A co-dominant SCAR marker, Mi23, for detection of the *Mi-1*.2 gene for resistance to root-knot nematode in tomato germplasm

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The *Mi-1* resistance gene was introgressed into cultivated tomato from *Solanum peruvianum* in the 1940's (Smith, 1940) and is currently the only source of root-knot nematode resistance in modern tomato cultivars. *Mi-1* confers resistance to three species of root-knot nematode, *Meloidogyne incognita, M. javanica*, and *M. arenaria*. The principle means of utilization of this gene for developing nematode resistant tomato cultivars is by traditional breeding aided by marker-assisted selection.

The Rex-1 CAPS marker is widely used to assay for the *Mi-1* gene in tomato (Williamson et al., 1994). The Rex-1 marker has proven relatively reliable. However, El Mehrach et al. (2005) found that the REX marker gave false positives for the presence of *Mi-1* with some of the begomovirus-resistant germplasm derived from Ih902, which has begomovirus-resistance genes reportedly introgressed from *Solanum habrochaites* (Vidavsky and Czosnek, 1998). Primers were designed that only amplified a PCR fragment, if the *Mi-1.2* gene is present, but these primers do not distinguish heterozygous plants (El Mehrach et al., 2005). This report describes, Mi23, a co-dominant SCAR marker for the *Mi-1.2* gene (Milligan et al., 1998), which is located within the *Mi-1* locus (Seah et al., 2007).

Material and Methods

Primer design: The region on the short arm of chromosome 6 where the *Mi-1* gene is located is well defined genetically and physically (see Seah et al., 2004; 2007). The *Mi-1* locus in both resistant and susceptible tomato consists of two clusters with three and four copies of *Mi-1* homologues (Seah et al., 2004), which in resistant tomato are approximately 300 kb apart (Vos et al., 1998). Comparison of the sequences flanking *Mi-1.2* and its homologues from resistant and susceptible tomato revealed regions of high conservation, but also complex rearrangements including inverted chromosomal segments (Seah et al., 2004; 2007). In searching for an appropriate maker, Seah and Williamson noted that between the functional copy of *Mi-1* gene (*Mi-1.2*) and its closest downstream homologue, the pseudogene, *Mi-1.3*, lies 5 kb of sequence that is not predicted to encode protein sequences but is > 97% identical to the sequence between homologues *Mi-1B* and the pseudogene *Mi-1A* in susceptible tomato (see green boxes in Fig. 1 of Seah et al., 2007). Alignment of these sequences confirmed the strong similarity and revealed the presence of an indel of 57 nt. Primers (Mi23F and Mi23R) that flanked the indel and were conserved between *S. lycopersicum* and *S. peruvianum*-derived regions were selected with the aid of the program Primer3 by Sean and Williamson. Mi23F is 5'-TGG AAA AAT GTT GAA TTT CTT TTG-3', and Mi23R is 5'- GCA TAC TAT ATG GCT TGT TTA CCC-3'.

PCR methods: DNA was extracted from fresh leaves of plants with PUREGENE® DNA Purification Kit (Gentra Systems, Inc., Minneapolis, MN) and DNA adjusted to approximately 10 ng/µl. PCR parameters were for 25-µl reactions containing 2.5 µl 2.5 mM dNTPs, 5 µl 5x buffer, 2.5 µl 2.5 mM $MgCl_2$, 0.1 µl (0.5 units) Go*Taq* DNA polymerase (Promega Corp., Madison, WI), 2.5 µl each forward and reverse primer at 10 µM, 2-5 µl of DNA extract, and water. PCR cycles were 94 C for 3 min, the 35 cycles of 94 C for 30 sec, 57 C for 1 min, and 72 C for 1 min. These cycles were followed by 72 C for 10 min, and then the reaction was held at 18 C. PCR reactions were performed in the MJ DNA Engine PT200 Thermocycler™ (MJ Research Inc., Waltham, MA). PCR-amplified fragments were separated by gel electrophoresis with 2% agarose in 0.5 X TBE buffer, stained with ethidium bromide, and visualized with UV light. ssDNA was digested in PCR reactions with shrimp alkaline phosphatase (Progmega Corp.) and exonuclease I (Epicentre, Madison, WI); and the PCR-fragments directly sequenced with Big Dye Sequencing Kit™ and analyzed by the Biotechnology Center, University of Wisconsin-Madison.

Germplasm: The line M82-1-8 (Ve, F1) and Gh13 (Mejía et al., 2005) were used as the *mi*/*mi* genotype (susceptible) and had the *S. lycopersicum* sequence for the REX-1 marker. Two lines, Motelle and Gh2, which are known to be resistant to root-knot nematode, were used as the *Mi*/*Mi* genotype and had the *S. peruvianum* sequence for the REX-1 marker. The F1 hybrid, Llanero (resistant to begomoviruses, GenTropic Seeds), which is known to be heterozygous (*Mi*/*mi*) (unpublished data), and Marwa (V, F2, N and tolerant to *Tomato yellow leaf curl virus*, Syngenta), which is presumably heterozygous, were used as the heterozygous controls. Other commercial F1 hybrids, which were determined to be heterozygous at the REX locus by sequence analysis, were Celebrity (Seminis Seeds), Charanda (Vilmorin), Crista (Harris Moran), Dominique (Hazera Genetics), Tequila (Vilmorin), and Viva Italia (Harris Moran). Rodeo (Heinz) was homozygous at the Mi locus, as determined by the REX-1 marker sequence. Titrit (F1, F2, Ve, TMV, FCRR, Royal Sluis) is not resistant to RKN, but tolerant to *Tomato yellow leaf curl virus*.

Results and discussion

The susceptible genotypes M82-1-8 and Gh13 (*mi*/*mi*), the resistant genotypes Motelle and Gh2 (*Mi*/*Mi*) gave PCR fragments of ca. 430 bp, and ca. 380 bp, respectively (Fig. 1). The heterozygous genotypes Llanero and Marwa gave three fragments, 380, 430 and 500 bp. The third, slower moving PCR-fragment from the heterozygous plants was shown to be a heteroduplex between the two fragments (380 and 430 bp), which migrated more slowly due to the presence of a 56 nucleotide loop in the heteroduplex molecules (Fig. 2).

The PCR fragments from M82-1-8 (AY596779) and Gh2 were sequenced and a BLAST search performed at the National Center for Biotechnology Information. The 432-bp fragment (GenBank no.) from M82-1-8 had 100% nt identity with *Solanum lycopersicum* (cv. Heinz 1706, DQ863289) for nt 9,545-9,976, which is located between two resistance-like protein ORFs in cluster 2e. The 377-bp fragment from Gh2 had 100% nt identity with the *Mi-1* locus from Motelle (U81378, *Solanum peruvianum* introgression for *Mi-1* locus) for nt 25,819-26,195, which is located between the *Mi-1.2* resistance gene and a pseudo-resistance gene (*Mi-1.3*) in cluster 1p. Thus, the sequence of the PCR fragments matched the areas of the *S. lycopersicum* and *S. peruvianum* genomes used to design the primers. When the two sequences were compared, there were indels of 1 nt and 56 nt, which accounted for the differences in the length of the two sequences. Besides these two indels, there were 13 SNPs between these two sequences.

When six commercial hybrids (Celebrity, Charanda, Crista, Dominique, Tequila and Viva Italia) with reported resistance to root-knot nematode were tested with the primers Mi23F/Mi23R, all produced the pattern associated with heterozygous plants for the *Mi-1* locus. Rodeo gave the expected single 380-bp fragment for the homozygous genotype (Mi/Mi). Titrit, which lacks the *Mi-1* locus, gave the 420-bp fragment for the susceptible genotype. These primers were also tested on 73 breeding lines and hybrids for begomovirus resistance from the Guatemalan project (Mejía et al., 2005) as well as 31 other inbreds and hybrids, and unambiguous PCR patterns were obtain (Fig. 3).

Previously, false positives indicating the presence of the *Mi-1* locus were obtained with two co-dominant CAPS markers, REX-1 (Williamson et al., 1994) and Cor-Mi (Contact Cornell University Foundation, Ithaca, NY) by El Mehrach et al. (2005) for the begomovirus-resistant breeding line, Ih902 (F1, F2, Ve, Vidavsky and Czosnek, 1998). The line Ih902, which was susceptible to root-knot nematode (Williamson, unpublished data), is one of the main sources of begomovirus-resistance in the tomato breeding program at San Carlos University, Guatemala. The REX fragments from Ih902 (mi/mi) and Motelle (Mi/Mi) were sequenced and had 100% nt identity. **Thus, the *REX-1* locus was not predictive of the presence of the *Mi-1.2* gene in this breeding line.** Comparison of the PCR-Cor-Mi fragment sequence from Motelle (*Mi*/*Mi*), Ih902 and Moneymaker (*mi*/*mi*) showed that they were not identical. Surprisingly, the sequence of the Cor-PCR fragment from Ih902 was identical with that from the TY52 line, which is homozygous for *Ty-1*/*Ty-1* (pers. com., H. Czosnek). The *Ty-1* begomovirus-resistance locus is derived from *Solanum chilense* LA1969 and was mapped to the short arm of chromosome 6 (Zamir et al., 1994). The *REX-1* marker sequence for the TY52 line, which has the *Ty-1* locus introgression from *S. chilense*, gives a distinct digestion pattern with *TaqI* restriction enzyme (Milo et al., 2001). This indicates that the *Ty-1* introgression exits in the region of the REX-1 marker for TY52. This has also been shown for many other lines derived from *S. chilense* LA2779 (unpublished data). Therefore, due to the limitations of these CAPS markers, it was of value to test Mi23 with tomato lines that gave false positives as well as lines known to have the *S. chilense* introgression for the *Ty-1* locus.

When Ih902, TY52 (*Ty-1/Ty-1*), and 2 breeding lines [Gc9 (EU033925) and Gc143-2, both are *S. chilense* LA2779 derived line] homozygous for the *Ty-1* locus were evaluated with the Mi23F/Mi23R primers, only the 430-bp PCR fragment, corresponding in size to that present in susceptible *S. lycopersicon* was amplified. This is what would be expected for these 4 lines, which are known to be susceptible to the root knot nematode. **In this case, the Mi23 primer pair did not give a false positive with Ih902 for the *Mi-1.2* gene.** Surprisingly, sequence of these PCR-fragments from the 4 lines were identical, and were distinguished by 16 SNPs and a 1-nt indel from the sequence from *S. lycopersicon*, M82-1-8. Besides the 56-nt indel, there were 6 SNPs between the Ih902 sequence and that from Gh2 (*Mi*/*Mi*). It was concluded that Ih902 had *S. chilense* in this region. These results indicate that these primers might be useful to detect genotypes with the *Ty-1* locus, i.e., introgression from *S. chilense* (see Protocol III, this web site).

It was of interest to evaluate the Mi23 marker with several wild species that are sources of disease resistance genes that have been introgressed into the short arm of chromosome 6. Three accessions of *S. peruvianum* (LA3858, LA3858, and LA0111) were tested. LA3858 (EU033932) and LA3900 gave 377-bp fragments, which had 100% nt identity with the fragment from Gh2 (*Mi/Mi*). *S. peruvianum* LA0111 yielded the heterozygous pattern with three fragments. *S. arcanum*, which is phylogentically closely related to *S. peruvianum* (F. Rodriguez and D. Spooner, pers. com.), yielded a 377-bp fragment (EU033928), which had 99% nt identity with the fragment from Gh2 (3 SNPs). Two *S. chilense* accessions [LA2779 (EU033931) and LA1932 (EU033929)], which are known sources of resistance genes for begomoviruses, gave 433-bp fragments and had

96% nt identity with the sequence from M82-1-8. The two accessions of *S. pimpinellifolium* [LA1606 and LA2184 EU033930)] produced 432-bp fragments that were 99.8% and 100% identical, respectively, with that produced by M82-1-8.

Conclusions

The co-dominant SCAR marker, Mi23, has the advantage over previous PCR-based markers in that the restriction enzyme digestion step is not required, and it is more tightly linked with the *Mi-1.2* gene. This marker does not give false positive fragments with the begomovirus-resistant breeding lines derived from *S. habrochaites* (Vidavsky and Czosnek, 1998) and *S. chilense* (*Ty-1* locus) (Agrama and Scott, 2006).

It is suggested from the analyses of these markers for the Ih902 germplasm that the order of markers is REX-1, Cor-Mi, Mi23, and Ty-1 (TG97). For other germplasm this order might be different. For the TY52 (Ty-1/Ty-1) line, REX-1, Mi23 and TY-1 (TG97) markers all had *S. chilense* sequences. In Gh2, the REX-1, Mi23, and Cor-Mi markers have *S. peruvianum* sequences and *Ty-1* (TG97) has *S. chilense* sequence. Thus, it is possible to break the linkage between the *Mi-1.2* gene and the *Ty-1* gene.

Sequences and their alignment are given below.

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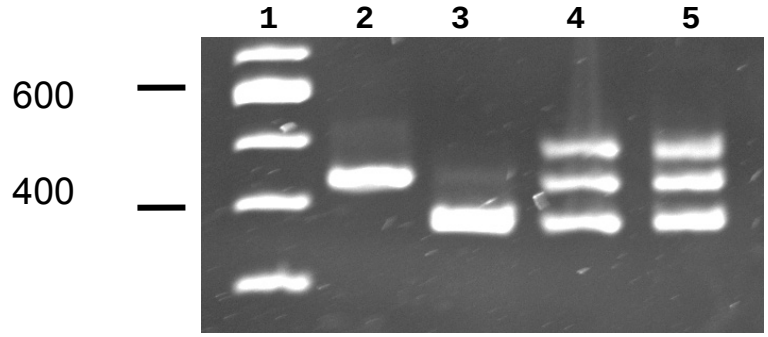

Fig. 1. PCR with primers Mi23F/Mi23R at annealing temperature of 57 C for detection of the *Mi-1* locus. Lane 1, 100-bp marker (Invitrogen); lane 2, M82-1-8 (*mi/mi*); lane 3, Motelle (*Mi/Mi*); lane 4, Marwa (VF2N and tolerance to *Tomato yellow leaf curl virus*); lane 5, Llanero (*Mi/mi*, as determined by genotype of parents).

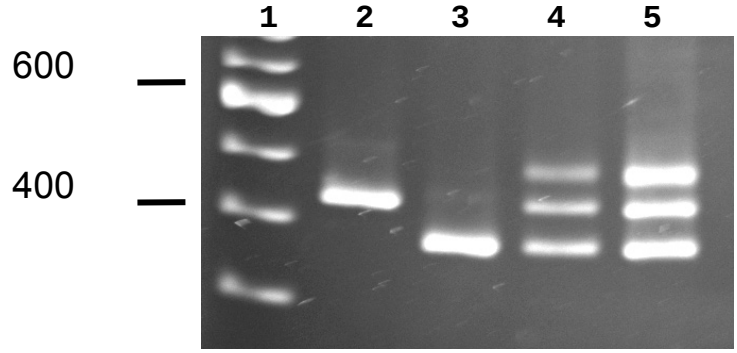

Fig. 2. PCR with primers Mi23F/Mi23R at annealing temperature of 57 C. Lane 1, 100-bp marker (Invitrogen); lane 2, M82-1-8; lane 3, Motelle; lane 4, Llanero (heterozygous), lane 5, equal amounts of the PCR fragments for M82-1-8 and Motelle mixed together and subjected to the standard PCR cycles. Note that three bands are present and that these correspond to the identical sizes of the bands from the heterozygous hybrid Llanero.

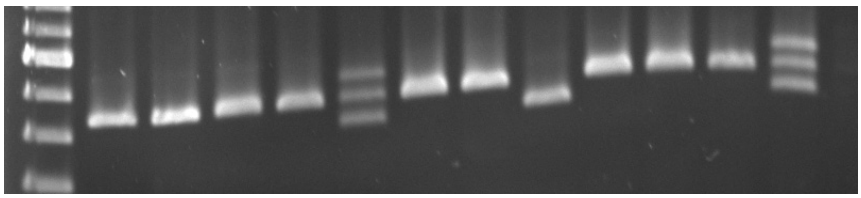

Fig. 3. PCR with primers Mi23F/Mi23R at annealing temperature of 57 C for detection of the *Mi-1* locus in tomato breeding lines.

Brenda Esperanza Garcia and Douglas P. Maxwell, University of Wisconsin-Madison, March 2007 (Contact: dpmax@plantpath.wisc.edu)

Alignment of sequences for the Mi23 locus for: M82 = M82-1-8, mi/mi; LA1606, *S. pimpinellifolium*; LA2184, *S. pimpinellifolium*; Gc9, resistant to begomoviruses, introgression from *S. chilense* LA2779; TY52, resistant to TYLCV, with introgression from *S. chilense* LA1969 for the *Ty-1* gene; LA2779, *S. chilense*; LA1932, *S. chilense*; LA0392, *S. arcanum*; LA3858, *S. peruvianum*; LA3900, *S. peruvianum*; Gh2, Mi/Mi, resistant to root-knot nematode and also resistant to begomoviruses from Ih902.

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M82         TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
LA1606      TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
LA2184      TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
Gc9         TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
TY52        TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
LA2779      TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
LA1932      TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTtAAAaTATGAAAACAAGTATT     60
LA0392      TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
LA3858      TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
LA3900      TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
Gh2         TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
Consensus   tggaaaaatgttgaatttcttttgtaagtgtacaaagtt aaa tatgaaaacaagtatt

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LA1606      TGGAGTTTCTAAAATTTTGGAATATTCTGGCAAAATTTGAGCGGAGAAATGTGACAGTTC    120
LA2184      TGGAGTTTCTAAAATTTTGGAATATTCTGGCAAAATTTGAGCGGAGAAATGTGACAGTTC    120
Gc9         TGGAGTTTCTAAAATTTTGGAATATTCTGGCtAAATTTGAGCGGAGAAATGTGACAGTTC    120
TY52        TGGAGTTTCTAAAATTTTGGAATATTCTGGCtAAATTTGAGCGGAGAAATGTGACAGTTC    120
LA2779      TGGAGTTTCTAAAATTTTGGAATATTCTGGCtAAATTTGAGCGGAGAAATGTGACAGTTC    120
LA1932      TGGAGTTTCTAAAATTTTGGAATATTCTaGCtAAATTTGAGCGGAGAAATGTGACAGTTC    120
LA0392      TGGAGTTTCTAAAATTTTGGAATATTCTGGCAAAATTTGAGCGGAGAAAT.........    110
LA3858      TGGAGTTTCTAAAATTTTGGAATATTCTGGCAAAATTTGAGCGGAGAAAT.........    110
LA3900      TGGAGTTTCTAAAATTTTGGAATATTCTGGCAAAATTTGAGCGGAGAAAT.........    110
Gh2         TGGAGTTTCTAAAATTTTGGAATATTCTGGCAAAATTTGAGCGGAGAAAT.........    110
Consensus   tggagtttctaaaattttggaatattct gc aaatttgagcggagaaat

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LA1606      ACGTCCAAATCTCCAGAGTCTTCATACATAGAAGTGTCAAACAAATTGGCAGGTTCTTAC    180
LA2184      ACGTCCAAATCTCCAGAGTCTTCATACATAGAAGTGTCAAACAAATTGGCAGGTTCTTAC    180
Gc9         ACGTCCAAATCTCCAGAGTCTTCATACATAGAAGTGTCAAACAAtTTaGCAGGTTCTTAC    180
TY52        ACGTCCAAATCTCCAGAGTCTTCATACATAGAAGTGTCAAACAAtTTaGCAGGTTCTTAC    180
LA2779      AtGTCCAAATCTCCAGAGTCTTCATACATAGAAGTGTCAAACAAATTGGCtGGTTCTTAC    180
LA1932      ACGTCCAAATCTCCAGAGTCTTCATACATAGAAGTGTCAAACAAATTGGCAGGTTCTTAC    180
LA0392      ..............................................TGGCAGGTTCTTAC    124
LA3858      ..............................................TGGCAGGTTCTTAC    124
LA3900      ..............................................TGGCAGGTTCTTAC    124
Gh2         ..............................................TGGCAGGTTCTTAC    124
Consensus                                                   t gc ggttcttac
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LA1606      A.CCTTTTACTGTTCTAAAAAGATGTCTACAATTcGTTTCATCAAAGCCCCGACGGAACT    239
LA2184      A.CCTTTTACTGTTCTAAAAAGATGTCTACAATTTGTTTCATCAAAGCCCCGACGGAACT    239
Gc9         AtCtTTTTACTGTTCTAAAAAGATGTCTACAATTcGTTTCATCAAAGCCCCGACGGAACT    240
TY52        AtCtTTTTACTGTTCTAAAAAGATGTCTACAATTcGTTTCATCAAAGCCCCGACGGAACT    240
LA2779      AtCtTTTTACTGTTCTAAAAAGATGTCTACAATTcGTTTCATCAAAGCCCCGACGGAACT    240
LA1932      AtCtTTTTACTGTTCTAAAAAGATGTCTACAATTcGTTTCATCAAAGCCCCGACGGAACT    240
LA0392      AtCtTTTTACTGTTCTAAAAAGATGTCTACAATTcGTTTCATCAAAGCCCCGACGGAACT    184
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LA3900      AtCtTTTTACTGTTCTAAAAAGATGTCTACAATTcGTTTgATCAAAGCCCCGACGGAACT    184
Gh2         AtCtTTTTACTGTTCTAAAAAGATGTCTACAATTcGTTTgATCAAAGCCCCGACGGAACT    184
Consensus   a c ttttactgttctaaaaagatgtctacaatt gttt atcaaagccccgacggaact

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LA2184      ATTAAGTAGACGACGTTAGTAAAATAACAAGCAACCAAAGCAGTTACGAGAGATCACTTT    299
Gc9         ATTAAGTAGACGAgGTTAGTAAAATAACAAGCAACCAAAtCAGTTtaGAGAGATCACTTT    300
TY52        ATTAAGTAGACGAgGTTAGTAAAATAACAAGCAACCAAAtCAGTTtaGAGAGATCACTTT    300
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LA0392      ATTAAGTAGACGACGTTAGTAAAATAACAAGCAACCAAAGCAGTTtaGAGAGATCACTTT    244
LA3858      ATTAAGTAGACGAgGTTAGTAAAATAACAAGCAACCAAAGCAGTTtaGAGAGATCACTTT    244
LA3900      ATTAAGTAGACGAgGTTAGTAAAATAACAAGCAACCAAAGCAGTTtaGAGAGATCACTTT    244
Gh2         ATTAAGTAGACGAgGTTAGTAAAATAACAAGCAACCAAAGCAGTTtaGAGAGATCACTTT    244
Consensus   attaagtagacga gttagtaaaataacaagcaaccaaa cagtt  gagagatcacttt

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LA1606      TTTCCCAGGGGATTTTTCTAGTAAGATTTTAATCAAGCACATTATCTACTAAATATATAG    359
LA2184      TTTCCCAGGGGATTTTTCTAGTAAGATTTTAATCAAGCACATTATCTACTAAATATATAG    359
Gc9         TTTCCCAcGGaATTTTTCTAGTAAGATTTTAAcCAgGCAtATTATCTtCTAAATATATAG    360
TY52        TTTCCCAcGGaATTTTTCTAGTAAGATTTTAAcCAgGCAtATTATCTtCTAAATATATAG    360
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LA1932      TTTCCCAcGGGATTTTTCTAGTAAGATTTTAAcCAgGCAtATTATCTtCTAAATATATAG    360
LA0392      TTTCCCAtGGGATTTTTCTAGTAAGATTTTAAcCAgGCAtATTATCTtCTAAATATgTAG    304
LA3858      TTTCCCAcGGGATTTTTCTAGTAAGATTTTAAcCAgGCAtATTATCTtCTAAATATgTAG    304
LA3900      TTTCCCAcGGGATTTTTCTAGTAAGATTTTAAcCAgGCAtATTATCTtCTAAATATgTAG    304
Gh2         TTTCCCAcGGGATTTTTCTAGTAAGATTTTAAcCAgGCAtATTATCTtCTAAATATgTAG    304
Consensus   tttccca gg atttttctagtaagattttaa ca  ca attatct ctaaatat tag

M82         CGAGTTAGTATCATTATACTTTGTGTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    419
LA1606      CGAGTTAGTATCATTATACTTTGTGTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    419
LA2184      CGAGTTAGTATCATTATACTTTGTGTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    419
Gc9         CGAGTTAGTATtATTATACTTTGTcTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    420
TY52        CGAGTTAGTATtATTATACTTTGTcTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    420
LA2779      CGAGTTAGTATCATTATACTTTGTcTACAAATTAAATTTCGATTACgCTGGGTAAACAAG    420
LA1932      CGAGTTAGTATCATTATACTTTGTcTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    420
LA0392      CGAGTTAGTATCATTATACTTTGTcTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    364
LA3858      CGAGTTAGTATCATTATACTTTGTcTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    364
LA3900      CGAGTTAGTATCATTATACTTTGTcTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    364
Gh2         CGAGTTAGTATCATTATACTTTGTcTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    364
Consensus   cgagttagtat attatactttgt tacaaattaaatttcgattac ctgggtaaacaag
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M82         CCATATAGTATGC                                                  432
LA1606      CCATATAGTATGC                                                  432
LA2184      CCATATAGTATGC                                                  432
Gc9         CCATATAGTATGC                                                  433
TY52        CCATATAGTATGC                                                  433
LA2779      CCATATAGTATGC                                                  433
LA1932      CCATATAGTATGC                                                  433
LA0392      CCATATAGTATGC                                                  377
LA3858      CCATATAGTATGC                                                  377
LA3900      CCATATAGTATGC                                                  377
Gh2         CCATATAGTATGC                                                  377
Consensus   ccatatagtatgc
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M82, mi/mi **GenBank no. EU033926**

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      SEQ: 432 bp;
Composition  152 A; 67 C; 74 G; 139 T; 0 OTHER
Percentage:  35.2%  A; 15.5%  C; 17.1%  G; 32.2%  T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 133.54 dsDNA: 266.25
ORIGIN
1      TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61     TGGAGTTTCT AAAATTTTGG AATATTCTGG CAAAATTTGA GCGGAGAAAT GTGACAGTTC
121    ACGTCCAAAT CTCCAGAGTC TTCATACATA GAAGTGTCAA ACAAATTGGC AGGTTCTTAC
181    ACCTTTTACT GTTCTAAAAA GATGTCTACA ATTTGTTTCA TCAAAGCCCC GACGGAACTA
241    TTAAGTAGAC GACGTTAGTA AAATAACAAG CAACCAAAGC AGTTACGAGA GATCACTTTT
301    TTCCCAGGGG ATTTTTCTAG TAAGATTTTA ATCAAGCACA TTATCTACTA AATATATAGC
361    GAGTTAGTAT CATTATACTT TGTGTACAAA TTAAATTTCG ATTACTCTGG GTAAACAAGC
421    CATATAGTAT GC
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***S. pimpinellifolium* LA1606**

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      SEQ: 432 bp;
Composition  152 A; 68 C; 74 G; 138 T; 0 OTHER
Percentage:  35.2%  A; 15.7%  C; 17.1%  G; 31.9%  T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 133.53 dsDNA: 266.25
ORIGIN
1      TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61     TGGAGTTTCT AAAATTTTGG AATATTCTGG CAAAATTTGA GCGGAGAAAT GTGACAGTTC
121    ACGTCCAAAT CTCCAGAGTC TTCATACATA GAAGTGTCAA ACAAATTGGC AGGTTCTTAC
181    ACCTTTTACT GTTCTAAAAA GATGTCTACA ATTCGTTTCA TCAAAGCCCC GACGGAACTA
241    TTAAGTAGAC GACGTTAGTA AAATAACAAG CAACCAAAGC AGTTACGAGA GATCACTTTT
301    TTCCCAGGGG ATTTTTCTAG TAAGATTTTA ATCAAGCACA TTATCTACTA AATATATAGC
361    GAGTTAGTAT CATTATACTT TGTGTACAAA TTAAATTTCG ATTACTCTGG GTAAACAAGC
421    CATATAGTAT GC
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***S. pimpinellifolium* LA2184** **GenBank no. EU033930**

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      SEQ: 432 bp;
Composition  152 A; 67 C; 74 G; 139 T; 0 OTHER
Percentage:  35.2%  A; 15.5%  C; 17.1%  G; 32.2%  T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 133.54 dsDNA: 266.25
ORIGIN
1      TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61     TGGAGTTTCT AAAATTTTGG AATATTCTGG CAAAATTTGA GCGGAGAAAT GTGACAGTTC
121    ACGTCCAAAT CTCCAGAGTC TTCATACATA GAAGTGTCAA ACAAATTGGC AGGTTCTTAC
181    ACCTTTTACT GTTCTAAAAA GATGTCTACA ATTTGTTTCA TCAAAGCCCC GACGGAACTA
241    TTAAGTAGAC GACGTTAGTA AAATAACAAG CAACCAAAGC AGTTACGAGA GATCACTTTT
301    TTCCCAGGGG ATTTTTCTAG TAAGATTTTA ATCAAGCACA TTATCTACTA AATATATAGC
361    GAGTTAGTAT CATTATACTT TGTGTACAAA TTAAATTTCG ATTACTCTGG GTAAACAAGC
421    CATATAGTAT GC
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Gc9, resistant to begomoviruses with introgression from *S. chilense* LA2779, susceptible to root-knot nematode, mi/mi, Ty1/Ty1; **GenBank no. EU033925**

SEQ: 433 bp;
Composition 150 A; 66 C; 71 G; 146 T; 0 OTHER
Percentage: 34.6% A; 15.2% C; 16.4% G; 33.7% T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 133.77 dsDNA: 266.87
ORIGIN
1 TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61 TGGAGTTTCT AAAATTTTGG AATATTCTGG CTAAATTTGA GCGGAGAAAT GTGACAGTTC
121 ACGTCCAAAT CTCCAGAGTC TTCATACATA GAAGTGTCAA ACAATTTAGC AGGTTCTTAC
181 ATCTTTTTAC TGTTCTAAAA AGATGTCTAC AATTCGTTTC ATCAAAGCCC CGACGGAACT
241 ATTAAGTAGA CGAGGTTAGT AAAATAACAA GCAACCAAAT CAGTTTAGAG AGATCACTTT
301 TTTCCCACGG AATTTTTCTA GTAAGATTTT AACCAGGCAT ATTATCTTCT AAATATATAG
361 CGAGTTAGTA TTATTATACT TTGTCTACAA ATTAAATTTC GATTACTCTG GGTAAACAAG
421 CCATATAGTA TGC

TY52, resistant to begomoviruses (TYLCV) with introgression from *S. chilense* LA1969, Ty1/Ty1 (near isogenic line from Dani Zamir, Hebrew University of Jerusaleum)

SEQ: 433 bp;
Composition 150 A; 66 C; 71 G; 146 T; 0 OTHER
Percentage: 34.6% A; 15.2% C; 16.4% G; 33.7% T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 133.77 dsDNA: 266.87
ORIGIN
1 TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61 TGGAGTTTCT AAAATTTTGG AATATTCTGG CTAAATTTGA GCGGAGAAAT GTGACAGTTC
121 ACGTCCAAAT CTCCAGAGTC TTCATACATA GAAGTGTCAA ACAATTTAGC AGGTTCTTAC
181 ATCTTTTTAC TGTTCTAAAA AGATGTCTAC AATTCGTTTC ATCAAAGCCC CGACGGAACT
241 ATTAAGTAGA CGAGGTTAGT AAAATAACAA GCAACCAAAT CAGTTTAGAG AGATCACTTT
301 TTTCCCACGG AATTTTTCTA GTAAGATTTT AACCAGGCAT ATTATCTTCT AAATATATAG
361 CGAGTTAGTA TTATTATACT TTGTCTACAA ATTAAATTTC GATTACTCTG GGTAAACAAG
421 CCATATAGTA TGC

LA2779, *S. chilense*, this accession was source of resistance for begomoviruses (J. W. Scott, University of Florida); **GenBank no. EU033931**

SEQ: 433 bp;
Composition 149 A; 66 C; 74 G; 144 T; 0 OTHER
Percentage: 34.4% A; 15.2% C; 17.1% G; 33.3% T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 133.83 dsDNA: 266.87
ORIGIN
1 TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61 TGGAGTTTCT AAAATTTTGG AATATTCTGG CTAAATTTGA GCGGAGAAAT GTGACAGTTC
121 ATGTCCAAAT CTCCAGAGTC TTCATACATA GAAGTGTCAA ACAAATTGGC TGGTTCTTAC
181 ATCTTTTTAC TGTTCTAAAA AGATGTCTAC AATTCGTTTC ATCAAAGCCC CGACGGAACT
241 ATTAAGTAGA CGAGGTTAGT AAAATAACAA GCAACCAAAG CAGTTTAGAG AGATCACTTT
301 TTTCCCACGG GATTTTTCTA GTAAGATTTT AACCAGACAT ATTATCTTCT AAATATATAG
361 CGAGTTAGTA TCATTATACT TTGTCTACAA ATTAAATTTC GATTACGCTG GGTAAACAAG
421 CCATATAGTA TGC

LA1932, *S. chilense*, this accession was source of resistance for begomoviruses (J. W. Scott, University of Florida); **GenBank no. EU033929**

SEQ: 433 bp;
Composition 150 A; 68 C; 71 G; 144 T; 0 OTHER
Percentage: 34.6% A; 15.7% C; 16.4% G; 33.3% T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 133.74 dsDNA: 266.87
ORIGIN
1 TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTT AAAATATGAA AACAAGTATT
61 TGGAGTTTCT AAAATTTTGG AATATTCTAG CTAAATTTGA GCGGAGAAAT GTGACAGTTC
121 ACGTCCAAAT CTCCAGAGTC TTCATACATA GAAGTGTCAA ACAAATTGGC AGGTTCTTAC
181 ATCTTTTTAC TGTTCTAAAA AGATGTCTAC AATTCGTTTC ATCAAAGCCC CGACGGAACT
241 ATTAAGTAGA CGACGTTAGT AAAATAACAA GCAACCAAAT CAGTTTAGAG AGATCACTTT
301 TTTCCCACGG GATTTTTCTA GTAAGATTTT AACCAGGCAT ATTATCTTCT AAATATATAG
361 CGAGTTAGTA TCATTATACT TTGTCTACAA ATTAAATTTC GATTACTCTG GGTAAACAAG
421 CCATATAGTA TGC

LA0392, *S. arcanum*, closely related to *S. peruvianum*; **GenBank no. EU033928**

SEQ: 377 bp;
Composition 129 A; 54 C; 65 G; 129 T; 0 OTHER
Percentage: 34.2% A; 14.3% C; 17.2% G; 34.2% T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 116.58 dsDNA: 232.35
ORIGIN
1 TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61 TGGAGTTTCT AAAATTTTGG AATATTCTGG CAAAATTTGA GCGGAGAAAT TGGCAGGTTC
121 TTACATCTTT TTACTGTTCT AAAAAGATGT CTACAATTCG TTTCATCAAA GCCCCGACGG
181 AACTATTAAG TAGACGACGT TAGTAAAATA ACAAGCAACC AAAGCAGTTT AGAGAGATCA
241 CTTTTTTCCC ATGGGATTTT TCTAGTAAGA TTTTAACCAG GCATATTATC TTCTAAATAT
301 GTAGCGAGTT AGTATCATTA TACTTTGTCT ACAAATTAAA TTTCGATTAC TCTGGGTAAA
361 CAAGCCATAT AGTATGC

LA3858, *S. peruvianum*, this species is the reported source of resistance for Mi gene; **GenBank no. EU033932**

SEQ: 377 bp;
Composition 129 A; 53 C; 67 G; 128 T; 0 OTHER
Percentage: 34.2% A; 14.1% C; 17.8% G; 34.0% T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 116.65 dsDNA: 232.35
ORIGIN
1 TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61 TGGAGTTTCT AAAATTTTGG AATATTCTGG CAAAATTTGA GCGGAGAAAT TGGCAGGTTC
121 TTACATCTTT TTACTGTTCT AAAAAGATGT CTACAATTCG TTTGATCAAA GCCCCGACGG
181 AACTATTAAG TAGACGAGGT TAGTAAAATA ACAAGCAACC AAAGCAGTTT AGAGAGATCA
241 CTTTTTTCCC ACGGGATTTT TCTAGTAAGA TTTTAACCAG GCATATTATC TTCTAAATAT
301 GTAGCGAGTT AGTATCATTA TACTTTGTCT ACAAATTAAA TTTCGATTAC TCTGGGTAAA
361 CAAGCCATAT AGTATGC

LA3900, *S. peruvianum*, this species is the reported source of resistance for Mi gene
SEQ: 377 bp;
Composition 129 A; 53 C; 67 G; 128 T; 0 OTHER
Percentage: 34.2% A; 14.1% C; 17.8% G; 34.0% T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 116.65 dsDNA: 232.35
ORIGIN
1 TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61 TGGAGTTTCT AAAATTTTGG AATATTCTGG CAAAATTTGA GCGGAGAAAT TGGCAGGTTC
121 TTACATCTTT TTACTGTTCT AAAAAGATGT CTACAATTCG TTTGATCAAA GCCCCGACGG
181 AACTATTAAG TAGACGAGGT TAGTAAAATA ACAAGCAACC AAAGCAGTTT AGAGAGATCA
241 CTTTTTTCCC ACGGGATTTT TCTAGTAAGA TTTTAACCAG GCATATTATC TTCTAAATAT
301 GTAGCGAGTT AGTATCATTA TACTTTGTCT ACAAATTAAA TTTCGATTAC TCTGGGTAAA
361 CAAGCCATAT AGTATGC

Gh2, Mi/Mi, resistant to root knot nematode and also begomoviruses with Ty1/Ty1, Ty3/Ty3; **GenBank no. EU033926**
SEQ: 377 bp;
Composition 129 A; 53 C; 67 G; 128 T; 0 OTHER
Percentage: 34.2% A; 14.1% C; 17.8% G; 34.0% T; 0.0%OTHER
Molecular Weight (kDa): ssDNA: 116.65 dsDNA: 232.35
ORIGIN
1 TGGAAAAATG TTGAATTTCT TTTGTAAGTG TACAAAGTTA AAATTATGAA AACAAGTATT
61 TGGAGTTTCT AAAATTTTGG AATATTCTGG CAAAATTTGA GCGGAGAAAT TGGCAGGTTC
121 TTACATCTTT TTACTGTTCT AAAAAGATGT CTACAATTCG TTTGATCAAA GCCCCGACGG
181 AACTATTAAG TAGACGAGGT TAGTAAAATA ACAAGCAACC AAAGCAGTTT AGAGAGATCA
241 CTTTTTTCCC ACGGGATTTT TCTAGTAAGA TTTTAACCAG GCATATTATC TTCTAAATAT
301 GTAGCGAGTT AGTATCATTA TACTTTGTCT ACAAATTAAA TTTCGATTAC TCTGGGTAAA
361 CAAGCCATAT AGTATGC

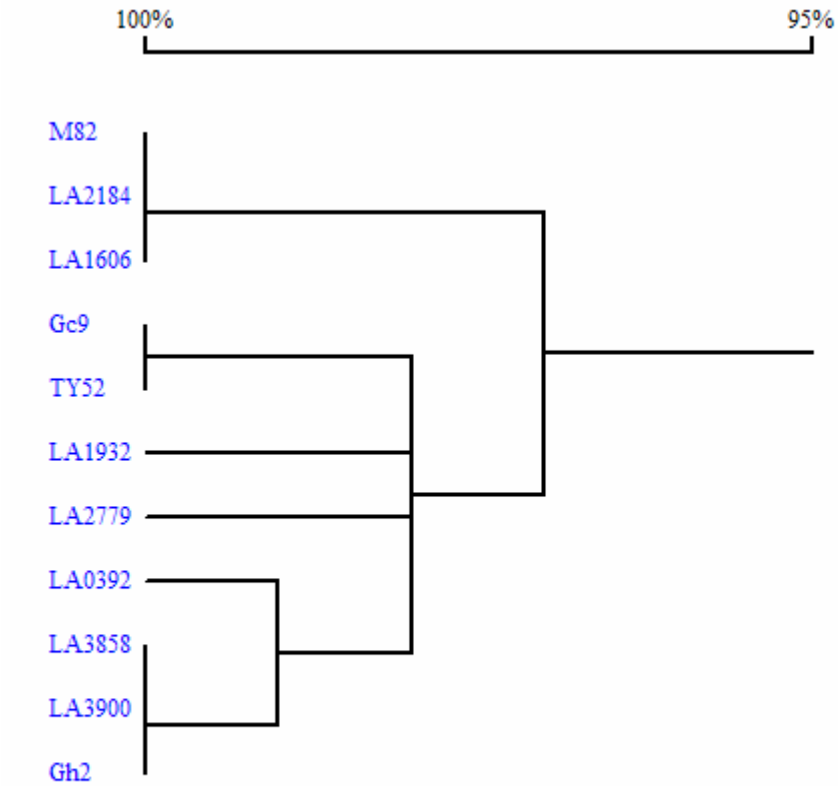
Calculations from DNAMAN software:

Distance matrix of 11 sequences

M82	0
LA1606	0.002 0
LA2184	0.000 0.002 0
Gc9	0.039 0.037 0.039 0
TY52	0.039 0.037 0.039 0.000 0
LA2779	0.037 0.035 0.037 0.021 0.021 0
LA1932	0.035 0.032 0.035 0.018 0.018 0.021 0
LA0392	0.029 0.027 0.029 0.021 0.021 0.019 0.019 0
LA3858	0.035 0.032 0.035 0.019 0.019 0.016 0.021 0.008 0
LA3900	0.035 0.032 0.035 0.019 0.019 0.016 0.021 0.008 0.000 0
Gh2	0.035 0.032 0.035 0.019 0.019 0.016 0.021 0.008 0.000 0.000 0

Homology matrix of 11 sequences (does not consider the large indel)

M82	100%
LA1606	99.8% 100%
LA2184	100.0% 99.8% 100%
Gc9	96.1% 96.3% 96.1% 100%
TY52	96.1% 96.3% 96.1% 100.0% 100%
LA2779	96.3% 96.5% 96.3% 97.9% 97.9% 100%
LA1932	96.5% 96.8% 96.5% 98.2% 98.2% 97.9% 100%
LA0392	97.1% 97.3% 97.1% 97.9% 97.9% 98.1% 98.1% 100%
LA3858	96.5% 96.8% 96.5% 98.1% 98.1% 98.4% 97.9% 99.2% 100%
LA3900	96.5% 96.8% 96.5% 98.1% 98.1% 98.4% 97.9% 99.2% 100.0% 100%
Gh2	96.5% 96.8% 96.5% 98.1% 98.1% 98.4% 97.9% 99.2% 100.0% 100.0% 100%



Homology Tree from DNAMAN software