A CAPS marker, FER-G8, for detection of *Ty3* and *Ty3a* alleles associated with *S. chilense* introgressions for begomovirus resistance in tomato breeding lines

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7 April 2007

Several different accessions of *S. chilense* have been used as sources of begomovirus resistance genes. Zamir et al. (1994) used LA1969 as a source of *Ty-1* gene in chromosome 6 (ca. 8 cM). LA1969 was also the source of resistance against *Tomato yellow leaf curl virus* (TYLCV) for new lines developed in Cuba (Piñón et al., 2005). Scott and his team (Agrama and Scott, 2006; Scott, 2001; Scott et al., 1995) have used several accessions of *S. chilense* as sources of resistance to TYLCV and *Tomato mottle virus* (ToMoV). Lines from Scott's program at the University of Florida were evaluated in Guatemala (Mejía et al., 2005); and the line Gc9 with resistance from LA2779 and line Gc171 from LA2779/LA1932 were highly resistant to bipartite begomoviruses. The begomovirus resistance *Ty-1* and *Ty-3* loci on chromosome 6 for advanced breeding lines derived from LA2779 were mapped between C2_At2g39690 (5.3 cM) to T0834 (32 cM) (Ji et al., 2007). The partially dominant *Ty-3* gene was mapped region between cLEG-31-P16 (20 cM) and T1079 (27 cM) (Ji and Scott, 2006). The line Gc9 has an introgression from on chromosome 6 from the *REX-1* locus (6 cM) to T0834 (32 cM), which would include the loci for *Ty-1* and *Ty-3* (Maxwell, unpublished data). SCAR or CAPS markers have been developed to detect the *Ty-1* and *Ty-3* loci (see this web site). Unfortunately, the co-dominant SCAR marker, FLUW25, only detected the Ty-3 introgression from LA2779 and not the introgression from LA1932, line Gc171. Thus, it was expected that this Gc171 might have a different introgression. The FLUW25 primers amplify DNA in the region of the FER BAC clone (56B23, AY678298) at 25 cM on chromosome 6. Primers were designed for several of the genes associated with this BAC clone. For the gene 8 and gene 10, three different sequences were obtained for Heinz 1706 (*S. lycopersicum*), Gc9 (*S. chilense* LA2779), and Gc171 (*S. chilense* LA1932) (Martin and Maxwell, unpublished data). This report describes the development of a CAPS marker to detect the alleles associated with *S. lycopersicum* (*ty-1*), *S. chilense* LA2779 (*Ty-3*), and *S. chilense* LA1932 (*Ty-3a*) at gene 8 of the FER BAC clone (AY678298).

Material and Methods

Primers: FER-G8F1 is 5'-cat ccc gtg cat cat cca aag tga c -3', and FER-G8R1 is 5'-cta agg gtg tac ccc aag gga ac -3'. These primers matched the sequence of the G8 from nt 171,604 to 172,113 of the FER BAC clone (56B23, AY678298).

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 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, 55 C for 1 min, and 72 C for 1.5 min. These cycles were followed by 72 C for 10 min, and then the reaction was held at 4 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 *Taq*I restriction enzyme (Promega Corp.) at 65 C for 2-4 hrs and fragments separated on 2% agarose gels in 0.5X TBE buffer, stained with ethidium bromide and visualized with UV light.

Results and Discussion

The primer pair FER-G8F1/FER-G8R1 gave a 500-bp fragment with M82-1-8 (*S. lycopersicum*), Gc171, and GIh902 (this line has the same introgression as Gc9 for this region (6.5 cM to 32 cM), unpublished data). The sequences were aligned (Fig. 1) and the sequence for M82-1-8 did not have a *Taq*I site, the one for Gc171 had one site (*Ty-3a* locus) and the for GIh902 two *Taq*I sites (*Ty-3* locus). The FER-G8 PCR fragments were digested with *Taq*I and the fragment pattern was as expected for the three alleles (Fig. 2).

The relative importance of *Ty-3* and *Ty-3a* loci to contribute to begomovirus resistance is not known. In much of the germplasm in the tomato breeding program at San Carlos University, Guatemala, such as Gc9 and Ih902 (see 902 in Vidavsky and Czosnek, 1998), the *Ty-1* and *Ty-3* loci are present. In the case of Gc171, the *Ty-3a* allele is present, but not the *Ty-1* locus. In the future, it will be important to determine how closely lined this CAPS marker, FER-G8, is to the functional gene and also the contribution of this region to the resistance level. On this web site, the protocol for the co-dominant SCAR marker, P6-25, is available. This SCAR marker was developed from sequences near the 3' prime end of the FER BAC clone. Thus, it might be valuable to follow these two markers in segregating populations to see which marker is most closely linked to the functional gene.

References:

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Fig. 1. Sequence of FER-G8 fragment for M82 (*S. lycopersicum, ty-1* allele), Gc171 (most likely *S. chilense* LA1932, *Ty-3a* allele), and Ih902 (same introgression as Gc9 from *S. chilense*, LA2779, *Ty-3* allele). Sequence lengths are incorrect. *Taq*I restriction sites are marked.

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M82                         CATCCCGTGCATCATCCAAAGTGACTTCCCATATCCCTATTTA     60
Gc171                       CATCCCGTGCATCATCCAAAGTGACTTCCCATATCCCTATTTA     60
GIh902 .........            CATCCCGTGCATCATCCAAAGTGACTTCCCATATCCCTATTcA     51
Consensus                   catcccgtgcatcatccaaagtgacttcccatatccctatt a

M82         TACAGTTAGCAATTAGACAGCAGGTATGGAACCAAATTGATTAGAACGATAAAATCGGGA    120
Gc171       TACAGTTAGCAATTAGACAGCAGaTATGGAACCAAATTGATTAGAACGATAAAATtGGGA    120
GIh902      TACAGTTAGCAATTAGACAGCAGaTATGGAACCAAATTGATTAGAACGATAAAATtGGGA    111
Consensus   tacagttagcaattagacagcag tatggaaccaaattgattagaacgataaaat ggga

M82         GACAAAATAGTTGAATTATCTATTAAATCATACTCATTGCCAACACATTTAAGAA.GAGG    179
Gc171       GACAAAATAGTTGAATTATCTATTAAATCATACTCATTGCCAACACATTaAAGAAaGAGG    180
GIh902      GgCAAAATAGTTGAATTATCTATTAAATCATACTCATTGCCAACACATTaAgaAA.GAGG    170
Consensus   g caaaatagttgaattatctattaaatcatactcattgccaacacatt a  aa gagg
                                              TaqI
M82         AGTTTCTTAGGGACATAAAGAACTGCAGAAGCAATCGGCAACAATGAGATGTAAGGCATG    239
Gc171       AGTTTCTTAGGGACAaAAAGAAaTGCAGAAGCAATTCGaCAACAATGAGATGTAAGGCATG    240
GIh902      AGTTTCTTAGGGACATAAAGAAaTGCAGAAGCAATTCGaCAAaAATGAGATGTAAGGCATG    230
Consensus   agtttcttagggaca aaagaa tgcagaagcaatcg caa aatgagatgtaaggcatg
                                 TaqI
M82         GATGCCCTTGAATGTTAAGTTAGTCAAGCAAGCTCAAGCTGCAGAGCTGATACAGTATCT    299
Gc171       GATGCCCTTGAATGTTAAGTTAGTCAAGCAAGCTCAAGCTGCAGAGCTGATACAGTATCT    300
GIh902      GATGCCCTTGAATGTTAAGTTAGTCgAGCAAGCTCAAGCTGCAGAGCTGATACAGTATCT    290
Consensus   gatgcccttgaatgttaagttagtc agcaagctcaagctgcagagctgatacagtatct

M82         TACATGCTAGTTTGCATTTTGATTGATATATATCAGCCTGTACAAGACCTTCCAACAAAC    359
Gc171       TACATGCTAGTTTGCATTTTGATTGATATATATCAGCCTGTACAAGACCTTCCAACAAAC    360
GIH902      TACATGCTAGcTTGCATTTTGATTGATATATATCAGCCTGTACAAGACCTTCCAACAAAC    350
Consensus   tacatgctag ttgcattttgattgatatatatcagcctgtacaagaccttccaacaaac

M82         ACTACATACTAATCATGATGTTAATGCACATAACTACGGATGCACCAGCATTCAGAAGTT    419
Gc171       ACTACATACTAATCATGATGTTAATGCACATAACTACGGATGCACCAGCATTCAGAAGTT    420
GIh902      ACTACATACTAATCATGATGTTAATGCACATAACTACGGATGCACCAGCATTCAGAAGTT    410
Consensus   actacatactaatcatgatgttaatgcacataactacggatgcaccagcattcagaagtt

M82         CAAACTAACCTGCATGTTGAATGCTACCATCTGAGCTCCATGCATCCAGCCAGTCATAGG    479
Gc171       CAAACTAACCTGCATGTTGAATGCTACCATCTGAGCTCCATGCATCCAGCCAGTCATAGG    480
GIh902      CAAACTAACCTGCATGTTGAATGCTACCATCTGAGCTCCATGCATCCAGCCAGTCATAGG    470
Consensus   caaactaacctgcatgttgaatgctaccatctgagctccatgcatccagccagtcatagg

M82         CTTGAAATTTGAGGAGGTCACTCTTGTTCCCTTGGGGTACACCCTTAG               527
Gc171       CTTGAAATTTGAGGAGGTCACTCTTGTTCCCTTGGGGTACACCCTTAG               528
GIh902      CTTGAAATTTGAGGAGGTCACTCTTGTTCCCTTGGGGTACACCCTTAG               518
Consensus   cttgaaatttgaggaggtcactcttgttcccttggggtacacccttag
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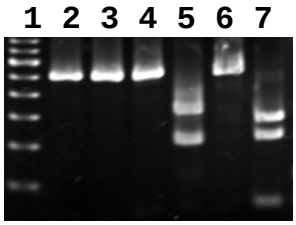

Fig. 2. *Taq*I digestion of the FER-G8 PCR fragments (500 bp). Lane 1, 100-bp marker (Invitrogen); lane 2, undigested Gc171; lane 3, undigested M82; lane 4, undigested GIh902; lane 5, digested Gc171; lane 6, digested M82; lane 7, digested GIh902.