Protocol III

The co-dominant SCAR marker, Mi23, for the *Mi-1* gene is a CAPS marker for the detection of *Ty-1* locus in tomato germplasm

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The *Ty-1* locus introgressed (4 to 9 cM) from *S. chilense* LA1969 was mapped to chromosome 6 (Zamir et al., 1994) and is associated within a region of resistance genes (see Pérez de Castro et al., 2007, Introduction: *Ty-1* locus, this web site). Since the *Ty-1* introgression overlaps the *Mi-1* introgression (root-knot nematode resistance), markers for the *Mi-1* gene have been used for the detection of the *Ty-1* introgression (El Mehrach et al., 2005; Milo, 2001). This report describes the development of a CAPS marker for Ty-1 introgression from the co-dominant SCAR marker, Mi23, for the *Mi-1* gene (ca. 6 cM). These markers amplify DNA between the *Mi-1.2* gene and *Mi-1.3* pseudogene in the cluster 2p (Sean and Williamson, pers. com.).

Material and Methods

Primers: 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' (Sean and Williamson, pers. com.).

PCR and Restriction Enzyme 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: 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. Approximately 10 µl of the PCR reaction was used in a 25 µl reaction mixture with *Rsa*I restriction enzyme (Promega Corp.) at 37 C for 2-4 hrs and fragments separated on 2% agarose gels in 0.5 XTBE buffer, stained with ethidium bromide, and visualized with UV light.

Results

Mi23-PCR fragment sizes were ca. 430 bp for the susceptible (*mi/mi*) genotypes M82-1-8 and Gh13, and ca. 380 bp for the root-root resistant (*Mi/Mi*) genotypes Motelle and Gh2. For heterozygous hybrids, Marwa and Llanero, three bands of 380, 430 and 550 bp were generally detected (Fig. 1). The larger band was a heteroduplex hybrid between the two smaller bands (first suggested by V. Williamson, UC-Davis). The 430-

bp Mi23-PCR fragments from M82 (mi/mi) and TY52 (*Ty-1/Ty-1*) were sequenced and aligned (Fig. 2). Sequences were checked for restriction sites and *Rsa*I would digest the M82 fragment into fragments of 31, 47 and 354 bp, where as only one restriction site was present in the fragment from TY52. This would result in fragments of 31 and 402 bp for TY52 introgression. Thus, after *Rsa*I digestion and electrophoresis, homozygous plants (*ty-1/ty-1*) would give the 354-bp fragment and this fragment is easily distinguished from the homozygous resistant plant (*Ty-1/Ty-1*) fragment of 402 bp. Heterozygous plants would have the two fragment sizes (Fig. 3).

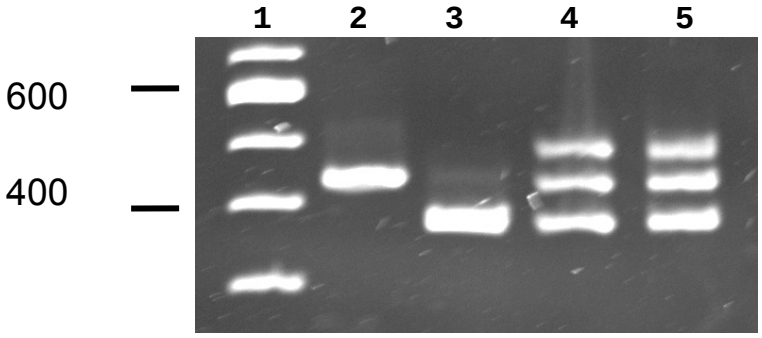

Fig. 1. PCR with primers Mi23F/Mi23R at annealing temperature of 57 C for detection of the *Mi-1* locus. Lane 1, 100-bp marker (Invitrogen); lane 2, M82-1-8 (*mi/mi*); lane 3, Motelle (*Mi/Mi*); lane 4, Marwa (VF2N and tolerance to *Tomato yellow leaf curl virus*); lane 5, Llanero (*Mi/mi*, as determined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

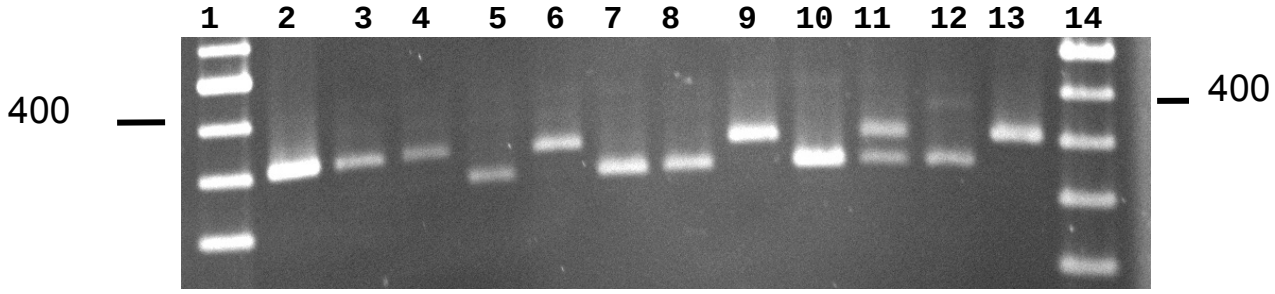

Fig. 3. Mi23-PCR fragments from tomato germplasm that were 430 bp (*mi/mi*) were then digested with *Rsa*I and separated on a 2% agarose gel. Lane 1, 100-bp marker (Invitrogen); lane 2, Gh25 (field code, 100a) (chil/chil); lane 3, Gc143-4 (chil/chil); lane 4, Ghp44-5 (chil/chil); lane 5, Gh23-1 (lyc/lyc); lane 6, Gc43-3 (chil/chil); lane 7, Ghp31-1 (lyc/lyc); lane 8, Ghp44-2 (lyc/lyc); lane 9, Gh25-1 (chil/chil); lane 10, GhT44-3 (lyc/lyc); lane 11, XA577 (F1) (lyc/chil); lane 12, *S. lycopersicum* var. *cerasiformae* (lyc/lyc); lane 13, Gc43 (field code, 68a) (chil/chil); lane 14, 100-bp marker (Invitrogen). Note: lyc = *S. lycopersicum*, *ty-1* allele; chil = *S. chilense*, *Ty-1* allele.

Other lines that gave the 430-bp fragment with Mi23F/R primers were digested with *Rsa*I. Heinz 1706 and the heritage tomato Purple Russian that have known *S. lycopersicum* sequences in this region gave the expected band at 354 bp. The TY52 line (Ty-1/Ty-1), which is homozygous for the Ty-1 introgression (*S. chilense*) gave a 402-bp fragment (TY52 was supplied by H. Czosnek, Hebrew University of Jerusalem, HUJ). The breeding lines Gc9 with a *S. chilense* introgression from LA2779 in this region and GIh902 from HUJ (see line 902 in Vidavsky and Czosnek, 1998), which had an identical introgression as Gc9 (Maxwell et al., unpublished data), also gave the same size fragment of 402 bp. The 430-bp fragment from GIh902 and Gc9 were sequenced and they had 100% nt identity with the sequence from TY52 introgression. The commercial hybrid Titrit (Royal Sluis) is sold in Morocco as *mi/mi* and tolerant to *Tomato yellow leaf curl virus*. It had a 430-bp fragment, which when digested gave the two fragment sizes for a heterozygous hybrid at this locus, and thus, it is suggested that it would have lyc/chil sequences for this Mi23 locus and the genotype *ty-1/Ty-1*.

This CAPS marker can also be used with heterozygous hybrids that are *Mi-1*/-- to determine the nature of the other allele. These hybrids would have the *S. peruvianum* introgression for the *Mi-1* locus, and then either *S. lycopersicum* or *S. chilense* sequences in this region for the other haplotype. The 380-bp fragment was sequenced for the Mi/Mi genotype (Gh2) and there was one *Rsa*I restriction site, which would result in fragment sizes of 31 and 346 bp (Fig. 2). For the heterozygous plants (Mi/--), the Mi23F/R primers give three fragments, 380 bp, 430 bp and 550 bp. The larger fragment results from the hybrid of the smaller two fragments. When the three fragments from the heterozygous *Mi/lyc* hybrid (Llanero) are digested with *Rsa*I, two fragments resulted, 350 and 400 bp. The 350-bp fragment is two fragments, one from the *S. lycopersicum* fragment (430 bp, two *Rsa*I sites) and one from the *S. peruvianum* fragment (380 bp, one *Rsa*I site). The 500-bp fragment resulted from digestion of the 550-bp fragment (the heteroduplex hybrid). For the three fragments from the heterozygous *Mi/chil* hybrid (Romelia), which would be the case for the hybrid with *Mi-1* gene and *Ty-1* gene in the uncoupling phase, three bands were produced (350, 400, and 500 bp) as each fragment only has one *Rsa*I site at nt 31 (Fig. 4). Marwa (*Mi/mi;Ty-1/ty-1*) had a digestion pattern similar to Romelia and this is what was expected from other sequence data for the TG97 CAPS marker (Garcia and Maxwell, unpublished).

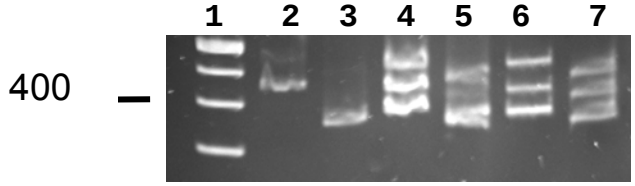

Fig. 4. Mi23F/R PCR fragments undigested and digested with *Rsa*I. Lane 1, 100-bp marker (Invitrogen); lane 2, plant (lyc/lyc) undigested; lane 3, plant (lyc/lyc) digested; lane 4, Llanero hybrid (per/lyc) undigested; lane 5, Llanero hybrid (per/lyc) digested; lane 6, Romelia hybrid (per/chl) undigested; lane 7, Romelia hybrid (per/lyc) digested. (Not the best of gels, sorry!) Note: lyc = *S. lycopersicum*, *ty-1* allele; chl = *S. chilense*, *Ty-1* allele, per = *S. peruvianum*.

Heterozygous plants with the Mi-1 locus present a problem with this Mi23 marker and digestion with *Rsa*I (see Fig. 4) as the digestion needs to be complete and also a good gel needs to be run. Since the heteroduplex hybrid is present, it is not possible to

obtain satisfactory sequence by direct sequencing of the PCR products. A better option is to use the CAPS marker for TG97 or CT21 (see other protocols at this web site). Since these CAPS markers produce fragments of the about the same size from the different genotypes, it is possible to also sequence these fragments and to use this information to confirm the restriction fragments. Thus, the CAPS marker, Mi23, is most useful to determine the genotype of homozygous plants (either, *ty-1/ty-1* or *Ty-1/Ty-1*). This CAPS marker would be useful in a breeding program where the genotype of the parents is known as then the heterozygous plants would not be an issue. **Also, it is important to note that this marker is not closely linked to the *Ty-1* gene, and thus, plants that are *Ty-1/Ty-1* could be scored as negative for this allele.** In our evaluations of germplasm from the tomato breeding program at San Carlos University, a plant with *S. lycopersicum* sequences at Mi23 and *S. chilense* sequences at TG97 has not been detected. But this genotype is possible.

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Reference:

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Fig. 2. Sequence alignment of Mi23F/R fragments from M82-1-8 (*mi/mi*), TY52 (*Ty1/Ty1*), and Gh2 (*Mi/Mi*;*Ty-1/Ty-1*). *Rsa*I restriction sites marked.

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                                        RsaI
M82         TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
TY52        TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
Gh2         TGGAAAAATGTTGAATTTCTTTTGTAAGTGTACAAAGTTAAAATTATGAAAACAAGTATT     60
Consensus   tggaaaaatgttgaatttcttttgtaagtgtacaaagttaaaattatgaaaacaagtatt

M82         TGGAGTTTCTAAAATTTTGGAATATTCTGGCAAAATTTGAGCGGAGAAATGTGACAGTTC    120
TY52        TGGAGTTTCTAAAATTTTGGAATATTCTGGCtAAATTTGAGCGGAGAAATGTGACAGTTC    120
Gh2         TGGAGTTTCTAAAATTTTGGAATATTCTGGCAAAATTTGAGCGGAGAAAT.........    110
Consensus   tggagtttctaaaattttggaatattctggc aaatttgagcggagaaat

M82         ACGTCCAAATCTCCAGAGTCTTCATACATAGAAGTGTCAAACAAATTGGCAGGTTCTTAC    180
TY52        ACGTCCAAATCTCCAGAGTCTTCATACATAGAAGTGTCAAACAAtTTaGCAGGTTCTTAC    180
Gh2         ............................................TGGCAGGTTCTTAC    124
Consensus                                                t gcaggttcttac
                  *
M82         ACCTTTT.ACTGTTCTAAAAAGATGTCTACAATTTGTTTCATCAAAGCCCCGACGGAACT    239
TY52        AtCTTTTtACTGTTCTAAAAAGATGTCTACAATTcGTTTCATCAAAGCCCCGACGGAACT    240
Gh2         AtCTTTTtACTGTTCTAAAAAGATGTCTACAATTcGTTTgATCAAAGCCCCGACGGAACT    184
Consensus   a ctttt actgttctaaaaagatgtctacaatt gttt atcaaagccccgacggaact

M82         ATTAAGTAGACGACGTTAGTAAAATAACAAGCAACCAAAGCAGTTACGAGAGATCACTTT    299
TY52        ATTAAGTAGACGAgGTTAGTAAAATAACAAGCAACCAAAtCAGTTtaGAGAGATCACTTT    300
Gh2         ATTAAGTAGACGAgGTTAGTAAAATAACAAGCAACCAAAGCAGTTtaGAGAGATCACTTT    244
Consensus   attaagtagacga gttagtaaaataacaagcaaccaaa cagtt  gagagatcacttt

M82         TTTCCCAGGGGATTTTTCTAGTAAGATTTTAATCAAGCACATTATCTACTAAATATATAG    359
TY52        TTTCCCAcGGaATTTTTCTAGTAAGATTTTAAcCAgGCAtATTATCTtCTAAATATATAG    360
Gh2         TTTCCCAcGGGATTTTTCTAGTAAGATTTTAAcCAgGCAtATTATCTtCTAAATATgTAG    304
Consensus   tttccca gg atttttctagtaagattttaa ca gca attatct ctaaatat tag
                                        RsaI
M82         CGAGTTAGTATCATTATACTTTGTGTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    419
TY52        CGAGTTAGTATtATTATACTTTGTcTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    420
Gh2         CGAGTTAGTATCATTATACTTTGTcTACAAATTAAATTTCGATTACTCTGGGTAAACAAG    364
Consensus   cgagttagtat attatactttgt tacaaattaaatttcgattactctgggtaaacaag

M82         CCATATAGTATGC                                                  432
TY52        CCATATAGTATGC                                                  433
Gh2         CCATATAGTATGC                                                  377
Consensus   ccatatagtatgc
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M82, mi/mi        GenBank no. EU033926
      SEQ: 432 bp;

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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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;

ORIGIN

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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
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Gh2, Mi/Mi, resistant to root knot nematode and also begomoviruses with Ty1/Ty1, Ty3/Ty3; **GenBank no. EU033926**

SEQ: 377 bp;

ORIGIN

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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
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