/2	3	5
	Anastasia	*Mi/mi*	*Ty-1/ty-1*	1/2	2	3	5
	TY197	*mi/mi*	*ty-1/ty-1*	1	1-2[h]	1	6
	LA3473	*mi/mi*	*Ty-1/Ty-1*	2	3	3	7
	UPV21008[i]	*mi/mi*	*ty-1/ty-1*	1	1	1	1
S. peruvianum	PI128657	*Mi/Mi*	*ty-1/ty-1*	2	2-2/3	3	8
	UPV20196	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20340	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20342	*mi/mi*	*ty-1/ty-1*	2	3	3	1
	UPV20345	*mi/mi*	*ty-1/ty-1*	2	2	3	1
S. chilense	LA1969	*mi/mi*	*Ty-1/Ty-1*	2	2	3	7
	LA2884	*mi/mi*	*ty-1/ty-1*	2	2	3	7
	UPV20304	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20306	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20310	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20320	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20328	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20329	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20336	*mi/mi*	*ty-1/ty-1*	2	2	3	1
S. habrochaites	LA0386	*mi/mi*	*ty-1/ty-1*	3	2	D_1^j	7
	LA1777	*mi/mi*	*ty-1/ty-1*	3	2	D_2	7
	UPV16910a	*mi/mi*	*ty-1/ty-1*	2	2	D_3	1
	UPV17046 E	*mi/mi*	*ty-1/ty-1*	2	2	D_4	1
S. pimpinellifolium	LA1636	*mi/mi*	*ty-1/ty-1*	2	1	2	7
	LA1670	*mi/mi*	*ty-1/ty-1*	2	1	2	7
	LA2182	*mi/mi*	*ty-1/ty-1*	2	1	1	7
	LA2188	*mi/mi*	*ty-1/ty-1*	2	1	1	7
	LA2725	*mi/mi*	*ty-1/ty-1*	2	1	2	7
	PI 390728	*mi/mi*	*ty-1/ty-1*	2	1	1	9
	PI 127807	*mi/mi*	*ty-1/ty-1*	2	1	2	9

[a] Alleles for the *Mi* gene: *M*i resistant allele, *mi* susceptible allele

[b] Alleles for the *Ty-1* gene: *Ty-1* resistant allele, *ty-1* susceptible allele

[c] Alleles for the Aps-1 marker (see results for description)

[d] Alleles for the REX-1 marker (see results for description)

[e] Allele for the JB-1 marker (see results for description)

[f] Source: 1: Genebank of the Institute for the Conservation and Improvement of Agrodiversity (COMAV), Valencia, Spain; 2: J. Gragera, Sevicio de Investigación y Desarrollo Tecnológico, (SIDT) Badajoz, Spain; 3: Semillas Fitó S.A., Barcelona, Spain; 4: Plant material with this genetic composition belongs to a seed company; 5: Seminis Vegetable Seeds, Murcia, Spain; 6: Dr. M. Pilowsky, Volcani Center, Rehovot, Israel; 7: Tomato Genetics Resource Center (TGRC), University of California, Davis; 8: United States Department of Agriculture (USDA); 9: Australian Plant Genetic Resource Information Service (AusPGRIS), corresponding genebank codes are AUSTRCF311996 (PI 390728) and AUSTRCF312128 (PI127807)

[g] Bars separate alleles present in heterozygous individuals

[h] Hyphens separate different patterns for different individuals of a concrete plant material

[i] This accession was formerly classified as *L. esculentum* var. *cerasiforme*

[j] D: Alleles different than the ones described for the rest of the species

Table 1. Between five and seven plants per accession were analysed. UPV21183 and UPV21745 are local tomato varieties and FC is a breeding line; none of them should have introgressions from any wild species. Gevora and H1124 are breeding lines homozygous for *Mi* and susceptible to TYLCD. Fitó 1, 2, 3 and 4 are breeding lines homozygous for *Ty-1* and/or *Mi*. SC is a breeding line homozygous for *Ty-1*. Boludo and Anastasia are commercial hybrids which are heterozygous for *Ty-1* and *Mi*. TY197 is a breeding line with resistance to TYLCD derived from *S. peruvianum* (Lapidot et al., 1997). LA3473 is a breeding line with resistance to TYLCD derived from LA1969, so carrying *Ty-1* (Michelson et al., 1994). UPV21008 is an accession of *S. lycopersicum* (formerly *Lycopersicon esculentum* var. *cerasiforme*). PI128657 and LA1969 are the sources of *Mi* and *Ty-1*, respectively. The remaining accessions of wild species are either resistant or susceptible to TYLCD and *Meloidogyne*, but in no case do they carry *Ty-1* or *Mi*.

DNA extraction

Plant DNA used for analysis was extracted from 75 mg of fresh tissue, following the procedure described by Doyle & Doyle (1990) with some modifications.

Markers

Markers employed, primer sequences and the basis of their design are listed in Table 2.

Amplification and restriction conditions

The PCR reaction was carried out in a total volume of 25 µl containing: 1× buffer recommended by suppliers, 2.5 mM $MgCl_2$, 0.5 µM of each primer, 0.4 mM dNTPs, 1 U of *Taq* polymerase and 40 ng of template DNA. The amplification was carried out in an Eppendorf Martercycler Thermal Cycler with the following conditions (except for marker JB-1): 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, followed by an extension step of 10 min at 72°C. For JB-1, the optimum conditions for amplification were: 20 cycles of 94°C for 10 s, 55°C for 30 s and 72°C for 70 s, 10 cycles of 94°C for 10 s, 53°C for 30 s and 72°C for 70 s, followed by an extension step of 10 min at 72°C. Restrictions of 10 µl of the amplified products were performed overnight, in a total volume of 25 µl with 5 U of the corresponding enzyme, using buffers recommended by the suppliers at the recommended temperature. Digestion products were analysed by agarose gel electrophoresis (2% agarose w/v with TBE 1× buffer) and visualized by ethidium bromide staining. All reagents employed were supplied by Roche Diagnostics (Manheim, Germany).

Results

Screening for markers linked to *Ty-1*

PCR amplification of DNA from tomato accessions (*S. lycopersicum* plant material in Table 1) and subsequent digestions, when possible, were

Table 2 Markers of the region of gene *Ty-1* assayed

Marker	Primer sequence	Design basis	Restriction enzymes
Aps-1	ApsF: 5′-GGCAGGAGAATATGCCAAAA-3′ ApsR: 5′-CGTTCCATTCTCAACCCATT-3′	Designed based on a genomic clone (Williamson & Colwell, 1991)	*Taq*I
REX-1	REX-F1: 5′-TCGGAGCCTTGGTCTGAATT-3′ REX-R3: 5′-ATGCCAGAGATGATTCGTGA-3′	Williamson et al. (1994)	*Taq*I
JB-1	JB1F: 5′-AACCATTATCCGGTTCACTC-3′ JB1R: 5′-TTTCCATTCCTTGTTTCTCTG-3′	Designed based on RFLP CT21[a]	*Taq*I
CT216	CT216F 5′-ATTCTCCGGCGAGCCAAATC-3′ CT216R 5′-TTGTCTTCTTCTTCTAGTCGAC-3′	Designed based on RFLP CT216[a]	*Taq*I and *Hinf*I
CT119	CT119F: 5′-TCAGGTATCGAACCAAAACC-3′ CT119R: 5′-TAAAAGGTTCATCCTAATAC-3′	Dixon et al. (1995)	–
GP79	GP79F: 5′-TGTTCTCTAGTATCTCATCC-3′ GP79R: 5′-GGATTGTGATGTCGAGTTGC-3′	Dixon et al. (1995)	*Taq*I and *Tru*9I

[a] Information about these markers can be found at: http://www.sgn.cornell.edu

carried out using the primers and enzymes listed in Table 2. Clear amplification products were obtained for all the markers tested, except for CT119. However, for markers CT216 and GP79, polymorphism was not detected with the restriction enzymes employed.

For Aps-1 marker, two different alleles appeared in *S. lycopersicum* plant material (Fig. 1a): both showed a common band of approximately 700 pb and differed in a band slightly smaller than 300 pb (allele 1) or slightly larger than 300 pb (allele 2). Both alleles were codominant. All *S. lycopersicum* plant material without introgressions from wild species that were tested in this assay showed allele 1, so this must be the allele that corresponds to the cultivated species. TY197 also showed the*S. lycopersicum* allele for the Aps-1 marker. Plant materials that were homozygous for *Mi* exhibited either allele 1 or allele 2 for this marker. It has already been reported that DNA fragments introgressed from *S. peruvianum* kept by plant materials with this gene are variable in length, depending on their origin. Generally, lines derived from VFN8 maintain the *S. peruvianum* allele for locus *Aps-1*, while in those derived from HES lines, recombination occurred between *Mi* and *Aps-1* and they have the *S. lycopersicum* allele for *Aps-1* (Messeguer et al., 1991). Lines which were homozygous for *Ty-1* showed allele 2 for the *Aps-1* marker. The commercial hybrids, which were heterozygous for *Ty-1* and *Mi*, showed the heterozygous pattern for the *Aps-1* marker.

For the REX-1 marker (Fig. 1b), three different alleles appeared in *S. lycopersicum* plant material. Two of these alleles were previously described by Williamson et al. (1994): allele 1 consisted of a band of 750 pb and allele 2 of two bands of approximately 570 and 160 pb. These two alleles were co-dominant. We found one more allele, allele 3, which presented three bands of 350, 220 and 160 pb. All plant material without introgressions from any wild species showed allele 1 and all lines homozygous for *Mi*, allele 2, as previously described by Williamson et al. (1994). TY197 presented alternatively alleles 1 and 2, i.e., some plants kept *S. lycopersicum* allele while others kept *S. peruvianum* allele. Boludo, heterozygous for *Mi*, showed both allele 1 and 2. However, Anastasia, which is also heterozygous for *Mi*, was homozygous for allele 2. In lines which were homozygous for *Ty-1* but which did not carry *Mi*, alleles 1, 2 and 3 appeared alternatively.

Three different alleles appeared for JB-1 marker (Fig. 1c). All three had a common band of approximately 400 pb, which was the only band present for allele 1; allele 2 also consisted

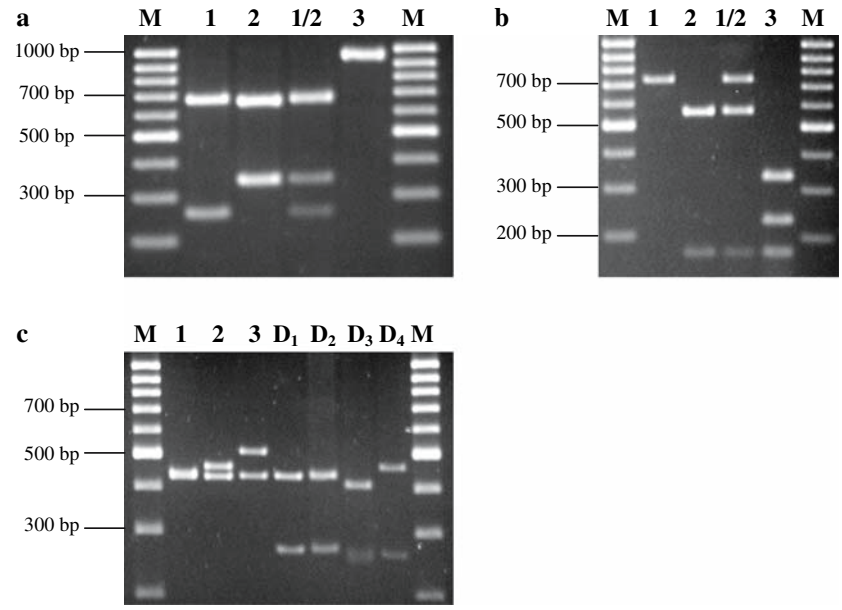

Fig. 1 Alleles for the markers Aps-1 (**a**), REX-1 (**b**) and JB-1 (**c**). Alleles are represented as coded in Table 1. M: DNA molecular weight marker (O'GeneRuler™ 100 bp DNA Ladder, Fermentas, Canada)

of a band slightly larger than 400 pb and allele 3 had a band of 500 pb. Allele 2 and allele 3 were co-dominant and dominant over allele 1. All *S. lycopersicum* lines without introgressions from wild species showed allele 1. This allele was also present in TY197 and in lines that, carrying *Mi*, showed allele 1 for the *Aps-1* marker. Lines that carried *Mi* and had the large introgression from *S. peruvianum* showed allele 2 for the JB-1 marker. All lines with *Ty-1* showed allele 3, independent of the presence of *Mi*.

Alleles for tomato and different wild tomato relatives

For the Aps-1 marker, *S. lycopersicum* accessions without introgressions showed allele 1. The rest of the species assayed showed allele 2, except for two *S. habrochaites* accessions, which showed a new allele (allele 3). This allele appeared as a result of the lack of a restriction site for *Taq*I in the amplification product (Fig. 1a).

Allele 1 for the REX-1 marker was present for all accessions of *S. lycopersicum* without introgressions and *S. pimpinellifolium*. Allele 2 was shown by all *S. chilense* and *S. habrochaites* accessions. Most *S. peruvianum* accessions had allele 2, as previously described (Williamson et al., 1994). However, we detected a new allele (allele 3) in some accessions of this species.

For the JB-1 marker, *S. lycopersicum* accessions without introgressions showed allele 1. *Solanum pimpinellifolium* accessions showed, alternatively, allele 1 or allele 2. All *S. chilense* accessions showed allele 3. This allele also appeared in all assayed *S. peruvianum* accessions. In *S. habrochaites*, several new alleles appeared in the different accessions (Fig. 1c), but all of them were distinguishable from the three alleles described for the rest of the species. The fact that allele 3 of the JB-1 marker is associated with *Ty-1* allows the use of this marker to tag the presence of this resistance allele. Moreover, the pattern obtained in different wild species shows that fragments in this region from these species will not interfere with the results for this marker.

Discussion

Molecular markers have many applications in plant breeding (Lörz & Wenzel, 2005; Nuez & Carillo, 2000). The availability of molecular markers linked to genes which confer desirable traits allows the shortening of breeding programmes. Several resistance genes identified in different wild tomato relatives have been introgressed into the cultivated species. Some of them map to chromosome 6 and are genetically very close. Identifying an allele of a marker associated specifically to one of these resistance genes can be complicated. Some breeding lines incorporate several genes from different wild species, and different wild species often share the same allele for a marker. This can lead to false positive results. *Ty-1* gene, which confers resistance to TYLCD, has been introgressed from *S. chilense* accession LA1969. We have identified a CAPS marker tightly linked to *Ty-1*.

Locus 1 of acid phosphatase (*Aps-1*) was the first isozyme employed as a marker for *Mi*. Knowledge of this locus at the sequence level (Williamson & Colwell, 1991) allowed us to develop a PCR-based marker. The results with this marker for plant material homozygous for *Mi* coincide with results previously reported (Messeguer et al., 1991); these plant materials showed, alternatively, allele 1 or 2, corresponding to alleles found in *S. lycopersicum* and *S. peruvianum*, respectively. For lines containing *Ty-1*, the alleles of this marker coincide with the alleles in plants carrying *Mi* along with the larger introgression of *S. peruvianum*. Analyses of alleles for the different wild tomato relatives support these results, given that *S. peruvianum* and *S. chilense* have the same allele for this marker. Furthermore, all species tested have this same allele, except *S. lycopersicum*, so introgressions from other species could lead to false positive results with this marker. Comparison at the sequence level of the amplification products obtained here from *S. chilense* and the sequence from *S. peruvianum* (GI:170369) showed that it would be possible to design a molecular marker that allowed the distinguishing of alleles for both species (data not shown). However, we also tested the Aps-1 marker on plants that did not present

the *Mi* locus. In some cases, recombination occurred between Aps-1 and *Ty-1*, given that some lines that were selected based on the presence of allele 2 of Aps-1 were susceptible to TYLCD when inoculated (data not shown). So, Aps-1 is not very useful as a marker for *Ty-1* given that it is not tightly linked to this gene, and the presence of other genes, in the same region from different wild species can lead to false positive results.

REX-1 marker has previously been reported as being tightly linked to *Mi* (Williamson et al., 1994). All homozygous plant materials carrying *Mi* showed allele 2. Six plants of accession PI128657, the donor of *Mi*, were analysed, and all but one showed allele 2 for the REX-1 marker; the exception was a heterozygous plant for alleles 2 and 3. Kaloshian et al. (1998) also found considerable polymorphism within accessions of *S. peruvianum* for many RFLP and PCR markers. Given that all plants carrying *Mi* showed allele 2 at the REX-1 locus, this must be the allele inherited from PI128657 in the single F_1 plant from which all plants carrying *Mi* descend. None of the *S. lycopersicum* plant materials without *Mi* showed allele 2, except for SC, which carried *Ty-1* but not *Mi*. Furthermore, it has been reported that begomovirus-resistant lines derived from *S. habrochaites* that are susceptible to *M. incognita* give false positive results for the REX-1 marker (El Mehrach et al., 2005). These authors studied the REX-1 marker at the sequence level, detecting the same single nucleotide polymorphism (SNP) associated with a *Taq*I restriction site in plant material with introgressions from *S. peruvianum* and *S. habrochaites*. Our results support this finding, given that all *S. habrochaites* accessions tested in this experiment showed allele 2 for the REX-1 marker. Resistance to TYLCV derived from *S. habrochaites* has been mapped to chromosome 11 (Hanson et al., 2000). However, introgressions in chromosome 6 must have been retained in resistant plant material developed from this source. Lines which were homozygous for *Ty-1* and did not carry *Mi*, showed, alternatively, alleles 1, 2 or 3. Allele 1 (*S. lycopersicum* allele) appeared in some breeding lines coded as Fitó 3. Allele 2 was present in SC, introgressed from *S. chilense*. Allele 3 was shown by LA3473; El Mehrach et al. (2005) found 2 *Taq*I restriction sites in the amplification product of the REX-1 locus of this accession, which would result in the three bands observed in our results. LA3474 is a breeding line derived from an initial cross between *S. lycopersicum* cv. M82-1-8 and *S. chilense* LA1969 (Michelson et al., 1994). Given that allele 3 is not the *S. chilense* allele for this marker, the presence of this allele in accession LA3474 must be due to the background of cv. M82-1-8. TY197 showed alternatively alleles 1 and 2, so indicating that along the breeding programme developed to derive this line, some plants have retained the *S. lycopersicum* allele while others have inherited the *S. peruvianum* allele. Anastasia and Boludo, which were heterozygous for *Mi* and *Ty-1*, showed different patterns. Anastasia was homozygous for allele 2, whereas Boludo showed alleles 1 and 2 for this marker. These are commercial cultivars, so we do not know their genealogy. However, these results can be explained. Anastasia must have been developed from the cross of one line carrying *Mi* and another one carrying *Ty-1*. Each of the alleles present in Anastasia would derive from one of these lines. The allele from the line carrying *Mi* would be the *S. peruvianum* allele, while the allele from the line carrying *Ty-1* would be the *S. chilense* allele. On the contrary, Boludo must have been derived from a cross between a line carrying both *Mi* and *Ty-1*, and another line with the *S. lycopersicum* alleles for both genes. It is not therefore possible to determine the *S. peruvianum* or *S. chilense* origin of allele 2 for REX-1 in Boludo. In any case, marker REX-1 is not useful in marker-assisted selection for *Ty-1*. Allele 2, which is present in *S. chilense* LA1969, the source of this gene, is not frequently introgressed along with *Ty-1*. So REX-1 marker and *Ty-1* are not tightly linked.

One allele of JB-1 marker is always associated to *Ty-1*. All *S. lycopersicum* plant material carrying *Ty-1*, whether homozygous or heterozygous, showed allele 3 for this marker. This allele is also present in all accessions tested belonging to *S. chilense*, among them LA1969, the source of *Ty-1*. None of the *S. lycopersicum* plant materials without *Ty-1* showed this allele. Among them, plant material which did not carry *Mi*, showed

allele 1, which is the *S. lycopersicum* allele. In plant material carrying *Mi*, alleles 1 and 2 appeared alternatively; allele 1 was present in lines with the small introgression from *S. peruvianum* (Gevora and Fitó 1), whereas allele 2 was shown by lines that retained the larger introgression (H1124 and Fitó 2). However, all accessions of *S. peruvianum*, including PI128657, showed allele 3. Therefore the JB-1 region has not been introgressed in *S. lycopersicum* along with *Mi*, so the presence of this gene in cultivated plant material will not interfere with the results of this marker which tags *Ty-1*. Allele 3 is not present in the rest of the wild species analysed—*S. pimpinellifolium* and *S. habrochaites*—so introgressions from these species will not interfere, either.

Regarding the order of the markers and genes involved in this study, the results reported in this paper allow the location of CT21 (the RFLP marker from which JB-1 was designed) to be identified. The relative positions of *Mi*, REX-1 and Aps-1 have been previously reported (Fig. 2a). The first studies that were developed in order to locate *Mi* established that this locus resided near the centromere in chromosome 6 and was tightly linked to *Aps-1* (Gilbert, 1958; Rick & Fobes, 1974). Later, a higher resolution map around the *Mi* gene was established, in which *Mi* and *Aps-1* could be separated (Messeguer et al., 1991). Further studies based on radiation-induced deletion mapping supported the conclusion that *Mi* is separated from *Aps-1* by the centromere, mapping *Aps-1* on the long arm of chromosome 6 (Liharska et al., 1997). The location of *Mi* below REX-1 was established by Kaloshian et al. (1998). The relative position of CT21, though, was not clear: this RFLP marker has been mapped together with TG231 and *Aps-1* (Tanksley et al., 1992). The lines tested here that carried *Mi* retained introgressions from *S. peruvianum* that were variable in length, maintaining either the *S. peruvianum* allele only for the REX-1 marker or for both REX-1 and Aps-1 markers. In no case did these plant materials contain the *S. peruvianum* PI128657 allele for JB-1 marker (Fig. 2b).

Messeguer et al. (1991) classified *Meloidogyne*-resistant cultivars into three categories with respect to the amount of linked *peruvianum* DNA retained around the *Mi* gene. Two of these groups of cultivars did not contain the *S. peruvianum* allele at *Aps-1* nor any of the markers past this point. The group of cultivars with the largest introgression from PI128657 retained *S. peruvianum* alleles for markers between TG297 and TG231. We have analysed some plant material belonging to this category. These plant materials contained the *peruvianum* allele for *Aps-1*. However, in no case did they retain the *S. peruvianum* allele for the JB-1 marker. Therefore, we conclude that JB-1 is located beyond the TG231 marker. This position of CT21 is consistent with the results found in lines carrying *Ty-1*. Introgressions from *S. chilense* in these lines are

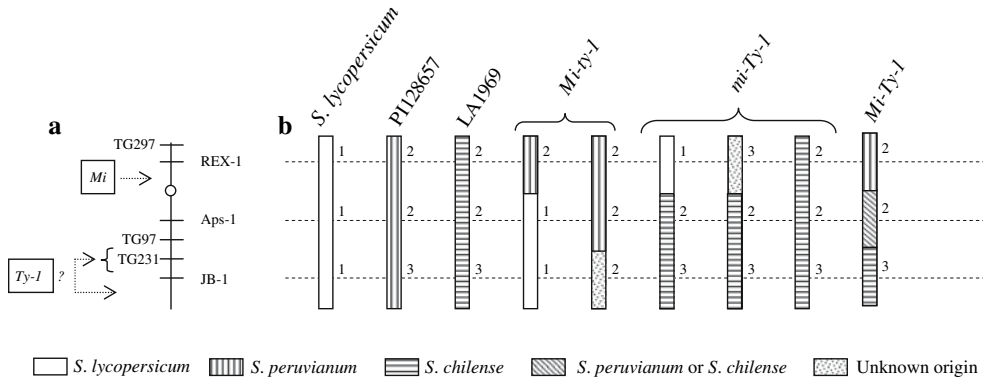

Fig. 2 (**a**) Diagram of *Mi/Ty-1* region of tomato chromosome 6, showing the relative order of markers and genes (framed). They are not on a genetic or physical scale. The centromere is indicated by a white circle. (**b**) Genetic composition of this region in the plant material used in this study. Introgressions from wild tomato relatives are represented by bars. Numbers on the right side of each bar indicate the allele as coded in Table 1

also variable in length. As stated above, introgressions always included the *S. chilense* allele for the JB-1 marker. In most of the plant material tested which carried *Ty-1*, the *S. chilense* allele for the *Aps-1* was also present. Moreover, even the allele for the REX-1 marker was introgressed from *S. chilense* along with *Ty-1* in some lines also carrying the *chilense* allele at *Aps-1*. The position of *Ty-1* with respect to JB-1 remains unclear. Zamir et al. (1994) located this locus below TG97, which is in accordance with the results obtained here.

The results reported here have allowed the identification of an allele of the JB-1 marker linked to *Ty-1*. This marker is more useful in marker-assisted selection than those previously used such as *Aps-1*, given that the presence of *Mi* or some other genes introgressed from wild tomato relatives will not interfere.

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