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 MgCl₂, 0.1 µl (0.5 units) GoTaq 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 (Promega 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 mil/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 (mil/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 phylogenetically 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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References:
Milo, J. 2001. The PCR-based marker REX-1, linked to the gene Mi, can be used as a marker to TYLCV tolerance. Tomato Breeders Roundtable www.oardc.ohio-state.edu/tomato/TBRT%202001%20Abstracts.pdf


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.
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*; 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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LA2184     CCATATAGTATGC                                                   432
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TY52       CCATATAGTATGC                                                   433
LA2779     CCATATAGTATGC                                                   433
LA1932     CCATATAGTATGC                                                   433
LA0392     CCATATAGTATGC                                                   377
LA3858     CCATATAGTATGC                                                   377
LA3900     CCATATAGTATGC                                                   377
Gh2        CCATATAGTATGC                                                   377
Consensus  ccatatagtatgc

M82, mi/mi             GenBank no. EU033926
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

S. pimpinellifolium LA1606
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

S. pimpinellifolium LA2184  GenBank no. EU0033930
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
**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**

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361  CGAGTAGTA TATATTACTT TTGTCTACAA ATTTAAATTTC GATTACTCTG GTAAACAAG
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 Jerusalem)

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 TACAAAGTGA AAATTATGAA AACAAGTATT
61   TGGAGTTTCT AAAATTTTGG AATATTCTGG CTAAATTTGA GCGGAGAAAT GTGACAGTTC
121  ACGTCCAAT ATCCAGAGTC TTTCATACATA GAAGTGTCAA ACAATTTAGC AGGTTCTTAC
181  ATCTTTTTAC TGTTCCTAAA GAATGTCTAC AATTCGTTTC ATCAAAGCCC CGACGGAACT
241  ATTAAGTAGA CGAGGTATTG AAAATAACCA ACAAAACACT CATTGTAGAG AGATCACCTT
301  TTTCCTACGG AATTTTCTTA GTAAATTTTT AAACAGGCAAT ATATTCTCT AAATATATAG
361  CGAGTAGTA TATATTACTT TTGTCTACAA ATTTAAATTTC GATTACTCTG GTAAACAAG
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 TACAAAGTGA AAATTATGAA AACAAGTATT
61   TGGAGTTTCT AAAATTTTGG AATATTCTGG CTAAATTTGA GCGGAGAAAT GTGACAGTTC
121  ACGTCCAAT ATCCAGAGTC TTTCATACATA GAAGTGTCAA ACAATTTAGC AGGTTCTTAC
181  ATCTTTTTAC TGTTCCTAAA GAATGTCTAC AATTCGTTTC ATCAAAGCCC CGACGGAACT
241  ATTAAGTAGA CGAGGTATTG AAAATAACCA ACAAAACACT CATTGTAGAG AGATCACCTT
301  TTTCCTACGG AATTTTCTTA GTAAATTTTT AAACAGGCAAT ATATTCTCT AAATATATAG
361  CGAGTAGTA TATATTACTT TTGTCTACAA ATTTAAATTTC GATTACTCTG GTAAACAAG
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 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 ACAAAATTTA TTTTCGATTAC TCTGGGTAAA
361    CAAGCCATAT AGTATGC

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 TTTGATCAAA GCCCCGACGG
181    AACTATTAAG TAGACGAGGT TAGTAAAATA ACAAGCAACC AAAGCAGTTT AGAGAGATCA
241    CTTTTTTCCC ACGGGATTTT TCTAGTAAGA TTTTAACCAG GCATATTATC TTCTAAATAT
301    GTAGCGAGTT AGTATCATTA TACTTTGTCT ACAAAATTTA TTTTCGATTAC 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 ACAAAATTTA TTTTCGATTAC TCTGGGTAAA
361    CAAGCCATAT AGTATGC
**LA3900**, *S. peruvianum*, this species is the reported source of resistance for Mi gene

SEQ: 377 bp;

Composition: 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 TTCTAATAAT
301    GTAGCGAGTT AGTATCATTA TACTTTGTCT ACAAAATTTAA TTTCGATTAC TCTGGTAAA
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 TTCTAATAAT
301    GTAGCGAGTT AGTATCATTA TACTTTGTCT ACAAAATTTAA TTTCGATTAC TCTGGTAAA
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 0.016 0.008 0.000 0
LA3900              0.035 0.032 0.035 0.019 0.019 0.016 0.016 0.008 0.000 0.000 0
Gh2                0.035 0.032 0.035 0.019 0.019 0.016 0.016 0.008 0.000 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